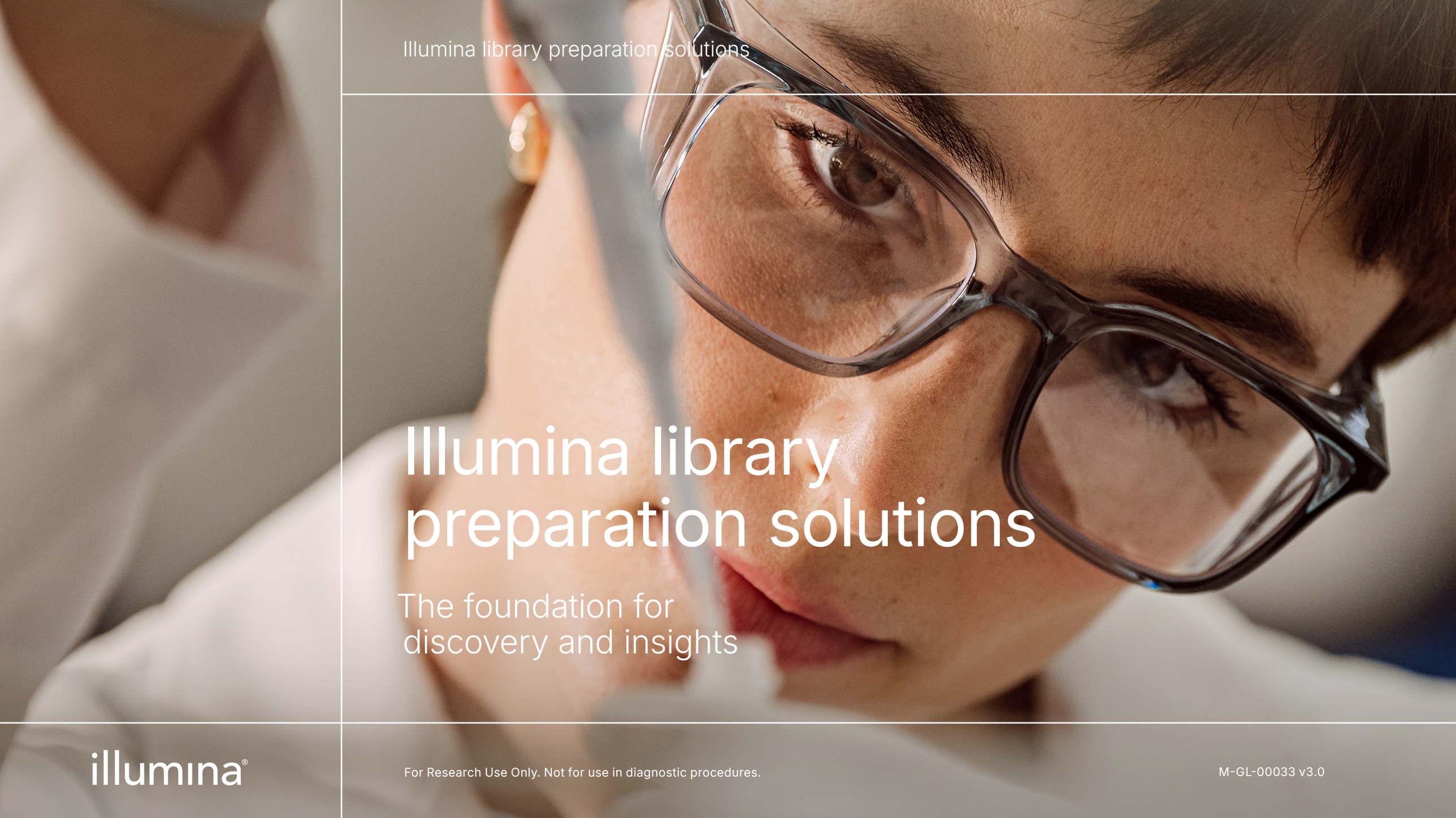


Illumina library preparation solutions

The foundation for discovery and insights

illumina®

M-GL-00033 v3.0

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 preparation 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.

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

Simple

On-bead tagmentation reduces manual steps and simplifies the workflow

Scalable

Compatibility with automation supports high-throughput

Fast

Streamlined workflow is completed in as little as 1.5 hrs* with minimal hands-on time

*For Illumina DNA PCR-Free Prep

Flexible library preparation solutions for DNA sequencing

The versatile Illumina DNA library preparation portfolio provides flexibility for examining small, targeted regions or the entire genome.

