TruSeq® Nano DNA Library Prep Kit

A low-input method that delivers a high-confidence, comprehensive view of the genome for virtually any sequencing application.

Highlights

- **Low Sample Input**
  Excellent data quality from as little as 100 ng input empowers interrogation of samples with limited available DNA
- **Excellent Coverage Quality**
  Significantly reduced library bias and gaps in coverage provide greater insight into the genome
- **Unprecedented Flexibility**
  Streamlined TruSeq workflow enables library preparation in less than 1 day, while supporting various read lengths
- **Inclusive Solution**
  Reliable solution includes master-mixed reagents, size-selection beads, and up to 96 indexes for the highest operational efficiency

Figure 1: TruSeq Nano DNA Library Prep Kit—TruSeq Nano DNA Library Prep Kits offer a low-input solution for preparing and indexing sample libraries. The TruSeq Nano DNA LT Kit provides up to 24 indexes for low-throughput studies (with both Sets A and B), while the TruSeq Nano DNA HT Kit includes 96 dual-index combinations for high-throughput studies.

Introduction

By offering a low-input method based on the industry's most widely adopted library preparation workflow, the TruSeq Nano DNA Library Prep Kit enables efficient interrogation of samples that have limited available DNA. This kit significantly reduces typical PCR-induced bias and provides detailed sequence information for traditionally challenging regions of the genome. Two kit types are available to accommodate a range of study designs: the TruSeq Nano DNA LT Library Prep Kit for low-throughput studies and the TruSeq Nano DNA HT Library Prep Kit for high-throughput studies (Figure 1).

Low Sample Input

The TruSeq Nano DNA protocol eliminates the typical requirement for micrograms of DNA, enabling researchers to study samples with limited available DNA (eg, tumor samples) and supporting preservation of samples for use in future or alternate studies. This kit offers the flexibility of 2 protocols for generating large (550 bp) or small (350 bp) insert sizes to support a diverse range of applications. In addition to accelerating the workflow, simple bead-based size selection avoids typical sample loss associated with gel-based selection. TruSeq Nano DNA Kits are validated for high-quality genomic coverage for virtually any whole-genome sequencing application.

Accelerated Library Preparation

The TruSeq DNA library preparation workflow has been streamlined by replacing gel-based size selection with bead-based selection (Figure 2), enabling researchers to prepare high-quality libraries in less than a day. Optimized for various read lengths, from 2 × 101 bp to 2 × 151 bp, the TruSeq Nano DNA Kit is designed to match the everincreasing read lengths of Illumina sequencing instruments. Master-mixed reagents, provided sample purification beads for cleanup and size selection, robust TruSeq indexes, and optimized protocols contribute to the simplified workflow, requiring minimal hands-on time and few cleanup steps for processing large sample numbers.

Innovative Library Preparation Chemistry

These kits are used to prepare DNA libraries for single-read, paired-end, and indexed sequencing. The TruSeq Nano DNA protocol supports shearing by Covaris ultrasonication, requiring 100 ng of input DNA for an average insert size of 350 bp or 200 ng DNA for an average insert size of 550 bp. Library construction begins with 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. Single- or dual-index adapters are ligated to the fragments (Figure 2E) and the ligated products are amplified with reduced-bias PCR (Figure 2F).

