illumina®

TruSeq™ DNA Nano

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**
  Very low library bias and fewer gaps in coverage enable deep insight into the genome
- **High flexibility**
  Streamlined workflow enables library preparation in less than one day, while supporting various read lengths
- **Inclusive solution**
  Reliable solution includes master-mixed reagents, size-selection beads, and up to 96 unique dual indexes (UDIs)

Figure 1: TruSeq DNA Nano—TruSeq DNA Nano offers a low-input solution for preparing and indexing sample libraries. TruSeq DNA Nano 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

By offering a low-input method based on the widely adopted TruSeq library preparation workflow, TruSeq DNA Nano enables efficient interrogation of samples that have limited available DNA. This workflow 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).

Low Sample Input

TruSeq DNA Nano reduces 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 workflow offers the flexibility of two 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 DNA Nano is validated for high-quality genomic coverage for a wide range of Illumina whole-genome sequencing (WGS) applications.

Accelerated Library Preparation

The TruSeq DNA library preparation protocol 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. TruSeq DNA Nano is optimized for various read lengths from 2 × 101 bp to 2 × 151 bp. Master-mixed reagents, sample purification beads for clean up and size selection, robust TruSeq indexes, and optimized protocols contribute to the simplified workflow, requiring a low number of cleanup steps for processing large sample numbers.

Innovative Library Preparation Chemistry

TruSeq DNA Nano can be used to prepare DNA libraries for single-read, paired-end, and indexed sequencing. TruSeq DNA Nano 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 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. Single- or dual-index adapters are ligated to the fragments (Figure 2E) and the ligated products are amplified with reduced-bias PCR (Figure 2F).

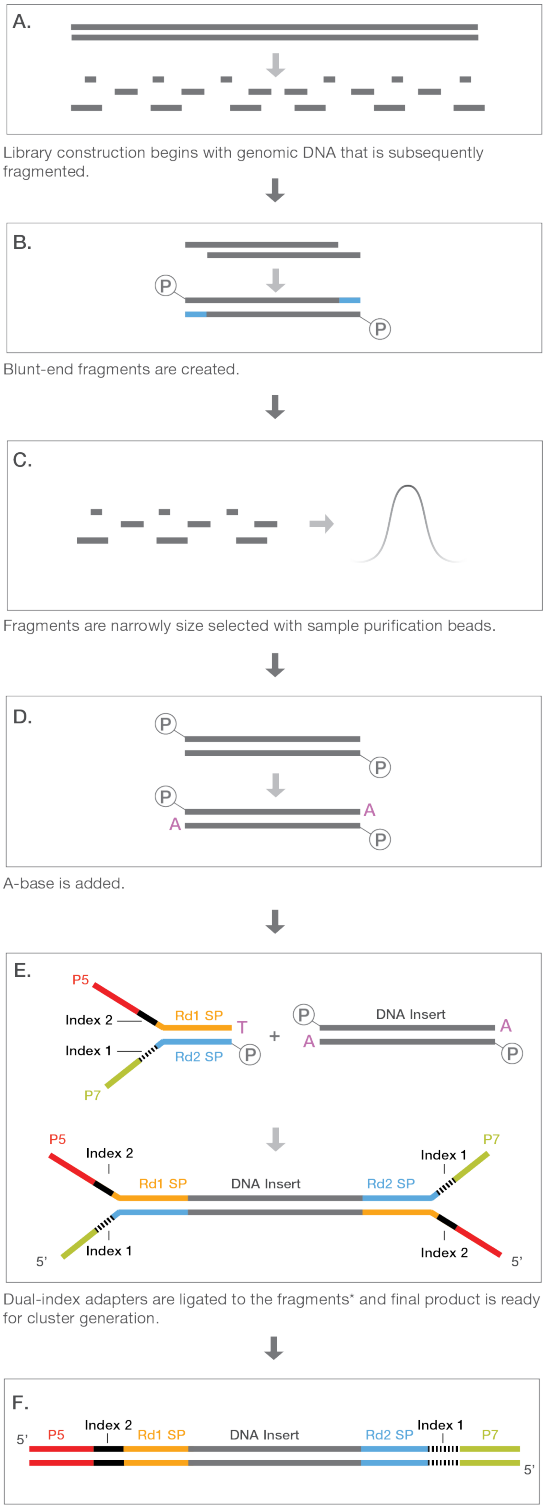


Figure 2: TruSeq DNA Nano Workflow—The TruSeq DNA Nano workflow features adapter ligation resulting in sequence-ready products with minimal PCR-induced bias. *The TruSeq DNA Nano LT indexing solution features a single-index adapter at Step E.

