FFPE Small RNA Library Preparation for SOLiD™ Sequencing

Part Number MAN0003613 Rev. A

Introduction

This instruction sheet provides a ligation-based protocol for isolating small RNA sequences from formalin-fixed, paraffin-embedded (FFPE) tissue samples and converting them into templates suitable for sequencing on the SOLiD™ System.

First, you isolate total RNA from FFPE tissues using the Ambion RecoverAll™ kit and enrich the small RNA using the PureLink® miRNA Isolation Kit. Then you use the SOLiD™ Total RNA-Seq Kit to prepare libraries from the enriched miRNAs, followed by sequencing on the SOLiD™ System.

Materials Required

- RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE (Applied Biosystems or Invitrogen Part no. AM1975)
- PureLink® miRNA Isolation Kit (Invitrogen Part no. K1570-01)
- SOLiD™ Total RNA-Seq Kit (Applied Biosystems Part no. 4445374)
- RNase AWAY™ Reagent (Invitrogen Part no. 10328-011)
- 100% ethanol
- Clean 1.5-mL tubes

For RNA analysis

- RNA quantification method, such as the Qubit® Fluorometer (Invitrogen Part no. Q32866 or Q32867) with a Qubit™ RNA Assay Kit (Invitrogen Part no. Q32852 or Q32855) or UV absorbance on a NanoDrop® spectrophotometer
- RNA qualification method, such as a denaturing agarose gel or the Agilent® 2100 Bioanalyzer™ Instrument

Precautions

For all steps, to avoid RNase contamination, we recommend cleaning the lab bench and pipettors with RNase AWAY™ Reagent. Wear gloves and use RNase-free pipette tips for all procedures.

Extract Total RNA from FFPE Samples

Use the RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE to isolate total RNA from FFPE samples, following the protocol provided in the kit. The protocol is also available online at:
http://tools.invitrogen.com/content/sfs/manuals/fm_1975.pdf

Quantitate the total RNA using a Qubit® Fluorometer with a Qubit™ RNA Assay Kit or a NanoDrop® spectrophotometer, then proceed to **Enrich the Small RNA** below.

Enrich the Small RNA

The *SOLiD™ Total RNA-Seq Kit Protocol* includes a procedure in Appendix B entitled "Small RNA enrichment," using the PureLink® miRNA Isolation Kit. The protocol is available online at:
http://tools.invitrogen.com/content/sfs/manuals/4452437A.pdf

Follow the PureLink® small RNA enrichment procedure in that appendix, ***except*** in step 1.a note the change in red below:

1. Prepare the sample:
   a. Resuspend 1–10 µg of the total RNA from your FFPE sample in 90 µL Nuclease-free Water (instead of 5–50 µg).

Proceed with the rest of the procedure as written, and then proceed to **Determine the Yield of Enriched Small RNA** below.

Determine the Yield of Enriched Small RNA

Use a Qubit® Fluorometer with a Qubit™ RNA Assay Kit to quantitate the yield of the enriched small RNA. Refer to the Qubit™ RNA Assay Kit protocol and the Qubit® Fluorometer user guide for instructions.

Note: The typical yield after small RNA enrichment is about 15–30% of the input amount. The recovery rate can vary due to the age and quality of the sample and differences in procedures for FFPE preparation.

Based on the amount of enriched small RNA in a 3-µL volume, proceed according to the table below:

Amount of Small RNA in 3 µL	Instructions
100–200 ng	Proceed with the following protocol using the SOLiD™ Total RNA-Seq Kit. Use 100–200 ng of enriched small RNA for ligation.
< 100 ng	Determine the volume containing 100–200 ng of small RNA. Dry down this volume of RNA completely in a centrifugal vacuum concentrator at low or medium heat (≤ 40°C); this should take 10–20 minutes. Resuspend the RNA in 3 µL nuclease-free water, then proceed with the following protocol using the SOLiD™ Total RNA-Seq Kit.

