

Analytical performance of a highly sensitive, distributable whole-genome sequencing (WGS) assay for molecular residual disease (MRD) detection research

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BACKGROUND

- Molecular residual disease (MRD) detection using cell-free DNA (cfDNA) for solid tumors is quickly evolving and gaining momentum for predicting disease recurrence and guiding treatment decisions. However, currently there is a lack of distributable solutions to allow flexible research analysis.
- Here we describe the development and analytical performance of a distributable Illumina WGS Oncology Prep (Research Use Only) kit for research on MRD detection using tumor-informed whole-genome sequencing (WGS) for multiple cancer types.

METHODS

- The Illumina WGS Oncology Prep kit was built using Illumina’s ligation-based WGS library preparation and high-throughput sequencing technologies.
- A single library prep kit is compatible with various sample input types, and supports multiple oncology-related research applications, including high analytical sensitivity MRD detection research.
- MRD assay is composed of two major steps, fingerprinting and circulating tumor DNA (ctDNA) detection (Figure 1).
- At the fingerprinting step, WGS of DNA from FFPE and matched buffy coat was performed at 100X and 50X raw depth respectively to generate a tumor specific somatic variant list (“fingerprint”).
- At the ctDNA detection step, WGS of cfDNA was performed at 50X raw depth, and ctDNA was detected using the matched fingerprint.
- Sequencing was performed on NovaSeq™ X or NovaSeq™ 6000 series.
- Data analysis can be conducted on Illumina’s DRAGEN MRD pipeline that is available both locally and on cloud via Illumina Connected Analytics.

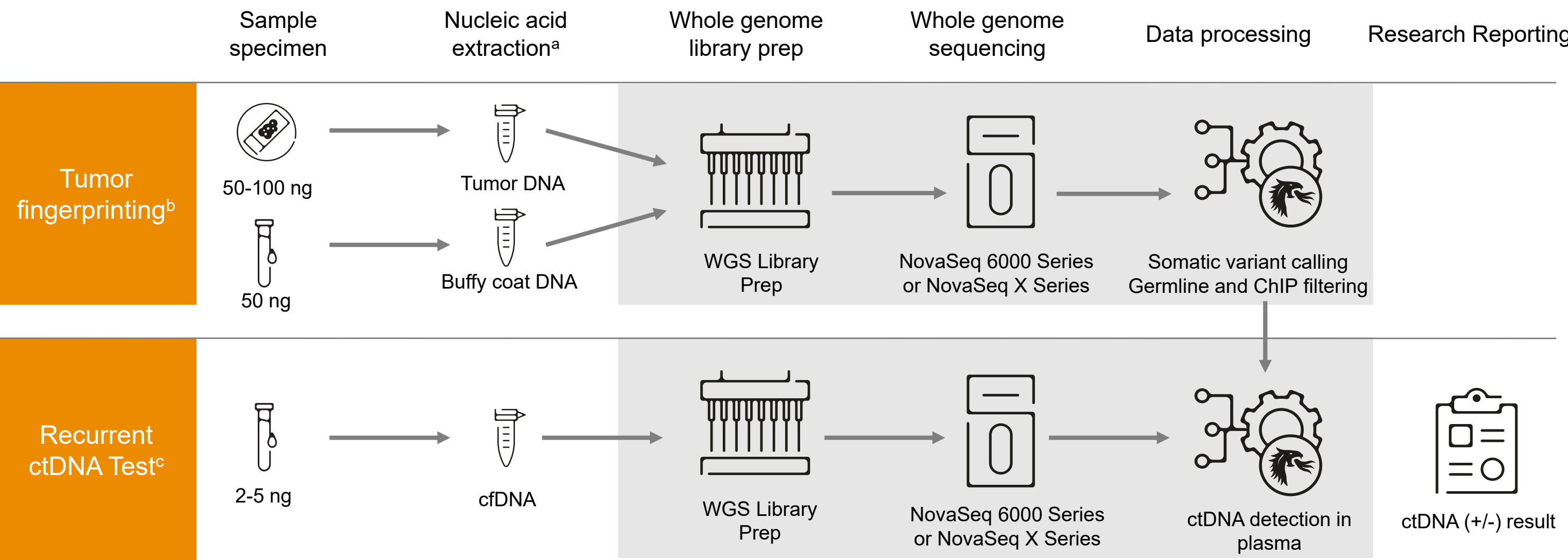


Figure 1. Tumor-informed ctDNA detection. **a**, Extraction is performed using commercially available extraction kits; **b**, Tumor fingerprinting is performed once at baseline; **c**, ctDNA testing can be performed at multiple longitudinal time points.

