RNA EXTRACTION FOR RNA-SEQ

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BACKGROUND

- For isolation of RNA from eukaryotic cells using Trizol (Invitrogen) and the RNeasy Mini Kit (Qiagen)

NOTES

- Wear goggles, gloves and coat

EQUIPMENT

- Equipment:
  - Fume Hood in MDCL 4077
  - Vortex
  - Centrifuge
  - Pipettes and Tips, Tubes
  - NanoDrop
- Materials:
  - Trizol (reagent cat# 15596-018), Invitrogen
  - RNeasy Mini Kit, Qiagen
  - Chloroform
  - 70% Ethanol (prepared with RNAse-free water)
  - RNAse-free water

PROTOCOL

1. Add Trizol to cells and freeze at -80°C until frozen (can store up to 1 month)
   a. If adherent: add enough to cover plate
   b. If pelleted: add 1mL to microcentrifuge tube
2. Thaw and resuspend samples
3. Incubate at room temperature for 5 minutes
4. Add 200µL chloroform per 1mL Trizol; shake/vortex
5. Incubate at room temperature for 2 minutes
6. Centrifuge for 20 minutes at max speed (preferably 4°C, but not necessarily)
7. Remove the aqueous (top) phase of the sample by pipetting the solution out
   a. Avoid drawing interphase or organic layer while pipetting
8. Add 1 volume 70% EtOH to 1 volume aqueous phase
9. Add 700µL of the EtOH/aqueous mix to the RNeasy column
10. Centrifuge for 30s at max speed; discard flowthrough
11. Add remainder of the EtOH/aqueous mix to the same RNeasy column

12. Centrifuge for 30s at max speed; discard flowthrough
13. Add 700µL RWI (wash); centrifuge for 30s; discard flowthrough
14. Add 500µL RPE (wash); centrifuge for 30s; discard flowthrough
15. Add 500µL RPE (wash); centrifuge for 30s; discard flowthrough
16. Discard last flowthrough followed by centrifugation for 2 minutes at max speed to dry column of EtOH
17. Add 50µL of RNase/DNase-free H_2O to column
18. Let stand for 1 minute
19. Spin for 2 minutes at max speed
20. Transfer eluate to new tube
21. Measure RNA concentration using the NanoDrop Spectrophotometer; note 260/280 as well
22. Freeze samples at -80°C
23. See “Sample Preparation for RNA-seq” for subsequent steps