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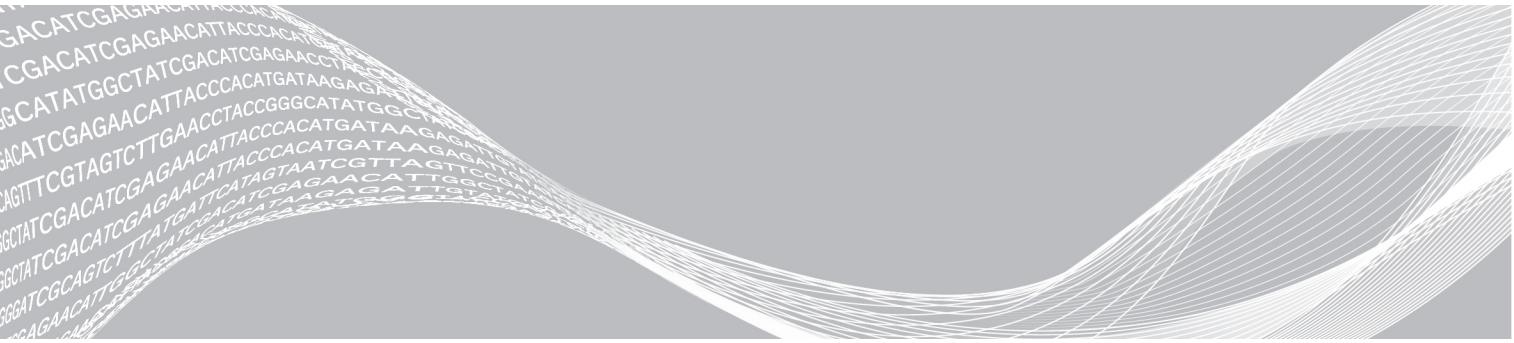
# Illumina DRAGEN Bio-IT Platform 3.7

## User Guide



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# Revision History

| Document | Date | Description of Change |
|---|---|---|
| Document # 1000000141465 | October 2020 | Adding information on the following features:<br>• Single-cell RNA<br>• Systematic noise filtering<br>• DRAGEN Graph Mapper<br>• Forced genotyping for SV calling<br>• Exome auto-detect for SV calling<br>• BCL Conversion<br>• Input streaming<br>• CYP2D6 options<br>• RNA sorting and duplicate marking<br>• CNV Coverage Uniformity<br>Added the following information:<br>• CNV metrics file<br>• Tumor-normal pairs input for Somatic mode<br>• Joint detection in small variant calling<br>• Sequencing input recommendations and quality checks for SV calling. |

# Table of Contents

# Getting Started

Before you begin, make sure that the Illumina® DRAGEN™ Bio-IT Platform server is turned on and that you are logged in.

This Getting Started section helps you to start processing data as quickly as possible. It provides instructions for the following:

DRAGEN provides tests you can run to make sure that your DRAGEN system is properly installed and configured. Before running the tests, make sure that the DRAGEN server has adequate power and cooling, and is connected to a network that is fast enough to move your data to and from the machine with adequate performance.

## DRAGEN Bio-IT Platform Overview

The Illumina DRAGEN™ Bio-IT Platform is based on the highly reconfigurable DRAGEN Bio-IT Processor, which is integrated on a Field Programmable Gate Array (FPGA) card and is available in a preconfigured server that can be seamlessly integrated into bioinformatics workflows. The platform can be loaded with highly optimized algorithms for many different NGS secondary analysis pipelines, including the following:

- Whole genome
- Exome
- RNA-Seq
- Methylome
- Cancer

All interaction is accomplished via DRAGEN software that runs on the host server and manages all communication with the DRAGEN board.

This user guide summarizes the technical aspects of the system and provides detailed information for all DRAGEN command line options.

If you are working with DRAGEN for the first time, Illumina recommends that you first read the *Getting Started* on page 1 section, which provides a short introduction to DRAGEN, including running a test of the server, generating a reference genome, and running example commands.

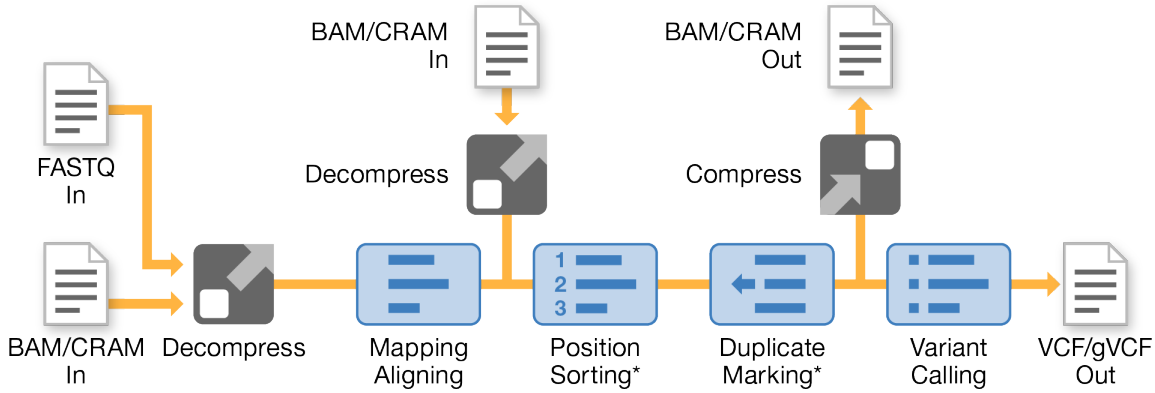
### Input Requirements

The following are the supported input specifications for DRAGEN.

| Specifications | Requirement |
|---|---|
| Supported Input Files | cBCL, FASTQ, BAM, CRAM, GVCF |
| Upper Limit | 300x coverage of human Whole Genome sequencing data.<br>200x/100x coverage of human T/N. |

## DRAGEN DNA Pipeline

Figure 1 DRAGEN DNA Pipeline




* Optional

The DRAGEN DNA Pipeline accelerates the secondary analysis of NGS data. For example, the time taken to process an entire human genome at 30x coverage is reduced from approximately 10 hours (using the current industry standard, BWA-MEM+GATK-HC software) to approximately 20 minutes. Time scales linearly with coverage depth.

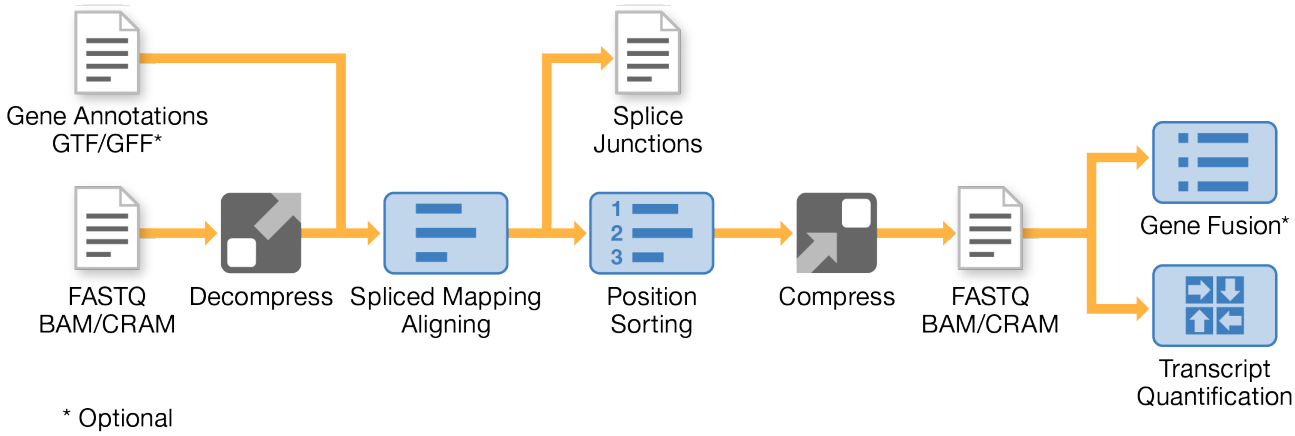
These pipelines harness the tremendous power of the DRAGEN Bio-IT Platform and include highly optimized algorithms for mapping, aligning, sorting, duplicate marking, and haplotype variant calling. They also use platform features such as hardware-accelerated compression and optimized BCL conversion, together with the full set of platform tools.

Unlike all other secondary analysis methods, DRAGEN DNA Applications do not reduce accuracy to achieve speed improvements. Accuracy for both SNPs and INDELs is improved over that of BWA-MEM+GATK-HC in side-by-side comparisons.

In addition to haplotype variant calling, the pipeline supports calling of copy number and structural variants as well as detection of repeat expansions.

### DRAGEN RNA Pipeline

DRAGEN includes an RNA-seq (splicing-aware) aligner, as well as RNA-specific analysis components for gene expression quantification and gene fusion detection.




The DRAGEN RNA Pipeline shares many components with the DNA Pipeline. Mapping of short seed sequences from RNA-Seq reads is performed similarly to mapping DNA reads. In addition, splice junctions (the joining of noncontiguous exons in RNA transcripts) near the mapped seeds are detected and incorporated into the full read alignments.

### DRAGEN Methylation Pipeline

The DRAGEN Methylation Pipeline provides support for automating the processing of bisulfite sequencing data to generate a BAM with the tags required for methylation analysis and reports detailing the locations with methylated cytosines.

## Software Installation

If you are already running the latest version of the DRAGEN software and hardware, you can skip ahead to *Run the Self Test* on page 1.

You can query the current version of software and hardware with the following command:

```
dragen_info -b
```

You can query only the software version with the following command:

```
dragen --version
```

To install a new version of software or hardware, first download the package from the DRAGEN Bio-IT Platform support pages on the Illumina website to your DRAGEN server. The preferred installation method is to use the self-extracting .run file, as follows:

```
sudo sh dragen-3.3.7.el7.x86_64.run
```

During installation, if you are prompted to switch to a new hardware version, enter 'y'. It is important that the hardware upgrade process is not interrupted. When it is complete, you must halt and power cycle the server. A reboot command does not update the hardware version. You must use the following halt command to power the server off and on:

```
sudo ipmitool chassis power cycle
```

DRAGEN periodically checks for license renewal by communicating with the license server at lus.edicogenome.com. For servers that are behind a firewall, a proxy can be configured by the network administrator in `/etc/environment`. For example:

```
http_proxy="http://proxy.customer.com:80/"
https_proxy="https://proxy.customer.com:80/"
ftp_proxy="http://proxy.customer.com:80/"
rsync_proxy="http://proxy.customer.com:80/"
no_proxy="localhost,127.0.0.1,localaddress,.localdomain.com,.customer.com"
```

# System Updates

DRAGEN is a flexible and extensible platform that is highly reconfigurable. Your DRAGEN subscription allows you to download updates to the DRAGEN processors and software. These updates provide speed, performance, throughput, and accuracy improvements.

# License Usage

To check current license usage and expiration date, use the following command:

```
dragen_lic -f genome
```

The license information is output, as follows:

```
LICENSE_MSG| ---- Board #0 (1234565) ----
LICENSE_MSG| License Genome: used 1000.0/100000 Gbases since 2019-Jan-01
(1000000000000 bases, 1.0%)
LICENSE_MSG| Issued=2019-Jan-01, start=2019-Jan-01, expiry=2020-Jan-01,
period=12 months

LICENSE_MSG| -- License dongle
LICENSE_MSG| STATUS : OK
LICENSE_MSG| DONGLE SN: 0012345678900
LICENSE_MSG| RELEASE : 2016.07p5-19358
LICENSE_MSG| CHIPID : 001234567890EAD
LICENSE_MSG| DNA: active, accelerators=DNA
LICENSE_MSG| issue=2019-Jan-01, start=2019-Jan-01, expiry=2020-Jan-01
LICENSE_MSG| RNA: active, accelerators=RNA
LICENSE_MSG| issue=2019-Jan-01, start=2019-Jan-01, expiry=2020-Jan-01
LICENSE_MSG| GZIP: active, accelerators=GZIP
LICENSE_MSG| issue=2019-Jan-01, start=2019-Jan-01, expiry=2020-Jan-01
LICENSE_MSG| GUNZ: active, accelerators=GUNZ
```

```
LICENSE_MSG| issue=2019-Jan-01, start=2019-Jan-01, expiry=2020-Jan-01
LICENSE_MSG| HMM: active, accelerators=HMM
LICENSE_MSG| issue=2019-Jan-01, start=2019-Jan-01, expiry=2020-Jan-01
LICENSE_MSG| SMW: active, accelerators=SMW
LICENSE_MSG| issue=2019-Jan-01, start=2019-Jan-01, expiry=2020-Jan-01
LICENSE_MSG| RANS: active, accelerators=RANS
LICENSE_MSG| issue=2019-Jan-01, start=2019-Jan-01, expiry=2020-Jan-01
LICENSE_MSG| GRAPH: active, accelerators=GRAPH
LICENSE_MSG| issue=2019-Jan-01, start=2019-Jan-01, expiry=2020-Jan-01
```

The above license output example is for the Genome license. The first line shows that 1000 gigabases have been consumed. The license installed is for 100000 gigabases and 1% of the gigabases been used. The second line shows the license data and it is the expiry date that is important. The licenses expires either at the expiry date or when 100% of the licensed gigabases are consumed.

Following the license data is the license information that is stored on the dongle or USB key attached to the server. These lines show the status of all the accelerators that are enabled and they are specific to the different pipelines. The accelerators also have an expiry date which should be similar to the license for each pipelines and similar to the genome license example.

To obtain a new license, contact your customer service representative at customerservice@illumina.com. If you encounter problems using your license, contact Illumina Technical Support at techsupport@illumina.com.

# Running the System Check

After turning on the server, you can make sure that your DRAGEN server is functioning properly by running `/opt/edico/self_test/self_test.sh`, which does the following:

- Automatically indexes chromosome M from the hg19 reference genome
- Loads the reference genome and index
- Maps and aligns a set of reads
- Saves the aligned reads in a BAM file
- Asserts that the alignments exactly match the expected results

Each server ships with the test input FASTQ data for this script, which is located in `/opt/edico/self_test`. The system check takes approximately 25–30 minutes.

The following example shows how to run the script and shows the output from a successful test.

```
[root@edico2 ~]# /opt/edico/self_test/self_test.sh
---------------------------------
test hash creating
test hash created
---------------------------------
reference loading /opt/edico/self_test/ref_data/chrM/hg19_chrM
reference loaded
```

```
--------------------------------

real0m0.640s
user0m0.047s
sys0m0.604s
not properly paired and unmapped input records percentages: PASS
--------------------------------
md5sum check dbam sorted: PASS
--------------------------------
SELF TEST COMPLETED
SELF TEST RESULT : PASS
```

If the output BAM file does not match expected results, then the last line of the above text is as follows:

```
SELF TEST RESULT : FAIL
```

If you experience a FAIL result after running this test script immediately after turning on your DRAGEN server, contact Illumina Technical Support.

## Running Your Own Test

When you are satisfied that your DRAGEN system is performing as expected, you are ready to run some of your own data through the machine, as follows:

- Load the reference table for the reference genome
- Determine location of input and output files
- Process input data

## Generate a Reference

If you do not have a reference, you can generate one by using the *dragen –build-hash-table* command and passing in the location of the reference FASTA file. You can specify a set of parameters when building your hash table (see the *DRAGEN Bio-IT Platform User Guide* (1000000070494)).

For testing purposes, you can run the example shell script or the one of commands shown in the examples in this guide. For these examples, the FASTA file is assumed to be located in `/staging/human/reference/hg19/hg19.fa`. Change the path in the script or command line to the correct directory, if needed. You must have change access to `/staging/human/reference` and its subdirectories.

Run the example shell script as follows:

```
/opt/edico/examples/build_hash_table.sh
```

Or, run the dragen command as follows:

```
mkdir -p /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149
cd /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
```

```
dragen --build-hash-table true --ht-reference
/staging/human/reference/hg19/hg19.fa \
--output-dir /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
--ht-alt-liftover /opt/edico/liftover/hg19_alt_liftover.sam
```

If you generate a hash table without including the *--ht-alt-liftover* option, an error similar to the following may occur (depending on the .fa reference file used):

```
ERROR: Detected hg19 alternate contigs in reference at:
/staging/hg19fa/hg19.fa
DRAGEN map quality is significantly improved by building a reference with a
liftover file to enable ALT aware mapping. Use the --ht-alt-lifeover option
to specify a liftover file.
You may ignore this error and continue using your existing reference by
adding --ht-alt-aware-validate=false to your command line. However, DRAGEN
map quality will be significantly affected.
Generate the hash table with either the --ht-alt-liftover or the –ht alt-
aware-validate=false option to avoid the error listed above.
```

The *dragen --build-hash-table* command is multithreaded and defaults to eight threads. This command takes approximately 15 minutes to run. You can use the *--ht-num-threads* option with a value up to 32 (depending on the number of threads your server supports) to reduce the run time.

The hash table directory name lists key default option values that were used during the hash table build. Illumina recommends following this best practice when you generate your own hash tables and change the directory name accordingly.

If you enabled the CNV function, generating a hash table takes ~2 hours.

## Generate an HG19 Reference

If you do not have a FASTA reference, you can get the hg19 FASTA files from UCSC and concatenate them into a single hg19.fa file as follows:

```
mkdir /staging/hg19fa
cd /staging/hg19fa
wget
hgdownload.cse.ucsc.edu/goldenPath/hg19/bigZips/chromFa.tar.gz
tar -zxvf chromFa.tar.gz
cat chr*.fa > hg19.fa
```

Generate the DRAGEN hash table reference using the following commands.

```
mkdir /staging/hg19/
dragen --ht-reference /staging/hg19fa/hg19.fa \
```

```
--output-directory /staging/hg19/ --build-hash-table true \
--ht-alt-liftover /opt/edico/liftover/hg19_alt_liftover.sam
```

### Load the Reference Genome

After the binary reference is loaded into memory on the DRAGEN board, it can be used for processing any number of input data sets. You do not need to reload the reference unless you restart the system, or need to use a different reference hash table.

The reference is loaded automatically the first time you process data with it. You can manually load the reference genome onto the board by using the following shell script or command. The reference directory in this example is /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149.

```
/opt/edico/examples/load_reference.sh
```

OR

```
dragen -l \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149
```

This command loads the binary reference genome into memory on the DRAGEN board, where it is used for processing any number of input data sets. You do not need to reload the reference genome unless you restart the system or need to switch to a different reference genome. It can take up to a minute to load a reference genome.

DRAGEN checks whether the specified reference genome is already resident on the board. If it is, then the upload of the reference genome is automatically skipped. You can force reloading of the same reference genome using the *force-load-reference (-l)* command line option.

The command to load the reference genome prints the software and hardware versions to standard output. For example:

```
DRAGEN Host Software Version 01.001.035.01.00.30.6682 and
Bio-IT Processor Version 0x1001036
```

After the reference genome has been loaded, the following message is printed to standard output:

```
DRAGEN finished normally
```

## Prepare a Reference Genome

Before a reference genome can be used with DRAGEN, it must be converted from FASTA format into a custom binary format for use with the DRAGEN hardware. The options used in this preprocessing step offer tradeoffs between performance and mapping quality.

The DRAGEN system is shipped with reference genomes hg19 and GRCh37. Both reference genomes are preinstalled based on recommended settings for general applications. If you find that performance and mapping quality are adequate, there is a good chance that you can simply work with these supplied reference genomes. Depending on your read lengths and other particular aspects of your application, you may be able to improve mapping quality and/or performance by tuning the reference preprocessing options.

## Hash Table Background

The DRAGEN mapper extracts many overlapping seeds (subsequences or K-mers) from each read, and looks up those seeds in a hash table residing in memory on its PCIe card, to identify locations in the reference genome where the seeds match. Hash tables are ideal for extremely fast lookups of exact matches. The DRAGEN hash table must be constructed from a chosen reference genome using the *dragen --build-hash-table* option, which extracts many overlapping seeds from the reference genome, populates them into records in the hash table, and saves the hash table as a binary file.

### Reference Seed Interval

The size of the DRAGEN hash table is proportionate to the number of seeds populated from the reference genome. The default is to populate a seed starting at every position in the reference genome, ie, roughly 3 billion seeds from a human genome. This default requires at least 32 GB of memory on the DRAGEN PCIe board.

To operate on larger, nonhuman genomes or to reduce hash table congestion, it is possible to populate less than all reference seeds using the *--ht-ref-seed-interval* option to specify an average reference interval. The default interval for 100% population is *--ht-ref-seed-interval 1*, and 50% population is specified with *--ht-ref-seed-interval 2*. The population interval does not need to be an integer. For example, *--ht-ref-seed-interval 1.2* indicates 83.3% population, with mostly 1-base and some 2-base intervals to achieve a 1.2 base interval on average.

### Hash Table Occupancy

It is characteristic of hash tables that they are allocated a certain size, but always retain some empty records, so they are less than 100% occupied. A healthy amount of empty space is important for quick access to the DRAGEN hash table. Approximately 90% occupancy is a good upper bound. Empty space is important because records are pseudo-randomly placed in the hash table, resulting in an abnormally high number of records in some places. These congested regions can get quite large as the percentage of empty space approaches zero, and queries by the DRAGEN mapper for some seeds can become increasingly slow.

### Hash Table / Seed Length

The hash table is populated with reference seeds of a single common length. This primary seed length is controlled with the *--ht-seed-len* option, which defaults to 21.

The longest primary seed supported is 27 bases when the table is 8 GB to 31.5 GB in size. Generally, longer seeds are better for run time performance, and shorter seeds are better for mapping quality (success rate and accuracy). A longer seed is more likely to be unique in the reference genome, facilitating fast mapping without needing to check many alternative locations. But a longer seed is also

more likely to overlap a deviation from the reference (variant or sequencing error), which prevents successful mapping by an exact match of that seed (although another seed from the read may still map), and there are fewer long seed positions available in each read.

Longer seeds are more appropriate for longer reads, because there are more seed positions available to avoid deviations.

Table 1 Seed Length Recommendations

| Value for *-ht-seed-len* | Read Length |
|---|---|
| 21 | 100 bp to 150 bp |
| 17 to 19 | shorter reads (36 bp) |
| 27 | 250+ bp |

## Hash Table / Seed Extensions

Due to repetitive sequences, some seeds of any given length match many locations in the reference genome. DRAGEN uses a unique mechanism called seed extension to successfully map such high-frequency seeds. When the software determines that a primary seed occurs at many reference locations, it extends the seed by some number of bases at both ends, to some greater length that is more unique in the reference.

For example, a 21-base primary seed may be extended by 7 bases at each end to a 35-base extended seed. A 21-base primary seed may match 100 places in the reference. But 35-base extensions of these 100 seed positions may divide into 40 groups of 1–3 identical 35-base seeds. Iterative seed extensions are also supported, and are automatically generated when a large set of identical primary seeds contains various subsets that are best resolved by different extension lengths.

The maximum extended seed length, by default equal to the primary seed length plus 128, can be controlled with the *--ht-max-ext-seed-len* option. For example, for short reads, it is advisable to set the maximum extended seed shorter than the read length, because extensions longer than the whole read can never match.

It is also possible to tune how aggressively seeds are extended using the following options (advanced usage):

- *--ht-cost-coeff-seed-len*
- *--ht-cost-coeff-seed-freq*
- *--ht-cost-penalty*
- *--ht-cost-penalty-incr*

There is a tradeoff between extension length and hit frequency. Faster mapping can be achieved using longer seed extensions to reduce seed hit frequencies, or more accurate mapping can be achieved by avoiding seed extensions or keeping extensions short, while tolerating the higher hit frequencies that result. Shorter extensions can benefit mapping quality both by fitting seeds better between SNPs, and

by finding more candidate mapping locations at which to score alignments. The default extension settings along with default seed frequency settings, lean aggressively toward mapping accuracy, with relatively short seed extensions and high hit frequencies.

The defaults for the seed frequency options are as follows:

| Option | Default |
|---|---|
| *--ht-cost-coeff-seed-len* | 1 |
| *--ht-cost-coeff-seed-freq* | 0.5 |
| *--ht-cost-penalty* | 0 |
| *--ht-cost-penalty-incr* | 0.7 |
| *--ht-max-seed-freq* | 16 |
| *--ht-target-seed-freq* | 4 |

## Seed Frequency Limit and Target

One primary or extended seed can match multiple places in the reference genome. All such matches are populated into the hash table, and retrieved when the DRAGEN mapper looks up a corresponding seed extracted from a read. The multiple reference positions are then considered and compared to generate aligned mapper output. However, *dragen* enforces a limit on the number of matches, or frequency, of each seed, which is controlled with the *--ht-max-seed-freq* option. By default, the frequency limit is 16. In practice, when the software encounters a seed with higher frequency, it extends it to a sufficiently long secondary seed that the frequency of any particular extended seed pattern falls within the limit. However, if a maximum seed extension would still exceed the limit, the seed is rejected, and not populated into the hash table. Instead, *dragen* populates a single High Frequency record.

This seed frequency limit does not tend to impact DRAGEN mapping quality notably, for two reasons. First, because seeds are rejected only when extension fails, only extremely high-frequency primary seeds, typically with many thousands of matches are rejected. Such seeds are not very useful for mapping. Second, there are other seed positions to check in a given read. If another seed position is unique enough to return one or more matches, the read can still be properly mapped. However, if all seed positions were rejected as high frequency, often this means that the entire read matches similarly well in many reference positions, so even if the read were mapped it would be an arbitrary choice, with very low or zero MAPQ.

Thus, the default frequency limit of 16 for *--ht-max-seed-freq* works well. However, it may be decreased or increased, up to a maximum of 256. A higher frequency limit tends to marginally increase the number of reads mapped (especially for short reads), but commonly the additional mapped reads have very low or zero MAPQ. This also tends to slow down DRAGEN mapping, because correspondingly large numbers of possible mappings are occasionally considered.

In addition to a frequency limit, a *target* seed frequency can be specified with *--ht-target-seed-freq* option. This target frequency is used when extensions are generated for high frequency primary seeds. Extension lengths are chosen with a preference toward extended seed frequencies near the target. The default of 4 for *--ht-target-seed-freq* means that the software is biased toward generating shorter seed extensions than necessary to map seeds uniquely.

## Handling Decoy Contigs

The behavior of DRAGEN with respect to the handling of decoy contigs in the reference has changed since version 2.6.

Starting with DRAGEN 3.x, DRAGEN's hash table builder automatically detects the absence of the decoy contigs from the reference and adds it to the FASTA file, prior to building the hash table. The decoys file is found at `/opt/edico/liftover/hs_decoys.fa`. If the reference is missing the decoy contigs, then the reads which map to the decoy contigs are artificially marked as unmapped in the output BAM (because the original reference does not have the decoy contig). This results in an artificially lower mapping rate, however, the accuracy of variant calling is improved thanks to removing false positive caused by decoy reads.

Illumina recommends using this feature by default. However, you can to set the *--htsuppress-decoys* option to true to suppress adding these decoys to the hash table.

The table below describes the difference in behavior between older DRAGEN versions (2.6 and earlier) and DRAGEN 3.x versions with respect to the handling of decoy contigs in the hash table builder:

| DRAGEN Behavior | DRAGEN 2.6 and earlier versions | DRAGEN 3.x |
|---|---|---|
| Reference does not include the decoy contigs (eg, GRCh37) | Decoy reads mismap elsewhere in the genome due to the lack of contigs in the reference.<br>• Artificially higher mapping rate.<br>• False positive calls in noisy regions to which the decoy contigs are mismapped. | DRAGEN automatically detects the absence of the decoy contig from the reference and adds it to the FASTA file.<br>• Artificially lower mapping rate (because decoy reads which map to the decoy contigs are artificially marked as unmapped in the output BAM (because the original reference does not have the decoy contig) .<br>• False positive calls are avoided thanks to adding the decoy contigs under the hood. Therefore this helps variant calling. |

| DRAGEN Behavior | DRAGEN 2.6 and earlier versions | DRAGEN 3.x |
|---|---|---|
| Reference includes the decoy contigs (eg, hs37d5) | Decoy reads map to the decoy contigs.<br>• High mapping rate<br>• No false positive calls caused by decoy reads because decoy reads map to the right place | Decoy reads map to the decoy contig.<br>• High mapping rate<br>• No false positive calls caused by decoy reads because decoy reads map to the right place |

## ALT-Aware Hash Tables

To enable ALT-aware mapping in DRAGEN, build GRch38 (and other references with ALT contigs) with a liftover file by using the *--ht-alt-liftover* option. The hash table builder classifies each reference sequence as primary or alternate based on the liftover file, and packs primaries before alternates in reference.bin. SAM liftover files for hg38DH and hg19 are in the `/opt/edico/liftover` folder. The *--ht-alt-liftover* option specifies the path to the liftover file to build an ALT-aware hash table.

To override the liftover file requirement, set the *--ht-alt-aware-validate* option to false when building the hash tables and when running dragen.

### Custom Liftover Files

Custom liftover files can be used in place of those provided with DRAGEN. Liftover files must be SAM format, but no SAM header is required. SEQ and QUAL fields can be omitted ('*'). Each alignment record should have an alternate haplotype reference sequence name as QNAME, indicating the RNAME and POS of its liftover alignment in a destination (normally primary assembly) reference sequence.

Reverse-complemented alignments are indicated by bit 0x10 in FLAG. Records flagged unmapped (0x4) or secondary (0x100) are ignored. The CIGAR may include hard or soft clipping, leaving parts of the ALT contig unaligned.

A single reference sequence cannot serve as both an ALT contig (appearing in QNAME) and a liftover destination (appearing in RNAME). Multiple ALT contigs can align to the same primary assembly location. Multiple alignments can also be provided for a single ALT contig (extras optionally be flagged 0x800 supplementary), such as to align one portion forward and another portion reverse-complemented. However, each base of the ALT contig only receives one liftover image, according to the first alignment record with an M CIGAR operation covering that base.

SAM records with QNAME missing from the reference genome are ignored, so that the same liftover file may be used for various reference subsets, but an error occurs if any alignment has its QNAME present but its RNAME absent.

## Command Line Options

Use the *--build-hash-table* option to transform a reference FASTA into the hash table for DRAGEN mapping. It takes as input a FASTA file (multiple reference sequences being concatenated) and a preexisting output directory and generates the following set of files:

| | |
|---|---|
| `reference.bin` | The reference sequences, encoded in 4 bits per base. Four-bit codes are used, so the size in bytes is roughly half the reference genome size. In between reference sequences, N are trimmed and padding is automatically inserted. For example, hg19 has 3,137,161,264 bases in 93 sequences. This is encoded in 1,526,285,312 bytes = 1.46 GB, where 1 GB means 1 GiB or $2^{30}$ bytes. |
| `hash_table.cmp` | Compressed hash table. The hash table is decompressed and used by the DRAGEN mapper to look up primary seeds with length specified by the *--ht-seed-len* option and extended seeds of various lengths. |
| `hash_table.cfg` | A list of parameters and attributes for the generated hash table, in a text format. This file provides key information about the reference genome and hash table. |
| `hash_table.cfg.bin` | A binary version of `hash_table.cfg` used to configure the DRAGEN hardware. |
| `hash_table_stats.txt` | A text file listing extensive internal statistics on the constructed hash including the hash table occupancy percentages. This table is for information purposes. It is not used by other tools. |

Build command usage is as follows:

```
dragen --build-hash-table true [options] --ht-reference <reference.fasta> --output-directory <outdir>
```

The sections that follow provide information on the options for building a hash table.

### Input/Output Options

The *--ht-reference* and *--output-directory* options are required for building a hash table. The *--ht-reference* option specifies the path to the reference FASTA file, while *--output-directory* specifies a preexisting directory where the hash table output files are written. Illumina recommends organizing various hash table builds into different folders. As a best practice, folder names should include any nondefault parameter settings used to generate the contained hash table. The sequence names in the reference FASTA file must be unique.

### Primary Seed Length

The *--ht-seed-len* option specifies the initial length in nucleotides of seeds from the reference genome to populate into the hash table. At run time, the mapper extracts seeds of this same length from each read, and looks for exact matches (unless seed editing is enabled) in the hash table.

The maximum primary seed length is a function of hash table size. The limit is k=27 for table sizes from 16 GB to 64 GB, covering typical sizes for whole human genome, or k=26 for sizes from 4 GB to 16 GB.

The minimum primary seed length depends mainly on the reference genome size and complexity. It needs to be long enough to resolve most reference positions uniquely. For whole human genome references, hash table construction typically fails with k < 16. The lower bound may be smaller for shorter genomes, or higher for less complex (more repetitive) genomes. The uniqueness threshold of *--ht-seed-len 16* for the 3.1Gbp human genome can be understood intuitively because log4(3.1 G) ≈ 16, so it requires at least 16 choices from 4 nucleotides to distinguish 3.1 G reference positions.

### Accuracy Considerations

For read mapping to succeed, at least one primary seed must match exactly (or with a single SNP when edited seeds are used). Shorter seeds are more likely to map successfully to the reference, because they are less likely to overlap variants or sequencing errors, and because more of them fit in each read. So for mapping accuracy, shorter seeds are mainly better.

However, very short seeds can sometimes reduce mapping accuracy. Very short seeds often map to multiple reference positions, and lead the mapper to consider more false mapping locations. Due to imperfect modeling of mutations and errors by Smith-Waterman alignment scoring and other heuristics, occasionally these noise matches may be reported. Run time quality filters such as *--Aligner.aln_min_score* can control the accuracy issues with very short seeds.

### Speed Considerations

Shorter seeds tend to slow down mapping, because they map to more reference locations, resulting in more work such as Smith-Waterman alignments to determine the best result. This effect is most pronounced when primary seed length approaches the reference genome's uniqueness threshold, eg, K=16 for whole human genome.

### Application Considerations

- **Read Length**—Generally, shorter seeds are appropriate for shorter reads, and longer seeds for longer reads. Within a short read, a few mismatch positions (variants or sequencing errors) can chop the read into only short segments matching the reference, so that only a short seed can fit between the differences and match the reference exactly. For example, in a 36 bp read, just one SNP in the middle can block seeds longer than 18 bp from matching the reference. By contrast, in a 250 bp read, it takes 15 SNPs to exceed a 0.01% chance of blocking even 27 bp seeds.

- **Paired Ends**—The use of paired end reads can make longer seeds yield good mapping accuracy. DRAGEN uses paired end information to improve mapping accuracy, including with rescue scans that search the expected reference window when only one mate has seeds mapping to a given reference region. Thus, paired end reads have essentially twice the opportunity for an exact matching seed to find their correct alignments.
- **Variant or Error Rate**—When read differences from the reference are more frequent, shorter seeds may be required to fit between the difference positions in a given read and match the reference exactly.
- **Mapping Percentage Requirement**—If the application requires a high percentage of reads to be mapped somewhere (even at low MAPQ), short seeds may be helpful. Some reads that do not match the reference well anywhere are more likely to map using short seeds to find partial matches to the reference.

## Maximum Seed Length

The *--ht-max-ext-seed-len* option limits the length of extended seeds populated into the hash table. Primary seeds (length specified by *--ht-seed-len*) that match many reference positions can be extended to achieve more unique matching, which may be required to map seeds within the maximum hit frequency (*--ht-max-seed-freq*).

Given a primary seed length k, the maximum seed length can be configured between k and k+128. The default is the upper bound, k+128.

### When to Limit Seed Extension

The *--ht-max-ext-seed-len* option is recommended for short reads, eg, less than 50 bp. In such cases, it is helpful to limit seed extension to the read length minus a small margin, such as 1–4 bp. For example, with 36 bp reads, setting *--ht-max-ext-seed-len* to 35 might be appropriate. This ensures that the hash table builder does not plan a seed extension longer than the read causing seed extension and mapping to fail at run time, for seeds that could have fit within the read with shorter extensions.

While seed extension can be similarly limited for longer reads, eg, setting *--ht-max-ext-seed-len* to 99 for 100 bp reads, there is little utility in this because seeds are extended conservatively in any event. Even with the default k+128 limit, individual seeds are only extended to the lengths required to fit under the maximum hit frequency (*--ht-max-seed-freq*), and at most a few bases longer to approach the target hit frequency (*--ht-target-seed-freq*), or to avoid taking too many incremental extension steps.

## Maximum Hit Frequency

The *--ht-max-seed-freq* option sets a firm limit on the number of seed hits (reference genome locations) that can be populated for any primary or extended seed. If a given primary seed maps to more reference positions than this limit, it must be extended long enough that the extended seeds subdivide into smaller groups of identical seeds under the limit. If, even at the maximum extended seed

length (*--ht-max-ext-seed-len*), a group of identical reference seeds is larger than this limit, their reference positions are not populated into the hash table. Instead, *dragen* populates a single High Frequency record.

The maximum hit frequency can be configured from 1 to 256. However, if this value is too low, hash table construction can fail because too many seed extensions are needed. The practical minimum for a whole human genome reference, other options being default, is 8.

### Accuracy Considerations

Generally, a higher maximum hit frequency leads to more successful mapping. There are two reasons for this. First, a higher limit rejects fewer reference positions that cannot map under it. Second, a higher limit allows seed extensions to be shorter, improving the odds of exact seed matching without overlapping variants or sequencing errors.

However, as with very short seeds, allowing high hit counts can sometimes hurt mapping accuracy. Most of the seed hits in a large group are not to the true mapping location, and occasionally one of these noise hits may be reported due to imperfect scoring models. Also, the mapper limits the total number of reference positions it considers, and allowing very high hit counts can potentially crowd out the actual best match from consideration.

### Speed Considerations

Higher maximum hit frequencies slow down read mapping, because seed mapping finds more reference locations, resulting in more work, such as Smith-Waterman alignments, to determine the best result.

## ALT-Aware Liftover File Options

The following options control See *ALT-Aware Hash Tables* on page 13 for more information on building a custom liftover file.

- *--ht-alt-liftover*

  The *--ht-alt-liftover* option specifies the path to the liftover file to build an ALT-aware hash table. This option is required when building from a reference with ALT contigs. SAM liftover files for hg38DH and hg19 are provided in `/opt/edico/liftover`.

- *--ht-alt-aware-validate*

  When building hash tables from a reference that contains ALT-contigs, building with a liftover file is required. To disable this requirement, set the *--ht-alt-aware-validate* option to false.

- *--ht-decoys*

  The DRAGEN software automatically detects the use of hg19 and hg38 references and adds decoys to the hash table when they are not found in the FASTA file. Use the *--ht-decoys* option to

specify the path to a decoys file. The default is `/opt/edico/liftover/hs_decoys.fa`.

- *--ht-suppress-decoys*

  Use the *--ht-suppress-decoys* option to suppress the use of the decoys file when building the hash table.

## Graph Mapper Hash Table Options

The following are additional ALT-aware file options to control building hash tables for the DRAGEN graph mapper.

- *--ht-pop-alt-contigs* —Specifies the path to the reference FASTA file with population alternate contigs. The standard reference FASTA is augmented with the population alternate contigs during hash table build. The population alternate contigs file must have a corresponding liftover SAM file. A population alternate contig file for hg38 reference is provided in `/opt/edico/liftover (pop_altContig.fa.gz)`.
- *--ht-pop-alt-liftover*—Specifies the path to the liftover file for the population alternate contigs. The liftover SAM file must have a corresponding population alternate contigs FASTA. A population alternate contig SAM liftover file for hg38 reference is provided in `/opt/edico/liftover (pop_liftover.sam.gz)`.
- *--ht-pop-snps*—Specifies the path to a VCF file containing unphased population SNPs. The standard reference FASTA is augmented with these SNPs as multibase codes during mapping-aligning. Each SNP entry in the VCF only requires the `CHROM`, `POS`, `REF`, `ALT` columns. The `ALT` column can have multiple comma-separated population SNP VCF for hg38 reference is provided in `/opt/edico/liftover (pop_snps.vcf.gz)`.

## DRAGEN Software Options

- *--ht-num-threads*

  The *--ht-num-threads* option determines the maximum number of worker CPU threads that are used to speed up hash table construction. The default for this option is 8, with a maximum of 32 threads allowed.

  If your server supports execution of more threads, it is recommended that you use the maximum. For example, the DRAGEN servers contain 24 cores that have hyperthreading enabled, so a value of 32 should be used. When using a higher value, adjust *--ht-max-table-chunks* needs to be adjusted as well. The servers have 128 GB of memory available.

- *--ht-max-table-chunks*

  The *--ht-max-table-chunks* option controls the memory footprint during hash table construction by limiting the number of ~1 GB hash table chunks that reside in memory simultaneously. Each additional chunk consumes roughly twice its size (~2 GB) in system memory during construction.

The hash table is divided into power-of-two independent chunks, of a fixed chunk size, X, which depends on the hash table size, in the range 0.5 GB < X ≤ 1 GB. For example, a 24 GB hash table contains 32 independent 0.75 GB chunks that can be constructed by parallel threads with enough memory and a 16 GB hash table contains 16 independent 1 GB chunks.

The default is *--ht-max-table-chunks* equal to *--ht-num-threads*, but with a minimum default *--ht-max-table-chunks* of 8. It makes sense to have these two options match, because building one hash table chunk requires one chunk space in memory and one thread to work on it. Nevertheless, there are build-speed advantages to raising *--ht-max-table-chunks* higher than *--ht-num-threads*, or to raising *--ht-num-threads* higher than *--ht-max-table-chunks*.

## Size Options

- *--ht-mem-limit*—Memory Limit

  The *--ht-mem-limit* option controls the generated hash table size by specifying the DRAGEN board memory available for both the hash table and the encoded reference genome. The *--ht-mem-limit* option defaults to 32 GB when the reference genome approaches WHG size, or to a generous size for smaller references. Normally there is little reason to override these defaults.

- *--ht-size*—Hash Table Size

  This option specifies the hash table size to generate, rather than calculating an appropriate table size from the reference genome size and the available memory (option *--ht-mem-limit*). Using default table sizing is recommended and using *--ht-mem-limit* is the next best choice.

## Seed Population Options

- *--ht-ref-seed-interval*—Seed Interval

  The *--ht-ref-seed-interval* option defines the step size between positions of seeds in the reference genome populated into the hash table. An interval of 1 (default) means that every seed position is populated, 2 means 50% of positions are populated, etc. Noninteger values are supported, eg, 2.5 yields 40% populated.

  Seeds from a whole human reference are easily 100% populated with 32 GB memory on DRAGEN boards. If a substantially larger reference genome is used, change this option.

- *--ht-soft-seed-freq-cap* and *--ht-max-dec-factor*—Soft Frequency Cap and Maximum Decimation Factor for Seed Thinning

  Seed thinning is an experimental technique to improve mapping performance in high-frequency regions. When primary seeds have higher frequency than the cap indicated by the *--ht-soft-seed-freq-cap* option, only a fraction of seed positions are populated to stay under the cap. The *--ht-max-dec-factor* option specifies a maximum factor by which seeds can be thinned. For example, *--ht-max-dec-factor 3* retains at least 1/3 of the original seeds. *--ht-max-dec-factor 1* disables any thinning.

Seeds are decimated in careful patterns to prevent leaving any long gaps unpopulated. The idea is that seed thinning can achieve mapped seed coverage in high frequency reference regions where the maximum hit frequency would otherwise have been exceeded. Seed thinning can also keep seed extensions shorter, which is also good for successful mapping. Based on testing to date, seed thinning has not proven to be superior to other accuracy optimization methods.

- *--ht-rand-hit-hifreq* and *--ht-rand-hit-extend*—Random Sample Hit with HIFREQ Record and EXTEND Record

  Whenever a HIFREQ or EXTEND record is populated into the hash table, it stands in place of a large set of reference hits for a certain seed. Optionally, the hash table builder can choose a random representative of that set, and populate that HIT record alongside the HIFREQ or EXTEND record.

  Random sample hits provide alternative alignments that are very useful in estimating MAPQ accurately for the alignments that are reported. They are never used outside of this context for reporting alignment positions, because that would result in biased coverage of locations that happened to be selected during hash table construction.

  To include a sample hit, set *--ht-rand-hit-hifreq* to 1. The *--ht-rand-hit-extend* option is a minimum pre-extension hit count to include a sample hit, or zero to disable. Modifying these options is *not* recommended.

## Seed Extension Control

DRAGEN seed extension is dynamic, applied as needed for particular K-mers that map to too many reference locations. Seeds are incrementally extended in steps of 2–14 bases (always even) from a primary seed length to a fully extended length. The bases are appended symmetrically in each extension step, determining the next extension increment if any.

There is a potentially complex seed extension tree associated with each high frequency primary seed. Each full tree is generated during hash table construction and a path from the root is traced by iterative extension steps during seed mapping. The hash table builder employs a dynamic programming algorithm to search the space of all possible seed extension trees for an optimal one, using a cost function that balances mapping accuracy and speed. The following options define that cost function:

- *--ht-target-seed-freq*—Target Hit Frequency

  The *--ht-target-seed-freq* option defines the ideal number of hits per seed for which seed extension should aim. Higher values lead to fewer and shorter final seed extensions, because shorter seeds tend to match more reference positions.

- *--ht-cost-coeff-seed-len*—Cost Coefficient for Seed Length

  The *--ht-cost-coeff-seed-len* option assigns the cost component for each base by which a seed is extended. Additional bases are considered a cost because longer seeds risk overlapping variants or sequencing errors and losing their correct mappings. Higher values lead to shorter final seed extensions.

- *--ht-cost-coeff-seed-freq*—Cost Coefficient for Hit Frequency

The *--ht-cost-coeff-seed-freq* option assigns the cost component for the difference between the target hit frequency and the number of hits populated for a single seed. Higher values result primarily in high-frequency seeds being extended further to bring their frequencies down toward the target.

- *--ht-cost-penalty*—Cost Penalty for Seed Extension

  The *--ht-cost-penalty* option assigns a flat cost for extending beyond the primary seed length. A higher value results in fewer seeds being extended at all. Current testing shows that zero (0) is appropriate for this parameter.

- *--ht-cost-penalty-incr*—Cost Increment for Extension Step

  The *--ht-cost-penalty-incr* option assigns a recurring cost for each incremental seed extension step taken from primary to final extended seed length. More steps are considered a higher cost because extending in many small steps requires more hash table space for intermediate EXTEND records, and takes substantially more run time to execute the extensions. A higher value results in seed extension trees with fewer nodes, reaching from the root primary seed length to leaf extended seed lengths in fewer, larger steps.

## Pipeline Specific Hash Tables

When building a hash table, DRAGEN configures the options to work for DNA-seq processing by default. To run RNA-Seq data, you must build an RNA-Seq hash table using the *--ht-build-rna-hashtable true* option. For an RNA-Seq alignment run, refer to the original *--output-directory*, not to the automatically generated subdirectory.

The CNV pipeline requires that the hash table be built with *--enable-cnv* set to true, which generates an additional k-mer hashmap that is used in the CNV algorithm. Illumina recommends that that you always use the *--enable-cnv* option, in case you wish to perform CNV calling with the same hash table that is used for mapping and aligning.

DRAGEN methylation runs require building a special pair of hash tables with reference bases converted from C->T for one table, and G->A for the other. When running the hash table generation with the *--ht-methylated* option, these conversions are done automatically, and the converted hash tables are generated in a pair of subdirectories of the target directory specified with *--output-directory*. The subdirectories are named CT_converted and GA_converted, corresponding to the automatic base conversions. When using these hash tables for methylated alignment runs, refer to the original *--output-directory* and not to either of the automatically generated subdirectories.

These base conversions remove a significant amount of information from the hashtables, so you may find it necessary to tune the hash table parameters differently than you would in a conventional hash table build. The following options are recommended for building hash tables for mammalian species:

```
dragen --build-hash-table=true --output-directory $REFDIR \
--ht-reference $FASTA --ht-max-seed-freq 16 \
--ht-seed-len 27 --ht-num-threads 40 --ht-methylated=true
```

## Determine Input and Output File Locations

The DRAGEN Bio-IT Platform is very fast, which requires careful planning for the locations of the input and output files. If the input or output files are on a slow file system, then the overall performance of the system is limited by the throughput of that file system. It is recommended that inputs and outputs are streamed directly from/to a mounted external storage system.

The DRAGEN system is preconfigured with at least one fast file system consisting of a set of fast SSD disks grouped with RAID-0 for performance. This file system is mounted at `/staging`. This name was chosen to emphasize the fact that this area was built to be large and fast, but is not redundant. Failure of any of the file system's constituent disks leads to the loss of all data stored there.

During processing, DRAGEN generates and reads back temporary files. With DRAGEN, it is highly recommended to always direct temporary files to the fast SSD (or `/staging`) by using the *--intermediate-results-dir* option. If the *--intermediate-results-dir* option is not provided, temporary files are written to the `--output-directory`. DRAGEN recommends streaming inputs and outputs using an mounted external storage system.

## Process Your Input Data

To analyze FASTQ data, use the *dragen* command. For example, the following command can be used to analyze a single-ended FASTQ file:

```
dragen \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-1 /staging/test/data/SRA056922.fastq \
--output-directory /staging/test/output \
--output-file-prefix SRA056922_dragen \
--RGID DRAGEN_RGID \
--RGSM DRAGEM_RGSM
```

For more information on the command line options, see *DRAGEN Host Software* on page 47

## Example Commands for Processing FASTQ Data

After you have loaded your reference, you can process input FASTQ data. Choose the example that best matches your data sets. These commands can take up to approximately 30 minutes to run on a 24 core server with SSD drives on a 30x coverage whole human genome when running end-to-end (FASTQ input to VCF output). The speed scales with input size, so a 60x coverage genome would take twice as long. Exome data takes a fraction of the time. A successful result is indicated by the following message (an application exit code of 0 when run from a script):

```
DRAGEN finished normally
```

This message is followed by a block of metrics such as read count and performance. If there is a problem with the command line options, an error is displayed, followed by help usage. You may need to scroll up to see the error.

The DRAGEN log can be redirected to a file, to keep the record for future reference.

To get help on dragen command line options, run the following command:

```
dragen -h
```

The example commands shown in this document are formatted for visual display and include line feed characters. To avoid copy-paste errors, each example command is contained in an individual shell script in `/opt/edico/examples/`. These examples have the following requirements:

- All commands accept either FASTQ or gzipped FASTQ (fastq.gz). DRAGEN automatically determines the file type.
- All commands include the *-f* option, which forces the output file to be overwritten if it exists.
- All commands assume that your DRAGEN reference (hash table) directory is /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149, and your FASTA reference file is /staging/human/reference/hg19/hg19.fa. Replace those with the correct references or directory paths, if needed.
- All command examples assume that the example data package is in /`staging/examples` (in particular, the .fastq and fastq.gz files are expected to be in `/staging/examples/reads`).
- To run these example commands, you must have write access to the `/staging/examples` folder.

## End-to-End Aligning and Variant Calling Examples

### Paired-End BAM Input, VCF Output

- Enter the following input:

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-b /staging/human/unsorted_SRA056922_30x_e10_50M.bam \
--enable-map-align true \
--enable-map-align-output true \
--enable-variant-caller true \
--vc-sample-name Unsorted_SRA056922_30x_e10_50M \
--output-directory /staging/examples/ \
--output-file-prefix SRA056922_30x_e10_50M \
--enable-duplicate-marking true
```

- Or, run `/opt/edico/examples/paired_fastq_in_dupmark_bam_and_vcf_out.sh`.

If the */staging/human/unsorted_SRA056922_30x_e10_50M.bam* input file for the example above is missing, run the `/opt/edico/examples/paired_fastq_in_unsorted_bam_out.sh` script o generate it.

### Paired-End FASTQ Input, VCF Output (Default)

- Enter the following input:

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-1 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_1.fastq.gz \
-2 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_2.fastq.gz \
--enable-variant-caller true \
--RGID Illumina_RGID \
--RGSM SRA056922_30x_e10_50M \
--output-directory /staging/examples/ \
--output-file-prefix SRA056922_30x_e10_50M \
```

- Or, run `/opt/edico/examples/paired_fastq_in_vcf_out.sh`.

This example shows the minimum options that must be specified to perform an end-to-end run. By default, duplicate-marking is not performed and no BAM output is produced.

### Paired-End Fastq Input, Sorted and Duplicate-Marked, VCF Output

- Enter the following input:

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-1 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_1.fastq.gz \
-2 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_2.fastq.gz \
--enable-variant-caller true \
--RGID Illumina_RGID \
--RGSM SRA056922_30x_e10_50M \
--output-directory /staging/examples/ \
--output-file-prefix SRA056922_30x_e10_50M \
--enable-duplicate-marking true
```

- Or, run `/opt/edico/examples/paired_fastq_in_dupmark_vcf_out.sh`.

### Paired-End FASTQ Input, Sorted BAM and VCF Output

- Enter the following input:

```
dragen -f
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-1 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_1.fastq.gz \
-2 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_2.fastq.gz \
```

```
--enable-variant-caller true \
--RGID Illumina_RGID \
--RGSM SRA056922_30x_e10_50M \
--output-directory /staging/examples/ \
--output-file-prefix SRA056922_30x_e10_50M \
--enable-duplicate-marking true \
--enable-map-align-output true
```

- Or, run `/opt/edico/examples/sorted_bam_in_vcf_out.sh`.

## Paired-End FASTQ Input, Sorted SAM and VCF Output

- Enter the following input:

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-1 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_1.fastq.gz \
-2 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_2.fastq.gz \
--enable-variant-caller true \
--RGID Illumina_RGID \
--RGSM SRA056922_30x_e10_50M \
--output-directory /staging/examples/ \
--output-file-prefix SRA056922_30x_e10_50M \
--enable-duplicate-marking true \
--enable-map-align-output true \
--output-format SAM
```

- Or, run `/opt/edico/examples/paired_fastq_in_dupmark_sam_and_vcf_out.sh`.

## Paired-End FASTQ Input, Sorted CRAM and VCF Output

- Enter the following input:

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-1 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_1.fastq.gz \
-2 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_2.fastq.gz \
--enable-variant-caller true \
--RGID Illumina_RGID \
--RGSM SRA056922_30x_e10_50M \
--output-directory /staging/examples/ \
--output-file-prefix SRA056922_30x_e10_50M \
--enable-duplicate-marking true \
--enable-map-align-output true \
--output-format CRAM \
```

- Or, run `/opt/edico/examples/paired_fastq_in_dupmark_cram_and_vcf_out.sh`.

### Paired-End FASTQ Input, Sorted BAM and VCF Output, plus Repeat Genotyping VCF

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-1 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_1.fastq.gz \
-2 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_2.fastq.gz \
--enable-variant-caller true \
--RGID Illumina_RGID \
--RGSM SRA056922_30x_e10_50M \
--output-directory /staging/examples/ \
--output-file-prefix SRA056922_30x_e10_50M \
--enable-duplicate-marking true \
--enable-map-align-output true \
--repeat-genotype-enable true \
--repeat-genotype-specs /opt/edico/repeat-specs/hg19 \
--repeat-genotype-sex female \
--repeat-genotype-ref-fasta /staging/human/reference/h19/hg19.fa
```

## Alignment Only Examples

All the variations for performing alignment shown in these examples can be used in the end-to-end case as well.

### Map/Align Single-Ended FASTQ Input, Sorted BAM output (Default)

- Enter the following input:

```
dragen –f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-1 /staging/examples/reads/SRA056922_30x_rand1_100K.fastq \
--output-directory /staging/examples/ \
--output-file-prefix SRA056922_30x_rand1_100K \
--RGID DRAGEN_RGID \
--RGSM DRAGEN_RGSM \
```

- Or, run `/opt/edico/examples/single_fastq_in_bam_out.sh`.

### Map/Align Single-ended FASTQ input, Sorted, Duplicate-Marked BAM Output

- Enter the following input:

```
dragen –f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-1 /staging/examples/reads/SRA056922_30x_rand1_100K.fastq \
```

```
--output-directory /staging/examples/ \
--output-file-prefix SRA056922_30x_rand1_100K_dup_marked \
--enable-duplicate-marking true \
--RGID DRAGEN_RGID \
--RGSM DRAGEN_RGSM
```

- Or, run `/opt/edico/examples/single_fastq_in_dupmark_bam_out.sh`.

## Map/Align Paired-End FASTQ Input, Sorted BAM Output (Default)

- Enter the following input:

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-1 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_1.fastq.gz \
-2 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_2.fastq.gz \
--output-directory /staging/examples/ \
--output-file-prefix SRA056922_30x_e10_50M \
--RGID DRAGEN_RGID \
--RGSM DRAGEN_RGSM
```

- Or, run `/opt/edico/examples/paired_fastq_in_bam_out.sh`.

## Map/Align Paired-End FASTQ Input, Sorted CRAM Output

- Enter the following input:

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-1 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_1.fastq.gz \
-2 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_2.fastq.gz \
--output-directory /staging/examples/ \
--output-file-prefix SRA056922_30x_e10_50M \
--output-format CRAM \
--RGID DRAGEN_RGID \
--RGSM DRAGEN_RGSM
```

- Or, run `/opt/edico/examples/paired_fastq_in_cram_out.sh`.

## Map/Align Paired-End FASTQ Input, Sorted Uncompressed BAM Output

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-1 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_1.fastq.gz \
-2 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_2.fastq.gz \
--output-directory /staging/examples/ \
--output-file-prefix uncompressed_SRA \
```

```
--enable-bam-compression false \
--RGID DRAGEN_RGID \
--RGSM DRAGEN_RGSM
```

### Map/Align Paired-End FASTQ Input, Sorted SAM Output

- Enter the following input:

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-1 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_1.fastq.gz \
-2 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_2.fastq.gz \
--output-directory /staging/examples/ \
--output-file-prefix SRA056922_30x_e10_50M \
--output-format SAM \
--RGID DRAGEN_RGID \
--RGSM DRAGEN_RGSM
```

- Or, run `/opt/edico/examples/paired_fastq_in_sam_out.sh`.

### Map/Align Paired -End FASTQ Input, UN-Sorted BAM output

- Enter the following input:

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-1 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_1.fastq.gz \
-2 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_2.fastq.gz \
--output-directory /staging/examples/ \
--output-file-prefix unsorted_SRA056922_30x_e10_50M \
--enable-sort false \
--RGID DRAGEN_RGID \
--RGSM DRAGEN_RGSM
```

- Or, run `/opt/edico/examples/paired_fastq_in_unsorted_bam_out.sh`.

### Map/Align Interleaved Paired-Ended FASTQ Input, BAM Output

- Enter the following input:

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-1 /staging/examples/reads/SRA056922_PE_30x_rand1_10K_interleaved.fastq \
--interleaved \
--output-directory /staging/examples/ \
```

```
--output-file-prefix SRA056922_PE_30x_rand1_10K_interleaved \
--RGID DRAGEN_RGID \
--RGSM DRAGEN_RGSM
```

- Or, run `/opt/edico/examples/interleaved_fastq_in_bam_out.sh`.

## RNA Map and Align Only Examples

Any of the Map/Align Only examples can be used for RNA. The only difference in the command is to set the *--enable-rna* option to true. DRAGEN automatically picks up the RNA-specific hash tables and uses the RNA spliced aligner in its processing.

The hash table used for these examples must be generated with the *--ht-build-rna-hashtable true* option. Otherwise, the run will fail with an error similar to the following:

```
ERROR: The specified hashtable directory cannot be used to run RNA:
/staging/examples/reference/hg19/hg19.fa.k_21.f_16.m_149
```

If this error occurs, regenerate the hash table with the *--ht-build-rna-hashtable true* option.

### RNA Map/Align Paired-Ended FASTQ Input, BAM Output

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-1 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_1.fastq.gz \
-2 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_2.fastq.gz \
--output-directory /staging/examples/ \
--output-file-prefix SRA056922_30x_e10_50M \
--enable-rna true \
--RGID DRAGEN_RGID \
--RGSM DRAGEN_RGSM
```

The following is example command-line to map-align RNA-seq data with additional command line options, including a path to a gtf file with gene annotations. The gene annotations file improves mapping by providing a list of known splice-junctions (rather than discovering them all de novo.)

```
dragen -f \
-r <HASHTABLE_DIR>
-1 <FASTQ1> \
-2 <FASTQ2> \
-a $/reference_genomes/annotation/GTF/$gencode.annotation.gtf
--enable-map-align true \
--enable-sort=true \
--enable-bam-indexing true \
--enable-map-align-output true \
--output-format=BAM \
--RGID=<READ_GROUP_ID> \
--RGSM=<Sample_NAME> \
```

```
--RGPL=<LIBRARY> \
--config-file /opt/edico/config/dragen-user-defaults.cfg \
--enable-rna=true \
--output-directory <OUT_DIR> \
--output-file-prefix <PREFIX>
```

### RNA Quantification

To run gene and transcript expression quantification add the following option:

```
--enable-rna-quantification true
```

When *--enable-rna-quantification* is set to true, GC bias correction is the default and *--rna-quantification-gc-bias* does not have to be enabled. In addition, the library type is automatically detected and *--rna-quantification-library-type* does not have to be set.

NOTE

Library-type auto-detection only works for paired-end data. If you have single-end data, you need to specify the library type by setting the *--rna-quantification-library-type* option.

### RNA Fusion

To run gene fusion detection add the following option:

```
--enable-rna-gene-fusion true
```

You do not need to specify the library because fusion does not use it.

## Epigenome Map and Align Examples

Prior to performing an epigenome (methylation) map and align run with bisulfite sequencing data, you must first create methylation-specific reference hash tables, as follows:

```
mkdir -p /staging/human/reference/hg19_epigenome
dragen --build-hash-table true \
--ht-reference /staging/human/reference/hg19/hg19.fa \
--ht-max-seed-freq 64 --ht-seed-len 27 --ht-methylated true \
--output-directory /staging/human/reference/hg19_epigenome \
--ht-alt-liftover /opt/edico/liftover/hg19_alt_liftover.sam
```

The above dragen command produces two hash table directories under `/staging/human/reference/hg19_epigenome`: `GA_converted` and `CT_converted`. The `CT_converted` hash table is produced by converting each C base to T in the reference sequences. Similarly, the `GA_converted` hash table is produced from the G->A base-converted reference sequences. The base-converted references have less complexity, and to compensate, the hash table seed length argument (*--ht-seed-len*) is typically increased to 27 for mammalian genomes (default seed length is 21).

The *--ht-alt-aware-validate false* option can be used in place of *--ht-alt-liftover*. However, the dragen map quality will be significantly affected due to the presence of alternate contigs in the hg19.fa reference.

## Epigenome Map/Align, Directional-protocol, Single-Ended FASTQ Input, BAM Output

The directional (Lister) protocol produces reads from two of the four possible bisulfite sequencing strands. Therefore, when the *--methylation-protocol=directional* option is used, DRAGEN aligns each read or read pair twice with different constraints corresponding to the two possible strands. The following DRAGEN command produces two separate BAM files:

```
mkdir –p /staging/epigenome/directional
dragen -f –r /staging/human/reference/hg19_epigenome \
-1 /staging/epigenome/reads/sample_1_R1.fastq.gz \
-2 /staging/epigenome/reads/sample_10_R2.fastq.gz \
--RGID Illumina_RGID \
--RGSM sample_10 \
--RGPL illumina \
--output-directory /staging/epigenome/directional \
--output-file-prefix sample_10 \
--methylation-protocol=directional \
--enable-sort false
```

## Epigenome Map/Align, Nondirectional-protocol, Paired-Ended FASTQ Input, BAM Output

The nondirectional protocol produces reads from all four possible bisulfite sequencing strands. Therefore, when the *--methylation-protocol=non-directional* argument is used, DRAGEN aligns each read four times and produces four separate BAM files.

```
mkdir –p /staging/epigenome/non-directional
dragen -f –r /staging/human/reference/hg19_epigenome \
-1 /staging/epigenome/reads/sample_10_R1.fastq.gz \
-2 /staging/epigenome/reads/sample_10_R2.fastq.gz \
--RGID Illumina_RGID \
--RGSM sample_10 \
--RGPL illumina \
--output-directory /staging/epigenome/non-directional \
--output-file-prefix sample_10 \
--methylation-protocol non-directional \
--enable-sort false
```

## Variant Calling Only Examples

The variant calling only examples show how you can pass an existing aligned BAM or CRAM file directly to the DRAGEN Variant Caller. By default, the BAM/CRAM file is passed through the sorting stage prior to variant calling.

To duplicate mark your BAM file before running the DRAGEN Variant Caller, you need to use a separate tool. The DRAGEN Duplicate Marker depends on information provided by the Mapper/Aligner that does not exist in BAM files. To take advantage of the DRAGEN Duplicate Marker, use DRAGEN in end-to-end mode.

The BAM/CRAM files that are used as input to these example commands are not included in the example data set. They are generated by example commands in *Alignment Only Examples* on page 26.

### Unsorted BAM Input, VCF Output (Default)

- Enter the following input:

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-b /staging/examples/unsorted_SRA056922_30x_e10_50M.bam \
--enable-variant-caller true \
--output-directory /staging/examples/ \
--output-file-prefix unsorted_output_SRA056922_30x_e10_50M \
--enable-map-align false
```

- Or, run `/opt/edico/examples/unsorted_bam_in_vcf_out.sh`.

### Sorted BAM Input, VCF Output

- Enter the following input:

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-b /staging/examples/SRA056922_30x_e10_50M.bam \
--enable-variant-caller true \
--output-directory /staging/examples/ \
--output-file-prefix sorted_output_SRA056922_30x_e10_50M \
--enable-map-align false
--enable-sort false
```

- Or, run `/opt/edico/examples/sorted_bam_in_vcf_out.sh`.

### Sorted CRAM Input, VCF Output

- Enter the following input:

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
--enable-variant-caller true \
--output-directory /staging/examples/ \
--output-file-prefix sorted_output_SRA056922_30x_e10_50M \
--enable-sort false \
--enable-map-align false \
--cram-input /staging/examples/SRA056922_30x_e10_50M.cram
```

- Or, run `/opt/edico/examples/sorted_cram_in_vcf_out.sh`.

## Somatic Small Variant Caller Examples

If you are using the Tumor-Normal BAM input option, and your BAM read groups have a shared RGID, DRAGEN cannot determine which read group the reads belong to. Ideally, you should have different RGIDs for each read group, but you can work around the problem by setting the *--prepend-filename-to-rgid* to true.

### Paired-End FASTQ Input

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
--tumor-fastq1 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_
1.fastq.gz \
--tumor-fastq2 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_
2.fastq.gz \
--enable-variant-caller true \
--RGID-tumor DRAGEN_RGID \
--RGSM-tumor DRAGEN_RGSM \
--output-directory /staging/examples/ \
--output-file-prefix SRA056922_30x_e10_50M
```

### Sorted BAM Input

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
--tumor-bam-input /staging/examples/SRA056922_30x_e10_50M.bam \
--enable-variant-caller true \
--output-directory /staging/examples/ \
--output-file-prefix sorted_output_SRA056922_30x_e10_50M \
--enable-map-align false \
--prepend-filename-to-rgid true
```

# gVCF and Genotyping Examples

## Paired-End FASTQ Input, gVCF Output

- Enter the following input:

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-1 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_1.fastq.gz \
-2 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_2.fastq.gz \
--enable-variant-caller true \
--vc-emit-ref-confidence GVCF \
--RGID Illumina_RGID \
--RGSM SRA056922_30x_e10_50M \
--output-directory /staging/examples/ \
--output-file-prefix SRA056922_30x_e10_50M
```

- Or, run `/opt/edico/examples/paired_fastq_in_gVCF_out.sh`.

## Join Calling with gVCF Input

- Enter the following input:

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
--enable-joint-genotyping true \
--output-directory /staging/examples/ \
--output-file-prefix Joint_SRA056922_30x_e10_50M \
--variant /staging/examples/SRA056922_30x_e10_50M.gvcf.gz
```

- Or, run `/opt/edico/examples/single_gVCF_in_jointVCF_out.sh`.

## Pedigree-Based Joint Genotyping with Three gVCF Files and a Pedigree File Input, Joint-Genotyped VCF Output

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
--enable-joint-genotyping true \
--output-directory /staging/examples/ \
--output-file-prefix Joint_SRA056922_30x_e10_50M \
--variant /staging/examples/mother.gvcf.gz \
--variant /staging/examples/father.gvcf.gz \
--variant /staging/examples/child.gvcf.gz \
--pedigree-file <PEGIGREE_FILE>
```

## One Step Population-Based Joint Genotyping with gVCF Input, Joint-Genotyped Multisample Output

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
--enable-joint-genotyping true \
--output-directory /staging/examples/ \
--output-file-prefix Joint_SRA056922_30x_e10_50M \
--variant /staging/examples/SRA056922_30x_e10_50M.gvcf.gz
```

## Two Step Population-Based Joint Genotyping with gVCF List Input, Joint-Genotyped Multisample VCF Output

The first step generates a multisample VCF as output using a gVCF list as input and the following command line option.

```
dragen -f \
--enable-gvcf-genotyper true \
--enable-map-align false \
--variant-list ${GVCF_LIST} \
--ht-reference ${FASTA_REF} \
--intermediate-results-dir ${TEMP_DIR} \
--output-directory ${OUTPUT_DIR} \
--output-file-prefix ${COHORT_NAME}
```

The second step generates a joint-genotyped multisample VCF using the multisample VCF produced from step one as input and the following command line option. To specify the multisample VCF, use *--variant*.

```
dragen -f \
--enable-joint-genotyping true \
--variant ${MULTISAMPLE_VCF} \
--ref-dir ${FASTA_REF} \
--output-directory ${OUTPUT_DIR}\
--output-file-prefix ${COHORT_NAME}.joint_genotyped
```

Table 2 Joint-calling modes, with associated input files and command line options.

| **VCF to generate** | Population joint-called multisample gVCF | Family joint-called multisample gVCF | Population joint-called multisample VCF | Family joint-called multisample VCF |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| **Input file** | Multisample combined gVCF file | Multisample combined gVCF file | Multi-sample combined gVCF file or X individual gVCF files | Multi-sample combined gVCF file or X individual gVCF files |
| **Use pedigree file** | No | Yes | No | Yes |
| **Command line option** | --enable-joint-genotyping true --enable-multi-sample-gvcf=TRUE | --enable-joint-genotyping true --enable-multi-sample-gvcf=TRUE --pedigree-file file.ped | --enable-joint-genotyping true | --enable-joint-genotyping true --pedigree-file file.ped |

## Coverage Metrics Report Example

By default, DRAGEN reports read coverage over the whole genome, and if available also for the target bed. You can specify up to three additional regions over which coverages are reported. In the following example, DRAGEN generates two coverage reports, `cov_report` and `full_res`, for the additional coverage region 1.

