illumina®

MiSeq™Dx Instrument

The first FDA-regulated, CE-IVD–marked, next-generation sequencing instrument for *in vitro* diagnostic use.

Highlights

- **Easy-to-Use Instrument**
  Simple instrument operation with intuitive touch screen interface and an automated workflow
- **Exceptional Data Quality**
  Demonstrated reliability through extensive system verification
- **Wide Range of Applications**
  Designed for use with a menu of *in vitro* diagnostic (IVD) assays and as an open platform for the development of custom IVD kits and assays
- **Flexible Options for Clinical Laboratory Environments**
  Offered with a menu of molecular diagnostic assays, and the ability to run a set of assays in Research Mode

Figure 1: MiSeqDx Instrument—The FDA-regulated, CE-IVD–marked MiSeqDx instrument offers a simple workflow, a user-friendly software interface, and enhanced user security.

Overview

The MiSeqDx instrument is the first Food and Drug Administration (FDA)–regulated and Conformité Européene *in vitro* diagnostic (CE-IVD)–marked platform for next-generation sequencing (NGS) (Figure 1). Designed specifically for the clinical laboratory environment, the MiSeqDx instrument offers a small footprint (0.3 square meters), an easy-to-use workflow, and data output tailored to the diverse needs of clinical labs. In addition, the on-instrument integrated software enables run setup, sample tracking, user management, audit trails, and results interpretation.* Taking advantage of proven Illumina sequencing by synthesis (SBS) chemistry, the MiSeqDx instrument provides accurate, reliable screening and diagnostic testing.

The NGS Advantage

Compared to capillary electrophoresis-based Sanger sequencing, NGS can detect a broader range of DNA variants, including low-frequency variants and adjacent phased variants, with a faster time to result and fewer hands-on steps.[1,2] Illumina SBS chemistry employs natural competition among all four labeled nucleotides, which reduces incorporation bias and allows more robust sequencing of repetitive regions and homopolymers compared to other sequencing systems.[3] Comprehensive results are delivered quickly, eliminating the need for time-consuming reflex testing.

Simple, Three-Step Workflow

Assays run on the MiSeqDx instrument follow a simple, three-step process (Figure 2) that starts with genomic DNA (gDNA) extracted from human peripheral whole blood specimens or formalin-fixed, paraffin embedded (FFPE) tissues. DNA samples are then prepared for sequencing through the addition of primers, generating indexed libraries for simultaneous capture and amplification of hundreds of targeted regions in multiple samples.

Prepared libraries are sequenced with a ready-to-use, prefilled, MiSeqDx reagent cartridge. Simply thaw the cartridge, load the library, insert into the MiSeqDx instrument, and start sequencing with the push of a button.

NGS on the MiSeqDx platform uses Illumina SBS chemistry, in which massively parallel sequencing of millions of DNA fragments occurs by a proprietary reversible terminator–based method. Single bases are detected as they are incorporated into growing DNA strands. Base calls are made directly from signal intensity measurements during each cycle. To learn more about SBS chemistry, visit www.illumina.com.

*Detailed results reports are available for target-specific assays, such as the TruSight Cystic Fibrosis 139-Variant Assay and TruSight Cystic Fibrosis Clinical Sequencing Assay.

