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Overview

The Local Run Manager CF 139 Variant analysis module is for use with Illumina MiSeqDx Cystic Fibrosis 139-Variant Assay. 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 the MiSeqDx Cystic Fibrosis 139-Variant Assay. See the package insert *MiSeqDx Cystic Fibrosis 139-Variant Assay (document # 15038347)*.

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 139 Variant 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 139**.
- 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 the following options:

▶ Enter samples manually

- 1 Select the number of sample from the Number of Samples drop-down box. Consider the following information when you make a selection.
 - ▶ Choose the number of samples that is nearest to the number of samples you are testing. The drop-down list contains samples in multiples of 8. If the exact number of samples is not in the list, select the number nearest, but less than the number you are testing, so that index diversity requirements are met. For example, if you want to test 18 samples, select 16 samples. Then add 2 additional samples. Make sure to select index adapters for extra wells.
 - ▶ For 48 samples, there are two different configurations available. One configuration is 48-Set 1, and the other is 48-Set 2. The configuration sets contain different i7 index primers and well positions. These sets can be used to set up two sequencing runs for a total of 96 samples.
- 2 Use the blank table on the Create Run screen. Suggested sample wells are highlighted.
 - ▶ **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 highlighted 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 highlighted 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	A503	A02	
Sample3		A701	A504	A03	
Sample4		A702	A504	A04	
Sample5		A702	A505	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 139 Variant 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 139-Variant Assay, see the package insert *MiSeqDx Cystic Fibrosis 139-Variant Assay (document # 15038347)*.

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, 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 MiSeqDxCF139VariantAssay.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.

Column Heading	Description
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"> • 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%
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, MiSeqDxCf139VariantAssay.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.

Sample Variant Information

Table 2 Variants Table

Column Heading	Description
Mutations Name 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.
Mutations 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
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 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 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	Variant genotype. For SNVs, DIVs, and DELs: <ul style="list-style-type: none"> • HET — Heterozygous • HOM — Homozygous For PolyTGPolyT variant, the actual genotype is reported. NOTE: PolyTGPolyT is reported only when the R117H variant is detected.

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 MiSeqDxCF139VariantAssay.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 MiSeqDxCF139VariantAssay.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 139 Variant analysis module that was used for analysis.

Revision History

Document	Date	Description of Change
Document # 1000000012184 v02	April 2020	Updated EU Authorized Representative address. Updated Australian Sponsor address.
Document # 1000000012184 v01	August 2017	<p>Changed the name of the Analysis Report section to Result Reports.</p> <p>Added information on field name differences between the Local Run Manager software and the *.txt file in the Sample Information and Sample Variant Information sections.</p> <p>Added a description of the sample ID.</p> <p>Removed Analysis Methods section information. Analysis methods are detailed in the package insert <i>MiSeqDx Cystic Fibrosis 139-Variant Assay (document # 15038347)</i>.</p> <p>In the Specify Samples for the Run section, added information that set 1 and 2 of the 48 sample configuration can be used for 96 samples and two sequencing runs.</p> <p>In the Enter Samples Manually section, revised the following:</p> <ul style="list-style-type: none"> • Changed step 1 from sample ID to sample name to be consistent with the software. • Changed step 2 to indicate that positive and negative control samples are required. • Changed step 3 to indicate that spaces cannot be used in sample descriptions. <p>Moved the Edit Run Information section to the <i>Local Run Manager Software Reference Guide for MiSeqDx (document # 1000000011880)</i>.</p> <p>In the example table of the Import Samples section, added zeros in front of the numbers in the Sample_Well column. Although you can create an import sample file without the leading zeros in the column, the table is now consistent with the template that comes with the software.</p> <p>In the Analysis Results File section, added information about the Alignment folder naming convention that N is a sequential number that increases if the analysis is repeated or requeued.</p> <p>Added this Revision History.</p>
Document # 1000000012184 v00	June 2017	Initial release.

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