DATA SHEET

Illumina FFPE DNA Prep with Exome 2.5 Enrichment

Fast, flexible, and scalable exome results with support for low-input, FFPE samples

- Detect rare variants with low allele frequencies from gDNA extracted from FFPE tissue
- Prepare sequencing-ready libraries in ≤ 10 hours with ~4 hours hands-on time
- Analyze data and call variants with high analytical sensitivity using DRAGEN™ secondary analysis
- Enable user-defined interpretation and research report generation with Illumina Connected Insights

illumina®

Sample-to-insights exome sequencing with a trusted partner

In the field of cancer research, archival formalin-fixed paraffin-embedded (FFPE) samples hold an abundance of invaluable information for cancer studies. Because FFPE samples generally yield highly degraded DNA, they can be challenging to analyze with next-generation sequencing (NGS) methods.

Illumina FFPE DNA Prep with Exome 2.5 Enrichment is a versatile library preparation solution (Table 1) that uses the power of NGS technology to achieve highly sensitive detection of low-abundance variants in low-input FFPE samples. This single-vendor solution comprises the library preparation kit, the Twist Bioscience for Illumina Exome 2.5 Panel, and Illumina mid- to high-throughput sequencing systems, including the NextSeq™ 2000 System and the NovaSeq™ X Series. Data analysis is performed using DRAGEN pipelines available in BaseSpace™ Sequence Hub and Illumina Connected Analytics. Illumina Connected Insights can be used to enable user-defined analysis and interpretation.

Streamlined workflow

Illumina FFPE DNA Prep with Exome 2.5 Enrichment is part of an integrated whole-exome sequencing (WES) tumor–normal workflow delivering excellent performance and data quality. The scalable workflow starts with extracted genomic DNA (gDNA) from FFPE samples, followed by library preparation and enrichment. Enriched libraries are sequenced on Illumina mid- and high-throughput systems and highly accurate variant calling is performed using DRAGEN secondary analysis (Figure 1). This user-friendly solution delivers high performance for exome sequencing, provides automation-friendly fill volumes, and accommodates sample multiplexing for efficient scaling (Table 1).

Table 1: Illumina FFPE DNA Prep with Exome 2.5 Enrichment at a glance

Parameter	Specification
DNA type	gDNA from FFPE tissue
DNA input	40 ng FFPE DNA
Sample multiplexing	96–192 unique dual indexes
Duplicate marking	Nonrandom unique molecular identifiers (UMIs)
Enrichment plexity	4-plex
Supported sequencing systems	NextSeq 2000 System, P3 or P4 flow cells NovaSeq 6000 System, SP, S1, or S2 flow cells NovaSeq X Series, 1.5B flow cell
Total workflow time[a]	≤ 10 hrs
Total hands-on time	~4 hrs

a. Includes library preparation, enrichment, and normalization steps.

Fast, flexible library preparation

Illumina FFPE DNA Prep with Exome 2.5 Enrichment is a ligation-based assay that uses a single hybridization step for rapid library preparation (Figure 2). It uses the Twist Bioscience for Illumina Exome 2.5 Panel and allows interrogation of mitochondrial or additional targets when supplemented with the Twist Bioscience for Illumina Mitochondrial Panel or Illumina Custom Enrichment Panel v2.

Sequencing-ready libraries are prepared in ≤ 10 hours, with only ~4 hours of hands-on time, enabling researchers to go from extracted DNA to sequencing in a single day. The QIAGEN AllPrep DNA/RNA FFPE Kit (QIAGEN, Catalog no. 80234) is recommended for DNA extraction from FFPE samples.

