Clontech Laboratories, Inc.

# Low Input Library Prep Kit v2 User Manual

Cat. # 634899
(061616)

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# Table of Contents



# Table of Figures



# Table of Tables

# I. Introduction

The Low Input Library Prep Kit v2 provides a highly sensitive, simple, fast (2 hour), and robust protocol for converting up to 12 samples of fragmented, double-stranded DNA (dsDNA) into a sequencing-ready library for the Illumina® platform. It utilizes a single-tube, three-step protocol for each barcoded sample and eliminates intermediate purification steps. The Low Input Library Prep Kit v2 enables efficient ligation-based library preparation from 50 pg to 20 ng of starting dsDNA.

Use the Low Input Library Prep Kit v2 to convert the cDNA output from SMARTer® Ultra® Low kits or the SMARTer Universal Low Input RNA Kit for Sequencing into sequencing templates suitable for cluster generation and high-throughput sequencing.

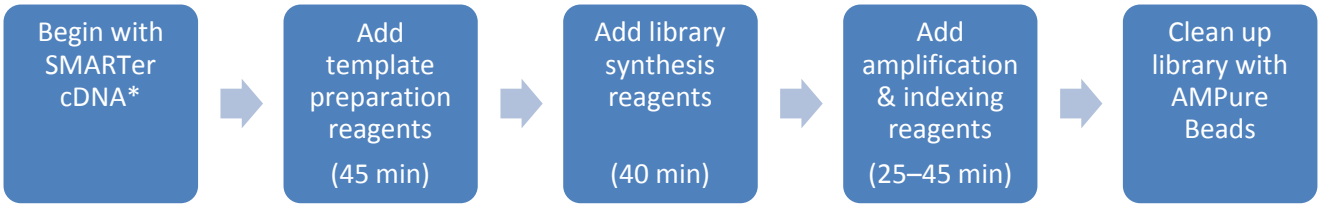


**Figure 1. The Low Input Library Prep Kit v2 workflow takes approximately two hours.** *Begin with Rsa I-digested SMARTer cDNA (from the SMARTer Universal Low Input RNA Kit) or Covaris-sheared SMARTer cDNA (from the SMARTer Ultra Low kits).

# II. Low Input Library Prep Kit v2 Specifications

## A. List of Components

### Low Input Library Prep Kit v2 (12 rxns; Cat. # 634899)

| Vol | Component Name | Cap Color |
|---|---|---|
| 24 µl | Template Preparation Buffer | Red |
| 12 µl | Template Preparation Enzyme | Red |
| 12 µl | Library Synthesis Buffer | Yellow |
| 12 µl | Library Synthesis Enzyme | Yellow |
| 300 µl | Library Amplification Buffer | Green |
| 12 µl | Library Amplification Enzyme | Green |
| 48 µl | Nuclease-Free Water | Clear |
| 40 µl | Indexing Reagent 1 | Blue |
| 40 µl | Indexing Reagent 2 | Blue |
| 40 µl | Indexing Reagent 3 | Blue |
| 40 µl | Indexing Reagent 4 | Blue |
| 40 µl | Indexing Reagent 5 | Blue |
| 40 µl | Indexing Reagent 6 | Blue |
| 40 µl | Indexing Reagent 7 | Blue |
| 40 µl | Indexing Reagent 8 | Blue |
| 40 µl | Indexing Reagent 9 | Blue |
| 40 µl | Indexing Reagent 10 | Blue |
| 40 µl | Indexing Reagent 11 | Blue |
| 40 µl | Indexing Reagent 12 | Blue |

## B. Index Sequences

Low Input Library Prep Kit v2 single indexes use Illumina-compatible 8-nt sequences developed by the Wellcome Trust Sanger Institute in Cambridge, UK. Each Indexing Reagent Tube contains a unique single index sequence. The Low Input Library Prep Kit v2 single indexes share the same sequences in the first six bases (in bold) as the Illumina TruSeq® LT indexes AD001 through AD012 (Table 1).

