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Confirming cystic fibrosis diagnosis in newborns using TruSight™ Cystic Fibrosis

Comprehensive profiling of cystic fibrosis (CF) variants with an *in vitro* diagnostic next-generation sequencing (NGS) solution.

Summary

- **Confirmatory diagnostic for CF in newborns**
  FDA-cleared NGS-based *in vitro* diagnostic (IVD) test for cystic fibrosis
- **Two consolidated assays in a single workflow**
  Comprehensive profiling of 139 common CF variants or all 27 exons and relevant intronic regions of the *CFTR* gene
- **Fast and easy sequencing and analysis workflow**
  Integrated with the MiSeq™Dx instrument for onboard data analysis and simplified reporting

Introduction

Cystic fibrosis is an autosomal recessive hereditary disease that affects approximately 70,000 children and adults worldwide.[1] CF affects a diverse population around the world, with the highest recognized incidence observed in individuals of European descent, with a rate of ~1 in 3000 individuals (Figure 1). Millions of people carry a single mutated gene and do not exhibit any symptoms.[2] Many of these people, known as carriers, are unaware of their mutation and their risk for passing it to their children. As lifelong treatment is required, early diagnosis is critical to improve both survival and quality of life.[3]

CF treatment

There is no cure for CF; however, there are multiple treatment options, with most aimed at symptoms of CF.[4] Exciting developments in the treatment of CF include medications that augment function of CFTR by targeting specific mutations in the gene sequence. While these molecular therapeutics can be highly effective, they are only helpful if the patient has the specific mutation, highlighting the importance of CF testing and variant identification. The comprehensive profiling offered with TruSight Cystic Fibrosis enables detection of variants associated with current and future pharmaceutical treatments.

CF screening and detection

CF screening has become a standard of care for newborn screening, and begins with a biochemical blood test for levels of immunoreactive trypsinogen (IRT) (Figure 2). IRT is normally found at small levels in the body, but can be elevated in individuals with CF. However, IRT levels can be elevated for other reasons, and confirmatory testing is required.

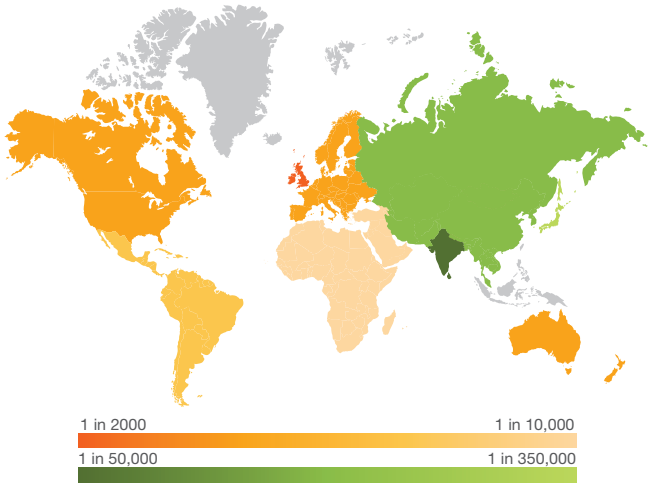

Figure 1: Cystic fibrosis is a global disease—The highest incidence of CF is observed in Europe and countries of European descent.

Genetic testing can be carried out as part of a newborn confirmatory diagnostic testing procedure to identify *CFTR* mutations in the affected individual (Figure 2). Studies have indicated that classification of CF patients according to genotype may aid in predicting clinical outcome of disease.[5] Conventional genetic tests for CF target known mutations and *CFTR* rearrangements by PCR-based methods.[6] Typically, these tests only screen for the most common variants, leaving the potential for missed detection.[7] TruSight Cystic Fibrosis provides the ability to profile 139 common CF variants, including 23 variants recommended by the American College of Medical Genetics (ACMG). Further variant analysis can be carried out by full *CFTR* gene sequencing with the TruSight Cystic Fibrosis Clinical Sequencing Assay (Figure 2). Incorporating TruSight Cystic Fibrosis with existing IRT testing methods provides a confirmatory diagnostic and enables comprehensive analysis of causal variants associated with CF.

TruSight Cystic Fibrosis

TruSight Cystic Fibrosis is a Food and Drug Administration (FDA)–cleared NGS-based IVD test for cystic fibrosis. It integrates the following assays into a single CF testing workflow:

- **TruSight Cystic Fibrosis 139-Variant Assay**—accurately detects 139 *CFTR* variants[8] with known clinical relevance (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.