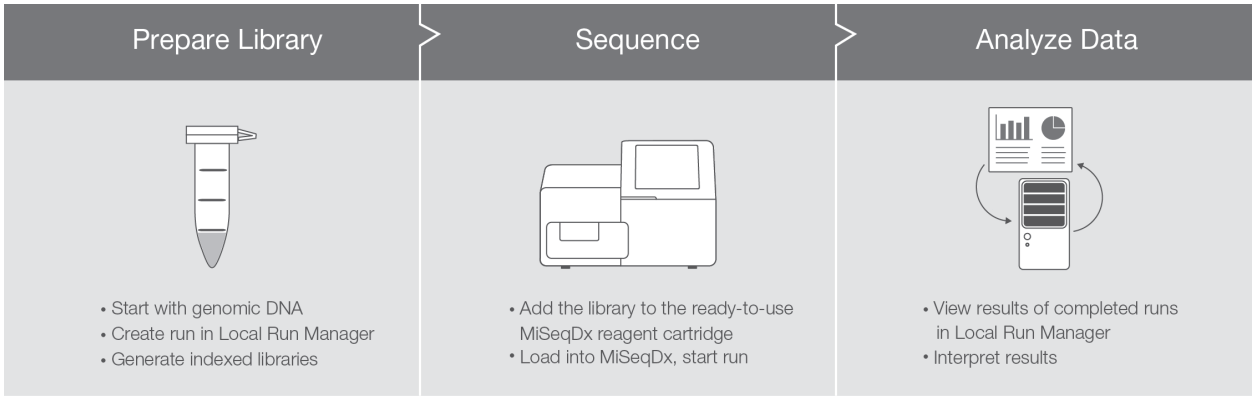

Figure 2: Three-Step MiSeqDx Assay Process—The MiSeqDx instrument is part of an integrated, 3-step process. Detailed results reports are only available with target-specific assays, such as the Extended RAS Panel, the TruSight Cystic Fibrosis 139-Variant Assay, and TruSight Cystic Fibrosis Clinical Sequencing Assay.

Integrated System Software

The MiSeqDx platform offers fully integrated on-board instrument software that can be accessed through a user-friendly touch screen interface. Sequencing runs can be planned and tracked with audit trails using the Local Run Manager software, which supports library tracking and specification of sequencing run parameters. The Local Run Manager software runs on the instrument computer, which allows users to monitor run progress and view analysis results from other computers connected to the same network. After sequencing is completed, Local Run Manager automatically starts data analysis using one of several available analysis modules. Assay-specific analysis modules are available to perform alignment and variant calling of specific variants or across the user-defined targeted regions.

User Management Software

To ensure proper system use, the MiSeqDx instrument is equipped with an integrated user management system between the Local Run Manager and the MiSeq Operating Software. This enables laboratories to control and trace system access, ensuring that only authorized personnel are running tests.

Available Assays and Reagents

Multiple IVD assays and reagents are currently available for use on the MiSeqDx instrument:†

- **The TruSight™ Cystic Fibrosis 139-Variant Assay** detects 139 clinically relevant and functionally verified variants in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, as defined by the CFTR2 database.[4]
- **The TruSight Cystic Fibrosis Clinical Sequencing Assay** detects mutations within the protein coding regions and intron/exon boundaries of the *CFTR* gene.
- **The TruSeq Custom Amplicon Kit Dx** is an amplicon-based sequencing solution that enables users to develop assays targeting the genetic variants most important to them. Users design their own oligonucleotide probes specific to their regions of interest and use validated MiSeqDx reagents to prepare libraries and sequence.
- **The Extended RAS Panel** is the first FDA-approved NGS-based IVD to aid in the identification of patients with colorectal cancer for treatment with Vectibix® by simultaneously evaluating 56 mutations in *RAS* genes contraindicated for Vectibix therapy. On-instrument software delivers an easy-to-interpret report. Currently available only in the US.

For added functionality, use Illumina library preparation assays designed for use on the MiSeq™ system on the MiSeqDx instrument when run in Research Mode.

†Detailed results reports are available for target-specific assays, such as the TruSight Cystic Fibrosis 139-Variant Assay and TruSight Cystic Fibrosis Clinical Sequencing Assay.

MiSeqDx Instrument Specifications

Instrument Specifications
Instrument Configuration
RFID tracking for consumables
MiSeq Operating Software
Local Run Manager Software
Instrument Control Computer (Internal)
Base Unit: Intel Core i7-2710QE 2.10 GHz
Memory: 2 × 8 GB DDR3 SO-DIMM
Hard Drive: None
Solid State Drives: 2 × 1TB SATA
Operating System: Windows 7 embedded standard
Light Emitting Diode (LED)
530 nm, 660 nm
Dimensions
W × D × H: 68.6 cm × 56.5 cm × 52.3 cm
(27.0 in × 22.2 in × 20.6 in)
Weight: 54.5 kg (120 lbs)
Crated Weight: 90.9 kg (200 lbs)
Power Requirements
100–240V AC @ 50/60Hz, 10A, 400 W
Radio Frequency Identifier (RFID)
Frequency: 13.56 MHz
Power: 100 mW
Throughput
1–96 samples/run, depending upon assay
Performance Parameters
Maximum Read Length: Up to 2 × 300 bp (Refer to package insert for assay dependent specifications)
Output (2 × 150 bp run): > 5 Gb
Reads passing filters > 15 million
Q30 score (at read length of 2 × 150 bp) ≥ 80%
Total overall accuracy: ≥ 99.66%
Total overall reproducibility: ≥ 99.70%

Ordering Information

Product	Catalog No.
MiSeqDx Instrument	DX-410-1001
MiSeqDx Reagent Kit v3[a]	20012552
MiSeqDx Reagent Kit v3[a]	20037124
TruSeq Custom Amplicon Kit Dx	20005718
TruSight Cystic Fibrosis Library Prep (supports the TruSight Cystic Fibrosis 139-Variant Assay and TruSight Cystic Fibrosis Clinical Sequencing Assay, 96 tests)	20036925
Extended RAS Panel (Configured for 2 runs with up to 10 samples plus 2 controls per run, or 20 samples per kit)	20012431

a. Catalog numbers 20012552 and 20037124 provide the same reagents. Catalog number used depends upon customer country or region.

