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Overview

The Local Run Manager CF Clinical Seq 2.0 Micro analysis module is for use with TruSight Cystic Fibrosis Clinical Sequencing Assay when processing 24–36 samples. The assay detects mutations in the protein coding regions and intron/exon boundaries of the cystic fibrosis transmembrane conductance regulator (CFTR) gene, including two large deletions and two-deep intronic mutations. The analysis module performs secondary analysis and report generation from sequencing runs that use TruSight Cystic Fibrosis. For more information on TruSight Cystic Fibrosis, refer to *TruSight Cystic Fibrosis Package Insert (Document # 1000000097720)*.

The analysis module evaluates short regions of amplified DNA, or amplicons, for variants. Focused sequencing of amplicons enables high coverage of particular regions across a large number of samples.

About This Guide

This guide provides instructions for setting up run parameters for sequencing and analysis for the CF Clinical Seq 2.0 Micro analysis module. Use of the software requires basic knowledge of the current Windows operating system and web browser-based user interface. For information about the Local Run Manager dashboard and system settings, refer to the *Local Run Manager Software Reference Guide for MiSeqDx (document # 1000000011880)*.

Enter Run Information

Set Parameters

- 1 Log in to Local Run Manager.
- 2 Select **Create Run**, and select **CF Clinical Seq 2.0 Micro**.
A confirmation window will display.selection
- 3 Select the check box, 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 fewer).
- 5 **[Optional]** Enter a run description.
Use alphanumeric characters, spaces, underscores, or dashes (150 characters or fewer).
- 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 on the Create Run screen.
- ▶ **Import samples**—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 fewer).
- 2 Right-click and select positive and negative control samples.

To save a run, you 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 fewer).
- 4 **[Optional]** Select an Index 1 adapter from the Index 1 (i7) drop-down list.
The default layout autopopulates i7 and i5 index combinations.
- 5 **[Optional]** Select an Index 2 adapter from the Index 2 (i5) drop-down list.
The default layout autopopulates i7 and i5 index combinations.
- 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 fewer than 24 samples have been entered, the Insufficient Sample window displays. Select **Proceed** to continue, or select **Cancel** to edit the samples.
If more than 36 samples have been entered, an error message displays and the run is not saved. Enter 36 samples or fewer.



CAUTION

Sequencing with pooled libraries that contain fewer than 24 samples is not validated by Illumina. Refer to TruSight Cystic Fibrosis Package Insert (Document # 1000000097720) for more information.

Import Samples

You can import sample information from two types of files:

- ▶ A sample information file that was previously exported from the CF Clinical Seq 2.0 Micro 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 importing and includes placeholder information in each column. Use an external editor to customize the template file as follows.
 - 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 **[Optional]** Select **Export** to export sample information to an external file.
- 5 Select **Save Run**.
If fewer than 24 samples have been entered, the Insufficient Sample window displays. Select **Proceed** to continue, or select **Cancel** to edit the samples.
If more than 36 samples have been entered, an error message displays and the run is not saved. Enter 36 samples or fewer.

**CAUTION**

Sequencing with pooled libraries that contain fewer than 24 samples is not validated by Illumina. Refer to TruSight Cystic Fibrosis Package Insert (Document # 1000000097720) for more information.

Edit a Run

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

Analysis Methods

For a description of the analysis methods for TruSight Cystic Fibrosis, refer to *TruSight Cystic Fibrosis Package Insert (Document # 1000000097720)*.

View Run and Results

- 1 From the Local Run Manager main screen, select the run name.
- 2 From the Run Overview tab, review the sequencing run metrics.
- 3 **[Optional]** Select the **Copy to Clipboard** icon to copy the output run folder path.
- 4 Select the Sequencing Information tab to review run parameters and consumables information.
- 5 Select the Samples and Results tab to view the analysis results.
The tab provides a sample list that summarizes the call rate, performance, coordinates not called, and information on controls.
 - ▶ If analysis was repeated, expand the Select Analysis drop-down list.
 - ▶ Select the appropriate analysis.
- 6 **[Optional]** Double-click the Comment field to enter a comment about a sample in the list. Select **Save Changes**.
- 7 Select samples in the list to display detailed variant information.
Sample variant information displays below the sample list.
- 8 **[Optional]** Double-click the field in the Interpretation column to select one of the following options:
 - ▶ **CF**—CF causing
 - ▶ **MVCC**—Mutation of Varying Clinical Consequence
 - ▶ **MOUS**—Mutation of Unknow Significance
 - ▶ **NCFCM**—Non CF Causing Mutation
 - ▶ **Unknown**
- 9 Select **Save Changes**.

