MiSeq™Dx Instrument Software Updates with Local Run Manager

Local Run Manager provides modular software support for MiSeqDx assays, with analysis equivalent to MiSeq Reporter.

Introduction

The MiSeqDx intrument offers an integrated sequencing ecosystem, including system software that is accessed through a user-friendly touchscreen interface. The updated MiSeqDx software architecture is called Local Run Manager (LRM). LRM is an on-instrument solution for creating a sequencing run, monitoring run status, analyzing sequencing data, and viewing results. It is easily accessed through a web browser and integrates with the instrument control software. LRM is the interface between the instrument control software and individual software applications for each assay, referred to as modules. Each module includes the functionality to perform secondary analysis for an assay.

The current MiSeqDx software is non-modular and the secondary analysis software is MiSeq Reporter (MSR). Updating the MiSeqDx software architecture to a modular framework with LRM (Table 1) will enable current and future MiSeqDx assays to be performed on a single commercial instrument/software configuration.

The integration of LRM into the MiSeqDx instrument was designed to provide the flexibility of modular analysis while avoiding any significant changes in workflow. Three assays currently affected by this change are the MiSeqDx Universal Kit, MiSeqDx Cystic Fibrosis (CF) 139-Variant Assay, and MiSeqDx CF Clinical Sequencing Assay. The modules for each of these assays are the same analysis pipelines which were previously available as components of the MiSeq Reporter software, and this technical note describes the testing that was performed to demonstrate equivalent results between LRM and MSR.

Comparison of LRM and MSR Analysis Results Using the MiSeqDx Universal Kit

The MiSeqDx Universal Kit is a validated, FDA-cleared sequencing solution that enables clinical laboratories to develop their own next-generation sequencing (NGS) assays for use on the MiSeqDx instrument. On-system software was designed to provide easy-to-interpret results. The primary analysis and secondary analysis methodologies are the same for Universal V1 Module for both MSR and LRM. To verify functional equivalency of MSR and LRM Software for MiSeqDx Universal Kit 1.0, 13 well-characterized reference samples (Platinum Genome, NA12877-NA12893) were tested independently on three MiSeqDx instruments. The variant call accuracy, as measured by negative percent agreement (NPA) and positive percent agreement (PPA) (Table 2) was identical for the three runs as compared to the data previously analyzed with MSR.[1] Sequencing run metrics for the Universal Kit 1.0 using MSR were also verified to be equivalent with the LRM.

Comparison of LRM and MSR Analysis Results Using the CF Assays

The MiSeqDx CF 139-Variant Assay and the MiSeqDx CF Clinical Sequencing Assay are FDA-cleared, *in vitro* diagnostic (IVD), NGS tests that provide a comprehensive view of the cystic fibrosis transmembrane receptor (*CFTR*) gene. Results from these assays are reported in an easy-to-read text file format that a board-certified geneticist (or equivalent) can easily interpret. The assay report includes IDs and nomenclature for each variant. The primary analysis and secondary analysis methodologies for the two assays are unchanged between MSR, and the CF 139 Analysis Module and CF Clinical Analysis Module, in LRM, .

Table 1: Local Run Manager Modules to Replace MiSeq Reporter Software

Software to be Retired	Replacement Software
MiSeq Reporter CF 139-Variant Assay Workflow	Local Run Manager CF 139 Analysis Module
MiSeq Reporter CF Clinical Sequencing Assay Workflow	Local Run Manager CF Clinical Analysis Module
MiSeq Reporter MiSeqDx Universal Workflow	Local Run Manager Universal V1 Analysis Module

Table 2: Base Call Results for 13 Characterized Samples Assessed with the MiSeqDx Universal Kit and Analyzed with MSR and LRM

	MSR Single Run		LRM (Instrument 1)		LRM (Instrument 2)		LRM (Instrument 3)	
Sample	PPA (%)	NPA (%)	PPA (%)	NPA (%)	PPA (%)	NPA (%)	PPA (%)	NPA (%)
NA12877	89.47	100	89.47	100	89.47	100	89.47	100
NA12878	89.47	100	89.47	100	89.47	100	89.47	100
NA12879	95.00	100	95.00	100	95.00	100	95.00	100
NA12880	90.00	100	90.00	100	90.00	100	90.00	100
NA12881	90.91	100	90.91	100	90.91	100	90.91	100
NA12882	93.75	100	93.75	100	93.75	100	93.75	100
NA12883	95.83	100	95.83	100	95.83	100	95.83	100
NA12884	95.24	100	95.24	100	95.24	100	95.24	100
NA12885	89.47	100	89.47	100	89.47	100	89.47	100
NA12886	90.91	100	90.91	100	90.91	100	90.91	100
NA12887	94.74	100	94.74	100	94.74	100	94.74	100
NA12888	95.83	100	95.83	100	95.83	100	95.83	100
NA12893	90.00	100	90.00	100	90.00	100	90.00	100

Positive percent agreement (PPA) = 100×TP/(TP+FN) where the true positives (TP) are positive variant calls at genomic coordinates where variants are present according to the reference sequence and the false negatives (FN) are negative variant calls where variants are present according to the reference sequence.

