TruSeq® DNA PCR-Free Sample Preparation Kit

Setting new standards for unbiased data quality and superior coverage.

Highlights

- **Superior Coverage**
  Elimination of PCR-induced bias and fewer coverage gaps provide greater access to the genome
- **Faster Sample Preparation**
  PCR-Free protocol accelerates the most widely adopted sample preparation chemistry
- **Unprecedented Flexibility**
  PCR-Free kits are optimized to support a variety of read lengths and applications
- **Inclusive Solution**
  Reliable solution includes master-mixed reagents, size-selection beads, and up to 96 indices for the highest operational efficiency

Introduction

The TruSeq DNA PCR-Free Sample Preparation Kit offers numerous enhancements to the industry's most widely adopted sample preparation workflow, providing an optimized, all-inclusive sample preparation for whole-genome sequencing applications. By eliminating PCR amplification steps, the PCR-Free protocol removes typical PCR-induced bias and streamlines the proven TruSeq workflow. This results in excellent data quality and detailed sequence information for traditionally challenging regions of the genome. Two kit types are available to accommodate a range of study designs: the TruSeq DNA PCR-Free LT Sample Preparation Kit for low-throughput studies and the TruSeq DNA PCR-Free HT Sample Preparation Kit for high-throughput studies (Figure 1).

Accelerated Sample Preparation

The TruSeq DNA sample preparation workflow has been streamlined further by removing the PCR step and replacing gel-based size selection with bead-based selection (Figure 2). This kit offers unprecendented flexibility with two protocol options for generating either large (550 bp) or small (350 bp) insert sizes to support a variety of applications, matching the ever-increasing read lengths of Illumina sequencing instruments. Master-mixed reagents, provided sample purification beads, and optimized protocols contribute to the simplified library construction workflow, requiring minimal hands-on time and few cleanup steps for processing large sample numbers. TruSeq DNA PCR-Free sample preparation decreases library preparation time, empowering applications from microbial sequencing to whole human genome sequencing.[1]

Figure 1: TruSeq DNA PCR-Free Sample Preparation Kit

TruSeq DNA PCR-Free kits are an efficient solution for preparing and indexing sample libraries. The TruSeq DNA PCR-Free LT kit provides up to 24 indices for low-throughput studies (with both Sets A and B), while the TruSeq DNA PCR-Free HT kit includes 96 dual-index combinations for high-throughput studies.