On-bead tagmentation chemistry 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.

Learn more →

DNA library preparation

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.

Features enhanced library preparation efficiency with integrated DNA extraction protocols for blood, saliva, and dried blood spots

Follows a flexible workflow to support a broad range of DNA input (1–500 ng), including low-input samples

Offers high performance for sequencing whole genomes, amplicons, plasmids, and microbial genomes

Learn more →

Summary of Illumina DNA library preparation solutions for whole-genome sequencing

Specifications	Illumina DNA PCR-Free Prep	Illumina DNA Prep
Application	Human whole-genome sequencing	Whole-genome sequencing for large or small genomes
Method	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 amounts	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 compatibility	No	Not demonstrated
Automation compatibility	Yes	Yes
Recommended index classes	Illumina DNA/RNA Unique Dual Indexes Tagmentation	Illumina DNA/RNA Unique Dual Indexes Tagmentation
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, NextSeq™ 550 System, NextSeq 1000 System, NextSeq 2000 System, NovaSeq™ 6000 System, NovaSeq X Series
Recommended analysis software	DRAGEN™ Germline, DRAGEN Somatic, Illumina Connected Insights, Emedgene™ software	DRAGEN Germline, DRAGEN Somatic, DRAGEN Metagenomics pipeline, CosmosID, SPAdes Genome Assembler
Consumables and equipment	Illumina DNA PCR-Free Prep consumables and equipment	Illumina DNA Prep consumables and equipment

DNA enrichment and library preparation

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. This solution provides extraordinary flexibility for input type and amount and supports 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
- Accommodates predesigned and customized enrichment panels; the Illumina Custom Enrichment Panel v2 is easily designed with the DesignStudio free online tool or our Concierge Design Services Team

Learn more →

Illumina DNA Prep with Exome 2.5 Enrichment

Illumina DNA Prep with Exome 2.5 Enrichment provides cost-efficient human whole-exome sequencing results with exceptional 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 preparation 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

Learn more →

Illumina FFPE DNA Prep with Exome 2.5 Enrichment

Illumina FFPE DNA Prep with Exome 2.5 Enrichment enables sensitive and comprehensive whole-exome sequencing with a tumor-normal workflow for detecting low-frequency variants from FFPE samples.

- Includes unique molecular identifiers (UMIs) for error correction and reduction of false positives to enable accurate detection of low-frequency mutations
- Features a fast, hybrid-capture workflow for preparing sequencing-ready libraries in ~10 hours, with only 4 hours of hands-on time
- Provides comprehensive coverage of exons and variants with high on-target rates and uniform coverage
- 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

Learn more →

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 cfDNA extracted from plasma, blood, and liquid biopsy samples.

