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TruSeq™ DNA PCR-Free

Simple, streamlined whole-genome sequencing library preparation that provides accurate and comprehensive coverage of complex genomes.

Highlights

- **Streamlined Library Preparation**
  PCR-free protocol accelerates the TruSeq DNA library preparation workflow
- **Excellent Coverage Quality**
  Very low library bias and fewer gaps in coverage enable deep insight into the genome
- **High Flexibility**
  Optimized PCR-free workflows support various read lengths and applications
- **Inclusive Solution**
  Reliable solution includes master-mixed reagents, size-selection beads, and up to 96 unique dual indexes (UDIs)

Figure 1: TruSeq DNA PCR-Free—TruSeq DNA PCR-Free offers an efficient solution for preparing and indexing sample libraries. TruSeq DNA PCR-Free accommodates up to 24 indexes for low-throughput studies, or up to either 96 dual indexes or 96 unique dual indexes (sold separately) for high-throughput studies.

Introduction

TruSeq DNA PCR-Free offers numerous enhancements to the widely adopted TruSeq DNA workflow, providing optimized library preparation for whole-genome sequencing (WGS) applications. By eliminating PCR amplification steps, the PCR-free protocol significantly reduces typical PCR-induced bias and provides detailed sequence information for traditionally challenging regions of the genome. Low-throughput and high-throughput protocols are available to accommodate a range of study designs (Figure 1).

Accelerated Library Preparation

The TruSeq DNA library preparation workflow has been streamlined by removing the PCR amplification step and replacing gel-based size selection with bead-based selection (Figure 2). This workflow offers exceptional flexibility with two protocol options for generating either large (550 bp) or small (350 bp) insert sizes to support various applications. Master-mixed reagents, provided sample purification beads for clean up and size selection, robust TruSeq indexes, and optimized protocols contribute to the simplified library preparation workflow, requiring a low number of cleanup steps for processing large sample numbers. TruSeq DNA PCR-Free decreases library preparation time, enabling researchers to perform applications from microbial sequencing to human WGS.[2]

Innovative Library Preparation Chemistry

TruSeq DNA PCR-Free can be used to prepare DNA libraries for single-read, paired-end, and indexed sequencing. TruSeq DNA PCR-Free supports shearing by Covaris ultrasonication, requiring 1 µg of input DNA for an average insert size of 350 bp or 2 µg DNA for an average insert size of 550 bp. Library construction begins with fragmented genomic DNA (gDNA) (Figure 2A). Blunt-end DNA fragments are generated using a combination of fill-in reactions and exonuclease activity (Figure 2B), and size selection is performed with provided sample purification beads (Figure 2C). An A-base is then added to the blunt ends of each strand, preparing them for ligation to the indexed adapters (Figure 2D). Each adapter contains a T-base overhang for ligating the adapter to the A-tailed fragmented DNA. These adapters contain the full complement of sequencing primer hybridization sites for single, paired-end, and indexed reads. With no need for additional PCR amplification, single or dual-index adapters are ligated to the fragments and products are ready for cluster generation (Figure 2E).