- The workflow features:

Low cfDNA input requirement: 5 ng recommended, 2 ng minimum, enabling high sample success rate

Single versatile library prep kit: compatible with all MRD sample types

Fast turn-around time (TAT): ≤ 5 days for tumor fingerprinting; ≤ 5 days for each ctDNA testing

RESULTS

- Analytical sensitivity was evaluated using dilution series generated from patient-derived cfDNA and contrived cell line-derived nucleosomal DNA, representing five cancer types.
- Four variant allele frequency (VAF) levels, 10 replicates per level per reagent kit lot across three days by two or more operators.
- Following CLSI guidelines, Limit of Detection (LoD) was estimated using empirical hit rate or probit regression model.
- LoD95 ranges from 10 to 40 PPM (Table 1). Both reagent lots demonstrated consistent detection performance in the dilution series from a melanoma patient sample (Figure 2).

Sample source	Cancer type	LoD95 (PPM)	
		Lot 1	Lot 2
Patient	Bladder	25 ^a	25 ^a
Contrived	Breast	30 ^a	40 ^b
Patient	Colorectal	15 ^a	20 ^b
Patient	Melanoma	10 ^a	10 ^a
Contrived	NSCLC	20 ^a	20 ^a

Table 1. Analytical Sensitivity for samples from five cancer types. **a**, Determined by empirical hit rate; **b**, Determined by probit regression.

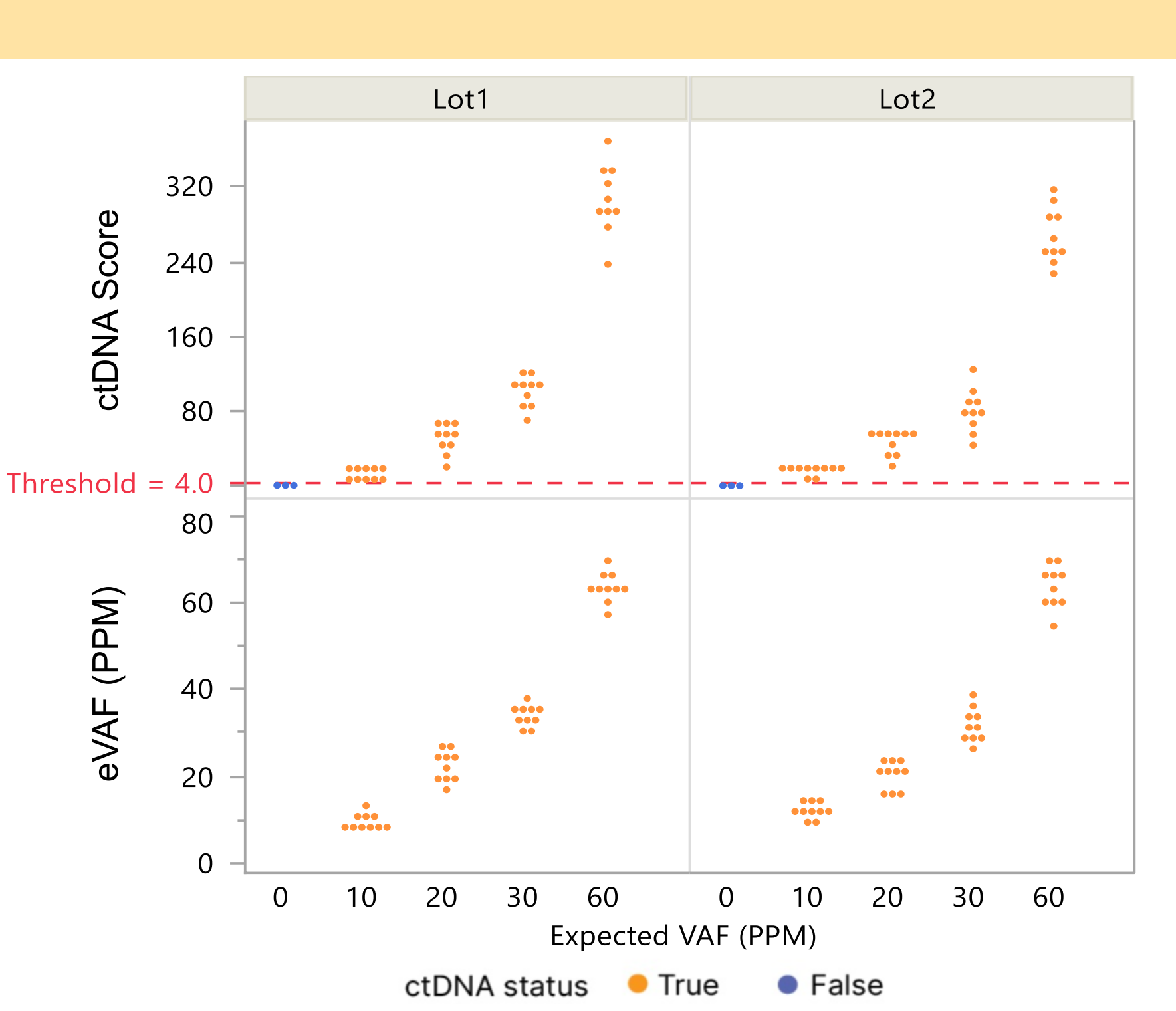


Figure 2. ctDNA detection for a melanoma patient sample across dilution series.

