

Fast, Flexible, and Self-Normalizing Library Prep with Nextera™ Flex Chemistry

Revolutionary library preparation chemistry enables fast, integrated workflows for a wide range of sequencing applications.

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 this challenge the release of the Nextera DNA Flex Library Preparation Kit introduced 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, Nextera Flex chemistry offers extraordinary flexibility for input type and input amount, including direct sample input of fresh blood or saliva, as well as a wide range of supported applications (Table 1). The Nextera DNA Flex Library Prep Kit 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, as well as whole-exome sequencing (WES), Illumina introduced the Nextera Flex for Enrichment solution, which features enrichment bead-linked transposomes (eBLTs) for enrichment compatible-library prep. Furthermore, Nextera Flex for Enrichment is compatible with Illumina and third-party enrichment probes/panels, which enables content portability for increased flexibility.

Table 1: Sample types supported by Nextera Flex chemistry

DNA input type	Nextera DNA Flex	Nextera Flex for 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 Nextera DNA Flex and with ≥ 50 ng DNA input for Nextera Flex for Enrichment.

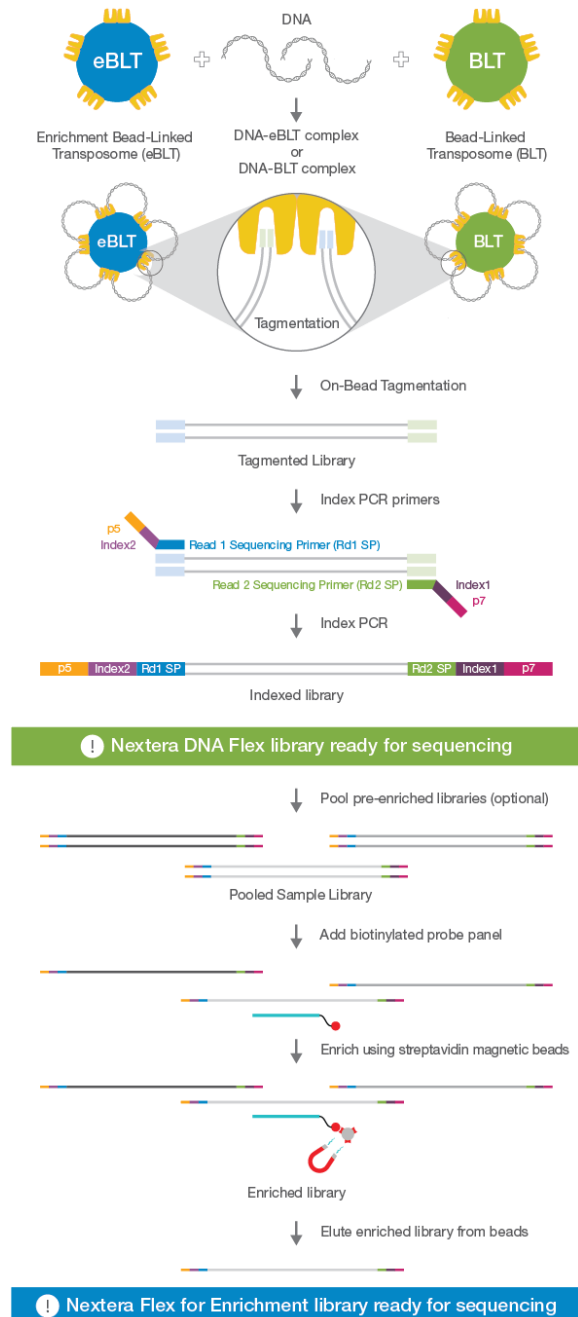


Figure 1: Nextera Flex bead-linked transposome chemistry—On-Bead Tagmentation mediates the simultaneous fragmentation of gDNA and the addition of Illumina sequencing primers. Reduced-cycle PCR amplifies DNA fragments and adds indexes and adapters. Sequencing-ready Nextera DNA Flex libraries are pooled.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

Traditional library prep and enrichment (Illumina exome)



Nextera Flex for enrichment (Illumina exome)



Traditional library prep (Illumina WGS)



Nextera DNA Flex (WGS)



Figure 2: Nextera Flex technology offers the fastest Illumina workflows—Nextera Flex technology 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.

