DATA SHEET

Illumina DNA PCR-Free Prep, Tagmentation

A high-performing, fast, integrated workflow for whole-genome sequencing applications

- Optimized library prep performance generates highly accurate and reliable results
- Flexible protocol accommodates a broad range of sample types for sensitive sequencing applications
- Fast, automation-compatible workflow of ~1.5 hours total time with low input DNA requirements

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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 the genome, especially in 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).

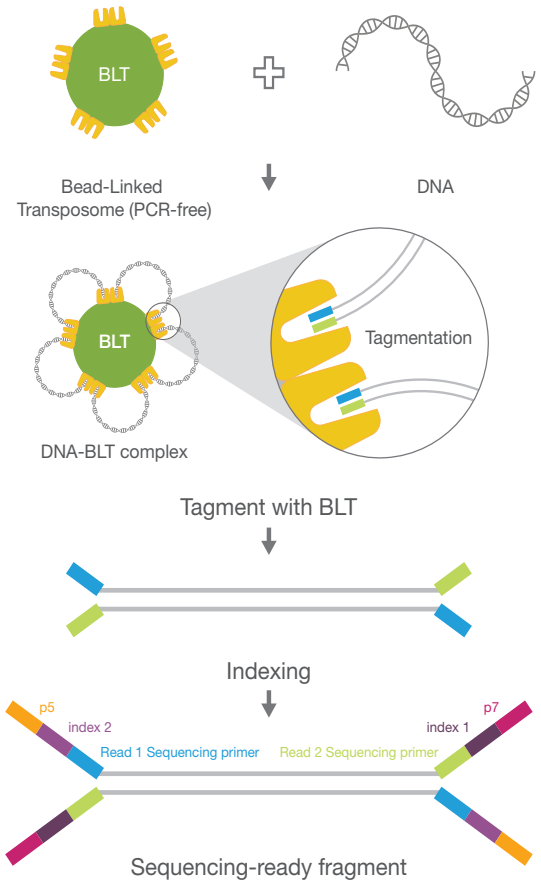

Figure 1: Illumina DNA PCR-Free chemistry—An efficient solution for preparing and indexing sample libraries.

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 2.5 hours from raw samples such as blood or saliva (Table 1).

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 300 ng[a]	1 to 2 µg
Fragmentation method	On-Bead Tagmentation	Covaris sonication
Sample multiplexing	384 dual indexes[b]	96 dual indexes
Supported sequencing systems	MiniSeq, MiSeq, NextSeq 550, NextSeq 1000, NextSeq 2000, NovaSeq 6000	All Illumina sequencing systems
Total workflow time[c]	~90 min[d] extracted gDNA ~2.5 hr raw blood or saliva	~11 hours
Insert size[e]	450 bp	350 bp or 550 bp

a. Maximum input amount for Illumina DNA PCR-Free is 2 µg
b. For index correction strategies to mitigate variability across multiplexed libraries, see Balancing sample coverage for whole-genome sequencing
c. Total workflow time includes DNA extraction and quantification, tagmentation, and library pooling steps.
d. Workflow time for saturating input gDNA (300 ng)
e. For more information on adjusting insert sizes to 350 bp or 550 bp, see Tunable insert sizes with Illumina DNA PCR-Free Prep, Tagmentation

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 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 wide 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.

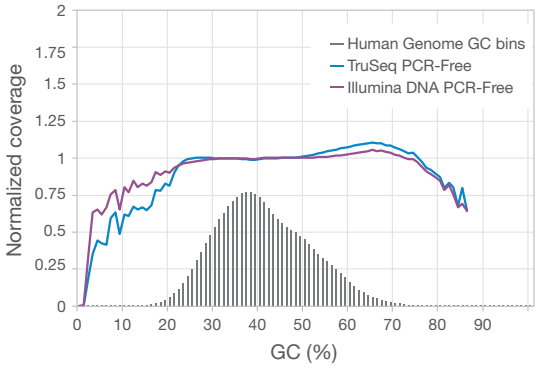

Figure 2: Illumina DNA PCR-Free coverage uniformity—Illumina DNA PCR-Free provides uniform coverage across a range of GC content in the human genome.