NOTE

Each time changes are saved, an analysis results file is generated in the **Alignment** folder. This file includes a time stamp appended to the file name, with the format **YYMMDD_HHMMSS**.

For more information on the Run Overview and Sequencing Information tabs and how to requeue analysis, refer to the *Local Run Manager Software Reference Guide for MiSeqDx (document # 1000000011880)*.

Lot Tracking Data File

Once analysis is complete, choose one of the following options to view the `LotTracking.txt` file.

- ▶ From the Sample and Results tab, select **Export Lot Tracking Data**.
- ▶ Open the file in the Alignment folder.
The path to the Alignment folder is displayed on the Samples and Results tab in the Analysis Folder field.
 - ▶ **[Optional]** Select the **Copy to Clipboard** icon to copy the analysis folder path.

When analysis is complete, the lot tracking data file is written to the Alignment folder for the run. For example: `MiSeqAnalysis\<Run Folder Name>\Alignment_N\YYMMDD_HHMMSS`. N is a sequential number that increases if the analysis was requeued. YYMMDD_HHMMSS is the timestamp of the run.

Results Report

After secondary analysis is complete, analysis results are summarized on the Samples and Results tab. An analysis results output file for the assay also summarizes results in a one tab-delimited text file called `TruSightCFClinicalSequencingAssay.txt`.

Results in the analysis results file include the information found on the Sample and Results tab. Choose one of the following options to view the analysis results file `TruSightCFClinicalSequencingAssay.txt`:

- ▶ From the Sample and Results tab, select **Export Data**.
- ▶ Open the file in the Alignment folder.
The path to the Alignment folder is displayed in the Analysis Folder field of the Samples and Results tab.
- ▶ Select the **Copy to Clipboard** icon to copy the analysis folder path.

The analysis files for each sequencing run are stored on-instrument. The files are saved to `MiSeqAnalysis\<Run Folder Name>\Data\Intensities\BaseCalls` and `MiSeqAnalysis\<Run Folder Name>\Alignment_N\YYMMDD_HHMMSS`. N is a sequential number that increments if the analysis was requeued. YYMMDD_HHMMSS is the timestamp of the run.

Analysis Result File Information

The analysis results file `TruSightClinicalSequencingAssay.txt` includes three sections: the file header, sample information, and sample variants information. The file header is listed first, and includes general information about the run. There is only one file header per run. Sample information and sample variants information are included for every sample used in the analysis. These sections are paired together for each sample.

The following table provides a description of each row in the file header, which includes information about the run:

Table 1 File Header (Run Information)

Row Heading	Description
Test	Describes the test that was performed.
Run ID	Run ID that was generated by the MCS at the beginning of the sequencing run.
Run Date	Date (YYMMDD) that the sequencing run was started in MOS.
Analysis Version	Software version that was used for analysis.

The following table provides a description of each row in the sample information sections:

Table 2 Sample Information

Row Heading	Description
Sample ID	The sample name provided when the run was created, combined with the analysis ID assigned by the Local Run Manager software. If you requeue a run, the sample name part of the sample ID stays the same, while the analysis ID changes. This field is in the *.txt file only.
Sample Name	The sample name provided when the run was created.
Control	The type of control as provided when the run was created Values are positive or negative. A blank field indicates sample only.
Comment	An optional text field for comments. Comments from a previous analysis run do not carry over to the next analysis run.
Performance	Pass or Fail rating based on the call rate. For a positive control sample: <ul style="list-style-type: none"> • PASS with a call rate \geq 99% • FAIL with a call rate $<$ 99% For a negative control sample: <ul style="list-style-type: none"> • PASS with a call rate \leq 10% • FAIL with a call rate $>$ 10% For a sample not labeled as a positive or negative control: <ul style="list-style-type: none"> • PASS with a call rate \geq 99% • FAIL with a call rate $<$ 99%
Sample Call Rate	The number of variant positions that meet a predefined confidence value threshold divided by the total variant positions interrogated. Call rate is described on a per-sample basis and reported as a percentage that is calculated as 1 minus [number of variant positions with incomplete calls divided by the total number of variant positions sequenced].

