DATA SHEET

Illumina DNA Prep with Exome 2.5 Enrichment

Focused, consistent exome results from a single partner

- Cost-effective exome coverage using a focused, comprehensive, up-to-date exome panel
- Easy-to-use library preparation kit with qualified automation methods
- High-quality end-to-end solution and support
- Flexible content options with mitochondrial panel and custom panel spike-ins

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End-to-end exome sequencing with a single reliable partner

Illumina DNA Prep with Exome 2.5 Enrichment delivers economical human whole-exome sequencing (WES) results with outstanding performance and data quality. The easy-to-use library preparation and enrichment solution is part of an end-to-end workflow that spans from samples to reporting (Figure 1). Illumina Qualified Methods are available on a range of automation platforms through our partners. Illumina DNA Prep with Exome 2.5 Enrichment starts with extracted genomic DNA (gDNA), or direct blood or saliva input,* and combines rapid on-bead tagmentation library preparation chemistry followed by hybrid–capture exome enrichment (Figure 2).[1] The Illumina DNA Prep with Enrichment chemistry supports integrated normalization of high-quality input DNA (≥ 50 ng), which enables simple volume-based pooling for hybridization and provides even sequencing output from each enriched exome library. Libraries are sequenced on the NovaSeq™ 6000, NovaSeq X, NextSeq™ 1000, NextSeq 2000, or NextSeq 550 Systems. Award-winning DRAGEN™ secondary analysis performs variant calling.[2] For genetic diseases, the Emedgene platform applies explainable artificial intelligence (XAI) and automation to streamline interpretation and reporting for exome panels.

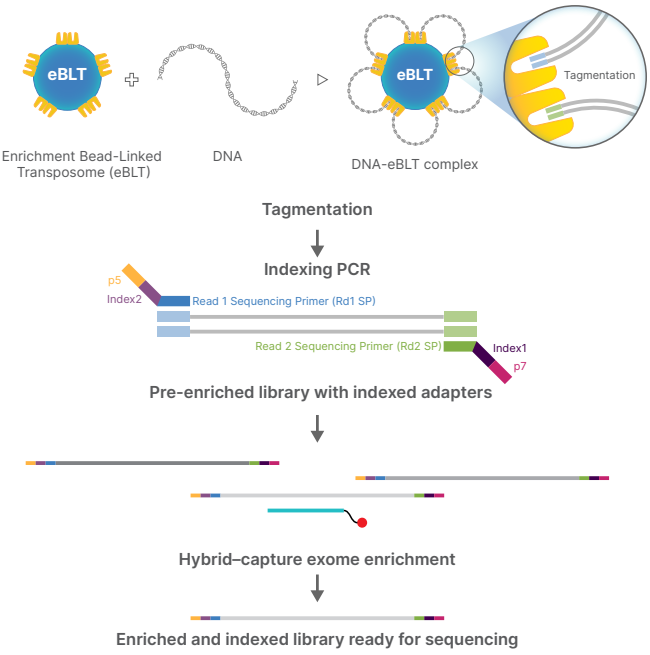


Figure 2: Streamlined tagmentation-based library preparation with exome enrichment—Enrichment bead-linked transposomes (eBLT) mediate a uniform tagmentation reaction with high tolerance to varying DNA sample input amounts.[1] Following hybrid–capture enrichment, exome libraries are ready for sequencing.

