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TruSeq® DNA Library Prep Kits

Prepare and index up to 96 samples. Minimal hands-on steps. Proven results.

TruSeq DNA Library Prep Kits Highlights

- **Simple Workflow for High-Quality DNA Library Preparation**
  Minimal hands-on steps using master-mixed reagents and an optimized protocol
- **Scalable and Cost-Effective Solution**
  Optimized formulations and plate-based processing enables large-scale studies at a lower cost
- **Enhanced Multiplex Performance**
  Reliable, accurate indexing solution for preparing up to 96 samples simultaneously

Introduction

TruSeq DNA Library Preparation Kits are a simple, cost-effective solution for generating high-quality libraries, compatible with unparalleled Illumina sequencing output. TruSeq library preparation has become the industry's most trusted and widely adopted library preparation method for next-generation sequencing (NGS). Illumina offers two kit types to accommodate a range of study designs: the TruSeq DNA LT Library Preparation Kits for low-throughput studies (< 48 samples) and the TruSeq DNA HT Library Preparation Kit for high-throughput studies (> 48 samples) (Figure 1).

Simplified Solution for Any Application

TruSeq DNA Library Prep kits enable researchers to simultaneously prepare up to 96 samples. Fast and efficient, the TruSeq DNA HT kit simplifies sample indexing for high-throughput studies by providing pre-loaded index adapters in 96-well plates, which eliminates the need for manual index mixing and minimizes the risk of error. With this pre-optimized indexing solution, researchers no longer need to develop, screen, source, and QC their own indices.

Figure 1: TruSeq DNA Library Preparation Kits

TruSeq Library Prep kits are an efficient, high-quality solution for preparing and indexing sample libraries. The TruSeq DNA LT kit provides 24 single indices for low-throughput studies, while the TruSeq DNA HT kit (shown here) provides 96 unique dual-index combinations for high-throughput studies.

Each index has been rigorously tested to ensure high performance across all Illumina sequencing platforms.

With the TruSeq DNA Library Prep kits, researchers can take on virtually any DNA sequencing application, including large-scale whole-genome resequencing, targeted resequencing, *de novo* sequencing, metagenomics, and low-throughput methylation studies. Prepared libraries are amenable to single-read, paired-end, and multiplexed sequencing on all Illumina sequencing instruments.

TruSeq DNA Library Prep kits are compatible with the TruSeq Exome Enrichment and TruSeq Custom Enrichment Assays, providing a seamless end-to-end library preparation solution for targeted resequencing. The library prep kits include gel-free protocols that eliminate the time-intensive gel purification step found in other enrichment workflows.

Table 1: High-Quality Indexing Results with the TruSeq DNA HT Library Prep Kit

Flow Cell Lane	Cluster Density (K/mm²)	% Clusters Passing Filter	% Perfect Match for Index 1	% Perfect Match for Index 2	% Reads Demultiplexed
1	774	93.1	98.0	97.9	97.7
2	784	93.1	98.0	97.9	97.7
3	892	92.1	97.9	97.4	97.5
4	888	91.6	97.7	97.4	97.5

A series of samples was prepared using the TruSeq DNA HT Library Preparation kit, then pooled and sequenced on the HiSeq 2000 system (flow cell lanes 1 and 2). A second series was prepared and pooled in the same manner, but was enriched using the TruSeq Custom Enrichment kit prior to sequencing (flow cell lanes 3 and 4). Following the sequencing run, the pooled libraries were demultiplexed for analysis. Both the standard and enriched libraries produced an equally high percentage of reads with perfect matches for index 1 and index 2, showing that each index was accurately sequenced and detected. Nearly 98% of reads across all libraries were successfully demultiplexed, demonstrating that the TruSeq DNA HT Library Prep kit produces superior indexing accuracy for both whole-genome and targeted libraries.

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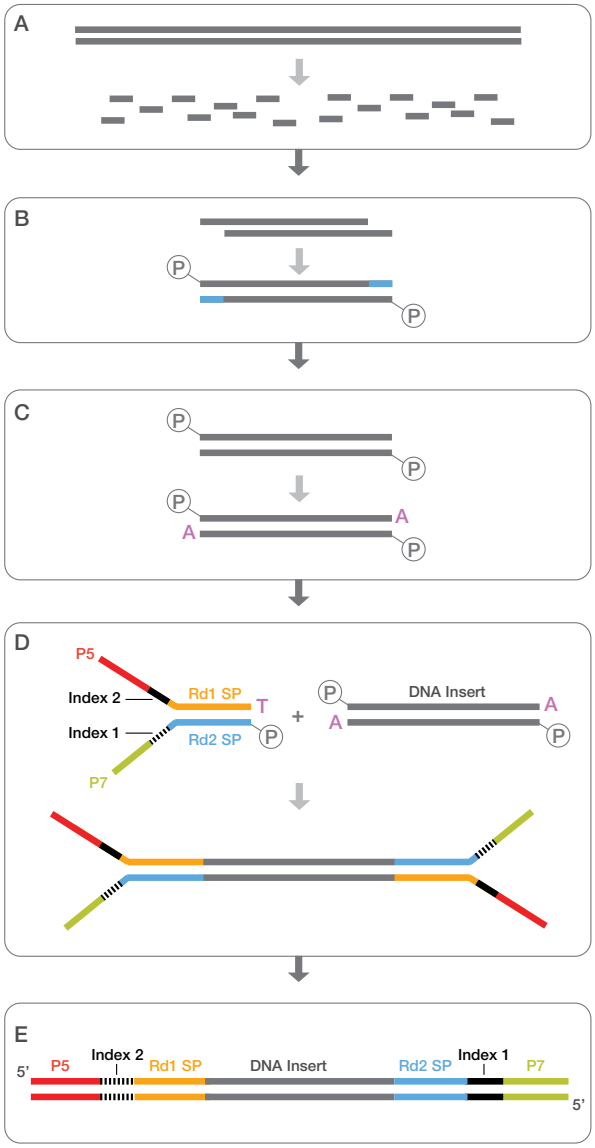
Figure 2: Dual-Index Library Preparation Using the TruSeq DNA HT Library Prep Kit



