MiSeqDx™ Cystic Fibrosis Clinical Sequencing Assay

The first FDA-cleared *in vitro* diagnostic next-generation sequencing assay to provide a complete view of the *CFTR* gene for increased confidence in cystic fibrosis variant detection.

Highlights

- **Complete View of the *CFTR* Gene**
  Capture all variants in the protein coding regions and intron/exon boundaries of the *CFTR* gene
- **Accurate Results**
  Deep coverage (> 3,000×) ensures detection accuracy with a Positive Agreement (PA) of 99.66%*
- **Removed Demographic Bias**
  Sequencing the *CFTR* gene removes the bias inherent in existing genotyping panels

* PA is the number of samples with agreeing variant calls divided by the total number of samples with that variant as identified by the reference method.

Introduction

Cystic fibrosis (CF) affects approximately 70,000 children and adults worldwide[1]. The disease appears when an individual inherits two mutated copies of the cystic fibrosis transmembrane receptor (*CFTR*) gene. Early diagnosis and treatment of CF can improve both survival and quality of life[2]. Unfortunately, current molecular genotyping tests target a limited number of mutations, omitting variants that may have some clinical relevance. As a result, families may endure long periods of additional testing and patients may experience a delay in receiving needed treatment.

To overcome this challenge, Illumina offers the MiSeqDx Cystic Fibrosis Clinical Sequencing Assay (Figure 1), the first FDA-cleared *in vitro* diagnostic (IVD) next-generation sequencing (NGS) test for obtaining a comprehensive view of the *CFTR* gene.

Figure 1: MiSeqDx Cystic Fibrosis Clinical Sequencing Assay. The MiSeqDx Cystic Fibrosis Clinical Sequencing Assay is the first FDA-cleared NGS test to provide a comprehensive view of the *CFTR* gene.

Looking at the *CFTR* Sequence

Using Illumina NGS technology and sequencing by synthesis (SBS) chemistry, the MiSeqDx Cystic Fibrosis Clinical Sequencing Assay sequences all protein coding regions and intron/exon boundaries of the *CFTR* gene. In addition, the assay detects two large deletions, two deep intronic mutations, and indels in homopolymeric regions such as the 2184delA deletion (Table 1). The assay also automatically detects polyTG/polyT variants. Looking at the full *CFTR* sequence minimizes the demographic bias inherent in existing CF genotyping panel tests. As a result, the MiSeqDx Cystic Fibrosis Clinical Sequencing Assay can uncover rare mutations not accounted for in standard tests.

How it Works

The MiSeqDx Cystic Fibrosis Clinical Sequencing Assay takes advantage of Illumina NGS technology and SBS chemistry to produce a reliable *CFTR* sequence. The streamlined sample preparation workflow replaces the multiple rounds of PCR required by Sanger sequencing

with a single PCR step. For higher throughput, the assay enables multiplexing capabilities for simultaneous sequencing of 8 samples (Figure 2). Additionally, Illumina NGS is highly accurate and reproducible with ≥ 99.7% overall agreement for all reported positions and a sample first pass rate of 99.7%.

Complete Kit Convenience

The MiSeqDx Cystic Fibrosis Clinical Sequencing Assay includes everything needed for library preparation, sample multiplexing, and sequencing in a single kit. All reagents are packaged in a convenient ready-to-use format, minimizing hands-on time and increasing uniformity in all tests.

Efficient Sample Preparation Increases Throughput

Library preparation begins with 250 ng genomic DNA (gDNA) isolated from a blood sample. The DNA is mixed with a pool of oligonucleotide probes. Each probe includes sequences designed to capture the designated variant and an adapter sequence used in a subsequent amplification reaction. The probes hybridize to the DNA, one upstream and one downstream of specific *CFTR* variants (Figure 3). A proprietary extension-ligation reaction extends across the region of interest, followed by ligation, to unite the two probes. This reaction creates a template strand and gives the assay excellent specificity.

To increase the number of samples analyzed in a single sequencing run, individual libraries are "tagged" with a unique identifier, or index. These unique sample-specific indices are added to each extensionligation template in a PCR amplification step. The final reaction product contains the *CFTR* variants with the necessary sequencing adapters and indices for sequencing on the MiSeqDx instrument. An automated three-read sequencing strategy identifies each tagged sample for individual downstream analysis. Using this approach, sample identification is highly accurate, maintaining the high integrity of the assay. Eight samples can be pooled in a single sequencing run.

