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# TruSeq™ RNA and DNA Sample Preparation Kits v2

Master-mixed reagents, optimized adapter design, and a flexible workflow provide a simple, cost-effective method for preparing RNA and DNA samples for scalable next-generation sequencing.

**Highlights**

- **Simple Workflow for RNA and DNA:** Master-mixed reagents and minimal hands-on steps.
- **Scalable and Cost-Effective Solution:** Optimized formulations and plate-based processing enables large-scale studies at a lower cost.
- **Enhanced Multiplex Performance:** Twenty-four adaptor-embedded indexes enable high-throughput processing and greater application flexibility.
- **High-Throughput Gene Expression Studies:** Gel-free, automation-friendly RNA sample preparation for rapid expression profiling.

## Introduction

Illumina next-generation sequencing (NGS) technologies continue to evolve, offering increasingly higher output in less time. Keeping pace with these developments requires improvements in sample preparation. To maximize the benefits of NGS and enable delivery of the highest data accuracy, Illumina offers the TruSeq RNA and DNA Sample Preparation Kits (Figure 1).

The TruSeq RNA and DNA Sample Preparation Kits provide a simple, cost-effective solution for generating libraries from total RNA or genomic DNA that are compatible with Illumina's unparalleled sequencing output. Master-mixed reagents eliminate the majority of pipetting steps and reduce the amount of clean-up, as compared to previous methods, minimizing hands-on time. New automation-friendly workflow formats enable parallel processing of up to 96 samples. This results in economical, high-throughput RNA or DNA sequencing studies achieved with the easiest-to-use sample preparation workflow offered by any NGS platform.

## Simple and Cost-Effective Solution

Whether processing samples for RNA-Seq, genomic sequencing, or exome enrichment, the TruSeq kits provide significantly improved library preparation over previously used methods. New protocols reduce the number of purification, sample transfer, and pipetting steps. The new universal, methylated adaptor design incorporates an index sequence at the initial ligation step for improved workflow efficiency and more robust multiplex sequencing. For maximum flexibility, the same TruSeq kit can be used to prepare samples for single-read, paired-end, and multiplexed sequencing on all Illumina sequencing instruments.

TruSeq DNA and RNA Sample Prep kits include gel-free protocols that eliminate the time-intensive gel purification step found in other methods, making the process more consistent and fully automatable.

The gel-free protocol for TruSeq DNA sample preparation is available for target enrichment using the TruSeq Exome Enrichment or TruSeq Custom Enrichment kits.

TruSeq sample preparation makes RNA sequencing for high-throughput experiments more affordable, enabling gene expression profiling studies to be performed with NGS at a lower cost than arrays. It also provides a cost-effective DNA sequencing solution for large-scale whole-genome resequencing, targeted resequencing, *de novo* sequencing, metagenomics, and methlyation studies.

## Enhanced Multiplex Performance

TruSeq kits take advantage of improved multiplexing capabilities to increase throughput and consistency, without compromising results. Both the RNA and DNA preparation kits include adapters containing unique index sequences that are ligated to sample fragments at the beginning of the library construction process. This allows the samples to be pooled and then individually identified during downstream analysis. The result is a more efficient, streamlined workflow that leads directly into a superior multiplexing solution. There are no additional PCR steps required for index incorporation, enabling a robust, easy-to-follow procedure. With 24 unique indexes available, up to 384 samples can be processed in parallel on a single HiSeq 2000 run.

## TruSeq RNA Sample Preparation

With TruSeq reagents, researchers can quickly and easily prepare samples for next-generation sequencing (Figure 2). Improvements in the RNA to cDNA conversion steps have significantly enhanced the overall workflow and performance of the assay (Figure 3).

**Figure 1: TruSeq Sample Preparation Kits**

TruSeq Sample Preparation Kits are available for both genomic DNA and RNA samples.

AGAATGATAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAAC
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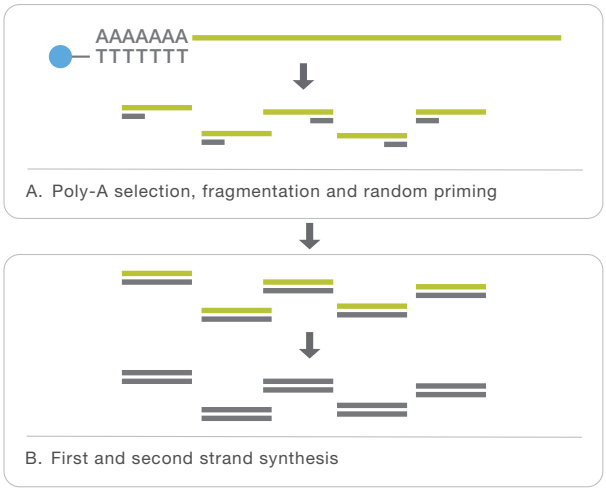
**Figure 2: Optimized TruSeq RNA Sample Preparation**




Starting with total RNA, mRNA is polyA-selected and fragmented. It then undergoes first- and second-strand synthesis to produce products ready for library construction (Figure 4).

Starting with total RNA, the messenger RNA is first purified using polyA selection (Figure 2A), then chemically fragmented and converted into single-stranded cDNA using random hexamer priming. Next, the second strand is generated to create double-stranded cDNA (Figure 2B) that is ready for the TruSeq library construction workflow (Figure 4).

Efficiencies gained in the polyA selection process, including reduced sample transfers, removal of precipitation steps, and combining of elution and fragmentation into a single step, enable parallel processing of up to 48 samples in approximately one hour. This represents a 75% reduction in hands-on time for this portion of library construction. Improving performance, the optimized random hexamer priming strategy provides the most even coverage across transcripts, while allowing user-defined adjustments for longer or shorter insert lengths.

Eliminating all column purification and gel selection steps from the workflow removes the most time-intensive portions, while improving the assay robustness. It also allows for decreased input levels of RNA—as low as 100 ng— and maintains single copy per gene sensitivity.

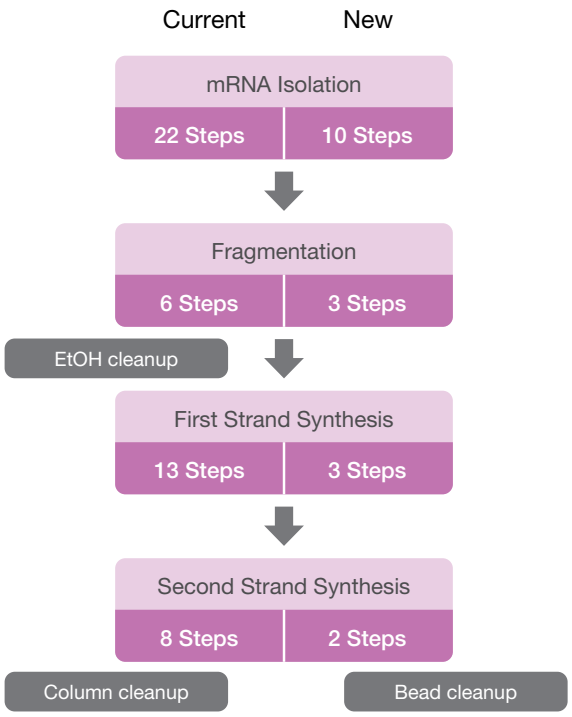
## TruSeq DNA Sample Preparation

The TruSeq DNA Sample Preparation Kits are used to prepare DNA libraries with insert sizes from 300–500 bp for single, paired-end, and multiplexed sequencing. The protocol supports shearing by either sonication or nebulization with a low input requirement of 1 ug of DNA.

## Sequence-Ready Libraries

Library construction begins with either double-stranded cDNA synthesized from RNA or fragmented gDNA (Figure 4A). Blunt-end DNA fragments are generated using a combination of fill-in reactions and exonuclease activity (Figure 4B). An 'A'- base is then added to the blunt ends of each strand, preparing them for ligation to the sequencing adapters (Figures 4C). Each adapter contains a 'T'-base overhang on 3'-end providing a complementary overhang for ligating the adapter

**Figure 3: TruSeq RNA Sample Preparation Reagents Provide Significant Savings in Time and Effort**




| | Current Methods | TruSeq Methods | Savings |
|---|---|---|---|
| No. of Steps | 49 | 18 | 31 |
| Time (hours) | 16 | 12 | 25% |

