APPLICATION NOTE

Accurate, IVDR-compliant NGS variant calling using the Illumina DRAGEN™ Server for NextSeq™ 550Dx Instruments

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For *In Vitro* Diagnostic Use. Not available in all regions and countries.

Introduction

Next-generation sequencing (NGS) applications are an indispensable part of diagnostic testing, including newborn screening, genetic disease testing, and oncology testing. Diagnostic NGS assays need to be easy for labs to adopt and use and must comply with essential regulations that are designed to ensure the highest standard of care and protect patients.

Illumina DRAGEN Server for NextSeq 550Dx Instruments enables fast and highly accurate variant calling for diagnostic workflows using flexible Illumina DNA Prep with Enrichment Dx targeted library preparation. Based on bead-linked transposome chemistry, Illumina DNA Prep with Enrichment Dx offers a flexible, easy targeted enrichment solution for use in *in vitro* diagnostic (IVD) applications. The secondary analysis application for Illumina DNA Prep with Enrichment Dx run on the Illumina DRAGEN Server for NextSeq 550Dx Instruments complies with European Union (EU), *In Vitro* Diagnostics Regulation (IVDR) 2017/746 for integration into diagnostic workflows.

This application note demonstrates a DNA-to-data solution that combines Illumina DNA Prep with Enrichment Dx library preparation, sequencing on the NextSeq 550Dx Instrument, and secondary analysis using the enrichment application on Illumina DRAGEN Server for NextSeq 550Dx Instruments (Figure 1). The advanced graphical user interface for Illumina Run Manager on the Illumina DRAGEN Server for NextSeq 550Dx Instruments delivers user-friendly sequencing run setup; automated secondary data analysis; and fast, efficient, lossless Original Read Archive (ORA) data compression.[1] Additionally, direct integration with NextSeq 550Dx Instruments reduces user interactions to a single touchpoint at run setup and decreases overall time to results. This solution produces high-quality sequencing data and accurate detection of germline and somatic variants in diagnostic testing.

Methods

Sample preparation

For evaluation of germline variant calling, genomic DNA (gDNA) was extracted from Coriell Institute reference samples (Catalog nos. NA24631, NA24385, NA12877, and NA12878). For evaluation of somatic variant calling, eight replicates of DNA were extrated and prepared using control samples from SeraCare.

Library preparation

Libraries were prepared using Illumina DNA Prep with Enrichment Dx with UD Indexes Set A (Illumina, Catalog no. 20051352) using 50 ng of input DNA for both germline and somatic variant detection. Target enrichment was carried out using an exome panel and a panel targeting 500+ cancer genes.

Sequencing

Sequencing on the NextSeq 550Dx Instrument was configured using Illumina Run Manager included on the Illumina DRAGEN Server for NextSeq 550Dx Instruments.

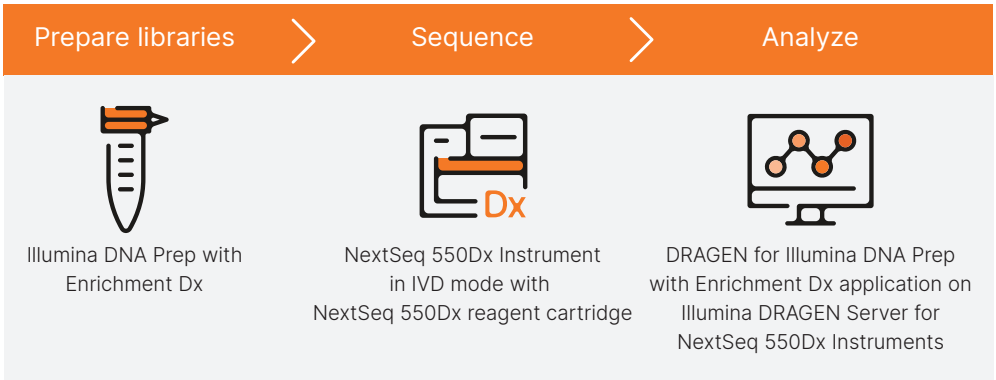

Figure 1: Accurate and efficient IVDR-compliant workflow incorporating Illumina DRAGEN Server for NextSeq 550Dx Instruments.

Prepared libraries were sequenced on the NextSeq 550Dx Instrument in IVD Mode with High Output Reagent Kit v2.5 (300 cycles) IVD (Illumina, Catalog no. 20028871). Paired-end read length was 2 × 151 bp.

