APPLICATION NOTE

Human WGS with Illumina DNA Prep

- Reduce library preparation time with a low number of steps and minimal hands-on time
- Obtain consistent results from DNA, blood, or saliva, without the need for normalization
- Increase workflow efficiency and data consistency with integrated DNA extraction
- Produce sequencing data with minimal bias comparable to mechanical fragmentation methods

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Introduction

The most comprehensive and unbiased method of interrogating the 3.2 billion bases of the human genome is whole-genome sequencing (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.

Illumina DNA Prep represents the latest evolution in library preparation, offering exceptional flexibility for sample input type, amount, and a wide range of supported applications, including human WGS (Figure 1). Compatible with all Illumina sequencing systems, Illumina DNA Prep provides even genome coverage with the proven accuracy of Illumina sequencing by synthesis (SBS) chemistry. As part of comprehensive NGS workflow that includes library prep, sequencing, and simplified data analysis using DRAGEN™ apps in BaseSpace™ Sequence Hub (Figure 2), Illumina DNA Prep delivers reliable results for human WGS applications.

Figure 1: Human WGS with Illumina DNA Prep—Delivering even coverage to produce reliable results for human WGS applications.

Furthermore, the user-friendly workflow reduces the number of hands-on steps and to support liquid-handling systems for library prep automation. These workflow advances combine to make Illumina DNA Prep the fastest workflow with the fewest number of steps in the Illumina library preparation portfolio (Figure 3).

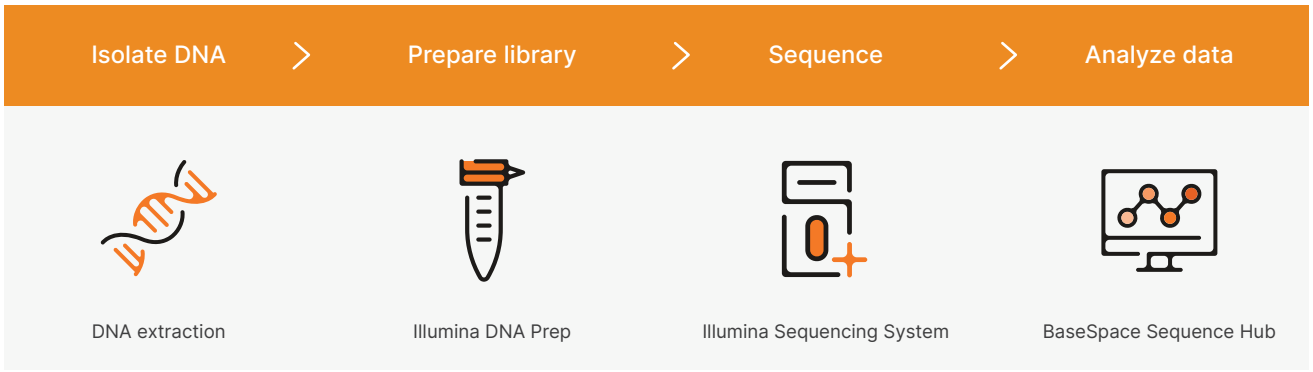

Figure 2: Illumina DNA Prep workflow.