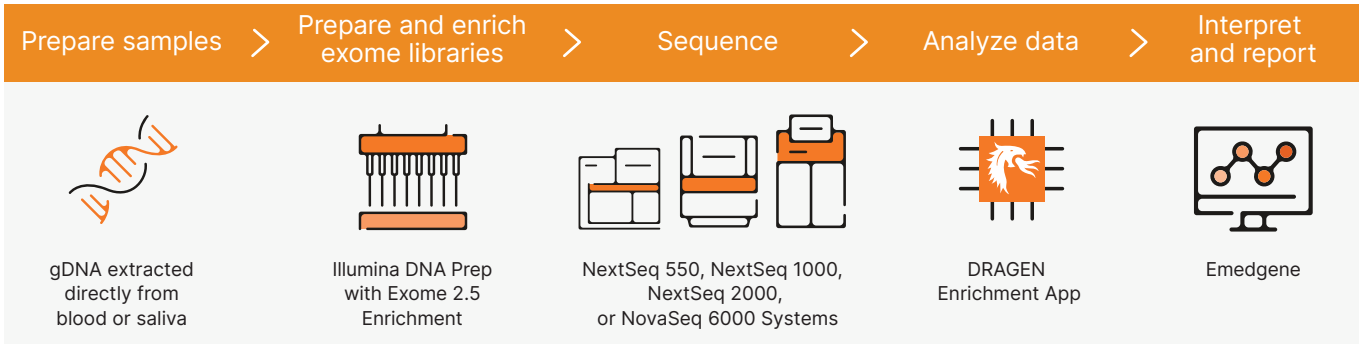


Figure 1: From samples to reporting from a single partner—Illumina supports an end-to-end workflow for WES. Extracted gDNA (or DNA from blood or saliva following direct extraction protocols) is input to library prep with Illumina DNA Prep with Exome 2.5 Enrichment. Qualified Methods are available on a range of automation platforms. Sequence according to scale and throughput needs on Illumina instruments. Accurate, rapid secondary analysis and variant calling is performed with the DRAGEN Enrichment App. For genetic diseases, Emedgene enables intuitive interpretation and reporting.

* Direct blood and saliva protocols require the Flex Lysis Reagent Kit. Data shown is generated from extracted gDNA, not blood or saliva. Blood and saliva performance may vary depending on sample quality.

Cost-effective, comprehensive coverage of disease-associated variants in public databases

Illumina DNA Prep with Exome 2.5 Enrichment uses a focused, comprehensive, up-to-date exome enrichment panel. The Twist Bioscience for Illumina Exome 2.5 Panel, included in the kit, is smaller than the Illumina Exome Panel and has improved target region coverage for variants reported in public databases (Table 1 and Table 2). This focused coverage enables a cost-effective WES solution at an optimal number of samples per sequencing run (Table 3).

The Twist Bioscience for Illumina Exome 2.5 Panel covers curated coding sequences (CDS) from RefSeq, Consensus Coding Sequence (CCDS), American College of Medical Genetics and Genomics (ACMG), the Cancer Gene Census from the Catalog of Somatic Mutations in Cancer (COSMIC), and the Online Mendelian Inheritance in Man (OMIM) (Table 1) with exceptional coverage of pathogenic or likely pathogenic variants reported in the ClinVar and ACMG databases (Table 2).[3-9]

Coverage of the mitochondrial genome (chrM) can be added easily by using the Twist Bioscience for Illumina Mitochondrial Panel as a spike-in panel in the Illumina DNA Prep with Exome 2.5 Enrichment protocol. The Mitochondrial Panel offers complete coverage of the 16,659 bp and 37 genes of the chrM, allowing enrichment and sequencing of mitochondrial DNA (mtDNA) variants.

Table 1: Improved target region coverage in a cost-effective size 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	45 Mb
RefSeq CDS[3]	99.1%	98.2%
CCDS CDS[4]	99.9%	99.5%
ACMG 73 genes CDS[5]	99.9%	99.3%
COSMIC Cancer Gene Census CDS[6,7]	99.9%	99.3%
OMIM[8]	99.1%	97.7%

Table 2: Clinical research–focused content of the Twist Bioscience for Illumina Exome 2.5 Panel

ClinVar pathogenic/likely pathogenic variants CDS[a,9]	98.6%
ACMG 73 pathogenic/likely pathogenic variants CDS[b,5]	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.