Optimized library prep performance

Nextera Flex 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 Nextera DNA Flex, 10-1000 ng for Nextera Flex for 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, including precious samples. 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 the Nextera DNA Flex Library Prep Kit, 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).

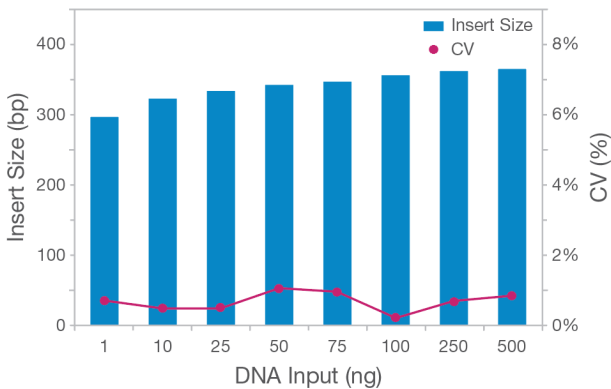


Figure 3: Uniform and consistent insert sizes—On-Bead Tagmentation delivers consistent insert sizes regardless of DNA input amount. From 1–500 ng DNA input, the total coefficient of variance (CV) is 6.09%. Libraries were produced with *E. coli* replicate samples using the Nextera DNA Flex Kit. Sequencing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

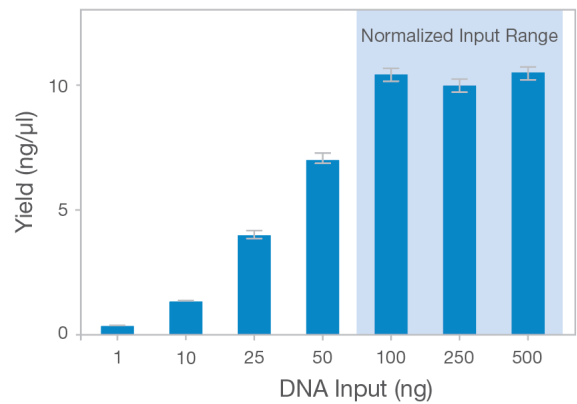


Figure 4: Tagmented and normalized libraries—Beads become saturated ≥ 100 ng, leading to normalized yield of tagmented DNA. Nextera DNA Flex libraries were produced with Human-NA12878 samples (Coriell Institute).

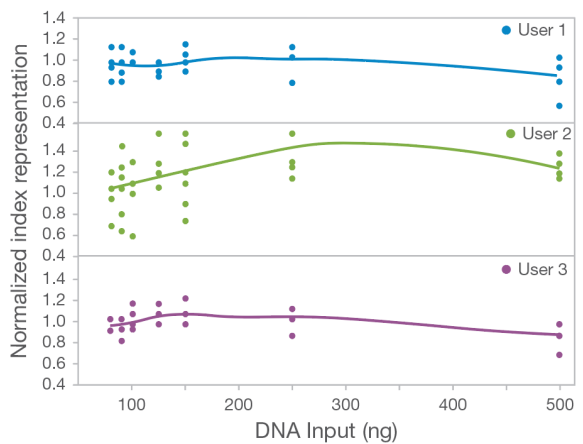


Figure 5: Library normalization is insensitive to input amount or user—On-Bead Tagmentation self-normalizes library yield and insert size across a range of DNA input amounts for libraries prepared by three different users. Normalized index representation is plotted as a function of input amount.

Flexible chemistry enables a broad range of applications

Nextera Flex chemistry supports a broad range of research interests and advanced study designs. The growing portfolio of Nextera Flex products supports various NGS methods including WGS, exome sequencing, and both fixed and custom sequencing panels of varying sizes.

