TruSight Cystic Fibrosis

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Package Insert

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Catalog # 20036925: 1-4 Runs, up to 96 Samples per Kit

Product Overview

The TruSight™ Cystic Fibrosis Library Prep is a library preparation kit that supports the TruSight Cystic Fibrosis 139-Variant Assay and the TruSight Cystic Fibrosis Clinical Sequencing Assay.

Intended Use for the TruSight Cystic Fibrosis 139-Variant Assay

The TruSight Cystic Fibrosis 139-Variant Assay (formerly known as 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)[1] and in 2011 by the American College of Obstetricians and Gynecologists (ACOG).[2] 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, preimplantation testing, or for stand-alone diagnostic purposes.

The test is intended to be used on the Illumina MiSeqDx Instrument.

Intended Use for the TruSight Cystic Fibrosis Clinical Sequencing Assay

The TruSight Cystic Fibrosis Clinical Sequencing Assay (formerly known as the Illumina MiSeqDx Cystic Fibrosis Clinical Sequencing Assay) is a targeted sequencing *in vitro* diagnostic system that resequences the protein coding regions and intron/exon boundaries of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene in

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genomic DNA isolated from human peripheral whole blood specimens collected in K_2EDTA. 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, for preimplantation testing, carrier screening, newborn screening, or population screening.

Cystic Fibrosis Background

Clinical Description

Cystic fibrosis (CF) is one of the most common genetic disorders of the Western world and the most common life-threatening autosomal recessive disorder in the non-Hispanic white population.[3-7] CF impacts the viscosity of mucus secretions and affects the epithelia of the respiratory tract, pancreas, intestine, hepatobiliary system, male genital tract, and the sweat glands, resulting in complex multiorgan, multisystem disease[4-6] with the lungs being the primary organ system associated with morbidity and mortality.[8] In many cases, nutritional decline presages progression of CF lung disease. A key focus of current interventional efforts is early diagnosis through newborn screening,[7] thereby facilitating timely access to vital medical services and allowing the best possible outcome for individuals with the disease.[4,7] Although there are sex differences in survival, with median survival reported to be greater for men than for women, overall median survival is 38.3 years in the US.[8]

CFTR Variants and Incidence

The cystic fibrosis transmembrane conductance regulator gene (CFTR) identified in 1989 is located on the long arm of chromosome 7 and contains 27 coding exons spread over 230 kb.[4] A 6.5 kb mRNA produced by the normal allele encodes CFTR, a 1490-amino acid integral membrane protein that functions as a regulated chloride channel in the epithelial cells of multiple organs.[4,5] More than 1900 variants of CFTR are currently described, the majority being point mutations.[9] The most common CFTR variant is the F508del allele,[5] which accounts for almost 70% of all CFTR variants.[3] However, other common CFTR variants often result in a CF phenotype and other CFTR-related disorders.[3-5]

Cystic fibrosis has a disease incidence estimated at one in 2,000–4,000 live births and a prevalence of approximately 30,000 individuals in the US population.[4] It occurs in all ethnic and racial populations, at various frequencies: one in 3,000 Caucasians, one in 9,200 Hispanic Americans, one in 10,900 Native Americans, one in 15,000 African Americans, and one in 31,000 Asian Americans.[4,6] Current estimates of CFTR mutation carrier frequency by ethnicity in the US, based on a cohort of 364,890 individuals referred for carrier testing with no family history of CF are provided in .

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General Cystic Fibrosis Mutation Carrier Frequency in Different Ethnic Groups in the US[10]

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
Other Ethnicity: > 1 Ethnicity	1 in 34
Other Ethnicity: Part African American	1 in 56
Other Ethnicity: Part Caucasian	1 in 32
Other Ethnicity: Part Hispanic	1 in 51
Not provided	1 in 37
All individuals	1 in 38

Summary and Explanation of the Cystic Fibrosis 139-Variant Assay

Overview of the CFTR2 Project

The CFTR2 project is an international initiative led by a team of researchers and clinicians and supported by a grant from National Institute of Health and the U.S. Cystic Fibrosis Foundation.[11,12] CFTR2 is intended to provide comprehensive and expert-reviewed functional and clinical information on CFTR variants. In an effort to clinically validate all CF variants with allele frequencies of 0.01% and greater, 25 CF registries and clinics from around the world[13] pooled together resources with the goal of matching clinical information from over 39,000 CF patients with the nearly 1,900 CF variants that had been recorded over the years in the CFTR1 database at the Hospital for Sick Children in Toronto.[11,13] Clinical characteristics, such as sweat chloride concentration, lung function (FEV1% predicted), and pancreatic status were analyzed alongside CFTR genotype information. The systematic approach of simultaneously analyzing these variants from clinical, functional, and genetic perspectives yielded 134 unique CF-causing variants at 129 unique genomic positions (since for five positions, two nucleotide changes appear at the same position) contained

in the CFTR2 database (as of August 2013). The use of a panel comprising all these variants is expected to account for 95.4% of cystic fibrosis-causing alleles and increase the identification of the couples at risk through detection of both alleles to ~91% from 72% using the ACMG recommended panel of 23 variants.

CFTR Variants in Panel

The variants reported by the Cystic Fibrosis 139-Variant Assay were specifically chosen because they represent the full set of clinically validated variants classified as CF-causing in the CFTR2 database (as of August 2013) at Johns Hopkins University, a product of the CFTR2 (Clinical and Functional Translation of CFTR) initiative.

The assay tests for: 134 CF-causing variants, one ACMG recommended panel variant (R117H, classified as a Mutation of Varying Clinical Consequence, MVCC, by CFTR2), one conditionally reported modifying variant (PolyTG/PolyT), and three conditionally reported benign variants (I506V, I507V, F508C)[14] for a total of 139 reported variants.

The 134 CF-causing variants correspond to 129 CF-causing variants in the CFTR2 database. The CFTR2 database includes five CF-causing variants for which the same protein level change can arise from two distinct nucleotide changes (eg, S466X(C>A) and S466X(C>G)). These five variants are listed according to the amino acid codon in the CFTR2 database (eg, S466X) while the assay reports each individual variant (eg, S466X(C>A) and S466X(C>G)). The list of 139 variants reported by the Cystic Fibrosis 139-Variant Assay is provided in . Bold=ACMG-23; Italics=Conditionally reported.

Cystic Fibrosis 139-Variant Assay Summary of Variants*

M1V (c.1A>G)	T338I (c.1013C>T)	**R553X (c.1657C>T)**	3272-26A>G (c.3140-26A>G)
CFTRdele2,3 (c.54-5940_ 273+10250del21kb)	S341P (c.1021T>C)	A559T (c.1675G>A)	L1065P (c.3194T>C)
Q39X (c.115C>T)	1154insTC (c.1022_ 1023insTC)	**R560T (c.1679G>C)**	R1066C (c.3196C>T)
E60X (c.178G>T)	R347H (c.1040G>A)	R560K (c.1679G>A)	R1066H (c.3197G>A)
P67L (c.200C>T)	**R347P (c.1040G>C)**	1811+1.6kbA>G (c.1679+1.6kbA>G)	L1077P (c.3230T>C)
R75X (c.223C>T)	R352Q (c.1055G>A)	1812-1G>A (c.1680-1G>A)	W1089X (c.3266G>A)
G85E (c.254G>A)	1213delT (c.1081delT)	E585X (c.1753G>T)	Y1092X(C>A) (c.3276C>A)
394delTT (c.262_263delTT)	1248+1G>A (c.1116+1G>A)	**1898+1G>A (c.1766+1G>A)**	Y1092X(C>G) (c.3276C>G)

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405+1G>A (c.273+1G>A)	1259insA (c.1127_1128insA)	1898+3A>G (c.1766+3A>G)	M1101K (c.3302T>A)
406-1G>A (c.274-1G>A)	W401X (c.1202G>A)	2143delT (c.2012delT)	E1104X (c.3310G>T)
E92X (c.274G>T)	W401X (c.1203G>A)	2183AA >G (c.2051_2052delAAinsG)	R1158X (c.3472C>T)
E92K (c.274G>A)	1341+1G>A (c.1209+1G>A)	**2184delA (c.2052delA)**	**R1162X (c.3484C>T)**
Q98X (c.292C>T)	1461ins4 (c.1329_1330insAGAT)	2184insA (c.2052_2053insA)	**3659delC (c.3528delC)**
457TAT>G (c.325_327delTATinsG)	**A455E (c.1364C>A)**	R709X (c.2125C>T)	S1196X (c.3587C>G)
D110H (c.328G>C)	1525-1G>A (c.1393-1G>A)	K710X (c.2128A>T)	W1204X (c.3611G>A)
R117C (c.349C>T)	S466X (C>A) (c.1397C>A)	2307insA (c.2175_2176insA)	W1204X (c.3612G>A)
R117H (c.350G>A)	S466X (C>G) (c.1397C>G)	L732X (c.2195T>G)	3791delC (c.3659delC)
Y122X (c.366T>A)	L467P (c.1400T>C)	2347delG (c.2215delG)	**3849+10kbC>T (c.3717+12191C>T)**
574delA (c.442delA)	1548delG (c.1418delG)[†]	R764X (c.2290C>T)	G1244E (c.3731G>A)
621+1G>T (c.489+1G>T)	S489X (c.1466C>A)	2585delT (c.2453delT)	3876delA (c.3744delA)
663delT (c.531delT)	S492F (c.1475C>T)	E822X (c.2464G>T)	S1251N (c.3752G>A)
G178R (c.532G>A)	Q493X (c.1477C>T)	2622+1G>A (c.2490+1G>A)	3905insT (c.3773_3774insT)
711+1G>T (c.579+1G>T)	**I507del (c.1519_1521delATC)**	E831X (c.2491G>T)	**W1282X (c.3846G>A)**
711+3A>G (c.579+3A>G)	**F508del (c.1521_1523delCTT)**	W846X (c.2537G>A)	4005+1G>A (c.3873+1G>A)
711+5G>A (c.579+5G>A)	1677delTA (c.1545_1546delTA)	R851X (c.2551C>T)	4016insT (c.3884_3885insT)
712-1G>T (c.580-1G>T)	V520F (c.1558G>T)	2711delT (c.2583delT)	**N1303K (c.3909C>G)**
H199Y (c.595C>T)	Q525X (c.1573C>T)[†]	**2789+5G>A (c.2657+5G>A)**	Q1313X (c.3937C>T)

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P205S (c.613C>T)	1717-8G>A (c.1585-8G>A)	Q890X (c.2668C>T)	4209TGTT>AA (c.4077_4080delTGTTinsAA)
L206W (c.617T>G)	**1717-1G>A (c.1585-1G>A)**	L927P (c.2780T>C)	CFTRdele22,23 (c.3964-78_4242+577del)
Q220X (c.658C>T)	**G542X (c.1624G>T)**	S945L (c.2834C>T)	4382delA (c.4251delA)
852del22 (c.720_741delAGGGAGAATGATGATGAAGTAC)	S549R (c.1645A>C)	3007delG (c.2875delG)	*PolyTG/PolyT*
1078delT (c.948delT)	S549N (c.1646G>A)	G970R (c.2908G>C)	*I506V (c.1516A>G)*
G330X (c.988G>T)	S549R (c.1647T>G)	3120G>A (c.2988G>A)	*I507V (c.1519A>G)*
R334W (c.1000C>T)	**G551D (c.1652G>A)**	**3120+1G>A (c.2988+1G>A)**	*F508C (c.1523T>G)*
I336K (c.1007T>A)	Q552X (c.1654C>T)	3121-1G>A (c.2989-1G>A)	

* Variants are listed in genomic coordinate order. The associated nucleotide level change for each variant is in parenthesis.

† Classified in the CFTR2 database[12] as a CF-causing variant, while the Sosnay paper[13] classifies the variant as indeterminate. The database classification is more current and reflects the completed functional testing, which was not available at the time of the Sosnay publication.

Summary and Explanation of the Cystic Fibrosis Clinical Sequencing Assay

Assay Design

All protein-coding regions in the CFTR gene, including 10 nt flanking intronic sequence, are detected for all exons except for three (exons 7, 10, and 20). For exon 7 and exon 10, only 5 nt flanking intronic sequence is included at the 5' end of the exon to avoid proximal homopolymeric indels. For exon 20, 30 nt flanking intronic sequence is included at the 5' end of the exon to enable detection of the mutation 3272-26A>G. In addition, the assay also detects ~100 nt flanking sequence at the 5' and 3' UTRs, two deep intronic mutations (1811+1.6kbA>G, 3489+10kbC>T), two large deletions (CFTRdele2,3, CFTRdele22,23), and the PolyTG/PolyT region. The full coverage of the assay is shown in the genomic coordinate positions listed in .

NOTE

Limitations exist for detection of deletions at specific genomic locations within the sequenced regions of this assay (see *Limitations of the Procedure Cystic Fibrosis Clinical Sequencing Assay*).

Cystic Fibrosis Clinical Sequencing Assay Genomic Coordinate Coverage

	hg19 Genomic coordinate start (chr7)	hg19 Genomic coordinate stop (chr7)	Length (base pair)
CFTR_Exon 1	117120041	117120211	171
CFTR_Exon 2	117144297	117144427	131
CFTR_Exon 3	117149078	117149206	129
CFTR_Exon 4	117170943	117171178	236
CFTR_Exon 5	117174320	117174429	110
CFTR_Exon 6	117175292	117175475	184
CFTR_Exon 7^	117176597	117176737	141
CFTR_Exon 8	117180144	117180410	267
CFTR_Exon 9	117182060	117182172	113
CFTR_Exon 10^	117188690	117188887	198
CFTR_Exon 11	117199508	117199719	212
CFTR_Exon 12	117227783	117227897	115
CFTR_Intron 12*	117229516	117229526	11
CFTR_Exon 13	117230397	117230503	107
CFTR_Exon 14	117231978	117232721	744
CFTR_Exon 15	117234974	117235122	149
CFTR_Exon 16	117242870	117242927	58
CFTR_Exon 17	117243576	117243846	271
CFTR_Exon 18	117246718	117246817	100
CFTR_Exon 19	117250563	117250733	171
CFTR_Exon 20[#]	117251605	117251872	268
CFTR_Exon 21	117254657	117254777	121
CFTR_Exon 22	117267566	117267834	269
CFTR_Intron 22*	117280010	117280020	11
CFTR_Exon 23	117282482	117282657	176

	hg19 Genomic coordinate start (chr7)	hg19 Genomic coordinate stop (chr7)	Length (base pair)
CFTR_Exon 24	117292886	117292995	110
CFTR_Exon 25	117304732	117304924	193
CFTR_Exon 26	117305503	117305628	126
CFTR_Exon 27	117306952	117307262	311
Total Bases			5203**

^ For Exon 7 and Exon 10, only 5 nt of flanking intronic sequence is included upstream of the exon to avoid homopolymeric stretches in these regions. In the case of Exon 10, this is the PolyT/Poly TG region in Intron 9. This region is treated specially and separately.

* For the deep intronic mutations, 5 nt flanking the SNV on either side are also included.

For Exon 20, 30 nt of flanking intronic sequence is included at the 5' end of the exon to enable detection of the mutation 3272-26A>G.

** With the two large deletions and the PolyTG/PolyT regions, the total positions/regions is 5206.

Principles of Procedure

The TruSight Cystic Fibrosis Library Prep is intended for manually preparing libraries used for the sequencing of DNA from peripheral whole blood specimens. Library prep consists of four key steps: Hybridization, Extension-Ligation, PCR Amplification, and Library Normalization.

NOTE

Library prep procedures for the Cystic Fibrosis 139-Variant Assay and the Clinical Sequencing Assay are identical.

Library Preparation
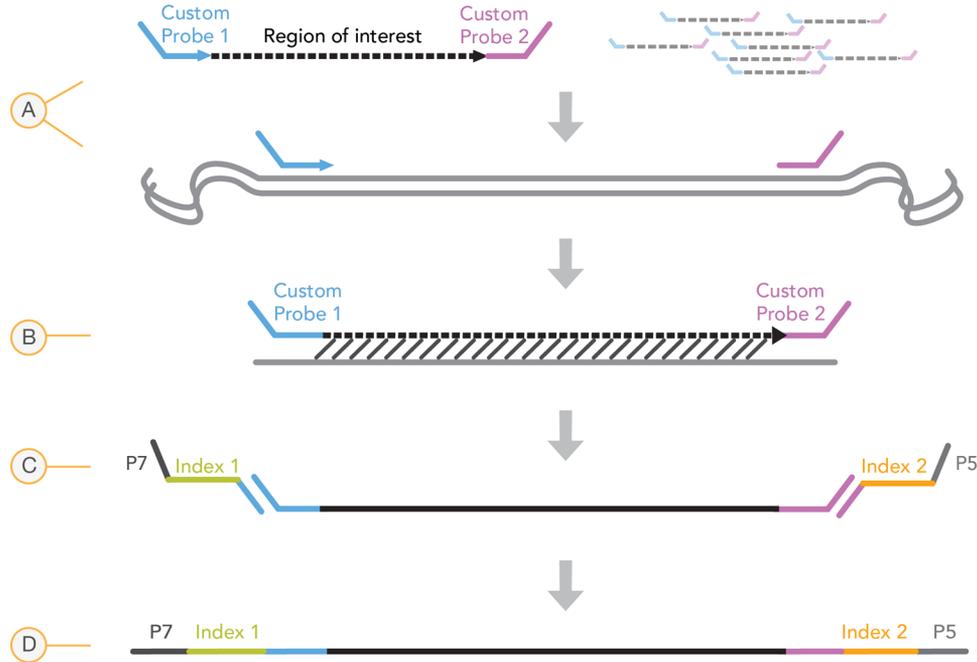
A. Hybridization—The first step, Hybridization, hybridizes a pool of upstream and downstream oligonucleotides specific to the cystic fibrosis gene to the input genomic DNA. At the end of this process, a three-step wash procedure with a filter capable of size selection removes unbound oligonucleotides from the genomic DNA.

B. Extension-Ligation—The second step, Extension-Ligation, connects the hybridized upstream and downstream oligonucleotides. A DNA polymerase extends from the upstream oligonucleotides through the targeted region, followed by ligation to the 5' end of the downstream oligonucleotide using a DNA ligase. The result is the formation of products that contain the CF-specific oligonucleotides flanked by sequences required for amplification.

C. PCR Amplification—The third step, PCR Amplification, amplifies the extension-ligation products using index adapters that add index sequences for sample multiplexing, and common adapters required for cluster generation on the MiSeqDx. At the end of this process, a PCR clean-up procedure purifies the PCR products (referred to as a library).

D. Library Normalization—The final step, Library Normalization, normalizes the quantity of each library to ensure more equal library representation in the final pooled library. At the end of this process, the pooled library is loaded onto the MiSeqDx for sequencing using SBS chemistry.

Sequencing

SBS chemistry uses a reversible-terminator method to detect single nucleotide bases as they are incorporated into growing DNA strands. During each sequencing cycle, a single fluorescently labeled deoxynucleotide triphosphate (dNTP) is added to the nucleic acid chain. The nucleotide label serves as a terminator for polymerization, so after each dNTP incorporation, the fluorescent dye is imaged to identify the base and then enzymatically cleaved to allow incorporation of the next nucleotide. Because all four reversible terminator-bound dNTPs (A, G, T, C) are present as single, separate molecules, natural competition minimizes incorporation bias. Base calls are made directly from signal intensity measurements during each sequencing cycle. The result is base-by-base sequencing.

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Data Analysis

The first step in data analysis is called primary analysis. This process is done by Real-Time Analysis (RTA) software and generates base calls and quality scoring. In the next step, called secondary analysis, the base calls generated during primary analysis are processed to produce information for each sample. Performed by the Local Run Manager software, secondary analysis includes demultiplexing, FASTQ file generation, alignment, variant calling, and generation of VCF files containing information about variants found at specific positions in the reference genome.

- Demultiplexing—If the run contains multiple samples and the run has index reads, this is the first step in secondary analysis. Demultiplexing separates data from pooled samples based on the unique sequence indexes that were added during the PCR amplification step.

- FASTQ File Generation—After demultiplexing, the Local Run Manager generates intermediate files in the FASTQ format, which is a text format used to represent sequences. FASTQ files contain the reads for each sample and the quality scores, excluding reads from any clusters that did not pass filter.

- Alignment—Alignment compares sequences against the reference to identify a relationship between the sequences and assigns a score based on regions of similarity. Aligned reads are written to files in BAM format. For the Cystic Fibrosis 139-Variant Assay and Cystic Fibrosis Clinical Sequencing Assay, a banded Smith-Waterman algorithm performs local sequence alignments to determine similar regions between two sequences.

- Variant Calling—This step records single nucleotide variants (SNV), insertions and deletions (indels), and other structural variants in a standardized text file called `TruSightCF139VariantAssay.txt` for the Cystic Fibrosis 139-Variant Assay or `TruSightCFClinicalSequencingAssay.txt` for the Cystic Fibrosis Clinical Sequencing Assay.

For more information on the analysis workflow, refer to the guides for the analysis software installed with your MiSeqDx. For the *Local Run Manager CF 139-Variant 2.0 Analysis Module Workflow Guide*, see *document # 1000000100945*. For the *Local Run Manager CF Clinical Seq 2.0 Analysis Module Workflow Guide*, see *document # 1000000100946*. For the *Local Run Manager CF 139-Variant 2.0 Micro Analysis Module Workflow Guide*, see *document # 200017946*. For the *Local Run Manager CF Clinical Seq 2.0 Micro Analysis Module Workflow Guide*, see *document # 200017945*.

Limitations of the Procedure Cystic Fibrosis 139-Variant Assay

- For *in vitro* diagnostic use.

- The results obtained using the Cystic Fibrosis 139-Variant Assay should be used and interpreted in the context of a full clinical evaluation.

- The assay is designed to identify a specific subset of known variants in the CFTR gene, but does not include all variants identified in the CFTR gene. Specifically, the assay only reports amino acid level changes if they are associated with the nucleotide changes as listed in . While other nucleotide level changes can lead to the same amino acid level changes, they are not reported by the assay. Therefore, the failure to identify a variant does not guarantee that other CFTR variants are not present in the samples being analyzed.

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- Variants identified by this assay vary in frequency among different populations.

- As with any hybridization-based assay, underlying polymorphisms or variants in oligonucleotide-binding regions can affect the alleles being probed and, consequently, the calls made.

- The assay cannot determine whether the orientation of the PolyTG/PolyT variant is in cis/trans to the R117H variant. For patients with an R117H variant, additional testing should be performed to determine whether a PolyTG/PolyT variant, which may affect the clinical phenotype (eg, 12-13(TG) or 5T), is in a cis/trans orientation to the R117H variant.

- PolyTG/PolyT are homopolymeric regions known to be difficult to interpret with sequence-based assays due to polymerase slippage. A 0.9% (4/448) miscall rate was observed for PolyTG/PolyT results demonstrating a ±1 TG discrepancy when compared to Sanger bidirectional sequencing in .

Limitations of the Procedure Cystic Fibrosis Clinical Sequencing Assay

- For *in vitro* diagnostic use.

- The results obtained using the Cystic Fibrosis Clinical Sequencing Assay should be used and interpreted in the context of a full clinical evaluation.

- The assay sequences the following regions within the CFTR gene:
  - All protein-coding regions in the CFTR gene across 27 exons.
  - Between 5-10 bases of flanking intronic sequence.
  - 100 nucleotides of intronic sequence at the 5' and 3'untranslated regions.
  - Two deep intronic mutations (1811+1.6kbA>G, 3489+10kbC>T).
  - The PolyTG/PolyT sequence located in intron 9.
  - A total of 5206 positions/regions of the possible 188,702 base pairs in the gene.

- The assay is designed to sequence protein coding regions and intron/exon boundaries of the CFTR gene and does not include all intronic regions and large deletions. Thus, an overall wild type result does not guarantee that other cystic fibrosis transmembrane conductance regulator (CFTR) mutations/variants are not present in the samples being analyzed.
  - The assay is designed to detect two specific large deletions: CFTRdele2,3 and CFTRdele22,23. The assay cannot detect or report on other large deletions. This assay is only validated for insertions and deletions up to and including 3 bp in size.

- All insertions/deletions are left aligned in homopolymeric regions as opposed to right aligned following HGVS nomenclature. For example, the variant c.313delA (with sequence context GAATC) is identified as a G-ATC deletion, but the deletion is reported in dbSNP as a GA-TC deletion. An exception to this are the 135 CF variations

listed in CFTR2 as disease-causing (based on variant database version 04/10/2012). All the indels in homopolymeric regions within this set of variations are reported to match the expected variant reporting as per CFTR2.[13]

- The assay has a limitation in detecting deletions at specific genomic locations within the sequenced regions. Genomic coordinates that the assay cannot report deletions are listed in . The assay cannot detect deletions that include the base or bases in the limitation column.

Genomic Coordinates Where Deletions Cannot be Detected

CFTR gene region	hg19 Genomic coordinates (chr7)
CFTR_Exon1	117120041; 117120211
CFTR_Exon3	117149091
CFTR_Exon4	117170953-117170954*; 117171082
CFTR_Exon5	117174362
CFTR_Exon6	117175417
CFTR_Exon7	117176621
CFTR_Exon8	117180176-117180177*
CFTR_Exon9	117182126
CFTR_Exon10	117188771
CFTR_Exon11	117199544-117199545*; 117199697
CFTR_Exon12	117227802
CFTR_Exon14	117232106-117232107*; 117232466-117232467*; 117232609
CFTR_Exon17	117243705; 117243843
CFTR_Exon18	117246751
CFTR_Exon19	117250688
CFTR_Exon20	117251788
CFTR_Exon22	117267721
CFTR_Exon23	117282597
CFTR_Exon24	117292953
CFTR_Exon25	117304740-117304741*; 117304869
CFTR_Exon26	117305518
CFTR_Exon27	117307178

* Only deletions that include both bases listed here cannot be detected. For example, in Exon8, only deletions ≥ 2 bp that include the bases at both genomic coordinates 117180176 and 117180177 cannot be detected. A single base deletion at 117180176 or 117180177 can be detected.

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- If the impacted coordinate listed in is the left-most base within a homopolymeric region, a deletion at any other position within the homopolymeric stretch cannot be detected because it cannot be distinguished from a deletion at the impacted coordinate.
- The assay cannot detect a total of five variants listed in the ClinVar clinical database (accessed database version December 2014). These five specific variants are included in . This assay limitation does not impact any variants listed in the Cystic Fibrosis database, CFTR2 (database version 04/10/2012). No frequency data was available for any of the variants.

