illumina®

DATA SHEET

Illumina DNA Prep with Exome 2.5 Enrichment v2

Enhanced WES performance with improved exome coverage

Achieve efficient exome enrichment with on-bead tagmentation

Analyze variant-rich and challenging genomic regions with an optimized panel design

Expand coverage using optional mitochondrial and custom spike-in content

Introduction

Whole-exome sequencing (WES) is an essential tool for uncovering the genetic basis of inherited disease and cancer.[1-3] As research needs expand, laboratories require WES solutions that deliver consistent enrichment, strong representation of GC-rich and variable regions, and efficient workflows that can scale without increasing sequencing demands.

The original Illumina DNA Prep with Exome 2.5 Enrichment assay provided a streamlined WES workflow using hybrid–capture enrichment and automation-friendly processes. Illumina DNA Prep with Exome 2.5 Enrichment v2 introduces updated chemistry for improved performance while using less sequencing capacity. The v2 assay delivers better on-target enrichment and minimizes off-target reads, while addressing lower GC coverage and enhancing coverage uniformity across targeted exons. Optional mitochondrial enrichment and custom spike-in content are supported, enabling laboratories to refine or extend target coverage within the same workflow.

The library preparation and enrichment solution is part of an end-to-end workflow that spans sample preparation to reporting (Figure 1). The assay uses the same tagmentation-based library preparation and hybrid–capture enrichment as the original assay, maintaining an automation-friendly workflow with reproducible performance (Figure 2).

Together, the advancements in Illumina DNA Prep with Exome 2.5 Enrichment v2 deliver enhanced enrichment performance, improved coverage of GC regions, and flexible content options, while also enabling scalable studies without added complexity.