WGS applications with Nextera DNA Flex

The Nextera DNA Flex Library Preparation Kit supports human WGS, 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, the Nextera DNA Flex Kit delivers comprehensive genomic coverage.

Microbial WGS

The Nextera DNA Flex Library Prep Kit 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 Nextera Flex chemistry for microbial genome assembly. The Nextera DNA Flex Library Prep Kit 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).

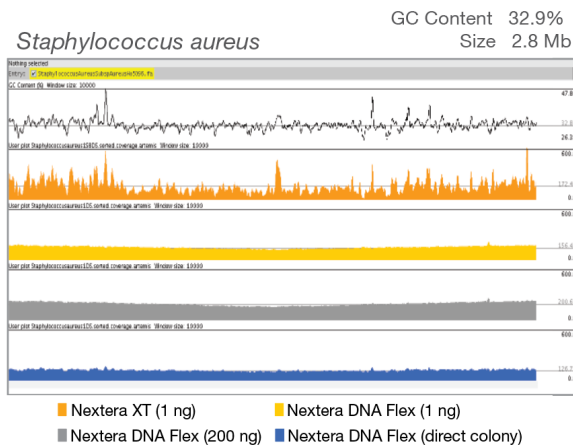


Figure 6: Improved coverage uniformity—The Nextera DNA Flex Library Preparation Kit achieves greater uniformity of coverage across the *Staphylococcus aureus* bacterial genome, as compared to the Nextera XT DNA Library Prep Kit. Plot shows a 4 Mb genome view of coverage.



Learn more about WGS applications with Nextera Flex:

[Human Whole-Genome Sequencing with the Nextera DNA Flex Library Preparation Kit Application Note](#)



[Microbial Whole-Genome Sequencing with the Nextera DNA Flex Library Preparation Kit Application Note](#)

[Direct Bacterial Colony Sequencing with the Nextera DNA Flex Library Preparation Kit Application Note](#)

[Nextera DNA Flex Library Preparation for Soil Shotgun Metagenomics Analysis](#)

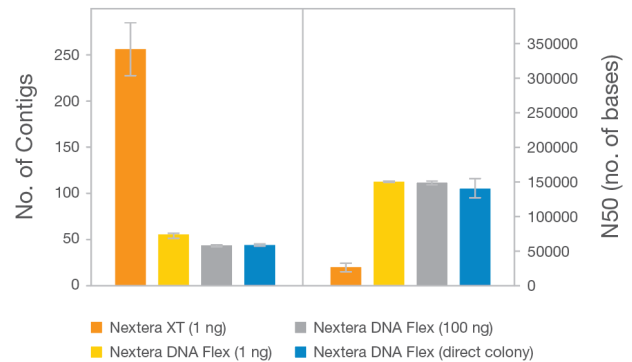


Figure 7: Improved genome assembly—*Staphylococcus aureus* libraries prepared with the Nextera DNA Flex Library Preparation Kit and sequenced using 2 × 150 bp reads on the NextSeq™ 550 System result in genomic assemblies with significantly lower numbers of contigs and higher N50 values, indicative higher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

Human WGS

The Nextera DNA Flex Library Prep Kit 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).

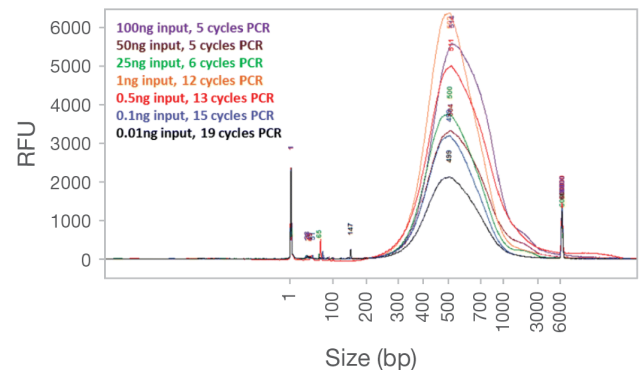


Figure 8: Nextera DNA Flex library preparation from very low input—Library traces for Nextera DNA Flex libraries prepared from DNA input amounts ranging from 0.01 ng to 100 ng show the expected size distribution for high-quality libraries.

