TruSight™ Cystic Fibrosis

An FDA-cleared *in vitro* diagnostic next-generation sequencing solution

- A single workflow enables users to run two assays for cystic fibrosis testing

- An ancestrally diverse variant panel combined with full *CFTR* sequencing provides comprehensive coverage

- Efficient library prep provides highly flexible through-put of 24-96 samples per run

- Highly accurate, easily interpreted results presented in a clear, concise report

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Introduction

Cystic fibrosis (CF) affects approximately 70,000 children and adults worldwide.[1] The disease appears when an individual inherits two disease causing variants in trans of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. Millions of people carry a single mutated gene and do not exhibit any symptoms. Many of these people, known as carriers, are unaware of their mutation and their risk for passing the mutation to their children. In the United States alone, the carrier frequency is estimated to be 3% of the population.[2]

CF affects a diverse population, with the highest recognized incidence observed in European populations.[3] Early diagnosis and treatment of CF can improve both survival and quality of life.[4] However, current CF testing methods focus on *CFTR* variants most commonly found in individuals of European ancestry, potentially missing CF causative variants in other demographics that may have clinical relevance. As a result, families may endure long periods of additional genetic testing and patients may experience a delay in receiving needed treatment.

To address these challenges, Illumina offered the MiSeq™Dx Cystic Fibrosis 139-Variant Assay and the MiSeqDx Cystic Fibrosis Clinical Sequencing Assay. These assays were the first Food and Drug Administration (FDA)-cleared next-generation sequencing (NGS)-based in vitro diagnostic (IVD) tests for cystic fibrosis. These preexisting assays have been consolidated into a single NGS solution for cystic fibrosis testing: TruSight Cystic Fibrosis (Figure 1).

TruSight Cystic Fibrosis consolidates existing CF assays

TruSight Cystic Fibrosis combines the MiSeqDx Cystic Fibrosis 139-Variant Assay and MiSeqDx Cystic Fibrosis Clinical Sequencing Assay into a single kit to increase versatility and sample throughput with updated sequencing reagents while maintaining the same workflow, product specifications, and performance as the original assays. As part of the integration into TruSight Cystic Fibrosis, the assay names have been changed to the TruSight Cystic Fibrosis 139-Variant Assay and the TruSight Cystic Fibrosis Clinical Sequencing Assay.

Figure 1: TruSight Cystic Fibrosis—TruSight Cystic Fibrosis combines preexisting assays into a single kit to increase versatility and sample throughput.

Integrated workflow

TruSight Cystic Fibrosis integrates both assays into a single CF testing workflow (Figure 2). Customers choose which assay to perform at the beginning of the test by selecting the appropriate analysis module in Local Run Manager (LRM). Users then prepare sample libraries, load them on to the MiSeqDx instrument for sequencing, and analyze the data with the appropriate software.

To help determine which assay may be best suited to testing objectives, users should consider the following:

- **TruSight Cystic Fibrosis 139-Variant Assay—**accurately detects 139 clinically relevant *CFTR* variants[5] (Table 1).
- **TruSight Cystic Fibrosis Clinical Sequencing Assay—**sequences all protein coding regions and intron/exon boundaries (Figure 3), providing a comprehensive view of the *CFTR* gene.

For more information, see the Intended Use Statements or read the package insert.

Optimized kit configuration

TruSight Cystic Fibrosis maintains the same workflow, same product specifications, and same data and performance quality as the MiSeqDx Cystic Fibrosis 139-Variant and MiSeqDx Cystic Fibrosis Clinical Sequencing Assays. TruSight Cystic Fibrosis offers a modular configuration with library prep reagents and sequencing reagents sold separately to provide greater flexibility with component ordering. Also, TruSight Cystic Fibrosis uses improved MiSeqDx v3 sequencing by synthesis (SBS) chemistry,