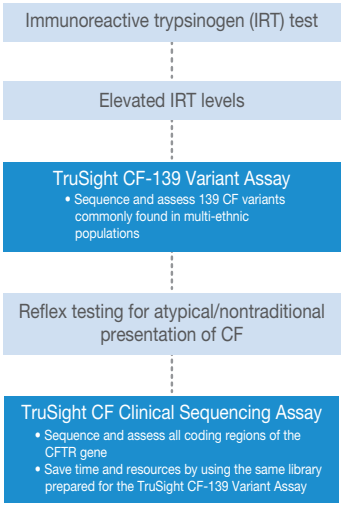

Figure 2: Newborn CF testing—Newborn screening for CF begins with a test for IRT levels in blood. Confirmatory testing involves a chloride sweat test and/or genetic tests for mutational analysis. The TruSight Cystic Fibrosis 139-Variant Assay is ideal for initial testing with an extensive panel of 139 *CFTR* variants. Subsequent variant analysis can be carried out by full *CFTR* sequencing with the TruSight Cystic Fibrosis Clinical Sequencing Assay.

TruSight CF workflow

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 and load them on to the MiSeqDx instrument for sequencing (Figure 4). Data are analyzed with the appropriate software automatically when the run is complete (Figure 5). TruSight Cystic Fibrosis enables reflex testing by providing an assay configuration whereby the same libraries prepared for one assay can be used for the other, saving time and resources. For more information, see the Intended Use Statements or read the package insert.

Table 1: TruSight Cystic Fibrosis 139-Variant Assay assesses 139 CF variants, including 23 variants recommended by ACMG

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.

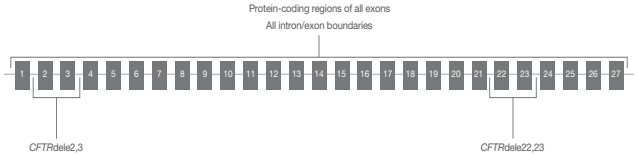

Figure 3: *CFTR* regions sequenced with the TruSight Cystic Fibrosis Clinical Sequencing Assay—*CFTR* regions sequenced by the assay include protein-coding regions across all exons, intron/exon boundaries, ~100 nt of flanking sequence at the 5' and 3' UTRs, two deep intronic mutations (1811+1.6kbA>G,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

Sequencing on the MiSeqDx Instrument

The MiSeqDx instrument is the first FDA-regulated IVD NGS system (Figure 4). Designed specifically for the clinical laboratory environment, the MiSeqDx instrument offers a small footprint (0.3 square meters), an easy-to-use workflow, and data output tailored to the diverse needs of clinical labs. Taking advantage of proven Illumina sequencing by synthesis (SBS) chemistry, the MiSeqDx instrument provides accurate, reliable screening and diagnostic testing.

Figure 4: MiSeqDx Instrument—The FDA-regulated MiSeqDx instrument offers a simple workflow, a user-friendly software interface, and highly accurate data.

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 139-Variant Assay provides the common name for mutations, in addition to the genomic location. The TruSight Cystic Fibrosis Clinical Sequencing Assay also provides variant type, allelic frequency, genomic coordinate, and sequencing depth for each identified variant (Figure 6). In addition to reports generated by Local Run Manager software, users have access to raw data files for convenient storage.