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-1 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_1.fastq.gz \
-2 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_2.fastq.gz \
--RGID Illumina_RGID \
--RGSM SRA056922_30x_shuffle16k \
--output-directory /staging/examples/ \
--output-file-prefix SRA056922_30x_e10_50M \
--qc-coverage-region-1 /staging/examples/reads/vc_smoke.callable.bed \
--qc-coverage-reports-1 cov_report full_res
```

The `full_res` report corresponds to the Bedtools coverage option, and includes a per base resolution read depth. The `cov_report` includes read depth summaries per region including mean, median, min and max depths.

## CNV Examples

These examples show how to use DRAGEN CNV to process already mapped and aligned BAM files using the two modes of normalization supported by the DRAGEN CNV pipeline: Self Normalization and Panel of Normals.

Self Normalization requires that the DRAGEN hash table be generated with the *enable-cnv=true* option. It is recommended that you always generate a CNV compatible hash table if you frequently run CNV.

The *enable-map-align option* is set to true by default in the configuration file. Set it to false if you do not need to map and align the input BAM.

The *--intermediate-results-dir* option should be set to a local directory (eg, `/staging/intermediate` or `/local` ssd). Otherwise, long processing time may occur during the CNV step.

## Running with Self Normalization

Self normalization is the preferred method for single sample WGS processing. The BAM goes through the entire CNV pipeline with a single command line.

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-b /staging/examples/SRA056922_30x_e10_50M.bam \
--intermediate-results-dir /staging/intermediate \
--output-directory /staging/examples/ \
--output-file-prefix dragen_cnv1 \
--enable-map-align false \
--enable-cnv true \
--cnv-enable-self-normalization true \
```

## Running with a Panel of Normals

The Panel of Normals approach requires pregenerating the target.counts file for each sample to be used, and then executing one final command to perform the normalization and copy number variant calling.

To calculate target counts with BAM Input, this example command extracts the signals, including read counts, from the alignments of the BAM file. It generates a *.target.counts file to be used in the normalization step. Target counts should be calculated for each input BAM file, including the case sample under analysis and the normals samples.

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-b /staging/examples/SRA056922_30x_e10_50M.bam \
--intermediate-results-dir /staging/intermediate \
--output-directory /staging/examples/ \
--output-file-prefix dragen_cnv1 \
--enable-map-align false \
--enable-cnv true
```

The following command performs the normalization and generates the CNV calls. The normals samples should be listed in a text file (`normal.txt` in this example) that provides the path to the *.target.counts files of the normal samples. The case sample *.target.counts file is specified with the *--cnv-input* option. In this example, gcbias correction of the input is disabled.

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
--intermediate-results-dir /staging/intermediate \
--output-directory /staging/examples/ \
--output-file-prefix dragen_cnv2 \
--enable-cnv true \
--cnv-input /staging/examples/dragen_cnv1.target.counts \
--cnv-normals-list normal.txt \
--cnv-enable-gcbias-correction false
```

## FASTQ Processing

This example runs the DRAGEN CNV caller in Self Normalization mode directly from FASTQ samples. It first maps and aligns the FASTQ and continues directly to CNV calling. This step can be combined with variant calling.

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-1 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_1.fastq.gz \
-2 /staging/examples/reads/SRA056922_30x_shuffle16k_e10_50M_2.fastq.gz \
--RGID Illumina_ID \
--RGSM SRA056922_30x_shuffle16k \
--intermediate-results-dir /staging/intermediate \
--output-directory /staging/examples/ \
--output-file-prefix dragen_cnv \
--enable-map-align true \
--enable-cnv true \
--cnv-enable-self-normalization true
```

## Running De Novo CNV Calling

De novo calling requires previously generated normalized signal files (*.tn.tsv) from the single sample analysis. If a pedigree file is supplied, then a de novo state and a de novo quality score will be annotated for the proband sample's records.

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
--cnv-input father.tn.tsv \
--cnv-input mother.tn.tsv \
--cnv-input child.tn.tsv \
--intermediate-results-dir /staging/intermediate \
```

```
--output-directory /staging/examples/ \
--output-file-prefix trio_cnv \
--pedigree-file trio.ped \
--enable-cnv true
```

### Running Somatic CNV Calling

Somatic CNV calling requires a tumor and matched normal sample. The matched normal sample must first go through the germline small variant caller to produce a *.hard-filtered.vcf.gz. If known, it is recommended that you specify the sample sex.

```
dragen -f \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
--tumor-bam-input tumor.bam \
--intermediate-results-dir /staging/intermediate \
--output-directory /staging/examples/ \
--output-file-prefix somatic_cnv \
--enable-map-align false \
--enable-cnv true \
--cnv-normal-b-allele-vcf normal.hard-filtered.vcf.gz \
--sample-sex female
```

## Structural Variant Calling Examples

Structural Variant calling can run in the following modes:

- Standalone—Runs from mapped BAM/CRAM input files. Requires the *--enable-map-align=false* and *--enable-sv=true* options.
- Integrated—Automatically runs on the output of the DRAGEN mapper/aligner. Requires the *--enable-map-align=true*, *--enable-sv=true*, and *--enable-map-align-output=true* options.

Structural Variant calling can be enabled along with any other caller as well.

### Integrated Execution Example

```
dragen -f \
--ref-dir <HASH_TABLE_DIR> \
--enable-map-align true \
--enable-map-align-output true \
--enable-sv true \
--output-directory <OUT_DIR> \
--output-file-prefix <PREFIX> \
-1 <FASTQ1> -2 <FASTQ2> \
--RGID <RGID> \
--RGSM <RGSM>
```

### Standalone Joint Diploid Calling Example

```
dragen -f \
--ref-dir <HASH_TABLE_DIR> \
--enable-map-align false \
--enable-sv true \
--bam-input <BAM1> \
--bam-input <BAM2> \
--bam-input <BAM3> \
--output-directory <OUT_DIR> \
--output-file-prefix <PREFIX>
```

### Standalone De Novo Quality Scoring Example

```
dragen -f \
--variant <TRIO_VCF_FILE> \
--pedigree-file <PED_FILE> \
--enable-map-align false \
--sv-denovo-scoring true \
--output-directory <OUT_DIR> \
--output-file-prefix <PREFIX>
```

## BCL to FASTQ Conversion with Minimal Settings

This example shows how to use DRAGEN to process Illumina BCL format files.

The BCL directory used in the example is not included in the example data package. Replace `/mnt/san/131022_hsxten008_0123_FC543` with your own BCL directory.

- Enter the following input:

  ```
  dragen --bcl-conversion-only=true \
  --bcl-input-directory /mnt/san/131022_hsxten008_0123_FC543 \
  -- output-directory /staging/examples/
  ```

- Or run `/opt/edico/examples/bcl_in_fastq_out.sh`.

## S3 and HTTP Streaming Input Examples

DRAGEN can process input files directly from an S3 bucket, or using HTTP presigned URLs, which is known as input streaming. The input files do not need to be downloaded to a local disk prior to being processed. Instead, the files are streamed over the network directly into the DRAGEN processor.

Streaming is supported for compressed FASTQ (*.fastq.gz) files. A future version of DRAGEN will also support streaming from BAM (*.bam) files. Input streaming can be used in all the configurations that use single-end FASTQs, paired-end FASTQs, and FASTQ lists. The following examples show some of the ways to use input streaming.

### Streaming FASTQ Input using S3

```
dragen -f
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-1 s3://s3-bucket-name/path/to/object_1.fastq.gz \
-2 s3://s3-bucket-name/path/to/object_2.fastq.gz \
--RGID object_ID \
--RGSM sample_name \
--output-directory /staging/examples/ \
--output-file-prefix streaming
```

### Streaming FASTQ Input using HTTP

```
dragen -f
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-1 https://bucket-name.amazonaws.com/path/to/object_1.fastq.gz?querystring
\
-2 https://bucket-name.amazonaws.com/path/to/object_2.fastq.gz$querystring
\
--RGID object_ID \
-RGSM sample_name \
--output-directory /staging/examples/ \
--output-file-prefix streaming
```

You need to have permission to access the remote files. If you have access to the file, then DRAGEN is capable of streaming the remote file. The S3 object requires AWS authentication and credentials. The authentication should already be set up on the instance you are running, for example, via IAM policies. An HTTP URL most likely has a query string attached to it, which provides the authentication credentials or necessary tokens to grant permission. The security method may be present in other parts of the URL, for example:

```
https://stagingdl.dnanex.us/security/string/sample_1.fastq.gz
```

## Multicaller Workflows

DRAGEN supports running multiple tools in a single workflow.

The *enable-component* flag controls which components are enabled or disabled. DRAGEN constructs a workflow using the enabled components and automatically resolves any component inconsistencies. When possible, DRAGEN runs components in parallel.

Each component has a set of options that configures input settings, internal algorithm parameters, or output files and filtering criteria. Refer to the individual component sections for more details.

Some options, such as *output-directory* and *sample-sex*, are shared amongst callers.

Each variant caller produces its own set of VCFs and metric output files.

## Example Component Commands

```
enable-map-align
enable-sort
enable-duplicate-marking
enable-variant-caller
enable-cnv
enable-sv
```

## Input Formats

DRAGEN accepts the following common standard NGS input formats:

- FASTQ (`fastq-file1` and `fastq-file2`)
- FASTQ List (`fastq-list`)
- BAM (`bam-input`)
- CRAM (`cram-input`)

Somatic workflows can use tumor equivalent input files (eg, `fastq-file1` and `fastq-file2`).

When running from unaligned reads, the reads first go through the map/align component to produce alignments which continue downstream to the variant callers. When running from prealigned reads, DRAGEN supports re-aligning with the map/align component or using the existing alignments from the source input.

## Multicaller Command Line Example

The following example demonstrates best practices for combining command line options from single caller scenarios to create a multicaller workflow. The example consists of the following steps:

- Configure the INPUT options.
- Configure the OUTPUT options.
- Configure MAP/ALIGN depending on if realignment is desired or not.
- Configure the VARIANT CALLERs based on the application.
- Build up the necessary options for each component separately, to enable reuse in the final command line.

```
INPUT_OPTIONS="
--ref-dir $DRAGEN_HASH_TABLE \
```

```
--fastq-file1 $FASTQ1 \
--fastq-file2 $FASTQ2 \
--RGSM $RGSM \
--RGID $RGID \
"
OUTPUT_OPTIONS="
--output-directory $OUTPUT \
--output-file-prefix $PREFIX \
"
MA_OPTIONS="
--enable-map-align true \
... <any other optional settings> \
"
CNV_OPTIONS="
--enable-cnv true \
... <any other optional settings> \
"
SNV_OPTIONS="
--enable-variant-caller true \
... <any other optional settings> \
"
SV_OPTIONS="
--enable-sv true \
... <any other optional settings> \
"
CMD="
dragen \
$INPUT_OPTIONS \
$OUTPUT_OPTIONS \
$MA_OPTIONS \
$CNV_OPTIONS \
$SNV_OPTIONS \
$SV_OPTIONS \
"
```

## Germline

The following table summarizes the support for some input formats and variant callers. Some supported features and callers are not listed in the table.

| GERMLINE | FASTQ with Map/Align | BAM/CRAM | BAM/CRAM with Map/Align |
|---|---|---|---|
| CNV + SNV | Supported | Supported | Supported |
| CNV + SV | Supported | Supported | Supported |
| SNV + SV | Supported | Supported | Supported |
| CNV + SNV + SV | Supported | Supported | Supported |

## Somatic

Somatic workflows specify both tumor and normal inputs. The need for potentially two input files (tumor and matched normal) as well as the need for a matched normal SNV VCF for the Somatic CNV caller means extra care has to be taken. For this reason, the recommended tumor/normal workflow first starts with running the matched normal through the Germline Workflow.

1. Run matched normal through Germline workflow (CNV + SNV + SV + ...). The workflow generates the matched normal SNV VCF.
2. Run tumor and matched normal through Somatic workflow (CNV + SNV + SV + ...)

```
INPUT_OPTIONS="
--ref-dir $DRAGEN_HASH_TABLE \
--tumor-bam-input $TUMOR_BAM \
--bam-input $NORMAL_BAM \
"
OUTPUT_OPTIONS="
--output-directory $OUTPUT \
--output-file-prefix $PREFIX \
"
MA_OPTIONS="
--enable-map-align false \
... <any other optional settings> \
"
CNV_OPTIONS="
--enable-cnv true \
--cnv-normal-b-allele-vcf $SNV_VCF \
... <any other optional settings> \
"
SNV_OPTIONS="
--enable-variant-caller true \
... <any other optional settings> \
"
SV_OPTIONS="
```

```
--enable-sv true \
... <any other optional settings> \
"
CMD="
dragen \
$INPUT_OPTIONS \
$OUTPUT_OPTIONS \
$MA_OPTIONS \
$CNV_OPTIONS \
$SNV_OPTIONS \
$SV_OPTIONS \
"
```

The following table lists the various combinations that are supported under the tumor/normal mode of operation.

| Tumor normal | FASTQ with Map/Align | BAM/CRAM | BAM/CRAM with Map/Align |
|---|---|---|---|
| CNV + SNV | Supported | Supported | Supported |
| CNV + SV | Supported | Supported | Supported |
| SNV + SV | Supported | Supported | Supported |
| CNV + SNV + SV | Supported | Supported | Supported |

To run in tumor only mode, remove the matched normal input from the INPUT options and configure each individual caller to run in tumor only mode. The following table lists the combinations that are supported under the tumor only mode of operation.

| Tumor normal | FASTQ with Map/Align | BAM/CRAM | BAM/CRAM with Map/Align |
|---|---|---|---|
| CNV + SNV | Supported | Supported | Supported |
| CNV + SV | Supported | Supported | Supported |
| SNV + SV | Supported | Supported | Supported |
| CNV + SNV + SV | Supported | Supported | Supported |

WES analysis is supported if the mode is supported in singe caller mode and there is no input configuration conflict.

# Troubleshooting

The DRAGEN software automatically resets the board if any problems are encountered. In the rare case that reset does not occur automatically, you can use the following command to reset:

```
/opt/edico/bin/dragen_reset
```

If resetting does not resolve the issue, contact Illumina Technical Support. Run the following command to collect diagnostic and configuration information for Technical Support.

```
sudo sosreport --batch --tmp-dir /staging/tmp
```

This tool takes several minutes to run and reports the location where it has saved the diagnostic information in `/staging/tmp`. Please include the report when you submit a support ticket for Illumina Technical Support.

For more information, refer to the *DRAGEN Bio-IT Platform User Guide*(1000000070494) on the Illumina Support Site.

# Additional Resources and Support

For additional information, resources, system updates, and support, please visit the DRAGEN support page on the Illumina website.

# DRAGEN Host Software

You use the DRAGEN host software program *dragen* to build and load reference genomes, and then to analyze sequencing data by decompressing the data, mapping, aligning, sorting, duplicate marking with optional removal, and variant calling.

Invoke the software using the *dragen* command. The command-line options are described in the following sections.

Command-line options can also be set in a configuration file. For more information on configuration files, see *Configuration Files* on page 61. If an option is set in the configuration file and is also specified on the command-line, the command-line option overrides the configuration file.

## Command-line Options

For a complete list of command line options, see *Command Line Options* on page 1.

### DRAGEN Command-Line Options

Perform the following command-line options using the *dragen*:

- Build Reference/Hash Table

```
dragen --build-hash-table true --ht-reference <REF_FASTA> \
--output-directory <REF_DIRECTORY> [options]
```

- Run Map/Align and Variant Caller *(*.fastq to *.vcf)*

```
dragen -r <REF_DIRECTORY> --output-directory <OUT_DIRECTORY> \
--output-file-prefix <FILE_PREFIX> [options] -1 <FASTQ1> \
[-2 <FASTQ2>] --RGID <RG0> --RGSM <SM0> --enable-variant-caller true
```

- Run Map/Align *(*.fastq to *.bam)*

```
dragen -r <REF_DIRECTORY> --output-directory <OUT_DIRECTORY> \
--output-file-prefix <FILE_PREFIX> [options] \
-1 <FASTQ1> [-2 <FASTQ2>] \
--RGID <RG0> --RGSM
```

- Run Variant Caller *(*.bam to *.vcf)*

```
dragen -r <REF_DIRECTORY> --output-directory <OUT_DIRECTORY> \
--output-file-prefix <FILE_PREFIX> [options] -b <BAM> \
--enable-variant-caller true
```

- Run BCL Converter *(BCL to *.fastq)*

```
dragen --bcl-conversion-only true --bcl-input-directory <BCL_DIRECTORY> \
--output-directory <OUT_DIRECTORY>
```

- Run RNA Map/Align *(*.fastq to *.bam)*

```
dragen -r <REF_DIRECTORY> --output-directory <OUT_DIRECTORY> \
--output-file-prefix <FILE_PREFIX> [options] -1 <FASTQ1> \
[-2 <FASTQ2>] --enable-rna true
```

## Operating Modes

DRAGEN has two primary modes of operation, as follows:

- Mapper/aligner
- Variant caller

DRAGEN is capable of performing each mode independently or as an end-to-end solution. DRAGEN also allows you to enable and disable decompression, sorting, duplicate marking, and compression along the DRAGENpipeline.

## Full Pipeline Mode

To execute full pipeline mode, set *--enable-variant-caller* to *true* and provide input as unmapped reads in *.fastq, *.bam, or *.cram formats.

DRAGEN performs decompression, mapping, aligning, sorting, and optional duplicate marking and feeds directly into the variant caller to produce a VCF file. In this mode, DRAGEN uses parallel stages throughout the pipeline to drastically reduce the overall run time.

## Map/Align Mode

Map/align mode is enabled by default. Input is unmapped reads in *.fastq, *.bam, or *.cram format. DRAGEN produces an aligned and sorted BAM or CRAM file. To mark duplicate reads at the same time, set *--enable-duplicate-marking* to *true*.

## Variant Caller Mode

To execute variant caller mode, set the *--enable-variant-caller* option to true.

Input is a mapped and aligned BAM file. DRAGEN produces a VCF file. If the BAM file is already sorted, you can skip sorting by setting *--enable-sort* to *false*. BAM files cannot be duplicate marked in the DRAGEN pipeline prior to variant calling if they have not already been marked. Use the end-to-end mode of operation to take advantage of the mark-duplicates feature.

## RNA-Seq Data

To enable processing of RNA-Seq–based data, set *--enable-rna* to *true*.

DRAGEN uses the RNA spliced aligner during the mapper/aligner stage. DRAGEN dynamically switches between the required modes of operation.

## Bisulfite MethylSeq Data

To enable processing of Bisulfite MethylSeq data, set the *--enable-methylation-calling* option to true. DRAGEN automates the processing of data for Lister and Cokus (aka directional and nondirectional) protocols, generating a single BAM with bismark-compatible tags.

Alternatively, you can run DRAGEN in a mode that produces a separate BAM file for each combination of the C->T and G->A converted reads and references. To enable this mode of processing, you need to build a set of reference hash tables with *--ht-methylated* enabled, and run *dragen* with the appropriate *--methylation-protocol* setting.

## Reference Genome Options

Before you can use the DRAGENsystem for aligning reads, you must load a reference genome and its associated hash tables onto the PCIe card. For information on preprocessing a reference genome's FASTA files into the native DRAGEN binary reference and hash table formats, see *Prepare a Reference Genome* on page 8. You must also specify the directory containing the preprocessed binary reference and hash tables with the *-r* [or *--ref-dir*] option. This argument is always required.

Use the following command to You can load the reference genome and hash tables to DRAGEN card memory separately from processing reads/, as follows.

```
dragen -r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149
```

You can Uuse the *-l (--force-load-reference)* option to force the reference genome to load even if it is already loaded, as follows.

```
dragen -l -r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149
```

The time needed to load the reference genome depends on the size of the reference, but for typical recommended settings, it takes approximately 30–60 seconds.

## Output Options

The following commandline options for output are mandatory:

- *--output-directory <out_dir>*—Specifies the output directory for generated files.
- *--output-file-prefix <out_prefix>*—Specifies the output file prefix. DRAGENappends the appropriate file extension onto this prefix for each generated file.
- *-r [ --ref-dir ]* specifies the reference hash table.

For brevity, the following examples do not include these mandatory options.

For mapping and aligning, the output is sorted and compressed into BAM format by default before saving to disk. You can control the output format from the map/align stage with the *--output-format <SAM|BAM|CRAM>* option. If the output file exists, DRAGEN issues a warning and exits. To force overwrite if the output file already exists, use the *-f [ --force ]* option.

For example, the following commands output to a compressed BAM file and forces overwrite:

```
dragen ... -f
dragen ... -f --output-format bam
```

To generate a BAI-format BAM index file (.bai file extension), set *--enable-bam-indexing* to true.

The following example outputs to a SAM file and forces overwrite:

```
dragen ... -f --output-format sam
```

The following example outputs to a CRAM file and forces overwrite:

```
dragen ... -f --output-format cram
```

DRAGEN can generate mismatch difference (MD) tags, as described in the BAM standard. The feature is turned off by default because there is a small performance cost to generate these strings. To generate MD tags, set *--generate-md-tags* to true.

To generate ZS:Z alignment status tags, set *--generate-zs-tags* to true. These tags are only generated in the primary alignment and when a read has suboptimal alignments qualifying for secondary output (even if none were output because *--Aligner.sec-aligns* was set to 0).

The following are valid tag values:

- ZS:Z:R—Multiple alignments with similar score were found.
- ZS:Z:NM—No alignment was found.
- ZS:Z:QL—An alignment was found, but it was below the quality threshold.

To generate SA:Z tags, set *--generate-sa-tags* to true (the default). These tags provide alignment information (position, cigar, orientation) of groups of supplementary alignments, which are useful in structural variant calling.

## Preservation or Stripping of BQSR Tags

The Picard Base Quality Score Recalibration (BQSR) tool produces output BAM files that include tags BI and BD. BQSR calculates these tags relative to the exact sequence for a read. If a BAM file with BI and BD tags is used as input to mapper/aligner with hard clipping enabled, the BI and/or BD tags can become invalid.

The recommendation is to strip these tags when using BAM files as input. To remove the BI and BD tags, set the *--preserve-bqsr-tags* option to *false*. If you preserve the tags, DRAGEN warns you to disable hard clipping.

## Read Group Options

DRAGEN assumes that all the reads in a given FASTQ belong to the same read group. The DRAGEN system creates a single @RG read group descriptor in the header of the output BAM file, with the ability to specify the following standard BAM attributes:

| Attribute | Argument | Description |
|---|---|---|
| ID | --RGID | Read group identifier. If you include any of the read group parameters, RGID is required. It is the value written into each output BAM record. |
| LB | --RGLB | Library. |

| Attribute | Argument | Description |
|---|---|---|
| PL | --RGPL | Platform/technology used to produce the reads. The BAM standard allows for values CAPILLARY, LS454, ILLUMINA, SOLID, HELICOS, IONTORRENT and PACBIO. |
| PU | --RGPU | Platform unit, eg, flowcell-barcode.lane. |
| SM | --RGSM | Sample. |
| CN | --RGCN | Name of the sequencing center that produced the read. |
| DS | --RGDS | Description. |
| DT | --RGDT | Date the run was produced. |
| PI | --RGPI | Predicted mean insert size. |

If any of these arguments are present, the DRAGEN software adds an RG tag to all the output records to indicate that they are members of a read group. The following example shows a command line that includes read group parameters:

```
dragen --RGID 1 --RGCN Broad --RGLB Solexa-135852 \
--RGPL Illumina --RGPU 1 --RGSM NA12878 \
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-1 SRA056922.fastq --output-directory /staging/tmp/ \
--output-file-prefix rg_example
```

When using the *--fastq-list* option to input multiple read groups, BAM tags (and others) are specified for each read group by adding columns to the `fastq_list.csv` file. Each column heading consists of four capital letters and each begins with 'RG'. For each column, each read group's values for that column are propagated to the output BAM file in an identically named tag.

## License Options

To suppress the license status message at the end of the run, use the *--lic-no-print* option. The following shows an example of the license status message:

```
LICENSE_MSG| ======================================================
LICENSE_MSG| License report
LICENSE_MSG|   Genome status [ACxxxxxxxxxxx] : used 1263.9 Gbases since
2018-Feb-15 (1263886160894 bases, unlimited)
LICENSE_MSG|   Genome  bases [ACxxxxxxxxxxx] : 202000000
LICENSE_MSG|   Genome  bases [total]         : 202000000
```

# Input Options

The DRAGEN system is capable of processing reads in FASTQ format or BAM/CRAM format. If your input FASTQ files end in .gz, DRAGEN automatically decompresses the files using hardware-accelerated decompression.

## Input File Types

Use the following command-line options for any input files you would like to use.

### FASTQ Input Files

FASTQ input files can be single-ended or paired-end. Use the following examples to input FASTQ files.

- **Single-ended in one FASTQ file** *(-1 option)*

```
dragen -r <REF_DIR> -1 <fastq> --output-directory <OUT_DIR> \
--output-file-prefix <OUTPUT_PREFIX> --RGID <RGID> --RGSM <RGSM>
```

- **Paired-end in two matched FASTQ files***(-1 and -2 options)*

```
dragen -r <REF_DIR> -1 <fastq1> -2 <fastq2> \
--output-directory <OUT_DIR> --output-file-prefix <OUT_PREFIX> \
--RGID <RGID> --RGSM <RGSM>
```

- **Paired-end in a single interleaved FASTQ file***(--interleaved (-i) option)*

```
dragen -r <REF_DIR> -1 <INTERLEAVED_FASTQ> -i \
--RGID <RGID> --RGSM <RGSM>
```

If using, *bcl2fastq* or the *DRAGEN BCL* command use the following common file naming convention:

```
<SampleID>_S<#>_<Lane>_<Read>_<segment#>.fastq.gz
```

The file naming convention is different for HiSeq X and NextSeq Sequencing Systems.

Older versions of bcl2fastq and DRAGEN could segment FASTQ samples into multiple files to limit file size or to decrease the time to generate them.

For Example:

```
RDRS182520_S1_L001_R1_001.fastq.gz
RDRS182520_S1_L001_R1_002.fastq.gz
...
RDRS182520_S1_L001_R1_008.fastq.gz
```

These files do not need to be concatenated to be processed together by DRAGEN. To map/align any sample, provide the first file in the series (*-1 <FileName>_001.fastq*). DRAGEN reads all segment files in the sample consecutively for both of the FASTQ file sequences specified using the *-1 and -2 options* for paired-end input and for compressed fastq.gz files. To turn the behavior off, set *--enable-auto-multifile* to false on the command line.

DRAGEN can also optionally read multiple files by the sample name given in the file name, which can be used to combine samples that have been distributed across multiple BCL lanes or flow cells. To enable this feature, set the *--combine-samples-by-name* option to true.

If the FASTQ files specified on the command-line use the Casava 1.8 file naming convention shown above and additional files in the same directory share that sample name, those files and all their segments are processed automatically. Note that sample name, read number, and file extension must match. Index barcode and lane number can differ.

To avoid impacting system performance, input files must be located on a fast file system.

## Multiple FASTQ Input Files

To provide multiple FASTQ input files, it is recommended to use the *--fastq-list <csv file name>* option to specify the name of a CSV file containing the list of FASTQ files, instead of using the *--combine-samples-by-name* option. For example:

```
dragen -r <ref_dir> --fastq-list <CSV_FILE> \
--fastq-list-sample-id <Sample_ID> \
--output-directory <OUT_DIR> --output-file-prefix <OUT_PREFIX>
```

Using a CSV file allows you to name the FASTQ input files, input from multiple subdirectories, and add BAM tags specified explicitly for each read group. DRAGENautomatically generates a CSV file of the correct format during BCL conversion to FASTQ. The CSV file is named `fastq_list.csv` and contains an entry for each FASTQ file or paired-end file pair produced during the run.

## FASTQ CSV File Format

The first line of the CSV file specifies the title of each column, and is followed by one or more data lines. All lines in the CSV file must contain the same number of comma-separated values and should not contain white space or other extraneous characters.

Column titles are case-sensitive. The following column titles are required:

- RGID—Read Group
- RGSM—Sample ID
- RGLB—Library
- Lane—Flow cell lane
- Read1File—Full path to a valid FASTQ input file
- Read2File—Full path to a valid FASTQ input file. Required for paired-end input. If not using paired-end input, leave empty.

Each FASTQ file can only be referenced once in the CSV list.. All values in the Read2File column must be reference valid files or must all be empty.

When generating a BAM file using fastq-list input, one read group is generated per unique RGID value. The BAM header contains RG tags for the following read groups:

- ID (from RGID)
- SM (from RGSM)
- LB (from RGLB)

Additional tags can be specified for each read group by adding a column title. The column title must be only four upper-case characters and begin with RG. For example, to add a PU (platform unit) tag, add a column named RGPU and specify the value for each read group in this column. All column titles must be unique.

A fastq-list file can contain files for more than one sample. If a fastq-list file contains only one unique RGSM entry, then no additional options need to be specified and DRAGEN processes all files listed in the fastq-list file. If there is more than one unique RGSM entry in a fastq-list file, one of the following must also be specified in addition to *--fastq-list <filename>*.

- To process a specific sample from the CSV file, use *--fastq-list-sample-id <SampleID>*. Only the entries in the fastq-list file with an RGSM value matching the specified SampleID are processed.
- To process all samples together in the same run, regardless of the RGSM value, set *--fastq-list-all-samples* to true.

For a single run, only one BAM and VCF output file are produced because all input read groups are expected to belong to the same sample. To process multiple samples from one BCL conversion run, run the DRAGEN secondary analysis multiple times using different values for the *--fastq-list-sample-id* option.

There is no option to specify groupings or subsets of RGSM values for more complex filtering, but the fastq-list file can be modified to achieve the same effect.

The following is an example FASTQ list CSV file with the required columns:

```
RGID,RGSM,RGLB,Lane,Read1File,Read2File
CACACTGA.1,RDSR181520,UnknownLibrary,1,/staging/RDSR181520_S1_L001_R1_
001.fastq, /staging/RDSR181520_S1_L001_R2_001.fastq
AGAACGGA.1,RDSR181521,UnknownLibrary,1,/staging/RDSR181521_S2_L001_R1_
001.fastq, /staging/RDSR181521_S2_L001_R2_001.fastq
TAAGTGCC.1,RDSR181522,UnknownLibrary,1,/staging/RDSR181522_S3_L001_R1_
001.fastq, /staging/RDSR181522_S3_L001_R2_001.fastq
AGACTGAG.1,RDSR181523,UnknownLibrary,1,/staging/RDSR181523_S4_L001_R1_
001.fastq, /staging/RDSR181523_S4_L001_R2_001.fastq
```

If you use the *--tumor-fastq-list* option for somatic input, use the *--tumor-fastq-list-sample-id <SampleID>* option to specify the sample ID for the corresponding FASTQ list, as shown in the following example:

```
dragen -r <ref_dir> --tumor-fastq-list <csv_file> \
--tumor-fastq-list-sample-id <Sample_ID> \
--output-directory <out_dir> \
--output-file-prefix <out_prefix> --fastq-list <csv_file_2> \
--fastq-list-sample-id <Sample_ID_2>
```

## Tumor-Normal Pairs Input

If using *fastq_lists* or *tumor_fastq_lists* comprising of multiple samples (RGSMs) in somatic mode, you can use a loop to iterate through the two lists to create tumor-normal pairs for testing. Create a *.txt file with the RGSM of each normal sample to be tested (one per line), and then create a separate *.txt file with the RGSM of the tumor samples to be tested. Make sure that the tumor sample RGSM is listed in the same order as the corresponding normal samples and to include a blank line after the last sample.

The following example script can then be used to perform testing in somatic mode. Each iteration takes one entry from the tumor samples list and one entry from the normal samples list (from top to bottom) to create a tumor-normal pair as input for the DRAGEN run.

```
#!/bin/bash
HT="/staging/HT/"
tumor_fastq_list="/staging/inputs/tumor_fastq_list.csv"
normal_fastq_list="/staging/inputs/normal_fastq_list.csv"
tumor_samples_list="/staging/inputs/tumor_samples_list.txt"
normal_samples_list="/staging/inputs/normal_samples_list.txt"
while read -u 3 -r tumor_RGSM && read -u 4 -r normal_RGSM; do
output_dir="/staging/results/${tumor_RGSM}_${normal_RGSM}"
mkdir -p ${output_dir}
dragen \
-r ${HT} \
--tumor-fastq-list ${tumor_fastq_list} \
--tumor-fastq-list-sample-id ${tumor_RGSM} \
--fastq-list ${normal_fastq_list} \
--fastq-list-sample-id ${normal_RGSM} \
--output-directory ${output_dir} \
--output-file-prefix ${tumor_RGSM}_${normal_RGSM}
done 3<${tumor_samples_list} 4<${normal_samples_list}
```

The following are examples of the FASTQ lists and samples lists used as input for the script.

Sample fastq_list.csv:

```
RGPL,RGID,RGSM,RGLB,Lane,Read1File,Read2File
DRAGEN_RGPL,DRAGEN_RGID_N1.1,normal-1,ILLUMINA,1,/staging/inputs/normal-1_
S1_L001_R1_001.fastq.gz,/staging/inputs/normal-1_S1_L001_R2_001.fastq.gz
```

```
      DRAGEN_RGPL,DRAGEN_RGID_N1.2,normal-1,ILLUMINA,2,/staging/inputs/normal-1_
      S1_L002_R1_001.fastq.gz,/staging/inputs/normal-1_S1_L002_R2_001.fastq.gz
      DRAGEN_RGPL,DRAGEN_RGID_N2.1,normal-2,ILLUMINA,1,/staging/inputs/normal-2_
      S1_L001_R1_001.fastq.gz,/staging/inputs/normal-2_S1_L001_R2_001.fastq.gz
      DRAGEN_RGPL,DRAGEN_RGID_N2.2,normal-2,ILLUMINA,2,/staging/inputs/normal-2_
      S1_L002_R1_001.fastq.gz,/staging/inputs/normal-2_S1_L002_R2_001.fastq.gz
      DRAGEN_RGPL,DRAGEN_RGID_N3.1,normal-3,ILLUMINA,1,/staging/inputs/normal-3_
      S1_L001_R1_001.fastq.gz,/staging/inputs/normal-3_S1_L001_R2_001.fastq.gz
      DRAGEN_RGPL,DRAGEN_RGID_N3.2,normal-3,ILLUMINA,2,/staging/inputs/normal-3_
      S1_L002_R1_001.fastq.gz,/staging/inputs/normal-3_S1_L002_R2_001.fastq.gz

Sample tumor_fastq_list.csv content:
      RGPL,RGID,RGSM,RGLB,Lane,Read1File,Read2File
      DRAGEN_RGPL,DRAGEN_RGID_T1.1,tumor-1,ILLUMINA,1,/staging/inputs/tumor-1_
      S1_L001_R1_001.fastq.gz,/staging/inputs/tumor-1_S1_L001_R2_001.fastq.gz
      DRAGEN_RGPL,DRAGEN_RGID_T1.2,tumor-1,ILLUMINA,2,/staging/inputs/tumor-1_
      S1_L002_R1_001.fastq.gz,/staging/inputs/tumor-1_S1_L002_R2_001.fastq.gz
      DRAGEN_RGPL,DRAGEN_RGID_T2.1,tumor-2,ILLUMINA,1,/staging/inputs/tumor-2_
      S1_L001_R1_001.fastq.gz,/staging/inputs/tumor-2_S1_L001_R2_001.fastq.gz
      DRAGEN_RGPL,DRAGEN_RGID_T2.2,tumor-2,ILLUMINA,2,/staging/inputs/tumor-2_
      S1_L002_R1_001.fastq.gz,/staging/inputs/tumor-2_S1_L002_R2_001.fastq.gz
      DRAGEN_RGPL,DRAGEN_RGID_T3.1,tumor-3,ILLUMINA,1,/staging/inputs/tumor-3_
      S1_L001_R1_001.fastq.gz,/staging/inputs/tumor-3_S1_L001_R2_001.fastq.gz
      DRAGEN_RGPL,DRAGEN_RGID_T3.2,tumor-3,ILLUMINA,2,/staging/inputs/tumor-3_
      S1_L002_R1_001.fastq.gz,/staging/inputs/tumor-3_S1_L002_R2_001.fastq.gz
Sample normal_samples_list
      normal-1
      normal-2
      normal-3
Sample tumor_samples_list content
      tumor-1
      tumor-2
      tumor-3
```

## BAM Input Files

To use BAM files as input to the mapper/aligner, set *--enable-map-align* to true. If you leave this option set to false (the default), you can use the BAM file as input to the variant caller.

When you specify a BAM file as input, DRAGEN ignores any alignment information contained in the input file, and outputs new alignments for all reads. If the input file contains paired-end reads, it is important to specify that the input data should be sorted so that pairs can be processed together. Other pipelines require you to resort the input data set by read name. DRAGEN vastly increases the speed of this operation by pairing the input reads, and sending on to the mapper/aligner when pairs are identified. Use the *--pair-by-name* option to enable or disable this feature (the default is true).

- Specify single-ended input in one BAM file with the *(-b)* and `--pair-by-name=false` options, as follows:
  ```
  dragen -r <ref_dir> -b <bam> --output-directory <out_dir> \
  --output-file-prefix <out_prefix> --pair-by-name false
  ```
- Specify paired-end input in one BAM file with the *(-b)* and `--pair-by-name=true` options, as follows:
  ```
  dragen -r <ref_dir> -b <bam> --output-directory <out_dir> \
  --output-file-prefix <out_prefix> --pair-by-name true
  ```

## CRAM Input Files

You can use CRAM files as input to the DRAGEN mapper/aligner and variant caller. The DRAGEN functionality available when using CRAM input is the same as when using BAM input.

The *--cram-reference* option is no longer needed. The CRAM compressor and decompressor uses the DRAGEN reference.

Use the following options to provide a CRAM input to either mapper/aligner or variant caller:

- *--cram-input*—The name and path for the CRAM file.
- *--cram-input*—One usage example is paired-end input in a single CRAM file. In addition, set the *--pair-by-name* option to true.
  ```
  dragen -r <ref_dir> --cram-input <cram> --output-directory <out_dir> \
  --output-file-prefix <out_prefix> --pair-by-name true
  ```

## BCL Input Files

BCL is the output format of Illumina sequencing systems. Under limited circumstances, DRAGEN can read directly from BCL for map-align operations, saving the time needed for conversion to FASTQ.

DRAGEN can read directly from BCL in the following circumstances:

- Only one lane is input as part of a run (specified on the command-line).
- The lane has only a single sample specified in the SampleSheet.csv file.

When converting BCL to FASTQ is required, DRAGEN provides a BCL to FASTQ converter (see *DRAGEN BCL Data Conversion* on page 238).

The following example command is for BCL input with only one lane of input:

```
dragen --bcl-input-dir <BCL_ROOT> --bcl-only-lane <num> -r <ref_dir> \
--output-directory <out_dir> --output-file-prefix <out_prefix>
```

For additional BCL conversion options, see *DRAGEN BCL Data Conversion* on page 238.

## Handling of N bases

One of the techniques that DRAGEN uses to optimize handling sequences can lead to the overwriting the base quality score assigned to N base calls.

When you use the *--fastq-n-quality* and *--fastq-offset* options, the base quality scores are overwritten with a fixed base quality. The default values for these options are 2 and 33 respectively to match the Illumina minimum quality of 35 (ASCII character '#').

## Read Names for Paired-End Reads

By a common convention, read names can include suffixes (such as `/1` or `/2`), which indicate the end of a pair the read represents. For BAM input using the *--pair-by-name option*, DRAGEN ignores these suffixes to find matching pair names. By default, DRAGEN uses the forward slash character as the delimiter for these suffixes and ignores the /1 and /2 when comparing names. By default, DRAGEN strips these suffixes from the original read names.

DRAGEN provides the following options to control how suffixes are used:

- To change the delimiter character for suffixes, use the *--pair-suffix-delimiter* option. Valid values for this option include forward-slash (/), dot (.), and colon (:).
- To preserve the entire name, including the suffixes, set *--strip-input-qname-suffixes* to false.
- To append a new set of suffixes to all read names, set *--append-read-index-to-name* to true, where the delimiter is determined by the *--pair-suffix-delimiter* option. By default the delimiter is a slash, so /1 and /2 are added to the names.

## Gene Annotation Input Files

When processing RNA-Seq data, you can supply a gene annotations file by using the *--annotation-file* option. Providing this file improves the accuracy of the mapping and aligning stage (see *Input Files* on page 214). The file should conform to the GTF/GFF format specification and should list annotated transcripts that match the reference genome being mapped against. The similar GFF3 format is currently not supported.

DRAGEN can take the `SJ.out.tab` file (see *SJ.out.tab* on page 217) as an annotations file to help guide the aligner in a two-pass mode of operation.

## Sample Sex

Use the *--sample-sex* option to specify the sample sex on the command line. The information is passed to all callers. The CNV caller contains a separate sex inference feature, but the specified *--sample-sex* takes precedence over the inferred sex. The following example specifies sample sex using the *--sample-sex* option:

```
--sample-sex MALE
--sample-sex FEMALE
```

If *--sample-sex* is not specified on the command line, the Ploidy Estimator runs by default to determine the sex.

## Stream Input Files

DRAGEN can stream input files directly from an AWS S3 bucket, or using HTTP presigned URLs. You do not need to download the input files to a local disk prior to being processed. The files are streamed over the network directly into the DRAGEN processor.

Input streaming is most beneficial for large input files. DRAGEN supports input streaming for BAMs and compressed FASTQ files. For FASTQ files, input streaming can be used in all the configurations that use single-end FASTQs, paired-end FASTQs, and FASTQ lists.

Input streaming is supported for the following use cases.

- Mapping/aligning of FASTQ and BAM.
- Germline and somatic small variant calling from BAM (without remapping).

For other file types that are significantly smaller in size, download them locally before running the analysis.

### Security and Permissions

To stream input files, you must have permission to access the remote files. The S3 object requires AWS authentication and credentials. The authentication should already be set up on the instance you are running, for example, via IAM policies. An HTTP URL most likely has a query string attached to it, which provides the authentication credentials or necessary tokens to grant permission.

### Examples

The following examples display possible methods to stream input files directly with DRAGEN.

### Streaming FASTQ Input Using S3

```
dragen -f
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-1 s3://s3-bucket-name/path/to/object_1.fastq.gz \
```

```
-2 s3://s3-bucket-name/path/to/object_2.fastq.gz \
--RGID object_ID \
--RGSM sample_name \
--output-directory /staging/examples/ \
--output-file-prefix streaming
```

### Streaming FASTQ Input Using HTTP

```
dragen -f
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-1 https://bucket-name.amazonaws.com/path/to/object_1.fastq.gz?querystring
\
-2 https://bucket-name.amazonaws.com/path/to/object_2.fastq.gz?querystring
\
--RGID object_ID \
--RGSM sample_name \
--output-directory /staging/examples/ \
--output-file-prefix streaming
```

### Streaming BAM Input Using S3

```
dragen -f
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-b s3://s3-bucket-name/path/to/object_1.bam \
--output-directory /staging/examples/ \
--output-file-prefix streaming
```

### Streaming BAM Input Using HTTP

```
dragen -f
-r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
-b https://bucket-name.amazonaws.com/path/to/object_1.bam?querystring \
--output-directory /staging/examples/ \
--output-file-prefix streaming
```

## Autogenerated MD5SUM for BAM and CRAM Output Files

An MD5SUM file is generated automatically for BAM and CRAM output files. The MD5SUM file has the same name as the output file with an .md5sum extension appended (eg, `whole_genome_run_123.bam.md5sum`). The MD5SUM file is a single-line text file that contains the md5sum of the output file, which exactly matches the output of the Linux md5sum command.

The MD5SUM calculation is performed as the output file is written, so there is no measurable performance impact (compared to the Linux md5sum command, which can take several minutes for a 30x BAM).

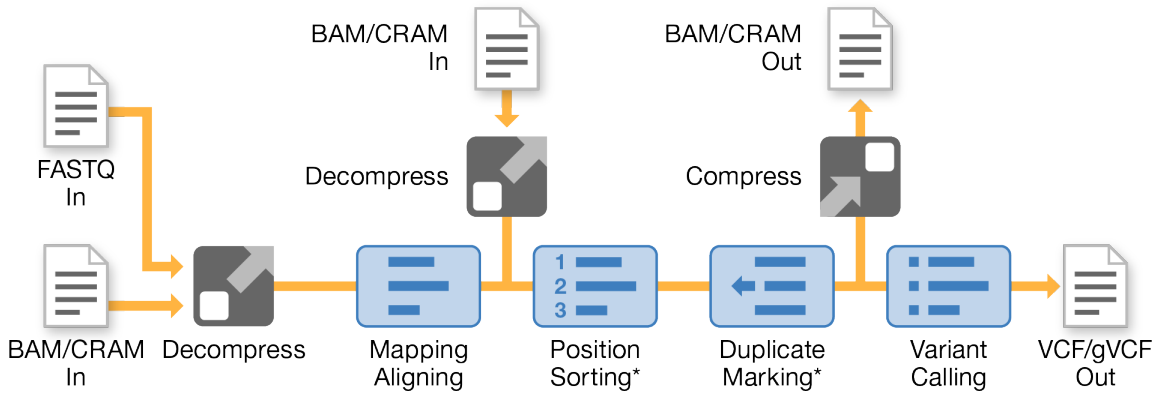
# Configuration Files

Command-line options can be stored in a configuration file. The location of the default configuration file is `/opt/edico/config/dragen-user-defaults.cfg`. To specify a different file, use the *--config-file (-c)* option. The configuration file used for a given run supplies the default settings for that run, any of which can be overridden by command-line options.

The recommended approach is to use the `dragen-user-defaults.cfg` file as a template to create default settings for different use cases. Copy `dragen-user-defaults.cfg`, rename the copy, and then modify the new file for the specific use-case. As a best practice, put options that rarely change into the configuration file and specify options that vary per run on the command-line.

# DRAGEN DNA Pipeline

Figure 2 DNA Pipeline for DRAGEN




* Optional

## DNA Mapping

### Seed Density

The *seed-density* option controls how many (normally overlapping) primary seeds from each read the mapper looks up in its hash table for exact matches. The maximum density value of 1.0 generates a seed starting at every position in the read, ie, (L-K+1) K-base seeds from an L-base read.

Seed density must be between 0.0 and 1.0. Internally, an available seed pattern equal or close to the requested density is selected. The most sparse pattern is one seed per 32 positions, or density 0.03125.

- **Accuracy Considerations**—Generally, denser seed lookup patterns improve mapping accuracy. However, for long reads ( 50 bp+) and low sequening error rates, there is minimum improvement beyond the default 50% seed lookup density..
- **Speed Considerations**—Denser seed lookup patterns generally slow down mapping, while more sparse seed patterns speed up mapping. However, when the seed-mapping stage runs faster than the aligning stage, a sparser seed pattern does not improve the mapper speed.

### Relationship to Reference Seed Interval

Functionally, a denser or more sparse seed lookup pattern has an impact similar to a shorter or longer reference seed interval (build hash table option *--ht-ref-seed-interval*). Populating 100% of reference seed positions and looking up 50% of read seed positions has the same effect as populating 50% of reference seed positions and looking up 100% of read seed positions. Either way, the expected density of seed hits is 50%.

More generally, the expected density of seed hits is the product of the reference seed density (the inverse of the reference seed interval) and the seed lookup density. For example, if 50% of reference seeds are populated and 33.3% (1/3) of read seed positions are looked up, then the expected seed hit density should be 16.7% (1/6).

Local Analysis Software automatically adjusts theseed lookup pattern to make sure it does not systematically miss the seed positions populated from the reference. For example, the mapper does not look up seeds matching only odd positions in the reference when only even positions are populated in the hash table, even if the reference seed interval is 2 and seed-density is 0.5.

## Map Orientations

The *--Mapper.map-orientations* option is used in mapping reads for bisulfite methylation analysis. The option is set automatically based on the value set for *--methylation-protocol*.

The *--Mapper.map-orientations* option can restrict the orientation of read mapping to only forward in the reference genome or only reverse-complemented. The following are the valid values for *--map-orientations*:

- 0—Either orientation (default).
- 1—Only forward mapping.
- 2—Only reverse-complemented mapping.

If mapping orientations are restricted and paired end reads are used, the expected pair orientation can only be FR, not FF, or RF.

## Seed Density Command-Line Options

Although Local Analysis Software primarily maps reads by finding exact reference matches to short seeds, it can also map seeds differing from the reference by one nucleotide by also looking up single-SNP edited seeds. Seed editing is usually not necessary with longer reads (100 bp+), because longer reads have a high probability of containing at least one exact seed match. This is especially true when paired ends are used, because a seed match from either mate can successfully align the pair. However, seed editing can be useful to increase mapping accuracy for short single-ended reads, with some cost in increased mapping time. The following options control seed editing:

Table 3 Seed Editing Options

| Command-Line Option Name | Configuration File Option Name |
|---|---|
| --Mappper.seed-density | seed-density |
| --Mapper.edit-mode | edit-mode |
| --Mapper.edit-seed-num | edit-seed-num |
| --Mapper.edit-read-len | edit-read-len |

| Command-Line Option Name | Configuration File Option Name |
|---|---|
| --Mapper.edit-chain-limit | edit-chain-limit |

### edit-mode and edit-chain-limit

The *edit-mode* and *edit-chain-limit* options control when seed editing is used. The following four edit-mode values are available:

| Mode Value | Description |
|---|---|
| 0 | No editing (default). |
| 1 | Chain length test. |
| 2 | Paired chain length test. |
| 3 | Full seed editing. |

Edit mode 0 requires all seeds to match exactly. Mode 3 is the most expensive because every seed that fails to match the reference exactly is edited. Modes 1 and 2 employ heuristics to look up edited seeds only for reads most likely to be salvaged to accurate mapping.

The main heuristic in edit modes 1 and 2 is a seed chain length test. Exact seeds are mapped to the reference in a first pass over a given read, and the matching seeds are grouped into chains of similarly aligning seeds. If the longest seed chain in the read exceeds a threshold edit-chain-limit, the read does not require seed editing, because there is already a mapping position.

Edit mode 1 triggers seed editing for a given read using the seed chain length test. If no seed chain exceeds *edit-chain-limit* (including if no exact seeds match), then a second seed-mapping pass is attempted using edited seeds. Edit mode 2 further optimizes the heuristic for paired-end reads. If either mate has an exact seed chain longer than *edit-chain-limit*, then seed editing is disabled for the pair because a rescue scan is likely to recover the mate alignment based on seed matches from one read. Edit mode 2 is the same as mode 1 for single-end reads.

### edit-seed-num and edit-read-len

For edit modes 1 and 2, when the heuristic triggers seed editing, these options control how many seed positions are edited in the second pass over the read. Although exact seed mapping can use a densely overlapping seed pattern, such as seeds starting at 50% or 100% of read positions, most of the value of seed editing can be obtained by editing a much more sparse pattern of seeds, even a nonoverlapping pattern. Generally, if a user application can afford to spend some additional amount of mapping time on seed editing, a greater increase in mapping accuracy can be obtained for the same time cost by editing seeds in sparse patterns for a large number of reads, than by editing seeds in dense patterns for a small number of reads.

Whenever seed editing is triggered, these two options request *edit-seed-num* seed editing positions, distributed evenly over the first *edit-read-len* bases of the read. For example, with 21-base seeds, *edit-seed-num*=6 and *edit-read-len*=100, edited seeds can begin at offsets {0, 16, 32, 48, 64, 80} from the 5' end, consecutive seeds overlapping by five bases. Because sequencing technologies often yield better base qualities nearer the (5') beginning of each read, this can focus seed editing where it is most likely to succeed. When a particular read is shorter than *edit-read-len*, fewer seeds are edited.

Seed editing is more expensive when the reference seed interval (build hash table option *--ht-ref-seed-interval*) is greater than 1. For edit modes 1 and 2, additional seed editing positions are automatically generated to avoid missing the populated reference seed positions. For edit mode 3, the time cost can increase dramatically because query seeds matching unpopulated reference positions typically miss and trigger editing.

# DNA Aligning

## Smith-Waterman Alignment Scoring Settings

The first stage of mapping is generates seeds from the read and looks for exact matches in the reference genome. The seed match results are then refined by running full Smith-Waterman alignments on the locations with the highest density of seed matches. The alignment algorithm compares each position of the read against all candidate positions of the reference. These comparisons correspond to a matrix of potential alignments between read and reference. For each of the candidate alignment positions, Smith-Waterman generates scores that are used to evaluate whether the best alignment passing through that matrix cell reaches it by a nucleotide match or mismatch (diagonal movement), a deletion (horizontal movement), or an insertion (vertical movement). A match between read and reference provides a bonus, on the score, and a mismatch or indel imposes a penalty. The overall highest scoring path through the matrix is the alignment chosen.

The specific values chosen for scores in this algorithm indicate how to balance, for an alignment with multiple possible interpretations, the possibility of an indel as opposed to one or more SNPs, or the preference for an alignment without clipping. The default DRAGENscoring values are reasonable for aligning moderate length reads to a whole human reference genome for variant calling applications. But any set of Smith-Waterman scoring parameters represents an imprecise model of genomic mutation and sequencing errors, and differently tuned alignment scoring values can be more appropriate for some applications.

The following options control Smith-Waterman Alignment:

| Command-Line Option Name | Configuration File Option Name |
|---|---|
| --Aligner.global | global |

| Command-Line Option Name | Configuration File Option Name |
|---|---|
| --Aligner.match-score | match-score |
| --Aligner.match-n-score | match-n-score |
| --Aligner.mismatch-pen | mismatch-pen |
| --Aligner.gap-open-pen | gap-open-pen |
| --Aligner.gap-ext-pen | gap-ext-pen |
| --Aligner.unclip-score | unclip-score |
| --Aligner.no-unclip-score | no-unclip-score |
| --Aligner.aln-min-score | aln-min-score |

- *global*

  The *global* option (value can be 0 or 1) controls whether alignment is forced to be end-to-end in the read. When set to 1, alignments are always end-to-end, as in the Needleman-Wunsch global alignment algorithm (although not end-to-end in the reference), and alignment scores can be positive or negative. When set to 0, alignments can be clipped at either or both ends of the read, as in the Smith-Waterman local alignment algorithm, and alignment scores are nonnegative.

  Generally, *global*=0 is preferred for longer reads, so significant read segments after a break of some kind (large indel, structural variant, chimeric read, and so forth) can be clipped without severely decreasing the alignment score. Setting *global=1* might not have the desired effect with longer reads because insertions at or near the ends of a read can function as pseudoclipping. Also, with *global*=0, multiple (chimeric) alignments can be reported when various portions of a read match widely separated reference positions.

  Using *global*=1 is sometimes preferable with short reads, which are unlikely to overlap structural breaks, unable to support chimeric alignments, and are suspected of incorrect mapping if they cannot align well end-to-end.

  Consider using the *unclip-score* option, or increasing it, instead of setting *global*=1, to make a soft preference for unclipped alignments.

- *match-score*

  The *match-score* option is the score for a read nucleotide matching a reference nucleotide (A, C, G, or T). Its value is an unsigned integer, from 0 to 15. *match_score*=0 can only be used when *global*=1. A higher match score results in longer alignments, and fewer long insertions.

- *match-2-score*

  The match-2-score option is the score for a read nucleotide matching a 2-base IUPAC-IUB code in the reference (K, M, R, S, W, or Y). This option is a signed integer, ranging from -16 to 15.

- *match-3-score*

The *match-3-score* option is the score for a read nucleotide matching a 3-base IUPAC-IUB code in the reference (B, D, H, or V). This option is a signed integer, from -16 to 15.

- *match-n-score*

  The *match-n-score* option is the score for a read nucleotide matching an N code in the reference. This option is a signed integer, from -16 to 15.

- *mismatch-pen*

  The *mismatch-pen* option is the penalty (negative score) for a read nucleotide mismatching any reference nucleotide or IUPAC-IUB code (except 'N', which cannot mismatch). This option is an unsigned integer, from 0 to 63. A higher mismatch penalty results in alignments with more insertions, deletions, and clipping to avoid SNPs.

- *gap-open-pen*

  The *gap-open-pen* option is the penalty (negative score) for opening a gap (ie, an insertion or deletion). This value is only for a 0-base gap. It is always added to the gap length times gap-ext-pen. This option is an unsigned integer, from 0 to 127. A higher gap open penalty causes fewer insertions and deletions of any length in alignment CIGARs, with clipping or alignment through SNPs used instead.

- *gap-ext-pen*

  The *gap-ext-pen* option is the penalty (negative score) for extending a gap (ie, an insertion or deletion) by one base. This option is an unsigned integer, from 0 to 15. A higher gap extension penalty causes fewer long insertions and deletions in alignment CIGARs, with short indels, clipping, or alignment through SNPs used instead.

- *unclip-score*

  The *unclip-score* option is the score bonus for an alignment reaching the beginning or end of the read. An end-to-end alignment receives twice this bonus. This option is an unsigned integer, from 0 to 127. A higher unclipped bonus causes alignment to reach the beginning and/or end of a read more often, where this can be done without too many SNPs or indels.

  A nonzero unclip-score is useful when *global=0* to make a soft preference for unclipped alignments. Unclipped bonuses have little effect on alignments when *global=1*, because end-to-end alignments are forced anyway (although 2 × unclip-score does add to every alignment score unless *no-unclip-score = 1*). Illuminarecommends using the default for unclip-score when *global=1*, because some internal heuristics consider how local alignments would have been clipped.

  For longer reads, setting *unclip-score* much higher than *gap-open-pen* can result in insertions at or near one end of a read being utilized as pseudoclipping, as happens with *global=1*.

- *no-unclip-score*

  The *no-unclip-score* option can be 0 or 1. The default is 1. When *no-unclip-score* is set to 1, any unclipped bonus (unclip-score) contributing to an alignment is removed from the alignment score before further processing, such as comparison with *aln-min-score*, comparison with other alignment scores, and reporting in AS or XS tags. However, the unclipped bonus still affects the

best-scoring alignment found by Smith-Waterman alignment to a given reference segment, biasing toward unclipped alignments.

If *unclip-score* > 0 causes a Smith-Waterman local alignment to extend out to one or both ends of the read, the alignment score stays the same or increases if *no-unclip-score*=0, whereas it stays the same or decreases if *no-unclip-score*=1.

The default, *no-unclip-score*=1, is recommended when *global*=1, because every alignment is end-to-end, and there is no need to add the same bonus to every alignment.

When changing *no-unclip-score*, consider whether *aln-min-score* should be adjusted. When *no-unclip-score*=0, unclipped bonuses are included in alignment scores compared to the *aln-min-score* floor, so the subset of alignments filtered out by *aln-min-score* can change significantly with *no-unclip-score*.

- *aln-min-score*

  The *aln-min-score* option specifies a minimum acceptable alignment score. Any alignment results scoring lower are discarded. Increasing or decreasing *aln-min-score* can reduce or increase the percentage of reads mapped. This option is a signed integer (negative alignment scores are possible with *global*=0).

  *aln-min-score* also affects MAPQ estimates. The primary contributor to MAPQ calculation is the difference between the best and second-best alignment scores, and *aln-min-score* serves as the suboptimal alignment score if nothing higher was found except the best score. Therefore, increasing *aln-min-score* can decrease reported MAPQ for some low-scoring alignments.

## Paired-End Options

DRAGEN can process paired-end data passed via a pair of FASTQ files or in a single interleaved FASTQ file. The hardware maps the two ends separately, and then determines a set of alignments that seem most likely to form a pair in the expected orientation and having roughly the expected insert size. The alignments for the two ends are evaluated for the quality of their pairing, with larger penalties for insert sizes far from the expected size. The following options control processing of paired-end data:

- *Reorientation*

  The *pe-orientation* option specifies the expected paired-end orientation. Only pairs with this orientation can be flagged as proper pairs. Valid values are as follows:

  - 0—FR (default)
  - 1—RF
  - 2—FF

- *unpaired-pen*

  For paired end reads, best mapping positions are determined jointly for each pair, according to the largest pair score found, considering the various combinations of alignments for each mate. A pair

score is the sum of the two alignment scores minus a pairing penalty, which estimates the unlikelihood of insert lengths further from the mean insert than this aligned pair.

The *unpaired-pen* option specifies how much alignment pair scores should be penalized when the two alignments are not in properly paired position or orientation. This option also serves as the maximum pairing penalty for properly paired alignments with extreme insert lengths.

The *unpaired-pen* option is specified in Phred scale, according to its potential impact on MAPQ. Internally, it is scaled into alignment score space based on Smith-Waterman scoring parameters.

- *pe-max-penalty*

  The *pe-max-penalty* option limits how much the estimated MAPQ for one read can increase because its mate aligned nearby. A paired alignment is never assigned MAPQ higher than the MAPQ that it would have received mapping single-ended, plus this value. By default, *pe-max-penalty* = *mapq-max* = 255, effectively disabling this limit.

  The key difference between *unpaired-pen* and *pe-max-penalty* is that *unpaired-pen* affects calculated pair scores and thus which alignments are selected and *pe-max-penalty* affects only reported MAPQ for paired alignments.

## Mean Insert Size Detection

When working with paired-end data, DRAGEN chooses among the highest-quality alignments for the two ends to try to choose likely pairs. To make this choice, DRAGEN uses a Gaussian statistical model to evaluate the likelihood that a pair of alignments constitutes a pair. This model is based on the intuition that a particular library prep tends to create fragments of roughly similar size, thus producing pairs whose insert lengths cluster well around some mean insert length.

If you know the statistics of your library prep for an input file (and the file consists of a single read group), you can specify the characteristics of the insert-length distribution: mean, standard deviation, and three quartiles. These characteristics can be specified with the *Aligner.pe-stat-mean-insert*, *Alinger.pe-stat-stddev-insert*, *Aligner.pe-stat-quartiles-insert*, and *Aligner.pe-stat-mean-read-len* options. However, it is typically preferable to allow DRAGEN to detect these characteristics automatically.

To enable automatic sampling of the insert-length distribution, set *--enable-sampling* to true. When the software starts execution, it runs a sample of up to 100,000 pairs through the aligner, calculates the distribution, and then uses the resulting statistics for evaluating all pairs in the input set.

The DRAGEN host software reports the statistics in its stdout log, as follows:

```
Final paired-end statistics detected for read group 0, based on 79935 high
quality pairs for FR orientation
   Quartiles (25 50 75) = 398 410 421
   Mean = 410.151
   Standard deviation = 14.6773
   Boundaries for mean and standard deviation: low = 352, high = 467
```

```
Boundaries for proper pairs: low = 329, high = 490

NOTE: DRAGEN's insert estimates include corrections for
clipping (so they are no identical to TLEN)
```

The insert length distribution for each sample is written to `fragment_length_hist.csv`. Each sample starts with the following lines

```
#Sample: sample name
FragmentLength,Count
```

These lines are followed by the histogram.

When the number of sample pairs is very small, there is not enough information to characterize the distribution with high confidence. In this case, DRAGEN applies default statistics that specify a very wide insert distribution, which tends to admit pairs of alignments as proper pairs, even if they may lie tens of thousands of bases apart. In this situation, DRAGEN outputs a message, as follows:

```
WARNING: Less than 28 high quality pairs found - standard deviation is
calculated from the small samples formula
```

The small samples formula calculates standard deviation as follows:

```
if samples < 3 then
     standard deviation = 10000
else if samples < 28 then
    standard deviation = 25 * (standard deviation + 1) / (samples – 2)
end if
if standard deviation < 12 then
     standard deviation = 12
end if
```

The default model is “standard deviation = 10000”. If the first 100,000 reads are unmapped or if all pairs are improper pairs, then the standard deviation is set to 10,000 and the mean and quartiles are set to 0. The minimum value for standard deviation is 12, which is independent of the number of samples.

For RNA-Seq data, the insert size distribution is not normal due to pairs containing introns. The DRAGENN software estimates the distribution using a kernel density estimator to fit a long tail to the samples. This estimate leads to a more accurate mean and standard deviation for RNA-Seq data and proper pairing.

DRAGEN writes detected paired-end stats into a tab-delimited log file in the output directory called `.insert-stats.tab`. This file contains the statistical distribution of detected insert sizes for each read group, including quartiles, mean, standard deviation, minimum, and maximum. The information matches the standard-out report above. Additionally, the log file includes the minimum and maximum insert limits that DRAGEN applied for rescue scans.

## Rescue Scans

For paired-end reads, where a seed hit is found for one mate but not the other, rescue scans hunt for missing mate alignments within a rescue radius of the mean insert length. DRAGEN host software sets the default rescue radius to 2.5 standard deviations of the empirical insert distribution. In cases where the insert standard deviation is large compared to the read length, the rescue radius is restricted to limit mapping slowdowns. In this case, a warning message is displayed, as follows:

```
Rescue radius = 220
Effective rescue sigmas = 0.5
     WARNING: Default rescue sigmas value of 2.5 was overridden by host software!
     The user may wish to set rescue sigmas value explicitly with --Aligner.rescue-sigmas
```

Although the user can ignore this warning, or specify an intermediate rescue radius to maintain mapping speed, it is recommended to use 2.5 sigmas for the rescue radius to maintain mapping sensitivity. To disable rescue scanning, set *max-rescues* to 0.

## Output Options

DRAGEN can track multiple independent alignments for each read. These alignments include the optimal (primary) one, as well as those mapping different subsegments of the read, (chimeric/supplementary), and sub-optimal (secondary) mappings of the read to different areas of the reference.

For DNA alignment by default, DRAGEN can emit one primary alignment for each read, up to three chimeric alignments (Aliger.supp-aligns=3), and no secondary alignments (Aligner.sec-aligns=0). The maximum user-specified value for supp-aligns or sec-aligns is 30. The maximum total number of emitted alignments per read is 31. If the sum of supp-aligns and sec-aligns is greater than 30, then chimeric alignments are tracked with higher priority.

Use the following configuration options to control how many of each type of alignment to include in DRAGEN output.

- *mapq-max*

  The *mapq-max* option specifies a ceiling on the estimated MAPQ that can be reported for any alignment, from 0 to 255. If the calculated MAPQ is higher, this value is reported instead. The default is 60.

- *supp-aligns*, *sec-aligns*

  The *supp-aligns* and *sec-aligns* options restrict the maximum number of supplementary (ie, chimeric and SAM FLAG 0x800) alignments and secondary (ie, suboptimal and SAM FLAG 0x100) alignments, respectively, that can be reported for each read.

  A maximum of 31 alignments are reported for any read total, including primary, supplementary, and secondary. Therefore, *supp-aligns* and *sec-aligns* each range from 0–30. Supplementary alignments are tracked and output with higher priority than secondary ones.

  High settings for these two options impact speed so it is advisable to increase only as needed.

- *sec-phred-delta*

  The *sec-phred-delta* option controls which secondary alignments are emitted based on the alignment score relative to the primary reported alignment. Only secondary alignments with likelihood within this Phred value of the primary are reported.

- *sec-aligns-hard*

  The *sec-aligns-hard* option suppresses the output of all secondary alignments if there are more secondary alignments than can be emitted. Set *sec-aligns-hard* to 1 to force the read to be unmapped when not all secondary alignments can be output.

- *supp-as-sec*

  When the *supp-as-sec* option is set to 1, then supplementary (chimeric) alignments are reported with SAM FLAG 0x100 instead of 0x800. The default is 0. The *supp-as-sec* option provides compatibility with tools that do not support FLAG 0x800.

- *hard-clips*

  The *hard-clips* option is used as a field of 3 bits, with values ranging from 0 to 7. The bits specify alignments, as follows:

  - Bit 0—primary alignments
  - Bit 1—supplementary alignments
  - Bit 2—secondary alignments

  Each bit determines whether local alignments of that type are reported with hard clipping (1) or soft clipping (0). The default is 6, meaning primary alignments use soft clipping and supplementary and secondary alignments use hard clipping.

# DRAGEN Graph Mapper

To improve variant calling accuracy in segmental duplications and other regions difficult to map with Illumina reads, you can use the graph mapper in DRAGEN. The graph-based method uses alt-aware mapping for population haplotypes stitched into the reference with known alignments to establish alternate graph paths that reads could seed-map and align to. The graph mapper reduces mapping ambiguity because reads that contain population variants are attracted to the specific regions where the variants are observed. Graph mapper is supported only for the hg38 reference.

DRAGEN augments the FASTA reference with around 900,000 short alternate contigs derived from population haplotypes of phased variants to evolve the FASTA reference to a graph reference. The DRAGEN mapper's alt-aware capabilities are used to project reads that match the population haplotypes to corresponding primary assembly alignments with a precise lift-over alignment.

When given a set of population variants (VCF) or haplotypes, the FASTA modification is categorized in the following two types:

- Alternate contigs represent population haplotypes. Alt-contigs can have a single variant or a combination of nearby phased variants.

- Ambiguous codes (IUPAC codes) to represent SNPs . To improve alignment, edit the reference FASTA with isolated population SNPs.

DRAGEN graph mapper requires hash tables that are built using a FASTA containing population alternate contigs, corresponding lift-over SAM and unphased SNP VCF files are specified. The following is an example DRAGEN command line to build a graph-based hash table:

```
dragen --build-hash-table true \
--ht-build-rna-hashtable true --enable-cnv true \
--ht-reference hg38.fa \
--output-directory /tmp/ --ht-num-threads 40 \
--ht-alt-liftover /opt/edico/liftover/bwa-kit_hs38DH_liftover.sam \
--ht-pop-alt-contigs /opt/edico/liftover/pop_altContig.fa.gz \
--ht-pop-alt-liftover /opt/edico/liftover/pop_liftover.sam.gz \
--ht-pop-snps /opt/edico/liftover/pop_snps.vcf.gz
```

# Read Trimming

DRAGEN can remove artifacts from reads using hardware accelerated read trimming. Hardware accelerated read trimming is available on U200 and AWS systems, as part of the DRAGEN mapper and adds no additional run time. To enable the trimmer in normal mode, use `--read-trimmers`. To enable the trimmer in soft mode, use `--soft-read-trimmers`.

In normal trimming mode, potential artifacts are removed from input reads. Reads which are trimmed to fewer than 20 bases are filtered and replaced with a dummy read consisting of 10 N bases. In addition, the filtered reads have 0x200 SAM flag set.