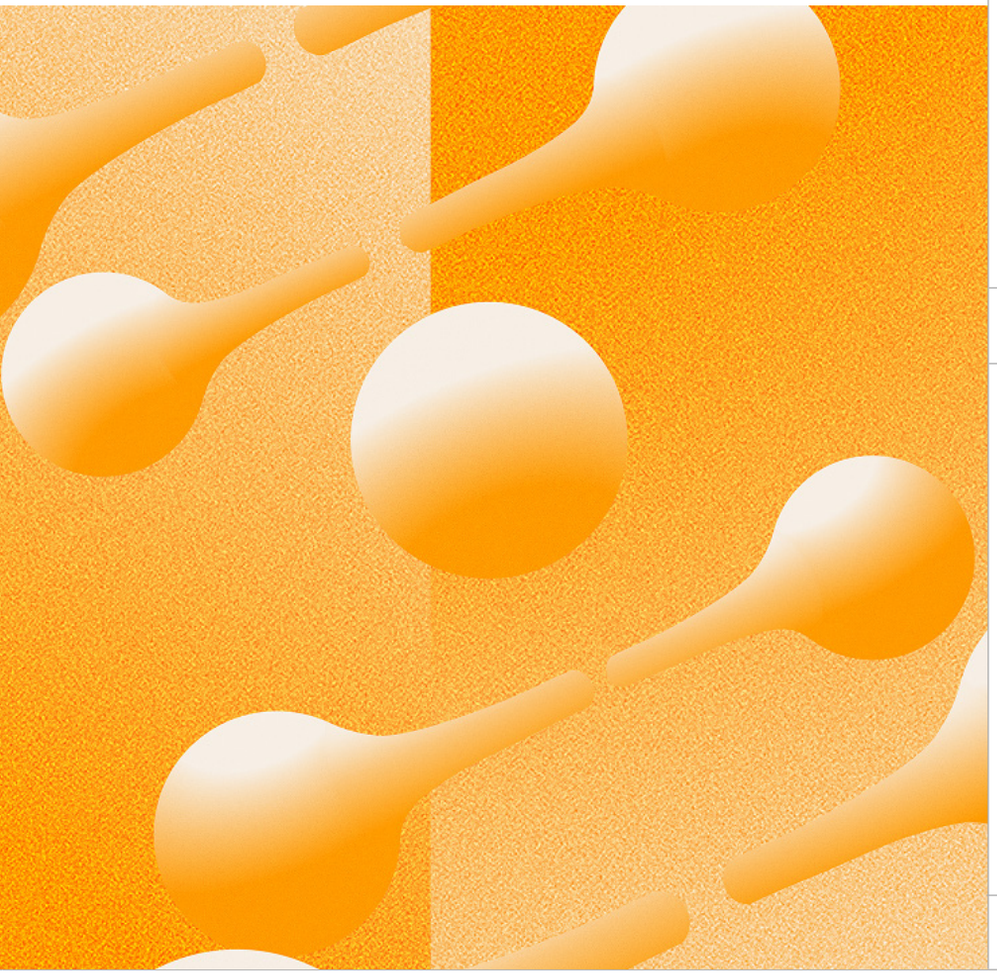
- Includes UMIs for error correction and reduction of false positives to enable accurate detection of low-frequency mutations
- Features a fast, flexible, and scalable workflow for preparing sequencing -ready libraries in 8.5–9.5 hours, with just 2.5–3 hours of hands-on time
- Accommodates user-designed and customized enrichment panels; the Illumina Custom Enrichment Panel v2 is easily designed with the DesignStudio free online tool or our Concierge Design Services Team

Learn more →

Summary of Illumina DNA enrichment and library preparation solutions

Specifications	Illumina DNA Prep with Enrichment	Illumina DNA Prep with Exome 2.5 Enrichment	Illumina FFPE DNA Prep with Exome 2.5 Enrichment	Illumina Cell-Free DNA Prep with Enrichment
Application	Whole-exome sequencing and targeted resequencing	Small genomes, PCR amplicons, Plasmids	Targeted sequencing from FFPE DNA samples	Targeted sequencing from cfDNA samples
Method	Bead-linked transposome and hybrid–capture chemistry	Bead-linked transposome and hybrid–capture chemistry	Hybrid–capture chemistry	Hybrid–capture chemistry
Specialized sample types	Blood, saliva, FFPE DNA	Blood, saliva	FFPE and low-input samples	cfDNA from plasma or blood
DNA input amounts	10–1000 ng (50 ng minimum for complex genomes or FFPE DNA)	50–1000 ng	40 ng FFPE DNA	10–30 ng (20 ng recommended)
Hands-on time	~2 hr	~2 hr	~4 hr	~2.5–3 hr
Assay time	~6.5 hr	~6.5 hr	~10 hr	~10–12 hr
PCR protocol	Yes	Yes	Yes	Yes
Library quantification needed	No	No	Yes	No
Fragmentation included	Yes, on-bead	Yes, on-bead	Not included, required	Not required
FFPE compatibility	Yes	No	Yes	Yes
Automation compatibility	Yes	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 Exome 2.5 Panel, Twist Bioscience for Illumina Mitochondrial Panel, Illumina Custom Enrichment Panel v2	Twist Bioscience for Illumina Exome 2.5 Panel, Twist Bioscience for Illumina Mitochondrial Panel	Twist Bioscience for Illumina Exome 2.5 Panel, Illumina Custom Enrichment Panel v2, Illumina Custom Enrichment Panel, user-defined panels
Recommended index classes	Illumina DNA/RNA Unique Dual Indexes, Tagmentation	Illumina DNA/RNA Unique Dual Indexes, Tagmentation	Illumina UMI DNA/RNA Unique Dual v3 Indexes, Ligation	Illumina UMI DNA/RNA Unique Dual v3 Indexes, Ligation
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	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
Recommended analysis software	DRAGEN Enrichment, DRAGEN Somatic, Illumina Connected Insights, Emedgene software	DRAGEN Enrichment, DRAGEN Somatic, Illumina Connected Insights, Emedgene software	DRAGEN Somatic, Illumina Connected Insights	DRAGEN for ILMN cfDNA Prep with Enrichment, Illumina Connected Insights
Consumables and equipment	Illumina DNA Prep with Enrichment consumables and equipment	Illumina DNA Prep with Exome 2.5 Enrichment consumables and equipment	Illumina FFPE DNA Prep with Exome 2.5 Enrichment consumables and equipment	Illumina Cell-Free DNA Prep with Enrichment consumables and equipment

