

Nextera™ DNA Flex Library Preparation Kit

A fast, integrated workflow for a wide range of applications from human whole-genome sequencing to amplicons, plasmids, and microbial species.

Highlights

- Fast Library Prep Workflow**
 Save time and reduce hands-on touch points with On-Bead Tagmentation, which reduces total library prep time to less than three hours
- Integrated Sample Input**
 Enhance library preparation efficiency with integrated DNA extraction protocols for blood, saliva, and dried blood spots
- Flexible Workflow with Broad DNA Input Range**
 Simplify daily operations with a kit that supports a broad DNA input range (1–500 ng), multiple DNA input types, and small to large genome sizes
- Wide Range of Applications**
 Sequence human or other large/complex genomes as well as amplicons and microbial, parasitic, or fungal species
- Optimized Library Prep Performance**
 Obtain consistent insert sizes and high coverage uniformity regardless of user experience level

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 before they are able to start the sequencing process. Pre-library preparation steps include DNA extraction, quantitation, and fragmentation, while post-library prep steps include library quality assessments, library quantitation, and normalization.

The release of the Nextera DNA Library Preparation Kits introduced tagmentation chemistry, which combined DNA fragmentation and adapter ligation steps into a single 15-minute reaction and reduced library prep time to 90 minutes.¹ With the launch of Nextera XT DNA Library Prep Kits, the need for library quantitation before library pooling and sequencing was eliminated.² Now the latest revolution in Illumina library prep chemistry is here — the Nextera DNA Flex Library Preparation Kit. The unique chemistry found in the Nextera DNA Flex Library Preparation Kit (Figure 1, Table 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, Table 2).

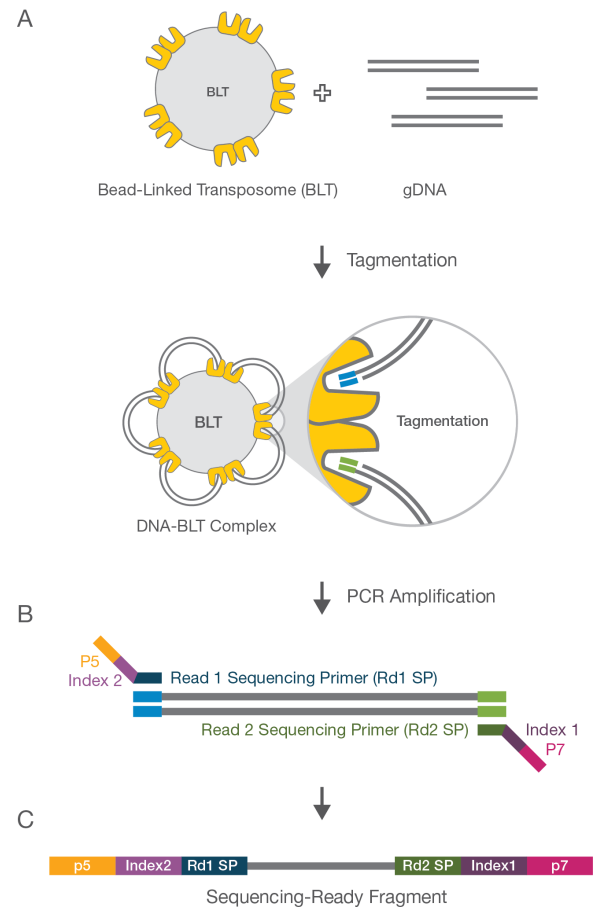


Figure 1: Nextera Bead-Linked Transposome Chemistry—(A) Bead-linked transposomes mediate the simultaneous fragmentation of gDNA and the addition of Illumina sequencing primers. (B) Reduced-cycle PCR amplifies sequencing ready DNA fragments and adds indexes and adapters. (C) Sequencing-ready fragments are washed and pooled.