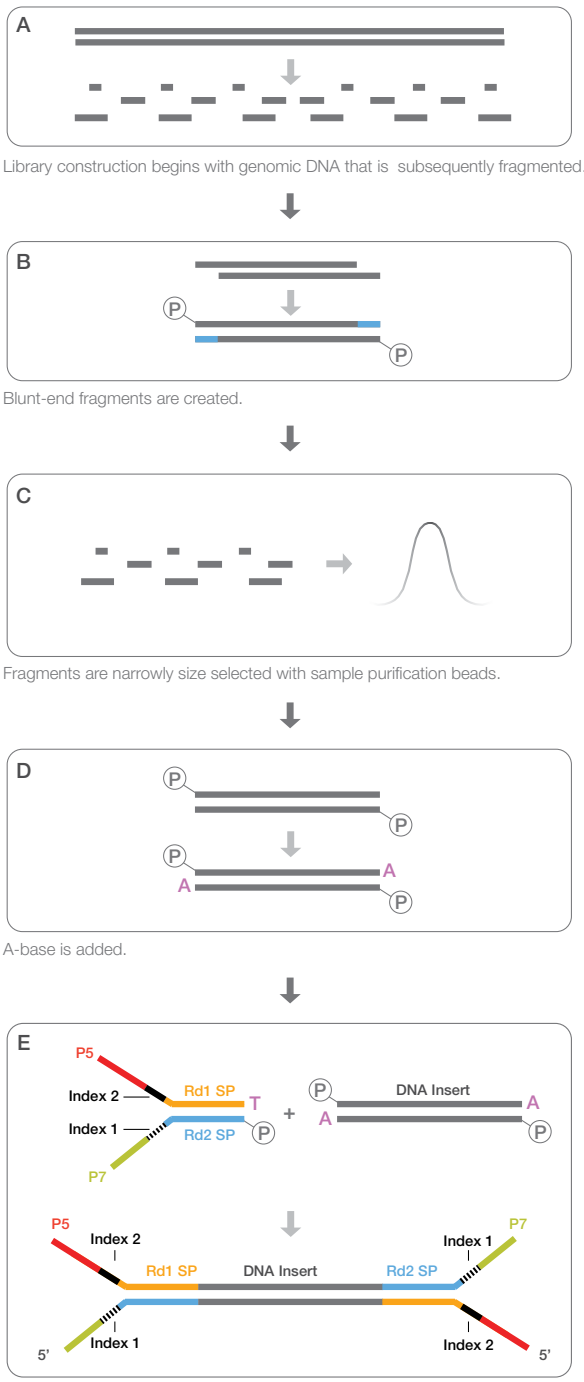
Innovative Sample Preparation Chemistry

TruSeq DNA PCR-Free Sample Preparation kits are used to prepare DNA libraries for single, paired-end, and indexed sequencing. The protocol supports shearing by Covaris ultrasonication, requiring 1 µg of input DNA for an average insert size of 350 bp or 2 µg for an average insert size of 550 bp. Library construction begins wtih fragmented 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 samples are ready for cluster generation (Figure 2E).

Superior Coverage

The TruSeq DNA PCR-Free Sample Preparation Kit optimizes sequencing data to provide greater insight into the genome, including coding, regulatory, and intronic regions. PCR-Free sample preparation generates reduced library bias and gaps (Figure 3). Exceptional data quality delivers base-pair resolution of somatic and *de novo* mutations, supporting accurate identification of causative variants. The removal of PCR amplification from the TruSeq workflow removes amplification biases to improve coverage uniformity across the genome (Figure 4).

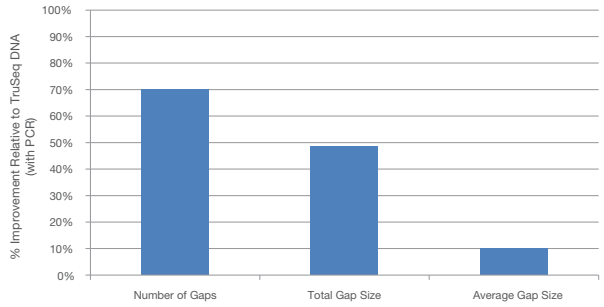
Figure 2: Adapter Ligation Results in Sequence-Ready Constructs without PCR



Dual-index adapters are ligated to the fragments* and final product is ready for cluster generation.

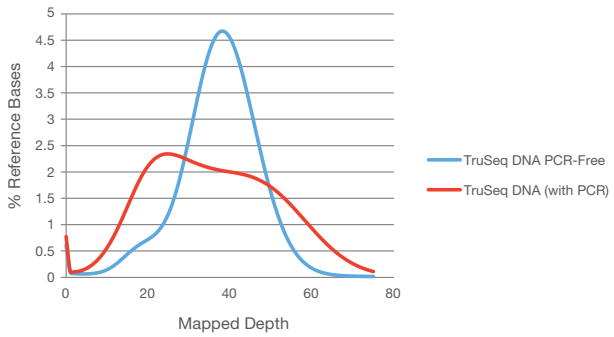
*The TruSeq DNA PCR-Free LT indexing solution features a single-index adapter at this step.