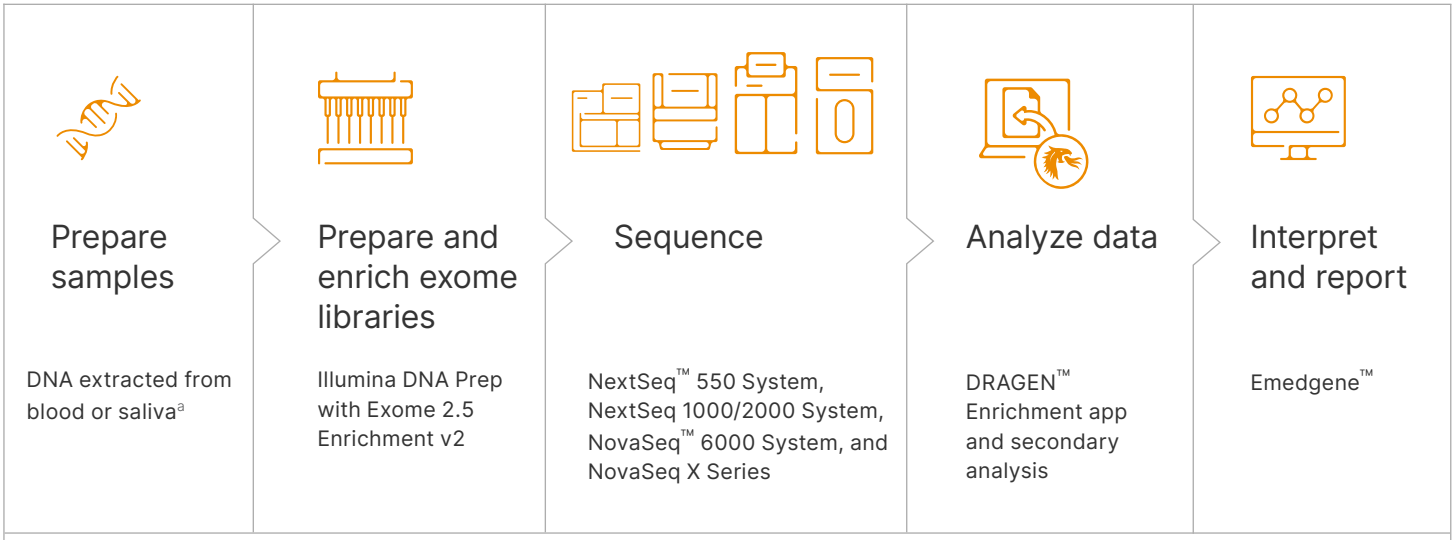

Figure 1: End-to-end WES workflow
Illumina DNA Prep with Exome 2.5 Enrichment v2 is part of a comprehensive workflow that supports whole-exome sequencing (WES) from sample preparation through data analysis and interpretation. Extracted genomic DNA is prepared and enriched using Illumina DNA Prep with Exome 2.5 Enrichment v2, with hands-on time reduced through automation-friendly workflows. Enriched libraries are sequenced and enrichment analysis is performed using the DRAGEN Enrichment app. Variant calling is performed using DRAGEN secondary analysis.[4] Interpretation can be performed with tools such as Emedgene.
a. DNA is extracted using the Flex Lysis Reagent Kit.

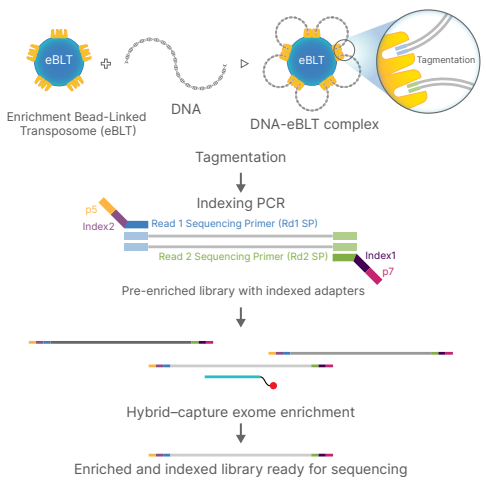

Figure 2: Streamlined tagmentation-based library preparation with exome enrichment
On-bead tagmentation chemistry mediates uniform fragmentation and adapter tagging with high tolerance to variation in DNA input amounts.[5] Following hybrid–capture enrichment, the prepared libraries are ready for sequencing, and the integrated normalization approach helps support even sequencing output across enriched libraries.

Comprehensive, focused coverage of disease-associated variants

Illumina DNA Prep with Exome 2.5 Enrichment v2 uses an up-to-date exome panel that balances breadth and efficiency by concentrating sequencing capacity on regions with the greatest relevance to human disease research. The complete kit includes the Twist Bioscience for Illumina Exome 2.5 Panel, which offers curated coverage of coding sequences from widely used public resources, including RefSeq, CCDS, ACMG, COSMIC, and OMIM* (Table 1).[6-11] This targeting strategy reflects current knowledge of disease-associated genes while maintaining a focused footprint that preserves sequencing efficiency.

In addition to comprehensive gene-level coverage, the panel targets exonic regions where pathogenic and likely pathogenic variants are frequently reported in databases such as ClinVar and ACMG (Table 2). This supports studies that rely on consistent representation of well-characterized variant sites across the exome.

The design also emphasizes performance in challenging regions, including GC-rich segments and difficult-to-cover exons. Improvements in probe placement and target selection help maintain coverage uniformity and minimize dropout, enabling reliable variant detection across a wide range of genomic contexts.

Table 1: Coverage of coding regions represented in key databases with the Twist Bioscience for Illumina Exome 2.5 Panel

Exome panel	Twist Bioscience for Illumina Exome 2.5 Panel	Illumina Exome Panel
Size	37.5 Mb	42.21 Mb
RefSeq CDS[6]	99.1%	98.2%
CCDS CDS[7]	99.9%	99.5%
ACMG 73 genes CDS[8]	99.9%	99.3%
COSMIC Cancer Gene Census CDS[9,10]	99.9%	99.3%
OMIM[11]	99.1%	97.7%

Table 2: Coverage of pathogenic and likely pathogenic variant sites included in disease-related genetic variant databases

ClinVar pathogenic/likely pathogenic variants CDS[a,12]	98.6%
ACMG 73 pathogenic/likely pathogenic variants CDS[b,8]	99.9%

a. The ClinVar public archive reports relationships among human variations and phenotypes with supporting evidence. Pathogenic/likely pathogenic variants are reported based on ClinVar classification guidelines.
b. The ACMG pathogenic/likely pathogenic variants list includes the overlaid variants between the curated coding sequences of ACMG genes and ClinVar pathogenic/likely pathogenic variants.