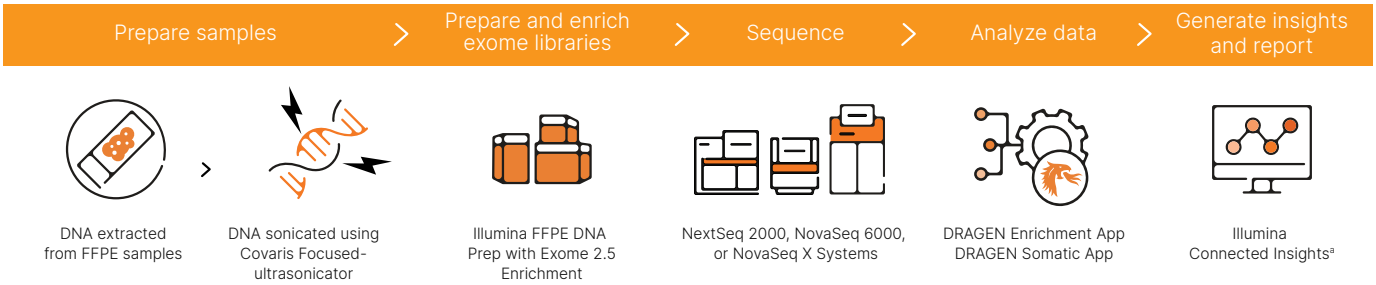

Figure 1: From samples to reporting with a single partner—Illumina supports a streamlined workflow for WES, spanning library preparation, sequencing, and data analysis.

a. The Illumina Connected Insights product line supports user-defined analysis through application programming interface (API) calls to third-party knowledge sources. Illumina Connected Insights integration will be available with a DRAGEN software update in Q2 2025.

Flexible, scalable coverage

Illumina FFPE DNA Prep with Exome 2.5 Enrichment uses a focused, comprehensive, up-to-date exome enrichment panel. This focused coverage enables a cost-effective WES tumor–normal solution at an optimal number of samples per sequencing run (Table 2). Coverage of the mitochondrial genome (chrM) can be added easily using the Twist Bioscience for Illumina Mitochondrial Panel as a spike-in panel in the Illumina FFPE DNA Prep with Exome 2.5 Enrichment protocol. Specific regions of interest can be added with custom spike-in panels of up to 10,000 probes using Illumina Custom Enrichment Panel v2.

High-quality performance

Illumina FFPE DNA Prep with Exome 2.5 Enrichment features the same library preparation and enrichment chemistry as Illumina Cell-Free DNA with Enrichment to enable exceptional performance with FFPE samples.

Read the Illumina Cell-Free DNA Prep with Enrichment data sheet to learn more.

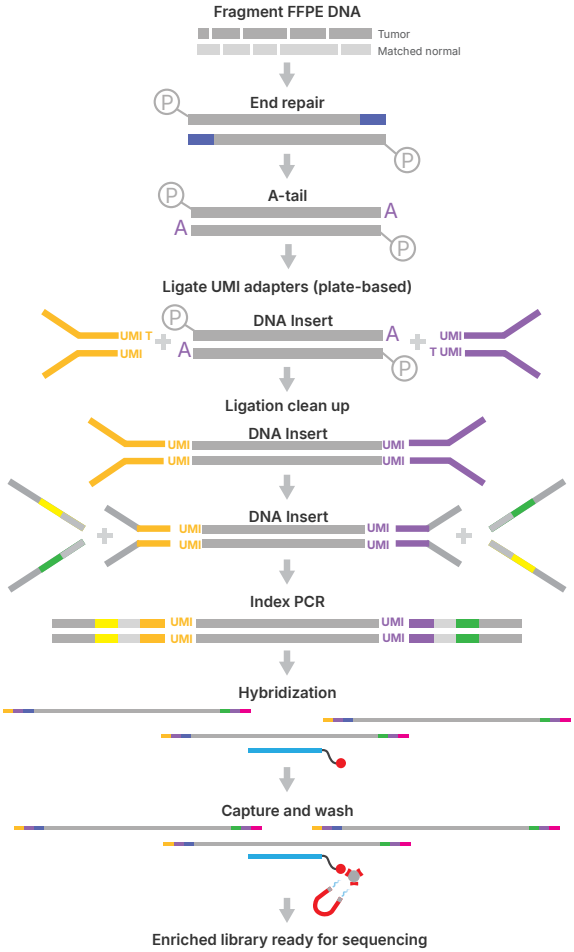

Figure 2: Streamlined ligation-based library preparation with exome enrichment—Following hybrid–capture enrichment, exome libraries are ready for sequencing.

