TECHNICAL NOTE

Illumina DNA Prep with Enrichment Dx enables highly accurate variant detection

Demonstrated performance with whole blood and FFPE samples using enrichment probe panels of varying sizes

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Introduction

Illumina DNA Prep with Enrichment Dx is a library prep and enrichment next-generation sequencing (NGS) solution that is compliant with European Union (EU) *In Vitro* Diagnostics Regulation (IVDR) 2017/746 and is regulated by the Food and Drug Administration (FDA). It supports a wide range of genomic DNA (gDNA) input amounts extracted from whole blood or formalin-fixed, paraffin-embedded (FFPE) tissue. When combined with the MiSeq™Dx, NextSeq™ 550Dx, or NovaSeq™ 6000Dx Instruments, Illumina DNA Prep with Enrichment Dx enables clinical laboratories to add targeted sequencing enrichment panels to their menu of diagnostic applications.

Illumina DNA Prep with Enrichment Dx supports both fixed and custom panels of varying sizes, including exome panels. The kit is compatible with Illumina and third-party enrichment DNA oligonucleotide panels for increased flexibility.

This technical note demonstrates the exceptional performance of Illumina DNA Prep with Enrichment Dx in the production of high-quality sequencing data and sensitive detection of germline and somatic variants with reference control and real-world whole blood and FFPE samples (Figure 1). Various Illumina and third-party enrichment probe panels that meet the specifications for validation with Illumina DNA Prep with Enrichment Dx (Table 1) were used for evaluation as proof of principle.

Table 1: Illumina DNA Prep with Enrichment Dx probe panel requirements[a]

Parameter	Specification
Probe type	Single- or double-stranded DNA
Probe length	80 bp or 120 bp
Panel size	500–675,000 probes
Total probe input[b]	≥ 3 pmols

a. Panels outside these specifications have not been validated and are considered off-label use.
b. For enrichment at plexities from 1-plex to 12-plex.

Methods

Samples and DNA extraction

Samples for evaluation included NA12878 reference material from the Coriell Institute, Horizon HD799 formalin-compromised reference DNA, and DNA extracted from real-world whole blood and FFPE tissue samples. Any validated extraction method can be used. Refer to the Illumina DNA Prep with Enrichment Dx data sheet to learn more about sample preparation.

Library preparation and enrichment

Libraries were prepared for all samples using Illumina DNA Prep with Enrichment Dx. Either 50 ng or 1000 ng of extracted DNA or the total volume was input to library prep. Hybridization was carried out with five different enrichment panels (Table 2). Libraries prepared from whole blood and FFPE samples were hybridized as 12-plex and 1-plex enrichment reactions, respectively.

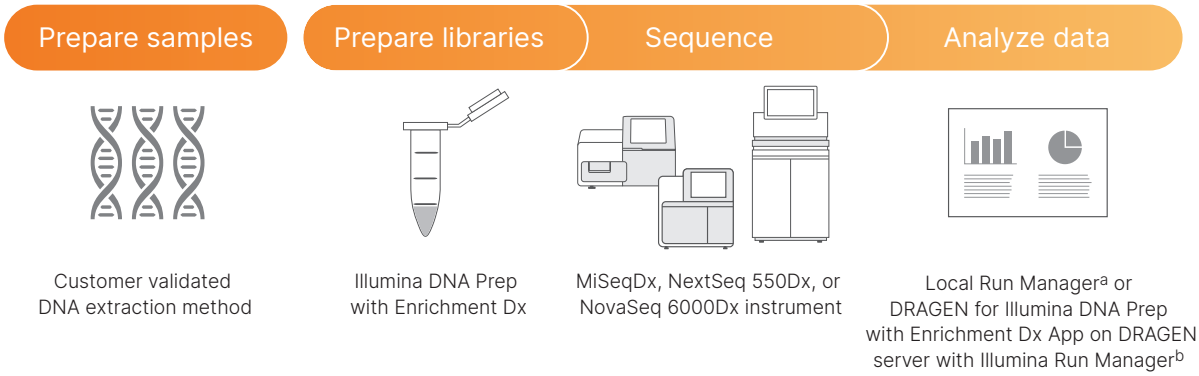

Figure 1: Illumina DNA Prep with Enrichment Dx workflow—DNA was extracted following standard protocols. Libraries were prepared using Illumina DNA Prep with Enrichment Dx and several hybrid–capture probe panels, followed by sequencing and data analysis.
a. Available on MiSeqDx and NextSeq 550Dx Instruments.
b. Available on NextSeq 550Dx and NovaSeq 6000Dx Instruments.

Table 2: Enrichment panel specifications used for evaluation

Panel feature	Exome panel I	Exome panel T	Large panel I	Midsize panel I	Small panel I
Panel size	45.2 Mb	33 Mb	12 Mb	1.94 Mb	255 kb
Probe size	80 bp	120 bp	80 bp	80 bp	80 bp

Panel size, total length of sequence in target regions; **probe size**, length of enrichment pulldown probe.

Sequencing

Prepared libraries were sequenced on the MiSeqDx or NextSeq 550Dx instrument using the MiSeqDx Reagent Kit v3 or NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles), respectively, at a read length of 2 × 151 bp.

