

# Local Run Manager CF Clinical Sequencing Analysis Module

## Workflow Guide

FOR IN VITRO DIAGNOSTIC USE

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## Overview

The Local Run Manager CF Clinical Sequencing analysis module is for use with Illumina MiSeqDx 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 the MiSeqDx Cystic Fibrosis Clinical Sequencing Assay. See the package insert *MiSeqDx Cystic Fibrosis Clinical Sequencing Assay (document # 15038344)*.

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 Sequencing analysis module. 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 Click **Create Run**, and select **CF Clinical**.
- 3 Enter a run name that identifies the run from sequencing through analysis.  
Use alphanumeric characters, spaces, underscores, or dashes.
- 4 [Optional] Enter a run description to help identify the run.  
Use alphanumeric characters.

### 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.

After you have populated the samples table, you can export the sample information to an external file, and use the file as a reference when preparing libraries or import the file for another run.

### Enter Samples Manually

- 1 Enter a unique sample name in the Sample Name field.  
Use alphanumeric characters, dashes, or underscores.
- 2 Right-click and select positive and negative control samples.
- 3 [Optional] Enter a sample description in the sample Description tab.  
Use alphanumeric characters, dashes, or underscores.
- 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 that autopopulate the wells already meet index diversity requirements.

- 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 that autopopulate the wells already meet index diversity requirements.
- 6 Click the  **Print** icon to display the plate layout.
- 7 Select **Print** to print the plate layout as a reference for preparing libraries.
- 8 [Optional] Click **Export** to export sample information to an external file.
- 9 Click **Save Run**.

## Import Samples

- 1 Click **Import Samples** and browse to the location of the sample information file. There are two types of files you can import.
  - ▶ Click **Template** on the Create Run screen to make a new plate layout. The template file contains the correct column headings for import. Enter sample information in each column for the samples in the run. Delete example information in unused cells, and then save the file.

Sample_Name	Description	I7_Index_ID	I5_Index_ID	Sample_Well	Control
Sample1		A701	A503	A01	
Sample2		A701	A504	A02	
Sample3		A701	A505	A03	
Sample4		A702	A503	A04	
Sample5		A702	A504	A05	
Sample6		A702	A505	A06	
Sample7		A710	A503	A07	Positive
Sample8		A710	A504	A08	Negative

- ▶ Use a file of sample information that was exported from the CF Clinical Sequencing module using the Export feature.
- 2 Click the  **Print** icon to display the plate layout.
- 3 Select **Print** to print the plate layout as a reference for preparing libraries.
- 4 [Optional] Click **Export** to export sample information to an external file.
- 5 Click **Save Run**.

## 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 the MiSeqDx Cystic Fibrosis Clinical Sequencing Assay, see the package insert *MiSeqDx Cystic Fibrosis Clinical Sequencing Assay (document # 15038344)*.

## View Run and Results

- 1 From the Local Run Manager dashboard, click the run name.
- 2 From the Run Overview tab, review the sequencing run metrics.
- 3 [Optional] Click the **Copy to Clipboard**  icon to copy the output run folder path.
- 4 Click the Sequencing Information tab to review run parameters and consumables information.
- 5 Click the Samples and Results tab to view the analysis results file location.  
The tab also 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.
- 7 Select samples in the list to display detailed variant information.  
Sample variant information displays below the sample list.

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)*.

## Result Reports

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 one tab-delimited text file called MiSeqDxCFClinicalSequencingAssay.txt.

## Sample Information

**Table 1** Sample List Information

Column Heading	Description
Sample Name	The sample name provided when the run was created.
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 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].
Performance	Pass or Fail rating based on the call rate. For a positive control sample: <ul style="list-style-type: none"> <li>• PASS — with a call rate <math>\geq</math> 99%</li> <li>• FAIL — with a call rate <math>&lt;</math> 99%</li> </ul> For a negative control sample: <ul style="list-style-type: none"> <li>• PASS — with a call rate <math>\leq</math> 10%</li> <li>• FAIL — with a call rate <math>&gt;</math> 10%</li> </ul> For a sample not labeled as a positive or negative control: <ul style="list-style-type: none"> <li>• PASS — with a call rate <math>\geq</math> 99%</li> <li>• FAIL — with a call rate <math>&lt;</math> 99%</li> </ul>

Column Heading	Description
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. To enter a comment, double-click in the comment field. Comments entered in this field are saved in the analysis report file, MiSeqDxCFClinicalSequencingAssay.txt. If analysis is requeued, a new report file is written. Comments from a previous analysis run do not carry over to the next analysis run.
Coordinates Not Called	Genomic coordinates within the targeted region where a call was not reported due to low confidence values.