Table 2: Sequencing plexity for Illumina FFPE DNA Prep with Exome 2.5 Enrichment libraries

Sample type	Desired mean target coverage depth[a]	Recommended single-end reads	Maximum number of libraries per flow cell					
			NextSeq 2000 System		NovaSeq 6000 System			NovaSeq X Series
			P3	P4	SP	S1	S2	1.5B
Tumor	≥ 130×	≥ 100M	8	12	4	12	28	12
Normal	≥ 30×	≥ 28M	8	12	4	12	28	12

a. Mean target coverage was calculated with the setting to not double-count overlapping mates.

Illumina FFPE DNA Prep with Exome 2.5 Enrichment shows outstanding enrichment assay performance with FFPE tissue samples. Libraries were prepared from 40 ng of input FFPE DNA from 12 tumor–normal pairs using the Illumina FFPE DNA Prep with Exome 2.5 Enrichment kit and the Twist Bioscience for Illumina Exome 2.5 Panel. Sequencing was performed on the NovaSeq 6000 System using the S1 flow cell with a targeted sequencing depth of ≥ 100M single-end reads for tumor and ≥ 28M single-end reads for matched normal samples. Tumor libraries from mid- to high-quality FFPE samples show mean target coverage depth above 130× (where mean target coverage was calculated with the setting to not double-count overlapping mates) and ≥ 90% of targets achieved ≥ 50× coverage, with minimal impact on other library quality control (QC) metrics (Figure 3).

Sensitive detection of low-frequency variants

Illumina FFPE DNA Prep with Exome 2.5 Enrichment library detects small DNA variants at low variant allele frequencies (VAF). Libraries were prepared using 40 ng Seraseq Tumor Mutation DNA Mix v2 AF10 (SeraCare, Catalog no. 0710-0094) as "tumor" sample and 40 ng Seraseq FFPE WT (DNA/RNA) Reference Material (SeraCare, Catalog no. 0710-0137) as the paired "normal" sample. The mutation mix was diluted to < 10% VAF. Twelve "tumor" and four "normal" library replicates per VAF level were prepared. The 13 4-plex enriched libraries with the Twist for Illumina Exome 2.5 panel were sequenced on two S2 flow cells on the NovaSeq 6000 system at 2 × 151 bp read length.

For analysis, the sequencing reads of the Tumor libraries were subsampled to a read depth of 100M, 70M, and 46M single-end reads to explore the relationship between UMI-collapsed on-target coverage and variant detection. The reads for normal libraries were subsampled to 28M single-end reads. Variant calling was performed using the DRAGEN Somatic App in BaseSpace Sequence Hub.

Illumina FFPE DNA Prep with Exome 2.5 Enrichment detects gene amplifications at 2.5 fold-change. Library replicates were prepared using 40 ng or 20 ng (four replicates each) of Seraseq Solid Tumor CNV Mix +3 copies (SeraCare, Catalog no. 0710-2866) as "tumor" sample, and 40 ng DNA from Seraseq FFPE WT (DNA/RNA) Reference Material (SeraCare, Catalog no. 0710-0137) as the paired "normal" sample. The libraries were sequenced on an S1 flow cell on the NovaSeq 6000 system at 2 × 151 bp read length, and sequencing reads subsampled to 100M single-end reads for the CNV Mix and 28M reads for the WT DNA Reference sample. Somatic CNV calling was performed with the DRAGEN Somatic App in BaseSpace Sequence Hub using a baseline file from a panel of normals constructed from 45 normal FFPE tissue samples and analyzed with the DRAGEN default segmentation algorithm. Reported values are the mean and standard deviation of the eight replicates of the CNV Mix and compared against the expected fold-change reported by SeraCare.

