Illumina DNA PCR-Free Prep, Tagmentation

High performance for sensitive applications such as human whole-genome sequencing.

Highlights

- **Highly accurate, whole-genome coverage**
  Results in fewer gaps in coverage, even in genomic regions with high-GC or high-AT content
- **Simplified workflow with reduced overall time**
  Supports easy volume-based library pooling and minimizes pre- and post-library quantification steps
- **Highly compatible with automation**
  Integrates with liquid-handling robotics to automate workflows for minimal touch points and significant time savings
- **Excellent performance with low DNA sample input**
  Delivers accurate base calling and variant identification for a broad range of DNA input amounts

Introduction

Next-generation sequencing (NGS) has revolutionized the way researchers perform genomic studies by dramatically increasing the amount and quality of data that can be generated per run and reducing cost and time to answer. While Illumina sequencing technology has advanced rapidly in recent years, PCR-dependent library preparation protocols still present significant challenges. PCR bias can lead to uneven coverage across regions of the genome, especially regions with extremely uneven base composition. To address this challenge, Illumina DNA PCR-Free Prep, Tagmentation (Illumina DNA PCR-Free) offers a unique combination of On-Bead Tagmentation with a PCR-free workflow (Figure 1).

How it works

Tagmentation is a transposome-mediated reaction that combines tagging and DNA fragmentation into a single, rapid reaction. On-Bead Tagmentation uses bead-linked transposomes to perform a more uniform tagmentation reaction compared to in-solution tagmentation. After the bead-linked transposomes are saturated with DNA, no additional tagmentation can occur, delivering consistent library yield and uniform library insert sizes.[1,2] Furthermore, by removing PCR amplification steps, Illumina DNA PCR-Free chemistry eliminates PCR-induced bias and provides highly accurate sequence information for sensitive applications such as tumor–normal variant identification or human whole-genome sequencing (WGS). The Illumina DNA PCR-Free assay can be completed in 90 minutes from extracted genomic DNA (gDNA) or just 3 hours from raw samples such as blood or saliva (Table 1).

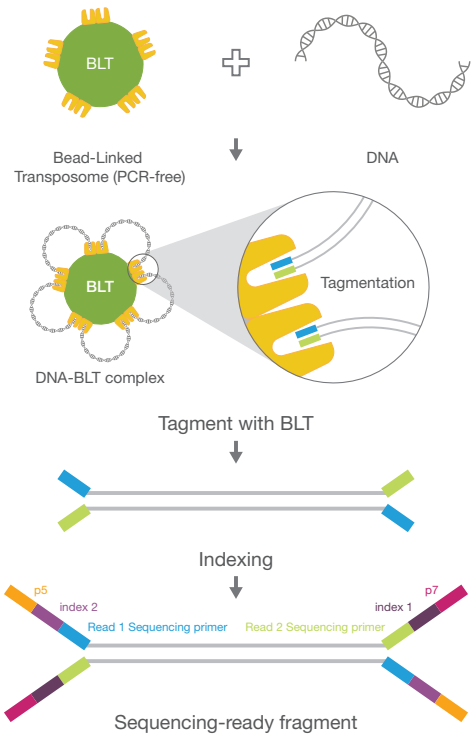

Figure 1: Illumina DNA PCR-Free chemistry—An efficient solution for preparing and indexing sample libraries.

