illumina®

Illumina library preparation solutions

The foundation for discovery and insights

Optimized library preparation for Illumina sequencing systems

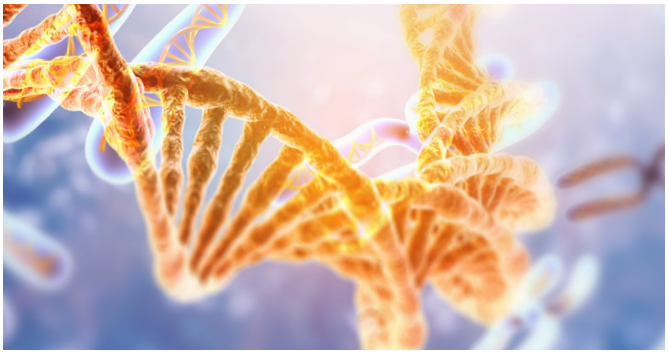
The Illumina next-generation sequencing (NGS) library preparation portfolio offers technology advancements that enable quality, precision, and ease of use for both DNA and RNA sequencing. As the foundation of an end-to-end NGS workflow, these kits are optimized for use with Illumina instruments and secondary data analysis tools.

Illumina library prep protocols accommodate a range of throughput needs, from lower-throughput protocols for small labs to fully automated workflows for large laboratories and genome centers. Library preparation solutions are available for a broad range of sample types, from cell culture to fresh tissue, formalin-fixed paraffin-embedded (FFPE) samples, blood, and other challenging sample types.

Versatile library prep for DNA sequencing

The versatile Illumina DNA library preparation portfolio provides flexibility for examining small, targeted regions or the entire genome. On-bead tagmentation technology allows labs to achieve the consistent insert sizes and high coverage uniformity needed for optimal sequencing results. Protocols are available for automated and manual sample processing. The concise workflows support easy volume-based library pooling while minimizing library quantification steps.

This brochure covers a representative selection of library preparation options. A comprehensive list of library preparation solutions is available at Illumina.com.



Illumina DNA PCR-Free Prep

Illumina DNA PCR-Free Prep offers a unique combination of on-bead tagmentation with a PCR-free workflow for sensitive applications, such as human whole-genome sequencing.

- Eliminates PCR-induced bias and provides high-performance data for applications that require uncompromised accuracy, such as human whole-genome sequencing
- Produces libraries in 90 minutes from extracted genomic DNA or in 2.5 hours from raw samples, such as blood, saliva, and dried blood spots
- Supports a broad range of DNA input (25–300 ng) and is compatible with automation

Illumina DNA Prep*

Illumina DNA Prep offers a unique chemistry that integrates DNA extraction, fragmentation, library preparation, and library normalization steps for an exceptionally fast and flexible workflow.

- Offers high performance for sequencing whole genomes, amplicons, plasmids, and microbial genomes
- Follows a flexible workflow to support a broad range of DNA input (1–500 ng), including low-input samples
- Features enhanced library preparation efficiency with integrated DNA extraction protocols for blood, saliva, and dried blood spots

*Formerly known as Nextera™ DNA Flex.

Illumina DNA Prep with Enrichment*

Illumina DNA Prep with Enrichment combines versatile and fast library preparation with enrichment functionality for targeted enrichment and exome sequencing applications. It offers extraordinary flexibility for input type and amount, and a wide range of enrichment sequencing applications.

- Supports a broad range of DNA input (10–1000 ng) and multiple sample types, including blood, saliva, and FFPE DNA
- Provides a rapid enrichment workflow for targeted resequencing using a single, 90-minute hybridization step
- Enables whole-exome sequencing and advanced study designs in cancer and genetic disease research
- Compatible with predesigned and customized enrichment panels such as the Illumina Custom Enrichment Panel v2, easily designed with the DesignStudio free online tool or our Concierge Design Services Team