The assay achieves 90% detection of single nucleotide variants (SNVs) (Figure 4A) and insertions/deletions (indels) (Figure 4B) at 5% VAF from 40 ng input with 100M single-end reads. The sensitivity at 6–7% VAF remains high with as low as 46M reads per tumor library. 100% sensitivity was observed for the detection of 12 gene amplifications at ~2.5 fold-change in a control sample with three additional copies per gene (Figure 5).

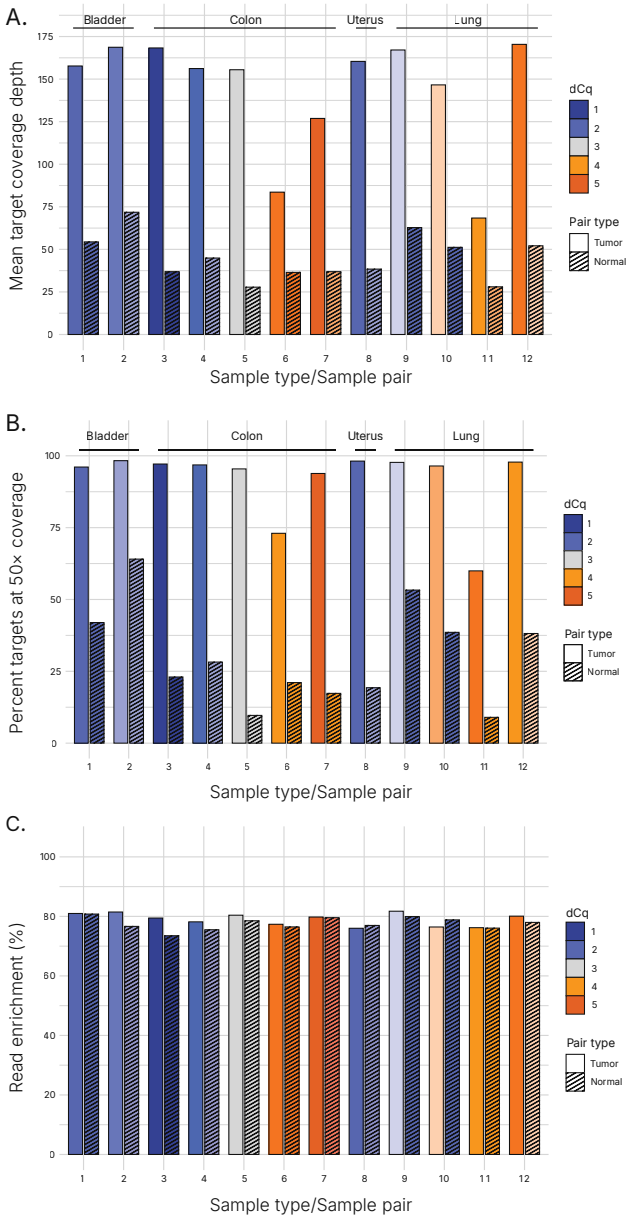

Figure 3: Performance metrics for FFPE tissue samples—Tumor–normal libraries from mid- to high-quality FFPE tissue samples achieved excellent enrichment metrics, including > 70% read enrichment, high mean target coverage depth, and > 90% of targets with ≥ 50× coverage.