Each of the prepared Nextera DNA Flex 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 PF, and smaller insert size for the 0.5 ng sample.

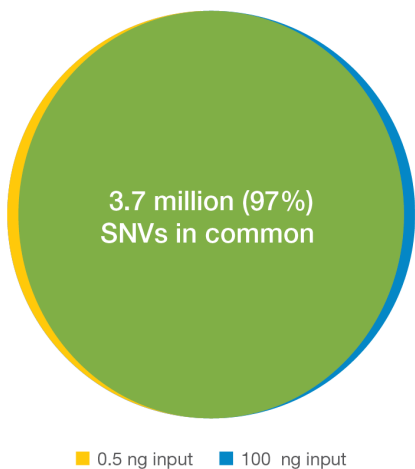


Figure 9: Strong concordance in SNV calling for low input amounts—3.7 million (97%) of SNV calls were shared between Nextera DNA Flex libraries prepared from DNA input amounts of 0.5 ng and 100 ng.

Targeted enrichment applications with Nextera Flex for Enrichment

Nextera Flex for Enrichment provides high coverage uniformity and padded read enrichment for custom, fixed, and exome panels (Figure 10).



Learn more about targeted applications with Nextera Flex:

[Somatic Variant Detection in FFPE Samples with Nextera Flex for Enrichment](#)

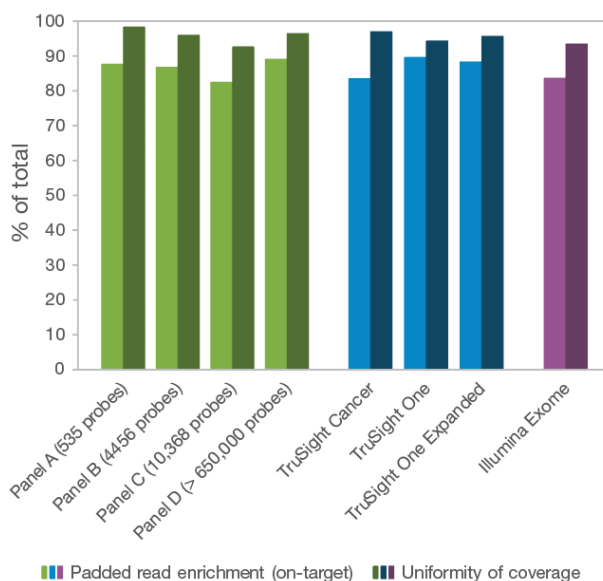


Figure 10: High coverage uniformity and padded read enrichment—Nextera Flex for Enrichment provides high coverage uniformity and on-target padded read enrichment for custom panels (A-D, varying sizes), fixed panels (TruSight™ Cancer, TruSight One, and TruSight One Expanded), and exome panels (Illumina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

Summary

Nextera Flex 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 Nextera Flex chemistry with a single hybridization reaction provides the fastest workflow in the Illumina enrichment portfolio. The user-friendly solution supports users of all experience levels and provides a common workflow for a variety of experimental designs. On-Bead Tagmentation chemistry enables support for a wide range of DNA input amounts, various sample types, and a broad range of applications, including human WGS, environmental metagenomics, plant and animal research, tumor profiling, fixed panels, custom panels, and WES, and more. See how the innovative Nextera Flex for Enrichment technology combined with the power of Illumina SBS chemistry can advance and accelerate your research goals today.

Learn More

To learn more about Nextera Flex technology, visit www.illumina.com/products/by-brand/nextera.html

Read the [Nextera Flex for Enrichment Data Sheet](#)

Read the [Nextera DNA Flex Library Prep Kit Data Sheet](#)

References

1. Illumina. (2018) [Nextera Flex for Enrichment Data Sheet](#). Accessed June 2019.

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