MiSeqDx® Instrument for Instruments with Dual Boot Configuration

FOR IN VITRO DIAGNOSTIC USE

Catalog # DX-410-1001

Intended Use

The Illumina MiSeqDx is a sequencing instrument that measures fluorescence signals of labeled nucleotides through the use of instrument specific reagents and flow cells (MiSeqDx Universal Kit 1.0), imaging hardware, and data analysis software. The MiSeqDx Platform is intended for targeted sequencing of human genomic DNA from peripheral whole blood samples. The MiSeqDx Platform is not intended for whole genome or de novo sequencing.

Principles of Procedure

The Illumina MiSeqDx is intended for targeted re-sequencing of human genomic DNA from peripheral whole blood specimens using Illumina supplied consumables. The genomic DNA is processed through the library preparation steps, which specifically amplifies the intended genomic regions of each sample using the custom oligonucleotides designed by the user, while also adding the indexes and flow cell capture sequences to the amplified products. Library preparation consists of four key steps: Hybridization, Extension-Ligation, PCR Amplification, and Library Normalization. The resulting normalized sample libraries are ready for sequencing on the Illumina MiSeqDx instrument using SBS (sequencing by synthesis) chemistry. SBS chemistry uses a reversible-terminator method to detect single nucleotide bases as they are incorporated into growing DNA strands. The real-time analysis (RTA) software performs image analysis and base calling, as well as assigns a quality score to each base for each sequencing cycle. When primary analysis finishes, the MiSeq Reporter software on the MiSeqDx instrument processes base calls through secondary analysis, which includes demultiplexing, FASTQ file generation, alignment, variant calling, and generation of VCF (files) containing information about variants found at specific positions in a reference genome.

Limitations of the Procedure

1. For *in vitro* diagnostic use.
2. This product is limited to delivering:
   - Sequencing output >1 Gb
   - Reads >3 million
   - Read length (in paired end run) 2 x 150 bp
   - Bases higher than Q30 >75% (Greater than 75% of bases have Phred scale quality score greater than 30, indicating base call accuracy greater than 99.9%)
3. Variants in homopolymer runs exceeding eight bases will be filtered out in the VCF files (R8 filter).
4. The system has been validated for the detection of SNVs and up to 3 base deletions. Evaluation of 1 base insertions has been limited to 3 different insertions on 3 separate chromosomes.
5. The system has problems detecting 1 base insertions or deletions in homopolymer tracts (e.g. polyA).
6. The MiSeqDx system is designed to deliver qualitative (i.e. genotype) results.
7. As with any hybridization-based workflow, underlying polymorphisms, mutations, insertions or deletions in oligonucleotide-binding regions can affect the alleles being probed and, consequently, the calls made.
8. Recommended minimal coverage per amplicon needed for accurate variant calling (Q(max_gt | poly_site) >= 100) is 75x.

Product Components

The Illumina MiSeqDx consists of the following:

- MiSeqDx Instrument (Catalog # DX-410-1001)

The following software components are required for MiSeqDx operation and data analysis:

Software Application	Function	Description
MOS - MiSeqDx Operating Software	Controls instrument operation	The MOS software application manages the operation of the instrument during sequencing and generates images for use by Real-Time Analysis (RTA) software.
RTA - Real-time Analysis Software	Performs primary analysis	The RTA software application converts the images generated by MOS for each tile per cycle of the sequencing run into base call files which are inputs for MiSeq Reporter software. The RTA software application does not contain a user interface.
MiSeq Reporter	Performs secondary analysis	The MiSeq Reporter software processes base calls through secondary analysis which includes de-multiplexing, FASTQ file generation, alignment, variant calling, and generation of VCF files containing information about variants found at specific positions in a reference genome.

Storage and Handling

Element	Specification
Temperature	Transportation and Storage: -10°C to 40°C (14°F to 104°F) Operating Conditions: 19°C to 25°C (66°F to 77°F)
Humidity	Transportation and Storage: Non-condensing humidity Operating Conditions: 30–75% relative humidity (non-condensing)

Equipment and Materials Required, Not Provided

Sequencing Consumables

MiSeqDx Universal Kit 1.0 (Catalog # DX-103-1001)

User-Supplied Consumables

Make sure that the following user-supplied consumables are available before beginning a run.

Consumable	Purpose
Alcohol wipes, 70% Isopropyl or Ethanol, 70%	Cleaning the flow cell holder
Lab tissue, low-lint	Cleaning the flow cell stage
Lens paper, 4 x 6 in.	Cleaning the flow cell
Tween 20	Washing the instrument
Tweezers, square-tip plastic (optional)	Removing flow cell from flow cell shipping container
Water, laboratory-grade	Washing the instrument

Guidelines for Laboratory-Grade Water

Always use laboratory-grade water to perform instrument procedures. Never use tap water. Any of the following are acceptable examples:

- Illumina PW1
- 18 Megaohm (MΩ) water
- Milli-Q water
- Super-Q water
- Molecular biology-grade water

Warnings and Precautions

CAUTION

Federal law restricts this device to sale by or on the order of a physician or other practitioner licensed by the law of the State in which he/she practices, to use or order the use of the device.

1. **Some components of** Illumina **provided reagents for use with the** MiSeqDx **instrument contain potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations.** For environmental, health, and safety information, see the SDS at support.illumina.com/sds.html. (See respective product inserts for more information.)
2. Some components of Illumina provided reagent kits contain 2-Mercaptoethanol, a reducing agent. (See respective product inserts for more information.) Personal injury can occur through inhalation, ingestion, skin contact and eye contact. Use in a well ventilated area and dispose of any containers and unused contents in accordance with applicable local governmental safety standards. For more information, contact Illumina Technical Support.
3. Handle all specimens as if they are potentially infectious agents.
4. Failure to follow the procedures as outlined may result in erroneous results or significant reduction in sample quality.
5. Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink or smoke in designated work areas. Wear disposable gloves and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.
6. Proper laboratory practices and good laboratory hygiene is required to prevent PCR products from contaminating reagents, instrumentation, and genomic DNA samples. PCR contamination may cause inaccurate and unreliable results.
7. To prevent contamination, ensure that pre-amplification and post-amplification areas have dedicated equipment (e.g., pipettes, pipette tips, vortexer, and centrifuge).
8. Index-sample pairing must match the sample sheet exactly. Mismatches between the sample sheet and plate layout will result in loss of positive sample identification and incorrect result reporting.
9. During the library normalization step of the respective reagent product insert, it is extremely critical to completely resuspend the library bead pellet. This is essential for achieving consistent cluster density on the MiSeqDx flow cell.
10. Adhere to the specified incubation times in the library normalization steps as described in the respective reagent package insert. Improper incubation can affect library representation and cluster density.
11. Installation of user-supplied anti-virus software is strongly recommended to protect the computer against viruses. Consult user manual for instructions on installation.
12. Do not operate the MiSeqDx with any of the panels removed. Operating the instrument with any of the panels removed creates potential exposure to line voltage as well as DC voltages.
13. Do not touch the flow cell state in the flow cell compartment. The heater in this compartment operates between 22°C and 95°C and may result in burns.
14. The instrument weighs approximately 126 lbs. and could cause serious injury if dropped or mishandled.

Procedural Notes

The throughput per MiSeqDx run can be between 8 to 48 samples. The indexing primers used during PCR amplification must be chosen based on desired final sample throughput to ensure diversity in index sequence.

NOTE
For maximum throughput efficiency, perform library preparation for up to 96 samples and then divide the samples into two sequencing runs with a maximum of 48 samples each.

The MiSeqDx uses a green LED to sequence G/T bases and a red LED to sequence A/C bases. At each cycle at least one of two nucleotides for each color channel need to be read to ensure proper registration. It is important to maintain color balance for each base of the index read being sequenced, otherwise registration failure could occur during sequencing of the Index Read.

If sequencing fewer than the 48 samples in a sequencing run, select the appropriate indexes based on their sequences to maintain color balance in the green and red channels. At a minimum, runs with 8 to 48 samples must include the indexing primer combinations identified in the MiSeqDx Universal Kit 1.0 package insert.

To accurately process smaller runs, at least eight samples must be present. If six unique samples (excluding the positive and negative controls) are not available, it is acceptable to fill the run with sample replicates or any human genomic DNA sample. See the MiSeqDx Universal Kit 1.0 package insert for the minimal set of color-balanced indexes to use for eight-sample sequencing runs.

Instructions for Use

The following instructions for use of the MiSeqDx instrument require reagents provided in the MiSeqDx Universal Kit 1.0.

MiSeqDx Sample Sheet Preparation

1 From the Illumina Worklist Manager Welcome screen, select **Create Worklist**.
2 In the Test Type field, select **MiSeqDx Universal**.
3 In the Worklist Name field, enter a name for the sample sheet.
   — If the alpha-numeric reagent cartridge barcode ID is used for the sample sheet name, the MiSeq Operating Software (MOS) will find the sample sheet automatically.
   — If any other name is used for the sample sheet, the **Browse** button in the MiSeq Operating Software (MOS) can be used to locate the appropriate sample sheet.
4 [Optional] Enter a description to identify the run.
5 Make sure that the date matches the start date of the run.
6 Select **Next**.

Enter Sample Information

1 From the Table tab or the Plate tab, enter the following information for each well containing sample:
   a **Sample ID**—Enter a unique sample ID.
   b **Index 1 and Index 2**—Specify the index adapter that will be used for each Index Read.
   c **Manifest**—Specify the name of the manifest file that contains information about the samples in that particular well.
2 [Optional] To record more detailed information about the samples, enter a sample name and description.
3 [Optional] To identify controls on the plate, select Negative or Positive from the **Control** drop-down menu.
4 Go to the Plate Graphic tab and use the **Copy to Clipboard** or **Print** option to capture an image of the sample plate.
5 Select **Finish**.

