

SOLiD™ RNA Barcoding Kit, Module 17–32

Insert PN 4452443 Rev. B

Component	Volume†	Cap color	Storage conditions
SOLiD™ 5′ PCR Primer	200 μL	White	Store all kit components at –20 °C. Do not store in a frost-free freezer.
SOLiD™ 3′ Primers: BC017–032	12 μL each	Blue	

† Sufficient 5′ and 3′ primers are supplied for the preparation of 3 cDNA libraries per barcoded primer, for a total of 48 libraries.



Note: For all components, read the Safety Data Sheet (SDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

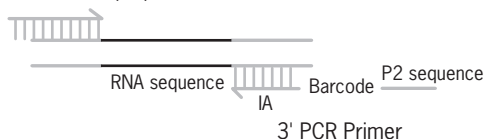
Product information

The SOLiD™ RNA Barcoding Kit, Module 17-32 (PN 4453189) is a set of PCR primers that are designed for use with the SOLiD™ Total RNA-Seq Kit (PN 4445374) to enable multiplex SOLiD™ System sequencing of libraries prepared from whole transcriptome or small RNA samples.

The 5′ PCR Primer in this kit is identical to that provided in the SOLiD Total RNA-Seq Kit, and its sequence corresponds to SOLiD emulsion PCR primer P1.

Sixteen 3′ (reverse) PCR primers are included in the SOLiD RNA Barcoding Kit, Module 17-32. Each SOLiD 3′ Primer contains the P2 sequence required for SOLiD emulsion PCR, a unique barcode sequence, and an internal adaptor (IA) sequence necessary for sequencing the barcode (Figure 1). Use the barcoded 3′ PCR primers from this kit in the cDNA amplification step of the SOLiD Total RNA-Seq Kit library preparation procedures to generate barcoded cDNA libraries that can be mixed for multiplex SOLiD System sequencing.

Figure 1 cDNA library amplification with SOLiD™ RNA Barcoding Kit primers
5′ PCR Primer (P1)



Preparation of barcoded RNA libraries for multiplex SOLiD™ System sequencing

Overview

Barcoded RNA libraries are generated at the cDNA amplification step in the whole transcriptome and small RNA library preparation procedures in the SOLiD Total RNA-Seq Kit protocol. Each barcoded library is handled separately through purification and completion of the library preparation procedure. Barcoded libraries are ready to be combined into a multiplex sequencing pool just prior to templated bead preparation by emulsion PCR.

cDNA library amplification

Preserving color balance in multiplex SOLiD™ System sequencing

Plan your experiments to include multiples of four barcoded libraries in every multiplex sequencing pool, to preserve color balance for the SOLiD System sequencing run. Color balance is the relative proportion of beads in a given cycle that are called as each of the four colors. For more information, refer to *Applied Biosystems SOLiD™ 4 System SETS User Guide* (PN 4448411).

The barcode sequences of the SOLiD 3′ Primers in the SOLiD RNA Barcoding Kit, Module 17-32 are optimized so that color balance is maintained within consecutive groups of four primers.

- For multiplex sequencing of four libraries, use one of these color-balanced groups of four barcoded primers: BC017–BC020, BC021–BC024, BC025–BC028, or BC029–BC032.
- To perform multiplex sequencing with eight libraries, use two different, color-balanced groups of four barcoded primers.
- Typically, use one barcoded primer for each sample, with duplicate in-gel PCRs per sample. However, you may need to use a different barcoded primer for each in-gel PCR to generate barcoded libraries in multiples of four. For example, if you have only two samples, use two different barcoded SOLiD 3′ Primers for each sample, one in each in-gel PCR.

Procedure

1. Follow the procedure for either whole transcriptome or small RNA library preparation described in the *SOLiD™ Total RNA-Seq Kit Protocol* (PN 4452437) through size selection of the cDNA.
2. At the step "Amplify the cDNA," substitute a barcoded SOLiD 3' Primer from this kit for the 3' PCR Primer provided with the SOLiD Total RNA-Seq Kit. (See "[Preserving color balance in multiplex SOLiD™ System sequencing](#)" on page 1.)
Use 2 µL of the SOLiD 3' Primer for each in-gel PCR reaction.

3. Continue the SOLiD Total RNA-Seq Kit library preparation procedure to completion.

Note: Do not combine different barcoded libraries during the SOLiD Total RNA-Seq Kit procedure, even if they are prepared from the same sample.

Pooling barcoded libraries for templated bead preparation

1. Using the library template molar concentration calculated as described in the *SOLiD™ Total RNA-Seq Kit Protocol*, combine barcoded libraries in color-balanced multiples of four at equimolar concentrations.
2. Follow the instructions for optimizing the emulsion PCR with the pooled library as described in the *SOLiD™ 4 System Templated Bead Preparation Guide* (PN 4448378).
Use starting multiplex sequencing pooled library template concentrations of 0.4 pM and 0.8 pM for workflow analysis (WFA) optimization of ePCR.

Limited Consumable Product Warranty

Life Technologies warrants the accompanying consumables will be free of defects in materials and workmanship for one year from the date of shipment.

Safety information

Obtaining SDSs

To obtain Safety Data Sheets for any chemical product supplied by Applied Biosystems or Ambion:

- At www.appliedbiosystems.com, select **Support** ▶ **MSDS**. Search by chemical name, product name, product part number, or MSDS part number. Right-click to print or download the MSDS of interest.
- At www.ambion.com, go to the web catalog page for the product of interest. Select **MSDS**, then right-click to print or download.
- E-mail (MSDS_Inquiry_CCRM@appliedbiosystems.com), telephone (650-554-2756; USA), or fax (650-554-2252; USA) your request, specifying the catalog or part number(s) and the name of the product(s). The associated SDSs will be e-mailed unless you request fax or postal delivery. Requests for postal delivery require 1 to 2 weeks for processing.



Note: For the SDSs of chemicals not distributed by Applied Biosystems or Ambion, contact the chemical manufacturer.

For research use only. Not intended for human or animal therapeutic or diagnostic use.

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Part Number 4452443 Rev. B 07/2010