**Table I. Index Sequences Barcode Key**

| Index | Sequence | TruSeq LT Index | TruSeq LT Sequence |
|---|---|---|---|
| 1 | **ATCACG**TT | AD001 | ATCACG |
| 2 | **CGATGT**TT | AD002 | CGATGT |
| 3 | **TTAGGC**AT | AD003 | TTAGGC |
| 4 | **TGACCA**CT | AD004 | TGACCA |
| 5 | **ACAGTG**GT | AD005 | ACAGTG |
| 6 | **GCCAAT**GT | AD006 | GCCAAT |
| 7 | **CAGATC**TG | AD007 | CAGATC |
| 8 | **ACTTGA**TG | AD008 | ACTTGA |
| 9 | **GATCAG**CG | AD009 | GATCAG |
| 10 | **TAGCTT**GT | AD010 | TAGCTT |
| 11 | **GGCTAC**AG | AD011 | GGCTAC |
| 12 | **CTTGTA**CT | AD012 | CTTGTA |

## C. Storage and Handling of Kit Components

- Store the Low Input Library Prep Kit v2 at –20°C.
- Immediately prior to use, spin down the Template Preparation Enzyme, Library Synthesis Enzyme, and Library Amplification Enzyme tubes and place them on ice.
- Thaw all other components on ice, vortex briefly, and spin down prior to use.

## D. Additional Materials Required

The following reagents are required but not supplied. These materials have been validated to work with this protocol. Please do not make any substitutions because you may not obtain the expected results.

**General Materials**

- Single-channel pipette: 10 µl, 20 µl, and 200 µl; one each
- Eight-channel pipette: 20 µl and 200 µl; one each
- Filter pipette tips: 10 µl, 20 µl, and 200 µl; one 96-tip box each
- 8-strip nuclease-free 0.2-ml thin-wall PCR tubes with caps

**SPRI Bead Purification Materials**

- Agencourt AMPure PCR Purification Kit
  (5-ml kit: Beckman Coulter, Part No. A63880; 60 ml kit: Beckman Coulter, Part No. A63881)
  Use this kit for the SPRI Purifications (Section III.D)
- Magnetic Stand-96 (Ambion, Part No. AM10027)
  Use this stand for the second purification (Section III.D)
- 96-Well V-bottom Plate (500 µl) (VWR, Cat. No. 47743-996)
- MicroAmp Clean Adhesive Seal (Applied Biosystems, Part No. 4306311)

- 80% ethanol
- Purification Buffer [included in the SMARTer Universal Low Input RNA Kit for Sequencing (Cat. # 634938 & 634940), the SMARTer Ultra Low RNA Kit for Illumina Sequencing (Cat. # 634936), and the SMARTer Ultra Low Input RNA for Illumina Sequencing - HV (Cat. # 634820, 634823, 634826, 634828 & 634830)] or Elution Buffer [included in the SMARTer Ultra Low Input Kit for Sequencing - v3 (Cat. Nos. 634854, 634855, 634856 & 634857) and the SMART-Seq® v4 Ultra Low Input RNA Kit for Sequencing (Cat. # 634888, 634889, 634890, 634891, 634892, 634893 & 634894)]

## E. Applications

- Singleplex or multiplex next-generation sequencing on Illumina systems
- Compatible with both single-read and paired-end sequencing

This kit is **not** recommended for use with:

- Greater than 20 ng input DNA per reaction
- Single-stranded DNA or RNA

## F. Input DNA Sample Requirements

| | Optimal | Not Recommended |
|---|---|---|
| **Nucleic Acid Type** | Fragmented, double-stranded DNA or cDNA | RNA or single-stranded DNA |
| **Input Length** | 100–600 bp | >1,000 bp |
| **Input Volume** | 10 µl | >10 µl |
| **Input Quantity** | 50 pg to 20 ng dsDNA | >20 ng dsDNA |

# III. Low Input Library Prep Kit v2 Preparation Protocol

## A. Protocol: Template Preparation

1. Add 10 µl of each DNA sample to a PCR tube or well.

   If you wish to run a negative control in parallel with your samples, substitute 10 µl nuclease-free $H_2O$ for the DNA sample in one tube.