Sample Preparation

The following steps should be conducted according to the Instructions for Use in the MiSeqDx Universal Kit 1.0 package insert:

- Hybridization of Oligonucleotide Pool
- Removal of Unbound Oligonucleotides
- Extension-Ligation of Bound Oligonucleotides
- PCR Amplification
- PCR Clean-Up
- Library Normalization
- Library Pooling

Prepare the Reagent Cartridge

1. Thaw the MiSeqDx Reagent Cartridge in a water bath containing enough room temperature deionized water to submerge the base of the reagent cartridge up to the water line printed on the reagent cartridge. Do not allow the water to exceed the maximum water line.
2. Allow the reagent cartridge to thaw in the room temperature water bath for approximately 1 hour or until completely thawed.
3. Remove the cartridge from the water bath and gently tap it on the bench to dislodge water from the base of the cartridge. Dry the base of the cartridge. Make sure that no water has splashed on the top of the reagent cartridge.

Inspect the Reagent Cartridge

1. Invert the reagent cartridge ten times to mix the thawed reagents, and then inspect that all positions are thawed.

   NOTE
   It is critical that the reagents in the cartridge are thoroughly thawed and mixed to ensure proper sequencing.

2. Inspect reagents in positions 1, 2, and 4 to make sure that they are fully mixed and free of precipitates.
3. Gently tap the cartridge on the bench to reduce air bubbles in the reagents.

   NOTE
   The MiSeqDx sipper tubes go to the bottom of each reservoir to aspirate the reagents, so it is important that the reservoirs are free of air bubbles.

4. Place the reagent cartridge on ice or set aside at 2°C to 8°C (up to 6 hours) until ready to set up the run. For best results, proceed directly to loading the sample and setting up the run.

Prepare Samples for Sequencing

1. Proceed with one **DAL** tube for sequencing.
2. If the **DAL** tube was stored frozen, thaw completely and mix by pipetting up and down.
3. Add 6 µl of 20 pM PhiX Internal Control to the **DAL** tube.
4. Pipette up and down 3–5 times to rinse the tip and ensure complete transfer.
5. Mix the **DAL** tube by vortexing the tube at top speed.
6. Centrifuge the **DAL** tube at 1000 × g at 20°C for 1 minute.
7. Incubate the **DAL** tube on a heat block at 96°C for 2 minutes.
8. After the incubation, invert the **DAL** tube 1–2 times to mix, then immediately place in the ice-water bath.
9. Keep the **DAL** tube in the ice-water bath for 5 minutes.

   NOTE
   Perform the heat denaturation step immediately before loading the **DAL** tube into the MiSeqDx reagent cartridge to ensure efficient template loading on the MiSeqDx flow cell.

Load Sample Libraries onto Cartridge

1. Use a separate, clean, and empty 1 ml pipette tip to pierce the foil seal over the reservoir on the reagent cartridge labeled **Load Samples**.
2. Pipette 600 µl of the **DAL** sample libraries into the **Load Samples** reservoir. Avoid touching the foil seal.
3. Check for air bubbles in the reservoir after loading sample. If air bubbles are present, gently tap the cartridge on the bench to release the bubbles.
4. Proceed directly to the run setup steps using the MiSeq Operating Software (MOS) interface.

Run Setup

1. Log in to the MiSeq Operating Software (MOS).
2. Select **Sequence**.
   A series of run setup screens open in the following order: Load Flow Cell, Load Reagents, Review, and Pre-Run Check.
3. When the Load Flow Cell screen appears, clean and then load the flow cell.
4. Close the flow cell latch and flow cell compartment door.
   Both the latch and compartment door must be closed before beginning the run. When the flow cell is loaded, the software reads and records the RFID. A confirmation that the RFID was successfully read appears in the lower-right corner of the screen.
5. When the Load Reagents screen appears, empty the waste bottle, load the MiSeqDx SBS Solution (PR2) bottle, and then load the reagent cartridge.
   When the MiSeqDx SBS Solution (PR2) bottle and reagent cartridge are loaded, the software reads and records the RFID. A confirmation that the RFID was successfully read appears in the lower-right corner of the screen.
6. Select the appropriate sample sheet.
   By default, the software will look for a sample sheet file with a name matching the barcode number of the reagent cartridge loaded on the instrument.
7. Confirm the run settings and results of the pre-run check.
8. Start the run.
   The Sequencing screen opens when the run begins. This screen provides a visual representation of the run in progress, including intensities and quality scores (Q-scores).

Results

The integrated primary analysis software (real-time analysis [RTA]) performs image analysis and base calling, as well as assigns a quality score to each base for each sequencing cycle. When primary analysis finishes, the MiSeq Reporter software on the MiSeqDx instrument begins secondary analysis, as described below.

Demultiplexing

Demultiplexing is the first step in analysis if the sample sheet lists multiple samples and the run has index reads. Demultiplexing separates data from pooled samples based on short index sequences that tag samples from different libraries. Each index read sequence is compared to the index sequences specified in the sample sheet. No quality values are considered in this step.

FASTQ File Generation

After demultiplexing, MiSeq Reporter generates intermediate files in the FASTQ format, which is a text format used to represent sequences. FASTQ files contain the reads for each sample and the quality scores, excluding reads from any clusters that did not pass filter. The quality score Q is calculated as $-10 \log_{10} P$, where P is the probability that base call is incorrect.

Alignment

Alignment compares sequences against the reference to identify a relationship between the sequences and assigns a score based on regions of similarity. Aligned reads are written to files in BAM format. For the MiSeqDx Universal Kit 1.0, MiSeq Reporter uses a banded Smith-Waterman algorithm that performs local sequence alignments to determine similar regions between two sequences.

Variant Calling

For the MiSeqDx Universal Kit 1.0, MiSeq Reporter uses Starling variant caller, which calls SNPs and small indels, as well as summarizes depth and probabilities for every site in the genome. Starling produces html-formatted reports of SNPs and indels and tab-delimited text files containing variants in the Variant Call Format (VCF). For information on how results can be calculated from VCF files, see the *MiSeq Reporter User Guide (document # 15039188)*.

Performance Characteristics

Accuracy

Three separate studies were conducted to assess the accuracy of the MiSeqDx Platform.

Study 1

This study used a representative assay designed to query a variety of genes covering 24,434 bases across 19 different chromosomes, and containing potentially clinically relevant exons. The 13 unique samples used in this study are from two parents and 11 children that have been frequently sequenced by multiple laboratories and sequencing methodologies. There are six samples from females and seven from males. Accuracy was determined for single nucleotide variants (SNVs) by comparing the study data to a well-characterized reference database. The reference database sequence was derived from the combination of multiple sequencing methodologies, publicly available data, and hereditary information. The following table to evaluate accuracy of the system was compiled based on data from the first run in the study. No repeat testing was done for this study.

The results from this study are presented below.

Table 1 Study 1 Amplicon-level Accuracy Data for the MiSeqDx Platform

Amplicon	Chromosome	Analyzed Fragment Size[1]	Amplicon Genomic Content	# Unique Samples	Total # Samples Analyzed[2]	# Calls Per Sample That Could Be Made[3]	# No Calls[4]	# Correct Calls Per Sample[5]	# Incorrect Calls[6]	% Correct Calls[7]
1	1	132	Poly C (5), 63% GC	13	15	132	0	132	0	100
2	1	128	Poly T (5)	13	15	128	0	128	0	100
3	2	133	-	13	15	133	0	133	0	100
4	2	119	-	13	15	119	0	119	0	100
5	2	127	Poly T (5)	13	15	127	0	127	0	100
6	2	135	Poly A (6)	13	15	135	0	135	0	100
7	2	122	Poly T (5), Poly C (5)	13	15	122	0	122	0	100
8	2	110	Poly T (5)	13	15	110	0	110	0	100
9[8]	2	131	Poly A (14)	13	15	130-131	0	130-131	9	99.54
10	2	117	-	13	15	117	0	117	0	100
11	2	121	-	13	15	121	0	121	0	100
12	2	114	-	13	15	114	0	114	0	100
13	2	129	Poly A (5)	13	15	129	0	129	0	100
14	3	131	Poly A (5), Poly T (5)	13	15	131	0	131	0	100
15	3	130	-	13	15	130	0	130	0	100
16	3	130	-	13	15	130	0	130	0	100
17	3	117	-	13	15	117	0	117	0	100

Amplicon	Chromosome	Analyzed Fragment Size[1]	Amplicon Genomic Content	# Unique Samples	Total # Samples Analyzed[2]	# Calls Per Sample That Could Be Made[3]	# No Calls[4]	# Correct Calls Per Sample[5]	# Incorrect Calls[6]	% Correct Calls[7]
18	3	136	Poly T (5)	13	15	136	0	136	0	100
19	3	131	Poly T (5), SNV	13	15	131	0	131	0	100
20	3	123	Poly A (5)	13	15	123	0	123	0	100
21	3	117	Poly A (6), Poly T (5), Homologous region on a different chromosome	13	15	117	0	117	0	100
22	3	119	Homologous region on a different chromosome	13	15	119	0	119	0	100
23	3	120	-	13	15	120	0	120	0	100
24	3	129	Poly T (5)	13	15	129	0	129	0	100
25	4	133	Poly C (7), 66% GC	13	15	133	0	133	0	100
26	4	135	Poly C (5), 69% GC	13	15	135	0	135	0	100
27	4	123	SNV	13	15	123	0	123	0	100
28	4	134	-	13	15	134	0	134	0	100
29	4	132	-	13	15	132	0	132	0	100
30	4	121	Poly A (5), SNV	13	15	121	0	121	0	100
31	4	125	-	13	15	125	0	125	0	100