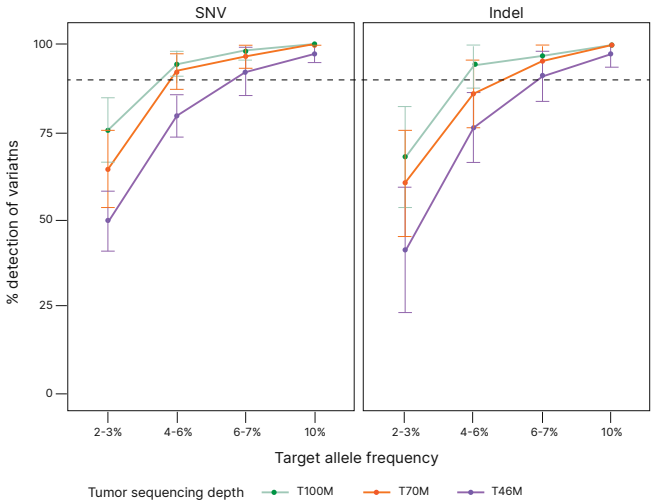

Figure 4: Variant detection at low VAF—Results demonstrate that Illumina FFPE DNA Prep with Exome 2.5 Enrichment achieves 90% detection of SNVs and indels at 5% VAF from 40 ng input with 100M single-end reads (green line). As expected, the detection rate for 4–5% VAF variants decreases with the number of reads. However, the sensitivity remains high for variants around 6–7% VAF with as few as 46M single-end reads (purple line) per tumor library. The dashed line represents 90% variant detection.

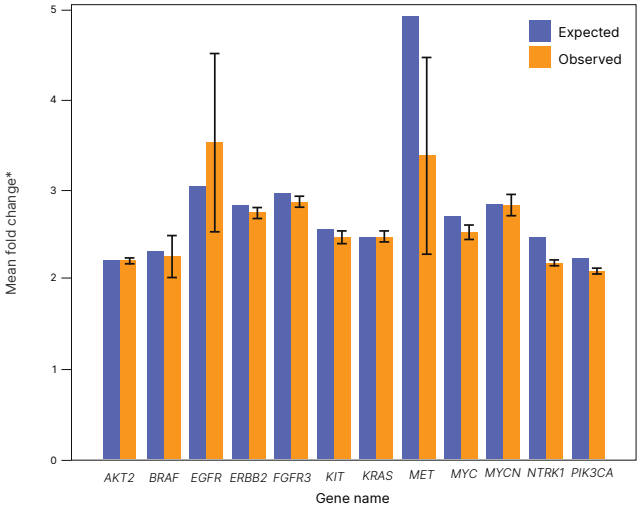

Figure 5: Gene amplification detection— 100% sensitivity was observed using Illumina FFPE DNA Prep with Exome 2.5 Enrichment for the detection of 12 gene amplifications in a control sample with three additional copies per gene. Reported values are the mean and standard deviation of the eight replicates of the CNV Mix and compared against the expected fold-change reported by Seracare. *Seracare reports that *BRAF*, *EGFR*, and *MET* were amplified using two synthetic constructs with small areas of overlap, therefore, CNV events spanning these overlap regions may show inflated amplification value.

Accurate evaluation of tumor mutational burden (TMB)

Illumina FFPE DNA Prep with Exome 2.5 Enrichment combines the comprehensive exome content of the Twist for Illumina Exome 2.5 Panel and the sophisticated DRAGEN Somatic bioinformatics pipeline to provide accurate TMB estimations. The capability of Illumina FFPE DNA Prep with Exome 2.5 Enrichment to assess TMB was evaluated using SeraCare TMB reference standards and FFPE DNA from tissue samples. Illumina FFPE DNA Prep with Exome 2.5 Enrichment libraries were prepared with 40 ng of Seraseq gDNA TMB Mix Score 7 and TMB Mix Score 13 standards and their respective "normal" samples (SeraCare, Catalog no. 0710-1326 and 0170-1586, respectively). Sequencing data were analyzed with the DRAGEN Enrichment app, and the BAM files imported as tumor-normal pairs to the DRAGEN Somatic app. Results show that observed TMB values meet expected values (Table 3).

Table 3: Accurate TMB evaluation with Illumina FFPE DNA Prep with Exome 2.5 Enrichment

Seraseq gDNA TMB mix	Expected TMB score	DRAGEN Somatic App TMB score
TMB - high	13	16.7 (0.1)
TMB - low	7	9.4 (0.1)

Reported values are the mean and standard deviation of six technical replicates.