Table 3: Estimated number of enriched Exome 2.5 Enrichment libraries per flow cell and sequencing system[a]

Desired mean target coverage depth	NextSeq 550 System[b]		NextSeq 2000 System		NovaSeq 6000 System			
	Mid-output	High-output	P2[c]	P3	SP	S1	S2	S4
50×	6	19	19	57	34	69	176	428[d]
100×	3	9	9	28	17	34	88	214
200×	1	4	4	14	8	17	45	109

a. Estimates are based on 2 × 101 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 using S4 flow cells 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 this many enriched libraries requires additional indexes.

Illumina DNA Prep with Exome 2.5 Enrichment sequencing data show excellent coverage consistency with a high percentage of targets covered at 20× sequence depth or higher using 5 Gb output and two different hybridization times for enrichment (Figure 3).

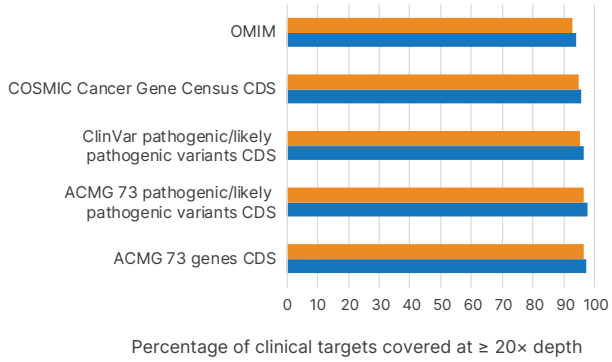


Figure 3: Extensive, comprehensive coverage of disease-associated variants in public databases—Illumina DNA Prep with Exome 2.5 Enrichment using a 1.5-hour (orange) or 16-hour (blue) hybridization time shows high average percent coverage at ≥ 20× of targets from public databases, including ACMG, ClinVar, OMIM, and COSMIC.[5-9]

High-quality performance

Illumina DNA Prep with Exome 2.5 Enrichment shows outstanding enrichment assay performance. Data were collected using the Illumina DNA Prep with Exome 2.5 Enrichment kit and the included Twist Bioscience for Illumina Exome 2.5 Panel with a 1.5-hour or 16-hour hybridization time. For comparison, the assay was also performed using the original Illumina Exome Panel, following the same protocol except the 1.5-hour hybridization and washing temperatures were reduced to 58°C due to the use of shorter probes (labeled 'IEP 1.5 hr' in Figure 4 and Figure 5).

Performance metrics from the DRAGEN Enrichment App and the Picard pipeline[10] reveal optimal percentage of mappable reads, reads on target (based on percent passing filter for unique reads aligned, percent padded unique read enrichment, and percent padded unique base enrichment), and outstanding mean target coverage for Illumina DNA Prep with Exome 2.5 Enrichment with the Twist Bioscience for Illumina Exome 2.5 Panel probes (Figure 4).

