1. PURPOSE

This protocol describes the extraction of total RNA from human and mouse cell lines.

2. SCOPE

This procedural format is utilized by Human Colon Cancer and Mouse microarray projects under the supervision of John Quackenbush within the Mammalian Genomics Dept.

3. MATERIALS

3.1 TRIzol® Reagent (Invitrogen; Cat# 15596018)
3.2 Dulbecco's Phosphate Buffered Saline (D-PBS) (Invitrogen; Cat# 14190144)
3.3 Ethanol (200 proof) (Pharmco; Cat# 111000200)
3.4 Isopropanol (2-Propanol) (Fisher Scientific; Cat# A451-1)
3.5 Chloroform (Fisher Scientific; Cat# C298-500)
3.6 RNase-free water (DEPC treated) (ResGen; Cat# 750023)
3.7 RNase ZAP™ (Invitrogen; Cat# 46-0434)
3.8 50 mL Centrifuge Tube (VWR; Cat# 21008-242)
3.9 Disposable Cell Scraper (Fisher Scientific; Cat# 08-773-2)
3.10 10CC syringe (Becton Dickinson; Cat# 301604)
3.11 18G syringe needles (Becton Dickinson; Cat# 305196)

4. PROCEDURE

4.1 Avoiding RNase contamination

4.1.1 To prevent microbial contamination (and subsequent RNase contamination), always wear disposable gloves and practice good sterile technique when handling samples and reagents.

4.1.2 Always use sterile, disposable plasticware and use pipettes which are dedicated only to RNA handling.

4.1.3 Before starting wipe down the work surface and pipettes with RNase ZAP™ to promote an RNase-free environment.

4.2 RNA Extraction

NOTE: This procedure is optimized for extracting total RNA from cell lines that have been grown in large cell culture plates (150 mm diameter) with coverage greater than 80%. Therefore, the amount of TRIzol® used herein is based on the estimated combined quantity of cells grown in two large plates. If isolating RNA from a different number of cells, consult Form No. 18057N (which accompanies TRIzol® Reagent) to make adjustments.

NOTE: TRIzol® Reagent is very toxic and should only be used with caution. Work only in a fume hood with proper personal protective equipment (gloves, eye protection, etc.). TRIzol® waste is hazardous and should be handled and disposed of according to institutional hazardous waste protocols.

4.2.1 Carefully aspirate into bleach the media from the large cell culture plates.

4.2.2 Add 5mL of Dulbecco's Phosphate Buffered Saline (D-PBS) to each large plate, swirl to wash and aspirate.

4.2.3 Add another 5mL D-PBS to each large plate.

4.2.4 With a cell scraper loosen the cells from the plate surface and transfer cell suspension to a 50mL conical tube (add 2 plates per tube).

4.2.5 To each plate add another 5mL of D-PBS, swirl to rinse and transfer to the 50mL tube.

4.2.6 Centrifuge at 2300 RPM for 3 minutes to pellet the cells.

4.2.7 Aspirate off supernatant being careful not to lose the pellet.

4.2.8 Add 10mL of TRIzol® to the cell pellet and pass through a syringe (10CC w/ 18 gauge needle) to break up the pellet.

4.2.9 Incubate at room temperature for 5 minutes.

4.2.10 Add 2mL chloroform (1/5 of TRIzol® volume), shake vigorously for 30 seconds, and let set for 2-3 minutes at room temperature.

NOTE: The VWR 50mL centrifuge tube (from **Materials**) can withstand high centrifugal speeds and is therefore recommended for the following centrifugation step. The Falcon® Blue Max™ 50mL polypropylene conical tubes (Becton Dickinson; Cat# 352070) are not recommended for the following centrifugation because they have been known to break.

4.2.11 Centrifuge at 10,000g for 20 minutes at 4^0C (Beckman Avanti J-25 Centrifuge with 16.250 rotor or comparable unit).

4.2.12 Transfer the top, aqueous layer (clear) to a clean 50mL conical tube (DO NOT TOUCH THE INTERPHASE).

4.2.13 To the aqueous solution add an equal volume of isopropanol, mix well, and let sit at room temperature for 10 minutes.

4.2.14 Centrifuge at 10,000g for 20 minutes at 4^0C to pellet the RNA.

4.2.15 Aspirate off the majority of the supernatant and then pipette off the remaining supernatant to avoid losing the pellet.

4.2.16 Wash the pellet with 2mL 75% ethanol (in DEPC-treated water). Pipette off ethanol.

4.2.17 Add 1mL ethanol, crush up the pellet, and transfer to an RNase-free 1.5mL tube.

4.2.18 Store at -80^0C until later use.