Concordance with TruSight™ Oncology 500

Illumina FFPE DNA Prep with Exome 2.5 Enrichment libraries were prepared with 40 ng FFPE DNA from tumor and matched normal samples. Libraries were sequenced on multiple S2 flow cells of the NovaSeq 6000 System at 2 x 151 bp read length, and sequencing reads for tumor and matched normal libraries subsampled to the recommended number of reads (Table 2). Data was analyzed with the DRAGEN Enrichment app, and the BAM files imported as tumor/normal pairs to the DRAGEN Somatic app in BaseSpace Sequence Hub. In parallel, 40 ng of the same FFPE DNA from the tumor samples was processed with TruSight Oncology 500, and data analyzed using TruSight Oncology 500 v2.2.1 local app software. Results show high concordance (R^2 = 0.999) with the TMB score reported by the TruSight Oncology 500 workflow, demonstrating excellent performance of Illumina FFPE DNA Prep with Exome 2.5 Enrichment for assessing this key oncology biomarker (Figure 6). An analysis pipeline for microsatellite instability (MSI) and homologous recombination deficiency (HRD) biomarkers is under development; results from the DRAGEN Somatic App should be considered preliminary. Contact your sales specialist to learn about these biomarkers.

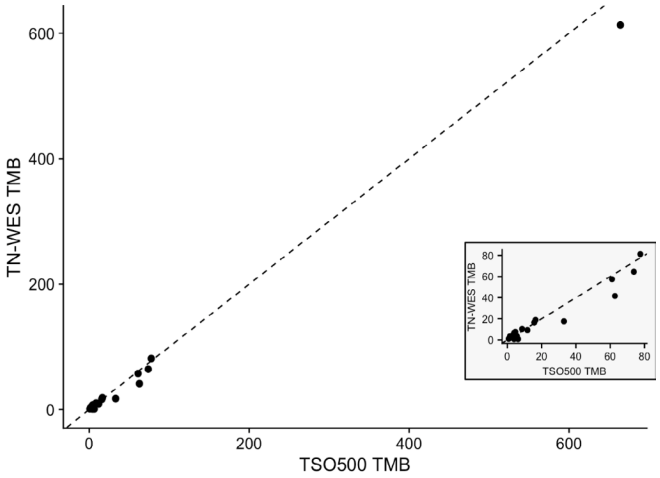

Figure 6: Accurate TMB evaluation—Evaluation of TMB with Illumina FFPE DNA Prep with Exome 2.5 Enrichment shows high concordance with TMB scores obtained with TruSight Oncology 500. Inset plot shows the TMB values zoomed to ≤ 80.

Content flexibility with Illumina FFPE DNA Prep with Exome 2.5 Enrichment

Illumina FFPE DNA prep with Exome 2.5 enrichment is compatible with custom probes designed to cover new targets or to boost coverage of higher interest regions. Panels are designed for a customized list of targets and added easily as spike-in probes in the Illumina FFPE DNA Prep with Exome 2.5 Enrichment protocol. Illumina custom panels v2 are designed through the DesignStudio™ online design tool, or with help from the Illumina Concierge team.

Table 4: Compatibility of Illumina FFPE DNA Prep with Exome 2.5 Enrichment with supplemental content

Panel	Twist for Illumina Mitochondrial	Custom Enrichment v2 Panel A	Custom Enrichment v2 Panel B	
Panel size (bases)	16,659	244,283	1,590,551	
Number of probes	139	2804	10,353	
Number of genes covered	37	79	1038	
Spike-in panel/Exome panel ratio	0.008[a]	0.4[a]	0.4[a]	
Sequencing reads[b]	100M	100M	100M	110M–220M[c]
Mean target coverage exome[d]	136×	137×	111×	151×
Mean target coverage spike-in region[d]	1397×	193×	52×	72×
Percent exome targets coverage ≥ 50×	93%	93%	85%	93%
Percent spike-in targets coverage ≥ 50×	99.70%	99%	42%	57%

a. 2 µl of 1:50 dilution from Twist for Illumina Mitochondrial Panel, or 2 µl of Custom Enrichment v2 Panel mixed with 3 µl of Twist for Illumina Exome 2.5 Panel.
b. Number of single-end sequencing reads in millions.
c. Indicates the range of sequencing reads observed for the different FFPE DNA tumor samples.
d. Mean target coverage was calculated with the setting to not double-count overlapping mates.