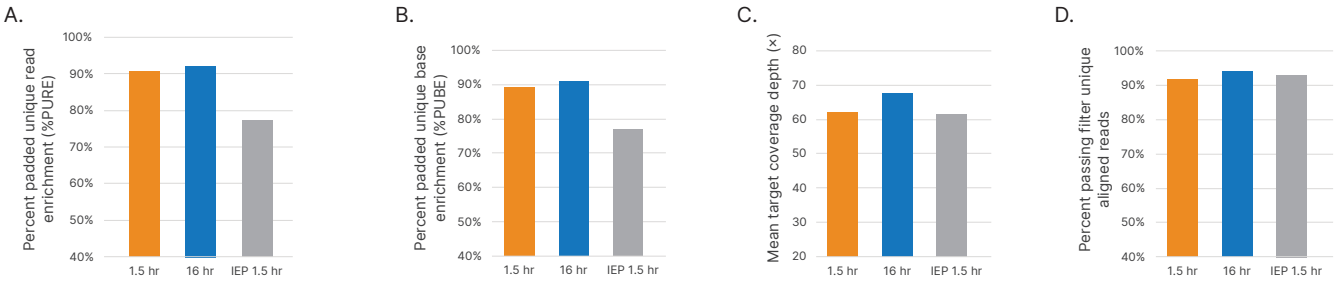


Figure 4: Excellent enrichment assay performance of Illumina DNA Prep with Exome 2.5 Enrichment—Illumina DNA Prep with Exome 2.5 Enrichment using a 1.5-hr (orange) or 16-hr (blue) hybridization time illustrates robust performance compared to the Illumina Exome Panel (IEP 1.5 hr, gray). DRAGEN metrics: (A) percent padded unique read enrichment (% PURE, 150-bp padded size); (B) percent padded unique base enrichment (% PUBE, 150-bp padded size); and (C) mean target coverage depth. Picard metrics: (D) percent passing filter unique reads aligned. Enriched libraries were sequenced on the NovaSeq 6000 System, S4 flow cell with 5 Gb output (50M paired-end reads, 25M clusters) and 2 × 101 bp read lengths.

Coverage depth and uniformity

Additional analysis demonstrates excellent coverage uniformity of the Illumina DNA Prep with Exome 2.5 Enrichment compared to the Illumina Exome Panel (Figure 5). Illumina DNA Prep with Exome 2.5 Enrichment ensures even coverage with a high percentage of bases at 20× or higher read depth, a low fold-80 base penalty, and a low percentage of zero coverage targets.

The consistent performance between the 1.5-hour and 16-hour hybridization times illustrates how labs can speed up their workflows with 1.5-hour hybridization times or extend their hybridizations overnight if that better suits their workflow.