Known Variants not Detected by the Cystic Fibrosis Clinical Sequencing Assay

Variant #	ClinVar ID	CFTR gene region	Genomic location (Chr 7)	cDNA Name (HGVS)	Protein Name (HGVS)	rs ID
1	RCV000046424	CFTR_ Exon3	117149091	c.168delA	p.Glu56Aspfs	rs397508269
2	RCV000046687	CFTR_ Exon17	117243703-117243704*	c.2775_ 2776delTT	p.Leu926Alafs	rs397508433
3	RCV000046688	CFTR_ Exon17	117243705	c.2777delT	p.Leu926Cysfs	rs397508434
4	RCV000046782	CFTR_ Exon19	117250690*	c.3106delA	p.Thr1036Profs	rs397508497
5	RCV000046857	CFTR_ Exon20	117251789*	c.3294delG	p.Trp1098Cysfs	rs397508534

* In these cases, the impacted coordinates fall within a homopolymeric region.

- Variants identified by this assay vary in frequency among different populations. It is not possible to validate all combinations of variants that could be detected in the CFTR gene by this assay. It is recommended that novel and rare variants be confirmed by the user using a validated reference method.
- As with any hybridization-based assay, underlying polymorphisms, mutations, insertions, or deletions in oligonucleotide-binding regions can affect the alleles being probed and, consequently, the calls made.
- For complex variants where a deletion and insertion occur at the same site, the assay can report it as two separate variants in close proximity. Variant phasing is not evaluated and other possible solutions to the detected sequence must be considered. Refer to for an example of a complex variant of this nature.

Complex Variant, Example

Sequence Context (Reference)	GAAGAAATT
Observed Sequence for Variant	GAAT - - ATT
Variant Expected	Deletion of GAA, Insertion of T (both changes on the same chromosome)
Variants Reported by the Assay	SNP (G>T); Deletion of AA

- If more than two variants are identified for a sample, it is recommended that the user verify the result by repeating the sample using the MiSeqDx Instrument with a fresh gDNA extract to rule out cross-contamination of the specimen.

NOTE

> Haplotype phasing should be considered when two or more variants are detected. This assay cannot determine whether variants are in cis/trans to other variants.

- The assay cannot determine whether the orientation of the PolyTG/PolyT variant is in cis/trans to other variants. For patients with an R117H variant, additional testing to determine whether a PolyTG/PolyT variant, which may affect the clinical phenotype (eg, 12-13(TG) or 5T), is in a cis/trans orientation should be performed. PolyTG/PolyT are homopolymeric regions known to be difficult to sequence due to polymerase slippage.

Product Components

The TruSight Cystic Fibrosis Kit consists of the following components:

- TruSight Cystic Fibrosis Library Prep (Catalog # 20036925)

Reagents Provided

Reagents for the TruSight Cystic Fibrosis Library Prep are provided by Illumina. The kit has been configured for 1 - 4 uses with a maximum of 96 samples per kit.

TruSight Cystic Fibrosis Library Prep, Box 1 # 20036244

Box 1 reagents are shipped frozen and are stable when stored at -25°C to -15°C. The reagents are stable for a maximum of six freeze-thaw cycles until the specified expiration date.

Box 1A Pre-Amp Reagents, # 20036207

Component	Quantity	Fill Volume	Active Ingredients	Storage
Cystic Fibrosis Oligo Pool	1 tube	600 µl	Buffered aqueous solution containing oligonucleotides targeting the *CFTR* gene.	-25°C to -15°C

Component	Quantity	Fill Volume	Active Ingredients	Storage
Hybridization Buffer	1 tube	4.32 ml	Buffered aqueous solution containing salts and formamide.	-25°C to -15°C
Extension-Ligation Mix	1 tube	4.8 ml	Buffered aqueous solution containing proprietary blend of DNA polymerases, DNA ligase, and dNTPs.	-25°C to -15°C
Index 2 Primers (A501-A508)	1 tube per primer	192 µl	PCR primers with index sequences and sequencing adapters.	-25°C to -15°C
Index 1 Primers (A701-A712)	1 tube per primer	128 µl	PCR primers with index sequences and sequencing adapters.	-25°C to -15°C
PCR Polymerase	1 tube	56 µl	Proprietary DNA polymerase.	-25°C to -15°C
PCR Master Mix	1 tube	2.8 ml	Buffered aqueous solution containing salts and dNTPs.	-25°C to -15°C

Box 1B Post-Amp Reagents, # 20036208

Component	Quantity	Fill Volume	Active Ingredients	Storage
Library Normalization Diluent	1 tube	4.6 ml	Buffered aqueous solution containing salts, 2-Mercaptoethanol, and formamide.	-25°C to -15°C
Library Dilution Buffer	1 tube	4.5 ml	Buffered aqueous solution.	-25°C to -15°C
PhiX Internal Control Library	1 tube	10 µl	Buffered aqueous solution containing PhiX genomic DNA.	-25°C to -15°C

TruSight Cystic Fibrosis Library Prep, Box 2 # 20036209

Box 2 reagents are shipped ambient and are stable when stored at 15°C to 30°C until the specified expiration date.

Box 2 Pre-Amp Reagents

Component	Quantity	Fill Volume	Active Ingredients	Storage
Filter Plate	4 plates	Not applicable	Polypropylene microplate with a modified polyethersulfone membrane.	15°C to 30°C

Box 2 Post-Amp Reagents

Component	Quantity	Fill Volume (ml)	Active Ingredients	Storage
Elution Buffer	1 tube	4.8	Buffered aqueous solution	15°C to 30°C
Library Storage Buffer	1 tube	3.5	Buffered aqueous solution	15°C to 30°C

TruSight Cystic Fibrosis Library Prep, Box 3 # 20036250

Box 3 reagents are shipped refrigerated and are stable when stored at 2°C to 8°C until the specified expiration date.

Box 3A Pre-Amp Reagents, # 20036251

Component	Quantity	Fill Volume (ml)	Active Ingredients	Storage
Stringent Wash Buffer	1 bottle	24	Buffered aqueous solution containing salts, 2-Mercaptoethanol, and formamide.	2°C to 8°C
Universal Wash Buffer	1 tube	4.8	Buffered aqueous solution containing salts.	2°C to 8°C

Box 3B Post-Amp Reagents, # 20036245

Component	Quantity	Fill Volume (ml)	Active Ingredients	Storage
PCR Clean-Up Beads	1 tube	5	Buffered aqueous solution containing solid phase paramagnetic beads and polyethylene glycol.	2°C to 8°C
Library Normalization Wash	2 tubes	4.8	Buffered aqueous solution containing salts, 2-Mercaptoethanol, and formamide.	2°C to 8°C
Library Beads	1 tube	1.2	Buffered aqueous solution containing solid phase paramagnetic beads.	2°C to 8°C

Reagents Required, Not Provided

Pre-Amp Reagents

- 10 N NaOH (prepare from tablets or use a standard solution)
- TE Buffer
- RNase/DNase-free water

Post-Amp Reagents

- 10 N NaOH (prepare from tablets or use a standard solution)
- Ethanol (EtOH), 200 proof for molecular biology
- TE Buffer
- RNase/DNase-free water

MiSeqDx Reagents

- MiSeqDx Reagent Kit v3 (Catalog # 20037124) or MiSeqDx Reagent Kit v3 Micro (Catalog # 20063860)
- 5% sodium hypochlorite
- Tween 20
- Laboratory-grade water

Storage and Handling

1. Room temperature is defined as 15°C to 30°C.
2. The Hybridization Buffer, Stringent Wash Buffer, and Library Normalization Diluent reagents might form visible precipitates or crystals. Before use, vortex vigorously, and then visually inspect to make sure that no precipitates are present.
3. Adhere to the following best practices when handling PCR Clean-Up Beads and Library Beads:
   - The beads should never be frozen.
   - Allow the beads to reach room temperature.
   - Immediately before use, vortex the beads until well-suspended and color appears homogeneous.
   - Thoroughly mix the sample after the beads are added by pipetting up and down 10 times. A shaker can be used to mix samples.

FOR IN VITRO DIAGNOSTIC USE.

    – Incubate the bead/sample mixture at room temperature for the entire duration indicated.

    – Follow instructions when using the magnetic stand. Wait for the solution to clear before aspirating. Keep the plate on the magnetic stand when slowly aspirating the supernatant, taking care not to disturb the separated beads.

4. Do not freeze the Library Beads or mix with the Library Normalization Diluent reagent if not used immediately.

Equipment and Materials

Equipment and Materials Provided, Sold Separately

- MiSeqDx Instrument, Catalog # DX-410-1001
- TruSeq Index Plate Fixture Kit, Catalog # FC-130-1005
- TruSeq Index Plate Fixture & Collar Kit, Catalog # FC-130-1007
- Index Adapter Replacement Caps, Catalog # DX-502-1003
- MiSeq Tube, Catalog # MS-102-9999

Equipment and Materials Required, Not Provided

Pre-Amp Equipment and Materials

- Heat Block—One heat block for a 96-well plate is required. Heat blocks with heated lids are acceptable for use. The use of thermal cyclers or heat blocks with active cooling (eg, Peltier, thermoelectric cooled) is not recommended for the hybridization step. The passive cooling step is critical for proper hybridization. The heat block must meet the following performance specifications:
  - Temperature range: Ambient +5°C to 99°C
  - Temperature regulation: ±0.1°C at 37°C; ±0.4°C at 60°C
- Sample Incubator—One incubator (hybridization oven) is required. The incubator must meet the following performance specifications:
  - Temperature range: Ambient +10°C to 100°C
  - Temperature regulation: ±0.2°C
- Tabletop Centrifuge—A temperature controlled tabletop centrifuge capable of maintaining 20°C is required. A separate centrifuge is required in the post-amp area. Any plate centrifuge that fits a 96-well plate with filter unit and attains the designated speeds of the protocol (280 to 2400 × g) is acceptable.

- Precision Pipettes—One set of precision pipettes is required. A separate set is required in the post-amp area. The use of precision pipettes is required to make sure accurate reagent and sample delivery. Single-channel or multichannel pipettes can be used if they are calibrated regularly and are accurate within 5% of stated volume.

- Consumables—The following consumables are required:
  - 96-well skirted PCR plates, 0.2 ml, polypropylene, or equivalent
  - 96-well storage plates, 0.8 ml (MIDI plates)
  - Solution basin, PVC, DNase, RNase-free (trough)
  - Adhesive aluminum foil seal
  - Appropriate PCR plate seal
  - Aerosol resistant pipette tips
  - Conical tubes, 15 ml

Post-Amp Equipment and Materials

- Thermal Cycler—One thermal cycler is required. The thermal cycler must have a heated lid and meet the following performance specifications:
  - Temperature control range: 4°C to 99°C
  - Control accuracy: ±0.25°C from 35°C to 99°C

- Microplate Shaker—One microplate shaker is required in the post-amp lab area. The plate shaker must meet the following performance specifications:
  - Max mixing speed: 3000 rpm
  - Mixing speed range: 200 rpm to 3000 rpm

- Tabletop Centrifuge—One tabletop centrifuge capable of maintaining 20°C is required. A separate centrifuge is required in the pre-amp area. Any plate centrifuge that attains the designated speeds of the protocol (280 to 2400 × g) is acceptable.

- Heat Block—One heat block for tubes is required. The heat block must meet the following performance specifications:
  - Temperature range: Ambient +5°C to 99°C
  - Temperature regulation: ±0.1°C at 37°C; ±0.4°C at 60°C

- Magnetic Stand—One magnetic stand for a 96-well plate is required. Better performance is seen when the magnets are on the side of the stand and not on the bottom.

- Precision Pipettes—One set of precision pipettes is required. A separate set is required in the pre-amp area. The use of precision pipettes is required to ensure accurate reagent and sample delivery. Single-channel or multichannel pipettes can be used if they are calibrated regularly and are accurate within 5% of stated volume.

- Tabletop Centrifuge—A temperature-controlled centrifuge capable of maintaining 20°C that accepts microcentrifuge tubes is required. Any centrifuge that attains the designated speeds of the protocol (280 to 1000 × g) is acceptable.

- Consumables—The following consumables are required:

- 96-well skirted PCR plates, 0.2 ml, polypropylene, or equivalent
- 96-well storage plates, 0.8 ml (MIDI plates)

NOTE

> Make sure that the 96-well plate is fit compatible with the magnetic stand.

- Conical tubes: 15 ml and 50 ml
- Microcentrifuge tubes (screw-top recommended)
- PCR 8-tube strips
- Solution basins, PVC, DNase, RNase-free (trough)
- Adhesive aluminum foil seals
- Adhesive single-use plate seals
- Aerosol resistant pipette tips

Specimen Collection, Transport, and Storage

WARNING

Handle all specimens as if they are potentially infectious agents.

- Whole blood specimens collected in K2EDTA tubes can be used.

- Whole blood specimens can be stored for no longer than 7 days at room temperature, up to 30 days at 2°C to 8°C, or up to 30 days if frozen at -25°C to -15°C.

- Transport whole blood for no longer than 7 days at room temperature, 30 days at 2°C to 8°C, or 30 days if frozen at -25°C to -15°C. Transportation of whole blood must comply with country, federal, state, and local regulations for the transport of etiologic agents.

- No adverse effect on assay performance was observed when genomic DNA was subjected to 6 freeze/thaw cycles.

- No adverse effect on assay performance was observed with whole blood specimens with elevated bilirubin, cholesterol, triglyceride, EDTA, or hemoglobin present.

Warnings and Precautions

⚠️ CAUTION

> Federal law restricts this device to sale by or on the order of a physician or other practitioner licensed by the law of the State in which he/she practices, to use or order the use of the device.

WARNING

Handle all specimens as if they are potentially infectious agents.

FOR IN VITRO DIAGNOSTIC USE.

TruSight Cystic Fibrosis Package Insert

WARNING

This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, refer to the SDS by searching the product code at support.illumina.com/sds.html. (Refer to *Reagents Provided* for more information.)

- Some components of this assay contain 2-Mercaptoethanol, a reducing agent. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Use in a well-ventilated area and dispose of any containers and unused contents in accordance with applicable local governmental safety standards. For additional environmental, health, and safety information, refer to the SDS by searching the product code at support.illumina.com/sds.html. (Refer to *Reagents Provided* for more information.)

- Some components of this assay contain formamide, an aliphatic amide that is a probable reproductive toxin. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat. Handle used reagents as chemical waste and discard in accordance with the governmental safety standards for your region. For additional environmental, health, and safety information, refer to the SDS by searching the product code at support.illumina.com/sds.html. (Refer to *Reagents Provided* for more information.)

- Immediately report any serious incidents related to this product to Illumina and the Competent Authority of the Member State in which the user and/or patient is established.

- Handle all specimens as if they are potentially infectious agents.

- Failure to follow the procedures as outlined may result in erroneous results or significant reduction in sample quality.

- Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink, or smoke in designated work areas. Wear disposable gloves and laboratory coats when handling specimens and assay reagents. Wash hands thoroughly after handling specimens and assay reagents.

- Do not use any assay components beyond their stated expiration date on the assay carton label. Do not interchange assay components from different assay lots. Assay lots are identified on the assay carton label.

- To prevent sample or reagent degradation, make sure that all sodium hypochlorite vapors have fully dissipated prior to starting the protocol.

- Proper laboratory practices and good laboratory hygiene are required to prevent PCR products from contaminating reagents, instrumentation, and genomic DNA samples. PCR contamination may cause inaccurate and unreliable results.

- Changes in the physical appearance of the reagents provided can indicate deterioration of the materials. If changes in the physical appearance occur (eg, obvious changes in reagent color or cloudiness apparent with microbial contamination), do not use the reagents.

- To prevent contamination, physically separate pre-amplification and post-amplification areas and make sure that pre-amp and post-amp areas have dedicated equipment (eg, pipettes, pipette tips, vortexer, and centrifuge).

FOR IN VITRO DIAGNOSTIC USE.

- Avoid cross-contamination. Use fresh pipette tips between samples and between dispensing reagents. Mix samples with a pipette and centrifuge the plate when indicated. Do not vortex the plates. Using aerosol-resistant tips reduces the risk of amplicon carry-over and sample-to-sample cross-contamination.

- Index-sample pairing must match sample information entered for the MiSeqDx run. Mismatches between the sample information and plate layout will result in loss of positive sample identification and incorrect result reporting.

- Always prepare fresh 80% ethanol for wash steps. Ethanol can absorb water from the air, impacting results.

- Adhere to the specified drying time following the magnetic stand step to make sure ethanol evaporates completely. Residual ethanol can impact the performance of subsequent reactions.

- Store the assay components at the specified temperature in designated pre-amplification and post-amplification areas.

- Repeated freeze-thaw cycles (up to 6) of the Box 1 components do not compromise the integrity of the assay.

- Do not mix the Cystic Fibrosis Oligo Pool and Hybridization Buffer for storage. When combined, the Cystic Fibrosis Oligo Pool becomes unstable, even when stored frozen.

- The use of thermal cyclers with active cooling (eg, Peltier, thermoelectric cooled) is not recommended for the hybridization step. The passive cooling step is critical for proper hybridization.

- Always add PCR Polymerase to the PCR Master Mix immediately prior to use. Never store the combined master mix.

- During the library normalization step, it is extremely critical to completely resuspend the library bead pellet. This is essential for achieving consistent cluster density on the MiSeqDx Instrument flow cell.

- Adhere to the specified incubation times in the library normalization step. Improper incubation can affect library representation and cluster density.

- Due to the number of plate transfers and subsequent potential for contamination, take extreme care to make sure that well contents remain fully in the well. Do not splash contents.

- The 250 ng DNA input recommendation allows for DNA quantity variation. Assay performance is driven by this input level.

- Sample variants with a No Call designation on the test report indicate that the data for that variant position did not meet defined sequencing thresholds. Do not report variants with a No Call designation unless repeat testing produces values that meet defined thresholds and are no longer designated as No Call.

Acronyms

TruSight Cystic Fibrosis Library Prep Acronyms

Acronym	Definition
AMP	AMplification Plate

FOR IN VITRO DIAGNOSTIC USE.

Acronym	Definition
CLP	CLean-up Plate
DAL	Diluted Amplicon Library
FPU	Filter Plate Unit
HYB	HYBridization Plate
LNP	Library Normalization Plate
NTC	No Template Control
PAL	Pooled Amplicon Library
SGP	StoraGe Plate

Additional Resources

The TruSight Cystic Fibrosis support pages on the Illumina website provide software, training resources, product compatibility information, and the following documentation. Always check support pages for the latest versions.

Resource	Description
Local Run Manager CF 139-Variant 2.0 Analysis Module Workflow Guide (document # 1000000100945)	Provides instructions for setting up run parameters for sequencing and analysis for the CF 139-Variant 2.0 analysis module.
Local Run Manager CF Clinical Seq 2.0 Analysis Module Workflow Guide (document # 1000000100946)	Provides instructions for setting up run parameters for sequencing and analysis for the CF Clinical Seq 2.0 analysis module.
Local Run Manager CF 139-Variant 2.0 Micro Analysis Module Workflow Guide (document # 200017946)	Provides instructions for setting up run parameters for sequencing and analysis for the CF 139-Variant 2.0 micro analysis module.
Local Run Manager CF Clinical Seq 2.0 Micro Analysis Module Workflow Guide (document # 200017945)	Provides instructions for setting up run parameters for sequencing and analysis for the CF Clinical Seq 2.0 micro analysis module.
Local Run Manager Software Reference Guide for MiSeqDx (document #1000000011880)	Provides instructions for creating a run, monitoring status, analyzing sequencing data, and viewing results on the MiSeqDx instrument.
MiSeqDx Instrument Reference Guide for MOS v2 (document #1000000021961)	Provides instructions for setting up and sequencing runs, including maintenance procedures for the MiSeqDx instrument.

Procedural Notes

- Illumina requires that one positive control DNA sample and a negative control (NTC or No Template Control) are included in every run. This is defined as a set of samples processed in parallel. The positive control DNA sample should be a well-characterized sample with one or more known CFTR variants. Illumina recommends the use of a wild type control. The wild type control should be run as a sample and should not replace the positive or negative control.
- Store the assay components at the specified temperature in designated pre-amplification and post-amplification areas.
- Repeated freeze-thaw cycles (up to 6) of the Box 1 components do not compromise the integrity of the assay.

Sample Preparation

Before beginning the Cystic Fibrosis 139-Variant Assay or the Cystic Fibrosis Clinical Sequencing Assay, extract and quantitate the DNA from whole blood.

- Any validated DNA extraction method can be used.
- Quantitate the DNA using a spectrophotometer. Make sure that the A260/A280 of the DNA sample is > 1.5. Normalize the DNA sample to 50 ng/µl. Each sample requires 5 µl genomic DNA (total of 250 ng).

Sample Throughput

For the Cystic Fibrosis 139-Variant Assay and the Cystic Fibrosis Clinical Sequencing Assay, the sample throughput can be 24–96 samples with the MiSeqDx Reagent Kit v3 and 24–36 samples with the MiSeqDx Reagent Kit v3 Micro. The indexing primers used during PCR amplification must be chosen based on desired final sample throughput to make sure each library uses a unique index combination.

NOTE
  Proceeding with fewer than 24 samples is not validated by Illumina.

Library Preparation Workflow

The following diagram illustrates the library preparation workflow for the Cystic Fibrosis 139-Variant Assay and Cystic Fibrosis Clinical Sequencing Assay. Pre-amplification steps include: Hybridization of Oligo Pool, Removal of Unbound Oligos, and Extension-Ligation of Bound Oligos. For the PCR amplification step, the PCR plate setup occurs in the pre-amp area, while PCR on the thermal cycler occurs in the post-amp area. Post-amplification steps include: PCR Clean-Up and Library Normalization and Pooling.

FOR IN VITRO DIAGNOSTIC USE.

Safe stopping points are marked between steps.

Cystic Fibrosis 139-Variant Assay and Cystic Fibrosis Clinical Sequencing Assay Library Preparation Workflow
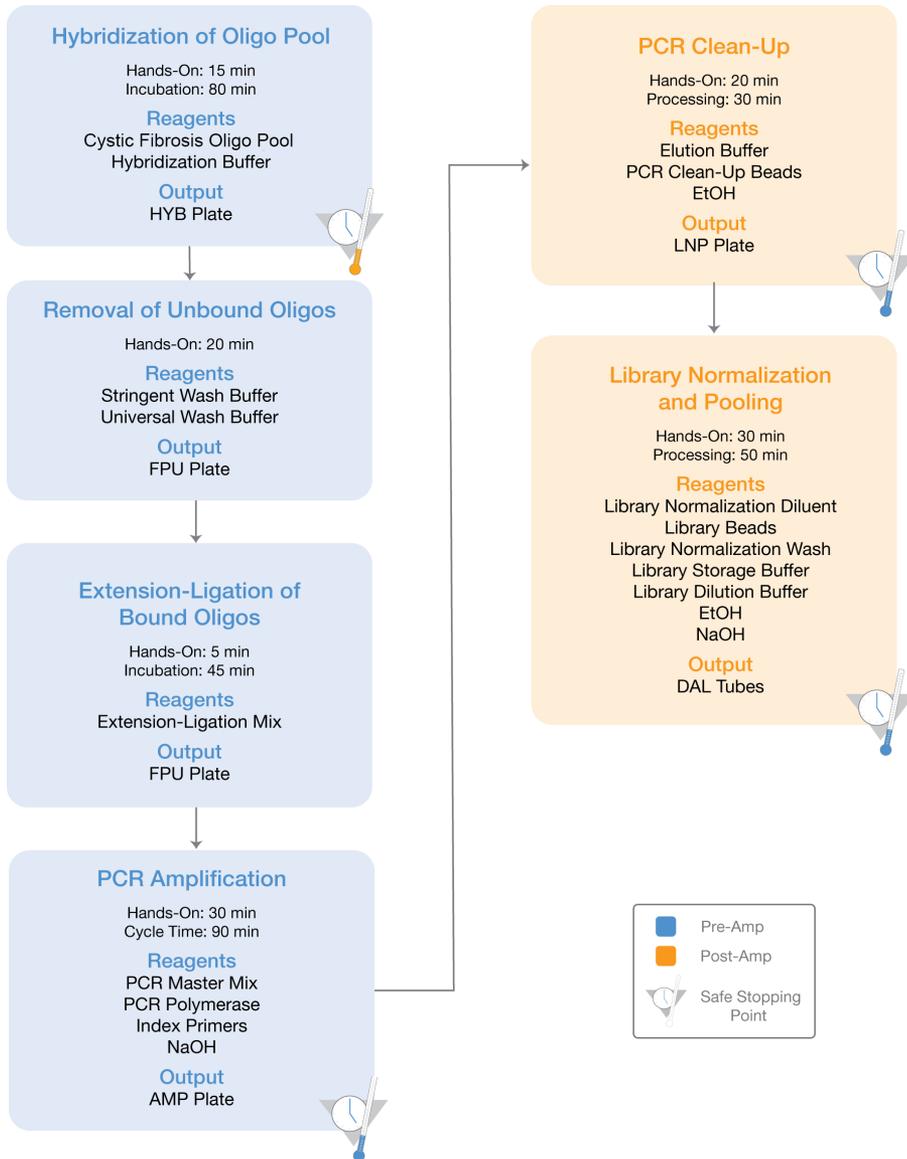
Instructions for Use

The TruSight Cystic Fibrosis Library Prep supports two assays, the Cystic Fibrosis 139-Variant Assay and Cystic Fibrosis Clinical Sequencing Assay, which can be run with either the MiSeqDx Reagent Kit v3 (24-96 samples with the non-micro analysis modules) or MiSeqDx Reagent Kit v3 Micro (24-36 samples with the micro analysis modules). The TruSight Cystic Fibrosis workflow includes assay selection, library preparation, sequencing, and post-run wash. Refer to the table below for more information about available workflows.

FOR IN VITRO DIAGNOSTIC USE.

Assay Selection	Throughput	Sequencing Reagent	Analysis Module
Cystic Fibrosis Clinical Sequencing Assay	24-36	MiSeqDx Reagent Kit v3 Micro	CF Clinical Seq 2.0 Micro
	24-96	MiSeqDx Reagent Kit v3	CF Clinical Seq 2.0
Cystic Fibrosis 139-Variant Assay	24-36	MiSeqDx Reagent Kit v3 Micro	CF 139-Variant 2.0 Micro
	24-96	MiSeqDx Reagent Kit v3	CF 139-Variant 2.0

Assay Selection and Run Setup

- If using Cystic Fibrosis 139-Variant Assay, refer to *Using the Local Run Manager CF 139-Variant 2.0 Analysis Module*.
  - You can also refer to this page for instructions on using the TruSight Cystic Fibrosis 139-Variant 2.0 Micro Analysis Module. If you do so, make sure to select CF 139-Variant 2.0 Micro when creating the run instead of CF 139-Variant 2.0.
- If using Cystic Fibrosis Clinical Sequencing Assay, refer to *Using the Local Run Manager CF Clinical Seq 2.0 Analysis Module*.
  - You can also refer to this page for instructions on using the TruSight Cystic Fibrosis Clinical Seq 2.0 Micro Analysis Module. If you do so, make sure to select CF Clinical Seq 2.0 Micro when creating the run instead of CF Clinical Seq 2.0.