Table 1: Illumina DNA PCR-Free specifications

Parameter	Illumina DNA PCR-Free	TruSeq DNA PCR-Free
DNA input type	gDNA, blood, saliva, plasmids, dried blood spots	gDNA
DNA input amount	25 ng to 1 µg gDNA	1-2 µg
Fragmentation method	On-Bead Tagmentation	Covaris sonication
Sample multiplexing	384 dual indexes	96 dual indexes
Supported sequencing systems	MiniSeq™, MiSeq™, NextSeq™ 550, NextSeq 1000, NextSeq 2000, HiSeq 3000/4000, NovaSeq 6000 Systems	All Illumina sequencing systems
Total workflow time[a]	~90 minutes extracted gDNA ~3 hours raw blood or saliva	~11 hours
Insert size	350 bp, 450 bp, or 550 bp	350 bp or 550 bp

a. Total workflow time includes DNA extraction and quantitation (or Flex Lysis), tagmentation, and library pooling steps

Highly uniform whole-genome coverage for human WGS

Coverage uniformity measures data comprehensiveness across the genome for a sequencing run. Uniform coverage enables more accurate calling of variants that are distant from the mean depth.[3] To assess coverage performance across a range of low, medium, and high GC content, normalized coverage data from Illumina DNA PCR-Free and TruSeq™ DNA PCR-Free were plotted against human genome content by GC percentage. The bulk of human genome data are comprised of 20-70% GC sequence. Both kits show even coverage levels across a range of GC content as represented by human WGS data (Figure 2), indicating that Illumina DNA PCR-Free is exceptionally well suited for human WGS applications.

Even coverage across high-GC or -AT regions

Due to structural elements in human genome transcription, human gene promotor regions are frequently GC-rich or GC-poor and can be difficult to amplify with PCR.[4] Therefore, human WGS libraries prepared with kits that exclude PCR may show improved coverage in certain GC-rich promotor regions. To compare coverage performance of Illumina DNA PCR-Free, TruSeq DNA PCR-Free, and TruSeq DNA Nano (includes PCR), libraries were prepared from human cell line NA12878 gDNA (Coriell Institute). All libraries were sequenced on a HiSeq™ X System with a run configuration of 2 × 150 bp. Data were down sampled to 32×-40× coverage. Compared to the TruSeq DNA Nano data, both Illumina DNA PCR-Free and TruSeq DNA PCR-Free data sets show superior coverage across a high-GC gap region in the human *RNPEPL1* gene (Figure 3). Using Illumina DNA PCR-Free improves coverage across challenging regions.

Excellent performance across a range of DNA input amounts

Illumina DNA PCR-Free was evaluated for performance across a range of DNA input amounts. Libraries were prepared from human cell line DNA (Coriell Institute, NA12878) using 600 ng and 20-200 ng* input amounts with TruSeq DNA PCR-Free and Illumina DNA PCR-Free, respectively. Libraries were sequenced on a NovaSeq™ 6000 System with a run configuration of 2 × 150 bp and down sampled to a mean coverage of 40×. Quality scores, base calling, and variant calling metrics were compared. Data from each library type exceed the 75% > Q30 quality specification for the NovaSeq 6000 System (Figure 4a). The data sets also show equivalent base calling performance within both autosomes and exons, and equivalent variant calling (Figure 4b). Data quality, base calling performance, and variant calling across all DNA inputs, including the low input of 20 ng,* were also equivalent.

On-Bead Tagmentation and PCR-free protocol

Illumina DNA PCR-Free provides a unique and powerful combination of benefits from On-Bead Tagmentation and PCR-free chemistry. The on-bead saturation point of Illumina DNA PCR-Free is ≥ 300 ng. On-bead saturation enables robust insert size control and normalized yields from DNA input amounts above 300 ng. This minimizes quantification steps both before and after library prep. Normalized libraries can be pooled by volume, avoiding time-consuming quantification of individual libraries. By eliminating quantification and

* The recommended input range for Illumina DNA PCR-Free is 25–300 ng.