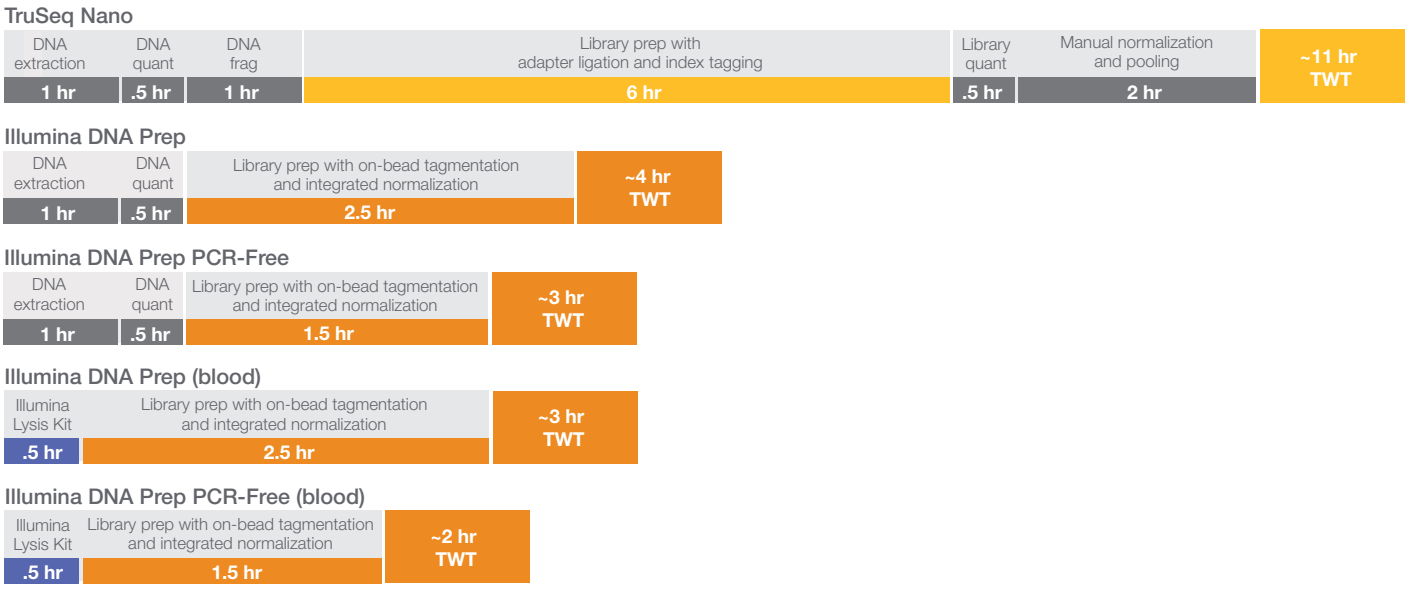

Figure 3: Illumina DNA Prep delivers the fastest Illumina library preparation workflow—Calculations based on processing 16 samples at a time with a multichannel pipette. TWT indicates total workflow time (TWT) from DNA extraction to library normalization and pooling. Calculations assumed specific methods: DNA extraction (QIAamp DNA Mini Kit or Illumina Lysis Kit), DNA quantitation (Qubit), DNA fragmentation (Covaris), and manual library normalization and pooling (Bioanalyzer System). 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.

Optimized library prep

A major advance of the Illumina DNA Prep 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 quantitation of the initial DNA sample saving time and costs associated with DNA quantitation and normalization reagents, kits, and equipment
- Eliminates the need for DNA fragmentation, saving time and costs associated with separate shearing instruments or enzymatic kits
- Eliminates the need for individual library quantitation and normalization before pooling and sequencing

Integrated sample input

With the Illumina Lysis Kit, DNA extraction can be processed directly from fresh blood samples, resulting in time and cost savings while improving data consistency. Illumina Lysis Kits have been optimized and validated for Illumina DNA Prep. 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. Note that saliva samples can be processed directly from the recommended collection tubes using Illumina Purification Beads without the need for additional lysis reagent kits.

To demonstrate the optimized performance of Illumina DNA 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 (Table 1 and Figure 5).

