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Preparing Small RNA Libraries for PGM™ Sequencing

Publication No. MAN0006951 **Rev. Date:** 28 June 2012

RNA Sequencing

The Total Exosome RNA and Protein Isolation Kit (Cat. no. 4478545) is recommended for purification of exosomal RNA. RNA content of exosomes derived from at least 5 mL of cell media using the Total Exosome Isolation (from cell culture media) reagent or at least 200 µL of serum with the Total Exosome Isolation (from serum) reagent can be sequenced using the techniques described below.

For exosomes isolated using ultracentrifugation protocols (with or without sucrose gradient), we recommend increasing the input volume by 10-fold, because of very low recovery with this method.

For the complete protocol, refer to the manual for the Total Exosome RNA and Protein Isolation Kit at www.lifetechnologies.com.

Small RNA libraries can be conveniently prepared with the Ion Total RNA-Seq Kit v2 (Cat. no. 4475936) and sequenced on the Ion Torrent™ PGM™.

The following version of the Total RNA-Seq Kit protocol has been modified to accommodate these specific characteristics of exosome samples:

- Relatively low amount of RNA
- Majority of the RNA cargo is short <200 nt

Note: This protocol is just a guide, and further modifications may be required, depending on the objectives of your study, the origin of the exosomes, the concentration and purity of RNA, the availability of certain kits/reagents, and other factors. For additional details on small RNA library construction, refer to the Ion Total RNA-Seq Kit v2 User Guide at www.lifetechnologies.com.

Experimental Overview

(*Optional*) RNase III fragmentation

RNase III fragmentation is recommended when performing analysis of long RNA. However, it is not necessary if the primary goal is analysis of short RNA (<200 nt). For additional details on fragmentation clean up, refer to the manual for the Purelink® RNA Micro Kit at www.lifetechnologies.com.

1. Incubate the reaction in a thermal cycler at 37°C for 30 seconds.
2. Clean up 100 µL of the fragmented RNA with the Purelink® RNA Micro Kit (Cat no. 12183-016).
3. Add 100 µL of Lysis Buffer and 400 µL of 100% ethanol, and then mix well.
4. Bind, wash, and elute the RNA (in 12 µL) from the column.

Small Library Construction

Hybridize and ligate the small RNA

1. Hybridize at 65°C for 10 minutes and 16°C for 5 minutes.
2. Perform ligation by incubating at 16°C for 16 hours (overnight).

Perform reverse transcription

1. Incubate the reverse transcription (RT) mix with the ligated RNA sample at 70°C for 10 minutes.
2. Perform RT reaction at 42°C for 30 minutes.

Purify cDNA using MagMAX™ Beads

1. Add 5 µL beads to one well of a 96-well plate for each sample.
2. Add 250 µL Binding Solution Concentrate to each well containing beads, and pipet up and down 10 times.
3. Add 60 µL of nuclease-free water to each of the 40 µL RT reactions and transfer to one of the wells of the 96-well plate.
4. Add 275 µL 100% ethanol to each well and mix by pipetting up and down.
5. Wash and elute cDNA (in 12 µL) from MagMAX™ beads.

Amplify the cDNA

1. Perform 18 cycles of PCR (total) with Platinum® PCR SuperMix High Fidelity.

Purify the amplified DNA

1. Add 5 µL beads to one well on a 96-well plate for each sample.
2. Add 280 µL Binding Solution Concentrate to each well containing beads, and pipet up and down 10 times.
3. Add 27 µL of nuclease-free water to each of the 53 µL PCR reactions.
4. Add 230 µL 100% ethanol to each well and mix by pipetting up and down.
5. Wash and elute cDNA (in 10 µL) in from MagMAX™ beads.

Analyze the cDNA

1. Run 1 µL of the sample on Agilent DNA High Sensitivity chip to assess the yield and size distribution.
2. Determine the molar concentration of the library with the Agilent® 2100 Bioanalyzer® Instrument Expert software.

Perform sequencing on the Ion PGM™ System

1. Enter settings and run sample. Small RNA libraries should run at 160 flows (40 cycles).
2. Enter the remaining information as needed, then follow the remaining prompts to start the run.

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