APPLICATION NOTE

Illumina DNA Prep chemistry

Fast, flexible, and self-normalizing library prep

- On-bead tagmentation chemistry supports a broad range of applications
- Simple workflow integrates DNA extraction, quantitation, fragmentation, and library normalization
- Optional, single hybridization reaction enables targeted enrichment applications

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Introduction

While advances in next-generation sequencing (NGS) technology have accelerated the pace of genomic research, many laboratories continue to experience bottlenecks during the library preparation phase of the NGS workflow. With multiple steps required both before and after library preparation, many labs contend with significant delays in starting the sequencing process. Pre-library prep steps include DNA extraction, quantitation, and fragmentation, while post-library prep steps include library quality assessments, library quantitation, and normalization.

To overcome these delays, Illumina DNA Prep* offers bead-linked transposome (BLT) chemistry for on-bead tagmentation. This unique chemistry (Figure 1) integrates the DNA extraction, fragmentation, library preparation, and library normalization steps to deliver the fastest, most flexible workflows in the Illumina library prep portfolio (Figure 2).

Beyond providing a rapid workflow, Illumina DNA Prep chemistry offers extraordinary flexibility for input type and input amount, including direct sample input of fresh blood or saliva, and supports a wide range of applications (Table 1). Illumina DNA Prep is compatible with whole-genome sequencing (WGS) applications, including human, small/microbial, and large, complex genome sequencing. For targeted DNA enrichment applications, including fixed and custom panels of varying sizes, and whole-exome sequencing (WES), Illumina offers Illumina DNA Prep with Enrichment,† which features enrichment bead-linked transposomes (eBLTs) for enrichment compatible library prep. Furthermore, Illumina DNA Prep with Enrichment is compatible with Illumina and third-party enrichment probes/panels, enabling content portability for increased flexibility.

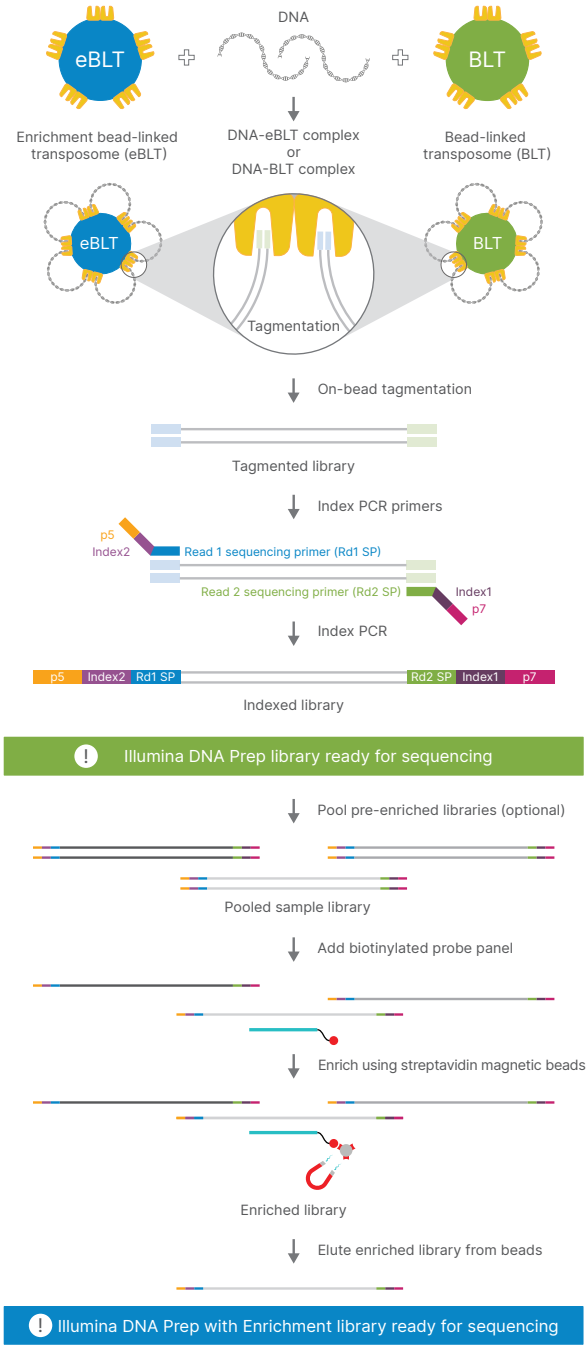

Figure 1: Illumina DNA Prep bead-linked transposome chemistry—On-bead tagmentation mediates the simultaneous fragmentation of gDNA and the addition of Illumina sequencing primers. Reduced-cycle PCR amplifies DNA fragments and adds indexes and adapters. Sequencing-ready Illumina DNA Prep libraries are pooled. Illumina DNA Prep with Enrichment libraries are pooled and undergo a single hybridization reaction to produce an enriched library ready for sequencing.