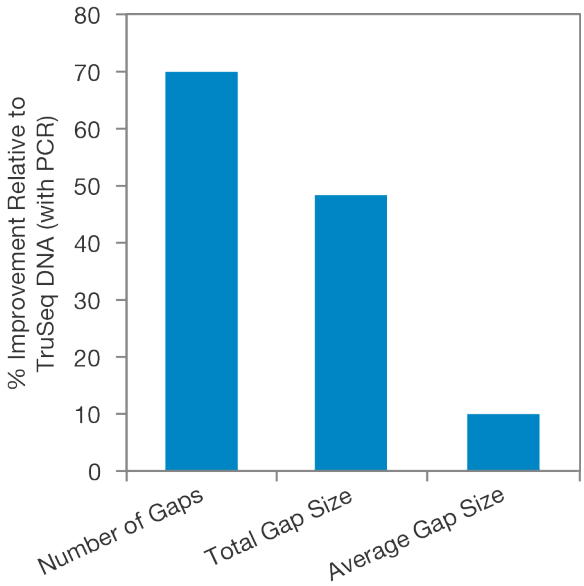


Figure 3: Fewer Gaps in Coverage—TruSeq DNA Nano libraries show significant reduction in the number and total size of gaps when compared to libraries prepared using the TruSeq DNA (with PCR) protocol. A gap is defined as a region ≥ 10 bp in length, where an accurate genotype cannot be determined due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

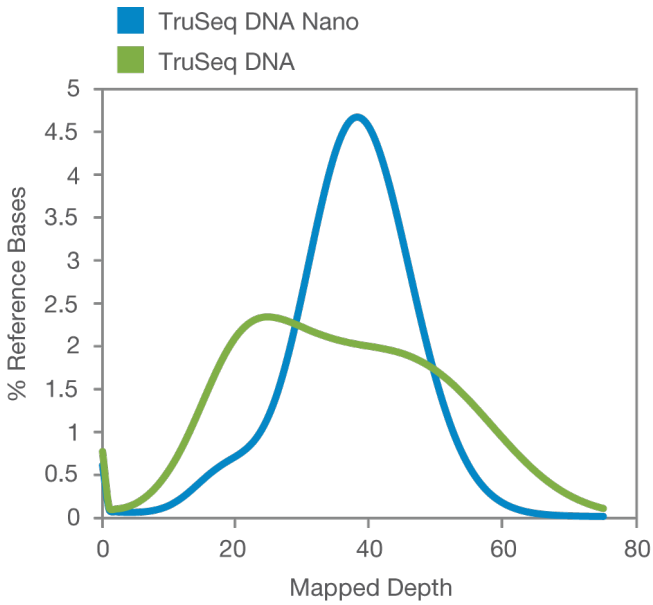


Figure 4: Greater Coverage Uniformity—TruSeq DNA Nano libraries provide greater coverage uniformity across the genome when compared to those generated using the TruSeq DNA (with PCR) protocol.

Table 1: TruSeq DNA Library Preparation

Specification	TruSeq DNA Nano	TruSeq DNA PCR-Free	Legacy 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]
Size-Selection Beads	Included	Included	Not Included
Applications	WGS applications, 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), (available soon)		
Compatible Illumina Sequencing Systems	HiSeq™ 1000, HiSeq 1500, HiSeq 2000, HiSeq 2500, HiSeq 3000, HiSeq 4000, HiSeq X Five. HiSeq X Ten, MiniSeq™, MiSeq™, MiSeqDx in research mode, NextSeq™ 500, NextSeq 550, NextSeq 1000, NextSeq 2000, and NovaSeq™ 6000 Systems		HiSeq, HiScanSQ, Genome Analyzer, and MiSeq, and MiniSeq Systems

a. LT, low-throughput; HT, high-throughput

Excellent Coverage Quality

TruSeq DNA Nano reduces 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). It 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 characterization of causative variants. TruSeq DNA Nano 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).

Flexible and Inclusive Library Preparation

The TruSeq family of library preparation solutions offers several workflows for sequencing applications, compatible with a range of research needs and study designs (Table 1). All TruSeq workflows support high- and low-throughput studies. TruSeq DNA Nano supports WGS and is ideal for sequencing applications that require sparsely available DNA. 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.

