TruSight™ Cystic Fibrosis

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. CF affects a diverse population, with the highest recognized incidence observed in European populations.[2,3] 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 patients of European ancestry, potentially missing CF causative variants in other demographics that may have clinical relevance.

To address this challenge, 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.

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General information

What are the differences between the MiSeqDx Cystic Fibrosis and TruSight Cystic Fibrosis products?

MiSeqDx Cystic Fibrosis consists of two separate FDA-cleared, CE-marked IVD CF assay testing kits: the MiSeqDx Cystic Fibrosis 139-Variant Assay (Catalog no. DX-102-1004) and the MiSeqDx Cystic Fibrosis Clinical Sequencing Assay (Catalog no. DX-102-1001). These products were launched in 2013 and use the MiSeqDx Sequencing System with the MiSeqDx Reagent Kit v1. Both assays use a coupled kit solution, providing library prep and sequencing reagents.

The MiSeqDx Cystic Fibrosis assays have since been replaced with TruSight Cystic Fibrosis (Catalog no. 20036925). TruSight Cystic Fibrosis supports both the TruSight Cystic Fibrosis 139-Variant Assay and the TruSight Cystic Fibrosis Clinical Sequencing Assay options with a single library prep product. TruSight Cystic Fibrosis requires the use of the MiSeqDx Sequencing System and either the MiSeqDx Reagent Kit v3 (Catalog no. 20037124) or MiSeqDx Reagent Kit v3 Micro (Catalog no. 20063860). MiSeqDx sequencing reagents are not provided with the library prep kit and must be purchased separately.

For more detailed information, refer to the TruSight Cystic Fibrosis Package Insert (Document no. 1000000097720) and TruSight Cystic Fibrosis data sheet.

Are there any regional restrictions in obtaining TruSight Cystic Fibrosis?

As of March 2022, TruSight Cystic Fibrosis is available for purchase in the United States, most European countries, Australia, New Zealand, and Canada. Starting in June 2022, the MiSeqDx Reagent Kit v3, Micro will be available for purchase in the United States and most European countries. Additional regions will be added as regulatory approvals are obtained.

What are the differences between the TruSight Cystic Fibrosis 139-Variant Assay and TruSight Cystic Fibrosis Clinical Sequencing Assay?

The TruSight Cystic Fibrosis 139-Variant Assay is a qualitative *in vitro* diagnostic assay that detects 139 clinically relevant CF-causing mutations and variants in the *CFTR* gene simultaneously in genomic DNA isolated from human peripheral whole blood specimens.

The TruSight Cystic Fibrosis Clinical Sequencing Assay is a targeted sequencing *in vitro* diagnostic assay that resequences the protein coding region and intron/exon boundaries of the *CFTR* gene in genomic DNA isolated from human peripheral whole blood.

For details on the intended use of each assay, see the complete Intended Use statements on page 7 of this document.

Sample and library preparation

What is the required sample type?

DNA extracted from whole blood is the required sample type.

What sample extraction method can be used?

Any validated sample extraction method may be used.

How do you quantify the DNA?

A spectrophotometer is recommended for quantifying extracted DNA. A fluorescent DNA intercalating agent can be used for quantification, but is not necessary.

How long can samples be stored and under what conditions?

Whole blood specimens can be stored no longer than the following:

- 7 days at room temperature
- 30 days at 2°C to 8°C
- 30 days at -25°C to -15°C

Can dried blood spots be used as input?

No, the assay is not validated for use with dried blood spots. Dried blood spots contain less than the recommended amount of DNA and are likely to result in lower call rates than whole blood.

What is the recommended DNA input? Is the input amount recommended or required?

The required sample input is 250 ng DNA extracted from whole blood. DNA inputs ranging from 25 ng to 1250 ng have been used to produce accurate results.

Can you automate TruSight Cystic Fibrosis library preparation?

No, library preparation is only offered in a manual format.

Are the target probes used in the TruSight Cystic Fibrosis assay the same as the target probes used in the MiSeqDx Cystic Fibrosis kits?

Yes, the probes are identical for the TruSight Cystic Fibrosis and MiSeqDx Cystic Fibrosis kits.

Can a single library prep be used for the TruSight Cystic Fibrosis 139-Variant Assay and TruSight Cystic Fibrosis Clinical Sequencing Assay?

Yes. Prepared libraries can be stored as diluted amplicon libraries (DALs) for up to 28 days at -25°C to -15°C after preparation. There is no difference between libraries prepared for the TruSight Cystic Fibrosis 139-Variant Assay and TruSight Cystic Fibrosis Clinical Sequencing Assay.

Can I run fewer than 24 samples?

At least 24 libraries must be prepared and pooled for sequencing. Fewer than 24 samples has not been validated by Illumina. Users may prepare a sample sheet that does not include some of those samples if reports are not desired for all libraries sequenced.

How long can I store the pooled library (ie, DAL)?