* Illumina DNA Prep was previously known as the Nextera™ DNA Flex Library Prep Kit. The two kits use the same tagmentation chemistry and have identical product performance specifications and kit configurations.

† Illumina DNA Prep with Enrichment was previously known as the Nextera DNA Flex for Enrichment Library Prep Kit. The two kits use the same tagmentation chemistry and have identical product performance specifications and kit configurations.

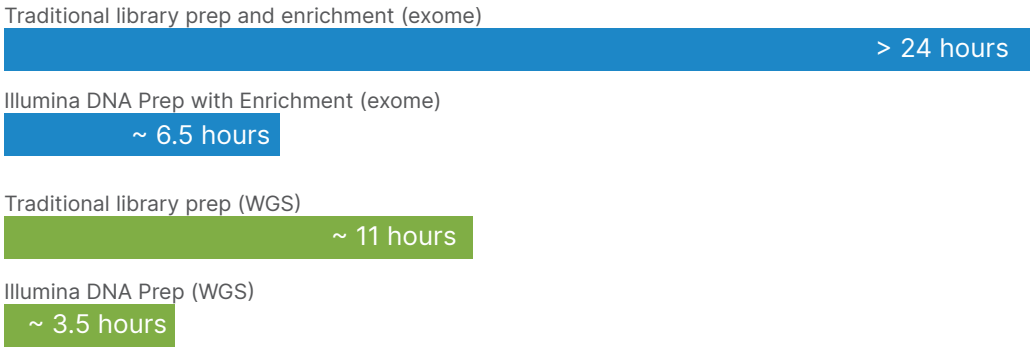

Figure 2: Illumina DNA Prep chemistry offers the fastest Illumina workflows—Illumina DNA Prep chemistry offers the lowest total workflow times for exome (library prep and enrichment) and whole-genome sequencing (library prep) applications, compared to traditional, nonbead-linked transposome–based chemistry. Times may vary depending on equipment used, number of samples processed, automation procedures, or user experience.

Table 1: Sample types supported by Illumina DNA Prep chemistry

DNA input type	Illumina DNA Prep	Illumina DNA Prep with Enrichment
gDNA	✓	✓
Blood	✓	✓
Saliva	✓	✓
Microbial genomes/plasmids	✓	Not validated[a]
DNA extracted from FFPE tissue	Not validated[a]	✓
DNA input verified[b]	1–500 ng	10–1000 ng

a. Technically feasible but not formally validated by Illumina. Results may vary.
b. Saturation-based normalization occurs with ≥ 100 ng DNA input for Illumina DNA Prep and with ≥ 50 ng DNA input for Illumina DNA Prep with Enrichment.

Optimized library prep performance

Illumina DNA Prep chemistry has enabled major improvements in library preparation performance. On-bead tagmentation produces highly uniform and consistent insert sizes (~350 bp for WGS, ~200 bp for targeted enrichment), across a wide DNA input range (1–500 ng for Illumina DNA Prep, 10–1000 ng for Illumina DNA Prep with Enrichment) (Figure 3),[1] eliminating the need for careful transposome:DNA ratio optimization. Furthermore, the wide DNA input range allows flexibility for experiments with various sample types. On-bead tagmentation also delivers uniform and consistent library yields across a wide DNA input range (Figure 4). At or near 100 ng DNA input, beads become saturated, eliminating the need for time-consuming library quantitation and normalization steps before pooling. In a comparison of multiple users preparing libraries across a range of DNA inputs on different days with Illumina DNA Prep, on-bead tagmentation enabled self-normalization of libraries with saturation above inputs of 100 ng and consistent index representation with a % CV for the entire data set > 15% (Figure 5).