To demonstrate the compatibility of Illumina FFPE DNA Prep with Exome 2.5 Enrichment with a wide range of spike-in panel sizes, the Illumina FFPE DNA Prep with Exome 2.5 Enrichment protocol was performed with the Twist for Illumina Mitochondrial Panelor two Illumina Custom Enrichment Panels v2 (Table 4). Results demonstrate a correlation between the size of the spike-in panel and the coverage of exome and spike-in targets. Higher coverage is achieved with the small mitochondrial panel, while excellent coverage is achieved for both the exome and the spike-in targets when using a mid-sized spike-in panel (Panel A). For the large spike-in Panel B, exome coverage remains high while the coverage for the region targeted by the spike-in panel is modest but can be increased with deeper sequencing. Deeper coverage of overlapping regions (Figure 7A) or sequencing coverage for new targets (Figure 7B) is observed in the presence of spike-in panels. Furthermore ~10-fold increase on mean target coverage depth and the percent of targets with ≥ 50× coverage was observed in the regions targeted by Custom Enrichment v2 Panel B when the spike-in panel was combined with Twist for Illumina Exome 2.5 Panel during the enrichment reaction (Table 5).

Table 5: Spike-in of Illumina Custom Enrichment v2 Panel improves enrichment metrics

Parameter	Twist for Illumina Exome 2.5	Twist for Illumina Exome 2.5 + spike-in Panel[a]
Mean target coverage depth for spike-in region[b]	5.5×	52×
Coverage uniformity spike-in	20%	89%
Percent spike-in targets with coverage ≥ 20×	5%	78%
Percent spike-in targets with coverage ≥ 50×	4%	42%

a. Custom Enrichment v2 Panel B, see Table 4 for panel details.
b. Mean target coverage was calculated with the setting to not double-count overlapping mates.