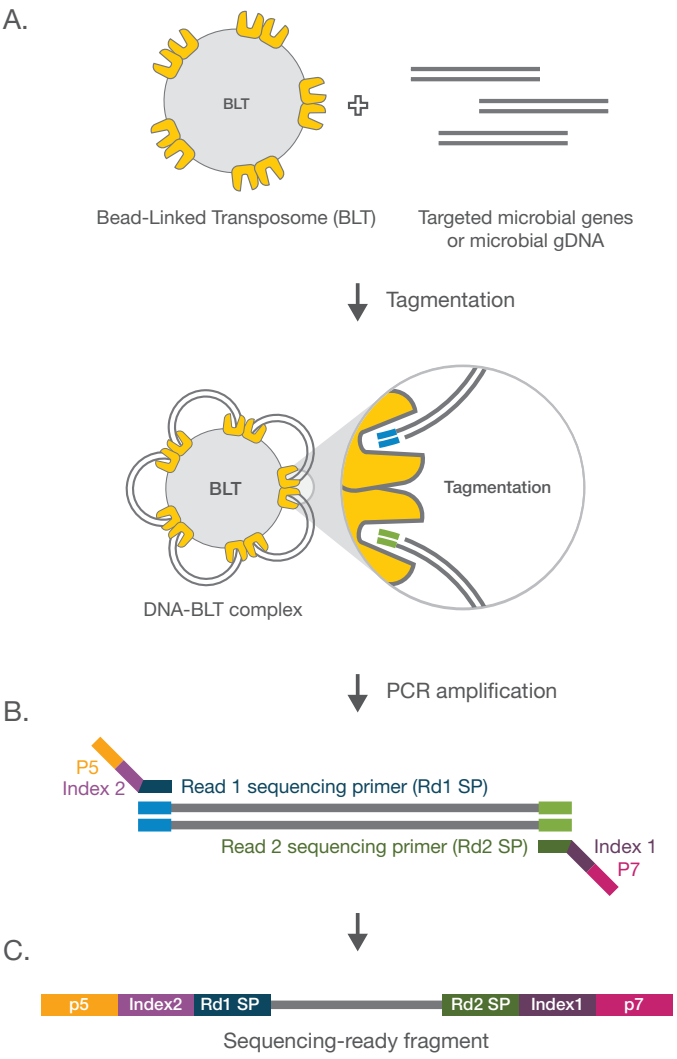

Figure 4: Illumina bead-linked transposome chemistry—(A) Bead-linked transposomes mediate the simultaneous fragmentation of gDNA and addition of sequencing primers. (B) Reduced-cycle PCR amplifies sequencing-ready DNA fragments and adds indexes and adapters. (C) Sequencing-ready fragments are washed and pooled.

Table 1: Quantitation and library fragment size for human blood and saliva samples

Parameter	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.

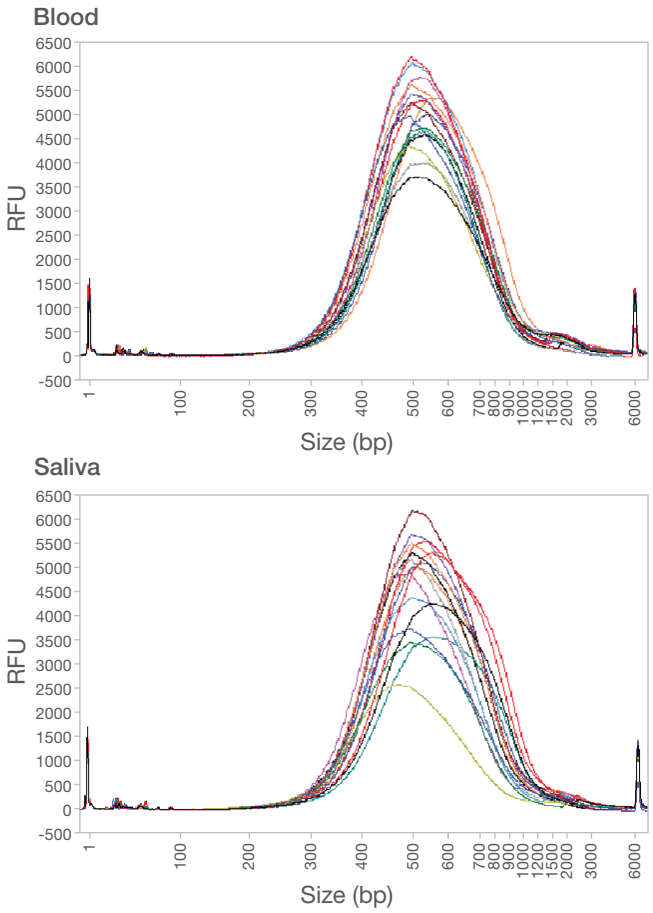

Figure 5: Quality assessment of Illumina DNA Prep libraries—Fragment Analyzer traces of prepared libaries from whole blood and saliva collected from eight individuals in duplicate (16 total samples).