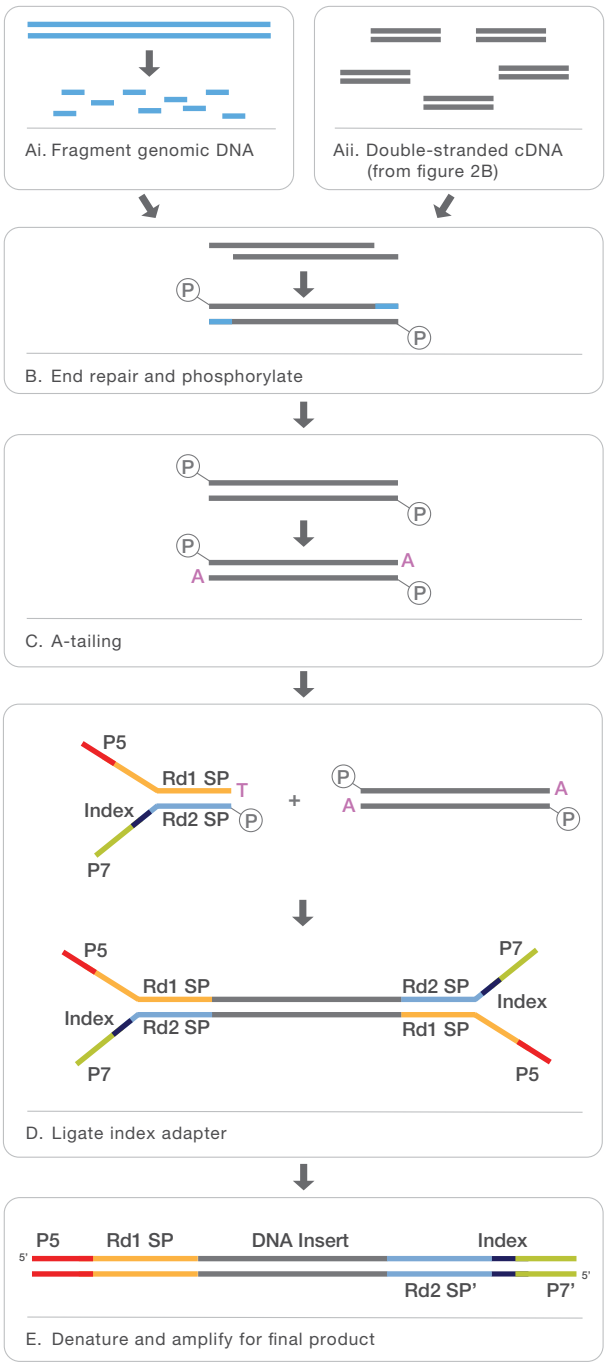
Compared to current methods for preparing mRNA samples for sequencing, use of the TruSeq reagents significantly reduces the number of steps and hands-on time.

**Table 1: Savings When Processing 96 Samples**

| |
|---|
| > 50% of pipetting steps eliminated |
| > 50% of reagent tubes eliminated |
| > 75% of clean-up steps eliminated |
| > 50% of sample transfer steps eliminated |

Compared to previous kits, processing multiple samples with the new TruSeq Sample Preparation Kits provides significant reductions in library construction costs, the number of steps, hands-on time, and PCR dependency.

**Figure 4: Adapter Ligation Results in Sequence-Ready Constructs without PCR**




Library construction begins with either fragmented genomic DNA or double-stranded cDNA produced from total RNA (Figure 4A). Blunt-end fragments are created (Figure 4B) and an A-base is then added (Figure 4C) to prepare for indexed adapter ligation (Figure 4D). Final product is created (Figure 4E), which is ready for amplification on either the cBot or the Cluster Station.

to the A-tailed fragmented DNA. These newly redesigned adapters contain the full complement of sequencing primer hybridization sites for single, paired-end, and multiplexed reads. This eliminates the need for additional PCR steps to add the index tag and multiplex primer sites (Figure 4D). Following the denaturation and amplification steps (Figure 4E), libraries can be pooled with up to 12 samples per lane (96 sample per flow cell) for cluster generation on either cBot or the Cluster Station.

