

# Local Run Manager CF 139-Variant 2.0 Micro Analysis Module

## Workflow Guide

FOR IN VITRO DIAGNOSTIC USE

Overview	3
Enter Run Information	3
Analysis Methods	5
View Run and Results	5
Lot Tracking Data File	5
Results Report	6
Revision History	7
Technical Assistance	8



This document and its contents are proprietary to Illumina, Inc. and its affiliates ("Illumina"), and are intended solely for the contractual use of its customer in connection with the use of the product(s) described herein and for no other purpose. This document and its contents shall not be used or distributed for any other purpose and/or otherwise communicated, disclosed, or reproduced in any way whatsoever without the prior written consent of Illumina. Illumina does not convey any license under its patent, trademark, copyright, or common-law rights nor similar rights of any third parties by this document.

The instructions in this document must be strictly and explicitly followed by qualified and properly trained personnel in order to ensure the proper and safe use of the product(s) described herein. All of the contents of this document must be fully read and understood prior to using such product(s).

FAILURE TO COMPLETELY READ AND EXPLICITLY FOLLOW ALL OF THE INSTRUCTIONS CONTAINED HEREIN MAY RESULT IN DAMAGE TO THE PRODUCT(S), INJURY TO PERSONS, INCLUDING TO USERS OR OTHERS, AND DAMAGE TO OTHER PROPERTY, AND WILL VOID ANY WARRANTY APPLICABLE TO THE PRODUCT(S).

ILLUMINA DOES NOT ASSUME ANY LIABILITY ARISING OUT OF THE IMPROPER USE OF THE PRODUCT(S) DESCRIBED HEREIN (INCLUDING PARTS THEREOF OR SOFTWARE).

© 2022 Illumina, Inc. All rights reserved.

All trademarks are the property of Illumina, Inc. or their respective owners. For specific trademark information, see [www.illumina.com/company/legal.html](http://www.illumina.com/company/legal.html).

## Overview

The Local Run Manager CF 139-Variant 2.0 Micro analysis module is for use with TruSight Cystic Fibrosis 139-Variant Assay when processing 24–36 samples. The assay detects 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 specimens. 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 targeted 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 139-Variant 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 then select **CF 139-Variant 2.0 Micro**.
- 3 Enter a run name that identifies the run from sequencing through analysis.  
Use alphanumeric characters, spaces, underscores, or dashes (40 characters or fewer).
- 4 **[Optional]** Enter a run description.  
Use alphanumeric characters, spaces, underscores, or dashes (150 characters or fewer).
- 5 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 139-Variant 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, 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.

**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 `TruSightCF139VariantAssay.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 `TruSightCF139VariantAssay.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 `TruSightCF139VariantAssay.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.

Row Heading	Description
Performance	<p>Pass or Fail rating based on the call rate.</p> <p>For a positive control sample:</p> <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> <p>For a negative control sample:</p> <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> <p>For a sample not labeled as a positive or negative control:</p> <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>
Sample Call Rate	<p>The number of variant positions that meet a predefined confidence value threshold divided by the total variant positions interrogated.</p> <p>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].</p>

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

**Table 3 Sample Variants Information**

Column Heading	Description
Mutations (Common Name)	Common name of the Cystic Fibrosis variant, as described in the CFTR2 database. The name of the field is Mutations (Common Name) in the *.txt file.
Mutation Type	<p>The type of variant.</p> <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>
dbSNP rsID	The dbSNP rsID of the variant, if applicable.
CFTR Gene Region	CFTR gene region (exon # or intron #) where variant is present.
Genomic Location	Genomic location of the variant within chromosome 7.
cDNA Name (HGVS)	Description of the variant at the DNA level using the coding DNA (cDNA) sequence nomenclature as recommended by the Human Genome Variation Society (HGVS). The name of the field is cDNA Name (HGVS) in the *.txt file.
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). The name of the field is Protein Name (HGVS) in the *.txt file.
Result	<p>Variant genotype.</p> <p>For SNVs, DIVs, and DELs:</p> <ul style="list-style-type: none"> <li>• HET—Heterozygous</li> <li>• HOM—Homozygous</li> </ul> <p>For PolyTGPolyT variant, the actual genotype is reported. PolyTGPolyT is reported only when the R117H variant is detected.</p>
Interpretation	This field is always left blank.

## Revision History

Document	Date	Description of Change
Document # 200017946 v00	May 2022	Initial release.

## Technical Assistance

For technical assistance, contact Illumina Technical Support.

**Website:** [www.illumina.com](http://www.illumina.com)  
**Email:** [techsupport@illumina.com](mailto: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](http://support.illumina.com/sds.html).

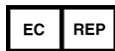
Product documentation—Available for download from [support.illumina.com](http://support.illumina.com).





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

**FOR IN VITRO DIAGNOSTIC USE**

© 2022 Illumina, Inc. All rights reserved.

**illumina®**