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Human Whole-Genome Sequencing with the Nextera™ DNA Flex Library Preparation Kit

Fast and flexible library prep from blood, saliva, or genomic DNA that provides uniform coverage for human whole-genome sequencing (WGS).

Highlights

- **Fast and Simple Workflow**
  Reduce library preparation time with a low number of steps and minimal hands-on time
- **Optimized Library Prep**
  Obtain consistent results from DNA, blood, or saliva input, without the need for normalization
- **Integrated Sample Input**
  Increase workflow efficiency and data consistency with integrated DNA extraction for multiple sample types
- **High-Quality Data**
  Produce sequencing data with minimal bias comparable to mechanical fragmentation methods

Figure 1: Human WGS with the Nextera DNA Flex Library Prep Kit—The Nextera DNA Flex Library Preparation Kit delivers even coverage to produce reliable results for human WGS applications.

Introduction

The most comprehensive and unbiased method of interrogating the 3.2 billion bases of the human genome is WGS.[1,2] The rapid drop in sequencing costs and the ability of WGS to produce large volumes of data quickly make it a powerful tool for human genomics research. However, many laboratories continue to experience bottlenecks during the library preparation phase of the next-generation sequencing (NGS) workflow. This slowdown is primarily caused by multiple steps required both before and after library preparation. Pre-library preparation steps include DNA extraction, quantitation, and fragmentation, while post-library preparation steps include library quality assessments, library quantitation, and normalization.

The Nextera DNA Flex Library Preparation Kit represents the latest evolution in Nextera library prep. Featuring unique chemistry that integrates multiple pre- and post-library preparation steps, the Nextera DNA Flex Library Preparation Kit delivers the fastest workflow with the fewest steps in the Illumina library prep portfolio (Figure 2). In addition to speed and efficiency gains, it offers exceptional flexibility for sample input type, amount, and a wide range of supported applications, including human WGS (Figure 1).

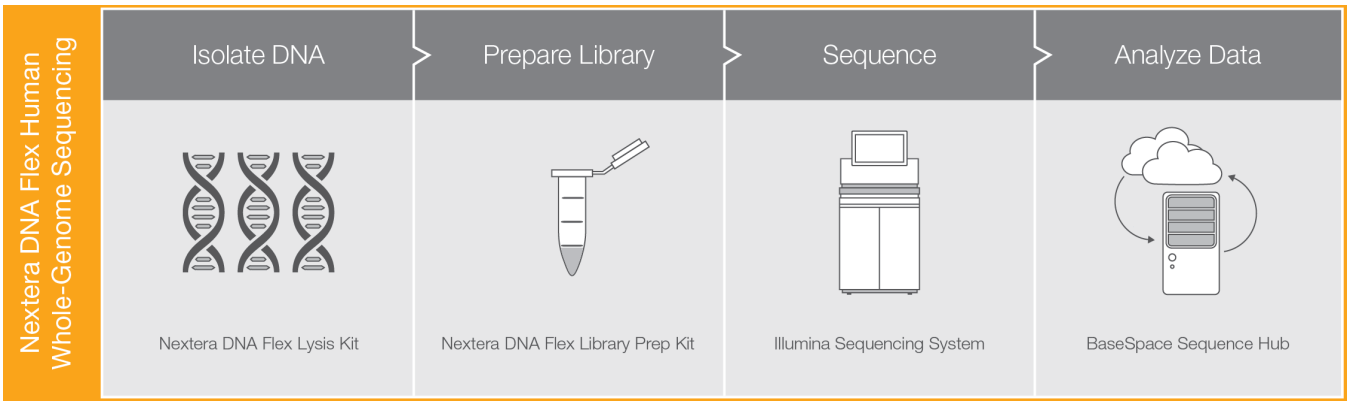

Figure 2: Nextera DNA Flex Library Prep Workflow—User-friendly Nextera DNA Flex library preparation is part of a streamlined workflow that includes integrated blood and saliva processing, sequencing, and data analysis.