Negative percent agreement (NPA) = 100×TN/(FP+TN) where the false positives (FP) are positive variant calls at genomic coordinates where variants are absent according to the reference sequence and the true negatives (TN) are negative variant calls where variants are absent according to the reference sequence.

To assess equivalency between MSR and LRM in performance of the CF assays, replicates of CF-positive DNA samples derived from Coriell cell lines were tested on three MiSeqDx instruments for the CF 139-Variant assay (Table 3) and two MiSeqDx instruments for the CF Clinical Sequencing assay (Table 4). The samples represent different types of sequence variations which could be present in the *CFTR* gene (single nucleotide variations, small insertions/deletions (indels), compound indels, indels in homopolymeric regions, and large deletions). The variant call accuracy and call rates were identical for all samples in the replicate runs as compared to data previously generated with MSR for both assays (Table 5). Sequencing run metrics using were also verified to be equivalent between LRM and MSR for each run.

Table 3: DNA Samples Used for CF Testing with Genotypes Reported by the CF 139-Variant Assay

Coriell Sample ID	Mutations (Common Names)
NA07381	F508del, 3849+10kbC>T
NA07857	M1101K (HOM)
NA11290	621+1G>T, A455E
NA12785	R347P, G551D
NA18668	CFTR dele2,3, F508del
NA18802	Y122X, R1158X
NA20836	3905insT
NA18803	F508del, 2183AA>G

All mutations are heterozygous unless indicated otherwise.

Table 4: DNA Samples Used for Testing with CF Genotypes Reported by the CF Clinical Sequencing Assay

Coriell Sample ID	Variations Genomic Coordinate (chr7,hg19), (Genotype Result)	
NA07381	117199533 (A/A)	117235055 (T/G)
	117199644 (ATCT/A)	117280015 (C/T)
	117232223 (C/T)	117307108 (G/A)
	117188661 ((TG)10(T)7/(TG)10(T)9)	
NA07857	117199533 (G/A)	117235055 (T/G)
	117199709 (G/A)	117251797 (A/A)
	117188661 ((TG)11(T)7/(TG)10(T)7)	
NA11290	117171169 (G/T)	117199533 (A/A)
	117188849 (C/A)	
	117188661 ((TG)10(T)9/(TG)10(T)9)	
NA20836	117282541 (C/CT)	
	117188661 ((TG)11(T)7/(TG)11(T)7)	
NA12785	117180324 (G/C)	117235055 (T/G)
	117199533 (G/A)	117306991 (C/T)
	117227860 (G/A)	117307108 (G/A)
	117188661 ((TG)11(T)7/(TG)10(T)7)	
NA18668	117199533 (G/A)	117138366 (Het Deletion)
	117199644 (ATCT/A)	
	117188661 ((TG)11(T)7/(TG)10(T)9)	
NA18802	117149147 (G/A)	117199533 (G/A)
	117171045 (T/A)	117235055 (T/G)
	117175347 (G/T)	117267579 (C/T)
	117188661 ((TG)11(T)7/(TG)10(T)7)	

Table 5: Test Results for CF 139-Variant Assay and CF Clinical Sequencing Assay Runs Analyzed With LRM

Run Metric	Specification	CF 139 Assay LRM runs (48 samples/run)			CF Clinical Assay LRM runs (8 samples/ run)	
		Instrument 1	Instrument 2	Instrument 3	Instrument 1	Instrument 2
Call Rate[a]	≥ 99%	100%	100%	100%	100%	100%
Accuracy[b]	Genotypes correctly called	100%	100%	100%	100%	100%

a. Call Rate for a given sample is the number of positions/regions with genotype calls as a percentage of the number of positions/regions sequenced.
b. Accuracy for a given sample is the percent agreement with a reference method (Sanger bi-directional sequencing, and PCR assay for large deletions), calculated for those base positions that receive a base call.

Data presented in the original package inserts for the CF 139-Variant Assay and CF Clinical Sequencing Assay were analyzed with MSR.[2,3] To demonstrate equivalency in analysis of variant calling, subsets of the data were reanalyzed with the CF 139 Analysis Module and CF Clinical Analysis Module in LRM..

The clinical accuracy study in the CF 139-Variant Assay package insert was reanalyzed with LRM to assess each of the 139 variants detected by the assay. A subset of the data from the reproducibility study, which assessed 92 samples in three independent sites, was reanalyzed. Thirdly, the DNA input study, which evaluated call rate calculations for samples at below 100%, was reanalyzed. From the CF Clinical Sequencing Assay package insert, runs from the DNA Input Study were reanalyzed. Apart from the minor annotation updates described in Table 6, reanalysis of all studies with LRM yielded identical results for variant call rates and genotype calls as were obtained previously with MSR.