Table 1: Nextera DNA Flex Library Prep Specifications

	Nextera DNA Flex
DNA Input Type	gDNA, Blood, Saliva, PCR Amplicons, Plasmids, Dried Blood Spots
DNA Input Required	1–500 ng, Small Genomes 100–500 ng, Large Genomes
Sample Multiplexing	24 Single Indexes, 96 Dual Indexes
Supported Sequencing Systems	All Illumina Systems
Total Library Prep Workflow Time (gDNA)^a	3–4 hours

a. Includes DNA extraction, library preparation, and library normalization/pooling steps

TruSeq™ Nano

DNA Extraction	DNA Quant	DNA Frag	Library Prep with Adapter Ligation and Index Tagging	Library Quant	Manual Normalization and Pooling	~11 Hours TWT
1 hr	.5 hr	1 hr	6 hr	.5 hr	2 hr	

Nextera XT

DNA Extraction	DNA Quant	Library Prep with Nextera Tagmentation	Bead-Based Normalization and Pooling	~5.5 Hours TWT
1 hr	.5 hr	2.5 hr	1.5 hr	

Nextera DNA Flex

DNA Extraction	DNA Quant	Library Prep with Nextera Tagmentation and Integrated Normalization	~4 Hours TWT
1 hr	.5 hr	2.5 hr	

Nextera DNA Flex (Blood, Saliva)

Flex Lysis Kit	Quant-Free Library Prep with Nextera Tagmentation and Integrated Normalization	~3 Hours TWT
.5 hr	2.5 hr	

Figure 2: Nextera DNA Flex Delivers the Fastest Illumina Workflow—Calculations made assuming 16 samples were processed at a time with a multichannel pipette. TWT= total workflow time from DNA extraction to library normalization and pooling. Workflow step times calculated assuming specific methods: DNA extraction (QIAamp DNA Mini Kit or Flex Lysis Kit), DNA Quantitation (Qubit), DNA Fragmentation (Covaris), and Manual Library Normalization and Pooling (Bioanalyzer). Times may vary depending on equipment used, number of samples processed, automation procedures, or user experience. Workflow steps colored in gray are not included in the library prep kits.

Table 2: Comparison of Illumina Prep Workflows

	TruSeq Nano	Nextera XT	Nextera DNA Flex ^{a,b}
Integrated DNA Lysis Included	—	—	✓
Flexible, Broad DNA Input Range	—	—	✓
Library Normalization Included	—	✓	✓
DNA Input Required	100–200 ng	1 ng	1–500 ng
Total Library Prep Time ^c	11 hours	5 hours	3–4 hours
Insert Size	350 bp or 550 bp	< 300	300–350 bp
Sample Multiplexing	96 dual indexes	384 dual indexes	24 Single Indexes, 96 dual indexes

a. Integrated DNA extraction protocols available for blood, saliva, and DBS samples

b. Library normalization occurs with ≥ 100 ng DNA input

c. Total Library Prep Time includes DNA extraction, library preparation, and library normalization/pooling steps

Beyond providing a rapid workflow, the Nextera DNA Flex Library Preparation Kit offers extraordinary flexibility for input type, input amount, and a wide range of supported applications. From human whole-genome sequencing (WGS) to small microbial plasmids, the Nextera DNA Flex Library Preparation Kit delivers even genome coverage with the proven accuracy of Illumina sequencing by synthesis (SBS) chemistry.³

Fast and Flexible Library Preparation Workflow

The Nextera DNA Flex Library Preparation Kit includes several features that combine to deliver the fastest library preparation workflow in the Illumina portfolio. A major advance in the Nextera DNA Flex chemistry is On-Bead Tagmentation, which uses bead-bound transposomes to mediate a more uniform tagmentation reaction compared to in-solution tagmentation reactions. After the bead-bound transposomes are saturated with DNA, no additional tagmentation can occur enabling a highly uniform saturation-based normalization process. This strategy provides several significant advantages:

- For DNA inputs between 100–500 ng, accurate quantification of the initial DNA sample is not required. DNA insert fragment size is

not affected by DNA input within this range, saving time and costs associated with cumbersome quantification processes.