Data analysis

Data analysis with the DRAGEN for Illumina DNA Prep with Enrichment Dx app was configured as part of sequencing run planning with Illumina Run Manager and launched automatically after the sequencing run completed. This configuration with a single touchpoint eliminates the need for manual steps to initiate secondary analysis after sequencing. After analysis is initiated, the NextSeq 550Dx Instrument is available for the next sequencing run (Figure 2).

Run times for the DRAGEN for Illumina DNA Prep with Enrichment Dx analysis application were tested using data generated with the NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles) IVD (Illumina, Catalog no. 20028871). Run time analysis included FASTQ generation, ORA compression, mapping/aligning, and variant calling. FASTQ files were compressed with DRAGEN ORA compression, resulting in up to ~5× smaller file sizes, faster file transfers, and reduced data storage and energy costs.

For the variant calling accuracy and run time comparison, samples were aligned with BWA-MEM (0.7.17),[2] processed with SAMtools (1.15.1)[3] and Picard (2.27.5),[4] and analyzed with GATK 4.3.0.[5] Variant calling accuracy was assessed with the Illumina Variant Calling Assessment Tool (VCAT 4.1.0) by comparing each sample to a truth set. Analysis was completed on a high-performance computer cluster with CPU nodes with optimized multithreading for the analysis.

Results

Data demonstrated that the DRAGEN for Illumina DNA Prep with Enrichment Dx application produced high-quality sequencing data analysis and accurate detection of variants that exceeds the performance observed with the established BWA-GATK analysis pipeline (Table 1). The included lossless ORA compression in the DRAGEN for Illumina DNA Prep with Enrichment Dx application produced substantially reduced file sizes (Table 2), supporting significant savings for data storage and energy resources. Additionally, Illumina DNA Prep with Enrichment Dx germline analysis, with or without ORA compression, takes place in fraction of the time required for BWA-GATK analysis (Figure 3).

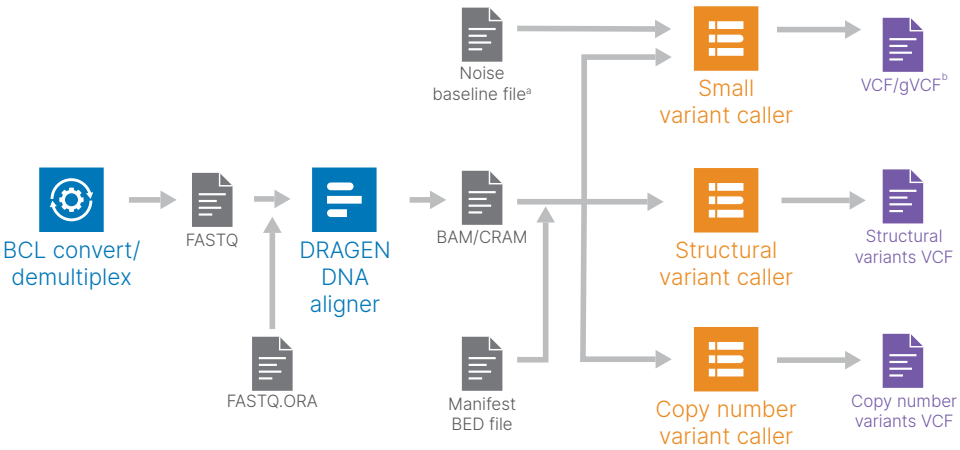

Figure 2: DRAGEN for Illumina DNA Prep with Enrichment Dx App workflow—Analysis can be configured in Illumina Run Manager to begin automatically upon sequencing run completion. The workflow includes FASTQ file generation, mapping/aligning, and both germline and somatic variant calling. Exceptionally efficient ORA compression can reduce the size of FASTQ files by up to 5×.

a. The DRAGEN Baseline Builder app can be used to build an optional custom noise baseline file for use in the somatic variant mode.
b. gVCF files are not generated with somatic variant calling.

Table 1: Comparison of germline variant calling accuracy in IVD Mode

Pipeline	SNV precision	SNV recall	SNV F1	Indel precision	Indel recall	Indel F1
BWA-GATK	97.36%	93.95%	95.62%	65.29%	79.83%	71.78%
DRAGEN for Illumina DNA Prep with Enrichment Dx application	99.14%	95.85%	97.46%	90.12%	85.43%	87.70%

Table 2: DRAGEN for Illumina DNA Prep with Enrichment Dx application ORA compression performance

	FASTQ file size (GB)	
Panel size	Fastq.gz[a]	Fastq.ora
Large panel (45 Mb)	86.8 GB	25.7 GB

a. Qzip is an alternative compression tool for sequence data outputting files with *.qz format.