One minor change observed for both assays was in the Analysis Version documented in the MiSeqDxCF139VariantAssay.txt for CF 139 runs and MiSeqDxCFClinicalSequencingAssay.txt for CF Clinical runs. The version differences simply identify the software version used (MiSeq Reporter v2.2.31.1, CF 139 Module v1.0.0.246, or CF Clinical Module v1.0.0.185) and are therefore expected (Table 3, Figure 1).

Additionally, a few annotation updates were made in the CF 139-Variant Assay (Table 6). These were made for the following reasons:

- to correct the genomic coordinates reported for four small indel variants which occur in homopolymeric regions
- to revise the common names for several mutations that have unecessary spaces or dashes removed from their names
- to correct the cDNA name for the PolyTG/PolyT variant
- to update the protein names for two variants

The revised annotations were observed in the LRM analysis results, as expected.

Summary

The MiSeqDx software architecture will be updated to a modular framework with LRM, enabling current and future MiSeqDx assays to be performed on a single commercial instrument/software configuration. Although the software framework was changed to improve workflow flexibility, the primary analysis and secondary analysis methodologies remain the same for both MSR and LRM modules. Comparison of secondary analysis results between MSR and LRM resulted in no difference in variants called using the MiSeqDx Universal Kit, the MiSeqDx CF 139-Variant Assay, and the MiSeqDx CF Clinical Sequencing Assay.

Learn More

To learn more about the MiSeqDx System and Local Run Manager software, visit: www.illumina.com/systems/sequencing-platforms/miseqdx.html

A

Test CF Clinical Sequencing Assay
For In Vitro Diagnostic Use.
Run ID 151007_M00507_0111_000000000-AHNHT
Run Date 151007
Analysis Version 1.0.0.185

Sample ID NA07904_0-1021
Sample Name NA07904_0
Control
Comment
Performance Pass
Sample Call Rate 100.00%

Variant Type	Coordinate	Chromosome	Frequency	Depth	cDNA Name (HGVS)	Protein Name (HGVS)	dbSNP ID	Reference	Result	Interpretation
Div	117282541	chr7	0.4661035	23670	c.3773_3774insT	p.Leu1258PhefsX7	rs121908789	C	C/CT	
PolyTGPolyT	117188661	chr7	N/A	N/A	N/A	PolyTG/PolyT	(TG)11(T)7	(TG)11(T)7/(TG)11(T)7		

Coordinates Not Called:

B

Test CF Clinical Sequencing Assay
For In Vitro Diagnostic Use.
Run ID 151007_M00507_0111_000000000-AHNHT
Run Date 151007
Analysis Version 2.2.31.1

Sample ID NA07904_0-1003
Sample Name NA07904_0
Control
Comment
Performance Pass
Sample Call Rate 100.00%

Variant Type	Coordinate	Chromosome	Frequency	Depth	cDNA Name (HGVS)	Protein Name (HGVS)	dbSNP ID	Reference	Result	Interpretation
Div	117282541	chr7	0.4661035	23670	c.3773_3774insT	p.Leu1258PhefsX7	rs121908789	C	C/CT	
PolyTGPolyT	117188661	chr7	N/A	N/A	N/A	PolyTG/PolyT	(TG)11(T)7	(TG)11(T)7/(TG)11(T)7		

Coordinates Not Called:

Figure 1: Report Files for LRM and MSR —The format and content of the final reports for LRM (A) and MSR (B) are identical except for the analysis version numbers.

Table 6: Description of Changes in Updated Variant Annotations from CFTRCarrierScreenPanelRev22 to CFTRCarrierScreenPanelRev23

Genomic Locations		
Mutations (common name)	**Previous genomic location (chr 7)**	**Corrected genomic locations (chr 7)**
1259insA	117182079	117182080
1548delG	117199541	117199542
3659delC	117267633	117267634
4382delA	117306968	117306969
Mutation Names (common names)		
Previous mutation name		**Corrected mutation name**
CFTR dele2, 3		CFTRdele2, 3
405+1 G->A		405+1G>A
457TAT->G		457TAT>G
711+5 G->A		711+5G>A
712-1 G->T		712-1G>T
1341+1G->A		1341+1G>A
S466X (C->A)		S466X (C>A)
S466X (C->G)		S466X (C>G)
1717-8G->A		1717-8G>A
1811+1.6kb A->G		1811+1.6kbA>G
1812-1 G->A		1812-1G>A
3121-1G->A		3121-1G>A
4005+1G->A		4005+1G>A
cDNA Names		
Variation		**Previous name**
PolyTGPolyT[a]		c.1210-12T[5_9]
Protein Names		
Mutations (common name)	**Previous protein name**	**Revised protein name**
3120G>A[b]	p.Gln996Gln	p.Gln996=
4209TGTT>AA	p.Val1360delfsX?/(CFTR1)	p.Val1360Thrfs

a. The previous cDNA name was deleted, since it only referenced PolyT, and did not include PolyTG
b. RefSeq ID rs121908797