Amplicon	Chromosome	Analyzed Fragment Size[1]	Amplicon Genomic Content	# Unique Samples	Total # Samples Analyzed[2]	# Calls Per Sample That Could Be Made[3]	# No Calls[4]	# Correct Calls Per Sample[5]	# Incorrect Calls[6]	% Correct Calls[7]
32	4	134	Poly T (5)	13	15	134	0	134	0	100
33	4	118	-	13	15	118	0	118	0	100
34	4	122	Poly A (5)	13	15	122	0	122	0	100
35	4	131	-	13	15	131	0	131	0	100
36	4	133	-	13	15	133	0	133	0	100
37	4	128	Poly T (6)	13	15	128	0	128	0	100
38	4	131	-	13	15	131	0	131	0	100
39	4	129	Poly A (5), Poly T (5), SNV	13	15	129	0	129	0	100
40	4	133	Poly T (5), SNV	13	15	133	0	133	0	100
41	4	112	SNV	13	15	112	0	112	0	100
42	4	133	-	13	15	133	0	133	0	100
43	4	135	-	13	15	135	0	135	0	100
44	4	122	-	13	15	122	0	122	0	100
45	4	117	-	13	15	117	0	117	0	100
46[9]	4	124	-	13	15	125	0	125	0	100
47	4	117	Poly T (5)	13	15	117	0	117	0	100
48	4	128	Poly A (7)	13	15	128	0	128	0	100
49	4	123	Poly A (6)	13	15	123	0	123	0	100

Amplicon	Chromosome	Analyzed Fragment Size[1]	Amplicon Genomic Content	# Unique Samples	Total # Samples Analyzed[2]	# Calls Per Sample That Could Be Made[3]	# No Calls[4]	# Correct Calls Per Sample[5]	# Incorrect Calls[6]	% Correct Calls[7]
50	4	133	-	13	15	133	0	133	0	100
51	4	112	-	13	15	112	0	112	0	100
52	4	129	-	13	15	129	0	129	0	100
53	4	126	-	13	15	126	0	126	0	100
54	4	132	-	13	15	132	0	132	0	100
55	5	131	-	13	15	131	0	131	0	100
56	5	119	-	13	15	119	0	119	0	100
57	5	120	Poly A (5)	13	15	120	0	120	0	100
58	5	119	-	13	15	119	0	119	0	100
59	5	118	-	13	15	118	0	118	0	100
60	5	112	-	13	15	112	0	112	0	100
61	5	120	-	13	15	120	0	120	0	100
62	5	120	Poly A (5)	13	15	120	0	120	0	100
63	5	115	CT(5)	13	15	115	0	115	0	100
64	5	112	SNV	13	15	112	0	112	0	100
65	5	135	Poly T (6)	13	15	135	0	135	0	100
66	5	131	63% GC	13	15	131	0	131	0	100
67	5	121	-	13	15	121	0	121	0	100
68	5	132	Poly A (6), Poly T (8)	13	15	132	0	132	0	100

Amplicon	Chromosome	Analyzed Fragment Size[1]	Amplicon Genomic Content	# Unique Samples	Total # Samples Analyzed[2]	# Calls Per Sample That Could Be Made[3]	# No Calls[4]	# Correct Calls Per Sample[5]	# Incorrect Calls[6]	% Correct Calls[7]
69	7	133	-	13	15	133	0	133	0	100
70	7	120	60% GC	13	15	120	0	120	0	100
71	7	135	-	13	15	135	0	135	0	100
72	7	126	Poly A (5), 59% GC	13	15	126	0	126	0	100
73	7	134	-	13	15	134	0	134	0	100
74	7	122	Poly C (5), 63% GC	13	15	122	0	122	0	100
75	7	127	59% GC; SNV	13	15	127	0	127	0	100
76	7	123	-	13	15	123	0	123	0	100
77	7	125	-	13	15	125	0	125	0	100
78	7	133	Poly A (5), Poly T (5)	13	15	133	0	133	0	100
79	7	116	-	13	15	116	0	116	0	100
80	7	135	-	13	15	135	0	135	0	100
81	7	118	-	13	15	118	0	118	0	100
82	7	136	67% GC	13	15	136	0	136	0	100
83	7	131	58% GC	13	15	131	0	131	0	100
84	7	119	Poly G (6), 61% GC	13	15	119	0	119	0	100

Amplicon	Chromosome	Analyzed Fragment Size[1]	Amplicon Genomic Content	# Unique Samples	Total # Samples Analyzed[2]	# Calls Per Sample That Could Be Made[3]	# No Calls[4]	# Correct Calls Per Sample[5]	# Incorrect Calls[6]	% Correct Calls[7]
85	7	122	Poly T (5)	13	15	122	0	122	0	100
86	7	123	Poly A (6)	13	15	123	0	123	0	100
87	8	127	60% GC	13	15	127	0	127	0	100
88	8	129	57% GC	13	15	129	0	129	0	100
89	9	130	Poly T (5)	13	15	130	0	130	0	100
90	9	116	-	13	15	116	0	116	0	100
91	9	119	Homologous region on a different chromosome	13	15	119	0	119	0	100
92	9	121	-	13	15	121	0	121	0	100
93	9	117	Homologous region on a different chromosome	13	15	117	0	117	0	100
94	9	114	-	13	15	114	0	114	0	100
95[10]	9	129	Poly A (14)	13	15	130	0	129 (of 130)	15	99.23
96	9	114	Homologous region on a different chromosome; SNV	13	15	114	0	114	0	100
97	9	122	-	13	15	122	0	122	0	100
98	9	127	Poly A (5), Poly C (5)	13	15	127	0	127	0	100
99	9	133	-	13	15	133	0	133	0	100

Amplicon	Chromosome	Analyzed Fragment Size[1]	Amplicon Genomic Content	# Unique Samples	Total # Samples Analyzed[2]	# Calls Per Sample That Could Be Made[3]	# No Calls[4]	# Correct Calls Per Sample[5]	# Incorrect Calls[6]	% Correct Calls[7]
100	9	138	64% GC	13	15	138	0	138	0	100
101	9	139	-	13	15	139	0	139	0	100
102	9	116	-	13	15	116	0	116	0	100
103	9	133	Poly A (5), 57% GC	13	15	133	0	133	0	100
104	9	138	57% GC	13	15	138	0	138	0	100
105	9	136	Poly C (5), 67% GC	13	15	136	0	136	0	100
106	9	118	70% GC	13	15	118	0	118	0	100
107	10	128	62% GC	13	15	128	0	128	0	100
108	10	120	60% GC	13	15	120	0	120	0	100
109	10	139	58% GC; SNV	13	15	139	0	139	0	100
110	10	118	57% GC	13	15	118	0	118	0	100
111	10	123	Poly T (5)	13	15	123	0	123	0	100
112	10	121	-	13	15	121	0	121	0	100
113	10	129	26% GC	13	15	129	0	129	0	100
114	10	122	-	13	15	122	0	122	0	100
115	10	124	Poly T (5); Homologous region on a different chromosome	13	15	124	0	124	0	100

Amplicon	Chromosome	Analyzed Fragment Size[1]	Amplicon Genomic Content	# Unique Samples	Total # Samples Analyzed[2]	# Calls Per Sample That Could Be Made[3]	# No Calls[4]	# Correct Calls Per Sample[5]	# Incorrect Calls[6]	% Correct Calls[7]
116	10	135	CA(4)	13	15	135	0	135	0	100
117	10	135	Poly A (6); Homologous region on a different chromosome	13	15	135	0	135	0	100
118	10	119	Poly C (5); SNV	13	15	119	0	119	0	100
119	10	125	-	13	15	125	0	125	0	100
120	10	131	-	13	15	131	0	131	0	100
121	10	117	-	13	15	117	0	117	0	100
122	10	116	-	13	15	116	0	116	0	100
123	10	129	58% GC	13	15	129	0	129	0	100
124	11	117	Poly T (10)	13	15	117	0	117	0	100
125	11	117	Poly T (5)	13	15	117	0	117	0	100
126	11	113	Poly A (5)	13	15	113	0	113	0	100
127	11	129	-	13	15	129	0	129	0	100
128	11	121	Poly T (5)	13	15	121	0	121	0	100
129	11	123	-	13	15	123	0	123	0	100
130	11	127	Poly A (6)	13	15	127	0	127	0	100
131	11	136	Poly T (6)	13	15	136	0	136	0	100
132	11	132	Poly T (5)	13	15	132	0	132	0	100

Amplicon	Chromosome	Analyzed Fragment Size[1]	Amplicon Genomic Content	# Unique Samples	Total # Samples Analyzed[2]	# Calls Per Sample That Could Be Made[3]	# No Calls[4]	# Correct Calls Per Sample[5]	# Incorrect Calls[6]	% Correct Calls[7]
133	11	115	-	13	15	115	0	115	0	100
134	11	117	Poly T (8); 19% GC	13	15	117	0	117	0	100
135	11	134	Poly A (5); Poly T (5)	13	15	134	0	134	0	100
136	11	131	Poly A (5)	13	15	131	0	131	0	100
137	11	133	26% GC; SNV	13	15	133	0	133	0	100
138	11	137	Poly T (8); SNV	13	15	137	0	137	0	100
139	11	131	Poly A (5)	13	15	131	0	131	0	100
140	12	131	-	13	15	131	0	131	0	100
141	12	128	-	13	15	128	0	128	0	100
142	12	133	Poly A (5)	13	15	133	0	133	0	100
143	12	136	-	13	15	136	0	136	0	100
144	12	124	-	13	15	124	0	124	0	100
145	12	122	59% GC	13	15	122	0	122	0	100
146	13	122	-	13	15	122	0	122	0	100
147	13	116	Poly C (5)	13	15	116	0	116	0	100
148	13	133	-	13	15	133	0	133	0	100
149	13	117	SNV	13	15	117	0	117	0	100

Amplicon	Chromosome	Analyzed Fragment Size[1]	Amplicon Genomic Content	# Unique Samples	Total # Samples Analyzed[2]	# Calls Per Sample That Could Be Made[3]	# No Calls[4]	# Correct Calls Per Sample[5]	# Incorrect Calls[6]	% Correct Calls[7]
150	13	124	Poly T (6)	13	15	124	0	124	0	100
151	13	123	Poly T (5); 26% GC	13	15	123	0	123	0	100
152	13	115	Poly A (5)	13	15	115	0	115	0	100
153	13	125	-	13	15	125	0	125	0	100
154	13	121	-	13	15	121	0	121	0	100
155	13	123	-	13	15	123	0	123	0	100
156	13	114	-	13	15	114	0	114	0	100
157	13	119	-	13	15	119	0	119	0	100
158	14	122	58% GC	13	15	122	0	122	0	100
159	16	122	-	13	15	122	0	122	0	100
160	16	121	-	13	15	121	0	121	0	100
161	16	123	Poly C (5)	13	15	123	0	123	0	100
162	17	119	-	13	15	119	0	119	0	100
163	17	119	61% GC	13	15	119	0	119	0	100
164	17	135	-	13	15	135	0	135	0	100
165	17	116	Poly C (6); 60% GC; SNV	13	15	116	0	116	0	100
166	17	123	-	13	15	123	0	123	0	100
167	17	116	62% GC	13	15	116	0	116	0	100