DRAGEN contains a novel lossless soft-trimming mode. In soft-trimming mode, reads are mapped as though they had been trimmed, but no bases are removed. The intention of soft trimming is to suppress systematic mismapping of reads containing trimmable artifacts, such as Poly-G artifacts, from getting mapped to reference G homopolymers or adapter sequences getting mapped to matching reference loci, without actually losing the trimmed bases in aligned output. Soft trimming for Poly-G artifacts is enabled by default on supported systems.

## Read Trimming Metrics

The trimmer generates a metrics file titled `<output prefix>.trimmer_metrics.csv`. Metrics are available on an aggregate level over all input data. The metrics units are in reads or bases.

- **Total input reads**—Total number of reads in the input files.
- **Total input bases**—Total number of bases in the input reads.
- **Total input bases R1**—Total number of bases in R1 reads.
- **Total input bases R2**—Total number of bases in R2 reads.
- **Average input read length**—Total number of input bases divided by the number of input reads.

- **Total trimmed reads**—Total number of reads trimmed by at least one base, not including soft-trimming.
- **Total trimmed bases**—Total number of bases trimmed, not including soft-trimming.
- **Average bases trimmed per read**—The number of trimmed bases divided by the number of input reads.
- **Average bases trimmed per trimmed read**—The number of trimmed bases divided by the number of trimmed reads.
- **Remaining poly-G K-mers R1 3prime**—The number of R1 3' read ends that contain likely Poly-G artifacts after trimming.
- **Remaining poly-G K-mers R2 3prime**—The number of R2 3' read ends thatcontain likely Poly-G artifacts after trimming.
- **Poly-G trimmed reads**—The number of reads with at least one base trimmed during Poly-G artifact trimming. This metric is reported for both mates (R1 and R2) and the filtering status (unfiltered and filtered) of the trimmed read. The metric includes reads which were trimmed during soft-trimming.
- **Poly-G trimmed bases**—The number of bases trimmed during Poly-G artifact trimming. This metric is reported for both mates (R1 and R2) and the filtering status (unfiltered and filtered) of the trimmed read. The metric includes bases from reads which were trimmed during soft-trimming.
- **Total filtered reads**—The number of reads that were filtered out during trimming.
- **Reads filtered for minimum read length R1**—The number of R1 reads that were filtered due to being trimmed below the minimum read length.
- **Reads filtered for minimum read length R2**—The number of R2 reads that were filtered due to being trimmed below the minimum read length.

## Poly-G Trimming

Poly-G artifacts appear on two-channel sequencing system when the dark base G is called after synthesis has terminated. This results in calling several erroneous high-confidence G bases on the ends of affected reads. For contaminated samples, a large number of affected reads can be mapped to reference regions with high G content. This can cause problems for processing downstream.

## Read Trimming Settings

Use the following options to configure read trimming:

- *--read-trimmers*—To enable Poly-G trimming, set to polyg. To disable Poly-G trimming, set to `none`. During mapping, artifacts are removed from all reads. Reads are mapped accordingly.
- *--soft-read-trimmers*—To enable soft Poly-G trimming, set to `polyg`. To disable Poly-G trimming, set to `none`. During mapping, reads are aligned as if they had been trimmed. No bases are removed from the reads. Soft-trimming is enabled by default.

# DRAGEN FastQC

DRAGEN's FastQC module is a tool for calculating common metrics used for quality control of high-throughput sequencing data. The tool is modeled after the metrics generated by Babraham Institute's FastQC tool.

The metrics are generated automatically on all DRAGEN map-align workflows with no additional run time and output in a CSV format file called `<PREFIX>.fastqc_metrics.csv`.

For users only interested in sample QC or would like to obtain FastQC results only, DRAGEN provides a mode to generate the `fastqc_metrics.csv` file directly.

By default DRAGEN FastQC and read-trimming are run as preprocessing steps to standard sequence alignment workflows. If DNA alignment is not needed or if QC results are needed more quickly, the mapping and BAM output portions of the workflow can be disabled. The workflow only outputs key metric files and runs ~70% faster. This option is available on the command-line by entering `--fastqc-only=true` after the DRAGEN command.

## Metric Granularity

It is not possible due to memory constraints to guarantee single-base resolution for all metrics. DRAGEN provides an algorithmic solution for binning via `--fastqc-granularity`. DRAGEN allocates 256 bins in memory for each size or position-based metric. The granularity value of 4–7 inclusive can be used to determine the bin size. High values use smaller bins for greater resolution. Lower values can be used to create larger bins for larger read-lengths.

| Granularity | Single Base Resolution (bp) | Resolution at 150 (bp) | Recommended Read-Lengths (bp) |
|---|---|---|---|
| 7 [default] | 1–255 | 1 | < 256 |
| 6 | 1–128 | 2 | ≥ 256 and < 507 |
| 5 | 1–64 | 4 | ≥ 507 and < 4031 |
| 4 | 1–32 | 8 | ≥ 4031 |

## Adapter and Kmer Sequence Files

To include metrics for adapter or other sequence content, DRAGEN FastQC needs to be provided with the desired sequences in FASTA format. DRAGEN provides two options for this purpose, `--fastqc-adapter-file` for adapter sequences and `--fastqc-kmer-file` for any additional kmers of interest so that users can add sequences of interest without changing the expected adapter results.

DRAGEN FastQC can accept up to a combined total of 16 adapters and kmer sequences. Each sequence can be a maximum of 12 bp in length. By default, DRAGEN uses the adapter file located at `/opt/edico/config/adapter_sequences.fasta`. The file contains the following same adapter sequences as Babraham's FastQC v 0.11.10 and later.

- Illumina Universal Adapter—`AGATCGGAAGAG`
- Illumina Small RNA 3' Adapter—`TGGAATTCTCGG`
- Illumina Small RNA 5' Adapter—`GATCGTCGGACT`
- Nextera Transposase Sequence—`CTGTCTCTTATA`

## FastQC Metrics Output

The FastQC metrics are output to a CSV file format in the run output directory called `<PREFIX>.fastqc_metrics.csv`.

The reported metrics are broken down into eight sections by metric type. Each section is broken down further into separate rows by either the length, position, or other relevant categorical variables. The following are the metric sections.

- **Read Mean Quality**—Total number of reads. Each average Phred-scale quality value is rounded to the nearest integer.
- **Positional Base Mean Quality**—Average Phred-scale quality value of bases with a specific nucleotide and at a given location in the read. Locations are listed first and can be either specific positions or ranges. The nucleotide is listed second and can be A, C, G, or T. N or ambiguous bases are assumed to have the system default value, usually QV2.
- **Positional Base Content**—Number of bases of each specific nucleotide at given locations in the read. Locations are given first and can be either specific positions or ranges. The nucleotide is listed second and can be A, C, G, T, N.
- **Read Lengths**— Total number of reads with each observed length. Lengths can be either specific sizes or ranges, depending on the settings specified using `--fastqc-granularity`.
- **Read GC Content**—Total number of reads with each GC content percentile between 0 % and 100 %.
- **Read GC Content Quality**—Average Phred-scale read mean quality for reads with each GC content percentile between 0% and 100%.
- **Sequence Positions**—Number of times an adapter or other kmer sequence is found, starting at a given position in the input reads. Sequences are listed first in the metric description in quotes. Locations are listed second and can be either specific positions or ranges.
- **Positional Quality**—Phred-scale quality value for bases at a given location and a given quantile of the distribution. Locations are listed first and can be either specific positions or ranges. Quantiles are listed second and can be any whole integer 0–100.

The following are examples rows from each section.

| Section | Mate | Metric | Value |
|---|---|---|---|
| READ MEAN QUALITY | Read1 | Q38 Reads | 965377 |
| ... | | | |
| POSITIONAL BASE MEAN QUALITY | Read1 | ReadPos 145-152 T Average Quality | 34.49 |
| POSITIONAL BASE MEAN QUALITY | Read1 | ReadPos 150 T Average Quality | 34.44 |
| POSITIONAL BASE MEAN QUALITY | Read1 | ReadPos 256+ T Average Quality | 36.99 |
| ... | | | |
| POSITIONAL BASE CONTENT | Read1 | ReadPos 145-152 A Bases | 113362306 |
| POSITIONAL BASE CONTENT | Read1 | ReadPos 150 A Bases | 14300589 |
| POSITIONAL BASE CONTENT | Read1 | ReadPos 256+ A Bases | 13249068 |
| ... | | | |
| READ LENGTHS | Read1 | 150bp Length Reads | 77304421 |
| READ LENGTHS | Read1 | 144-151bp Length Reads | 77304421 |
| READ LENGTHS | Read1 | >=255bp Length Reads | 1000000 |
| ... | | | |
| READ GC CONTENT | Read1 | 50% GC Reads | 140878674373 |
| ... | | | |
| READ GC CONTENT QUALITY | Read1 | 50% GC Reads Average Quality | 36.20 |
| ... | | | |
| SEQUENCE POSITIONS | Read1 | 'AGATCGGAAGAG' 137bp Starts | 20 |
| SEQUENCE POSITIONS | Read1 | 'AGATCGGAAGAG' 137-144bp Starts | 23 |
| ... | | | |
| POSITIONAL QUALITY | Read1 | ReadPos 150 50% Quantile QV | 37 |
| POSITIONAL QUALITY | Read1 | ReadPos 145-152 50% Quantile QV | 37 |
| ... | | | |

# ALT-Aware Mapping

The GRCh38 human reference contains many more alternate haplotypes (ALT contigs) than previous versions of the reference. Generally, including ALT contigs in the mapping reference improves mapping and variant calling specificity, because misalignments are eliminated for reads matching an ALT contig but scoring poorly against the primary assembly. However, mapping with GRCh38's ALT contigs without special treatment can substantially degrade variant calling sensitivity in corresponding regions, because many reads align equally well to an ALT contig and to the corresponding position in the primary assembly. DRAGEN ALT-aware mapping eliminates this issue, and instead obtains both sensitivity and specificity improvements from ALT contigs.

ALT-aware mapping requires hash tables that are built with ALT liftover alignments specified (see *ALT-Aware Hash Tables* on page 13). If a hash table built with liftover alignments is provided, DRAGEN automatically runs with ALT-aware mapping. Set the *--alt-aware* option to false to disable ALT-Aware mapping with a liftover reference.

DRAGEN requires ALT-Aware hash tables for any hg19 or GRCh38 reference where ALT contigs are detected. To disable this requirement in DRAGEN, set the *--ht-alt-aware-validate* option to false.

When ALT-aware mapping is enabled, the mapper and aligner are aware of the liftover relationship between ALT contig positions and corresponding primary assembly positions. Seed matches within ALT contigs are used to obtain corresponding primary assembly alignments, even if the latter score poorly. Liftover groups are formed, each containing a primary assembly alignment candidate, and zero or more ALT alignment candidates that lift to the same location. Each liftover group is scored according to its best-matching alignments, taking properly paired alignments into account. The winning liftover group provides its primary assembly representative as the primary output alignment, with MAPQ calculated based on the score difference to the second-best liftover group. Emitting primary alignments within the primary assembly maintains normal aligned coverage and facilitates variant calling there. If the *--Aligner.en-alt-hap-aln* option is set to 1 and *--Aligner.supp-aligns* is greater than 0, then corresponding alternate haplotype alignments can also be output, flagged as supplementary alignments.

The following is a comparison of alternative options for dealing with alternate haplotypes.

- Mapping without ALT contigs in the reference:
  - False-positive variant calls result when reads matching an alternate haplotype misalign somewhere else.
  - Poor mapping and variant calling sensitivity where reads matching an ALT contig differ greatly from the primary assembly.
- Mapping with ALT contigs but no ALT awareness:
  - False-positive variant calls from misaligned reads matching ALT contigs are eliminated.
  - Low or zero aligned coverage in primary assembly regions covered by alternate haplotypes, due to some reads mapping to ALT contigs.
  - Low or zero MAPQ in regions covered by alternate haplotypes, where they are similar or identical to the primary assembly.

  - Variant calling sensitivity is dramatically reduced throughout regions covered by alternate haplotypes.
- Mapping with ALT contigs and alt awareness:
  - False-positive variant calls from misaligned reads matching ALT contigs are eliminated.
  - Normal aligned coverage in regions covered by alternate haplotypes because primary alignments are to the primary assembly.
  - Normal MAPQs are assigned because alignment candidates within a liftover group are not considered in competition.
  - Good mapping and variant calling sensitivity where reads matching an ALT contig differ greatly from the primary assembly.

# Sorting

The map/align system produces a BAM file sorted by reference sequence and position by default. Creating this BAM file typically eliminates the requirement to run *samtools sort* or any equivalent postprocessing command. The *--enable-sort* option can be used to enable or disable creation of the BAM file, as follows:

- To enable, set to true.
- To disable, set to false.

On the reference hardware system, running with sort enabled increases run time for a 30x full genome by about 6–7 minutes.

# Duplicate Marking

Marking or removing duplicate aligned reads is a common best practice in whole-genome sequencing. Not doing so can bias variant calling and lead to incorrect results.

The DRAGEN system can mark or remove duplicate reads, and produce a BAM file with duplicates either marked in the FLAG field or entirely removed.

## Algorithm

The DRAGEN duplicate-marking algorithm is modeled on the Picard toolkit MarkDuplicates feature. All the aligned reads are grouped into subsets in which all the members of each subset are potential duplicates.

For two pairs to be duplicates, they must have the following:

- Identical alignment coordinates (position adjusted for soft- or hard-clips from the CIGAR) at both ends.
- Identical orientations (direction of the two ends, with the left-most coordinate being first).

In addition, an unpaired read may be marked as a duplicate if it has identical coordinate and orientation with either end of any other read, whether paired or not.

Unmapped or read pairs are never marked as duplicates.

When DRAGEN identifies a group of duplicates, it picks one as the best of the group, and marks the others with the BAM PCR or optical duplicate flag (0x400, or decimal 1024). For this comparison, duplicates are scored based on the average sequence Phred quality. Pairs receive the sum of the scores of both ends, while unpaired reads get the score of the one mapped end. The score is intended to preserve the reads with the highest-quality base calls.

If two reads (or pairs) have exactly matching quality scores, DRAGEN breaks the tie by choosing the pair with the higher alignment score. If there are multiple pairs that also tie on this attribute, then DRAGEN chooses a pairarbitrarily.

The score for an unpaired read R is the average Phred quality score per base, calculated as follows:

$$score\,(R) = \frac{\sum_i (R.QUAL[i] \ where \ R.QUAL[i] \geq dedup\_min\_qual)}{sequence\_length(R)}$$

Where:

- R is a BAM record
- QUAL is its array of Phred quality scores
- *dedup-min-qual* is a DRAGEN configuration option with default value of 15.

For a pair, the score is the sum of the scores for the two ends.

This score is stored as a one-byte number, with values rounded down to the nearest one-quarter. This rounding may lead to different duplicate marks from those chosen by Picard, but because the reads were very close in quality this has negligible impact on variant calling results.

## Limitations

The limitations to DRAGEN duplicate marking implementation are as follows:

- When there are two duplicate reads or pairs with very close Phred sequence quality scores, DRAGEN might choose a different winner from that chosen by Picard. These differences have negligible impact on variant calling results.
- If using a single FASTQ file as input, DRAGEN accepts only a single library ID as a command-line argument (PGLB). For this reason, the FASTQ inputs to the system must be already separated by library ID. Library ID cannot be used as a criterion for distinguishing non-duplicates.

## Settings

The following options can be used to configure duplicate marking in DRAGEN:

- *--enable-duplicate-marking*

  Set to true to enable duplicate marking. When *--enable-duplicate-marking* is enabled, the output is

sorted, regardless of the value of the *enable-sort* option.

- *--remove-duplicates*

  Set to true to suppress the output of duplicate records. If set to false, set the 0x400 flag in the FLAG field of duplicate BAM records. When *--remove-duplicates* is enabled, then *enable-duplicate-marking* is forced to enabled as well.

- *--dedup-min-qual*

  Specifies the Phred quality score below which a base should be excluded from the quality score calculation used for choosing among duplicate reads.

# Small Variant Calling

The DRAGEN Small Variant Caller is a high-speed haplotype caller implemented with a hybrid of hardware and software. The caller performs localized *de novo* assembly in regions of interest to generate candidate haplotypes and then performs read likelihood calculations using a hidden Markov model (HMM).

Variant calling is disabled by default. You can enable variant calling by setting the *--enable-variant-caller* option to true.

## The Variant Caller Algorithm

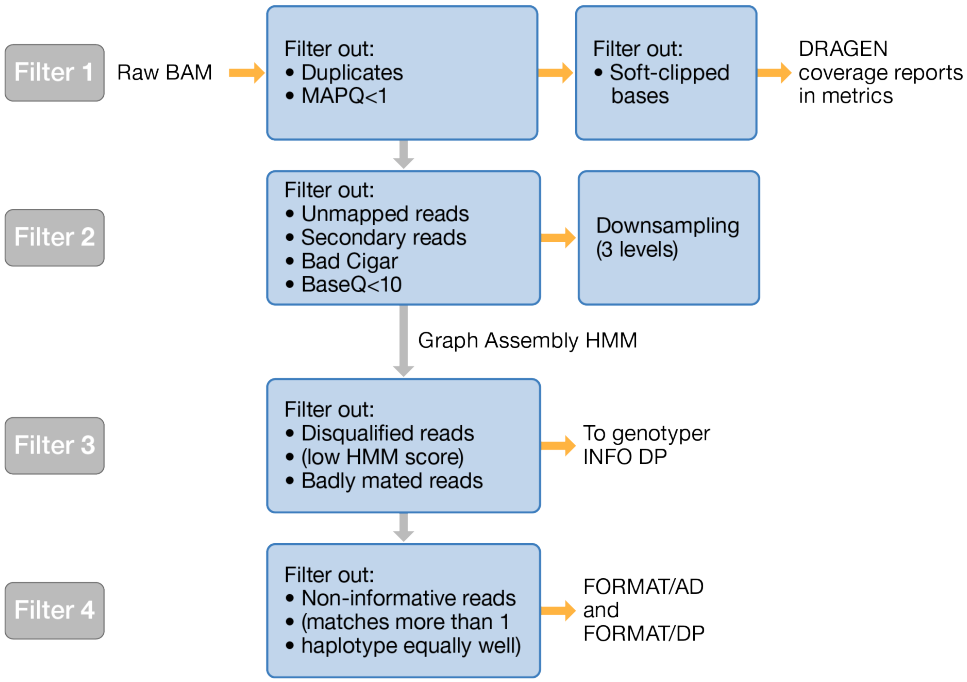
The DRAGEN Haplotype Caller performs the following steps:

- **Active Region Identification**—Identifies areas where multiple reads disagree with the reference are identified, and selects windows around them (active regions) for processing.
- **Localized Haplotype Assembly**— For each active region, assembles all overlapping reads in each active region into a de Bruijn graph (DBG). A DBG is a directed graph based on overlapping K-mers (length K sub-sequences) in each read or multiple reads. When all reads are identical, the DBG is linear. Where there are differences, the graph forms bubbles of multiple paths diverging and rejoining. If the local sequence is too repetitive and K is too small, cycles can form, which invalidate the graph. Values of K=10 and 25 are tried by default. If those values produce an invalid graph, then additional values of K = 35, 45, 55, 65 are tried until a cycle-free graph is obtained. From this cycle-free DBG, every possible path is extracted to produce a complete list of candidate haplotypes, ie, hypotheses for what the true DNA sequence may be on at least one strand.
- **Haplotype Alignment**—Uses the Smith-Waterman algorithm to align each extracted haplotype to the reference genome to determine what variations from the reference it implies.
- **Read Likelihood Calculation**—Tests each read against each haplotype, to estimate a probability of observing the read assuming the haplotype was the true original DNA sampled. This calculation is performed by evaluating a pair hidden Markov model (HMM), which accounts for the various possible ways the haplotype might have been modified by PCR or sequencing errors into the read observed. The HMM evaluation uses a dynamic programming method to calculate the total probability of any series of Markov state transitions arriving at the observed read.

- **Genotyping**—Forms the possible diploid combinations of variant events from the candidate haplotypes and, for each combination, calculates the conditional probability of observing the entire read pileup. Calculations use the constituent probabilities of observing each read, given each haplotype from the pair HMM evaluation. These calculations feed into the Bayesian formula to calculate a likelihood that each genotype is the truth, given the entire read pileup observed. Genotypes with maximum likelihood are reported.

## Filter BAM Input

Information in the IGV BAM pileup differs from the INFO/DP and FORMAT/DP in the VCF/gVCF as a result of filtering steps applied throughout variant calling. There are four filters used to exclude reads from the genotyping calculations. The following figure summarizes the four filters.




- Filter 1 filters out the following reads from the IGV BAM input:
  - Duplicate reads.
  - Reads with MAPQ=0.
  - Soft-clipped bases. DRAGEN filters out soft-clipped bases only when calculating coverage reports.
  - **[Somatic]** Reads with MAPQ < vc-min-tumor-read-qual, where vc-min-tumor-read-qual >1.
- Filter 2 trims bases with BQ < 10 and filters out the following reads:
  - Unmapped reads.
  - Secondary reads.
  - Reads with bad cigars.

- Filter 3 occurs after down-sampling and HMM. Filter 3 filters out the following reads:
  - Reads that are badly mated. A badly mated read is a read where the pair is mapped to two different reference contigs.
  - Disqualified reads. Reads are disqualified if their HMM score is below a threshold.
- Filter 4 occurs after the genotyper runs. The genotyper adds annotation information to the FORMAT field. Filter 4 filters out reads that are not informative. For example, if the HMM scores of the read against two different haplotypes are almost equal, the read is filtered out because it does not provide enough information to distinguish which of the two haplotypes are more likely.
  INFO/DP includes both informative and non-informative reads.
  FORMAT/AD and FORMAT/DP include only informative reads.

## Variant Caller Options

The following options control the variant caller stage of the DRAGEN host software.

- *--enable-variant-caller*

  Set *--enable-variant-caller* to *true* to enable the variant caller stage for the DRAGEN pipeline.

- *--vc-target-bed*

  This is an optional command line input that restricts processing of the small variant caller, target bed related coverage, and callability metrics to regions specified in a BED file. The BED file is a text file containing at least three tab-delimited columns. The first three columns are chromosome, start position, and end position, respectively. The positions are zero-based. For example:

  For example:

  ```
  # header information
  chr11 0 246920
  chr11 255660 255661
  ```

- *--vc-target-bed-padding*

  This is an optional command line input that can be used to pad all of the target BED regions with the specified value. For example, if a BED region is 1:1000-2000 and a padding value of 100 is used, it is equivalent to using a BED region of 1:900-2100 and a padding value of 0. Any padding added to *--vc-target-bed-padding* is used by the small variant caller and by the target bed coverage/callability reports. The default padding is 0.

- *--vc-sample-name*

  The *--vc-sample-name* option is deprecated. In end-to-end germline mode (with FASTQ input), the variant caller uses the RGSM value as the sample name. In end-to-end somatic mode, *--RGSM-tumor* can be used to specify the sample name of the tumor sample. In stand-alone mode (with BAM input), the variant caller uses the RGSM value from the BAM header as the sample name. In somatic mode, the RGSM value from a tumor BAM is as the tumor sample name.

- *--vc-target-coverage*

  The *--vc-target-coverage* option specifies the target coverage for down-sampling. The default

value is 500 for germline mode and 50 for somatic mode.

- *--vc-enable-gatk-acceleration*

  If *--vc-enable-gatk-acceleration* is set to true, the variant caller runs in GATK mode (concordant with GATK 3.7 in germline mode and GATK 4.0 in somatic mode).

- *--vc-remove-all-soft-clips*

  If *--vc-remove-all-soft-clips* is set to true, the variant caller does not use soft clips of reads to determine variants.

- *--vc-decoy-contigs*

  The *--vc-decoy-contigs* option specifies a comma-separated list of contigs to skip during variant calling. This option can be set in the configuration file.

- *--vc-enable-decoy-contigs*

  If *--vc-enable-decoy-contigs* is set to true, variant calls on the decoy contigs are enabled. The default value is false.

- *--vc-enable-phasing*

  The *–vc-enable-phasing* option enables variants to be phased when possible. The default value is true.

- *--vc-enable-phasing*

  The *–vc-enable-phasing* option enables variants to be phased when possible. The default value is true.

- *--vc-enable-vcf-output*

  The *–vc-enable-vcf-output* option enables VCF file output during a gVCF run. The default value is false.

## Down-sampling Options for Small Variant Calling

The options for down-sampling reads in the small variant calling pipeline are as follows:

- *--vc-target-coverage* specifies the maximum number of reads with a start position overlapping any given position.
- *--vc-max-reads-per-active-region* specifies the maximum number of reads covering a given active region.
- *--vc-max-reads-per-raw-region* specifies the maximum number of reads covering a given raw region.
- *--vc-min-reads-per-start-pos* specifies the minimum number of reads with a start position overlapping any given position.

For mitochondrial small variant calling, the down-sampling options can be set separately because the mitochondrial contig contains a higher depth than the rest of the contigs in a WGS dataset. The following are the down-sampling options for the mitochondrial contig.

- *--vc-target-coverage-mito*
- *--vc-max-reads-per-active-region-mito*
- *--vc-max-reads-per-raw-region-mito*

The target coverage and max/min reads in raw/active region options are not directly related and could be triggered independently.

The target coverage option runs first and is meant to limit the number of reads that share the same start position at any given position. It is not a limit on the total coverage at a given position.

The following are the default down-sampling values for each small variant calling mode.

| Mode | Down-sampling Option | Default Value |
|---|---|---|
| Germline | *--vc-target-coverage* | 500 |
| Germline | *--vc-max-reads-per-active-region* | 10000 |
| Germline | *--vc-max-reads-per-raw-region* | 30000 |
| Somatic | *--vc-target-coverage* | 50 |
| Somatic | *--vc-max-reads-per-active-region* | 10000 |
| Somatic | *--vc-max-reads-per-raw-region* | 30000 |
| High Coverage | *--vc-target-coverage* | 100000 |
| High Coverage | *--vc-max-reads-per-active-region* | 200000 |
| High Coverage | *--vc-max-reads-per-raw-region* | 200000 |
| Mitochondrial | *--vc-target-coverage-mito* | 40000 |
| Mitochondrial | *--vc-max-reads-per-active-region-mito* | 40000 |
| Mitochondrial | *--vc-max-reads-per-raw-region-mito* | 40000 |

The following example that shows that the DP reported in a variant record can far exceed the *--vc-target-coverage* default value of 500 in Germline mode:

For example, assume the default value of *--vc-target-coverage* is 500. If there are 400 reads starting at position 1, another 400 starting at position 2, and another 400 starting at position 3, the target coverage option is not triggered (because 400 < 500). If there is a variant at position 4, its reported depth could be as high as 1200. This example shows that the DP reported in a variant record can far exceed the *--vc-target-coverage* value.

After the target coverage step, the maximum number of reads that share the same position is 500 (if *--vc-target-coverage* is set to 500).

The next down-sampling step is to apply the *--vc-max-reads-per-raw-region* and *--vc-max-reads-per-active-region* limits. In this step, the maximum number of reads that share the same position can be further reduced from the 500 maximum value from the first step. These options are used to limit the total number of reads in an entire region using a leveling down-sampling method.

The down-sampling mechanism scans each start position from the start boundary of the region and discards one read from that position, then moves on to the next position, until the total number of reads falls below the threshold. It can potentially take several passes across the entire region for the total number of reads in the entire region to fall below the threshold. After the threshold is met, the down-sampling step is stopped regardless of which position was considered last in the region.

If the number of reads at any position with same start position is equal to or lower than the *--vc-min-reads-per-start-pos*, that position is skipped to ensure that there is always at least a minimum number of reads (set to *--vc-min-reads-per-start-pos*) at any start position.

When down-sampling occurs, the choice of which reads to keep or remove is somewhat random. However, the random number generator is seeded to a default value to ensure that it produces the same set of values in each run. This ensures exactly reproducible results, which means there is no run to run variation when using the same input data.

## Down-sampling Options for Mitochondrial Small Variant Calling

The options for down-sampling reads in the small variant calling pipeline for the mitochondrial contig are as follows:

- *--vc-target-coverage-mito* specifies the maximum number of reads with a start position overlapping any given position.
- *--vc-max-reads-per-active-region-mito* specifies the maximum number of reads covering a given active region.
- *--vc-max-reads-per-raw-region-mito* specifies the maximum number of reads covering a given raw region.

The default value for all three options is 40000. Their function is the same as described in *Down-sampling Options for Small Variant Calling* on page 84. You can set the down-sampling options for the mitochondrial contig separately from the rest of the contigs because the mitochondrial contig has much higher depth than the rest of the contigs in a WGS dataset.

## Phasing and Phased Variants

DRAGEN supports output of phased variant records in the germline VCF and gVCF file. When two or more variants are phased together, the phasing information is encoded in a sample-level annotation, FORMAT/PS, that identifies which set the phased variant is in. The value in the field in an integer representing the position of the first phased variant in the set. All records in the same contig with matching PS values belong to the same set.

```
##FORMAT=<ID=PS,Number=1,Type=Integer,Description="Physical phasing ID
information, where each unique ID within a given sample (but not across
samples) connects records within a phasing group">
```

The following is an example of a DRAGEN single sample gVCF, where two SNPs are phased together.

```
chr1    1947645 .       C       T,<NON_REF>     48.44   PASS
DP=35;MQ=250.00;MQRankSum=4.983;ReadPosRankSum=3.217;FractionInf
ormativeReads=1.000;R2_5P_bias=0.000
GT:AD:AF:DP:F1R2:F2R1:GQ:PL:SPL:ICNT:GP:PRI:SB:MB:PS
0|1:20,15,0:0.429:35:9,7,0:11,8,0:47:83,0,50,572,758,622:255,0,2
55:19,0:4.844e+01,8.387e-
05,5.300e+01,4.500e+02,4.500e+02,4.500e+02:0.00,34.77,37.77,34.7
7,69.54,37.77:11,9,10,5:12,8,8,7:1947645

chr1    1947648 .       G       A,<NON_REF>     50.00   PASS
DP=36;MQ=250.00;MQRankSum=5.078;ReadPosRankSum=2.563;FractionInf
ormativeReads=1.000;R2_5P_bias=0.000
GT:AD:AF:DP:F1R2:F2R1:GQ:PL:SPL:ICNT:GP:PRI:SB:MB:PS
1|0:16,20,0:0.556:36:8,9,0:8,11,0:48:85,0,49,734,613,698:255,0,2
55:16,0:5.000e+01,7.067e-
05,5.204e+01,4.500e+02,4.500e+02,4.500e+02:0.00,34.77,37.77,34.7
7,69.54,37.77:10,6,11,9:8,8,12,8:1947645
```

During the genotyping step, all haplotypes and all variants are considered over an active region. For each pair of variants, if both variants occur on all of the same haplotypes, or if either is a homozygous variant, then they are phased together. If the variants only occur on different haplotypes, then they are phased opposite to each other. If any heterozygous variants are present on some of the same haplotypes but not others, phasing is aborted and no phasing information is output for the active region.

## Ploidy Support

The small variant caller currently only supports either ploidy 1 or 2 on all contigs within the reference except for the mitochondrial contig, which uses a continuous allele frequency approach (see *Mitochondrial Calling* on page 88). The selection of ploidy 1 or 2 for all other contigs is determined as follows.

- If *--sample-sex* is not specified on the command line, the Ploidy Estimator determines the sex. If the Ploidy Estimator cannot determine the sex karyotype or detects sex chromosome aneuploidy, all contigs are processed with ploidy 2.
- If *--sample-sex* is specified on the command line, contigs are processed as follows.
  - For female samples, DRAGEN processes all contigs with ploidy 2, and marks variant calls on chrY with a filter PloidyConflict.

– For male samples, DRAGEN processes all contigs with ploidy 2, except for the sex chromosomes. DRAGEN processes chrX with ploidy 1, except in the PAR regions, where it is processed with ploidy 2. chrY is processed with ploidy 1 throughout.

DRAGEN detects sex chromosomes by the naming convention, either X/Y or chrX/chrY. No other naming convention is supported.

| --sample-sex | Ploidy Estimation | Sample Sex in Small VC |
|---|---|---|
| male | Not relevant | Male |
| female | Not relevant | Female |
| Not specified | XY | Male |
| Not specified | XX | Female |
| Not specified | Everything else | None |

## Overlapping Mates in Small Variant Calling

DRAGEN v3.6 and later no longer treats overlapping mates as independent evidence for a given event. This improves how overlapping mates in a read pair are handled in the variant caller and accuracy. DRAGEN handles overlapping mates as follows in both the germline and somatic pipelines.

- When the two overlapping mates agree with each other on the allele with the highest HMM score, the genotyper uses the mate with the greatest difference between the highest and the second highest HMM score. The HMM score of the other mate becomes zero.
- When the two overlapping mates disagree, the genotyper sums the HMM score between the two mates, assigns the combined score to the mate that agrees with the combined result, and changes the HMM score of the other mate to zero.
- The base qualities of overlapping mates are no longer adjusted.

## Mitochondrial Calling

Typically, there are approximately 100 mitochondria in each mammalian cell, and each mitochondrion harbors 2–10 copies of mitochondrial DNA (mtDNA). For example, if 20% of the chrM copies have a variant, then the allele frequency (AF) is 20%. This is also referred to as continuous allele frequency. The expectation is that the AF of variants on chrM is anywhere between 0% and 100%.

DRAGEN now processes chrM through a continuous AF pipeline. In this case, a single ALT allele is considered, and the AF is estimated, and can be anywhere between 0% and 100%.

QUAL is not output in the chrM variant records. Instead, the confidence score is INFO/LOD

```
##INFO=<ID=LOD,Number=1,Type=Float,Description="Variant LOD score">
```

which gives the confidence that a variant is present at a given locus.

GQ is not output in the chrM variant records because DRAGEN doesn't test for multiple diploid genotype candidates. Instead, an ALT allele is considered as a candidate variant, and if INFO/LOD > *vc-lod-call-threshold* (default = 4), then the FORMAT/GT is hard-coded to 0/1, and the FORMAT/AF yields an estimate on the variant allele frequency, which ranges anywhere within [0,1].

The following filters can be applied to mitochondrial variant calls.

- *--vc-lod-call-threshold*

  LOD threshold for emitting calls in the VCF. The default is 4.

- *--vc-lod-filter-threshold*

  LOD threshold to mark emitted VCF calls as filtered. The default is 6.3.

- If INFO/LOD < *vc-lod-call-threshold*, the variant is not emitted in the VCF.
- If INFO/LOD > *vc-lod-call-threshold* but INFO/LOD < vc-lod-filter-threshold the variant is emitted in the VCF but FILTER=lod_fstar.
- If INFO/LOD > *vc-lod-call-threshold* and INFO/LOD > vc-lod-filter-threshold, the variant is emitted in the VCF and FILTER=PASS.

The following are example VCF records on the chrM, showing one call with very high AF and another with very low AF. In both cases FORMAT/LOD > *emit_threshold*. FORMAT/LOD is also > lod_fstar threshold, so the FILTER annotation is PASS.

```
chrM 2259 . C T . PASS
DP=9791;MQ=60.00;LOD=38838.40;FractionInformativeReads=0.994
GT:AD:AF:F1R2:F2R1:DP:SB:MB
0/1:5,9729:0.999:1,5007:4,4722:9734:3,2,4885,4844:1,4,4807,4922
chrM 16192 . C T . PASS
DP=9644;MQ=60.00;LOD=26.12;FractionInformativeReads=0.992
GT:AD:AF:F1R2:F2R1:DP:SB:MB
0/1:9537,26:0.003:5530,16:4007,10:9563:4484,5053,13,13:4961,4576,19,
```

## gVCF and Joint VCF Mode

In gVCF mode, which is available for the germline pipeline, the NON_REF regions are output alongside the variant records. The following is an example of NON_REF and variant regions output in chrM gVCF mode:

```
chrM 751 . A <NON_REF> . PASS END=1437 GT:AD:DP:GQ:MIN_DP:PL:SPL:ICNT
0/0:6920,9:6929:99:4077:0,120,1800:0,255,255:40,4
chrM 1438 . A G,<NON_REF> . PASS
DP=8500;MQ=57.39;LOD=30441.87;FractionInformativeReads=0.871
GT:AD:AF:F1R2:F2R1:DP:SB:MB
0/1:0,7400,0:1.000:0,3765,0:0,3635,0:7400:0,0,3994,3406:0,0,3633,3767
chrM 1439 . A <NON_REF> . PASS END=2258 GT:AD:DP:GQ:MIN_DP:PL:SPL:ICNT
0/0:6120,10:6130:99:4190:0,120,1800:0,255,255:40,14
```

### FORMAT/GT

In NON_REF regions, the FORMAT/GT is hard-coded to 0/0 and at variant loci, the FORMAT/GT is hard-coded to 0/1.

The FORMAT/GT for chrM is not determined by FORMAT/AF. It is determined by whether a variant is emitted at a position or not. The decision to emit the variant or not is determined by comparing the INFO/LOD score to a threshold. All variants with FORMAT/LOD > emit_threshold get emitted. Variants with FORMAT/LOD < emit_threshold are not emitted in the gVCF and get banded in a NON_REF region with FORMAT/GT=0/0.

One of the following two scenarios can occur at a given position.

- A variant is detected by the genotyper at a given position, and the FORMAT/LOD > emit_threshold. In this case, the FORMAT/GT is hard-coded to 0/1, and DRAGEN outputs FORMAT/AD, FORMAT/DP and FORMAT/AF computed at that position.
- No variant is detected at the given position, or, a variant is detected but the FORMAT/LOD < emit_threshold. In this case, the FORMAT/GT is hard-coded to 0/0, and the position gets banded with contiguous positions if they are in the same scenario. All positions with FORMAT/GT = 0/0 get banded together. The FORMAT/DP reported for the band is computed as the median DP across all positions within the band. The FORMAT/AD values reported in the band are the AD values selected at the position where FORMAT/DP = median DP. In the joint VCF, FORMAT/AF is computed based on FORMAT/AD.

The following are examples of variant record on chrM in a trio joint VCF.

The first sample shows a variant, but FORMAT/FT is lod_fstar because FORMAT/LOD < lod_fstar_threshold.

The second sample does not have a variant and the FORMAT/AF=0.

The third sample does not have a variant even though the FORMAT/AF>0, this means that that position for the sample is in a NON_REF region over which no variant was detected with sufficient confidence.

```
chrM 2622 . G A . . DP=11199;MQ=59.73 GT:AD:AF:DP:FT:LOD:F1R2:F2R1
0/1:3375,8:0.002:3383:lod_fstar:4.4:1689,2:1686,6
0/0:5629,1:0:5118:PASS:.:.:. 0/0:3505,8:0.003:2645:PASS:.:.:.
```

### QUAL, QD, and GQ Formulation

In single sample VCF and gVCF, the QUAL follows the definition of the VCF specification (https://samtools.github.io/hts-specs/VCFv4.3.pdf).

- QUAL is the Phred-scaled probability that the site has no variant and is computed as:

  ```
  QUAL = -10*log10 (posterior genotype probability of a homozygous-
  reference genotype (GT=0/0))
  ```

  That is, QUAL = GP (GT=0/0), where GP = posterior genotype probability in Phred scale.

QUAL = 20 means there is 99% probability that there is a variant at the site. The GP values are also given in Phred-scale in the VCF file.

- GQ is the Phred-scaled Probability that the call is incorrect.

  GQ=-10*log10(p), where p is the probability that the call is incorrect.

  GQ=-10*log10(sum(10.^(-GP(i)/10))) where the sum is taken over the GT that did not win.

  So, GQ of 3 means there's a 50 percent chance that the call is incorrect, and GQ of 20 means there's a 1 percent chance that the call is incorrect.
- QD is the QUAL normalized by the read depth, DP.

| **Metric** | QUAL | GQ | QD |
|---|---|---|---|
| **Description** | Probability that the site has no variant | Probability that the call is incorrect | Qual normalized by Depth |
| **Formulation** | QUAL = GP(GT=0/0) | GQ=-10*log10(p) | QUAL/DP |
| **Scale** | Unsigned Phred | Unsigned Phred | Unsigned Phred |
| **Numerical example** | QUAL=20: 1 % chance that there is no variant at the site<br>QUAL=50: 1 in 1e5 chance that there is no variant at the site | GQ=3, 50% chance that the call is incorrect<br>GQ=20, 1% chance that the call is incorrect | |

## Change in the Range of Values Between DRAGEN 2.6+ and Earlier Versions of DRAGEN/GATK

The range of the QUAL (and therefore GQ and QD) values has changed between older DRAGEN versions (and/or GATK) and DRAGEN 2.6 and later versions. In older DRAGEN versions, the QUAL calculations followed the GATK approach. In newer DRAGEN versions (2.6 and later), the algorithms for small variant detection were improved and they result in more realistic QUAL (and therefore GQ and QD) values (ie, smaller values than GATK).

The QUAL values changed because the probability calculations used by GATK and earlier DRAGEN versions assume that errors are uncorrelated across reads. This is assumed for both mapping errors and base-call errors. In a real world with correlated errors, this leads to wildly inflated QUAL scores as well as suboptimal detection performance. The algorithms in DRAGEN 2.6 and later incorporate the hypothesis of correlated errors from within the variant caller, leading to more accurate detection and more realistic QUAL scores. The more realistic QUAL scores in DRAGEN 2.6+ are smaller than the inflated QUAL scores in GATK.

## Histogram of QUAL, QD, and GQ

When plotting the histogram of QUAL (and QD and GQ) values across the calls of a DRAGEN 2.6+ VCF, a peak can occur at a certain value (eg, 50) with a smaller portion of QUAL values below and above this level. This is because the Foreign Read Detection (FRD) algorithm sets a limit on the QUAL (and indirectly on GQ and QD) value of heterozygous variants. See *Foreign Read Detection* on page 92. The exact value depends on the mapping between the maximum MAPQ from the mapper and a phred confidence that a read is correctly mapped. Homozygous variants are not subject to this limit, because the FRD hypothesis is applied against heterozygous variants only (this in turn is because the *--vc-frd-beta-max* option that specifies the maximum allele frequency for the foreign read hypothesis is set to 0.5).

The high-level summary is that the QUAL of heterozygous variants is bounded, and the QUAL of homozygous variants is not. However, the QUAL scale is the same across all variants (het or hom), only the bound is different.

## Modeling of Correlated Errors Across Reads

DRAGEN has two algorithms that model correlated errors across reads in a given pileup.

- Foreign read detection (FRD) detects mismapped reads. FRD modifies the probability calculation to account for the possibility that a subset of the reads were mismapped. Instead of assuming that mapping errors occur independently per read, it estimates the probability that a burst of reads is mismapped, incorporating such evidence as MAPQ and skewed AF. For more information, see *Foreign Read Detection* on page 92.
- The base quality drop off (BQD) algorithm detects correlated base call errors using a mechanism that detects systematic and correlated base call errors caused by the sequencing instrument. The detection mechanism exploits certain properties of those errors (strand bias, position of the error in the read, base quality) to estimate the probability that the alleles are the result of a systematic error event rather than a true variant. For more information, see *Base Quality Dropoff* on page 93.

### Foreign Read Detection

Mapping errors typically occur in bursts, but variant callers typically treat mapping errors as independent error events per read, which can result in high confidence scores in spite of low MAPQ and/or skewed AF. To mitigate overestimation of confidence scores some variant callers include a threshold on the minimum MAPQ to include in the calculation, however this method can discard evidence and result in false positives.

DRAGEN includes Foreign Read Detection (FRD), which is an extends the legacy genotyping algorithm by incorporating an additional hypothesis that reads in the pileup might be foreign reads (ie, their true location is elsewhere in the reference genome). The algorithm exploits multiple properties (skewed allele frequency and low MAPQ) and incorporates this evidence into the probability calculation.

Sensitivity is improved by rescuing FN, correcting genotypes, and enabling lowering of the MAPQ threshold for incoming reads into the variant caller. Specificity is improved by removing FP and correcting genotypes.

### Base Quality Dropoff

Conventional variant callers treat sequencing errors as independent across reads, however bursts of errors can occur at a specific locus and increase the number of false positives.

These errors have distinct characteristics differentiating them from true variants. The base quality drop off (BQD) algorithm is a detection mechanism that exploits certain properties of those errors (strand bias, position of the error in the read, low mean base quality over said subset of reads at the locus of interest) and incorporates them into the probability calculation.

## Joint Detection of Overlapping Variants

Genotyping can benefit if variants at multiple loci in a single active region are detecting jointly. Joint Detection feature groups loci in a Joint Detection region if the following conditions are met:

- Loci have alleles that overlap each other.
- Loci are in the STR region or less than 10 bases apart of the STR region.
- Loci are less than 10 bases apart of each other.

Joint Detection alters the variant caller algorithm in the part of localized haplotype assembly and genotyping. It generates a haplotype list where all possible combinations of the alleles in the Joint Detection regions are represented. This leads to larger number of haplotypes. During genotyping, Joint Detection calculates likelihoods that each haplotype pair is the truth, given the read pileup is observed. Genotypes likelihoods are calculated as sum of the likelihoods of haplotype pairs that support the alleles in the genotypes. Genotypes with maximum likelihood are reported.

You can enable Joint Detection by setting the --vc-enable-joint-detection option to true. Run time slightly increases when Joint Detection feature is enabled.

## ROH Caller

Regions of homozygosity (ROH) are detected as part of the small variant caller. The caller detects and outputs the runs of homozygosity from whole genome calls on autosomal human chromosomes. Sex chromosomes are ignored. ROH output allows downstream tools to screen for and predict consanguinity between the parents of the proband subject.

A region is defined as consecutive variant calls on the chromosome with no large gap in between these variants. In other words, regions are broken by chromosome or by large gaps with no SNV calls. The gap size is set to 3 Mbases.

### ROH Algorithm

The ROH algorithm runs on the small variant calls. It excludes variants with multiallelic sites, indels, complex variants, non-PASS filtered calls, and homozygous reference sites. The variant calls are then filtered further using a blacklist bed, and finally depth filtering is applied after the blacklist filter. The

default value for the fraction of filtered calls is 0.2, filtering the calls with the highest 10% and lowest 10% in DP values. The resulting calls are then used to find regions.

The ROH algorithm first finds seed regions that contain at least 50 consecutive homozygous SNV calls with no heterozygous SNV or gaps of 500,000 bases between the variants. The regions can be extended using a scoring system that functions as follows.

- Score increases with every additional homozygous variant (0.025) and decreases with a large penalty (1–0.025) for every heterozygous SNV. This provides some tolerance of presence of heterozygous SNV in the region.
- Each region expands on both ends until the regions reach the end of a chromosome, a gap of 500,000 bases between SNVs occurs, or the score becomes too low (0).

Overlapping regions are merged into a single region. Regions can be merged across gaps of 500,000 bases between SNVs if a single region would have been called from the beginning of the first region to the end of the second region without the gap. There is no maximum size for regions, but regions always end at chromosome boundaries.

## ROH Options

- *--vc-enable-roh*

  Enable or disable the ROH caller by setting this option to true or false. Enabled by default for human autosomes only.

- *--vc-roh-blacklist-bed*

  If provided, the ROH caller ignores variants that are contained in any region in the blacklist BED file. DRAGEN distributes blacklist files for all popular human genomes and automatically selects a blacklist to match the genome in use, unless this option is used explicitly select a file.

## ROH Output

The ROH caller produces an ROH output file named `<output-file-prefix>.roh.bed` in which each row represents one region of homozygosity. The bed file contains the following columns:

```
Chromosome Start End Score #Homozygous #Heterozygous
```

Where

- Score is a function of the number of homozygous and heterozygous variants, where each homozygous variant increases the score by 0.025, and each heterozygous variant reduces the score by 0.975.
- Start and end positions are a 0-based, half-open interval.
- #Homozygous is number of homozygous variants in the region.
- #Heterozygous is number of heterozygous variants in the region.

The caller also produces a metrics file named `<output-file-prefix>.roh_metrics.csv` that lists the number of large ROH and percentage of SNPs in large ROH (> 3 MB).

## B-Allele Frequency Output

B-Allele frequency (BAF) output is enabled by default in germline and somatic VCF and gVCF runs.

The BAF value is equal to either AF or (1 – AF), where

- AF = (alt_count / (ref_count + alt_count))
- BAF = 1 – AF, only when ref base < alt base, order of priority for bases is A < T <G < C < N

For each small variant VCF entry with exactly one SNP alternate allele, the output contains a corresponding entry in the BAF output file.

- <NON_REF> lines are excluded
  - ForceGT variants (as marked by the "FGT" tag in the INFO field) are not included in the output, unless the variant also contains the "NML" tag in the INFO field.
  - Variants where the ref_count and alt_count are both zero are not included in the output.

### BAF Options

*--vc-enable-baf*

Enable or disable B-allele frequency output. Enabled by default.

### BAF Output

The BF generates are BigWig-compressed files, named `<output-file-prefix>.baf.bw` and `<output-file-prefix>.hard-filtered.baf.bw`. The hard-filtered file only contains entries for variants that pass the filters defined in the VCF (ie, PASS entries).

Each entry contains the following information:

```
Chromosome Start End BAF
```

Where:

- Chromosome is a string matching a reference contig.
- Start and end values are zero-based, half open intervals.
- BAF is a floating point value.

## Somatic mode

The DRAGEN Somatic Pipeline allows ultrarapid analysis of NGS data to identify cancer-associated mutations in somatic chromosomes. DRAGEN calls SNVs and indels from both matched tumor-normal pairs and tumor-only samples.

For the tumor-normal pipeline, both samples are analyzed jointly such that germline variants are excluded, generating an output specific to tumor mutations. The tumor-only pipeline produces a VCF file containing both germline and somatic variants that can be further analyzed to identify tumor mutations. For the tumor sample, both pipelines make no ploidy assumptions, enabling detection of low-frequency alleles.

The output, after multiple filtering *dbSNP Annotation* on page 115steps, is in the form of a VCF file. Variants that fail the filtering steps are kept in the output VCF, with a FILTER annotation indicating which filtering steps have failed.

You can use somatic quality (SQ) as the primary metric to describe the confidence with which the caller made a somatic call. SQ is reported as a format field for the tumor sample. Variants with SQ score below the SQ filter threshold are filtered out using the `weak_evidence` tag. To trade off sensitivity against specificity, adjust the SQ filter threshold. Lower thresholds produce a more sensitive caller and higher thresholds produce a more conservative caller.

## Somatic Mode Options

Somatic mode has the following command line options:

- *--tumor-fastq1* and *--tumor-fastq2*

  The *--tumor-fastq1* and *--tumor-fastq2* options are used to input a pair of FASTQ files into the mapper aligner and somatic variant caller. These options can be used with OTHER FASTQ options to run in tumor-normal mode. For example:

  ```
  dragen -f -r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
  --tumor-fastq1 <TUMOR_FASTQ1> \
  --tumor-fastq2 <TUMOR_FASTQ2> \
  --RGID-tumor <RG0-tumor> --RGSM-tumor <SM0-tumor> \
  -1 <NORMAL_FASTQ1> \
  -2 <NORMAL_FASTQ2> \
  --RGID <RG0> –RGSM <SM0> \
  --enable-variant-caller true \
  --output-directory /staging/examples/ \
  --output-file-prefix SRA056922_30x_e10_50M
  ```

- *--tumor-fastq-list*

  The *--tumor-fastq-list* option is used to input a list of FASTQ files into the mapper aligner and somatic variant caller. This option can be used with other FASTQ options to run in tumor-normal mode. For example:

  ```
  dragen -f \
  -r /staging/human/reference/hg19/hg19.fa.k_21.f_16.m_149 \
  --tumor-fastq-list <TUMOR_FASTQ_LIST> \
  --fastq-list <NORMAL_FASTQ_LIST> \
  ```

```
--enable-variant-caller true \
--output-directory /staging/examples/ \
--output-file-prefix SRA056922_30x_e10_50M
```

- *--tumor-bam-input* and *--tumor-cram-input*

  The *--tumor-bam-input* or *--tumor-cram-input* option is used to input a mapped BAM or CRAM file into the somatic variant caller. These options can be used with other BAM/CRAM options to run in tumor-normal mode.

- *--vc-min-tumor-read-qual*

  The *--vc-min-tumor-read-qual* option specifies the minimum read quality (MAPQ) to be considered for variant calling. The default value is 3 for tumor-normal analysis or 20 for tumor-only analysis.

- *--vc-callability-tumor-thresh* and *--vc-callability-normal-thresh*

  The *--vc-callability-tumor-thresh* option specifies the callability threshold for tumor samples, and the *--vc-callability-normal-thresh* option specifies the callability threshold for normal samples, if present. The somatic callable regions report includes all regions with tumor coverage above the tumor threshold and if applicable, normal coverage above the normal threshold. For more information on the somatic callable regions report, see *Somatic Callable Regions Report* on page 203. The default value for the tumor-only threshold is 15 and the tumor-normal threshold is 5.

- *--vc-somatic-hotspots* and *--vc-hotspot-log10-prior-boost*

  The somatic hotspots option allows an input VCF to specify the positions where the risk for somatic mutations are assumed to be significantly elevated. DRAGEN genotyping priors are boosted for all postions specified in the VCF, so it is possible to call a variant at one of these sites with fewer supporting reads. The cosmic database in VCF format can be used as one source of prior information to boost sensitivity for known somatic mutations. The size of the adjustment can be controlled via vc-hotspot-log10-prior-boost, which has a default value of 4 (log10 scale) corresponding to an increase of 40 phred.

- *--vc-enable-liquid-tumor-mode* and *vc-tin-contam-tolerance*

  In a tumor-normal analysis, DRAGEN accounts for tumor-in-normal (TiN) contamination by running liquid tumor mode. Liquid tumor mode is disabled by default. When liquid tumor mode is enabled, DRAGEN is able to call variants in the presence of TiN contamination up to a specified maximum tolerance level. `--vc-enable-liquid-tumor-mode` enables liquid tumor mode with a default maximum contamination TiN tolerance of 0.15. If using the default maximum contamination TiN tolerance, somatic variants are expected to be observed in the normal sample with allele frequencies up to 15% of the corresponding allele in the tumor sample. `vc-tin-contam-tolerance` enables liquid tumor mode and allows you to set the maximum contamination TiN tolerance. The maximum contamination TiN tolerance must be greater than zero. For example, `vc-tin-contam-tolerance=-0.1`.

  Liquid tumors in liquid tumor mode refers to hematological cancers, such as leukemia. For liquid tumors it is not feasible to use blood as a normal control because the tumor is present in the blood. Skin or saliva is typically used as the normal sample. However, skin and saliva samples can still

contain blood cells, so that the matched normal control sample contains some traces of the tumor sample and somatic variants are observed at low frequencies in the normal sample. If the contamination is not accounted for, it can severely impact sensitivity by suppressing true somatic variants.

In typical use cases of liquid tumor mode, the library is WGS or WES, with medium depth for example (100x T/ 40xN), and the lowest VAF detected for these types of depths is ~5%. In typical use cases of liquid biopsy, the library is a targeted gene panel (eg 500 genes), with very high raw depth (> 2000-5000x), and uses UMI indexing to enable sensitivity at VAF down to 0.5 % LoD.

## Unique Molecular Identifiers Support

To optimize variant calling for different UMI use cases, use the following two batch modes. Both options are false by default. Enable UMI variant calling by setting one of them to true.

- *--vc-enable-umi-solid*—The VC UMI solid mode is optimized for solid tumors with post collapsed coverage rates of ~200—300X and target allele frequencies of 5% and higher.
- *--vc-enable-umi-liquid*—The liquid biopsy pipeline is not equivalent to liquid tumor mode. The liquid biopsy pipeline starts from a regular blood sample and looks for low VAF somatic variants from tumor cell free DNA floating in the blood. This type of test enables tumor profiling (diagnosis/biomarker identification) from plasma rather than from tissue, which requires an invasive biopsy. The VC UMI liquid mode is optimized for a liquid biopsy pipeline with post collapsed coverage rates of ~2000–2500X and target allele frequencies of 0.4% and higher.

These batch settings do not include the *vc-systematic-noise filter*. DRAGEN recommends adding the filter. For more information, see *Systematic Noise Filtering* on page 115.

In UMI pipelines, DRAGEN can estimate nucleotide mutation biases, such as oxidation and deamination artifacts, that might exist upstream of the sequencing system on a per sample basis. During variant calling, DRAGEN corrects the biases.

To enable this feature, use --vc-target-bed, which also specifies the target regions for variant calling, or --vc-snp-error-cal-bed. If using --vc-snp-error-cal-bed, specify the regions to estimate nucleotide substitution bias from if different from the target bed file or if a target bed file is not specified. If a third-party tool is used to produce the collapsed reads, then configure the tool so that the base call quality scores quantify the error shown on the sequencing system only. DRAGEN uses this error estimation to account for errors upstream of the sequencing system.

## Post Somatic Calling Filtering

The following options are available for post somatic calling filtering:

- *--vc-sq-call-threshold*

  Emits calls in the VCF. The default is 3.0 for tumor-normal and 0.1 for tumor-only. If the value for *vc-sq-filter-threshold* is lower than *vc-sq-call-threshold*, the filter threshold value is used instead of the call threshold value.

- *--vc-sq-filter-threshold*

  Marks emitted VCF calls as filtered. The default is 17.5 for tumor-normal and 6.5 for tumor-only.

- *--vc-enable-triallelic-filter*

  Enables the multiallelic filter. The default is true.

- *--vc-enable-af-filter*

  Enables the allele frequency filter. The default value is false. When set to true, the VCF excludes variants with allele frequencies below the AF call threshold or variants with an allele frequency below the AF filter threshold and tagged with low AF filter tag. The default AF call threshold is 1% and the default AF filter threshold is 5%. To change the threshold values, use the following command line options: vc-af-call-threshold and vc-af-filter-threshold.

- *--vc-enable-non-homref-normal-filter*

  Enables the non-homref normal filter. The default value is true. When set to true, the VCF filters out variants if the normal sample genotype is not a homozygous reference.

- *--vc-enable-vaf-ratio-filter*

  Adds one condition to be filtered out by the alt_allele_in_normal filter. The default value is false. When set to true, the VCF filters out variants if the normal sample AF is greater than 20% of tumor sample AF.

| Somatic Mode | Filter ID | Description |
|---|---|---|
| Tumor-Only & Tumor-Normal | clustered_events | Clustered events were observed in a given active region. Threshold for clustered events is configurable (default is ≥ 3). Enabled only when using *--vc-enable-gatk-acceleration=true*. |
| Tumor-Only & Tumor-Normal | weak_evidence | Variant does not meet likelihood threshold. The likelihood ratio for SQ tumor-normal is < 17.5 or < 3.0 for SQ tumor-only. |
| Tumor-Only & Tumor-Normal | multiallelic | Site filtered if there are two or more alt alleles at this location in the tumor. |
| Tumor-Only & Tumor-Normal | str_contraction | Suspected PCR error where the alt allele is one repeat unit less than the reference. Enabled only when using *--vc-enable-gatk-acceleration=true*. |

| Somatic Mode | Filter ID | Description |
|---|---|---|
| Tumor-Only & Tumor-Normal | base_quality | Median base quality of alt reads at this locus is < 20. |
| Tumor-Only & Tumor-Normal | mapping_quality | Median mapping quality of alt reads at this locus is < 20 (tumor-normal) or < 30 (tumor-only). |
| Tumor-Only & Tumor-Normal | fragment_length | Absolute difference between the median fragment length of alt reads and median fragment length of ref reads at a given locus > 10000. |
| Tumor-Only & Tumor-Normal | read_position | Median of distances between the start and end of read and a given locus < 5 (the variant is too close to edge of all the reads). |
| Tumor-Only & Tumor-Normal | panel_of_normals | Seen in at least one sample in the panel of normal VCF. |
| Tumor-Only & Tumor-Normal | low_af | Allele frequency is below the threshold specified *with --vc-af-filter-threshold* (default is 5%). Enabled only when using *--vc-enable-af-filter=true*. |
| Tumor-Only & Tumor-Normal | systematc_noise | If AQ score is < 10 (default) for tumor-normal or < 60 (default) for tumor-only, the site is filtered. |

## Matched Normal Control Sample Post Somatic Filtering

| Somatic Mode | Filter ID | Description |
|---|---|---|
| Tumor-Normal | noisy_normal | More than three alleles are observed in the normal sample at allele frequency above 9.9%. |

| Somatic Mode | Filter ID | Description |
|---|---|---|
| Tumor-Normal | alt_allele_in_normal | Alt allele frequency in the normal sample is above 0.2 plus the maximum contamination tolerance. For solid tumor mode the value is 0. For liquid tumor mode the default value is 0.15. See vc-enable-vaf-ratio-filter for optional conditions. |
| Tumor-Normal | filtered_reads | More than 90% of reads have been filtered out. |
| Tumor-Normal | no_reliable_supporting_read | No reliable supporting read was found in the tumor sample. A reliable supporting read is a read supporting the alt allele with mapping quality ≥ 40, fragment length ≤ 10,000, basecall quality ≥ 25, and distance from start/end of read ≥ 5. |
| Tumor-Normal | strand_artifact | Severe strand bias. Alt allele supported by at least four reads, but only on one strand. |
| Tumor-Normal | too_few_supporting_reads | Variant is supported by < 3 reads in the tumor sample. |
| Tumor-Normal | non-homref-normal | Normal sample genotype is not a homozygous reference. |
| Tumor-Normal | germline_risk | Likelihood of allele being present in normal > 0.025. Enabled only when using *--vc-enable-gatk-acceleration=true*. |
| Tumor-Normal | artifact_in_normal | TLOD of the normal read set (Normal artifact LOD) is > 0.0. Not called normal artifact if allele fraction in normal is much smaller than allele fraction in tumor (normalAlleleFraction < (0.1 * tumorAlleleFraction). Enabled only when using *--vc-enable-gatk-acceleration=true*. |

QUAL is not output in the somatic variant records. Instead, the confidence score is FORMAT/SQ.

```
##FORMAT=<ID=SQ,Number=1,Type=Float,Description="Somatic quality">
```

The field is specific to the sample. For the tumor samples, it quantifies the evidence that a somatic variant is present at a given locus.

If a normal sample is also available, the corresponding FORMAT/SQ value quantifies the evidence that the normal sample is a homozygous reference at a given locus.

GQ is not output in the somatic variant records, because DRAGEN does not test for multiple diploid genotype candidates. Instead, an ALT allele is considered as a candidate somatic variant. If tumor SQ > vc-sq-call-threshold (default is 3), then the FORMAT/GT for the tumor sample is hard-coded to 0/1, and the FORMAT/AF yields an estimate on the somatic variant allele frequency, which ranges anywhere within [0,1].

- If tumor SQ < vc-sq-call-threshold, the variant is not emitted in the VCF.
- If tumor SQ > vc-sq-call-threshold but tumor SQ < vc-sq-filter-threshold, the variant is emitted in the VCF, but FILTER=weak_evidence.
- If tumor SQ > vc-sq-call-threshold and tumor SQ > vc-sq-filter-threshold, the variant is emitted in the VCF and FILTER=PASS (unless the variant is filtered by a different filter).
- The default vc-sq-filter-threshold is 17.5 for tumor-normal and 3.0 for tumor-only analysis.

The following is an example somatic T/N VCF record. Tumor SQ > vc-sq-call-threshold but tumor SQ < vc-sq-filter-threshold, so the FILTER is marked as weak_evidence.

```
2 593701 . G A . weak_evidence
DP=97;MQ=48.74;SQ=3.86;NLOD=9.83;FractionInformativeReads=1.000
GT:SQ:AF:F1R2:F2R1:DP:SB:MB 0/0:9.83:33,0:0.000:14,0:19,0:33
0/1:3.86:61,3:0.047:29,2:32,1:64:35,26,0,3:39,22,1,2
```

The clustered-events penalty is an exception to the above rule for emitting variants. By default, the clustered-events penalty replaces the clustered-events filter in tumor-normal mode. Instead of applying a hard filter when too many events are clustered together, DRAGEN applies a penalty to the SQ scores of co-phased clustered events. Clustered events with weak evidence are no longer called, but clustered events with strong evidence can still be called. This is equivalent to lowering the prior probability of observing clustered co-phased variants. The penalty is applied after the decision to emit variants, so that penalized variants still appear in the VCF if their unpenalized score is high enough.

## Joint Analysis for Multiple Samples

DRAGEN supports pedigree-based and population-based joint analysis for multiple samples. In pedigree-based analysis, samples from the same species are related to each other. In population-based analysis, samples of the same species are unrelated to each other.

Joint analysis requires a gVCF file for each sample. To create a gVCF file, run the germline small variant caller with the *--vc-emit-ref-confidence gVCF* option.

The gVCF file contains information on the variant positions and positions determined to be homozygous to the reference genome. For homozygous regions, the gVCF file includes statistics that indicate how well reads support the absence of variants or alternative alleles. Contiguous homozygous runs of bases with similar levels of confidence are grouped into blocks, referred to as hom-ref blocks. Not all entries in the gVCF are contiguous. A reference might contain gaps that are not covered by either variant line or a hom-ref block. Gaps correspond to regions that are not callable. A region is not

callable if there is not at least one read mapped to the region with a MAPQ score above zero. The following example shows a joint VCF where one sample has a variant, and the other two samples are in a gVCF gap. Gaps are represented by "./.:.:".

```
1 605262 . G A 13.41 DRAGENHardQUAL
AC=2;AF=1.000;AN=2;DP=2;FS=0.000;MQ=14.00;QD=6.70;SOR=0.693
GT:AD:AF:DP:GQ:FT:F1R2:F2R1:PL:GP ./.:.:.:.:.:.:LowDepth
1/1:0,2:1.000:2:4:PASS:0,0:0,2:50,6,0:1.383e+01,4.943e+00,1.951e+00
./.:.:.:.:.:.:LowDepth
```

## Pedigree Mode

Use pedigree mode to jointly analyze samples from related individuals and to perform *de novo* calling.

To invoke pedigree mode set the *--enable-joint-genotyping* option to true and use the *--pedigree-file* option to specify the path to a pedigree file that describes the relationship between panels.

The pedigree file must be a tab-delimited text file with the file name ending in the .ped extension. The following information is required.

| Column Header | Description |
|---|---|
| Family_ID | The pedigree identifier. |
| Individual_ID | The ID of the individual. |
| Paternal_ID | The ID of the individual's father. If the founder, the value is 0. |
| Maternal_ID | The ID of the individual's mother. If the founder, the value is 0. |
| Sex | The sex of the sample. If male, the value is 1. If female, the value is 2. |
| Phenotype | The genetic data of the sample. If unknown, the value is 0. If unaffected, the value is 1. If affected, the value is 2. |

The following is an example of an input pedigree file.

```
#Family_ID Individual_ID   Paternal_ID    Maternal_ID    Sex
Phenotype
FAM001     NA12877_Father  0              0              1   1
FAM001     NA12878_Mother  0              0              2   1
FAM001     NA12882_Proband NA12877_Father NA12878_Mother 2   2
FAM001     NA12883_Proband NA12877_Father NA12878_Mother 1   0
```

To compare multiple pedigrees, you can run gVCF Genotyper on the output of Joint Genotyper and merge multiple joint-called pedigrees into a single multisample VCF.

Use the `--vc-emit-ref-confidence` gVCF option to configure the Joint Genotyper to write a multisample GVCF.

## De Novo Calling

The De Novo Caller identifies all the trios within the pedigree and generate a *de novo* score for each child. The *De Novo* Caller supports multiple trios within a single pedigree. Pedigree Mode supports *de novo* calling for small, structural, and copy number variants.

Pedigree Mode is run in multiple steps. The following is an example workflow for a trio from FASTQ.

1. Run single sample alignment and variant calling to generate per sample output using the following inputs for Pedigree Mode.
   - gVCF files for Small Variant Caller.
   - *.tn.tsv files for the Copy Number Caller.
   - BAM files for the Structural Variant Caller.
2. Run Pedigree Mode for Small Variant Caller.
   For more information, see *Small Variant De Novo Calling* on page 104.
3. Run Pedigree Mode for Copy Number Caller.
   For more information, see *Multisample CNV Calling* on page 1.
4. Run Pedigree Mode for Structural Variant Caller.
   For more information, see *Structural Variant De Novo Quality Scoring* on page 176.
5. Run DeNovo Variant Small Variant Filtering.
   For more information, see *De Novo Small Variant Filtering* on page 111.

## Small Variant De Novo Calling

The Small Variant De Novo callers considers a trio of samples at a time, where samples are related via a pedigree file. The Small Variant De Novo Caller determines all positions that have a Mendelian conflict based on the genotype from the individual sample gVCFs. Sex chromosomes in males are treated as haploid apart from the PAR regions, which are treated as diploid.

Each of those positions is then processed through the Pedigree Caller to compute a joint posterior probability matrix for the possible genotypes. The probabilities are used to determine whether the proband has a *de novo* variant with a DQ confidence score. All three subjects are assumed to have an independent error probability.

At positions where the original genotype from the gVCFs shows a double Mendelian conflict (eg, 0/0+0/0->1/1 or 1/1+1/1->0/0), the trio samples genotypes can be adjusted to the highest joint posterior probability that has at least one Mendelian conflict.

The DQ formula is DQ = -10log10(1 - Pdenovo).

Pdenovo is the sum of all indexes in the joint posterior probability matrix with one of more Mendelian conflicts.

In the GT overwrite step, it is possible that the parents GT to get overwritten. In the case of multiple trios, the parents GT is based on the last trio processed. The trios are processed in the order they are listed in the pedigree file. DRAGEN currently does not add an annotation in the VCF in cases where the GT was overwritten.

The multisample VCF file is annotated with FORMAT/DQ and FORMAT/DN fields to the output a VCF file that represents a *de novo* Quality Score and an associated *de novo* call. The DN field in the VCF is used to indicate the *de novo* status for each segment.

The following are the possible values:

- **Inherited**—The called trio genotype is consistent with Mendelian inheritance.
- **LowDQ**—The called trio genotype is inconsistent with Mendelian inheritance and DQ is less than the *de novo* quality threshold.
- **DeNovo**—The called trio genotype is inconsistent with Mendelian inheritance and DQ is greater than or equal to the *de novo* quality threshold.

