UW Genome Sciences
RNA: TX extraction
Revised 02/02/11

Total RNA Extraction From Tx Samples

Purpose:

To extract total RNA from tx samples stored in RNAlater using Qiagen's TissueLyser and RNeasy Lipid Tx Mini kit.

Materials/Equipment:

1. 1.5ml/2ml tubes
2. 5-7mm steel beads
3. Qiagen TissueLyser LT
4. Qiagen RNeasy Lipid Tx Mini Kit
5. Chloroform
6. 70% Ethanol
7. Micro/Centrifuge
8. Agilent bioanlayzer

Procedure:

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

Tx disruption and homogenization via the Tissue Lyser LT

Note: Do not overload columns. Load no more than 30-100mg of tx sample, depending on tx type.

1. Add one 5mm stainless steel bead (or one 7mm bead for tough tx) in a 2 ml tube.
2. Remove 30-100mg of tx sample stored in RNAlater and place into 2 ml tube.
3. Add 1ml of QIAzol lysis reagent.
4. Place tubes into Tissue Lyser adaptor insert. Attach adaptor to Tissue Lyser and screw lid shut.
5. Run Tissue Lyser for 2-5 min at 50 Hz. *Note: Processing times will vary based on tx type and can be extended until sample is completely homogenized. Complete disruption of cells allows for the release of nucleic acids. Complete homogenization allows proper binding to spin columns.*

RNA extraction

Note: Add 4 volumes of 100% ethanol to Buffer RPE before use.

1. Pipet lysate into new 1.5ml tube.
2. Incubate at room temp for 5min to allow nucleoproteins to dissociate.
3. Add 200ul of chloroform and vortex for to mix.
6. Incubate at room temp for 5 min.
7. Centrifuge at 12000rcf for 15 min at 4C for phase separation.
8. Transfer ~500ul of RNA (top aqueous layer) into a new 1.5ml tube.
9. Perform following steps one/two samples at a time:
   A. Add equal vol of 70% ethanol. Vortex immediately at max speed for 5 sec to prevent precipitation.
   B. Transfer up to 700ul of sample to column. Spin at 12000rcf for 15sec at room temp.
   C. Repeat above with any remaining sample.
   D. Add 700ul of Buffer RW1 to column and spin at 12000rcf for 15 sec at room temp.
   E. Add 500ul of Buffer RPE to column and spin at 12000rcf for 15 sec at room temp.
   F. Add 500ul of Buffer RPE to column and spin at 12000rcf for 2min at room temp.
   G. Place column in a new collection tube and spin at max speed for 1 min to remove residual ethanol.
10. Transfer column to labeled 1.5ml tube. Add 30ul of RNAse free H20 directly to the membrane. Incubate for 3-5 min and spin for 1 min at 12000rcf at RT.
11. Repeat elution step with an additional 30ul of RNAse free H20.
12. Store samples at -80C.

Quantification/QC

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