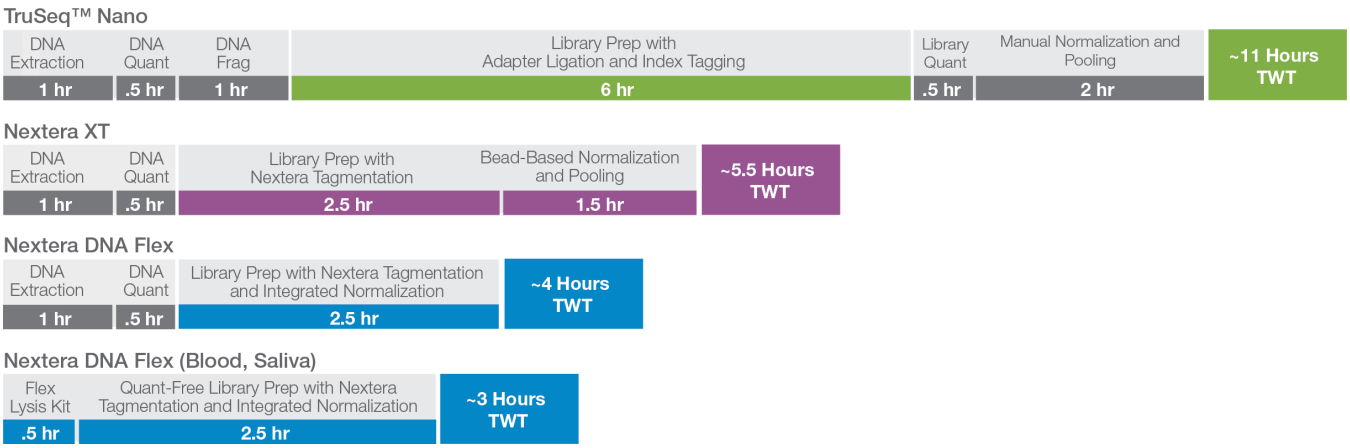

Figure 3: Nextera DNA Flex Delivers the Fastest Workflow—Calculations made assuming 16 samples were processed at a time with a multichannel pipette. TWT= total workflow time from DNA extraction to library normalization and pooling. Workflow step times calculated assuming specific methods: DNA extraction (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.

Compatible with all Illumina sequencing systems, the Nextera DNA Flex Library Preparation Kit delivers even genome coverage with the proven accuracy of Illumina sequencing by synthesis (SBS) chemistry, used to generate more than 90% of the world's sequencing data.* As part of an integrated NGS workflow that includes library prep, sequencing, and simplified data analysis in BaseSpace™ Sequence Hub (Figure 2), the Nextera DNA Flex Library Preparation Kit delivers reliable results for human WGS applications.

Optimized Library Prep

A major advance of the Nextera DNA Flex Library Preparation Kit is On-Bead Tagmentation, which uses bead-linked transposomes (BLTs) to mediate simultaneous DNA fragmentation and the tagging of Illumina sequencing primers (Figure 4).

On-Bead Tagmentation provides several significant advantages:

- Eliminates the need for accurate quantitation of the initial DNA sample, across a wide DNA input range (100–500 ng), saving time and costs associated with DNA quantitation and normalization reagents, kits, and equipment.
- Eliminates the need for separate DNA fragmentation steps, saving time and costs associated with separate shearing instruments or enzymatic kits.
- Eliminates the need for individual library quantitation and normalization, across a wide DNA input range (100–500 ng), before pooling and sequencing.

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 workflow advances combine to make Nextera DNA Flex the fastest workflow with the fewest number of steps in the Illumina library preparation portfolio (Figure 3).

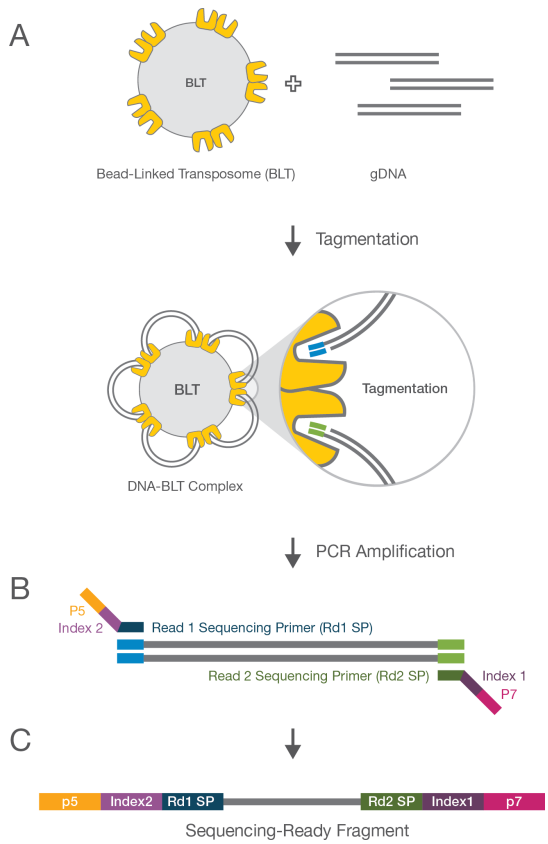

Figure 4: Nextera On-Bead Tagmentation Chemistry—(A) BLTs mediate tagmentation. (B) Reduced-cycle PCR amplifies sequencing ready DNA fragments and adds indexes and adapters. (C) Sequencing-ready fragments are washed and pooled.