The following is an example VCF line for a trio:

```
1 16355525. G A 34.46 PASS
AC=1;AF=0.167;AN=6;DP=45;FS=6.69;MQ=108.04;MQRankSum=0.156;QD=2.46;ReadPos
RankSum=0;SOR=0.01
GT:AD:AF:DP:GQ:FT:F1R2:F2R1:PL:GP:PP:DPL:DN:DQ
0/1:11,3:0.214:14:39:PASS:8,2:3,1:74,0,47:39.454,0.00053613,49.99:0,1,104:
74,0,47:DeNovo:0.6737
0/0:18,0:0:16:48:PASS:.:.:0,48,605:.:0,12,224:0,48,255:.:0/0:14,0:0:14:42:
PASS:.:.:0,42,490:.:0,5,223:0,42,255:.:.
```

### De Novo Small Variant Options

The following command line options are available for *de novo* small variant calling.

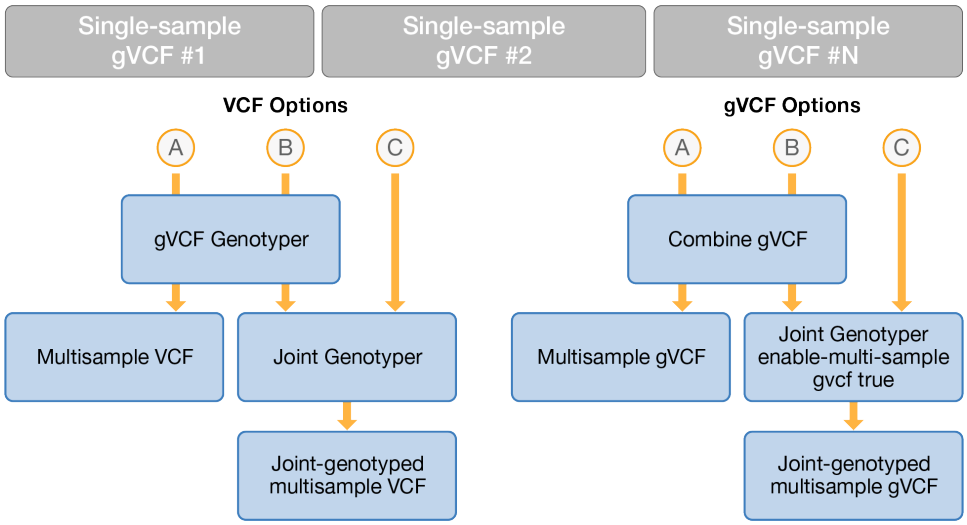
- *--enable-joint-genotyping*—Run the joint genotyping caller.
- *--variant*—Specify the gVCF input to the workflow.
- *--pedigree-file*—Specify the pedigree file to enable De Novo Calling if there is a trio present.
- *--qc-snp-denovo-quality-threshold*—Specify the minimum DQ value for a SNP to be considered *de novo*. The default value is 0.05.
- *--qc-indel-denovo-quality-threshold*—Specify the minimum DQ value for an indel to be considered *de novo*. The default value is 0.02.

## Population Mode

DRAGEN provides a population-based analysis option to jointly analyze samples from unrelated individuals. To initiate population mode, use the following genotypers.

- **gVCF Genotyper**—Uses a set of single or multisample gVCFs as input and outputs a multisample VCF, which contains one entry for any variant seen in any of the input gVCFs. The variants are genotyped across all input samples using information from the hom-ref blocks as necessary. The gVCF genotyper does not adjust genotypes based on population information. See *gVCF Genotyper Options* on page 1 for information on the available command line options.
- **Joint Genotyper**—Uses information from the whole cohort to improve the accuracy of individual genotypes. You can input multisample VCF, multisample gVCF, or a set of single sample gVCFs. To receive output as a multisample gVCF, set --enable-multi-sample-gVCF to true. See *Joint Genotyper Options* on page 1 for information on the available command line options.
- **Combine gVCFs**—Uses single-sample gVCF and multisample gVCFs to generate one multisample gVCF. Variants present in any input sample are genotyped across all samples similarly to gVCF genotyper. Combine gVCF accepts any combination of single-sample gVCFs and multisample gVCFs as input, but generating output might be slow if merging a large number of samples. For large scale population calling, use gVCF Genotyper instead of combine gVCF. See *Combine gVCF Options* on page 1 for information on the available command line options.

The following figure displays the different pathways and data flows between the gVCF Genotyper, Combine gVCFs, and Joint Genotyper.




To receive a list of variants present in the cohort and the genotypes of that variant in each of the cohort members, run the gVCF Genotyper. Optionally, you can run the Joint Genotyper after to build a second multisample VCF. The Joint Genotyper output refines the sample genotypes based on the population information. If using the gVCF Genotyper output only, you can filter out infrequent variants to prevent noise if the variants contain low depth or genotype quality. Use an open-source utility like bcftools on the output file to filter the variants.

To compare multiple pedigrees, you can run gVCF Genotyper on the output of Joint Genotyper and merge multiple joint-called pedigrees into a single multisample VCF.

Use the `--vc-emit-ref-confidence` gVCF option to configure the Joint Genotyper to write a multisample GVCF.

## Process gVCF Files From GATK

Both gVCF Genotyper and Joint Genotyper can process gVCF files written by the GATK variant caller when using GATK v4.1. To enable this option, set `--vc-enable-gatk-acceleration` for both gVCF Genotyper and Joint Genotyper.

## Genotyper Options

This section provides information on the options available for each of the genotypers.

### gVCF Options

In addition to the standard parameters for the variant caller stage of the DRAGEN host software, the following parameters are available to create a gVCF:

- *--vc-emit-ref-confidence*
  To enable banded gVCF generation, set to GVCF. To enable base pair resolution gVCF generation, set to BP_RESOLUTION. Banded gVCF generation is enabled by default and recommended. BP_Resolution results in large files that slow down any subsequent analysis.
- *--vc-gvcf-gq-bands*
  The *--vc-gvcf-gq-bands* option defines genotype quality (GQ) bands values. If the GQ values for positions homozygous to the reference lie within the same band, the positions homozygous to the reference are grouped into the same block.
- *--vc-max-alternate-alleles*
  The *--vc-max-alternate-alleles* option specifies the maximum number of ALT alleles that are output in VCF or gVCF. The default value is 6.

### gVCF Genotyper Options

The gVCF Genotyper uses a set of single sample gVCFs to output a multisample VCF that contains one entry per variant seen in any of the input gVCFs. Genotypes cannot be adjusted using population information.

gVCF Genotyper can also read gVCF files from an S3 bucket. For gVCF files in a public bucket, URLs with the prefix *s3://* or *https://* can be used in `--variant` or `--variant-list`. If the bucket requires authentication, environment variables or config files can be used. See the Samtools website for information on the htslib AWS S3 plugin.

gVCF Genotyper needs access to the index file for each gVCF input. The URLs for each gVCF and index file needs to be combined as `https://url1.gvcf.gz##idx##https://url2.gvcf.gz.tbi`, and then passed to *--variant* or *--variant-list* on the command line.

The following parameters are available for gVCF Genotyper.

- *--enable-gvcf-genotyper*
  To enable the gVCF Genotyper, set to true.
- *--ht-reference*
  The file containing the reference sequence in FASTA format. *--ht-reference* is required.
- *--output-directory*
  The output directory. *--output-directory* is required.
- *--output-file-prefix*
  The prefix used to label all output files. *--output-file-prefix* is required.
- *--gg-output-format*
  The output file format. The default value is vcf.gz. The permitted output file formats are vcf.gz, vcf, or bcf. Only the vcf.gz format is compatible with the joint genotyper. If using a different format, you can convert the format using the open-source bcftools utility.
- *--gg-regions*
  The file specifying the regions to run the gVCF Genotyper in. Variants outside these regions are ignored. The file can either be a bed file or a list of genomic regions specified using chromosome:start-end. Genomic regions can be separated by commas or line breaks. If using exome or enrichment data, specify the list of regions targeted by the probes to limit additional time spent processing unreliable genotype variants that lie outside the targeted regions.
- *--gg-enable-concat*
  Concat output for genomic regions into a single output file. By default, the value is set to true.
- *--gg-max-alternate-alleles*
  Maximum number of alternate alleles. By default, the value is set to 50. If there are more alleles than the set limit, alleles are ranked by frequency of occurrence in the input samples. The most common k alleles are output.
- *--gg-enable-indexing*
  Build a tabix index for the output file. By default, the value is set to false. The *--gg-output-format* must be set to vcf.gz to use *--gg-enable-indexing*.
- *--gg-extra-params=--min-depth=X*
  **[Optional]**Remove false-positive variant calls. Values for genotypes (FORMAT/GT) in samples with read depth (FORMAT/DP) below the threshold are set to missing. The default threshold is 8.
- *--gg-drop-genotypes*
  Select to output only the alleles for each variant. By default, the value is set to false. *--gg-drop-genotypes* is equivalent to running bcftools view -G on the default output.
- *--gg-allele-list*
  **[Optional]** Force the output of genotypes at specified sites. The path of a vcf.gz or bcf file containing the sites must be included.
- *--gg-extra-params=--remove-nonref*

**[Optional]**Removes the <NON_REF> symbolic allele from the output of gVCF Genotyper. This option should be used to support downstream tools which cannot process VCF lines with <NON_REF>.

## Joint Genotyper Options

You can run the Joint Genotyper from a multisample VCF, a multisample gVCF, or directly from a set of single sample gVCFs.

The following parameters are available for Joint Genotyper.

- *--enable-joint-genotyping*
  To run the Joint Genotyper, set to true.
- *--output-directory*
  The output directory. *--output-directory* is required.
- *--output-file-prefix*
  The prefix used to label all output files. *--output-file-prefix* is required.
- *-r*
  The directory where the hash table resides.
- *--variant* or *--variant-list*
  Specifies the path to a single gVCF file. You can specify multiple gVCF files using multiple *--variant* options. A maximum of 200 gVCFs are supported. Use *--variant-list* to specify a file containing a list of gVCF files that need to be combined using one variant file path per line.
- *--pedigree-file*
  Specify the path to a pedigree file describing the relationship between samples. For more information, see *Pedigree Mode* on page 103.

## Combine gVCF Options

You can run Combine gVCFs on a set of single sample gVCFs and multisample gVCFs to create a single multisample gVCF as output. For large-scale population calling, use gVCF Genotyper instead of the Combine gVCF Genotyper.

The following parameters are available for combine gVCFs:

- *--enable-combinegvcfs*
  To run Combine gVCFS, set to true.
- *--intermediate-results-dir*
  **[Optional]** Set the intermediate results to a directory different from the output directory, such as /staging/temp. If running Combine gVCFs, *--intermediate-results-dir* is recommended. For more information, see *Determine Input and Output File Locations* on page 22.
- *--output-directory*

The output directory. *--output-directory* is required.

- *--output-file-prefix*
  The prefix used to label all output files. *--output-file-prefix* is required.
- *-r*
  The directory where the hash table resides.
- *--variant* or *--variant-list*
  Specifies the path to a single gVCF file. You can specify multiple gVCF files using multiple *--variant* options. 200 gVCFs are supported, but it is not recommended to combine more than 10 gVCFS. Use *--variant-list* to specify a file containing a list of gVCF files that need to be combined using one variant file path per line.

## Joint Analysis Output Format

There are two available joint analysis output files:

- **Multisample VCF**—A VCF file containing a column with genotype information for each of the input samples according to the input variants.
- **Multisample gVCF**—A gVCF file augmenting the content of a multisample VCF file, similar to how a gVCF file augments a VCF file for a single sample. In between variant sites, the multisample gVCF contains statistics that describe the level of confidence that each sample is homozygous to the reference genome. Multisample gVCF is a convenient format for combining the results from a pedigree or small cohort into a single file. If using a large number of samples, fluctuation in coverage or variation in any of the input samples creates a new hom-ref block, which causes a highly fragmented block structure and a large output file that can be slow to create.

### Hom-ref Blocks FORMAT Fields

In hom-ref blocks, the following FORMAT fields are calculated uniquely.

- **FORMAT/DP**—Values represent the minimum DP across all positions within the band.
- **FORMAT/AD**—Values represent the position in the band where DP=median DP.
- **FORMAT/AF**—Values are based on FORMAT/AD.
- **FORMAT/PL**—Values represent the Phred likelihoods per genotype hypothesis. For hom-ref blocks, each value in FORMAT/PL represents the minimum value across all positions within the band.
- **FORMAT/SPL** and **FORMAT/ICNT**—Parameters reported in the gVCF records, including both hom-ref blocks and variant records. The parameters are used to compute the confidence score of a variant being *de novo* in the proband of a trio. For SNP, FORMAT/PL and FORMAT/SPL are both used as input to the DeNovo caller. FORMAT/PL represents Phred likelihoods obtained from the genotyper, if the genotyper is called. FORMAT/SPL represents Phred likelihoods obtained from column-wise estimation, pre-graph. Each value in FORMAT/SPL represents the minimum across all positions within the band. For INDEL, the PL value is computed in the joint pedigree calling step

based on the FORMAT/ICNT reported in the gVCF file. FORMAT/ICNT consist of two values. The first value is the number of reads with no indels at the position, and the second value is the number of reads with indels at the position. Each value in FORMAT/ICNT represent the maximum of the value across all positions within the band.

In the following example hom-ref block, ICNT provides information on if each sample contains an Indel at the position of interest. If the proband contains an indel at the position and the ICNT of the parents does not indicate any read supporting an indel, then the confidence score is high for the proband to have an indel *de novo* call at the position.

```
chr1 10288 . C <NON_REF> . PASS END=10290 GT:AD:DP:GQ:MIN_DP:PL:SPL:ICNT
0/0:131,4:135:69:132:0,69,1035:0,125,255:23,1

chr1 10291 . C
T,<NON_REF> 38.45 PASS
DP=100;MQ=24.72;MQRankSum=0.733;ReadPosRankSum=4.112;FractionInformativeRe
ads=0.600;R2_5P_bias=0.000
GT:AD:AF:DP:F1R2:F2R1:GQ:PL:SPL:ICNT:GP:PRI:SB:MB
0/1:28,32,0:0.533,0.000:60:20,21,0:8,11,0:15:73,0,12,307,157,464:255,0,255
:23,10:3.8452e+01,1.3151e-
01,1.5275e+01,3.0757e+02,1.9173e+02,4.5000e+02:0.00,34.77,37.77,34.77,69.5
4,37.77:4,24,7,25:8,20,14,18
```

The SPL and ICNT is specific to DRAGEN. The GATK variant caller does not output the SPL and ICNT values.

## De Novo Small Variant Filtering

The filtering step identifies de novo variants calls of the joint calling workflow in regions with ploidy changes. Since de novo calling can have reduced specificity in regions where at least one of the pedigree members shows non-diploid genotypes, the de novo variant filtering marks relevant variants and thus can improve specificity of the call set.

Based on the structural and copy number variant calls of the pedigree, the FORMAT/DN field in the proband is changed from the original DeNovo value to DeNovoSV or DeNovoCNV if the de novo variant overlaps with a ploidy-changing SV or CNV, respectively. All other variant details remain unchanged, and all variants of the input VCF will also be present in the filtered output VCF. Structural or copy number variants which result in no change of ploidy, such as inversions, are not considered in the filtering. As an example, a de novo SNV calls in the input VCF

```
chr1 234710899 . T C 44.74 PASS
AC=1;AF=0.167;AN=6;DP=73;FS=4.720;MQ=250.00;MQRankSum=5.310;QD=1.15;ReadPo
sRankSum=1.366;SOR=0.251
GT:AD:AF:DP:GQ:FT:F1R2:F2R1:PL:GL:GP:PP:DQ:DN
0/1:21,18:0.462:39:48:PASS:14,10:7,8:84,0,50:-8.427,0,-5:4.950e+01,7.041e-
```

```
05,5.300e+01:15,0,120:3.2280e-01:DeNovo
0/0:13,0:0.000:11:30:PASS:.:.:0,30,450:.:.:10,0,227
0/0:25,0:0.000:22:60:PASS:.:.:0,60,899:.:.:0,33,227
```

Overlapping with an SV duplication in the proband, mother or father would be represented in the filtered output VCF as follows:

```
chr1 234710899 . T C 44.74 PASS
AC=1;AF=0.167;AN=6;DP=73;FS=4.720;MQ=250.00;MQRankSum=5.310;QD=1.15;ReadPo
sRankSum=1.366;SOR=0.251
GT:AD:AF:DP:GQ:FT:F1R2:F2R1:PL:GL:GP:PP:DQ:DN
0/1:21,18:0.462:39:48:PASS:14,10:7,8:84,0,50:-8.427,0,-5:4.950e+01,7.041e-
05,5.300e+01:15,0,120:3.2280e-01:DeNovoSV
0/0:13,0:0.000:11:30:PASS:.:.:0,30,450:.:.:10,0,227
0/0:25,0:0.000:22:60:PASS:.:.:0,60,899:.:.:0,33,227
```

The following is an example command line for running the de novo filtering, based on the files returned by the joint calling workflows:

```
dragen \
--dn-enable-denovo-filtering true \
--dn-input-joint-vcf <JOINT_SMALL_VARIANT_VCF> \
--dn-output-joint-vcf <OUTPUT_VCF> \
--dn-sv-vcf <JOINT_SV_VCF> \
--dn-cnv-vcf <JOINT_CNV_VCF> \
--enable-map-align false
```

### De Novo Small Variant Filtering Options

The following options are used for de novo variant filtering:

- *--dn-input-vcf*—Joint small variant VCF from the de novo calling step to be filtered.
- *--dn-output-vcf*—File location to which the filtered VCF should be written. If not specified, the input VCF is overwritten.
- *--dn-sv-vcf*—Joint structural variant VCF from the SV calling step. If omitted, checks with overlapping structural variants are skipped.
- *--dn-cnv-vcf*— Joint structural variant VCF from the CNV calling step. If omitted, checks with overlapping copy number variants are skipped.

## Germline Variant Small Hard Filtering

DRAGEN provides post-VCF variant filtering based on annotations present in the VCF records. Default and non-default variant hard filtering are described below. However, due to the nature of DRAGEN's algorithms, which incorporate the hypothesis of correlated errors from within the core of variant caller,

the pipeline has improved capabilities in distinguishing the true variants from noise, and therefore the dependency on post-VCF filtering is substantially reduced. For this reason, the default post-VCF filtering in DRAGEN is very simple.

## Default Small Variant Hard Filtering

The default filters in the germline pipeline are as follows:

- ##FILTER=<ID=DRAGENSnpHardQUAL,Description="Set if true:QUAL < 10.41">
- ##FILTER=<ID=DRAGENIndelHardQUAL,Description="Set if true:QUAL < 7.83">
- ##FILTER=<ID=LowDepth,Description="Set if true:DP <= 1">
- ##FILTER=<ID=PloidyConflict,Description="Genotype call from variant caller not consistent with chromosome ploidy">
- ##FILTER=<ID=base_quality,Description="Site filtered because median base quality of alt reads at this locus does not meet threshold">
- ##FILTER=<ID=lod_fstar,Description="Variant does not meet likelihood threshold (default threshold is 6.3)">
- DRAGENSnpHardQUAL and DRAGENIndelHardQUAL: For all contigs other than the mitochondrial contig, the default hard filtering consists of thresholding the QUAL value only. A different default QUAL threshold value is applied to SNP and INDEL.
- lod_fstar: For the mitochondrial contig, the default hard filtering consists of thresholding the LOD score only.
- base_quality: For the mitochondrial contig, this filter applies to sites where the median base quality of alt reads falls below a threshold.
- LowDepth: This filter is applied to all variants call with FORMAT/DP <= 1/.
- PloidyConflict: This filter is applied to all variant calls on chrY of a female subject, if female is specified on the DRAGEN command line, of if female is detected by the Ploidy Estimator.

## Non-Default Small Variant Hard Filtering

DRAGEN supports basic filtering of variant calls as described in the VCF standard. You can apply any number of filters with the *--vc-hard-filter* option, which takes a semicolon-delimited list of expressions, as follows:

*<filter ID>:<snp|indel|all>:<list of criteria>*,

where the list of criteria is itself a list of expressions, delimited by the || (OR) operator in this format:

*<annotation ID> <comparison operator> <value>*

The meaning of these expression elements is as follows:

- **filterID**—The name of the filter, which is entered in the FILTER column of the VCF file for calls that are filtered by that expression.

- **snp/indel/all**—The subset of variant calls to which the expression should be applied.
- **annotation ID**—The variant call record annotation for which values should be checked for the filter. Supported annotations include FS, MQ, MQRankSum, QD, and ReadPosRankSum.
- **comparison operator**—The numeric comparison operator to use for comparing to the specified filter value. Supported operators include <, ≤, =, ≠, ≥, and >.

For example, the following expression would mark with the label "SNP filter" any SNPs with FS < 2.1 or with MQ < 100, and would mark with "indel filter" any records with FS < 2.2 or with MQ < 110:

```
--vc-hard-filter="SNP filter:snp:FS < 2.1 || MQ < 100; indel
filter:indel:FS < 2.2 || MQ < 110"
```

This example is for illustration purposes only and is NOT recommended for use with DRAGEN V3 output. Illumina recommends using the default hard filters.

The only supported operation for combining value comparisons is OR, and there is no support for arithmetic combinations of multiple annotations. More complex expressions may be supported in the future.

## Orientation Bias Filter

The orientation bias filter is designed to reduce noise typically associated with the following:

- Pre-adapter artifacts introduced during genomic library preparation (eg, a combination of heat, shearing, and metal contaminates can result in the 8-oxoguanine base pairing with either cytosine or adenine, ultimately leading to G→T transversion mutations during PCR amplification), or
- FFPE (formalin-fixed paraffin-embedded) artifact. FFPE artifacts stem from formaldehyde deamination of cytosines, which results in C to T transition mutations.

The orientation bias filter can only be used on somatic pipelines. To enable the filter, set the *--vc-enable-orientation-bias-filter* option to true. The default is false.

The artifact type to be filtered can be specified with the *--vc-orientation-bias-filter-artifacts* option. The default is C/T,G/T, which correspond to OxoG and FFPE artifacts. Valid values include C/T, or G/T, or C/T,G/T,C/A.

An artifact (or an artifact and its reverse compliment) cannot be listed twice. For example, C/T,G/A is not valid, because C→G and T→A are reverse compliments.

The orientation bias filter adds the following information.

- *##FORMAT=<ID=F1R2,Number=R,Type=Integer,Description=*"Count of reads in F1R2 pair orientation supporting each allele">
- *##FORMAT=<ID=F2R1,Number=R,Type=Integer,Description=*"Count of reads in F2R1 pair orientation supporting each allele">
- *##FORMAT=<ID=OBC,Number=1,Type=String,Description=*"Orientation Bias Filter base context">
- *##FORMAT=<ID=OBPa,Number=1,Type=String,Description=*"Orientation Bias prior for artifact">

- *##FORMAT=<ID=OBParc,Number=1,Type=String,Description=*"Orientation Bias prior for reverse compliment artifact">
- *##FORMAT=<ID=OBPsnp,Number=1,Type=String,Description=*"Orientation Bias prior for real variant">

## dbSNP Annotation

In Germline, Tumor-Normal somatic, or Tumor-Only somatic modes, DRAGEN can look up variant calls in a dbSNP database and add annotations for any matches that it finds there. To enable the dbSNP database search, set the *--dbsnp* option to the full path to the dbSNP database VCF or .vcf.gz file, which must be sorted in reference order.

For each variant call in the output VCF, if the call matches a database entry for CHROM, POS, REF, and at least one ALT, then the rsID for the matching database entry is copied to the ID column for that call in the output VCF. In addition, DRAGEN adds a DB annotation to the INFO field for calls that are found in the database.

DRAGEN matches variant calls based on the name of the reference sequence/contig, but there is no additional way to assert that the reference used for constructing the dbSNP is the same as the reference used for alignment and variant calling. Make sure that the contigs in the selected annotation database match those in the alignment/variant calling reference.

## Panel of Normals VCF

When DRAGEN is used in the Tumor-Normal or Tumor-Only somatic mode, a panel of normals (PON) VCF can be specified for the purpose of filtering out systematic errors. The PON VCF must be generated ahead of time and represents a set of variants that were detected by the DRAGEN Somatic Pipeline when run on normal samples that are not matched to the subject from whom the tumor sample was taken. The PON VCF may contain several dozen samples. The samples should ideally be normal samples collected on the same library prep/sequencing instrument, so that if there are systematic errors that occur during sequencing/library prep, they get captured in the PON VCF.

- *--panel-of-normals*

  Specifies a PON VCF file. When a PON VCF file is used as input and if a somatic variant is found in at least one sample in the file, it is marked as panel_of_normals in the FILTER column of the output VCF.

## Systematic Noise Filtering

When Local Analysis Software is used in the Tumor-Normal or Tumor-Only somatic mode, a BED file with site-specific noise level can be specified for the purpose of filtering out sequencing / systematic noise. The site-specific noise level is used to calculate an AQ score, similar to the Phred-scale. If the AQ score is smaller than the defined threshold, the variant is filtered as systematic noise. The systematic noise BED file is built using VCFs that were generated by the Local Analysis Software Somatic Pipeline

when run on normal samples that do not necessarily match to the subject the tumor sample was taken from. The file might contain several dozen samples, ideally normal samples collected on the same library prep kit and sequencing system, so that if there are systematic errors that occur during library prep or sequencing, they are captured in the systematic noise BED file.

The following are the available systematic noise command-line options

- *--vc-systematic-noise*—Specifies a systematic noise BED file. If a somatic variant does not pass the AQ threshold, the variant is marked as systematic_noise in the FILTER column of the output VCF.
- *--vc-systematic-noise-filter-threshold*—Set the AQ threshold. By default the threshold value for tumor-normal is 10 and 60 for tumor-only.

Several prebuilt systematic noise files for WGS and WES can be downloaded. For best performance normal samples collected on the same library prep kit and sequencing system should be used to build the systematic noise BED file.

## Generate Systematic Noise BED File

You can generate systematic noise BED files from normal samples collected using library prep, sequencing system, and panels. If using panel sequencing, 50 samples are recommended.

To generate a BED file, do as follows.

1. Run DRAGEN somatic tumor-only on normal samples with *--vc-systematic-noise* set to `true` to generate VCF output per normal sample.
2. Build the BED file using the VCFs and the following options.
   - *--vc-systematic-noise-raw-input-list*—List of input VCFs. Enter one VCF per line.
   - *--vc-systematic-noise-germline-vaf-threshold*—Minimum VAF to remove potential germlines from systematic noise file building. Variants with a VAF greater than the threshold are not considered systematic noise. The default is none.

     If using small panels, the recommended threshold is 0.3.
   - *--vc-systematic-noise-use-germline-tag*—Use DRAGEN internal germline tagging to remove potential germlines. Mutually exclusive with --vc-systematic-noise-germline-vaf-threshold. The default is false.

     If using WGS or WES, the recommended setting is `true`.
   - *--vc-systematic-noise-method*—Method to calculate the systematic noise level (noise allele frequency) across samples. Enter `mean` to calculate the average noise allele frequency, `max` to calculate the maximum, or `aggregate` to calculate total alleles / total depth per locus across samples. The default is `mean`.

     If using WGS the recommended setting is `max`. If using WES, the recommended setting is `aggregate`. If using small panels for higher sensitivity, the recommended setting is `mean` or `aggregate`.

You can also build systematic noise BED files in the cloud using the BaseSpace Sequence Hub DRAGEN CNV Baseline Builder App.

### Prebuilt Systematic Noise BED Files

The following prebuilt systematic noise files for WGS and WES are available for download on the Illumina DRAGEN BIo-IT Platform Support Site page.

| Pre-built Systematic Noise File | Comment | Number of Normal Samples |
|---|---|---|
| WGS_hg38_v1.0_systematic_noise.bed.gz | WGS hg38 | 28 Samples, mixture of PCRFree NovaSeq, PCRFree HiSeqX, TruSeq Nano HiSeqX |
| WGS_hs37d5_v1.0_systematic_noise.bed.gz | WGS hs37d5 | 31 Samples, mixture of PCRFree NovaSeq, PCRFree HiSeqX, TruSeq Nano HiSeqX |
| WGS_hg19_v1.0_systematic_noise.bed.gz | WGS hg19 | 31 Samples, mixture of PCRFree NovaSeq, PCRFree HiSeqX, TruSeq Nano HiSeqX |
| WES_Nextera_IDT_hg38_v1.0_systematic_noise.bed.gz | Nextera library prep; IDT exome; hg38 | 47 Samples |
| WES_Nextera_IDT_hs37d5_v1.0_systematic_noise.bed.gz | Nextera library prep; IDT exome; hs37d5 | 47 Samples |
| WES_Nextera_IDT_hg19_v1.0_systematic_noise.bed.gz | Nextera library prep; IDT exome; hg19 | 47 Samples |
| WES_TruSeq_IDT_hg38_v1.0_systematic_noise.bed.gz | TruSeq library prep; IDT exome; hg38 | 53 Samples |
| WES_TruSeq_IDT_hs37d5_v1.0_systematic_noise.bed.gz | TruSeq library prep; IDT exome; hs37d5 | 53 Samples |
| WES_TruSeq_IDT_hg19_v1.0_systematic_noise.bed.gz | TruSeq library prep; IDT exome; hg19 | 53 Samples |

## Autogenerated MD5SUM for VCF Files

An MD5SUM file is generated automatically for VCF output files. This file is in the same output directory and has the same name as the VCF output file, but with an .md5sum extension appended. For example, whole_genome_run_123.vcf.md5sum. The MD5SUM files is a single-line text file that contains the md5sum of the VCF output file. This md5sum exactly matches the output of the Linux md5sum command.

## Force Genotyping

DRAGENsupports force genotyping (ForceGT) for Germline SNV variant calling. To use ForceGT, use the *--vc-forcegt-vcf* option with a list of small variants to force genotype. The input list of small variants can be a .vcf or .vcf.gz file.

The current limitations of ForceGT are as follows:

- ForceGT is supported for Germline SNV variant calling in the V3 mode. The V1, V2, and V2+ modes are not supported.
- ForceGT is not supported for Somatic SNV variant calling.
- ForceGT variants do not propagate through Joint Genotyping.

### ForceGT Input

DRAGEN software supports only a single ForceGT VCF input file, which must meet the following requirements:

- Have the same reference contigs as the VCF used for variant calling.
- Be sorted by reference contig name and position.
- Be normalized (parsimonious and left-aligned).
- Contain no complex variants (variants that require more than one substitution / insertion / deletion to go from ref allele to alt allele). For example, any variant in the ForceGT VCF similar to the following results in undefined behavior in the DRAGEN software:

  ```
  chrX 153592402 GTTGGGGATGCTGAC CACCCTGAAGGG
  ```

The following nonnormalized variants cause undefined behavior in DRAGEN software:

- Not parsimonious: `chrX 153592402 GC GCG`
- Parsimonious representation: `chrX 153592403 C CG`

### ForceGT Operation and Expected Outcome

When running Germline SNV variant calling with ForceGT, DRAGEN generates a single sample gVCF using the ForceGT VCF as input on the command line. The single sample gVCF output file contains all regular calls and the forceGT calls, as follows:

- For a ForceGT call that was not called by the variant caller (not common), the call is tagged with FGT in the INFO field.
- For a ForceGT call that was also called by the variant caller and filter field is PASS (common), the call is tagged with NML:FGT in the INFO field (NML denotes normal)
- For a normal call (and PASS) by the variant caller, with no ForceGT call (normal), no extra tags are added (no NML tag, no FGT tag)

This scheme distinguishes among calls that are present due to FGT only, common in both ForceGT input and normal calling, and normal calls.

All the variants in the input ForceGT VCF are genotyped and present in the output single sample gVCF file. The following table lists the reported GTs for the variants.

| Condition | Reported GT |
|---|---|
| At a position with no coverage | ./. |
| At a position with coverage but no reads supporting ALT allele | 0/0 |
| At a position with coverage and reads supporting ALT allele | 0/0 or 0/1 or 1/1 or 1/2 |

At a position where the variant call made by default DRAGEN software is different from the one specified in the input ForceGT vcf, there are multiple entries at the same position in the output gVCF, as follows:

- One entry for the default DRAGEN variant call, and
- One entry each for every variant call present in the input ForceGT VCF at that position.

```
chrX 100 G C [Default DRAGEN variant call]
chrX 100 G A [Variant in ForceGT vcf]
```

If a target bed file is provided along with the input ForceGT VCF, then the output gVCF file only contains ForceGT variants that overlap the bed file positions.

# Copy Number Variant Calling

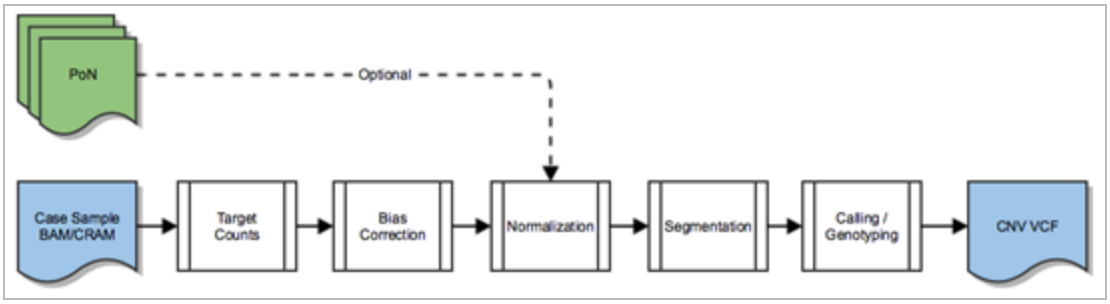
The DRAGEN Copy Number Variant (CNV) pipeline can call CNV events using next-generation sequencing (NGS) data. This pipeline supports multiple applications in a single interface via the DRAGEN Software, including processing of whole-genome sequencing data and whole-exome sequencing data for germline analysis.

The DRAGEN CNV pipeline supports two normalization modes of operation. The two modes apply different normalization techniques to handle biases that differ based on the application, for example, WGS versus WES. While the default option settings attempt to provide the best trade-off in terms of speed and accuracy, a specific workflow may require more finely tuned option settings.

## CNV Workflow

The DRAGEN CNV pipeline follows the workflow shown in the following figure.

Figure 3 DRAGEN CNV Pipeline Workflow




This pipeline uses many aspects of the DRAGEN platform available in other pipelines, such as hardware acceleration and efficient I/O processing. To enable CNV processing in the DRAGEN Host Software, set the *--enable-cnv* command line option to true.

The CNV pipeline has the following processing modules:

- Target Counts—binning of the read counts and other signals from alignments.
- Bias Correction—correction of intrinsic system biases.
- Normalization—detection of normal ploidy levels and normalization of the case sample.
- Segmentation—breakpoint detection via segmentation of the normalized signal.
- Calling / Genotyping—thresholding, scoring, qualifying, and filtering of putative events as copy number variants.

The normalization module can optionally take in a panel of normals (PoN), which is used when a cohort or population samples are readily available. All other modules are shared between the different CNV algorithms.

## Signal Flow Analysis

The following figures show a high-level overview of the steps in the DRAGEN CNV Pipeline as the signal traverses through the various stages. These figures are examples and are not identical to the plots that are generated from the DRAGEN CNV Pipeline.

The first step in the DRAGEN CNV Pipeline is the target counts stage, which extracts signals such as read count and improper pairs and puts them into target intervals.

Figure 4 Read Count Signal

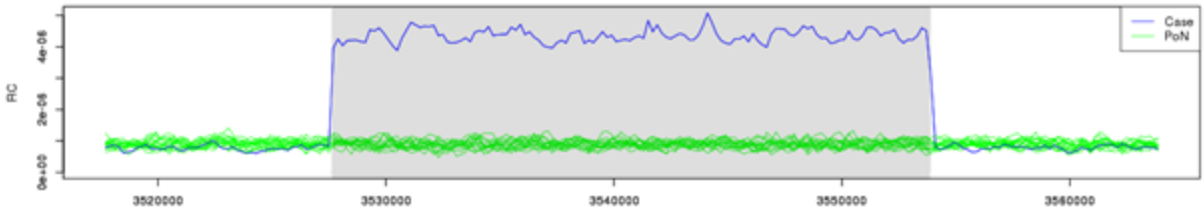
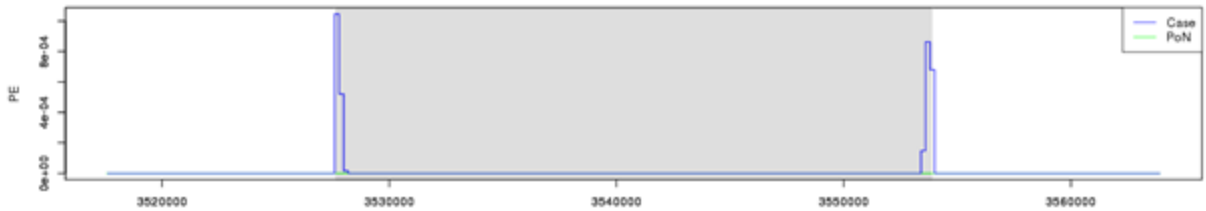
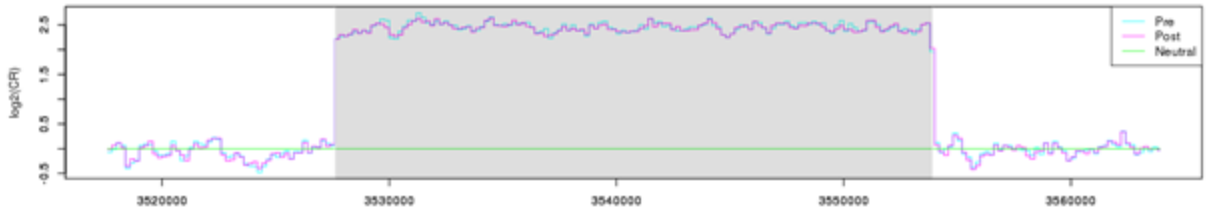



Figure 5 Improper Pairs Signal




Next, the case sample is normalized against the panel of normals, or against the estimated normal ploidy level, and any other biases are subtracted out of the signal to amplify any event level signals.

Figure 6 Pre/Post Tangent Normalization




The normalized signal is then segmented using one of the available segmentation algorithms, and events are called from the segments.

Figure 7 Segments

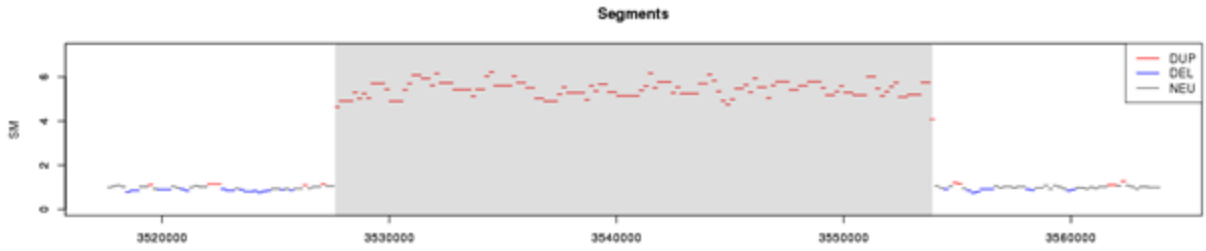



Figure 8 Called Events


The events are then scored and emitted in the output VCF.

## CNV Pipeline Options

The following are the top-level options that are shared with the DRAGEN Host Software to control the CNV pipeline. The input into the DRAGEN CNV can be a BAM or CRAM file. If you are using the DRAGEN mapper and aligner, FASTQ files can be used.

- *--bam-input*—The BAM file to be processed.
- *--cram-input*—The CRAM file to be processed.
- *--enable-cnv*—Enable or disable CNV processing. Set to true to enable CNV processing.
- *--enable-map-align*—Enables the mapper and aligner module. The default is true, so all input reads are remapped and aligned unless this option is set to false.
- *--fastq-file1*, *--fastq-file2*—FASTQ file, or files, to be processed.
- *--output-directory*—Output directory where all results are stored.
- *--output-file-prefix*—Output file prefix that will be prepended to all result file names.
- *--ref-dir*—The DRAGEN reference genome hashtable directory.

## CNV Pipeline Input

The DRAGEN CNV pipeline supports multiple input formats. The most common format is an already mapped and aligned BAM or CRAM file. If you have data that has not yet been mapped and aligned, see *Generate an Alignment File* on page 123.

To run the DRAGEN CNV Pipeline directly with FASTQ input without generating a BAM or CRAM file, then see *Streaming Alignments* on page 124, which outlines steps for streaming alignment records directly from the DRAGEN map/align stage.

## Reference Hashtable

For the DRAGEN CNV pipeline, the hashtable must be generated with the *--enable-cnv* option set to true, in addition to any other options required by other pipelines. When *--enable-cnv* is true, *dragen* generates an additional k-mer uniqueness map that the CNV algorithm uses to counteract mapability biases. The k-mer uniqueness map file only needs to be generated once per reference hashtable and takes about 1.5 hours per whole human genome.

The reference hashtable is a pregenerated binary representation of the reference genome. For information on generating a hashtable, see *Prepare a Reference Genome* on page 8.

The following is an example of a command to generate a hashtable.

```
dragen \
--build-hash-table true \
--ht-reference <FASTA> \
--output-directory <OUTPUT> \
--enable-cnv true \
--enable-rna true
```

## Generate an Alignment File

The following command line examples show how to run the DRAGEN map/align pipeline depending on your input type. The map/align pipeline generates an alignment file in the form of a BAM or CRAM file that can then be used in the DRAGEN CNV Pipeline.

You need to generate alignment files for all samples that have not already been mapped and aligned, including any samples to be used as references for normalization. Each sample must have a unique sample identifier, which is specified with the *--RGSM* option. For BAM and CRAM input files, the sample identifier is taken from the file, so the *--RGSM* option is not required.

Example command to map/align a FASTQ file:

```
dragen \
-r <HASHTABLE> \
-1 <FASTQ1> \
-2 <FASTQ2> \
--RGSM <SAMPLE> \
--RGID <RGID> \
--output-directory <OUTPUT> \
--output-file-prefix <SAMPLE> \
--enable-map-align true
```

Example command to map/align an existing BAM file:

```
dragen \
-r <HASHTABLE> \
--bam-input <BAM> \
--output-directory <OUTPUT> \
--output-file-prefix <SAMPLE> \
--enable-map-align true
```

Example command to map/align an existing CRAM file:

```
dragen \
-r <HASHTABLE> \
--cram-input <CRAM> \
--output-directory <OUTPUT> \
--output-file-prefix <SAMPLE> \
--enable-map-align true
```

### Streaming Alignments

DRAGEN can map and align FASTQ samples and then directly stream them to downstream callers. Examples of downstream callers include the CNV Caller and the Haplotype Variant Caller. This process allows you to skip generation of a BAM or CRAM file, bypassing the need to store additional files.

To stream alignments directly to the DRAGEN CNV pipeline, run the FASTQ sample through a regular DRAGEN map/align workflow, and then provide additional arguments to enable CNV. The following shows an example command line to map/align a FASTQ file and send it to the Germline CNV WGS pipeline.

```
dragen \
-r <HASHTABLE> \
-1 <FASTQ1> \
-2 <FASTQ2> \
--RGSM <SAMPLE> \
--RGID <RGID> \
--output-directory <OUTPUT> \
--output-file-prefix <SAMPLE> \
--enable-map-align true \
--enable-cnv true \
--cnv-enable-self-normalization true
```

For information on running CNV concurrently with the Haplotype Variant Caller, see *Concurrent CNV and Small Variant Calling* on page 140.

## Target Counts

The target counts stage is the first processing stage for the DRAGEN CNV pipeline. This stage bins the alignments into intervals. The primary analysis format for CNV processing is the target counts file, which contains the feature signals that are extracted from the alignments to be used in downstream

processing. The binning strategy, interval sizes, and their boundaries are controlled by the target counts generation options, and the normalization technique used.

When working with whole genome sequence data, the intervals are autogenerated from the reference hashtable. Only the primary contigs from the reference hashtable are considered for binning. You can specify additional contigs to bypass with the *--cnv-skip-contig-list* option.

With whole exome sequence data, the target BED file supplied with the *--cnv-target-bed* option is used to determine the intervals for analysis.

The target counts stage generates a .target.counts.gz file, which can be later used in place of any BAM or CRAM by specifying it with the *--cnv-input* option for the normalization stage. The .target.counts.gz file is an intermediate file for the DRAGEN CNV pipeline and should not be modified.

The .target.counts.gz file is a tab-delimited compressed text file with the following columns:

- Contig identifier
- Start position
- End position
- Target interval name
- Count of alignments in this interval
- Count of improperly paired alignments in this interval

An example of a *.target.counts.gz file is shown below.

```
contig  start   stop    name                SampleName
improper_pairs
1       565480  565959  target-wgs-1-565480  7           6
1       566837  567182  target-wgs-1-566837  9           0
1       713984  714455  target-wgs-1-713984  34          4
1       721116  721593  target-wgs-1-721116  47          1
1       724219  724547  target-wgs-1-724219  24          21
1       725166  725544  target-wgs-1-725166  43          12
1       726381  726817  target-wgs-1-726381  47          14
1       753243  753655  target-wgs-1-753243  31          2
1       754322  754594  target-wgs-1-754322  27          0
1       754594  755052  target-wgs-1-754594  41          0
```

## Whole Genome

If the samples are whole genome, then the effective target intervals width is specified with the *--cnv-interval-width* option. The higher the coverage of a sample, the higher the resolution that can be

detected. This option is important when running with a panel of normals because all the samples must have matching intervals. For self normalization, the effective width may be larger than the specified value.

The default value for WGS is 1000 bp with a sample coverage of ≥ 30x.

| WGS Coverage per Sample | Recommended Resolution* (bp) |
|---|---|
| 5 | 10000 |
| 10 | 5000 |
| ≥30 | 1000 |

*Using a `cnv-interval-width` of ≤250 bp for WGS analysis can drastically increase run time

The intervals are autogenerated for every contig in the reference. You can specify a list of contigs to skip by using the *--cnv-skip-contig-list* option. This option takes comma-separated list of contig identifiers. The contig identifiers must match the reference hashtable that you are using. By default, only the mitochondrial chromosomes are skipped. Non-primary contigs are never processed.

For example, to skip chromosome M, X, and Y, use the following option:

```
--cnv-skip-contig-list "chrM,chrX,chrY"
```

## Whole Exome

If the samples are whole exome samples, a target BED file should be supplied with the *--cnv-target-bed $TARGET_BED* option.

The intervals in target BED file indicate regions where alignments are expected based on the target capture kit. The BED file intervals are further split into intervals of smaller size, depending on the value of *cnv-interval-width*.

To use a standard BED file, make sure that there is no header present in the file. In this case, all columns after the third column are ignored, similar to the operation of DRAGEN Variant Caller.

## Target Counts Options

The following options control the generation of target counts.

- *--cnv-counts-method*—Specifies the counting method for an alignment to be counted in a target bin. Values are midpoint, start, or overlap. The default value is overlap when using the panel of normal approach, which means if an alignment overlaps any part of the target bin, it is counted for that bin. In the self normalization mode, the default counting method is start.

- *--cnv-min-mapq*—Specifies the minimum MAPQ for an alignment to be counted during target counts generation. The default value is 3 for self normalization and 20 otherwise. When generating counts for panel of normals, all MAPQ0 alignments are counted.
- *--cnv-target-bed*—Specifies a properly formatted BED file that indicates the target intervals to sample coverage over. For use in WES analysis.
- *--cnv-interval-width*—Specifies the width of the sampling interval for CNV processing. This option controls the effective window size. The default is 1000 for WGS analysis and 500 for WES analysis.
- *--cnv-skip-contig-list*—Specifies a comma-separated list of contig identifiers to skip when generating intervals for WGS analysis. The default contigs that are skipped, if not specified, are "chrM,MT,m,chrm".

### Target Counts Dropout Regions

In the WGS case where a BED file is not specified for a given reference, the same intervals should be generated each time. The intervals created take into account the mappability of the reference genome using a k-mer uniqueness map created during hashtable generation. A dropout region is a complex region that does not count alignments and results in an interval missing from the analysis. Dropout regions include centromeres, telomeres, and low complexity regions. If there is sufficient signal in the flanking regions, an event can still span these dropout regions, even if alignment counting does not occur in the regions. The event is handled by the segmentation stage.

## GC Bias Correction

GC Biases measure the relationship between GC content and read coverage across a genome. Biases can occur in library prep, capture kits, sequencer differences, and mapping, resulting in difficulties calling CNV events. The Consumable Prefix GC bias correction module attempts to correct these biases.

The GC bias correction module immediately follows the target counts stage and operates on the .target.count file. GC bias correction generates a GC bias corrected version of the file, which has a .target.counts.gc-corrected.gz extension in the file name. The GC bias corrected versions are recommended for any downstream processing when working with WGS data. For WES, if there are enough target regions, then the GC bias corrected counts can also be used.

Typical capture kits have over 200,000 targets spanning the regions of interest. If your BED file has fewer than 200,000 targets, or if the target regions are localized to a specific region in the genome (such that GC bias statistics may be skewed), then GC bias correction should be disabled.

The following options control the GC bias correction module.

- *--cnv-enable-gcbias-correction*—Enable or disable GC bias correction when performing target counts generation. The default is true.
- *--cnv-enable-gcbias-smoothing*—Enable or disable smoothing the GC bias correction across adjacent GC bins with an exponential kernel. The default is true.

- *--cnv-num-gc-bins*—Specifies the number of bins for GC bias correction. Each bin represents the GC content percentage. Allowed values are 10, 20, 25, 50, or 100. The default is 25.

## Normalization

The DRAGEN CNV pipeline supports two normalization algorithms:

- Self Normalization—Estimates the autosomal diploid level from the sample under analysis to determine the baseline level to normalize by. Sex chromosomes and PAR regions are handled based on the sample sex.
- Panel of Normals—A reference-based normalization algorithm that uses additional matched normal samples to determine a baseline level from which to call CNV events. The matched normal samples in this case means it has undergone the same library prep and sequencing workflow as the case sample.

Which algorithm to use depends on the available data and the application. Use the following guidelines to select the mode of normalization.

### Self Normalization

- Whole genome sequencing
- Single sample analysis
- Additional matched samples are not readily available
- Simpler workflow via a single invocation

### Panel of Normals

- Whole genome sequencing
- Whole exome sequencing
- Targeted panels, including somatic panels
- Additional matched samples are available
- Tumor/Matched-Normal analysis
- Non-human samples

### Self Normalization

The DRAGEN CNV pipeline provides the self normalization mode that does not require a reference sample or a panel of normals. Enable this mode by setting *--cnv-enable-self-normalization* to true. This operating mode bypasses the need to run two stages and can save time. It uses the statistics within the case sample itself to determine the baseline from which to make a call.

Because self normalization uses the statistics within the case sample, this mode is not recommended for WES or targeted sequencing analysis due to the potential for insufficient data.

The self normalization mode is the recommended approach for whole-genome sequencing single sample processing. The pipeline continues through to the segmentation and calling stage, producing the final called events.

```
dragen \
-r <HASHTABLE> \
--bam-input <BAM> \
--output-directory <OUTPUT> \
--output-file-prefix <SAMPLE> \
--enable-map-align false \
--enable-cnv true \
--cnv-enable-self-normalization true
```

If you are running from a FASTQ sample, then the default mode of operation is self normalization.

When operating in self normalization mode, the *--cnv-interval-width* option that is used during the target counts stage becomes the effective interval width based on the number of unique k-mer positions. You typically do not have to modify this option.

Self normalization auto-generates the target intervals for use during analysis based on the reference genome and only works for standard human references. Non-standard human references require a BED file to process and the Panel of Normals approach.

## Panel of Normals

The Panel of Normals approach uses a set of matched normal samples to determine the baseline level from which to call CNV events. These matched normal samples should be derived from the same library prep and sequencing workflow that was used for the case sample. This allows the algorithm to subtract out system level biases that are not sample specific.

In this mode of operation, the DRAGEN CNV pipeline is broken down into two distinct stages. The target counts stage is performed on each sample, case, and normals, to bin the alignments. The normalization and call detection stage is then performed with the case sample against the panel of normals to determine the events.

### Target Counts Stage

Target counts should be performed for all your samples, whether they are to be used as references or are the case samples under investigation. The case sample and all samples to be used as a panel of normals sample must have identical intervals and therefore should be generated with identical settings. The target counts stage also performs GC Bias correction, which is enabled by default.

The examples below are for WGS processing. For exome processing, see *Whole Exome* on page 126.

The following is an example command for processing a BAM file.

```
dragen \
-r <HASHTABL> \
--bam-input <BAM> \
--output-directory <OUTPUT> \
--output-file-prefix <SAMPLE. \
--enable-map-align false \
--enable-cnv true
```

The following is an example command for processing a CRAM file.

```
dragen \
-r <HASHTABLE> \
--cram-input <CRAM> \
--output-directory <OUTPUT> \
--output-file-prefix <SAMPLE> \
--enable-map-align false \
--enable-cnv true
```

### Normalization and Call Detection Stage

The next step in the CNV pipeline when using a panel of normals is to perform the normalization and to make the calls. This step involves selecting a panel of normals, which is a list of target counts files to be used for reference-based median normalization.

You can run the analysis in other workflow combinations, keeping in mind that the CNV events are called for the reference samples used. Ideally the panel of normals samples follow library prep and sequencing workflows that are identical to the workflows of the case sample under analysis. For calling on sex chromosomes, it is recommended that you use sex matched references in the panel. Because the normalization is performed on a per-target basis against the panel of normals median, having sex matched references is critical to detecting copy number events on the sex chromosomes.

For optimal bias correction, a minimum of 50 samples is recommended as a panel. DRAGEN can run with a single-sample panel, but single-sample panels can result in artifactual calls in the test sample where the panel sample has copy number changes.

To generate a Panel of Normals (PON), create a plain text file in which each line in the file contains a path pointing to a target.counts.gz file generated from the target counts stage. Relative paths are supported provided the paths are relative to the current working directory. Absolute paths are recommended in case the workflow is used later or shared with other users.

The following is an example PON file, which uses a subset of the GC corrected files from the target counts stage.

```
/data/output_trio1/sample1.target.counts.gc-corrected.gz
/data/output_trio1/sample2.target.counts.gc-corrected.gz
/data/output_trio2/sample4.target.counts.gc-corrected.gz
```

```
/data/output_trio2/sample5.target.counts.gc-corrected.gz
/data/output_trio3/sample7.target.counts.gc-corrected.gz
/data/output_trio3/sample8.target.counts.gc-corrected.gz
....
```

Alternatively, the files to be used in the panel of normals can be specified with the *--cnv-normals-file* option. This option takes a single file name, and can be specified multiple times.

After you have created a PON file, you can run the caller by specifying your case sample with the *--cnv-input* option and the PON file with the *--cnv-normals-list* option. Because we recommend using the GC bias corrected counts from the previous stage, there is no need to run GC bias correction again.
GC bias correction can be disabled by setting *--cnv-enable-gcbias-correction* to false. For example:

```
dragen \
-r <HASHTABLE> \
--output-directory <OUTPUT> \
--output-file-prefix <SAMPLE> \
--enable-map-align false \
--enable-cnv true \
--cnv-input <CASE_COUNTS> \
--cnv-normals-list <NORMALS> \
--cnv-enable-gcbias-correction false
```

This command normalizes the case sample against the panel of normals.

### Panels of Normals for Somatic Analysis

For WGS applications, DRAGEN supports somatic analysis for both Tumor Matched Normal mode as well as Tumor Only mode, including allele specific copy number calling. For more information, see *Somatic CNV Calling* on page 146.

For somatic targeted panels, the use of a panel of normals as the reference baseline can provide insight into copy number variants. The reported events are based solely on the normalized copy ratio values and the deviation from the expected reference baseline levels. This might be sufficient for some applications requiring only the detection of gains and losses in targeted genes.

## Normalization Options

These options control the preconditioning of the panel of normals and the normalization of the case sample.

- *--cnv-enable-self-normalization*—Enable/disable self normalization mode, which does not require a panel of normals.
- *--cnv-extreme-percentile*—Specifies the extreme median percentile value at which to filter out samples. The default is 2.5.

- *--cnv-input*—Specifies a target counts file for the case sample under investigation when using a panel of normals.
- *--cnv-normals-file*—Specifies a target.counts.gz file to be used in the panel of normals. This option can be specified multiple times, once for each file.
- *--cnv-normals-list*—Specifies a text file containing paths to the list of reference target counts files to be used as a panel of normals. Absolute paths are recommended in case the workflow is used later or shared with other users. Relative paths are supported provided the paths are relative to the current working directory.
- *--cnv-max-percent-zero-samples*—Specifies a threshold for filtering out targets with too many zero coverage samples. The default is 5%.
- *--cnv-max-percent-zero-targets*—Specifies a threshold for filtering out samples with too many zero coverage targets. The default is 2.5%.
- *--cnv-target-factor-threshold*—Specifies the bottom percentile of panel-of-normals medians to filter out useable targets. The default is 1% for whole genome processing and 5% for targeted sequencing processing.
- *--cnv-truncate-threshold*—Specifies a percentage threshold for truncating extreme outliers. The default is 0.1%.

## Segmentation

After a case sample has been normalized, it goes through a segmentation stage. There are multiple segmentation algorithms implemented in DRAGEN, including the following:

- CBS (Circular Binary Segmentation)
- SLM (Shifting Level Models)

The SLM algorithm has three variants, SLM, HSLM, and ASLM. HSLM (Heterogeneous SLM) is for use in exome analysis and handles target capture kits that are not equally spaced. ASLM (Adaptive SLM) includes additional sample-specific estimation of technical variability of depth of coverage (as opposed to changes in copy number), based on the median variance within fixed windows or a preliminary set of segments based on b-allele ratios, and can provide more robustness to "noisy" or "wavy" samples.

The default segmentation algorithm in use is SLM for germline whole genome processing, ASLM for somatic whole genome processing, and CBS for whole exome processing.

- *--cnv-segmentation-mode*—Specifies the segmentation algorithm to perform. The default value is determined by the type of analysis. The following are the available default values.

| Analysis | Default |
|---|---|
| Germline WGS | SLM |
| Somatic WGS | ASLM |
| Targeted/WES | HSLM |

- *--cnv-merge-distance*—Specifies the maximum number of base pairs between two segments that would allow them to be merged. The default value is 0 for WGS, which means the segments must be directly adjacent. For WES analysis this parameter is disabled by default due to the spacing of targeted intervals.
- *--cnv-merge-threshold*—Specifies the maximum segment mean difference at which two adjacent segments should be merged. The segment mean is represented as a linear copy ratio value. The default is 0.2 for WGS and 0.4 for WES. To disable merging, set the value to 0.

## Circular Binary Segmentation

Circular Binary Segmentation is implemented directly in DRAGEN and is based on *A faster circular binary segmentation for the analysis of array CGH data* with enhancements to improve sensitivity for NGS data.

The following options control Circular Binary Segmentation.

- *--c-alpha*—Specifies the significance level for the test to accept change points. The default is 0.01.
- *--cnv-cbs-eta*—Specifies the Type I error rate of the sequential boundary for early stopping when using the permutation method. The default is 0.05.
- *--cnv-cbs-kmax*—Specifies maximum width of smaller segment for permutation. The default is 25.
- *--cnv-cbs-min-width*—Specifies the minimum number of markers for a changed segment. The default is 2.
- *--cnv-cbs-nmin*—Specifies the minimum length of data for maximum statistic approximation. The default is 200.
- *--cnv-cbs-nperm*—Specifies the number of permutations used for p-value computation. The default is 10000.
- *--cnv-cbs-trim*—Specifies the proportion of data to be trimmed for variance calculations. The default is 0.025.

## Shifting Level Models Segmentation

The Shifting Level Models (SLM) segmentation mode follows from the R implementation of SLMSuite: a suite of algorithms for segmenting genomic profiles.

- *--cnv-slm-eta*—Baseline probability that the mean process changes its value. The default is 1e-5.
- *--cnv-slm-fw*—Minimum number of data points for a CNV to be emitted. The default is 0, which means segments with one design probe could in effect be emitted.
- *--cnv-slm-omega*—Scaling parameter modulating relative weight between experimental/biological variance. The default is 0.3.
- *--cnv-slm-stepeta*—Distance normalization parameter. The default is 10000. This option is only valid for HSLM.

Regardless of the segmentation method, initial segments are split across large gaps where depth data is unavailable, such as across centromeres.

## Quality Scoring

Quality scores are computed using a probabilistic model that uses a mixture of heavy tailed probability distributions (one per integer copy number) with a weighting for event length. Noise variance is estimated. The output VCF contains a phred-scale metric measuring confidence in called amplification (CN > 2 for diploid locus), deletion (CN < 2 for diploid locus), or copy neutral (CN=2 for diploid locus) events.

The scoring algorithm also calculates exact-copy-number quality scores that are inputs to the DeNovo CNV detection pipeline.

## Blacklist Filtering

You can input blacklist BED to the CNV caller to filter out regions from analysis. This is useful if there are certain regions in the genome, which are known to be problematic. You can also blacklist larger intervals that specify common CNVs to aid in downstream analysis. DRAGEN does not provide a prebuilt blacklist, but you can use the *cnv-blacklist-bed* option to specify the intervals to blacklist. The intervals should be formatted in standard three-column BED format.

The intervals in the blacklist are compared with the original target counts intervals. If the overlap is greater than *cnv-blacklist-min-overlap*, the target counts interval are excluded from analysis. The `*.target.counts.gz` file still includes the interval, so you can inspect the original read counts. The normalization stage removes intervals and is reflected in the `*.tn.tsv.gz` file.

A blacklist interval does not guarantee that a CNV call does not span the interval. If there is sufficient data flanking the region, the segmentation stage along with any merging might still generate a call spanning the blacklisted interval. However, the call would not take read counts from blacklisted intervals into account.

## Output Files

The DRAGEN host software generates many intermediate files. The final call file that contains the amplification and deletion events is the *.seg.called.merged file.

In addition to the segment file, DRAGEN emits the calls in the standard VCF format. By default, the VCF file includes only copy number gain and loss events. For copy neutral segments, refer to the *.seg.called.merged file. To have copy neutral (REF) calls included in the output VCF, set *--cnv-enable-ref-calls* to true.

For more information about the .seg.called.merged file, and how to use the output files to aid in debug and analysis, see *Signal Flow Analysis* on page 120.

### CNV VCF File

The CNV VCF file follows the standard VCF format. Due to the nature of how CNV events are represented versus how structural variants are represented, not all fields are applicable. In general, if more information is available about an event, then it is annotated. Some fields in the DRAGEN CNV VCF are unique to CNVs.

The following is an example of the header lines that are specific to CNV.

```
##fileformat=VCFv4.2
##CoverageUniformity=0.402517
##contig=<ID=1,length=249250621>
##contig=<ID=2,length=243199373>
##contig=<ID=3,length=198022430>
##contig=<ID=4,length=191154276>
##contig=<ID=5,length=180915260>
…
##reference=file:///reference_genomes/Hsapiens/hs37d5/DRAGEN
##ALT=<ID=CNV,Description="Copy number variant region">
##ALT=<ID=DEL,Description="Deletion relative to the reference">
##ALT=<ID=DUP,Description="Region of elevated copy number
relative to the reference">
##INFO=<ID=REFLEN,Number=1,Type=Integer,Description="Number of
REF positions included in this record">
##INFO=<ID=SVLEN,Number=.,Type=Integer,Description="Difference
in length between REF and ALT alleles">
##INFO=<ID=SVTYPE,Number=1,Type=String,Description="Type of
structural variant">
##INFO=<ID=END,Number=1,Type=Integer,Description="End position
of the variant described in this record">
##INFO=<ID=CIPOS,Number=2,Type=Integer,Description="Confidence
interval around POS">
##INFO=<ID=CIEND,Number=2,Type=Integer,Description="Confidence
interval around END">
##FILTER=<ID=cnvQual,Description="CNV with quality below 10">
##FILTER=<ID=cnvCopyRatio,Description="CNV with copy ratio
within +/- 0.2 of 1.0">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=SM,Number=1,Type=Float,Description="Linear copy
ratio of the segment mean">
##FORMAT=<ID=CN,Number=1,Type=Integer,Description="Estimated
```

```
copy number">
##FORMAT=<ID=BC,Number=1,Type=Integer,Description="Number of
bins in the region">
##FORMAT=<ID=PE,Number=2,Type=Integer,Description="Number of
improperly paired end reads at start and stop breakpoints">
```

The ID column is used to represent the event.

The REF column contains an N for all CNV events.

The ALT column specifies the type of CNV event. Because the VCF contains only CNV events, only the DEL or DUP entry is used.

The QUAL column contains an estimated quality score for the CNV event, which is used in hard filtering.

The FILTER column contains PASS if the CNV event passes all filters, otherwise it contains the name of the failed filter.

The INFO column contains information representing the event, mostly identical to the ID column. The REFLEN entry indicates the length of the event. The SVTYPE entry is always CNV. The END entry indicates the end position of the event. The CIPOS and CIEND entries are currently not used.

The FORMAT fields are described in the header.

- GT—Genotype
- SM—Linear copy ratio of the segment mean
- CN—Estimated copy number
- BC—Number of bins in the region
- PE—Number of improperly paired end reads at start and stop breakpoints

Since germline copy number calling determines overall copy number rather than the copy number on each haplotype, the genotype type field contains missing values for diploid regions when CN is greater than or equal to 2. The following are examples of the GT field for various VCF entries:

| Diploid or Haploid? | ALT | FORMAT:CN | FORMAT:GT |
|---|---|---|---|
| Diploid | . | 2 | ./. |
| Diploid | <DUP> | > 2 | ./1 |
| Diploid | <DEL> | 1 | 0/1 |
| Diploid | <DEL> | 0 | 1/1 |
| Haploid | . | 1 | 0 |
| Haploid | <DUP> | > 1 | 1 |
| Haploid | <DEL> | 0 | 1 |

## Coverage Uniformity

The DRAGEN CNV pipeline provides a measure of the quality of the data for a sample. The CNV pipeline assumes that post-normalization target counts are independently and identically distributed (IID). Coverage in most high-quality WGS samples is uniform enough for the CNV caller to produce accurate calls, but some samples violate the IID assumption in a manner and to a degree that leads to unusually high numbers of false positive calls. For such samples, there are local correlations of coverage bias extending across multiple target intervals so that most or all the target counts in a given genomic region will be artifactually elevated (or, alternatively, depressed), and the strength of the bias is sufficient for the region to be identified as a distinct copy number segment and assigned an incorrect copy number. The `CoverageUniformity` metric quantifies the degree of local coverage correlation in the sample to help identify poor-quality samples. CoverageUniformity is present in the VCF header when WGS self-normalization method is selected. This metric is only available for germline samples.

A larger value for this metric means the coverage in a sample is less uniform, which indicates that the sample has more nonrandom noise, and could be considered poor quality. The CoverageUniformity metric depends on factors other than sample quality, such as the *cnv-interval-width* setting and sample mean coverage. DRAGEN recommends using this score to compare the quality of samples from similar mean coverage and the same command line options. Because of this, DRAGEN CNV only provides the metric and does not take any action based on it.

## Visualization and BigWig Files

To perform analysis on a known truth set, you can use the intermediate output files from the pipeline stages. These files can be parsed to aid in fine-tuning options.

All files have a structure similar to a BED file, with an optional header line.

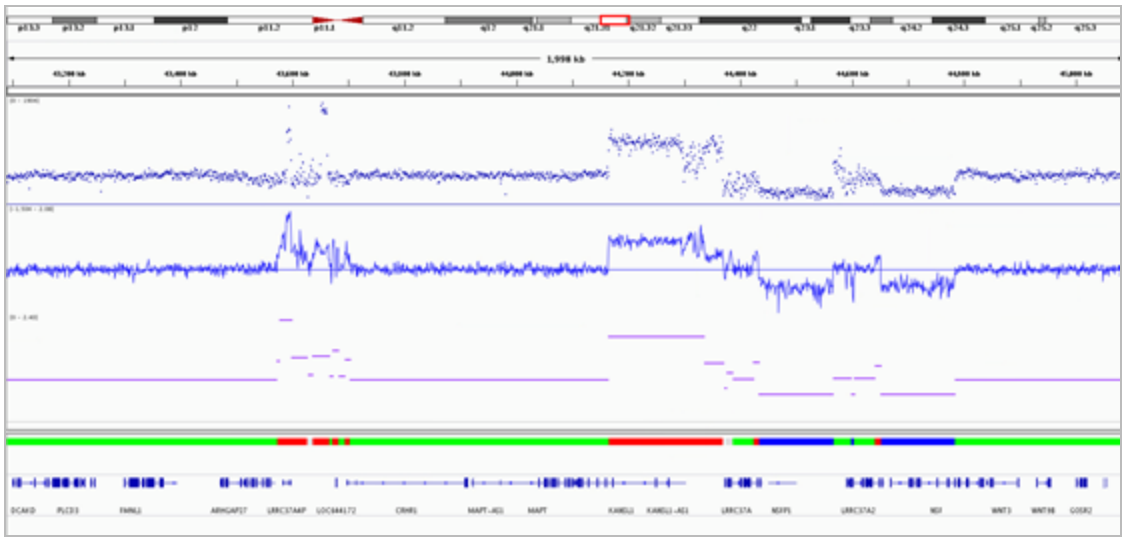
- **.target.counts.gz*—Contains the number of read counts per target interval. This is the raw signal as extracted from the alignments of the BAM or CRAM file. The format is identical for both the case sample and any panel of normals samples. There is also a bigWig representation of a target.counts.diploid file, which is normalized to the normal ploidy level of 2 instead of raw counts.
- **.tn.tsv.gz*—The case sample's tangent normalized signal, per target interval. This file contains the log-normalized copy ratio signal. A strong signal deviation from 0.0 indicates a potential for a CNV event.
- **.seg.called.merged*—Contains the segments produced from the segmentation algorithm.
- **.cnv.vcf*—Output CNV VCF file indicating events.

To generate additional equivalent bigWig and gff files, set the *--enable-cnv-tracks* option to true. These files can be loaded into IGV along with other tracks that are available, such as RefSeq genes. Using these tracks alongside publicly available tracks allows for easier interpretation of calls. DRAGEN autogenerates IGV session XML file if tracks are generated by DRAGEN CNV. The *.cnv.igv_ session.xml can be loaded directly into IGV for analysis.