- On-Bead Tagmentation eliminates the need for separate mechanical or enzymatic DNA fragmentation steps, saving time and costs associated with shearing instruments or enzymatic kits.
- For DNA inputs between 100–500 ng, On-Bead Tagmentation results in a saturation-based DNA normalization, eliminating the need for time-consuming individual library quantitation and normalization before pooling

Furthermore, the user-friendly workflow is designed to reduce the number of hands-on steps and to support liquid-handling systems for library prep automation. These advances combine to produce a workflow with the lowest number of steps and the fastest total workflow time in the Illumina portfolio (Figure 2).

Integrated DNA Input

With Nextera DNA Flex Library Preparation Kits and Flex Lysis Reagent Kits, DNA extraction can be processed directly from fresh blood or saliva samples. The optional Nextera DNA Flex Lysis Kits have been optimized and validated for Nextera DNA Flex library

preparation and the workflow steps, reagents, and user guide instructions are fully integrated for maximum efficiency. The lysis protocols are carried out with convenient bead-based reagents, require less than 30 minutes of hands-on time, and feed directly into the Nextera DNA Flex tagmentation reaction.

Optimized Library Preparation Performance

The properties of On-Bead Tagmentation have enabled major improvements in library preparation performance. The Nextera DNA Flex Library Preparation Kit produces highly uniform and consistent insert sizes (300–350 bp), across a wide DNA input range (1-500 ng) (Figure 3). Because On-Bead Tagmentation enables generation of uniform insert sizes across a broad input range, the need for careful transposome:DNA ratio optimization as a means of controlling fragment length has been eliminated. Furthermore, the wide DNA input range allows flexibility for experiments with various sample types, including precious samples. In addition to uniform insert sizes, On-Bead Tagmentation delivers uniform and consistent library yields across a wide DNA input range (100-500 ng) (Figure 4). At or near 100 ng DNA input, beads become saturated, leading to consistent, normalized yields, eliminating the need for time-consuming library quantitation and normalization steps before pooling. In a comparison of Nextera DNA Flex and TruSeq Nano DNA Library Prep Kit performance, the Nextera DNA Flex Library Preparation Kit produced results comparable to or, for certain metrics, better than mechanical fragmentation (Table 3).

Beyond the workflow improvements supported by bead-based technology, the most significant advantage of consistent and uniform insert sizes and library yields is more even and uniform coverage across the genome for both human and nonhuman species (Figure 5). Even genomes with high or low GC content show remarkably even coverage without region-specific bias (Figure 5B).

Table 3: Nextera DNA Flex Library Prep Performance

Parameter ^a	Nextera DNA Flex	TruSeq Nano
Paired-End Reads Passing Filter	3.7 × 10 ⁸	3.7 × 10 ⁸
Autosome Callability	96.5%	96.9%
Autosome Exon Callability	98.4%	98.4%
Autosome Coverage > 10x	98.5%	98.6%
SNV Recall	98.7%	98.7%
SNV Precision	99.8%	99.7%
Indel Recall	93.7%	92.9%
Indel Precision	97.0%	94.9%

a. The analysis was run on 20 samples (all NA12878 Corriell samples), spread across 5 runs, to approximate 30x human genome builds. Data analysis was performed using BaseSpace Apps [Whole Genome Sequencing v6.0.0](#) and [Variant Calling Assessment Tool v3.0.0](#). SNV = Single Nucleotide Variant, Indel = Insertion-Deletion Variant.