*Data calculations on file. Illumina, Inc., 2015.

Integrated Sample Input

With Nextera DNA Flex Lysis Kits, DNA extraction can be processed directly from fresh blood or saliva samples, resulting in time and cost savings while improving data consistency. Nextera DNA Flex Lysis Kits have been optimized and validated for the Nextera DNA Flex Library Preparation Kit. 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.

To demonstrate the optimized performance of Nextera DNA Flex library prep, eight samples of human blood and saliva were collected in duplicate, stored at 4°C, and processed within 24 hours of collection. Quality assessment with the Fragment Analyzer shows the consistent size and concentration of the prepared libraries (Figure 5 and Table 1).

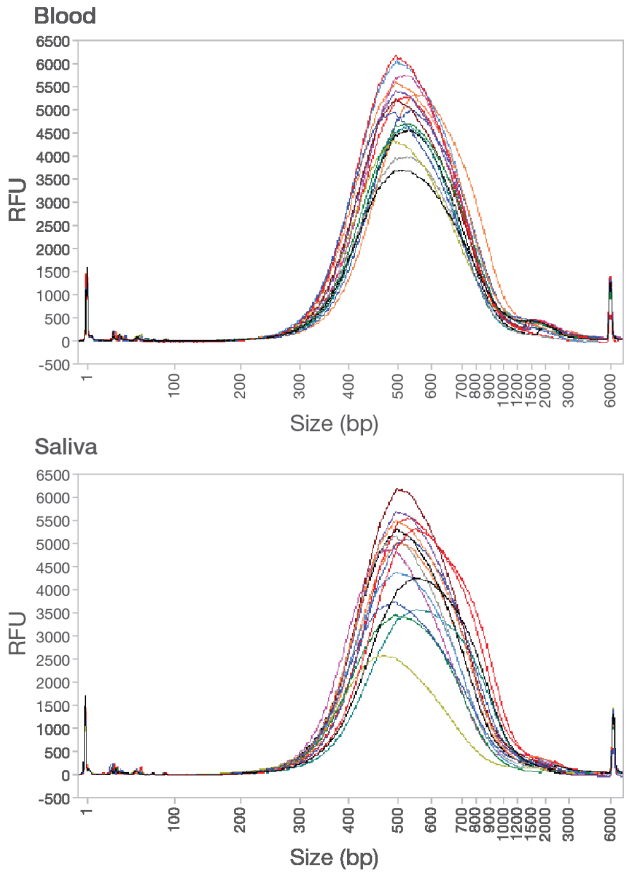

Figure 5: Quality Assessment of Nextera DNA Flex Libraries—Fragment Analyzer traces of prepared libaries from whole blood and saliva collected from eight individuals in duplicate (16 total samples).

Table 1: Quantitation and Library Fragment Size for Human Blood and Saliva Samples

	Blood	Saliva
Average Yield (16 samples)[a]	9.99 ng/µl	8.96 ng/µl
Fragment Size	324	317

a. Quantitation performed with Qubit dsDNA HS Kit.

High Quality Data

The Nextera DNA Flex Library Preparation Kit minimizes bias and provides uniform coverage across the human genome (Figure 6), and delivers exceptional coverage of challenging regions (Figure 7), at levels comparable to the TruSeq Nano DNA LIbrary Preparation Kit. The Nextera DNA Flex Library Preparation Kit produces results comparable to mechanical DNA fragmentation methods, such as those used in the TruSeq Nano DNA Library Preparation Kit (Table 2). Further assessment of data quality by various sequencing run metrics demonstrates the performance of the Nextera DNA Flex Library Preparation Kit in generating high-quality libraries from multiple, varying sample types that deliver exceptional data quality across Illumina sequencing systems (Table 3).