High-quality data

Illumina DNA Prep minimizes bias, 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. Results generated with Illumina DNA Prep are comparable to those obtained with mechanical DNA fragmentation methods, as 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 Illumina DNA Prep in generating high-quality libraries from multiple, varying sample types that deliver exceptional data quality across Illumina sequencing systems (Table 3).

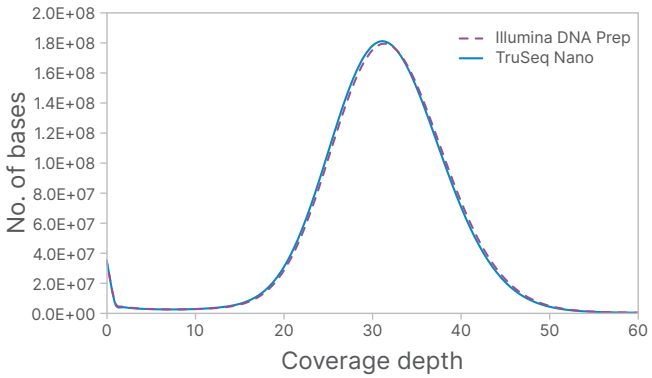

Figure 6: Human whole-genome coverage uniformity—Illumina DNA Prep delivers uniform coverage across the genome comparable to the TruSeq Nano DNA Library Prep Kit.

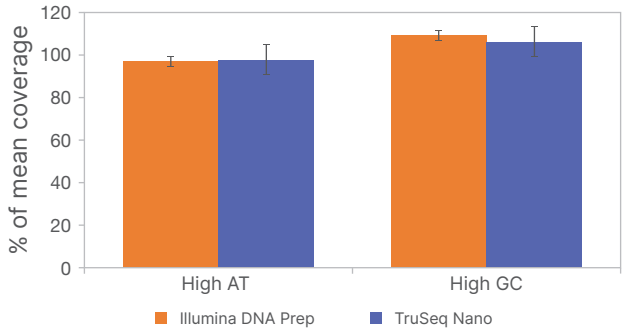

Figure 7: Illumina DNA Prep delivers excellent coverage of challenging regions—Includes coverage of regions of high AT and high GC content. High AT and High GC are defined as 100 bases with ≥ 75% AT or GC content, respectively.

Table 2: Comparison of Illumina DNA Prep and TruSeq Nano performance

Parameter[a]	Illumina DNA Prep	TruSeq Nano
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.
PF, passing filter; SNV, single nucleotide variant; Indel, insertion/deletion.

Table 3: Illumina DNA Prep performance[a] by sample type

Parameter	HiSeq™ X gDNA	NovaSeq™ 6000 gDNA	NovaSeq 6000 blood	NovaSeq 6000 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.
gDNA, genomic DNA; bp, base pairs.

Summary

Illumina DNA Prep 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. Illumina DNA Prep delivers sequencing results equivalent to mechanical fragmentation methods with minimal bias across the genome. The simple, user-friendly workflow and optimized performance make Illumina DNA Prep an ideal solution for human WGS.

Ordering information

Product	Catalog no.
Illumina DNA Prep, (M) Tagmentation (24 Samples, IPB)	20060060
Illumina DNA Prep, (M) Tagmentation (96 Samples, IPB)	20060059
Illumina Lysis Reagent Kit	20042221
Illumina DNA/RNA UD Indexes Set A, Tagmentation (96 Indexes, 96 Samples)	20091654
Illumina DNA/RNA UD Indexes Set B, Tagmentation (96 Indexes, 96 Samples)	20091656
Illumina DNA/RNA UD Indexes Set C, Tagmentation (96 Indexes, 96 Samples)	20091658
Illumina DNA/RNA UD Indexes Set D, Tagmentation (96 Indexes, 96 Samples)	20091660

Learn more

Illumina DNA Prep

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. doi:10.1038/ng.2983
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. Published 2017 May 24. doi:10.1186/s12864-017-3671-0

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M-GL-02084 v1.0