Can Next-Generation Sequencing Replace Sanger Sequencing? A Review of the Illumina Cystic Fibrosis Diagnostic Test on the MiSeqDx™ Instrument


Introduction

Cystic Fibrosis is a considered to be a common genetic disease, especially among the European Union (EU) and United States (US). The WHO currently states that the incidence rate in newborns is between 1 in 2000-3000 in the EU and 1 in 3500 in the US. Current practice for confirmatory diagnostic testing of individuals suspected of having cystic fibrosis typically involves the use of single-plex PCR and subsequent Sanger sequencing of numerous amplicons spanning the protein coding region of the CFTR gene. This process is both time and labor intensive.

Here we describe the development of a new next-generation sequencing assay, the Illumina MiSeqDx Cystic Fibrosis Diagnostic Assay, for the detection of all known and novel CFTR variants on the MiSeqDx instrument. The assay is designed to sequence the full protein coding region of CFTR and intron-exon boundaries to detect variations within the CFTR gene as an aid in diagnosis of cystic fibrosis in genomic DNA extracted from whole blood samples. The assay is designed to also detect two deep intronic mutations (3869+10kbC>T and 1811+1.6kbA>G), and two large deletions (CFTRdel5.3, and CFTRdel22.23).

Assay Workflow

The MiSeqDx Cystic Fibrosis Diagnostic Assay allows 8 samples to be processed in less than 48 hours from extracted DNA through completed data analysis. For the library preparation, the samples can be processed from extracted DNA to normalized samples (ready to be loaded on the sequencing instrument) within 7 hrs with less than 2.5 hrs of hands on time. All 8 normalized samples can be pooled and sequenced in a single MiSeqDx run.

Sequencing on the MiSeqDx instrument

The Illumina MiSeqDx system is a benchtop personal sequencer which utilizes Synthesis (SBS) technology. The MiSeqDx has an integrated fluidics architecture and a built-in CPU which enables cluster generation, sequencing, data analysis, and mutation report generation to be integrated on a single instrument.

- After library preparation, the pooled, normalized and indexed library is loaded on to the MiSeq reagent cartridge which contains all of the reagents required for cluster generation and SBS. The library is first hybridized, then covalently attached (through bridge amplification) onto the flow cell surface, and amplified to generate millions of clusters which can then be sequenced using SBS.

- The SBS process uses four fluorescently labeled nucleotides; during each sequencing cycle, a single dNTP with reversible terminator is added, the fluorescent dye imaged to identify the base and the terminator enzymatically cleaved to allow incorporation of the next nucleotide.

- The imaging subcomponent of the instrument consists of two cameras and two LEDs. Each LED is able to capture fluorescence in two channels (530 nm and 660 nm), which together allow for the system to recognize the four base pairs.

- The Illumina MiSeqDx Cystic Fibrosis System performs paired end 2 x 150 cycle sequencing to allow sequencing for 150 cycles from both directions, and 2x8 cycle sequencing to determine the sequences of both indexes in each cluster.

Assay Performance

The Illumina MiSeqDx Cystic Fibrosis Diagnostic Assay had an average call rate of 99.99% when tested on N=400 unrelated blood samples (393 samples had a 100% call rate, 7 samples had a call rate of 99.98% due to No call for PolyTG/PolyT) and a call rate >99.9% when tested on >1500 HapMap and human variation samples of multiple ethnicities.

Accuracy and Reproducibility

A set of 47 Coriell DNA samples, chosen to be representative of the different types of mutations within CFTR gene, were tested using the Illumina MiSeqDx Cystic Fibrosis Diagnostic assay by 3 operators on each of 3 MiSeqDx instruments. The results across 9 MiSeqDx runs indicated excellent reproducibility (100%) and accuracy (100%) when compared to results obtained with bi-directional Sanger sequencing (for all variations except the 2 large deletions) and PCR assay (for the 2 large deletions).

miSeqDx Software Components

The Illumina MiSeqDx Cystic Fibrosis Diagnostic Assay can accurately sequence the entire CFTR gene in a single run with up to 8 samples. This will provide significant workflow benefits versus current Sanger Sequencing methods. The design of the instrument and the preliminary performance of the Illumina assay make this system appropriate for clinical use.*