Amplicon	Chromosome	Analyzed Fragment Size[1]	Amplicon Genomic Content	# Unique Samples	Total # Samples Analyzed[2]	# Calls Per Sample That Could Be Made[3]	# No Calls[4]	# Correct Calls Per Sample[5]	# Incorrect Calls[6]	% Correct Calls[7]
168	17	118	Poly C (5); 65% GC	13	15	118	0	118	0	100
169	17	129	-	13	15	129	0	129	0	100
170	17	131	Poly G (6); 67% GC; SNV	13	15	131	0	131	0	100
171	17	127	61% GC	13	15	127	0	127	0	100
172	17	118	Poly C (5)	13	15	118	0	118	0	100
173	17	138	61% GC	13	15	138	0	138	0	100
174	17	131	58% GC	13	15	131	0	131	0	100
175	18	112	-	13	15	112	0	112	0	100
176	18	124	-	13	15	124	0	124	0	100
177	18	134	Poly A (6)	13	15	134	0	134	0	100
178	18	129	-	13	15	129	0	129	0	100
179	18	133	-	13	15	133	0	133	0	100
180	18	118	-	13	15	118	0	118	0	100
181	18	114	60% GC	13	15	114	0	114	0	100
182	18	118	-	13	15	118	0	118	0	100
183	19	122	Poly G (6); 66% GC	13	15	122	0	122	0	100
184	19	139	64% GC	13	15	139	0	139	0	100

Amplicon	Chromosome	Analyzed Fragment Size[1]	Amplicon Genomic Content	# Unique Samples	Total # Samples Analyzed[2]	# Calls Per Sample That Could Be Made[3]	# No Calls[4]	# Correct Calls Per Sample[5]	# Incorrect Calls[6]	% Correct Calls[7]
185	19	131	67% GC	13	15	131	0	131	0	100
186	19	141	59% GC; Homologous region on a different chromosome	13	15	141	0	141	0	100
187	19	121	Poly C (5); 72% GC; Homologous region on a different chromosome	13	15	121	0	121	0	100
188	19	138	58% GC	13	15	138	0	138	0	100
189	19	123	64% GC	13	15	123	0	123	0	100
190	19	138	-	13	15	138	0	138	0	100
191	20	117	Poly T (5)	13	15	117	0	117	0	100
192	22	136	Poly A (7)	13	15	136	0	136	0	100
193	22	122	Poly A (5); Poly C (5)	13	15	122	0	122	0	100
194	22	122	62% GC; SNV	13	15	122	0	122	0	100
195	22	119	66% GC	13	15	119	0	119	0	100

[1] Analyzed fragment means the size of the sequenced genomic region in bases, not including target-specific primers.
[2] Total # of samples listed is 15 because two of the 13 unique samples analyzed were run in two independent replicates.
[3] # calls/sample that could be made is the number of bases that had adequate quality to be called by the system.
[4] # of no calls is the number of bases in an amplicon that results in a no call in the run.
[5] # correct calls per sample is number of bases in the amplicon that were called that had results that matched the human genome reference sequence build 19 and the well characterized composite reference.
[6] # incorrect calls were the total number of incorrect calls for the SNV or indel in that amplicon; additional details on incorrect calls are presented in footnotes below.

[7] % correct calls equals the correct call rate for all of the bases in the amplicon, where the correct call for the SNV or indel is based on the well characterized composite reference information and the correct call for the bases in the remainder of the amplicon sequence is based on comparison to human genome reference sequence build 19. This column may have more than one expected result for a given amplicon if some samples contain an indel while some do not, e.g., amplicon 9. The % correct calls for the samples with incorrect result is presented in the table.

[8] Amplicon 9 has a homopolymer run of 14 A's according to the human genome reference sequence build 19. However, the well characterized composite reference information for 7 out of 13 samples have 13 A's in this homopolymer run. In these 7 samples, this one base pair deletion represents a false negative in the MiSeqDx sequencing accuracy study.

[9] Amplicon 46 has a one base insertion which is reported in 9 samples in the well characterized reference database and is correctly detected in all analyzed samples.

[10] Amplicon 95 has a homopolymer run of 14 A's according to human genome reference sequence build 19. However, the well characterized composite reference sequences for 13 out of 13 samples have 15 A's in this homopolymer run. In these 13 samples, this one base pair insertion is a false negative in the MiSeqDx sequencing accuracy study.

The following table contains data from Study 1 presented with positive and negative percent agreement, where the variant results are compared to the well-characterized composite reference information for PPA calculations. Since the composite reference information only provides results for the single nucleotide variants and insertions/deletions, non-variant base results are compared to human genome reference sequence build 19 for NPA calculations. All non-variant bases had 100% agreement with the reference sequence. All SNVs had 100% agreement with the reference sequence. Variants that were missed were either 1 base insertions or 1 base deletions in the homopolymer regions.

Table 2 Agreement of the MiSeqDx Platform Base Call Results with Reference Data for 13 Well-characterized Samples

Sample	# Amplicons	% Amplicon Coverage[1]	Variants expected per sample[2]	Variants Correctly Called	Variants Missed[3]	Non-variant bases called correctly	PPA[4] (%)	NPA[5] (%)
NA12877	195	100	19	17	2	24418	89.47	100
NA12878	195	100	19	17	2	24417	89.47	100
NA12879	195	100	20	19	1	24416	95.00	100
NA12880	195	100	20	18	2	24417	90.00	100
NA12881	195	100	22	20	2	24415	90.91	100
NA12882	195	100	16	15	1	24419	93.75	100
NA12883	195	100	24	23	1	24412	95.83	100
NA12884	195	100	21	20	1	24415	95.24	100
NA12885	195	100	19	17	2	24417	89.47	100
NA12886	195	100	22	20	2	24415	90.91	100
NA12887	195	100	19	18	1	24416	94.74	100
NA12888	195	100	24	23	1	24412	95.83	100
NA12893	195	100	20	18	2	24417	90.00	100

[1] % Amplicon coverage is number of bases in the amplicons sequenced with confidence.
[2] Variants expected per sample includes both SNVs and indels.
[3] For the variants missed, please see the first table for study 1 and the footnotes 8-10.
[4] Positive percent agreement (PPA) = 100xTP/(TP+FN) where the true positives (TP) are the number of positive variant calls at genomic coordinates where variants are present according to the reference sequence and mutant allele called is concordant with reference sequence (column named "Variants called correctly") and the false negatives (FN) are the number of negative variant calls at genomic coordinates where variants are present according to the reference sequence (column named "Variants missed").
[5] Negative percent agreement (NPA) = 100xTN/(FP+TN) where the false positives (FP) are the number of positive variant calls at genomic coordinates where variants are absent according to the reference sequence, or if mutant allele called is discordant with reference sequence (not in the table; no false positive variants calls were made in this study) and true negatives (TN) are the number of negative variant calls at genomic coordinates where variants are absent according to the reference standard (column named "non-variant bases called correctly").

Study 2

The sequencing results for the amplicon panel above were compared to a highly confident genotype established for NA12878 by the National Institutes of Standards and Technology (NIST) (v.2.15[1]). Out of the 195 amplicons, 184 amplicons were within highly confident reference calls in the NIST sequence and were compared. Non-variant base calls were compared to human genome reference sequence build 19.

Table 3 Comparison of the MiSeqDx Platform Sequencing Results for NA12878 Sample with NIST Database

Sample	# Amplicons	% Amplicon Coverage[2]	Variants expected	Variants Correctly Called	Variants Missed	Non-variant bases called correctly	PPA[3] (%)	NPA[4] (%)
NA12878	184	100	17	16	1[5]	23066	94.12	100

[1] Zook, JM et al. Integrating sequencing datasets to form highly confident SNP and indel genotype calls for a whole human genome. arXiv:1307.4661 [q-bio.GN].
[2] % Amplicon coverage is number of bases in the amplicons sequenced with confidence.
[3] Positive percent agreement (PPA) = 100xTP/(TP+FN).
[4] Negative percent agreement (NPA) = 100xTN/(FP+TN).
[5] The missed variant is the one base pair deletion in amplicon 9 in the homopolymer run of 14 A's not called by the MiSeqDx that is present in the NIST sequence. Note that the NIST sequence does not include the one base pair insertion in the other homopolymer of A's that was present in the other reference database used above in study 1.

Study 3

An additional accuracy study was performed to assess the performance of small insertions and deletions within a representative assay, the Illumina MiSeqDx Cystic Fibrosis 139-Variant Assay, that included a subset of *CFTR* clinically significant genetic variations analyzed with the MiSeq Reporter software using the MiSeqDx Platform targeted DNA sequencing workflow. The queried insertions and deletions were detected where expected with high confidence. These samples were characterized by bidirectional Sanger sequencing as a reference method to establish the expected sequence.