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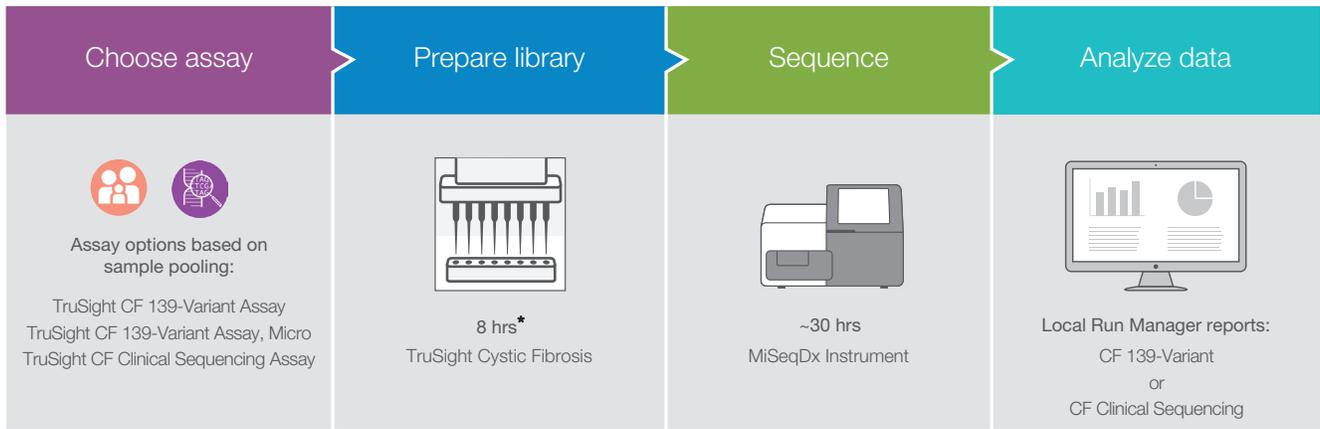
Figure 2: TruSight Cystic Fibrosis workflow—TruSight Cystic Fibrosis offers a streamlined, integrated workflow that includes library prep, sequencing, and data analysis and reporting for the TruSight Cystic Fibrosis 139-Variant and Clinical Sequencing Assays.
*Library prep time depends on sample throughput and may vary.

resulting in higher cluster densities and read lengths, as well as providing higher sample throughputs. TruSight Cystic Fibrosis enables use of the same library prep kit four times to process 24 samples per run, or one time to process 96 samples in a single run. Additional flexibility is provided by two sequencing kit configurations: the standard MiSeqDx v3 kit (24-96 samples per flow cell) and the lower priced MiSeqDx v3 Micro kit (24-36 samples per flow cell). All reagents are packaged in a ready-to-use format, minimizing hands-on time and increasing uniformity in all tests.
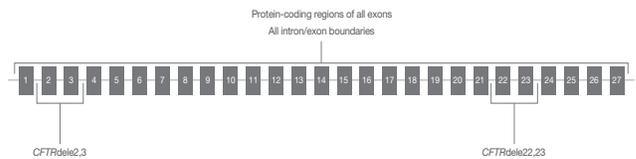

Figure 3: *CFTR* regions sequenced with the TruSight Cystic Fibrosis Clinical Sequencing Assay—*CFTR* regions sequenced by the assay include protein coding regions across all exons, intron/exon boundaries, ~100 nt of flanking sequence at the 5' and 3' UTRs, two deep intronic mutations (1811+1.6kbA>G, 3489+10kbC>T), two large deletions (CFTRdele2,3, CFTRdele22,23) and the PolyTG/PolyT region.

Efficient library prep increases throughput

Library preparation begins with 250 ng genomic DNA (gDNA) isolated from a blood sample. The DNA is mixed with an oligonucleotide pool of probes. Each probe includes sequence designed to capture the designated variant and an adapter sequence used in a subsequent amplification reaction. The probes hybridize to the DNA, one upstream and one downstream of specific *CFTR* variants (Figure 4). A proprietary extension–ligation reaction extends across the region of interest, followed by ligation, to unite the two probes. This reaction creates a template strand, giving the assay excellent specificity (Table 2).