Illumina DNA Prep with Exome 2.5 Enrichment

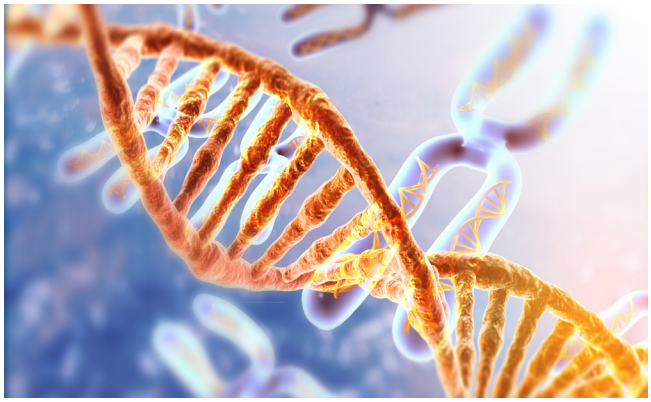
Illumina DNA Prep with Exome 2.5 Enrichment provides economical human whole-exome sequencing results with outstanding performance and data quality. The easy-to-use library preparation and enrichment solution is part of an end-to-end workflow from preparing samples to reporting results.

- Includes library prep and hybridization reagents, Exome 2.5 probe panel, beads, and indexes in a high-performance, complete whole-exome sequencing kit
- Enables a 6.5-hour whole-exome sequencing workflow with fast, simple protocol and built-in library normalization
- Provides comprehensive coverage of exons and variants with high on-target rates and uniform coverage, enabling high multiplexing, increased throughput, and decreased costs
- Allows addition or enhancement of exome content with a supplementary enrichment panel. Illumina Custom Enrichment Panel v2 spike-ins are easily designed in DesignStudio free online tool or by Concierge Design Services Team

Illumina Cell-Free DNA Prep with Enrichment

Illumina Cell-Free DNA Prep with Enrichment is a versatile library preparation solution optimized for use with low-input cell-free DNA (cfDNA) extracted from plasma samples. It enables fast, flexible, and scalable cfDNA library preparation with high analytical sensitivity detection of low-abundance mutations in liquid biopsy samples.

- Accommodates user-supplied customized enrichment panels from Illumina or third-party providers, including Illumina Custom Enrichment Panel v2, which is easily designed with the DesignStudio free online tool
- Includes unique molecular identifiers (UMIs) for error correction and reduction of false positives
- Delivers sequencing-ready libraries with user-supplied panels in 8.5–9.5 hours with just 2.5–3 hours of hands-on time



*Formerly known as Nextera Flex for Enrichment.

Product	Illumina DNA PCR-Free Prep	Illumina DNA Prep
Applications	Human whole-genome sequencing	Whole-genome sequencing for large or small genomes
Mechanism of action	Bead-linked transposome	Bead-linked transposome
Specialized sample types	Blood Dried blood spots Saliva	Blood Dried blood spots Saliva Bacterial colonies Low-input samples
DNA input amount	25–300 ng	1–500 ng
Hands-on time	~45 min	1–1.5 hr
Assay time	~1.5 hr	~3–4 hr
PCR protocol	No	Yes
Library quantification needed	No	No
Fragmentation included	Yes, on bead	Yes, on bead
FFPE compatible	No	Not demonstrated
Automation friendly	Yes	Yes
Compatible panels	N/A	N/A
Recommended index classes	Illumina DNA/RNA UD Indexes Tagmentation	Illumina DNA/RNA UD Indexes Tagmentation
Recommended analysis software	DRAGEN™ Germline DRAGEN Somatic Illumina Connected Insights Emedgene™ software	DRAGEN Germline DRAGEN Somatic DRAGEN Metagenomics Pipeline CosmosID SPAdes Genome Assembler
Recommended sequencing systems	NextSeq™ 550 System NextSeq 1000 System NextSeq 2000 System NovaSeq™ 6000 System NovaSeq X Series	iSeq™ 100 System MiniSeq™ System MiSeq™ Series NestSeq 550 System NextSeq 1000 System NextSeq 2000 System NovaSeq 6000 System NovaSeq X Series
Link to consumables and equipment list	support.illumina.com/downloads/illumina-dna-pcr-free-consumables-and-equipment.html	support.illumina.com/downloads/illumina-dna-prep-consumables--equipment-list-1000000033564.html



