DATA SHEET

Illumina DNA Prep

A fast, integrated library prep workflow for a wide range of sequencing applications

- Prepare libraries in < 3 hours with minimal hands-on touch points
- Enhance library preparation efficiency with integrated DNA extraction protocols for blood
- Support a broad DNA input range (1-500 ng) and multiple DNA input types
- Access a wide range of applications with the ability to sequence large and small genomes and amplicons

illumina®

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 a sequencing run. Prelibrary preparation steps include DNA extraction, quantitation, and fragmentation, while post-library prep steps include library quality assessments, library quantitation, and normalization.

The Nextera™ DNA Library Preparation Kit 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. The Nextera XT DNA Library Prep Kit eliminated the need for library quantitation before library pooling and sequencing.[1] Building on these innovations, the Illumina DNA Prep Kit* offers a unique chemistry (Figure 1, Table 1) that integrates the DNA extraction, fragmentation, library preparation, and library normalization steps to deliver the fastest, most flexible workflow in the Illumina library prep portfolio (Figure 2, Table 2).

Beyond providing a rapid workflow, the Illumina DNA Prep 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 Illumina DNA Prep Kit delivers even genome coverage and exceptional data quality.

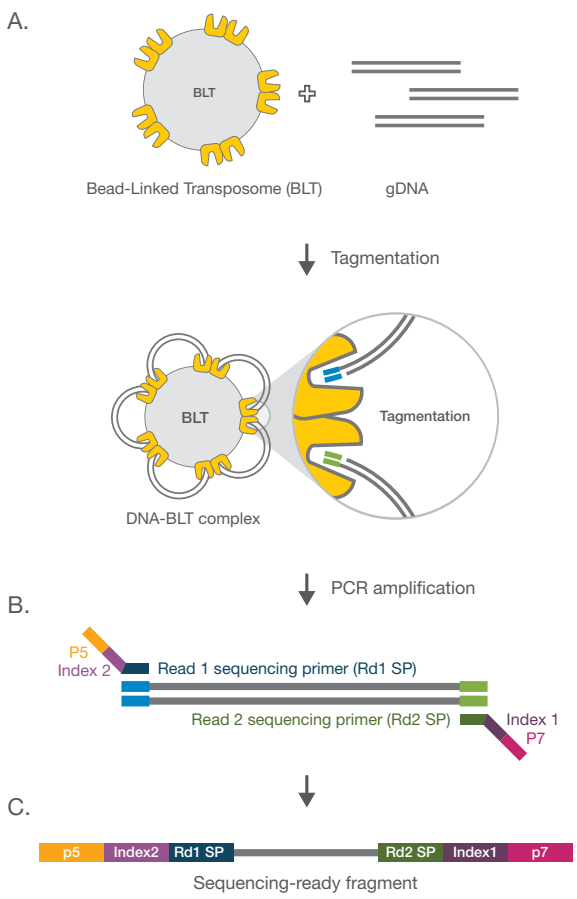

Figure 1: Illumina bead-linked transposome chemistry—(A) Bead-linked transposomes mediate the simultaneous fragmentation of gDNA and the addition of sequencing primers. (B) Reduced-cycle PCR amplifies sequencing-ready DNA fragments and adds indexes and adapters. (C) Sequencing-ready fragments are washed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

Fast library preparation workflow

The Illumina DNA Prep Kit combines several features to deliver the fastest library preparation workflow in the Illumina portfolio. One major advance 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.

Table 1: Illumina DNA Prep specifications

Parameter	Specification
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, 384 dual indexes
Supported sequencing systems	All Illumina systems
Total workflow time[a] (gDNA)	3-4 hr

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

* Formerly Nextera DNA Flex Library Preparation Kit.

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 reduces the number of hands-on steps and supports liquid-handling systems for library prep automation. These advances produce a workflow with the least number of steps and the fastest total workflow time in the Illumina portfolio (Figure 2).