The following IGV tracks are automatically populated in the output IGV session file:

- *.target.counts.bw*—BigWig representation of the target counts bins. Setting the track view in IGV to barchart or points is recommended.
- **.improper_pairs.bw*—Bigwig representation of the improper pairs counts. Setting the track view in IGV to barchart is recommended.
- **.tn.bw*—Bigwig representation of the tangent normalized signal. Setting the track view in IGV to points is recommended.
- **.seg.bw*—Bigwig representation of the segments. Setting the track view in IGV to points is recommended.
- **.cnv.gff3*—GFF3 representation of the CNV events. DEL events show up as blue and DUP events show as red. Filtered events are a light gray. Selecting an event brings up a window for viewing annotation details.

Figure 9 IGV Example



## Exclude Interval Files

To improve accuracy, the DRAGEN CNV Pipeline excludes genomic intervals if one or more the target intervals failed at least one quality requirement. The excluded intervals are reported to *.excluded_ intervals.bed.gz file. The file identifies the regions of the genome that are not callable for CNV analysis and describes the reason intervals were excluded in the fourth column. The following are the possible reasons for exclusion.

| Exclusion Reason | Description | Command Line Option |
|---|---|---|
| NON_KMER_ UNIQUE | Nonunique Kmer bases are larger than 50% of interval. | Not applicable. This reason only applies to self-normalization mode. |
| BLACKLIST | Interval overlaps with blacklist larger than threshold. | --cnv-blacklist-min-overlap |
| PON_MAX_ PERCENT_ZERO_ SAMPLES | Number of PON samples with 0 coverage is larger than threshold. | --cnv-max-percent-zero-targets |
| PON_TARGET_ FACTOR_ THRESHOLD | Median coverage of interval is lower than threshold of overall median coverage. | --cnv-target-factor-threshold |
| PON_MISSING_ INTERVAL | Target interval not found in PON | Not applicable. |

## Output and Filtering Options

The output and filtering options control the CNV output files.

- *--cnv-blacklist-bed*—Specifies a BED file indicating intervals to exclude from the from CNV analysis. If a target interval overlaps regions specified from blacklist BED file more than*cnv-blacklist-min-overlap*, it is suppressed.
- *--cnv-blacklist-min-overlap*—Specifies a fraction for filtering threshold of overlap amount between target interval and blacklist region (0.5).
- *--cnv-enable-plots*—Generate plots as part of the CNV pipeline. The default is false.
- If you perform WGS CNV analysis with high-resolution intervals (less than 1000 bp), then plot generation can take longer to complete. Illumina recommends that you use the default (disabled).
- *--cnv-enable-ref-calls*—Emit copy neutral (REF) calls in the output VCF file. The default is true for single WGS CNV analysis.
- *--cnv-enable-tracks*—Generate track files that can be imported into IGV for viewing. When this option is enabled, a *.gff file for the output variant calls is generated, as well as *.bw files for the tangent normalized signal. The default is true.
- *--cnv-filter-bin-support-ratio*—Filters out a candidate event if the span of supporting bins is less than the specified ratio with respect to the overall event length. The default ratio is 0.2 (20% support). As an example, if an event is called and has a length of 100,000 bp, but the target interval bins that support the call only spans a total of 15,000 bp (15,000/100,000 = 0.15), then it will be filtered out.

- *--cnv-filter-copy-ratio*—Specifies the minimum copy ratio threshold value centered about 1.0 at which a reported event is marked as PASS in the output VCF file. The default value is 0.2, leading to calls less than CR=0.8 or greater than CR=1.2.
- *--cnv-filter-length*—Specifies the minimum event length in bases at which a reported event is marked as PASS in the output VCF file. The default is 10000.
- *--cnv-filter-qual*—Specifies the QUAL value at which a reported event is marked as PASS in the output VCF file. The default is 10.
- *--cnv-min-qual*—Specifies the minimum reported QUAL. The default is 3.
- *--cnv-max-qual*—Specifies the maximum reported QUAL. The default is 200.
- *--cnv-ploidy*—Specifies the normal ploidy value. This option is used only for estimation of the copy number value emitted in the output VCF file. The default is 2.
- *--cnv-qual-length-scale*—Specifies the bias weighting factor to adjust QUAL estimates for segments with longer lengths. This is an advanced option and should not need to be modified. The default is 0.9303 (2-0.1).
- *--cnv-qual-noise-scale*—Specifies the bias weighting factor to adjust QUAL estimates based on sample variance. This is an advanced option and should not need to be modified. The default is 1.0.

## Concurrent CNV and Small Variant Calling

DRAGEN can perform mapping and aligning of FASTQ samples and then directly stream the data to downstream callers. A single sample can run through both the CNV and the small VC if the input is a FASTQ sample. This triggers self normalization by default.

Run the FASTQ sample through a regular DRAGEN map/align workflow, and then provide additional arguments to either enable the CNV or VC, or both. The options that apply to CNV in the standalone workflows are also applicable here.

The following examples show different commands.

### Map/align FASTQ with CNV

```
dragen \
-r <HASHTABLE> \
-1 <FASTQ1> \
-2 <FASTQ2> \
--RGSM <SAMPLE> \
--RGID <RGID> \
--output-directory <OUTPUT> \
--output-file-prefix <SAMPLE> \
--enable-map-align true \
--enable-cnv true \
--cnv-enable-self-normalization true
```

### Map/Align FASTQ w/ VC

```
dragen \
-r <HASHTABLE> \
-1 <FASTQ1> \
-2 <FASTQ2> \
--RGSM <SAMPLE> \
--RGID <RGID> \
--output-directory <OUTPUT> \
--output-file-prefix <SAMPLE> \
--enable-map-align true \
--enable-variant-caller true
```

### Map/Align FASTQ w/ CNV and VC

```
dragen \
-r <HASHTABLE> \
-1 <FASTQ1> \
-2 <FASTQ2> \
--RGSM <SAMPLE> \
--RGID <RGID> \
--output-directory <OUTPUT> \
--output-file-prefix <SAMPLE> \
--enable-map-align true \
--enable-cnv true \
--cnv-enable-self-normalization true \
--enable-variant-caller true
```

### BAM Input to CNV and VC

```
dragen \
-r <HASHTABLE> \
--bam-input <BAM> \
--output-directory <OUTPUT> \
--output-file-prefix <SAMPLE> \
--enable-map-align false \
--enable-cnv true \
--cnv-enable-self-normalization true \
--enable-variant-caller true
```

### Sample Correlation and Sex Genotyper

When running the target counts stage or the normalization stage, the DRAGEN CNV pipeline also provides the following information about the samples in the run.

- A correlation metric of the read count profile between the case sample and any panel of normals samples. A correlation metric greater than 0.90 is recommended for confident analysis, but there is no hard restriction enforced by the software.
- The predicted sex of each sample in the run. The sex is predicted based on the read count information in the sex chromosomes and the autosomal chromosomes. The median value for the counts is printed to the screen for the autosomal chromosomes, the X chromosome, and the Y chromosome.

The results are printed to the screen when running the pipeline. For example:

```
==========================================
Correlation Table
==========================================
Correlation of case sample PlatinumGenomes_50X_NA12877 against
PlatinumGenomes_50X_NA12878: 0.984092

Sex Genotyper
==========================================
Predicted sex of samples
PlatinumGenomes_50X_NA12877: MALE XY 0.99737
PlatinumGenomes_50X_NA12878: FEMALE XX 0.968929
```

The predicted sexes for samples in use are also printed to the *.cnv_metrics.csvoutput file.

To perform analysis on the sex chromosomes using a panel of normals, it is recommended that you use sex matched samples in the panel of normals.

You can override the sex of the sample with the *–sample-sex* option .

## Multisample CNV Calling

Multisample CNV calling is possible starting from tangent normalized counts files (*.tn.tsv.gz) specified with the *--cnv-input* option (one per sample). Multisample CNV analysis benefits from using joint segmentation to increase the sensitivity of detection of copy number variable segments. For each copy number variable segment identified, the copy number genotype of each sample is emitted in a single VCF entry to facilitate annotation and interpretation.

Multisample CNV calling is recommended for only WGS analysis.

The following is an example command line for running a trio analysis:

```
dragen \
-r <HASHTABLE> \
--output-directory <OUTPUT> \
--output-file-prefix <SAMPLE> \
--enable-cnv true \
--cnv-input <FATHER_TN_TSV> \
--cnv-input <MOTHER_TN_TSV> \
--cnv-input <PROBAND_TN_TSV> \
--pedigree-file <PEDIGREE_FILE>
```

## De Novo CNV Calling Options

All input samples should have gone through the same single sample WGS workflow and must have identical intervals.

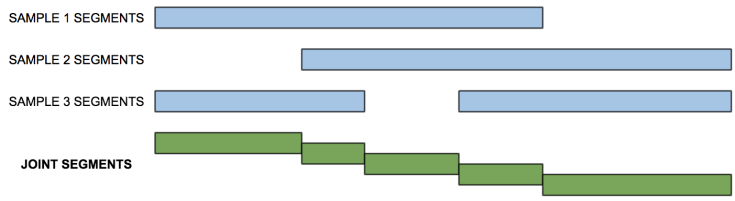
The following options are used in DeNovo CNV calling:

- *--cnv-input*—For DeNovo CNV calling, this specifies the input tangent-normalized signal files (*.tn.tsv) from the single sample runs. This option can be specified multiple times, once for each input sample.
- *--cnv-filter-de-novo-qual*—Phred-scaled threshold at which a putative event in the proband sample is marked as DeNovo. Default value is 0.10.
- *--pedigree-file*—Pedigree file specifying the relationship between the input samples.

## Joint Segmentation

First, CNV calling is performed on each sample independently. Joint segmentation then uses the copy number variable segments from each single sample analysis to derive a set of joint copy number variable segments. This set of joint segments is determined simply by taking the union of all breakpoints from the copy number variable segments of all samples. This results in the splitting of any partially overlapping segments across different samples.

Figure 10 Overlapping Segments




Following joint segmentation, copy number calling is again performed independently on each sample using the joint segments. Segments can be merged as with the single sample analysis, but each joint segment is emitted in the mutlisample VCF as a single entry. The quality score (QS in the VCF) from the sample's merged segment, if applicable, is used for filtering the call. Sample calls are filtered using the sample's FT field in the multisample VCF. The QUAL column of the multisample VCF is always missing (ie, "."). The FILTER column of the mutlisample VCF is "SampleFT" if none of the sample's FT fields are "PASS", and "PASS" if any of the sample's FT fields are "PASS".

## De Novo Calling Stage

A de novo event is defined as the existence of a genotype at a particular locus in a proband's genome that did not result from standard Mendelian inheritance from the parents. The de novo calling stage identifies putative de novo events in the proband of each trio of a multisample analysis. In some cases, these putative de novo events may be real, but they can also arise from sequencing or analysis artifacts. Consequently, a de novo quality score is assigned to each putative de novo event and used to filter out low-quality de novo events. Trios are specified by specifying a .ped file with the *--pedigree-file* option. Multiple trios can be specified (eg, quad analysis), and all valid trios will be processed.

For each joint segment in a trio, the de novo caller determines if there is a Mendelian inheritance conflict for the called copy number genotypes. The CNV caller does not identify the copy number for each allele of a given diploid segment, which means assumptions are made about the possible allelic composition of the parent genotypes.

The assumption is that the copy number 0 allele is not present for diploid regions of a parent's genome (sex dependent) when the assigned copy number is 2 or greater. This results in simplifications, as follows:

| Parent Copy Number Genotype | Possible Copy Number Alleles | Assumed Possible Copy Number Alleles |
|---|---|---|
| 2 | 0/2, 1/1 | 1/1 |
| 3 | 0/3, 1/2 | 1/2 |
| 4 | 0/4, 1/3, 2/2 | 1/3, 2/2 |
| N | x/(N-x) for x <= N/2 | x/(N-x) for 1 <= x <= N/2 |

The following are examples of consistent and inconsistent copy number genotypes for diploid regions using these assumptions:

| Mother Copy Number | Father Copy Number | Proband Copy Number | Mendelian Consistent? |
|---|---|---|---|
| 2 | 2 | 2 | Yes |
| 2 | 2 | 1 | No |
| 3 | 2 | 4 | No |
| 3 | 2 | 2 | Yes |
| 2 | 0 | 2 | No |

If a joint segment has a Mendelian inheritance conflict, a Phred-scaled de novo quality score (DQ field in the VCF) is calculated using the likelihoods for each copy number state (see Quality Scoring section) of each sample in the trio, combined with a prior for the trio genotypes:

```
DQ = -10log(1-Sum over conflicting genotypes(p(CNm|data)*p(CNf|data)*p
(CNp|data)*p(CNm,CNf,CNp))/Sum over all genotypes(p(CNm|data)*p(CNf|data)*p
(CNp|data)*p(CNm,CNf,CNp)))
```

Where

- CNm = Mother copy number
- CNf = Father copy number
- CNp = Proband copy number
- p(CNm,CNf,CNp) = the prior for the trio genotype

The DN field in the VCF is used to indicate the de novo status for each segment. Possible values are:

- Inherited - the called trio genotype is consistent with Mendelian inheritance
- LowDQ - the called trio genotype is inconsistent with Mendelian inheritance and DQ is less than the de novo quality threshold (default 0.1)
- DeNovo - the called trio genotype is inconsistent with Mendelian inheritance and DQ is greater than or equal to the de novo quality threshold (default 0.1)

## Multisample CNV VCF Output

The records in a multisample CNV VCF differ slightly from the single sample case. The major differences are as follows:

- The per-record entries are broken down into the segments among the union of all the input samples breakpoints, which means there are more entries in the overall VCF.
- The QUAL column is not used and its value is ".". The per-sample quality is carried over into the SAMPLE columns with the QS tag.
- The FILTER column indicates PASS if any of the individual SAMPLE columns PASS. Otherwise, it indicates SampleFT.

- The per-sample annotations are carried over from their originating calls. The single sample filters are applied at the sample level and are emitted in the FT annotation.

Additionally, if a valid pedigree is used, then de novo calling is performed, which adds the following two annotations to the proband sample.

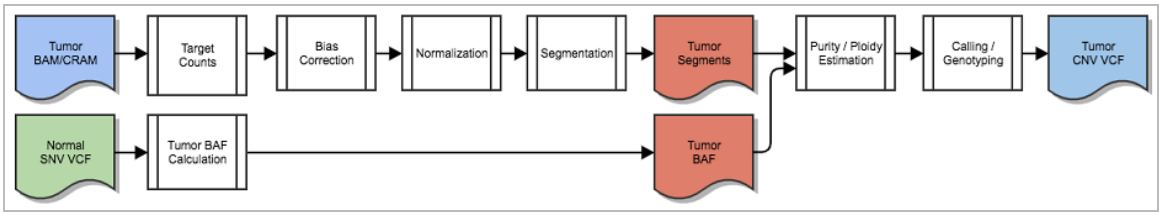
```
##FORMAT=<ID=DQ,Number=1,Type=Float,Description="De novo quality">
##FORMAT=<ID=DN,Number=1,Type=String,Description="Possible values are
'Inherited', 'DeNovo' or 'LowDQ'. Threshold for a passing de novo call is
DQ > 0.100000">
```

While the VCF contains many entries, due to the joint segmentation stage, the number of de novo events can be found by extracting entries that have a DN and DQ annotation. These records are also extracted and are converted to GFF3 in the de novo calling case.

# Somatic CNV Calling

To detect somatic copy number aberrations and regions with loss of heterozygosity, run the DRAGEN CNV caller on a tumor sample in combination with a VCF containing germline SNVs. The output file is a VCF file. Components of the germline CNV caller are reused in the somatic algorithm with the addition of a somatic modeling component, which estimates tumor purity and ploidy.

Figure 11 Somatic CNV Caller Workflow




The germline SNVs are used to compute b-allele ratios in the tumor, which allows for allele-specific copy number calling on the tumor sample. Where possible, use of the small-variant VCF from a matched normal sample is preferred ("tumor/normal" mode), but a catalog of population SNPs can be used when a matched normal sample is not available ("tumor-only" mode).

When a matched normal sample is available, it should first be processed using the germline small variant caller. In this case, only germline-heterozygous SNV sites are used for determining b-allele ratios. If no matched normal is available, population SNP b-allele ratios are computed as for matched normal heterozygous loci, but are treated as variants of unknown germline genotype; possible genotype assignments are statistically integrated to determine allele specific copy number.

In matched normal mode, a VCF containing germline copy number changes for the individual may optionally be input. This ensures that germline CNVs are output as separate segments in the somatic CNV VCF, and annotated with the germline copy number so that it is clear whether there are specifically-somatic copy number changes in the region.

## Somatic CNV Calling Options

- *--tumor-bam-input* or *--tumor-cram-input*
- *--cnv-normal-b-allele-vcf* or *--cnv-population-b-allele-vcf*
- *--sample-sex*
- *--cnv-normal-cnv-vcf*
- *--cnv-use-somatic-vc-vaf*

To trigger the somatic CNV caller, the input alignments must use the tumor equivalent options, such as *--tumor-bam-input* or *--tumor-cram-input*. Support for running from FASTQ input (*--tumor-fastq1*, *--tumor-fastq2*, and *--tumor-fastq-list*) is not available in the somatic CNV caller. In addition, the somatic CNV caller does not support running directly from the mapper/aligner.

Use the *--cnv-normal-b-allele-vcf* option to specify a matched normal SNV VCF or *--cnv-population-b-allele-vcf* to specify a population SNP catalog. For more information on specifying b-allele loci, see *Specify B-Allele Loci* on page 148

If the sample sex is known, use the *--sample-sex* option to specify the sex. If it is not specified, the caller attempts to estimate the sample sex from the tumor alignments.

Use *--cnv-normal-cnv-vcf* to specify germline CNVs from a matched normal sample.

Use *--cnv-use-somatic-vc-vaf* to control whether the variant allele frequencies (VAFs) or somatic SNVs are used to help select the tumor model for the sample. For more information, see *VAF Aware Mode* on page 149

The following is an example command line for running the minimalsomatic CNV caller.

```
dragen \
-r <HASHTABLE> \
--output-directory <OUTPUT> \
--output-file-prefix <SAMPLE> \
--enable-map-align false \
--enable-cnv true \
--tumor-bam-input <TUMOR_BAM> \
--cnv-normal-b-allele-vcf <SNV_NORMAL_VCF> \
--sample-sex <SEX>
```

You can enable additional features when a matched-normal sample is also available. If a matched-normal sample is available, enable germline-aware mode and VAF-aware mode using the following example command-line. For more information on germline-aware mode and VAF-aware mode, see *Germline-aware Mode* on page 149 and *VAF Aware Mode* on page 149.

```
dragen \
-r <HASHTABLE> \
--output-directory <OUTPUT> \
```

```
--output-file-prefix <SAMPLE> \
--enable-map-align false \
--enable-cnv true \
--tumor-bam-input <TUMOR_BAM> \
--cnv-normal-b-allele-vcf <SNV_NORMAL_VCF> \
--enable-vc true \
--bam-input <NORMAL_BAM>
--cnv-normal-cnv-vcf <CNV_NORMAL_VCF> \
--sample-sex <SEX>
```

If you provide an unmatched normal in place of a matched normal, you need to disable CNV calling or run in tumor-only mode. You can force tumor-only mode by omitting *--cnv-normal-cnv-vcf*, specifying *--cnv-population-b-allele-vcf* instead of *--cnv-normal-b-allele-vcf*, and adding *--cnv-use-somatic-vc-vaf=false*.

## Target Counts and B-allele Counts

The target counting stage and its output are the same for the germline CNV calling case. The target intervals with the read counts are output in a *.target.counts file. The b-allele counting occurs in parallel with the read counting phase, and the values are output in a *.baf.bedgraph.gz file. This file can be loaded into IGV along with other bigwig files generated by DRAGEN for visualization.

## Specify B-Allele Loci

Use the *--cnv-normal-b-allele-vcf* option to specify a matched normal SNV VCF. Ideally, this VCF file comes from processing the matched normal sample through the DRAGEN germline small variant caller with filters applied. Typically, this file name has a *.hard-filtered.vcf.gz extension. All records marked as PASS that are determined to be heterozygous in the normal sample are used to measure the b-allele counts of the tumor sample . You can also use equivalent gVCF file (*.hard-filtered.gvcf.gz), but the processing time is significantly longer due to the number of records, most of which are not heterozygous sites. It is recommended that you use the VCF file.

Use the *--cnv-population-b-allele-vcf* option to specify a population SNP VCF. To obtain a population SNP VCF, process an appropriate catalog of population variation, such as from dbSNP, the 1000G project, or other large cohort discovery efforts. Only high-frequency SNPs should be included. For example, include SNPs with minor allele population frequency ≥ 10% to limit run time impact and reduce artifacts. Specify the ALT allele frequency by adding AF=<alt frequency> to the INFO section of each record. Additional INFO fields might be present, but DRAGEN only parses and uses the AF field. Sites specified with *--cnv-population-b-allele-vcf* can be either heterozygous or homozygous in the germline genome from which the tumor genome derives.

The following is an example valid population SNP record:

```
chr1 51479 . T A 1000 PASS AF=0.3253
```

DRAGEN considers the following requirements when parsing records from the b-allele VCF:

- Only simple SNV sites.
- Records must be marked PASS in the FILTER field.
- If there are duplicate records (same CHROM and POS) in the VCF, then the first occurring record is used.

## Germline-aware Mode

To specify germline CNVs from a matched normal sample, use `--cnv-normal-cnv-vcf` . When specified, CNV records marked as `PASS` in the normal sample are used during tumor-sample segmentation to ensure that confident germline CNV boundaries are also boundaries in the somatic output. Segments with germline copy number changes relative to reference ploidy are excluded from somatic model selection.

During somatic copy number calling and scoring, the germline copy number is used to modify the expected depth contribution from the normal contamination fraction of the tumor sample, leading to more accurate assignment of somatic copy number in regions of germline CNV. Finally, somatic CNV VCF entries are annotated with germline copy number (NCN) and the somatic copy number difference relative to germline (SCND) for those segments that have germline CNVs.

## VAF Aware Mode

When both the small variant caller and the CNV caller are enabled in a tumor-matched normal run, the somatic SNV results can affect the estimated purity and ploidy of the tumor sample. The somatic SNV variant allele frequencies (VAFs), as captured by the allele depth values from passing somatic SNVs, reflect the combination of tumor purity, total tumor copy number at a somatic SNV locus, and the number of tumor copies bearing the somatic allele. Clusters of somatic SNVs with similar allele depths inform the tumor model.

Somatic SNV variant allele frequency (VAF) information is especially useful when a tumor has limited copy number variation and/or CNVs are mostly subclonal, as, for instance, in many liquid tumors. Without VAF information, the estimated tumor model may be incorrect or low-confidence, leading either to wrong or filtered calls. VAF information can also help determine the presence or absence of a genome duplication even in clear, clonal CNVs.

To take advantage of VAF signal, you must run somatic CNV calling with small variant calling on tumor and matched-normal read and alignment inputs. For example, you could use the following options: `--enable-vc=true --enable-cnv=true --tumor-bam-input T_BAM --bam-input N_BAM` together. By default, VAF-based modeling is enabled. You can disable VAF-based modeling by setting `--cnv-use-somatic-vc-vaf` to `false`.

## Somatic CNV Model

Selecting a tumor purity and diploid coverage level (ploidy) is a key component of the somatic CNV caller. It attempts to fit the observed data, the read counts and b-allele counts, across all segments in the tumor sample, using a grid-search approach that evaluates many candidate models. A log

likelihood score is emitted for each candidate. These scores are output in the *.cnv.purity.coverage.models.tsv file. The somatic CNV caller chooses the (purity, coverage) pair with the highest log likelihood, and computes several measures of model confidence based on the relative likelihood of the chosen model vs alternative models.

If the confidence in the chosen model is low, then the output VCF marks all records with a lowModelConfidence FILTER.

## Somatic CNV Smoothing

The segmentation stage might produce adjacent or nearby segments that are assigned the same copy number and have similar depth and BAF data. This can result in fragmentation of a region with consistent true copy number into several pieces, which may be undesirable for downstream use of copy number estimates. Additionally, for some uses, it may be preferable to smooth short segments that would be assigned different copy number whether due to a true copy number change or an artifact. To reduce undesirable fragmentation, initial segments may be merged during a post-calling segment smoothing step.

After initial calling, segments shorter than the specified value of `--cnv-filter-length` are deemed negligible. Among the remaining non-negligible segments, successive pairs are evaluated for merging. Two successive segments that are within `--cnv-merge-distance` of one another and have the same CN and MCN assignments are combined, along with any intervening neglibible segments, on a trial basis, into a single segment that is recalled and rescored. If the merged segment receives the same CN and MCN as its constituent non-neglible pieces, with a sufficiently high quality score, the original segments are replaced with the merged segment. The merged segment might be further merged with other initial or merged segments to either side. Merging proceeds until there are not any segment pairs that meet the merging criteria.

## Somatic CNV VCF Output

The somatic CNV VCF file follows the standard VCF format and has the following differences from the germline CNV VCF output.

The following header lines are specific to somatic CNV calling.

- **[ModelSoure]**—The primary basis on which the final tumor model was chosen. The following values are included:
  - **[DEPTH+BAF]**—Depth+BAF signal is used to determine tumor model.
  - **[DEPTH+BAF_DOUBLED]**—The initial depth+BAF model is additionally duplicated based on VAF signal or excess segments at half the expected depth change.
  - **[DEPTH+BAF_DEDUPLICATED]**—The depth+BAF model is deduplicated based on VAF signal or insufficient segments supporting a duplication.
  - **[DEPTH+BAF_WEAK]**—Depth+BAF signal is used to determine (lower-confidence) tumor model.
  - **[VAF]**—VAF signal is used to determine tumor model due to insufficient depth+BAF signal.

  - **[DEGENERATE_DIPLOID]**—Sample is treated as high-purity diploid in absence of adequate signal from depth+BAF and VAF. The diploid coverage is set to lowest value observed in a substantial number of bases in segments with BAF=50%. All VCF records have `lowModelConfidence` added to `FILTER` value.
  - **[SAMPLE_MEDIAN]**—Sample is treated as high-purity diploid in absence of adequate signal from depth+BAF and VAF. Diploid coverage set to sample median. All VCF recordsl have `lowModelConfidence` added to `FILTER` value.
- **EstimatedTumorPurity**—Estimated fraction of cells in the sample due to tumor. The range of this field is [0, 1].
- **DiploidCoverage**—Expected read count for a target bin in a diploid region. The numeric value is unlimited.
- **OverallPloidy**—Length weighted average of tumor copy number for PASS events. The numeric value is unlimited.
- **AlternativeModelDedup**—An alternative to the best model corresponding to one less whole-genome duplication is given as a pair of values (tumor purity, diploid coverage). This may be useful for manual investigation where the best model may involve a spurious genome duplication.
- **AlternativeModelDup**—An alternative to the best model corresponding to one more whole-genome duplication is given as a pair of values (tumor purity, diploid coverage). This may be useful for manual investigation where the best model may have missed a true genome duplication.

The ID column represents the type of event. In addition to representing GAIN, LOSS, and REF events, the CNLOH (copy neutral loss of heterozygosity) and GAINLOH (copy number gain with LOH) entries represent LOH (loss of heterozygosity) events.

The ALT field may have two alleles, such as <DEL>,<DUP>, which allows representation of allele specific copy numbers if they differ in copy number states.

The FILTER field has the following additional applied filters.

- **binCount**—CNV events with a bin count lower than the threshold are filtered.
- **lowModelConfidence**—A low confidence in the model estimate marks all records as non-PASSING.

The FORMAT fields are described in the header section. The following fields are specific to somatic CNV:

- AS—Number of allelic read count sites.
- BC—Number of read count bins.
- CN—Estimated total copy number in tumor fraction of sample.

- CNF—Floating point estimate of tumor copy number.
- CNQ—Exact total copy number Qscore.
- MAF—Maximum a posteriori estimate of minor allele frequency.
- MCN—Estimated minor-haplotype copy number.
- MCNF—Floating point estimate of tumor minor-haplotype copy number.
- MCNQ—Minor copy number Qscore.
- NCN—Normal-sample copy number (present only in germline-aware mode).
- SCND—Difference between CN and GCN (present only in germline-aware mode).
- SD—Best estimate of segment's bias-corrected read count.

### Allele Specific Copy Number Example

By estimating tumor purity, the total tumor copy number can be reported. The BAF estimates, whether from matched normal SNVs or population SNPs, allows for allele specific copy number calling.

The following table provides examples for a DUP in a reference diploid region.

| Total Copy Number | Minor Copy Number | ASCN Scenario |
|---|---|---|
| 4 | 2 | 2+2 |
| 4 | 1 | 3+1 |
| 4 | 0 | 4+0 |

The last entry is a loss of heterozygosity (LOH) case. The total copy number is still considered a DUP, so this is annotated as GAINLOH to distinguish it from copy neutral LOH CNLOH, which would be 2+0.

## Repeat Expansion Detection with Expansion Hunter

Short tandem repeats (STRs) are regions of the genome consisting of repetitions of short DNA segments called repeat units. STRs can expand to lengths beyond the normal range and cause mutations called repeat expansions. Repeat expansions are responsible for many diseases, including Fragile X syndrome, amyotrophic lateral sclerosis, and Huntington's disease.

DRAGEN includes a repeat expansion detection method called ExpansionHunter. ExpansionHunter performs sequence-graph based realignment of reads that originate inside and around each target repeat. It then genotypes the length of the repeat in each allele based on these graph alignments. More information and analysis is available in the following ExpansionHunter papers:

- ExpansionHunter (http://www.genome.org/cgi/doi/10.1101/gr.225672.117)
- Graph ExpansionHunter (https://doi.org/10.1101/572545)

These methods work only for whole human genome samples in PCR-free libraries. Repeats are only genotyped if the coverage at the locus is at least 10x.

## Repeat Expansion Detection Options

To enable DRAGEN repeat expansion detection, the following command-line options are required.

- --repeat-genotype-enable = true
- --repeat-genotype-specs=<path to spec file>

In addition, the sex of the sample should be set using the *--sample-sex* option.

The following options are optional.

- --repeat-genotype-region-extension-length=<length of region around repeat to examine> (default 1000bp)
- --repeat-genotype-min-baseq=<Minimum base quality for 'high confidence' bases> (default 20)

For more information on the spec file specified by *--repeat-genotype-specs* option, see *Repeat Expansion Specification Files* on page 1.

The main output of repeat expansion detection is a VCF file, containing the variants found via this analysis.

## Repeat Expansion Specification Files

The repeat-specification (also called variant catalog) JSON file defines the repeat regions for ExpansionHunter to analyze. Default repeat-specification for some pathogenic repeats are in the `/opt/edico/repeat-specs/_directory` (based on the reference genome used with DRAGEN).

You can create specification files for new repeat regions by using one of the provided specification files as a template. See the ExpansionHunter documentation for details on the format.

`--repeat-genotype-specs` is required for ExpansionHunter. If the option is not provided, DRAGEN will attempt to auto-detect the applicable catalog file from /opt/edico/repeat-specs/ based on the reference provided.

## Repeat Expansion Detection Output Files

## VCF Output File

The results of repeat genotyping are output as a separate VCF file, giving the length of each allele at each callable repeat defined in the repeat-specification catalog file. The name is <outputPrefix>.repeats.vcf (.gz).

The VCF output file begins with the following fields.

Table 4 Core VCF Fields

| Field | Description |
|---|---|
| CHROM | Chromosome identifier |
| POS | Position of the first base before the repeat region in the reference |
| ID | Always . |
| REF | The reference base at position POS |
| ALT | List of repeat alleles in format <STRn> where n is the number of repeat units |
| QUAL | Always . |
| FILTER | LowDepth filter is applied when the overall locus depth is below 10x or the number of reads that span one or both breakends is below 5. |

Table 5 Additional INFO Fields

| Field | Description |
|---|---|
| SVTYPE | Always STR |
| END | Position of the last base of the repeat region in the reference |
| REF | Number of repeat units spanned by the repeat in the reference |
| RL | Reference length in bp |
| RU | Repeat unit in the reference orientation |
| REPID | Repeat id from the repeat-specification file |

Table 6 GENOTYPE (Per Sample) Fields

| Field | Description |
|---|---|
| GT | Genotype |
| SO | Type of reads that support the allele; can be SPANNING, FLANKING, or INREPEAT meaning that the reads span, flank, or are fully contained in the repeat |
| CI | Confidence interval called repeat length of each allele |
| AD_SP | Number of spanning reads consistent with the allele |
| AD_FL | Number of flanking reads consistent with the allele |
| AD_IR | Number of in-repeat reads consistent with the allele |

For example, the following VCF entry describes the state of C9orf72 repeat in a sample with ID LP6005616-DNA_A03.

```
QUAL     FILTER  INFO    FORMAT  LP6005616-DNA_A03
chr9     27573526        .       C       <STR2>,<STR349> .
```

```
PASS
SVTYPE=STR;END=27573544;REF=3;RL=18;RU=GGCCCC;REPID=ALS
GT:SO:CN:CI:AD_SP:AD_FL:AD_IR
1/2:SPANNING/INREPEAT:2/349:2-2/323-376:19/0:3/6:0/459
```

In this example, the first allele spans 2 repeat units while the second allele spans 349 repeat units. The repeat unit is GGCCCC (RU INFO field), so the sequence of the first allele is GGCCCCGGCCCC and the sequence of the second allele is GGCCCC x 349. The repeat spans three repeat units in the reference (REF INFO field).

The length of the short allele was estimated from spanning reads (SPANNING) while the length of the expanded allele was estimated from in-repeat reads (INREPEAT). The confidence interval for the size of the expanded allele is (323,376). There are 19 spanning and 3 flanking reads consistent with the repeat allele of size 2 (that is 19 reads fully contain the repeat of size 2 and 2 flanking reads overlap at most 2 repeat units). Also, there are 6 flanking and 459 in-repeat reads consistent with the repeat allele of size 349.

### Additional Output Files

The sequence-graph alignments of reads in the targeted repeat regions are output in a BAM file. You can use a specialized GraphAlignmentViewer tool (github.com/Illumina/GraphAlignmentViewer/) to visualize the alignments. Programs like Integrative Genomics Viewer (IGV) are not designed for displaying graph-aligned reads and cannot visualize these BAMS.

The BAMs store graph alignments in custom XG tags using the format `<LocusName>,<StartPosition>,<GraphCIGAR>`.

- **LocusName**—A locus identifier that matches the corresponding entry in the repeat expansion specification file.
- **StartPosition**—The starting alignment position of a read on the first graph node.
- **GraphCIGAR**—The alignment of a read against the graph starting from that position. GraphCIGAR consists of a sequence of graph node identifiers and linear CIGARS describing the alignment of the read to each node.

Quality scores in the BAM file are binary. High-scoring bases are assigned a score of 40, and low-scoring bases are assigned a score of 0.

## Spinal Muscular Atrophy Calling

Disruption of all copies of the SMN1 gene in an individual causes spinal muscular atrophy (SMA). SMN1 has a very high identity paralog, SMN2, with differs only in approximately 10 SNVs and small indels. One of these (hg19 chr5:70247773 C->T) affects splicing and largely disrupts the production of functional SMN protein from SMN2. Standard WGS analysis does not produce complete variant calling results for

SMN due to this high-similarity duplication combined with common copy-number variation. However, approximately 95% of SMA cases can be detected by determining the absence of the functional C (SMN1) allele in any copy of SMN.

DRAGEN SMA calling uses sequence-graph realignment to align reads to a single reference representing SMN1 and SMN2. In addition to the standard diploid genotype call, DRAGEN uses a direct statistical test to check for presence of any C allele. If no C allele is detected, the sample is called affected, otherwise unaffected.

SMA calling is only supported for human whole-genome sequencing samples in PCR-free libraries.

### Usage

SMA calling is implemented together with repeat expansion detection. For information on graph-alignment and options, see *Repeat Expansion Detection with Expansion Hunter* on page 152.

SMA calling is enabled, along with repeat expansion detection, by setting the *--repeat-genotype-enable* option to true. To activate SMA calling, the variant specification catalog file must include a description of the targeted SMN1/2 variant. Example files are available in the `/opt/edico/repeat-specs/experimental` folder.

SMN output is included along with any targeted repeats in <outputPrefix>.repeat.vcf. SMN output is represented as a single SNV call at the key (splice-affecting) position in SMN1, with SMA status in custom fields:

Table 7 SMA Result in repeat.vcf Output

| Field | Description |
|---|---|
| VARID | SMN marks the SMN call. |
| GT | Genotype call at this position using a normal (diploid) genotype model. |
| DST | SMA status call: + indicates detected, - indicates undetected, ? indicates undetermined. |
| AD | Total read counts supporting the C and T allele. |
| RPL | Log10 Likelihood ratio between the affected and unaffected models. Positive scores are in favor of unaffected. |

## CYP2D6 Caller

The CYP2D6 caller is capable of genotyping the CYP2D6 gene from whole-genome sequencing (WGS) data and is derived from the method implemented in Cyrius[1]. Due to high sequence similarity with its pseudogene paralog CYP2D7 and a wide variety of common structural variants (SVs), a specialized caller is necessary to resolve variants and identify likely star allele haplotypes.

The CYP2D6 Caller performs the following steps:

1. Determines total CYP2D6 and CYP2D7 copy number from read depth.
2. Determines CYP2D6-derived copy number at CYP2D6/CYP2D7 differentiating sites.

3. Detects SV breakpoints by calculating the changes in CYP2D6-derived copy number along the CYP2D6 gene.
4. Calls small variants in CYP2D6 copies.
5. Identifies star alleles from the detected SV breakpoints and small variants.
6. Identifies the most likely genotype for the called star alleles.

The CYP2D6 Caller requires whole-genome sequencing (WGS) data aligned to a human reference genome with at least 30x coverage.

### Total CYP2D6 and CYP2D7 Copy Number

The first step of CYP2D6 calling is to determine the combined copy number of CYP2D6 and CYP2D7. Reads aligned to regions in either CYP2D6 or CYP2D7 are counted. The counts in each region are corrected for GC-bias, and then normalized to a diploid baseline. The GC-bias correction and normalization factors are determined from read counts in 3000 preselected 2 kb regions across the genome. The combined CYP2D6 and CYP2D7 copy number is then calculated from the average sequencing depth across the CYP2D6 and CYP2D7 regions.

### Differentiating Sites

The CYP2D6-derived copy number is calculated at 117 predefined differentiating sites across the CYP2D6 gene. The differentiating sites are selected at positions with sequence differences in CYP2D6 and CYP2D7 where calling the CYP2D6-derived copy number was most likely to be correct based on sequencing data from the 1000 Genomes Project. The figure below shows the frequency of correctly calling the CYP2D6-derived copy number as a function of the position having a sequence difference between CYP2D6 and CYP2D7. The sequence differences showing calling accuracy > 98% are used as differentiating sites.

For each differentiating site, CYP2D6-specific and CYP2D7-specific alleles are counted in reads mapping to either CYP2D6 or the homologous region in CYP2D7. The CYP2D6-derived copy number is then calculated from this two gene-specific allele counts using the total CYP2D6 and CYP2D7 copy number calculated from the previous step.

### Structural Variant Calling

The CYP2D6-derived copy number along the CYP2D6 gene is used to identify known population structural variants (SVs), including whole gene deletions and duplications as well as certain gene conversions and gene fusions. The following fusion variants are detected:

| Fusion Breakpoint | Hybrid Gene Structure | Associated Star Alleles |
|---|---|---|
| exon 9 | 2D6-2D7 | 4.013, 36, 57, 83 |
| exon 9 | 2D7-2D6 | 13 |

| Fusion Breakpoint | Hybrid Gene Structure | Associated Star Alleles |
|---|---|---|
| intron 4 | 2D7-2D6 | 13 |
| intron 1 | 2D7-2D6 | 13 |
| intron 1 | 2D6-2D7 | 68 |

In addition to the exon 9 fusion breakpoints, exon 9 can participate in CYP2D7 gene conversion resulting in an embedded CYP2D7 sequence instead of a true hybrid. The exon 9 gene conversions are also detected by the structural variant caller. Because only changes in CYP2D6-derived copy number yield structural variant calls, there might be rare cases where two hybrid copies result in no structural variant calls. For example, when both *36 and *13 with fusion breakpoint in exon 9 are present. However, the structural variant caller is capable of detecting multiple copies of the same fusion type (eg, *36x2) or cases where both an exon 9 gene conversion copy and an exon 9 2D6-2D7 hybrid are present.

### Small Variant Calling

118 small variants that define various star alleles are detected from the read alignments. 96 of these variants are in unique (nonhomologous) regions of CYP2D6 with high mapping quality. Only reads mapping to CYP2D6 are used for calling variants in nonhomologous regions. The other 22 variants occur in homologous regions of CYP2D6 where reads mapping to either CYP2D6 or CYP2D7 are used for variant calling.

For each variant, reads containing either the variant allele or the nonvariant alleles are counted. A binomial model that incorporates the sequencing errors is then used to determine the most likely variant copy number (0 for nonvariant). A strand bias filter is applied for certain noisy variants that tend to have false positive calls.

In some cases, when the allele counts are noisy or the total CYP2D6 copy number is greater than five, the most likely variant copy number can be wrong. To handle these cases, the small variant caller also indicates alternate, less likely variant copy numbers.

### Star Allele Identification

The called SVs and small variant genotypes are matched against the definitions of 124 different star alleles. In some cases, different sets of star alleles might match the called variant genotypes. The matching sets are emitted. When the small variant caller indicates alternate variant copy numbers with significant likelihood, then these alternate sets of variant genotypes are also matched to the star allele definitions. The number of matched star alleles must match the number of CYP2D6-derived gene copies determined from previous steps. When there are fewer than two CYP2D6-derived gene copies, then one or more *5 deletion haplotypes are included in the output set of star alleles. If all variant genotypes cannot be matched to a set of star alleles, the CYP2D6 caller returns a no call during the genotyping step with filter value `No_call`.

## Genotyping

Given a possible set of star alleles, the genotyping step attempts to identify the two likely haplotypes that contain all star alleles in the set. The deletion haplotype\(*5) is considered as a possible haplotype during this process. The likelihood of any given genotype is determined from a table of population frequencies determined from the 1000 Genomes Project and the most likely genotype is selected. When two or more possible genotypes are identified with similar likelihoods, then all genotypes are emitted. This results in a call with filter value `More_than_one_possible_genotype`.

## CYP2D6 Output File

The CYP2D6 Caller generates a `<output-file-prefix>.cyp2d6.tsv file` in the output directory. The output file contains the following tab-delimited fields:

- Sample name.
- One or more semicolon-delimited CYP2D6 genotypes or `None` for no call.
- The filter status. The value can include: `PASS`, `No_call`, or `More_than_one_possible_genotype`.

Each CYP2D6 genotype contains two haplotypes separated by a slash (eg *1/*2). Each haplotype consists of one or more star alleles separated by a plus sign (eg *10+*36). When a haplotype contains more than one copy of the same star allele, that star allele only appears once and is followed by a multiplication sign, and then the number of copies (eg *1x2 for two copies of *1).

## Output File Examples

The following are example output files:

```
NA18632 *10+*36x2/*52 PASS
HG01190 *4+*68/*5 PASS
NA17244 *2/*4x2+*13+*83;*2/*4+*4.013+*13+*39 More_than_one_possible_
genotype
NA19908 *1/*46;*43/*45 More_than_one_possible_genotype
NA18611 None No_call
```

## Command-line Examples

To enable the CYP2D6 Caller, `use --enable-cyp2d6=true`. The CYP2D6 Caller is disabled by default. The CYP2D6 Caller can run directly with the mapper or from prealigned BAM/CRAM input. You can also enable the CYP2D6 Caller in parallel with other components as part of a germline analysis workflow.

## FASTQ Input

The following is an example command line with FASTQ input:

```
dragen \
```

```
-r /staging/human/reference/hg38_alt_aware/DRAGEN/8 \
--fastq-file1 /staging/test/data/NA12878_R1.fastq \
--fastq-file2 /staging/test/data/NA12878_R2.fastq \
--output-directory /staging/test/output \
--output-file-prefix NA12878_dragen \
--RGID DRAGEN_RGID \
--RGSM NA12878 \
--enable-map-align=true \
--enable-cyp2d6=true
```

### Prealigned BAM Input

The following is an example command line with prealigned BAM input:

```
dragen \
-r /staging/human/reference/hg38_alt_aware/DRAGEN/8 \
--bam-input /staging/test/data/NA12878.bam \
--output-directory /staging/test/output \
--output-file-prefix NA12878_dragen \
--enable-map-align=false \
--enable-cyp2d6=true
```

# Structural Variant Calling

The DRAGEN Structural Variant (SV) Caller integrates and extends Manta structural variant caller methods to provide structural variant (SV) and indel calls 50 bases or larger. SVs and indels are called from mapped paired-end sequencing reads. The SV caller is optimized for analysis of germline variation in small sets of individuals .

The SV caller performs the following actions:

- Discovers, assembles, and scores large-scale SVs, medium-sized indels, and large insertions within a single efficient workflow.
- Combines paired and split-read evidence during SV discovery and scoring to improve accuracy, but does not require split-reads or successful breakpoint assemblies to report a variant in cases where there is strong evidence otherwise.
- Scores known SV deletions and insertions from an input VCF file against one or more input samples, either as a standalone procedure or together with standard SV discovery.
- Provides scoring models for germline variants in small sets of diploid samples .

All SV and indel inferences are output in VCF 4.1 format.

## DRAGEN SV Caller Overview

The DRAGEN SV Caller divides the SV and indel discovery process into the following two primary steps:

1. Scans the genome to build various genome-wide data structures, including a breakend association graph of all SV associated regions. The graph contains edges connecting all regions of the genome that have a possible breakend association. Edges can connect two different regions of the genome to represent evidence of a long-range association, or an edge may connect a region to itself to capture a local indel/small SV association. These associations are more general than a specific SV hypothesis and many breakend candidates may be found on one edge. Typically only one or two candidates are found per edge.
2. Analyzes breakend association graph to discover candidate SVs, then scores discovered candidate SVs and any known SVs from the input. Analysis and scoring is performed as follows.
   a. Inference of SV candidates associated with the given graph edge.
   b. Attempted assembly of the SVs breakends.
   c. Scoring/genotyping and filtration of the SV under various biological models (currently diploid, germline, and somatic).
   d. Output scored SVs to VCF.

## DRAGEN SV Caller Capabilities

The Consumable Prefix SV Caller can discover all structural variant types that are identifiable in the absence of copy number analysis and large-scale de novo assembly. For more information on detectable types, see *Detected Variant Classes* on page 162.

For each structural variant and indel, the SV Caller attempts to assemble the breakends to base pair resolution and report the left-shifted breakend coordinate (per the VCF 4.1 SV reporting guidelines), together with any breakend homology sequence and/or inserted sequence between the breakends. It is often the case that the assembly will fail to provide a confident explanation of the data. In this case, the variant is reported as IMPRECISE, and scored according to the paired-end read evidence only.

You can provide known SVs to be scored as input. This known SV input can be scored either standalone or together with the standard SV discovery workflow, in which case the known and discovered SVs are merged.

The sequencing reads provided as input to the SV Caller are expected to be from a paired-end sequencing assay that results in an "innie" orientation between the two reads of each sequence fragment, each presenting a read from the outer edge of the fragment insert inward.

The SV Caller is primarily tested for whole-genome and whole-exome (or other targeted enrichment) sequencing assays on DNA. For these assays the following applications are supported:

- Joint analysis of 10 or fewer diploid individuals
- Subtractive analysis of a matched tumor/normal sample pair
- Analysis of an individual tumor sample

For joint analysis, there is no specific restriction against larger cohorts, but this has not been extensively tested so there might be stability or call quality issues.

Tumor samples can be analyzed without a matched normal sample. In this case, no scoring function is available, but the supporting evidence counts are available and many filters can still be usefully applied.

## Detected Variant Classes

The SV Caller can discover all variation classes that can be explained as novel DNA adjacencies in the genome. Novel DNA adjacencies are classified into the following categories based on the breakend pattern:

- Deletions
- Insertions. SV insertions can be divided into the following two subclasses depending on if the inserted sequence can be fully assembled.
  - Fully-assembled insertions
  - Partially-assembled (ie. inferred) insertions
- Tandem Duplications
- Unclassified breakend pairs corresponding to intra- and inter-chromosomal translocations, or complex structural variants.

## Known Limitations

The SV Caller should not be able to directly discover the following variant types:

- Dispersed duplications.
- Most expansion/contraction variants of a reference tandem repeat.
- Breakends corresponding to small inversions.
  - The limiting size is not tested, but in theory detection falls off below ~200 bases. So-called micro-inversions might be detected indirectly as combined insertion/deletion variants.
- Fully-assembled large insertions.
  - The maximum fully-assembled insertion size should correspond to approximately twice the read-pair fragment size, but power to fully assemble the insertion should fall off to impractical levels before this size.
  - The SV Caller does detect and report very large insertions when the breakend signature of such an event is found, even though the inserted sequence cannot be fully assembled.

More general repeat-based limitations exist for all variant types:

- Power to assemble variants to breakend resolution falls to zero as breakend repeat length approaches the read size.
- Power to detect any breakend falls to (nearly) zero as the breakend repeat length approaches the fragment size.

While the SV Caller classifies certain novel DNA-adjacencies into variant classes, it has limited ability to infer high-level events resulting from complex rearrangements, so certain calls summarized as deletions, duplications, and insertions

might be better described by looking at the full system of breakends and copy number changes associated with a given event.

## Forced Genotyping Capability

The DRAGEN SV caller is capable of force genotyping a set of SVs input from a VCF file. Forced genotyping means that the input SVs are scored and emitted in the output of the SV Caller even if the variant is not supported in the sample data. For example, given a germline analysis, the input variants are processed and written to the output VCF, even if the variant quality threshold falls below the normally required for an SV to be emitted.

Forced genotyping typically enables known SVs to be detected at higher recall than standard SV discovery (particularly for SV discovery on a lower-depth sample). Forced genotyping can also be useful to assert against the presence of an SV allele. For example, you can use forced genotyping to distinguish a confident homozygous reference genotype from a lack of sequencing coverage over the SV locus.

Forced genotyping SVs are processed according to the current SV analysis being run. For example if a germline analysis is configured by providing one or more normal samples as input, then the input SVs are scored under a germline model.

Forced genotyping alleles are always emitted in the output and might have modified scoring and filtering rules applied compared to SVs only discovered from the sample data.

### Forced Genotyping Modes

Forced Genotyping can be run in two modes.

- Standalone—Only SVs described in an input VCF are scored and emitted.
- Integrated—The standard SV discovery analysis is run and the results are merged with SVs scored from the forced genotyping input. The workflow outputs the union of SVs discovered from the sample data and any additional forced genotyping alleles. The workflow is run whenever the *--sv-discovery* option is true.

### Forced Genotyping Inputs

You can specify forced genotyping input using the *--sv-forcegt-vcf* option. The input must be a VCF of SV alleles. The SV allele types are restricted to insertions and deletions, which are not labeled with the `VCF IMPRECISE` flag. The following are the filtering criteria required for the VCF record to be processed as an input SV allele. Any of the filtration criteria lead to the VCF record being removed from the set of input SVs for forced genotyping. When a forced genotyping VCF is specified on the command line, the SV caller reports the total number of SV records used as input SVs and the total number of

records filtered (if any) due to the above criteria.

- Describes an insertion or deletion. Deletions can optionally be described using the symbolic `ALT` format (`<DEL>`) when a value is also provided for `INFO/END`. Insertions must have an `ALT` entry describing the complete insertion sequence.
- Cannot contain the `INFO/IMPRECISE` flag.
- Cannot contain multiple ALT alleles.
- Has a `FILTER` value of `PASS` or `.`
- All indels are greater than the minimum scored variant size (default is 50).
- Cannot contain a previous SV allele.
- The `REF` field cannot be empty or unknown (`.`)

### Forced Genotyping Output

Forced genotyping SVs are always output to the standard VCF output of the SV Caller, regardless of whether the forced genotyping is standalone or integrated with SV calling. When the same SV allele is independently discovered from the sample data, only the discovered SV appears in the final output. The discovered SV allele is annotated to indicate the match to a forced genotyping input SV, and the scoring and filtration rules are changed to match.

VCF output records influenced by forced genotyping have the following associated fields.

- The flag `INFO/NotDiscovered` is set for any VCF record that was not independently discovered from the sample data. When forced genotyping is run in standalone, all output records contain the flag. When integrated with SV calling, the flag can distinguish the SV alleles that would not have been discovered in a standard SV analysis.
  - For these variants only, the usual SV caller ID field generated from the SV Locus graph is not available, instead the ID is taken from the corresponding user input VCF. The suffix `UserInput${InputVCFRecordNumber}` is appended to the ID, separated by an underscore.
- Any output VCF record that corresponds to a forced genotyping input VCF record has the value `INFO/UserInputId=${ID}` set to reflect the VCF ID value of the input VCF record. The corresponding record might have also been discovered independently from the sample data and might not have the `INFO/NotDiscovered` flag set.
- Any output VCF record that corresponds to a forced genotyping input VCF record containing forced genotyping alleles that match exactly to an input SV have the flag `INFO/KnownSVScoring`. VCF records with this flag are always emitted in the output of the SV Caller. Several filters, such as MaxDepth, are not applied.

## Input Requirements

When running the SV Caller, the input sequencing reads must be from a standard Illumina paired-end sequencing assay with an FR read pair orientation, where for each sequence fragment, a read proceeds from each end of the fragment inwards.

The SV Caller is optimized for paired end libraries where the fragment size is typically larger than the size of both reads. Overlapping read pairs can be used to discover SVs, but might not always be handled optimally. For libraries where the typical fragment size is less than the read length, the SV caller attempts to differentiate reads sequencing into adapter sequence from variant signal. In such cases, the SV Caller's input quality checks may fail and cause SV analysis to be skipped.

### Input Quality Checks

The SV Caller runs quality checks on the input sequencing reads for each sample to make sure that the input corresponds to a paired read assay with the expected FR orientation, prior to estimating the fragment size distribution. To check consensus read pair orientation, a subset of high quality read pairs is sampled. At least 90% of these must have the expected FR orientation for SV analysis to continue, otherwise the SV caller issues a warning, skips any further analysis, and the resulting output files display empty results.

The SV Caller can tolerate nonpaired reads in the input, if sufficient paired-end reads exist to estimate the fragment size distribution. To estimate the fragment size distribution, the SV Caller requires at least 100 read pairs which meet the quality requirements of the estimation routine. Both reads of the pair must have a non-zero mapping quality to the same chromosome, are not filtered or part of a split read mapping, and do not contain indels or soft-clipping. If a sample does not contain a sufficient number of such read pairs, the SV Caller issues a warning, skips any further analysis, and writes empty results to its output files.

### Read Groups

The SV Caller disregards any read group labels applied to the input sequences. Each input sample is treated as a separate library with a single fragment size distribution.

### File Format

In standalone mode, input sequencing reads must be mapped and provided as input in either BAM or CRAM format. Each input file must be coordinate sorted and indexed to produce an asamtools/htslib-style index in a file named to match the input BAM or CRAM file with an additional '.bai', '.crai' or '.csi' file name extension.

At least one BAM or CRAM file must be provided for the normal or tumor sample. A matched tumor-normal sample pair can be provided as well. If multiple input files are provided for the normal sample, each file is treated as a separate sample as part of a joint diploid sample analysis.

In standalone mode, input BAM or CRAM files contain the following limitations:

- Alignments cannot have an unknown read sequence (SEQ="*")

- Alignments cannot contain the "=" character in the SEQ field.
- Alignments cannot use the sequence match/mismatch ("="/"X"). CIGAR notation RG (read group) tags in the alignment records are ignored. Each alignment file is treated as representing one sample.
- Alignments with base call quality values greater than 70 are rejected. These are not supported on the assumption that this indicates an offset error.

### Exome/Targeted Mode

The SV caller can be configured for targeted sequencing inputs, which disables high depth filters. Exome mode can be directly set to true or false with the command line option *--sv-exome*. If not directly set, exome mode defaults to false unless the you run the SV caller in integrated mode and there is not more than 50 Gb of sequencing input.

## Structural Variant Calling Options

The following command line options are supported for the Structural Variant Caller:

- *--enable-sv*—Enable or disable the structural variant caller. The default is false.
- *--sv-call-regions-bed*—Specifies a BED file containing the set of regions to call. Optionally, you can compress the file in gzip or bgzip format.
- *--sv-region*—Limit the analysis to a specified region of the genome for debugging purposes. This option can be specified multiple times to build a list of regions. The value must be in the format “chr:startPos-endPos”.
- *--sv-exome*—Set to true to configure the variant caller for targeted sequencing inputs, which includes disabling high depth filters. In integrated mode, the default is to autodetect targeted sequencing input, and in standalone mode the default is false.
- *--sv-output-contigs*—Set to true to have assembled contig sequences output in a VCF file. The default is false.
- *--sv-forcegt-vcf*—Specify a VCF of structural variants for forced genotyping. The variants are scored and emitted in the output VCF even if not found in the sample data. The variants are merged with any additional variants discovered directly from the sample data.
- *--sv-discovery*—Flag to enable SV discovery. This flag can be set to false only when *--sv-forcegt-vcf* is used. When set to false, SV discovery is disabled and only the forced genotyping input variants are processed. The default is true.

## Modes of Operation

Structural Variant calling can run in the following modes:

- Standalone—Uses mapped BAM/CRAM input files. This mode requires the following options:
  - *--enable-map-align false*
  - *--enable-sv true*
- Integrated—Automatically runs on the output of the DRAGEN mapper/aligner. This mode requires the following options:
  - *--enable-map-align true*
  - *--enable-sv true*
  - *--enable-map-align-output true*
  - *--output-format bam*

Structural Variant calling can be enabled along with any other caller as well.

The following is an example command line for Integrated mode:

```
dragen -f \
 --ref-dir=<HASH_TABLE> \
--enable-map-align true \
--enable-map-align-output true \
--enable-sv true \
--output-directory <OUT_DIR> \
--output-file-prefix <PREFIX> \
--RGID Illumina_RGID \
--RGSM <sample name> \
-1 <FASTQ1> \
-2 <FASTQ2>
```

The following is an example command line for Joint Diploid calling in standalone mode:

```
dragen -f \
--ref-dir <HASH_TABLE> \
--bam-input <BAM1> \
--bam-input <BAM2> \
--bam-input <BAM3> \
--enable-map-align false \
--enable-sv true \
--output-directory <OUT_DIR> \
--output-file-prefix <PREFIX>
```

## Structural Variant VCF Output

The structural variants VCF output file is available in the output directory. The file is named *<output-file-prefix>.sv.vcf.gz*. The contents of the file depend on the type of analysis.

For each major analysis category (germline, tumor/normal and tumor-only), the appropriate VCF output file is output, reflecting variant calls made under the variant calling mode corresponding to the given analysis type.

The Structural Variant caller produces additional output in the `<output-directory>/sv/` directory. The `<output-directory>/sv/results` folder contains additional variants and statistics output files. Additional subdirectories contain logs and intermediate outputs from the variant calling process.

### Structural Variant Predictions

In `<output-directory>/sv/results/variants`, the SV Caller outputs a set of VCF files. Currently, two VCF files are created for a germline analysis, and an additional somatic VCF is produced for a tumor/normal subtraction. These files are:

- `diploidSV.vcf.gz`

  SVs and indels scored and genotyped under a diploid model for the set of samples in a joint diploid sample analysis or for the normal sample in a tumor/normal subtraction analysis. In the case of a tumor/normal subtraction, the scores in this file do not reflect any information from the tumor sample.

- `somaticSV.vcf.gz`

  SVs and indels scored under a somatic variant model. This file is only produced if a tumor sample alignment file is supplied during configuration

- `candidateSV.vcf.gz`

  Unscored SV and indel candidates. Only a minimal amount of supporting evidence is required for a variant to be entered as a candidate in this file. A variant must be a candidate to be considered for scoring, therefore a variant does not appear in the other VCF outputs if it is not present in the file. The file includes indels of size 8 and larger. The smallest indels are provided for workflow flexibility, but are not scored. Indel scoring starts at size 50.

For tumor-only analysis, the SV Caller produces the following additional output VCF:

- `tumorSV.vcf.gz`

  Subset of the `candidateSV.vcf.gz` file after removing redundant candidates and small indels less than the minimum scored variant size (50). The SVs are not scored, but include the following additional details: (1) paired and split read supporting evidence counts for each allele, (2) a subset of the filters from the scored tumor-normal model are applied to the single tumor case to improve precision.

### VCF Output

VCF output follows the VCF 4.1 specification for describing structural variants. It uses standard field names wherever possible. All custom fields are described in the VCF header. The following sections provide information on the variant representation details and the primary VCF field values.

### VCF Sample Names

Sample names output in the VCF output are extracted from each input alignment file from the first read group (@RG) record found in the header. Any spaces found in the name are replaced with underscores. If no sample name is found, a default (SAMPLE1, SAMPLE2, etc.) label is used instead.

### Small Indel Representation

All variants are reported in the VCF using symbolic alleles unless they are classified as a small indel, in which case full sequences are provided for the VCF REF and ALT allele fields. A variant is classified as a small indel if all of the following criteria are met:

- The variant can be entirely expressed as a combination of inserted and deleted sequence.
- The deletion or insertion length is not 1000 or greater.
- The variant breakends and/or the inserted sequence are not imprecise.

When VCF records are output in the small indel format, they also include the CIGAR INFO tag describing the combined insertion and deletion event.

### Insertions with Incomplete Insert Sequence Assembly

Large insertions are reported in some cases even when the insert sequence cannot be fully assembled. In this case, the SV Caller reports the insertion using the <INS> symbolic allele and includes the special INFO fields LEFT_SVINSSEQ and RIGHT_SVINSSEQ to describe the assembled left and right ends of the insert sequence. The following is an example of such a record from the joint diploid analysis of NA12878, NA12891 and NA12892 mapped to hg19:

```
chr1 11830208 MantaINS:1577:0:0:0:3:0 T <INS> 999 PASS
END=11830208;SVTYPE=INS;CIPOS=0,12;CIEND=0,12;HOMLEN=12;HOMSEQ=TAAATTTTTCT
T;LEFT_
SVINSSEQ=TAAATTTTTCTTTTTTCTTTTTTTTTTAAATTTATTTTTTTATTGATAATTCTTGGGTGTTTCTC
ACAGAGGGGGATTTGGCAGGGTCACGGGACAACAGTGGAGGGAAGGTCAGCAGACAAACAAGTGAACAAAGGTC
TCTGGTTTTCCCAGGCAGAGGACCCTGCGGCCTTCCGCAGTGTTCGTGTCCCTGATTACCTGAGATTAGGGATT
TGTGATGACTCCCAACGAGCATGCTGCCTTCAAGCATCTGTTCAACAAAGCACATCTTGCACTGCCCTTAATTC
ATTTAACCCCGAGTGGACACAGCACATGTTTCAAAGAG;RIGHT_
SVINSSEQ=GGGGCAGAGGCGCTCCCCACATCTCAGATGATGGGCGGCCAGGCAGAGACGCTCCTCACTTCCTA
GATGTGATGGCGGCTGGGAAGAGGCGCTCCTCACTTCCTAGATGGGACGGCGGCCGGGCGGAGACGCTCCTCAC
TTTCCAGACTGGGCAGCCAGGCAGAGGGGCTCCTCACATCCCAGACGATGGGCGGCCAGGCAGAGACACTCCCC
ACTTCCCAGACGGGGTGGCGGCCGGGCAGAGGCTGCAATCTCGGCACTTTGGGAGGCCAAGGCAGGCGGCTGCT
```

```
CCTTGCCCTCGGGCCCCGCGGGGCCCGTCCGCTCCTCCAGCCGCTGCCTCC GT:FT:GQ:PL:PR:SR
0/1:PASS:999:999,0,999:22,24:22,32 0/1:PASS:999:999,0,999:18,25:24,20
0/0:PASS:230:0,180,999:39,0:34,0
```

### Normalizing Small Tandem Duplications

Tandem duplications may also be represented as insertions. This representation creates ambiguity in how such variants are represented in the VCF output, particularly for small tandem duplications, and can lead to complications such as unrecognized call duplication.

To better normalize the SV caller output such that the same variant type is not represented in two different VCF formats, small tandem duplications (< 1000 bases) are converted to insertions in the VCF output. Insertions converted from such tandem duplications have a formatting similar to incomplete insertions, using the symbolic allele <INS> for the ALT field. An example of such an insertion is:

```
chr2 2520057 MantaDUP:TANDEM:53645:0:1:0:0:0 T <INS> 813 PASS
END=2520057;SVTYPE=INS;SVLEN=52;DUPSVLEN=52 GT:FT:GQ:PL:PR:SR
0/1:PASS:393:863,0,390:25,0:19,25
```

Converted insertions include copies of certain output fields as they would have appeared in a tandem duplication record, such as INFO/DUPSVINSSEQ providing a copy of the breakpoint insertion value computed for the duplication. In the context of a duplication such a value would normally be written to INFO/SVINSSEQ. An example of a converted insertion with such a value is:

```
chr2 2645730 MantaDUP:TANDEM:53649:0:1:0:0:0 C <INS> 367 PASS
END=2645730;SVTYPE=INS;SVLEN=97;DUPSVLEN=86;DUPSVINSLEN=11;DUPSVINSSEQ=CTC
ACCTTCAT GT:FT:GQ:PL:PR:SR 0/1:PASS:367:417,0,386:19,0:20,15
```

For more information about copied INFO fields, see *VCF INFO Fields* on page 171.

### Inversions

Inversions are reported as a set of breakends. For example, given a simple reciprocal inversion, four breakends are reported, sharing the same EVENT INFO tag. The following is an example breakend records representing a simple reciprocal inversion:

```
chr1 17124941 MantaBND:1445:0:1:1:3:0:0 T [chr1:234919886[T 999 PASS
SVTYPE=BND;MATEID=MantaBND:1445:0:1:1:3:0:1;CIPOS=0,1;HOMLEN=1;
HOMSEQ=T;INV5;EVENT=MantaBND:1445:0:1:0:0:0:0;JUNCTION_QUAL=254;BND_
DEPTH=107;
MATE_BND_DEPTH=100 GT:FT:GQ:PL:PR:SR 0/1:PASS:999:999,0,999:65,8:15,51
chr1 17124948 MantaBND:1445:0:1:0:0:0:0 T T]chr1:234919824] 999 PASS
SVTYPE=BND;MATEID=MantaBND:1445:0:1:0:0:0:1;INV3;EVENT=MantaBND:1445:0:1:0
:0:0:0;
JUNCTION_QUAL=999;BND_DEPTH=109;MATE_BND_DEPTH=83 GT:FT:GQ:PL:PR:SR
0/1:PASS:999:999,0,999:60,2:0,46
chr1 234919824 MantaBND:1445:0:1:0:0:0:1 G G]chr1:17124948] 999 PASS
```

```
SVTYPE=BND;MATEID=MantaBND:1445:0:1:0:0:0:0;INV3;EVENT=MantaBND:1445:0:1:0
:0:0:0;
JUNCTION_QUAL=999;BND_DEPTH=83;MATE_BND_DEPTH=109 GT:FT:GQ:PL:PR:SR
0/1:PASS:999:999,0,999:60,2:0,46
chr1 234919885 MantaBND:1445:0:1:1:3:0:1 A [chr1:17124942[A 999 PASS
SVTYPE=BND;MATEID=MantaBND:1445:0:1:1:3:0:0;CIPOS=0,1;HOMLEN=1;
HOMSEQ=A;INV5;EVENT=MantaBND:1445:0:1:0:0:0:0;JUNCTION_QUAL=254;BND_
DEPTH=100;
MATE_BND_DEPTH=107 GT:FT:GQ:PL:PR:SR 0/1:PASS:999:999,0,999:65,8:15,51
```

### VCF INFO Fields

| ID | Description |
|---|---|
| IMPRECISE | Flag indicating that the structural variation is imprecise, ie, the exact breakpoint location is not found |
| SVTYPE | Type of structural variant |
| SVLEN | Difference in length between REF and ALT alleles |
| END | End position of the variant described in this record |
| CIPOS | Confidence interval around POS |
| CIEND | Confidence interval around END |
| CIGAR | CIGAR alignment for each alternate indel allele |
| MATEID | ID of mate breakend |
| EVENT | ID of event associated to breakend |
| HOMLEN | Length of base pair identical homology at event breakpoints |
| HOMSEQ | Sequence of base pair identical homology at event breakpoints |
| SVINSLEN | Length of insertion |
| SVINSSEQ | Sequence of insertion |
| LEFT_SVINSSEQ | Known left side of insertion for an insertion of unknown length |
| RIGHT_SVINSSEQ | Known right side of insertion for an insertion of unknown length |
| PAIR_COUNT | Read pairs supporting this variant where both reads are confidently mapped |
| BND_PAIR_COUNT | Confidently mapped reads supporting this variant at this breakend (mapping may not be confident at remote breakend) |
| UPSTREAM_PAIR_COUNT | Confidently mapped reads supporting this variant at the upstream breakend (mapping may not be confident at downstream breakend) |

| ID | Description |
|---|---|
| DOWNSTREAM_PAIR_COUNT | Confidently mapped reads supporting this variant at this downstream breakend (mapping may not be confident at upstream breakend) |
| BND_DEPTH | Read depth at local translocation breakend |
| MATE_BND_DEPTH | Read depth at remote translocation mate breakend |
| JUNCTION_QUAL | If the SV junction is part of an EVENT (ie, a multi-adjacency variant), this field provides the QUAL value for the adjacency in question only |
| SOMATIC | Flag indicating a somatic variant |
| SOMATICSCORE | Somatic variant quality score |
| JUNCTION_SOMATICSCORE | If the SV junction is part of an EVENT (ie, a multi-adjacency variant), this field provides the SOMATICSCORE value for the adjacency in question only |
| CONTIG | Assembled contig sequence, if the variant is not imprecise (with *--outputContig*) |
| DUPSVLEN | Length of duplicated reference sequence |
| DUPHOMLEN | Length of base pair identical homology at event breakpoints excluding duplicated reference sequence |
| DUPHOMSEQ | Sequence of base pair identical homology at event breakpoints excluding duplicated reference sequence |
| DUPSVINSLEN | Length of inserted sequence after duplicated reference sequence |
| DUPSVINSSEQ | Inserted sequence after duplicated reference sequence |
| NotDiscovered | Variant candidate specified by the user and not discovered from input sequencing data |
| UserInputId | Variant ID from user input VCF |
| KnownSVScoring | Variant is associated with a user specified input variant, therefore scoring and filtration criteria are relaxed under a stronger prior assumption of truth |

### VCF FORMAT Fields

| ID | Description |
|---|---|
| GT | Genotype |
| FT | Sample filter, 'PASS' indicates that all filters have passed for this sample |
| GQ | Genotype Quality |

| ID | Description |
|---|---|
| PL | Normalized, Phred-scaled likelihoods for genotypes as defined in the VCF specification |
| PR | Number of spanning read pairs which strongly (Q30) support the REF or ALT alleles |
| SR | Number of split-reads which strongly (Q30) support the REF or ALT alleles |

### VCF FILTER Fields

#### Germline

The following table lists the VCF FILTER fields applied to germline VCF output.

| ID | Level | Description |
|---|---|---|
| MinQUAL | Record | QUAL score is less than 20 (not applied to records with KnownSVScoring flag) |
| MinGQ | Sample | GQ score is less than 15 (filter applied at sample level; not applied to records with KnownSVScoring flag) |
| Ploidy | Record | For DEL & DUP variants, the genotypes of overlapping variants (with similar size) are inconsistent with diploid expectation (not applied to records with KnownSVScoring flag) |
| MaxDepth | Record | Depth is greater than 3x the median chromosome depth near one or both variant breakends (not applied to records with KnownSVScoring flag) |
| MaxMQ0Frac | Record | For a small variant (<1000 bases), the fraction of reads in all samples with MAPQ0 around either breakend exceeds 0.4 (not applied to records with KnownSVScoring flag) |
| NoPairSupport | Record | For variants significantly larger than the paired read fragment size, no paired reads support the alternate allele in any sample (not applied to records with KnownSVScoring flag) |
| SampleFT | Record | No sample passes all the sample-level filters |
| HomRef | Sample | Homozygous reference call |

#### Tumor-Normal Somatic

The following table lists the VCF FILTER fields applied to tumor-normal somatic VCF output.

| ID | Level | Description |
|---|---|---|
| MinSomaticScore | Record | SOMATICSCORE is less than 30 |
| MaxDepth | Record | Normal sample site depth is greater than 3x the median chromosome depth near one or both variant breakends (not applied to records with KnownSVScoring flag) |
| MaxMQ0Frac | Record | For a small variant (< 1000 bases) in the normal sample, the fraction of reads with MAPQ0 around either breakend exceeds 0.4 (not applied to records with KnownSVScoring flag) |

### Tumor-Only

The following table lists the VCF FILTER fields applied to tumor-only VCF output.

| ID | Level | Description |
|---|---|---|
| MaxDepth | Record | Depth is greater than 3x the median chromosome depth near one or both variant breakends (not applied to records with KnownSVScoring flag) |
| MaxMQ0Frac | Record | For a small variant (<1000 bases), the fraction of reads in all samples with MAPQ0 around either breakend exceeds 0.4 (not applied to records with KnownSVScoring flag) |

## Interpretation of VCF Filters

There are two levels of filters: record level (FILTER) and sample level (FORMAT/FT).