Product	Illumina DNA Prep with Enrichment	Illumina DNA Prep with Exome 2.5 Enrichment	Illumina Cell-Free DNA Prep with Enrichment
Applications	Whole-exome sequencing Targeted resequencing	Small genomes PCR amplicons Plasmids	Targeted sequencing from cell-free DNA samples
Mechanism of action	Bead-linked transposome and hybrid–capture chemistry	Bead-linked transposome and hybrid–capture chemistry	Hybrid–capture chemistry
Specialized sample types	Blood Saliva FFPE DNA	Blood Saliva	cfDNA from plasma or whole blood
DNA input amount	10–1000 ng 50 ng minimum for complex genomes or FFPE DNA	50–1000 ng	10–30 ng (20 ng recommended)
Hands-on time	~2 hr	~2 hr	~2.5–3 hr
Assay time	~6.5 hr	~6.5 hr	~8.5–9 hr
PCR protocol	Yes	Yes	Yes
Library quantification needed	No	No	No
Fragmentation included	Yes, on bead	Yes, on bead	Not required
FFPE compatible	Yes	No	Demonstrated compatibility only
Automation friendly	Yes	Yes	Yes
Compatible panels	Twist Bioscience for Illumina Exome 2.5 Panel Illumina Exome Panel TruSight Hereditary Cancer TruSight One TruSight One Expanded Illumina Custom Enrichment Panel Illumina Custom Enrichment Panel v2	Twist Bioscience for Illumina Mitochondrial Panel Illumina Custom Enrichment Panel v2	Illumina Custom Enrichment Panel v2 Twist Bioscience for Illumina Exome 2.5 Panel[a] Illumina Custom Enrichment Panel User-defined panels
Recommended index classes	Illumina DNA/RNA UD Indexes Tagmentation	Illumina DNA/RNA UD Indexes Tagmentation	Illumina DNA UD Indexes Tagmentation
Recommended analysis software	DRAGEN Enrichment DRAGEN Somatic Illumina Connected Insights Emedgene software	DRAGEN Enrichment DRAGEN Somatic Illumina Connected Insights Emedgene	DRAGEN for ILMN cfDNA Prep with Enrichment
Recommended sequencing systems	MiniSeq System MiSeq Series NextSeq 550 System NextSeq 1000 System NextSeq 2000 System NovaSeq 6000 System NovaSeq X Series	NextSeq 550 System NextSeq 1000 System NextSeq 2000 System NovaSeq 6000 System NovaSeq X Series	NextSeq550 System NextSeq 1000 System NextSeq 2000 System NovaSeq 6000 System NovaSeq X Series
Link to consumables and equipment list	support.illumina.com/downloads/illumina-dna-prep-with-enrichment-consumables-equipment-1000000048602.html	support.illumina.com/downloads/Illumina-dna-prep-with-exome-v2-plus-enrichment.html	support.illumina.com/downloads/illumina-cell-free-dna-prep-with-enrichment-analysis.html

a. Compatibility with Twist Bioscience for Illumina Exome 2.5 Panel demonstrated in application note.

High-performance library prep for RNA sequencing

Advances in RNA-Seq library prep are revolutionizing the study of the transcriptome. The Illumina RNA library prep portfolio includes a range of solutions to support various applications and sample types. Illumina RNA library prep offers flexibility, scalability, and performance with a rapid, automation-friendly workflow option to prepare sequencing-ready libraries in a single day.

The RNA library preparation products mentioned in this brochure are representative of the portfolio. A comprehensive list of all available options is available at Illumina.com.

Illumina Stranded Total RNA Prep with Ribo-Zero™ Plus, Illumina Stranded Total RNA with Ribo-Zero Plus Microbiome

Illumina Stranded Total RNA Prep offers streamlined, rapid, ligation-based library preparation that supports low sample inputs and a wide range of RNA-Seq applications. The Illumina Ribo-Zero Plus rRNA Depletion Kit is included for efficient removal of ribosomal RNA (rRNA) from multiple species, including human, mouse, rat, and bacteria, allowing researchers to focus studies on high-value sequences.

- Detects coding and noncoding transcripts for whole-transcriptome sequencing
- Supports a wide range of RNA inputs (1–1000 ng) and delivers robust performance in low-quality or FFPE samples
- Includes Ribo-Zero Plus for efficient removal of abundant RNA from multiple species, including human, mouse, rat, gram +/-bacteria, and globin mRNA in a single step
- Provides an option for Ribo-Zero Plus Microbiome for depletion of undesirable host and pan-bacterial rRNA from complex microbial samples (eg, stool) for metatranscriptomics research

Illumina Stranded mRNA Prep

Illumina Stranded mRNA Prep is an advanced solution that offers rapid, ligation-based library preparation that supports low sample input and high accuracy for mRNA-Seq applications.