Table 1: *CFTR* Regions Sequenced in the MiSeqDx Cystic Fibrosis Clinical Sequencing Assay.

All protein coding regions
Intron/exon boundaries
Large deletions (exon 2,3 and exon 22,23)
Deep intronic mutations (1811+1.6Kb A>G, 3849+10Kb C>T)
Indels in homopolymeric regions
PolyTG/PolyT

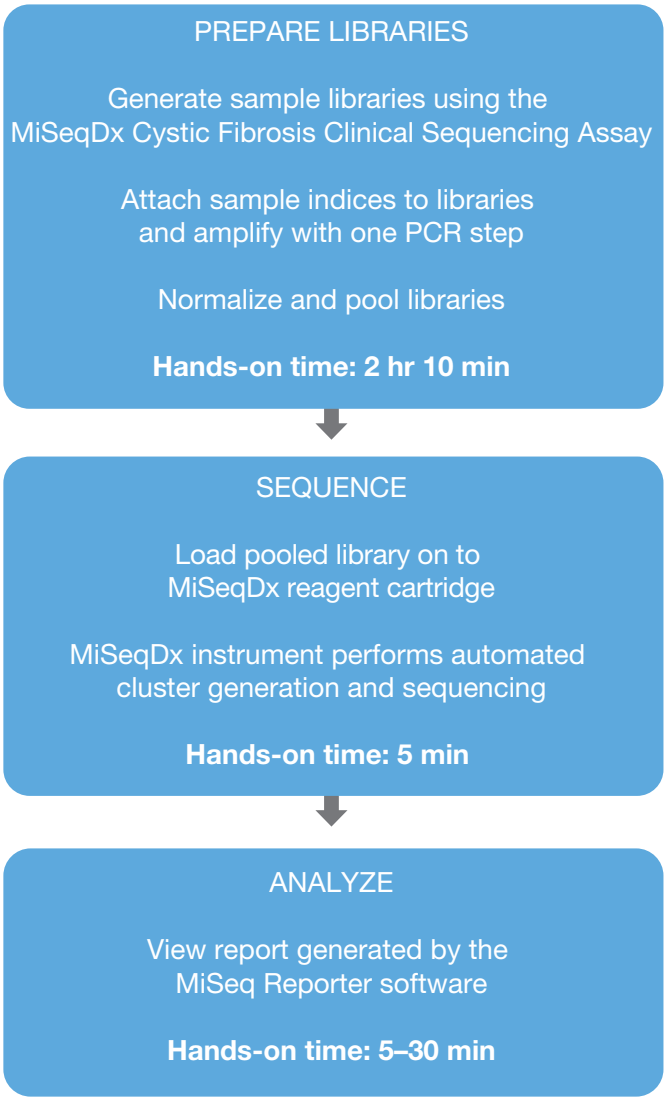

Figure 2: Integrated Clinical Sequencing Assay Workflow. The MiSeqDx Cystic Fibrosis Clinical Sequencing Assay offers a fully integrated end-to-end solution for sequencing the protein coding regions and intron/exon boundaries of the *CFTR* gene.

