MicroRNA Quantification:
Guideline for MicroRNA extraction

Introduction

Quality of the RNA has always been a crucial step for transcriptomic analyses. Moreover this difficulty, the conservation of the representativeness of the small RNAs and in particular of the mature miRNAs has become a "one-step behind challenge".

At present, several protocols have been developed to enrich the fraction of the miRNAs. At DNAVision, we have decided to validate protocols without enrichment of the miRNAs. Indeed, one of the big challenge of tomorrow will be to integrate the data obtained by microarray with these obtained by miRNAs. Therefore, we thought that it was crucial to use the same RNA samples to avoid any bias.

We thus recommend to use the following methods:

a) Total RNA Isolation with the miRNeasy Mini kit from Qiagen

NB Use only the protocol 1

b) Total RNA Isolation with TRIzol Reagent

1. See specific sample type for homogenization:

   Frozen tissues:
   Frozen tissues can be powdered on dry ice or liquid nitrogen with a mortar and pestle, and then homogenized in 1mL of Trizol reagent. Alternatively, they can also be placed directly in 1mL of cold Trizol and immediately homogenized with a rotor power homogenizer to quickly dissociate the tissue.

   Fresh tissues:
   Rinse the tissue in cold PBS, place in 1mL of Trizol and immediately homogenized with a rotor power homogenizer to quickly dissociate the tissue.

   Monolayer cell cultures:
   Wash cells 1x with cold PBS, and add an appropriate volume of Trizol for the size of culture dish used. For 6-well plates & 6cm dishes, 1mL of Trizol is appropriate. For 10cm dishes, 2mL is appropriate, and can be aliquoted into smaller 1mL volumes for the remainder of the protocol. If large amounts of RNA are needed, scale up the following protocol as needed.

2. Incubate the homogenized sample at room temperature for 5 minutes.

3. Add 200 uL of chloroform (per 1mL Trizol), shake vigorously (not vortex) for 15 seconds to mix well, and incubate at room temperature for 3 minutes.

4. Centrifuge samples at 8,000-11,000 x g at 4°C for 15 minutes to separate phases.

5. Transfer upper (clear) aqueous layer to a fresh 1.7mL tube.

6. Add 0.5mL of isopropanol to the aqueous layer, mix thoroughly by shaking for 15 seconds, and incubate at room temperature for 10 minutes.

7. Centrifuge samples at 8,000-11,000 x g at 4°C for 10-30 minutes to pellet RNA.

8. Carefully remove the supernatant & add 1mL 75% DEPC-ethanol and vortex on low for 5-10 seconds to wash the pellet thoroughly.

9. Centrifuge at 6,000 x g at 4°C for 5 minutes to re-pellet RNA.

10. Carefully remove the supernatant and air-dry the pellet at room temperature for 5-10 minutes.

11. Dissolve the pellet in DEPC-dH2O (30-100uL, depending on yield) by gentle pipetting.

12. Store the RNA at -80°C if directly used.

NB The precipitation step is crucial to avoid any lost of miRNAs.

If you are not familiar or equipped for RNA extraction, we can also provided this service (if you are interested, please inquire).