Using the Local Run Manager CF 139-Variant 2.0 Analysis Module

Set Parameters

1. Log in to Local Run Manager.
2. Select Create Run, and then select CF 139-Variant 2.0.
3. Enter a run name that identifies the run from sequencing through analysis.
   Use alphanumeric characters, spaces, underscores, or dashes (40 characters or less).
4. [Optional] Enter a run description.
   Use alphanumeric characters, spaces, underscores, or dashes (150 characters or less).
5. Enter the lot number and expiration date for the library prep kit.

Specify Samples for the Run

Specify samples for the run using one of the following options:

- Enter samples manually—Use the blank table at the bottom of the Create Run screen. Suggested sample wells are highlighted.

- Import sample sheet—Navigate to an external file in a comma-separated values (*.csv) format. A template is available for download on the Create Run screen.

Enter Samples Manually

1. Enter a unique sample name in the Sample Name field.
   Use alphanumeric characters, dashes, or underscores (40 characters or less).

2. Right-click and select positive and negative control samples.
   To save a run, it must have at least one positive and one negative control.

3. [Optional] Enter a sample description in the sample description tab.
   Use alphanumeric characters, dashes, or underscores (50 characters or less).

4. [Optional] Select an Index 1 adapter from the Index 1 (i7) drop-down list.
   This step is optional because the i7 and i5 index combinations autopopulate with a default layout.

5. [Optional] Select an Index 2 adapter from the Index 2 (i5) drop-down list.
   This step is optional because the i7 and i5 index combinations autopopulate with a default layout.

6. Select the Print icon to display the plate layout.

7. Select Print to print the plate layout as a reference for preparing libraries.

8. [Optional] Select Export to export sample information file.

9. Select Save Run.
   If less than 24 samples have been entered, the Insufficient Sample window will display. Select Proceed to continue, or select Cancel to edit the samples.

⚠️ CAUTION

Sequencing with pooled libraries that contain less than 24 or more than 96 samples is not validated by the Illumina Local Run Manager CF 139-Variant 2.0 Analysis Module. Sequencing with pooled libraries that contain less than 24 or more than 36 samples is not validated by Illumina TruSight Cystic Fibrosis 139-Variant 2.0 Micro Analysis Module.

Import Sample Sheet

Sample information can be imported from two types of files:

- A sample information file that was previously exported from the Local Run Manager CF 139-Variant 2.0 Analysis Module using the Export feature.

- A template file, which can be generated by selecting Template on the Create Run screen. The template file contains the correct column headings for import, with placeholder information in each column. Use an external editor to customize the template file:

   1. Add sample information for each sample in the run.

   2. After all sample information has been added, delete any remaining placeholder information in unused cells.

FOR IN VITRO DIAGNOSTIC USE.

3. Save template file

To import sample information:

1. Select Import Samples, and then browse to the file and select it.

2. Select the Print icon to display the plate layout.

3. Select Print to print the plate layout as a reference for preparing libraries.

4. Select Save Run.

   If less than 24 samples have been entered, the Insufficient Sample window will display. Select Proceed to continue, or select Cancel to edit the samples.

   ⚠ CAUTION

   Sequencing with pooled libraries that contain less than 24 or more than 96 samples is not validated by the Illumina Local Run Manager CF 139-Variant 2.0 Analysis Module. Sequencing with pooled libraries that contain less than 24 or more than 36 samples is not validated by Illumina TruSight Cystic Fibrosis 139-Variant 2.0 Micro Analysis Module.

Edit a Run

For instructions on editing the information in your run before sequencing, see *Local Run Manager Software Reference Guide for MiSeqDx (document # 1000000011880)*.

Using the Local Run Manager CF Clinical Seq 2.0 Analysis Module

Set Parameters

1. Log in to Local Run Manager.

2. Select Create Run, and select CF Clinical Seq 2.0.
   A confirmation window will display selection.

3. Select the checkbox and select Confirm to continue, or select Cancel to return to the main screen.

4. Enter a run name that identifies the run from sequencing through analysis.
   Use alphanumeric characters, spaces, underscores, or dashes (40 characters or less).

5. [Optional] Enter a run description.
   Use alphanumeric characters, spaces, underscores, or dashes (150 characters or less).

6. Enter the lot number and expiration date for the library prep kit.

Specify Samples for the Run

Specify samples for the run using one of the following options:

- Enter samples manually—Use the blank table at the bottom of the Create Run screen. Suggested sample wells are highlighted.
- Import sample sheet—Navigate to an external file in a comma-separated values (*.csv) format. A template is available for download on the Create Run screen.

Enter Samples Manually

1. Enter a unique sample name in the Sample Name field.
   Use alphanumeric characters, dashes, or underscores (40 characters or less).
2. Right-click and select positive and negative control samples.
   To save a run, it must have at least one positive and one negative control.
3. [Optional] Enter a sample description in the sample description tab.
   Use alphanumeric characters, dashes, or underscores (50 characters or less).
4. [Optional] Select an Index 1 adapter from the Index 1 (i7) drop-down list.
   This step is optional because the i7 and i5 index combinations autopopulate with a default layout.
5. [Optional] Select an Index 2 adapter from the Index 2 (i5) drop-down list.
   This step is optional because the i7 and i5 index combinations autopopulate with a default layout.
6. Select the Print icon to display the plate layout.
7. Select Print to print the plate layout as a reference for preparing libraries.
8. [Optional] Select Export to export sample information file.
9. Select Save Run.
   If less than 24 samples have been entered, the Insufficient Sample window will display. Select Proceed to continue, or select Cancel to edit the samples.

⚠ CAUTION

Sequencing with pooled libraries that contain less than 24 or more than 96 samples is not validated by the Illumina Local Run Manager CF Clinical Seq 2.0 Analysis Module. Sequencing with pooled libraries that contain less than 24 or more than 36 samples is not validated by Illumina TruSight Cystic Fibrosis Clinical Seq 2.0 Micro Analysis Module.

Import Sample Sheet

Sample information can be imported from two types of files:

- A sample information file that was previously exported from the Local Run Manager CF Clinical Seq 2.0 Analysis Module using the Export feature.

- A template file, which can be generated by selecting Template on the Create Run screen. The template file contains the correct column headings for import, with placeholder information in each column. Use an external editor to customize the template file:

    1. Add sample information for each sample in the run.

    2. After all sample information has been added, delete any remaining placeholder information in unused cells.

    3. Save template file.

To import sample information:

1. Select Import Samples, and then browse to the file and select it.

2. Select the Print icon to display the plate layout.

3. Select Print to print the plate layout as a reference for preparing libraries.

4. Select Save Run.

    If less than 24 samples have been entered, the Insufficient Sample window will display. Select Proceed to continue, or select Cancel to edit the samples.

    ⚠️ CAUTION

    Sequencing with pooled libraries that contain less than 24 or more than 96 samples is not validated by the Illumina Local Run Manager CF Clinical Seq 2.0 Analysis Module. Sequencing with pooled libraries that contain less than 24 or more than 36 samples is not validated by Illumina TruSight Cystic Fibrosis Clinical Seq 2.0 Micro Analysis Module.

Edit a Run

For instructions on editing the information in your run before sequencing, see *Local Run Manager Software Reference Guide for MiSeqDx (document # 1000000011880)*.

Library Preparation

NOTE

The library preparation workflow for Cystic Fibrosis 139-Variant Assay and Cystic Fibrosis Clinical Sequencing Assay is identical.

FOR IN VITRO DIAGNOSTIC USE.

Hybridization of Oligonucleotide Pool

Consumables

- 96-well PCR plate
- Genomic DNA (gDNA) samples
- Hybridization Buffer
- Positive control sample
- Cystic Fibrosis Oligo Pool
- TE Buffer
- Adhesive aluminum foil seal

Preparation

1. Prepare the following consumables:

Reagent	Storage	Instructions
Hybridization Buffer	-25°C to -15°C	Bring to room temperature. Vortex vigorously to make sure that all precipitates have completely dissolved, and then briefly centrifuge the tubes to collect liquid.
Cystic Fibrosis Oligo Pool	-25°C to -15°C	Bring to room temperature. Vortex vigorously to make sure that all precipitates have completely dissolved, and then briefly centrifuge the tubes to collect liquid.

2. Bring gDNA samples and positive control sample to room temperature.
3. Set a 96-well heat block to 95°C.
4. Pre-heat an incubator to 37°C.

Procedure

1. Label a new 96-well PCR plate "HYB_Plate_ID".
2. Create the sample plate according to the plate graphic printed from Local Run Manager.
3. Following the plate layout generated from the Local Run Manager, add 5 µl negative control (e.g. TE Buffer) to the appropriate well of the HYB plate.
4. Add 5 µl sample or control at 50 ng/µl (250 ng total) to the appropriate wells of the HYB plate.
5. Add 5 µl Cystic Fibrosis Oligo Pool to each sample well.
6. Add 40 µl Hybridization Buffer to each sample in the HYB plate.
7. Gently pipette up and down 3–5 times to mix.
8. Seal the **HYB** plate and centrifuge 1000 × g at 20°C for 1 minute.

9. Place the HYB plate in the pre-heated block at 95°C and incubate for 1 minute.

10. Reduce the heat block to 40°C and continue incubating until the heat block reaches 40°C (~80 minutes). Gradual cooling is critical for proper hybridization.

SAFE STOPPING POINT

After the heat block reaches 40°C, the HYB plate is stable holding at 40°C for 2 hours.

Removal of Unbound Oligonucleotides

Consumables

- Extension-Ligation Mix
- Filter plate
- Stringent Wash Buffer
- Universal Wash Buffer
- MIDI plate

Preparation

1. Prepare the following consumables:

Reagent	Storage	Instructions
Extension-Ligation Mix	-25°C to -15°C	Bring to room temperature. Vortex to mix.
Stringent Wash Buffer	2°C to 8°C	Bring to room temperature. Vortex vigorously. Make sure that all precipitate has dissolved.
Universal Wash Buffer	2°C to 8°C	Bring to room temperature. Vortex to mix.

2. Assemble the filter plate assembly unit (FPU) *from top to bottom*:

    - Lid
    - Filter plate
    - Adapter collar
    - MIDI plate

3. Pre-wash the filter plate membrane as follows.

    a. Add 45 µl Stringent Wash Buffer to each well.

    b. Cover the filter plate with the lid and centrifuge at 2400 × g at 20°C for 5 minutes.

4. Check to verify that all wells of the filter plate are draining completely. If the wash buffer does not drain completely, centrifuge again at 2400 × g at 20°C until all liquid has gone through (an additional 5-10 minutes).

⚠️ CAUTION

It is critical to control the centrifuge temperature during the washing steps. Make sure the centrifuge is pre-cooled to 20°C before each use. If the temperature reaches 25°C or higher, the higher temperature may lead to higher stringency in primer binding. In rare cases, if samples have SNVs in primer binding regions, the higher stringency may lead to allele dropout.

Procedure

1. Remove the HYB plate from the heat block and centrifuge at 1000 × g at 20°C for 1 minute.
2. Using a multi-channel pipette set to 55 µl, transfer the entire volume of each sample to the corresponding wells of the filter plate.
3. Cover the filter plate with the lid and centrifuge at 2400 × g at 20°C for 5 minutes.
4. Wash the filter plate as follows.
   a. Add 45 µl Stringent Wash Buffer to each sample well.
   b. Cover the filter plate with the lid and centrifuge at 2400 × g at 20°C for 5 minutes.
5. Wash the plate a second time.
6. If the wash buffer does not drain completely, centrifuge again at 2400 × g at 20°C until all liquid has drained (an additional 5-10 minutes).
7. Discard all the flow-through and reassemble the FPU.
8. Add 45 µl Universal Wash Buffer to each sample well.
9. Cover the filter plate with the lid and centrifuge at 2400 × g at 20°C for 10 minutes.
10. Make sure that all liquid has drained after centrifugation. Repeat centrifugation if necessary.

Extension-Ligation of Bound Oligonucleotides

Consumables

- Extension-Ligation Mix
- Adhesive aluminum foil seal

Procedure

1. Add 45 µl of Extension-Ligation Mix to each sample well of the filter plate.
2. Seal the filter plate and then cover with the lid.
3. Incubate the **FPU** in the pre-heated 37°C incubator for 45 minutes.
4. While the FPU plate is incubating, prepare the AMP (Amplification Plate) as described in the following section.

PCR Amplification

Consumables

- 96-well PCR plate
- PCR plate seal
- Index Primers (A501-A508 and A701-A712)
- 10 N NaOH
- PCR Master Mix
- PCR Polymerase
- 15 mL conical tube

Preparation

1. Determine the index primers to be used according to the graphical plate layout in Local Run Manager.
2. Prepare the following consumables:

Reagent	Storage	Instructions
Index Primers (A501-A508 and A701-A712)	-25°C to -15°C	Bring to room temperature. Vortex to mix, and then briefly centrifuge.
PCR Polymerase	-25°C to -15°C	Leave in freezer until needed to prepare PCR working solution.
PCR Master Mix	-25°C to -15°C	Bring to room temperature. Vortex to mix, and then briefly centrifuge.

3. Prepare fresh 0.05 N NaOH by adding 25 µl 10 N NaOH to 4975 µl RNase/DNase-free water.
4. Label a new 96-well PCR plate AMP.
5. Add index primers to the AMP plate as follows.
   a. Add 4 µl of the selected Index 2 Primers (A501-A508) to the appropriate well in the AMP plate.
   b. Discard the original white caps, and then apply new white caps.
   c. Add 4 µl of the selected Index 1 Primers (A701-A712) to the appropriate row of the AMP plate.
   d. Discard the original orange caps, and then apply new orange caps.
6. Prepare the following consumables:

Reagent	Storage	Instructions
PCR Polymerase	-25°C to -15°C	Remove from storage and briefly centrifuge. Proceed immediately to next step. If PCR polymerase will be used for additional preparations, return to storage after creating PCR working solution.

7. Prepare the PCR Working Solution as follows.

> NOTE   Instructions below include volumes required to process 96 samples. If fewer samples are being processed, adjust volumes accordingly to preserve reagents.

   a.   For 96 samples, add 56 µl PCR Polymerase to 2.8 ml PCR Master Mix.
   b.   Invert 20 times to mix.
   The PCR Working Solution is stable at room temperature for 10 minutes.

Procedure

1. Remove the FPU from the incubator, and then remove the seal.
2. Cover the filter plate with the lid, and then centrifuge at 2400 × g at 20°C for 2 minutes.
3. Add 25 µl 0.05 N NaOH to each well on the filter plate.
4. Pipette up and down 5-6 times.
5. Cover the filter plate with the lid, and incubate at room temperature for 5 minutes.
6. While the filter plate is incubating, transfer 22 µl PCR Master Mix to each well of the AMP plate containing index primers.
7. Transfer samples eluted from the filter to the AMP plate as follows.
   a.   Pipette the samples in the first column of the filter plate up and down 5-6 times.
   b.   Transfer 20 µl from the filter plate to the corresponding column of the AMP plate.
   c.   Gently pipette up and down 5-6 times to thoroughly combine the DNA with the PCR Master Mix.
   d.   Repeat transfer steps for the remaining columns from the filter plate to the AMP plate.
8. Seal the AMP plate and secure with a rubber roller.
9. Centrifuge at 1000 × g at 20°C for 1 minute.
10. Transfer the AMP plate to the post-amplification area.
11. Perform PCR using the following program on a thermal cycler:
    - 95°C for 3 minutes
    - 25 cycles of:
        - 95°C for 30 seconds
        - 62°C for 30 seconds
        - 72°C for 60 seconds
    - 72°C for 5 minutes
    - Hold at 10°C

SAFE STOPPING POINT

If not proceeding immediately to PCR Clean-Up, the AMP plate can remain on the thermal cycler overnight, or can be stored at 2°C to 8°C up to 48 hours.

PCR Clean-Up

Consumables

- 50 mL conical tube
- Adhesive single-use plate seals
- Two MIDI plates
- Elution Buffer
- PCR Clean-Up Beads

Preparation

1. Prepare the following consumables:

Reagent	Storage	Instructions
PCR Clean-Up Beads	2°C to 8°C	Let stand for 30 minutes to bring to room temperature.

2. For 96 samples, prepare fresh 80% EtOH using 36 mL absolute EtOH and 9 mL DNase/RNase-free water. Mix thoroughly.

NOTE   If fewer than 96 samples are being processed, adjust volumes accordingly to preserve reagents.

Procedure

1. Centrifuge the AMP plate at 1000 × g at 20°C for 1 minute.
2. Label a new MIDI plate "CLP_Plate_ID" (Clean-up Plate).
3. Invert PCR Clean-Up Beads 10 times. Vortex vigorously and then invert 10 more times. Visually inspect solution to make sure that beads are resuspended.
4. Add 45 µl PCR Clean-Up Beads to each well of the CLP plate.
5. Transfer the entire PCR product from each well of the AMP plate to the corresponding well of the CLP plate.
6. Seal and shake on a microplate shaker at 1800 rpm for 2 minutes.
7. Incubate at room temperature without shaking for 10 minutes.
8. Place the plate on a magnetic stand and wait until the liquid is clear (~2 minutes).
9. With the CLP plate on the magnetic stand, carefully remove and discard the supernatant.
10. Wash beads as follows.
    a. Keep on the magnetic stand and add 200 µl fresh 80% EtOH to each well.
    b. Wait a minimum of 30 seconds or until the supernatant is clear.
    c. Remove and discard all supernatant from each well.
11. Wash beads a second time.

12. Use a P20 multi-channel pipette set to 20 µl to remove excess EtOH.

13. Remove the CLP plate from the magnetic stand and air-dry the beads for 10 minutes.

14. Add 30 µl Elution Buffer to each sample.

15. Seal the CLP plate and shake on a microplate shaker at 1800 rpm for 2 minutes. After shaking, verify if samples were resuspended. If not, repeat this step.

16. Incubate at room temperature for 2 minutes.

17. Place the CLP plate on the magnetic stand and wait until the supernatant is clear (~2 minutes).

18. Label a new MIDI plate LNP.

19. Transfer 20 µl supernatant from each well of the CLP plate to the corresponding well of the LNP plate.

20. [Optional] Transfer the remaining 10 µl of supernatant from the CLP plate to a new plate and label the plate with the run name and date. Store this plate at -25°C to -15°C until completion of the sequencing run and data analysis. The cleaned up PCR products can be used for troubleshooting efforts in the event of sample failures.

SAFE STOPPING POINT

If stopping at this point, seal the LNP plate and centrifuge at 1000 × g at 20°C for 1 minute. The plate is stable for up to 3 hours at 2°C to 8°C.

Library Normalization and Pooling

Consumables

- 15 ml conical tube
- 96-well PCR plate
- Microcentrifuge tubes
- Library Beads
- Library Dilution Buffer
- Library Normalization Diluent
- Library Normalization Wash
- 10 N NaOH
- RNase/DNase-free water

Preparation

1. Prepare the following consumables:

Reagent	Storage	Instructions
Library Beads	2°C to 8°C	Bring to room temperature.

Reagent	Storage	Instructions
Library Dilution Buffer	-25°C to -15°C	Bring to room temperature. Vortex vigorously. Make sure that all precipitate has dissolved.
Library Normalization Diluent	-25°C to -15°C	Bring to room temperature. Vortex vigorously. Make sure that all precipitate has dissolved.
Library Normalization Wash	2°C to 8°C	Bring to room temperature. Vortex vigorously.

2. Prepare fresh 0.1 N NaOH by adding 50 μl 10 N NaOH to 4950 μl RNase/DNase-free water.

Procedure

1. Mix Library Normalization Diluent and Library Beads in a fresh 15 ml conical tube as follows.

   NOTE Instructions below include volumes required to process 96 samples. If fewer samples are being processed, adjust volumes accordingly to preserve reagents.
   Volumes must be adjusted for 36 samples maximum when preparing libraries for use with MiSeqDx Reagent Kit v3 Micro and Micro Analysis Modules.

   a. For 96 samples, add 4.4 ml of Library Normalization Diluent.

   b. Vortex Library Beads vigorously for 1 minute with intermittent inversion until the beads are resuspended and no pellet is found at the bottom of the tube when the tube is inverted.

   c. Pipette Library Beads up and down 10 times to resuspend.

   ⚠️ CAUTION

   It is extremely critical to completely resuspend the library bead pellet at the bottom of the tube. The use of a P1000 ensures that the beads are homogeneously resuspended and that there is no bead mass at the bottom of the tube. This is essential for achieving consistent cluster density on the flow cell.

   d. For 96 samples, pipette 800 μl Library Beads to the conical tube containing Library Normalization Diluent.

   e. Mix by inverting the tube 15-20 times.

2. Add 45 μl Library Normalization Diluent/Library Beads Working Solution to each well of the LNP plate.

3. Seal and shake on a microplate shaker at 1,800 rpm for 30 minutes.

   NOTE If proceeding with sequencing on the same day, begin thawing the reagent cartridge. Follow the instructions to thaw the MiSeqDx Reagent cartridge in the section entitled *Prepare for Sequencing*.

4. Place LNP plate on the magnetic stand and wait until the liquid is clear (~2 minutes).

5. With the LNP plate on the magnetic stand, carefully remove and discard the supernatant.

6. Remove the LNP plate from the magnetic stand and wash beads with Library Normalization Wash as follows:

    a. Add 45 µl Library Normalization Wash to each sample well.

    b. Seal the LNP plate and shake on a microplate shaker at 1,800 rpm for 5 minutes.

    c. Place the plate on the magnetic stand for a minimum of 2 minutes or until the supernatant is clear.

    d. Carefully remove and discard the supernatant.

7. Repeat the Library Normalization Wash procedure as described in the previous step.

8. Use a P20 multi-channel pipette set to 20 µl to remove excess Library Normalization Wash.

9. Remove the LNP plate from the magnetic stand, and then add 30 µl 0.1 N NaOH to each well.

10. Seal the LNP plate and shake on a microplate shaker at 1,800 rpm for 5 minutes.

11. During the 5 minute elution, label a new 96-well PCR plate SGP.

12. Add 30 µl Library Storage Buffer to each well.

13. Make sure all samples in the LNP plate are completely resuspended. If the samples are not completely resuspended, gently pipette samples up and down or lightly tap the plate on the bench, and, then shake for another 5 minutes.

14. Place the LNP plate on the magnetic stand for a minimum of 2 minutes.

15. Using a multi-channel pipette set to 30 µl, transfer the supernatant from the LNP plate to the SGP plate. Gently pipette up and down 5 times to mix.

16. Seal the SGP plate, and then centrifuge at 1,000 x g at 20°C for 1 minute.

17. Vortex the Library Dilution Buffer and make sure that all the precipitates have dissolved completely. Briefly centrifuge to collect contents.

18. Label a new microcentrifuge tube PAL.

19. Determine the samples to be pooled for sequencing. A maximum of 96 samples can be pooled for sequencing using the MiSeqDx Reagent Kit v3 and non-micro Analysis Modules. A maximum of 36 samples can be pooled for sequencing using the MiSeqDx Reagent Kit v3 Micro with Micro Analysis Modules.

20. Transfer 5 µl of each library to be sequenced from each well of the SGP plate column-by-column to the corresponding well of the PCR eight-tube strip.

21. Transfer the contents of the PCR eight-tube strip into the PAL tube. Vortex PAL tube until fully mixed.

22. Seal the SGP plate with an adhesive plate seal and label with run name and date.

NOTE   The SGP plate can be stored at -25°C to -15°C for up to 3 days and used to re-pool libraries if necessary.

23. Label 2-3 new microcentrifuge tubes DAL.

24. Add 585 µl Library Dilution Buffer to the DAL tubes.

25. Transfer 9 µl PAL to each DAL tube containing Library Dilution Buffer.

26. Pipette up and down 3-5 times to rinse the tip and to make sure the transfer is complete.

SAFE STOPPING POINT

If not proceeding immediately to sequencing on the MiSeqDx, the DAL tubes can be stored at -25°C to -15°C for up to 28 days.

Sequencing

Prepare for Sequencing

Consumables

- MiSeqDx Reagent Kit v3 or MiSeqDx Reagent Kit v3 Micro
- Library Dilution Buffer
- PhiX Internal Control Library

Preparation

1. Set a heat block suitable for 1.5 ml centrifuge tubes to 96°C.
2. In an ice bucket, prepare an ice water bath.
3. Prepare the following consumables:

Reagent	Storage	Instructions
Library Dilution Buffer	-25°C to -15°C	Thaw at room temperature. Vortex to mix. Make sure that all precipitate has dissolved. Briefly centrifuge and place in ice water bath. Additional Library Dilution Buffer is provided with the MiSeqDx Reagent Kit v3or the MiSeqDx Reagent Kit v3 Micro, if needed.
PhiX Internal Control Library	-25°C to -15°C	Thaw at room temperature. Place in ice water bath.
MiSeqDx Reagent Kit v3 cartridge or MiSeqDx Reagent Kit v3 Micro	-25°C to -15°C	Allow the reagent cartridge to thaw in the room temperature water bath for approximately 90 minutes or until it is thawed completely. For additional information regarding reagent cartridge preparation, see MiSeqDx Instrument Reference Guide for MOS v2 (document # 1000000021961).
MiSeqDx Flow Cell	2°C to 8°C	Bring to room temperature. For additional information regarding flow cell preparation, see MiSeqDx Instrument Reference Guide for MOS v2 (document # 1000000021961).
MiSeqDx SBS Solution(PR2)	2°C to 8°C	Bring to room temperature. For additional information regarding SBS solution preparation, see MiSeqDx Instrument Reference Guide for MOS v2 (document # 1000000021961).

Denature and Dilute PhiX Internal Control Library

Consumables

- DNase/RNase-free water
- 10 N NaOH
- Library Dilution Buffer
- PhiX Internal Control Library
- TE buffer
- 15 mL conical tube
- Microcentrifuge tubes

Preparation

1. In a conical tube, combine the following volumes to prepare 0.1N NaOH:
   - DNase/RNase-free water (2475 µl)
   - Stock 10 N NaOH (25 µl)

2. Invert the tube several times to mix.

   ⚠ CAUTION

   Using freshly diluted NaOH is essential to completely denature samples for cluster generation on the MiSeqDx.

   If PhiX is prepared the same day as Library Normalization, the same stock of 0.1N NaOH can be used.

3. Combine the following volumes to dilute the PhiX Internal Control Library to 2 nM:
   - 10 nM PhiX Internal Control Library (2 µl)
   - 1X TE Buffer (8 µl)

4. Combine the following volumes to prepare a 1 nM PhiX Internal Control Library:
   - 2 nM PhiX Internal Control Library (10 µl)
   - 0.1 N NaOH (10 µl)

5. Vortex briefly to mix.

6. Centrifuge the 1 nM PhiX Internal Control at 280 × g at 20°C for 1 minute.

7. Incubate for 5 minutes at room temperature to denature the PhiX Internal Control library solution into single strands.