Construct the Amplified Small RNA library

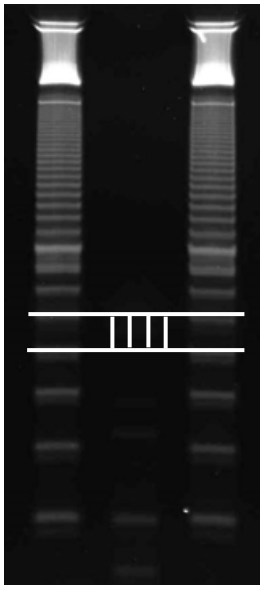
Follow the protocol entitled "Construct the amplified small RNA library" in the *SOLiD™ Total RNA-Seq Kit Protocol*, ***except*** for the steps highlighted in red below. Modifications to these procedures are described below:

1. Hybridize and ligate the RNA
2. Perform reverse transcription
3. Purify the cDNA
4. Size select the cDNA
5. Amplify the cDNA
6. Purify the amplified DNA
7. Assess the yield and size distribution of the amplified DNA
8. Proceed with SOLiD™ System templated bead preparation

Size select the cDNA (starting page 41 of Rev. A of the *SOLiD™ Total RNA-Seq Kit Protocol*)

Follow this procedure as written, ***except*** in step 8 remove the region of the gel between the 60-nt and 70-nt bands:

8. Illuminate the stained gel, then excise the gel containing 60 to 70 nt of cDNA (instead of 60–80 nt).
   a. Using a clean razor blade, make horizontal cuts directly on the 60-nt and 70-nt bands to excise the gel between 60 and 70 nt of cDNA.



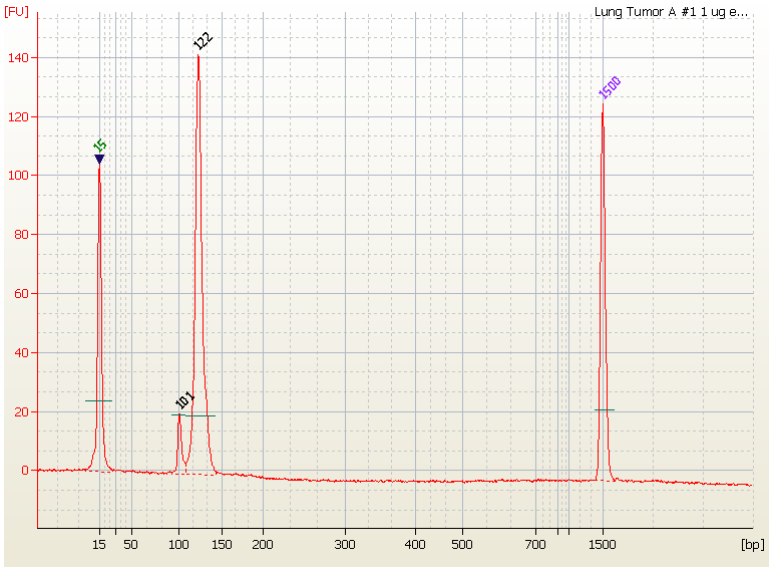
Amplify the cDNA (starting page 44 of Rev. A of the *SOLiD™ Total RNA-Seq Kit Protocol*)

Follow this procedure as written, ***except*** in step 2 run the PCR reaction for **18 cycles** (instead of 15 cycles).

Stage	Temp	Time
Hold	95°C	5 min
Cycle (**18 cycles**)	95°C	30 sec
	62°C	30 sec
	72°C	30 sec
Hold	72°C	7 min

Size Profile of the Amplified Library

The following Agilent 2100 Bioanalyzer™ Instrument profile was generated from 120 ng of enriched small RNA from a lung-tumor FFPE sample. The peak at 122 bp is the adaptor-ligated miRNA. The peak around 101 bp is from adaptor self-ligation by-products.



Trademarks

For Research Use Use Only. Not intended for any animal or human therapeutic or diagnostic use.

Notice to Purchaser: Please refer to the Ambion RecoverAll™ Kit, PureLink® miRNA Isolation Kit, and SOLiD™ Total RNA-Seq Kit protocols and manuals for limited label license or disclaimer information.

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