Long Lab, Stanford University
http://cmgm.stanford.edu/biology/long/

# RNA Purification--TRIZOL

Uses TRIZOL, a proprietary formulation from BRL, which contains phenol, guanidine isothiocyanate (Based on Chomczynski method)

1. Transfer 100 ml of saturated culture into precooled centrifuge bottle. Cool well before spin (using liquid nitrogen.) Discard supernatant and quick-freeze pellet.
2. Quick thaw pellet. Add 12 ml TRIZOL. Dissolve pellet and aliquot into two plastic tubes containing 1 ml acid-washed, baked glass beads.
3. Vortex thoroughly. Incubate 5 minutes room temp. Prespin 12,000 x g 10 minutes at 4°C to remove "crud" and glass beads.
4. Phase separation as per BRL protocol. Use 1.2 ml chloroform per tube.
5. Isopropanol precipitation and ethanol wash as per BRL protocol.
6. Air dry pellet, resuspend in 0.5 ml water. Transfer to microfuge tube.
7. Add 0.3 ml 8M LiCl. Allow at least 2 hours on ice for RNA to precipitate.
8. Pellet RNA. Resuspend in water. Ethanol precipitate and wash 2X with 75% ethanol. Resuspend in water and freeze at -80°C.

Yield from 100 ml culture is enough for 2 to 4 primer extension reactions.