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MiSeqDx™ Universal Kit

A validated, FDA-cleared kit enabling clinical laboratories to design their own assays for use on the MiSeqDx instrument.

Highlights

- **Flexible Assay Design**
  Develop assays to meet specific customer needs
- **Validated Kit**
  Guaranteed performance specifications for supplied library preparation and sequencing reagents
- **Fast Results**
  Go from genomic DNA to fully analyzed data in < 2 days
- **High Assay Reproducibility**
  Consistent results between multiple operators and systems

Introduction

The MiSeqDx Universal Kit is a validated, FDA-cleared amplicon sequencing solution that enables clinical laboratories to develop their own next-generation sequencing (NGS) assays for use on the FDA-cleared MiSeqDx instrument (Figure 1). Together, the MiSeqDx Universal Kit and instrument offer clinical labs the accuracy and reliability needed to bring NGS capabilities into their facility. The result is an expansion in the number of diagnostic applications these labs can provide their customers.

Validated DNA-to-Data Solution

The MiSeqDx Universal Kit offers an integrated DNA-to-data solution that includes high-quality reagents, a streamlined workflow, and automated data analysis. Validation of library preparation and sequencing reagents ensures consistent performance from one lot to the next, enabling long-term use of designed assays and facilitated change management. There's no need to spend valuable lab resources and time revalidating reagents or assays for future runs. The fast, efficient workflow requires just 250 ng genomic DNA (gDNA) to produce high-quality sequencing data in < 2 days.

Customized Assay Design

With the MiSeqDx Universal Kit, clinical labs develop assays using oligonucleotide probes they have designed on their own. This provides users with the flexibility to target specific regions of interest. Target specifications (Table 1) allow the potential for sequencing > 90% of the exons in RefSeq genes, depending upon assay design.

Figure 1: MiSeqDx Universal Kit

The FDA-cleared MiSeqDx Universal Kit provides library preparation and sequencing reagents that allow clinical laboratories to develop their own diagnostic tests intended for use on the MiSeqDx instrument.

Table 1: Parameters of Targets Reliably Sequenced with the MiSeqDx Universal Kit

Target Specification	Value
GC content	
Minimum	19%
Maximum	72%
PolyA length	7
PolyT length	8
PolyG length	6
PolyC length	7
Dinucleotide repeat lengths	5×
Trinucleotide repeat lengths	4×

Data from a representative custom amplicon assay designed to query various genes and regions. Exact performance will depend on assay design.

AGAATGATAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAAC
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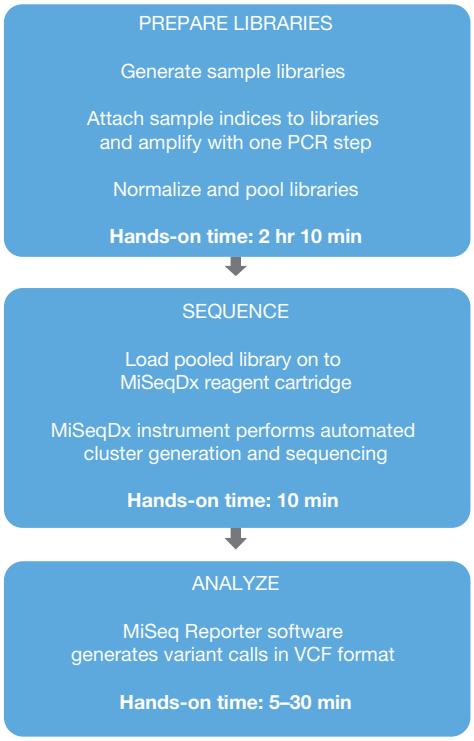
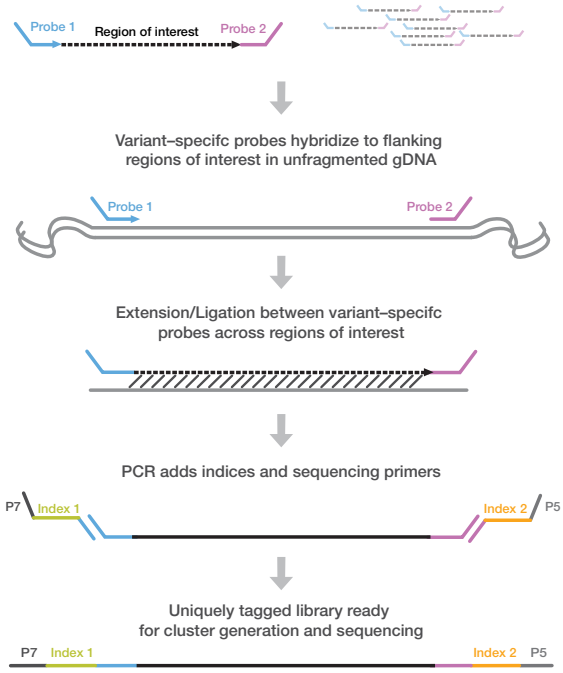
Figure 2: Streamlined Custom Assay Workflow



The MiSeqDx Universal Kit enables clinical laboratories to develop their own NGS assays for use on the MiSeqDx instrument.