* ACMG, American College of Medical Genetics and Genomics; CCDS, Consensus Coding Sequence; COSMIC, Catalogue of Somatic Mutations in Cancer; OMIM, Online Mendelian Inheritance in Man.

By focusing coverage on the most informative portion of the exome and limiting representation of low-value or low-yield regions, the panel enables efficient use of sequencing capacity while maintaining depth where it matters most. This targeted footprint supports flexible batching across Illumina sequencing platforms, allowing laboratories to optimize throughput and cost per sample according to project size and experimental needs (Table 3).

Extensive coverage of curated targets

Illumina DNA Prep with Exome 2.5 Enrichment v2 delivers broad coverage across curated clinical and research resources under both rapid and extended hybridization conditions (Figure 3). This level of coverage helps ensure that genes and regions commonly used in human disease research are consistently represented, reducing the likelihood of gaps in key exonic content.

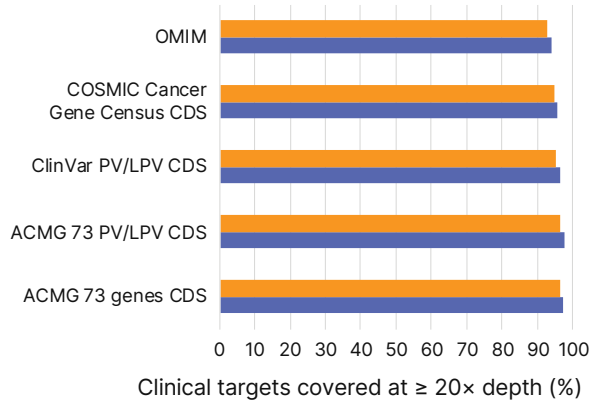

Figure 3: Consistent coverage of disease-associated targets across hybridization conditions

Illumina DNA Prep with Exome 2.5 Enrichment v2 achieves high percentages of targets reaching ≥ 20× coverage under both 1.5-hr (orange) and 16-hr (blue) hybridization conditions. Representative results are shown for genes and variants listed in major public databases, including ACMG, ClinVar, OMIM, and COSMIC.[8-13]

ACMG, American College of Medical Genetics and Genomics; CDS, coding sequence; COSMIC, Catalogue of Somatic Mutations in Cancer; OMIM, Online Mendelian Inheritance in Man; PV/LPV, pathogenic/likely pathogenic variant.

Table 3: Sample capacity for WES by sequencing system and flow cell[a]

Desired mean target coverage depth	No. of samples										
	NextSeq 550 System[b]		NextSeq 2000 System		NovaSeq 6000 System				NovaSeq X System		
	Mid-output	High-output	P2[c]	P3	SP	S1	S2	S4	1.5B	10B	25B
50×	6	19	19	57	25	50	128	310[d]	62	380	944
100×	3	9	9	28	12	25	64	155	31	190	497
200×	1	4	4	14	6	12	32	77	15	95	248

a. Estimates are based on 2 × 100 bp read length, calculated based on typical internal experiments. Number of samples may vary depending on workflow handling, input sample or library quality, and actual sequencing output of each platform and flow cell. Actual data was acquired on the NovaSeq 6000 System and NovaSeq X System using S4 and 10B flow cells, respectively, and extrapolated to other instruments and flow cells.
b. NextSeq 550 reagent kits support 2 × 150 bp read lengths.
c. P2 flow cells with the same sample throughput are also available on the NextSeq 1000 System.
d. Pooling libraries at this scale requires additional indexes.

Enrichment performance

Illumina DNA Prep with Exome 2.5 Enrichment v2 demonstrates strong enrichment performance for both 1.5-hour and 16-hour hybridization conditions (Figure 4). The refined probe design supports efficient hybrid capture, reflected in high proportions of padded unique reads and padded unique bases aligning to the targeted regions.

Mean target coverage is similarly stable under both conditions, indicating reliable representation of targeted exons. The percentage of passing filter unique reads aligned to target regions also remains high, supporting reliable detection of variants across biologically relevant loci.

