Local Run Manager
CF Clinical Seq 2.0 Analysis Module

Workflow Guide

FOR IN VITRO DIAGNOSTIC USE

Overview

The Local Run Manager CF Clinical Seq 2.0 analysis module is for use with TruSight Cystic Fibrosis Clinical Sequencing Assay. 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. See the package insert *TruSight Cystic Fibrosis (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 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, see 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**.
    A pop up window will display to confirm that CF Clinical Seq 2.0 is the intended selection.
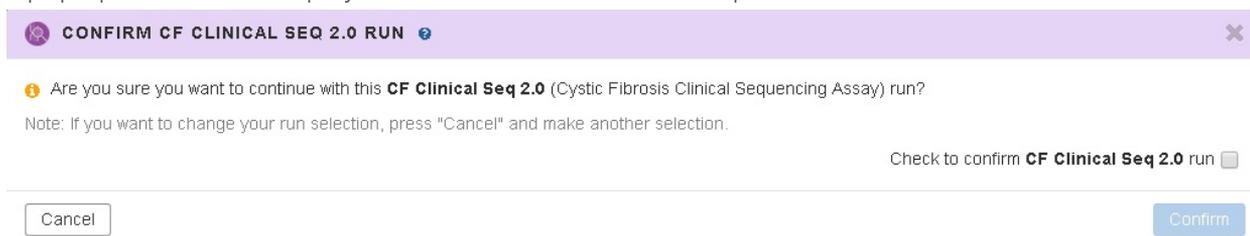
3   Select the check box, and select **Confirm** to continue (or, select **Cancel** to return to the main screen).

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

2   Right-click and select positive and negative control samples.
    In order 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**
> Proceeding with less than 24 samples is not validated by Illumina. See TruSight Cystic Fibrosis Package Insert (Document # 1000000097720) for more information.

Import Samples

Sample information can be imported from two types of files:

▶   A sample information file that was previously exported from the CF Clinical Seq 2.0 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   Once 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**.

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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

Proceeding with less than 24 samples is not validated by Illumina. See TruSight Cystic Fibrosis Package Insert (Document # 1000000097720) for more information.

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)*.

Analysis Methods

For a description of the analysis methods for TruSight Cystic Fibrosis, see the package insert *TruSight Cystic Fibrosis (Document # 1000000097720)*.

View Run and Results

1   From the Local Run Manager main screen, click 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 and select the appropriate analysis.

6   **[Optional]** Double-click the Comment field to enter a comment about a sample in the list. Click **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
    Click **Save Changes**.

NOTE

Each time changes are saved, an analysis results file will be 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, see 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, click **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, click **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.

> **NOTE**
> Select the **Copy to Clipboard** icon to copy the analysis folder path.

The analysis files for each sequencing run are stored on instrument, with files 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. Note that there is only one file header per run. In contrast, 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 MiSeq Operating Software (MOS) 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: • PASS — with a call rate ≥ 99% • FAIL — with a call rate < 99% For a negative control sample: • PASS — with a call rate ≤ 10% • FAIL — with a call rate > 10% For a sample not labeled as a positive or negative control: • PASS — with a call rate ≥ 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. • 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. The name of the field is Coordinate in the *.txt file.
Chromosome	The reference chromosome ID. The name of the field is Chromosome in the *.txt file.

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Column Heading	Description
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. The name of the field is dbSNP ID in the *.txt file.
Reference	The reference base at the genomic coordinate at which the variant appears. Reference in the *.txt file.
Result	Variant genotype. • 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. The name of the field is Result in the *.txt file.
Interpretation	This field enables the Medical Geneticist to provide clinical interpretation of the mutation for each sample. • 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 # 1000000100946 v01	August 2021	Updated EU Authorized Representative address.
Document # 1000000100946 v00	December 2019	Initial release.

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Technical Assistance

For technical assistance, contact Illumina Technical Support.

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

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

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