Most record-level filters are independent of sample-level filters. However, in a germline analysis, if none of the samples pass all sample-level filters, the SampleFT filter record-level filter is applied.

## Interpretation of INFO/EVENT Field

Some structural variants reported in the VCF, such as translocations, represent a single novel sequence junction in the sample. The INFO/EVENT field indicates that two or more such junctions are hypothesized to occur together as part of a single variant event. All individual variant records belonging to the same event share the same INFO/EVENT string. Note that although such an inference could be applied after SV calling by analyzing the relative distance and orientation of the called variant breakpoints, the SV Caller incorporates this event mechanism into the calling process to increase sensitivity towards such larger-scale events. Given that at least one junction in the event has already passed standard variant candidacy thresholds, sensitivity is improved by lowering the evidence thresholds for additional junctions which occur in a pattern consistent with a multijunction event (such as a reciprocal translocation pair).

Although this mechanism could generalize to events including an arbitrary number of junctions, it is currently limited to two. Thus, at present it is most useful for identifying and improving sensitivity towards reciprocal translocation pairs.

### VCF ID Field

The VCF ID, or identifier, field can be used for annotation, or in the case of BND (breakend) records for translocations, the ID value is used to link breakend mates or partners. The following is an example of a VCF ID field from the SV caller.

```
MantaINS:1577:0:0:0:3:0
```

The value provided in the ID field reflects the SV association graph edge(s) from which the SV or indel was discovered. The value is unique within any single VCF output file produced by the SV Caller, and is used to link associated breakend records using the standard VCF MATEID key.

### Convert SV VCF to BEDPE Format

It can sometimes be convenient to express structural variants in BEDPE format. For such applications we recommend the script vcfToBedpe available from:

```
https://github.com/ctsa/svtools
```

This repository is forked from @hall-lab with modifications to support VCF 4.1 SV format.

BEDPE format greatly reduces structural variant information compared to the SV Caller VCF output. In particular, breakend orientation, breakend homology, and insertion sequence are lost, in addition to the ability to define fields for locus and sample specific information. For this reason, Illumina only recommends BEDPE as a temporary output for applications that require it.

## Statistics Output File

Statistics are provided in the files in `<output-directory>/sv/results/stats`.

- `alignmentStatsSummary.txt`

  Fragment length quantiles for each input alignment file.

- `svLocusGraphStats.tsv`

  Statistics and runtime information pertaining to the SV locus graph.

- `svCandidateGenerationStats.tsv`

  Statistics and runtime information pertaining to the SV candidate generation.

- `svCandidateGenerationStats.xml`

  XML data backing the svCandidateGenerationStats.tsv report.

- `diploidSV.sv_metrics.csv`

  The number of passing SV calls under a diploid model. This file is only produced in the germline analysis, or tumor/normal analysis.

- `somaticSV.sv_metrics.csv`

  The number of passing SV calls under a somatic variant model. This file is only produced in the tumor/normal analysis.

- `tumorSV.sv_metrics.csv`

  The number of passing SV calls in the tumor-only analysis. This file is only produced in the tumor-only analysis.

## Structural Variant De Novo Quality Scoring

De novo quality scoring can be enabled for structural variant joint diploid calling, by setting *--sv-denovo-scoring* to true and supplying a pedigree file. This adds FORMAT/DQand FORMAT/DN fields to the output VCF file to represent a De Novo Quality Score and an associated De Novo call.

The following example shows a command line for enabling the de novo quality scoring for a joint diploid run.

```
dragen -f
--ref-dir <HASH_TABLE> \
--bam-input <BAM1> \
--bam-input <BAM2> \
--bam-input <BAM3> \
--enable-map align=false \
--enable-sv=true \
--output-directory <OUT_DIR> \
--output-file-prefix <PREFIX> \
--sv-denovo-scoring true \
--RGID DRAGEN_RGID \
--RGSM <sample name>
--pedigree-file <PED_FILE>
```

Consumable Prefix can also be run on an existing Structural Variant output VCF containing multiple samples (ie, a Trio with a Proband and Parents) to generate a modified VCF file that contains FORMAT/DQ and FORMAT/DN fields (the original file is not changed).

The following example shows a command line for deriving the de novo quality score from an existing SV trio.

```
dragen -f \
--variant <TRIO_VCF_FILE> \
--pedigree-file <PED_FILE> \
--enable-map-align false \
--sv-denovo-scoring true \
--output-directory <OUT_DIR> \
--output-file-prefix <PREFIX>
```

The DQ field is defined as follows:

```
##FORMAT=<ID=DQ,Number=1,Type=Float,Description="Denovo quality">
```

The DQ field represents a score of the posterior probability of the variant being denovo in the proband. If it can be calculated, the score in Phred scale is added to the proband, while the other samples are marked with a period ( . ) to indicate missing.

For example, DQ scores of 13 and 20 would correspond to a posterior probability of a de novo variant of 0.95 and 0.99, respectively.

The DN Field is defines as follows:

```
##FORMAT=<ID=DN,Number=1,Type=String,Description="Possible values are
'DeNovo' or 'LowDQ'. Threshold for a passing de novo call is DQ >= 20">
```

DRAGEN compares valid (> 0) DQ scores with a threshold with default score of 20. A score greater than or equal to the threshold results in DN field of the sample set to DeNovo, while a score below the threshold is declared to be LowDQ. If there is not a valid DQ score (ie, DQ equals “0” or “.”,) the DN field is set to “.”

The threshold can be changed by using the *--sv-denovo-threshold* command line option. For example, if the threshold needs to be reduced to 10, add *--sv-denovo-threshold 10* to the DRAGEN command line.

The inputs to this function are the VCF file and the Pedigree file that specifies which sample in the trio is the proband, mother, or father. In the scenario where there are multiple trios specified in the pedigree file (eg, multi-generation pedigree), DRAGEN automatically detects the trios and assesses the DeNovo variants on the proband sample of each trio.

# Ploidy Calling

## Ploidy Estimator

The Ploidy Estimator runs by default. The Ploidy Estimator uses reads from the mapper/aligner to calculate the sequencing depth of coverage for each autosome and allosome in the human genome. The sex karyotype of the sample is then estimated using the ratios of the median sex chromosome coverages to the median autosomal coverage. The sex karyotype is estimated based on the range the ratios fall in. If the ratios are outside all expected ranges ,then the Ploidy Estimator does not determine a sex karyotype.

| Sex Karyotype | X Ratio Min | X Ratio Max | Y Ratio Min | Y Ratio Max |
|---|---|---|---|---|
| XX | 0.75 | 1.25 | 0.00 | 0.25 |
| XY | 0.25 | 0.75 | 0.25 | 0.75 |
| XXY | 0.75 | 1.25 | 0.25 | 0.75 |

| Sex Karyotype | X Ratio Min | X Ratio Max | Y Ratio Min | Y Ratio Max |
|---|---|---|---|---|
| XYY | 0.25 | 0.75 | 0.75 | 1.25 |
| X0 | 0.25 | 0.75 | 0.00 | 0.25 |
| XXXY | 1.25 | 1.75 | 0.25 | 0.75 |
| XXX | 1.25 | 1.75 | 0.00 | 0.25 |

Ploidy estimation can fail if the type of input sequencing data cannot be determined or if there is not sufficient sequencing coverage in the autosomes. When ploidy estimation fails the estimated median coverage values will be zero.

When both tumor and matched normal reads are provided as input, the Ploidy Estimator only estimates sequencing coverage and sex karyotype for the matched normal sample and ignores the tumor reads. If only tumor reads are provided as input, the Ploidy Estimator estimates sequencing coverage and sex karyotype for the tumor sample.

## Ploidy Estimator Reference Sex Karyotype

The estimated sex karyotype (if determined), is made available to downstream components (eg variant callers). For components that support using the estimated sex karyotype, refer to the corresponding section for more information. To overwrite the sex karyotype used by downstream components, use the `--sample-sex` command line option. If the value specified is `male`, then the sex karyotype is `XY`. If the value specified is `female`, then the sex karyotype is `XX`. The sex karyotype, whether provided via the command line or estimated, is converted to a reference sex karyotype for use as a baseline in variant calling.

The following table provides a summary of reference sex karyotypes and variant callers

| Sex Karyotype | CNV Caller | ExpansionHunter | Ploidy Caller | Small Variant Caller | SV Caller |
|---|---|---|---|---|---|
| XX | XX | XX | XX | XXYY | XXYY |
| XY | XY | XY | XY | XY | XXYY |
| XXY | XY | XX | XY | XXYY | XXYY |
| XYY | XY | XX | XY | XXYY | XXYY |
| X0 | XX | XX | XX | XXYY | XXYY |
| XXXY | XY | XX | XY | XXYY | XXYY |
| XXX | XX | XX | XX | XXYY | XXYY |
| Undetermined | XX | XX | XX | XXYY | XXYY |

- The Copy Number Variant Caller utilizes its own estimation module that is largely consistent with the Ploidy Estimator. Any differences can be attributed to the read counting implementation.
- The Small Variant Caller marks chrY calls with the PloidyConflict filter when the sex karyotype is XX.
- The Structural Variant Caller always treats the sex chromosomes as diploid and does not take into account the sex karyotype.

### Ploidy Estimator Output Metrics

The Ploidy Estimator results, including each normalized per-contig median coverage, is reported in the `<output-file-prefix>.ploidy_estimation_metrics.csv` file and in standard output.

The following is an example of the results.

```
PLOIDY ESTIMATION Autosomal median coverage 44.79
PLOIDY ESTIMATION X median coverage 42.47
PLOIDY ESTIMATION Y median coverage 20.82
PLOIDY ESTIMATION 1 median / Autosomal median 0.95
PLOIDY ESTIMATION 2 median / Autosomal median 1.05
PLOIDY ESTIMATION 3 median / Autosomal median 1.01
PLOIDY ESTIMATION 4 median / Autosomal median 0.99
...
PLOIDY ESTIMATION 22 median / Autosomal median 0.99
PLOIDY ESTIMATION X median / Autosomal median 0.95
PLOIDY ESTIMATION Y median / Autosomal median 0.46
PLOIDY ESTIMATION Ploidy estimation XXY
```

## Ploidy Caller

The Ploidy Caller uses the per contig median coverage values from the Ploidy Estimator to detect aneuploidy and chromosomal mosaicism in a human germline sample from whole genome sequencing data.

The Ploidy Caller runs by default except in the following circumstances:

- The Ploidy Estimator cannot determine if the input data is from whole genome sequencing. For example, data from exome or targeted sequencing.
- The reference genome does not contain the expected 22 autosomes and two allosomes for humans.
- There is no germline sample. For example, tumor-only analysis.

### Calling Model

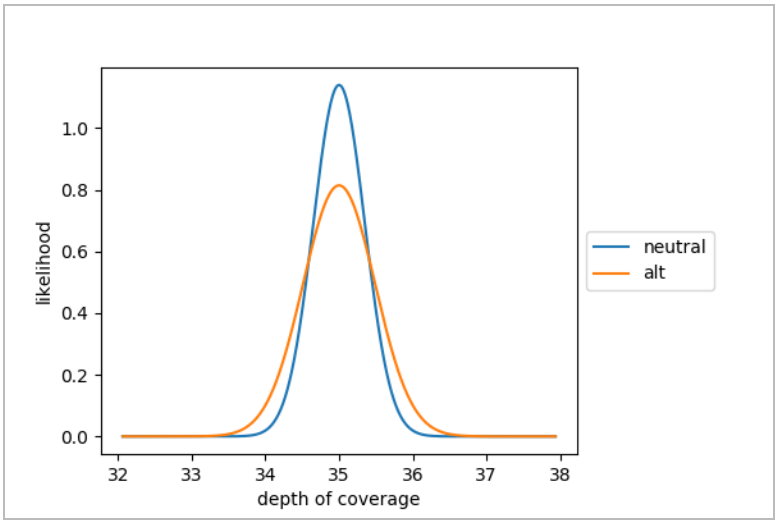
Chromosomal mosaicism is detected when there is a significant shift in median coverage of a chromosome compared to the overall autosomal median coverage.

The following table displays some examples of expected shifts in coverage for a give aneuploidy and mosaic fraction.

| Neutral Copy Number | Variant Copy Number | Mosaic Fraction | Expected Coverage Shift |
|---|---|---|---|
| 2 | 1 | 10 % | -5% |
| 2 | 1 | 5 % | -2.5% |
| 2 | 3 | 5 % | +2.5% |
| 2 | 3 | 10 % | +5% |

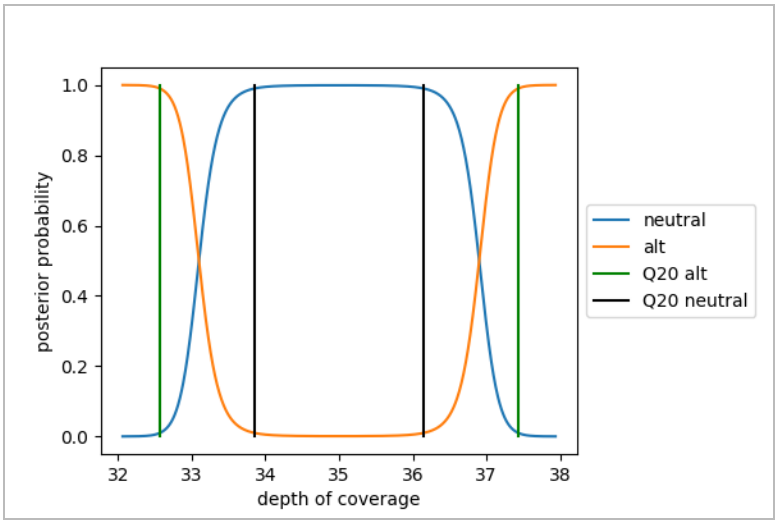
The Ploidy Caller models coverage as a normal distribution for both the null (neutral) and the alternative (mosaic) hypotheses. The two normal distributions have equal mean at the median autosomal coverage for the sample, but the variance of the alternative normal distribution is greater than that of the null normal distribution. The baseline variance of the two models at 30x coverage was determined empirically from a cohort of ~2500 WGS samples. The actual variance used for the two models is calculated from the baseline variance at 30x coverage, adjusting for the median autosomal coverage of the sample. Below are the likelihood distributions for the null and alternative hypotheses for a sample with 35x median autosomal coverage.

Figure 12 Null and Alternative Hypotheses Likelihood Distributions




After apply an empirically estimated prior for chromosomal mosaicism, the Ploidy Caller generates ploidy calls according to the posterior probability of null and alternative hypotheses as shown below for a sample with 35x median autosomal sequencing coverage.

Figure 13 Null and Alternative Hypotheses Posterior Probability




A 35x median autosomal coverage, the threshold for deciding between a neutral (REF) and an alternative (DEL or DUP) call is roughly at +/- 5% shift in coverage for an autosome. A 100x median autosomal coverage, the threshold is at roughly +/0 3% shift in coverage for an autosome. A Q20 threshold is used to filter low quality calls.

## Ploidy Caller Reference Sex Karyotype

In addition to detecting aneuploidy and chromosomal mosaicism in autosomes where the expected reference ploidy is two, the Ploidy Caller can also detect these variants in allosomes.

The reference sex karyotype used for making calls on the allosomes is determined from the sex karyotype of the sample either provided on the command line using `--sample-sex` or from the Ploidy Estimator. If the sex karyotype of the sample is not provided on the command line and not determined by the Ploidy Estimator, then the sex karyotype is assumed to be XX. Whenever the sex karyotype contains at least one Y chromosome, the reference sex karyotpye is XY. If the sex karyotype does not contain at least one Y chromosome, then the sex karyotype is XX.

The following table displays each of the possible sex karyotypes for a sample. If the Y chromosome reference ploidy is zero, then ploidy calling is not performed on the Y chromosome.

| Sex Karyotype | X Reference Ploidy | Y Reference Ploidy |
|---|---|---|
| XX | 2 | 0 |
| XY | 1 | 1 |
| XXY | 1 | 1 |
| XYY | 1 | 1 |

| Sex Karyotype | X Reference Ploidy | Y Reference Ploidy |
|---|---|---|
| X0 | 2 | 0 |
| XXXY | 1 | 1 |
| XXX | 2 | 0 |

## Ploidy Caller Output File

The Ploidy Caller generates a <output-file-prefix>.ploidy.vcf.gz output file in the output directory. The output file follows the VCF 4.2 Specification. A single record is reported for each reference autosome and allosome, except for the Y chromosome if the reference sex karotype is XX. Calls are not made for other sequences in the reference genome, such as mitochondrial DNA, unlocalized or unplaced sequences, alternate contigs, decoy contigs, or the Epstein-Barr virus sequence.

The following information is provided in the VCF file.

- **Meta information**—The VCF output file contains common meta-information such as `DRAGENVersion` and `DRAGEN CommandLine`, as well as Ploidy Caller specific information. The VCF header contains the meta-information for median autosome depth of coverage, the provided sex karyotype if available, the estimated sex karyotype from the Ploidy Estimator if available, and the reference sex karyotype. The following is an example of the header lines:

  ```
  ##autosomeDepthOfCoverage=36.635
  ##providedSexKaryotype=XY
  ##estimatedSexKaryotype=X0
  ##referenceSexKaryotype=XY
  ```

- **FILTER fields**—The VCF output file includes the LowQual filter, which filters results with quality score below 20.
- **INFO Fields**—The VCF output INFO fields include the following:
  - END—End position of the variant described in this record.
  - SVTYPE—Type of structural variant.
- **Format fields**—The VCF output file includes the following format fields. There is no `GT` FORMAT field. A variant call in the VCF displays either `<DUP>` or `<DEL>` in the ALT column. A non-variant call displays . in the ALT column. If using the output file for downstream use, a GT field can be added for variant calls using ./1 for a diploid contig and `1` for a haploid contig. For non-variant calls, use `0/0` for diploid and `0` for haploid.
  - DC—Depth of coverage.
  - NDC—Normalized depth of coverage.

The following is an example output file.

```
##fileformat=VCFv4.2
...
##autosomeDepthOfCoverage=36.635
##providedSexKaryotype=XY
##estimatedSexKaryotype=X0
##referenceSexKaryotype=XY
##INFO=<ID=END,Number=1,Type=Integer,Description="End position of the
variant described in this record">
##INFO=<ID=SVTYPE,Number=1,Type=String,Description="Type of structural
variant">
##ALT=<ID=DEL,Description="Deletion relative to the reference">
##ALT=<ID=DUP,Description="Region of elevated copy number relative to the
reference">
##FILTER=<ID=LowQual,Description="QUAL below 20">
##FORMAT=<ID=DC,Number=1,Type=Float,Description="Depth of coverage">
##FORMAT=<ID=NDC,Number=1,Type=Float,Description="Normalized depth of
coverage">
#CHROM POS ID REF ALT QUAL FILTER INFO FORMAT MySampleName
chr1 1 . N . 31.1252 PASS END=248956422 DC:NDC 36.836:1.00549
chr2 1 . N . 31.451 PASS END=242193529 DC:NDC 36.668:1.0009
...
chr21 1 . N . 31.4499 PASS END=46709983 DC:NDC 36.6:0.999045
chr22 1 . N . 28.8148 PASS END=50818468 DC:NDC 37.2:1.01542
chrX 1 . N . 29.7892 PASS END=156040895 DC:NDC 18:0.982667
chrY 1 . N <DEL> 150 PASS END=57227415;SVTYPE=DEL DC:NDC 5.7:0.311178
```

### Cell Line Artifacts

Samples derived from cell lines frequently have coverage artifacts that might result in variant ploidy calls on some chromosomes. Chromosomes 17, 19, and 22 are the most common for the cell line coverage artifacts. When performing accuracy assessments of ploidy calls on cell line samples, filter out chromosomes with known cell line artifacts.

## QC Metrics and Coverage/Callability Reports

DRAGEN generates pipeline-specific metrics coverage reports during each run. There are four different groups of metrics that are generated at different stages of the pipeline:

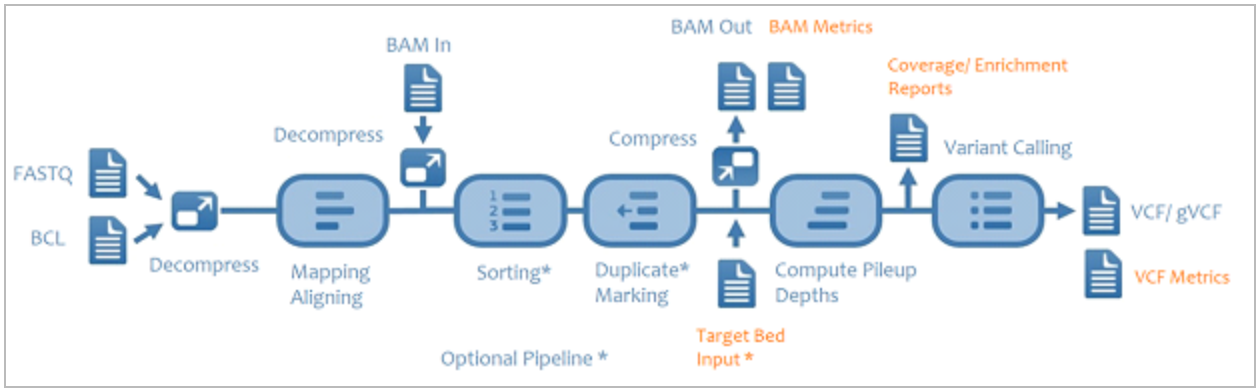
- Mapping and Aligning metrics
- VCF metrics
- Duration (or run time) metrics

- Coverage (or enrichment) metrics and reports

The mapping/aligning metrics, VCF metrics, Duration metrics, and a subset of available coverage reports are autogenerated and do not require any activation or specific commands. Additional coverage metrics can be enabled, and additional coverage regions can be specified.

Metric calculation is performed during analysis so that it does not impact the DRAGEN run time.

Figure 14 Generation of Metrics and Reports




## QC Metrics Output Format

The QC metrics are printed to the standard out humanand CSV files are written to the run output directory.

- <output prefix>.mapping_metrics.csv
- <output prefix>.vc_metrics.csv
- <output prefix>.time_metrics.csv
- <output prefix>.<coverage region prefix>_coverage_metrics.csv
- <output prefix>.<other coverage reports>.csv

| Section | RG/Sample | Metric | Count/Ration/Time | Percentage/Seconds |
|---|---|---|---|---|
| MAPPING/ALIGNING SUMMARY | | Total input reads | 816360354 | |
| MAPPING/ALIGNING SUMMARY | | Number of duplicate reads (marked not removed) | 15779031 | 1.93 |

...

| Section | RG/Sample | Metric | Count/Ration/Time | Percentage/Seconds |
|---|---|---|---|---|
| MAPPING/ALIGNING PER RG | RGID_1 | Total reads in RG | 816360354 | 100 |
| MAPPING/ALIGNING PER RG | RGID_1 | Number of duplicate reads (marked) | 15779031 | 1.93 |
| ... | | | | |
| VARIANT CALLER SUMMARY | | Number of samples | 1 | |
| VARIANT CALLER SUMMARY | | Reads Processed | 738031938 | |
| ... | | | | |
| VARIANT CALLER PREFILTER | SAMPLE_1 | Total | 4918287 | 100 |
| VARIANT CALLER PREFILTER | SAMPLE_1 | Biallelic | 4856654 | 98.75 |
| ... | | | | |
| RUN TIME | | Time loading reference | 00:18.6 | 18.65 |
| RUN TIME | | Time aligning reads | 19:24.4 | 1164.42 |

## Mapping and Aligning Metrics

Mapping and aligning metricslike the metrics computed are available on an aggregate level (over all input data), and on a per read group level. Unless explicitly stated, the metrics units are in reads (ie, not in terms of pairs or alignments).

- **Total input reads**—Total number of reads in the input FASTQ files.
- **Number of duplicate marked reads**—Reads marked as duplicates as a result of the *--enable-duplicate-marking* option being used.
- **Number of duplicate marked and mate reads removed**—Reads marked as duplicates, along with any mate reads, that are removed when the *--remove-duplicates* option is used.
- **Number of unique reads**—Total number of reads minus the duplicate marked reads.

- **Reads with mate sequenced**—Number of reads with a mate.
- **Reads without mate sequenced**—Total number of reads minus number of reads with mate sequenced.
- **QC-failed reads**—Reads not passing platform/ vendor quality checks (SAM flag 0x200).
- **Mapped reads**—Total number of mapped reads minus number of unmapped reads
- **Number of unique and mapped reads**—Number of mapped reads minus number of duplicate marked reads.
- **Unmapped reads**—Total number of reads that could not be mapped.
- **Singleton reads**—Number of reads where the read could be mapped, but the paired mate could not be read.
- **Paired reads**—Count of reads in which both reads in the pair are mapped.
- **Properly paired reads**—Both reads in the pair are mapped and fall within an acceptable range from each other based on the estimated insert length distribution.
- **Not properly paired reads (discordant)**—The number of paired reads minus the number of properly paired reads.
- **Paired reads mapped to different chromosomes**—The number of reads with a mate, where the mate was mapped to a different chromosome
- **Paired reads mapped to different chromosomes (MAPQ >= 10)**—The number of reads with a MAPQ>10 and with a mate, where the mate was mapped to a different chromosome
- **Reads with indel R1**—The percentage of R1 reads containing at least 1 indel.
- **Reads with indel R2**—The percentage of R2 reads containing at least 1 indel.
- **Soft-clipped bases R1**—The percentage of bases in R1 reads that are soft clipped.
- **Soft-clipped bases R2**—The percentage of bases in R2 reads that are soft clipped.
- **Mismatched bases R1**—The number of mismatched bases on R1, which is the sum of SNP count and indel lengths. It does not count anything within soft clipping, or RNA introns. It also does not count a mismatch if either the reference base or read base is N.
- **Mismatched bases R2**—The number of mismatched bases on R2, which is the sum of SNP count and indel lengths. It does not count anything within soft clipping, or RNA introns. It also does not count a mismatch if either the reference base or read base is N.
- **Mismatched bases R1 (excluding indels)**—The number of mismatched bases on R1. The indels lengths are ignored. It does not count anything within soft clipping, or RNA introns. It also does not count a mismatch if either the reference base or read base is N.
- **Mismatched bases R2 (excluding indels)**—The number of mismatched bases on R2. The indels lengths are ignored. It does not count anything within soft clipping, or RNA introns. It also does not count a mismatch if either the reference base or read base is N.

- **Q30 Bases**—The total number of bases with a BQ ≥ 30.
- **Q30 Bases R1**—The total number of bases on R1 with a BQ ≥ 30.
- **Q30 Bases R2**—The total number of bases on R2 with a BQ >= 30.
- **Q30 Bases (excluding dups & clipped bases)**—The number of bases on non-duplicate, and non-clipped bases with a BQ ≥ 30.
- **Histogram of reads map qualities**
  - Reads with MAPQ [40:inf)
  - Reads with MAPQ [30:40)
  - Reads with MAPQ [20:30)
  - Reads with MAPQ [10:20)
  - Reads with MAPQ [0:10)
- **Total alignments**—Total number of loci reads aligned to with > 0 quality.
- **Secondary alignments**—Number of secondary alignment loci.
- **Supplementary (chimeric) alignments**—A chimeric read is split over multiple loci (possibly due to structural variants). One alignment is referred to as the representative alignment, the other are supplementary.
- **Estimated read length**—Total number of input bases divided by the number of reads.
- **Histogram**—See *Histogram Coverage Report* on page 196.
- **PCT of bases aligned that fell inside the interval region**—Number of bases inside the interval region and the target region divided by the total number of bases aligned.
- **Estimated sample contamination**—The estimated fraction of reads in a sample that may be from another human source.

  The prediction accuracy of variant calling is affected by cross-sample contamination. Even small levels of contamination can lead to many FP calls, especially in pipelines where the aim is to detect variants with low allele frequencies.

  The DRAGEN cross-sample contamination module uses a probabilistic mixture model to estimate the fraction of reads in a sample that may be from another human source. This sample contamination fraction is estimated as the parameter value in the mixture model that maximizes the likelihood of the observed reads at multiple pileup locations. The mixture model accounts for the population allele frequencies and the inferred sample genotypes.

  To enable this metric in germline mode, you must provide the file path on the command line to a VCF that includes marker sites (RSIDs) with population allele frequencies.

  ```
  --qc-cross-cont-vcf /opt/edico/config/sample_cross_contamination_
  resource_hg19/GRCh37/GRCh38.vcf
  ```

In somatic mode the contamination algorithm first tries to avoid biases that could be introduced by CNV or LoH. The algorithm then estimates nucleotide noise from the sample to adjust for FFPE samples.

To enable somatic contamination detection, use the following setting.

```
--qc-somatic-contam-vcf /opt/edico/config/somatic_sample_cross_
contamination_resource_hg19/GRCh37/GRCh38.vcf.gz
```

The VCF resource files that are included with DRAGEN can be reconstructed from the Ensembl database. The VCFs included in the DRAGEN config folder contain ~5000 marker locations where the population AFs are close to 0.5. The files are reference specific (hg19/GRCh37/hg38). DRAGEN will abort if an incompatible resource and reference file is used (eg, CRCh37 resource file and hg19 reference).

The following shows example output for a sample with 1.1% contamination. This value is provided as a fraction, so a value of 0.011 the same as 1.1% estimated contamination.

```
MAPPING/ALIGNING SUMMARY Estimated sample contamination 0.011
```

## Somatic Mode Mapping and Aligning Metrics

In somatic tumor-normal mode, the mapping and aligning metrics are generated separately for the tumor and normal samples, with each line beginning with TUMOR or NORMAL to indicate the sample. The metrics for the tumor sample are output first, followed by the metrics for the normal sample. Metrics per read group are also separated into tumor and normal read groups.

In somatic tumor-only mode, the mapping and aligning metrics follow the same conventions as germline mode, without metrics labeled as TUMOR or Normal.

## Variant Calling Metrics

The generated variant calling metrics are similar to the metrics computed by RTG vcfstats. Metrics are reported for each sample in multi sample VCF and gVCF files. Based on the run case, metrics are reported either as standard VARIANT CALLER or JOINT CALLER. Metrics are reported both for the raw (PREFILTER) and hard filtered (POSTFILTER) VCF file.

PON (Panel of Normals) and COSMIC filtered variants are counted as PASS variants in the POSTFILTER VCF metrics. These PASS variants can cause higher than expected variant counts in the POSTFILTER VCF metrics.

- **Number of samples**—Number of samples in the population/ joint VCF.
- **Reads Processed**—The number of reads used for variant calling, excluding any duplicate marked reads and reads falling outside of the target region.
- **Total**—The total number of variants (SNPs + MNPs + indels).
- **Biallelic**—Number of sites in a genome that contains two observed alleles, counting the reference as one, and therefore allowing for one variant allele.

- **Multiallelic**—Number of sites in the VCF that contain three or more observed alleles. The reference is counted as one, therefore allowing for two or more variant alleles.
- **SNPs**—A variant is counted as an SNP when the reference, allele 1, and allele2 are all length 1.
- **Insertions (Hom)**—Number of variants that contains homozygous insertions
- **Insertions (Het)**—Number of variants where both alleles are insertions, but not homozygous
- **Deletions (Het)** —Number of variants that contains homozygous deletions
- **INDELS (Het)**—Number of variants where genotypes are either [insertion+deletion], [insertion+SNP] or [deletion+SNP].
- **De Novo SNPs**—*De novo* marked SNPs, with DQ > 0.05. Sett the *--qc-snp-denovo-quality-threshold* option to the required threshold. The default is 0.05.
- **De Novo INDELs**—*De novo* marked indels with DQ values > 0.02. This DQ threshold can be specified by Setting the *--qc-indel-denovo-quality-threshold* option to the required DQ threshold. The default is 0.02.
- **De Novo MNPs**—Same as the option for SNPs. Set the *--qc-snp-denovo-quality-threshold* to the required threshold. The default is 0.05.
- **(Chr X SNPs)/(Chr Y SNPs) ratio in the genome (or the target region)**—Number of SNPs in chromosome X (or in the intersection of chromosome X with the target region) divided by the number of SNPs in chromosome Y (or in the intersection of chromosome Y with the target region). If there was no alignment to either chromosome X or chromosome Y, this metric shows as NA.
- **SNP Transitions**—An interchange of two purines (A<->G) or two pyrimidines (C<->T).
- **SNP Transversions**—An interchange of purine and pyrimidine bases Ti/Tv ratio: ratio of transitions to transitions.
- **Heterozygous**—Number of heterozygous variants.
- **Homozygous**—Number of homozygous variants.
- **Het/Hom ratio**—Heterozygous/ homozygous ratio.
- **In dbSNP**—Number of variants detected that are present in the dbSNP reference file. If no dbSNP file is provided via the *--bsnp* option, then both the **In dbSNP** and **Novel** metrics show as NA.
- **Novel**—Total number of variants minus number of variants in dbSNP.
- **Percent Callability**—Available only in germline mode with gVCF output. The percentage of non-N reference positions having a PASSing genotype call. Multi-allelic variants are not counted. Deletions are counted for all the deleted reference positions only for homozygous calls. Only autosomes and chromosomes X, Y, and M are considered.
- **Percent Autosome Callability**—Only autosomes are considered.

- **Percent QC Region Callability in Region i (i is equivalent to regions 1,2, or 3)**—Available if callability for custom regions is requested via the *--qc-coverage-region-i* option and the callability output is specified with *--qc-coverage-reports-i*. All contigs are considered.

### Duration Metrics

The duration metrics section includes a breakdown of the run duration for each process. For example, the following metrics are generated for the mapper and variant caller pipeline:

- Time loading reference
- Time aligning reads
- Time sorting and marking duplicates
- Time DRAGStr calibration
- Time partial reconfiguration
- Time variant calling
- Total runtime

## Callability Report

DRAGEN automatically generates a callability report as part of variant caller metrics when running the germline small variant caller with gVCF output mode.DRAGEN appends the percent callability for each region included in the BED file specified by the test and generates a full-callability-report in both BED and CSV formats. Callability is defined as the fraction of non-N reference positions having a PASSing genotype call. The following criteria are used to calculate callability metrics:

- Callability is calculated over the gVCF.
- Multi-allelic variants are not counted.
- Decoy contigs are ignored.
- Unplaced and unlocalized contigs are ignored.
- N positions are considered non-callable.
- For regions where no variant calling was performed, callability is 0.
- A homozygous deletion counts as a PASSing genotype call for all the reference positions spanned by the deletion.

If the *--vc-target-bed* option is specified, the output is a `target_bed_callability.bed` file that contains the overall and autosome callability over the input target bed region. The padding size specified by the *--vc-target-bed-padding* option is used and overlapping regions are merged.

Callability can also be output with the custom region coverage/callability reports.

## Coverage/Callability Reports Over Custom Regions

DRAGEN generates the following coverage reports:

- A set of default reports for either the whole genome, or, if the *--vc-target-bed* option is specified, for the target region.
- Optionally, additional reports for up to three regions of interest (coverage regions).

  For each specified region, DRAGEN generates the default reports, and any optional report requested for the region.

To generate coverage region reports, use the *–qc-coverage-region-i* option, where i is 1, 2, or 3.

- Each *–qc-coverage-region-i* option requires a bed file argument.
- Regions in each bed file can be optionally padded using the *--qc-coverage-region-padding-i* option, which defaults to 0.
- A set of default reports is generated for each region.
- Optionally, additional reports can be specified for each region by using the *–qc-coverage-reports-i* option.

The following example shows the options required to generate coverage reports.

```
$ dragen … \
--qc-coverage-region-1 <bed file 1> \
--qc-coverage-reports-1 full_res \
--qc-coverage-region-2 <bed file 2> \
--qc-coverage-region-3 <bed file 3> \
--qc-coverage-reports-3 full_res cov_report
```

## Counting Reads and Bases

All default and optional coverage reports listed in Table 8 and Table 9 use the following default rules for counting reads and bases:

- Duplicate reads are ignored.
- Soft and/or hard-clipped bases are ignored.
- Reads with MAPQ=0 are ignored.
- Overlapping mates are double-counted.

Nondefault settings:

The reports are available with or without running either the mapper and aligner, or the variant caller. However, the *--enable-sort* options must be set to true (the default is true).

By default overlapping mates are double counted. Set *--qc-coverage-ignore-overlaps=true* to resolve all of the alignments for each fragment and avoid double-counting any overlapping bases. This might result in marginally longer run times. This option also requires setting *--enable-map-align=true*. *--qc-coverage-ignore-overlaps* is a global setting and updates all qc-coverage-reports.

By default soft-clipped bases are not counted towards coverage. Set *--qc-coverage-count-soft-clipped-bases=true* to include soft-clipped bases in the coverage calculations. *--qc-coverage-count-soft-clipped-bases* is a global setting and updates all qc-coverage reports.

Any combination of the optional reports can be requested for each region. If multiple report types are selected per region, they should be space-separated.

It is possible to override the min MAPQ and min BQ to apply for a given region using the qc-coverage-filters.

A coverage filter is enabled by using one of the *--qc-coverage-filters-i* options (where i is 1, 2, or 3), in combination with the associated *--qc-coverage-region-i* option:

- *--qc-coverage-region-i=<targetedregions.bed>*
- *--qc-coverage-filters-i <filters string>*

For example, the following options are used to enable 1 bp resolution coverage output with filtering:

```
--qc-coverage-region-1 <targetedregions.bed>
--qc-coverage-filters-1 'mapq<10,bq<30'
--qc-coverage-reports-1 full_res
```

- The argument syntax is mapq<value,bq<value, which means that reads that have a mapping quality less than the specified value are not counted, and/or bases with a base call quality below the specified value.
- Valid filter arguments are mapq and bq only. Either, or both, can be specified.
- Only one operator < is supported. <=, >, >=, = are not supported.
- When filtering is enabled for a targeted region, DRAGEN outputs the filtered report files for this region. Unfiltered report files are not output for the targeted filtered region.

## Callability for the Custom Regions

If the *--qc-coverage-region-i* option is used with *--qc-coverage-reports-i* (where i is 1, 2, or 3), callability can be added as a report type for that region. The output is a `qc-coverage-region-i_callability.bed` file. For each specified `qc-coverage-region-i` file, the average callability is reported in the variant calling metrics file. The padding size specified by the *--qc-coverage-region-padding-i* is used and overlapping regions are merged.

The optional min MAPQ and min BQ filters only influence read and base counting and do not influence the callability reports.

Contig lengths and region of interest lengths (used as denominators) do not include regions with N in the FASTA.

## Available Report Types

Table 8 Default Report

| File Name | Description |
|---|---|
| _coverage_ metrics.csv | *Coverage Metrics Report* on page 193 |
| _fine_hist.csv | *Fine Histogram Coverage Report* on page 196 |
| _hist.csv | *Histogram Coverage Report* on page 196 |
| _overall_mean_ cov.csv | *Overall Mean Coverage Report* on page 196 |
| _contig_mean_ cov.csv | *Per Contig Mean Coverage Report* on page 196 |
| _ploidy.csv | *Predicted Ploidy Report* |

Table 9 Optional Reports

| File Name | Description |
|---|---|
| _full_res.bed | *Full Res Report* on page 196 |
| _cov_report.bed | *Coverage Report* on page 197 |
| _callability.bed | *Callability Report* on page 190 |

## Coverage Metrics Report

The coverage metrics report outputs a `_coverage_metrics.csv` file, which provides metrics over a region, where the region can be the genome, a target region, or a QC coverage region. The first column of the output file contains the section name COVERAGE SUMMARY and the second column is empty for all metrics.

The following criteria are used when calculating coverage:

- Duplicate reads and clipped bases are ignored.
- Only reads with MAPQ > min MAPQ and bases with BQ > min BQ are considered.

The following table lists the calculated metrics:

| Metric | Description |
|---|---|
| **Aligned bases in region** | Number of uniquely mapped bases to region and the percentage relative to the number of uniquely mapped bases to the genome. |
| **Average alignment coverage over region** | Number of uniquely mapped bases to region divided by the number of sites in region. |

| Metric | Description |
|---|---|
| **Uniformity of coverage (PCT > 0.2*mean) over region** | Percentage of sites with coverage greater than 20% of the mean coverage in region. |
| **PCT of region with coverage [ix, inf)** | Percentage of sites in region with at least ix coverage, where i can equal 100, 50, 20, 15, 10, 3, 1, or 0. |
| **PCT of region with coverage [ix, jx)** | Percentage of sites in region with at least ix but less than jx coverage, where (i, j) can equal (50, 100), (20, 50), (15, 20), (10, 15), (3, 10), (1, 3), or (0, 1). |
| **Average chromosome X coverage over region** | Total number of bases that aligned to the intersection of chromosome X with region divided by the total number of loci in the intersection of chromosome X with region. If there is no chromosome X in the reference genome or the region does not intersect chromosome X, this metric shows as NA. |
| **Average chromosome Y coverage over region** | Total number of bases that aligned to the intersection of chromosome Y with region divided by the total number of loci in the intersection of chromosome Y with region. If there is no chromosome Y in the reference genome or the region does not intersect chromosome Y, this metric shows as NA. |
| **XAvgCov/YAvgCov ratio over genome/target region** | Average chromosome X alignment coverage in region divided by the average chromosome Y alignment coverage in region. If there is no chromosome X or chromosome Y in the reference genome or the region does not intersect chromosome X or Y, this metric shows as NA. |
| **Average mitochondrial coverage over region** | Total number of bases that aligned to the intersection of the mitochondrial chromosome with region divided by the total number of loci in the intersection of the mitochondrial chromosome with region. If there is no mitochondrial chromosome in the reference genome or the region does not intersect mitochondrial chromosome, this metric shows as NA. |
| **Average autosomal coverage over region** | Total number of bases that aligned to the autosomal loci in region divided by the total number of loci in the autosomal loci in region. If there is no autosome in the reference genome, or the region does not intersect autosomes, this metric shows as NA. |
| **Median autosomal coverage over region—** | Median alignment coverage over the autosomal loci in region. If there is no autosome in the reference genome or the region does not intersect autosomes, this metric shows as NA. |

| Metric | Description |
|---|---|
| **Mean/Median autosomal coverage ratio over region** | Mean autosomal coverage in region divided by the median autosomal coverage in region. If there is no autosome in the reference genome or the region does not intersect autosomes, this metric shows as NA. |
| **Aligned reads in region—** | Number of uniquely mapped reads to region and percentage relative to the number of uniquely mapped reads to the genome. When region is the target BED, this metric is equivalent to and replaces Capture Specificity based on target region. |

The following is an example of the contents of the _coverage_metrics.csv file:

```
COVERAGE SUMMARY,,Aligned bases,148169295474
COVERAGE SUMMARY,,Aligned bases in genome,148169295474,100.00
COVERAGE SUMMARY,,Average alignment coverage over genome,46.08
COVERAGE SUMMARY,,Uniformity of coverage (PCT > 0.2*mean) over genome,91.01
COVERAGE SUMMARY,,PCT of genome with coverage [100x: inf),0.25
COVERAGE SUMMARY,,PCT of genome with coverage [ 50x: inf),50.01
COVERAGE SUMMARY,,PCT of genome with coverage [ 20x: inf),89.46
COVERAGE SUMMARY,,PCT of genome with coverage [ 15x: inf),90.51
COVERAGE SUMMARY,,PCT of genome with coverage [ 10x: inf),91.01
COVERAGE SUMMARY,,PCT of genome with coverage [  3x: inf),91.69
COVERAGE SUMMARY,,PCT of genome with coverage [  1x: inf),92.10
COVERAGE SUMMARY,,PCT of genome with coverage [  0x: inf),100.00
COVERAGE SUMMARY,,PCT of genome with coverage [ 50x:100x),49.76
COVERAGE SUMMARY,,PCT of genome with coverage [ 20x: 50x),39.45
COVERAGE SUMMARY,,PCT of genome with coverage [ 15x: 20x),1.04
COVERAGE SUMMARY,,PCT of genome with coverage [ 10x: 15x),0.51
COVERAGE SUMMARY,,PCT of genome with coverage [  3x: 10x),0.67
COVERAGE SUMMARY,,PCT of genome with coverage [  1x:  3x),0.42
COVERAGE SUMMARY,,PCT of genome with coverage [  0x:  1x),7.90
COVERAGE SUMMARY,,Average chr X coverage over genome,24.70
COVERAGE SUMMARY,,Average chr Y coverage over genome,20.96
COVERAGE SUMMARY,,Average mitochondrial coverage over genome,20682.19
COVERAGE SUMMARY,,Average autosomal coverage over genome,47.81
COVERAGE SUMMARY,,Median autosomal coverage over genome,48.62
COVERAGE SUMMARY,,Mean/Median autosomal coverage ratio over genome,0.98
COVERAGE SUMMARY,,XAvgCov/YAvgCov ratio over genome,1.18
COVERAGE SUMMARY,,XAvgCov/AutosomalAvgCov ratio over genome,0.52
COVERAGE SUMMARY,,YAvgCov/AutosomalAvgCov ratio over genome,0.44
COVERAGE SUMMARY,,Aligned reads,1477121058
COVERAGE SUMMARY,,Aligned reads in genome,1477121058,100.00
```

## Fine Histogram Coverage Report

The fine histogram report outputs a `_fine_hist.csv` file, which contains two columns: Depth and Overall. The value in the Depth column ranges from 0 to 1000+ and the Overall column indicates the number of loci covered at the corresponding depth.

## Histogram Coverage Report

The histogram report outputs a `_hist.csv` file, which provides the following information:

- Percentage of bases in the coverage bed/target bed/wgs region that fall within a certain range of coverage.
- Duplicate reads are ignored if DRAGEN is run with *--enable-duplicate-marking true*.

The following ranges are used:

```
"[100x:inf)", "[1x:3x)", "[0x:1x)"
```

## Overall Mean Coverage Report

The Overall Mean Coverage report generates an `_overall_mean_cov.csv` file, which contains the average alignment coverage over the coverage bed/target bed/wgs, as applicable.

The following is an example of the contents of the `_overall_mean_cov.csv` file:

```
Average alignment coverage over target_bed,80.69
```

## Per Contig Mean Coverage Report

The Contig Mean Coverage report generates a `_contig_mean_cov.csv` file, which contains the estimated coverage for all contigs, and an autosomal estimated coverage. The file includes the following three columns:

| Column 1 | Column 2 | Column 3 |
|---|---|---|
| Contig name | Number of bases aligned to that contig, which excludes bases from duplicate marked reads, reads with MAPQ=0, and clipped bases. | The estimated covered, calculated as follows. Number of bases aligned to the contig (ie, Col2) divided by length of the contig or (if a target bed is used) the total length of the target region spanning that contig. |

## Full Res Report

The Full Res Report outputs a _full_res.bed file. This file is tab-delimited file and has as its first three columns the standard BED fields, and as its fourth column the depth. Each record in the file is for a given interval that has a constant depth. If the depth changes, then a new record is written to the file. Alignments that have a mapping quality value of 0, duplicate reads, and clipped bases are not counted towards the depth.

Only base positions that fall under the user-specified coverage-region bed regions are present in the _ full_res.bed output file.

The _full_res.bed file structure is similar to the output file of *bedtools genomecov -bg*. The contents are identical if the bedtools command line is executed after filtering out alignments with mapping quality value of 0, and possibly filtering by a target BED (if specified).

The following is an example of the contents of the _full_res.bed file:

```
chr1 121483984 121483985 10
chr1 121483985 121483986 9
chr1 121483986 121483989 8
chr1 121483989 121483991 7
chr1 121483991 121483992 6
chr1 121483992 121483993 4
chr1 121483993 121483994 2
chr1 121483994 121484039 1
chr1 121484039 121484043 2
chr1 121484043 121484048 3
chr1 121484048 121484050 7
chr1 121484050 121484051 11
chr1 121484051 121484052 17
chr1 121484052 121484053 149
chr1 121484053 121484054 323
chr1 121484054 121484055 2414
```

## Coverage Report

The cov_report report generates a _cov_report.bed file in a tab-delimited formatfull-coverage-report file in both BED and CSV formats. The first three columns are standard BED fields. The remaining column fields are statistics calculated over the interval region specified on the same record line.

The following table lists the appended columns.

| Column | Description |
|---|---|
| **total_cvg** | The total coverage value. |

| Column | Description |
|---|---|
| **mean_cvg** | The mean coverage value. |
| **Q1_cv** | The lower quartile (25th percentile) coverage value. |
| **median_cvg** | The median coverage value. |
| **Q3_cvg** | The upper quartile (75th percentile) coverage value. |
| **min_cvg** | The minimum coverage value. |
| **max_cvg** | The maximum coverage value. |
| **pct_above_X** | Indicates the percentage of bases over the specified interval region that had a depth coverage greater than X. |

By default, if an interval has a total coverage of 0, then the record is written to the output file. To filter out intervals with zero coverage, set *vc-emit-zero-coverage-intervals* to false in the configuration file.

The following is an example of the contents of the _cov_report.bed file:

```
chrom  start      end        total_cvg  mean_cvg  Q1_cvg  median_cvg  Q3_cvg  min_cvg  max_cvg  pct_above_5
…
chr5   34190121   34191570   76636      52.89     44.00   54.00       60.00   32       76       100.00
…
chr5   34191751   34192380   39994      63.58     57.00   61.00       69.00   50       85       100.00
…
chr5   34192440   34192642   10074      49.87     47.00   49.00       51.00   44       62       100.00
…
chr9   66456991   66457682   31926      46.20     39.00   45.00       52.00   27       65       100.00
…
chr9   68426500   68426601   4870       48.22     42.00   48.00       54.00   39       58       100.00
…
chr17  41465818   41466180   24470      67.60     4.00    66.00       124.00  2        153      66.30
…
chr20  29652081   29652203   5738       47.03     40.00   49.00       52.00   34       58       100.00
…
chr21  9826182    9826283    4160       41.19     23.00   52.00       58.00   5        60       99.01
…
```

## Coverage/Callability Reports Use Cases and Expected Output

The following table shows which outputs are generated when default options (*--vc-target-bed*) versus optional coverage region options (*--coverage-region*) are used.

| --vc-target-bed specified? Y/N | --qc- coverage-region-i (i equal to 1, 2, or 3) specified? Y/N | Expected Output Files |
|---|---|---|
| N | N | wgs_coverage_metrics.csv<br>wgs_fine_hist.csv<br>wgs_hist.csv<br>wgs_overall_mean_cov.csv<br>wgs_contig_mean_cov.csv |
| N | Y | wgs_coverage_metrics.csv<br>wgs_fine_hist.csv<br>wgs_hist.csv<br>wgs_overall_mean_cov.csv<br>wgs_contig_mean_cov.csv<br><br>For each coverage region specified by the user:<br>qc-coverage-region-i_coverage_metrics.csv<br>qc-coverage-region-i_fine_hist.csv<br>qc-coverage-region-i_hist.csv<br>qc-coverage-region-i_overall_mean_cov.csv<br>qc-coverage-region-i_contig_mean_cov.csv<br>qc-coverage-region-i_full_res.bed if full_res report type is requested for qc-coverage-region-i<br>qc-coverage-region-i_cov_report.bed if cov_report report type is requested for qc-coverage-region-i<br>qc-coverage-region-i_callability.bed if GVCF mode is enabled and the callability or exome-callability report type is requested |

| --vc-target-bed specified? Y/N | --qc- coverage-region-i (i equal to 1, 2, or 3) specified? Y/N | Expected Output Files |
|---|---|---|
| Y | N | wgs_coverage_metrics.csv<br>wgs_fine_hist.csv<br>wgs_hist.csv<br>wgs_overall_mean_cov.csv<br>wgs_contig_mean_cov.csv<br><br>target_bed_coverage_metrics.csv<br>target_bed_fine_hist.csvtarget_bed_hist.csv<br>target_bed_overall_mean_cov.csv<br>target_bed_contig_mean_cov.csv<br><br>target_bed_callability.bed if GVCF mode is enabled |

| --vc-target-bed specified? Y/N | --qc- coverage-region-i (i equal to 1, 2, or 3) specified? Y/N | Expected Output Files |
|---|---|---|
| Y | Y | wgs_coverage_metrics.csv<br>wgs_fine_hist.csv<br>wgs_hist.csv<br>wgs_overall_mean_cov.csv<br>wgs_contig_mean_cov.csv<br><br>target_bed_coverage_metrics.csv<br>target_bed_fine_hist.csv<br>target_bed_hist.csv<br>target_bed_overall_mean_cov.csv<br>target_bed_contig_mean_cov.csv<br>target_bed_callability.bed if GVCF mode is enabled<br><br>For each coverage region specified by the user:<br>qc-coverage-region-i _coverage_metrics.csv<br>qc-coverage-region-i_fine_hist.csv<br>qc-coverage-region-i_hist.csv<br>qc-coverage-region-i_overall_mean_cov.csv<br>qc-coverage-region-i_contig_mean_cov.csv<br><br>qc-coverage-region-i_full_res.bed if full_res report type is requested for qc-coverage-region-i<br>qc-coverage-region-i_cov_report.bed if cov_report report type is requested for qc-coverage-region-i<br>qc-coverage-region-i_callability.bed if GVCF mode is enabled and the callability or exome-callability report type is requested |

### BigWig Compression of Coverage Metrics

When *--enable-metrics-compression* is set to true, the 1 bp resolution coverage metrics output bed file (`_full_res.bed`) are compressed to bigwig format.

## GC Bias Report

The GC bias report provides information on GC content and the associated read coverage across a genome. DRAGEN GC bias metric is modeled after the Picard implementation and adapted to preexisting internal measures. The DRAGEN GC bias correction module attempts to correct these biases following the target count stage. For more information, see *GC Bias Correction* on page 127

The GC bias metric is computed as follows.

1. Calculates GC content using a 100 bp wide, per-base rolling window over all chromosomes in the reference genome, excluding any decoys and alternate contigs. Windows containing more than four masked (N) bases in the reference are discarded.
2. Calculates the average coverage for each window, excluding any non-PF, duplicate, secondary, and supplementary reads.
3. Calculates the average global coverage across the whole genome.
4. Groups valid windows based on the percentage of GC content, both at individual percentages and five 20% ranges as summary.
5. Calculates the normalized coverage for each group by dividing the average coverage for the bin by the global average coverage across the genome. Values below 1.0 indicate a lower than expected coverage at the given GC percent or range. Coverages significantly deviating from 1.0 at greater GC values are an expected result.
6. Calculates dropout metrics as the sum of all positive values of (percentage of windows at GC X- percentage aligned reads at GC X) for each GC ≤ 50% and > 50% for AT and GC dropout.

By default, the GC bias metric report is not calculated. To enable GC Bias calculations, enter the *--gc-metrics-enable* command line option. The following is an example command:

```
$ dragen -b <BAM file> -r <reference genome> --gc-metrics-enable=true
```

The GC metrics report generates a `gc_metrics.csv` file. The file is structured as follows.

```
GC BIAS DETAILS,,Windows at GC [0-100],<number of windows>,<fraction of all
windows>
GC BIAS DETAILS,,Normalized coverage at GC [0-100],<average coverage of all
windows at given GC divided by average coverage of whole genome>
GC METRICS SUMMARY,,Window size,<window size in base, typically 100>
GC METRICS SUMMARY,,Number of valid windows,<total number of windows used
in calculations>
GC METRICS SUMMARY,,Number of discarded windows,<total number windows
discarded due to more than 4 masked bases>
GC METRICS SUMMARY,,Average reference GC,<average GC content over all valid
windows>
GC METRICS SUMMARY,,Mean global coverage,<average genome coverage over all
valid windows>
GC METRICS SUMMARY,,Normalized coverage at GCs <GC range>,<average coverage
of all windows at given GC range divided by average coverage of whole
genome>
GC METRICS SUMMARY,,AT Dropout,<Calculated AT dropout value>
GC METRICS SUMMARY,,GC Dropout,<Calculated GC dropout value>
```

The GC bias report also includes the following command line options, but they are not recommended.

- *--gc-metrics-window-size*—Overrides the default rolling window size of 100 bp.

- *--gc-metrics-num-bins*—Overrides the number of summary bins.

## Somatic Metrics Report

In somatic tumor-normal mode, DRAGEN generates separate reports for the tumor and normal samples. Each report is labeled according to the sample type. Tumor sample reports include `tumor` at the end of the file name, and normal sample reports include `normal` at the end of the file name. To include both tumor and normal sample results in one file, set the *--vc-enable-separate-t-n-metrics* option to false. DRAGEN then reports on the aggregate of both samples instead.

DRAGEN generates the following reports:

- coverage_metrics
- contig_mean_cov
- fine_hist
- hist
- overall_mean_cov
- ploidy
- cov_report (if requested)
- full_res (if requested)
- gc_metrics (if requested)

If a target bed is included in the run or the option *--qc-region-coverage-region-i* (where i is 1, 2, or 3) is included, then the corresponding coverage reports are also split into tumor and normal files.

In somatic tumor-only mode, the reports are identical to those in germline mode and do not include tumor or normal in the file names.

## Somatic Callable Regions Report

In somatic mode, DRAGEN automatically generates a somatic callable regions report as a bed file. The somatic callable regions report includes all regions with tumor coverage at least as high as the tumor threshold and (if applicable) normal coverage at least as high as the normal threshold. If only the tumor sample is provided, then the report includes all regions with tumor coverage at least as high as the tumor threshold. Each line in the bed output file is formatted as follows.

```
chromosome region_start region_end
```

You can specify the threshold values using the *--vc-callability-tumor-thresh* or *--vc-callability-normal-thresh* options. The default value for the tumor threshold is 15. The default value for the normal threshold is 5. For more information on each option, see *Somatic Mode Options* on page 96.

If the target bed or the *--qc-region-coverage-region-i*(where i is 1, 2, or 3) option is included in the run. The DRAGEN then generates corresponding somatic callable regions bed files in addition to the whole genome somatic callable region bed file.

# Virtual Long Read Detection

DRAGEN Virtual Long Read Detection (VLRD) is an alternate and more accuratevariant caller focused on processing homologous/similar regions of the genome. A conventional variant caller relies on the mapper/aligner to determine which reads likely originated from a given location. It also detects the underlying sequence at that location independently of other regions not immediately adjacent to it. Conventional variant calling works well when the region of interest does not resemble any other region of the genome over the span of a single read (or a pair of reads for paired-end sequencing).

However, a significant fraction of the human genome does not meet this criterion. Many regions of the genome have near-identical copies elsewhere, and as a result, the true source location of a read might be subject to considerable uncertainty. If a group of reads is mapped with low confidence, a typical variant caller might ignore the reads, even though they contain useful information. If a read is mismapped (ie, the primary alignment is not the true source of the read), it can result in detection errors. Short-read sequencing technologies are especially susceptible to these problems. Long-read sequencing can mitigate these problems, but it typically has much higher cost and/or higher error rates, or other shortcomings.

DRAGEN VLRD attempts to tackle the complexities presented by the genome's redundancy from a perspective driven by the short-read data. Instead of considering each region in isolation, VLRD considers all locations from which a group of reads may have originated and attempts to detect the underlying sequences jointly using all available information.

## Running DRAGEN VLRD

Like the DRAGEN variant caller, VLRD takes either FASTQ or sorted BAM files as input and produces an output VCF file. VLRD supports processing a set of only two homologous regions.

VLRD is not enabled by default. To run VLRD, set the *--enable-vlrd* option to true. The following is an example DRAGEN command to run VLRD.

```
dragen \
-r <REF> \
-1 <FQ1> \
 -2 <FQ2> \
--RGID <RG> --RGSM <SM> \
--output-dir <OUTPUT> \
--output-file-prefix <PREFIX> \
--enable-map-align true \
--enable-sort=true \
--enable-duplicate-marking true \
--enable-vlrd true
--vc-target-bed similar_regions.bed
```

## VLRD Updated Map/Align Output

DRAGEN VLRD can output a remapped BAM/SAM file in addition to the regular DRAGEN Map/Align output. To enable output of a remapped BAM/SAM file, set the *--enable-vlrd-map-align-output* option to true. The default for this option is false.

The additional map/align output by VLRD only contains reads mapped to the regions that were processed by VLRD.

VLRD considers the information available from all the homologous regions jointly to update the read alignments. The updated VLRD map/align output is primarily useful for pile-up analysis involving homologous regions.

## VLRD Settings

The following options are specific to VLRD in the DRAGEN host software.

- *--enable-vlrd*

  If set to true, VLRD is enabled for the DRAGEN pipeline.

- *--vc-target-bed*

  Specifies the input bed file. DRAGEN requires an input target bed file specifying the homologous regions to be processed by VLRD. The input bed file id formatted to correctly process the homologous regions. The maximum region span processed by VLRD is 900 bp.

  For example:

```
chr1    161497562   161498362   0   0
chr1    161579204   161580004   0   0
chr1    21750837     21751637   1   0
chr1    21809355     21810155   1   1
```

  - The first three columns are like traditional bed files: column 1 is chromosome description, column 2 is region start, and column 3 is region end.
  - Column 4 is homologous region Group ID. This groups regions that are homologous to each other.
  - Rows 1 and 2 have the same value in column 4 indicating that these should be processed as a set of homologous regions, independent of the next group in row 3 and 4. If not set correctly, software might group regions that are not homologous to each other, leading to incorrect variant calls.
  - Column 5 indicates whether a region is reverse complemented with respect to the other homologous region. A value of 1 denotes that the region is reverse complemented with respect to other regions in the same group.
  - Row 4, column 5 is set to 1. This indicates that the region is homologous to the region in row 3 only if it is reverse complemented.

The DRAGEN installation package contains two VRLD bed files for hg19 and hs37d5 reference genomes under `/opt/edico/examples/VLRD`. You can use these bed files as is for running VLRD, or as an example to generate a custom bed file.

- *--enable-vlrd-map-align-output*

  If set to true, VLRD outputs a remapped BAM/SAM file that only contains reads mapped to the regions that were processed by VLRD.

# Unique Molecular Identifiers

DRAGEN can process data from whole genome and hybrid-capture assays with unique molecular identifiers (UMI). UMIs are molecular tags added to DNA fragments before amplification to determine the original input DNA molecule of the amplified fragments. UMIs help reduce errors and biases introduced by DNA damage such as deamination before library prep, PCR error, or sequencing errors.

To use the UMI Pipeline, the input reads files must be from a paired-end run. Input can be pairs of FASTQ files or aligned/unaligned BAM input.

DRAGEN supports the following UMI types:

- Dual, nonrandom UMIs, such as TruSight Oncology (TSO) UMI Reagents or IDT xGen Prism.
- Dual, random UMIs, such as Agilent SureSelect XT HS2 molecular barcodes (MBC) or IDT xGen Duplex Seq Adapters.
- Single-ended, random UMIs, such as Agilent SureSelect XT HS molecular barcodes (MBC) or IDT xGen dual index UMI Adapters.

DRAGEN uses the UMI sequence to group the read pairs by their original input fragment and generates a consensus read pair for each such group, or family. The consensus reduces error rates to detect rare and low frequency somatic variants in DNA samples with high accuracy. DRAGEN generates a consensus as follows.

1. Aligns reads.
2. Groups reads into groups with matching UMI and pair alignments. These groups are referred to as families.
3. Generates a single consensus read pair for each read family.

These generated reads have higher quality scores than the input reads and reflect the increased confidence gained by combining multiple observations into each base call.

UMI workflow is only compatible with small variant calling and SV in DRAGEN

## UMI Input

Enter UMIs in one of the following formats:

- Read name—The UMI sequence is located in the eighth colon-delimited field of the read name (QNAME). For example, NDX550136:7:H2MTNBDXX:1:13302:3141:10799:AAGGATG+TCGGAGA

- BAM tag—The UMI is present as an RX tag in prealigned or aligned BAM file (standard SAM format).
- FASTQ file—The UMI is located in a third FASTQ file using the same read order as the read pairs.

To create FASTQ, append the UMI to the read name, and then specify the appropriate OverrideCycles setting in the DRAGEN BCL conversion tool (see *DRAGEN BCL Data Conversion* on page 238). DRAGEN supports UMIs with two parts each with a maximum of 8 bp and separated by `+`, or a single UMI with a maximum of 15 bp.

## UMI Input Correction Table

For dual, nonrandom UMIs, you can provide a predefined UMI correction table or a list of valid UMI sequences as input. To create the UMI correction table, use a tab-delimited file, include a header, and add the following fields.

| Field | Value |
|---|---|
| UMI | The UMI sequence. For example, `ACGTAC`. |
| IsValid | Specify if the UMI sequence is valid. Enter either: `TRUE` or `FALSE`. |
| NearestCodes | Colon-separated list of nearest UMI sequences. For example, `ACGTAA:ACGTAT`. |
| SecondNearestCodes | Colon-separated list of second nearest sequences. For example, `ACGGAA:ACGGAT`. |

If customized correction table is not specified, DRAGEN uses the default table for TruSight Oncology (TSO) UMI Reagents. Alternatively, you can provide a file for whitelisted nonrandom UMI with valid UMI sequence one per line. DRAGEN then autogenerates a UMI correction table with hamming distance of one.

## UMI Options

- *--umi-library-type*—Set the batch option for different UMIs correction. Three batch modes are available that optimize collapsing configurations for different UMI types. Use one of the following modes:
  - *random-duplex*—Dual, random UMIs.
  - *random-simplex*—Single-ended, random UMIs.
  - *nonrandom-duplex*—Dual, nonrandom UMIs. To use this option, provide a target manifest file using *--umi-metrics-interval-file*.
- *--umi-min-supporting-reads*—Specify the number of matching UMI inputs reads required to generate a consensus read. Any family with insufficient supporting reads is discarded. For example, the following are the recommended settings for FFPE and ctDNA.

  - **[FFPE]** If the variant > 1%, use *--umi-min-supporting-reads=1* with the *--vc-enable-umi-solid* variant caller parameter. For more information on variant caller options, see *Variant Caller Options* on page 83.
  - **ctDNA** If the variant < 1%, use *--umi-min-supporting-reads=2*. with the *--vc-enable-umi-liquid* variant caller parameter. For more information on variant caller options, see *Variant Caller Options* on page 83
- *--umi-enable*—To enable read collapsing, set the *--umi-enable* option to true. This option is not compatible with *–enable-duplicate-marking* because the UMI pipeline generates a consensus read from a set of candidate input reads, rather than choosing the best nonduplicate read. If using the *--umi-library-type* option, *--umi-enable* is not required.
- *--umi-emit-multiplicity*—Set the consensus sequence type to output. DRAGEN UMI allows you to collapse duplex sequences from the two strands of the original molecules. Duplex sequence is typically ~20–60% of total library, depending on library kit, input material, and sequencing depth. Enter one of the following consensus sequence types:
  - *both*—Output both simplex and duplex sequences. This option is the default.
  - *simplex*—Output only simplex sequences.
  - *duplex*—Output only duplex sequences.
- *--umi-source*—Specify the input type for the UMI sequence. The following are valid values: `qname`, `bamtag`, `fastq`. If using *--umi-source=fastq*, provide the UMI sequence from FASTQ file using *--umi-fastq*.
- *--umi-correction-table*—Enter the path to a customized correction table. By default, Local Run Manager uses lookup correction with a built-in table for the Illumina TruSight Oncology and Illumina for IDT UMI Index Anchor kits.
- *--umi-nonrandom-whitelist*—Enter the path for a customized, valid UMI sequence.
- *--umi-metrics-interval-file*—Enter the path for target region in BED format.