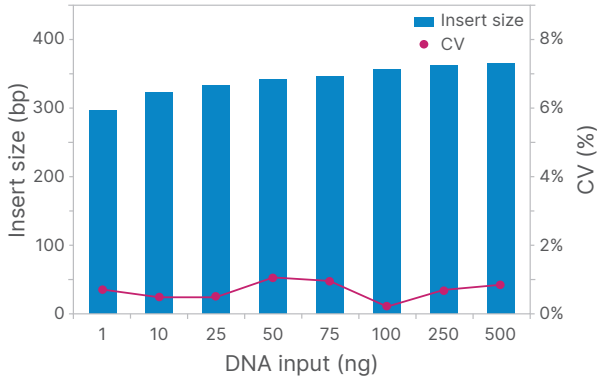

Figure 3: Uniform and consistent insert sizes—On-bead tagmentation delivers consistent insert sizes regardless of DNA input amount. From 1–500 ng DNA input, the total coefficient of variance (CV) is 6.09%. Libraries were produced with *E. coli* replicate samples using the Illumina DNA Prep. Sequencing was performed on a MiSeq™ System (2 × 76 bp run).

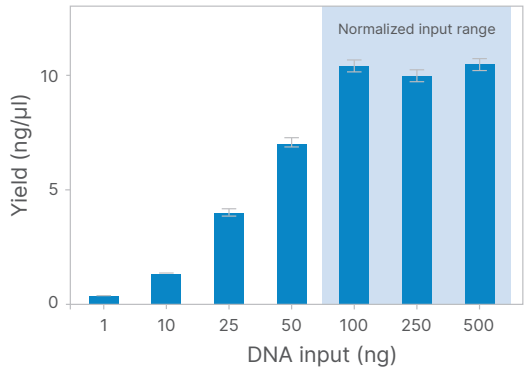

Figure 4: Tagmented and normalized libraries—Beads become saturated at ≥ 100 ng, leading to normalized yield of tagmented DNA. Illumina DNA Prep libraries were produced with Human-NA12878 samples (Coriell Institute).

Flexible chemistry enables a broad range of applications

Illumina DNA Prep chemistry supports a broad range of research interests, advanced study designs, and various NGS methods, including WGS, exome sequencing, and both fixed and custom sequencing panels of varying sizes.

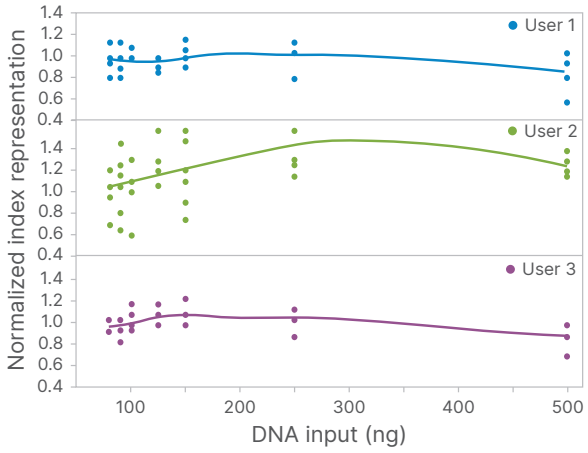

Figure 5: Library normalization is insensitive to input amount or user—On-bead tagmentation self-normalizes library yield and insert size across a range of DNA input amounts for libraries prepared by three different users. Normalized index representation is plotted as a function of input amount.

WGS applications with Illumina DNA Prep

Illumina DNA Prep supports cancer genomics research, environmental metagenomics, infectious disease research, agrigenomics, and more. Whether sequencing large complex genomes, small genomes, plasmids, amplicons, gram positive/gram negative bacteria, fungi, or a range of plant and animal species, Illumina DNA Prep delivers comprehensive genomic coverage.

Microbial WGS

Illumina DNA Prep achieves greater uniformity of coverage across a bacterial genome, as compared to the Nextera XT DNA Library Prep Kit, particularly at low DNA input amounts (Figure 6). Furthermore, two measurements of genome assembly quality, the N50 value and the number of contigs in an assembly, support the superior performance of Illumina DNA Prep chemistry for microbial genome assembly. Illumina DNA Prep produces libraries with high N50 values (increased average contig length) and fewer total numbers of contigs, compared to libraries prepared with the Nextera XT DNA Library Prep Kit (Figure 7).

