

RNA extraction and Isolation:

- Ice Bucket/ Dry Ice
- Qiagen Kit
- Pestles and pestle rotator
- RNA-free water
- β -ME 2ul per 200ul of RLT Buffer
- RNase-free DNase Set: inject 550ul RNase-free water into DNase 1 vial using an RNase-free needle and syringe. Do not vortex, gently invert

Homogenize

- 1) Retrieve dry ice, clean pestles, motorized pestle and bench space with RNase disinfectant followed by EtOH.
- 2) Chill pestles in dry ice.
- 3) Remove RNAlater solution
- 4) 350 μ l **β -ME RLT** Buffer in each sample
- 5) Press pestle and grind in tube for 30-40 seconds
- 6) Allow 10 min for complete lysis
- 7) Vortex samples and pulse

Remove Protein

- 8) Centrifuge at max speed for 3 minutes (13,000-14,000 rpm)
- 9) Check for pellet, pipette super nat or whole sample to new tube, might leave a little liquid on the bottom of the tube

Filter Process

- 10) Prepare pink columns
- 11) Add 350 μ l **70% ethanol** made fresh to lysate and mix gently by pipetting or vortex, always change pipettes
- 12) Transfer total 700 μ l to RNeasy Mini spin column fixed inside 2 ml collection tube (no lid tube).
- 13) Close column lid and centrifuge 15 ~30 seconds at >10,000 rpg
- 14) Discard the flow through, replace pink column in tube
- 15) **350 μ l of RW1** buffer
- 16) Centrifuge >10,000 rpm 15 s, discard the flow through

On Column DNase digestion

- 17) Make DNase Master Mix (DNMM): 10 μ l DNase (DO NOT VORTEX) and 70 μ l RDD buffer using filter pipette, centrifuge down
- 18) Directly add 80 μ l to column and let sit at RT for 30 minutes
- 19) Centrifuge for 15s at 10,000 rpm, discard flow through
- 20) Transfer the column to a new 2ml collection tube

Wash Steps

- 21) (2x) Pipette **500 μ l of RPE** buffer to the column
- 22) Centrifuge (1st 15 sec, 2nd 2 min) RT 10,000 rpm, discard the flow through
- 23) Air Drying: Transfer column to a new 2ml collection tube, centrifuge 1 min RT at full 14,000 rpm. Remove column and check for drops. Centrifuge again to remove drops
- 24) Place column into new 1.5 ml Eppendorf
- 25) Pipette 35 μ l of **RNase free water** at **65°C** directly on the center of the column, sit 2min
- 26) Centrifuge 1 min > 10,000 rpm at RT, elute contains your RNA