8. In a new microcentrifuge tube, combine the following volumes to prepare a 20 pM PhiX Internal Control library:
   - Denatured PhiX Internal Control Library (2 µl)
   - Pre-chilled Library Dilution Buffer (98 µl)

⚠ CAUTION

The denatured 20 pM PhiX Internal Control Library can be stored up to 3 weeks at -25°C to -15°C as single-use aliquots.

Prepare Samples for Sequencing

1. Proceed with one DAL tube for sequencing.
2. If the DAL tube was stored frozen, thaw completely and mix by pipetting up and down.
3. If 20 pM PhiX Internal Control Library was stored frozen, remove single use aliquot, thaw completely, mix by vortex, and then briefly centrifuge.
4. Add 6 µl 20 pM PhiX Internal Control Library to the DAL tube.
5. Pipette up and down 3-5 times to rinse the tip and make sure the transfer completes.
6. Mix the DAL tube by vortexing the tube at top speed.
7. Centrifuge the DAL tube at 1000 x g at 20°C for 1 minute.
8. Incubate the DAL tube on a heat block at 96°C for 2 minutes.
9. After the incubation, invert the DAL tube 1-2 times to mix, and then immediately place in the ice-water bath.
10. Keep the DAL tube (pooled libraries) in the ice-water bath for 5 minutes.

Load Pooled Libraries onto Cartridge

1. Use a new 1 ml pipette tip to pierce the foil seal over the reservoir on the reagent cartridge labeled Load Samples.
2. Pipette 600 µl from DAL tube into the Load Samples reservoir. Avoid touching the foil seal.
3. Check for air bubbles in the reservoir after loading sample. If air bubbles are present, gently tap the cartridge on the bench to release the bubbles.
4. Proceed directly to the run setup steps using the MiSeq Operating Software (MOS) interface. For additional information regarding run setup on the MiSeqDx, refer to the *MiSeqDx Instrument Reference Guide for MOS v2 (document # 1000000021961)*.

Post-Run Wash with Template Line Wash

Following sequencing, it is highly recommended to do a post-run wash with template line wash.

⚠ CAUTION

If template line wash is not conducted, negative control call rates could be impacted in subsequent run.

NOTE   The post-run wash workflow for Cystic Fibrosis 139-Variant Assay and Cystic Fibrosis Clinical Sequencing Assay is identical.

Consumables

- Microcentrifuge tubes
- Laboratory-grade water
- Tween 20
- 5% Sodium hypochlorite
- MiSeq Tube

WARNING

This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, see the SDS at support.illumina.com/sds.html.

Preparation

1. Prepare fresh wash solution with Tween 20 and laboratory-grade water as follows.

   a. Add 5 ml 100% Tween 20 to 45 ml laboratory-grade water. These volumes result in 10% Tween 20.

   b. Add 25 ml 10% Tween 20 to 475 ml laboratory-grade water. These volumes result in a 0.5% Tween 20 wash solution.

   c. Invert five times to mix.

2. Prepare fresh sodium hypochlorite wash solution with laboratory-grade water as follows.

   a. Add 36 µl 5% sodium hypochlorite to 864 µl laboratory-grade water. These volumes result in a 1:25 sodium hypochlorite dilution.

   b. Add 50 µl 1:25 sodium hypochlorite dilution to 950 µl of laboratory-grade water in a MiSeq tube.

3. Using the correct concentration of sodium hypochlorite is important. Make sure to check the percentage of sodium hypochlorite on the product label. If the concentration is too high, it can make cluster generation fail in subsequent runs. If 5% sodium hypochlorite is not available, make a 1 ml solution of 0.01% sodium hypochlorite in laboratory-grade water. Do not use sodium hypochlorite with a maintenance wash or a standby wash.

4. Prepare the wash components with fresh wash solution as follows.

   a. Add 6 ml wash solution to each reservoir of the wash tray.

   b. Add 350 ml wash solution to the 500 ml wash bottle.

Procedure

1. Insert the MiSeq tube containing 0.01% sodium hypochlorite wash solution into position 17 of the wash tray. Make sure that the neck of the tube is flush with the tray.

   The tube displaces the Tween 20 and laboratory-grade water wash solution from position 17.

FOR IN VITRO DIAGNOSTIC USE.

MiSeq Tube in Position 17 of the Wash Tray
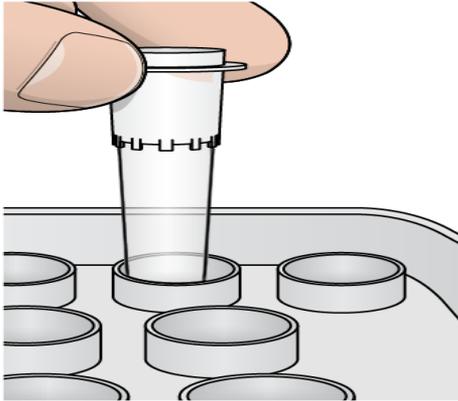
> ⚠ **CAUTION**
>
> Make sure to insert the MiSeq tube with sodium hypochlorite into tray position 17 only. Inserting the tube in another position can make cluster generation fail in subsequent runs.

2. When the run is complete, select Start Wash.
   The software automatically raises the sippers in the reagent chiller.

3. Select Perform optional template line wash on the Post-Run Wash screen.

4. Open the reagent compartment door and reagent chiller door, and then slide the used reagent cartridge from the chiller.

5. Slide the wash tray into the reagent chiller until it stops, and then close the reagent chiller door.

6. Raise the sipper handle in front of the MiSeqDx SBS Solution bottle and waste bottle until it locks into place.

7. Remove the MiSeqDx SBS Solution bottle and replace it with the wash bottle.

8. Remove the waste bottle and discard the contents appropriately. Return the waste bottle to the reagent compartment.

9. Slowly lower the sipper handle. Make sure that the sippers lower into the wash bottle and waste bottle.

10. Close the reagent compartment door.

11. Select Next. The post-run wash begins.

12. When the wash is complete, leave the used flow cell, wash tray, and wash bottle containing the remaining wash solution on the instrument.

13. The sippers remain in the down position, which is normal. Leave the unused wash solution in the wash tray and wash bottle to prevent the sippers from drying out and air from entering the system.

Reanalysis of Sequenced Libraries

Following a sequencing run, reanalysis of the same sequencing dataset may be performed by following the *Requeue Analysis* procedure of the *Local Run Manager Software Reference Guide for MiSeqDx (document # 1000000011880)*. Requeue analysis is restricted to the module originally used to perform sequencing. Requeue analysis will allow edits to

FOR IN VITRO DIAGNOSTIC USE.

sample information and generate new reports.

NOTE   Pooled libraries used for sequencing must have 24-96 samples if using the MiSeqDx Reagent Kit v3, or 24-36 samples if using the MiSeqDx Reagent Kit v3 Micro. Reports for a subset of samples can be obtained by entering fewer samples during requeue setup. Reports will only be generated for samples entered during requeue setup.

Retesting Options for Pooled Libraries

The TruSight Cystic Fibrosis 139-Variant Assay uses the same library preparation workflow and reagents as the TruSight Cystic Fibrosis Clinical Sequencing Assay. The library preparation procedure requires the selection of an assay before starting. However, in cases where pooled libraries (DAL tubes) require additional testing (eg, repeating a sequencing run or reflex testing with another TruSight CF Assay), DAL tubes can be used as needed without repeating library preparation. For retesting follow the procedure below:

1. Set up run using instructions in *Assay Selection and Run Setup*.
2. Sequence libraries by following instructions in *Sequencing*.
3. After sequencing run completes, wash MiSeqDx by following instructions in *Post-Run Wash with Template Line Wash*.

NOTE   Pooled libraries used for sequencing must have at least 24-96 samples if using the MiSeqDx Reagent Kit v3, or 24-36 samples if using the MiSeqDx Reagent Kit v3 Micro. Reports for a subset of samples can be obtained by entering fewer samples during sequencing run setup. All pooled samples will be sequenced, but reports will only be generated for samples entered during sequencing run setup.

Interpretation of Results for the Cystic Fibrosis 139-Variant Assay

- The Cystic Fibrosis 139-Variant Assay is designed to detect 139 CFTR variants, including those recommended by ACMG ().
- The assay report lists the sample names and genotype for each variant detected for a sample.
  - All samples are interrogated for 134 CF-causing variants and the ACMG recommended R117H variant. Only detected mutant alleles are listed in the assay report.
  - The PolyTG/PolyT variant is reported only if the R117H variation is identified for a sample. For patients with an R117H variant, additional testing to determine whether a PolyTG/PolyT variant which may affect the clinical phenotype [eg 12-13 (TG) or 5T] is in cis/trans orientation to the R117H variant should be performed.

NOTE   The PolyTG/PolyT genotype is determined by the Cystic Fibrosis 139-Variant Assay based on read counting of the most common genotypes. Due to the digital nature of next-generation sequencing, the assay is able to achieve high accuracy from multiple observations. Other sequencing-based technologies use just a few observations.

– When a sample has homozygous F508del or I507del genotype, if one or more among the three benign polymorphisms I506V, I507V and F508C are detected, this is reported for the sample. If all three benign polymorphisms are wild type, the report indicates that I506V, I507V, and F508C variants are not present for the sample.

NOTE   Because the Cystic Fibrosis 139-Variant Assay is a sequencing-based assay, there is no interference to F508del or I507del reporting due to the three benign polymorphisms. Hence, no corrections will be made to the detected result.

– When a sample is identified as heterozygous and both wild type and mutant alleles are detected for the sample, the genotype result is reported as HET.
– When a sample is identified as homozygous and only the mutant allele is detected for the sample, the genotype result is reported as HOM.
– If no variant is identified for a sample, the report indicates that no panel variants are detected.

- The assay report provides sample call rate information for each sample. Call rate is calculated as the number of variant positions/regions that meet a predefined confidence value threshold divided by the total positions/regions interrogated.
  – For samples requiring conditional reporting, the additional variants interrogated are also accounted for in the call rate calculation.
  – Any variant with a predefined confidence value below the threshold is reported as a No call. It is recommended that you repeat the sample.
- A sample result is considered valid only if the call rate is ≥ 99%. If the call rate is < 99%, the performance is reported as Fail, and the sample must be repeated.

⚠️ CAUTION

If the sample call rate is < 50%, the performance will be reported as Fail and a Sample Failed comment is indicated on the report. Variant information is not displayed. This sample must be repeated.

- It is recommended that variants that were validated using synthetic specimens (see *Accuracy*) be verified by the user using a validated reference method prior to reporting the first patient result with those variants
- If more than two variants are identified for a sample, it is recommended that the user verify the result by repeating the sample using the TruSight Cystic Fibrosis 139-Variant Assay with a fresh gDNA extract to rule out cross-contamination of the specimen.

FOR IN VITRO DIAGNOSTIC USE.

NOTE   Haplotype phasing should be considered when two or more variants are detected.

- All variant interpretations should be made by a certified clinical molecular geneticist or equivalent following local procedures and guidelines.[15] Potential interpretation references include but are not limited to: CFTR2 database,[11] Sosnay paper,[13] ACMG 2004 guidelines,[1] and 2011 ACOG committee opinion.[2] For information on how results are calculated and presented, or for a description of the contents in text file report, see the guides for the analysis software installed with your MiSeqDx. For Local Run Manager, see Local Run Manager Software Reference Guide for MiSeqDx (document # 1000000011880) and Local Run Manager CF 139-Variant 2.0 Analysis Module Workflow Guide (document # 1000000100945) or Local Run Manager CF 139-Variant 2.0 Micro Analysis Module Workflow Guide (document # 200017946).

Interpretation of Results for the Cystic Fibrosis Clinical Sequencing Assay

The Cystic Fibrosis Clinical Sequencing Assay is designed to sequence all protein coding regions in the CFTR gene across the 27 exons, 5–30 bases of flanking intronic sequence, ~100 nt of flanking sequence at the 5' and 3' UTRs, and two deep intronic mutations (1811+1.6kbA>G, 3489+10kbC>T). The exact regions sequenced are listed in . Additionally the assay also reports on the PolyTG/PolyT variant, and two large deletions (CFTRdele2,3, CFTRdele22,23).

- The assay report lists the sample names and genotype for each variant detected for a sample.
  - The genomic coordinate, Human Genome Variation Society (HGVS) cDNA name, and protein name (if available) are reported for each variant.
  - The variant type is identified as single nucleotide variant (SNV), deletion/insertion variant (DIV), PolyTG/PolyT variant (PolyTGPolyT), or large deletion (DEL).
  - The genotype call (whether heterozygous or homozygous) can be inferred from the 'reference' base information, which provides the reference sequence at that genomic coordinate, and the 'result' description which provides the two alleles at the genomic position in the sample. For example, if the Reference is 'G' and the Result is 'A/G', this indicates a G>A change at that genomic coordinate and that the genotype is heterozygous for the variant allele. Likewise, if the Reference is 'G', and the Result is 'T/T', this indicates a G>T change at that genomic coordinate and that the genotype is homozygous for the variant allele.
  - The sequencing depth at the variant position is provided in the "Depth" field and the allelic frequency in the "Frequency" section.
- The assay report provides sample call rate information for each sample. Call rate is calculated as the number of variant positions/regions that meet a predefined confidence value threshold divided by the total positions/regions interrogated.
  - The genomic coordinate for any position or region for which the confidence value is below the threshold is listed separately in the "Coordinates not called" section. Users should evaluate the positions not called against relevant variant information to identify variants that may be missed and their corresponding population frequencies to determine if sample repeat is required.

FOR IN VITRO DIAGNOSTIC USE.

- A sample result is considered valid only if the call rate is ≥ 99%. If the call rate is below 99%, the performance will be reported as "Fail", and the sample must be repeated.

- It is recommended that any variants outside of what is validated in the accuracy study (see *Accuracy*) be verified by the user using a validated reference method prior to reporting the first patient result with those variants.

    NOTE   Haplotype phasing should be considered when two or more variants are detected.

- All variant interpretations should be made by a certified clinical molecular geneticist or equivalent following local procedures and guidelines[15]. Potential interpretation references include but are not limited to: CFTR2 database[11,12], Sosnay paper[13], ACMG 2004 guidelines[1], and 2011 ACOG committee opinion[2].
    For information on how results are calculated and presented, or for a description of the contents in text file report, see the guides for the analysis software installed with your *MiSeqDx*. For Local Run Manager, see *Local Run Manager Software Reference Guide for MiSeqDx (document # 1000000011880)* and the *Local Run Manager CF Clinical Seq 2.0 Analysis Module Workflow Guide (document # 1000000100946)* or Local Run Manager CF Clin Seq 2.0 Micro Analysis Module Workflow Guide (document # 200017945).

- The geneticist will use the Local Run Manager software to enter an interpretation value for each variant reported on a sample using a drop down menu. The interpretation value choices are: CF-causing, Mutation of varying clinical consequence, Mutation of unknown significance, or Non-CF causing. The value entered will be appended to the results file and display in the interpretation column on the Clinical Sequencing Assay report.

Quality Control Procedures

Good laboratory practices dictate that control material should be evaluated to detect differences in blood processing and technical procedures in the user's laboratory that may produce significant variability in results.

- Negative Control (NTC or No Template Control)—The use of a negative control is required on every run to detect possible incidences of contamination. The call rate for the negative control should be less than 10%. If a negative control generates a call rate > 10% and template line wash was conducted for previous run, contamination during assay processing may have occurred. The assay is considered as having failed and the entire assay must be repeated, starting from library preparation. The negative control sample is reported as Pass if it generates a call rate ≤ 10% and Fail if the call rate is > 10%.

    ⚠ CAUTION

    It is critical to run the template line wash after each sequencing run to prevent elevated negative control call rate. If negative control call rate is > 10%, and template line wash was not conducted in previous run, it is recommended that the operator complete a post-run wash with template line wash and repeat sequencing run.

- Positive Controls—A positive DNA control sample is required on every run. The positive control DNA sample should be a well-characterized sample with at least one known CFTR variant.[16] Illumina recommends the use of rotating positive controls consistent with the 2008 ACMG Technical Standards and Guidelines for CF mutation testing[17] and

the 2013 ACMG clinical laboratory standards for next-generation sequencing.[18] The positive control sample must generate the expected genotype. If the positive control generates a genotype different from what is expected, then an error in sample tracking or incorrect recording of indexing primers may have occurred. The entire assay must be repeated, starting from library preparation. The positive control sample is reported as Pass if it generates a call rate ≥ 99% and Fail if the call rate is < 99%.

- Wild Type Control–The wild type DNA control sample is recommended on every run. The wild type control sample should be a well characterized sample that does not contain any CFTR variants. The wild type control sample must generate the expected genotype. If the wild type control generates a genotype different from what is expected, then an error in sample tracking or incorrect recording of indexing primers may have occurred. The entire assay must be repeated, starting from library preparation.

- A sample result is considered valid only if the call rate is ≥ 99%. If the call rate is below 99%, the performance will be reported as "Fail", and the sample must be repeated.

- Prior to initial use of this product in the user's laboratory, the performance of the assay should be verified by testing a number of positive and negative samples with known performance characteristics.

- All quality control requirements should be performed in accordance with local, state, and/or federal regulations or accreditation requirements.

Performance Characteristics for the Cystic Fibrosis 139-Variant Assay

The performance characteristics for the Cystic Fibrosis 139-Variant Assay are based on studies that utilize the MiSeqDx Cystic Fibrosis 139-Variant Assay. Equivalency between the TruSight and MiSeqDx assays is provided in *Performance Equivalence with Illumina MiSeqDx Cystic Fibrosis 139-Variant Assay*.

Accuracy

Accuracy of the Cystic Fibrosis 139-Variant Assay was assessed by evaluating 500 samples representing a wide variety of CFTR variants from four separate sources. The primary source of accuracy data was a clinical accuracy study conducted using a panel of 366 samples. The majority (n= 355) of samples consisted of archived, anonymized clinical gDNA specimens isolated from human blood. The remaining 11 samples were obtained from commercially available cell line specimens.

Data from this study was supplemented with accuracy data from 68 cell line samples evaluated in the reproducibility study, 14 clinical samples from the extraction method evaluation analytical study, and 52 synthetic plasmid samples. The synthetic plasmids were designed to include the genomic context of the rare variants and contained anywhere from one to nine variants within the same construct. They were linearized, diluted to genomic DNA equivalent copy numbers, and blended with human genomic DNA samples of wild type genotype at equivalent copy numbers to mimic a heterozygous sample.

The genotyping results for 137 SNV/small InDel sites, including the PolyTG/PolyT region were compared to Sanger bidirectional sequence analysis. Two validated PCR based assays were used as the reference method for the two large deletions in the panel. Each duplex PCR assay made use of two primer sets to discriminate between wild type, heterozygous, and homozygous genotypes. One of the primer sets was designed to flank the deletion breakpoints, whereas the other amplified a region internal to the deletion. The two products were detected by size separation on an agarose gel.

The PCR assays were validated using a panel of 28 samples (22 samples for each deletion) consisting of cell line and blood derived genomic DNA samples and synthetic plasmids, which encompassed the WT, HET, and HOM genotypes for each large deletion. The PCR assays were confirmed to have 100% specificity and reproducibility for all samples tested, by evaluation of PCR products on an agarose gel. The accuracy of the PCR assays was confirmed using Sanger Sequencing and found to be 100% for all samples.

Accuracy was determined for each genotype through three statistical measures. Positive Agreement (PA) was calculated for each variant genotype by dividing the number of samples with agreeing variant calls by the total number of samples with that variant as identified by the reference methods. Negative Agreement (NA) was 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. Overall Agreement (OA) was calculated across all reported positions by dividing the number of concordant WT and variant positions by the total number of reported positions as determined by the reference methods.

The Cystic Fibrosis 139-Variant Assay had a genotype-level PA of 100%. The NA for all WT positions was > 99.99%, and the OA for all reported positions was > 99.99%. All test results are based on initial testing.

FOR IN VITRO DIAGNOSTIC USE.

Overall Accuracy of the Cystic Fibrosis 139-Variant Assay

Variant (Common Name)	Variant Type	cDNA Name	Total Calls per Variant	Positive Calls (Variants)			Negative Calls (Wild Type)	# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
				Clinical Samples	Cell Line Samples	Synthetic Samples						
CFTR dele2, 3	DEL	c.54-5940_ 273+10250 del21kb	500	4	1	0	495	0	0	100	100	100
E60X	SNV	c.178G>T	500	6	1	0	493	0	0	100	100	100
P67L	SNV	c.200C>T	500	1	0	1	498	0	0	100	100	100
R75X	SNV	c.223C>T	500	3	1	0	496	0	0	100	100	100
G85E	SNV	c.254G>A	500	6	2	0	492	0	0	100	100	100
394delTT	DIV	c.262_263 delTT	500	3	1	0	496	0	0	100	100	100
406-1G>A	SNV	c.274-1G>A	500	4	0	0	496	0	0	100	100	100
E92X	SNV	c.274G>T	500	0	1	1	498	0	0	100	100	100
D110H	SNV	c.328G>C	500	1	0	1	498	0	0	100	100	100
R117C	SNV	c.349C>T	500	4	0	0	496	0	0	100	100	100
R117H	SNV	c.350G>A	500	17	2	0	481	0	0	100	100	100
Y122X	SNV	c.366T>A	500	0	1	0	499	0	0	100	100	100
621+1G>T	SNV	c.489+1G>T	500	7	5	0	488	0	0	100	100	100
663delT	DIV	c.531delT	500	1	0	1	498	0	0	100	100	100
G178R	SNV	c.532G>A	500	1	1	0	498	0	0	100	100	100
711+1G>T	SNV	c.579+1G>T	500	3	1	0	496	0	0	100	100	100
P205S[*]	SNV	c.613C>T	500	1	0	1	498	0	0	100*	100	100
L206W	SNV	c.617T>G	500	8	1	0	491	0	0	100	100	100

Variant (Common Name)	Variant Type	cDNA Name	Total Calls per Variant	Positive Calls (Variants)			Negative Calls (Wild Type)	# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
				Clinical Samples	Cell Line Samples	Synthetic Samples						
1078delT	DIV	c.948delT	500	1	1	0	498	0	0	100	100	100
G330X	SNV	c.988G>T	500	1	1	0	498	0	0	100	100	100
R334W	SNV	c.1000C>T	500	6	1	0	493	0	0	100	100	100
I336K	SNV	c.1007T>A	500	0	1	0	499	0	0	100	100	100
1154insTC	DIV	c.1022_1023 insTC	500	0	1	0	499	0	0	100	100	100
R347H	SNV	c.1040G>A	500	6	1	1	492	0	0	100	100	100
R347P	SNV	c.1040G>C	500	3	2	0	495	0	0	100	100	100
R352Q	SNV	c.1055G>A	500	5	0	0	495	0	0	100	100	100
A455E	SNV	c.1364C>A	500	4	2	0	494	0	0	100	100	100
S466X (C>G)	SNV	c.1397C>G	500	1	0	1	498	0	0	100	100	100
1548delG	DIV	c.1418delG	500	1	0	1	498	0	0	100	100	100
Q493X	SNV	c.1477C>T	500	4	2	0	494	0	0	100	100	100
I507del	DIV	c.1519_1521 delATC	500	4	2	0	494	0	0	100	100	100
F508del	DIV	c.1521_1523 delCTT	500	84	29	0	387	0	0	100	100	100
1677delTA	DIV	c.1545_1546 delTA	500	1	0	0	499	0	0	100	100	100
V520F	SNV	c.1558G>T	500	2	0	0	498	0	0	100	100	100
1717-1G>A	SNV	c.1585- 1G>A	500	4	1	0	495	0	0	100	100	100

Variant (Common Name)	Variant Type	cDNA Name	Total Calls per Variant	Positive Calls (Variants)			Negative Calls (Wild Type)	# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
				Clinical Samples	Cell Line Samples	Synthetic Samples						
G542X	SNV	c.1624G>T	500	12	3	0	485	0	0	100	100	100
S549N	SNV	c.1646G>A	500	2	2	1	495	0	0	100	100	100
S549R (c.1647T>G)	SNV	c.1647T>G	500	3	1	0	496	0	0	100	100	100
G551D	SNV	c.1652G>A	500	8	3	0	489	0	0	100	100	100
R553X	SNV	c.1657C>T	500	8	2	0	490	0	0	100	100	100
A559T	SNV	c.1675G>A	500	4	0	1	495	0	0	100	100	100
R560T	SNV	c.1679G>C	500	6	1	0	493	0	0	100	100	100
1812-1 G>A	SNV	c.1680-1G>A	500	0	2	0	498	0	0	100	100	100
1898+1G>A	SNV	c.1766+1G>A	500	2	1	0	497	0	0	100	100	100
2143delT	DIV	c.2012delT	500	2	1	0	497	0	0	100	100	100
2183AA>G	DIV	c.2051_2052delAAinsG	500	3	1	0	496	0	0	100	100	100
2184insA	DIV	c.2052_2053insA	500	3	0	1	496	0	0	100	100	100
2184delA	DIV	c.2052delA	500	1	1	0	498	0	0	100	100	100
R709X	SNV	c.2125C>T	500	1	0	2	497	0	0	100	100	100
K710X	SNV	c.2128A>T	500	3	0	0	497	0	0	100	100	100