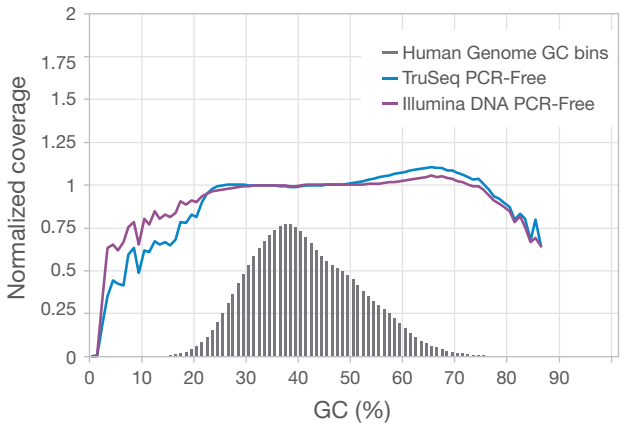

Figure 2: Illumina DNA PCR-Free coverage uniformity—Illumina DNA PCR-Free provides uniform coverage across a range of GC content in the human genome.

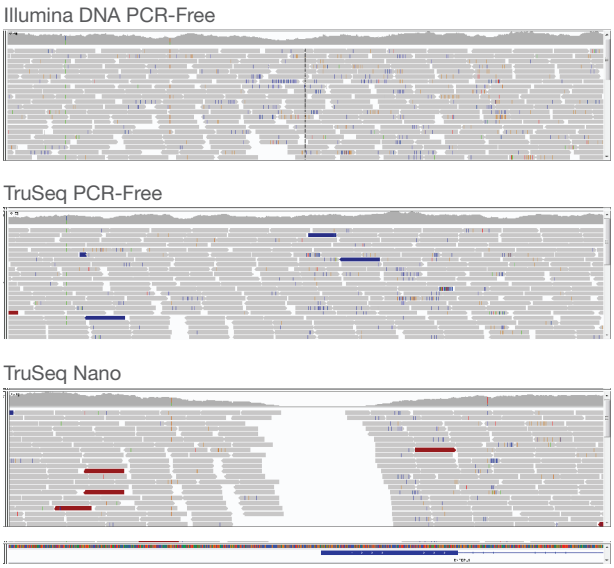

Figure 3: Comparison of read coverage across GC-rich regions—The Illumina DNA PCR-Free LIbrary Prep Kit provides superior read coverage across the GC-rich promoter region of the human *RNPEPL1* gene, as compared to TruSeq DNA PCR-Free and TruSeq DNA Nano Library Prep Kits. Read maps were visualized with the Integrative Genomics Viewer (IGV) App, available in BaseSpace™ Sequence Hub.

PCR steps, Illumina DNA PCR-Free offers a streamlined, 90-minute assay (Figure 5). Although normalization is achieved with inputs ≥ 150 ng, viable and high-performing libraries can be generated with as little as 20 ng* input DNA. The ability to run PCR-free library preps from low DNA inputs enables new applications such as WGS from dried blood spots.

Efficient sample multiplexing for high-throughput applications

Illumina DNA PCR-Free is compatible with IDT for Illumina DNA Unique Dual Indexes, which enable accurate sample demultiplexing on Illumina sequencing systems. Up to 384 indexes provide maximum flexibility for high-throughput sequencing projects.

Figure 4: Illumina DNA PCR-Free performance across a range of DNA inputs—Illumina DNA PCR-Free libraries prepared from a range of DNA inputs demonstrate (A) passing quality specifications for all DNA inputs and (B) equivalent callability performance. Q30 score = an inferred base call accuracy of 99.9%, autosome callability = the percentage of non-N reference positions in autosomal chromosomes with a passing genotype call, exon callability = the percentage of non-N reference positions in exons with a passing genotype call, SNPs = single nucleotide polymorphisms, indel = insertion-deletion mutation, precision (accuracy) = calculated as the ratio of [# of True Positive Calls/(# of True Positive Calls + # of False Positive Calls)], recall (sensitivity) = calculated as the ratio of [# of True Positive Calls/(# of True Positive Calls + # of False Negative Calls)]. Note: the lower limit sample input specifications for Illumina DNA PCR-Free have not been finalized.

