Trizol/RNeasy hybrid RNA extraction protocol
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1. Homogenize the starting material using an appropriate volume of Trizol (1 ml per 0.1g, check Trizol instructions). Store the homogenate at room temperature for 5 min (Use 10 ml round-bottom centrifuge tubes).

Optional Step: Centrifuge for 10 min at 12,000xg at 4C to eliminate debris and polysaccharides.

2. Add chloroform to the homogenate (0.2 ml chloroform per ml Trizol used) and shake vigorously for 20 sec, then allow the sample to sit at room temperature for 2-3 min.

3. Spin at 10,000xg for 18 min at 4C.

4. Carefully remove aqueous phase (top) by aspiration (use sterile disposable fine plastic pipette) and transfer to new sterile RNase-free tube (15 ml tube).

IMPORTANT: Stay away from the aqueous/organic interphase. This is where the DNA and RNases are. It is suggested to sacrifice aqueous material rather than risk taking this precipitate.

5. **Slowly** add an equal volume of 100% RNA-free EtOH, mixing it as needed.

6. Load the sample (up to 700ml) 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 8,000g. Discard flow-through.

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 1.5µl 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.

12. Store RNA at -80C until use.

IMPORTANT Recommendations:
-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!