Clontech Laboratories, Inc.

Low Input Library Prep Kit User Manual

Cat. Nos. 634947, 634945, & 634946
(010516)

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I. Introduction

The Low Input Library Prep Kit provides a highly sensitive, simple, fast (2 hr), 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 enables efficient ligation-based library preparation from 50 pg to 20 ng of starting dsDNA.

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

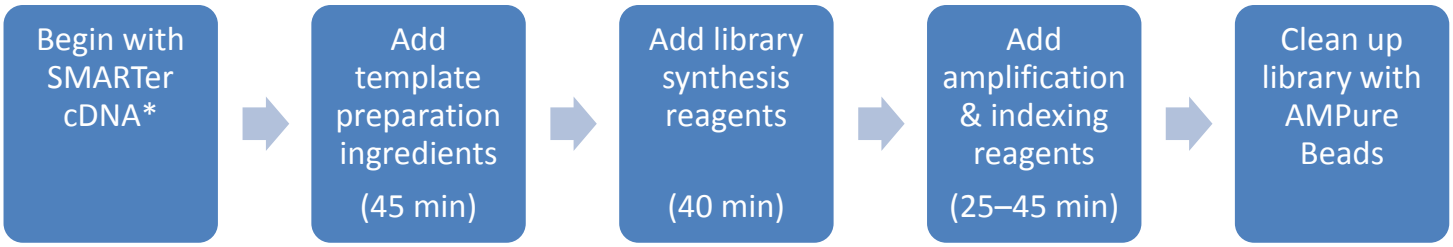

Figure 1. The Low Input Library Prep Kit workflow takes approximately 2 hours. *Begin with Rsa I-digested SMARTer cDNA (from the SMARTer Universal Low Input RNA Kit) or Covaris-sheared SMARTer cDNA (from the SMARTer Ultra Low RNA Kit for Illumina Sequencing).

II. Low Input Library Prep Kit Specifications

A. List of Components

Low Input Library Prep Kit (12 rxns; Cat. No. 634947)

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
582 µl	Library Amplification Buffer	Green
18 µl	Library Amplification Enzyme	Green
96 µl	Nuclease-Free Water	Clear
24 µl	Indexing Reagent 1	Violet
24 µl	Indexing Reagent 2	Violet
24 µl	Indexing Reagent 3	Violet
24 µl	Indexing Reagent 4	Violet
24 µl	Indexing Reagent 5	Violet
24 µl	Indexing Reagent 6	Violet
24 µl	Indexing Reagent 7	Violet
24 µl	Indexing Reagent 8	Violet
24 µl	Indexing Reagent 9	Violet
24 µl	Indexing Reagent 10	Violet
24 µl	Indexing Reagent 11	Violet
24 µl	Indexing Reagent 12	Violet

B. Index Sequences*

Table 1. Index Sequences Barcode Key

Index	Barcode	Index	Barcode
1	ATCACG	**7**	CAGATC
2	CGATGT	**8**	ACTTGA
3	TTAGGC	**9**	GATCAG
4	TGACCA	**10**	TAGCTT
5	ACAGTG	**11**	GGCTAC
6	GCCAAT	**12**	CTTGTA

* These index sequences correspond to Illumina index sequences for multiplexing.

C. Storage and Handling of Kit Components

- Store the Low Input Library Prep Kit 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:

- 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 box each
- One bag of 8-strip nuclease-free 0.2 ml thin wall PCR tubes with caps

For SPRI Bead Purification:

- 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 (Protocol III.D)
- Magnetic Stand-96 (Ambion Part No. AM10027)
  Use this stand for the second purification (Protocol 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. Nos. 634938 & 634940), the SMARTer Ultra Low RNA Kit for Illumina Sequencing (Cat. No. 634936), and the SMARTer Ultra Low Input RNA for Illumina Sequencing - HV (Cat. Nos. 634820, 634823, 634826, 634828, & 634830)]

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
Molecular Weight	100–600 bp	> 1,000 bp
Input Volume	10 µl	> 10 µl
Input Quantity	50 pg to 20 ng ds DNA	> 20 ng ds DNA

III. Low Input Library Prep Kit 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

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 (Protocol 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

2. Add 2 µl of Library Synthesis Premix to each sample. 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
4ºC	hold until next addition (Protocol 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.

8 µl	Nuclease-Free Water (clear cap)
48.5 µl	Library Amplification Buffer (green cap)
1.5 µl	Library Amplification Enzyme (green cap)
58 µl	Total Volume

2. Add 58 µl of Library Amplification Premix to each library.
3. Add 2 µl of the desired Indexing Reagent 1–12 to each sample to be barcoded. Mix 3–4 times with a pipette set to 50 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 2.

Table 2 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 2. Recommended Number of Amplification Cycles for Different Amounts of Input DNA

Input DNA (ng)	Amplification Cycles*	Output DNA (ng) Per 75 µl Reaction
2	5	15–50
1	6	15–30
0.5	7	15–30
0.1	10	20

* See PCR cycling information directly above (Protocol III.C.5). The four amplification cycles in Step C.5 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 even, 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 Protocol III.C. 5 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; suck 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 for 30 sec and carefully pipette out the supernatant. The ds 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, and then remove all the remaining ethanol.
9. Let the plate rest at room temperature for ~3–5 min, until the pellet appears 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. Nos. 634938 & 634940), the SMARTer Ultra Low RNA Kit for Illumina Sequencing (Cat. No. 634936), and the SMARTer Ultra Low Input RNA for Illumina Sequencing – HV kits (Cat. Nos. 634820, 634823, 634826, 634828, & 634830)] or Elution Buffer [included in SMARTer Ultra Low Input RNA for Illumina Sequencing - HV kits (Cat. Nos. 634820, 634823, 634826, 634828, & 634830) and SMART-Seq® v4 Ultra Low Input RNA Kit for Sequencing (Cat. Nos. 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, nonsticky tube.

E. Protocol: Library Quantification

cDNA libraries produced with the Low Input Library Prep Kit can be quantified using the Quant-iT PicoGreen dsDNA kit (Life Technologies), Agilent BioAnalyzer, Nanodrop (Thermo Scientific), Qubit (Life Technologies), or a similar UV absorption- or fluorescence-based protocol.

NOTE: To avoid interference, make sure that cDNA libraries are purified away from primers and proteins (see Protocol III.D) before pooling them at the desired molar ratio as described in Protocol III.F.

F. Protocol: Pooling Libraries Prepared with Separate Indices

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

G. Protocol: Sequencing Low Input Library Prep Kit Libraries

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

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Customer Service/Ordering	**Technical Support**
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Notice to Purchaser

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