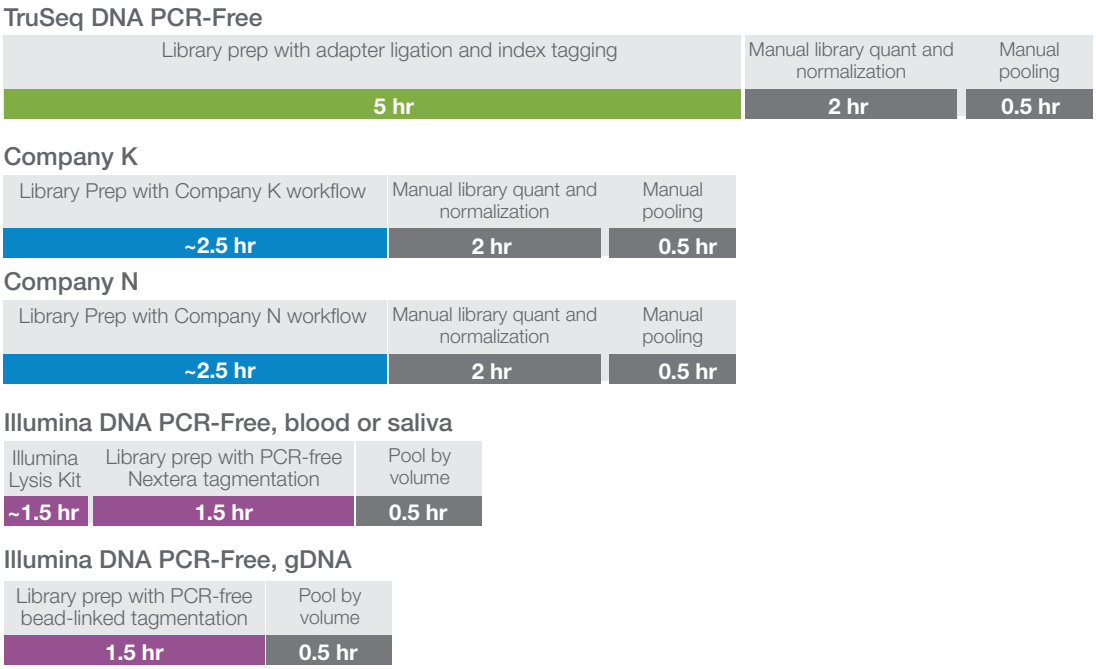

Figure 5: Illumina DNA PCR-Free workflow—The Illumina DNA PCR-Free workflow delivers a rapid total assay time of 90 minutes from fragmentation or tagmentation through library clean-up. Data on file, Illumina Inc., 2019. Note: Company N uses proprietary reagents combined with Illumina forked adapters.

Table 2: Automation consumables for 96 samples

Method	Sample type	Touch points	96 sample plates	Tips	Time
TruSeq DNA PCR-Free	gDNA	20	20	5504	10 hr 10 min
Company K	gDNA	13	19	4076	6 hr 21 min
Company N	gDNA	13	17	3266	5 hr 42 min
Illumina DNA PCR-Free (+ optional qPCR quantitation of pools)	blood, saliva	2 (6)	10 (12)	2016 (2072)	2 hr 32 min (4 hr 7 min)
Illumina DNA PCR-Free (+ optional qPCR quantitation of pools)	gDNA	2 (6)	8 (10)	1604 (1660)	1 hr 32 min (3 hr 7 min)

Modeled using Hamilton software for Hamilton Star with 96 core head + 8-channel. qPCR is included in automation modeling for all workflows on a sample by sample basis). Workflows other than Illumina DNA PCR-Free assume each sample is qPCR measured, adjusted, and pooled. Sample pooling is based on 4 pools of 24 samples. Data on file, Illumina Inc., 2019. Note: Company N uses proprietary reagents combined with Illumina forked adapters.