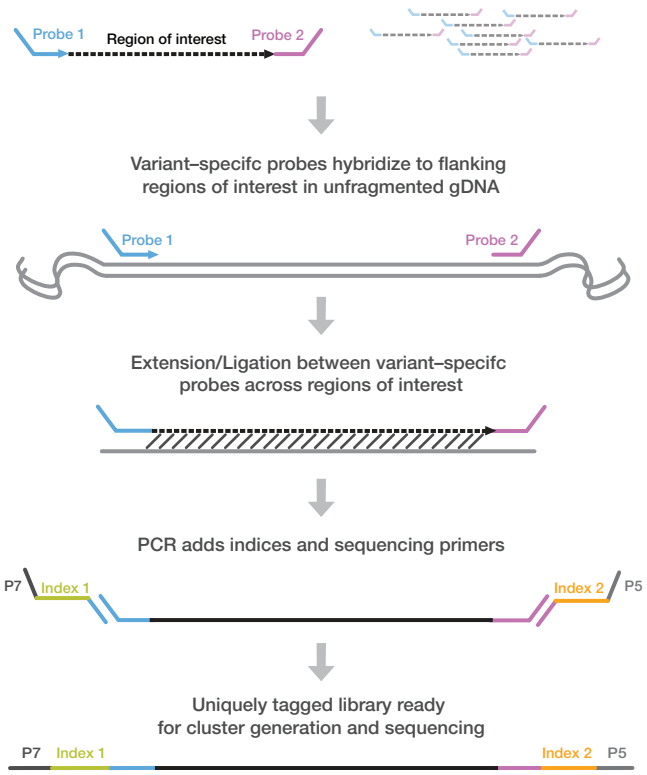

Figure 3: MiSeqDx Cystic Fibrosis Clinical Sequencing Assay Chemistry. The MiSeqDx Cystic Fibrosis Clinical Sequencing Assay offers a highly specific method for sequencing the *CFTR* gene.

Widely Adopted NGS Platform

Illumina SBS technology is a widely adopted NGS platform in the sequencing community. Through massively parallel sequencing using a proprietary reversible terminator-based method, it enables detection of single bases as they are incorporated into growing DNA strands. A fluorescently labeled terminator is imaged as each dNTP (deoxyribonucleotide triphosphates: dATP, dCTP, dGTP, or dTTP) is added and then cleaved to allow incorporation of the next base. Since all four reversible terminator-bound dNTPs are present during each sequencing cycle, natural competition minimizes incorporation bias. The result is base-by-base sequencing that enables highly accurate data.

Easy Results Interpretation

Results from the MiSeqDx Cystic Fibrosis Clinical Sequencing Assay are presented in an easy-to-read fashion that a board-certified molecular geneticist or equivalent can easily interpret. The assay report includes assay name, sample ID, dbSNP ID, HGVS nomenclature, and the call rates for each base (Figure 4). Call rates for each sample must be ≥ 99% to be considered valid.

Data Quality

The MiSeqDx Cystic Fibrosis Clinical Sequencing Assay supports 8 samples per run, while providing excellent specificity and uniformity. To assess performance, accuracy, and reproducibility, studies were conducted using Sanger sequencing and PCR as reference methods. Total accuracy and reproducibility were ≥ 99.7%.

Summary

The MiSeqDx Cystic Fibrosis Clinical Sequencing Assay is the first FDA-cleared NGS diagnostic test for CF. Sequencing the *CFTR* gene provides a comprehensive genetic view not available using standard molecular genotyping panels. The additional data eliminates demographic bias and enables accurate detection of two large deletions, two deep intronic mutations, and indels in homopolymeric regions. This single assay can lead to faster identification of clinically relevant mutations.