References

1. Illumina (2015) MiSeqDx Universal Kit 1.0 Package Insert. (support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/dx/miseqdx_universal_kit/miseqdx-universal-kit-package-insert-15039608-b.pdf).
2. Illumina (2017) MiSeqDx Cystic Fibrosis 139-Variant Assay Package Insert. (support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/dx/miseqdx_cystic_fibrosis_139-variant_assay/miseqdx-cystic-fibrosis-139-variant-package-insert-15038347-02.pdf).
3. Illumina (2017) MiSeqDx Cystic Fibrosis Clinical Sequencing AssayPackage Insert. (support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/dx/miseqdx_cystic_fibrosis_clinical_sequencing_assay/miseqdx-cystic-fibrosis-clinical-sequencing-package-insert-15038344-01.pdf).

Intended Use Statements

MiSeq™Dx Instrument Intended Use

The MiSeqDx instrument is intended for targeted sequencing of DNA libraries from human genomic DNA extracted from peripheral whole blood or formalin-fixed, paraffin-embedded (FFPE) tissue, when used for *in vitro* diagnostic (IVD) assays performed on the instrument. The MiSeqDx instrument is not intended for whole genome or *de novo* sequencing. The MiSeqDx instrument is to be used with registered and listed, cleared, or approved IVD reagents and analytical software.

MiSeq™Dx Cystic Fibrosis 139-Variant Assay Intended Use

The Illumina MiSeqDx Cystic Fibrosis 139-Variant Assay is a qualitative *in vitro* diagnostic system used to simultaneously detect 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 variants include those recommended in 2004 by the American College of Medical Genetics (ACMG)[1] and in 2011 by the American College of Obstetricians and Gynecologists (ACOG).[2] The test is intended for carrier screening in adults of reproductive age, in confirmatory diagnostic testing of newborns and children, and as an initial test to aid in the diagnosis of individuals with suspected cystic fibrosis. The results of this test are intended to be interpreted by a board-certified clinical molecular geneticist or equivalent and should be used in conjunction with other available laboratory and clinical information. This test is not indicated for use for newborn screening, fetal diagnostic testing, pre-implantation testing, or for stand-alone diagnostic purposes. The test is intended to be used on the Illumina MiSeqDx instrument.

References for MiSeqDx Cystic Fibrosis 139-Variant Assay

1. Watson MS, Cutting GR, Desnick RJ, et al. Cystic fibrosis population carrier screening: 2004 revision of American College of Medical Genetics mutation panel. *Genet Med* 2004;6(5):387–391.
2. American College of Obstetricians and Gynecologists Committee on Genetics. ACOG Committee Opinion No. 486: Update on carrier screening for cystic fibrosis. *Obstet Gynecol*. 2011;117(4):1028-1031.

MiSeq™Dx Cystic Fibrosis Clinical Sequencing Assay Intended Use

The Illumina MiSeqDx Cystic Fibrosis Clinical Sequencing Assay is a targeted sequencing *in vitro* diagnostic system that re-sequences the protein coding regions and intron/exon boundaries of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene in genomic DNA isolated from human peripheral whole blood specimens collected in K2EDTA. The test detects single nucleotide variants and small indels within the region sequenced, and additionally reports on two deep intronic mutations and two large deletions. The test is intended to be used on the Illumina MiSeqDx instrument.

The test is intended to be used as an aid in the diagnosis of individuals with suspected cystic fibrosis (CF). This assay is most appropriate when the patient has an atypical or non-classic presentation of CF or when other mutation panels have failed to identify both causative mutations. The results of the test are intended to be interpreted by a board-certified clinical molecular geneticist or equivalent and should be used in conjunction with other available information including clinical symptoms, other diagnostic tests, and family history. This test is not indicated for use for stand-alone diagnostic purposes, fetal diagnostic testing, pre-implantation testing, carrier screening, newborn screening, or population screening.

MiSeq™Dx Universal Kit Intended Use

The MiSeqDx Universal Kit 1.0 is a set of reagents and consumables used in the processing of human genomic DNA samples derived from peripheral whole blood, and in the subsequent targeted sequencing of the resulting sample libraries. User-supplied analyte-specific reagents are required for the preparation of libraries targeting specific genomic regions of interest. The MiSeqDx Universal Kit 1.0 is intended for use with the MiSeqDx instrument.

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