Automation-compatible workflows

Illumina DNA PCR-Free is highly compatible with automation due to the fast and simplified workflow. Because of the consistent and self-normalizing nature of the bead-based workflow, users can begin with raw blood or saliva samples, run the Flex Lysis protocol, and proceed to library prep without any quantification steps. These features enable an easy workflow for automated raw sample batch processing on liquid-handling platforms.

To demonstrate compatibility, automated workflows for TruSeq DNA PCR-Free and two competitor enzyme-based PCR-free workflows were compared to Illumina DNA PCR-Free. Touch points, labware, tip count, and time required for library preparation of 96 sample batches on a Hamilton liquid-handling robot were calculated for each workflow and showed Illumina DNA PCR-Free offers significant time savings (Table 2).

Reduced costs with Illumina DNA PCR-Free

Labware, tips, and qPCR reagents contribute to hidden costs when preparing libraries for NGS. A key advantage of bead-based technology is the automatic, bead-based normalization of all libraries prepared in a batch, which eliminates the need for individual library quantification, and allows simple library pooling by equal volume.

As PCR-free libraries are usually quantified by qPCR, Illumina DNA PCR-Free eliminates or dramatically reduces the amount of qPCR involved in the overall library preparation protocol (eg, PCR library amplification and post-library prep quantification). A model of hidden costs, including qPCR reagents, labware, tips, quantification reagents, and third-party extraction kits, reveals that the Illumina DNA PCR-Free workflow offers substantial savings.[5] For example, the hidden costs can account for ~56% of total costs for the TruSeq PCR-Free workflow, or ~44% for competitor enzyme-based PCR-free kits.† For the Illumina DNA PCR-Free workflow, hidden costs are just ~21%, which is a substantial reduction compared to other library preparation kits.†

† Library prep kit costs are matched for this calculation. Hidden costs are variable and calculated as a proportion of the total cost based on workflow assumptions (Table 1).

Summary

Illumina DNA PCR-Free offers a unique combination of benefits from On-Bead Tagmentation and PCR-free chemistry steps. On-Bead Tagmentation supports bead-based normalization, easy volume-based library pooling, and elimination of pre- and post-library quantification steps. The PCR-free workflow simplifies and reduces the overall workflow time while providing highly uniform coverage across repetitive or uneven genome regions. With the integrated Flex Lysis Reagent Kit, the workflow is compatible with blood, saliva, and dried blood spots as raw sample inputs. For sensitive applications such as human WGS, *de novo* assembly of microbial genomes, or tumor–normal variant calling, Illumina DNA PCR-Free delivers exceptional ease-of-use, uniform coverage, and high-accuracy data.

Learn more

To learn more, visit www.illumina.com/products/by-type/sequencing-kits/library-prep-kits/dna-pcr-free-prep.html

Ordering information

Product	Catalog no.
Illumina DNA PCR-Free Library Prep Kit (24 samples)	20041794
Illumina DNA PCR-Free Library Prep Kit (96 samples)	20041795
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 DNA/RNA UD Indexes Set C, Tagmentation (96 indexes, 96 samples)	20042666 Coming soon
IDT for Illumina DNA/RNA UD Indexes Set D, Tagmentation (96 indexes, 96 samples)	20042667 Coming soon
Illumina DNA PCR-Free Primer Kit R1 Sequencing	20041796
Illumina Lysis Reagent Kit	20042221

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

References

1. Illumina (2018). Nextera DNA Flex Library Preparation Kit. Accessed April 10, 2020.
2. Bruinsma S, Burgess J, Schlingman D, Czyz A, Morrell N, et al. Bead-linked transposomes enable a normalization-free workflow for NGS library preparation. *BMC Genomics*. 2018;19(1):722.
3. Illumina (2013). Comparison of TruSeq Sample Preparation Kits Technical Note. Accessed May 10, 2020.
4. Bajic VB, Choudhary V, Hock CK. Content analysis of the core promoter region of human genes. *In Silico Biol*. 2004;4(2):109-25.
5. Data calculations on file. Illumina, Inc., 2019.

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