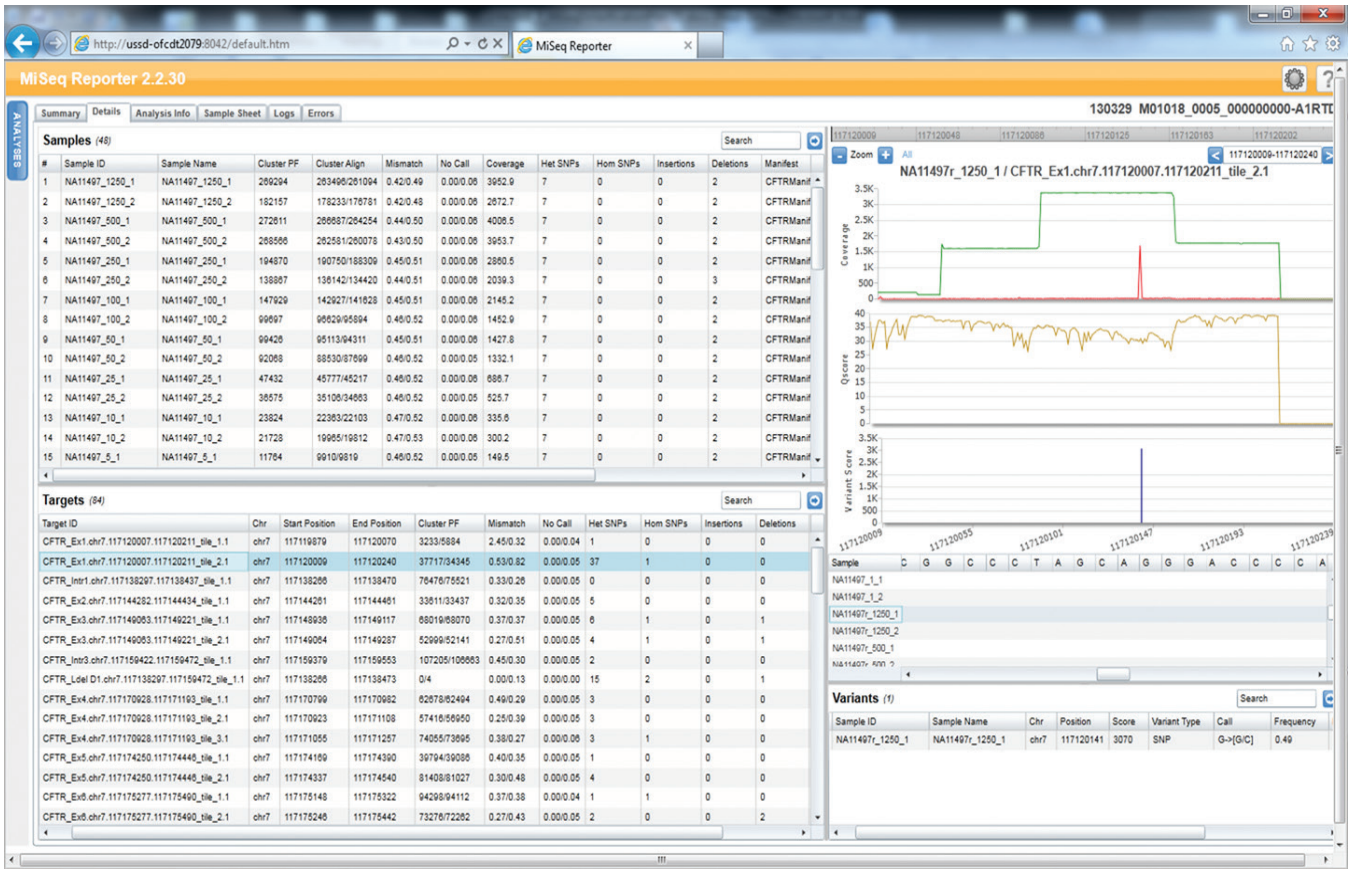

Figure 4: Easy Visualization Using the MiSeq Reporter Software.

Learn More

To learn more about the MiSeqDx Cystic Fibrosis Clinical Sequencing Assay, visit www.illumina.com/cysticfibrosis.

References

1. Cystic Fibrosis Foundation (www.cff.org/AboutCF/Faqs/)
2. U.S. National Library of Medicine. PubMed Health (www.ncbi.nlm.nih.gov/pubmedhealth/PMH0001167/)

Ordering Information.

Product	Catalog No.
MiSeqDx Cystic Fibrosis Clinical Sequencing Assay (6 runs, up to 48 samples)	DX-102-1001

Intended Use

The Illumina MiSeqDx Cystic Fibrosis Clinical Sequencing Assay is a targeted sequencing *in vitro* diagnostic system that re-sequences the protein coding regions and intron/exon boundaries of the cystic fibrosis transmembrane conductance regulator (CFTR) gene in genomic DNA isolated from human peripheral whole blood specimens collected in K2EDTA. The test detects single nucleotide variants and small indels within the region sequenced, and additionally reports on two deep intronic mutations and two large deletions. The test is intended to be used on the Illumina MiSeqDx instrument.

The test is intended to be used as an aid in the diagnosis of individuals with suspected cystic fibrosis (CF).This assay is most appropriate when the patient has an atypical or non-classic presentation of CF or when other mutation panels have failed to identify both causative mutations. The results of the test are intended to be interpreted by a board-certified clinical molecular geneticist or equivalent and should be used in conjunction with other available information including clinical symptoms, other diagnostic tests, and family history. This test is not indicated for use for stand-alone diagnostic purposes, fetal diagnostic testing, pre-implantation testing, carrier screening, newborn screening, or population screening.

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