5-base DNA library preparation



Illumina 5-Base DNA Prep

Illumina 5-Base DNA Prep enables comprehensive discovery of the 5-base genome (A, T, G, C, and 5mC), providing dual insights into the whole genome and methylome.

Uses novel chemistry that retains DNA sequence complexity for efficient alignment and sensitive variant and methylation detection

Expands insights with genome-wide detection of DNA variants and methylation events in a single sequencing run

Produces sequencing-ready libraries in less than one day with as little as 1 ng cell-free DNA (cfDNA) and 50 ng genomic DNA (gDNA)

Learn more →

Illumina 5-Base DNA Prep with Enrichment

Illumina 5-Base DNA Prep with Enrichment offers a sensitive, flexible approach for targeted genome and methylome insights from a single sample preparation and sequencing run.

Detects rare variants from low-input samples using integrated UMIs for error correction and reducing false positives

Produces sequencing-ready libraries in less than 11 hours with minimal touchpoints

Uses custom hybrid–capture probe panels for targeted sequencing applications

Learn more →

Summary of Illumina 5-Base DNA library preparation solutions

Specifications	Illumina 5-Base DNA Prep	Illumina 5-Base DNA Prep with Enrichment
Application	5-base whole-genome sequencing	5-base targeted sequencing
Method	Enzymatic conversion of 5mC to T Ligation-based addition of adapters and indexes	Enzymatic conversion of 5mC to T Ligation-based addition of adapters and indexes
Specialized sample types	cfDNA, gDNA	cfDNA, gDNA
DNA input amounts	1–20 ng cfDNA, 50–100 ng gDNA	1–20 ng cfDNA, 50–100 ng gDNA
Hands-on time	~2.5 hr	Varies
Assay time	~6–7.5 hr	~10–12 hr
PCR protocol	Yes	Yes
Library quantification needed	Yes	Yes
Fragmentation included	No, mechanical shearing required	No, mechanical shearing required
FFPE compatibility	Not demonstrated	Not demonstrated
Automation compatibility	Coming soon	Coming soon
Compatible panels	N/A	Illumina Custom Enrichment Panel v2
Recommended index classes	Illumina Unique Dual Indexes	Illumina Unique Dual Indexes
Recommended sequencing systems	NovaSeq 6000 System, NovaSeq X Series	NextSeq 1000 System, NextSeq 2000 System, NovaSeq 6000 System, NovaSeq X Series
Recommended analysis software	DRAGEN Germline, DRAGEN Somatic, Illumina Connected Multiomics	DRAGEN Enrichment
Consumables and equipment	Illumina 5-Base DNA Prep consumables and equipment	Illumina 5-Base DNA Prep with Enrichment consumables and equipment

High-performance library preparation solutions for RNA sequencing

Advances in RNA sequencing (RNA-Seq) library preparation have revolutionized the study of the transcriptome. The Illumina RNA library preparation portfolio supports various applications and sample types, offering efficient, comprehensive transcriptome coverage.

Illumina RNA library preparation combines 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.

RNA library preparation

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 sequencing, and more.

- Enriches for targeted transcripts of interest, including the RNA exome or RNA virus detection
- Offers exceptional capture efficiency and coverage uniformity even with low-input or FFPE samples
- Uses a simple tagmentation-based library preparation and RNA enrichment workflow with less than 2 hours of hands-on time

Learn more →

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

Learn more →

Illumina miRNA Prep

Illumina miRNA Prep is a streamlined, cost-effective solution for generating high-quality microRNA (miRNA) and small RNA libraries directly from total RNA or isolated miRNA from any species.