Master-mixed reagents and an optimized protocol improve the library construction workflow, significantly decreasing hands-on time and reducing the number of clean-up steps when processing samples for large-scale studies (Table 1). The simple and scalable workflow allows for high-throughput and automation-friendly solutions, as well as simultaneous manual processing for up to 96 samples. In addition, enhanced troubleshooting features are incorporated into each step of the workflow, with quality control sequences supported by Illumina RTA software.

## Enhanced Quality Controls

Specific Quality Control (QC) sequences, consisting of double-stranded DNA fragments, are present in each enzymatic reaction of the TruSeq sample 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.

## Designed For Automation

The TruSeq Sample Preparation Kits are compatible with high-throughput, automated processing workflows. Sample 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.

## Part of an Integrated Sequencing Solution

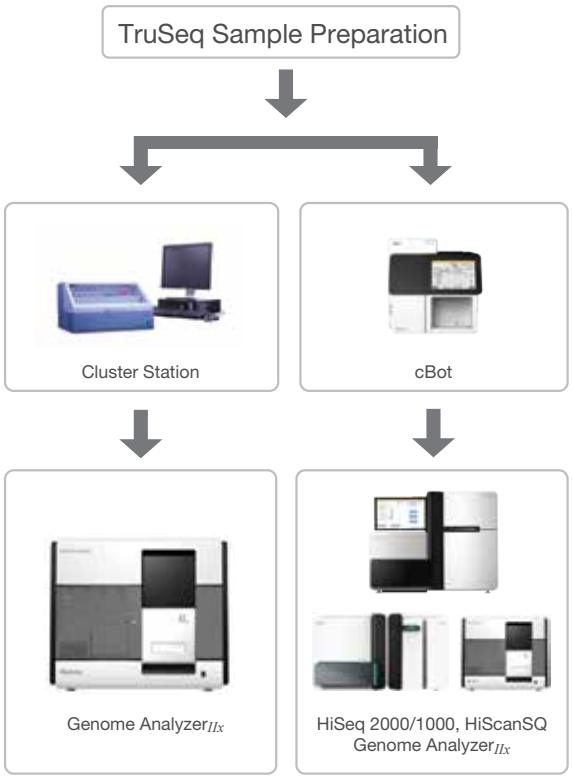
Samples processed with the TruSeq Sample Preparation Kits can be amplified on either the cBot Automated Cluster Generation System or the Cluster Station and used with any of Illumina's next-generation sequencing instruments, including HiSeq™ 2000, HiSeq 1000, HiScan™SQ, Genome Analyzer$_{IIx}$ (Figure 5).

## Summary

Illumina's new TruSeq Sample Preparation Kits enable simplicity, convenience, and affordability for library preparation. Enhanced multiplexing with 24 unique indexes allows efficient high-throughput processing. The pre-configured reagents, streamlined workflow, and automation-friendly protocol save researchers time and effort in their next-generation sequencing pursuits, ultimately leading to faster discovery and publication.

Learn more about Illumina's next-generation sequencing solutions at www.illumina.com/sequencing.

## Figure 5. Illumina's Complete Sequencing Solution




The TruSeq Sample Preparation Kits readily fit in with Illumina's advanced next-generation sequencing solutions.

## Ordering Information

| Product | Catalog No. |
|---|---|
| **For RNA Preparation** | |
| TruSeq RNA Sample Preparation Kit v2, Set A (12 indexes, 48 samples) | RS-122-2001 |
| TruSeq RNA Sample Preparation Kit v2, Set B (12 indexes, 48 samples) | RS-122-2002 |
| **For DNA Preparation** | |
| TruSeq DNA Sample Preparation Kit v2, Set A (12 indexes, 48 samples) | FC-121-2001 |
| TruSeq DNA Sample Preparation Kit v2, Set B (12 indexes, 48 samples) | FC-121-2002 |
| **For Cluster Generation on cBot and Sequencing on the HiSeq 2000/1000 and HiScanSQ** | |
| TruSeq Paired-End Cluster Kit v3—cBot—HS (1 flow cell) | PE-401-3001 |
| TruSeq Single-Read Cluster Kit v3—cBot—HS (1 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 (1 flow cell) | PE-300-2001 |
| TruSeq Single-Read Cluster Kit v2—cBot—GA (1 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 (1 flow cell) | PE-203-5001 |
| TruSeq Single-Read Cluster Kit v5—CS—GA (1 flow cell) | GD-203-5001 |

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