Table 1: TruSight Cystic Fibrosis 139-Variant Assay offers a panel of clinically relevant *CFTR* variants

Mutations in the ACMG-23 list for CF screening		
R347P	1717-1G>A	3849+10kbC>T
G85E	G542X	W1282X
R117H	G551D	711+1G>T
621+1G>T	R553X	R560T
R334W	2184delA	1898+1G>A
A455E	2789+5G>A	N1303K
I507del	3120+1G>A	R1162X
F508del	3659delC	

Only a subset of variants included in the assay are listed. To view the full list of variants in the TruSight Cystic Fibrosis 139-Variant Assay, visit www.illumina.com/TruSightCysticFibrosis.
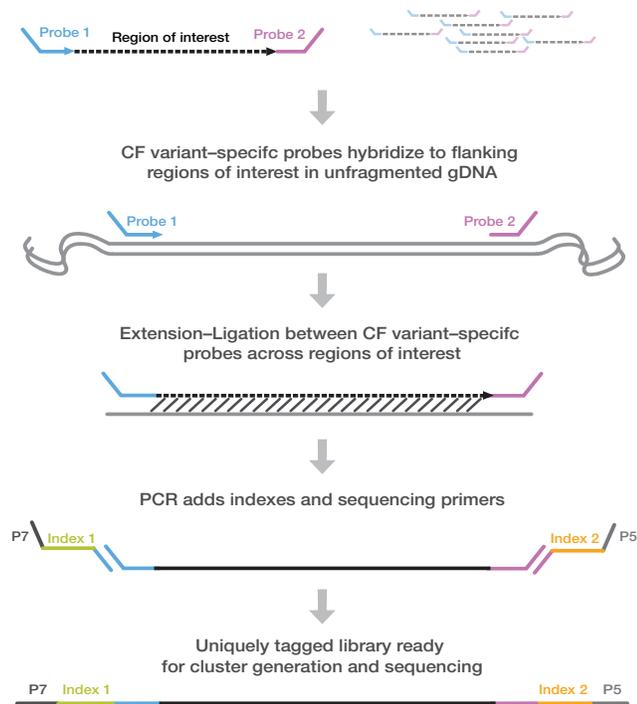
Figure 4: TruSight Cystic Fibrosis chemistry—TruSight Cystic Fibrosis enables multiplexing of up to 96 samples in a single run. Both the TruSight Cystic Fibrosis 139-Variant Assay and the TruSight Cystic Fibrosis Clinical Sequencing Assay use the same chemistry to produce high-quality sequencing libraries.

To increase the number of samples analyzed in a single sequencing run, individual libraries are "tagged" with a unique identifier, or index. These unique sample-specific indexes are added to each extension–ligation template in a PCR amplification step. The final reaction product contains the *CFTR* variants with the necessary sequencing adapters and indexes for sequencing on the MiSeqDx instrument. An automated four-read sequencing strategy identifies each tagged sample for individual downstream analysis. Using this approach, sample identification is highly accurate, maintaining the high integrity of the assay. For TruSight Cystic Fibrosis, 24–96 samples can be pooled and run using a standard MiSeqDx v3 kit for either the TruSight Cystic Fibrosis 139-Variant Assay or the Clinical Sequencing Assay in a single sequencing run. Alternatively, a smaller batch size of 24-36 samples can be run using the MiSeqDx v3 Micro kit for either assay along with the respective Micro assay module in Local Run Manager.

Highly accurate, easily interpreted results

Results from TruSight Cystic Fibrosis are presented in an easy-to-read fashion that a board-certified molecular geneticist or equivalent can readily interpret. Both assay reports include assay name, sampleID, variant identification, genotypes, and call rate for each sample (≥ 99% of positions must be called for a sample to be considered valid). The TruSight Cystic Fibrosis Clinical Sequencing Assay also provides variant type, allelic frequency, genomic coordinate, and sequencing depth for each identified variant. In addition to reports generated by Local Run Manager software, users have access to raw data files for convenient storage.

