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TruSeq™ Custom Amplicon Kit Dx

A validated, FDA-regulated, CE-IVD–marked kit enabling clinical laboratories to design custom assays for the MiSeq™Dx and NextSeq™ 550Dx sequencing instruments.

Highlights

- **Flexible Assay Design**
  Developed to meet unique, user-defined needs
- **Validated Kit**
  Validated performance specifications for library preparation when used with Illumina Dx sequencing instruments and reagents
- **Compatible With a Variety of Sample Types**
  Optimized library prep for DNA extracted from formalin-fixed, paraffin-embedded (FFPE) tissue and whole blood samples
- **High Assay Reproducibility**
  Consistent results between multiple operators and systems

Figure 1: TruSeq Custom Amplicon Kit Dx—The FDA-regulated, CE-IVD–marked TruSeq Custom Amplicon Kit Dx provides library preparation reagents that allow clinical laboratories to develop their own diagnostic tests intended for use on Illumina Dx sequencing instruments.

Introduction

The TruSeq Custom Amplicon Kit Dx (Figure 1) is a validated, Food and Drug Administration (FDA)-regulated, Conformité Européene *in vitro* diagnostic (CE-IVD)–marked, amplicon sequencing kit that allows clinical laboratories to develop custom next-generation sequencing (NGS) assays. These sequencing assays can be used with the FDA-regulated, CE-IVD–marked MiSeqDx and NextSeq 550Dx instruments. Together, the TruSeq Custom Amplicon Kit Dx and Illumina Dx sequencing instruments deliver a comprehensive DNA-to-data solution with the accuracy and reliability needed in a regulated environment. With the power of NGS, clinical labs can provide an expanding menu of diagnostic applications to their customers.

Validated Library Prep Solution

The TruSeq Custom Amplicon Kit Dx offers a validated library preparation solution that includes high-quality reagents and a streamlined workflow. Validation of library preparation reagents ensures consistent performance from one lot to the next, enabling long-term use of designed assays, and facilitating change management. Use of validated reagents eliminates the need to spend valuable lab resources revalidating reagents or assays for future runs. The fast, efficient workflow requires just 50 ng genomic DNA (gDNA) or 10 µl of qualified FFPE DNA to produce high-quality sequencing libraries in less than two days.

Customized Assay Design

With the TruSeq Custom Amplicon Kit Dx, clinical labs develop assays using custom-designed oligonucleotide probes. This customization gives users the flexibility to target specific regions of interest. Target specifications (Table 1) support sequencing greater than 90% of the exons in RefSeq,[1] depending on assay design. Customization of kit configuration also enables flexibility with sample throughput.

Table 1: Detection Capability of TruSeq Custom Amplicon Kit Dx

Parameter	Germline	Somatic
Genomic Context		
GC content	19–78%	19–73%[a]
PolyA length	≤ 8	≤ 8
PolyT length	≤ 10	≤ 10
PolyG length	≤ 7	≤ 6
PolyC length	≤ 6	≤ 6
Dinucleotide repeat length	≤ 11×	≤ 4×
Trinucleotide repeat length	≤ 5×	≤ 5×
Variants		
Single nucleotide polymorphisms	Yes	Yes, 5% limit of detection
Insertion length	≤ 24 bp	≤ 24 bp
Deletion length	≤ 25 bp	≤ 25 bp

a. Up to 78% with NextSeq 550Dx instrument.

Library Preparation Workflow

The TruSeq Custom Amplicon Kit Dx generates sequencing-ready libraries that can be loaded onto an Illumina Dx sequencing instrument for production of reliable sequencing data in less than two days.