Variant (Common Name)	Variant Type	cDNA Name	Total Calls per Variant	Positive Calls (Variants)			Negative Calls (Wild Type)	# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
				Clinical Samples	Cell Line Samples	Synthetic Samples						
2307insA	DIV	c.2175_2176insA	500	3	0	2	495	0	0	100	100	100
R764X	SNV	c.2290C>T	500	1	0	2	497	0	0	100	100	100
W846X	SNV	c.2537G>A	500	0	1	0	499	0	0	100	100	100
2789+5G>A	SNV	c.2657+5G>A	500	9	1	0	490	0	0	100	100	100
Q890X	SNV	c.2668C>T	500	1	0	0	499	0	0	100	100	100
3120G>A	SNV	c.2988G>A	500	1	0	0	499	0	0	100	100	100
3120+1G>A	SNV	c.2988+1G>A	500	7	1	0	492	0	0	100	100	100
3272-26A>G	SNV	c.3140-26A>G	500	0	1	0	499	0	0	100	100	100
R1066C	SNV	c.3196C>T	500	6	0	0	494	0	0	100	100	100
R1066H	SNV	c.3197G>A	500	1	0	1	498	0	0	100	100	100
W1089X	SNV	c.3266G>A	500	4	0	0	496	0	0	100	100	100
Y1092X (C>A)	SNV	c.3276C>A	500	3	1	0	496	0	0	100	100	100
M1101K	SNV	c.3302T>A	500	2	2	0	496	0	0	100	100	100
R1158X	SNV	c.3472C>T	500	7	1	0	492	0	0	100	100	100
R1162X	SNV	c.3484C>T	500	5	1	0	494	0	0	100	100	100
3659delC	DIV	c.3528delC	500	4	1	0	495	0	0	100	100	100
S1196X	SNV	c.3587C>G	500	1	0	0	499	0	0	100	100	100
3791delC	DIV	c.3659delC	500	2	0	0	498	0	0	100	100	100

| Variant (Common Name) | Variant Type | cDNA Name | Total Calls per Variant | Positive Calls (Variants) | | | Negative Calls (Wild Type) | # Miscalls | # No Calls | Positive Agreement (%) | Negative Agreement (%) | Overall Agreement (%) |
				Clinical Samples	Cell Line Samples	Synthetic Samples						
3849+10kbC>T	SNV	c.3717+12191C>T	500	11	2	0	487	0	0	100	100	100
3876delA	DIV	c.3744delA	500	6	1	0	493	0	0	100	100	100
S1251N	SNV	c.3752G>A	500	1	0	1	498	0	0	100	100	100
3905insT	DIV	c.3773_3774insT	500	3	1	0	496	0	0	100	100	100
W1282X	SNV	c.3846G>A	500	9	1	0	490	0	0	100	100	100
N1303K	SNV	c.3909C>G	500	9	1	0	490	0	0	100	100	100
CFTR dele22,23[§]	DEL	c.3964-78_4242+577del	500	1	0	1	498	1[§]	0	100	99.80	99.80
M1V	SNV	c.1A>G	500	0	0	1	499	0	0	100	100	100
Q39X	SNV	c.115C>T	500	0	0	1	499	0	0	100	100	100
405+1 G>A	SNV	c.273+1G>A	500	0	0	1	499	0	0	100	100	100
E92K	SNV	c.274G>A	500	0	0	1	499	0	0	100	100	100
Q98X	SNV	c.292C>T	500	0	0	2	498	0	0	100	100	100
457TAT>G	DIV	c.325_327delTATinsG	500	0	0	1	499	0	0	100	100	100
574delA	DIV	c.442delA	500	0	0	2	498	0	0	100	100	100
711+3A>G	SNV	c.579+3A>G	500	0	0	1	499	0	0	100	100	100
711+5 G>A	SNV	c.579+5G>A	500	0	0	1	499	0	0	100	100	100
712-1 G>T	SNV	c.580-1G>T	500	0	0	1	499	0	0	100	100	100
H199Y	SNV	c.595C>T	500	0	0	1	499	0	0	100	100	100

Variant (Common Name)	Variant Type	cDNA Name	Total Calls per Variant	Positive Calls (Variants)			Negative Calls (Wild Type)	# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
				Clinical Samples	Cell Line Samples	Synthetic Samples						
Q220X	SNV	c.658C>T	500	0	0	1	499	0	0	100	100	100
852del22	DIV	c.720741 delAGGG AGAAT GATGAT GAAGTAC	500	0	0	1	499	0	0	100	100	100
T338I	SNV	c.1013C>T	500	0	0	1	499	0	0	100	100	100
S341P	SNV	c.1021T>C	500	0	0	1	499	0	0	100	100	100
1213delT	DIV	c.1081delT	500	0	0	1	499	0	0	100	100	100
1248+1G>A	SNV	c.1116+1G>A	500	0	0	1	499	0	0	100	100	100
1259insA	DIV	c.1127_1128insA	500	0	0	2	498	0	0	100	100	100
W401X (c.1202G>A)	SNV	c.1202G>A	500	0	0	1	499	0	0	100	100	100
W401X (c.1203G>A)	SNV	c.1203G>A	500	0	0	1	499	0	0	100	100	100
1341+1G>A	SNV	c.1209+1G>A	500	0	0	2	498	0	0	100	100	100
1461ins4	DIV	c.1329_1330insAGAT	500	0	0	1	499	0	0	100	100	100
1525-1G>A	SNV	c.1393-1G>A	500	0	0	1	499	0	0	100	100	100

Variant (Common Name)	Variant Type	cDNA Name	Total Calls per Variant	Positive Calls (Variants)			Negative Calls (Wild Type)	# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
				Clinical Samples	Cell Line Samples	Synthetic Samples						
S466X (C>A)	SNV	c.1397C>A	500	0	0	1	499	0	0	100	100	100
L467P	SNV	c.1400T>C	500	0	0	1	499	0	0	100	100	100
S489X	SNV	1466C>A	500	0	0	2	498	0	0	100	100	100
S492F	SNV	c.1475C>T	500	0	0	1	499	0	0	100	100	100
Q525X	SNV	c.1573C>T	500	0	0	1	499	0	0	100	100	100
1717-8G>A	SNV	c.1585-8G>A	500	0	0	1	499	0	0	100	100	100
S549R (c.1645A>C)	SNV	c.1645A>C	500	0	0	1	499	0	0	100	100	100
Q552X	SNV	c.1654C>T	500	0	0	1	499	0	0	100	100	100
R560K	SNV	c.1679G>A	500	0	0	1	499	0	0	100	100	100
1811+1.6 kb A>G	SNV	c.1679+1.6 kbA>G	500	0	0	1	499	0	0	100	100	100
E585X	SNV	c.1753G>T	500	0	0	1	499	0	0	100	100	100
1898+3A>G	SNV	c.1766+3A>G	500	0	0	1	499	0	0	100	100	100
L732X	SNV	c.2195T>G	500	0	0	2	498	0	0	100	100	100
2347delG	DIV	c.2215delG	500	0	0	2	498	0	0	100	100	100
2585delT	DIV	c.2453delT	500	0	0	2	498	0	0	100	100	100
E822X	SNV	c.2464G>T	500	0	0	2	498	0	0	100	100	100
2622+1G>A[¶]	SNV	c.2490+1G>T[¶]	500	0	0	2	498	0	0	100	100	100

Variant (Common Name)	Variant Type	cDNA Name	Total Calls per Variant	Positive Calls (Variants)			Negative Calls (Wild Type)	# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
				Clinical Samples	Cell Line Samples	Synthetic Samples						
E831X	SNV	c.2491G>T	500	0	0	1	499	0	0	100	100	100
R851X	SNV	c.2551C>T	500	0	0	1	499	0	0	100	100	100
2711delT	DIV	c.2583delT	500	0	0	1	499	0	0	100	100	100
L927P	SNV	c.2780T>C	500	0	0	1	499	0	0	100	100	100
S945L	SNV	c.2834C>T	500	0	0	1	499	0	0	100	100	100
3007delG	DIV	c.2875delG	500	0	0	1	499	0	0	100	100	100
G970R	SNV	c.2908G>C	500	0	0	1	499	0	0	100	100	100
3121-1G>A	SNV	c.2989-1G>A	500	0	0	1	499	0	0	100	100	100
L1065P	SNV	c.3194T>C	500	0	0	1	499	0	0	100	100	100
L1077P^	SNV	c.3230T>C	500	0	0	1	499	0^	0	100	100	100
Y1092X (C>G)	SNV	c.3276C>G	500	0	0	1	499	0	0	100	100	100
E1104X	SNV	c.3310G>T	500	0	0	1	499	0	0	100	100	100
W1204X (c.3611G>A)	SNV	c.3611G>A	500	0	0	1	499	0	0	100	100	100
W1204X (c.3612G>A)	SNV	c.3612G>A	500	0	0	1	499	0	0	100	100	100
G1244E	SNV	c.3731G>A	500	0	0	1	499	0	0	100	100	100
4005+1G>A	SNV	c.3873+1G>A	500	0	0	1	499	0	0	100	100	100
4016insT	DIV	c.3884_3885insT	500	0	0	1	499	0	0	100	100	100

Variant (Common Name)	Variant Type	cDNA Name	Total Calls per Variant	Positive Calls (Variants)			Negative Calls (Wild Type)	# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
				Clinical Samples	Cell Line Samples	Synthetic Samples						
Q1313X	SNV	c.3937C>T	500	0	0	1	499	0	0	100	100	100
4209TG TT>AA	DIV	c.4077_ 4080delT GTTinsAA	500	0	0	1	499	0	0	100	100	100
4382delA	DIV	c.4251delA	500	0	0	1	499	0	0	100	100	100
PolyTG/ PolyT€	PolyTGPolyT	c.1210-12T [5_9]	19	17	2	0	0	0	0	100	Not applicable	100
I506V¥	SNV	c.1516A>G	1	0	0	0	1	0	0	Not applicable	100	100
I507V¥	SNV	c.1519A>G	1	0	0	0	1	0	0	Not applicable	100	100
F508C¥	SNV	c.1523T>G	1	0	0	0	1	0	0	Not applicable	100	100
Total			67522	557			66965	1	0	100	> 99.99	> 99.99

DIV is an acronym for Deletion/Insertion Variant.

* The Sanger report indicated the P205S variant as heterozygous for the clinical sample. A review of the Sanger trace data however indicated that the variant was in fact homozygous and incorrectly reported. The MiSeqDx reported the variant as homozygous.

§ A synthetic sample heterozygous for exon 8 was reported as heterozygous for the variant CFTR dele22, 23. Further investigation revealed that this result was likely from low-level contamination.

^ The original synthetic heterozygous specimen was determined to be improperly prepared. When it was subsequently tested after it was reprepared, using the same plasmid, it was detected.

€ When R117H is positive, the PolyTG/PolyT variant is additionally reported.

¥ In the case of one homozygous F508del variant, three additional wild type bases (ie, variants I506V, I507V, F508C) that were not identified in the specimen were additionally reported.

¶ The original validation study for the assay included two synthetic samples containing the nucleotide change c.2490+1G>T for variant 2622+1 G>A (data are included in this table). A second validation study was later done with a synthetic sample containing the nucleotide change c.2490+1G>A to support the actual nucleotide change (c.2490+1G>A) associated with the variant.

Accuracy of the Cystic Fibrosis 139-Variant Assay for I506V, I507V, and F508C.

Variant (Common Name)	Total Calls per Variant	Positive Calls (Variants)			Negative Calls (Wild Type)	# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
		Clinical Samples	Cell Line Samples	Synthetic Samples						
I506V	500	7	0	0	493	0	0	100	100	100
I507V	500	0	1	0	499	0	0	100	100	100
F508C	500	1	1	0	498	0	0	100	100	100

Accuracy of the Cystic Fibrosis 139-Variant Assay for PolyTG/PolyT Variants

PolyTGPolyT Genotype	# Clinical Samples	# Cell Line Samples	# Synthetic Samples	# Miscalls	# No Calls*	% Accuracy
(TG)9(T)7/(TG)11(T)7	2	0	0	0	1	50
(TG)9(T)9/(TG)10(T)7	1	0	0	0	0	100
(TG)9(T)9/(TG)11(T)7	5	1	0	0	0	100
(TG)9(T)9/(TG)11(T)9	1	0	0	0	0	100
(TG)10(T)7/(TG)10(T)7	25	8	0	0	0	100
(TG)10(T)7/(TG)10(T)9	39	16	0	0	0	100
(TG)10(T)7/(TG)11(T)5	2	0	0	0	0	100
(TG)10(T)7/(TG)11(T)7	72	11	0	0	0	100
(TG)10(T)7/(TG)12(T)5	1	0	0	0	0	100
(TG)10(T)7/(TG)12(T)7	10	1	0	0	1	90.9

PolyTGPolyT Genotype	# Clinical Samples	# Cell Line Samples	# Synthetic Samples	# Miscalls	# No Calls*	% Accuracy
(TG)10(T)9/(TG)10(T)9	7	6	0	0	0	100
(TG)10(T)9/(TG)11(T)5	5	0	0	0	0	100
(TG)10(T)9/(TG)11(T)7	76	20	0	0	0	100
(TG)10(T)9/(TG)11(T)9	3	0	0	0	0	100
(TG)10(T)9/(TG)12(T)5	3	2	0	0	0	100
(TG)10(T)9/(TG)12(T)7	13	0	0	0	1	92.3
(TG)11(T)5/(TG)11(T)7	6	0	0	1	0	83.3
(TG)11(T)7/(TG)11(T)7	52	8	0	0	0	100
(TG)11(T)7/(TG)11(T)9^	2	1	0	3^	0	0
(TG)11(T)7/(TG)12(T)5	2	0	0	0	0	100
(TG)11(T)7/(TG)12(T)7	37	3	0	0	0	100
(TG)11(T)9/(TG)12(T)7	3	0	0	0	0	100
(TG)12(T)7/(TG)12(T)7	2	2	0	0	0	100
Total**		448		4	3	98.44

* Samples were not retested.

^ One of the discordant results was from the reproducibility study. The PolyTG/PolyT result for the sample was concordant across all 18 replicates, but discordant with Sanger bidirectional sequencing.

** The total sample count for the PolyTG/PolyT variant is 448. All synthetic samples (n = 52) were built by blending linearized plasmids with one of two cell line samples, which were part of the reproducibility study. Because reporting the PolyTG/PolyT variant for these additional synthetic samples would result in the variant being overreported, the synthetic samples were excluded from this analysis.

Reproducibility

The reproducibility of the Cystic Fibrosis 139-Variant Assay was determined through a blinded study using three trial sites and two operators at each site. Two well-characterized panels of 46 samples each were tested by each operator at each site for a total of 810 calls per site. The panels contained a mix of genomic DNA from lymphoblastoid cell lines with known variants in the *CFTR* gene, as well as leukocyte-depleted blood spiked with lymphoblastoid cell lines with known variants in the *CFTR* gene. The blood samples were provided to allow incorporation of the extraction steps used to prepare gDNA that serves as the primary input for the assay workflow.

The sample pass rate, defined as the number of samples passing QC metrics on the first attempt, was 99.9%.

The genotype-level Positive Agreement for all variants was 99.77%. The Negative Agreement for all WT positions was 99.88% and the Overall Agreement for all reported positions was 99.88%. All test results are based on initial testing. No repeat testing was done for the reproducibility study.

Reproducibility of the Cystic Fibrosis 139-Variant Assay

Panel	Sample #	Sample Genotype	Variants	Total Calls per Site	Positive Agreeing Calls (Variants)			Negative Agreeing Calls (Wild Type)			# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
					Site 1	Site 2	Site 3	Site 1	Site 2	Site 3					
A	1	S549N (Het)		810	6	6	6	804	804	804	0	0	100	100	100
A	2	1812-1 G>A (Het)		810	6	6	6	804	804	804	0	0	100	100	100
A	3	Q493X/F508del (Het)		810	12	12	12	798	798	798	0	0	100	100	100
A	4*	F508del/2184delA (Het)		810	12	12	12	797	798	798	0	1*	100	100	100
A	5^	Y122X/R1158X (Het)		810	12	10	12	798	665	798	0	135^	94.44	94.44	94.44
A	6	F508del/2183AA>G (Het)		810	12	12	12	798	798	798	0	0	100	100	100
A	7	R75X (Het)		810	6	6	6	804	804	804	0	0	100	100	100
A	8	I507del/F508del (Het)		810	12	12	12	798	798	798	0	0	100	100	100
A	9**	F508del/W1282X (Het)		810	12	11	12	798	797	798	2**	0	97.22	99.96	99.92
A	10**	F508del/3272-26A>G (Het)		810	12	11	12	798	797	798	2**	0	97.22	99.96	99.92
A	11	F508del/3849+10kbC >T (Het)		810	12	12	12	798	798	798	0	0	100	100	100
A	12	621+1G>T/3120+1G >A (Het)		810	12	12	12	798	798	798	0	0	100	100	100
A	13	E60X/F508del (Het)		810	12	12	12	798	798	798	0	0	100	100	100
A	14	M1101K (Het)		810	6	6	6	804	804	804	0	0	100	100	100
A	15	M1101K (Hom)		810	6	6	6	804	804	804	0	0	100	100	100

Panel	Sample #	Sample Genotype	Variants	Total Calls per Site	Positive Agreeing Calls (Variants)			Negative Agreeing Calls (Wild Type)			# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
					Site 1	Site 2	Site 3	Site 1	Site 2	Site 3					
A	16	F508del (Hom)	I506V, I507V, F508C not present	828	6	6	6	822	822	822	0	0	100	100	100
A	17	F508del/3659delC (Het)		810	12	12	12	798	798	798	0	0	100	100	100
A	18	R117H/F508del (Het)	(TG)10 (T)9/ (TG)12 (T)5	816	18	18	18	798	798	798	0	0	100	100	100
A	19	621+1G>T/711+1G>T (Het)		810	12	12	12	798	798	798	0	0	100	100	100
A	20	G85E/621+1G>T (Het)		810	12	12	12	798	798	798	0	0	100	100	100
A	21	A455E/F508del (Het)		810	12	12	12	798	798	798	0	0	100	100	100
A	22	F508del/R560T (Het)		810	12	12	12	798	798	798	0	0	100	100	100
A	23	F508del/Y1092X (C>A) (Het)		810	12	12	12	798	798	798	0	0	100	100	100
A	24	N1303K (Het)		810	6	6	6	804	804	804	0	0	100	100	100
A	25	G542X (Hom)		810	6	6	6	804	804	804	0	0	100	100	100
A	26	G542X (Het)		810	6	6	6	804	804	804	0	0	100	100	100

Panel	Sample #	Sample Genotype	Variants	Total Calls per Site	Positive Agreeing Calls (Variants)			Negative Agreeing Calls (Wild Type)			# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
					Site 1	Site 2	Site 3	Site 1	Site 2	Site 3					
A	27	G551D/R553X (Het)		810	12	12	12	798	798	798	0	0	100	100	100
A	28	3849+10kbC>T (Hom)		810	6	6	6	804	804	804	0	0	100	100	100
A	29	WT		810	0	0	0	810	810	810	0	0	Not applicable	100	100
A	30	F508del (Het)		810	6	6	6	804	804	804	0	0	100	100	100
A	31	1717-1G>A (Het)		810	6	6	6	804	804	804	0	0	100	100	100
A	32	R1162X (Het)		810	6	6	6	804	804	804	0	0	100	100	100
A	33	R347P/G551D (Het)		810	12	12	12	798	798	798	0	0	100	100	100
A	34	R334W (Het)		810	6	6	6	804	804	804	0	0	100	100	100
A	35	WT		810	0	0	0	810	810	810	0	0	Not applicable	100	100
A	36	G85E (Het)		810	6	6	6	804	804	804	0	0	100	100	100
A	37	I336K (Het)		810	6	6	6	804	804	804	0	0	100	100	100
A	38	WT		810	0	0	0	810	810	810	0	0	Not applicable	100	100
A	39	F508del/3849+10kbC>T (Het)		810	12	12	12	798	798	798	0	0	100	100	100

Panel	Sample #	Sample Genotype	Variants	Total Calls per Site	Positive Agreeing Calls (Variants)			Negative Agreeing Calls (Wild Type)			# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
					Site 1	Site 2	Site 3	Site 1	Site 2	Site 3					
A	40	621+1G>T/3120+1G >A (Het)		810	12	12	12	798	798	798	0	0	100	100	100
A	41	F508del/3659delC (Het)		810	12	12	12	798	798	798	0	0	100	100	100
A	42	R117H/F508del (Het)	(TG)10 (T)9/ (TG)12 (T)5	816	18	18	18	798	798	798	0	0	100	100	100
A	43	G85E/621+1G>T (Het)		810	12	12	12	798	798	798	0	0	100	100	100
A	44	A455E/F508del (Het)		810	12	12	12	798	798	798	0	0	100	100	100
A	45	N1303K (Het)		810	6	6	6	804	804	804	0	0	100	100	100
A	46	G551D/R553X (Het)		810	12	12	12	798	798	798	0	0	100	100	100
B	47	2789+5G>A (Hom)		810	6	6	6	804	804	804	0	0	100	100	100
B	48	CFTR dele2, 3/F508del (Het)		810	12	12	12	798	798	798	0	0	100	100	100
B	49	F508del/1898+1G>A (Het)		810	12	12	12	798	798	798	0	0	100	100	100
B	50	WT		810	0	0	0	810	810	810	0	0	Not applicable	100	100
B	51	F508del/2143delT (Het)		810	12	12	12	798	798	798	0	0	100	100	100

Panel	Sample #	Sample Genotype	Variants	Total Calls per Site	Positive Agreeing Calls (Variants)			Negative Agreeing Calls (Wild Type)			# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
					Site 1	Site 2	Site 3	Site 1	Site 2	Site 3					
B	52	3876delA (Het)		810	6	6	6	804	804	804	0	0	100	100	100
B	53	3905insT (Het)		810	6	6	6	804	804	804	0	0	100	100	100
B	54	394delTT (Het)		810	6	6	6	804	804	804	0	0	100	100	100
B	55	F508del (Het)		810	6	6	6	804	804	804	0	0	100	100	100
B	56	WT		810	0	0	0	810	810	810	0	0	Not applicable	100	100
B	57	WT		810	0	0	0	810	810	810	0	0	Not applicable	100	100
B	58	F508del (Het)		810	6	6	6	804	804	804	0	0	100	100	100
B	59	WT		810	0	0	0	810	810	810	0	0	Not applicable	100	100
B	60	L206W (Het)		810	6	6	6	804	804	804	0	0	100	100	100
B	61	WT		810	0	0	0	810	810	810	0	0	Not applicable	100	100
B	62	G330X (Het)		810	6	6	6	804	804	804	0	0	100	100	100
B	63	WT		810	0	0	0	810	810	810	0	0	Not applicable	100	100
B	64	R347H (Het)		810	6	6	6	804	804	804	0	0	100	100	100

Panel	Sample #	Sample Genotype	Variants	Total Calls per Site	Positive Agreeing Calls (Variants)			Negative Agreeing Calls (Wild Type)			# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
					Site 1	Site 2	Site 3	Site 1	Site 2	Site 3					
B	65	1078delT (Het)		810	6	6	6	804	804	804	0	0	100	100	100
B	66	G178R/F508del (Het)		810	12	12	12	798	798	798	0	0	100	100	100
B	67	S549R (c.1647T>G) (Het)		810	6	6	6	804	804	804	0	0	100	100	100
B	68	S549N (Het)		810	6	6	6	804	804	804	0	0	100	100	100
B	69	W846X (Het)		810	6	6	6	804	804	804	0	0	100	100	100
B	70	WT		810	0	0	0	810	810	810	0	0	Not applicable	100	100
B	71	E92X/F508del (Het)		810	12	12	12	798	798	798	0	0	100	100	100
B	72[§]	621+1G>T/1154insTC (Het)		810	12	12	12	798	798	797	0	1[§]	100	99.96	99.96
B	73	G542X (Het)		810	6	6	6	804	804	804	0	0	100	100	100
B	74	F508del (Het)		810	6	6	6	804	804	804	0	0	100	100	100
B	75[^]	F508del (Het)		810	6	5	6	804	670	804	0	135[^]	94.44	94.44	94.44
B	76	F508del (Het)		810	6	6	6	804	804	804	0	0	100	100	100
B	77	621+1G>T/A455E (Het)		810	12	12	12	798	798	798	0	0	100	100	100
B	78	1812-1 G>A (Het)		810	6	6	6	804	804	804	0	0	100	100	100
B	79	WT		810	0	0	0	810	810	810	0	0	Not applicable	100	100

Panel	Sample #	Sample Genotype	Variants	Total Calls per Site	Positive Agreeing Calls (Variants)			Negative Agreeing Calls (Wild Type)			# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
					Site 1	Site 2	Site 3	Site 1	Site 2	Site 3					
B	80	F508del/R553X (Het)		810	12	12	12	798	798	798	0	0	100	100	100
B	81	F508del/G551D (Het)		810	12	12	12	798	798	798	0	0	100	100	100
B	82	R347P/F508del (Het)		810	12	12	12	798	798	798	0	0	100	100	100
B	83	R117H/F508del (Het)	(TG)10 (T)9/ (TG)12 (T)5	816	18	18	18	798	798	798	0	0	100	100	100
B	84	I507del (Het)		810	6	6	6	804	804	804	0	0	100	100	100
B	85	2789+5G>A (Hom)		810	6	6	6	804	804	804	0	0	100	100	100
B	86[§]	CFTR dele2, 3/F508del (Het)		810	12	12	12	798	797	798	0	1[§]	100	99.96	99.96
B	87	F508del/1898+1G>A (Het)		810	12	12	12	798	798	798	0	0	100	100	100
B	88	WT		810	0	0	0	810	810	810	0	0	Not applicable	100	100
B	89	F508del/2143delT (Het)		810	12	12	12	798	798	798	0	0	100	100	100
B	90	3905insT (Het)		810	6	6	6	804	804	804	0	0	100	100	100
B	91	394delTT (Het)		810	6	6	6	804	804	804	0	0	100	100	100
B	92	F508del (Het)		810	6	6	6	804	804	804	0	0	100	100	100
		Total		74556	2209			221182			4	273	99.77	99.88	99.88

* The wild type location corresponding to the N1303K variant for one replicate resulted in a No Call due to insufficient coverage.

^ One replicate of samples 5 and 75 had a 0% call rate. Further investigation indicates that samples may not have been added to the sample plate before library preparation because the sample volumes remaining in the tubes were consistent with no volume having been removed.

** Evidence indicates that samples 9 and 10 were likely switched by the operator before library preparation.

§ The wild type location corresponding to the M1V variant for one replicate of each of two samples resulted in a No Call due to insufficient coverage.

Supplemental Information on Reproducibility Study Variants

Variation (Common Name)	Variant Type	CFTR Gene Region
PolyTG/PolyT	Compound DIV*	Intron 9
2183AA>G	Compound DIV*	Exon 14
CFTR dele2, 3	DEL	Intron1-Intron3
1154insTC	DIV*	Exon 8
I507del	DIV*	Exon 11
F508del	DIV*	Exon 11
2143delT	DIV*	Exon 14
3659delC	DIV*	Exon 22
3876delA	DIV*	Exon 23
394delTT	DIV in homopolymeric region*	Exon 3
1078delT	DIV in homopolymeric region*	Exon 8
2184delA	DIV in homopolymeric region*	Exon 14
3905insT	DIV in homopolymeric region*	Exon 23
E60X	SNV	Exon 3
R75X	SNV	Exon 3
G85E	SNV	Exon 3
E92X	SNV	Exon 4
R117H	SNV	Exon 4
Y122X	SNV	Exon 4
621+1G>T	SNV	Intron 4

Variation (Common Name)	Variant Type	CFTR Gene Region
G178R	SNV	Exon 5
711+1G>T	SNV	Intron 5
L206W	SNV	Exon 6
G330X	SNV	Exon 8
R334W	SNV	Exon 8
I336K	SNV	Exon 8
R347P	SNV	Exon 8
R347H	SNV	Exon 8
A455E	SNV	Exon 10
Q493X	SNV	Exon 11
1717-1G>A	SNV	Intron 11
G542X	SNV	Exon 12
S549N	SNV	Exon 12
S549R (c.1647T>G)	SNV	Exon 12
G551D	SNV	Exon 12
R553X	SNV	Exon 12
R560T	SNV	Exon 12
1812-1 G>A	SNV	Intron 12
1898+1G>A	SNV	Intron 13
W846X	SNV	Exon 15
2789+5G>A	SNV	Intron 16

Variation (Common Name)	Variant Type	CFTR Gene Region
3120+1G>A	SNV	Intron 18
3272-26A>G	SNV	Intron 19
Y1092X (C>A)	SNV	Exon 20
M1101K	SNV	Exon 20
R1158X	SNV	Exon 22
R1162X	SNV	Exon 22
3849+10kbC>T	SNV	Intron 22
W1282X	SNV	Exon 23
N1303K	SNV	Exon 24

* DIV is an acronym for Deletion/Insertion Variant.