- Delivers accurate, unbiased detection of the protein-coding transcriptome with precise measurement of strand information
- Offers exceptional polyA capture efficiency and coverage uniformity, minimizing sequencing requirements
- Provides wide dynamic range for accurate gene expression profiling using as little as 25 ng of high-quality RNA samples

Illumina RNA Prep with Enrichment

Illumina RNA Prep with Enrichment provides accurate and efficient library preparation for targeted RNA-Seq studies. The kit is highly flexible for sample input type and quantity, making it suitable for a range of applications, including allele-specific expression, fusion detection, biomarker screening, exome, and more.

- Enriches for targeted transcripts of interest, including the RNA exome or RNA virus detection
- Offers exceptional capture efficiency and coverage uniformity, compatible with low-input or FFPE samples
- Uses simple tagmentation-based library prep and RNA enrichment workflow with minimal hands-on time (< 2 hours)

Product	Illumina Stranded Total RNA Prep with Ribo-Zero Plus or Illumina Ribo-Zero Plus Microbiome rRNA Depletion Kit	Illumina Stranded mRNA Prep	Illumina RNA Prep with Enrichment
Applications	Whole-transcriptome sequencing	mRNA-Seq Gene expression profiling	RNA exome enrichment mRNA-Seq/gene expression profiling for low-input or FFPE samples Virus detection
Mechanism of action	Enzymatic rRNA depletion Ligation-based addition of adapters and indexes	PolyA capture Ligation-based addition of adapters and indexes	Bead-linked transposome and hybrid–capture chemistry
Detection	Coding and noncoding transcriptome	Coding transcriptome with polyA tail	Targeted coding regions
Strand specificity	Stranded	Stranded	Nonstranded
Specialized sample types	Blood FFPE tissue Low-input samples	High-quality mRNA Low-input samples Not FFPE compatible	Blood FFPE tissue Low-input samples Saliva Nasal swabs
RNA input amount	1–1000 ng standard-quality RNA 10 ng minimum for optimal performance and FFPE samples	25–1000 ng standard-quality RNA	10 ng standard-quality RNA 20 ng RNA for low-quality/FFPE samples
Hands-on time	< 3 hr	< 3 hr	< 2 hr
Assay time	~7 hr	< 7 hr	< 9 hr
Library quantification needed	Yes	Yes	Yes
Fragmentation included	Yes	Yes	Not required
FFPE compatible	Yes	No	Yes
Automation friendly	Yes	Yes	Yes
Compatible panels	N/A	N/A	Illumina Exome Panel Respiratory Virus Oligo Panel v2 Respiratory Pathogen ID/AMR Enrichment Panel TruSight RNA Pan-Cancer Panel TruSight RNA Fusion Panel
Recommended index classes	Illumina RNA UD Indexes Ligation	Illumina RNA UD Indexes Ligation	Illumina DNA/RNA UD Indexes, Tagmentation
Recommended analysis software	DRAGEN RNA Pipeline DRAGEN Differential Expression	DRAGEN RNA Pipeline DRAGEN Differential Expression BaseSpace Correlation Engine	DRAGEN RNA Pipeline DRAGEN Differential Expression DRAGEN RNA Pathogen Detection
Recommended sequencing systems	NextSeq 550 System NextSeq 1000 System NextSeq 2000 System NovaSeq 6000 System NovaSeq X Series	NextSeq 550 System NextSeq 1000 System NextSeq 2000 System NovaSeq 6000 System NovaSeq X Series	iSeq 100 System MiSeq Series NextSeq 550 System NextSeq 1000 System NextSeq 2000 System NovaSeq 6000 System NovaSeq X Series
Link to consumables and equipment list	support.illumina.com/downloads/illumina-stranded-total-rna-consumables-1000000124517.html	support.illumina.com/downloads/illumina-stranded-mrna-consumables-1000000124520.html	support.illumina.com/downloads/illumina-rna-prep-consumables-1000000124437.html

Summary

The Illumina NGS library preparation portfolio delivers high quality, precision, and ease of use for both DNA and RNA sequencing. Protocols accommodate a range of throughput needs, from lower-throughput sequencing studies to fully automated library preparation for large laboratories. Available kits support a broad range of sample types, from cell lines to fresh tissue, FFPE samples, blood, and other challenging sample types. This brochure provides an overview of popular applications with additional solutions and applications available on the Illumina website.

