MiSeqDx™ Cystic Fibrosis 139-Variant Assay

The first FDA-cleared next-generation sequencing assay with the largest panel of functionally validated and clinically relevant cystic fibrosis variants in a demographically diverse population.

Highlights

- **Improved Detection Rates of Cystic Fibrosis–Causing Variants**
  Detection of couples at risk increased from 72% to ~91%*
- **Higher Confidence in Test Results**
  139-variant panel reduces residual risk
- **Reduced Additional Testing**
  Highly accurate and reproducible sequencing technology delivers the right answer the first time

* Compared to the current ACMG recommended panel.

MiSeqDx Cystic Fibrosis 139-Variant Assay. The MiSeqDx Cystic Fibrosis 139-Variant Assay is the first FDA-cleared NGS test to reliably detect 139 *CFTR* variants.[3]

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. Millions carry a single mutated gene and do not exhibit any symptoms.[2] Many of these people are unaware of their mutation and that they are at risk for passing the gene for CF to their children.

CF affects a diverse population with the highest recognized incidence observed in individuals of European descent (Table 1). Current CF testing methods focus on *CFTR* variants most commonly found in Caucasians, potentially missing CF causative variants in other demographics. To overcome this challenge, Illumina offers the MiSeqDx Cystic Fibrosis 139-Variant Assay (Figure 1). This FDA-cleared *in vitro* diagnostic (IVD) test accurately detects 139 *CFTR* variants[3] (Table 2) using Illumina next-generation sequencing (NGS) technology. Combining comprehensive detection with proven Illumina NGS technology provides the most complete answer the first time, reducing residual risk.

Complete Kit Convenience

The MiSeqDx Cystic Fibrosis 139-Variant 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. To accommodate a broad spectrum of testing needs, from small to large volumes, the assay is available in two kit configurations (2 or 20 runs).

Fully Integrated Workflow

The MiSeqDx Cystic Fibrosis 139-Variant Assay is a fully integrated CF testing solution (Figure 2). Simply prepare sample libraries, load on to the MiSeqDx instrument for sequencing, and analyze the data. Each step is easy to perform, with most of the workflow automated.

Efficient Sample Preparation Increases Throughput

Library preparation begins with 250 ng genomic DNA (gDNA) isolated from a blood sample. The DNA is mixed with an oligo pool of probes. Each probe includes sequence designed to capture the designated variant and an adapter sequence used in a subsequent

Table 1: CF Carrier Frequency in Different Ethnic Groups[a]

Ethnic Group	Observed Carrier Frequency
African American	1 in 84
Ashkenazi Jewish	1 in 29
Asian	1 in 242
Caucasian	1 in 28
Hispanic	1 in 59
Jewish	1 in 32
Middle Eastern	1 in 91
Native American	1 in 70
South Asian	1 in 118
Other Ethnicity	1 in 111
> 1 Ethnicity	1 in 34
Part African American	1 in 56
Part Caucasian	1 in 32
Part Hispanic	1 in 51
Not Provided	1 in 37
All Individuals	1 in 38

[a] Source: Rohlfs EM, Zhou Z, Heim RA, Nagan N, Rosenblum LS, et al. (2011) Cystic fibrosis carrier testing in an ethnically diverse US population. Clin Chem. 57: 841–848.

Table 2: Performance Characteristics of the MiSeqDx Cystic Fibrosis 139-Variant Assay

Characteristic	PA[a]	NA[b]	OA[c]
Accuracy	100%	> 99.99%	> 99.99%
Reproducibility	99.77%	99.88%	99.88%

[a] Positive Agreement (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.

[b] Negative Agreement (NA) calculated across all wild type (WT) positions by dividing the number of concordant WT positions by the total number of WT positions as defined by the reference methods.

[c] Overall Agreement (OA) calculated across all reported positions by dividing the number of concordant wild-type and variant positions by the total number of reported positions as determined by the reference methods.

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, giving the assay excellent specificity.