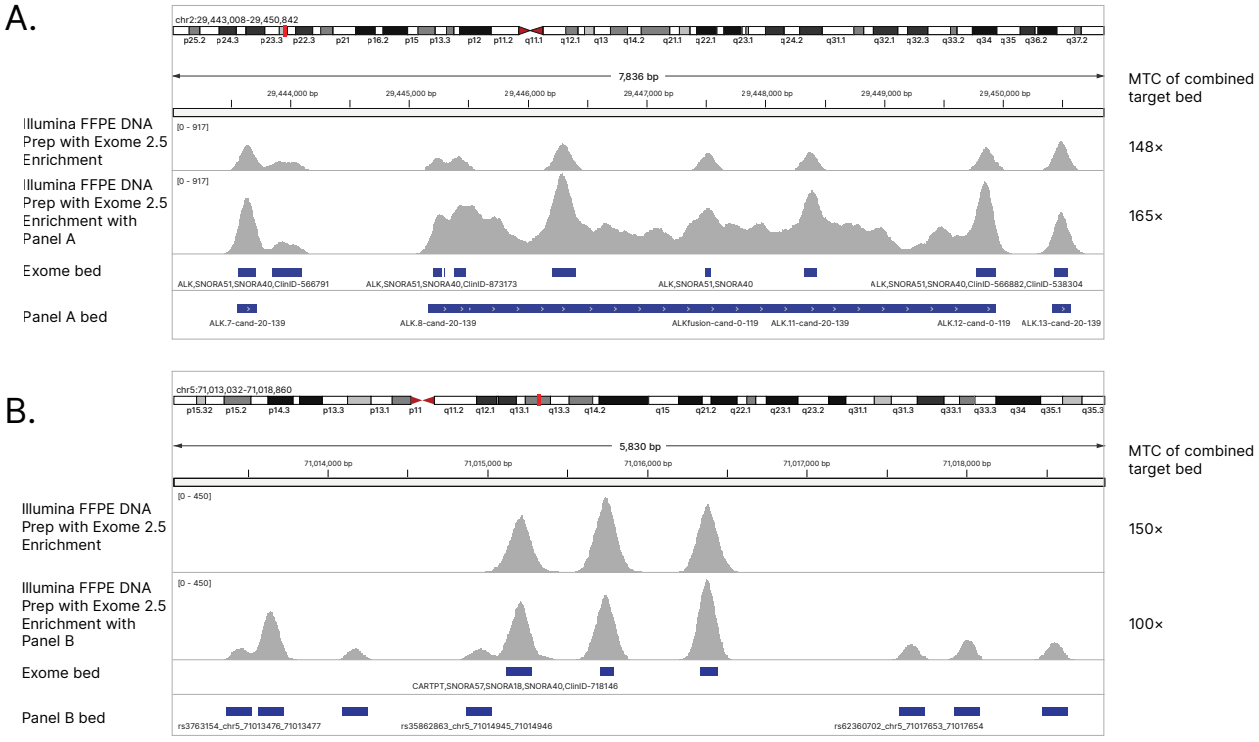

Figure 7: Deeper sequencing coverage with Illumina Custom Enrichment v2 spike-in panels—(A) Comparing mean target coverage (MTC, calculated with the setting to not double-count overlapping mates) of overlapping regions between the Illumina FFPE DNA Prep Exome 2.5 Enrichment Panel and Custom Enrichment v2 Panel A (see Table 4) with or without a spike-in panel shows more than 2-fold increase in coverage in the presence of the spike-in Panel A. (B) Comparing coverage of new regions targeted by Custom Enrichment v2 Panel B (see Table 4) with or without spike-in panel shows coverage in the presence of the spike-in Panel B.

Summary

Illumina FFPE DNA Prep with Exome 2.5 Enrichment offers a single-vendor, versatile library preparation solution optimized for use with low-input DNA from FFPE tissue samples. With the Illumina FFPE DNA Prep with Exome 2.5 Enrichment solution, researchers can detect low-frequency somatic variation with exceptional analytical sensitivity. The high-performance of the Illumina FFPE DNA Prep with Exome 2.5 Enrichment solution combined with sequencing on powerful Illumina sequencing systems and accelerated data analysis delivers a high-quality NGS tumor–normal workflow for FFPE DNA, spanning sample processing to data analysis, from a single trusted partner.

Learn more

Illumina FFPE DNA Prep with Exome 2.5 Enrichment

DRAGEN secondary analysis

Illumina Connected Insights

Ordering information

Product	Catalog no.
Illumina Cell-Free and FFPE DNA Prep with Enrichment, Ligation (192 samples, 4-plex)	20104103
Illumina Cell-Free and FFPE DNA Prep with Enrichment, Ligation (192 samples, 4-plex), on-premises[a]	20104104
Illumina Cell-Free and FFPE DNA Prep, Ligation (16 Samples)	20104105
Illumina Cell-Free and FFPE DNA Prep, Ligation (96 Samples)	20104106
Illumina Cell-Free and FFPE DNA Prep, Enrichment (16 Reactions)	20104107
IDT for Illumina UMI DNA/RNA UD Indexes Set A, Ligation (96 Indexes, 96 Samples)	20034701
IDT for Illumina UMI DNA/RNA UD Indexes Set B, Ligation (96 Indexes, 96 Samples)	20034702
Illumina UMI DNA/RNA UD v3 indexes Set A, Ligation (96 indexes, 96 samples)	20126235
Illumina UMI DNA/RNA UD v3 indexes Set B, Ligation (96 indexes, 96 samples)	20126237
IDT for Illumina UMI DNA/DNA Index Anchors Set A for Automation	20066404
IDT for Illumina UMI DNA/DNA Index Anchors Set B for Automation	20063213
Twist Bioscience for Illumina Exome 2.5 Panel	20076914
Twist Bioscience for Illumina Mitochondrial Panel	20093180

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