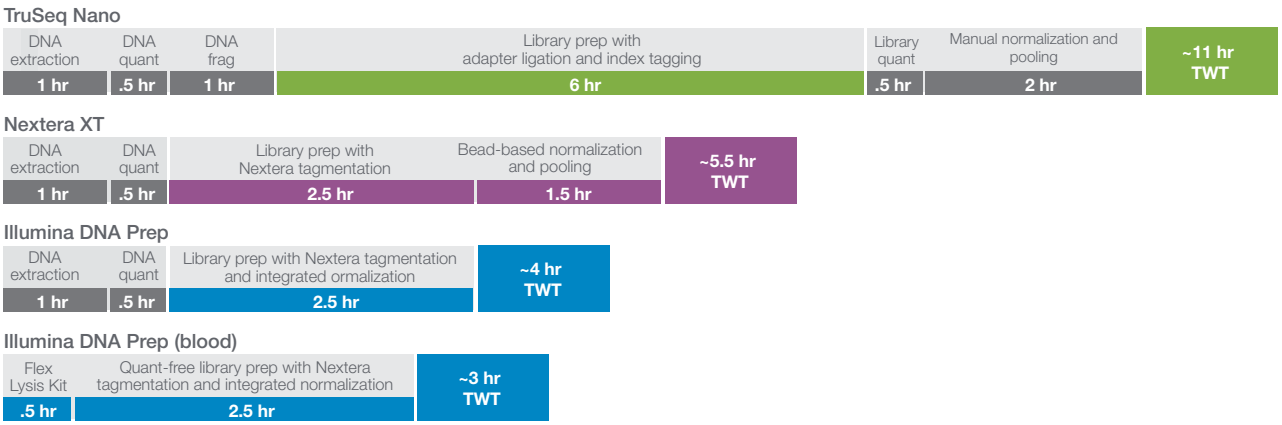

Figure 2: Illumina DNA Prep delivers the fastest Illumina workflow—Calculations based on processing 16 samples at a time with a multichannel pipette. TWT, total workflow time from DNA extraction to library normalization and pooling. Workflow step times calculated assumed specific methods: DNA extraction (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 library prep workflows

	TruSeq DNA Nano	Nextera XT	Illumina DNA Prep[a,b]
Integrated DNA lysis included	—	—	✓
Flexible, broad DNA input range	—	—	✓
Library normalization included	—	✓	✓
DNA input	100-200 ng	1 ng	1-500 ng
Total library prep time[c]	11 hr	5 hr	3-4 hr
Insert size	350 bp or 550 bp	< 300 bp	300-350 bp
Sample multiplexing	96 dual indexes	384 dual indexes	24 single indexes; 384 dual indexes

a. Integrated DNA extraction protocols available for blood and DBS samples.
b. Library normalization occurs with ≥ 100 ng DNA input.
c. Includes DNA extraction, library preparation, and library normalization/pooling steps.

Integrated DNA input

With Illumina DNA Prep Kits and Flex Lysis Reagent Kits, DNA extraction can be processed directly from fresh blood samples. The optional Flex Lysis Reagent Kits are optimized and validated for use with Illumina DNA Prep 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 Illumina DNA Prep tagmentation reaction.

Optimized performance

The properties of on-bead tagmentation have enabled major improvements in library preparation performance. The Illumina DNA Prep Kit produces highly uniform and consistent insert sizes (300-350 bp), across a wide DNA input range (1-500 ng) (Figure 3). On-bead tagmentation enables generation of uniform insert sizes across a broad input range, eliminating the need for careful transposome:DNA ratio optimization as a means of controlling fragment length. 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 and eliminating the need for time-consuming library quantitation and normalization steps before pooling. In a comparison of Illumina DNA Prep and TruSeq™ DNA Nano Library Prep Kit performance, the Illumina DNA Prep 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 5A). Even genomes with high- or low-GC content show remarkably even coverage without region-specific bias (Figure 5B).