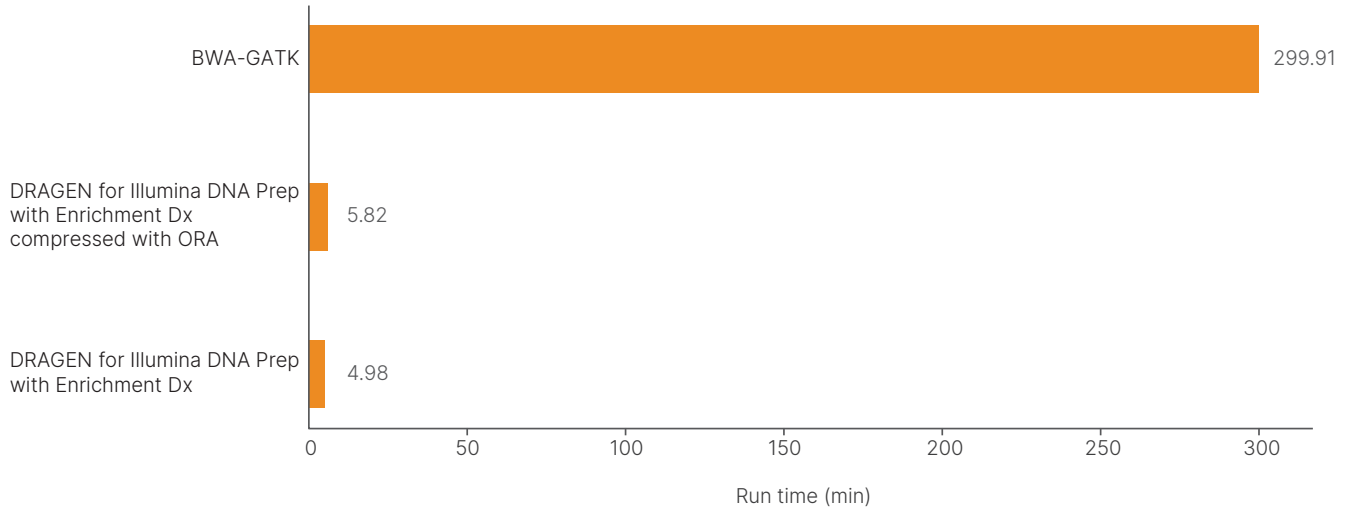

Figure 3: Run time comparison for DRAGEN for Illumina DNA Prep with Enrichment Dx run on Illumina DRAGEN Server for NextSeq 550Dx Instruments vs BWA-GATK analysis on a 16-core, high-performance computing platform—Average run times for germline analysis of eight samples are shown. Sequencing data was generated using a 42.5 Mb exome panel with a read depth of 200×.

Summary

The Illumina DRAGEN Server for NextSeq 550Dx Instruments expands and simplifies the Illumina IVD NGS offering with integrated secondary analysis using powerful and intuitive DRAGEN software. The server includes Illumina Run Manager to pair it with the NextSeq 550Dx Instrument, allowing users to configure the sequencing run and secondary analysis as part of the run setup minimizing potential user error by reducing the number of touchpoints. Analysis begins automatically following the sequencing run and the instrument is available immediately to initiate another sequencing run, minimizing downtime. In combination with the DRAGEN for Illumina DNA Prep with Enrichment Dx application for secondary analysis, the Illumina DRAGEN Server for NextSeq 550Dx Instruments offers diagnostic labs streamlined, accurate, IVDR-compliant secondary analysis for variant calling.

Ordering information

Product	Catalog no.
DRAGEN Server for NextSeq 550Dx Instruments	20086130
NextSeq 550Dx Instrument	20005715

Learn more

DRAGEN Server for NextSeq 550Dx Instruments

NextSeq 550Dx Instrument

Illumina DNA Prep with Enrichment Dx

DRAGEN for Illumina DNA Prep with Enrichment Dx application

In Vitro Diagnostic Medical Devices Regulation (EU) 2017/746

References

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2. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2009;25(14):1754-1760. doi:10.1093/bioinformatics/btp324
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6. Illumina. Variant Calling Assessment Tool. www.illumina.com/products/by-type/informatics-products/basespace-sequence-hub/apps/variant-calling-assessment-tool.html. Accessed December, 12 2023.

Intended use statements

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.

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.

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 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.

Illumina NextSeq 550Dx High Output Reagent Kit v2.5 (300 Cycles) (European Union)

The Illumina NextSeq 550Dx High Output Reagent Kit v2.5 (300 Cycles) 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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