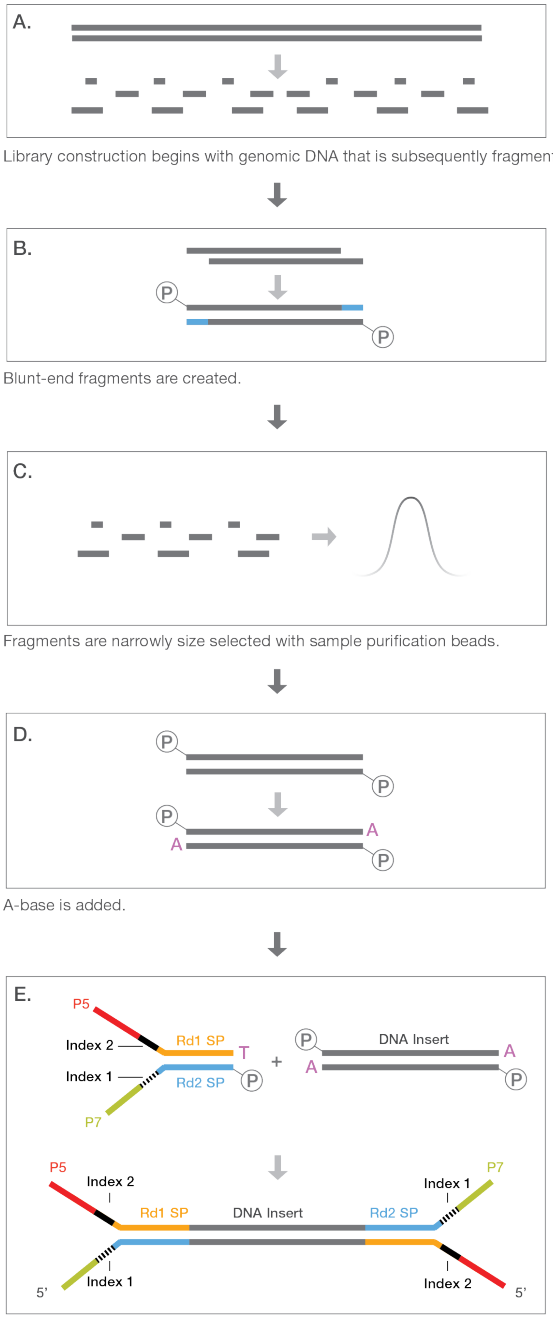

Figure 2: TruSeq DNA PCR-Free Workflow—The TruSeq DNA PCR-Free workflow features adapter ligation resulting in sequence-ready products without PCR amplification. *The TruSeq DNA PCR-Free LT indexing solution features a single-index adapter at Step E.

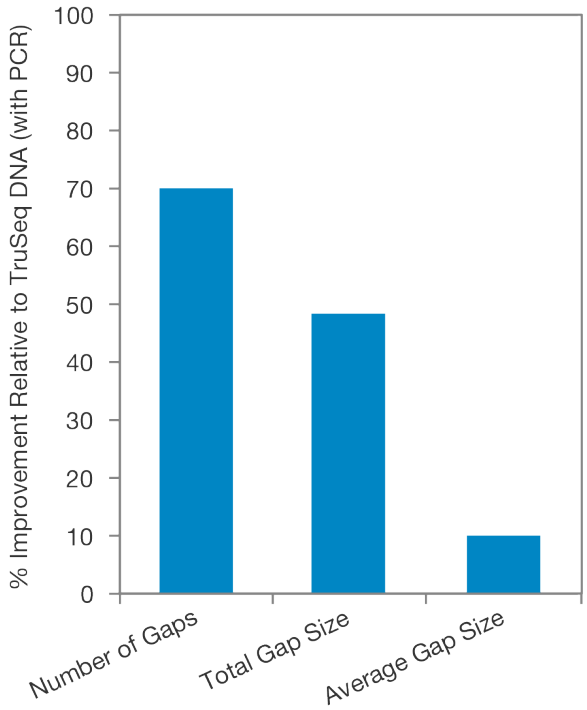

Figure 3: Fewer Gaps in Coverage—TruSeq DNA PCR-Free libraries show significant reduction in the number and total size of gaps when compared to libraries prepared using the TruSeq DNA (with PCR) protocol. A gap is defined as a region ≥ 10 bp in length, where an accurate genotype cannot be determined due to low depth, low alignment scores, or low base quality.