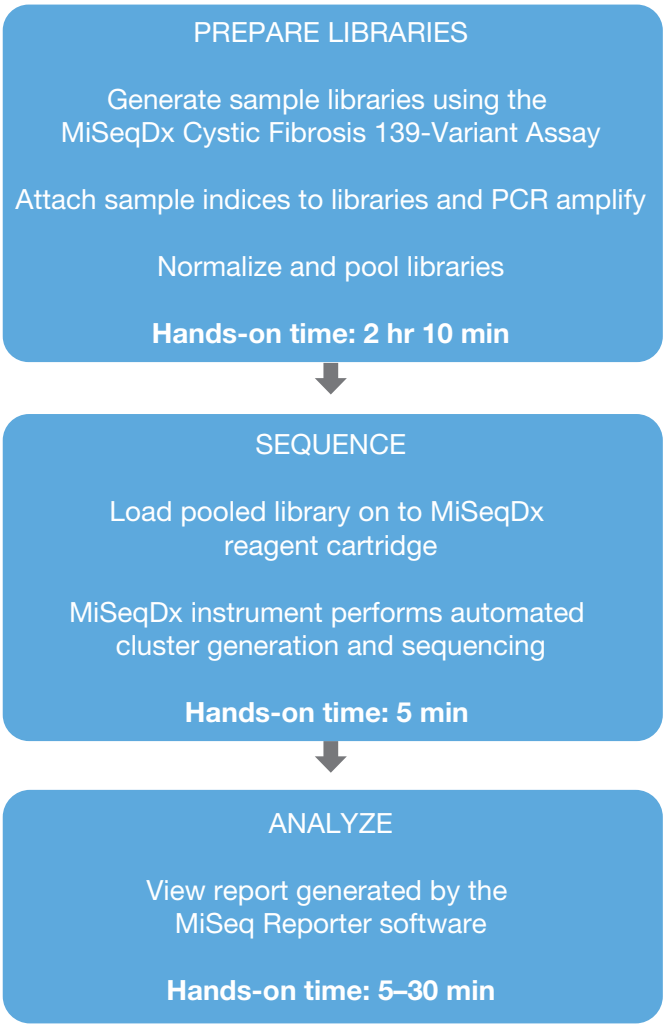

Figure 2: Integrated 139-Variant Assay Workflow. The MiSeqDx Cystic Fibrosis 139-Variant Assay offers a streamlined workflow for increased operational efficiency.

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 indexes are added to each extension-ligation template in a PCR amplification step. The final reaction product contains the *CFTR* variants with the necessary sequencing adapters and indexes for sequencing on the MiSeqDx instrument. An automated four-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. Up to 48 samples can be pooled in a single sequencing run.

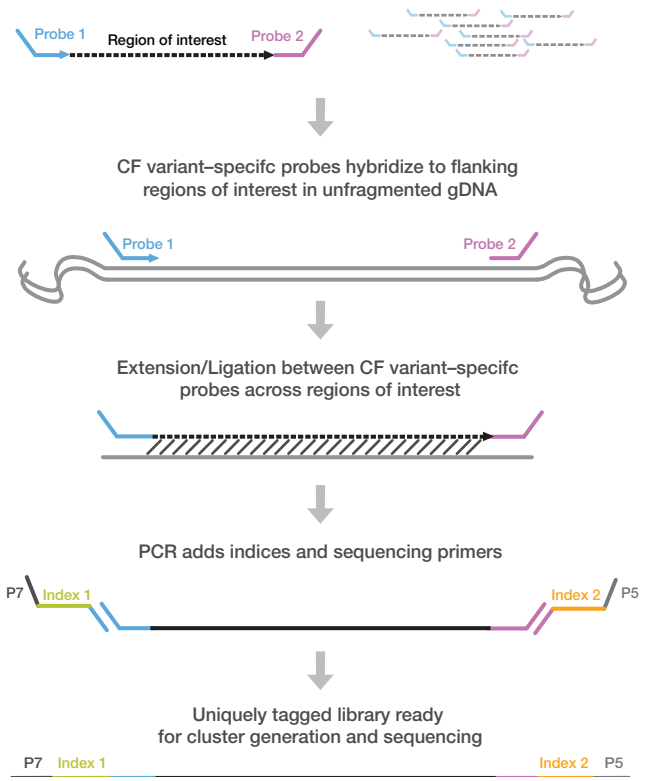

Figure 3: Multiplexing Capabilities of the MiSeqDx Cystic Fibrosis 139-Variant Assay. A highly multiplexed method simultaneously sequences up to 48 samples in a single sequencing run.

Widely Adopted NGS Platform

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

Easy Results Interpretation

Results from the MiSeqDx Cystic Fibrosis 139-Variant Assay are presented in an easy-to-read fashion that a board-certified molecular geneticist or equivalent can readily interpret. The report includes assay name, sample ID, dbSNP ID, and the call rate for each sample (Figure 4). Call rates must be ≥ 99% to be considered valid.