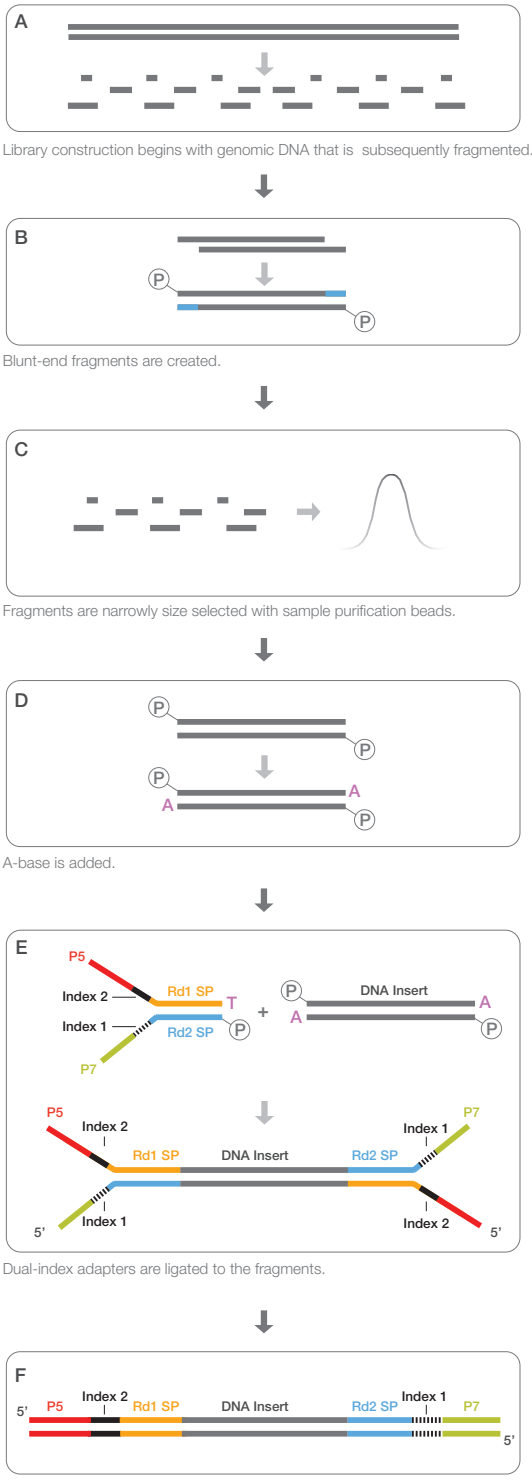

Figure 3: TruSeq Nano DNA Workflow — The TruSeq Nano DNA LT indexing solution features a single-index adapter at Step E.

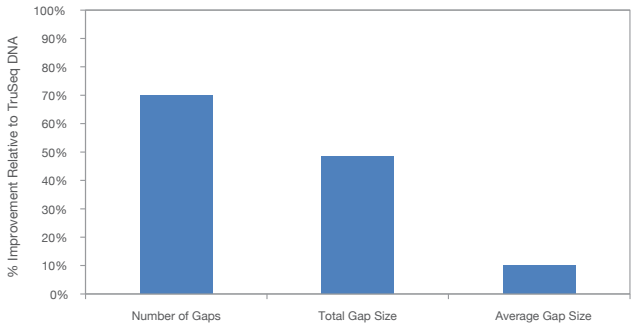

Figure 4: Fewer Gaps in Coverage — TruSeq Nano DNA libraries show significant reduction in the number and total size of gaps when compared to libraries prepared using the TruSeq DNA protocol. A gap is defined as a region ≥ 10 bp in length, where an accurate genotype cannot be determined due to low depth, low alignment scores, or low base quality.

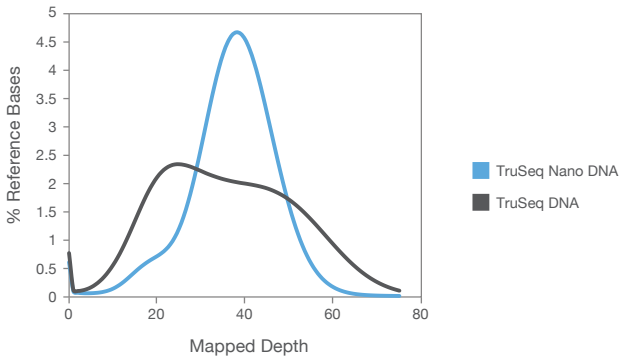

Figure 5: Greater Coverage Uniformity — TruSeq Nano DNA libraries provide greater coverage uniformity across the genome when compared to those generated using the TruSeq DNA protocol.

Table 1: Next-Generation Sequencing Simplified

Specification	TruSeq Nano DNA	TruSeq DNA PCR-Free	TruSeq DNA
Description	Based on widely adopted TruSeq library prep, with lower input and improved data quality	Superior 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) 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 indexes or 96 dual-index combinations		
Compatible Illumina sequencing systems	HiSeq®, HiScanSQ™, Genome Analyzer™, MiSeq®, and MiniSeq™ systems		

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

Excellent Coverage Quality

TruSeq Nano DNA Kits reduce the number and average size of typical PCR-induced gaps in coverage (Figure 3), delivering exceptional data quality. The enhanced workflow reduces library bias and improves coverage uniformity across the genome (Figure 4). These kits also provide 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 Nano DNA Kits provide a comprehensive view of the genome, including coding, regulatory, and intronic regions, enabling researchers to access more information from each sequencing run (Figure 6).

Flexible and Inclusive Library Preparation

The TruSeq family of library 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 Nano DNA Kit supports whole-genome sequencing and is ideal for sequencing applications that require sparsely available DNA. These kits provide numerous enhancements to the industry's most widely adopted DNA library preparation method, empowering all sequencing applications.