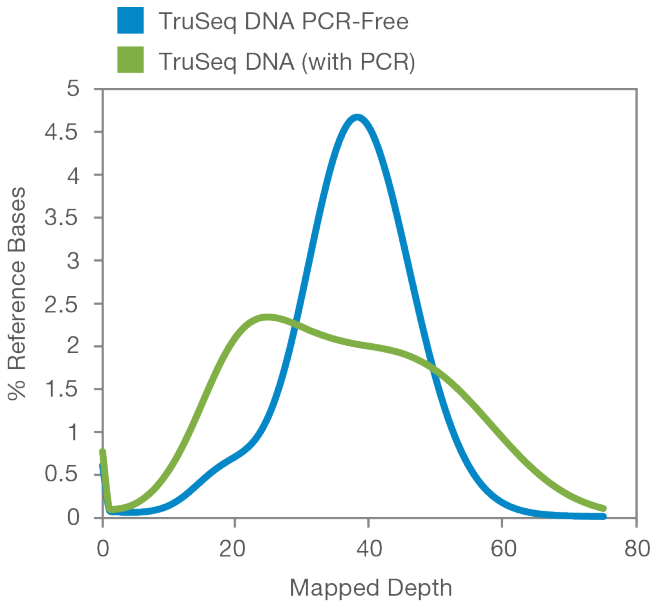

Figure 4: Greater Coverage Uniformity—TruSeq DNA PCR-Free libraries provide greater coverage uniformity across the genome when compared to those generated using the TruSeq DNA (with PCR) protocol.

Excellent Coverage Quality

TruSeq DNA PCR-Free reduces the number and average size of typical PCR-induced gaps in coverage (Figure 3), delivering exceptional data quality. The removal of PCR amplification from the TruSeq DNA PCR-Free workflow reduces library bias[2] and improves coverage uniformity across the genome (Figure 4). This workflow also provides excellent coverage of traditionally challenging genomic content, including GC-rich regions, promoters, and repetitive regions (Figure 5). High data quality delivers base-pair resolution, providing a detailed view of somatic and *de novo* mutations and supporting accurate identification of causative variants. TruSeq DNA PCR-Free provides a comprehensive view of the genome, including coding, regulatory, and intronic regions, enabling researchers to access more information from each sequencing run (Figure 6).

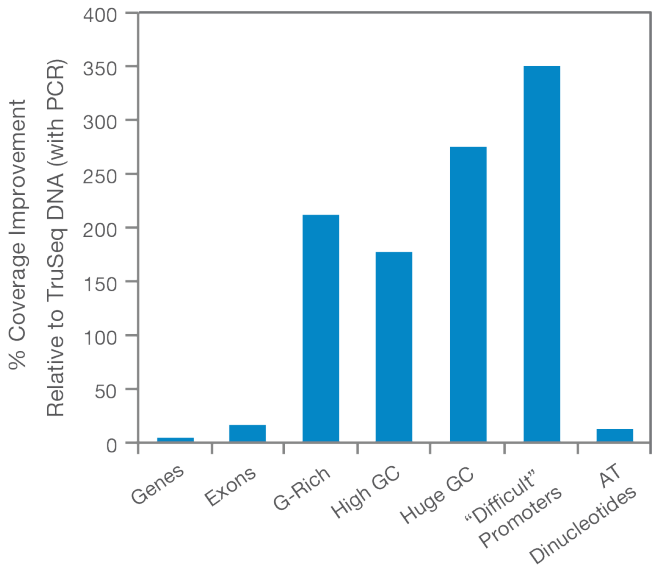

Figure 5: Increased Coverage of Challenging Regions—TruSeq DNA PCR-Free libraries demonstrate improved coverage of challenging genomic content. These regions include known human protein coding and nonprotein coding exons and genes defined in the RefSeq Genes track in the UCSC Genome Browser. G-Rich regions denote 30 bases with ≥ 80% G. High GC regions are defined as 100 bases with ≥ 75% GC content. Huge GC regions are defined as 100 bases with ≥ 85% GC content. "Difficult" Promoters denote the set of 100 promoter regions that are insufficiently covered, which have been empirically defined by the Broad Institute of MIT and Harvard. AT Dinucleotides indicate 30 bases of repeated AT dinucleotides.

