Total RNA Isolation Procedure

Total RNA samples submitted to the Gene Expression Resource for Affymetrix GeneChip analysis should be isolated using TRIzol Reagent or TRIzol LS Reagent (Invitrogen, Inc.). The isolation method we recommend is a slightly modified version of the manufacturer's protocol.

Recommendations:

- Ensure that all apparatus and reagents to be used in the isolation procedure are RNase-free. For tips on good RNA technique, refer to the links on the GER protocol page.
- TRIzol Reagent should be used for cultured cells and tissues. TRIzol LS Reagent should be used for biological fluids.
- Recommended minimum quantities of TRIzol to use with cells and tissue are described below. Keep in mind that RNA yields may improve if slightly more TRIzol Reagent is used.
- The proportion of chloroform used is important to the purification and phase separation. Be careful not to use less than the recommended amount.

Procedure:

- For isolation from **cells cultured in a monolayer**, aspirate the media and lyse the cells directly in the culture dish or flask by adding at least 1mL TRIzol Reagent per $10cm^2$ area. Ensure complete lysis by passing suspension through a pipette several times. Transfer to appropriate centrifuge tubes.
- For isolation from **cells cultured in suspension**, centrifuge the cells and discard the media. Add at least 1mL of TRIzol Reagent per 5×10^6 animal, plant, or yeast cells (1×10^7 bacterial cells) and ensure complete lysis by passing suspension through a pipette several times. Transfer to appropriate centrifuge tubes.
- For isolation from **tissue**, homogenize samples in at least 1mL TRIzol Reagent per 50-100mg of tissue. If tissue is frozen, avoid thawing tissue before homogenization. If tissue is fresh, homogenize in TRIzol as soon as possible. Remember that TRIzol can only inactivate RNases with which it is in direct contact—therefore tissue samples are not safe from RNA degradation until completely homogenized. For homogenization, we recommend using a power homogenizer (such as a Polytron) or a glass-Teflon homogenizer. Transfer to appropriate centrifuge tubes.
- For isolation from **biological fluids**, add 0.75mL of TRIzol LS Reagent to 0.25mL sample (no more than 5×10^6 cells). Ensure complete lysis by passing suspension through a pipette several times. Transfer to appropriate centrifuge tubes. Please note that biological fluids which contain a high level of contaminating material (such as whole blood) may be diluted 1:1 with RNase-free or DEPC-treated H_2O.

- [ ] For tissue samples only, we recommend an additional centrifugation step after homogenization to remove insoluble material from the sample. Centrifuge the sample at >12,000 x *g* for 10 minutes, at 2 to 8°C. Transfer the supernatant to a fresh tube (taking care not to transfer any fat that may form on top as a layer) and proceed with the isolation.
- [ ] After sample lysis/homogenization, incubate samples at room temperature for 5 minutes.
- [ ] Add 0.2mL chloroform per 1mL of TRIzol used **or** per 0.75mL TRIzol LS used.
- [ ] Cap tubes securely and shake vigorously by hand for 15 seconds.
- [ ] Incubate at room temperature for 2-3 minutes.

- Centrifuge samples at 12,000 x *g* for 15 minutes, at 2 to 8ºC.
- Transfer the colorless upper phase (aqueous phase) into fresh tubes.
- To the samples, add an equal volume of phenol:chloroform:isoamyl alcohol (125:24:1), **pH 4.3**, and shake vigorously by hand for 15 seconds.
  - For increased yields and minimization of organic contamination, Eppendorf phase-lock-gel tubes (heavy form) can be used to separate the phases in this second organic separation. For this procedure, follow the instructions below in conjunction with the manufacturer's recommendations.
  - Prepare an Eppendorf phase-lock-gel tube (heavy) by briefly centrifuging tube to pellet gel.
  - Transfer the sample to the prepared phase-lock-gel tube. For better phase separation, add RNase free water in the amount of 5% of the total volume of the sample. Do not vortex. Centrifuge sample at 16,000 x g for 5 min at room temperature.
- Transfer the upper phase (aqueous phase) into fresh tubes.
- Add 0.5mL isopropyl alcohol (room temperature) per 1mL of TRIzol used **or** per 0.75mL TRIzol LS used. Invert several times to mix well.
- Incubate samples at room temperature for 10 minutes.
- Centrifuge samples at 12,000 x *g* for 10 minutes, at 2 to 8ºC.
- Remove the supernatant, being careful not to disturb the RNA pellet.
- Wash the RNA pellet by adding at least 1mL of 75% EtOH per 1mL of TRIzol used **or** per 0.75mL TRIzol LS used.
- Centrifuge samples at 12,000 x *g* for 10 minutes, at 2 to 8ºC.
- Remove the supernatant, again taking care not to disturb the RNA pellet. Remove as much of the EtOH as possible (if necessary, spin samples briefly again to collect excess EtOH ; remove by pipetting), and then air-dry the pellets for about 10-30 minutes.
- Redissolve the pellets in RNase-free or DEPC-treated H_2O such that the RNA concentration will be greater than 0.3 μg/μL.
- Quantitate RNA, measuring absorbance at $\lambda = 260$ and $\lambda = 280$. Measure absorbance at dilutions such that the OD is between 0.1 and 1.0.
- Run 0.5 - 1 μg of each RNA sample (run the same amount of each sample) on a 1X TBE/0.8% agarose gel, stained with ethidium bromide.