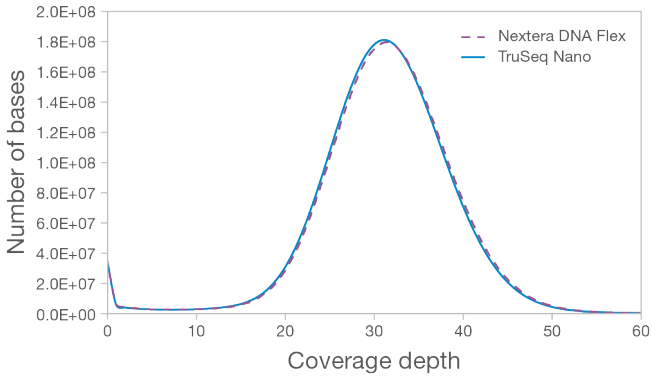

Figure 6: Human Whole-Genome Coverage Uniformity—The Nextera DNA Flex Library Preparation Kit delivers uniform coverage across the genome comparable to the TruSeq Nano DNA Library Prep Kit.

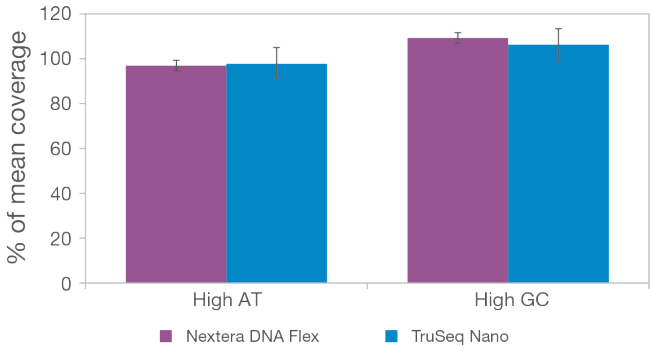

Figure 7: Excellent Coverage of Challenging Regions—The Nextera DNA Flex Library Preparation Kit delivers exceptional coverage of challenging genomic content, including regions of high AT and high GC content, at levels comparable to the TruSeq Nano DNA Library Prep Kit. High AT and High GC are defined as 100 bases with ≥ 75% AT or GC content, respectively.

Table 2: Comparison of Nextera DNA Flex and TruSeq Nano DNA Library Prep Kit Performance

Parameter[a]	Nextera DNA Flex Library Prep Kit	TruSeq Nano DNA Library Prep Kit
No. of Samples	20	20
No. of Runs	5	4
Total No. of Paired-End Reads PF	3.7×10^8	3.7×10^8
Percent Autosome Callability	96.5%	96.9%
Percent Exon Callability	98.4%	98.4%
Percent Autosome > 10×	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. Data presented is an average of 20 samples run with the respective library prep kit.

Abbreviations: PF, passing filter; SNV, single nucleotide variant; Indel, insertion/deletion.

Table 3: Nextera DNA Flex Performance[a] by Sample Type

	HiSeq X™ gDNA	NovaSeq™ gDNA	NovaSeq Blood	NovaSeq Saliva
Autosome Callability[b]	94.95	95.28	95.40	95.49
Autosome Exome Callability[b]	97.33	98.20	98.00	98.20
Autosome Coverage at 15×	95.12	95.12	95.30	95.38
Exon Coverage at 15×	98.20	98.86	98.56	98.77
Mean Coverage	29.95	29.92	30.12	30.79
Insert Size (bp)	338	308	310	311
Reads Passing Filter	732,982,146	764,427,030	754,207,938	931,648,654

a. Data analysis performed using the BaseSpace Sequence Hub Whole Genome Sequencing v5.0 App.
b. The percent of non-N reference positions with a "PASS" genotype call. Callability describes the percentage of base calls in the data set that pass the quality metrics required for making a genotype call. Base quality, alignment quality, and minimum coverage levels are taken into account.

Abbreviations: gDNA, genomic DNA; bp, base pairs.

Summary

The Nextera DNA Flex Library Preparation Kit features an innovative workflow that combines DNA extraction, quantitation, fragmentation, and library normalization to deliver the fastest workflow with the fewest number of steps in the Illumina library prep portfolio. On-Bead Tagmentation chemistry enables support for a wide range of DNA input amounts, various sample types, and a broad range of applications. The Nextera DNA Flex Library Preparation Kit delivers sequencing results equivalent to mechanical fragmentation methods with minimal bias across the genome. The simple, user-friendly workflow and optimized performance make the Nextera DNA Flex Library Preparation Kit an ideal solution for human WGS.

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

Learn More

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

References

1. Wang K, Yuen ST, Xu J, et al. Whole-genome sequencing and comprehensive molecular profiling identify new driver mutations in gastric cancer. *Nat Genet*. 2014;46(6):573–582.
2. Zahir FR, Mwenifumbo JC, Chun HE, et al. Comprehensive whole genome sequence analyses yields novel genetic and structural insights for Intellectual Disability. *BMC Genomics*. 2017;18(1):403.

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