DNA Extraction

Three commonly used, commercially available extraction methods representing magnetic bead extraction, alcohol precipitation, and silica filter column isolation methods were evaluated using EDTA anti-coagulated whole blood. A total of 14 unique blood samples were used in the study representing wild type and three mutant genotypes (three samples with F508del, one sample with I506V, and one sample with D110H). The three DNA extraction methods were tested independently by two different operators who each performed three runs per extraction method. Each extraction was performed by each operator on different days. The DNA concentration and A260/A280 ratio of the extracted gDNA samples was determined using spectrophotometry. The total sample size for each extraction method in this study was 168 (14 samples x 2 operators/extraction method x 3 runs/operator x 2 replicates/extracted gDNA sample).

Extraction Method	Number of Samples Tested	Call Rate	Accuracy	Sample First Pass Rate*
Alcohol Precipitation	168	100%	100%	100%
Silica Filter Column Isolation	168	100%	100%	100%
Magnetic Bead Extraction	168	100%	100%	100%

* Percent of samples having call rate of > 99% in first run.

DNA Input

The DNA input range of the Cystic Fibrosis 139-Variant Assay was evaluated by performing a serial dilution study using 14 representative DNA samples containing 16 unique CF variants.

Each sample was tested in duplicate at nine DNA input levels ranging from 1250 – 1 ng (1250 ng, 500 ng, 250 ng, 100 ng, 50 ng, 25 ng, 10 ng, 5 ng, and 1 ng). For determination of accuracy, sample genotypes were compared to bidirectional Sanger sequencing data and the deletions were compared to PCR assay. 1250 ng and 25 ng were identified as the upper and lower bound for DNA input respectively as they had ≥ 95% sample first pass rate with no incorrect calls (100% accuracy and call rate).

DNA inputs of 1250 ng, 250 ng, and 100 ng were further tested with four representative DNA samples and at least 20 replicates per DNA input level for each sample (n=4x20=80 samples), while the lower bound of 25 ng was tested with 14 samples, 20 replicates for each sample (n=14x20=280 samples). The accuracy and sample first pass rate was 100% at all DNA input levels.

The results indicate that the Cystic Fibrosis 139-Variant Assay can be used in the DNA input range from 1250-25 ng to produce accurate results.

Interfering Substances

To assess the impact of interfering substances on the Cystic Fibrosis 139-Variant Assay, the performance of the assay was evaluated in the presence and absence of potential interferents. Eight whole blood specimens were tested in the study including three CF positive samples with unique genotypes. Four endogenous interfering substances (bilirubin, cholesterol, hemoglobin, and triglyceride) were tested by spiking them into blood specimens prior to DNA extraction. The concentration limits for each substance are shown in the following table. Additionally, to assess interference resulting from blood collection (short draw), EDTA was spiked into blood samples, and to assess interference resulting from sample preparation, the final wash buffer from a silica filter column isolation method was added to purified genomic DNA.

The Cystic Fibrosis 139-Variant Assay achieved 100% call rate for all samples tested and 100% reproducibility in genotype calls between samples in the presence and absence of interfering substances.

To access the impact of multiplexing index primer interference, a cross-contamination study using two samples, each with unique homozygous genotypes at four different genomic positions, and two respective index primers was performed. No change in variant calling was observed with contamination levels < 40%. The sample genotype became heterozygous when contamination levels were ≥ 40%.

No interference was observed from any of the endogenous or exogenous interferents.

Test Substance	Total Number of Replicates	Concentration Tested in Blood (Upper Limit)	Concentration Tested in Blood (Lower Limit)	Call Rate
Bilirubin	16	684 µmol/L	137 µmol/L	100%
Cholesterol	16	13 mmol/L	2.6 mmol/L	100%
Hemoglobin	16	2 g/L	0.4 g/L	100%
Triglyceride	16	37 mmol/L	7.4 mmol/L	100%
EDTA	16	7.0 mg/mL	2.8 mg/mL	100%

Sample Indexing

Sample index primers are used in the assay to assign a unique barcode to each sample DNA, allowing the ability to pool multiple samples together into a single sequencing run. A total of 96 samples indexes were tested using eight unique DNA samples to verify the ability of the assay to consistently make a genotyping call for a given sample across different indexing primer combinations. Each sample was tested with 12 different indexing primer combinations. Sample results were compared against bidirectional Sanger sequencing data for all positions/variants except the two large deletions, which were confirmed using a duplex PCR assay. Reproducibility and accuracy were 100% for all sample/index primer combinations.

FOR IN VITRO DIAGNOSTIC USE.

Performance Equivalence with Illumina MiSeqDx Cystic Fibrosis 139-Variant Assay

The TruSight Cystic Fibrosis 139-Variant Assay (TruSight CF139) uses the same library preparation workflow and reagents as the Illumina MiSeqDx Cystic Fibrosis Assay (MiSeqDx CF139). TruSight CF139 uses the MiSeqDx Reagent Kit v3 while the MiSeqDx CF139 uses sequencing reagents included with the Assay. To demonstrate equivalency between TruSight CF139 and MiSeqDx CF139, results from nine TruSight CF139 runs were compared to a single MiSeqDx CF139 run as the gold standard. The TruSight CF139 runs were conducted at 96 sample throughput (maximum sample throughput for TruSight CF139) and the MiSeqDx CF139 run at 48 sample throughput (maximum sample throughput for MiSeqDx CF139). Sources of variability included in TruSight CF139 runs included three library preparation events (each with a unique lot of TruSight Cystic Fibrosis), three operators, three MiSeqDx instruments, and three lots of MiSeqDx Reagent Kit v3.

Variant calls from TruSight CF139 runs were compared to calls made by the MiSeqDx CF139 run. 47 unique samples were included in each TruSight CF139 run, with 2-3 replicates per sample (95 DNA samples and 1 NTC per run). For the MiSeqDx CF139 run, the same 47 samples were sequenced as singleton (47 DNA samples + 1 NTC per run). The sample panel was comprised of Coriell DNA samples extracted from immortalized cell lines and included samples representing every allele of the ACMG 23 mutations, deletion-insertion variants (including insertion/deletions in homopolymeric regions and insertion-with-deletion in the same region), homozygous variants, compound heterozygous variants, one of the targeted large deletions, a common PolyTG/PolyT variant, numerous single nucleotide variants, and a sample with no variants detected. Summary of results by genotype is provided in . Agreement between assays by variant type is presented in . Overall (Total) agreement between assays was > 99.99%.

Variant-calling Performance of TruSight CF 139-Variant Assay compared to MiSeqDx CF 139-Variant Assay

		MiSeqDx CF 139-Variant Assay				
		Hom Variant	Het Variant	Wild Type	No Call	Total
TruSight CF 139-Variant Assay	Hom Variant	87	-	-	-	87
	Het Variant	-	1,098	-	-	1,098
	Wild Type	-	-	113,889	-	113,889
	No Call	-	-	-	-	-
	Total	87	1,098	113,889	-	115,074

FOR IN VITRO DIAGNOSTIC USE.

Performance by Variant Type of TruSight CF 139-Variant Assay compared to MiSeqDx CF 139-Variant Assay

Variant Type	Correct calls	Incorrect calls	No calls	Agreement with MiSeqDx CF 139 Assay
SNV	672	0	0	100.00% (672/672)
DEL	18	0	0	100.00% (18/18)
DIV	495	0	0	100.00% (495/495)
PolyTG/PolyT	17	1	0	94.44% (17/18)
None (Wild Type)	113,889	0	0	100.00% (113,889/113,889)
Total	115,091	1	0	>99.99% (115,091/115,092)

A single discordant call was observed between TruSight CF139 and MiSeqDx CF139. The specific incorrect call was a PolyTG/PolyT variant. Summary of PolyTG/PolyT concordance is provided in . Since the PolyTG/PolyT genotype is only reported if the R117H variant is also detected, the data set only includes PolyTG/PolyT calls from a single DNA source.

PolyTG / PolyT Variant-calling Performance of TruSight CF 139-Variant Assay compared to MiSeqDx CF 139-Variant Assay

		MiSeqDx CF 139-Variant Assay			
		(TG)12(T)5 / (TG)10(T)9	(TG)12(T)5 / (TG)12(T)5	No call	Total
TruSight CF 139-Variant Assay	(TG)12(T)5 / (TG)10(T)9	17	-	-	17
	(TG)12(T)5 / (TG)12(T)5	1	-	-	1
	No call	-	-	-	-
	Total	18	-	-	18

Performance Characteristics for the Cystic Fibrosis Clinical Sequencing Assay

The performance characteristics for the Cystic Fibrosis Clinical Sequencing Assay are based on studies that utilize the MiSeqDx Cystic Fibrosis 139-Variant Assay. Equivalency between the TruSight and MiSeqDx assays is provided in *Performance Equivalence with Illumina MiSeqDx Cystic Fibrosis Clinical Sequencing Assay*.

Accuracy

Accuracy of the Cystic Fibrosis Clinical Sequencing Assay was assessed by evaluating 500 samples representing a wide variety of CFTR variants from four separate sources. The primary source of accuracy data was a clinical accuracy study conducted using a panel of 366 samples. The majority (n= 355) of samples consisted of archived, anonymized clinical gDNA specimens isolated from human blood. The remaining 11 samples were obtained from commercially available cell line specimens.

Data from this study was supplemented with accuracy data from 68 cell line samples evaluated in the reproducibility study, 14 clinical samples from the extraction method evaluation analytical study, and 52 synthetic plasmid samples. The synthetic plasmids were designed to include the genomic context of rare variants and contained anywhere from 1 to 10 variants within the same construct. They were linearized, diluted to genomic DNA equivalent copy numbers, and blended with human genomic DNA samples of wild type genotype at equivalent copy numbers to mimic a heterozygous sample.

For the Cystic Fibrosis Clinical Sequencing Assay, a total of 5,206 positions were compared to the reference methods of Sanger bidirectional sequencing and PCR testing. The genotyping results for SNV and small InDel sites, including the PolyTG/PolyT region, were compared to Sanger bidirectional sequence analysis.

Two validated PCR based assays were used as the reference method for the two large deletions in the panel. Each duplex PCR assay made use of two primer sets to discriminate between wild type, heterozygous, and homozygous genotypes. One of the primer sets was designed to flank the deletion breakpoints, whereas the other amplified a region internal to the deletion. The two products were detected by size separation on an agarose gel. The PCR assays were validated using a panel of 28 samples in all (22 samples for each deletion) consisting of cell line and blood derived genomic DNA samples, and synthetic plasmids which encompassed the WT, HET, and HOM genotypes for each large deletion. The PCR assays were confirmed to have 100% specificity and reproducibility for all samples tested, by evaluation of PCR products on an agarose gel. The accuracy of the PCR assays was confirmed using Sanger Sequencing and found to be 100% for all samples.

Accuracy was determined for each genotype through three statistical measures. Positive Agreement (PA) was calculated for each variant genotype by dividing the number of samples with agreeing variant calls by the total number of samples with that variant as identified by the reference methods. Negative Agreement (NA) was 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. Overall Agreement (OA) was calculated across all reported positions by dividing the number of concordant WT and variant positions by the total number of reported positions as determined by the reference methods.

The Cystic Fibrosis Clinical Sequencing Assay had a genotype-level PA of 99.66%, including PolyTG/PolyT variants (100% excluding PolyTG/PolyT variants). The NA for all WT positions was > 99.99% and the OA for all reported positions was > 99.99%.

Overall Accuracy for the Cystic Fibrosis Clinical Sequencing Assay

Genotype (Common Name/cDNA Name/Coordinate)	cDNA Name	Variant Type	CFTR Gene Region (hg19)	Positive Calls (Variants)			No Calls*	Miscalls	Positive Agreement
				Clinical Samples	Cell Line Samples	Synthetic Samples			
117120141	c.-8G>C^	SNV	Exon1	25	3	0	0	0	100
117120145	c.-4G>C^	SNV	Exon1	3	2	0	0	0	100
M1V	c.1A>G	SNV	Exon1	0	0	1	0	0	100
CFTR dele2, 3	c.54-5940_ 273+10250 del21kb	Del	Intron1	4	1	0	0	0	100
R31C	c.91C>T	SNV	Exon2	3	1	0	0	0	100
Q39X	c.115C>T	SNV	Exon2	0	0	1	0	0	100
E60X	c.178G>T	SNV	Exon3	6	1	0	0	0	100
P67L	c.200C>T	SNV	Exon3	1	0	1	0	0	100
R74W	c.220C>T	SNV	Exon3	0	2	0	0	0	100
R74Q	c.221G>A	SNV	Exon3	2	0	0	0	0	100
R75X	c.223C>T	SNV	Exon3	3	1	0	0	0	100
R75Q	c.224G>A	SNV	Exon3	20	1	0	0	0	100
G85E	c.254G>A	SNV	Exon3	6	2	0	0	0	100
394delTT	c.262_263 delTT	DIV	Exon3	3	1	0	0	0	100
405+1G>A	c.273+1G>A	SNV	Intron3	0	0	1	0	0	100
406-1G>A	c.274-1G>A	SNV	Exon4	4	0	0	0	0	100
E92K	c.274G>A	SNV	Exon4	0	0	1	0	0	100

Genotype (Common Name/cDNA Name/Coordinate)	cDNA Name	Variant Type	CFTR Gene Region (hg19)	Positive Calls (Variants)			No Calls*	Miscalls	Positive Agreement
				Clinical Samples	Cell Line Samples	Synthetic Samples			
E92X	c.274G>T	SNV	Exon4	0	1	1	0	0	100
Q98X	c.292C>T	SNV	Exon4	0	0	2	0	0	100
444delA	c.312delA	DIV	Exon4	0	2	0	0	0	100
457TAT>G	c.325_327 delTAT insG	DIV	Exon4	0	0	1	0	0	100
D110H	c.328G>C	SNV	Exon4	1	0	1	0	0	100
R117C	c.349C>T	SNV	Exon4	4	0	0	0	0	100
R117H	c.350G>A	SNV	Exon4	17	2	0	0	0	100
Y122X	c.366T>A	SNV	Exon4	0	1	0	0	0	100
F143LfsX10	c.425delT	DIV	Exon4	0	1	0	0	0	100
574delA	c.442delA	DIV	Exon4	0	0	2	0	0	100
Q151K	c.451C>A	SNV	Exon4	1	0	0	0	0	100
621+1G>T	c.489+1G>T	SNV	Intron4	7	5	0	0	0	100
621+3A>G	c.489+3A>G	SNV	Intron4	1	0	0	0	0	100
663delT	c.531delT	DIV	Exon5	1	0	1	0	0	100
G178R	c.532G>A	SNV	Exon5	1	1	0	0	0	100
711+1G>T	c.579+1G>T	SNV	Intron5	3	1	0	0	0	100
711+3A>G	c.579+3A>G	SNV	Intron5	0	0	1	0	0	100

Genotype (Common Name/cDNA Name/Coordinate)	cDNA Name	Variant Type	CFTR Gene Region (hg19)	Positive Calls (Variants)			No Calls*	Miscalls	Positive Agreement
				Clinical Samples	Cell Line Samples	Synthetic Samples			
711+5 G>A	c.579+5G>A	SNV	Intron5	0	0	1	0	0	100
712-1 G>T	c.580-1G>T	SNV	Exon6	0	0	1	0	0	100
H199Y	c.595C>T	SNV	Exon6	0	0	1	0	0	100
P205S	c.613C>T	SNV	Exon6	1	0	1	0	0**	100
L206W	c.617T>G	SNV	Exon6	8	1	0	0	0	100
A209S	c.625G>T	SNV	Exon6	0	1	0	0	0	100
Q220X	c.658C>T	SNV	Exon6	0	0	1	0	0	100
L227R	c.680T>G	SNV	Exon6	0	0	1	0	0	100
852del22	c.720_741 delAGGG AGAATG ATGATG AAGTAC	DIV	Exon6	0	0	1	0	0	100
E279D	c.837A>T	SNV	Exon7	1	0	0	0	0	100
R297Q	c.890G>A	SNV	Exon8	2	0	0	0	0	100
1078delT	c.948delT	DIV	Exon8	1	1	0	0	0	100
L320V	c.958T>G	SNV	Exon8	1	0	0	0	0	100
G330X	c.988G>T	SNV	Exon8	1	1	0	0	0	100
R334W	c.1000C>T	SNV	Exon8	6	1	0	0	0	100

Genotype (Common Name/cDNA Name/Coordinate)	cDNA Name	Variant Type	CFTR Gene Region (hg19)	Positive Calls (Variants)			No Calls*	Miscalls	Positive Agreement
				Clinical Samples	Cell Line Samples	Synthetic Samples			
I336K	c.1007T>A	SNV	Exon8	0	1	0	0	0	100
T338I	c.1013C>T	SNV	Exon8	0	0	1	0	0	100
1154insTC	c.1022_1023insTC	DIV	Exon8	0	1	0	0	0	100
S341P	c.1021T>C	SNV	Exon8	0	0	1	0	0	100
R347H	c.1040G>A	SNV	Exon8	6	1	1	0	0	100
R347P	c.1040G>C	SNV	Exon8	3	2	0	0	0	100
R352Q	c.1055G>A	SNV	Exon8	5	0	0	0	0	100
Q359K/ T360K	c.[1075C>A ;1079C>A]	SNV	Exon8	0	0	1	0	0	100
1213delT	c.1081delT	DIV	Exon8	0	0	1	0	0	100
1248+1G>A	c.1116+1G>A	SNV	Intron8	0	0	1	0	0	100
1259insA	c.1127_1128insA	DIV	Exon9	0	0	2	0	0	100
W401X (c.1202G>A)	c.1202G>A	SNV	Exon9	0	0	1	0	0	100
W401X (c.1203G>A)	c.1203G>A	SNV	Exon9	0	0	1	0	0	100
1341+1G>A	c.1209+1G>A	SNV	Intron9	0	0	2	0	0	100
PolyTGPolyT	Not applicable	PolyTG PolyT	Intron9	369	79	52	3	4[#]	98.60

Genotype (Common Name/cDNA Name/Coordinate)	cDNA Name	Variant Type	CFTR Gene Region (hg19)	Positive Calls (Variants)			No Calls*	Miscalls	Positive Agreement
				Clinical Samples	Cell Line Samples	Synthetic Samples			
1461ins4	c.1329_1330insAGAT	DIV	Exon10	0	0	1	0	0	100
A455E	c.1364C>A	SNV	Exon10	4	2	0	0	0	100
1525-1G>A	c.1393-1G>A	SNV	Exon11	0	0	1	0	0	100
S466X (C>A)	c.1397C>A	SNV	Exon11	0	0	1	0	0	100
S466X (C>G)	c.1397C>G	SNV	Exon11	1	0	1	0	0	100
L467P	c.1400T>C	SNV	Exon11	0	0	1	0	0	100
V470M	c.1408G>A	SNV	Exon11	311	71	0	0	0	100
1548delG	c.1418delG	DIV	Exon11	1	0	1	0	0	100
P477S	c.1429C>T	SNV	Exon11	0	1	0	0	0	100
S485T	c.1454G>C	SNV	Exon11	1	0	0	0	0	100
S489X	c.1466C>A	SNV	Exon11	0	0	2	0	0	100
S492F	c.1475C>T	SNV	Exon11	0	0	1	0	0	100
Q493X	c.1477C>T	SNV	Exon11	4	2	0	0	0	100
I506V	c.1516A>G	SNV	Exon11	7	0	0	0	0	100
I507del	c.1519_1521delATC	DIV	Exon11	4	2	0	0	0	100

Genotype (Common Name/cDNA Name/Coordinate)	cDNA Name	Variant Type	CFTR Gene Region (hg19)	Positive Calls (Variants)			No Calls*	Miscalls	Positive Agreement
				Clinical Samples	Cell Line Samples	Synthetic Samples			
F508del	c.1521_1523 delCTT	DIV	Exon11	84	29	0	0	0	100
I507V	c.1519A>G	SNV	Exon11	0	1	0	0	0	100
F508C	c.1523T>G	SNV	Exon11	1	1	0	0	0	100
1677delTA	c.1545_1546 delTA	DIV	Exon11	1	0	0	0	0	100
V520F	c.1558G>T	SNV	Exon11	2	0	0	0	0	100
Q525X	c.1573C>T	SNV	Exon11	0	0	1	0	0	100
E527E	c.1581A>G	SNV	Exon11	3	2	0	0	0	100
E528E	c.1584G>A	SNV	Exon11	6	2	0	0	0	100
1717-8G>A	c.1585-8G>A	SNV	Intron11	0	0	1	0	0	100
1717-1G>A	c.1585-1G>A	SNV	Exon12	4	1	0	0	0	100
G542X	c.1624G>T	SNV	Exon12	12	3	0	0	0	100
S549R (c.1645A>C)	c.1645A>C	SNV	Exon12	0	0	1	0	0	100
S549N	c.1646G>A	SNV	Exon12	2	2	1	0	0	100
S549R (c.1647T>G)	c.1647T>G	SNV	Exon12	3	1	0	0	0	100
G551D	c.1652G>A	SNV	Exon12	8	3	0	0	0	100
Q552X	c.1654C>T	SNV	Exon12	0	0	1	0	0	100
R553X	c.1657C>T	SNV	Exon12	8	2	0	0	0	100

Genotype (Common Name/cDNA Name/Coordinate)	cDNA Name	Variant Type	CFTR Gene Region (hg19)	Positive Calls (Variants)			No Calls*	Miscalls	Positive Agreement
				Clinical Samples	Cell Line Samples	Synthetic Samples			
I556V	c.1666A>G	SNV	Exon12	1	0	0	0	0	100
L558S	c.1673T>C	SNV	Exon12	0	0	1	0	0	100
A559T	c.1675G>A	SNV	Exon12	4	0	1	0	0	100
R560K	c.1679G>A	SNV	Exon12	0	0	1	0	0	100
R560T	c.1679G>C	SNV	Exon12	6	1	0	0	0	100
1811+1.6 kb A>G	c.1679+1.6 kbA>G	SNV	Intron12	0	0	1	0	0	100
1812-1 G>A	c.1680-1G>A	SNV	Exon13	0	2	0	0	0	100
A561T	c.1681G>A	SNV	Exon13	1	0	0	0	0	100
V562I	c.1684G>A	SNV	Exon13	1	0	0	0	0	100
Y569D	c.1705T>G	SNV	Exon13	0	0	1	0	0	100
P574H	c.1721C>A	SNV	Exon13	0	1	0	0	0	100
G576A	c.1727G>C	SNV	Exon13	4	1	0	0	0	100
D579G	c.1736A>G	SNV	Exon13	0	0	1	0	0	100
E585X	c.1753G>T	SNV	Exon13	0	0	1	0	0	100
1898+1G>A	c.1766+1G>A	SNV	Intron13	2	1	0	0	0	100
1898+3A>G	c.1766+3A>G	SNV	Intron13	0	0	1	0	0	100
H609R	c.1826A>G	SNV	Exon14	0	1	0	0	0	100
D614G	c.1841A>G	SNV	Exon14	0	0	2	0	0	100

Genotype (Common Name/cDNA Name/Coordinate)	cDNA Name	Variant Type	CFTR Gene Region (hg19)	Positive Calls (Variants)			No Calls*	Miscalls	Positive Agreement
				Clinical Samples	Cell Line Samples	Synthetic Samples			
R668C	c.2002C>T	SNV	Exon14	5	2	0	0	0	100
R668H	c.2003G>A	SNV	Exon14	1	0	0	0	0	100
2143delT	c.2012delT	DIV	Exon14	2	1	0	0	0	100
K684TfsX4	c.2046_2047 delAA	DIV	Exon14	0	0	1	0	0	100
2183AA>G	c.2051_2052 delAAinsG	DIV	Exon14	3	1	0	0	0	100
2184delA	c.2052delA	DIV	Exon14	1	1	0	0	0	100
2184insA	c.2052_2053 insA	DIV	Exon14	3	0	1	0	0	100
S686Y	c.2057C>A	SNV	Exon14	0	1	0	0	0	100
R709X	c.2125C>T	SNV	Exon14	1	0	2	0	0	100
K710X	c.2128A>T	SNV	Exon14	3	0	0	0	0	100
E725K	c.2173G>A	SNV	Exon14	2	0	0	0	0	100
2307insA	c.2175_2176 insA	DIV	Exon14	3	0	2	0	0	100
L732X	c.2195T>G	SNV	Exon14	0	0	2	0	0	100
2347delG	c.2215delG	DIV	Exon14	0	0	2	0	0	100
P750L	c.2249C>T	SNV	Exon14	1	0	0	0	0	100
V754M	c.2260G>A	SNV	Exon14	2	1	0	0	0	100
R764X	c.2290C>T	SNV	Exon14	1	0	2	0	0	100

Genotype (Common Name/cDNA Name/Coordinate)	cDNA Name	Variant Type	CFTR Gene Region (hg19)	Positive Calls (Variants)			No Calls*	Miscalls	Positive Agreement
				Clinical Samples	Cell Line Samples	Synthetic Samples			
2585delT	c.2453delT	DIV	Exon14	0	0	2	0	0	100
E822X	c.2464G>T	SNV	Exon14	0	0	2	0	0	100
2622+1G>A	c.2490+1G>T	SNV	Intron14	0	0	2	0	0	100
E831X	c.2491G>T	SNV	Exon15	0	0	1	0	0	100
D836Y	c.2506G>T	SNV	Exon15	0	1	0	0	0	100
W846X	c.2537G>A	SNV	Exon15	0	1	0	0	0	100
R851X	c.2551C>T	SNV	Exon15	0	0	1	0	0	100
T854T	c.2562T>G	SNV	Exon15	212	44	0	0	0	100
2711delT	c.2583delT	DIV	Exon15	0	0	1	0	0	100
V868V	c.2604A>G	SNV	Exon15	2	0	0	0	0	100
c.2657+2_2657+3insA	c.2657+2_2657+3insA	DIV	Intron16	0	0	1	0	0	100
2789+5G>A	c.2657+5G>A	SNV	Intron16	9	1	0	0	0	100
Q890X	c.2668C>T	SNV	Exon17	1	0	0	0	0	100
A923A	c.2769C>T	SNV	Exon17	1	0	0	0	0	100
L927P	c.2780T>C	SNV	Exon17	0	0	1	0	0	100
S945L	c.2834C>T	SNV	Exon17	0	0	1	0	0	100
M952T	c.2855T>C	SNV	Exon17	1	0	0	0	0	100
3007delG	c.2875delG	DIV	Exon17	0	0	1	0	0	100
T966T	c.2898G>A	SNV	Exon17	5	0	0	0	0	100