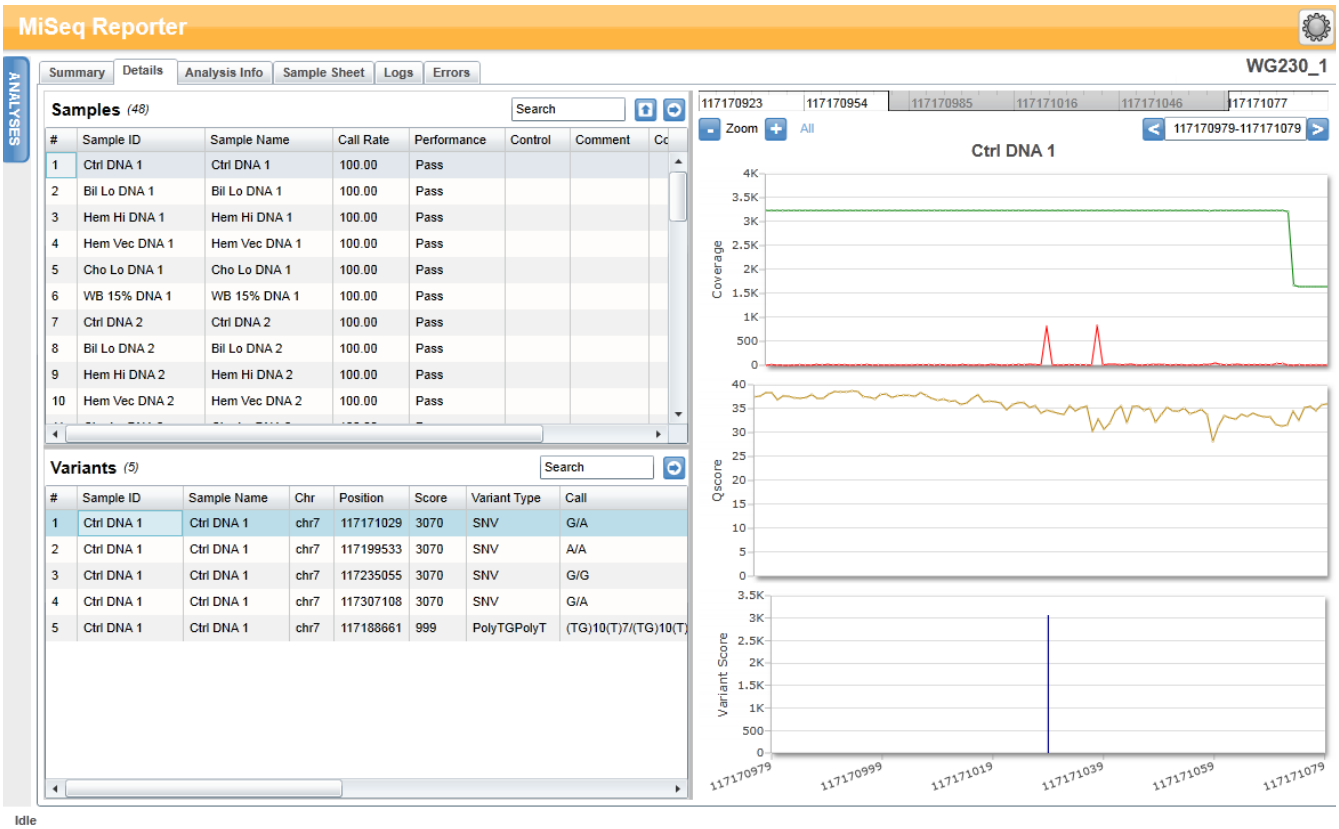

Figure 4: Easy Visualization Using the MiSeq Reporter Software.

Table 3: The MiSeqDx Cystic Fibrosis 139-Variant Assay Offers the Largest Panel of Clinically Relevant *CFTR* Variants

General Population[a]				
M1V	1213delT	1898+3A>G	2347delG	M1101K
CFTRdele2,3	1248+1G>A	1717-8G>A	R764X	E1104X
Q39X	1259insA	**1717-1G>A**	2585delT	**3659delC**
G85E	W401X(c.1202G>A)	**G542X**	2622+1G>A	**3849+10kbC>T**
E92X	W401X(c.1203G>A)	S549R(c.1645A>C)	E831X	**W1282X**
Q98X	1341+1G>A	S549R(c.1647T>G)	R851X	Q1313X
R117H	1461ins4	S549N	**2789+5G>A**	4209TGTT>AA
621+1G>T	**A455E**	**G551D**	L927P	CFTRdele22,23
711+3A>G	L467P	**R553X**	3007delG	4382delA
R334W	S489X	R560K	G970R	*I506V*
S341P	**I507del**	R709X	3120G>A	*I507V*
R347H	**F508del**	**2184delA**	**3120+1G>A**	*F508C*
R347P	Q525X[b]	L732X	3121-1G>A	