Table 4 Summary of Indel Detection with the MiSeqDx Platform

Amplicon	Insert size	Amplicon Genomic Content	# calls/ sample that could be made	# of bases called/ sample	# of no calls	# of correct calls/ sample	# of incorrect calls	% correct calls
1	129	1 base insertion	130	130	0	130	0	100
2	154	3 base deletion	151	151	0	151	0	100
3	167	2 base deletion	165	165	0	165	0	100
4	134	1 base deletion	133	133	0	133	0	100
5	132	1 base deletion	131	131	0	131	0	100
6	129	1 base deletion	128	128	0	128	0	100

The data provided by these accuracy studies supports the claim that the MiSeqDx Platform can accurately sequence:

- GC content ≥ 19% (all bases in 135 out of 135 sequenced amplicons with 19% GC content called correctly)
- GC content ≤ 72% (all bases in 135 out of 135 sequenced amplicons with 72% GC content called correctly)
- PolyA lengths ≤ 7 (PolyA repeat of 7 nucleotides was called correctly in 270 out of 270 sequenced amplicons containing PolyA =7)
- PolyT lengths ≤ 8 (PolyT repeat of 8 nucleotides was called correctly in 270 out of 270 sequenced amplicons containing PolyT =8)
- PolyG lengths ≤ 6 (PolyG repeat of 6 nucleotides was called correctly in 405 out of 405 sequenced amplicons containing PolyG =6)
- PolyC lengths ≤ 7 (PolyC repeat of 7 nucleotides was called correctly in 135 out of 135 sequenced amplicons containing PolyC =7)

- Dinucleotide repeat lengths ≤ 5x (all bases in 135 out of 135 sequenced amplicons with 5x dinucleotide repeat were called correctly)
- Trinucleotide repeat lengths ≤ 4x (all bases in 810 out of 810 sequenced amplicons with 4x trinucleotide repeats were called correctly)
- 1 base insertions and 3 or fewer base deletions
  - 2 out of 3 1-base insertions tested were called correctly. Correct calls were made for two 1-base insertions in non-homopolymer regions in 82 amplicons. One 1-base insertion was not called in a homopolymer run of 14 A's on chromosome 2 in 135 amplicons.
  - 3 out of 4 1-base deletions called correctly. All correct calls were made in non-homopolymer regions in 4 amplicons. One 1-base deletion was not called in a homopolymer run of 14 A's on chromosome 9 in 63 amplicons.
  - 2-base deletion were called correctly in one sample.
  - 3-base deletions were called correctly in 21 samples.

Reproducibility

The reproducibility of the MiSeqDx Platform was determined using two representative assays.

Study 1

A representative assay was designed to query a variety of genes covering 24,434 bases across 19 different chromosomes, and containing potentially clinically relevant exons. The study examined 13 samples over nine runs using three different MiSeqDx instruments and three different operators (Table 5). A single lot of library preparation reagents and two lots of sequencing consumables were used. The 13 samples were from two parents and 11 children that had been frequently sequenced by multiple laboratories and sequencing methodologies. Two samples were run in duplicate, so each run generated results for 15 samples.

For the evaluation of lot-to-lot reproducibility, 94 samples and two non-template controls were tested across three lots. Each lot was split into two 48-sample runs to test all reagents and possible index primer combinations. All sequencing runs were completed by a single operator and on a single MiSeqDx instrument to remove any potential variance contributed from operator or instrument (Table 6).

Correct calls were determined for single nucleotide variants (SNVs) by comparing the study data to well characterized reference information. There were no failed runs or re-runs for the reproducibility study. The following tables show the results of the studies to evaluate reproducibility of the system.

Table 5 Study 1 Instrument-to-instrument Reproducibility Results for the MiSeqDx Platform (Amplicon-level)

Amplicon	Chr.	Analyzed Fragment Size[1]	Amplicon Genomic Content	# of samples run[2]	MiSeqDx 1			MiSeqDx 2			MiSeqDx 3		
					Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]	Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]	Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]
1	1	132	Poly C (5); 63% GC	135	0	0	100	23[6]	0	99.61[7]	39[6]	0	99.34[7]
2	1	128	Poly T (5)	135	0	0	100	0	0	100	0	0	100
3	2	133	-	135	0	0	100	0	0	100	0	0	100
4	2	119	-	135	0	0	100	0	0	100	0	0	100
5	2	127	Poly T (5)	135	0	0	100	0	0	100	0	0	100
6	2	135	Poly A (6)	135	0	0	100	0	0	100	0	0	100
7	2	122	Poly T (5); Poly C (5)	135	0	0	100	0	0	100	0	0	100
8	2	110	Poly T (5)	135	0	0	100	0	0	100	0	0	100
9	2	131	Poly A (14)	135	0	27[8]	99.54	0	27[8]	99.54	0	27[8]	99.54
10	2	117	-	135	0	0	100	0	0	100	0	0	100
11	2	121	-	135	0	0	100	0	0	100	0	0	100
12	2	114	-	135	0	0	100	0	0	100	0	0	100
13	2	129	Poly A (5)	135	0	0	100	0	0	100	0	0	100
14	3	131	Poly A (5); Poly T (5)	135	0	0	100	0	0	100	0	0	100
15	3	130	-	135	0	0	100	0	0	100	0	0	100
16	3	130	-	135	0	0	100	0	0	100	0	0	100

Amplicon	Chr.	Analyzed Fragment Size[1]	Amplicon Genomic Content	# of samples run[2]	MiSeqDx 1			MiSeqDx 2			MiSeqDx 3		
					Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]	Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]	Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]
17	3	117	-	135	0	0	100	0	0	100	0	0	100
18	3	136	Poly T (5)	135	0	0	100	0	0	100	0	0	100
19	3	131	Poly T (5); SNV	135	0	0	100	0	0	100	0	0	100
20	3	123	Poly A (5)	135	0	0	100	0	0	100	0	0	100
21	3	117	Poly A (6); Poly T (5); Homologous region on a different chromosome	135	0	0	100	0	0	100	0	0	100
22	3	119	Homologous region on a different chromosome	135	0	0	100	0	0	100	0	0	100
23	3	120	-	135	0	0	100	0	0	100	0	0	100
24	3	129	Poly T (5)	135	0	0	100	0	0	100	0	0	100
25	4	133	Poly C (7); 66% GC	135	0	0	100	0	0	100	0	0	100
26	4	135	Poly C (5); 69% GC	135	0	0	100	0	0	100	0	0	100
27	4	123	SNV	135	0	0	100	0	0	100	0	0	100
28	4	134	-	135	0	0	100	0	0	100	0	0	100

Amplicon	Chr.	Analyzed Fragment Size[1]	Amplicon Genomic Content	# of samples run[2]	MiSeqDx 1			MiSeqDx 2			MiSeqDx 3		
					Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]	Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]	Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]
29	4	132	-	135	0	0	100	0	0	100	0	0	100
30	4	121	Poly A (5); SNV	135	0	0	100	0	0	100	0	0	100
31	4	125	-	135	0	0	100	0	0	100	0	0	100
32	4	134	Poly T (5)	135	0	0	100	0	0	100	0	0	100
33	4	118	-	135	0	0	100	0	0	100	0	0	100
34	4	122	Poly A (5)	135	0	0	100	0	0	100	0	0	100
35	4	131	-	135	0	0	100	0	0	100	0	0	100
36	4	133	-	135	0	0	100	0	0	100	0	0	100
37	4	128	Poly T (6)	135	0	0	100	0	0	100	0	0	100
38	4	131	-	135	0	0	100	0	0	100	0	0	100
39	4	129	Poly A (5); Poly T (5); SNV	135	0	0	100	0	0	100	0	0	100
40	4	133	Poly T (5); SNV	135	0	0	100	0	0	100	0	0	100
41	4	112	SNV	135	0	0	100	0	0	100	0	0	100
42	4	133	-	135	0	0	100	0	0	100	0	0	100
43	4	135	-	135	0	0	100	0	0	100	0	0	100
44	4	122	-	135	0	0	100	0	0	100	0	0	100

Amplicon	Chr.	Analyzed Fragment Size[1]	Amplicon Genomic Content	# of samples run[2]	MiSeqDx 1			MiSeqDx 2			MiSeqDx 3		
					Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]	Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]	Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]
45	4	117	-	135	0	0	100	0	0	100	0	0	100
46	4	124	-	135	0	0	100	0	0	100	0	0	100
47	4	117	Poly T (5)	135	0	0	100	0	0	100	0	0	100
48	4	128	Poly A (7)	135	0	0	100	0	0	100	0	0	100
49	4	123	Poly A (6)	135	0	0	100	0	0	100	0	0	100
50	4	133	-	135	0	0	100	0	0	100	0	0	100
51	4	112	-	135	0	0	100	0	0	100	0	0	100
52	4	129	-	135	0	0	100	0	0	100	0	0	100
53	4	126	-	135	0	0	100	0	0	100	0	0	100
54	4	132	-	135	0	0	100	0	0	100	0	0	100
55	5	131	-	135	0	0	100	0	0	100	0	0	100
56	5	119	-	135	0	0	100	0	0	100	0	0	100
57	5	120	Poly A (5)	135	0	0	100	0	0	100	0	0	100
58	5	119	-	135	0	0	100	0	0	100	0	0	100
59	5	118	-	135	0	0	100	0	0	100	0	0	100
60	5	112	-	135	0	0	100	0	0	100	0	0	100
61	5	120	-	135	0	0	100	0	0	100	0	0	100
62	5	120	Poly A (5)	135	0	0	100	0	0	100	0	0	100
63	5	115	CT(5)	135	0	0	100	0	0	100	0	0	100

Amplicon	Chr.	Analyzed Fragment Size[1]	Amplicon Genomic Content	# of samples run[2]	MiSeqDx 1			MiSeqDx 2			MiSeqDx 3		
					Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]	Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]	Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]
64	5	112	SNV	135	0	0	100	0	0	100	0	0	100
65	5	135	Poly T (6)	135	0	0	100	0	0	100	0	0	100
66	5	131	63% GC	135	0	0	100	0	0	100	0	0	100
67	5	121	-	135	0	0	100	0	0	100	0	0	100
68	5	132	Poly A (6); Poly T (8)	135	0	0	100	0	0	100	0	0	100
69	7	133	-	135	0	0	100	0	0	100	0	0	100
70	7	120	60% GC	135	0	0	100	0	0	100	0	0	100
71	7	135	-	135	0	0	100	0	0	100	0	0	100
72	7	126	Poly A (5); 59% GC	135	0	0	100	0	0	100	0	0	100
73	7	134	-	135	0	0	100	0	0	100	0	0	100
74	7	122	Poly C (5); 63% GC	135	0	0	100	0	0	100	0	0	100
75	7	127	59% GC; SNV	135	0	0	100	0	0	100	0	0	100
76	7	123	-	135	0	0	100	0	0	100	0	0	100
77	7	125	-	135	0	0	100	0	0	100	0	0	100
78	7	133	Poly A (5); Poly T (5)	135	0	0	100	0	0	100	0	0	100