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



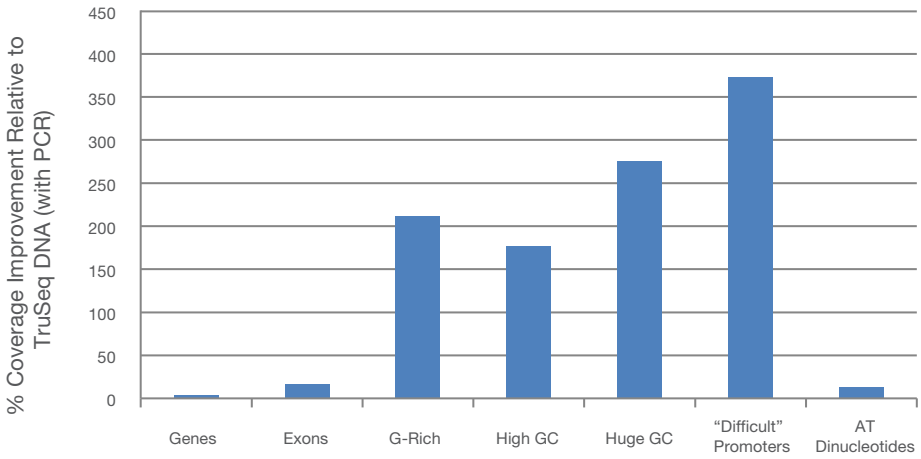
TruSeq DNA PCR-Free libraries provide greater coverage uniformity across the genome when compared to those generated using the TruSeq DNA protocol.

The PCR-Free kit also provides superior coverage of traditionally challenging genomic content, including GC-rich regions, promoters, and repetitive regions (Figure 5), allowing researchers to access more genomic information from each sequencing run (Figure 6).

Efficient Sample Multiplexing

TruSeq DNA PCR-Free Sample Preparation kits provide an innovative solution for sample multiplexing. Indices are added to sample gDNA fragments using a simple PCR-Free procedure. For the greatest operational efficiency, up to 96 pre-plated, uniquely indexed samples can be pooled and sequenced together in a single flow cell lane on any Illumina sequencing platform. After sequencing, the indices are used to demultiplex the data and accurately assign reads to the proper sample in the pool. The TruSeq DNA PCR-Free LT kit uses a single index for demultiplexing, while the TruSeq DNA PCR-Free HT kit employs a dual-indexing strategy, using a unique combination of two indices to demultiplex.

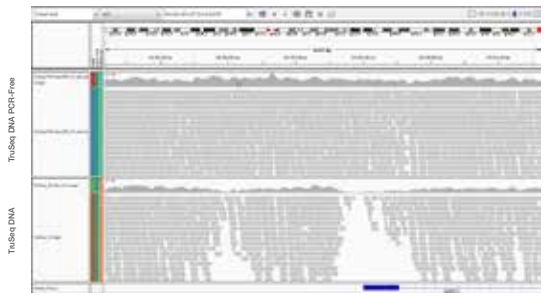
Figure 5: Increased Coverage of Challenging Regions



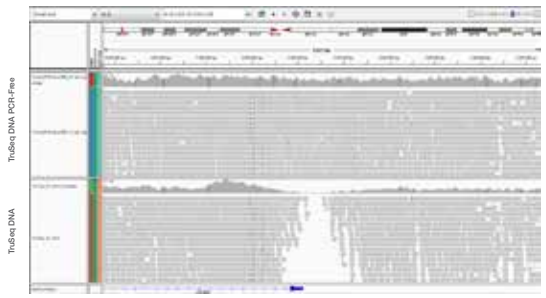
When compared to libraries generated by PCR-based workflows, such as TruSeq DNA Sample Preparation, PCR-Free libraries show improved coverage for challenging regions of the genome. These regions include known human protein coding and non-protein coding exons and genes defined in the RefSeq Genes track in the UCSC Genome Browser.[2] 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.[3] AT dinucleotides indicate 30 bases of repeated AT dinucleotide.