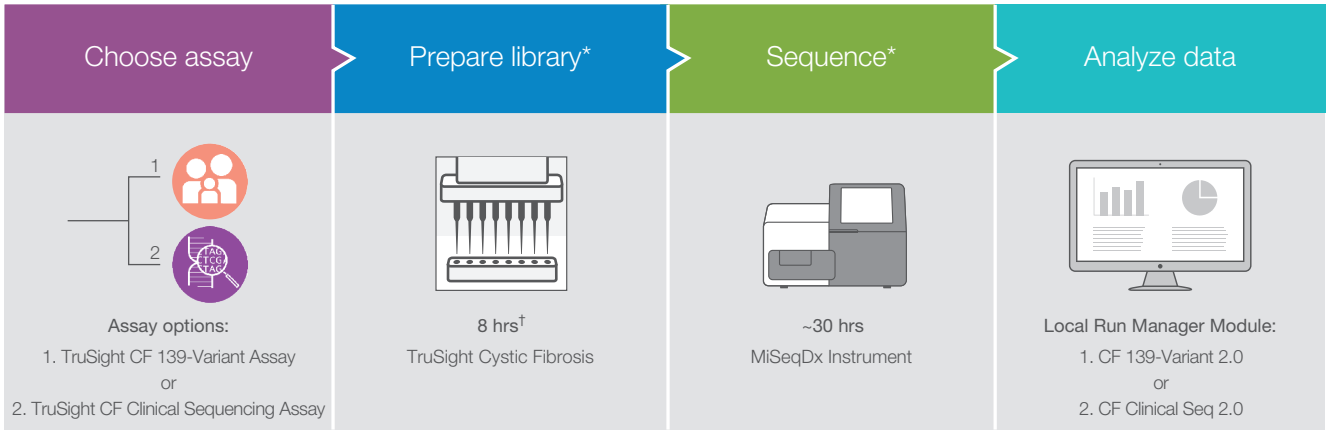

Figure 5: TruSight Cystic Fibrosis workflow—TruSight Cystic Fibrosis offers a streamlined, integrated workflow that includes library preparation, sequencing, and data analysis and reporting for the TruSight Cystic Fibrosis 139-Variant and Clinical Sequencing Assays.
* TruSight Cystic Fibrosis enables reflex testing by providing an assay configuration whereby the same libraries prepared for one assay can be used for the other.
† Library prep time depends on sample throughput and may vary.

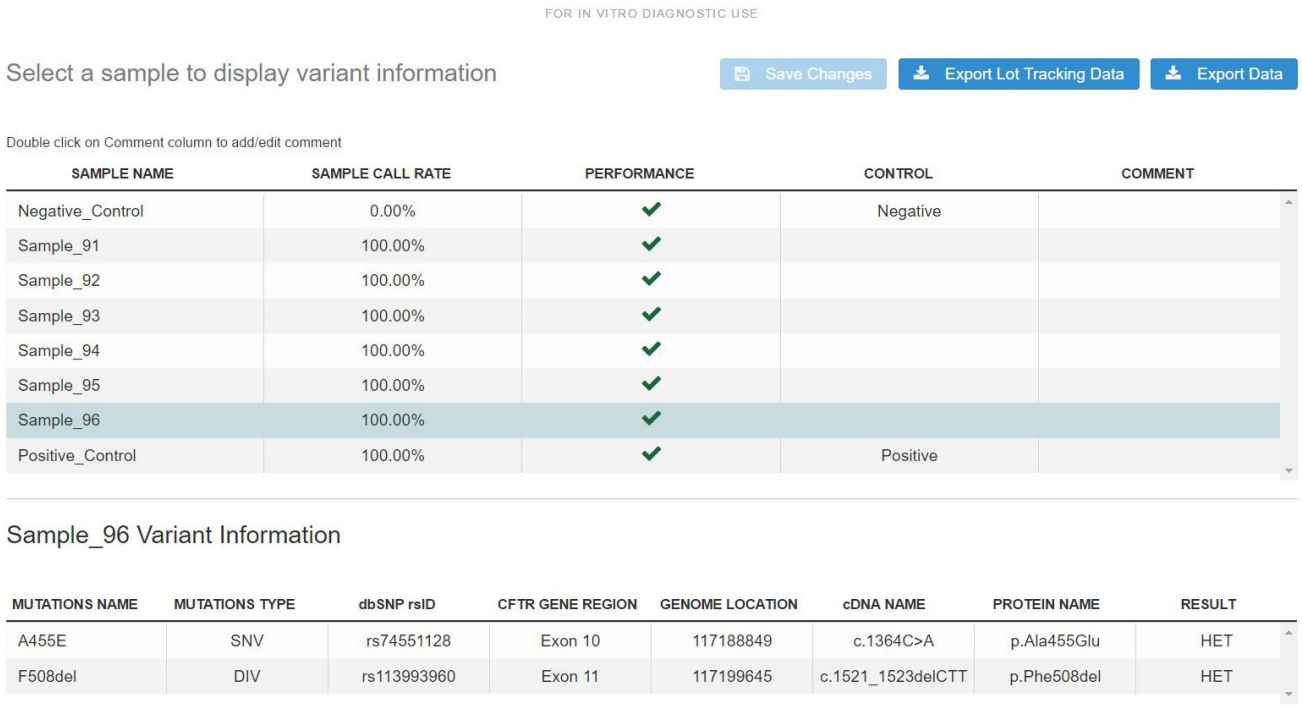

Figure 6: Data analysis output with LRM—Results from TruSight Cystic Fibrosis are presented in an easy-to-read fashion.

Summary

TruSight Cystic Fibrosis offers the TruSight Cystic Fibrosis 139-Variant Assay and the TruSight Cystic Fibrosis Clinical Sequencing Assay in a single solution. TruSight Cystic Fibrosis can be integrated into newborn CF testing for mutational analysis. The TruSight Cystic Fibrosis 139-Variant Assay provides an extensive panel of *CFTR* variants, including 23 common ACMG variants. 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.

Learn more

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

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)[9] and in 2011 by the American College of Obstetricians and Gynecologists (ACOG).[10] 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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