DALs can be stored for up to 28 days at -25°C to -15°C.

What consumables and equipment do I need to run TruSight Cystic Fibrosis?

Required materials and equipment are listed in the TruSight Cystic Fibrosis Package Insert (Document no. 1000000097720). Important equipment:

- Passively cooled heat block
- Refrigerated centrifuge

Sequencing

What sequencing system is TruSight Cystic Fibrosis designed for? Can it be used with the standard MiSeq System?

TruSight Cystic Fibrosis has been designed for use with the MiSeqDx Sequencing System and MiSeqDx v3 sequencing reagents. It is not compatible for use with the standard research use only MiSeq System.

How many samples per flow cell can I run for a TruSight Cystic Fibrosis 139-Variant Assay or TruSight Cystic Fibrosis Clinical Sequencing Assay? Is there sufficient coverage on a MiSeqDx v3 flow cell to have a throughput of 96 samples?

Both TruSight Cystic Fibrosis assays have been designed to run with the MiSeqDx Reagent Kit v3 and MiSeqDx Reagent Kit v3, Micro. The standard MiSeqDx Reagent Kit v3 has been validated to support sequencing of 24-96 samples for each assay per sequencing run. The MiSeqDx Reagent Kit v3, Micro supports batches of 24-36 samples. TruSight Cystic Fibrosis has been validated to support a minimum of 24 samples per flow cell run for both kit configurations.

Can I do reflex testing from the TruSight Cystic Fibrosis 139-Variant Assay to the TruSight Cystic Fibrosis Clinical Sequencing Assay?

Yes, the TruSight Cystic Fibrosis Clinical Sequencing Assay can be used when a patient has atypical or nonclassical presentation of Cystic Fibrosis or when other mutation panels, such as the TruSight Cystic Fibrosis 139-Variant Assay, have failed to identify both causative mutations.

How do you perform reflex testing using an existing library pool? Can I have a report that provides fewer than 24 samples?

If you plan to do reflex testing, we recommend preparing 3-5 DALs at the end of the library prep. After the TruSight Cystic Fibrosis 139-Variant Assay sequencing run is complete, prepare a new sample sheet for a TruSight Cystic Fibrosis Clinical Sequencing Assay run that only includes the samples for which you want results. Use the DAL that contains all of the libraries of interest with the TruSight Cystic Fibrosis Clinical Sequencing Assay run; only the samples entered on the sample sheet will be reported.

How do I troubleshoot sample failures?

Leftover PCR cleanup product from the clean-up plate (CLP) can be run on a 2-4% agarose gel to confirm the success of PCR. If no band is detected for a given sample, library preparation for that sample should be repeated. For more information, refer to the TruSight Cystic Fibrosis Package Insert (Document no. 1000000097720).

Which MiSeqDx reagents do I need to order for use with TruSight Cystic Fibrosis?

The MiSeqDx Reagent Kit v3 (Catalog no. 20037124) or the MiSeqDx Reagent Kit v3, Micro (Catalog no. 20063860) is required for sequencing TruSight Cystic Fibrosis libraries.

Can I use the TruSight Cystic Fibrosis library prep kit (Catalog no. 20036925) with MiSeq v3 RUO reagents?

No. The instructions for TruSight Cystic Fibrosis library preparation require use of MiSeqDx v3 sequencing reagents.

Do I need to run a minimum number of samples for index diversity? What restrictions are there regarding index combinations?

At least 24 libraries must be prepared and pooled for sequencing but a subset can be selected for requeue analysis. Each sample must have a unique index combination but there are no restrictions on which index combinations are used.

What is the typical expected cluster density range for a successful run? Is there a required cluster density?

Sequencing with MiSeqDx Reagent Kit v3 chemistry provides robust tolerance of a wide range of cluster densities without sequencing failure. Approximately 800-1500 K/mm^2 is a typical range of cluster density for TruSight Cystic Fibrosis assays.

What software is needed to run TruSight Cystic Fibrosis?

All software required to run TruSight Cystic Fibrosis, including Local Run Manager, is preinstalled on the MiSeqDx instrument (for instruments installed after January 2019). The module used in a sequencing run will depend on the assay being performed and the sequencing kit being used. The specific analysis modules to be installed on an instrument is based on customer needs and will be installed on an as-needed basis. For instruments purchased prior to January 2019, you may need to work with your local services teams to obtain the appropriate upgrades for TruSight Cystic Fibrosis compatibility.

Data analysis

Where is TruSight Cystic Fibrosis data analysis performed?

Data analysis for the TruSight Cystic Fibrosis Assays is conducted on-instrument.

Can data be stored externally? Where is the data from a run stored?

Data for each sequencing run is stored on the MiSeqDx instrument at MiSeqAnalysis\<Run Folder Name>. Data can be stored externally by changing the default output folder within the MiSeq Operating System User Interface. For instructions on how to set default output folder location, refer to the MiSeqDx Instrument Reference Guide for MOS v2 (Document no. 1000000021961).