Data analysis

FASTQ files were generated from base calls using the GenerateFASTQ Dx Module in Local Run Manager. Analysis of FASTQ files was carried out with custom scripts. Additional analysis and visualization of data was performed using Microsoft Excel or JMP statistical software. The DRAGEN for Illumina DNA Prep with Enrichment Dx app is an option for secondary analysis.

Results

To evaluate Illumina DNA Prep with Enrichment Dx, libraries prepared with different probe panels were sequenced and analyzed. Performance was measured by various sequencing metrics, including coverage uniformity, padded read enrichment, and fragment length median. The ability to call single nucleotide variants (SNV) and insertions/deletions (indels) accurately was determined by recall and precision metrics. To begin, 50 ng and 1000 ng of NA12878 reference DNA was used to prepare libraries with four panels. High-quality sequencing data was obtained, enabling accurate germline variant calling across all panels included for evaluation (Table 3).

Table 3: Assay performance with control DNA across enrichment panels

Panel	DNA input amount (ng)	Padded unique read enrichment	Uniformity of coverage	Fragment length median	SNV recall[a]	SNV precision[b]	Indel recall[a]	Indel precision[b]
Exome panel I	50	78.65	95.37%	175	96.11%	98.16%	89.84%	84.19%
	1000	80.81	96.35%	197	96.47%	99.60%	91.10%	94.05%
Exome panel T	50	93.29	97.50%	178	96.26%	99.34%	92.18%	90.27%
	1000	92.69	97.78%	198	96.40%	99.63%	91.30%	96.01%
Large panel I	50	82.43	95.78%	188	97.91%	98.99%	89.68%	83.89%
	1000	83.91	96.92%	213	98.13%	99.82%	90.70%	94.66%
Small panel I	50	78.34	98.10%	180	98.54%	99.88%	N/A	N/A
	1000	76.86	98.26%	202	98.54%	100%	N/A	N/A

a. Recall = true positives/(true positives + false negatives).
b. Precision = true positives/(true positives + false positives).
N/A, not applicable

These results demonstrate the ability of on-bead tagmentation used by Illumina DNA Prep with Enrichment Dx to normalize samples with varying inputs with minimal effects on data quality, enabling removal of normalization steps from the protocol for substantial time savings.

High padded read enrichment and coverage uniformity were seen when using DNA extracted from whole blood samples, the Illumina DNA Prep with Enrichment Dx solution, and both Exome panel I and Small panel I (Table 4). Similarly, high coverage uniformity was seen with FFPE libraries enriched with Midsize panel, and HD799 libraries enriched with the same panel, enabling highly accurate somatic variant calling (Table 5).

Table 4: Performance with whole blood input

Panel	Padded unique read enrichment	Uniformity of coverage	Fragment length median
Exome panel I	81.14	95.68%	203
Small panel I	77.64	98.13%	193

Table 5: Performance with formalin-compromised reference and real-world FFPE input

Panel	Input source	Input amount (ng)	Uniformity of coverage	SNV recall
Midsize panel I	FFPE	50	99.24%	N/A
		1000	99.14%	N/A
	HD799	50	96.69%	100%

Summary

Illumina DNA Prep with Enrichment Dx delivers an EU IVDR 2017/746 and FDA–regulated solution for targeted sequencing enrichment methods, including fixed panels, custom panels, and exome panels. This technical note demonstrates the optimal performance of the assay for targeted enrichment sequencing and both germline and somatic variant calling.

Learn more

Illumina DNA Prep with Enrichment Dx

Intended use statements

Illumina DNA Prep with Enrichment Dx (CE-IVD)

The Illumina DNA Prep with Enrichment Dx Kit is a set of reagents and consumables used to prepare sample libraries from genomic DNA derived from human cells and tissue to develop in vitro diagnostic assays. User supplied probe panels are required for the preparation of libraries targeting specific genomic regions of interest. The generated sample libraries are intended for use on Illumina sequencing systems. The Illumina DNA Prep with Enrichment Dx includes software for sequencing run setup, monitoring, and analysis.

Illumina DNA Prep with Enrichment Dx (United States)

Illumina DNA Prep with Enrichment 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 tissue. User-supplied probe panels are required for the preparation of libraries targeting specific genomic regions of interest. The generated sample libraries are intended for use on Illumina sequencing systems.

MiSeqDx Instrument

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.

NextSeq 550Dx Instrument (United States)

The NextSeq 550Dx 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 NextSeq 550Dx Instrument is not intended for whole genome or de novo sequencing. The NextSeq 550Dx Instrument is to be used with registered and listed, cleared or approved, IVD reagents and analytical software.

NextSeq 550Dx Instrument (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.

NovaSeq 6000Dx Instrument (United States)

The NovaSeq 6000Dx 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 with *in vitro* (IVD) diagnostic assays. The NovaSeq 6000Dx Instrument is not intended for whole-genome or *de novo* sequencing. The NovaSeq 6000Dx Instrument is intended for use with specific registered, certified, or approved IVD reagents and analytical software.

NovaSeq 6000Dx instrument (European Union/other)

The NovaSeq 6000Dx Instrument is intended for sequencing of DNA libraries when used with *in vitro* diagnostic (IVD) assays. The NovaSeq 6000Dx Instrument is intended for use with specific registered, certified, or approved IVD reagents and analytical software.

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