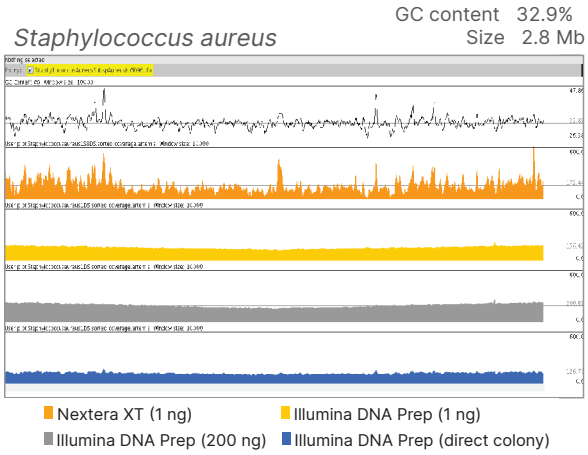

Figure 6: Improved coverage uniformity—Illumina DNA Prep achieves greater uniformity of coverage across the *Staphylococcus aureus* bacterial genome, as compared to the Nextera XT DNA Library Prep Kit. Plot shows a 4 Mb genome view of coverage.

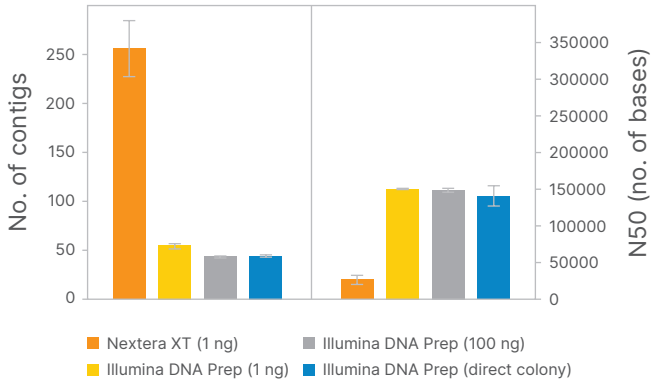

Figure 7: Improved genome assembly—*Staphylococcus aureus* libraries prepared with Illumina DNA Prep and sequenced using 2 × 150 bp reads on the NextSeq™ 550 System result in genomic assemblies with significantly lower numbers of contigs and higher N50 values, indicative higher quality assemblies, as compared to libraries prepared with the Nextera XT DNA Library Prep Kit.

Learn more about WGS applications with Illumina DNA Prep:

- Human whole-genome sequencing
- Microbial whole-genome sequencing
- Direct bacterial colony sequencing
- Soil shotgun metagenomics analysis

Human WGS

Illumina DNA Prep was used to generate a set of libraries from human DNA (NA12878), varying the input from 0.01 ng to 100 ng. Libraries were successfully generated for each input amount by increasing PCR cycle number according to DNA input, with a minimum yield of 100 ng from the 0.01 ng input. All libraries showed approximately the expected size distribution (Figure 8).

Each of the prepared Illumina DNA Prep libraries was sequenced on one lane of a HiSeq™ X Ten System with data analysis performed using the Whole Genome Sequencing App (version 8.0.1) and Variant Calling Assessment Tool (3.0.0), available in BaseSpace™ Sequence Hub. Samples were aligned and variant calling at each input amount was compared to Platinum Genome NA12878 data. At 0.1 ng input, greater than 99% of genome bases were covered at least 1× (data not shown). In fact, comparing single nucleotide variant (SNV) calls between 0.5 ng input and 100 ng input, 97% of calls were shared, demonstrating strong concordance (Figure 9). Sequencing coverage for the 0.5 ng and 100 ng samples were 22.8× and 40.1×, respectively. The difference in coverage is due primarily to higher duplicates, lower reads passing filter (PF), and smaller insert size for the 0.5 ng sample.

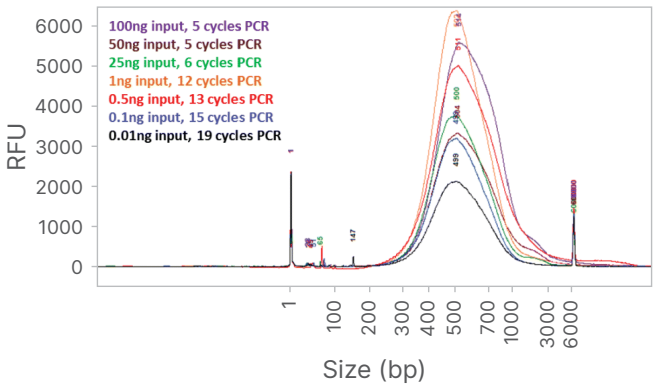

Figure 8: Illumina DNA Prep library preparation from very low input—Library traces for Illumina DNA Prep libraries prepared from DNA input amounts ranging from 0.01 ng to 100 ng show the expected size distribution for high-quality libraries.