## Nonrandom and Random UMI Correction

DRAGEN processes UMIs by grouping reads by UMI and alignment position. If there are sequencing errors in the UMIs, DRAGEN can correct and detect small sequencing errors by using a lookup table or by using sequence similarity and read counts. You specify the type of correction with the *--umi-correction-scheme* option using the values “lookup,” “random,” or “none.”

For sparse sets of nonrandom UMIs, it is possible to create a lookup table that specifies which sequence can be corrected and how to correct it. This correct file scheme works best on UMI sets where sequences have a minimum hamming/edit distance between them. By default, DRAGEN uses lookup correction with a built-in correct table for the Illumina TruSight Oncology and Illumina for IDT

UMI Index Anchor kits. Specify the path for your correction file using the *--umi-library-type* or *--umi-correction-file* option. If you are using a different set of nonrandom UMIs, contact Illumina Technical Support for information on generating the corresponding correction file.

In the random UMI correction scheme, DRAGEN must infer which UMIs at a given position are likely to be errors relative to other UMIs observed at the same position. The error modes include small UMI errors, such as one mismatch or UMI jumping or hopping artifact from library prep. DRAGEN accomplishes this as follows.

- Groups reads by fragment alignment position.
- Within a small fuzzy window at each position, groups the reads first by the exact UMI sequence, which forms a family.
- Estimate UMI jumping or hopping probability through insert size distribution and number of distinct UMI at certain positions.
- Within a fuzzy window, calculates pair-wise likelihood ratio to assess if two families with different UMI sequences and genomic positions are derived from same original molecule.
- Merges families with likelihood lower than threshold. The default threshold is 1.

### Merge Duplex UMIs

Duplex UMI adapters simultaneously tag both strands of double-stranded DNA fragments. It is then possible to identify reads resulting from amplification of each strand of the original fragment.

Consumable Prefix considers two collapsed read pairs to be the sequence of two strands of the same original fragment of DNA if they have the same alignment position (within a fuzzy window), complementary orientations, and their UMIs are swapped from Read 1 and Read 2. If there is only single-ended UMI, DRAGEN compares the start-end position of families from two strands and computes pair-wise likelihood to determine if they are likely originated from two distinct families or should be merged as a duplex sequence. By default, DRAGEN outputs both simplex and duplex consensus sequences. To change the consensus sequence output type, use *--umi-emit-multiplicity*.

### Example UMI Command

The following are example UMI commands:

### Generate Consensus BAM from FASTQ

The following is an example DRAGEN command for generating a consensus BAM file from input reads with Illumina UMIs:

```
dragen \
-r <REF> \
-1 <FQ1> \
-2 <FQ2> \
--output-dir <OUTPUT> \
```

```
--output-file-prefix <PREFIX> \
--enable-map-align true \
--enable-sort true \
--umi-enable true \
--umi-correction-scheme=lookup \
--umi-min-supporting-reads 2
```

### Use FASTQ UMI Input

To run with other random UMI library types, change *--umi-library-type* to `random-simplex` or `random-duplex`.

```
dragen \
-r <REF> \
-1 <FQ1> \
-2 <FQ3> \
--umi-source=fastq \
--umi-fastq <FQ2> \
--output-dir <OUTPUT> \
--output-file-prefix <
PREFIX> \
 --enable-map-align true \
--enable-sort true \
 --umi-library-type nonrandom-duplex \
 --umi-metrics-interval-file [valid target BED file]
```

### Use Customized Correction Table

```
dragen \
-r <REF> \
-1 <FQ1> \
-2 <FQ2> \
--umi-correction-table <valid umi correction table> \
--output-dir <OUTPUT> \
--output-file-prefix <PREFIX> \
--enable-map-align true \
--enable-sort true \
--umi-library-type nonrandom-duplex \
--umi-metrics-interval-file <valid target BED file>
```

### UMI Metrics

Consumable Prefix outputs an <output_prefix>.umi_metrics.csv file that describes the statistics for

UMI collapsing. This file summarizes statistics on input reads, how they were grouped into families, how UMIs were corrected, and how families-generated consensus reads. The following metrics can be useful when tuning the pipeline for your application:

- **Discarded families**—Any families having fewer than *--umi-min-supporting-reads* input or having a different duplex/simplex status than specified by *--umi-emit-multiplicity* are discarded. These reads are logged as **Reads filtered out**. The families are logged as **Families discarded**.
- **UMI correction**—Families may be combined in various ways. The number of such corrections are reported as follows.
  - **Families shifted**—Families with a very closefragment alignment coordinates (up to the distance specified in the *umi-fuzzy-window-size* parameter). The default *umi-fuzzy-window-size* parameter is 3.
  - **Families contextually corrected**—Families with the same fragment alignment coordinates and compatible UMIs are merged.
  - **Duplex families**—Families with close alignment coordinates and complementary UMIs are merged.

When you specify a valid path for *--umi-metrics-interval-file*, Consumable Prefix outputs a separate set of on target UMI statistics that contains only families within the specified BED file.

If you need to analyze the extent to which the observed UMIs cover the full space of possible UMI sequences, the histogram of unique UMIs per fragment position metric may be helpful. It is a zero-based histogram, where the index indicates a count of unique UMIs at a particular fragment position, and the value represents the number of positions with that count.

The following table describes available UMI metrics.

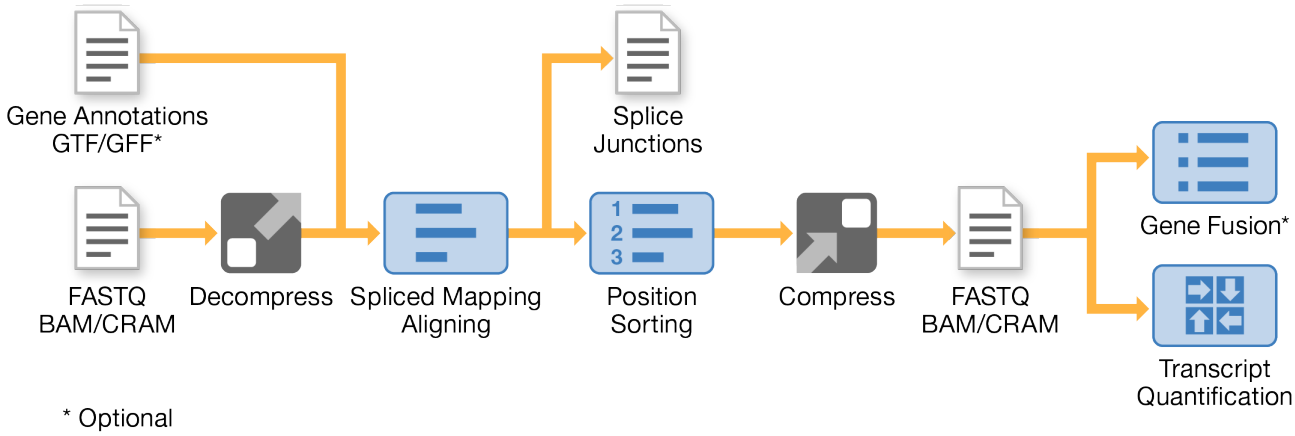
| Metric | Description |
|---|---|
| Number of reads | Total number of reads. |
| Number of reads with valid or correctable UMIs | Number of reads for which the UMIs could be corrected based on the lookup table. |
| Number of reads in discarded families | Number of reads in discarded families. Families are discarded when there are not enough raw reads to support the family. |
| Reads with all-G UMIs filtered out | Number of reads filtered out due to all-G in UMI sequence. |
| Reads filtered out | Number of reads filtered out in total either for properties or in a discarded family. |

| Metric | Description |
|---|---|
| Reads with uncorrectable UMIs | Number of reads where the UMI could not be corrected. |
| Total number of families | Number of simplex collapsed reads, |
| Families contextually corrected | Number of families that have some contextual correction. UMI correction is based on other families at the same mapping location. |
| Families shifted | Number of families that have some shift correction (merging of UMI families with very similar UMI and very close mapping locations. |
| Families discarded | Number of families filtered out by failing min supporting reads criteria or umi-emit type of simplex/duplex. |
| Families discarded by min-support-reads | Number of families filtered out by failing min supporting reads criteria. |
| Families discarded by duplex/simplex | Number of families filtered out by failing umi-emit type of simplex/duplex. |
| Duplex families | Number of families that are merged as duplex (both strands). For percentage, the denominator is the number of consensus pairs. |
| Consensus pairs emitted | Number of collapsed reads in output BAM. |
| Mean family depth | Average number of reads per family |
| Histogram of num supporting fragments | Number of families with zero raw reads, one raw read, two raw reads, three raw reads, etc |
| Number of collapsible regions | Number of regions. |
| Min collapsible region size (num reads) | Number of reads in the most populated region. |
| Max collapsible region size (num reads) | Number of reads in the least populated region |
| Mean collapsible region size (num reads) | Average number of reads per region. |

| Metric | Description |
|---|---|
| Collapsible region size standard deviation | Standard deviation of the number of reads per region. |
| On target number of reads | Number of reads that overlapped with UMI target interval *--umi-metrics-interval-file*. |
| On target number of reads with valid or correctable UMIs | Number of reads with a UMI that matched a UMI in the lookup table, including error allowance, and overlapped with UMI target interval. |
| On target duplex families | Number of families that are merged as duplex among all the families that are overlapped with UMI target interval. For percentage, denominator is the number of on-target consensus pairs. |
| On target mean family depth | Average number of reads per family that overlapped with UMI target interval. |
| Histogram of unique UMIs per fragment position | Number of positions with zero UMI sequences, one UMI sequence, two UMI sequences, etc. |
| Total families in probability model estimation | Total number of families used in estimation of UMI jumping rate and fragment size distribution used for probabilistic family merging. |
| Number of potential Jumping Families | Total number of families that are potential UMI jumping candidates and the corresponding ratio. |

# DRAGEN RNA Pipeline

DRAGEN includes an RNA-seq (splicing-aware) aligner, as well as RNA specific analysis components for gene expression quantification and gene fusion detection.




Most of the functionality and options described in Host Software Options and DNA Mapping also apply to RNA applications. Additional RNA-specific aspects are described in this section.

## Input Files

### Gene Annotation File

In addition to the standard input files (reads from fastq or bam, reference genome, etc.), DRAGEN can also take a gene annotations file as input. A gene annotations file aids in the alignment of reads to known splice junctions,and is required for gene expression quantification and gene fusion calling.

To specify a gene annotation file, use the *-a* (*--annotation-file*) command line option. The input file must conform to the GTF/GFF specification (http://uswest.ensembl.org/info/website/upload/gff.html). The file must contain features of type exon, and the record must contain attributes of type gene_id and transcript_id. An example of a valid GTF file is shown below.

```
chr1    HAVANA  transcript  11869   14409   .   +   .   gene_
id "ENSG00000223972.4"; transcript_id "ENST00000456328.2"; …
chr1    HAVANA  exon        11869   12227   .   +   .   gene_
id "ENSG00000223972.4"; transcript_id "ENST00000456328.2"; …
chr1    HAVANA  exon        12613   12721   .   +   .   gene_
id "ENSG00000223972.4"; transcript_id "ENST00000456328.2"; …
chr1    HAVANA  exon        13221   14409   .   +   .   gene_
id "ENSG00000223972.4"; transcript_id "ENST00000456328.2"; …
chr1    ENSEMBL transcript  11872   14412   .   +   .   gene_
id "ENSG00000223972.4"; transcript_id "ENST00000515242.2"; …
chr1    ENSEMBL exon        11872   12227   .   +   .   gene_
```

```
id "ENSG00000223972.4"; transcript_id "ENST00000515242.2"; …
chr1    ENSEMBL exon        12613   12721   .   +   .   gene_
id "ENSG00000223972.4"; transcript_id "ENST00000515242.2"; …
chr1    ENSEMBL exon        13225   14412   .   +   .   gene_
id "ENSG00000223972.4"; transcript_id "ENST00000515242.2"; …
…
```

Similarly, a GFF file can be used. Each exon feature must have as a Parent a transcript identifier that is used to group exons. An example of a valid GFF file is shown below.

```
1   ensembl_havana  processed_transcript    11869   14409
 .   +   .   ID=transcript:ENST00000456328;
1   havana          exon                    11869   12227
 .   +   .   Parent=transcript:ENST00000456328; …
1   havana          exon                    12613   12721
 .   +   .   Parent=transcript:ENST00000456328; …
1   havana          exon                    13221   14409
 .   +   .   Parent=transcript:ENST00000456328; …
…
```

The DRAGEN host software parses the file for exons within the transcripts and produces splice junctions. The following output displays the number of splice junctions detected.

```
============================================================
====
Generating annotated splice junctions
============================================================
====
Input annotations file: ./gencode.v19.annotation.gtf
Splice junctions database file:
output/rna.sjdb.annotations.out.tab

 Number of genes: 27459

 Number of transcripts: 196520
 Number of exons: 1196293
 Number of splice junctions: 343856
```

The splice junctions that are detected from the annotation file are also written to `*.sjdb.annotations.out.tab`. Splice junctions below a minimum length are excluded, which helps filter annotation artifacts that do not meet the minimum required length. This helps to lower the false detection rate for falsely annotated junctions. This minimum annotation splice junction length is controlled by the *--rna-ann-sj-min-len* option, which has a default value of 6.

### Two-Pass Splice Junction Alignment

Instead of using a GTF file for annotated splice junctions, the DRAGEN software is also capable of reading in an `SJ.out.tab` file (see *SJ.out.tab* on page 217). This file enables DRAGEN to run in a two-pass mode, where the splice junctions discovered in the first pass (output as SJ.out.tab file) are used to guide the mapping and alignment reads during a second run through DRAGEN. This mode of operation is useful to increase sensitivity for spliced alignments in cases when a gene annotations file is not readily available for the target genome.

## RNA Alignment

The DRAGEN RNA pipeline uses the DRAGEN RNA-Seq spliced aligner. Mapping of short seed sequences from RNA-Seq reads is performed similarly to mapping DNA reads. In addition, splice junctions (the joining of noncontiguous exons in RNA transcripts) near the mapped seeds are detected and incorporated into the full read alignments.

## Alignment Output

The output files generated when running DRAGEN in RNA mode are similar to those generated in DNA mode. RNA mode also produces extra information related to spliced alignments. Details regarding the splice junctions are present both in the SAM alignment record and an additional file, the SJ.out.tab file.

### BAM

The output BAM file meets the SAM specification and is compatible with downstream RNA-Seq analysis tools.

### RNA-Seq BAM Tags

The following BAM tags are emitted alongside spliced alignments.

- **XS:A**—The XS tag denotes the strand orientation of an intron. See *Compatibility with Cufflinks* on page 217.
- **jM:B**—The jM tag lists the intron motifs for all junctions in the alignments. It has the following definitions:
  - 0: non-canonical
  - 1: GT/AG

- 2: CT/AC
- 3: GC/AG
- 4: CT/GC
- 5: AT/AC
- 6: GT/AT

If a gene annotations file is used during the map/align stage, and the splice junction is detected as an annotated junction, then 20 is added to its motif value.

**NH:i**—A standard SAM tag indicating the number of reported alignments that contains the query in the current record. This tag may be used for downstream tools such as featureCounts.

**HI:i**—A standard SAM tag denoting the query hit index, with its value indicating that this alignment is the *i*-th one stored in the SAM. Its value ranges from 1 ... NH. This tag may be used for downstream tools such as featureCounts.

## Compatibility with Cufflinks

Cufflinks might require spliced alignments to emit the XS:A strand tag. This tag is present in the SAM record if the alignment contains a splice junction. The values for XS:A strand tag are as follows:

'.' (undefined), '+' (forward strand), '-' (reverse strand), or '*' (ambiguous).

If the spliced alignment has an undefined strand or a conflicting strand, then the alignment can be suppressed by setting the *--no-ambig-strand* option to 1.

Cufflinks also expects that the MAPQ for a uniquely mapped read is a single value. This value is specified by the *--rna-mapq-unique* option. To force all uniquely mapped reads to have a MAPQ equal to this value, set *--rna-mapq-unique* to a nonzero value.

## SJ.out.tab

Along with the alignments emitted in the SAM/BAM file, an additional SJ.out.tab file summarizes the high confidence splice junctions in a tab-delimited file. The columns for this file are as follows:

1. contig name
2. first base of the splice junction (1-based)
3. last base of the splice junction (1-based)strand (0: undefined, 1: +, 2: -)
4. strand (0: undefined, 1: +, 2: -)
5. intron motif: 0: noncanonical, 1: GT/AG, 2: CT/AC, 3: GC/AG, 4: CT/GC, 5: AT/AC, 6: GT/AT
6. 0: unannotated, 1: annotated, only if an input gene annotations file was used
7. number of uniquely mapping reads spanning the splice junction
8. number of multimapping reads spanning the splice junction
9. maximum spliced alignment overhang

The maximum spliced alignment overhang (column 8) field in the SJ.out.tab file is the anchoring alignment overhang. For example, if a read is spliced as ACGTACGT----------ACGT, then the overhang is 4. For the same splice junction, across all reads that span this junction, the maximum overhang is reported. The maximum overhang is a confidence indicator that the splice junction is correct based on anchoring alignments.

There are two SJ.out.tab files generated by the DRAGEN host software, an unfiltered version and a filtered version. The records in the unfiltered file are a consolidation of all spliced alignment records from the output SAM/BAM. However, the filtered version has a much higher confidence for being correct due to the use of the following filters.

A splice junction entry in the SJ.out.tab file is filtered out if *any* of these conditions are met:

- SJ is a noncanonical motif and is only supported by < 3 unique mappings.
- SJ of length > 50000 and is only supported by < 2 unique mappings.
- SJ of length > 100000 and is only supported by < 3 unique mappings.
- SJ of length > 200000 and is only supported by < 4 unique mappings.
- SJ is a noncanonical motif and the maximum spliced alignment overhang is < 30.
- SJ is a canonical motif and the maximum spliced alignment overhang is < 12.

The filtered SJ.out.tab is recommended for use with any downstream analysis or post processing tools. Alternatively, you can use the unfiltered SJ.out.tab and apply your own filters (for example, with basic awk commands).

Note that the filter does not apply to the alignments present in the BAM or SAM file.

## Mapping Metrics

The RNA Pipeline reports summary and per read group statistics pertaining to read mapping in the `mapping_metrics.csv file`. The metrics calculation accounts for spliced alignments in RNA. The following are some example metrics, Insert length: median, Supplementary (chimeric) alignments, etc.

## Chimeric.out.junction File

If there are chimeric alignments present in the sample, then a supplementary Chimeric.out.junction file is also output. This file contains information about split-reads that can be used to perform downstream gene fusion detection. Each line contains one chimerically aligned read. The columns of the file are as follows:

1. Chromosome of the donor.
2. First base of the intron of the donor (1-based).
3. Strand of the donor.
4. Chromosome of the acceptor.
5. First base of the intron of the acceptor (1-based).
6. Strand of the acceptor.

7. N/A—not used, but is present to be compatible with other tools. It will always be 1.
8. N/A—not used, but is present to be compatible with other tools. It will always be *.
9. N/A—not used, but is present to be compatible with other tools. It will always be *.
10. Read name.
11. First base of the first segment, on the + strand.
12. CIGAR of the first segment.
13. First base of the second segment.
14. CIGAR of the second segment.

CIGARs in this file follow the standard CIGAR operations as found in the SAM specification, with the addition of a gap length L that is encoded with the operation p. For paired end reads, the sequence of the second mate is always reverse complemented before determining strandedness.

The following is an example entry that shows two chimerically aligned read pairs, in which one of the mates is split, mapping segments of chr19 to chr12. Also shown are the corresponding SAM records associated with these entries.

```
chr19 580462 + chr12 120876182 + 1 * * R_15448 571532
49M8799N26M8p49M26S 120876183 49H26M
chr19 580462 + chr12 120876182 + 1 * * R_15459 571552
29M8799N46M8p29M46S 120876183 29H46M

R_15448:1   99    chr19   571531      60  49M8799N26M  =
580413
R_15448:2   147   chr19   580413      60  49M26S       =
571531
R_15448:2   2193  chr12   120876182   15  49H26M       chr19
571531

R_15459:1   99    chr19   571551      60  29M8799N46M  =
580433
R_15459:2   147   chr19   580433      4   29M46S       =
571551
R_15459:2   2193  chr12   120876182   15  29H46M       chr19
571551
```

## RNA Alignment Options

The aligner stage of the RNA spliced aligner uses Smith-Waterman Alignment Scoring options and Splicing Scoring Options.

## Smith-Waterman Alignment Scoring Options

Refer to *Smith-Waterman Alignment Scoring Settings* on page 1 for more details about the alignment algorithm used within DRAGEN. The following scoring options are specific to the processing of canonical and noncanonical motifs within introns.

- *--Aligner.intron-motif12-pen*

  The *--Aligner.intron-motif12-pen* option controls the penalty for canonical motifs 1/2 (GT/AG, CT/AC). The default value calculated by the host software is 1*(match-score + mismatch-pen).

- *--Aligner.intron-motif34-pen*

  The *--Aligner.intron-motif34-pen* option controls the penalty for canonical motifs 3/4 (GC/AG, CT/GC). The default value calculated by the host software is 3*(match-score + mismatch-pen).

- *--Aligner.intron-motif56-pen*

  The *--Aligner.intron-motif56-pen* option controls the penalty for canonical motifs 5/6 (AT/AC, GT/AT). The default value calculated by the host software is 4*(match-score + mismatch-pen).

- *--Aligner.intron-motif0-pen*

  The *--Aligner.intron-motif0-pen* option controls the penalty for noncanonical motifs. The default value calculated by the host software is 6*(match-score + mismatch-pen).

## Splicing Scoring Options

- *--Mapper.min-intron-bases*

  For RNA-Seq mapping, a reference alignment gap can be interpreted as a deletion or an intron. In the absence of an annotated splice junction, the min-intron-bases option is a threshold gap length separating this distinction. Reference gaps at least this long are interpreted and scored as introns, and shorter reference gaps are interpreted and scored as deletions. However, alignments can be returned with annotated splice junctions shorter than this threshold.

- *--Mapper.max-intron-bases*

  The max-intron-bases option controls the largest possible intron that is reported, which useful for preventing false splice junctions that would otherwise be reported. Set this option to a value that is suitable to the species you are mapping against.

- *--Mapper.ann-sj-max-indel*

  For RNA-seq, seed mapping can discover a reference gap in the position of an annotated intron, but with slightly different length. If the length difference does not exceed this option, the mapper investigates the possibility that the intron is present exactly as annotated, but an indel on one side or the other near the splice junction explains the length difference. Indels longer than this option and very near annotated splice junctions are not likely to be detected. Higher values may increase mapping time and false detections.

# Duplicate Marking

DRAGEN RNA can detect duplicate reads, which are defined as fragments that have both ends mapping to the same (clipping-adjusted) position during alignment. In RNA-Seq data, the reads can represent PCR duplicates during library prep or as a result from deep coverage of highly expressed regions. If *--enable-duplicate-marking* is set to true, duplicate fragments are marked in the BAM file and the total number of duplicate reads is reported as a mapping metric. Marking of duplicates does not affect gene expression quantification and gene fusion calling.

# MAPQ Scoring

By default, the MAPQ calculation for RNA-Seq is identical to DNA-Seq. The primary contributor to MAPQ calculation is the difference between the best and second-best alignment scores. Therefore, adjusting the alignment scoring parameters impacts the MAPQ estimate. These adjustments are outlined in *Smith-Waterman Alignment Scoring Settings* on page 1.

The *--mapq-strict-sjs* option is specific to RNA, and applies where at least one exon segment is aligned confidently, but there is ambiguity regarding possible splice junctions. When this option is set to 0, a higher MAPQ value is returned, expressing confidence that the alignment is at least partially correct. When this option is set to 1, a lower MAPQ value is returned, reflecting the splice junction ambiguity.

Some downstream tools, such as Cufflinks, expect the MAPQ value to be a unique value for all uniquely mapped reads. This value is specified with the *--rna-mapq-unique* option. Setting this option to a nonzero value overrides all MAPQ estimates based on alignment score. Instead, all uniquely mapped reads have a MAPQ set to the value of *--rna-mapq-unique*. All multimapped reads have a MAPQ value of int(-10*log10(1 - 1/NH), where the NH value is the number of hits (primary and secondary alignments) for that read.

# Gene Fusion Detection

The DRAGEN Gene Fusion module uses the DRAGEN RNA spliced aligner for detection of gene fusion events. It performs a split-read analysis on the supplementary (chimeric) alignments to detect potential breakpoints. The putative fusion events then go through various filtering stages to mitigate potential false positives. In addition to the final results, all potential candidates (unfiltered) are output, which can be used to maximize sensitivity.

## Running DRAGEN Gene Fusion

The following is an example command line for running an end to end RNA-Seq experiment.

```
/opt/edico/bin/dragen \
-r <HASHTABLE> \
-1 <FASTQ1> \
-2 <FASTQ2> \
-a <GTF_FILE> \
```

```
--output-dir <OUT_DIRECTORY> \
--output-file-prefix <PREFIX> \
--RGID <READ_GROUP_ID> \
--RGSM <Sample_NAME> \
--enable-rna true \
--enable-rna-gene-fusion true
```

At the end of a run, a summary of detected gene fusion events is output, which is similar to the following example.

```
================================================================
Loading gene annotations file
================================================================
  Input annotations file: ref_annot.gtf
  Number of genes: 27459
  Number of transcripts: 196520
  Number of exons: 1196293

================================================================
Launching DRAGEN Gene Fusion Detection
================================================================
annotation-file:            ref_annot.gtf
rna-gf-blast-pairs:         blast_pairs.outfmt6
rna-gf-exon-snap:           50
rna-gf-min-anchor:          25
rna-gf-min-neighbor-dist:   15
rna-gf-max-partners:        3
rna-gf-min-score-ratio:     0.15
rna-gf-min-support:         2
rna-gf-min-support-be:      10
rna-gf-restrict-genes       true

================================================================
Completed DRAGEN Gene Fusion Detection
================================================================
Chimeric alignments: 107923
Total fusion candidates: 38 (2116 before filters)

Time loading annotations:                              00:00:08.543
Time running gene fusion:                              00:00:18.470
Total runtime:                                         00:00:27.760
**********************************************************
DRAGEN finished normally
```

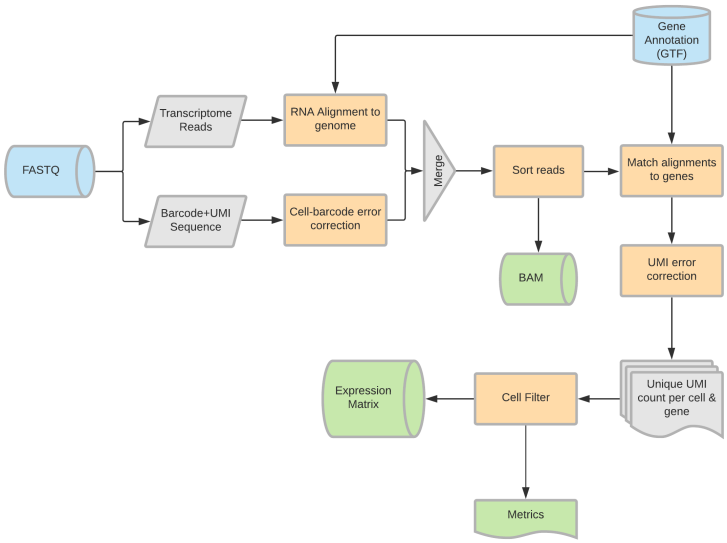
# Gene Expression Quantification

The DRAGEN RNA pipeline contains a gene expression quantification module, that estimates the expression of each transcript and gene in an RNA-seq dataset. First, it internally translates the genomic mapping of each read (read pair) to the corresponding transcript mappings. Then it uses an Expectation-Maximization (EM) algorithm to infer the transcript expression values that best match all the observed reads. The EM algorithm can also model GC-bias and correct for it in the reported quantification results.

# DRAGEN Single-Cell RNA Pipeline

The DRAGEN Single-Cell RNA (scRNA) Pipeline can process multiplexed single-cell RNA-Seq data sets from reads to a cell-by-gene UMI count gene expression matrix. The pipeline is compatible with library designs that have one read in a fragment match to a transcript and the other containing a cell-barcode and UMI. The pipeline includes the following functions:

- RNA-Seq (splice-aware) alignment and matching to annotated genes for the transcript reads.
- Cell-barcode and UMI error correction for the barcode read.
- UMI counting per cell and gene to measure gene expression.
- Sparse matrix output and QC metrics.




The functionality and options related to alignment and gene annotation are identical to the RNA-Seq pipeline. For information, see *DRAGEN RNA Pipeline* on page 214. Other RNA-Seq modules, such as gene fusion calling or transcript-level gene expression quantification are not supported for Single-Cell RNA.

## Input Files

### Alignment Reference

Use a standard DRAGEN RNA reference genome or hashtable for the DRAGENscRNA Pipeline. For example, build using `--ht-build-rna-hashtable=true`). The pipeline also requires a gene annotation file in GTF format, provided with the *--annotation* (*-a*) option.

### Read Input

The DRAGEN scRNA Pipeline requires both the transcript sequence and the barcode+UMI sequence for each fragment (read) as input. The transcript sequence is aligned to the genome to determine the expressed gene, the barcode+UMI sequence is split into the single-cell barcode to identify the unique cell, and a single-cell UMI for unique molecule quantification.

When starting from FASTQ, you can either include the UMI in the read name or provide separate UMI FASTQ files.

### UMI in Read Name

Provide the transcriptome reads as a single-end FASTQ file with the Barcode+UMI sequence in the eighth field of the read-name line. Separate sequences using a colon. The following example uses read2 (`sample.R2.fastq.gz`) as the transcript read.

```
@D00626:253:CAT5WANXX:4:1302:8433:85343:GAAACTCGTTCAGCGCTCATCTCGGC
ACAGGTCAGCTGGGAGCTTCTGCCCCCACTGCCTAGGGACCAACAGGGGCAGGAGGCAGTCACTGACCCCGAGA
AGTTTGCATCCTGCACAGCTAGAGATC
+
CCCCBFGGGGGGGGGGGGGGGGGGBDCGGGE>1BGDFGG0F@FBGGGBCDGGGGGGGGGGGGGGGGGGGGGGD
GGGGGGGGGGGGGFGGGGFGEGGGGGE
```

In the example, the GAA sequence is the barcode+UMI read and the ACAG sequence is the transcriptome read.

These FASTQ files can be generated by bclConvert and bcl2fastq using the UMI settings to define the single-cell barcode+UMI read. If using bclConvert, enter the barcode/UMI information using the `OverrideCycles` setting. For more information, see the *BCL Convert Software Guide (document # 1000000094725)*.

bclConvert refers to the entire single-cell barcode+UMI sequence as UMI.

Enter the following command line option to use the generated FASTQ files from bclConvert:

```
dragen -1 <file name> --umi-source=qname
```

The option is also compatible with the `--fastq-list` input options and with read input from BAM files.

### Separate UMI FASTQ Files

An alternative option is to provide the transcript and barcode+UMI sequences as two separate FASTQ files. One file contains only the transcriptome reads and one contains the corresponding barcode-reads in the same order. This file is similar to how read-pairs are normally handled. If using separate UMI files, the sequencing system run setup and bclConvert are not aware of the UMI at all and treat it as normal read sequence by default.

Enter the following command line option to use the separate UMI FASTQ files:

```
dragen -1 <file name> --umi-fastq=<file name> --umi-source=fastq
```

To use this method with multiple FASTQ files, do as follows.

1. Enter the barcode+UMI FASTQ files as read1 in the `fastq-list` file, and then enter the transcriptome read FASTQ files matching the default `fastq_list.csv` generated by bclConvert as read2.
2. Enter the following command:

```
dragen --fastq-list fastq_list.csv --umi-source=read1
```

### Using Multiple Libraries

The scRNA pipeline can process a single biological sample per DRAGEN run. To process multiple single-cell libraries together, split the single sample into multiple single-cell libraries with a unique set of cells in each DRAGEN keeps the cells (barcodes and UMIs) from each library separate and provides merged outputs across all. Read groups are used to specify the library for each FASTQ file using the RGLB attribute.

## Single-Cell RNA Settings

To use the scRNA workflow, enter `--enable-rna=true --enable-single-cell-rna=true`. This section includes information on additional scRNA settings.

### Barcode Options

By default the scRNA workflow assumes that the overall barcode/UMI sequence is made up of a single-cell barcode (possibly split into multiple blocks) and a single UMI. Enter the following command to identify the location of the single-cell barcode and single-cell UMI in the barcode read:

```
--single-cell-barcode-position <blockPos>[+<blockPos2>+<blockPos3>...] --
single-cell-umi-position <blockPos>
```

blockPos describes the offset of the first and last inclusive base of the block and is formatted as `<startPos>_<endPos>`. For example for a library with a 16 bp cell-barcode followed by a 10 bp UMI, enter: `--single-cell-barcode-position 0_15 --single-cell-umi-position 16_25`. For a

library with the cell-barcode split into three blocks of 9 bp separated by fixed linker sequences and an 8 bp UMI, enter `--single-cell-barcode-position=0_8+21_29+43_51 --single-cell-umi-position=52_59`.

## Cell filtering

DRAGEN uses a threshold on the number of unique UMIs per cell barcode to determine which barcodes likely correspond to single-cells in the original sample from background noise. The threshold is determined based on the distribution of UMIs per barcode and an expected number of true cells in the sample. For more information on how cell filtering is performed, see *Cell Filtering* on page 227 .

- *single-cell-number-cells*—**[Optional]** Set the expected number of cells. The default is 3000. Adjust only if the number of cells is far from the default.
- *single-cell-threshold*— Set the UMI count threshold value or use the value `fixed`. If using fixed, the UMI count threshold is set so that the expected number of cells pass, rather than estimated dynamically. The exact number of passing cells might be slightly larger than the number of single-cells because of the connection between the UMI count and the different cell barcodes.

To set a specific number of cells ahead of time, use the following command:

```
--single-cell-threshold=fixed --single-cell-number-cells=X
```

The command forces the UMI threshold value to pass the top X cells and any extra cells with the same number of unique UMIs.

## Additional Options

The following are additional options you can use to configure the Single-Cell RNA Pipeline settings.

- *rna-libary-type*—Set the orientation of transcript reads relative to the genomes. Enter `SF` for forward, `SR` for reverse, or `U` for unstranded. The default is SF.
- *single-cell-count-introns*—Include intronic reads in gene expression estimation. The default false.
- *qc-enable-depth-metrics*—Set to `false` to disable depth metrics for faster run times. The default is true.
- *bypass-anchor-mapping*—Set to `true` to disable RNA anchor (two-pass) mapping for increased performance. The default false.

## Command-line Example

The following is an example command line to run the DRAGEN Single Cell RNA Pipeline.

```
dragen --enable-rna=true --enable-single-cell-rna=true --umi-source=fastq
--single-cell-barcode 0_15 --single-cell-umi 16_25 -r reference_
genomes/Mus_musculus/mm10/DRAGEN/8 -a reference_genomes/Mus_
```

```
musculus/mm10/gtf/gencode.vM23.annotation.gtf.gz -1 lib1_S7_L001_R2_
001.fastq.gz --umi-fastq lib1_S7_L001_R1_001.fastq.gz --RGID=1 --
RGSM=sample1 --output-dir=/staging/out --output-file-prefix=sample1
```

## Cell Filtering

DRAGEN uses a UMI count threshold to separate cell-barcodes corresponding to single-cells in the original sample from background noise. Cell-barcodes with UMI counts beneath the threshold are considered background noise. To set the threshold, DRAGEN sorts all cell-barcodes by their UMI count in descending order and then determines the counts of the following two reference cell-barcodes:

- **topCount**—The maximum corresponding to the count of the cell-barcode at the first percentile of the UMI count distribution.
- **minCount**—An expected minimum corresponding to the count of the last expected cell. The minCount is represented as the _N_th cell, where N is the number of expected cells.

The final threshold is then set as `max(topCount * 0.1, minCount * 0.5`.

## Barcode Error Correction

Cell-barcode sequences from the input reads are error corrected based on the frequency with which each one is seen and an optional whitelist of expected cell-barcode sequences. A cell-barcode sequence is considered a neighbor of another cell-barcode if there is at most one mismatch. A cell-barcode sequence is corrected to its neighbor in the following circumstances. When corrected, all reads with the cell-barcode are assigned instead to the neighboring cell-barcode. The sequence error correction scheme is similar to the directional algorithm described in (Smith, Heger and Sudbery, 2020)[1].

- The neighboring cell-barcode is at least two times more frequent across all input reads.
- The neighboring cell-barcode is on the cell-barcode whitelist, but the original cell-barcode is not.

To avoid overcounting UMIs based on sequence errors, UMI error correction is performed among all reads with the same cell-barcode mapping to the same gene. UMI sequences that are likely errors of another UMI are not counted.

[1]Smith, T., Heger, A. and Sudbery, I., 2020. UMI-Tools: Modeling Sequencing Errors In Unique Molecular Identifiers To Improve Quantification Accuracy. [PDF] Cold Spring Harbor Laboratory Press. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5340976/> [Accessed 15 October 2020].

## Outputs

Single-cell RNA outputs are found in the standard DRAGEN output location using the prefix scRNA.

### Counts

The following three files provide information per-cell gene expression level in matrix market (*.mtx)

format:

- `<prefix>.scRNA.matrix.mtx`
  Count of unique UMIs for each cell/gene pair in sparse matrix format.
- `<prefix>.scRNA.barcodes.tsv`
  Cell-barcode sequence for each cell from the matrix.
- `<prefix>.scRNA.genes.tsv`
  Gene name and ID for each gene in the matrix. This includes all cell barcodes. The subset corresponding to passing cells can be found under the Filter column in `scRNA.barcodeSummary.tsv`.

## Alignments

Alignments of the transcript reads are sorted by coordinate and output as a BAM file. Each alignment is annotated with an `XB` tag containing the cell barcode and an `RX` tag containing the UMI. The alignments use the original sequences without any errors corrected. Fragments that did not have an associated barcode read, for example fragments trimmed on the input data, do not XB and RX tags.

## Overall Metrics

The `<prefix>.scRNA.metrics.csv` file contains per sample scRNA metrics.

## Barcode Read Metrics

| Metric | Description |
|---|---|
| Invalid barcode read | Overall barcode sequence (cell barcode + UMI) failed basic checks. For example, the barcode read was missing or too short. |
| Error free cell-barcode | Reads with cell-barcode sequences that were not altered during error correction. For example, if the read was an exact match to the whitelist. |
| Error corrected cell-barcode | Reads with cell-barcode sequences successfully corrected to a valid sequence. |
| Filtered cell-barcode | Reads with cell-barcode sequences that could not be corrected to a valid sequence. For example, the sequence does not match whitelist with at most one mismatch. |

## Transcript Read Metrics

| Metric | Description |
|---|---|
| Unique exon match | Reads match to exons of exactly one gene. |
| Unique intron match | Reads do not match exons, but introns of exactly one gene. For example, if using the command `--single-cell-count-introns=true`. |
| Ambiguous match | Reads match to multiple genes. |
| Wrong strand | Reads overlap a gene on the opposite strand defined by library type. |
| Mitochondrial reads | Reads map to the mitochondrial example, if there is a matching gene. |
| No gene | Reads do not match to any gene. Includes intronic reads unless using `--single-cell-count-introns=true`. |
| Filtered multimapper | Reads excluded due to multiple alignment positions in the genome. |

## UMI Count Metrics)

| Metric | Description |
|---|---|
| Total counted reads | Reads with valid cell-barcode and UMI that match a unique gene. |
| Reads with error-corrected UMI | Counted reads where the UMI was error-corrected to match another similar UMI sequence. |
| Reads with invalid UMI | Reads that were not counted due to invalid UMI sequence. For example, pure homopolymer reads or reads containing Ns. |
| Sequencing saturation | Fraction of reads with duplicate UMIs. 1 - (UMIs / Reads). |
| Unique cell-barcodes | Overall number of unique cell-barcode sequences in counted reads only. |
| Unique UMIs | Overall number of unique cell-barcode and UMI combinations counted. |

## Cell Metrics

| Metric | Description |
|---|---|
| UMI threshold for passing cells | Number of UMIs required for a cell-barcode to pass filtering. |
| Passing cells | Number of cell-barcodes that passed. |
| Fraction genic reads in cells | Counted reads assigned to cells that passed. |
| Median reads per cells | Total counted reads per cell that passed the filters. |
| Median UMIs per cells | Total counted UMIs per cell that passed the filters. |
| Median genes per cells | Genes with at least one UMI per cell that passed the filters. |
| Total genes detected | Genes with at least one UMI in at least one cell that passed the filters. |

## Per-Cell Metrics

The `<prefix>.scRNA.barcodeSummary.tsv` contains summary statistics for each unique cell-barcode per cell after error correction.

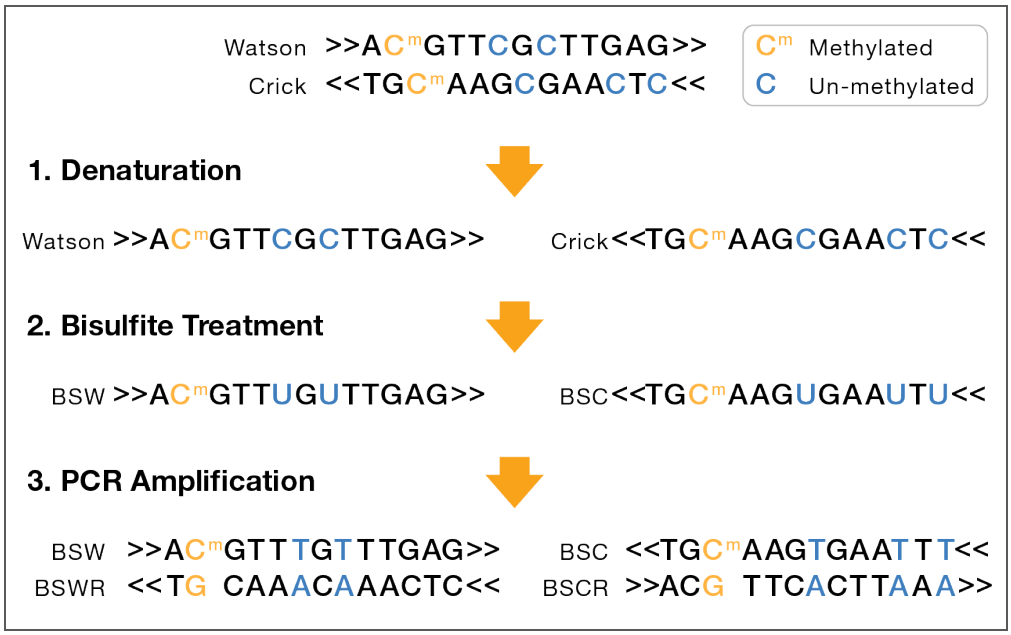
| Metric | Description |
|---|---|
| ID | Unique numeric ID for the cell-barcode. The ID matches the line in UMI count matrix (*.mtx) output. |
| Barcode | The cell-barcode sequence. |
| TotalReads | Total reads with the cell-barcode sequence. This includes error corrected reads. |
| GeneReads | Reads counted towards a gene. |
| UMIs | Total number of UMIs in counted reads. |
| Genes | Unique genes detected. |
| MitochondrialReads | Reads mapped to mitochondrial genome. |
| Filter | The following are the available filter values:<br>• PASS—Cell-barcode passes the filter.<br>• LOW—UMI count is below the threshold. |

# DRAGEN Methylation Pipeline

The epigenetic methylation of cytosine bases in DNA can have a dramatic effect on gene expression, and bisulfite sequencing is the gold standard for detecting epigenetic methylation patterns at single-base resolution. This technique involves chemically treating DNA with sodium bisulfite, which converts unmethylated cytosine bases to uracil, but does not alter methylated cytosines. Subsequent PCR amplification converts any uracils to thymines.

A bisulfite sequencing library can either be nondirectional or directional. For nondirectional, each double-stranded DNA fragment yields four distinct strands for sequencing, post-amplification, as shown in the following figure:

Figure 15 Nondirectional Bisulfite Sequencing




- Bisulfite Watson (BSW), reverse complement of BSW (BSWR),
- Bisulfite Crick (BSC), reverse complement of BSC (BSCR)

For directional libraries, the four strand types are generated, but adapters are attached to the DNA fragments such that only the BSW and BSC strands are sequenced (Lister protocol). Less commonly, the BSWR and BSCR strands are selected for sequencing (eg, PBAT).

BSW and BSC strands:

- A, G, T: unchanged
- Methylated C remains C
- Unmethylated C converted to T

BSWR and BSCR strands:

- Bases complementary to original Watson/Crick A, G, T bases remain unchanged

- G complementary to original Watson/Crick methylated C remains G
- G complementary to original Watson/Crick unmethylated C becomes A

Standard DNA sequencing is used to produce sequencing reads. Reads containing more methylated C's or G's complementary to methylated C's are less drastically affected by the bisulfite treatment, and have a higher likelihood of mapping to the reference than reads with more bases affected. The standard protocol to minimize this mapping bias is to perform multiple alignments per read, where specific combinations of read and reference genome bases are converted *in-silico* prior to each alignment run. Each alignment run has a set of constraints and base-conversions that corresponds to one of the bisulfite+PCR strand types expected from the protocol. By comparing the per read (pair) alignments across runs, DRAGEN determines the best alignment, and most likely, strand type for each read or read pair. DRAGEN can then use the alignments and strands for downstream methylation calling.

# DRAGEN Methylation Calling

Different methylation protocols require the generation of two or four alignments per input read, followed by an analysis to choose a best alignment and determine which cytosines are methylated. DRAGEN can automate this process, generating a single output BAM file with Bismark-compatible tags (XR, XG, and XM) that can be used for methylation calling and other downstream workflow.

When the *--methylation-protocol* option is set to a valid value other than none, DRAGEN automatically produces the required set of alignment runs, each with the appropriate base conversions on the reads, base conversions on the reference, and constraints on whether reads must be forward-aligned or reverse complement (RC) aligned with the reference.

The following options are automatically configured.

- --preserve-map-align-order true \
- --generate-md-tags true \
- --Aligner.global 1 \
- --Aligner.no-unpaired 1 \
- --Aligner.aln-min-score 0 \
- --Aligner.min-score-coeff -0.2 \
- --Aligner.match-score 0 \
- --Aligner.mismatch-pen 4 \
- --Aligner.gap-open-pen 6 \
- --Aligner.gap-ext-pen 1 \
- --Aligner.supp-aligns 0 \
- --Aligner.sec-aligns 0

Because global alignments (end-to-end in the reads) are generated, DRAGEN recommends trimming any artifacts introduced by library prep and adapter sequences.

When *--enable-methylation-calling* is set to true, DRAGEN analyzes the multiple alignments to produce a single methylation-tagged BAM file. When *--enable-methylation-calling* is set to false, DRAGEN outputs a separate BAM file per alignment run.

The following table describes these alignment runs:

| Protocol | BAM | Reference | Read 1 | Read 2 | Orientation Constraint |
|---|---|---|---|---|---|
| **directional** | | | | | |
| | 1 | C->T | C->T | G->A | Forward-only |
| | 2 | G->A | C->T | G->A | RC-only |
| **nondirectional, or directional-complement** | | | | | |
| | 1 | C->T | C->T | G->A | Forward-only |
| | 2 | G->A | C->T | G->A | RC-only |
| | 3 | C->T | G->A | C->T | RC-only |
| | 4 | G->A | G->A | C->T | Forward-only |
| **PBAT** | | | | | |
| | 3 | C->T | G->A | C->T | RC-only |
| | 4 | G->A | G->A | C->T | Forward-only |

In directional protocols, the library is prepared such that only the BSW and BSC strands are sequenced. Thus, alignment runs are performed with the two combinations of base conversions and orientation constraints best suited for these strands (directional runs 1 and 2 above).

With nondirectional protocols, reads from each of the four strands are equally likely, so alignment runs must be performed with two more combinations of base conversions and orientation constraints (nondirectional runs 3 and 4 above).

In PBAT protocols, the library is prepared so only the BSWR and BSCR strands are sequenced. Only two alignment runs are performed with the combinations of base conversions and orientation constraints best suited for these strands (runs 3 and 4).

The directional-complement protocol can also be used for PBAT or similar libraries where mainly the BSWR and BSCR strands are sequenced. With this protocol, all four aligner runs are performed, but relatively few good alignments are expected from the runs for the BSW and BSC strands, so DRAGEN is automatically tuned to a faster analysis mode for those runs.

For any protocol, you must use a reference hash table that was produced with *--ht-methylated true*. For more information, see *Pipeline Specific Hash Tables* on page 21.

The following is an example DRAGEN command line for the directional protocol:

```
dragen --enable-methylation-calling true \
--methylation-protocol directional \
--ref-dir /staging/ref/mm10/methylation --RGID RG1 --RGCN CN1 \
--RGLB LIB1 --RGPL illumina --RGPU 1 --RGSM Samp1 \
--intermediate-results-dir /staging/tmp \
-1 /staging/reads/samp1_1.fastq.gz \
-2 /staging/reads/samp1_2.fastq.gz \
--output-directory /staging/outdir \
--output-file-prefix samp1_directional_prot
```

## Methylation-Related BAM Tags

When *--enable-methylation-calling* is set to true, DRAGEN analyzes the alignments produced for the configured *--methylation-protocol*, and generates a single output BAM file that includes methylation-related tags for all mapped reads. As in Bismark, reads without a unique best alignment are excluded from the output BAM. The added tags are as follows:

| Tag | Brief Description | Description |
|---|---|---|
| XR:Z | Read conversion | For the best alignment, which base-conversion was performed on the read: CT or GA. |
| XG:Z | Reference conversion | For the best alignment, which base-conversion was performed on the reference: CT or GA |
| XM:Z | Methylation call | A byte-per-base methylation string. |

The XM:Z (methylation call) tag contains a byte corresponding to each base in the read's sequence. There is a period ('.') for each position not involving a cytosine, and a letter at cytosine positions. The letter indicates the context (CpG, CHG, CHH, or unknown). The case indicates methylation, with upper-case positions being methylated and lower-case unmethylated. The letters used at cytosine positions are as follows:

| Character | Methylated? | Context |
|---|---|---|
| . | not cytosine | not cytosine |
| z | No | CpG |
| Z | Yes | CpG |
| X | No | CHG |
| X | Yes | CHG |

| Character | Methylated? | Context |
|---|---|---|
| h | No | CHH |
| H | Yes | CHH |
| u | No | Unknown |
| U | Yes | Unknown |

## Methylation Cytosine and M-Bias Reports

To generate a genome-wide cytosine methylation report, set *--methylation-generate-cytosine-report* to true. The position and strand of each C in the genome are given in the first three fields of the report. A record with a '-' in the strand field is for a G in the reference FASTA. The counts of methylated and unmethylated C's covering the position are given in the fourth and fifth fields, respectively. The C-context in the reference (CG, CHG, or CHH) is given in the sixth field, and the trinucleotide sequence context is given in the last field (eg, CCC, CGT, CGA, etc). The cytosine report only includes records forThe following is an example cytosine report record:

```
chr2    24442367    +    18    0    CG    CGC
```

To generate an M-bias report, set *--methylation-generate-mbias-report* to true. This report contains three tables for single-ended data with one table for each C-context, and six tables for paired-end data. Each table is a series of records, with one record per read base position. For example, the first record for the CHG table contains the counts of methylated C's (field 2) and unmethylated C's (field 3) that occur in the first read base position, restricting to those reads in which the first base is aligned to a CHG location in the genome. Each record of a table also includes the percent methylated C bases (field 4) and the sum of methylated and unmethylated C counts (field 5).

The following is an example M-bias record for read base position 10:

```
10    7335    2356    75.69    9691
```

For data sets with paired-end reads that overlap, both the Cytosine and M-bias reports skip reporting any C's in the second read that overlaps the first read. In addition, 1-based coordinates are used for positions in both reports.

To match the bismark_methylation_extractor cytosine and M-bias reports generated by Bismark version 0.19.0, set the *--methylation-match-bismark* option to true. The ordering of records in Bismark and DRAGEN cytosine reports may differ. DRAGEN reports are sorted by genomic position.

## Using Bismark for Methylation Calling

The recommended approach to methylation calling is to automate the multiple required alignment runs, and add the XM, XR, XG tags. To generate a separate BAM file for each of the constraints and conversions needed by the methylation protocol, set *--enable-methylation-calling* to false. The BAM files can be used as input into Bismark for methylation calling. Contact Illumina Technical Support for more information on this use case if desired.

In this mode, a single DRAGEN run produces multiple BAM files in the directory specified by *--output-directory*, each containing alignments in the same order as the input reads. It is not possible to enable sorting or duplicate marking for these runs. Alignments include MD tags and have /1 or /2 appended to the names of paired-end reads for Bismark-compatibility. The BAM files use the following naming conventions:

- Single-end reads—*output-directory/output-file-prefix*.{CT,GA}read{CT,GA}reference.bam
- Paired-end-reads—*output-directory/output-file-prefix*.{CT,GA}read1{CT,GA}read2 {CT,GA}reference.bam,

Where *output-directory* and *output-file-prefix* are specified by the corresponding options, and CT and GA correspond to the base conversions listed in the table above.

Bismark does not have a directional-complement mode, but you can process such samples using Bismark's nondirectional mode with the expectation that runs 1 and 2 produce very few good alignments.

## Using TAPS Support

TET-Assisted Pyridine Borane Sequencing (TAPS) is a new assay which directly converts methylated C to T, whereas typical bisulfite conversion converts unmethylated C to T. This approach preserves genomic complexity and uses less destructive chemicals to enable lower input DNA.

To enable analysis of FASTQ data generated through TAPS, set `--methylation-TAPS` to true. By default, the option is false. This option is performed only during the alignment step and is not necessary when generating methylation cytosine and M-Bias reports from an existing BAM.

## Sort and Duplicate Reads Options

DRAGEN supports sorting and duplicate marking/removal for methylated reads during the alignment phase. To enable sort and duplicate read options, perform two separate runs as follows.

1. Set the options for the first run as follows.
   - To generate sorted alignment output (in BAM format), set `--enable-sort` to true.
   - To detect duplicate reads, set `--enable-duplicate-marking` to true.
   - **[Optional]**To remove duplicate reads, set `--remove-duplicates` to true.
   - Set `--methylation-generate-cytosine-report` and `--methylation-generate-mbias-report` to false.

   These options behave the same as in DNA alignment. For example, if `--enable-duplicate-marking` is set to true, `--enable-sort` is true.
2. Set the options for the second run as follows.
   - Use the sort/markdup/dedup alignment output from the previous run for *-b/--bam-input*.
   - Set *--methylation-reports-only* to true.

- Set --*enable-sort* to false.
- To generate cytosine report, set --*methylation-generate-cytosine-report* to true.
- To generate mbias report, set --*methylation-generate-mbias-report* to true.

# DRAGEN BCL Data Conversion

BCL format is the native output format of Illumina sequencing systems and consists of a directory hierarchy containing data files and metadata. The data files are organized according to the flow cell layout of the sequencing system. The software converts this data to sample separated FASTQ files.

DRAGEN provides BCL conversion software that uses hardware acceleration on the DRAGEN platform, which results in improved run times compared to a pure software execution. To run the conversion software, use the *--bcl-input-directory <BCL_ROOT>*, *--output-directory <DIR>*, and *--bcl-conversion-only true* options.

The DRAGEN BCL conversion is designed to output FASTQ files that match bcl2fastq2 v2.20 output.

DRAGEN BCL conversion supports the following features.

- Demultiplexing samples by barcode with optional mismatch tolerance.
- Adapter sequence masking or trimming with adjustable matching stringency.
- UMI sequence tagging and optional trimming.
- Output of FASTQ files for index reads.
- **[Optional]** Combine all lanes to the same FASTQ output files.
- High sample count support (100,000).
- UMI sequences in index reads.
- Eliminate skew as the result of adapter sequence trimming by using the MinimumAdapterOverlap setting.
- Outputs metrics to detect index-hopping.

## Command Line Options

The following example command contains the required BCL conversion options:

```
dragen --bcl-conversion-only true --bcl-input-directory <...> --output-
directory <...>
```

The following additional options can be specified on the command line:

- *--sample-sheet*—Specifies the path to SampleSheet.csv file. *--sample-sheet* is optional if the SampleSheet.csv file is in the *--bcl-input-directory* directory.
- *--run-info*—Sets the path to the `RunInfo.xml` file. By default, the file is located in the *--bcl-input-directory* directory.
- *--strict-mode*—If set to true, DRAGEN aborts if any files are missing. The default is false.

- *--first-tile-only*—If set to true, DRAGEN only converts the first tile of input (for testing and debugging). The default is false.
- *--bcl-only-lane <#>*—Convert only the specified lane in this conversion run.
- *-f*—Convert to output directory even if the directory exists (force).
- *--bcl-use-hw false*—Do not use DRAGEN FPGA acceleration during BCL conversion. This allows concurrent execution of BCL conversion with DRAGEN analysis.
- *--bcl-sampleproject-subdirectories true*—Output FASTQ files to subdirectories based on sample sheet `Sample_Project` column.
- *--no-lane-splitting true*—Output all lanes of a flow cell to the same FASTQ files consecutively.
- *--bcl-only-matched-reads true*—Disable outputting unmapped reads to files marked as Undetermined.

The following additional options can be used to manually control performance. Use of these options might reduce performance or result in analysis failure. Contact Illumina Technical Support if issues occur.

- *--shared-thread-odirect-output true* —Switch to an alternate file output method that is optimized for sample counts greater than 100,000. This option is not recommended for lower sample counts and/or if using distributed file system targets such as GPFS or Lustre.
- *--bcl-num-parallel-tiles <#>*—Number of tiles processed in parallel. The default is determined dynamically.
- *--bcl-num-conversion-threads <#>*—Number of conversion threads per tile. The default is determined dynamically.
- *--bcl-num-compression-threads <#>*—Number of CPU threads for compressing FASTQ output. The default is determined dynamically.
- *--bcl-num-decompression-threads <#>*—Number of CPU threads for decompressing input BCL files. The default is determined dynamically.

The input BCL root directory and the output directory must be specified. The specified input path is three levels higher than the BaseCalls directory and should contain the `RunInfo.xml` file.

To specify the directory to store output FASTQ files, use the *--output-directory* option.

## Sample Sheet Options

In addition to the command line options that control the behavior of BCL conversion, you can use the [Settings] section in the sample sheet configuration file to specify how the samples are processed. The following are the sample sheet settings for BCL conversion.

DRAGEN does not support the following sample sheet settings from bcl2fastq

- FindAdapterWithIndels

- ReverseComplement

| Option | Default | Value | Description |
|---|---|---|---|
| AdapterBehavior | trim | trim, mask | Whether adapter should be trimmed or masked. |
| AdapterRead1 | None | Read 1 adapter sequence containing A, C, G, or T | The sequence to trim or mask from the end of Read 1. |
| AdapterRead2 | None | Read 2 adapter sequence containing A, C, G, or T | The sequence to trim or mask from the end of Read 2. |
| AdapterStringency | 0.9 | Float between 0.5 and 1.0 | The stringency for matching the read to the adapter using the sliding window algorithm. |

| Option | Default | Value | Description |
|---|---|---|---|
| BarcodeMismatchesIndex1 | 1 | 0, 1, or 2 | The number of allowed mismatches between the first Index Read and index sequence. |
| BarcodeMismatchesIndex2 | 1 | 0, 1, or 2 | The number of allowed mismatches between the second Index Read and index sequence. |
| MinimumTrimmedReadLength | The minimum of 35 and the shortest non-indexed read length. | 0 to the shortest non-indexed read length | Reads trimmed below this point become masked at that point. |

| Option | Default | Value | Description |
|---|---|---|---|
| MinimumAdapterOverlap | 1 | 1, 2, or 3 | Do not trim detected adapter sequences shorter than this value. |
| MaskShortReads | The minimum of 22 and MinimumTrimmedReadLength. | 0 to MinimumTrimmedReadLength | Reads trimmed below this point become masked out. |
| TrimUMI | 1 | 0 or 1 | If set to 0, UMI sequences are not trimmed from output FASTQ reads. The UMI is still placed in sequence header. |
| CreateFastqForIndexReads | 0 | 0 or 1 | If set to 1, output FASTQ files for index reads and genomic reads. |

| Option | Default | Value | Description |
|---|---|---|---|
| OverrideCycles | None | Y: Specifies a sequencing read<br>I: Specifies an indexing read<br>U: Specifies a UMI length to be trimmed from read | String used to specify UMI cycles and mask out cycles of a read. |

## Override Cycles

The OverrideCycles mask elements are semicolon separated. For example:

```
OverrideCycles,U7N1Y143;I8;I8;U7N1Y143
```

DRAGEN supports flexible UMI processing during BCL conversion to support more third-party assays, including UMI sequences in index reads and multiple UMI regions per read. UMI sequences are trimmed from FASTQ read sequences and placed in the sequence identifier for each read, as normal.

The following are examples of OverrideCycles settings using 2x151 reads:

| Setting | Description |
|---|---|
| OverrideCycles,U7N1Y143;I8;I8;U7N1Y143 | UMI is comprised of the first 7 bps of each genomic read, linked by 1 bps of ignored sequence. This is the format for Illumina nonrandom UMIs, used in the following products:<br>• TruSight Oncology 170 RUO<br>• TruSight Oncology 500 RUO<br>• IDT for Illumina - UMI Index Anchors |
| OverrideCycles,Y151;I8;U10;Y151 | Index Read 2 is a 10 bps UMI. This is the format for Agilent XT HS. |
| OverrideCycles,Y151;I8U9;I8;Y151 | Index Read 1 contains both an index and a 9 bps UMI. This is the format for IDT Dual Index Adapters with UMIs. |
| OverrideCycles,U3N2Y146;I8;I8;U3N2Y146 | UMI is comprised of the first 3 bps of each genomic read, linked by 2 bps of ignored sequence. This is the format for UMIs in SureSelect XT HS 2 and IDT xGen Duplex Seq Adapter. |

| Setting | Description |
|---|---|
| OverrideCycles,Y151;I8;I8;U10N12Y127 | UMI is at the beginning of Read 2, attached with a linker sequence of length 12. |

## No lane splitting

When using *--no-lane-splitting true*, DRAGEN FASTQ file name convention and FASTQ contents match bcl2fastq2 for the same feature.

DRAGEN only supports this mode when the `Lane` column is not specified in the sample sheet to make sure that all samples are present in all lanes in the same order listed. This is generally expected for flow cells with no fluidic boundaries between lanes.

## BCL Metrics

DRAGEN BCL conversion outputs metrics in CSV format to the Reports/ output subfolder. Information provided includes metrics files for demultiplexing, adapter sequence trimming, index-hopping (for unique-dual indexes only), and the top unknown barcodes for each lane. In addition, the sample sheet and RunInfo.xml file used during conversion is copied into the Reports/ subdirectory for reference.

## Demultiplex Output File

The following information is included in the `Demultiplex_Stats.csv` output file.

| Column | Description |
|---|---|
| Lane | The lane for each metric. |
| SampleID | The contents of `Sample_ID` in the sample sheet for this sample. |
| Index | The contents of `index` in sample sheet for this sample. For dual-index, the value concatenated with `index2`. |
| # Reads | The total number of pass-filter reads mapping to this sample for the lane. |
| # Perfect Index Reads | The number of mapped reads with barcodes that match the indexes provided in the sample sheet. |
| # One Mismatch Index Reads | The number of mapped reads with barcodes matched with one base mismatched. |
| # of >= Q30 Bases (PF) | The total number of bases mapped to this sample with a quality score greater than or equal to 30. |
| Mean Quality Score (PF) | The mean quality score of all bases mapping to this sample. |

## Adapter Metrics Output File

The following information is included in the `Adapter_Metrics.csv` output file.

| Column | Description |
|---|---|
| Lane | The lane for each metric. |
| Sample_ID | The contents of Sample_ID in the sample sheet for this sample. |
| index | The contents of index in sample sheet for this sample. |
| index2 | The contents of index2 in the sample sheet for this sample. |
| R1_ AdapterBases | The total number of bases trimmed as adapter from read 1 reads. |
| R1_ SampleBases | The total number of bases not trimmed from read 1. |
| R2_ AdapterBases | The total number of bases trimmed as adapter from read 2 reads. |
| R2_ SampleBases | The total number of bases not trimmed from read 2. |
| # Reads | The total number of pass-filter reads mapping to this sample in this lane. |

## Index Hopping Counts Output File

For unique dual index inputs, the `Index_Hopping_Counts.csv` file provides the number of reads mapping to every possible combination of provided index and index2 values, including via mismatch tolerance. The metrics provide visibility into any index-hopping behavior that might be occurring. The samples with both index and index2 values present in the sample sheet are present in the index hopping file for reference. The following information is included in the `Index_Hopping_Counts.csv` output file.

| Column | Description |
|---|---|
| Lane | The lane for each metric. |
| SampleID | If the index combination corresponds to a sample, the contents of Sample_IDin the sample sheet for this sample. |
| index | The contents of index in sample sheet for the sample. |
| index2 | The contents of index 2i n sample sheet for the sample. |
| # Reads | The total number of pass-filter reads mapping to the index and index2 combination. |

## Top Unknown Barcodes Output File

The `Top_Unknown_Barcodes.csv` file lists the most commonly-encountered barcode sequences in the flow cell input that are not listed in the sample sheet. The 100 most common unlisted sequences are listed, along with any other sequences with a frequency equivalent to the 100th most commonly encountered sequence. The following information is included in the `Top_Unknown_Barcodes.csv` output file.

| Column | Description |
|---|---|
| Lane | The lane for each metric. |
| index | The first index value of this unlisted sequence |
| index2 | The second index value of this unlisted sequence |
| # Reads | The total number of pass-filter reads mapping to the index and index2 combination |

## FASTQ List Output File

The `fastq_list.csv` output file is located in the output folder with the FASTQ files. The files provides the associations between the sample indexes, lane, and the output FASTQ file names. For information on running DRAGEN using `fastq_list.csv`, see, lane, and the output FASTQ file names. The columns of each row are documented below, along with example entries from a test run. For more information on running DRAGEN using fastq_list.csv, see FASTQ CSV File Format.

| Column | Description |
|---|---|
| RGID | Read Group |
| RGSM | Sample ID |
| RGLB | Library |
| Lane | Flow cell lane |
| Read1File | Full path to a valid FASTQ input file |
| Read2File | Full path to a valid FASTQ input file. Required for paired-end input. If not using paired-end input, leave empty, |

The following is an example fastq_list.csv output file.

```
RGID,RGSM,RGLB,Lane,Read1File,Read2File
AACAACCA.ACTGCATA.1,1,UnknownLibrary,1,/home/user/dragen_bcl_out/1_S1_
L001_R1_001.fastq.gz,/home/user/dragen_bcl_out/1_S1_L001_R2_001.fastq.gz
AATCCGTC.ACTGCATA.1,2,UnknownLibrary,1,/home/user/dragen_bcl_out/2_S2_
L001_R1_001.fastq.gz,/home/user/dragen_bcl_out/2_S2_L001_R2_001.fastq.gz
```

```
CGAACTTA.GCGTAAGA.1,3,UnknownLibrary,1,/home/user/dragen_bcl_out/3_S3_
L001_R1_001.fastq.gz,/home/user/dragen_bcl_out/3_S3_L001_R2_001.fastq.gz
GATAGACA.GCGTAAGA.1,4,UnknownLibrary,1,/home/user/dragen_bcl_out/4_S4_
L001_R1_001.fastq.gz,/home/user/dragen_bcl_out/4_S4_L001_R2_001.fastq.gz
```

# Monitoring System Health

When you power up your DRAGEN system a daemon (*dragen_mond*) is started that monitors the card for hardware issues. This daemon is also started when your DRAGEN system is installed or updated. The main purpose of this daemon is to monitor DRAGEN Bio-IT Processor temperature and abort DRAGEN when the temperature exceeds a configured threshold.

To manually start, stop, or restart the monitor, run the following as root:

```
sudo service dragen_mond [stop|start|restart]
```

By default, the monitor polls for hardware issues once per minute and logs temperature once every hour.

The `/etc/sysconfig/dragen_mond` file specifies the command line options used to start *dragen_mond* when the service command is run. Edit DRAGEN_MOND_OPTS in this file to change the default options. For example, the following changes the poll time to 30 seconds and the log time to once every 2 hours:

```
DRAGEN_MOND_OPTS="-d -p 30 -l 7200"
```

The *-d* option is required to run the monitor as a daemon.

The *dragen_mond* command line options are as follows:

| Option | Description |
|---|---|
| *-m --swmaxtemp <n>* | Maximum software alarm temperature (Celsius). Default is 85. |
| *-i --swmintemp <n>* | Minimum software alarm temperature (Celsius). Default is 75. |
| *-H --hwmaxtemp <n>* | Maximum hardware alarm temperature (Celsius). Default is 100. |
| *-p --polltime <n>* | Time between polling chip status register (seconds). Default is 60. |

| Option | Description |
|---|---|
| *-l --logtime <n>* | Log FPGA temp every n seconds. Default is 3600. Must be a multiple of polltime |
| *-d --daemon* | Detach and run as a daemon. |
| *-h --help* | Print help and exit. |
| *-V --version* | Print the version and exit. |

To display the current temperature of the DRAGEN Bio-IT Processor, use the *dragen_info -t* command. This command does not execute if *dragen_mond* is not running.

```
% dragen_info -t
FPGA Temperature: 42C  (Max Temp: 49C, Min Temp: 39C)
```

## Logging

All hardware events are logged to `/var/log/messages` and `/var/log/dragen_mond.log`. The following shows an example in `/var/log/messages` of a temperature alarm:

```
Jul 16 12:02:34 komodo dragen_mond[26956]: WARNING: FPGA software over temperature alarm has been triggered
-- temp threshold: 85 (Chip status: 0x80000001)
Jul 16 12:02:34 komodo dragen_mond[26956]: Current FPGA temp: 86, Max temp: 88, Min temp: 48
Jul 16 12:02:34 komodo dragen_mond[26956]: All dragen processes will be stopped until alarm clears
Jul 16 12:02:34 komodo dragen_mond[26956]: Terminating dragen in process 1510 with SIGUSR2 signal
```

By default, temperature is logged to /var/log/dragen_mond.log every hour:

```
Aug 01 09:16:50 Setting FPGA hardware max temperature threshold to 100
Aug 01 09:16:50 Setting FPGA software max temperature threshold to 85
Aug 01 09:16:50 Setting FPGA software min temperature threshold to 75
Aug 01 09:16:50 FPGA temperatures will be logged every 3600 seconds
Aug 01 09:16:50 Current FPGA temperature is 52 (Max temp = 52, Min temp = 52)
Aug 01 10:16:50 Current FPGA temperature is 53 (Max temp = 56, Min temp = 49)
Aug 01 11:16:50 Current FPGA temperature is 54 (Max temp = 56, Min temp = 49)
```

If DRAGEN is executing when a thermal alarm is detected, the following is displayed in the terminal window of the DRAGEN process:

```
**********************************************************
**  Received external signal -- aborting dragen.        **
**  An issue has been detected with the dragen card.    **
**  Check /var/log/messages for details.                **
**                                                      **
**  It may take up to a minute to complete shutdown.    **
```

**********

If you see this message, stop running the DRAGEN software. Do the following to alleviate the overheating condition on the card:

- Be sure that there is ample air flow over the card. Consider moving the card to a slot where there is more air flow, adding another fan or increasing the fan speed.
- Give the card more space in the box. If there are available PCIe slots, move the card so that it has empty slots on either side.