Coverage uniformity and target representation

Illumina DNA Prep with Exome 2.5 Enrichment v2 also demonstrates uniform and reliable coverage across targeted exons for both 1.5-hour and 16-hour hybridization conditions (Figure 5). The panel design supports consistent read distribution across targeted regions, yielding low fold-80 base penalty values. GC-rich segments, which are traditionally difficult to enrich using hybrid–capture methods, show improved representation, with reduced GC dropout and a low percentage of zero-coverage targets across diverse genomic contexts.

Compared to the original version, Illumina DNA Prep with Exome 2.5 Enrichment v2 shows higher coverage uniformity, lower GC dropout, and reduced fold-80 base penalty values (Figure 6). These metrics demonstrate a more balanced performance and reliable variant detection across exons with varied sequence composition.

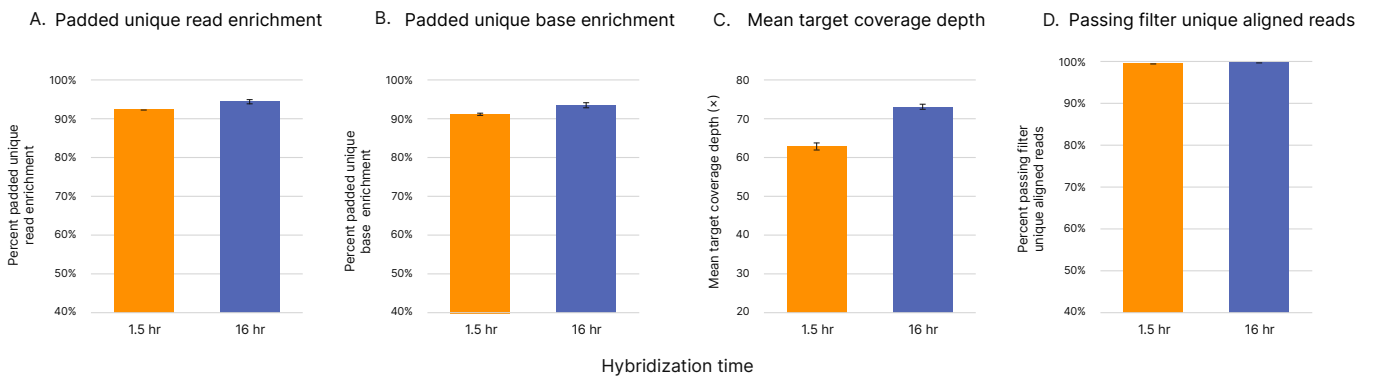

Figure 4: Enrichment performance across hybridization times

Illumina DNA Prep with Exome 2.5 Enrichment v2 demonstrates consistent enrichment performance for both 1.5-hr and 16-hr hybridization times. (A) consistently high padded unique read enrichment, (B) strong padded unique base enrichment across targets, (C) stable mean target coverage depth, (D) high percentages of passing filter unique reads aligned to targeted regions.