Amplicon	Chr.	Analyzed Fragment Size[1]	Amplicon Genomic Content	# of samples run[2]	MiSeqDx 1			MiSeqDx 2			MiSeqDx 3		
					Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]	Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]	Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]
79	7	116	-	135	0	0	100	0	0	100	0	0	100
80	7	135	-	135	0	0	100	0	0	100	0	0	100
81	7	118	-	135	0	0	100	0	0	100	0	0	100
82	7	136	67% GC	135	0	0	100	0	0	100	0	0	100
83	7	131	58% GC	135	0	0	100	0	0	100	0	0	100
84	7	119	Poly G (6); 61% GC	135	0	0	100	0	0	100	0	0	100
85	7	122	Poly T (5)	135	0	0	100	0	0	100	0	0	100
86	7	123	Poly A (6)	135	0	0	100	0	0	100	0	0	100
87	8	127	60% GC	135	0	0	100	0	0	100	0	0	100
88	8	129	57% GC	135	0	0	100	0	0	100	0	0	100
89	9	130	Poly T (5)	135	0	0	100	0	0	100	0	0	100
90	9	116	-	135	0	0	100	0	0	100	0	0	100
91	9	119	Homologous region on a different chromosome	135	0	0	100	0	0	100	0	0	100
92	9	121	-	135	0	0	100	0	0	100	0	0	100
93	9	117	Homologous region on a different chromosome	135	0	0	100	0	0	100	0	0	100

Amplicon	Chr.	Analyzed Fragment Size[1]	Amplicon Genomic Content	# of samples run[2]	MiSeqDx 1			MiSeqDx 2			MiSeqDx 3		
					Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]	Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]	Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]
94	9	114	-	135	0	0	100	0	0	100	0	0	100
95	9	129	Poly A (14)	135	0	45[9]	99.22	0	45[9]	99.22	0	45[9]	99.22
96	9	114	Homologous region on a different chromosome; SNV	135	0	0	100	0	0	100	0	0	100
97	9	122	-	135	0	0	100	0	0	100	0	0	100
98	9	127	Poly A (5); Poly C (5)	135	0	0	100	0	0	100	0	0	100
99	9	133	-	135	0	0	100	0	0	100	0	0	100
100	9	138	64% GC	135	0	0	100	0	0	100	0	0	100
101	9	139	-	135	0	0	100	0	0	100	0	0	100
102	9	116	-	135	0	0	100	0	0	100	0	0	100
103	9	133	Poly A (5); 57% GC	135	0	0	100	0	0	100	0	0	100
104	9	138	57% GC	135	0	0	100	0	0	100	0	0	100
105	9	136	Poly C (5); 67% GC	135	0	0	100	0	0	100	0	0	100
106	9	118	70% GC	135	0	0	100	0	0	100	0	0	100
107	10	128	62% GC	135	0	0	100	0	0	100	0	0	100

Amplicon	Chr.	Analyzed Fragment Size[1]	Amplicon Genomic Content	# of samples run[2]	MiSeqDx 1			MiSeqDx 2			MiSeqDx 3		
					Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]	Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]	Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]
108	10	120	60% GC	135	0	0	100	0	0	100	0	0	100
109	10	139	58% GC; SNV	135	0	0	100	0	0	100	0	0	100
110	10	118	57% GC	135	0	0	100	0	0	100	0	0	100
111	10	123	Poly T (5)	135	0	0	100	0	0	100	0	0	100
112	10	121	-	135	0	0	100	0	0	100	0	0	100
113	10	129	26% GC	135	0	0	100	0	0	100	0	0	100
114	10	122	-	135	0	0	100	0	0	100	0	0	100
115	10	124	Poly T (5); Homologous region on a different chromosome	135	0	0	100	0	0	100	0	0	100
116	10	135	CA(4)	135	0	0	100	0	0	100	0	0	100
117	10	135	Poly A (6); Homologous region on a different chromosome	135	0	0	100	0	0	100	0	0	100
118	10	119	Poly C (5); SNV	135	0	0	100	0	0	100	0	0	100
119	10	125	-	135	0	0	100	0	0	100	0	0	100
120	10	131	-	135	0	0	100	0	0	100	0	0	100

Amplicon	Chr.	Analyzed Fragment Size[1]	Amplicon Genomic Content	# of samples run[2]	MiSeqDx 1			MiSeqDx 2			MiSeqDx 3		
					Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]	Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]	Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]
121	10	117	-	135	0	0	100	0	0	100	0	0	100
122	10	116	-	135	0	0	100	0	0	100	0	0	100
123	10	129	58% GC	135	0	0	100	0	0	100	0	0	100
124	11	117	Poly T (10)	135	0	0	100	0	0	100	0	0	100
125	11	117	Poly T (5)	135	0	0	100	0	0	100	0	0	100
126	11	113	Poly A (5)	135	0	0	100	0	0	100	0	0	100
127	11	129	-	135	0	0	100	0	0	100	0	0	100
128	11	121	Poly T (5)	135	0	0	100	0	0	100	0	0	100
129	11	123	-	135	0	0	100	0	0	100	0	0	100
130	11	127	Poly A (6)	135	0	0	100	0	0	100	0	0	100
131	11	136	Poly T (6)	135	0	0	100	0	0	100	0	0	100
132	11	132	Poly T (5)	135	0	0	100	0	0	100	0	0	100
133	11	115	-	135	0	0	100	0	0	100	0	0	100
134	11	117	Poly T (8); 19% GC	135	0	0	100	0	0	100	0	0	100
135	11	134	Poly A (5); Poly T (5)	135	0	0	100	0	0	100	0	0	100
136	11	131	Poly A (5)	135	0	0	100	0	0	100	0	0	100
137	11	133	SNV; 26% GC	135	0	0	100	0	0	100	0	0	100

Amplicon	Chr.	Analyzed Fragment Size[1]	Amplicon Genomic Content	# of samples run[2]	MiSeqDx 1			MiSeqDx 2			MiSeqDx 3		
					Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]	Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]	Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]
138	11	137	Poly T (8); SNV	135	0	0	100	0	0	100	0	0	100
139	11	131	Poly A (5)	135	0	0	100	0	0	100	0	0	100
140	12	131	-	135	0	0	100	0	0	100	0	0	100
141	12	128	-	135	0	0	100	0	0	100	0	0	100
142	12	133	Poly A (5)	135	0	0	100	0	0	100	0	0	100
143	12	136	-	135	0	0	100	0	0	100	0	0	100
144	12	124	-	135	0	0	100	0	0	100	0	0	100
145	12	122	59% GC	135	0	0	100	0	0	100	0	0	100
146	13	122	-	135	0	0	100	0	0	100	0	0	100
147	13	116	Poly C (5)	135	0	0	100	0	0	100	0	0	100
148	13	133	-	135	0	0	100	0	0	100	0	0	100
149	13	117	SNV	135	0	0	100	0	0	100	0	0	100
150	13	124	Poly T (6)	135	0	0	100	0	0	100	0	0	100
151	13	123	Poly T (5); 26% GC	135	0	0	100	0	0	100	0	0	100
152	13	115	Poly A (5)	135	0	0	100	0	0	100	0	0	100
153	13	125	-	135	0	0	100	0	0	100	0	0	100
154	13	121	-	135	0	0	100	0	0	100	0	0	100

Amplicon	Chr.	Analyzed Fragment Size[1]	Amplicon Genomic Content	# of samples run[2]	MiSeqDx 1			MiSeqDx 2			MiSeqDx 3		
					Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]	Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]	Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]
155	13	123	-	135	0	0	100	0	0	100	0	0	100
156	13	114	-	135	0	0	100	0	0	100	0	0	100
157	13	119	-	135	0	0	100	0	0	100	0	0	100
158	14	122	58% GC	135	0	0	100	0	0	100	0	0	100
159	16	122	-	135	0	0	100	0	0	100	0	0	100
160	16	121	-	135	0	0	100	0	0	100	0	0	100
161	16	123	Poly C (5)	135	0	0	100	0	0	100	0	0	100
162	17	119	-	135	0	0	100	0	0	100	0	0	100
163	17	119	61% GC	135	0	0	100	0	0	100	0	0	100
164	17	135	-	135	0	0	100	0	0	100	0	0	100
165	17	116	Poly C (6); 60% GC; SNV	135	0	0	100	0	0	100	0	0	100
166	17	123	-	135	0	0	100	0	0	100	0	0	100
167	17	116	62% GC	135	0	0	100	0	0	100	0	0	100
168	17	118	Poly C (5); 65% GC	135	0	0	100	0	0	100	0	0	100
169	17	129	-	135	0	0	100	0	0	100	0	0	100

Amplicon	Chr.	Analyzed Fragment Size[1]	Amplicon Genomic Content	# of samples run[2]	MiSeqDx 1			MiSeqDx 2			MiSeqDx 3		
					Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]	Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]	Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]
170	17	131	Poly G (6); 67% GC; SNV	135	0	0	100	0	0	100	0	0	100
171	17	127	61% GC	135	0	0	100	0	0	100	0	0	100
172	17	118	Poly C (5)	135	0	0	100	0	0	100	0	0	100
173	17	138	61% GC	135	0	0	100	0	0	100	0	0	100
174	17	131	58% GC	135	0	0	100	0	0	100	0	0	100
175	18	112	-	135	0	0	100	0	0	100	0	0	100
176	18	124	-	135	0	0	100	0	0	100	0	0	100
177	18	134	Poly A (6)	135	0	0	100	0	0	100	0	0	100
178	18	129	-	135	0	0	100	0	0	100	0	0	100
179	18	133	-	135	0	0	100	0	0	100	0	0	100
180	18	118	-	135	0	0	100	0	0	100	0	0	100
181	18	114	60% GC	135	0	0	100	0	0	100	0	0	100
182	18	118	-	135	0	0	100	0	0	100	0	0	100
183	19	122	Poly G (6); 66% GC	135	0	0	100	0	0	100	0	0	100
184	19	139	64% GC	135	0	0	100	0	0	100	0	0	100
185	19	131	67% GC	135	0	0	100	0	0	100	0	0	100