Learn More

Learn more about the MiSeqDx instrument at www.illumina.com/miseqdx.

References

1. Shokralla S, Porter TM, Gibson JF, et al. Massively parallel multiplex DNA sequencing for specimen identification using an Illumina MiSeq platform. *Sci Rep*. 2015;5:9687.
2. Precone V, Monaco VD, Esposito MV, Cracking the Code of Human Diseases Using Next-Generation Sequencing: Applications, Challenges, and Perspectives. *Biomed Res Int*. 2015;161648.
3. Bentley DR, Balasubramanian S, Swerdlow HP, et al. Accurate Whole Human Genome Sequencing using Reversible Terminator Chemistry. *Nature*. 2008;456(7218):53-59.
4. Clinical and Functional Translation of CFTR. www.cftr2.org. Accessed August 15, 2017.

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 Reagent Kit v3 Intended Use

The Illumina MiSeqDx Reagent Kit v3 is a set of reagents and consumables intended for sequencing of sample libraries when used with validated assays. The MiSeqDx Reagent Kit v3 is intended for use with the MiSeqDx instrument and analytical software.

TruSight Cystic Fibrosis 139-Variant Assay Intended Use

The Illumina TruSight 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) and in 2011 by the American College of Obstetricians and Gynecologists (ACOG). 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 TruSight 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.

TruSight Cystic Fibrosis Clinical Sequencing Assay Intended Use

The Illumina TruSight 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.

TruSeq™ Custom Amplicon Kit Dx intended use

The Illumina TruSeq Custom Amplicon Kit Dx is a set of reagents and consumables used to prepare sample libraries from DNA extracted from peripheral whole blood and formalin-fixed, paraffin-embedded (FFPE) tissue. User-supplied analyte specific reagents are required for the preparation of libraries targeting specific genomic regions of interest. The generated sample libraries are intended for use on Illumina's high-throughput DNA sequence analyzers.

Extended RAS Panel Brief Statement

Intended Use

The Praxis™ Extended RAS Panel is a qualitative *in vitro* diagnostic test using targeted high-throughput parallel sequencing for the detection of 56 specific mutations in *RAS* genes [*KRAS* (exons 2, 3, and 4) and *NRAS* (exons 2, 3, and 4)] in DNA extracted from formalin-fixed, paraffin-embedded (FFPE) colorectal cancer (CRC) tissue samples. The Praxis™ Extended RAS Panel is indicated to aid in the identification of patients with CRC for treatment with Vectibix® (panitumumab)[1] based on a no mutation detected test result. The test is intended to be used on the Illumina MiSeqDx® instrument.

Relevant Warnings and Limitations

- For prescription use only.
- Optimal performance of the test requires appropriate sample handling as described in the instructions for use.
- The Extended RAS Panel Report only determines the presence or absence of the *KRAS* and *NRAS* mutations listed in the package insert.[2,3]
- For samples that pass the DNA qualification step based on requirements described in the instructions for use, the assay has an estimated invalid rate of 6.9%–14.5% as assessed in the retrospective clinical and accuracy studies, respectively.
- The risks of the Extended RAS Panel are associated with the potential mismanagement of patients resulting from false results of the test. A false positive test result may lead to Vectibix treatment being withheld from a patient who might have benefited. A false negative test result may lead to Vectibix treatment being administered to a patient who is not expected to benefit, therefore potentially exposing them to any adverse side effects associated with this therapy and also causing the patient to possibly fail to receive a different drug that might be more appropriate for them than Vectibix.
- Laboratories should follow safety procedures, as some components contain potentially hazardous chemicals and personal injury can occur through inhalation, ingestion, skin contact, and eye contact.

References for Extended RAS Panel

1. Vectibix full Prescribing Information, including Boxed Warnings.
2. Extended RAS Panel Package Insert.
3. Summary of Safety and Effectiveness Data.

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