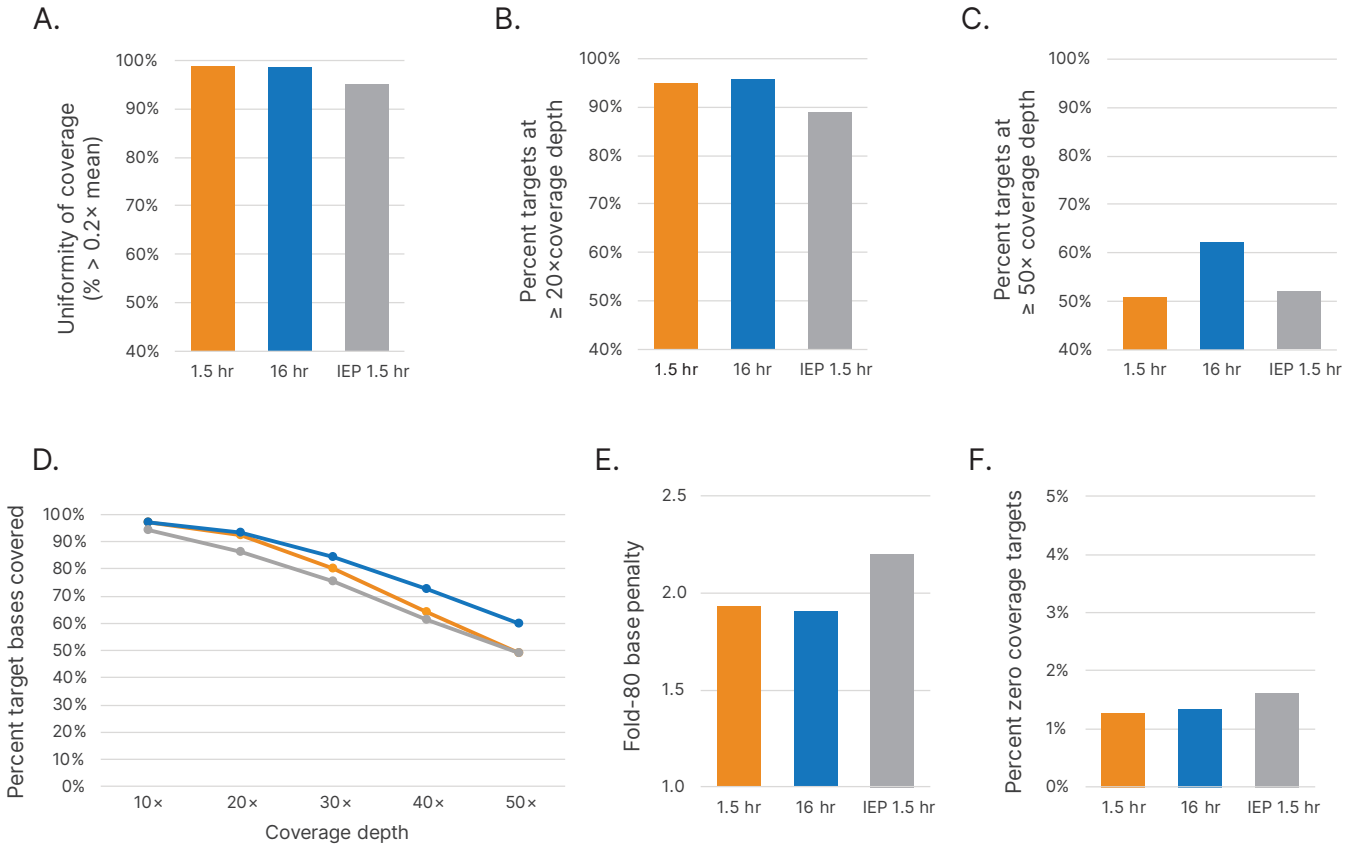


Figure 5: Excellent coverage and uniformity independent of the hybridization time—Illumina DNA Prep with Exome 2.5 Enrichment using a 1.5-hr (orange) or 16-hr (blue) hybridization time shows excellent coverage uniformity compared to the Illumina Exome Panel (IEP 1.5 hr, gray) run in parallel. DRAGEN metrics: (A) coverage uniformity (percent > 0.2× mean coverage); (B) percent targets covered at ≥ 20×; and (C) percent targets covered at ≥ 50×. Picard metrics: (D) percent target bases with 10×, 20×, 30×, 40×, and 50× coverage depth; (E) fold-80 base penalty (fold over-coverage necessary to raise 80% of bases in nonzero coverage targets to the mean coverage level in those targets); and (F) percent zero coverage targets. Enriched libraries sequenced on the NovaSeq 6000 System, S4 flow cell with 5 Gb output (50M paired-end reads, 25M clusters) and 2 × 101 bp read lengths.

Mitochondrial DNA is present in greater abundance relative to nuclear DNA in the cell. The Twist Bioscience for Illumina Mitochondrial Panel can be used at different concentrations relative to the exome panel, demonstrating flexibility in varying mtDNA coverage without impacting exome mean target coverage or coverage uniformity (Figure 6).

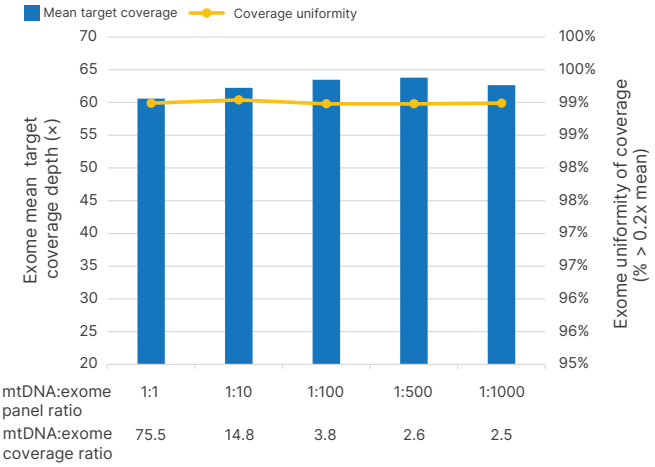


Figure 6: Uniform exome coverage with added mitochondrial coverage—Varying ratios of Twist Bioscience for Illumina Exome 2.5 Panel and Twist Bioscience for Illumina Mitochondrial Panel show consistent exome mean target coverage (blue bars) and coverage uniformity (yellow line). A total of 72 human cell line DNA samples from the Coriell Institute (NA24143, NA24149, and NA24385) were enriched (6 × 12-plex pools) with mtDNA:exome panel ratios varying from 1:1 to 1:1000 in 16-hour hybridization reactions. All 72 enriched libraries were sequenced on a NovaSeq 6000 System using a single S4 flow cell with 5 Gb output (50M paired-end reads, 25M clusters) and enrichment analysis was performed using the DRAGEN Enrichment app.