Amplicon	Chr.	Analyzed Fragment Size[1]	Amplicon Genomic Content	# of samples run[2]	MiSeqDx 1			MiSeqDx 2			MiSeqDx 3		
					Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]	Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]	Total # no calls[3]	Total # incorrect calls[4]	% correct calls[5]
186	19	141	59% GC; Homologous region on a different chromosome	135	0	0	100	0	0	100	0	0	100
187	19	121	Poly C (5); 72% GC; Homologous region on a different chromosome	135	0	0	100	0	0	100	0	0	100
188	19	138	58% GC	135	0	0	100	0	0	100	0	0	100
189	19	123	64% GC	135	0	0	100	0	0	100	0	0	100
190	19	138	-	135	0	0	100	0	0	100	0	0	100
191	20	117	Poly T (5)	135	0	0	100	0	0	100	0	0	100
192	22	136	Poly A (7)	135	0	0	100	0	0	100	0	0	100
193	22	122	Poly A (5); Poly C (5)	135	0	0	100	0	0	100	0	0	100
194	22	122	62% GC; SNV	135	0	0	100	0	0	100	0	0	100
195	22	119	66% GC	135	0	0	100	0	0	100	0	0	100

[1] Analyzed fragment is the size of the sequenced genomic region in bases, not including target-specific primers.
[2] Number of samples is calculated from 9 runs of 15 samples (11 samples run once and 2 samples run twice).
[3] Total number of no calls is the combined number of no calls obtained for all 45 runs analyzing the specific amplicon using a specified MiSeqDx instrument.
[4] Total number of incorrect calls is the combined number of incorrect calls obtained for all 45 runs analyzing the specific amplicon using a specified MiSeqDx instrument.

[5] % correct calls equals the correct call rate for all of the bases in the amplicon, where the correct call for the SNV or indel is based on the well characterized reference database and the correct call for the bases in the remainder of the amplicon sequence is based on comparison to human genome reference sequence build 19. This column may have more than one expected result for a given amplicon if some samples are expected to have an indel and some are not, e.g., amplicon 9.

[6] Amplicon 1 had a number of bases whose genotype could not be called: 12 bases in 1/9 runs in NA12881; 1 base in 2/9 runs and 3 bases in 1/9 runs in NA12886; 20 bases in 1/9 runs and 26 bases in 1/9 runs in NA12888. This is due to low coverage at no call bases in those runs, where the average sequencing depth was 33.2, with a minimum of 21 and maximum of 52.

[7] When no-calls are not included in the calculation, the correct call rate is 100%.

[8] Amplicon 9 has a homopolymer run of 14 A's according to the human genome reference sequence build 19. However, the well characterized reference information for 7 out of 13 samples have 13 A's in this homopolymer run. In these 7 samples, this one base pair deletion is called a false negative, and is called as false negative reproducibly in all nine runs.

[9] Amplicon 95 has a homopolymer run of 14 A's according to human genome reference sequence build 19. However, the well characterized reference information sequences for 13 out of 13 samples have 15 A's in this homopolymer run. In these 13 samples, this one base pair insertion is 100% reproducibly not called (i.e., it is false negative).

Reproducibility study 1 results for each sample are shown compounded from all nine runs into one column. The results displayed are solely for just the single nucleotide variants and insertions/deletions results versus the reference database sequence for three runs on three instruments. This analysis demonstrated that the results for the variants were reproducible across nine runs for these samples.

Table 6 Summary of the MiSeqDx Platform Reproducibility Results for 13 Well-characterized Samples

DNA #	DNA Sample Id	# Runs per sample	# of SNVs	Single Nucleotide Variants (SNVs)			# of indels	Insertions\Deletions (Indels)		
				# Called Correctly	# of False Positives[1]	# of False Negatives[2]		# Called Correctly	# of False Positives[1]	# of False Negatives[2]
1	NA12877[3]	18	16	16	0	0	3	1	0	2
2	NA12878[3]	18	17	17	0	0	2	0	0	2
3	NA12879	9	18	18	0	0	2	1	0	1
4	NA12880	9	17	17	0	0	3	1	0	2
5	NA12881	9	19	19	0	0	3	1	0	2
6	NA12882	9	15	15	0	0	1	0	0	1
7	NA12883	9	22	22	0	0	2	1	0	1
8	NA12884	9	19	19	0	0	2	1	0	1
9	NA12885	9	17	17	0	0	2	0	0	2
10	NA12886	9	19	19	0	0	3	1	0	2
11	NA12887	9	18	18	0	0	1	0	0	1
12	NA12888	9	22	22	0	0	2	1	0	1
13	NA12893	9	17	17	0	0	3	1	0	2

[1] False Positive = Variant called by MiSeqDx sequencing run but not in reference database.
[2] False Negative = Variant in reference database but not called in MiSeqDx sequencing run.
[3] Samples NA12877 and NA12878 were run in duplicate. Replicate samples generated identical results.

Study 2

A site-to-site reproducibility study performed with a representative assay, the Illumina MiSeqDx Cystic Fibrosis 139 Variant Assay, included a subset of *CFTR* clinically significant genetic variations analyzed with the MiSeq Reporter software using the MiSeqDx Platform targeted DNA sequencing workflow. The blinded study used 3 trial sites and 2 operators at each site. Two well-characterized panels of 46 samples each were tested by each of the operators at each site for a total of 810 calls per site. The panels contained a mix of genomic DNA from cell lines with known variants in the *CFTR* gene, as well as leukocyte-depleted blood spiked with cell lines with known variants in the *CFTR* gene. The blood samples were provided to allow incorporation of the extraction steps used to prepare gDNA that serves as the primary input for the assay workflow. The sample pass rate, defined as the number of samples passing QC metrics on the first attempt, was 99.88%. All test results are based on initial testing.

Table 7 Summary of Reproducibility Study Results Performed with a Representative MiSeqDx Cystic Fibrosis 139-Variant Assay

Panel	Sample #	Sample Genotype	Variants	Total Calls per Site	Positive Agreeing Calls (Variants)			Negative Agreeing Calls (Wild Type)			# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
					Site 1	Site 2	Site 3	Site 1	Site 2	Site 3					
A	1	S549N (HET)		810	6	6	6	804	804	804	0	0	100	100	100
A	2	1812-1 G>A (HET)		810	6	6	6	804	804	804	0	0	100	100	100
A	3	Q493X/F508del (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	4[1]	F508del/2184delA (HET)		810	12	12	12	797	798	798	0	1[1]	100	100	100
A	5[2]	Y122X/R1158X (HET)		810	12	10	12	798	665	798	0	135[2]	94.44	94.44	94.44
A	6	F508del/2183AA>G (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	7	R75X (HET)		810	6	6	6	804	804	804	0	0	100	100	100
A	8	I507del/F508del (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	9[3]	F508del/W1282X (HET)		810	12	11	12	798	797	798	2[3]	0	97.22	99.96	99.92
A	10[3]	F508del/3272-26A>G (HET)		810	12	11	12	798	797	798	2[3]	0	97.22	99.96	99.92

Panel	Sample #	Sample Genotype	Variants	Total Calls per Site	Positive Agreeing Calls (Variants)			Negative Agreeing Calls (Wild Type)			# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
					Site 1	Site 2	Site 3	Site 1	Site 2	Site 3					
A	11	F508del/3849+10kbC>T (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	12	621+1G>T/3120+1G>A (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	13	E60X/F508del (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	14	M1101K (HET)		810	6	6	6	804	804	804	0	0	100	100	100
A	15	M1101K (HOM)		810	6	6	6	804	804	804	0	0	100	100	100
A	16	F508del (HOM)	I506V, I507V, F508C not present	828	6	6	6	822	822	822	0	0	100	100	100
A	17	F508del/3659delC (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	18	R117H/F508del (HET)	(TG)10 (T)9/ (TG)12 (T)5	816	18	18	18	798	798	798	0	0	100	100	100
A	19	621+1G>T/711+1G>T (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	20	G85E/621+1G>T (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	21	A455E/F508del (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	22	F508del/R560T (HET)		810	12	12	12	798	798	798	0	0	100	100	100

Panel	Sample #	Sample Genotype	Variants	Total Calls per Site	Positive Agreeing Calls (Variants)			Negative Agreeing Calls (Wild Type)			# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
					Site 1	Site 2	Site 3	Site 1	Site 2	Site 3					
A	23	F508del/Y1092X (C>A) (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	24	N1303K (HET)		810	6	6	6	804	804	804	0	0	100	100	100
A	25	G542X (HOM)		810	6	6	6	804	804	804	0	0	100	100	100
A	26	G542X (HET)		810	6	6	6	804	804	804	0	0	100	100	100
A	27	G551D/R553X (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	28	3849+10kbC>T (HOM)		810	6	6	6	804	804	804	0	0	100	100	100
A	29	WT		810	0	0	0	810	810	810	0	0	N/A	100	100
A	30	F508del (HET)		810	6	6	6	804	804	804	0	0	100	100	100
A	31	1717-1G>A (HET)		810	6	6	6	804	804	804	0	0	100	100	100
A	32	R1162X (HET)		810	6	6	6	804	804	804	0	0	100	100	100
A	33	R347P/G551D (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	34	R334W (HET)		810	6	6	6	804	804	804	0	0	100	100	100
A	35	WT		810	0	0	0	810	810	810	0	0	N/A	100	100
A	36	G85E (HET)		810	6	6	6	804	804	804	0	0	100	100	100
A	37	I336K (HET)		810	6	6	6	804	804	804	0	0	100	100	100
A	38	WT		810	0	0	0	810	810	810	0	0	N/A	100	100
A	39	F508del/3849+10kbC>T (HET)		810	12	12	12	798	798	798	0	0	100	100	100