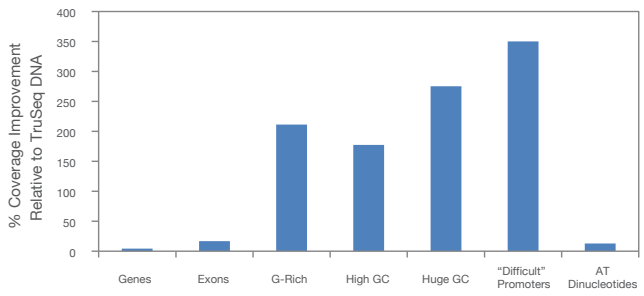

Figure 6: Increased Coverage of Challenging Regions — TruSeq Nano DNA libraries demonstrate improved coverage of challenging genomic content. These regions include known human protein coding and nonprotein coding exons and genes defined in the RefSeq Genes track in the UCSC Genome Browser.[1] G-Rich 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.[2] AT dinucleotides indicate 30 bases of repeated AT dinucleotide.

Efficient Sample Multiplexing

Using a simple procedure, indexes are added to sample genomic DNA fragments 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. The TruSeq Nano DNA LT Kit uses a single index for demultiplexing, while the TruSeq Nano DNA HT Kit employs a dual-indexing strategy, using a unique combination of 2 indexes to demultiplex. The TruSeq Nano DNA LT Kit includes up to 24 indexes with 2 sets of 12 each, and the TruSeq Nano DNA HT Kit offers 96 indexes.

Streamlined Solution

This inclusive kit contains library preparation reagents, sample purification beads, and robust TruSeq indexes for multiplexing, providing a complete preparation method optimized for the highest performance on all Illumina sequencing platforms. The TruSeq Nano DNA Kit harnesses the flexibility of 2 kit options, 24-sample and 96-sample, for scalable experimental design. With a simplified workflow and flexible multiplexing options, the TruSeq Nano DNA protocol offers a streamlined library preparation method that delivers high-quality sequencing data.

Summary

The TruSeq Nano DNA Library Prep Kit optimizes the TruSeq workflow to deliver a low-input 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, the TruSeq Nano DNA Library Prep Kit provides an all-inclusive sample preparation method for genome sequencing applications.

References

1. genome.ucsc.edu
2. www.broadinstitute.org

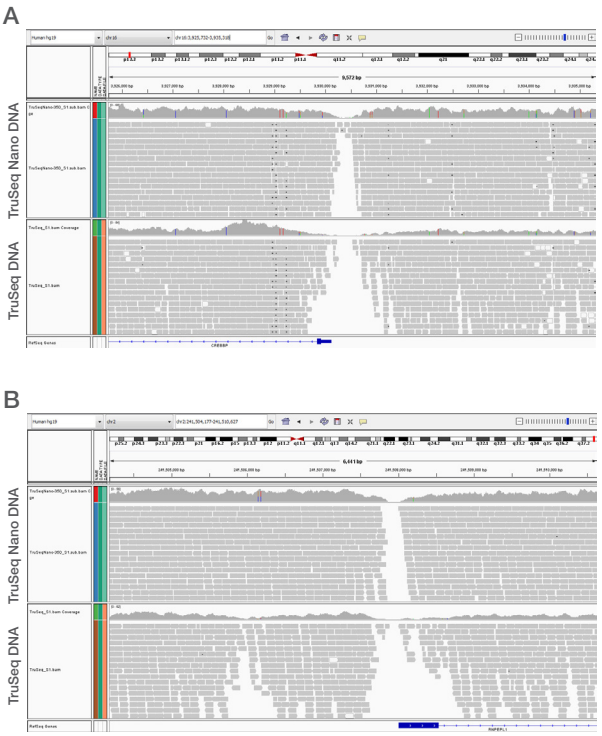

Figure 2: Figure 6: TruSeq Nano DNA Protocol Reduces Number of Coverage Gaps — Increased coverage of TruSeq Nano DNA libraries results in fewer coverage gaps, demonstrated here in the GC-rich coding regions of the *RNPEPL1* promoter (A) and the *ZBTB34* promoter (B). Sequence information generated by TruSeq Nano DNA prep is shown in the top panels of A and B, while sequence data generated using TruSeq DNA protocol are shown in the lower panels.

Ordering Information

Product	Catalog No.
TruSeq Nano DNA LT Library Prep Kit Set A (24 samples)	FC-121-4001
TruSeq Nano DNA LT Library Prep Kit Set B (24 samples)	FC-121-4002
TruSeq Nano DNA HT Library Prep Kit (96 samples)	FC-121-4003

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

Pub. No. 770-2013-012 Current as of 07 January 2016

illumina®