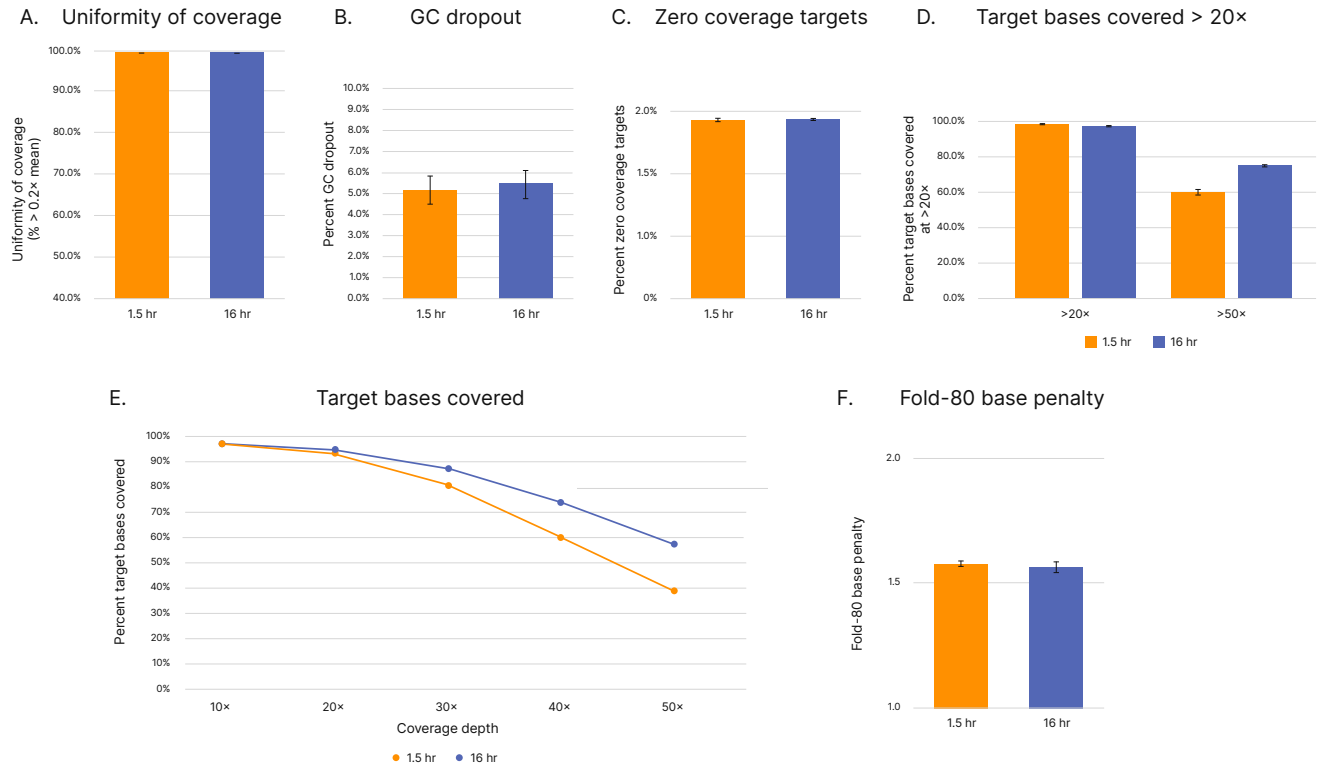

Figure 5: Coverage uniformity across hybridization times

Illumina DNA Prep with Exome 2.5 Enrichment v2 provides balanced and consistent coverage for both 1.5-hr and 16-hr hybridization times. (A) uniformity of coverage across targeted regions, (B) low percent GC dropout, (C) low percent zero-coverage targets, (D) high percent of targets with ≥ 50× coverage depth, (E) high percent of target bases covered at the indicated depth thresholds, (F) low fold-80 base penalty values.

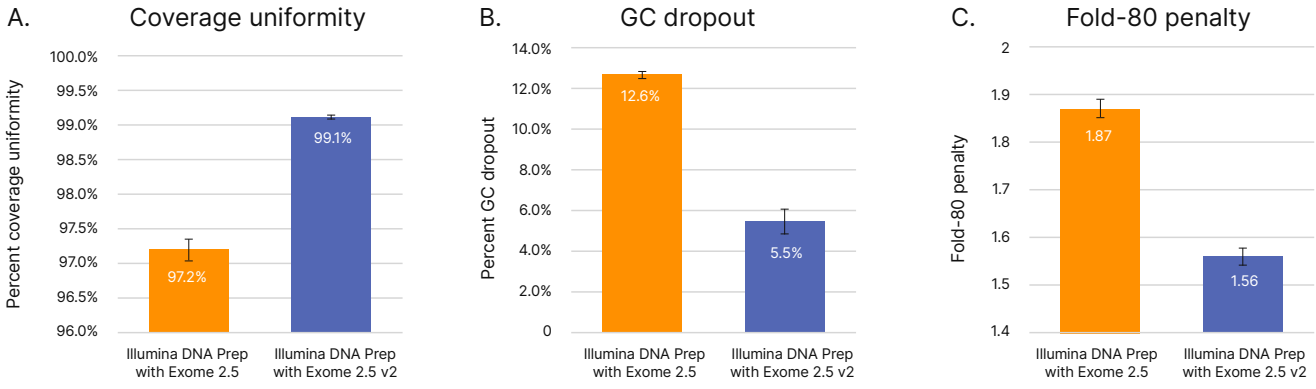

Figure 6: Improved coverage metrics with Illumina DNA Prep with Exome 2.5 Enrichment v2

Illumina DNA Prep with Exome 2.5 Enrichment v2 shows (A) higher coverage uniformity, (B) reduced GC dropout, and (C) lower fold-80 values, reflecting improved performance across both standard and GC-rich targets compared to the original version.