Even coverage across high-GC or -AT regions

Due to structural elements in human genome transcription, human gene promoter regions are frequently GC-rich or GC-poor and can be difficult to amplify with PCR.[4] Human WGS libraries prepared with kits that exclude PCR may show improved coverage in certain GC-rich promoter 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™ 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.

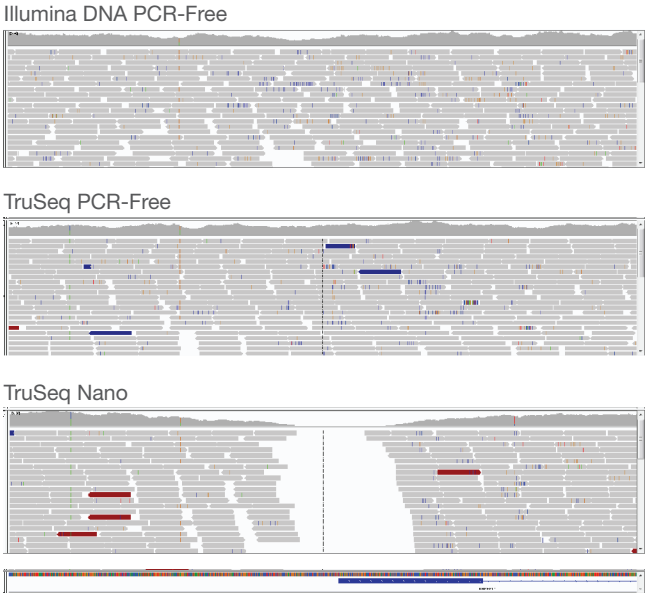

Figure 3: Comparison of read coverage across GC-rich regions—Illumina DNA PCR-Free provides superior read coverage across the GC-rich promoter region of the human *RNPEPL1* gene, compared to TruSeq DNA PCR-Free and TruSeq DNA Nano Library Prep Kits. Read maps were visualized with the Integrative Genomic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

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 are highly accurate, with more than 85% of bases scoring Q30 or above on 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 of gDNA. On-bead saturation enables robust insert size control and normalized yields from DNA input amounts above 300 ng. This protocol 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 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.

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, you can begin with raw blood or saliva samples, run the Illumina 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. These comparisons showed that Illumina DNA PCR-Free offers significant time savings (Table 2).

Reduced costs with Illumina DNA PCR-Free

Labware, tips, and qPCR reagents contribute to additional 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. This self-normalization eliminates the need for individual library quantification, and allows simple library pooling by equal volume. For strategies to correct for index-specific variation in performance across multiplexed libraries, refer to our technical note on Balancing sample coverage for whole-genome sequencing.