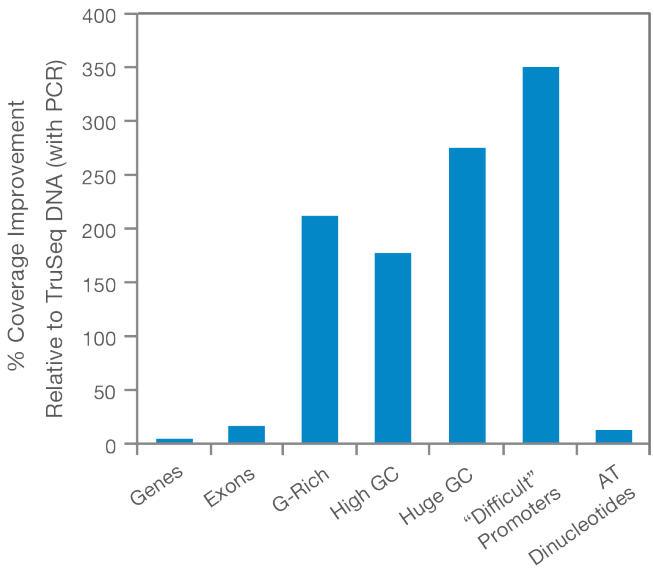


Figure 5: Increased Coverage of Challenging Regions—TruSeq DNA Nano libraries demonstrate improved coverage of challenging genomic content. These regions include known human protein coding and nonprotein coding exons and genes defined in the RefSeq Genes track in the UCSC Genome Browser.[1] G-Rich regions 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 dinucleotides.

Efficient Sample Multiplexing

Using a simple procedure, indexes are added to sample gDNA 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.

TruSeq DNA Nano 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 were developed in a collaboration between Integrated DNA Technologies, Inc. (IDT) and Illumina to employ unique pairs of indexes to demultiplex. The newly introduced UDIs (24 and 96, available separately) offer increased plexity that enables accurate assignment of reads and efficient use of flow cells. Using UDI combinations is a best practice to make sure that reads with incorrect indexes do not impact variant calling.

Streamlined Solution

TruSeq DNA Nano 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 Nano uses the flexibility of two options, 24-sample and 96-sample, for scalable experimental design. With a simplified protocol and flexible multiplexing options, TruSeq DNA Nano offers a streamlined library preparation method that delivers high-quality sequencing data.

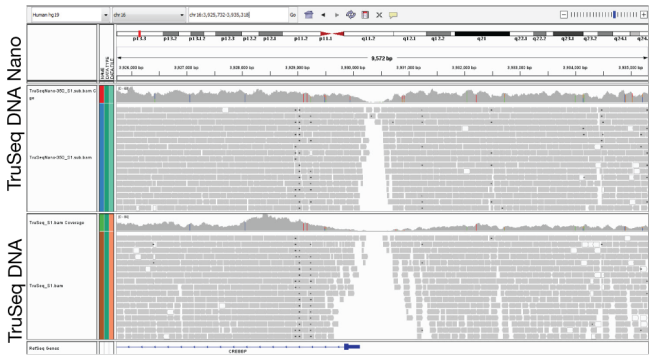
Summary

TruSeq DNA Nano optimizes the TruSeq protocol to deliver a low-input library preparation method for virtually 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 insights into the genome. By harnessing a faster workflow and enhanced data quality, TruSeq DNA Nano provides a comprehensive sample preparation method for genome sequencing applications.

Learn More

To learn more about TruSeq DNA Nano, visit www.illumina.com/products/truseq-nano-dna-sample-prep-kits.html

A.



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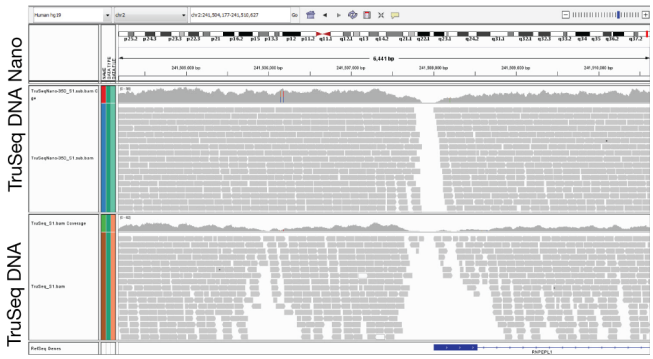

Figure 6: TruSeq DNA Nano Reduces Number of Coverage Gaps—Increased coverage of TruSeq DNA Nano libraries results in fewer coverage gaps, demonstrated here in the GC-rich coding regions of the (A) *RNPEPLI1* promoter and (B) *ZBTB34* promoter. Sequence information generated by TruSeq DNA Nano prep is 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 DNA Nano Low Throughput Library Prep Kit (24 samples)	20015964
TruSeq DNA Nano High Throughput Library Prep Kit (96 samples)	20015965
TruSeq DNA Single Indexes Set A (12 indexes, 24 samples)	20015960
TruSeq DNA Single Indexes Set B (12 indexes, 24 samples)	20015961
TruSeq DNA CD Indexes 96 samples	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

References

1. University of California, Santa Cruz (UCSC) Genome Browser. genome.ucsc.edu. Accessed July 2013.
2. The Broad Institute of MIT and Harvard. www.broadinstitute.org. Accessed July 2013.

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