Efficient Sample Multiplexing

Indexes are added to sample gDNA fragments using a simple PCR-free procedure to provide an innovative solution for sample multiplexing. For the greatest operational efficiency, up to 96 preplated, uniquely indexed samples can be pooled and sequenced together in a single flow cell lane on any Illumina sequencing platform. After sequencing, the indexes are used to demultiplex the data and accurately assign reads to the proper samples in the pool. TruSeq DNA PCR-Free can use a single indexing strategy or a dual-indexing strategy that uses a unique combination of two indexes to demultiplex. The unique dual index (UDI) adapters (available separately) were developed in a collaboration between Integrated DNA Technologies, Inc. (IDT) and Illumina to employ unique pairs to demultiplex.

The TruSeq DNA Single Indexes contain up to 24 indexes with two sets of 12 each, and the TruSeq DNA CD Indexes contain 96 indexes. Multisample studies can be conveniently managed using the Illumina Experiment Manager, a freely available software tool that provides easy reaction set up for plate-based processing. It allows researchers to configure the index sample sheet (ie, sample multiplexing matrix) for the instrument run, enabling automatic demultiplexing.

To learn more about Illumina Experiment Manager, visit www.illumina.com/informatics/research/experimental-design/illumina-experiment-manager.html.

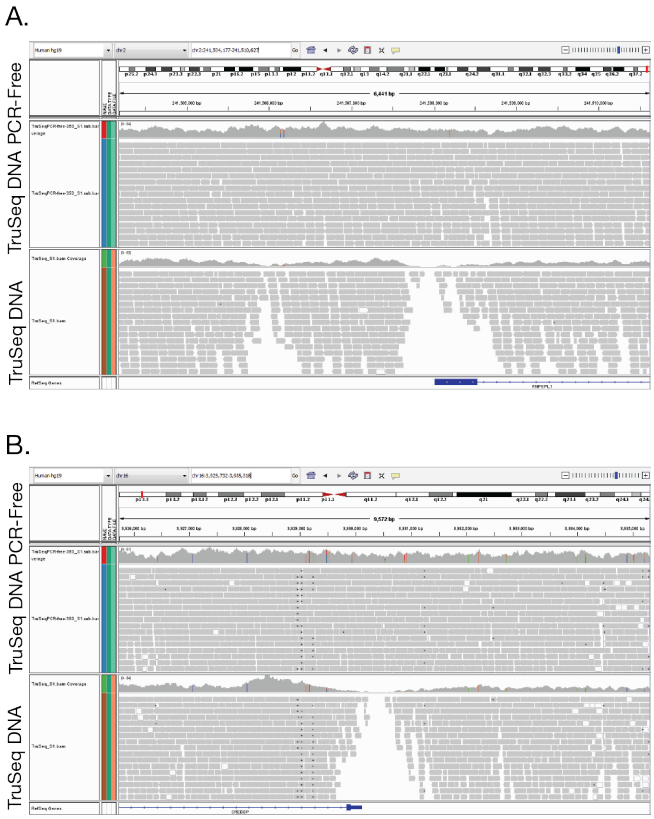

Figure 6: TruSeq DNA PCR-Free Reduces Number of Coverage Gaps—Increased coverage of TruSeq DNA PCR-Free libraries results in fewer coverage gaps, demonstrated here in the GC-rich coding regions of the (A) *RNPEPLI1* promoter and the (B) *CREBBP* promoter. Sequence information generated by TruSeq DNA PCR-Free is shown in the top panels of (A) and (B), while sequence data generated using TruSeq DNA protocol are shown in the lower panels.