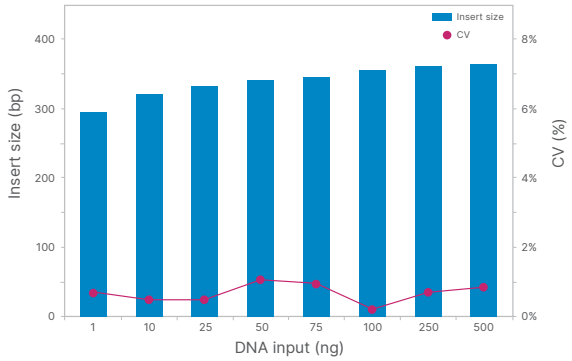

Figure 3: Uniform and consistent insert sizes—On-bead tagmentation delivers consistent insert sizes regardless of DNA input amount. From 1-500 ng DNA input, the total coefficient of variance (CV) is 6.09%. Libraries produced with *E. coli* replicate samples and run on a MiSeq™ System (2 × 76 bp).

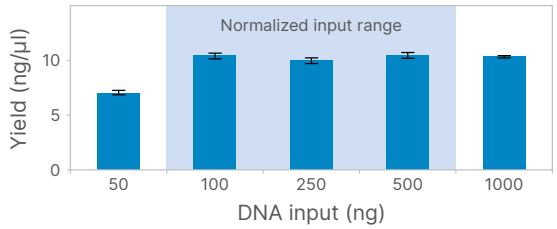

Figure 4: Tagmented and normalized libraries—Beads become saturated at or over 100 ng, leading to normalized yield of tagmented DNA, eliminating the need for downstream library normalization steps. Libraries produced with Human-NA12878 samples (Coriell Institute) and run on a MiSeq System (2 × 76 bp).

Table 3: Illumina DNA Prep performance

Parameter[a]	Illumina DNA Prep	TruSeq Nano
Paired-end reads PF	3.7×10^8	3.7×10^8
Autosome callability	96.5%	96.9%
Autosome exon callability	98.4%	98.4%
Autosome coverage > 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. Analysis was run on 20 samples (all NA12878 Corriel samples) spread across 5 runs to approximate 30× 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.

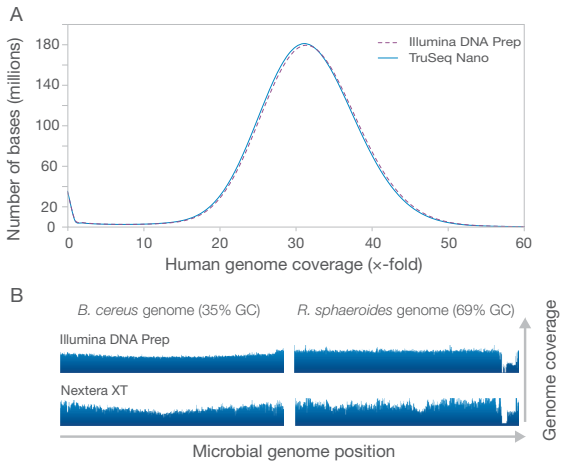

Figure 5: Improved coverage uniformity—(A) Illumina DNA Prep delivers uniform coverage across the genome comparable to the TruSeq DNA Nano kit. Libraries produced with Human-NA12878 samples (Coriell Institute) using the Illumina DNA Prep or TruSeq DNA Nano kits. Sequencing performed on a HiSeq X™ System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

Flexible workflow enables a broad range of applications

Perhaps the greatest advantage of Illumina DNA Prep 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, Illumina DNA Prep delivers comprehensive genomic coverage. The flexible, user-friendly workflow is adaptable for users of various experience levels, multiple applications, and multiple sample input types.

