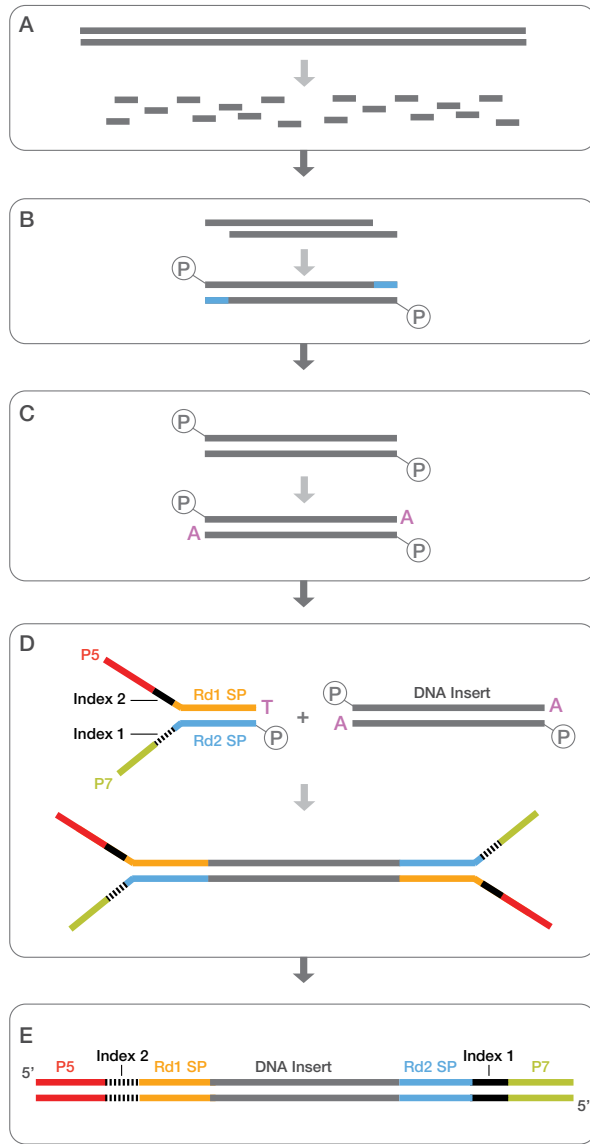


Figure 2: Dual-Index Library Preparation Using the TruSeq DNA HT Library Prep Kit



- 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.

