W.M. Keck Foundation Biotechnology Microarray Resource Laboratory at Yale University

Total RNA MinElute Cleanup with DNase Digestion using Qiagen RNeasy Protocol

Please do not make copies of or distribute this protocol.

A. Required reagents:

$\leq$ 45 µg RNA isolated by any method other than Qiagen RNeasy method
DEPC-treated water (Ambion)
RNeasy MinElute RNA Isolation Kit (Qiagen)
RNase-Free Dnase Set (Qiagen)
ß-Mercaptoethanol (ß-ME) (Sigma)
Absolute ethanol (Ultrapure) (American Bioanalytical)

B. Equipment and supplies:

Microcentrifuge with 1.5 ml tube rotor
Vortex mixer
Micropipettors
Aerosol-barrier tips
Microcentrifuge tubes
Vortex mixer
Powder-free gloves

I. DNase Digestion of RNA before RNA Cleanup

- **Do not vortex reconstituted DNase I.** Mix by gently inverting the tube.
- Prepare DNase I stock solution before using RNase-Free DNase set for the first time. Dissolve the solid Dnase (1500 Krunitz units) in 550 µl of RNase-free water. Mix gently by inverting the tube. Aliquots can be stored at -20C for up to 9 months. Thawed aliquots can be stored at 2-8C for up to 6 weeks. ***Do not freeze the aliquots after thawing.***

1. Mix the following in a microcentrifuge tube:

   - $\leq$ 87.5 µl RNA in solution
   - 10 µl Buffer RDD
   - 2.5 µl DNase I Stock solution

   Adjust volume to 100 µl with RNase-free water.

2. Incubate at room temp (20-25C) for 10 min.

3. Proceed to Total RNA MinElute Cleanup.

II. Total RNA MinElute Cleanup

- Maximum binding capacity of RNeasy MinElute spin column is 45 µg of RNA.
- Buffer RLT may form precipitate upon storage. If necessary, warm to redissolve.
- Add 10 µl ß-ME per 1 ml of buffer RLT just before use.
- Buffer RPE is supplied as a concentrate. Before using the first time, add 4 volumes of absolute ethanol as indicated on the bottle to obtain working solution.

1. Add 350 µl Buffer RLT to the sample. Mix well by pipetting 2 to 3 times.
2. Add 250 µl absolute ethanol. Mix well by pipetting 2 to 3 times.
3. Apply 700 µl of sample, including any precipitate, to an RNeasy MinElute spin column sitting in a 2 ml collection tube. Incubate column for 2 min. Centrifuge for 15 sec at ≥10,000 rpm.
4. Transfer RNeasy MinElute column into a new 2 ml collection tube. Save flow through until sample quantitation is completed.
5. Pipet 500 µl of Buffer RPE onto column. Spin for 15 sec at ≥10,000 rpm. Discard flow-through.
6. Pipette 500 µl of 80% ethanol to column. Centrifuge for 2 min at ≥10,000 rpm. Discard flow through and collection tube.
7. Place column in a new 2 ml collection tube. Centrifuge with caps open at full speed for 5 min. Discard flow-through and collection tube.

   *It is important to dry the RNeasy membrane since residual ethanol may reduce the recovery of RNA and also may interfere with subsequent reactions.

   *To avoid damage to the caps, place the columns into the centrifuge with at least one empty position between each column. Place the caps so that they point in the opposite direction to the rotation of the motor.

8. Add 14 µl of DEPC-treated water carefully and directly onto the center of column membrane. Incubate column 2 min. Centrifuge for 1 min at maximum speed to elute. Check volume recovered to determine if an additional spin is required.

9. Use spectrophotometric analysis to measure RNA yield. Take OD at 260 nm and 280 nm to determine sample concentration and purity. The A_{260}/A_{280} ratio should be above 1.8. Apply the convention that 1 OD at 260 equals 40 µg/ml RNA.

10. Run 0.5-1 µg of RNA on native 1 % agarose gel or 0.1-0.5 µg on an Agilent Bioanalyzer chip to assess the quality of RNA.

11. **Important: It is extremely important to start microarray experiments with very good quality of RNA. The A_{260}/A_{280} ratio should be above 1.8. The gel electrophoresis pattern should reveal two major bands of 28 S and 18 S RNA and no smear from genomic DNA (See Figure 1 below).**

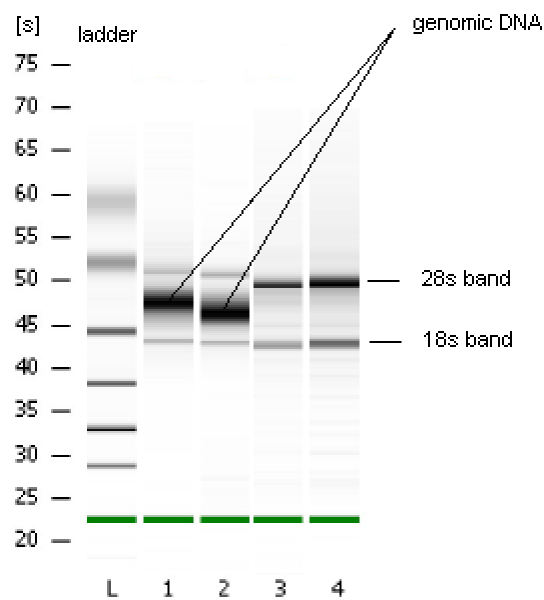

Figure 1: Bioanalyzer gel-image pattern of total RNA