## Sample Variant Information

Table 2 Variants Table

Column Heading	Description
Variant Type	The type of variant. <ul style="list-style-type: none"> <li>• SNV — Single Nucleotide Variant</li> <li>• DIV — Deletion Insertion Variant</li> <li>• DEL — Large deletion</li> <li>• PolyTGPolyT — PolyTG/PolyT genotype in CF gene</li> </ul>
Position Coordinate	The genomic position at which the variant was found. The name of the field is Coordinate in the *.txt file.
Chr Chromosome	The reference chromosome ID. The name of the field is Chromosome in the *.txt file.
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 dbSNP ID	The dbSNP ID of the variant. The name of the field is dbSNP ID in the *.txt file.
RefGene Reference	The reference base at the genomic coordinate at which the variant appears. Reference in the *.txt file.
Call Result	Variant genotype. <ul style="list-style-type: none"> <li>• 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.</li> <li>• Small Deletions — The variant allele describes the allele with deletion. ATCT/A, for example.</li> <li>• Insertions — The variant allele describes the allele with insertion. C/CT, for example.</li> <li>• DEL — Result is reported as Het Deletion or Hom Deletion.</li> <li>• PolyTGPolyT — The actual genotype is reported.</li> </ul> The name of the field is Result in the *.txt file.

Column Heading	Description
Interpretation	This field enables the Medical Geneticist to provide clinical interpretation of the mutation for each sample. Double-click on the Interpretation column to edit the value. To save changes to the Interpretation column, select the Save Changes icon. The following options are included in the Interpretation drop-down list: <ul style="list-style-type: none"> <li>• CF — CF causing</li> <li>• MVCC — Mutation of Varying Clinical Consequence</li> <li>• MOUS — Mutation of Unknow Significance</li> <li>• NCFCM — Non CF Causing Mutation</li> <li>• Unknown</li> </ul>
Filter	The criteria for a filtered variant. This field is not present in the *.txt file.

## Analysis Results File

Results in the analysis results file are identical to what is found on the Sample and Results tab. See [Sample Information on page 5](#) and [Sample Variant Information on page 6](#).

- 1 Choose one of the following options to view the analysis results file `MiSeqDxCFClinicalSequencingAssay.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 on the Samples and Results tab in the Analysis Folder field.  
When analysis is complete, the analysis results file is written to the Alignment folder for the run.  
For example: `MiSeqAnalysis\<Run Folder Name>\Alignment_N\YYYYMMDD_X`.  
X represents the Run ID. YYYYMMDD is the date of the run. N is a sequential number that increases if the analysis was repeated or requeued.

**Table 3 MiSeqDxCFC139VariantAssay.txt File Header**

Column 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 (DDMMYY) that the sequencing run was started in MOS.
Analysis Version	Version of Local Run Manager CF Clinical Sequencing analysis module that was used for analysis.

## Revision History

Document	Date	Description of Change
Document # 1000000010786 v01	April 2020	Updated EU Authorized Representative address. Updated Australian Sponsor address.
Document # 1000000010786 v00	August 2017	Initial release.

## Technical Assistance

For technical assistance, contact Illumina Technical Support.

Website: [www.illumina.com](http://www.illumina.com)  
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Safety data sheets (SDSs)—Available on the Illumina website at [support.illumina.com/sds.html](http://support.illumina.com/sds.html).

Product documentation—Available for download in PDF from the Illumina website. Go to [support.illumina.com](http://support.illumina.com), select a product, then select **Documentation & Literature**.



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