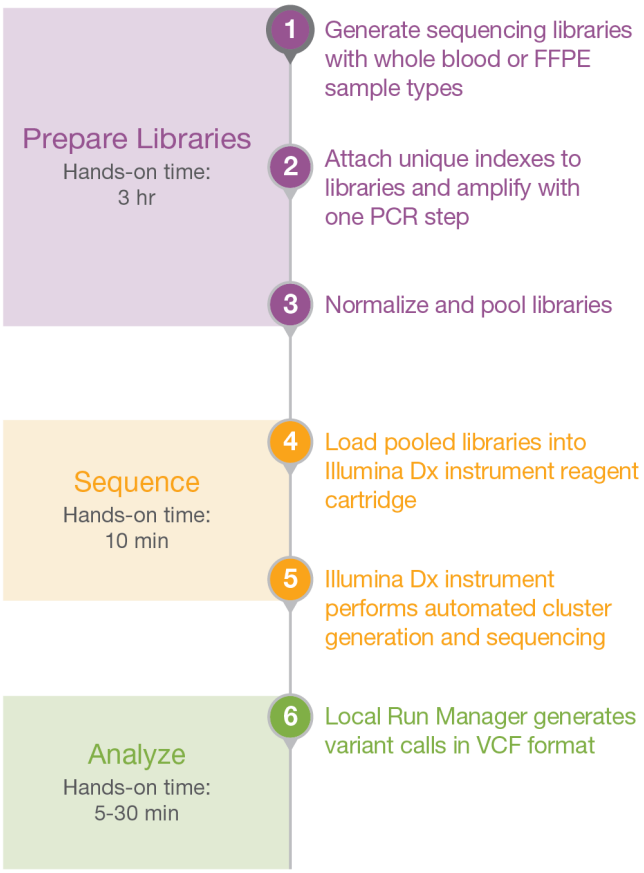

Figure 2: Streamlined Custom Assay Workflow—The TruSeq Custom Amplicon Kit Dx workflow provides DNA-to-data with a streamlined six-step process.

Streamlined Workflow

The TruSeq Custom Amplicon Kit Dx workflow includes six basic steps (Figure 2). Oligonucleotide probes targeting the regions of interest are mixed with the DNA sample during library preparation. The libraries are pooled and then sequenced on an Illumina Dx sequencing instrument. Data are gathered in real time and analyzed using Local Run Manager software.

gDNA or FFPE DNA Input

The TruSeq Custom Amplicon Kit Dx is optimized for use with 50 ng gDNA or 10 µl qualified FFPE tissue–derived DNA. FFPE DNA can be qualified by the TruSeq Custom Amplicon Dx–FFPE QC Kit, which uses a simple qPCR reaction to determine FFPE DNA quality. The results of the FFPE QC Kit are used to provide guidance on the quality and amount of input FFPE DNA.

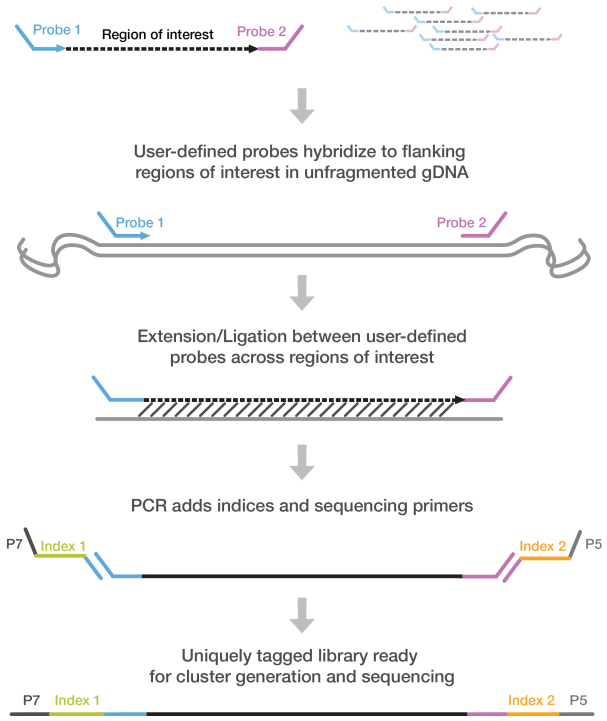

Figure 3: TruSeq Custom Amplicon Kit Dx Library Prep Chemistry—The TruSeq Custom Amplicon Kit Dx uses custom-designed probes to capture and sequence defined targets of interest.

Assay Chemistry

The TruSeq Custom Amplicon Kit Dx combined with proven sequencing by synthesis (SBS) chemistry allows the capture and sequencing of only the regions of interest (Figure 3). The gDNA or FFPE 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 DNA sequence. 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 provides excellent assay specificity.

To enable simultaneous analysis of multiple samples within a single sequencing run (multiplexing), individual libraries are "tagged" with a unique identifier or index. These unique, sample-specific indexes are added during the PCR amplification step in the library prep protocol. The final product includes the user-defined regions, the adapters, and the indexes necessary for sequencing on an Illumina Dx sequencing instrument.