Genotype (Common Name/cDNA Name/Coordinate)	cDNA Name	Variant Type	CFTR Gene Region (hg19)	Positive Calls (Variants)			No Calls*	Miscalls	Positive Agreement
				Clinical Samples	Cell Line Samples	Synthetic Samples			
G970R	c.2908G>C	SNV	Exon17	0	0	1	0	0	100
S977F	c.2930C>T	SNV	Exon18	0	0	1	0	0	100
3120G>A	c.2988G>A	SNV	Exon18	1	0	0	0	0	100
3120+1G>A	c.2988+1G>A	SNV	Intron18	7	1	0	0	0	100
3121-1G>A	c.2989-1G>A	SNV	Exon19	0	0	1	0	0	100
L997F	c.2991G>C	SNV	Exon19	2	1	0	0	0	100
I1027T	c.3080T>C	SNV	Exon19	1	2	0	0	0	100
3272-26A>G	c.3140-26A>G	SNV	Intron19	0	1	0	0	0	100
F1052V	c.3154T>G	SNV	Exon20	0	1	0	0	0	100
L1065P	c.3194T>C	SNV	Exon20	0	0	1	0	0	100
R1066C	c.3196C>T	SNV	Exon20	6	0	0	0	0	100
R1066H	c.3197G>A	SNV	Exon20	1	0	1	0	0	100
G1069R	c.3205G>A	SNV	Exon20	0	1	0	0	0	100
R1070W	c.3208C>T	SNV	Exon20	0	2	0	0	0	100
R1070Q	c.3209G>A	SNV	Exon20	0	1	0	0	0	100
L1077P	c.3230T>C	SNV	Exon20	0	0	1	0	0¥	100
W1089X	c.3266G>A	SNV	Exon20	4	0	0	0	0	100
Y1092X (C>A)	c.3276C>A	SNV	Exon20	3	1	0	0	0	100

Genotype (Common Name/cDNA Name/Coordinate)	cDNA Name	Variant Type	CFTR Gene Region (hg19)	Positive Calls (Variants)			No Calls*	Miscalls	Positive Agreement
				Clinical Samples	Cell Line Samples	Synthetic Samples			
Y1092X (C>G)	c.3276C>G	SNV	Exon20	0	0	1	0	0	100
T1095T	c.3285A>T	SNV	Exon20	7	0	0	0	0	100
M1101K	c.3302T>A	SNV	Exon20	2	2	0	0	0	100
E1104X	c.3310G>T	SNV	Exon20	0	0	1	0	0	100
c.3368-2A>T	c.3368-2A>T	SNV	Intron20	0	1	0	0	0	100
D1152H	c.3454G>C	SNV	Exon21	10	1	0	0	0	100
V1153E	c.3458T>A	SNV	Exon21	1	0	0	0	0	100
R1158X	c.3472C>T	SNV	Exon22	7	1	0	0	0	100
R1162X	c.3484C>T	SNV	Exon22	5	1	0	0	0	100
R1162L	c.3485G>T	SNV	Exon22	0	2	0	0	0	100
3659delC	c.3528delC	DIV	Exon22	4	1	0	0	0	100
S1196X	c.3587C>G	SNV	Exon22	1	0	0	0	0	100
W1204X (c.3611G>A)	c.3611G>A	SNV	Exon22	0	0	1	0	0	100
W1204X (c.3612G>A)	c.3612G>A	SNV	Exon22	0	0	1	0	0	100
3791delC	c.3659delC	DIV	Exon22	2	0	0	0	0	100
I1234V	c.3700A>G	SNV	Exon22	1	0	1	0	0	100
S1235R	c.3705T>G	SNV	Exon22	9	1	0	0	0	100

Genotype (Common Name/cDNA Name/Coordinate)	cDNA Name	Variant Type	CFTR Gene Region (hg19)	Positive Calls (Variants)			No Calls*	Miscalls	Positive Agreement
				Clinical Samples	Cell Line Samples	Synthetic Samples			
3849+10 kbC>T	c.3717+ 12191C>T	SNV	Intron22	11	2	0	0	0	100
G1244E	c.3731G>A	SNV	Exon23	0	0	1	0	0	100
3876delA	c.3744delA	DIV	Exon23	6	1	0	0	0	100
S1251N	c.3752G>A	SNV	Exon23	1	0	1	0	0	100
3905insT	c.3773_3774 insT	DIV	Exon23	3	1	0	0	0	100
D1270N	c.3808G>A	SNV	Exon23	0	2	0	0	0	100
W1282X	c.3846G>A	SNV	Exon23	9	1	0	0	0	100
P1290P	c.3870A>G	SNV	Exon23	10	3	0	0	0	100
4005+1G>A	c.3873+1G>A	SNV	Intron23	0	0	1	0	0	100
4016insT	c.3884_3885 insT	DIV	Exon24	0	0	1	0	0	100
T1299T	c.3897A>G	SNV	Exon24	3	0	0	0	0	100
N1303K	c.3909C>G	SNV	Exon24	9	1	0	0	0	100
Q1313X	c.3937C>T	SNV	Exon24	0	0	1	0	0	100
G1349D	c.4046G>A	SNV	Exon25	0	1	0	0	0	100
4209TG TT>AA	c.4077_4080 delTGTT insAA	DIV	Exon25	0	0	1	0	0	100

Genotype (Common Name/cDNA Name/Coordinate)	cDNA Name	Variant Type	CFTR Gene Region (hg19)	Positive Calls (Variants)			No Calls*	Miscalls	Positive Agreement
				Clinical Samples	Cell Line Samples	Synthetic Samples			
CFTR dele22,23	c.3964-78_ 4242+577del	Del	Intron24	1	0	1	0	0	100
4382delA	c.4251delA	DIV	Exon27	0	0	1	0	0	100
Y1424Y	c.4272C>T	SNV	Exon27	6	2	0	0	0	100
Q1463Q	c.4389G>A	SNV	Exon27	150	32	0	0	0	100
Total All Variants (PA)†					2072		3	4	99.66
Total All WT (NA)					2600928		1	2§	> 99.99
Total All WT and Variants (OA)					2603000		4	6	> 99.99

DIV is an acronym for Deletion/Insertion Variant.

* Samples were not retested.

^ Software does not report cDNA name for this genomic coordinate.

** The Sanger report listed the P205S variant as heterozygous for the clinical sample. A review of the Sanger trace data however indicated that the variant was in fact homozygous and incorrectly reported. MiSeqDx reported the variant as homozygous.

One of the discordant results was from the reproducibility study. The PolyTG/PolyT result for the sample was concordant across all 18 replicates, but discordant with Sanger bidirectional sequencing.

¥ The original synthetic heterozygous specimen was determined to be improperly prepared. When it was subsequently tested after it was reprepared, using the same plasmid, it was detected.

† PA excluding PolyTG/PolyT calls was 100%.

§ A synthetic sample heterozygous for exon 8 was reported as heterozygous for the variant CFTR dele22, 23. Further investigation revealed that this result was likely from low-level contamination. Also, for a second sample, Sanger primers could not fully detect the variant Q1463Q due to indels both upstream and downstream of the variant site.

PolyTG/PolyT variant accuracy for the Cystic Fibrosis Clinical Sequencing Assay

PolyTGPolyT Genotype	# Clinical Samples	# Cell Line Samples	# Synthetic Samples	# Miscalls	# No Calls*	% Accuracy
(TG)9(T)7/(TG)11(T)7	2	0	0	0	1	50.00
(TG)9(T)9/(TG)10(T)7	1	0	0	0	0	100
(TG)9(T)9/(TG)11(T)7	5	1	0	0	0	100
(TG)9(T)9/(TG)11(T)9	1	0	0	0	0	100
(TG)10(T)7/(TG)10(T)7	25	8	0	0	0	100
(TG)10(T)7/(TG)10(T)9	39	16	0	0	0	100
(TG)10(T)7/(TG)11(T)5	2	0	0	0	0	100
(TG)10(T)7/(TG)11(T)7	72	11	0	0	0	100
(TG)10(T)7/(TG)12(T)5	1	0	0	0	0	100
(TG)10(T)7/(TG)12(T)7	10	1	0	0	1	90.91
(TG)10(T)9/(TG)10(T)9	7	6	0	0	0	100
(TG)10(T)9/(TG)11(T)5	5	0	0	0	0	100
(TG)10(T)9/(TG)11(T)7	76	20	0	0	0	100
(TG)10(T)9/(TG)11(T)9	3	0	0	0	0	100
(TG)10(T)9/(TG)12(T)5	3	2	0	0	0	100
(TG)10(T)9/(TG)12(T)7	13	0	0	0	1	92.31
(TG)11(T)5/(TG)11(T)7	6	0	0	1	0	83.33
(TG)11(T)7/(TG)11(T)7	52	8	0	0	0	100
(TG)11(T)7/(TG)11(T)9^	2	1	0	3	0	0
(TG)11(T)7/(TG)12(T)5	2	0	0	0	0	100
(TG)11(T)7/(TG)12(T)7	37	3	0	0	0	100

PolyTGPolyT Genotype	# Clinical Samples	# Cell Line Samples	# Synthetic Samples	# Miscalls	# No Calls*	% Accuracy
(TG)11(T)9/(TG)12(T)7	3	0	0	0	0	100
(TG)12(T)7/(TG)12(T)7	2	2	0	0	0	100
Total		448		4	3	98.44

* Samples were not retested.

^ One of the discordant results was from the reproducibility study. The PolyTG/PolyT result for the sample was concordant across all 18 replicates, but discordant with Sanger bidirectional sequencing.

Reproducibility

The reproducibility of the Cystic Fibrosis Clinical Sequencing Assay was determined through a blinded study using three trial sites and two operators at each site. Two well-characterized panels of 46 samples each were tested by each of the operators at each site for a total of 276 sample results per operator. The panel contained a mix of genomic DNA from lymphoblastoid cell lines with known mutations in the *CFTR* gene, as well as leukocyte-depleted blood spiked with lymphoblastoid cell lines with known mutations in the *CFTR* gene. The blood samples were provided to allow incorporation of the extraction steps used to prepare gDNA that serves as the primary input for the assay workflow.

The sample pass rate, defined as the number of samples passing QC metrics on the first attempt, was 99.7%. All results are based on initial testing.

The genotype-level PA for all variants including the PolyTG/PolyT variant was 99.22% and excluding the PolyTG/PolyT variant was 99.60%. The NA for all WT was 99.70% and the OA for all reported positions was 99.70%. The PolyTG/PolyT variant PA was 97.83%.

Reproducibility of the Cystic Fibrosis Clinical Sequencing Assay (excluding PolyTG/PolyT variants)

Sample	HGVS Name (or Location if no HGVS)	Variant Name	Total Results		Agreeing Calls			Total* (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls$^\epsilon$	Miscalls	
1	c.1408G>A	V470M	6	18	6	6	6	0	0	100
1	c.1646G>A	S549N	6	18	6	6	6	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Variant Name	Total Results		Agreeing Calls			Total* (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls$^€$	Miscalls	
1	c.2562T>G	T854T	6	18	6	6	6	0	0	100
2	c.1408G>A	V470M	6	18	6	6	6	0	0	100
2	c.1581A>G	E527E	6	18	6	6	6	0	0	100
2	c.1680-1G>A	1812-1 G>A	6	18	6	6	6	0	0	100
2	c.2562T>G	T854T	6	18	6	6	6	0	0	100
2	c.312delA	444delA	6	18	6	6	6	0	0	100
2	c.3870A>G	P1290P	6	18	6	5	6	0	1	94.44
2	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
3	c.1408G>A	V470M	6	18	6	6	6	0	0	100
3	c.1477C>T	Q493X	6	18	6	6	6	0	0	100
3	c.1521_1523delCTT	F508del	6	18	6	6	6	0	0	100
3	c.2562T>G	T854T	6	18	6	6	6	0	0	100
3	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
4	c.1408G>A	V470M	6	18	5	6	6	1	0	94.44
4	c.1521_1523delCTT	F508del	6	18	5	6	6	1	0	94.44
4	c.2052delA	2184delA	6	18	5	6	6	1	0	94.44
5	c.1408G>A	V470M	6	18	6	5	6	1^	0	94.44
5	c.224G>A	R75Q	6	18	6	5	6	1^	0	94.44
5	c.2562T>G	T854T	6	18	6	5	6	1^	0	94.44

Sample	HGVS Name (or Location if no HGVS)	Variant Name	Total Results		Agreeing Calls			Total* (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls$^\epsilon$	Miscalls	
5	c.3472C>T	R1158X	6	18	6	5	6	1^	0	94.44
5	c.366T>A	Y122X	6	18	6	5	6	1^	0	94.44
5	c.625G>T	A209S	6	18	6	5	6	1^	0	94.44
6	c.1408G>A	V470M	6	18	6	6	6	0	0	100
6	c.1521_ 1523delCTT	F508del	6	18	6	6	6	0	0	100
6	c.2051_ 2052delAAinsG	2183AA>G	6	18	6	6	6	0	0	100
7	c.1408G>A	V470M	6	18	6	6	6	0	0	100
7	c.223C>T	R75X	6	18	6	6	6	0	0	100
7	c.2562T>G	T854T	6	18	6	6	6	0	0	100
8	c.1408G>A	V470M	6	18	6	6	6	0	0	100
8	c.1519_ 1521delATC	I507del	6	18	6	6	6	0	0	100
8	c.1521_ 1523delCTT	F508del	6	18	6	6	6	0	0	100
8	c.2562T>G	T854T	6	18	6	6	6	0	0	100
8	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
9	c.1408G>A	V470M	6	18	6	6	6	0	0	100
9	c.1521_ 1523delCTT	F508del	6	18	6	6	6	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Variant Name	Total Results		Agreeing Calls			Total* (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls€	Miscalls	
9	c.2562T>G	T854T	6	18	6	6	6	0	0	100
9	c.3846G>A	W1282X	6	18	6	5	6	0	1*	94.44
9	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
10	c.1408G>A	V470M	6	18	6	6	6	0	0	100
10	c.1521_1523delCTT	F508del	6	18	6	6	6	0	0	100
10	c.2562T>G	T854T	6	18	6	6	6	0	0	100
10	c.3140-26A>G	3272-26A>G	6	18	6	5	6	0	1*	94.44
10	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
11, 39	c.1408G>A	V470M	12	36	12	12	12	0	0	100
11, 39	c.1521_1523delCTT	F508del	12	36	12	12	12	0	0	100
11, 39	c.2002C>T	R668C	12	36	12	12	12	0	0	100
11, 39	c.2562T>G	T854T	12	36	12	12	12	0	0	100
11, 39	c.3717+12191C>T	3849+10kbC>T	12	36	12	12	12	0	0	100
11, 39	c.4389G>A	Q1463Q	12	36	12	12	12	0	0	100
12, 40	c.1408G>A	V470M	12	36	12	12	12	0	0	100
12, 40	c.2562T>G	T854T	12	36	12	12	12	0	0	100
12, 40	c.2988+1G>A	3120+1G>A	12	36	12	12	12	0	0	100
12, 40	c.4389G>A	Q1463Q	12	36	12	12	12	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Variant Name	Total Results		Agreeing Calls			Total* (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls€	Miscalls	
12, 40	c.489+1G>T	621+1G>T	12	36	12	12	12	0	0	100
13	c.1408G>A	V470M	6	18	6	6	6	0	0	100
13	c.1521_ 1523delCTT	F508del	6	18	6	6	6	0	0	100
13	c.178G>T	E60X	6	18	6	6	6	0	0	100
13	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
14	c.1408G>A	V470M	6	18	6	6	6	0	0	100
14	c.1584G>A	E528E	6	18	6	6	6	0	0	100
14	c.2562T>G	T854T	6	18	6	6	6	0	0	100
14	c.3302T>A	M1101K	6	18	6	6	6	0	0	100
15	c.1408G>A	V470M	6	18	6	6	6	0	0	100
15	c.1584G>A	E528E	6	18	6	6	6	0	0	100
15	c.2562T>G	T854T	6	18	6	6	6	0	0	100
15	c.3302T>A	M1101K	6	18	6	6	6	0	0	100
16	c.1408G>A	V470M	6	18	6	6	6	0	0	100
16	c.1521_ 1523delCTT	F508del	6	18	6	6	6	0	0	100
16	c.3080T>C	I1027T	6	18	6	6	6	0	0	100
17, 41	c.1408G>A	V470M	12	36	12	12	12	0	0	100
17, 41	c.1521_ 1523delCTT	F508del	12	36	12	12	12	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Variant Name	Total Results		Agreeing Calls			Total* (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls$^€$	Miscalls	
17, 41	c.3528delC	3659delC	12	36	12	12	12	0	0	100
18, 42	c.-4G>C	117120145	12	36	12	12	12	0	0	100
18, 42	c.1408G>A	V470M	12	36	12	12	12	0	0	100
18, 42	c.1521_1523delCTT	F508del	12	36	12	12	12	0	0	100
18, 42	c.350G>A	R117H	12	36	12	12	12	0	0	100
19	c.1408G>A	V470M	6	18	6	6	6	0	0	100
19	c.489+1G>T	621+1G>T	6	18	6	6	6	0	0	100
19	c.579+1G>T	711+1G>T	6	18	6	6	6	0	0	100
20, 43	c.1408G>A	V470M	12	36	12	12	12	0	0	100
20, 43	c.254G>A	G85E	12	36	12	12	12	0	0	100
20, 43	c.489+1G>T	621+1G>T	12	36	12	12	12	0	0	100
21, 44	c.1364C>A	A455E	12	36	12	12	12	0	0	100
21, 44	c.1408G>A	V470M	12	36	12	12	12	0	0	100
21, 44	c.1521_1523delCTT	F508del	12	36	12	12	12	0	0	100
22	c.1408G>A	V470M	6	18	6	6	6	0	0	100
22	c.1521_1523delCTT	F508del	6	18	6	6	6	0	0	100
22	c.1679G>C	R560T	6	18	6	6	6	0	0	100
22	c.2562T>G	T854T	6	18	6	6	6	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Variant Name	Total Results		Agreeing Calls			Total* (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls€	Miscalls	
22	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
23	c.1408G>A	V470M	6	18	6	6	6	0	0	100
23	c.1521_ 1523delCTT	F508del	6	18	6	6	6	0	0	100
23	c.3276C>A	Y1092X (C>A)	6	18	6	6	6	0	0	100
24, 45	c.1408G>A	V470M	12	36	12	12	12	0	0	100
24, 45	c.3909C>G	N1303K	12	36	12	12	12	0	0	100
24, 45	c.4046G>A	G1349D	12	36	12	12	12	0	0	100
25	c.1408G>A	V470M	6	18	6	6	6	0	0	100
25	c.1624G>T	G542X	6	18	6	6	6	0	0	100
26	c.-8G>C	117120141	6	18	6	6	6	0	0	100
26	c.1408G>A	V470M	6	18	6	6	6	0	0	100
26	c.1624G>T	G542X	6	18	6	6	6	0	0	100
27, 46	c.1408G>A	V470M	12	36	12	12	12	0	0	100
27, 46	c.1652G>A	G551D	12	36	12	12	12	0	0	100
27, 46	c.1657C>T	R553X	12	36	12	12	12	0	0	100
27, 46	c.2562T>G	T854T	12	36	12	12	12	0	0	100
27, 46	c.4389G>A	Q1463Q	12	36	12	12	12	0	0	100
28	c.1408G>A	V470M	6	18	6	6	6	0	0	100
28	c.2562T>G	T854T	6	18	6	6	6	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Variant Name	Total Results		Agreeing Calls			Total* (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls€	Miscalls	
28	c.3717+12191C>T	3849+10kbC>T	6	18	6	6	6	0	0	100
28	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
29	c.1408G>A	V470M	6	18	6	6	6	0	0	100
29	c.2562T>G	T854T	6	18	6	6	6	0	0	100
29	c.91C>T	R31C	6	18	6	6	6	0	0	100
30	c.1408G>A	V470M	6	18	6	6	6	0	0	100
30	c.1521_1523delCTT	F508del	6	18	6	6	6	0	0	100
30	c.2562T>G	T854T	6	18	6	6	6	0	0	100
30	c.3485G>T	R1162L	6	18	6	6	6	0	0	100
30	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
31	c.1408G>A	V470M	6	18	6	6	6	0	0	100
31	c.1585-1G>A	1717-1G>A	6	18	6	6	6	0	0	100
31	c.2562T>G	T854T	6	18	6	6	6	0	0	100
31	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
32	c.1408G>A	V470M	6	18	6	6	6	0	0	100
32	c.2562T>G	T854T	6	18	6	6	6	0	0	100
32	c.3484C>T	R1162X	6	18	6	6	6	0	0	100
32	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
33	c.1040G>C	R347P	6	18	6	6	6	0	0	100

TruSight Cystic Fibrosis Package Insert

Sample	HGVS Name (or Location if no HGVS)	Variant Name	Total Results		Agreeing Calls			Total* (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls€	Miscalls	
33	c.1408G>A	V470M	6	18	6	6	6	0	0	100
33	c.1652G>A	G551D	6	18	6	6	6	0	0	100
33	c.2562T>G	T854T	6	18	6	6	6	0	0	100
33	c.4272C>T	Y1424Y	6	18	6	6	6	0	0	100
33	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
34	c.1000C>T	R334W	6	18	6	6	6	0	0	100
34	c.3368-2A>T	c.3368-2A>T	6	18	6	6	6	0	0	100
35	c.1523T>G	F508C	6	18	6	6	6	0	0	100
36	c.254G>A	G85E	6	18	6	6	6	0	0	100
36	c.3454G>C	D1152H	6	18	6	6	6	0	0	100
37	c.1007T>A	I336K	6	18	6	6	6	0	0	100
37	c.1408G>A	V470M	6	18	6	6	6	0	0	100
37	c.2562T>G	T854T	6	18	6	6	6	0	0	100
37	c.3705T>G	S1235R	6	18	6	6	6	0	0	100
38	c.1408G>A	V470M	6	18	6	6	6	0	0	100
38	c.1727G>C	G576A	6	18	6	6	6	0	0	100
38	c.2002C>T	R668C	6	18	6	6	6	0	0	100
38	c.2057C>A	S686Y	6	18	6	6	6	0	0	100
38	c.2562T>G	T854T	6	18	6	6	6	0	0	100
38	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100

TruSight Cystic Fibrosis Package Insert

Sample	HGVS Name (or Location if no HGVS)	Variant Name	Total Results		Agreeing Calls			Total* (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls[€]	Miscalls	
47, 85	c.1408G>A	V470M	12	36	12	12	12	0	0	100
47, 85	c.2562T>G	T854T	12	36	12	12	12	0	0	100
47, 85	c.2657+5G>A	2789+5G>A	12	36	12	12	12	0	0	100
47, 85	c.4389G>A	Q1463Q	12	36	12	12	12	0	0	100
48, 86	c.54-5940_ 273+10250del21kb	CFTRdele2,3	12	36	12	11	12	1	0	97.22
48, 86	c.1408G>A	V470M	12	36	12	11	12	1	0	97.22
48, 86	c.1521_ 1523delCTT	F508del	12	36	12	11	12	1	0	97.22
49, 87	c.1408G>A	V470M	12	36	12	12	12	0	0	100
49, 87	c.1521_ 1523delCTT	F508del	12	36	12	12	12	0	0	100
49, 87	c.1766+1G>A	1898+1G>A	12	36	12	12	12	0	0	100
50, 88	c.1408G>A	V470M	12	36	12	12	12	0	0	100
50, 88	c.220C>T	R74W	12	36	12	12	12	0	0	100
50, 88	c.2562T>G	T854T	12	36	12	12	12	0	0	100
50, 88	c.3808G>A	D1270N	12	36	12	12	12	0	0	100
51, 89	c.1408G>A	V470M	12	36	12	12	12	0	0	100
51, 89	c.1521_ 1523delCTT	F508del	12	36	12	12	12	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Variant Name	Total Results		Agreeing Calls			Total* (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls$^{€}$	Miscalls	
51, 89	c.2012delT	2143delT	12	36	12	12	12	0	0	100
52	c.3744delA	3876delA	6	18	6	6	6	0	0	100
53, 90	c.3773_3774insT	3905insT	12	36	12	12	12	0	0	100
54, 91	c.1408G>A	V470M	12	36	12	12	12	0	0	100
54, 91	c.262_263delTT	394delTT	12	36	12	12	12	0	0	100
55, 92	c.1408G>A	V470M	12	36	12	12	12	0	0	100
55, 92	c.1519A>G	I507V	12	36	12	12	12	0	0	100
55, 92	c.1521_ 1523delCTT	F508del	12	36	12	12	12	0	0	100
55, 92	c.2562T>G	T854T	12	36	12	12	12	0	0	100
55, 92	c.3080T>C	I1027T	12	36	12	12	12	0	0	100
55, 92	c.4389G>A	Q1463Q	12	36	12	12	12	0	0	100
56	c.1408G>A	V470M	6	18	6	6	6	0	0	100
56	c.2562T>G	T854T	6	18	6	6	6	0	0	100
56	c.3154T>G	F1052V	6	18	6	6	6	0	0	100
56	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
57	c.-8G>C	117120141	6	18	6	6	6	0	0	100
57	c.1408G>A	V470M	6	18	6	6	6	0	0	100
57	c.2562T>G	T854T	6	18	6	6	6	0	0	100
57	c.3209G>A	R1070Q	6	18	6	6	6	0	0	100