Contact Illumina Technical Support if you are having trouble resolving the thermal alarm on your system.

## Hardware Alarms

The following table lists the hardware events logged by the monitor when an alarm is triggered:

| ID | Description | Monitor Action |
|---|---|---|
| 0 | Software overheating | Terminate usage until DRAGEN Bio-IT Processor cools to software minimum temperature. |
| 1 | Hardware overheating | Fatal. Aborts dragen software; system reboot required |
| 2 | Board SPD overheating | Logged as nonfatal |
| 3 | SODIMM overheating | Logged as nonfatal |
| 4 | Power 0 | Fatal. Aborts dragen software; system reboot required |
| 5 | Power 1 | Fatal. Aborts dragen software; system reboot required |
| 6 | DRAGEN Bio-IT Processor power | Logged as nonfatal |
| 7 | Fan 0 | Logged as nonfatal |
| 8 | Fan 1 | Logged as nonfatal |
| 9 | SE5338 | Fatal. Aborts dragen software; system reboot required |
| 10–30 | Undefined (Reserved) | Fatal. Aborts dragen software; system reboot required |

Fatal alarms prevent the DRAGEN host software from running and require a system reboot. When a software overheating alarm is triggered, the monitor looks for and aborts any running DRAGEN processes. The monitor continues to abort any new DRAGEN processes until the temperature decreases to the minimum threshold and the hardware clears the chip status alarm. When the software overheating alarm clears, DRAGEN jobs can resume executing.

Contact Illumina Technical Support with details from the log files if any of these alarms are triggered on your system.

# Nirvana (Variant Annotator)

Nirvana provides clinical-grade annotation of genomic variants, such as SNVs, MNVs, insertions, deletions, indels, STRs, SV, and CNVs. Use a VCF as input. The output is a structured JSON representation of all annotations and sample information extracted from the VCF. Nirvana can handle multiple alternate alleles and multiple samples.

DRAGEN provides a standalone implementation of the variant annotation software.

The following steps are required to run Nirvana.

1. Download external data sources, gene models, and reference genome.
2. Annotate the resulting JSON file.

By default, the Nirvana binaries are located in the `/opt/edico/share/nirvana` directory. This directory includes two files: the Downloader and Nirvana.

## Limitations

The Nirvana Variant Annotator and the Downloader are compatible with the following platforms:

- CentOS 6 using x64 processors.
- CentOS 7 and other modern Linux distributions using x64 processors.

## Download Data Files

To store annotation data files, create a top-level directory. The created directory contains three subdirectories:

- Cache contains gene models.
- SupplementaryAnnotation contains external data sources like dbSNP and gnomAD.
- References contains the reference genome.

The following command-line options are used:

| Option | Value | Example | Description |
|---|---|---|---|
| --ga | GRCh37, GRCh38, or both | GRCh38 | Genome assembly |
| --out | Output directory | ~/Data | Top-level output directory |

Download data files as follows.

1. To create a data directory, enter the following command.
   This example creates the Data directory in your home directory.
   ```
   mkdir ~/Data
   ```
2. Download the files for a genome assembly.
   This example downloads the genome assembly GRCh38.
   ```
   /opt/edico/share/nirvana/Downloader --ga GRCh38 --out ~/Data
   ```

You can use the same command to resynchronize the data sources with the Nirvana servers, including the following actions:

- Remove obsolete files, such as old versions of data sources, from the output directory.
- Download newer files.

The following is the created output:

```
--------------------------------------------------------------------------------
Downloader (c) 2020 Illumina, Inc.
Stromberg, Roy, Lajugie, Jiang, Li, and Kang 3.9.1-0-gc823805
--------------------------------------------------------------------------------
- downloading manifest... 37 files.
- downloading file metadata:
- finished (00:00:00.8).
- downloading files (22.123 GB):
- downloading 1000_Genomes_Project_Phase_3_v3_plus_refMinor.rma.idx
(GRCh38)
- downloading MITOMAP_20200224.nsa.idx (GRCh38)
- downloading ClinVar_20200302.nsa.idx (GRCh38)
- downloading REVEL_20160603.nsa.idx (GRCh38)
- downloading phyloP_hg38.npd.idx (GRCh38)
- downloading ClinGen_Dosage_Sensitivity_Map_20200131.nsi (GRCh38)
- downloading MITOMAP_SV_20200224.nsi (GRCh38)
- downloading dbSNP_151_globalMinor.nsa.idx (GRCh38)
- downloading ClinGen_Dosage_Sensitivity_Map_20190507.nga (GRCh38)
- downloading PrimateAI_0.2.nsa.idx (GRCh38)
- downloading ClinGen_disease_validity_curations_20191202.nga (GRCh38)
```

```
- downloading 1000_Genomes_Project_Phase_3_v3_plus.nsa.idx (GRCh38)
- downloading SpliceAi_1.3.nsa.idx (GRCh38)
- downloading dbSNP_153.nsa.idx (GRCh38)
- downloading TOPMed_freeze_5.nsa.idx (GRCh38)
- downloading MITOMAP_20200224.nsa (GRCh38)
- downloading gnomAD_2.1.nsa.idx (GRCh38)
- downloading ClinGen_20160414.nsi (GRCh38)
- downloading gnomAD_gene_scores_2.1.nga (GRCh38)
- downloading 1000_Genomes_Project_(SV)_Phase_3_v5a.nsi (GRCh38)
- downloading MultiZ100Way_20171006.pcs (GRCh38)
- downloading 1000_Genomes_Project_Phase_3_v3_plus_refMinor.rma (GRCh38)
- downloading ClinVar_20200302.nsa (GRCh38)
- downloading OMIM_20200409.nga (GRCh38)
- downloading Both.transcripts.ndb (GRCh38)
- downloading REVEL_20160603.nsa (GRCh38)
- downloading PrimateAI_0.2.nsa (GRCh38)
- downloading dbSNP_151_globalMinor.nsa (GRCh38)
- downloading Both.sift.ndb (GRCh38)
- downloading Both.polyphen.ndb (GRCh38)
- downloading Homo_sapiens.GRCh38.Nirvana.dat
- downloading 1000_Genomes_Project_Phase_3_v3_plus.nsa (GRCh38)
- downloading phyloP_hg38.npd (GRCh38)
- downloading SpliceAi_1.3.nsa (GRCh38)
- downloading TOPMed_freeze_5.nsa (GRCh38)
- downloading dbSNP_153.nsa (GRCh38)
- downloading gnomAD_2.1.nsa (GRCh38)
- finished (00:04:10.1).
Description Status
--------------------------------------------------------------------------------
1000_Genomes_Project_(SV)_Phase_3_v5a.nsi (GRCh38) OK
1000_Genomes_Project_Phase_3_v3_plus.nsa (GRCh38) OK
1000_Genomes_Project_Phase_3_v3_plus.nsa.idx (GRCh38) OK
1000_Genomes_Project_Phase_3_v3_plus_refMinor.rma (GRCh38) OK
1000_Genomes_Project_Phase_3_v3_plus_refMinor.rma.idx (... OK
Both.polyphen.ndb (GRCh38) OK
Both.sift.ndb (GRCh38) OK
Both.transcripts.ndb (GRCh38) OK
ClinGen_20160414.nsi (GRCh38) OK
ClinGen_Dosage_Sensitivity_Map_20190507.nga (GRCh38) OK
ClinGen_Dosage_Sensitivity_Map_20200131.nsi (GRCh38) OK
```

```
ClinGen_disease_validity_curations_20191202.nga (GRCh38) OK
ClinVar_20200302.nsa (GRCh38) OK
ClinVar_20200302.nsa.idx (GRCh38) OK
Homo_sapiens.GRCh38.Nirvana.dat OK
MITOMAP_20200224.nsa (GRCh38) OK
MITOMAP_20200224.nsa.idx (GRCh38) OK
MITOMAP_SV_20200224.nsi (GRCh38) OK
MultiZ100Way_20171006.pcs (GRCh38) OK
OMIM_20200409.nga (GRCh38) OK
PrimateAI_0.2.nsa (GRCh38) OK
PrimateAI_0.2.nsa.idx (GRCh38) OK
REVEL_20160603.nsa (GRCh38) OK
REVEL_20160603.nsa.idx (GRCh38) OK
SpliceAi_1.3.nsa (GRCh38) OK
SpliceAi_1.3.nsa.idx (GRCh38) OK
TOPMed_freeze_5.nsa (GRCh38) OK
TOPMed_freeze_5.nsa.idx (GRCh38) OK
dbSNP_151_globalMinor.nsa (GRCh38) OK
dbSNP_151_globalMinor.nsa.idx (GRCh38) OK
dbSNP_153.nsa (GRCh38) OK
dbSNP_153.nsa.idx (GRCh38) OK
gnomAD_2.1.nsa (GRCh38) OK
gnomAD_2.1.nsa.idx (GRCh38) OK
gnomAD_gene_scores_2.1.nga (GRCh38) OK
phyloP_hg38.npd (GRCh38) OK
phyloP_hg38.npd.idx (GRCh38) OK
--------------------------------------------------------------------------------
Peak memory usage: 52.3 MB
Time: 00:04:12.2
```

## Annotate Files

1. If you have not generated a VCF file, download a VCF file using the following command

   ```
   curl -O
   https://raw.githubusercontent.com/HelixGrind/DotNetMisc/master/TestFiles/H
   iSeq.10000.vcf.gz
   ```

2. To annotate the file, enter the following command:

   ```
   /opt/edico/share/nirvana/Nirvana -c ~/Data/Cache/GRCh38/Both \ -r
   ~/Data/References/Homo_sapiens.GRCh38.Nirvana.dat \ --sd
   ~/Data/SupplementaryAnnotation/GRCh38 -i HiSeq.10000.vcf.gz -o HiSeq.10000
   ```

The following are the available command line options:

| Option | Value | Example | Description |
|---|---|---|---|
| -c | Directory | ~/Data/Cache/GRCh38/Both | Cache directory |
| -r | Directory | ~/Data/References/Homo_sapiens.GRCh38.Nirvana.dat | Reference directory |
| --sd | Directory | ~/Data/SupplementaryAnnotation/GRCh38 | Supplementary annotation directory |
| -i | path | HiSeq.10000.vcf.gz | Input VCF path |
| -o | prefix | HiSeq.10000 | Output path prefix |

Using the example above, Nirvana generates the following output called `HiSeq.10000.json.gz`.

```
-------------------------------------------------------------------------------
Nirvana (c) 2020 Illumina, Inc.
Stromberg, Roy, Lajugie, Jiang, Li, and Kang 3.9.1-0-gc823805
-------------------------------------------------------------------------------
Initialization Time Positions/s
-------------------------------------------------------------------------------
Cache 00:00:01.9
SA Position Scan 00:00:00.4 23,867
Reference Preload Annotation Variants/s
-------------------------------------------------------------------------------
chr1 00:00:00.4 00:00:03.7 2,651
Summary Time Percent
-------------------------------------------------------------------------------
Initialization 00:00:02.3 25.7 %
Preload 00:00:00.4 5.4 %
Annotation 00:00:03.7 41.5 %
Peak memory usage: 1.284 GB
Time: 00:00:08.0
```

## JSON Output file

Nirvana produces an output file in JSON format that includes the following three sections:

| Section | Content |
|---|---|
| Header | Configuration, data source versions, and sample names. |
| Positions | Variant level annotation. |
| Genes | Gene level annotation. |

The following are outputs using the example commands specified in the sections above. Each of the following sections contains only a subset of information. The output file contains more information.

## Header

The following is an example of the Header section.


```
"header": {
"annotator": "Nirvana 3.9.0",
"creationTime": "2020-06-03 08:05:06",
"genomeAssembly": "GRCh38",
"schemaVersion": 6,
"dataVersion": "91.26.57",
"dataSources": [
{
"name": "VEP",
"version": "91",
"description": "BothRefSeqAndEnsembl",
"releaseDate": "2018-03-05"
},
{
"name": "ClinVar",
"version": "20200302",
"description": "A freely accessible, public archive of reports of the
relationships among human variations and phenotypes, with supporting
evidence",
"releaseDate": "2020-03-02"
},
{
"name": "dbSNP",
"version": "153",
"description": "Identifiers for observed variants",
"releaseDate": "2019-07-22"
},
{
"name": "gnomAD",
"version": "2.1",
"description": "gnomAD allele frequency data remapped to GRCh38 with
CrossMap by Ensembl",
"releaseDate": "2019-03-25"
},
```

```
{
"name": "PrimateAI",
"version": "0.2",
"description": "PrimateAI percentile scores.",
"releaseDate": "2018-11-07"
},
{
"name": "OMIM",
"version": "20200409",
"description": "An Online Catalog of Human Genes and Genetic Disorders",
"releaseDate": "2020-04-09"
}
],
"samples": [
"NA12878"
]
},
```


## Positions

Each position represents one row of the VCF file. Each position contains a samples section and a variants section. Reference and alternate alleles shown here matches the VCF content exactly.

The samples section lists the sample-specific information, such as genotype, in the same order as they appear in the VCF and in the JSON header above.

The variants section provides annotations for each alternate allele that appear in the VCF row. This includes allele-specific annotation from external data sources as well as transcript-level annotation. Reference and alternate alleles shown here are shown in their most shortened representation. For example, padding bases have been removed and the variant are left-aligned.


```
"positions": [
{
"chromosome": "chr1",
"position": 1043248,
"refAllele": "C",
"altAlleles": [
"T"
],
"quality": 441.42,
"filters": [
"PASS"
],
```

```
"strandBias": -425.94,
"cytogeneticBand": "1p36.33",
"samples": [
{
"genotype": "0/1",
"variantFrequencies": [
0.537
],
"totalDepth": 54,
"genotypeQuality": 99,
"alleleDepths": [
25,
29
]
}
],
"variants": [
{
"vid": "1-1043248-C-T",
"chromosome": "chr1",
"begin": 1043248,
"end": 1043248,
"refAllele": "C",
"altAllele": "T",
"variantType": "SNV",
"hgvsg": "NC_000001.11:g.1043248C>T",
"phylopScore": 0.1,
"clinvar": [
{
"id": "RCV000872112.1",
"variationId": 263161,
"reviewStatus": "criteria provided, single submitter",
"alleleOrigins": [
"germline"
],
"refAllele": "C",
"altAllele": "T",
"phenotypes": [
"not provided"
],
```

```
"medGenIds": [
"CN517202"
],
"significance": [
"likely benign"
],
"lastUpdatedDate": "2019-12-17",
"pubMedIds": [
"28492532"
],
"isAlleleSpecific": true
},
{
"id": "VCV000263161.2",
"reviewStatus": "criteria provided, multiple submitters, no conflicts",
"significance": [
"likely benign"
],
"refAllele": "C",
"altAllele": "T",
"lastUpdatedDate": "2019-12-17",
"isAlleleSpecific": true
}
],
"dbsnp": [
"rs116586548"
],
"globalAllele": {
"globalMinorAllele": "T",
"globalMinorAlleleFrequency": 0.004393
},
"gnomad": {
"coverage": 38,
"allAf": 0.000681,
"allAn": 264462,
"allAc": 180,
"allHc": 0,
"afrAf": 0.006216,
"afrAn": 23648,
"afrAc": 147,
```

```
"afrHc": 0,
"amrAf": 0.000689,
"amrAn": 33404,
"amrAc": 23,
"amrHc": 0,
"easAf": 0,
"easAn": 18830,
"easAc": 0,
"easHc": 0,
"finAf": 0,
"finAn": 22870,
"finAc": 0,
"finHc": 0,
"nfeAf": 5e-05,
"nfeAn": 120576,
"nfeAc": 6,
"nfeHc": 0,
"asjAf": 0.000304,
"asjAn": 9882,
"asjAc": 3,
"asjHc": 0,
"sasAf": 0,
"sasAn": 28456,
"sasAc": 0,
"sasHc": 0,
"othAf": 0.000147,
"othAn": 6796,
"othAc": 1,
"othHc": 0,
"maleAf": 0.000564,
"maleAn": 143614,
"maleAc": 81,
"maleHc": 0,
"femaleAf": 0.000819,
"femaleAn": 120848,
"femaleAc": 99,
"femaleHc": 0,
"controlsAllAf": 0.000626,
"controlsAllAn": 113456,
"controlsAllAc": 71
```

```
},
"oneKg": {
"allAf": 0.004393,
"afrAf": 0.016641,
"amrAf": 0,
"easAf": 0,
"eurAf": 0,
"sasAf": 0,
"allAn": 5008,
"afrAn": 1322,
"amrAn": 694,
"easAn": 1008,
"eurAn": 1006,
"sasAn": 978,
"allAc": 22,
"afrAc": 22,
"amrAc": 0,
"easAc": 0,
"eurAc": 0,
"sasAc": 0
},
"primateAI": [
{
"hgnc": "AGRN",
"scorePercentile": 0.12
}
],
"revel": {
"score": 0.136
},
"spliceAI": [
{
"hgnc": "AGRN",
"acceptorGainScore": 0.1,
"acceptorGainDistance": 23,
"acceptorLossScore": 0,
"acceptorLossDistance": -9,
"donorGainScore": 0,
"donorGainDistance": -5,
"donorLossScore": 0,
```

```
"donorLossDistance": 16
}
],
"topmed": {
"allAf": 0.002055,
"allAn": 125568,
"allAc": 258,
"allHc": 1
},
"transcripts": [
{
"transcript": "ENST00000379370.6",
"source": "Ensembl",
"bioType": "protein_coding",
"codons": "cCg/cTg",
"aminoAcids": "P/L",
"cdnaPos": "1444",
"cdsPos": "1394",
"exons": "8/36",
"proteinPos": "465",
"geneId": "ENSG00000188157",
"hgnc": "AGRN",
"consequence": [
"missense_variant"
],
"hgvsc": "ENST00000379370.6:c.1394C>T",
"hgvsp": "ENSP00000368678.2:p.(Pro465Leu)",
"isCanonical": true,
"polyPhenScore": 0.065,
"polyPhenPrediction": "benign",
"proteinId": "ENSP00000368678.2",
"siftScore": 0.05,
"siftPrediction": "tolerated"
},
{
"transcript": "NM_198576.3",
"source": "RefSeq",
"bioType": "protein_coding",
"codons": "cCg/cTg",
"aminoAcids": "P/L",
```

```
"cdnaPos": "1444",
"cdsPos": "1394",
"exons": "8/36",
"proteinPos": "465",
"geneId": "375790",
"hgnc": "AGRN",
"consequence": [
"missense_variant"
],
"hgvsc": "NM_198576.3:c.1394C>T",
"hgvsp": "NP_940978.2:p.(Pro465Leu)",
"isCanonical": true,
"polyPhenScore": 0.065,
"polyPhenPrediction": "benign",
"proteinId": "NP_940978.2",
"siftScore": 0.05,
"siftPrediction": "tolerated"
}
]
}
]
}
],
```


## Genes

For each gene referenced in the transcripts in the positions section, there is a matching entry in the genes section. The following example shows gene-level annotations from gnomAD, ClinGen Dosage Sensitivity Map, and OMIM.


```
"genes": [
{
"name": "AGRN",
"gnomAD": {
"pLi": 5.47e-07,
"pRec": 1,
"pNull": 1.41e-12,
"synZ": -3.96,
"misZ": 0.226,
"loeuf": 0.435
},
"clingenDosageSensitivityMap": {
```

```
"haploinsufficiency": "gene associated with autosomal recessive phenotype",
"triplosensitivity": "no evidence to suggest that dosage sensitivity is
associated with clinical phenotype"
},
"omim": [
{
"mimNumber": 103320,
"geneName": "Agrin",
"description": "The AGRN gene encodes agrin, a large and ubiquitous
proteoglycan with multiple isoforms that have diverse functions in
different tissues. Agrin was originally identified as an essential neural
regulator that induces the aggregation of acetylcholine receptors (AChRs)
and other postsynaptic proteins on muscle fibers and is crucial for the
formation and maintenance of the neuromuscular junction (NMJ) (Campanelli
et al., 1991; Burgess et al., 1999; summary by Maselli et al., 2012).",
"phenotypes": [
{
"mimNumber": 615120,
"phenotype": "Myasthenic syndrome, congenital, 8, with pre- and
postsynaptic defects",
"description": "Congenital myasthenic syndromes are genetic disorders of
the neuromuscular junction (NMJ) that are classified by the site of the
transmission defect: presynaptic, synaptic, and postsynaptic. CMS8 is an
autosomal recessive disorder characterized by prominent defects of both the
pre- and postsynaptic regions. Affected individuals have onset of muscle
weakness in early childhood; the severity of the weakness and muscles
affected is variable (summary by Maselli et al., 2012).\n\nFor a discussion
of genetic heterogeneity of CMS, see CMS1A.",
"mapping": "molecular basis of the disorder is known",
"inheritances": [
"Autosomal recessive"
]
}
]
}
]
}
```

# Hardware-Accelerated Compression and Decompression

Gzip compression is ubiquitous in bioinformatics. FASTQ files are often gzipped, and the BAM format itself is a specialized version of gzip. For that reason, the DRAGEN BioIT processor provides hardware support for accelerating compression and decompression of gzipped data. If your input files are gzipped, DRAGEN detects that and decompresses the files automatically. If your output is BAM files, then the files are automatically compressed.

DRAGEN provides standalone command-line utilities to enable you to compress or decompress arbitrary files. These utilities are analogous to the Linux gzip and gunzip commands, but are named *dzip* and *dunzip* (dragen zip and dragen unzip). Both utilities are able to accept as input a single file, and produce a single output file with the .gz file extension removed or added, as appropriate. For example:

```
dzip file1      # produces output file file1.gz
dunzip file2.gz # produces output file file2
```

Currently, *dzip* and *dunzip* have the following limitations and differences from gzip/gunzip:

- Each invocation of these tools can handle only a single file. Additional file names (including those produced by a wildcard * character) are ignored.
- They cannot be run at the same time as the DRAGEN host software.
- They do not support the command line options found in gzip and gunzip (eg, --recursive, --fast, --best, --stdout).

# Usage Reporting

As part of the install process, a daemon (dragen_licd) is created (or stopped then restarted). This background process self-activates at the end of each day to upload DRAGEN host software usage to anIllumina server. Information includes date, duration, size (number of bases), status of each run, and software version used.

Communication to theIllumina server is secured by encryption. If there is a communication error, the daemon retries until the next morning. If the upload continues to fail, communication is tried again the following night until communication succeeds. This means that during working hours, the system resources remain fully available and are not in any way hampered by this background activity.

To check the current license usage, use the *dragen_lic* command.

To generate a usage report, your server must meet the following requirements:

- 256 GB RAM and 2 TB HDD for 300x germline single sample coverage.
- T/N analysis coverage.
- 6 TB and 512 GB RAM.

The usage report does not contain the following information:

- Number of joint genotyper input files.

- GATK gVCF input to gVCF genotyper.
- Mixing gVCFs from different callers, such as joint calling and gVCF genotyper.

# Troubleshooting

If the DRAGEN system does not seem to be responding, do the following:

1. To determine if the DRAGEN system is hanging, follow the instructions in *How to Determine if the System is Hanging*.
2. Collect diagnostic information after a hang, or a crash, as described in *Sending Diagnostic Information to Illumina Support*.
3. After all information has been collected, reset your system. if needed, as described in *Resetting Your System after a Crash or Hang*.

## How to Determine if the System is Hanging

The DRAGEN system has a watchdog to monitor the system for hangs. If a run seems to be taking longer than it should, the watchdog may not be detecting the hang. Here are some things to try:

- Run the *top* command to find the active DRAGEN process. If your run is healthy, you should expect to see it consuming over 100% of the CPU. If it is consuming 100% or less, then your system may be hanging.
- Run the *du -s* command in the directory of the output BAM/SAM file. During a normal run, this directory should be growing with either intermediate output data (when sort is enabled) or BAM/SAM data.

## Sending Diagnostic Information to Illumina Support

Illumina would like your feedback on your DRAGEN system, including any reports of system malfunction. In the event of a crash, hang, or watchdog fault, run the *sosreport* command to collect diagnostic and configuration information, as follows:

```
sudo sosreport --batch --tmp-dir /staging/tmp
```

This command takes several minutes to execute and reports the location where it has saved the diagnostic information in `/staging/tmp`. Please include the report when you submit a support ticket for Illumina Technical Support.

## Resetting Your System after a Crash or Hang

If the DRAGEN system crashes or hangs, the *dragen_reset* utility must be run to reinitialize the hardware and software. This utility is automatically executed by the host software any time it detects an unexpected condition. In this case, the host software shows the following message:

```
Running dragen_reset to reset DRAGEN Bio-IT processor and software
```

If the software is hanging, please collect diagnostic information as described in subsection *Sending Diagnostic Information to Illumina Support* on page 266 and then execute *dragen_reset* manually, as follows:

```
/opt/edico/bin/dragen_reset
```

Any execution of *dragen_reset* requires the reference genome to be reloaded to the DRAGEN board. The host software automatically reloads the reference on the next execution.

# Command Line Option Reference

This section provides information on all the DRAGEN command line options, including the name used in the configuration file, the command line equivalent, a description, and the range of values.

## General Software Options

The following options are in the default section of the configuration file. The default section does not have a section name (eg, [Aligner]) associated with it. The default section is at the top of the configuration file. Note that some mandatory fields must be specified on the command line and are not present in configuration files.

| Name | Description | Command Line Equivalent | Value |
|---|---|---|---|
| alt-aware | Enables special processing for alt contigs, if alt liftover was used in hash table. Enabled by default if reference was built with liftover. | --alt-aware | true/false |
| append-read-index-to-name | By default, DRAGEN names both mate ends of pairs the same. When set to true, DRAGEN appends /1 and /2 to the two ends. | | true/false |
| bam-input | Aligned BAM file for input to the DRAGEN variant caller. | -b, --bam-input | |
| bcl-conversion-only | Perform Illumina BCL conversion to FASTQ format. | --bcl-conversion-only | true/false |
| bcl-input-directory | Input BCL directory for BCL conversion. | --bcl-input-directory | |
| bcl-only-lane | For BCL input, convert only specified lane number (default all lanes). | --bcl-only-lane | 1–8 |
| sample-sheet | For BCL input, path to SampleSheet.csv file. Default location is the BCL root directory. | --sample-sheet | |
| strict-mode | For BCL input, abort if any files are missing (false by default) | --strict-mode | true/false |

| Name | Description | Command Line Equivalent | Value |
|---|---|---|---|
| first-tile-only | Only convert the first tile of each lane during BCL conversion (for testing/debugging) | --first-tile-only | true/false |
| run-info | Set the path to `RunInfo.xml` file. Default is <flow cell>/RunInfo.xml | --run-info | |
| bcl-sampleproject-subdirectories | For BCL conversion, output to subdirectories based upon sample sheet 'Sample_Project' column | --bcl-sampleproject-subdirectories | |
| no-lane-splitting | Set whether to output all lanes of a flow cell to the same FASTQ files consecutively. Default is false. | --no-lane-splitting | true/false |
| bcl-only-matched-reads | Set whether to output unmapped reads to files marked as Undetermined. Default is false. | bcl-only-matched-reads | true/false |
| bcl-use-hw | Set to false to prevent use of DRAGEN FPGA acceleration during BCL conversion. Default is true. | --bcl-use-hw | true/false |
| bcl-num-parallel-tiles | Number of tiles to process in parallel. Default is dynamically determined. | --bcl-num-parallel-tiles | 1-<nproc> |
| bcl-num-conversion-threads | Number of conversion threads per tile. Default is dynamically determined. | --bcl-num-conversion-threads | 1-<nproc> |
| bcl-num-compression-threads | Number of CPU threads for output `fastq.gz` compression. Default is dynamically determined. | --bcl-num-compression-threads | 1-<nproc> |
| bcl-num-decompression-threads | Number of CPU threads for BCL input decompression. Default is dynamically determined. | --bcl-num-decompression-threads | 1-<nproc> |

| Name | Description | Command Line Equivalent | Value |
|---|---|---|---|
| shared-thread-odirect-output | Use alternative shared-thread ODIRECT file output. Default is false. | --shared-thread-odirect-output | true/false |
| build-hash-table | Generate a reference/hash table. | --build-hash-table | true/false |
| cram-input | CRAM file for input to the DRAGEN variant caller. | --cram-input | |
| dbsnp | Path to the variant annotation database VCF (or .vcf.gz) file. | --dbsnp | |
| enable-auto-multifile | Import subsequent segments of the *_001.{dbam,fastq} files. | --enable-auto-multifile | true/false |
| enable-bam-indexing | Enable generation of a BAI index file. | --enable-bam-indexing | true/false |
| enable-cnv | Enable copy number variant (CNV). | --enable-cnv | true/false |
| enable-duplicate-marking | Enable the flagging of duplicate output alignment records. | --enable-duplicate-marking | true/false |
| enable-map-align-output | Enables saving the output from the map/align stage. Default is true when only running map/align. Default is false if running the variant caller. | --enable-map-align-output | true/false |
| enable-methylation-calling | Whether to automatically add methylation-tags and output a single BAM for methylation protocols. | | true/false |
| enable-sampling | Automatically detect paired-end parameters by running a sample through the mapper/aligner. | | true/false |
| enable-sort | Enable sorting after mapping/alignment. | | true/false |
| enable-variant-caller | Enables the variant caller. | --enable-variant-caller | true/false |

| Name | Description | Command Line Equivalent | Value |
|---|---|---|---|
| enable-vcf-compression | Enable compression of VCF output files. Default is true. | | true/false |
| fastq-file1 | FASTQ file to input to the DRAGEN pipeline (may be gzipped). | -1, --fastq-file1 | |
| fastq-file2 | Second FASTQ file with paired-end reads for input. | -2, --fastq-file2 | |
| fastq-list | CSV file that contains a list of FASTQ files to process. | --fastq-list | |
| fastq-list-sample-id | Only process entries where the RGSM entry matches the given Sample ID parameter (for fastq-list.csv input). | --fastq-list-sample-id | true/false |
| fastq-list-all-samples | Enable/disable processing of all samples together, regardless of the RGSM value. | --fastq-list-all-samples | true/false |
| fastq-n-quality | Base call quality to output for N bases. Automatically added to fastq-n-quality for all output N's. | --fastq-n-quality | 0 to 255 |
| fastq-offset | FASTQ quality offset value. | --fastq-offset | 33 or 64 |
| filter-flags-from-output | Filter output alignments with any bits set in val present in the flags field. Hex and decimal values accepted. | --filter-flags-from-output | |
| force | Force overwrite of existing output file. | -f | |
| force-load-reference | Force loading of the reference and hash tables before starting the DRAGEN pipeline. | -l | |
| generate-md-tags | Whether to generate MD tags with alignment output records. Default is false. | --generate-md-tags | true/false |

| Name | Description | Command Line Equivalent | Value |
|---|---|---|---|
| generate-sa-tags | Whether to generate SA:Z tags for records that have chimeric/supplemental alignments. | --generate-sa-tags | true/false |
| generate-zs-tags | Whether to generate ZS tags for alignment output records. Default is false. | --generate-sz-tags | true/false |
| ht-alt-liftover | SAM format liftover file of alternate contigs in reference. | --ht-alt-liftover | |
| ht-pop-alt-contigs | Reference FASTA file with population alternate contigs to augment the standard reference. | --ht-pop-alt-contigs | |
| ht-pop-alt-liftover | SAM format liftover file of population alternate contigs. | --ht-pop-alt-liftover | |
| ht-pop-snps | VCF format file with unphased population SNPs used to augment the standard reference. | --ht-pop-snps | |
| ht-alt-aware-validate | Disable requirement for a liftover file when building a hash table from a reference that contains alt-contigs. | --ht-alt-aware-validate | true/false |
| ht-build-rna-hashtable | Enable generation of RNA hash table. Default is false. | --ht-build-rna-hashtable | true/false |
| ht-cost-coeff-seed-freq | Cost coefficient of extended seed frequency. | --ht-cost-coeff-seed-freq | |
| ht-cost-coeff-seed-len | Cost coefficient of extended seed length. | --ht-cost-coeff-seed-len | |
| ht-cost-penalty-incr | Cost penalty to incrementally extend a seed another step. | --ht-cost-penalty-incr | |
| ht-cost-penalty | Cost penalty to extend a seed by any number of bases. | --ht-cost-penalty | |
| ht-decoys | Specifies the path to a decoys file. | --ht-decoys | |

| Name | Description | Command Line Equivalent | Value |
|---|---|---|---|
| ht-max-dec-factor | Maximum decimation factor for seed thinning. | --ht-max-dec-factor | |
| ht-max-ext-incr | Maximum bases to extend a seed by in one step. | --ht-max-ext-incr | |
| ht-max-ext-seed-len | Maximum extended seed length. | -- ht-max-ext-seed-len | |
| ht-max-seed-freq | Maximum allowed frequency for a seed match after extension attempts. | --ht-max-seed-freq | 1–256 |
| ht-max-table-chunks | Maximum ~1 GB thread table chunks in memory at once. | --ht-max-table-chunks | |
| ht-mem-limit | Memory limit (hash table + reference), units B\|KB\|MB\|GB. | --ht-mem-limit | |
| ht-methylated | Automatically generate C->T and G->A converted reference hashtables. | --ht-methylated | true/false |
| ht-num-threads | Maximum worker CPU threads for building hash table. | --ht-num-threads | |
| ht-rand-hit-extend | Include a random hit with each EXTEND record of this freq record. | --ht-rand-hit-extend | |
| ht-rand-hit-hifreq | Include a random hit with each HIFREQ record. | --ht-rand-hit-hifreq | |
| ht-ref-seed-interval | Number of positions per reference seed. | --ht-ref-seed-interval | |
| ht-reference | Reference file in .fasta format to be used to build a hash table. | --ht-reference | |
| ht-seed-len | Initial seed length to store in hash table. | --ht-seed-len | |
| ht-size | Size of hash table, units B\|KB\|MB\|GB. | --ht-size | |
| ht-soft-seed-freq-cap | Soft seed frequency cap for thinning | --ht-soft-seed-freq-cap | |

| Name | Description | Command Line Equivalent | Value |
|---|---|---|---|
| ht-suppress-decoys | Suppress the use of a decoys file when building a hash table. | --ht-suppress-decoys | |
| ht-target-seed-freq | Target seed frequency for seed extension. | --ht-target-seed-freq | |
| input-qname-suffix-delimiter | Controls the delimiter used for append-read-index-to-name and for detecting matching pair names with BAM input. | | / or<br>. or<br>: |
| interleaved | Interleaved paired-end reads in single FASTQ. | -i | |
| intermediate-results-dir | Directory to store intermediate results in (eg, sort partitions). | | |
| lic-no-print | Suppress the license status message at the end of a run. | --lic-no-print | true/false |
| methylation-generate-cytosine-report | Generate a genomewide cytosine methylation report. | --methylation-generate-cytosine-report | true/false |
| methylation-generate-mbias-report | Generate a per-sequencer-cycle methylation bias report. | | true/false |
| methylation-TAPS | If input assays are generated by TAPS, set to true. | --methylation-TAPS | true/false |
| methylation-match-bismark | If true, match bismark's tags exactly, including bugs. | --methylation-match-bismark | true/false |
| methylation-protocol | Library protocol for methylation analysis. | --methylation-protocol | none / directional / nondirectional / directional-complement |
| num-threads | The number of processor threads to use. | -n, --num-threads | |
| output-directory | Output directory. | --output-directory | |

| Name | Description | Command Line Equivalent | Value |
|---|---|---|---|
| output-file-prefix | Output file name prefix to use for all files generated by the pipeline. | --output-file-prefix | |
| output-format | The format of the output file from the map/align stage. Valid values are bam (the default), sam, or dbam (a proprietary binary format). | --output-format | BAM / SAM / DBAM |
| pair-by-name | Whether to shuffle the order of BAM input records such that paired-end mates are processed together. | | |
| pair-suffix-delimiter | Change the delimiter character for suffixes. | --pair-suffix-delimiter | / . : |
| preserve-bqsr-tags | Whether to preserve input BAM file's BI and BD flags. Note this may cause problems with hard clipping. | | true/false |
| preserve-map-align-order | Produce output file that preserves original order of reads in the input file. | | true/false |
| qc-coverage-region-1 | First bed file to report coverage on. | --qc-coverage-region-1 | |
| qc-coverage-region-2 | Second bed file to report coverage on. | --qc-coverage-region-2 | |
| qc-coverage-region-3 | Third bed file to report coverage on. | --qc-coverage-region-3 | |
| qc-coverage-reports-1 | Types of reports requested for qc-coverage-region-1. | --qc-coverage-reports-1 | full_res / cov_report |
| qc-coverage-reports-2 | Types of reports requested for qc-coverage-region-2. | --qc-coverage-reports-2 | full_res / cov_report |
| qc-coverage-reports-3 | Types of reports requested for qc-coverage-region-3. | --qc-coverage-reports-3 | full_res / cov_report |

| Name | Description | Command Line Equivalent | Value |
|---|---|---|---|
| ref-dir | Directory containing the reference hash table. This reference is automatically loaded to the DRAGEN card, if it is not already there. | -r, --ref-dir | |
| ref-sequence-filter | Output only reads mapping to this reference sequence. | --ref-sequence-filter | |
| remove-duplicates | If true, remove duplicate alignment records instead of just flagging them. | | true/false |
| RGCN | Read group sequencing center name. | --RGCN | |
| RGCN-tumor | Read group sequencing center name for tumor input. | --RGCN-tumor | |
| RGDS | Read group description. | --RGDS | |
| RGDS-tumor | Read group description for tumor input. | --RGDS-tumor | |
| RGDT | Read group run date. | --RGDT | |
| RGDT-tumor | Read group run date for tumor input. | --RGDT-tumor | |
| RGID | Read group ID. | --RGID | |
| RGID-tumor | Read group ID for tumor input. | --RGID-tumor | |
| RGLB | Read group library. | --RGLB | |
| RGLB-tumor | Read group library for tumor input. | --RGLB-tumor | |
| RGPI | Read group predicted insert size. | --RGPI | |
| RGPI-tumor | Read group predicted insert size for tumor input. | --RGPI-tumor | |
| RGPL | Read group sequencing technology. | --RGPL | |
| RGPL-tumor | Read group sequencing technology for tumor input. | --RGPL-tumor | |
| RGPU | Read group platform unit. | --RGPU | |

| Name | Description | Command Line Equivalent | Value |
|---|---|---|---|
| RGPU-tumor | Read group platform unit for tumor input. | --RGPU-tumor | |
| RGSM | Read group sample name. | --RGSM | |
| RGSM-tumor | Read group sample name for tumor input. | --RGSM-tumor | |
| sample-size | Number of reads to sample when enable-sampling is true. | | |
| sample-sex | Sex of the sample. | --sample-sex | |
| strip-input-qname-suffixes | Whether to strip read-index suffixes (eg, /1 and /2) from input QNAMEs. | | true/false |
| tumor-bam-input | Aligned BAM file for the DRAGEN variant caller in somatic mode. | --tumor-bam-input | |
| tumor-cram-input | Aligned CRAM file for the DRAGEN variant caller in somatic mode. | --tumor-cram-input | |
| tumor-fastq-list | A CSV file containing a list of FASTQ files for the mapper, aligner, and somatic variant caller. | --tumor-fastq-list | |
| tumor-fastq-list-sample-id | The sample ID for the list of FASTQ files specified by tumor-fastq-list. | --tumor-fastq-list-sample-id | |
| tumor-fastq1 | FASTQ file for the DRAGEN pipeline using the variant caller in somatic mode (may be gzipped). | --tumor-fastq1 | |
| tumor-fastq2 | Second FASTQ file with reads paired to tumor-fastq1 reads for the DRAGEN pipeline using the variant caller in somatic mode (may be gzipped). | --tumor-fastq2 | |
| verbose | Enable verbose output from DRAGEN. | -v | |
| version | Print the version and exit. | -V | |

# Mapper Options

The following options are in the [Mapper] section of the configuration file. For more detailed information on these options, see *DNA Mapping* on page 62.

| Name | Description | Command Line Equivalent | Range |
|---|---|---|---|
| ann-sj-max-indel | Maximum indel length to expect near an annotated splice junction. | --Mapper.ann-sj-max-indel | 0 to 63 |
| edit-chain-limit | For edit-mode 1 or 2: Maximum seed chain length in a read to qualify for seed editing. | --Mapper.edit-chain-limit | edit-chain-limit >= 0 |
| edit-mode | 0 = No edits, 1 = Chain len test, 2 = Paired chain len test, 3 = Edit all std seeds. | --Mapper.edit-mode | 0 to 3 |
| edit-read-len | For edit-mode 1 or 2: Read length in which to try edit-seed-num edited seeds. | --Mapper.edit-read-len | edit-read-len > 0 |
| edit-seed-num | For edit-mode 1 or 2: Requested number of seeds per read to allow editing on. | --Mapper.edit-seed-num | edit-seed-num >= 0 |
| enable-map-align | Enable use of BAM input files for mapper/aligner. | --enable-map-align | true/false |
| map-orientations | 0=Normal, 1=No Rev Comp, 2=No Forward (paired end requires Normal). | --Mapper.map-orientations | 0 to 2 |
| max-intron-bases | Maximum intron length reported. | --Mapper.max-intron-bases | |
| min-intron-bases | Minimum reference deletion length reported as an intron. | --Mapper.min-intron-bases | |
| seed-density | Requested density of seeds from reads queried in the hash table | --Mapper.seed-density | 0 > seed-density > 1 |

# Aligner Options

The following options are in the [Aligner] section of the configuration file. For more information, see *DNA Aligning* on page 65

| Name | Description | Command Line Equivalent | Value |
|---|---|---|---|
| aln-min-score | (signed) Minimum alignment score to report; baseline for MAPQ.<br>When using local alignments (global = 0), aln-min-score is computed by the host software as "22 * match-score".<br>When using global alignments (global = 1), aln-min-score is set to -1000000.<br>Host software computation may be overridden by setting aln-min-score in configuration file. | --Aligner.aln-min-score | –2,147,483,648 to 2,147,483,647 |
| dedup-min-qual | Minimum base quality for calculating read quality metric for deduplication. | --Aligner.dedup-min-qual | 0–63 |
| en-alt-hap-aln | Allows chimeric alignments to be output, as supplementary. | --Aligner.en-alt-hap-aln | 0–1 |
| en-chimeric-aln | Allows chimeric alignments to be output, as supplementary. | --Aligner.en-chimeric-aln | 0–1 |
| gap-ext-pen | Score penalty for gap extension. | --Aligner.gap-ext-pen | 0–15 |
| gap-open-pen | Score penalty for opening a gap (insertion or deletion). | gap-open-pen | 0–127 |
| global | If alignment is global (Needleman-Wunsch) rather than local (Smith-Waterman). | --Aligner.global | 0–1 |
| hard-clips | Flags for hard clipping: [0] primary, [1] supplementary, [2] secondary. | --Aligner.hard-clips | 3 bits |

| Name | Description | Command Line Equivalent | Value |
|---|---|---|---|
| map-orientations | Constrain orientations to accept forward-only, reverse-complement only, or any alignments. | --Aligner.map-orientations | 0 (any)<br>1 (forward only)<br>2 (reverse only) |
| mapq-max | Ceiling on reported MAPQ. | --Aligner.mapq-max | 0 to 255 |
| mapq-strict-js | Specific to RNA. When set to 0, a higher MAPQ value is returned, expressing confidence that the alignment is at least partially correct. When set to 1, a lower MAPQ value is returned, expressing the splice junction ambiguity. | --mapq-strict-js | 0/1 |
| match-n-score | (signed) Score increment for matching a reference 'N' nucleotide IUB code. | --Aligner.match-n-score | -16–15 |
| match-score | Score increment for matching reference nucleotide. | --Aligner.match-score | When global = 0, match-score > 0; When global = 1, match-score >= 0 |
| max-rescues | Maximum rescue alignments per read pair. Default is 10. | --max-rescues | 0–1023 |
| min-score-coeff | Adjustment to aln-min-score per read base. | --Aligner. min-score-coeff | -64–63.999 |
| mismatch-pen | Score penalty for a mismatch. | --Aligner.mismatch-pen | 0–63 |

| Name | Description | Command Line Equivalent | Value |
|---|---|---|---|
| no-unclip-score | When no-unclip-score is set to 1, any unclipped bonus (unclip-score) contributing to an alignment is removed from the alignment score before further processing. | --Aligner.no-unclip-score | 0-1 |
| no-unpaired | If only properly paired alignments should be reported for paired reads. | --Aligner. no-unpaired | 0–1 |
| pe-max-penalty | Maximum pairing score penalty, for unpaired or distant ends. | --Aligner.pe-max-penalty | 0–255 |
| pe-orientation | Expected paired-end orientation: 0=FR, 1=RF, 2=FF. | --Aligner.pe-orientation | 0, 1, 2 |
| rescue-sigmas | Deviations from the mean read length used for rescue scan radius. Default is 2.5. | --Aligner.rescue-sigmas | |
| sec-aligns | Maximum secondary (suboptimal) alignments to report per read. | --Aligner.sec-aligns | 0–30 |
| sec-aligns-hard | Set to force unmapped when not all secondary alignments can be output. | --Aligner.sec-aligns-hard | 0–1 |
| sec-phred-delta | Only secondary alignments with likelihood within this Phred of the primary are reported. | --Aligner.sec-phred-delta | 0–255 |
| sec-score-delta | Secondary aligns allowed with pair score no more than this far below primary. | --Aligner. sec-score-delta | |
| supp-aligns | Maximum supplementary (chimeric) alignments to report per read. | --Aligner.supp-aligns | 0–30 |

| Name | Description | Command Line Equivalent | Value |
|---|---|---|---|
| supp-as-sec | If supplementary alignments should be reported with secondary flag. | --Aligner.supp-as-sec | 0–1 |
| supp-min-score-adj | Amount to increase minimum alignment score for supplementary alignments. This score is computed by host software as "8 * match-score" for DNA, and is default 0 for RNA. | --Aligner. supp-min-score-adj | |
| unclip-score | Score bonus for reaching each edge of the read. | --Aligner.unclip-score | 0–127 |
| unpaired-pen | Penalty for unpaired alignments in Phred scale. | --Aligner.unpaired-pen | 0–255 |

If you disable automatic detection of insert-length statistics via the *--enable-sampling* option, you must override all the following options to specify the statistics. For more information, see *Mean Insert Size Detection* on page 1. These options are part of the [Aligner] section of the configuration file.

| Option | Description | Command Line Equivalent | Value |
|---|---|---|---|
| pe-stat-mean-insert | Average template length. | | 0–65535 |
| pe-stat-mean-read-len | Average read length. | | 0–65535 |
| pe-stat-quartiles-insert | A comma-delimited trio of numbers specifying 25$^{th}$, 50$^{th}$, and 75$^{th}$ percentile template lengths. | | 0–65535 |
| pe-stat-stddev-insert | Standard deviation of template length distribution. | | 0–65535 |

# Variant Caller Options

The following options are in the Variant Caller section of the configuration file. For more information on these options, see *Variant Caller Options* on page 83.

| Name | Description | Command Line Equivalent | Value |
|---|---|---|---|
| dn-cnv-vcf | Joint structural variant VCF from the CNV calling step. If omitted, checks with overlapping copy number variants are skipped. | --dn-cnv-vcf | |
| dn-input-vcf | Joint small variant VCF *de novo* calling step to be filtered | --dn-input-vcf | |
| dn-output-vcf | File location to which the filtered VCF should be written. If not specified, the input VCF is overwritten. | --dn-output-vcf | |
| dn-sv-vcf | Joint structural variant VCF from the SV calling step. If omitted, checks with overlapping structural variants are skipped. | --dn-sv-vcf | |
| enable-combinegvcfs | Enable/disable combine gVCF run. | --enable-combinegvcfs | true/false |
| enable-joint-genotyping | To enable combine gVCF run, set to true. | --enable-joint-genotyping | true/false |
| enable-multi-sample-gvcf | Enable/disable generation of a multisample gVCF file. If set to true, requires a combined gVCF file as input. | --enable-multi-sample-gvcf | true/false |
| enable-sv | Enable/disable structural variant caller. Default is false. | --enable-sv | true/false |
| enable-vlrd | Enable/disable Virtual Long Read Detection. | --enable-vlrd | true/false |

| Name | Description | Command Line Equivalent | Value |
|---|---|---|---|
| panel-of-normals | The path to the panel of normals VCF file. | --panel-of-normals | |
| pedigree-file | Specific to joint calling. The path to a PED pedigree file containing a structured description of the familial relationships between samples. The pedigree file can contain trios. Only pedigree files that contain trios are supported. | --pedigree-file | |
| qc-snp-DeNovo-quality-threshold | The threshold for counting and reporting De Novo SNP variants. | --qc-snp-DeNovo-quality-threshold | |
| qc-indel-DeNovo-quality-threshold | The threshold for counting and reporting De Novo INDEL variants. | --qc-indel-DeNovo-quality-threshold | |
| variant | The path to a single gVCF file. Multiple --*variant* options can be used on the command line, one for each gVCF. Up to 500 gVCFs are supported. | --variant | |
| variant-list | The path to a file containing a list of input gVCF files, one file per line, that need to be combined. | --variant-list | |
| vc-af-call-threshold | Set the allele frequency call threshold to emit a call in the VCF if the AF filter is enabled. The default is 0.01. | --vc-af-call-threshold | |

| Name | Description | Command Line Equivalent | Value |
|---|---|---|---|
| vc-af-filter-threshold | Set the allele frequency filter threshold to mark emitted VCF calls as filtered if the AF filter is enabled. The default is 0.05. | --vc-af-filter-threshold | |
| vc-callability-normal-threshold | The normal sample coverage threshold for a site to be considered callable in the somatic callable regions report. | --vc-callability-normal-thresh | |
| vc-callability-tumor-threshold | The tumor sample coverage threshold for a site to be considered callable in the somatic callable regions report. | --vc-callability-tumor-thresh | |
| vc-decoy-contigs | The path to a comma-separated list of contigs to skip during variant calling. | --vc-decoy-contigs | |
| vc-detect-systematic-noise | Sensitive VCF run mode used when building a systematic noise file. | --vc-detect-systematic-noise | true/**false** |
| vc-emit-ref-confidence | To enable base pair gVCF generation, set to BP_RESOLUTION. To enable banded gVCF generation, set to GVCF. | --vc-emit-ref-confidence | BP_RESOLUTION / GVCF |
| vc-enable-af-filter | Enable/disable the allele frequency filter for somatic mode. Default is false. | --vc-enable-af-filter | true/false |
| vc-enable-baf | Enable or disable B-allele frequency output. The default is true (enabled). | --vc-enable-baf | true/false |

| Name | Description | Command Line Equivalent | Value |
|---|---|---|---|
| vc-enable-decoy-contigs | Enable/disable variant calls on decoy contigs. Default is false. | --vc-enable-decoy-contigs | true/false |
| vc-enable-gatk-acceleration | Enable/disable running variant caller in GATK mode. | --vc-enable-gatk-acceleration | true/false |
| vc-enable-liquid-tumor-mode | Enable/disable liquid tumor mode, which takes account of tumor-in-normal contamination. Default is false (disabled). | --vc-enable-liquid-tumor-mode | true/false |
| vc-enable-non-homref-normal-filter | Enable/disable the non-homref normal filter for filtering out somatic variants if the normal sample genotype is not homozygous reference. Default is true (enabled). | --vc-enable-non-homref-normal-filter | true/false |
| vc-enable-orientation-bias-filter | Enable/disable the orientation bias filter. | --vc-enable-orientation-bias-filter | true/false |
| vc-enable-phasing | Enable variants to be phased when possible. Default is true. | --vc-enable-phasing | true/false |
| vc-enable-roh | Enable or disable ROH caller and output. Default is true (enabled). | --vc-enable-roh | true/false |
| vc-enable-triallelic-filter | Enable/disable the multi-allelic filter for somatic mode. Default is true. | --vc-enable-triallelic-filter | true/false |
| vc-enable-vcf-output | Enables VCF file output during a gVCF run. Default is false. | --vc-enable-vcf-output | true/false |

| Name | Description | Command Line Equivalent | Value |
|---|---|---|---|
| vc-forcegt-vcf | Force genotyping for Germline small variant calling. A .vcf or .vcf.gz file containing a list of small variants is required. | --vc-forcegt-vcf | A .vcf or vcf.gz file specifying the small variants to force genotype. |
| vc-gvcf-gq-bands | Define GQ bands for gVCF output. Default is `10 20 30 40 60 80`. | --vc-gvcf-gq-bands | |
| vc-hard-filter | Boolean expression for filtering variant calls. Default expression is: DRAGENHardQUAL:all: QUAL < 10.4139;LowDepth:all: DP < 1 | | Parameters in the expression can include QD, MQ, FS, MQRankSum, ReadPosRankSum, QUAL, DP, and GQ. |
| vc-max-reads-per-active-region | Maximum number of reads per active region for downsampling. Default is 10000. | --vc-max-reads-per-active-region | |
| vc-max-reads-per-active-region-mito | Maximum number of reads covering a given active region for mitochondrial small variant calling. The default is 40000. | --vc-max-reads-per-active-region-mito | |
| vc-max-reads-per-raw-region | Maximum number of reads per raw region for downsampling; default is 30000. | --vc-max-reads-per-raw-region | |
| vc-max-reads-per-raw-region-mito | Maximum number of reads covering a specified raw region for mitochondrial small variant calling. The default is 40000. | --vc-max-reads-per-raw-region-mito | |

| Name | Description | Command Line Equivalent | Value |
|---|---|---|---|
| vc-min-base-qual | Minimum base quality to be considered for variant calling. Default is 10. | --vc-min-base-qual | |
| vc-min-call-qual | Minimum variant call quality for emitting a call. Default is 3. | --vc-min-call-qual | |
| vc-min-read-qual | Minimum read quality (MAPQ) to be considered for small variant calling. Default is 1 for germline, 3 for somatic T/N, and 20 for somatic T-only. | --vc-min-read-qual | |
| vc-min-reads-per-start-pos | Minimum number of reads per start position for downsampling. Default is 10. | --vc-min-reads-per-start-pos | |
| vc-min-tumor-read-qual | Minimum tumor read quality (MAPQ) to be considered for variant calling. | | |
| vc-orientation-bias-filter-artifacts | Artifact type to be filtered. An artifact (or an artifact and its reverse compliment) cannot be listed twice. | --vc-orientation-bias-filter-artifacts | C/T, G/T,or C/T, G/T, C/A |
| vc-remove-all-soft-clips | If set to true, variant caller does not use soft clips of reads to determine variants. Default is false. | --vc-remove-all-soft-clips | true/false |
| vc-roh-blacklist-bed | Blacklist BED file for ROH. | --vc-roh-blacklist-bed | |

| Name | Description | Command Line Equivalent | Value |
|---|---|---|---|
| vc-sq-call-threshold | Set the SQ call threshold to emit a call in the VCF. The default is 3 for both tumor-normal and tumor-only. | --vc-sq-call-threshold | |
| vc-sq-filter-threshold | Set the SQ filter threshold to mark emitted VCF calls as filtered. The default is 17.5 for tumor-normal and 6.5 for tumor-only. | --vc-sq-filter-threshold | |
| vc-systematic-noise | BED file with site-specific systematic noise level to calculate AQ score (systematic noise score). | --vc-systematic-noise | |
| vc-systematic-noise-filter-threshold | AQ threshold for applying the systematic-noise filter. Default 10 for tumor-normal and 60 for tumor-only. | --vc-systematic-noise-filter-threshold | 0–100. 10 for tumor-normal, 60 for tumor-only |
| vc-target-bed | Target regions BED file. | --vc-target-bed | |
| vc-target-bed-padding | Can be used to pad all of the target BED regions with the specified value (optional). If specified, is used by the small variant caller. | --vc-target-bed-padding | |
| vc-target-coverage | Target coverage for downsampling. Default value is 500 for germline and 50 for somatic mode. | --vc-target-coverage | |

| Name | Description | Command Line Equivalent | Value |
|---|---|---|---|
| vc-target-coverage-mito | Maximum number of reads with a start position overlapping any given position for mitochondrial small variant calling. Default value is 40000. | --vc-target-coverage-mito | |
| vc-tin-contam-tolerance | Maximum tumor-in-normal contamination expected. Setting this to a nonzero value enables liquid tumor mode. The default value is 0.15 if liquid tumor mode is enabled or 0 if it is disabled. | --vc-tin-contam-tolerance | |

## CNV Caller Options

The following options are available for the CNV Caller.

| Name | Description | Command Line Equivalent | Value |
|---|---|---|---|
| cnv-blacklist-bed | Regions to blacklist for CNV processing. | --cnv-blacklist-bed | |
| cnv-cbs-alpha | Significance level for the test to accept change points. Default is 0.01. | --cnv-cbs-alpha | |
| cnv-cbs-eta | Type I error rate of the sequential boundary for early stopping when using the permutation method. Default is 0.05. | --cnv-cbs-eta | |
| cnv-cbs-kmax | Maximum width of smaller segment for permutation. Default is 25. | --cnv-cbs-kmax | |

| Name | Description | Command Line Equivalent | Value |
|---|---|---|---|
| cnv-cbs-min-width | Minimum number of markers for a changed segment. Default is 2. | --cnv-cbs-min-width | |
| cnv-cbs-nmin | Minimum length of data for maximum statistic approximation. Default is 200. | --cnv-cbs-nmin | |
| cnv-cbs-nperm | Number of permutations used for p-value computation. Default is 10000. | --cnv-cbs-nperm | |
| cnv-cbs-trim | Proportion of data to be trimmed for variance calculations. Default is 0.025. | --cnv-cbs-trim | |
| cnv-counts-method | Overlap method for counting alignment. | --cnv-counts-method | midpoint / start / overlap |
| cnv-enable-gcbias-correction | Controls GC bias correction. | --cnv-enable-gcbias-correction | true/false |
| cnv-enable-gcbias-smoothing | Controls smoothing of the GC bias correction across adjacent GC bins with an exponential kernel. Default is true. | --cnv-enable-gcbias-smoothing | true/false |
| cnv-enable-plots | Enable/disable generation of plots. Default is false. | --cnv-enable-plots | true/false |
| cnv-enable-ref-calls | When true, copy neutral (REF) calls are included in the output VCF. | --cnv-enable-ref-calls | true/false |
| cnv-enable-self-normalization | Enable/disable self-normalization. | --cnv-enable-self-normalization | true/false |
| cnv-enable-tracks | Enable/disable generation of track files that can be imported into IGV for viewing. Default is true. | --cnv-enable-tracks | true/false |
| cnv-extreme-percentile | Extreme median percentile value at which to filter out samples. Default is 2.5. | --cnv-extreme-percentile | |

| Name | Description | Command Line Equivalent | Value |
|---|---|---|---|
| cnv-filter-bin-support-ratio | Filters out a candidate event if the span of supporting bins is less than the specified ratio with respect to the overall event length. The default ratio is 0.2 (20% support). | --cnv-filter-bin-support-ratio | |
| cnv-filter-copy-ratio | Minimum copy ratio threshold value centered about 1.0 at which a reported event is marked as PASS in the output VCF file. Default is 0.2 | --cnv-filter-copy-ratio | |
| cnv-filter-de-novo-quality | Phred-scale threshold for calling an event as de novo in the proband. | --cnv-filter-de-novo-quality | |
| cnv-filter-length | Minimum event length in bases at which a reported event is marked as PASS in the output VCF file. Default is 10000. | --cnv-filter-length | |
| cnv-filter-qual | The QUAL value at which a reported event is marked as PASS in the output VCF file. | --cnv-filter-qual | |
| cnv-input | CNV input file instead of a BAM. Either target.counts.gz or tn.tsv.gz (for *de novo*). | --cnv-input | |
| cnv-interval-width | Width of the sampling interval for CNV WGS processing. | --cnv-wgs-interval-width | |
| cnv-matched-normal | Target counts file of the matched normal sample. | --cnv-matched-normal | |
| cnv-max-percent-zero-samples | Threshold for filtering out targets with too many zero coverage samples. Default is 5%. | --cnv-max-percent-zero-samples | |
| cnv-max-percent-zero-targets | Threshold for filtering out samples with too many zero coverage targets. Default is 5%. | --cnv-max-percent-zero-targets | |

| Name | Description | Command Line Equivalent | Value |
|---|---|---|---|
| cnv-merge-distance | Maximum segment gap allowed for merging segments. | --cnv-merge-distance | |
| cnv-merge-threshold | The maximum segment mean difference at which two adjacent segments should be merged. The segment mean is represented as a linear copy ratio value. | --cnv-merge-threshold | |
| cnv-min-mapq | Minimum MAPQ for alignment to be counted. | --cnv-min-mapq | |
| cnv-normals-file | A single file to be used in the panel of normals. Can be specified multiple times, once for each file. | --cnv-normals-file | |
| cnv-normals-list | A panel of normals file. | --cnv-normals-list | |
| cnv-num-gc-bins | Number of bins for GC bias correction. Each bin represents the GC content percentage. Default is 25. | --cnv-num-gc-bins | 10 / 20 / 25/ 50 / 100 |
| cnv-ploidy | The normal ploidy value. Used for estimation of the copy number value emitted in the output VCF file. Default is 2. | --cnv-ploidy | |
| cnv-qual-length-scale | Bias weighting factor to adjust QUAL estimates for segments with longer lengths. Advanced option that should not have to be modified. Default is 0.9303 (2-0.1). | --cnv-qual-length-scale | |
| cnv-qual-noise-scale | Bias weighting factor to adjust QUAL estimates based on sample variance. Advanced option that should not have to be modified. Default is 1.0. | --cnv-qual-noise-scale | |
| cnv-segmentation-mode | Segmentation algorithm to perform. | --cnv-segmentation-mode | cbs / slm / aslm |

| Name | Description | Command Line Equivalent | Value |
|---|---|---|---|
| cnv-skip-contig-list | A comma-separated list of contig identifiers to skip when generating intervals for WGS analysis. The default contigs that are skipped, if not specified, are "chrM,MT,m,chrm". | --cnv-wgs-skip-contig-list | |
| cnv-slm-eta | Baseline probability that the mean process changes its value. Default is 1e-5. | --cnv-slm-eta | |
| cnv-slm-fw | Minimum number of data points for a CNV to be emitted. Default is 0. | --cnv-slm-fw | |
| cnv-slm-omega | Scaling parameter modulating relative weight between experimental/biological variance. Default is 0.3. | --cnv-slm-omega | |
| cnv-slm-stepeta | Distance normalization parameter. The default value is 10000. Only valid for “HSLM”. | --cnv-slm-stepeta | |
| cnv-target-bed | A properly formatted BED file that specifies the target intervals to sample coverage over. For use in WES analysis. | --cnv-target-bed | |
| cnv-target-factor-threshold | Percentile of panel-of-normals medians used to filter out targets. The default value is 1% for whole genome processing and 10% for targeted sequencing processing. | --cnv-target-factor-threshold | |
| cnv-truncate-threshold | Extreme outliers are truncated based on this percent threshold. Default is 0.1%. | --cnv-truncate-threshold | |
| cnv-use-somatic-vc-vaf | Use somatic SNV VAFs from VC to help determine purity and ploidy. | -cnv-use-somatic-vc-vaf | |

## Systematic Noise Creation Options

The following options are available to create systematic noise BED files from normal VCF files.

| Name | Description | Command Line Equivalent | Value |
|---|---|---|---|
| vc-systematic-noise-raw-input-list | List of VCFs to be used. One VCF per line. | --vc-systematic-noise-raw-input-list | |
| vc-systematic-noise-germline-vaf-threshold | Minimal variant allele frequency to remove potential germlines from the systematic noise file building. If not specified, all variants are used. | --vc-systematic-noise-germline-vaf-threshold | 0–1 |
| vc-systematic-noise-use-germline-tag | Whether to use DRAGEN internal germline tagging to remove potential germlines. Mutually exclusive with *--vc-systematic-noise-germline-vaf-threshold* . | --vc-systematic-noise-use-germline-tag | true/**false** |
| vc-systematic-noise-method | Method to calculate the systematic noise level across samples. | --vc-systematic-noise-method | **mean** / max / aggregate |

## Structural Variant Caller Options

| Name | Description | Command Line Equivalent | Range |
|---|---|---|---|
| enable-sv | Enable/disable structural variant caller. Default is false. | --enable-sv | true/false |
| sv-call-regions-bed | Specifies a BED file containing the set of regions to call. Optionally, you can compress the file in gzip or bzip format. | --sv-call-regions-bed | |
| sv-denovo-scoring | Enable/disable de novo quality scoring for for structural variant joint diploid calling. Pedigree file must also be provided. | --sv-denovo-scoring | |

| Name | Description | Command Line Equivalent | Range |
|---|---|---|---|
| sv-forcegt-vcf | Specify a VCF of structural variants for forced genotyping. The variants are scored and included in the output VCF even if not found in the sample data. The variants are merged with any additional variants discovered directly from the sample data. | --sv-forcegt-vcf | |
| sv-discovery | Enable SV discovery. This flag can be set to false when using --sv-forcegt-vcf to indicate that SV discovery should be disabled and only the forced genotyping input should be used. | --sv-discovery | true/false |
| sv-exome | When set to true, configures the variant caller for targeted sequencing inputs, which includes disabling high depth filters. Default is false. | --sv-exome | true/false |
| sv-output-contigs | Set to true to have assembled contig sequences output in a VCF file. Default is false. | --sv-output-contigs | true/false |
| sv-region | Limit the analysis to a specified region of the genome for debugging purposes. Can be specified multiple times to build a list of regions. | --sv-region | Must be in the format chr:startPos-endPos. |

## CYP2D6_CommandLine_fDG

| Name | Description | Command Line Equivalent | Range |
|---|---|---|---|
| enable-cyp2d6 | Enable CYP2D6 diplotyping. Default is false | --enable-cyp2d6 | true/false |

## Repeat Expansion Detection Options

The following options can be set in the RepeatGenotyping section of the configuration file or on the command line. For more information, see *Repeat Expansion Detection with Expansion Hunter* on page 152.

| Parameter Name | Description | Command Line Equivalent | Range |
|---|---|---|---|
| enable | Enable or disable repeat expansion detection. | --repeat-genotype-enable | true/false |
| specs | The full path to the JSON file that contains the repeat variant catalog (specification) describing the loci to call. | --repeat-genotype-specs | |

## RNA-Seq Command Line Options

| Name | Description | Command Line Equivalent | Range |
|---|---|---|---|
| enable-rna | Enable processing of RNA-seq data. | --enable-rna | true/false |
| annotation-file | Gene annotation file. Required for quantification and gene-fusion | --annotation-file, -a | Path to GTF (or .gtf.gz) file |
| enable-rna-quantification | Enable/disable RNA quantification. | --enable-rna-quantification | true/false |
| rna-quantification-library-type | Specifies the type of the RNA-seq library. Default is autodetect. | --rna-quantification-library-type | IU, ISR, ISF, U, SR, SF, orA. |
| rna-quantification-gc-bias | Correct for GC bias in fragment counts. | --rna-quantification-gc-bias | true/false |
| enable-rna-gene-fusion | Enable/disable RNA gene fusion calling. | --enable-rna-gene-fusion | true/false |
| rna-gf-restrict-genes | Ignore genes with biotype other than protein coding or lncRNA for gene fusions. | --rna-gf-restrict-genes | true/false |

# UMI Options

| Name | Description | Command Line Equivalent | Range |
|---|---|---|---|
| umi-library-type | Batch option for correcting UMIs. | --umi-library-type | Not required. The following are valid options: random-duplex / random-simplex / nonrandom-duplex |
| umi-enable | Enable UMI-based read processing. | --umi-enable | true / false |
| umi-correction-scheme | The methodology to use for correcting sequencing errors in UMIs. | --umi-correction-scheme | lookup / random / none / positional |
| umi-correction-table | Specify the correction table for lookup correction scheme. | --umi-correction-table | Path to table file |
| umi-emit-multiplicity | Consensus read output type. | --umi-emit-multiplicity | both/duplex/simplex |
| umi-min-supporting-reads | Number of input reads with matching UMI and position required to generate a consensus read. | --umi-min-supporting-reads | Integer ≥ 1. The default is 2. |
| umi-metrics-interval-file | Path to target regions file used for UMI on target metrics. | --umi-metrics-interval-file | Path to valid BED file |
| umi-source | The location to read UMIs from. | --umi-source | qname/bamtag/fastq |
| umi-fastq | Path to a separate FASTQ file with UMI sequences for each read | --umi-fastq | Path to valid FASTQ file |
| umi-nonrandom-whitelist | File listing valid nonrandom UMIs, one per line | --umi-nonrandom-whitelist | Path to file containing valid nonrandom UMI sequences |

| Name | Description | Command Line Equivalent | Range |
|---|---|---|---|
| umi-fuzzy-window-size | Collapse reads with matching UMIs and alignment positions +/ this distance | --umi-fuzzy-window-size | Integer ≥ 1. The default is 3. |

# Technical Assistance

For technical assistance, contact Illumina Technical Support.

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**Safety data sheets (SDSs)**—Available on the Illumina website at support.illumina.com/sds.html.

**Product documentation**—Available for download from support.illumina.com.