The following table provides a description of each column in the sample variants sections:

Table 3 Sample Variants Information

Column Heading	Description
Variant Type	The type of variant. <ul style="list-style-type: none"> • SNV—Single Nucleotide Variant • DIV—Deletion Insertion Variant • DEL—Large deletion • PolyTGPolyT— PolyTG/PolyT genotype in CF gene
Coordinate	The genomic position at which the variant was found.
Chromosome	The reference chromosome ID.
Frequency	The fraction of reads for the sample that include the variant. For example, if the reference base at a particular position is A and sample 1 has 60 A reads and 40 T reads, then the SNV has a variant frequency of 0.4.
Depth	The number of reads for a sample covering a particular position.
cDNA Name (HGVS)	Description of a variant at the DNA-level using the coding DNA (cDNA) sequence nomenclature as recommended by the Human Genome Variation Society (HGVS).
Protein Name (HGVS)	Description of a variant at the protein-level using the protein sequence nomenclature as recommended by the Human Genome Variation Society (HGVS).
dbSNP ID	The dbSNP ID of the variant.
Reference	The reference base at the genomic coordinate at which the variant appears. This field is in the *.txt file.

Column Heading	Description
Result	Variant genotype. <ul style="list-style-type: none"> • SNV or DIV—The two alleles observed are described. For example, A/T describes a SNV where both A and T alleles are observed and is heterozygous. The wild-type allele is listed first, followed by variant allele. • Small Deletions—The variant allele describes the allele with deletion. ATCT/A, for example. • Insertions—The variant allele describes the allele with insertion. C/CT, for example. • DEL—Result is reported as Het Deletion or Hom Deletion. • PolyTGPolyT—The actual genotype is reported.
Interpretation	This field enables the medical geneticist to provide clinical interpretation of the mutation for each sample. <ul style="list-style-type: none"> • CF—CF causing • MVCC—Mutation of Varying Clinical Consequence • MOUS—Mutation of Unknow Significance • NCFCM—Non CF Causing Mutation • Unknown

The Sample Variants Information section also includes a `Coordinates Not Called` entry, which lists genomic coordinates within the targeted region where a call was not reported due to low confidence values.

Revision History

Document	Date	Description of Change
Document # 200017945 v00	May 2022	Initial release.

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Website: www.illumina.com
Email: techsupport@illumina.com

Illumina Customer Support Telephone Numbers

Region	Toll Free	Regional
North America	+1.800.809.4566	
Australia	+1.800.775.688	
Austria	+43 800006249	+43 19286540
Belgium	+32 80077160	+32 34002973
China	400.066.5835	
Denmark	+45 80820183	+45 89871156
Finland	+358 800918363	+358 974790110
France	+33 805102193	+33 170770446
Germany	+49 8001014940	+49 8938035677
Hong Kong, China	800960230	
Ireland	+353 1800936608	+353 016950506
Italy	+39 800985513	+39 236003759
Japan	0800.111.5011	
Netherlands	+31 8000222493	+31 207132960
New Zealand	0800.451.650	
Norway	+47 800 16836	+47 21939693
Singapore	+1.800.579.2745	
South Korea	+82 80 234 5300	
Spain	+34 911899417	+34 800300143
Sweden	+46 850619671	+46 200883979
Switzerland	+41 565800000	+41 800200442
Taiwan, China	00806651752	
United Kingdom	+44 8000126019	+44 2073057197
Other countries	+44.1799.534000	

Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download from support.illumina.com.



Illumina
5200 Illumina Way
San Diego, California 92122 U.S.A.
+1.800.809.ILMN (4566)
+1.858.202.4566 (outside North America)
techsupport@illumina.com
www.illumina.com

CE



Illumina Netherlands B.V.
Steenoven 19
5626 DK Eindhoven
The Netherlands

Australian Sponsor

Illumina Australia Pty Ltd
Nursing Association Building
Level 3, 535 Elizabeth Street
Melbourne, VIC 3000
Australia

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