Table 2: TruSight Cystic Fibrosis performance

TruSight Cystic Fibrosis 139-Variant Assay			
Characteristic	PA[a]	NA[b]	OA[c]
Accuracy	100%	> 99.99%	> 99.99%
Reproducibility	99.77%	99.88%	99.88%
TruSight Cystic Fibrosis Clinical Sequencing Assay			
Characteristic	PA[a]	NA[b]	OA[c]
Accuracy	99.66%	> 99.99%	> 99.99%
Reproducibility	99.22%	99.70%	99.70%

a. Positive Agreement (PA) is the number of samples with agreeing variant calls divided by the total number of samples with that variant as identified by the reference method.
b. Negative Agreement (NA) calculated across all wild-type (WT) positions by dividing the number of concordant WT positions by the total number of WT positions as defined by the reference methods.
c. Overall Agreement (OA) calculated across all reported positions by dividing the number of concordant wild-type and variant positions by the total number of reported positions as determined by the reference methods.

Summary

TruSight Cystic Fibrosis offers a new kit configuration that combines the TruSight Cystic Fibrosis 139-Variant Assay and the TruSight Cystic Fibrosis Clinical Sequencing Assay (formerly MiSeqDx CF Assays) into a single solution. TruSight Cystic Fibrosis maintains the same workflow, same product specifications, and same data and performance quality as the MiSeqDx Cystic Fibrosis 139-Variant and Clinical Sequencing Assays. The TruSight Cystic Fibrosis 139-Variant Assay combines an expanded panel of 139 variants with advanced NGS technology. The assay provides accurate results for an ancestrally diverse population and improves detection of couples at risk of having an affected child. For deeper insights into CF, the TruSight Cystic Fibrosis Clinical Sequencing Assay enables sequencing of the *CFTR* gene to provide a comprehensive genetic view not available using standard molecular genotyping panels. The additional data eliminates demographic bias and enables accurate detection of two large deletions, two deep intronic mutations, and indels in homopolymeric regions. TruSight Cystic Fibrosis provides an integrated solution for CF testing that empowers clinicians to screen for known variants before interrogating the *CFTR* gene for novel mutations.

Learn more

To learn more about TruSight Cystic Fibrosis, visit illumina.com/TruSightCysticFibrosis

Ordering information

Product	Catalog no.
TruSight Cystic Fibrosis Library Prep	20036925
MiSeqDx instrument	DX-410-1001
MiSeqDx Reagent Kit v3	20037124
MiSeqDx Reagent Kit v3, Micro	20063860

Flexible NGS platform for IVD testing

In addition, a growing portfolio of FDA-cleared and FDA-approved IVD assays are available for use on the MiSeqDx instrument, in addition to TruSight Cystic Fibrosis:

- TruSeq™ Custom Amplicon Kit Dx—A validated, FDA-regulated and CE-IVD–marked kit enabling clinical laboratories to design custom NGS assays.

- Praxis™ Extended RAS Panel—The first FDA-approved NGS in vitro diagnostic for evaluating RAS mutations in colorectal cancer to determine patient eligibility for treatment with Vectibix.

References

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Intended use statements

TruSight Cystic Fibrosis 139-Variant Assay Intended Use

The Illumina TruSight Cystic Fibrosis 139-Variant Assay is a qualitative in vitro diagnostic system used to detect 139 clinically relevant cystic fibrosis disease-causing mutations and variants of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene simultaneously 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)[6] and in 2011 by the American College of Obstetricians and Gynecologists (ACOG).[7] 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.

TruSight Cystic Fibrosis Clinical Sequencing Assay Intended Use

The Illumina TruSight 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 genomic DNA isolated from human peripheral whole blood specimens collected in K2EDTA. The test detects single nucleotide variants and small indels within the region sequenced, and additionally reports on two deep intronic mutations and two large deletions. The test is intended to be used on the Illumina MiSeqDx instrument.

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

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