Optional enrichment content

Illumina DNA Prep with Exome 2.5 Enrichment v2 supports additional enrichment content that enables researchers to extend or refine WES coverage based on specific study requirements. These additions integrate directly into the standard workflow without requiring changes to library preparation or hybridization steps. The flexibility to include custom genomic content makes the workflow suitable for diverse research applications, including disease gene discovery, cancer genomics, and mechanistic studies of complex traits.

Mitochondrial genome enrichment

The Twist Bioscience for Illumina Mitochondrial Panel can be added as a spike-in to extend coverage to the mitochondrial genome within the Illumina DNA Prep with Exome 2.5 Enrichment v2 workflow. Incorporating this panel increases representation of mitochondrial targets without reducing coverage of the standard exome panel (Figure 7). Both data types can be generated from a single library preparation and sequencing run.

Custom spike-in content

Researchers can expand WES coverage using the Illumina Custom Enrichment Panel v2, which allows user-defined genomic regions to be incorporated into the hybrid–capture reaction. This is valuable for studies that require emerging disease genes, candidate regions from discovery projects, institution-specific targets, or other loci not included in standard exome designs. The custom panel is added as a spike-in to the existing hybridization step and does not alter the core workflow.

The spike-in content increases coverage across selected regions while maintaining performance across the base exome panel (Table 4). This provides an efficient way to incorporate additional targets into a WES workflow without running a separate assay, supporting flexible study designs while preserving the coverage performance of the primary panel.