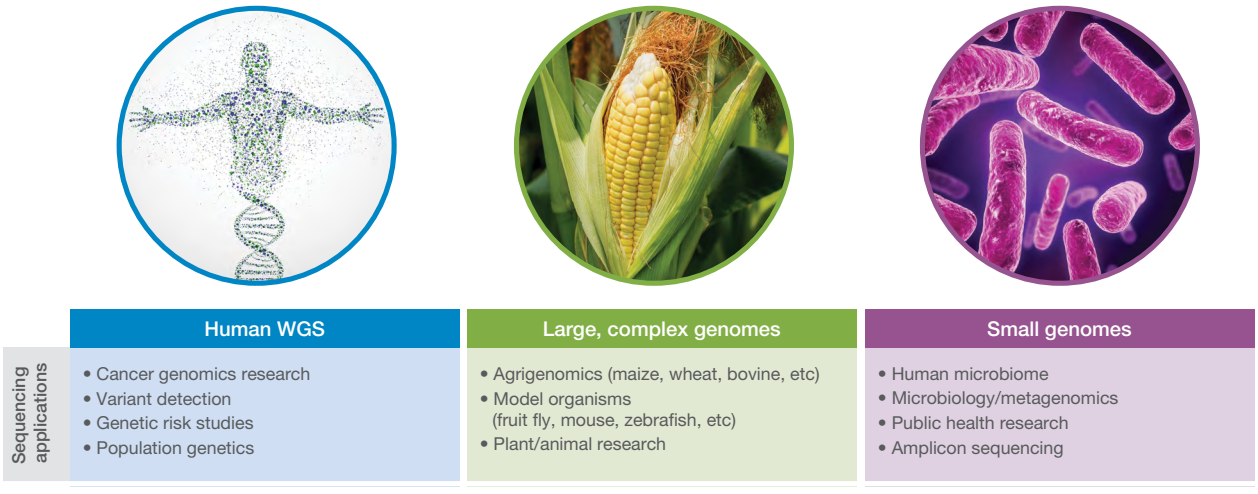

Figure 6: Broad range of applications with Illumina DNA Prep—From human WGS and large/complex genomes to small microbial genomes, Illumina DNA Prep provides experimental flexibility.

Summary

The Illumina DNA Prep Kit features a streamlined 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 supports 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 Illumina DNA Prep workflow combined with the power of Illumina SBS chemistry can advance and accelerate your research goals today.

Learn more

Illumina DNA Prep, illumina.com/products/by-brand/nextera.html

Ordering information

Product	Catalog no.
Illumina DNA Prep, (M) Tagmentation (24 samples)	20018704
Illumina DNA Prep, (M) Tagmentation (96 samples)	20018705
Flex Lysis Reagent Kit	20018706
IDT® for Illumina DNA/RNA UD Indexes Set A, Tagmentation (96 indexes, 96 samples)	20027213
IDT for Illumina DNA/RNA UD Indexes Set B, Tagmentation (96 indexes, 96 samples)	20027214
IDT for Illumina Nextera DNA UD Indexes Set C (96 indexes, 96 samples)	20027215
IDT for Illumina Nextera DNA UD Indexes Set D (96 indexes, 96 samples)	20027216
IDT for Illumina Nextera DNA UD Indexes Set A-D (384 indexes, 384 samples)	20027217
IDT for Illumina DNA/RNA UD Indexes Set C, Tagmentation (96 indexes, 96 samples)	20042666
IDT for Illumina DNA/RNA UD Indexes Set D, Tagmentation (96 indexes, 96 samples)	20042667
Nextera DNA CD Indexes (24 indexes, 24 samples)	20018707
Nextera DNA CD Indexes (96 indexes, 96 samples)	20018708

"IDT for Illumina DNA/RNA UD Indexes" are new names for "IDT for Illumina Nextera DNA UD Indexes"; kit contents remain the same.

Reference

1. Illumina (2014). Nextera XT DNA Library Preparation Kit Data Sheet. Accessed April 14, 2020.

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