Customized WES content

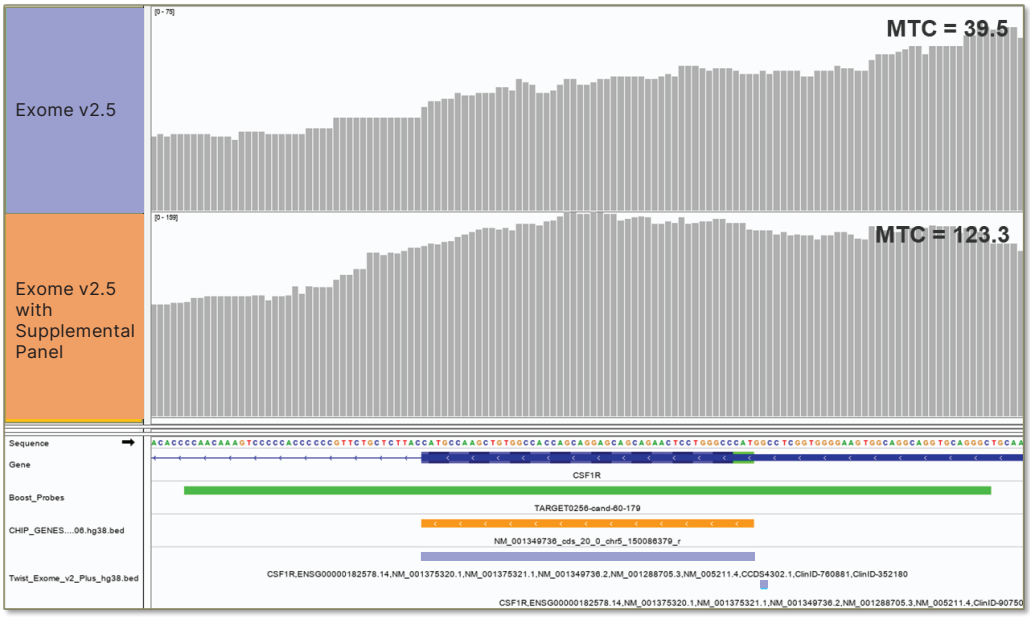
The Twist Bioscience for Illumina Exome 2.5 Panel can be customized with a supplemental panel to add targets or boost target coverage for WES experiments. The Illumina Custom Enrichment Panel v2 can be designed to extend sequencing coverage to new targets, or deepen coverage of existing WES target regions (Figure 7, Table 4). Panels are designed for a customized target list and added easily as a spike-in panel in the Illumina DNA Prep with Exome 2.5 Enrichment protocol, with or without the addition of mitochondrial panel spike-in. Custom panels are designed through the DesignStudio™ online design tool, or with help from the Illumina Concierge design team.

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

Parameter	Illumina DNA Prep with Exome 2.5 Enrichment	Illumina DNA Prep with Exome 2.5 Enrichment plus spike-in panel
Mean target coverage	63×	124×
Coverage uniformity	98.3%	98.4%
Percent targets covered at 20×	96.7%	98.6%
Percent targets covered at 50×	66.6%	96.3%
AT dropout	0.01%	1.08%
GC dropout	9.9%	2.3%

a. The Illumina DNA Prep with Exome 2.5 Enrichment protocol was performed with and without a custom spike-in panel targeting regions covered by the Twist Bioscience for Illumina Exome 2.5 Panel and coverage of the regions targeted by the spike-in panel was analyzed.