Figure 3: SBS Assay Chemistry is the Basis for the MiSeqDx Universal Kit



The MiSeqDx Universal Kit uses the highly specific SBS chemistry to capture and sequence your specific targets.

How it Works

The MiSeqDx Universal Kit takes advantage of Illumina sequencing by synthesis (SBS) NGS chemistry to produce reliable sequencing data in < 2 days.

Streamlined Workflow

A sequencing assay developed using the MiSeqDx Universal kit requires three basic steps: prepare libraries, sequence, and analyze (Figure 2). Oligonucleotide probes targeting the regions of interest are mixed with the DNA sample to generate libraries. The libraries are pooled and then sequenced on the MiSeqDx instrument. Data are gathered in real time and analyzed using MiSeq® Reporter software.

Specific Assay Chemistry

The MiSeqDx Universal Kit leverages SBS chemistry and long paired-end read capabilities to capture and sequence just the region of interest (Figure 3). Library preparation begins with 250 ng gDNA isolated from a blood sample. The DNA is mixed with a pool of user-defined oligonucleotide probes. Each probe includes sequences designed to capture regions of interest and an adapter sequence used in a subsequent amplification reaction. The probes hybridize to the DNA, one upstream and one downstream of the target. A proprietary extension-ligation reaction extends across the region of interest, followed by ligation to unite the two probes. This creates a template strand and gives the assay excellent specificity.

To enable simultaneous analysis of multiple samples, or multiplexing, within a single sequencing run, individual libraries are "tagged" with a unique identifier, or index. These unique sample-specific indices are added to each extension-ligation template in a single PCR amplification step. The final reaction product contains the desired amplicons with adapters and indices necessary for sequencing on the MiSeqDx instrument.

Widely Adopted NGS Technology

Illumina SBS chemistry is a widely adopted NGS technology in the sequencing community. Through massively parallel sequencing using a proprietary reversible terminator–based method, single bases are detected as they are incorporated into growing DNA strands. A fluorescently labeled terminator is imaged as each dNTP (dATP, dCTP, dGTP, or dTTP) is added and then cleaved to allow incorporation of the next base. Because all four reversible terminator bound dNTPs are present during each sequencing cycle, natural competition minimizes incorporation bias. The result is base-by-base sequencing that enables highly accurate data capture.

Results Visualization

After the sequencing run on the MiSeqDx instrument, data are automatically aligned and visualized using the MiSeq Reporter software. Users can view large genomic regions containing multiple amplicons or zoom in on individual amplicons. Quality scores are tracked and variant calls are visualized in an easy, intuitive manner.

Highly Reproducible Data

To demonstrate the high reproducibility of data produced using the MiSeqDx Universal Kit, a site-to-site reproducibility study was performed with a representative assay. The blinded study used three trial sites and two operators at each site. The sample first pass rate was 99.9%*. The results of this study are shown in Table 2.

Table 2: High Assay Reproducibility

Parameter	Value (%)
Positive Agreement	99.77
Negative Agreement	99.88
Overall Agreement	99.88

The representative assay was designed to query a subset of clinically relevant *CFTR* genetic variations.

Summary

The MiSeqDx Universal Kit provides clinical labs with a validated FDA-cleared reagent kit for designing their own NGS assays for the MiSeqDx instrument. This enables labs to more easily incorporate the speed and accuracy of NGS into their service offering.

Learn More

Visit www.illumina.com/MiSeqDx to learn more about the MiSeqDx Universal Kit and MiSeqDx instrument.

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.

Ordering Information

Product	Catalog No.
MiSeqDx Universal Kit (2 runs, up to 96 samples)	DX-103-1001
MiSeqDx instrument	DX-410-1001
TruSeq® Index Plate Fixture Kit	FC-130-1005
TruSeq Index Plate Fixture & Collar Kit	FC-130-1007

* Sample pass rate is defined as the number of samples passing QC metrics on the first attempt.

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