RNA Isolation and Purification

***M. tb.* RNA isolation.**

1. Centrifuge 20-30 ml of early- to mid-log culture (O.D. 0.1-0.2) 4 min at 3700 rpm, at RT-37C. Pipette off supernatant and immediately freeze on dry ice and store at –80C.
2. Add 1 ml Trizol (Gibco-BRL) to each of 4-8 pellets, suspend first pellet by vortexing, while other pellets are still frozen. Add suspension to 0.4 ml glass beads in a 2 ml screw cap tube.
3. Shake 30s at maximum speed in bead beater. Suspend next pellet and add to glass beads. Apply next sample to bead beater. Repeat bead beating two more times with each sample. Continue to periodically invert samples for at least 5 min in Trizol.
4. Centrifuge samples 45s at max speed, remove Trizol solution to a 2 ml screw cap tube containing half of the Heavy Phase Lock Gel I, supplied by 5 Prime 3 Prime, Inc. in 1.5 ml tubes and transferred with sterile stick to 2 ml tubes, and 300 µl Chloroform:isoamyl alcohol (24:1). Invert rapidly for 15s, and continue inverting periodically for 2 min.[a]
5. Centrifuge 5-10m, remove aqueous layer (540 µl) and add to a 1.5 ml tube containing 270 µl isopropanol then add 270 µl high salt solution (0.8M Na Citrate, 1.2M NaCl). Invert several times and spray with Staphene and remove from the P3. Typically ppted at 4C O/N.
6. Centrifuge 10m at 4C and remove isopropanol. Add 1 ml 75% EtOH[b], invert several times and centrifuge 5m.
7. Remove EtOH by aspiration. Then, dry under vacuum for 2 min [Do not over dry].
8. Suspend RNA in 90 ml RNase free water (don't suspend in DNaseI buffer), may need to heat 10m at 55-60C to dissolve RNA. (Optional: obtain RNA concentration)
9. Add 10 µl 10X DNaseI buffer to RNA (use no more than 80 µg RNA) and add 4 µl DNaseI (Ambion). Incubate 30m at 37C.

Qiagen RNeasy purification.

1. Add 350 µl RLT buffer (add 10 µl BME to 1 ml RLT before using) and vortex. Add 260 µl 95% EtOH (or 250 µl 100% EtOH) to each sample and vortex.
2. Add to RNeasy spin column, centrifuge 15s, transfer column to a new 2 ml collection tube. Add 500 µl RPE, centrifuge 15s, discard flow-through, add 500 µl additional RPE and centrifuge 2m. If column still wet on sides, remove wash solution from tube and spin 1m to dry.
3. Transfer to a 1.5 ml collection tube, elute with 40 µl RNase free water, centrifuge 1m.
4. Determine RNA concentration with A_{260}/A_{280} readings. Dilute 1 µl in 199 µl TE. [200(dilution factor)x 40 µg/A_{260}x A_{260} = µg/ml].
5. Run 1 µl RNA on 2% Agarose TAE gel. Run gel 45m at 100 volts.

[a]If Phase Lock Gel is not used, decrease Chloroform:isoamyl alcohol to 200µl. The yield will be less. Be careful not to remove any of the interface layer.
[b]Using 100% EtOH should be avoided in all steps of preparation of RNA intended for array analysis. Instead use 95% EtOH in preparation of solutions. Benzene contamination may fluoresce.
[c]Instead of using the RNeasy purification, the DNaseI can be inactivated at 65-70C for 15m and then EtOH precipitated. The RNeasy purification may not be necessary in all applications. We find the purification gives more consistent results and is less time consuming, compared to precipitation. In addition, other purifications can be used. Note: The RNeasy column will remove much of the small tRNA and degraded RNA.