TruSight Cystic Fibrosis Package Insert

| Sample | HGVS Name (or Location if no HGVS) | Variant Name | Total Results | | Agreeing Calls | | | Total* (All Sites) | | % Agreement |
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls$^{€}$	Miscalls	
58	c.1408G>A	V470M	6	18	6	6	6	0	0	100
58	c.1521_ 1523delCTT	F508del	6	18	6	6	6	0	0	100
58	c.2991G>C	L997F	6	18	6	6	6	0	0	100
59	c.1408G>A	V470M	6	18	6	6	6	0	0	100
59	c.2562T>G	T854T	6	18	6	6	6	0	0	100
59	c.3205G>A	G1069R	6	18	6	6	6	0	0	100
60	c.1408G>A	V470M	6	18	6	6	6	0	0	100
60	c.2562T>G	T854T	6	18	6	6	6	0	0	100
60	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
60	c.617T>G	L206W	6	18	6	6	6	0	0	100
61	c.1408G>A	V470M	6	18	6	6	6	0	0	100
61	c.2260G>A	V754M	6	18	6	6	6	0	0	100
61	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
62	c.1408G>A	V470M	6	18	6	6	6	0	0	100
62	c.2562T>G	T854T	6	18	6	6	6	0	0	100
62	c.988G>T	G330X	6	18	6	6	6	0	0	100
64	c.1040G>A	R347H	6	18	6	6	6	0	0	100
64	c.1408G>A	V470M	6	18	6	6	6	0	0	100
64	c.2562T>G	T854T	6	18	6	6	6	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Variant Name	Total Results		Agreeing Calls			Total* (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls$^\epsilon$	Miscalls	
64	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
65	c.948delT	1078delT	6	18	6	6	6	0	0	100
66	c.1408G>A	V470M	6	18	6	6	6	0	0	100
66	c.1521_1523delCTT	F508del	6	18	6	6	6	0	0	100
66	c.532G>A	G178R	6	18	6	6	6	0	0	100
67	c.1408G>A	V470M	6	18	6	6	6	0	0	100
67	c.1647T>G	S549R (c.1647T>G)	6	18	6	6	6	0	0	100
68	c.1408G>A	V470M	6	18	6	6	6	0	0	100
68	c.1646G>A	S549N	6	18	6	6	6	0	0	100
68	c.2562T>G	T854T	6	18	6	6	6	0	0	100
68	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
69	c.2506G>T	D836Y	6	18	6	6	6	0	0	100
69	c.2537G>A	W846X	6	18	6	6	6	0	0	100
70	c.1408G>A	V470M	6	18	6	6	6	0	0	100
70	c.2562T>G	T854T	6	18	6	6	6	0	0	100
70	c.3485G>T	R1162L	6	18	6	6	6	0	0	100
70	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
71	c.1408G>A	V470M	6	18	6	6	6	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Variant Name	Total Results		Agreeing Calls			Total* (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls$^€$	Miscalls	
71	c.1521_ 1523delCTT	F508del	6	18	6	6	6	0	0	100
71	c.2562T>G	T854T	6	18	6	6	6	0	0	100
71	c.274G>T	E92X	6	18	6	6	6	0	0	100
71	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
72	c.1022_1023insTC	1154insTC	6	18	6	6	5	1	0	94.44
72	c.1408G>A	V470M	6	18	6	6	5	1	0	94.44
72	c.2562T>G	T854T	6	18	6	6	5	1	0	94.44
72	c.4389G>A	Q1463Q	6	18	6	6	5	1	0	94.44
72	c.489+1G>T	621+1G>T	6	18	6	6	5	1	0	94.44
73	c.1408G>A	V470M	6	18	6	6	6	0	0	100
73	c.1624G>T	G542X	6	18	6	6	6	0	0	100
73	c.1826A>G	H609R	6	18	6	6	6	0	0	100
74	c.1408G>A	V470M	6	18	6	6	5	0	1	94.44
74	c.1429C>T	P477S	6	18	6	6	6	0	0	100
74	c.1521_ 1523delCTT	F508del	6	18	6	6	6	0	0	100
75	c.1408G>A	V470M	6	18	6	5	6	1^	0	94.44
75	c.1521_ 1523delCTT	F508del	6	18	6	5	6	1^	0	94.44
75	c.1721C>A	P574H	6	18	6	5	6	1^	0	94.44

Sample	HGVS Name (or Location if no HGVS)	Variant Name	Total Results		Agreeing Calls			Total* (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls$^{€}$	Miscalls	
76	c.1408G>A	V470M	6	18	6	6	6	0	0	100
76	c.1521_1523delCTT	F508del	6	18	6	6	6	0	0	100
76	c.2562T>G	T854T	6	18	6	6	6	0	0	100
76	c.425delT	F143LfsX10	6	18	6	6	6	0	0	100
76	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
77	c.1364C>A	A455E	6	18	6	6	6	0	0	100
77	c.1408G>A	V470M	6	18	6	6	6	0	0	100
77	c.489+1G>T	621+1G>T	6	18	6	6	6	0	0	100
78	c.1408G>A	V470M	6	18	6	6	6	0	0	100
78	c.1581A>G	E527E	6	18	6	6	6	0	0	100
78	c.1680-1G>A	1812-1 G>A	6	18	6	6	6	0	0	100
78	c.2562T>G	T854T	6	18	6	6	6	0	0	100
78	c.312delA	444delA	6	18	6	6	6	0	0	100
78	c.3870A>G	P1290P	6	18	6	6	6	0	0	100
78	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
79	c.1408G>A	V470M	6	18	6	6	6	0	0	100
79	c.220C>T	R74W	6	18	6	6	6	0	0	100
79	c.2562T>G	T854T	6	18	6	6	6	0	0	100
79	c.3808G>A	D1270N	6	18	6	6	6	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Variant Name	Total Results		Agreeing Calls			Total* (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls$^{€}$	Miscalls	
80	c.-8G>C	117120141	6	18	6	6	6	0	0	100
80	c.1408G>A	V470M	6	18	6	6	6	0	0	100
80	c.1521_1523delCTT	F508del	6	18	6	6	6	0	0	100
80	c.1657C>T	R553X	6	18	6	6	6	0	0	100
80	c.2562T>G	T854T	6	18	6	6	6	0	0	100
81	c.1408G>A	V470M	6	18	6	6	6	0	0	100
81	c.1521_1523delCTT	F508del	6	18	6	6	6	0	0	100
81	c.1652G>A	G551D	6	18	6	6	6	0	0	100
81	c.2562T>G	T854T	6	18	6	6	6	0	0	100
81	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
82	c.1040G>C	R347P	6	18	6	6	6	0	0	100
82	c.1408G>A	V470M	6	18	6	6	6	0	0	100
82	c.1521_1523delCTT	F508del	6	18	6	6	6	0	0	100
82	c.4272C>T	Y1424Y	6	18	6	6	6	0	0	100
83	c.-4G>C	11720145	6	18	6	6	6	0	0	100
83	c.1408G>A	V470M	6	18	6	6	6	0	0	100
83	c.1521_1523delCTT	F508del	6	18	6	6	6	0	0	100

Sample	HGVS Name (or Location if no HGVS)	Variant Name	Total Results		Agreeing Calls			Total* (All Sites)		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls$^€$	Miscalls	
83	c.350G>A	R117H	6	18	6	6	6	0	0	100
84	c.1408G>A	V470M	6	18	6	6	6	0	0	100
84	c.1519_1521delATC	I507del	6	18	6	6	6	0	0	100
84	c.2562T>G	T854T	6	18	6	6	6	0	0	100
84	c.4389G>A	Q1463Q	6	18	6	6	6	0	0	100
Total All Variants (PA)** (including PolyTG/PolyT data in)			2580	7740	2562	2553	2565	37	23	99.22
Total All WT (NA)			2871132	8613396	2865930	2855526	2865932	26006	2	99.70
Total All WT and variants (OA)			2873712	8621136	2868492	2858079	2868497	26043	25	99.70

$^€$ Samples were not retested.

^ One replicate each of samples 5 and 75 had a 0% call rate. Further investigation indicated that the samples had likely not been added to the sample plate before library preparation.

* Upon review, samples 9 and 10 were likely switched by the operator before library preparation.

** Excluding PolyTG/PolyT variants, the PA was 99.60%.

PolyTG/PolyT Reproducibility for the Cystic Fibrosis Clinical Sequencing Assay

Panel	Sample	Genotype	# of Results		Agreeing Calls			Total All Sites		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls	Miscalls	
A	1	(TG)12(T)7/(TG)12(T)7	6	18	6	6	6	0	0	100%
A	2	(TG)10(T)9/(TG)10(T)7	6	18	6	6	6	0	0	100%

Panel	Sample	Genotype	# of Results		Agreeing Calls			Total All Sites		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls	Miscalls	
A	3	(TG)10(T)7/(TG)10(T)9	6	18	6	6	6	0	0	100%
A	4	(TG)10(T)9/(TG)11(T)7	6	18	5	6	6	1	0	94.44%
A	5	(TG)10(T)7/(TG)11(T)7	6	18	6	5	6	1	0	94.44%
A	6	(TG)10(T)9/(TG)10(T)7	6	18	6	6	6	0	0	100%
A	7	(TG)10(T)9/(TG)11(T)7	6	18	6	6	6	0	0	100%
A	8	(TG)10(T)7/(TG)10(T)9	6	18	6	6	6	0	0	100%
A	9	(TG)10(T)9/(TG)10(T)7	6	18	6	6	6	0	0	100%
A	10	(TG)10(T)9/(TG)10(T)7	6	18	6	6	6	0	0	100%
A	11, 39	(TG)10(T)9/(TG)10(T)7	12	36	12	12	12	0	0	100%
A	12, 40	(TG)10(T)9/(TG)11(T)7	12	36	12	12	12	0	0	100%
A	13	(TG)10(T)9/(TG)11(T)7	6	18	6	6	6	0	0	100%
A	14	(TG)10(T)7/(TG)11(T)7	6	18	6	6	6	0	0	100%
A	15	(TG)10(T)7/(TG)11(T)7	6	18	6	5	6	1	0	94.44%
A	16	(TG)10(T)9/(TG)10(T)9	6	18	6	6	6	0	0	100%
A	17, 41	(TG)10(T)9/(TG)11(T)7	12	36	12	12	12	0	0	100%
A	18, 42	(TG)10(T)9/(TG)12(T)5	12	36	12	12	12	0	0	100%
A	19	(TG)10(T)9/(TG)11(T)7	6	18	6	6	6	0	0	100%
A	20, 43	(TG)10(T)9/(TG)11(T)7	12	36	12	12	12	0	0	100%
A	21, 44	(TG)10(T)9/(TG)10(T)9	12	36	12	12	12	0	0	100%
A	22	(TG)10(T)9/(TG)10(T)7	6	18	6	6	6	0	0	100%

Panel	Sample	Genotype	# of Results		Agreeing Calls			Total All Sites		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls	Miscalls	
A	23	(TG)10(T)9/(TG)11(T)7	6	18	6	6	6	0	0	100%
A	24, 45	(TG)10(T)9/(TG)11(T)7	12	36	12	12	12	0	0	100%
A	25	(TG)10(T)9/(TG)10(T)9	6	18	6	6	6	0	0	100%
A	26	(TG)10(T)9/(TG)11(T)7	6	18	6	6	6	0	0	100%
A	27, 46	(TG)10(T)7/(TG)11(T)7	12	36	11	12	12	0	1	97.22%
A	28	(TG)10(T)7/(TG)10(T)7	6	18	6	6	6	0	0	100%
A	29	(TG)10(T)7/(TG)12(T)7	6	18	6	4	4	4	0	77.78%
A	30	(TG)10(T)9/(TG)10(T)7	6	18	6	6	6	0	0	100%
A	31	(TG)10(T)7/(TG)11(T)7	6	18	6	6	6	0	0	100%
A	32	(TG)10(T)7/(TG)10(T)7	6	18	6	6	6	0	0	100%
A	33	(TG)10(T)7/(TG)11(T)7	6	18	5	6	6	1	0	94.44%
A	34	(TG)11(T)7/(TG)12(T)7	6	18	6	6	6	0	0	100%
A	35	(TG)11(T)7/(TG)11(T)7	6	18	6	6	6	0	0	100%
A	36	(TG)11(T)7/(TG)11(T)7	6	18	6	6	6	0	0	100%
A	37	(TG)11(T)7/(TG)12(T)7	6	18	6	6	6	0	0	100%
A	38	(TG)10(T)7/(TG)11(T)7	6	18	6	6	6	0	0	100%
B	47, 85	(TG)10(T)7/(TG)10(T)7	12	36	12	12	12	0	0	100%
B	48, 86	(TG)10(T)9/(TG)11(T)7	12	36	11	11	12	2	0	94.44%
B	49, 87	(TG)10(T)9/(TG)11(T)7	12	36	12	12	12	0	0	100%
B	50, 88	(TG)10(T)9/(TG)11(T)7	12	36	12	12	12	0	0	100%

Panel	Sample	Genotype	# of Results		Agreeing Calls			Total All Sites		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls	Miscalls	
B	51, 89	(TG)10(T)9/(TG)10(T)9	12	36	12	12	12	0	0	100%
B	52	(TG)11(T)7/(TG)11(T)7	6	18	6	6	6	0	0	100%
B	53, 90	(TG)11(T)7/(TG)11(T)7	12	36	12	12	12	0	0	100%
B	54, 91	(TG)10(T)9/(TG)11(T)7	12	36	12	12	12	0	0	100%
B	55, 92	(TG)10(T)9/(TG)10(T)7	12	36	12	12	12	0	0	100%
B	56	(TG)10(T)7/(TG)10(T)9	6	18	6	6	6	0	0	100%
B	57	(TG)12(T)7/(TG)12(T)7	6	18	6	6	6	0	0	100%
B	58	(TG)10(T)9/(TG)10(T)9	6	18	6	6	6	0	0	100%
B	59	(TG)11(T)7/(TG)12(T)7	6	18	5	6	6	1	0	94.44%
B	60	(TG)9(T)9/(TG)11(T)7	6	18	6	6	6	0	0	100%
B	61	(TG)10(T)9/(TG)11(T)7	6	18	6	6	6	0	0	100%
B	62	(TG)10(T)7/(TG)11(T)7	6	18	5	6	6	1	0	94.44%
B	63	(TG)11(T)7/(TG)11(T)7	6	18	6	6	6	0	0	100%
B	64	(TG)10(T)7/(TG)11(T)7	6	18	5	6	6	1	0	94.44%
B	65	(TG)11(T)7/(TG)11(T)7	6	18	6	6	6	0	0	100%
B	66	(TG)10(T)9/(TG)11(T)7	6	18	6	6	6	0	0	100%
B	67	(TG)11(T)7/(TG)11(T)7	6	18	6	6	6	0	0	100%
B	68	(TG)10(T)7/(TG)11(T)7	6	18	6	6	6	0	0	100%
B	69	(TG)11(T)7/(TG)11(T)7	6	18	6	6	6	0	0	100%
B	70	(TG)10(T)7/(TG)10(T)7	6	18	6	6	6	0	0	100%

Panel	Sample	Genotype	# of Results		Agreeing Calls			Total All Sites		% Agreement
			Per Site	All Sites	Site 1	Site 2	Site 3	No Calls	Miscalls	
B	71	(TG)10(T)9/(TG)11(T)7	6	18	6	6	6	0	0	100%
B	72	(TG)10(T)7/(TG)10(T)9	6	18	5	6	5	2	0	88.89%
B	73	(TG)10(T)9/(TG)11(T)7	6	18	6	6	6	0	0	100%
B	74	(TG)10(T)9/(TG)11(T)7	6	18	6	6	6	0	0	100%
B	75	(TG)10(T)7/(TG)10(T)9	6	18	6	5	6	1	0	94.44%
B	76	(TG)10(T)7/(TG)10(T)9	6	18	6	6	6	0	0	100%
B	77	(TG)10(T)9/(TG)10(T)9	6	18	6	6	6	0	0	100%
B	78	(TG)10(T)7/(TG)10(T)9	6	18	5	6	6	1	0	94.44%
B	79	(TG)10(T)7/(TG)11(T)7	6	18	6	6	6	0	0	100%
B	80	(TG)11(T)7/(TG)11(T)9	6	18	0	0	0	0	18*	0%
B	81	(TG)10(T)7/(TG)10(T)9	6	18	6	6	6	0	0	100%
B	82	(TG)10(T)9/(TG)11(T)7	6	18	6	6	6	0	0	100%
B	83	(TG)10(T)9/(TG)12(T)5	6	18	6	6	6	0	0	100%
B	84	(TG)10(T)7/(TG)10(T)7	6	18	6	6	6	0	0	100%
Total PolyTG/PolyT Variants (PA)			552	1656	537	540	543	17	19	97.83%

* All 18 samples were concordant with each other but discordant with Sanger bidirectional sequencing.

DNA Extraction

Three commonly used, commercially available extraction methods representing magnetic bead extraction, alcohol precipitation and silica filter column isolation methods, were evaluated using K2EDTA anti-coagulated whole blood. A total of 14 blood samples were used during the study; two were wild type, while the remaining samples carried unique genotypes representing nine different variants, including both common and rare variants. For the polyTG/polyT variation, samples with (T)5-9 and (TG)10-12 were included. The three DNA extraction methods were tested independently by two different operators who each performed three runs per extraction method. Each extraction was performed by each operator on different days. The DNA concentration and A260/A280 ratio of the extracted gDNA samples was determined using spectrophotometry. The total sample size for each extraction method in this study was 168 (14 samples x 2 operators/extraction method x 3 runs/operator x 2 replicates/extracted gDNA sample).

Extraction Method	Number of Samples Tested	Call Rate	Accuracy	Sample First Pass Rate*
Alcohol Precipitation	168	> 99.99%	> 99.99%	100%
Silica Filter Column Isolation	168	> 99.99%	> 99.99%	100%
Magnetic Bead Extraction	168	> 99.99%	> 99.99%	100%

* Percent of samples having call rate of > 99% in first run.

DNA Input

The DNA input range of the Cystic Fibrosis Clinical Sequencing Assay was evaluated by performing a serial dilution study using 14 representative DNA samples containing 16 unique CF variants.

Each sample was tested in duplicate at nine DNA input levels ranging from 1250 - 1 ng (1250 ng, 500 ng, 250 ng, 100 ng, 50 ng, 25 ng, 10 ng, 5 ng, and 1 ng). For determination of accuracy, sample genotypes were compared to bidirectional Sanger sequencing data and the deletions were compared to PCR assay. 1250 ng and 25 ng were identified as the upper and lower bound for DNA input respectively as they had ≥ 95% sample first pass rate with no incorrect calls (100% accuracy and call rate).

DNA inputs of 1250 ng, 250 ng, and 100 ng were further tested with four representative DNA samples and at least 20 replicates per DNA input level for each sample (n=4x20=80 samples), while the lower bound of 25 ng was tested with 14 samples and 20 replicates for each sample (n=14x20=280 samples). The accuracy and sample first pass rate was 100% at all DNA input levels.

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Interfering Substances

To assess the impact of interfering substances on the Illumina MiSeqDx Cystic Fibrosis System, the performance of the assay was evaluated in the presence and absence of potential interferents. Sixteen whole blood specimens with unique CF genotypes were tested in the study. Four endogenous interfering substances (bilirubin, cholesterol, hemoglobin, and triglycerides) were tested by spiking them into blood specimens prior to DNA extraction. The concentration limits for each substance is shown in the following table. Additionally, to assess interference resulting from blood collection (short draw), EDTA was spiked into blood samples, and to assess interference resulting from sample preparation, the final wash buffer from a silica filter column isolation method was added to purified genomic DNA.

The Cystic Fibrosis Clinical Sequencing Assay achieved 100% call rate for all samples tested, and 100% reproducibility in genotype calls between samples in the presence and absence of interfering substances. No interference was observed from any of the endogenous or exogenous interferents.

To assess the impact of multiplexing index primer interference, a cross contamination study using two samples, each with unique homozygous genotypes at four different genomic positions, and two respective index primers was performed. No change in variant calling was observed with contamination levels < 40%. The sample genotype became heterozygous when contamination levels were ≥ 40%.

Test Substance	Total Number of Replicates	Concentration Tested in Blood (Upper Limit)	Concentration Tested in Blood (Lower Limit)	Call Rate
Bilirubin	16	684 µmol/L	137 µmol/L	100%
Cholesterol	16	13 mmol/L	2.6 mmol/L	100%
Hemoglobin	16	2 g/L	0.4 g/L	100%
Triglyceride	16	37 mmol/L	7.4 mmol/L	100%
EDTA	16	7.0 mg/mL	2.8 mg/mL	100%

Performance Equivalence with Illumina MiSeqDx Cystic Fibrosis Clinical Sequencing Assay

The TruSight Cystic Fibrosis Clinical Sequencing Assay (TruSight CFCS) uses the same library preparation workflow and reagents as the Illumina MiSeqDx Cystic Fibrosis Assay (MiSeqDx CFCS). TruSight CFCS uses the MiSeqDx Reagent Kit v3. The MiSeqDx CFCS uses sequencing reagents included with the Assay. To demonstrate equivalency between TruSight CFCS and MiSeqDx CFCS, results from nine TruSight CFCS runs were compared to a single MiSeqDx CFCS run as the gold standard. The TruSight CFCS runs were conducted at 96-sample throughput (maximum sample throughput for TruSight CFCS). The MiSeqDx CFCS run was conducted at 48-sample throughput (maximum sample throughput for MiSeqDx CFCS). Sources of variability included in TruSight CFCS runs included three library preparation events (each with a unique lot of TruSight Cystic Fibrosis), three operators, three MiSeqDx instruments, and three lots of MiSeqDx Reagent Kit v3.

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TruSight Cystic Fibrosis Package Insert

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Variant calls from TruSight CFCS runs were compared to calls made by the MiSeqDx CFCS run. 47 unique samples were included in each TruSight CFCS run, with 2-3 replicates per sample (95 DNA samples and 1 NTC per run). For the MiSeqDx CFCS run, the same 47 samples were sequenced as singleton (47 DNA samples + 1 NTC per run). The sample panel was comprised of Coriell DNA samples extracted from immortalized cell lines, and included samples representing every allele of the ACMG 23 mutations.[1] The panel included deletion-insertion variants (including insertion/deletions in homopolymeric regions and insertion-with-deletion in the same region). The panel also included homozygous variants, compound heterozygous variants, and one of the targeted large deletions. It also included PolyTG/PolyT variants, single nucleotide variants, and a sample with no variants detected. Summary of results by genotype is provided in . Agreement between assays by variant type is presented in . Overall (Total) agreement between assays was > 99.99%.

Variant-calling Performance of TruSight CFCS-Variant Assay compared to MiSeqDx CFCS-Variant Assay

		MiSeqDx CF Clinical Assay				
		Hom Variant	Het Variant	Wild Type	No Call	Total
TruSight CF Clinical Assay	Hom Variant	551	-	-	-	551
	Het Variant	-	2,664	-	-	2,664
	Wild Type	-	-	4,426,182	-	4,426,182
	No Call	-	-	58	-	58
	Total	551	2,664	4,426,420	-	4,429,455

Performance by Variant Type of TruSight CF Clinical Sequencing Assay compared to MiSeqDx CF Clinical Sequencing Assay

Variant Type	Correct calls	Incorrect calls	No calls	Agreement with MiSeqDx CF Clinical Sequencing Assay
SNV	2,684	0	0	100.00% (2,684/2,684)
DEL	18	0	0	100.00% (18/18)
DIV	513	0	0	100.00% (513/513)
PolyTG/PolyT	847	1	3	99.88% (847/851)
None (Wild Type)	4,426,182	0	58	100.00% (4,426,182/4,426,240)
Total	4,430,244	1	61	>99.99% (4,430,244/4,430,306)

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A single discordant call was observed between TruSight CFCS and MiSeqDx CFCS. The specific incorrect call was a PolyTG/PolyT variant. Summary of PolyTG/PolyT concordance is provided in .

PolyTG / PolyT Variant-calling Performance of TruSight CF Clinical Sequencing Assay compared to MiSeqDx CF Clinical Sequencing Assay

	MiSeqDx CF Clinical Assay Table												
	(TG)10 (T)7/ (TG)10 (T)7	(TG)10 (T)7/ (TG)10 (T)9	(TG)10 (T)7/ (TG)12 (T)5	(TG)10 (T)7/ (TG)12 (T)7	(TG)10 (T)9/ (TG)10 (T)9	(TG)10 (T)9/ (TG)12 (T)5	(TG)11 (T)7/ (TG)10 (T)7	(TG)11 (T)7/ (TG)10 (T)9	(TG)11 (T)7/ (TG)11 (T)7	(TG)11 (T)7/ (TG)12 (T)7	(TG)12 (T)5/ (TG)12 (T)5	No Call	Total

														Total
					MiSeqDx CF Clinical Assay Table									
TruSight CF Clinical Assay	(TG)10(T)7/ (TG)10(T)7	50	-	-	-	-	-	-	-	-	-	-	-	50
	(TG)10(T)7/ (TG)10(T)9	-	189	-	-	-	-	-	-	-	-	-	-	189
	(TG)10(T)7/ (TG)12(T)5	-	-	18	-	-	-	-	-	-	-	-	-	18
	(TG)10(T)7/ (TG)12(T)7	-	-	-	18	-	-	-	-	-	-	-	-	18
	(TG)10(T)9/ (TG)10(T)9	-	-	-	-	72	-	-	-	-	-	-	-	72
	(TG)10(T)9/ (TG)12(T)5	-	-	-	-	-	17	-	-	-	-	-	-	17
	(TG)11(T)7/ (TG)10(T)7	-	-	-	-	-	-	126	-	-	-	-	-	126
	(TG)11(T)7/ (TG)10(T)9	-	-	-	-	-	-	-	249	-	-	-	-	249
	(TG)11(T)7/ (TG)11(T)7	-	-	-	-	-	-	-	-	72	-	-	-	72
	(TG)11(T)7/ (TG)12(T)7	-	-	-	-	-	-	-	-	-	36	-	-	36
	(TG)12(T)5/ (TG)12(T)5	-	-	-	-	-	1	-	-	-	-	-	-	1
	No Call	-	-	-	-	-	-	-	3	-	-	-	-	3
	Total	50	189	18	18	72	18	126	252	72	36	-	-	851

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Revision History

Document	Date	Description of Change
Document # 1000000097720 v04	October 2023	• Updated MiSeqDx Reagent Kit v3 catalog number. • Added kit catalog numbers to Reagents section. • Caution symbol removed from Notes and Warnings. • Clarified the use of MiSeqDx v3 Reagent Kit vs MiSeqDx v3 Reagent Kit Micro throughout. • Clarified Caution statement under Removal of Unbound Oligonucleotides section to ensure the centrifuge is appropriately cooled before each use. • Removed trademark statement in favor of URL where specific patents may be searched. • Updated CE mark with notified body number and EC|REP address with Importer symbol.
Document # 1000000097720 v03	May 2022	• Updated content throughout document to account for the MiSeqDx Reagent Kit v3 Micro (Catalog # 20063860) and its workflow. • Created Sample Preparation subsection within Procedural Notes section and moved its DNA extraction and quantitation information to this subsection. • Added Warning and Precautions statement for reporting serious incidents related to this product to Illumina and the Competent Authority of the Member State. • Added Summary of Safety and Performance statement with Basic UDI-DI to Product Labeling section. • Corrected placement of ™ icon in product overview. • Updated note, caution, and warning icons. • Completed Revision History table.
Document # 1000000097720 v02	August 2021	Updated EU Authorized Representative address.
Document # 1000000097720 v01	June 2020	Added new part number 20037124 for the MiSeqDx Reagent Kit v3.

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Document	Date	Description of Change
Document # 1000000097720 v00	March 2020	Initial release.

Patents and Trademarks

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Product Labeling

For a complete reference to symbols that may appear on product packaging and labeling, refer to the symbol key for your kit at support.illumina.com.

A Summary of Safety and Performance (SSP) is located at https://ec.europa.eu/tools/eudamed, after launch of the European Database on Medical Devices (Eudamed). It is linked to the Basic UDI-DI (0081627002CYSTFIB8C).