

Prepping Total RNA from Gene-Trapped ES Cells:

Be extremely sterile!!! This means to wear gloves, wipe down bench, and all pipettes w/ 70% EtOH, and clean pipette barrels. Do all work w/ barrier tips to minimize likelihood of Rnase contamination.

Reagents:

TRI ZOL - This reagent denatures cells on contact. Contains phenol which denatures/dissolves proteins.

PBS - washes cells

Choloform - will help remove residual phenol so it will not denature what is added next. Also stabilizes boundary btwn aq. phase and phenol (organic) phase.

0.2 x TE Buffer - contains H₂O, Tris, & EDTA which chelates Mg²⁺ so it is not available for enzymes (nucleases) to use. (Protects NAs).

Procedure:

1. To each 6cm plate of cells, discard media and rinse gently w/ PBS. (Shoot PBS stream against side of plate to avoid injuring cells).
2. Add 3 mls TRI ZOL and scrape cells up w/ cell scraper. (Can use same scraper for all cells even though they came from different colonies. Just rinse in PBS and wipe first).
3. Split each sample into 2 - 2 ml tubes w/ 1.5 ml sample each. (or 3 - 1.5 ml tubes w/ 1 ml sample each). Let sit 5 min. to dissociate complexes.
4. Add **0.2 ml Chloroform/1 ml Trizol**. So if using 2 ml tubes w/ 1.5ml sample, 1.5 x 0.2 = 0.3 ml chloroform into each tube. Shake and incubate 2-3 min. at RT. (Will notice separation of phases).
5. Centrifuge for 15 min. @ 4°C at no more than 12,000 x g.
6. Transfer aq. phase to new tube (contains RNA). Save organic phase for DNA isolation (store at 4°C).
7. Precipitate RNA by adding **0.5ml Isopropanol/initial 1 ml Trizol** (ex. 1.5 ml Trizol per 2 ml tube gets 0.75 ml Isopropanol).
8. Incubate at RT for 10 min.
9. Spin for 10 min. at 4°C at no more than 12,000 x g.

10. Discard supernatant if pellet is visible as gel on base of tube. (If not visible use 1 λ glycogen and spin 10 min. Note: glycogen is a *carrier* and will pull down NAs).
11. Wash pellet w/ 1ml 70% EtOH. Vortex and centrifuge at 4°C for 5 min. at no more than **7,500** x g.
12. Air dry briefly. Do not overdry pellet or else it will be harder to resuspend later.
13. Resuspend in 50 λ 0.2 x TE buffer. Vortex and place in 60°C for 1 hour w/ occasional vortexing to resuspend.