

Total RNA Extraction

Purpose:

To extract total RNA from cells stored in RNAlater using Ambion's Ribopure kit for exon analysis.

Materials/Equipment:

Note: Ensure supplies and equipment are RNase free prior to use.

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|------------------------|---------------------|------------------------|
| 1. RNase Zap | 4. 100% Ethanol | 7. Fume hood |
| 2. RNase free-tubes | 5. Chloroform | 8. Agilent bioanalyzer |
| 3. Ambion Ribopure kit | 6. Micro/Centrifuge | |

Procedure:

Note: Wipe down bench area, pipettes and any other equipment with RNase zap. Use designated RNase free supplies/equipment.

RNA extraction

1. Locate cells stored in RNAlater in -20C freezer in tx culture room.
2. Aliquot ~5million cells into a labeled 1.5ml RNase free tube.
3. Pellet cells at 14000 rpm for 5 min at 4C
4. Remove supernatant. Add 1ml of Tri-Reagent and vortex briefly to lyse cells. *Note: Ensure proper handling of tri-reagent solution as it contains phenol and guanidine thiocyanate.*
5. Incubate at room temp for 5 min.
6. Add 200ul of chloroform and vortex to mix.
7. Incubate at room temp for 5 min.
8. Centrifuge at 12000 rcf for 10 min at 4C for phase separation.
9. Transfer 400 ul of aqueous phase (top layer) containing the RNA into a new tube.
10. Perform following steps one/two samples at a time:
 - A. Add 200ul of 100% ethanol. Vortex immediately for 5 sec at max speed to prevent precipitation.
 - B. Transfer sample to an Ambion Ribopure collection tube with filter cartridge in place.
 - C. Centrifuge at 12000 rcf for 30 sec at room temp to bind RNA to filter.
 - D. Discard flow through and add 500ul of wash solution.
 - E. Centrifuge for 30 sec. Discard flow through and repeat wash step.
 - F. Discard flow through and centrifuge for an additional 30 sec to remove residual ethanol.
11. Transfer filter cartridge to a new labeled collection tube.
12. Add 100ul of elution buffer and incubate at room temp for 3-5 min.
13. Centrifuge at room temp for 30 sec at 12000 rcf.
14. Store samples at -80C.

Quantification/QC

1. Use Agilent's 2100 Bioanalyzer to check quality of samples.
2. Aliquot 1ug of sample and reserve for further processing on Affymetrix exon arrays. .