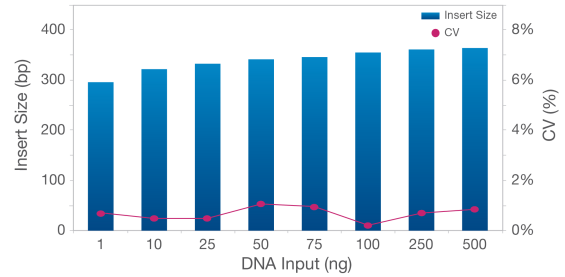


Figure 3: Uniform and Consistent Insert Sizes—On-Bead Tagmentation delivers consistent insert sizes regardless of DNA input amount. From 1–500 ng DNA input, the total coefficient of variance (CV) is 6.09%. Libraries produced with *E. coli* replicate samples using Nextera DNA Flex Kit. Run performed on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

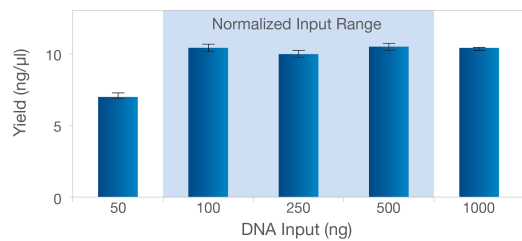


Figure 4: Tagmented and Normalized Libraries—Beads become saturated at or over 100 ng, leading to normalized yield of tagmented DNA. The normalization of tagmented DNA eliminates the need for downstream library normalization steps. Libraries produced with Human-NA12878 samples (Coriell Institute) using the Nextera DNA Flex Kit. Run performed on a MiSeq System (2 × 76 bp).

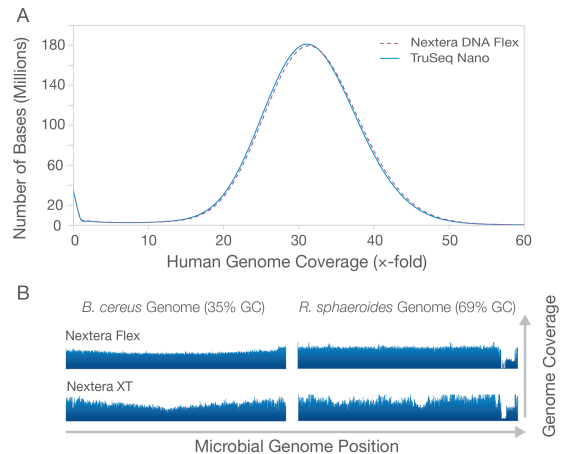


Figure 5: Nextera DNA Flex Improves Coverage Uniformity— (A) The Nextera DNA Flex Kit delivers uniform coverage across the genome comparable to the TruSeq Nano DNA Kit. Libraries produced with Human-NA12878 samples (Coriell Institute) using the Nextera DNA Flex or TruSeq Nano Kits. Sequencing performed on a HiSeq X™ System (2 × 151 bp). (B) Coverage is shown for microorganisms with extremely high or low GC content. Due to improved on-bead library prep chemistry, Nextera DNA Flex shows more even coverage than Nextera XT. Libraries were prepared with Nextera XT or Nextera DNA Flex Kits. Data was generated on a HiSeq™ 2500 System (Rapid Run v2, 2 × 151 bp).