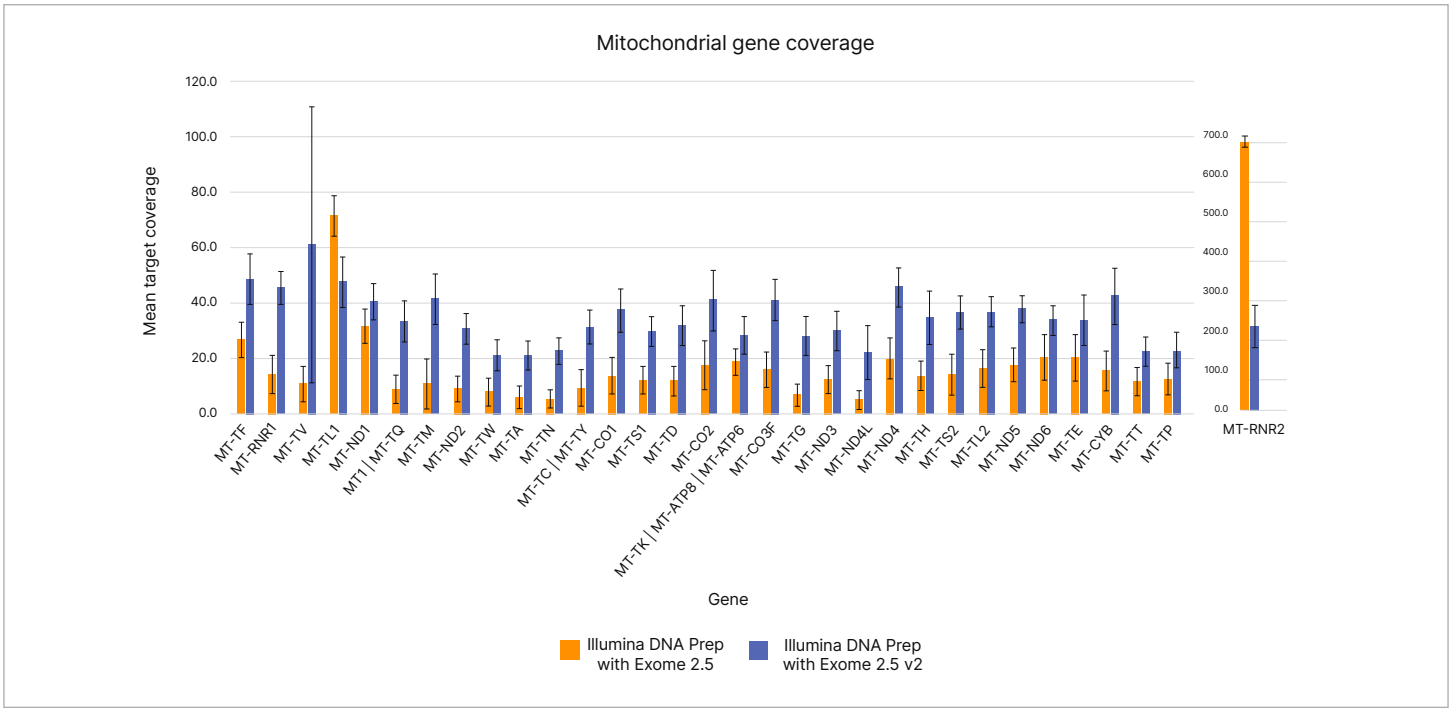

Figure 7: Gene-level coverage with Illumina DNA Prep with Exome 2.5 Enrichment v2
Illumina DNA Prep with Exome 2.5 Enrichment v2 provides consistent coverage across mitochondrial genes, demonstrating stable target representation across the updated exome panel.

Table 4: Illumina Custom Enrichment Panel v2 spike-in panel increases target coverage[a]

Parameter	Illumina DNA Prep with Exome 2.5 Enrichment v2	Illumina DNA Prep with Exome 2.5 Enrichment v2 plus custom spike-in panel
Mean target coverage	66×	154×
Percent targets covered at 30×	86.90%	98.60%
Percent targets covered at 50×	71.10%	94.40%

a. The Illumina DNA Prep with Exome 2.5 Enrichment v2 protocol was performed with and without a custom spike-in panel targeting regions covered by the Twist Bioscience for Illumina Exome 2.5 Panel. Coverage was then assessed across the regions targeted by the spike-in panel. Enriched libraries were sequenced on a NovaSeq 6000 System using a S4 flow cell (50M paired-end reads, 25M clusters) and enrichment analysis was performed using the DRAGEN Enrichment app.

Summary

Illumina DNA Prep with Exome 2.5 Enrichment v2 delivers a streamlined, flexible WES workflow with strong performance across the human exome. The panel design provides current coverage of biologically relevant genes within a focused footprint that optimizes sequencing capacity. Efficient target enrichment, consistent enrichment metrics, and uniform coverage under both rapid and extended hybridization conditions help ensure reliable representation of targeted exons, including GC-rich and traditionally challenging regions. This performance is reflected in low fold-80 base penalty values and minimal dropout. Optional mitochondrial enrichment and support for custom spike-in content enable laboratories to extend or refine coverage within the same workflow. Together, these features make Illumina DNA Prep with Exome 2.5 Enrichment v2 a scalable and reliable solution for WES studies in inherited disease, cancer, and other genome-based research.

Learn more →

Illumina DNA Prep with Exome 2.5 Enrichment v2

Library prep automation

DRAGEN secondary analysis

Emedgene tertiary analysis

DesignStudio Assay Design Tool

Ordering information

Product	Catalog no.
Illumina DNA Prep with Exome 2.5 Enrichment v2, (S) Tagmentation Set B (19 samples, 12-plex)[a]	20155541
Illumina DNA Prep with Exome 2.5 Enrichment, (S) Tagmentation Set D (19 samples, 12-plex)[a]	20155543
Flex Lysis Reagent Kit (96 reactions)[b]	20018706
Illumina DNA/RNA UD Indexes Set A, Tagmentation (96 indexes, 96 samples)[c]	20091654
Illumina DNA/RNA UD Indexes Set B, Tagmentation (96 indexes, 96 samples)[c]	20091656
Illumina DNA/RNA UD Indexes Set C, Tagmentation (96 indexes, 96 samples)[c]	20091658
Illumina DNA/RNA UD Indexes Set D, Tagmentation (96 indexes, 96 samples)[c]	20091650
Twist Bioscience for Illumina Mitochondrial Panel (96 samples, 12-plex)[d]	20093180
Illumina Custom Enrichment Panel v2 (32 µl, 120 bp)[e]	20073953
Illumina Custom Enrichment Panel v2 (384 µl, 120 bp)[e]	20073952
Illumina Custom Enrichment Panel v2 (1536 µl, 120 bp)[e]	20111339