- Generates reliable, miRNA-specific libraries, minimizes reaction biases, and eliminates adapter dimers, even from low-input of total RNA
- Incorporates UMIs for unbiased, accurate quantification of mature miRNAs
- Enables multiplexing by using up to 384 unique dual indexes for scalable sequencing based on experimental needs

Learn more →

Illumina Stranded Total RNA Prep with Ribo-Zero™ Plus or 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 types,including blood, saliva, and FFPE DNA
- Supports a wide range of RNA inputs from 1–1000 ng and delivers excellent 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

*Users can design and use custom depletion probes for other organisms or additional unwanted transcripts. Contact your Illumina sequencing specialist for more information.

Learn more →

Summary of Illumina RNA library preparation solutions

Specifications	Ilumina Stranded Total RNA Prep with Ribo-Zero Plus or Ribo-Zero Plus Microbiome	Illumina Stranded mRNA Prep	Illumina RNA Prep with Enrichment	Illumina miRNA Prep
Application	Whole-transcriptome sequencing	mRNA-Seq, gene expression profiling	RNA exome enrichment, mRNA-Seq, gene expression profiling for low-input or FFPE samples, virus detection	miRNA-Seq and small RNA sequencing
Method	Ligation-based addition of adapters and indexes, enzymatic rRNA depletion	PolyA capture, ligation-based addition of adapters and indexes	Bead-linked transposome, hybrid–capture chemistry	Ligation-based addition of adapters and indexes
Detection	Coding and noncoding transcriptome	Coding transcriptome with polyA tail	Targeted coding regions	miRNA
Strand specificity	Stranded	Stranded	Nonstranded	Stranded
Specialized sample types	Blood, FFPE tissue, low-input samples	High-quality mRNA, low-input samples	Blood, FFPE tissue, low-input samples, saliva, nasal swabs	Blood, FFPE tissue, low-input samples
RNA input amounts	1–1000 ng standard-quality RNA 10 ng minimum for optimal performance/FFPE samples	25–1000 ng standard-quality RNA	10 ng standard-quality RNA 20 ng RNA for low-quality/FFPE samples	1–500 ng RNA
Hands-on time	< 3 hr	< 3 hr	< 2 hr	3 hr
Assay time	~7 hr	< 7 hr	< 9 hr	7 hr
Library quantification needed	Yes	Yes	Yes	Yes
Fragmentation included	Yes	Yes	Not required	Not required
FFPE compatibility	Yes	No	Yes	Yes
Automation compatibility	Yes	Yes	Yes	Yes
Compatible panels	Illumina DNA/RNA Unique Dual Indexes, Tagmentation	Illumina DNA/RNA Unique Dual Indexes, Tagmentation	Illumina UMI DNA/RNA Unique Dual v3 Indexes, Ligation	Illumina UMI DNA/RNA Unique Dual v3 Indexes, Ligation
Recommended index classes	Illumina RNA Unique Dual Indexes, Ligation	Illumina RNA Unique Dual Indexes, Ligation	Illumina DNA/RNA Unique Dual Indexes, Tagmentation	Illumina UMI DNA/RNA Unique Dual v3 Indexes, Ligation
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, MiSeq Series, NextSeq 550 System, NextSeq 1000 System, NextSeq 2000 System, NovaSeq 6000 System, NovaSeq X Series	MiSeq Series, NextSeq 550 System, NextSeq 1000 System, NextSeq 2000 System, NovaSeq 6000 System, NovaSeq X Series
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	DRAGEN miRNA
Consumables and equipment	Illumina Stranded RNA Prep with Ribo-Zero Plus or Ribo-Zero Plus Microbiome consumables and equipment	Illumina Stranded mRNA Prep consumables and equipment	Illumina RNA Prep with Enrichment consumables and equipment	Illumina miRNA Prep with Enrichment consumables and equipment

Summary

The Illumina NGS library preparation portfolio delivers high quality, precision, and ease of use for both DNA and RNA sequencing. User-friendly protocols accommodate a range of experimental 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.

Maximize insights from samples, generate results you can trust, reduce hands-on time, and access expert support when you need it with Illumina.

DNA library preparation →

RNA library preparation →

Illumina 5-base solution →

DRAGEN secondary analysis →

Illumina Connected Insights →

Automation for library preparation →

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