

Protocol for RNA Isolation from Murine Reproductive Tissue
A501 – Techniques in Reproductive Diversity
28 November 2006

Please bring to class:

Clean lab coat

Items provided at your bench:

Gloves
70% ethanol
RNase Away
Nanopure (dd) H₂O
Paper towels
Pipettes
RNase-free pipette tips
Arrowhead tissue homogenizer tips
2.0 ml Phase Lock Gel™ (PLG) tubes
RNase-free 1.5 ml tubes
100% isopropanol
Liquid and pipette tip waste containers
RNase-free (DEPC) water
Ice buckets and ice

Locations of other required reagents:

TRIzol® Reagent, 4°C
Chloroform, room temperature in fume hood
75% ethanol made with DEPC water, -20°C

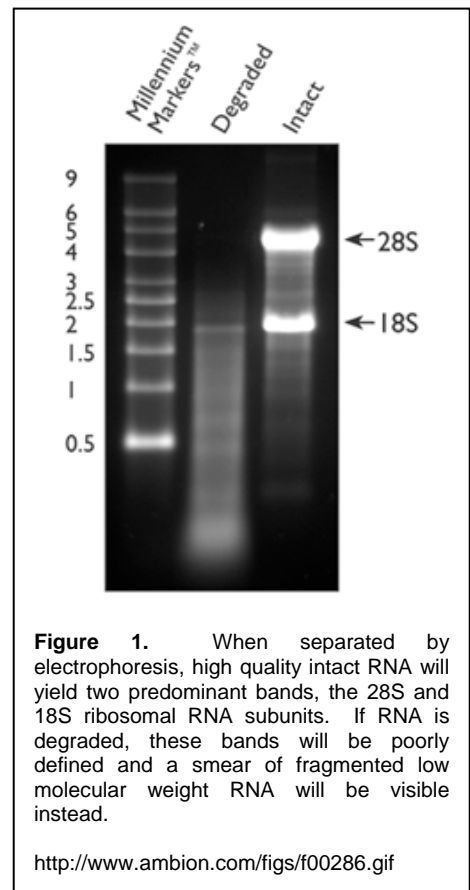
RNA Extraction Protocol:

Clean work area to remove RNases

- Spray work surface, pipettes, outside of pipette tip boxes, and tube rack with 70% ethanol and wipe clean with paper towels
- Repeat using RNase Away
- Repeat using Nanopure H₂O
- Use RNase Away followed by Nanopure H₂O continually throughout extraction procedure to clean your gloves and anything else you feel you may have contaminated

Homogenize tissue

- For each group member, obtain one mouse ovary or testis sample in a 1.5 mL tube from your group leader
- In fume hood, add 150 µL TRIzol® Reagent to each tube and homogenize by hand with a tissue homogenizer tip until tissue is completely dissociated
 - o Discard TRIzol-contaminated pipette tips in Biohazard Waste container
 - o Save blue arrowhead tissue homogenizer tips, which can be cleaned and reused
- Add an additional 350 µL TRIzol® to each tube, cap, vortex to mix, and incubate 5 min at room temperature
 - o If tissue is not completely dissociated, vortex periodically throughout the 5 min incubation until no more tissue is visible



Separate aqueous and organic phases

- In fume hood, add 300 μ L chloroform to each tube, cap tightly and shake vigorously for 15 s
 - o Discard tips in Biohazard Waste container
- Incubate 3 min at room temperature
- Meanwhile, label and pellet the gel in one PLG tube/sample at 12,000xg for 30 s at room temperature
- Transfer TRIzol/chloroform/tissue mixture to a PLG tube and centrifuge at 12,000xg for 5 min at 4°C
 - o The gel should form a barrier between the organic (pink) and aqueous (clear) phases upon centrifugation. If the upper aqueous layer is still pink, repeat the entire phase separation protocol.

Precipitate RNA

- Decant aqueous (top) phase to a labeled RNase-free 1.5 mL tube
 - o Discard PLG tubes in Biohazard Waste container in fume hood
- Add 400 μ L room temperature 100% isopropanol, cap tube tightly, and shake vigorously to mix
- Incubate 10 min at room temperature
- Pellet RNA by centrifugation at 12,000xg for 10 min at 4°C

Wash RNA

- Remove supernatant being careful not to disturb the RNA pellet
 - o Discard supernatant in liquid waste container at your bench
- Wash pellet with 600 μ L ice cold 75% ethanol (made with DEPC H₂O)
- Vortex briefly until pellet detaches from wall of tube
- Pellet RNA by centrifugation at 7,500xg for 5 min at 4°C

Redissolve RNA

- Remove as much supernatant as possible being careful not to disturb the RNA pellet
 - o Discard supernatant in liquid waste container at your bench
- Air dry pellet until a halo forms (~15 min)
- Resuspend pellet in 20 μ L DEPC H₂O by pipetting up and down and finger-flicking to mix
- Move tubes to ice and keep on ice for remainder of procedure

Determine RNA concentration

- For each RNA sample, make a 1:100 dilution of RNA by adding 1 μ L RNA to 99 μ L DEPC H₂O in a labeled RNase-free 1.5 mL tube
- Determine RNA concentration using an Eppendorf BioPhotometer according to the instructions of your group leader

Verify RNA integrity

- For each RNA sample, calculate the volume necessary to equal 1 μ g
- Label one new RNase-free 1.5 mL tube for each sample
- To each tube add 1 μ L gel loading buffer, 1 μ g RNA, and a volume of DEPC H₂O so that the total volume in each tube equals 10 μ L
- Load each 10 μ L complex into a separate well of a precast 1% agarose + ethidium bromide gel and run the gel at 100 V for ~1 h
- Take a picture of the gel using the camera/UV box in the darkroom (JH234) according to the instructions of your group leader
- Determine whether RNA is intact by noting the intensity of the 28S and 18S ribosomal RNA bands
 - o See Figure 1 for illustration of intact and degraded RNA samples