2. In a separate tube, prepare the Template Preparation Premix. Combine the following reagents in the order specified. Gently pipette up and down to incorporate.

| | |
|---|---|
| 2 µl | Template Preparation Buffer (red cap) |
| 1 µl | Template Preparation Enzyme (red cap) |
| 3 µl | Total Volume (per well) |

3. Add 3 µl of Template Preparation Premix to each DNA sample. Mix by pipetting 4–5 times with a pipette set to 8 µl. Spin down to collect the liquid at the bottom of the tube or plate.

4. Incubate samples in a thermal cycler using the following sequential conditions:

| | |
|---|---|
| 22°C | 25 min |
| 55°C | 20 min |
| 22°C | hold until next addition (Section III.B, Step 2)* |

* We recommend holding the sample at 22°C for less than 10 min before adding the Library Synthesis Premix.

## B. Protocol: Library Synthesis

1. In a separate tube, prepare the Library Synthesis Premix. Combine the following reagents in the order specified. Gently pipette up and down to incorporate.

| | |
|---|---|
| 1 µl | Library Synthesis Buffer (yellow cap) |
| 1 µl | Library Synthesis Enzyme (yellow cap) |
| 2 µl | Total Volume (per well) |

2. Add 2 µl of Library Synthesis Premix to each sample (Section III.A, Step 4). Mix by pipetting 4–5 times with a pipette set to 10 µl. Spin down to collect the liquid at the bottom of the tube or plate.

3. Incubate the samples in a thermal cycler using the following conditions:

| | |
|---|---|
| 22°C | 40 min |
| 4°C | hold until next addition (Section III.C, Step 2) |

## C. Protocol: Library Amplification

1. In a separate tube, prepare the Library Amplification Premix. Combine the following reagents in the order specified. Gently pipette up and down to incorporate.

| | |
|---|---|
| 4 µl | Nuclease-Free Water (clear cap) |
| 25 µl | Library Amplification Buffer (green cap) |
| 1.0 µl | Library Amplification Enzyme (green cap) |
| 30 µl | Total Volume (per well) |

2. Add 30 µl of Library Amplification Premix to each library (Section III.B, Step 3).

3. Add 5 µl of the desired Indexing Reagent 1–12 to each sample to be barcoded. Mix 3–4 times with a pipette set to 40 µl. Spin down to collect the liquid at the bottom of the tube or plate.

4. Transfer the PCR tubes or plate to a preprogrammed thermal cycler and incubate as follows:

| | |
|---|---|
| 72°C | 3 min |
| 85°C | 2 min |
| 98°C | 2 min |
| 4 cycles: | |
| 98°C | 20 sec |
| 67°C | 20 sec |
| 72°C | 40 sec |
| 4–10* cycles: | |
| 98°C | 20 sec |
| 72°C | 50 sec |

*Number of cycles depends on the amount of DNA. Refer to Table II.

Table II provides guidelines for the number of PCR cycles necessary for optimal amplification, depending on the input quantities of different biological samples. This table is intended to serve only as a baseline for approximation due to the large variation in molecular weight and quality of biological samples.

**Table II. Recommended Number of Amplification Cycles for Different Amounts of Input DNA**

| Input DNA (ng) | Amplification Cycles* | Output DNA (ng) Per 50 µl Reaction |
|---|---|---|
| 2 | 6 | 15–50 |
| 1 | 7 | 15–30 |
| 0.5 | 8 | 15–30 |
| 0.1 | 11 | 20 |

* See PCR cycling information on the previous page (Section III.C.4). The four amplification cycles in Step 4 are not included in this chart.