- Analytical specificity was evaluated using cfDNA sample derived from healthy donors.
- 104 libraries were successfully prepared across two reagent kit lots, two operators, and six testing days.
- Each cfDNA sample was evaluated with ten unique tumor fingerprints (two per cancer type).
- The assay achieved 99.5% analytical specificity (Table 2), demonstrating robust specificity across reagent kit lots, operators and testing days.

No. normal cfDNA libraries	104
No. unique tumor fingerprints	10
Total no. observations	1040
No. ctDNA negative observations	1035
No. ctDNA positive observations	5
Analytical specificity (95% CI)	99.5% (98.9% - 99.8%)

Table 2. Analytical Specificity across five cancer types.

- Assay precision was evaluated using matched patient-derived trios and cfDNA from healthy donors.
- Patient-derived cfDNA samples were diluted to challenging VAF levels.
- End-to-end testing was performed with all sample types processed across all testing conditions.
- The assay achieved 100% Percent Positive Concordance (PPC) and Percent Negative Concordance (PNC) (Figure 3).

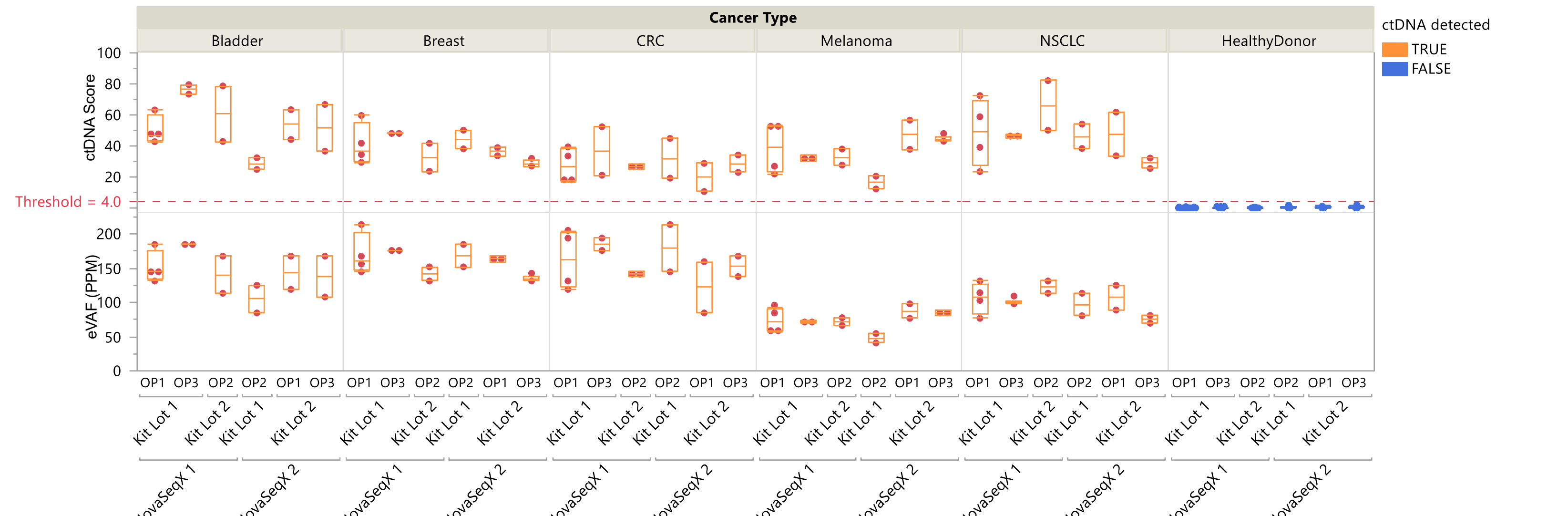


Figure 3. Assay precision with testing conditions across operators (OP), reagent kit lots, and NovaSeq X Plus units.

CONCLUSIONS

The Illumina WGS Oncology Prep offers a versatile, high-performance WGS multi-sample workflow for scalable deployment of MRD analysis research. It demonstrated high analytical sensitivity, analytical specificity and precision in detecting the presence of ctDNA from low input plasma samples with fast TAT and low operational complexity.

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