Regional European[a]				
E60X	G178R	1525-1G>A	2184insA	W1089X
P67L	**711+1G>T**	Q493X	E822X	Y1092X(C>A)
R75X	712-1G>T	1677delTA	W846X	Y1092X(C>G)
394delTT	Q220X	V520F	2711delT	R1158X
405+1G>A	852del22	Q552X	Q890X	S1196X
E92K	1078delT	**R560T**	S945L	G1244E
457TAT>G	I336K	E585X	3272-26A>G	S1251N
D110H	T338I	**1898+1G>A**	L1065P	3905insT
R117C	1154insTC	2143delT	R1066C	4005+1G>A
Y122X	R352Q	K710X	R1066H	**N1303K**
574delA	*PolyTG/PolyT*	2183AA>G	L1077P	4016insT

Middle Eastern[a]	US Hispanic[a]	Hispanic[a]	African American[a]	Native American[a]
S466X(C>A)	406-1G>A	663delT	G330X	**R1162X**
S466X(C>G)	711+5G>A	H199Y	A559T	
1548delG[b]	1812-1G>A	P205S	2307insA	
	S492F	L206W	3791delC	
	W1204X (c.3611G>A)	1811+1.6kb A>G		
	W1204X (c.3612G>A)	3876delA		

Listed within each demographic by genomic coordinate order. **Bold i**ndicates that these mutations are part of the ACMG-23 list recommended for CF screening. *Italics* indicates that these mutations are conditionally reported.

[a] Demographic data source: Castellani C, Cuppens H, Macek Jr M, Cassiman JJ, Kerem E, et al. (2008) Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice. J Cyst Fibros 7: 179–196.

[b] Mutation is classified in the CFTR2 database (www.cftr2.org) as a CF-causing variant while Sosnay et al. (Sosnay PR, Siklosi KR, Van Goor F, Kaniecki K, Yu H, et al. (2013) Defining the disease liability of variants in the cystic fibrosis transmembrane conductance regulator gene. Nat Genet. 45: 1160–1167.) classifies the variant as a mutation of unknown significance. The database classification is more current and reflects the completed functional testing, which was not available at the time of the Sosnay publication.

Best-in-Class Performance

The MiSeqDx Cystic Fibrosis 139-Variant Assay supports up to 48 samples per run with excellent specificity and uniformity. To assess the assay, performance, accuracy, and reproducibility studies were conducted using Sanger sequencing and PCR as reference methods. Highly accurate and reproducible results were achieved for all 139 variants (Table 2).

Summary

The MiSeqDx Cystic Fibrosis 139-Variant Assay is the first and only FDA cleared NGS assay for CF testing. This assay provides accurate results for an ethnically diverse population and improves detection of couples at risk of having an affected child. Combining an expanded panel of 139 variants with advanced NGS technology provides confidence in your CF testing.

Learn More

To learn more about the MiSeqDx Cystic Fibrosis 139-Variant Assay, visit www.illumina.com/cysticfibrosis.

Intended Use

The Illumina MiSeqDx Cystic Fibrosis 139-Variant Assay is a qualitative *in vitro* diagnostic system used to simultaneously detect 139 clinically relevant cystic fibrosis disease-causing mutations and variants of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene in genomic DNA isolated from human peripheral whole blood specimens. The variants include those recommended in 2004 by the American College of Medical Genetics (ACMG)[4] and in 2011 by the American College of Obstetricians and Gynecologists (ACOG)[5]. The test is intended for carrier screening in adults of reproductive age, in confirmatory diagnostic testing of newborns and children, and as an initial test to aid in the diagnosis of individuals with suspected cystic fibrosis. The results of this test are intended to be interpreted by a board-certified clinical molecular geneticist or equivalent and should be used in conjunction with other available laboratory and clinical information. This test is not indicated for use for newborn screening, fetal diagnostic testing, pre-implantation testing, or for stand-alone diagnostic purposes. The test is intended to be used on the Illumina MiSeqDx instrument.

References

1. Cystic Fibrosis Foundation (www.cff.org/AboutCF/Faqs/)
2. Cystic fibrosis. National Library of Medicine. PubMed Health. (www.ncbi.nlm.nih.gov/pubmedhealth/PMH0001167/)
3. Variants defined by the CFTR2 database (www.cftr2.org)
4. Watson MS, Cutting GR, Desnick RJ, Driscoll DA, Klinger K, et al. (2004) Cystic fibrosis population carrier screening: 2004 revision of American College of Medical Genetics mutation panel. Genet Med 6(5): 387–391.
5. Committee on Genetics. (April 2011) The American College of Obstetricians and Gynecologists Committee Opinion. Update on Carrier Screening for Cystic Fibrosis 486: 1–4.

Ordering Information

Product	Catalog No.
MiSeqDx Cystic Fibrosis 139-Variant Assay (20 runs, up to 960 tests)	DX-102-1003
MiSeqDx Cystic Fibrosis 139-Variant Assay (2 runs, up to 96 tests)	DX-102-1004

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