What raw data files are included for long-term storage?

BCL, FASTQ, and VCF files are included for long-term storage.

Where can I find the raw sequencing data from a run (ie, FASTQ or VCF files)?

When a sequencing run is complete, there will be an output folder containing all of the sequencing files. The path for this can be found in the "Sequencing Information" tab of Local Run Manager. In that path, there is an "Alignment_#" folder that contains a zipped file. After unzipping the file, the VCF and BAM files will be available. FASTQ files are available on the local drive (on the MiSeqDx computer). The default path for this is D:\Illumina\MiSeqAnalysis\{RunID}\Data\Intensities\BaseCalls.

What are the differences in data storage requirements between MiSeqDx Cystic Fibrosis and TruSight Cystic Fibrosis?

If storing files per sample (ie, VCF or FASTQ files), there is not much difference per sample. If storing whole run files, files from TruSight Cystic Fibrosis sequencing runs with v3 reagents can be up to 2× larger size than files from the MiSeqDx Cystic Fibrosis Assay. Run Folder sizes can be minimized by not saving image files.

Troubleshooting

Why is a template line wash recommended after the completion of each sequencing run? What impact will there be if I forget to wash the line?

Template line washes are essential to limit run-to-run carryover. If a wash is not conducted, elevated call rates are possible in the negative control sample.

If my run fails (ie, a run suitability failure), do I need to do a new library prep? Can I use my existing library prep?

If a sequencing run failure can be attributed to an instrument issue, existing libraries can be resequenced. If the negative control call rate is > 10% and a template line wash was not conducted in the previous run, it is recommended that the operator complete a post-run wash with a template line wash and repeat the sequencing run. Other failures, such as contamination during library preparation causing a negative control call rate that is > 10% or incorrect variant calls in positive or wild-type controls, will require new library preparation.

What is the typical concentration of a library? What is the typical size of my final library?

Due to bead normalization, measurement of pooled library concentrations is not needed. Cleaned-up PCR products can be used for troubleshooting efforts in the event of sample failures by performing gel electrophoresis for each sample of interest with a 2-4% TBE agarose gel and 100-bp ladder to confirm presence of an 300-400 bp library product. If needed to correct any pooling errors, the storage plate (SGP) can be used up to three days later for repooling and resequencing.

Intended use

TruSight Cystic Fibrosis 139-Variant Assay

The Illumina TruSight 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 samples. The variants include those recommended in 2004 by the American College of Medical Genetics (ACMG)[5] and in 2011 by the American College of Obstetricians and Gynecologists (ACOG).[6]

The test is intended for carrier screening in adults of reproductive age, in confirmatory diagnostic testing of newborns and children, and as an initial test to aid in the diagnosis of individuals with suspected cystic fibrosis. The results of this test are intended to be interpreted by a board-certified clinical molecular geneticist or equivalent and should be used in conjunction with other available laboratory and clinical information. This test is not indicated for use for newborn screening, fetal diagnostic testing, pre-implantation testing, or for stand-alone diagnostic purposes.

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

TruSight Cystic Fibrosis Clinical Sequencing Assay

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

The test is intended to be used as an aid in the diagnosis of individuals with suspected cystic fibrosis (CF). This assay is most appropriate when the patient has an atypical or non-classic presentation of CF or when other mutation panels have failed to identify both causative mutations. The results of 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 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.

For *In Vitro* Diagnostic Use.

Learn more

TruSight Cystic Fibrosis, illumina.com/TruSightCysticFibrosis

References

1. Cystic Fibrosis Foundation. www.cff.org/What-is-CF/About-Cystic-Fibrosis/. Accessed March 1, 2022.
2. Strom CM, Crossley B, Buller-Buerkle A, et al. Cystic fibrosis testing 8 years on: lessons learned from carrier screening and sequencing analysis. *Genet Med*. 2011;13(2):166–172. doi:10.1097/GIM.0b013e3181fa24c4
3. Mirtajani SB, Farnia P, Hassanzad M, et al. Geographical distribution of cystic fibrosis; the past 70 years of data analysis. *Biomed and Biotech Res J*. 2017;1(2):105–112. doi:10.4103/bbrj.bbrj_81_17
4. Rock MJ, Levy H, Zaleski C, Farrell PM. Factors accounting for a missed diagnosis after newborn screening. *Pediatr Pulmonol*. 2011;46(12):1166–1174. doi:10.1002/ppul.21509.
5. Watson MS, Cutting GR, Desnick RJ, et al. Cystic fibrosis population carrier screening: 2004 revision of American College of Medical Genetics mutation panel. *Genet Med*. 2004;6(5):387-391. doi:10.1097/01.gim.0000139506.11694.7c
6. ACOG Committee Opinion No. 486: Update on carrier screening for cystic fibrosis. *Obstet Gynecol*. 2011;117(4):1028-1031. doi:10.1097/AOG.0b013e31821922c2

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