Figure 6: PCR-Free Protocol Eliminates Coverage Gaps in GC-Rich Content

A



B



Increased coverage of TruSeq DNA PCR-Free libraries results in fewer coverage gaps, demonstrated here in the GC-rich coding regions of the *RNPEPL1* promoter (A) and the *CREBBP* promoter (B). PCR-Free sequence information is shown in the top panels of A and B, while sequence data generated using TruSeq DNA protocol (with PCR) are shown in the lower panels.

The TruSeq LT kit includes up to 24 indices with two sets of 12each, and the TruSeq HT kit offers 96 indices for efficient experimental design.

Multi-sample studies can be conveniently managed using the Illumina Experiment Manager, a freely available software tool that provides easy reaction setup for plate-based processing. It allows researchers to quickly configure the index sample sheet (i.e., sample multiplexing matrix) for the instrument run, enabling automatic demultiplexing.

Flexible and Inclusive Sample Preparation

The TruSeq family of sample preparation solutions offers several kits for sequencing applications, compatible with a range of research needs and study designs (Table 1). All TruSeq kits support high- and low-throughput studies. The TruSeq DNA PCR-Free kit provides superior coverage quality and drastically reduces library bias and coverage gaps, without requiring PCR amplification. These kits enhance the industry's most widely adopted DNA sample preparation method, empowering next-generation sequencing applications.

Simplified Solution

The comprehensive solution includes sample preparation reagents, sample purification beads, and robust TruSeq barcodes for sample multiplexing, providing a complete preparation method optimized for the highest performance on all Illumina sequencing platforms. The TruSeq DNA PCR-Free kit leverages the flexibility of two kit options, 24-sample and 96-sample, for a scalable experimental approach. With a simplified workflow and multiplexing options, the TruSeq DNA PCR-Free protocol offers the fastest library preparation method for the highest data quality.

Table 1: TruSeq DNA Sample Preparation Kits

Specification	TruSeq Nano DNA	TruSeq DNA PCR-Free	TruSeq DNA
Description	Based upon widely adopted TruSeq sample prep, with lower input and improved data quality	Superior genomic coverage with radically reduced library bias and gaps	Original TruSeq next-generation sequencing sample 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) samples	24 (LT) or 96 (HT) samples	48 (LT) or 96 (HT) samples
Supports enrichment	No*	No*	Yes
Size-selection beads	Included	Included	Not included
Applications	Whole-genome sequencing applications, including whole-genome resequencing, *de novo* assembly, and metagenomics studies		
Sample multiplexing	24 single indices or 96 dual-index combinations		
Compatible Illumina sequencers	HiSeq®, HiScanSQ™, Genome Analyzer™, and MiSeq® systems		

*Nextera Rapid Capture products support a variety of enrichment applications. For more information, visit www.illumina.com/NRC.

Summary

The TruSeq DNA PCR-Free Sample Preparation Kit optimizes the TruSeq workflow to deliver a faster sample preparation method for any species. The choice between protocol options provides greater flexibility to support a variety of applications and genomic studies. The PCR-Free kit also removes PCR-induced bias to facilitate detailed and accurate insight into the genome. By leveraging a faster workflow and superior data quality, the TruSeq DNA PCR-Free Sample Preparation Kit enables researchers to obtain high-quality genomic data, faster.

References

1. Saunders CJ, Miller NA, Soden SE, Dinwiddie DL, Noll A, et al. (2012) Rapid whole-genome sequencing for genetic disease diagnosis in neonatal intensive care units. Science Translational Medicine 4(154): 154ra135.
2. genome.ucsc.edu
3. www.broadinstitute.org

Ordering Information

Product	Catalog No.
TruSeq DNA PCR-Free LT Sample Preparation Kit Set A (24 samples)	FC-121-3001
TruSeq DNA PCR-Free LT Sample Preparation Kit Set B (24 samples)	FC-121-3002
TruSeq DNA PCR-Free HT Sample Preparation Kit (96 samples)	FC-121-3003

Illumina • 1.800.809.4566 toll-free (U.S.) • +1.858.202.4566 tel • techsupport@illumina.com • www.illumina.com

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