A. Library construction begins with genomic DNA that is subsequently fragmented
B. Blunt-end fragments are created
C. A-base is added
D. Dual-index adapters are ligated to the fragments*
E. Final product is ready for amplification

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

Accurate and Efficient Sample Multiplexing

TruSeq DNA Library Prep kits provide an innovative solution for uniquely multiplexing samples. Indices are added to sample gDNA fragments using an easy-to-follow, PCR-free procedure. Up to 96 uniquely indexed samples can be pooled and sequenced together in a single flow cell lane on any Illumina sequencer. After sequencing, the indices are used to demultiplex the data and accurately assign reads to the proper sample in the pool (Table 1). The TruSeq DNA LT kit uses a single index for demultiplexing, while the TruSeq DNA HT kit employs a dual-indexing strategy, using a unique combination of two indices to demultiplex (Figure 2).

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

Innovative Library Preparation Chemistry

The TruSeq DNA Library Preparation Kits are used to prepare DNA libraries with insert sizes from 300–500 bp for single, paired-end, and indexed sequencing. The protocol supports shearing by either sonication or nebulization with an input requirement of 1 µg of DNA. 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). An 'A'-base is then added to the blunt ends of each strand, preparing them for ligation to the sequencing adapters (Figures 2C). Each adapter contains a 'T'-base overhang on the 3'-end, providing a complementary 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. This eliminates the need for additional PCR steps to add the index tag and index primer sites (Figure 2D). Following the denaturation and amplification steps (Figure 2E), libraries can be pooled for sequencing.

Master-mixed reagents and an optimized protocol contribute to a simple library construction workflow, requiring minimal hands-on time and few cleanup steps for processing large sample numbers. The workflow allows for high-throughput and automation-friendly solutions, as well as simultaneous manual processing of hundreds of samples. In addition, enhanced troubleshooting features are incorporated into each step of the workflow, with quality control sequences supported by Illumina RTA software.

Quality Controls

Specific quality control (QC) sequences, consisting of double-stranded DNA fragments, are present in each enzymatic reaction of the TruSeq library preparation protocol: end repair, A-tailing, and ligation. During analysis, the QC sequences are recognized by the RTA software (versions 1.8 and later) and isolated from the sample data. The presence of these controls indicates that its corresponding step was successful. If a step was unsuccessful, the control sequences will be substantially reduced. QC controls assist in comparison between experiments and greatly facilitate troubleshooting.

AATGATAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTA
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CGTACCATTAAGAGCTACCGTGCAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTGCAACGACGAAAAGAATGATAACA
AATGATAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTA
TTACTTGATCCACTGATTCAACGTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAGCAACGACGAA
TATCAATTGAGACTAAATATTAACGTACCATTAAGAGTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTGCAACGAAAAGAATGATAACAGTAAC

Kit Specifications

	TruSeq DNA LT Kit	TruSeq DNA HT Kit
Recommended sample throughput	< 48 samples	> 48 samples
Available indices	24 single indices	96 dual-index combinations
Kit configuration for indices	Individual tubes	Pre-loaded 96-well plate
DNA input	1 µg	1 µg
Library preparation time	8 hours	8 hours
Compatible Illumina sequencers	HiSeq®, HiScanSQ™, Genome Analyzer™, and MiSeq® systems	

Designed For Automation

The TruSeq DNA Library Preparation Kits are compatible with high-throughput, automated processing workflows. Library preparation can be performed in standard 96-well microplates with master-mixed reagent pipetting volumes optimized for liquid-handling robots. Barcodes on reagents and plates allow end-to-end sample tracking and ensure that the correct reagents are used for the correct protocol, mitigating potential tracking errors.

Summary

TruSeq DNA Library Preparation Kits provide simplicity, convenience, and trusted results for library preparation. The kits feature an optimal indexing solution for either high-throughput or low-throughput studies. Master-mixed reagents and a simple, automation-friendly workflow minimize hands-on time and reduce human error. TruSeq DNA Library Prep kits are ideal for virtually any DNA sequencing application, providing researchers a single solution for generating the highest-quality data for any study.

Ordering Information

Product	Catalog No.
For DNA Preparation	
TruSeq DNA Library Prep LT, Set A (48 samples)	FC-121-2001
TruSeq DNA Library Prep LT, Set B (48 samples)	FC-121-2002
TruSeq DNA Library Prep Kit HT (96 Samples; Index Plate)	FC-121-2003
For Cluster Generation on cBot and Sequencing on a HiSeq or HiScanSQ System	
TruSeq Paired-End Cluster Kit v3—cBot—HS (one flow cell)	PE-401-3001
TruSeq Single-Read Cluster Kit v3—cBot—HS (one flow cell)	GD-401-3001
For Cluster Generation on cBot and Sequencing on the Genome Analyzer IIx	
TruSeq Paired-End Cluster Kit v2—cBot—GA (one flow cell)	PE-300-2001
TruSeq Single-Read Cluster Kit v2—cBot—GA (one flow cell)	GD-300-2001
For Cluster Generation on the Cluster Station and Sequencing on the Genome Analyzer IIx	
TruSeq Paired-End Cluster Kit v5—CS—GA (one flow cell)	PE-203-5001
TruSeq Single-Read Cluster Kit v5—CS—GA (one flow cell)	GD-203-5001

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