Learn more

DNA library preparation

RNA library preparation

Illumina DRAGEN secondary analysis

Emedgene tertiary analysis

Automation methods that span our library prep portfolio

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Ordering information

	Product	Catalog no.
Whole-genome DNA library prep kits	Illumina DNA PCR-Free Prep, Tagmentation (24 samples)	20041794
	Illumina DNA PCR-Free Prep, Tagmentation (96 samples)	20041795
	Illumina DNA LP, (M) Tagmentation (24 samples, IPB)	20060060
	Illumina DNA LP, (M) Tagmentation (96 samples, IPB)	20060059
Target enrichment DNA prep kits	Illumina DNA Prep, (S) Tagmentation (16 samples)	20025519
	Illumina DNA Prep, (S) Tagmentation (96 samples)	20025520
	Illumina DNA Prep with Enrichment, (S) Tagmentation (16 samples)	20025523
	Illumina DNA Prep with Enrichment, (S) Tagmentation (96 samples)	20025524
	Illumina cfDNA Prep with Enrichment, Ligation (192 samples, 4-plex), Cloud Analysis	20104103
	Illumina cfDNA Prep with Enrichment, Ligation (192 samples, 4-plex) On- premises	20104104
	Illumina cfDNA Prep, Ligation (16 samples)	20104105
	Illumina cfDNA Prep, Ligation (96 samples)	20104106
	Illumina cfDNA Enrichment (16 reactions)	20104107
	Illumina DNA Prep with Exome 2.5 Enrichment, (S) Tagmentation Set B (96 samples, 12-plex)	20077595
	Illumina DNA Prep with Exome 2.5 Enrichment, (S) Tagmentation Set D (96 samples, 12-plex)	20077596
RNA library prep kits	Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus (16 samples)	20040525
	Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus (96 samples)	20040529
	Illumina Stranded Total RNA Prep with Ligation, Ribo-Zero Plus Microbiome (96 samples)	20072063
	Illumina Stranded mRNA Prep, Ligation (16 samples)	20040532
	Illumina Stranded mRNA Prep, Ligation (96 samples)	20040534
	Illumina RNA Prep with Enrichment, (L) Tagmentation (16 samples)	20040536
	Illumina RNA Prep with Enrichment, (L) Tagmentation (96 samples)	20040537
	Flex Lysis Reagent Kit (96 reactions)	20018706
Indexes	Illumina DNA/RNA UD Indexes Set A, Tagmentation (96 indexes, 96 samples)	20091654
	Illumina DNA/RNA UD Indexes Set B, Tagmentation (96 indexes, 96 samples)	20091656
	Illumina DNA/RNA UD Indexes Set C, Tagmentation (96 indexes, 96 samples)	20091658
	Illumina DNA/RNA UD Indexes Set D, Tagmentation (96 indexes, 96 samples)	20091660
	Illumina RNA UD Indexes Set A, Ligation (96 indexes, 96 samples)	20091655
	Illumina RNA UD Indexes Set B, Ligation (96 indexes, 96 samples)	20091657
	Illumina RNA UD Indexes Set C, Ligation (96 indexes, 96 samples)	20091659
	Illumina RNA UD Indexes Set D, Ligation (96 indexes, 96 samples)	20091661
Enrichment panels	Illumina Exome Panel – Enrichment oligos only	20020183
	Respiratory Virus Oligos Panel V2	20044311
	TruSight Cancer – Enrichment oligos only (4 or 8 enrichment reactions)	FC-121-0202
	TruSight Hereditary Cancer – Enrichment oligos only (8 enrichment reactions)	20029551
	TruSight One – Enrichment oligos only (6 enrichment reactions)	20029227
	TruSight One Expanded – Enrichment oligos only (6 enrichment reactions)	20029226
	Illumina Custom Enrichment Panel v2 (32 µl, 120 bp)[a]	20073953
	Illumina Custom Enrichment Panel v2 (384 µl, 120 bp)	20073952
	Illumina Custom Enrichment Panel v2 (1536 µl, 120 bp)	20111339
	Illumina Custom Enrichment Panel (8 enrichment reactions)	20025371

a. 80-bp probe panels also available for design through Illumina Concierge Services design team. Contact your Illumina Sales Representative for further information.

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