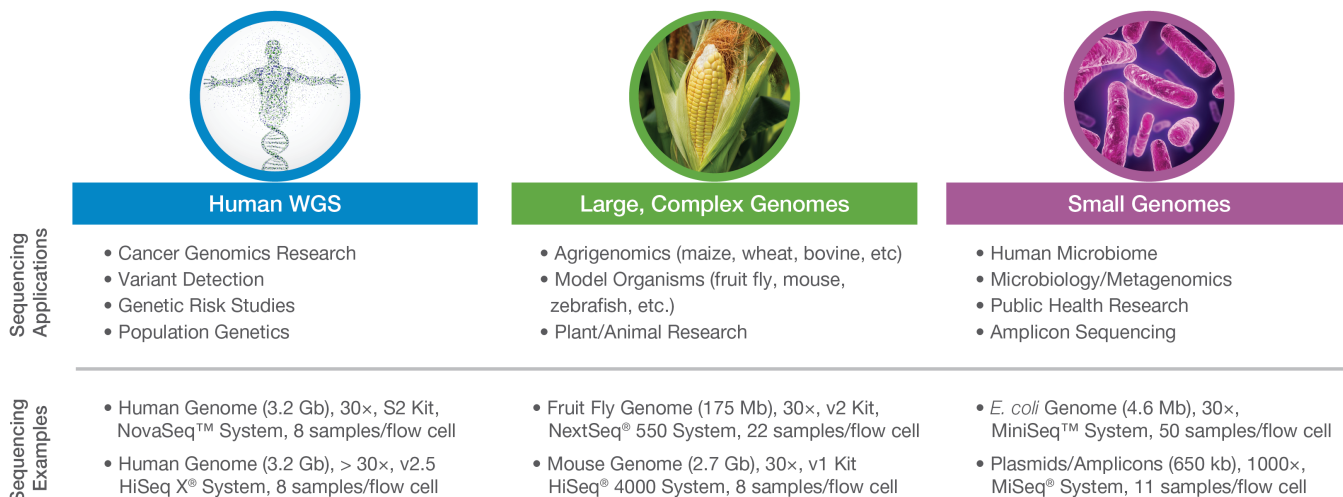


Figure 6: Broad Range of Applications with Nextera DNA Flex—The Nextera DNA Flex Library Preparation Kit can be used to support a broad range of applications. From human WGS and large/complex genomes to small microbial genomes, the Nextera DNA Flex Library Preparation Kit provides experimental flexibility.

Flexible Workflow Enables a Broad Range of Applications

Perhaps the greatest advantage of the Nextera DNA Flex Library Preparation Kit is the flexibility it provides for a broad range of research interests and applications. The kit supports human WGS, cancer genomics research, environmental metagenomics, infectious disease research, agrigenomics, and more (Figure 6). 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 Library Preparation Kit delivers comprehensive genomic coverage. The flexible, user-friendly workflow is adaptable for users of various experience levels, multiple applications, and multiple sample input types.

Summary

The Nextera DNA Flex Library Preparation Kit features a revolutionary workflow that combines DNA extraction, quantitation, fragmentation, and library normalization to deliver the fastest and most flexible library prep workflow in the Illumina portfolio. The user-friendly, automation-compatible workflow 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, and more. See how the innovative Nextera DNA Flex Library Prep workflow combined with the power of Illumina SBS chemistry can advance and accelerate your research goals today.

Ordering Information

Product	Catalog No.
Nextera DNA Flex Library Prep Kit (24 samples)	20018704
Nextera DNA Flex Library Prep Kit (96 samples)	20018705
Flex Lysis Reagent Kit	20018706
Nextera DNA CD Indexes (24 indexes, 24 samples)	20018707
Nextera DNA CD Indexes (96 indexes, 96 samples)	20018708

CD Indexes: Combinatorial Dual Indexes. 24 dual indexes provided to support up to 24 samples or 96 dual indexes provided to support up to 96 samples.
Single Indexes: 24 single indexes provided to support up to 96 samples.

Learn More

To learn more about the Nextera DNA Flex Library Prep Kit, visit www.illumina.com/nextera-dna-flex.

For more on human WGS with the Nextera DNA Flex Library Prep Kit, read the [Human WGS with Nextera DNA Flex Application Note](#).

For more on microbial genome sequencing with the Nextera DNA Flex Library Prep Kit read the [Microbial WGS with Nextera DNA Flex Application Note](#).

References

1. Illumina (2016). [Nextera DNA Library Preparation Kit Data Sheet](#). Accessed July 10 2017.
2. Illumina (2014). [Nextera XT DNA Library Preparation Kit Data Sheet](#). Accessed July 10 2017.
3. Bentley DR, Balasubramanian S, Swerdlow HP, et al. [Accurate whole human genome sequencing using reversible terminator chemistry](#). *Nature*. 2008;456:53-59.