Table 2: Variant Calling Study Shows High Assay Reproducibility[2]

Analysis	Metric	Variant Type	MiSeqDx (8 runs)	NextSeq 550Dx (9 runs)
Germline Variant Calling	PPA	SNV	99.8%	> 99.9%
	PPA	Insertion	98.1%	98.9%
	PPA	Deletion	99.8%	100%
	NPA		100%	100%
Somatic Variant Calling	PPA	SNV	100%	99.9%
	PPA	Insertion	100%	99.9%
	PPA	Deletion	100%	99.9%
	NPA		> 99.9%	> 99.9%

Percentages represent the lowest value across runs. Abbreviations: positive percent agreement (PPA), negative percent agreement (NPA), single nucleotide variant (SNV).

Widely Adopted NGS Technology

Illumina SBS chemistry is the most widely adopted NGS technology in the sequencing community.[3] 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.[4]

Highly Reproducible Data

To demonstrate the high reproducibility of data produced using the TruSeq Custom Amplicon Kit Dx and SBS chemistry, a variant calling precision study was performed with a representative assay. The study used multiple operators, instruments, and lots across 8–9 runs (Table 2).

Summary

The TruSeq Custom Amplicon Kit Dx delivers a validated FDA-regulated solution for the design of NGS assays targeting user-defined genomic regions of interest. This kit enables clinical labs to harness the speed and accuracy of NGS and to provide a growing range of diagnostic service offerings to their customers.

Learn More

To learn more about TruSeq Custom Amplicon Kit Dx, visit www.illumina.com/TSCAKitDx

To learn more about the MiSeqDx instrument, visit www.illumina.com/MiSeqDx.

To learn more about the NextSeq 550Dx instrument, visit www.illumina.com/nextseq550dx.

Ordering Information

Product	Catalog no.
TruSeq Custom Amplicon Kit Dx[a]	20005718
TruSeq Custom Amplicon Dx–FFPE QC Kit[a]	20006259
MiSeqDx Instrument	DX-410-1001
MiSeqDx Reagent Kit v3[a,b]	20012552
MiSeqDx Reagent Kit v3[a,b]	20037124
NextSeq 550Dx Instrument	20005715
NextSeq 550Dx High-Output Reagent Kit v2 (300 cycle)[a]	20019554
TruSeq Index Plate Fixture Kit	DX-130-1005
TruSeq Index Plate Fixture and Collar Kit	DX-130-1007

a. Class I sequencing consumables have single lot shipment, kit lot testing, advance change notification, and a Certificate of Analysis available for each lot. Reagents are developed under design control principles, manufactured under current good manufacturing practices (cGMP), and verified to ensure specification compliance.
b. Catalog numbers 20012552 and 20037124 provide the same reagents. Catalog number used depends upon customer country or region.

References

1. NCBI Reference Sequence Database (RefSeq). www.ncbi.nlm.nih.gov/refseq/. Accessed October 10, 2017.
2. Data on file. Illumina, Inc. 2017.
3. Data calculations on file. Illumina, Inc. 2015.
4. Bentley DR, Balasubramanian S, Swerdlow HP, Smith GP, Milton J, Brown CG, et al. Accurate whole human genome sequencing using reversible terminator chemistry. *Nature*. 2008;456(7218):53–9.

Intended Use Statements

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.

TruSeq™ Custom Amplicon Dx – FFPE QC Kit intended use

The Illumina TruSeq Custom Amplicon Dx – FFPE QC Kit is a set of reagents used to determine the amplification potential of genomic DNA (gDNA) extracted from formalin-fixed, paraffin-embedded (FFPE) samples.

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.

NextSeq™ 550Dx instrument intended use (European Union/Other)

The NextSeq 550Dx instrument is intended for sequencing of DNA libraries when used with *in vitro* diagnostic (IVD) assays performed on the instrument. The NextSeq 550Dx instrument is to be used with specific registered, certified or approved IVD reagents and analytical software.

NextSeq™ 550Dx High-Output Reagent Kit v2 (300 cycles) intended use

The Illumina NextSeq 550Dx High-Output Reagent Kit v2 (300 cycle) is a set of reagents and consumables intended for sequencing of sample libraries when used with validated assays. The kit is intended for use with the NextSeq 550Dx instrument and analytical software.

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