CSF1R
chr5:150086379-150086427



BCORL1
chrX:13001295-130016213

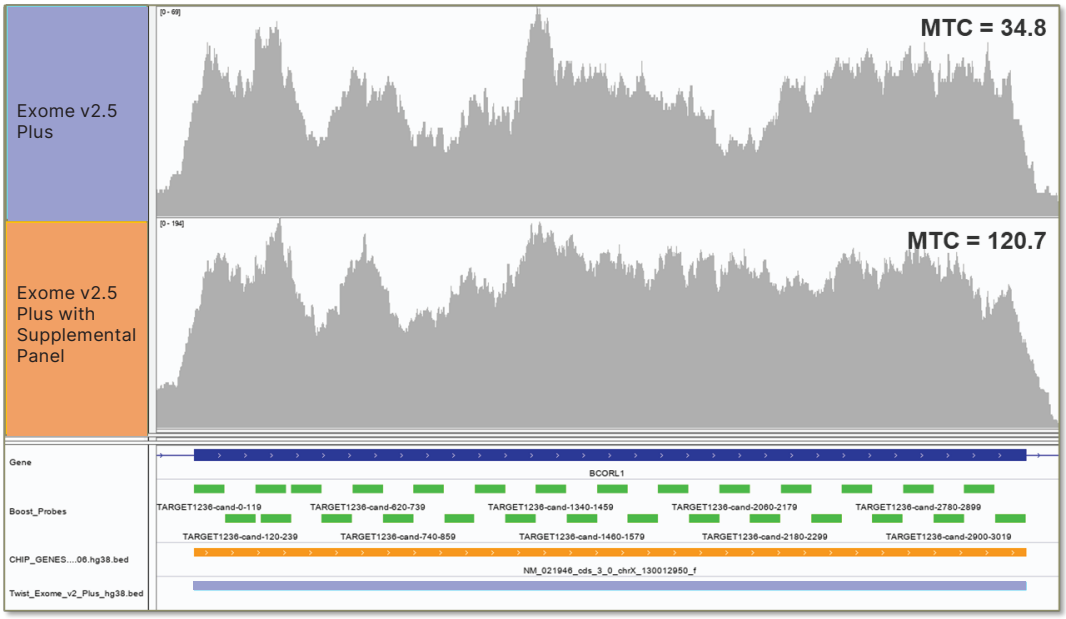


Figure 7: Illumina Custom Enrichment Panel v2 enhances WES target coverage—An Illumina Custom Enrichment Panel v2 consisting of 2689 probes covering 2689 exonic targets in the Twist Bioscience for Illumina Exome 2.5 Panel was designed. The Illumina DNA Prep with Exome 2.5 Enrichment protocol was performed with and without the custom spike-in panel, and coverage of the regions targeted by the spike-in panel was analyzed. Mean target coverage (MTC) of the covered regions improved by ~2-fold and percent targets covered at 50× increased significantly with the spike-in panel.

Summary

Illumina DNA Prep with Exome 2.5 Enrichment offers a well-designed, reliable human WES solution that is effective and efficient. The included Twist Bioscience for Illumina Exome 2.5 Panel provides comprehensive, up-to-date content covering disease-associated variants within the public databases, and optional Twist Bioscience for Illumina Mitochondrial Panel adds comprehensive coverage of chrM. The optimized, customizable enrichment panel enables high sample throughput for economical exome sequencing. Excellent, uniform coverage facilitates downstream analysis and interpretation. Additional efficiency gains can be achieved by adopting Illumina Qualified Methods on a range of automation platforms, available through our partners. With Illumina DNA Prep with Exome 2.5 Enrichment, labs can now benefit from a high-quality end-to-end exome sequencing workflow—from samples to reporting—from a single partner.

Learn more

Illumina DNA Prep with Exome 2.5 Enrichment

Illumina Qualified Methods for automation

DRAGEN secondary analysis

Emedgene tertiary analysis

DesignStudio assay design tool

Ordering information

Product	Catalog no.
Illumina DNA Prep with Exome 2.5 Enrichment, (S) Tagmentation Set B (96 samples, 12-plex)[a]	20077595
Illumina DNA Prep with Exome 2.5 Enrichment, (S) Tagmentation Set D (96 samples, 12-plex)[a]	20077596
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 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

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