## D. Protocol: Library Purification Using SPRI AMPure Beads

1. Cover all the wells of a 96-well Axygen V-bottom plate with a MicroAmp Clear Adhesive Seal. Use a razor blade or scalpel to uncover only the wells that you want to use. Vortex SPRI AMPure Beads until evenly suspended, then add 75 µl of beads to each well that you are using on the 96-well plate.
2. Transfer the entire PCR product from Section III.C, Step 4 to the wells of the plate containing the SPRI beads. Pipette the entire volume up and down 10 times to mix thoroughly. Incubate at room temperature for 8 min to let the DNA bind to the beads.

   **NOTE:** The beads are viscous; pipette the entire volume up, and push it out slowly.

3. Place the 96-well plate on the Ambion Magnetic Stand-96 for ~5 min or longer (until the liquid appears completely clear and there are no beads left in the supernatant).
4. Leave the plate on the magnetic stand and pipette out the supernatant.
5. While the plate is still on the magnetic stand, add 200 µl of freshly made 80% ethanol to each sample (without disturbing the beads) to wash away contaminants. Wait 30 sec and carefully pipette out the supernatant. The double-stranded cDNA will remain bound to the beads during the wash process.
6. Repeat Step 5.
7. Seal the sample wells on the plate and briefly spin down for 10 sec at 1,000 rpm to collect the liquid at the bottom of the well.
8. Set the plate on the magnetic stand for 30 sec, then remove all of the remaining ethanol.
9. Let the plate rest at room temperature for ~3–5 min, until the pellet looks dry. You may see a tiny crack in the pellet.

   **NOTE:** If you over-dry the beads, you will see many cracks in the pellet. If you under-dry the beads, the DNA recovery rate will be lower due to the remaining ethanol.

10. Once the beads are dried, add 12 µl of Purification Buffer [included in the SMARTer Universal Low Input RNA Kit for Sequencing (Cat. # 634938 & 634940), the SMARTer Ultra Low RNA Kit for Illumina Sequencing (Cat. # 634936), and the SMARTer Ultra Low Input RNA for Illumina Sequencing - HV (Cat. # 634820, 634823, 634826, 634828 & 634830)] or Elution Buffer [included in the SMARTer Ultra Low Input Kit for Sequencing - v3 (Cat. # 634854, 634855, 634856, & 634857) and the SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing (Cat. # 634888, 634889, 634890, 634891, 634892, 634893 & 634894)] to each sample well in order to cover the beads. Remove the plate from the magnetic stand and incubate at room temperature for 2 min to rehydrate.

11. Mix the pellet by pipetting up and down 10 times to elute the cDNA from the beads. Then put the plate back on the magnetic stand for 1 min or longer, until the solution is completely clear.
12. Transfer the clear supernatant containing your purified cDNA library from each well to a nuclease-free, non-sticky tube.

## E. Protocol: Library Quantification

cDNA libraries produced with the Low Input Library Prep Kit v2 can be quantified using the Quant-iT PicoGreen dsDNA kit (Life Technologies), the Agilent Bioanalyzer, or Qubit (Life Technologies). The Library Quantification Kit (Cat. # 638324) can also be used for library quantification.

## F. Protocol: Pooling Libraries Prepared with Separate Indexes

After performing library quantification, pool the library products at the desired molar ratio for sequencing. If samples will be pooled, they must have separate indexes (Table I, Section III).

## G. Protocol: Sequencing Low Input Library Prep Kit v2 Libraries

The Low Input Library Prep Kit v2 generates libraries ready for cluster amplification and sequencing on the Illumina Genome Analyzer™, HiSeq®, NextSeq®, or MiSeq® platforms using standard Illumina reagents and protocols for multiplexed libraries. Follow Illumina loading recommendations.

| Contact Us For Assistance | |
|---|---|
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## Notice to Purchaser

RUBICON GENOMICS

The Low Input Library Prep Kit contains ThruPLEX technology developed and manufactured by Rubicon Genomics, Inc., Ann Arbor, Michigan, USA and protected by US Patent 7,803,550 and others.



This document has been reviewed and approved by the Clontech Quality Assurance Department.