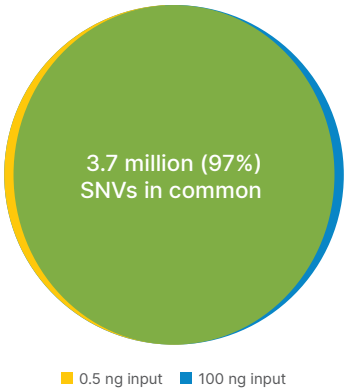

Figure 9: Strong concordance in SNV calling for low input amounts—3.7 million (97%) of SNV calls were shared between Illumina DNA Prep libraries prepared from DNA input amounts of 0.5 ng and 100 ng.

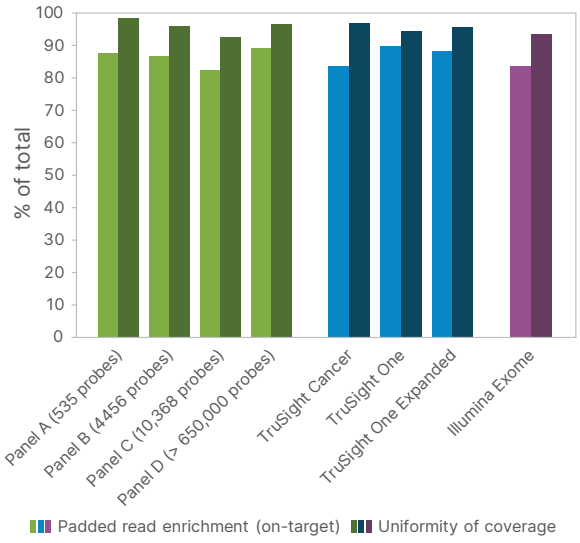

Figure 10: High coverage uniformity and padded read enrichment—Illumina DNA Prep with Enrichment provides high coverage uniformity and on-target padded read enrichment for custom panels (A–D, varying sizes), fixed panels (TruSight™ Cancer, TruSight One, and TruSight One Expanded), and exome panels (Illumina exome).

Targeted enrichment applications with Illumina DNA Prep with Enrichment

Illumina DNA Prep with Enrichment provides high coverage uniformity and padded read enrichment for custom, fixed, and exome panels (Figure 10).

Learn more about targeted enrichment applications with Illumina DNA Prep with enrichment:

- Somatic variant detection in FFPE samples

Summary

Illumina DNA Prep chemistry enables a revolutionary workflow that integrates DNA extraction, quantitation, fragmentation, and library normalization to deliver the fastest and most flexible library prep workflow in the Illumina portfolio. Integrating Illumina DNA Prep chemistry with a single hybridization reaction provides the fastest workflow in the Illumina enrichment portfolio. The user-friendly solution supports researchers of all experience levels and provides a common workflow for various experimental designs. On-bead tagmentation chemistry supports a wide range of DNA input amounts, various sample types, and a broad range of methods and applications, including human WGS, environmental metagenomics, plant and animal research, tumor profiling, fixed panels, custom panels, and WES, and more. See how the innovative Illumina DNA Prep chemistry combined with the power of Illumina sequencing by synthesis (SBS) chemistry can advance and accelerate your research goals today.

Learn More

On-bead tagmentation

Illumina DNA Prep

Illumina DNA Prep with Enrichment

References

1. Illumina. Illumina DNA Prep with Enrichment Data Sheet. https://www.illumina.com/content/dam/illumina/gcs/assembled-assets/marketing-literature/illumina-dna-prep-enrichment-data-sheet-m-gl-02147/ilmn-dna-prep-enrich-data-sheet-m-gl-02147.pdf. Published 2020. Updated 2023. Accessed October 26, 2023.

Ordering information

Product	Catalog no.
Illumina DNA Prep (M) Tagmentation (24 samples, IPB)	20060060
Illumina DNA Prep (M) Tagmentation (96 samples, IPB)	20060059
Illumina DNA Prep with Enrichment, (S) Tagmentation (96 samples)	20025524
Illumina DNA Prep with Enrichment, (S) Tagmentation (16 samples)	20025523
Illumina DNA Prep, (S) Tagmentation (96 samples)	20025520
Illumina DNA Prep, (S) Tagmentation (16 samples)	20025519

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