a. Kits include Illumina DNA Prep with Enrichment v2 library preparation and hybridization reagents, Illumina Purification Beads for cleanup/size selection, the Twist Bioscience for Illumina Exome 2.5 Panel enrichment probes, and an index adapter plate.
b. Kit required for direct blood input.
c. Choose a different index set if preferred.
d. Twist Bioscience for Illumina Mitochondrial Panel contains 32 µl of oligo panel, sufficient material for 8 hybridization reactions at 4 µl each.
e. Custom enrichment panels for human samples can be designed through the Illumina DesignStudio tool. Design support for nonhuman content is enabled through the Illumina Concierge design team. Contact your Illumina sales representative for more information about Concierge design services.

References

1. Clark MM, Stark Z, Farnaes L, et al. Meta-analysis of the diagnostic and clinical utility of genome and exome sequencing and chromosomal microarray in children with suspected genetic diseases. *NPJ Genom Med*. 2018;3:16. doi:10.1038/s41525-018-0053-8
2. Heydt C, Rehker J, Pappesch R, et al. Analysis of tumor mutational burden: correlation of five large gene panels with whole exome sequencing. *Sci Rep*. 2020;10(1):11387. doi:10.1038/s41598-020-68394-4
3. Kopetz S, Mills Shaw KR, Lee JJ, et al. Use of a targeted exome next-generation sequencing panel offers therapeutic opportunity and clinical benefit in a subset of patients with advanced cancers. *JCO Precis Oncol*. 2019;3:PO.18.00213. doi:10.1200/PO.18.00213
4. Mehio R, Ruehle M, Catreux S, et al. DRAGEN wins at PrecisionFDA Truth Challenge V2 showcase accuracy gains from alt-aware mapping and graph reference genomes. illumina.com/science/genomics-research/articles/dragen-wins-precisionfda-challenge-accuracy-gains. Published June 2, 2021. Accessed November 19, 2025.
5. Illumina. Illumina DNA Prep with Enrichment Data Sheet. illumina.com/content/dam/illumina/gcs/assembled-assets/marketing-literature/illumina-dna-prep-for-enrichment-770-2020-010/illumina-dna-prep-enrichment-data-sheet-770-2020-010.pdf. Published October 29, 2020. Accessed November 19, 2025.
6. NIH National Library of Medicine. RefSeq: NCBI Reference Sequence Database. NCBI website. ncbi.nlm.nih.gov/refseq. Updated July 18, 2023. Accessed November 19, 2025.
7. CCDS - Consensus CDS (CCDS) Database. NCBI website. ncbi.nlm.nih.gov/projects/CCDS/CcdsBrowse.cgi. Updated November 9, 2022. Accessed November 19, 2025.
8. NIH National Library of Medicine. ACMG Recommendations for Reporting of Secondary Findings in Clinical Exome and Genome Sequencing. NCBI website. ncbi.nlm.nih.gov/clinvar/docs/acmg. Updated June 27, 2023. Accessed November 19, 2025.
9. Catalog of Somatic Mutations in Cancer (COSMIC). COSMIC website. cancer.sanger.ac.uk/cosmic/download. Published May 23, 2023. Accessed November 19, 2025.
10. Cancer Gene Census. COSMIC website. cosmickb.org/about. Published 2025. Accessed November 19, 2025.
11. Catalog of Human Genes and Genetic Disorders. OMIM website. omim.org. Updated July 26, 2023. Accessed November 19, 2025.
12. NIH National Library of Medicine. ClinVar Database. ncbi.nlm.nih.gov/clinvar. Updated July 24, 2023. Accessed November 19, 2025.
13. GATK Team. CollectHsMetrics (Picard). Published January 7, 2020. Accessed November 19, 2025.

illumina®

1.800.809.4566 toll-free (US) | +1.858.202.4566 tel
techsupport@illumina.com | www.illumina.com

M-GL-03952 v1.0