Table 1: TruSeq DNA Library Preparation

Specification	TruSeq DNA Nano	TruSeq DNA PCR-Free	TruSeq DNA
Description	Based on widely adopted TruSeq library prep, with lower input and improved data quality	Excellent genomic coverage with radically reduced library bias and gaps	Original TruSeq next-generation sequencing library preparation method
Input Quantity	100–200 ng	1–2 µg	1 µg
Includes PCR	Yes	No	Yes
Assay Time	~ 6 hours	~ 5 hours	1–2 days
Hands-On Time	~ 5 hours	~ 4 hours	~ 8 hours
Target Insert Size	350 bp or 550 bp	350 bp or 550 bp	300 bp
Gel-Free	Yes	Yes	No
Number of Samples Supported	24 (LT) or 96 (HT)[a]	24 (LT) or 96 (HT)[a]	48 (LT) or 96 (HT)[a]
Supports Enrichment	No[b]	No[b]	Yes
Size-Selection Beads	Included	Included	Not Included
Applications	WGS, including whole-genome resequencing, *de novo* assembly, and metagenomics studies		
Sample Multiplexing	24 single indexes, 96 combinatorial dual indexes, 24 and 96 unique dual indexes (UDIs)		
Compatible Illumina Sequencing Systems	HiSeq™, HiScanSQ™, Genome Analyzer, and MiSeq™, and MiniSeq™ Systems		

a. LT, low-throughput; HT, high-throughput.
b. Nextera™ Rapid Capture products support various enrichment applications. For more information, visit www.illumina.com/NRC.

Flexible and Inclusive Library Preparation

The TruSeq family of library preparation solutions offers several options for sequencing applications, compatible with a range of research needs and study designs (Table 1). All TruSeq products support high- and low-throughput studies. These workflows provide numerous enhancements to the TruSeq DNA library preparation method, empowering various sequencing applications. Library prep reagents and sequencing indexes are now offered separately, enabling researchers to tailor these workflows to their experimental needs.

Streamlined Solution

TruSeq DNA PCR-Free contains library preparation reagents, sample purification beads, and robust TruSeq indexes for multiplexing, providing a comprehensive preparation method optimized for high performance on all Illumina sequencing platforms. TruSeq DNA PCR-Free offers the flexibility of two options, 24-sample and 96-sample, for scalable experimental design. With a simplified protocol and flexible multiplexing options, TruSeq DNA PCR-Free offers a streamlined library preparation method that delivers high-quality sequencing data.

Summary

TruSeq DNA PCR-Free optimizes the TruSeq workflow to deliver a streamlined library preparation method for any sequencing application. Low- and high-throughput options and varied insert sizes provide greater flexibility to support various applications and genomic studies. Workflow innovations reduce PCR-induced bias to facilitate detailed and accurate insight into the genome. By harnessing a faster workflow and enhanced data quality, TruSeq DNA PCR-Free provides a comprehensive sample preparation method for genome sequencing applications.

Learn More

To learn more about TruSeq DNA PCR-Free, visit www.illumina.com/products/by-type/sequencing-kits/library-prep-kits/truseq-dna-pcr-free.html

References

1. Saunders CJ, Miller NA, Soden SE, et al. Rapid whole-genome sequencing for genetic disease diagnosis in neonatal intensive care units. *Sci Translational Med.* 2012;4(154):154ra135.
2. Aird D, Ross MG, Chen WS, et al. Analyzing and minimizing PCR amplification bias in Illumina sequencing libraries. *Genome Biol.* 2011;12:R18.
3. University of California, Santa Cruz (UCSC) Genome Browser. genome.ucsc.edu. Accessed July 2013.
4. The Broad Institute of MIT and Harvard. www.broadinstitute.org. Accessed July 2013.

Ordering Information

Product	Catalog No.
TruSeq DNA PCR-Free Library Prep 24 samples	20015962
TruSeq DNA PCR-Free Library Prep 96 samples	20015963
TruSeq DNA Single Indexes Set A	20015960
TruSeq DNA Single Indexes Set B	20015961
TruSeq DNA CD Indexes	20015949
IDT for Illumina–TruSeq DNA UD Indexes (24 indexes, 96 samples)	20020590
IDT for Illumina–TruSeq DNA UD Indexes (96 indexes, 96 samples)	20022370

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