Panel	Sample #	Sample Genotype	Variants	Total Calls per Site	Positive Agreeing Calls (Variants)			Negative Agreeing Calls (Wild Type)			# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
					Site 1	Site 2	Site 3	Site 1	Site 2	Site 3					
A	40	621+1G>T/3120+1G>A (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	41	F508del/3659delC (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	42	R117H/F508del (HET)	(TG)10 (T)9/ (TG)12 (T)5	816	18	18	18	798	798	798	0	0	100	100	100
A	43	G85E/621+1G>T (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	44	A455E/F508del (HET)		810	12	12	12	798	798	798	0	0	100	100	100
A	45	N1303K (HET)		810	6	6	6	804	804	804	0	0	100	100	100
A	46	G551D/R553X (HET)		810	12	12	12	798	798	798	0	0	100	100	100
B	47	2789+5G>A (HOM)		810	6	6	6	804	804	804	0	0	100	100	100
B	48	CFTR dele2, 3/F508del (HET)		810	12	12	12	798	798	798	0	0	100	100	100
B	49	F508del/1898+1G>A (HET)		810	12	12	12	798	798	798	0	0	100	100	100
B	50	WT		810	0	0	0	810	810	810	0	0	N/A	100	100
B	51	F508del/2143delT (HET)		810	12	12	12	798	798	798	0	0	100	100	100
B	52	3876delA (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	53	3905insT (HET)		810	6	6	6	804	804	804	0	0	100	100	100

Panel	Sample #	Sample Genotype	Variants	Total Calls per Site	Positive Agreeing Calls (Variants)			Negative Agreeing Calls (Wild Type)			# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
					Site 1	Site 2	Site 3	Site 1	Site 2	Site 3					
B	54	394delTT (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	55	F508del (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	56	WT		810	0	0	0	810	810	810	0	0	N/A	100	100
B	57	WT		810	0	0	0	810	810	810	0	0	N/A	100	100
B	58	F508del (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	59	WT		810	0	0	0	810	810	810	0	0	N/A	100	100
B	60	L206W (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	61	WT		810	0	0	0	810	810	810	0	0	N/A	100	100
B	62	G330X (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	63	WT		810	0	0	0	810	810	810	0	0	N/A	100	100
B	64	R347H (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	65	1078delT (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	66	G178R/F508del (HET)		810	12	12	12	798	798	798	0	0	100	100	100
B	67	S549R (c.1647T>G) (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	68	S549N (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	69	W846X (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	70	WT		810	0	0	0	810	810	810	0	0	N/A	100	100

Panel	Sample #	Sample Genotype	Variants	Total Calls per Site	Positive Agreeing Calls (Variants)			Negative Agreeing Calls (Wild Type)			# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
					Site 1	Site 2	Site 3	Site 1	Site 2	Site 3					
B	71	E92X/F508del (HET)		810	12	12	12	798	798	798	0	0	100	100	100
B	72[4]	621+1G>T/1154insTC (HET)		810	12	12	12	798	798	797	0	1[4]	100	99.96	99.96
B	73	G542X (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	74	F508del (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	75[2]	F508del (HET)		810	6	5	6	804	670	804	0	135[2]	94.44	94.44	94.44
B	76	F508del (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	77	621+1G>T/A455E (HET)		810	12	12	12	798	798	798	0	0	100	100	100
B	78	1812-1 G>A (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	79	WT		810	0	0	0	810	810	810	0	0	N/A	100	100
B	80	F508del/R553X (HET)		810	12	12	12	798	798	798	0	0	100	100	100
B	81	F508del/G551D (HET)		810	12	12	12	798	798	798	0	0	100	100	100
B	82	R347P/F508del (HET)		810	12	12	12	798	798	798	0	0	100	100	100
B	83	R117H/F508del (HET)	(TG)10 (T)9/ (TG)12 (T)5	816	18	18	18	798	798	798	0	0	100	100	100
B	84	I507del (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	85	2789+5G>A (HOM)		810	6	6	6	804	804	804	0	0	100	100	100

Panel	Sample #	Sample Genotype	Variants	Total Calls per Site	Positive Agreeing Calls (Variants)			Negative Agreeing Calls (Wild Type)			# Miscalls	# No Calls	Positive Agreement (%)	Negative Agreement (%)	Overall Agreement (%)
					Site 1	Site 2	Site 3	Site 1	Site 2	Site 3					
B	86[4]	CFTR dele2, 3/F508del (HET)		810	12	12	12	798	797	798	0	1[4]	100	99.96	99.96
B	87	F508del/1898+1G>A (HET)		810	12	12	12	798	798	798	0	0	100	100	100
B	88	WT		810	0	0	0	810	810	810	0	0	N/A	100	100
B	89	F508del/2143delT (HET)		810	12	12	12	798	798	798	0	0	100	100	100
B	90	3905insT (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	91	394delTT (HET)		810	6	6	6	804	804	804	0	0	100	100	100
B	92	F508del (HET)		810	6	6	6	804	804	804	0	0	100	100	100
Total				74556	2209			221182			4	273	99.77	99.88	99.88

[1] The wild type location corresponding to the N1303K variant for one replicate resulted in a No Call due to insufficient coverage.
[2] One replicate of samples 5 and 75 had a 0% call rate. Further investigation indicates that samples may not have been added to the sample plate prior to library preparation because the sample volumes remaining in the tubes were consistent with no volume having been removed.
[3] Evidence indicates that samples 9 and 10 were likely switched by the operator prior to library preparation.
[4] The wild type location corresponding to the M1V variant for one replicate of each of two samples resulted in a No Call due to insufficient coverage.

DNA Extraction

Three different extraction methods, magnetic bead extraction, alcohol precipitation and silica filter column isolation were evaluated using K_2EDTA anticoagulated whole blood. Fourteen unique blood samples were used in the study representing a range of genotypes from one representative gene. The three DNA extraction methods were tested independently by 2 different operators who each performed 3 runs per extraction method. Each extraction was performed by each operator on different days. The DNA concentration and A260/A280 ratio of the extracted gDNA samples was determined using spectrophotometry. The total sample size for each extraction method in this study was 168 (14 samples x 2 operators/extraction method x 3 runs/operator x 2 replicates/extracted gDNA sample).

Extraction Method	Number of samples tested	Call Rate	Accuracy[1]	Sample First Pass Rate[2]
Alcohol Precipitation	168	100%	100%	100%
Silica Filter Column Isolation	168	100%	100%	100%
Magnetic Bead extraction	168	100%	100%	100%

[1]Accuracy - The percent agreement with a reference test method (bi-directional sequencing by Sanger) calculated for those base positions that receive a base call.

[2]Sample First Pass Rate - The number of samples that meet the specified call rate the first time they are processed (i.e., without the need for a re-run or additional processing) as a percentage of the total number of samples run during a single MiSeqDx sequencing experiment.

DNA Input

The DNA input range for the MiSeqDx Platform was evaluated by performing a serial dilution study using 14 representative DNA samples containing 16 unique single gene variants. Each sample was tested in duplicate at 9 DNA input levels ranging from 1250 ng to 1 ng (1250 ng, 500 ng, 250 ng, 100 ng, 50 ng, 25 ng, 10 ng, 5 ng, and 1 ng). For determination of accuracy, sample genotypes were compared to bidirectional Sanger sequencing data. 1250 ng and 25 ng were identified as the upper and lower bound for DNA input respectively as they had ≥95% sample first pass rate with no incorrect calls (100% accuracy and call rate).

DNA inputs of 1250 ng, 250 ng, and 100 ng were further tested with 4 representative DNA samples and 20 replicates per DNA input level for each sample (n=4*20=80 samples), while the lower bound of 25 ng was tested with 14 samples, 20 replicates for each sample (n=14*20=280 samples). The accuracy and sample first pass rate was 100% at all DNA input levels and sample call rates of >99%.

Interfering Substances

To assess the impact of interfering substances on the MiSeqDx Platform, a representative assay designed to query a single gene covering 11,529 bases was evaluated in the presence and absence of potential interferents. Eight whole blood samples representing eight unique genotypes were utilized in the study. Four endogenous interfering substances (bilirubin, cholesterol, hemoglobin, and triglyceride) were tested by spiking them into the blood specimens prior to DNA extraction. To assess interference resulting from blood collection (short draw), EDTA was spiked into blood samples at two concentrations. The concentration limits for each substance is shown in the following table. Additionally, to assess interference resulting from sample preparation, 15% wash buffer was added to 8 purified genomic DNA. A 100% call rate was achieved for all samples tested in addition to 100% reproducibility in genotype calls between samples in the presence and absence of interfering substances.

Test Substance	Total Number of Replicates	Concentration Tested in Blood (Upper Limit)	Concentration Tested in Blood (Lower Limit)	Call rate
Bilirubin	16	684 µmol/L	137 µmol/L	100%
Cholesterol	16	13 mmol/L	2.6 mmol/L	100%
Hemoglobin	16	2 g/L	0.4 g/L	100%
Triglyceride	16	37 mmol/L	7.4 mmol/L	100%
EDTA	16	7 mg/mL	2.8 mg/mL	100%

Sample Indexing

Sample index primers are used in the kit to assign a unique barcode to each sample DNA, allowing the ability to pool multiple samples together into a single sequencing run.

A total of 96 samples indexes were tested with a representative assay designed to query a single gene covering 11,529 bases using 8 unique DNA samples to verify the ability of the assay to consistently make a genotyping call for a given sample across different indexing primer combinations. Each sample was tested with 12 different indexing primer combinations. Forty-eight (48) index combinations were tested in one sequencing run. Sample results were compared against bidirectional Sanger sequencing data for all positions/variants. Reproducibility and accuracy were 100% for all sample/index primer combinations.

Revision History

Document #	Date	Description of Change
Document # 15070068 v05	August 2021	Updated EU Authorized Representative address. Added Revision History table.

Patents and Trademarks

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Product Labeling

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