Protocol Name: RNA Bx v1 – Isolation of RNA from Bx (from Invitrogen Trizol Protocol and RNeasy Protect Mini Kit Handbook with option to include small RNAs) updated 2/17/2011.

Notes:

- Bring Trizol reagent to room temperature.
- Buffer RPE is supplied as a concentrate in RNeasy kit. Add appropriate volume of 100% Ethanol before using for the first time.
- Work in the fume hood. Do not worry about RNases in steps 1-3. Your sample is full of them anyway. They are inhibited as long as they are in Trizol.
- From step 4 you should be careful of not contaminating the samples with RNases. Keep cleaning your gloves with RNase Zap (Ambion) through the whole process. The main source of contamination comes from your fingers by accidentally touching the inner part of the tube caps.
- While discarding flow-through in steps 6-9, avoid touching the mouth of the collection tubes with anything!

1. Homogenize tissue samples (up to 100mg) in 0.2ml of Trizol reagent with battery operated hand homogenizer (VWR Pellet Mixer Cat # 47747-370 and Fisherbrand 1.5 ml Pestle RNase and DNase free Cat #V7339-901). Then bring to 1ml with Trizol. Aspirate through 21g syringe several times. Incubate for 5 minutes at room temperature.
2. Add 200ul of Chloroform. Shake vigorously for 15 seconds and incubate at room temperature for 3 minutes.
3. Centrifuge at 12,000xg for 15 minutes at 4-8C.
4. Transfer the top aqueous phase to fresh tube and save remaining sample for DNA/Protein extraction.
5. Slowly add an equal volume of 100% RNase-free EtOH, mixing it as needed. **For micro RNA inclusion add 1.4 volumes of 100% EtOH instead of equal volume. Need at least 60%EtOH.**
6. Load the sample (up to 700ul) into an RNeasy column (Qiagen kit) seated in a collection tube and spin for 30 sec at 8,000xg. Discard flow-through.
7. Add 700µl buffer RW1 onto column and spin 30 sec at 8,000xg. Discard flow-through. **For microRNA DO NOT USE RW1 use only RPE**. Make sure ethanol has been added to the RPE buffer.
8. Transfer column into a new collection tube, add 500µl buffer RPE and spin for 30 sec at 8,000xg. Discard flow-through. Make sure ethanol has been added to the RPE buffer before use.
9. Add 500µl buffer RPE and spin 2 min at 8,000xg. Discard flow-through.
10. Spin the column for 1 min at 8,000xg to get rid of remaining buffer in the column.
11. Transfer the column to a new 1.5ml collection tube and pipet 30-50µl of RNase-free water directly onto the column membrane. Allow the sample to sit at room temperature for 1-2 min, and then spin 1 min at 8,000xg to elute RNA. Check on Nanodrop and Bioanalyzer.
12. Store RNA at -80C until use.