* Maximum input amount for Illumina DNA PCR-Free is 2 µg

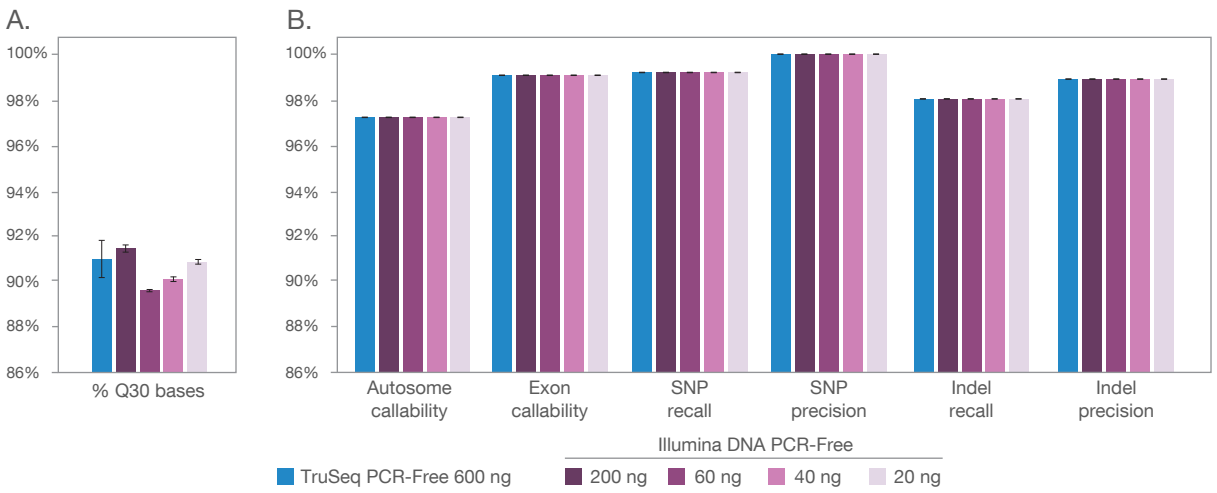

Figure 4: Illumina DNA PCR-Free Prep performance across a range of DNA inputs—Illumina DNA PCR-Free libraries prepared from a range of DNA inputs demonstrate (A) passing quality specifications for all DNA inputs and (B) equivalent callability performance. Q30 score, an inferred base call accuracy of 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 [No. of true positive calls/(No. of true positive calls + No. of false positive calls)]; recall (sensitivity), calculated as the ratio of [No. of true positive calls/(No. of true positive calls + No. of false negative calls)].

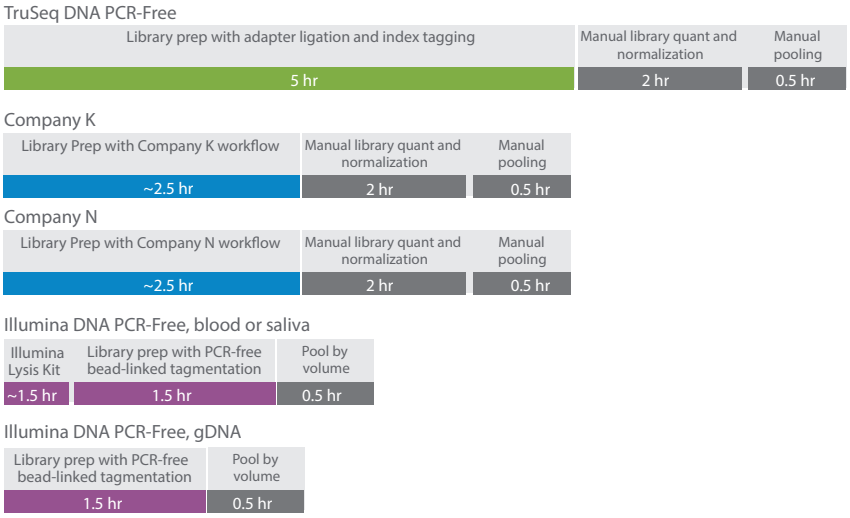

Figure 5: Illumina DNA PCR-Free workflow—The Illumina DNA PCR-Free workflow delivers a rapid total assay time of 90 minutes from fragmentation or tagmentation through library clean-up. Data on file, Illumina Inc., 2019. Note: Company N uses proprietary reagents combined with Illumina forked adapters.

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 quantification of pools)	blood, saliva	2 (6)	10 (12)	2016 (2072)	2 hr 32 min (4 hr 7 min)
Illumina DNA PCR-Free (+ optional qPCR quantification 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 liquid handling system. qPCR is included in automation modeling for all workflows on a sample-by-sample basis. Workflows other than Illumina DNA PCR-Free assume that each sample is qPCR measured, adjusted, and pooled. Sample pooling is based on four pools of 24 samples. Data on file, Illumina Inc., 2019. Note: Company N uses proprietary reagents combined with Illumina forked adapters.

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 additional costs, including qPCR reagents, labware, quantification reagents, tips, and third-party extraction kits, reveals that the Illumina DNA PCR-Free workflow offers substantial savings.[5] For example, additional 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, additional costs are just ~21%, which is a substantial reduction compared to other library preparation kits.†

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

Illumina DNA PCR-Free, illumina.com/products/by-type/sequencing-kits/library-prep-kits/dna-pcr-free-prep

† Library prep kit costs are matched for this calculation. Additional costs are variable and calculated as a proportion of the total cost based on workflow assumptions (Table 2).

Ordering information

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

References

1. Illumina. Illumina DNA Prep. illumina.com/content/dam/illumina-marketing/documents/products/datasheets/illumina-dna-prep-data-sheet-770-2020-009.pdf Published 2020. Accessed February 7, 2022.
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. doi: 10.1186 /s12864-018-5096-9.
3. Illumina. Comparison of TruSeq Sample Preparation Kits. illumina.com/content/dam/illumina-support/documents/products/technotes/technote_truseq_comparison.pdf Published 2013. Accessed January 31, 2022.
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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