

## Perou Lab 2007

### Simultaneous RNA and DNA extraction from human tissue

#### Pat1 RNA Extraction

Kit: Qiagen RNeasy mini kit Cat#74104

1. Use less than 30mg tissue, cut into smaller pieces, put the tissue into a 2ml or 5ml tube.
2. Add 300 to 600 $\mu$ l buffer RLT (Ensure that  $\beta$ -ME is added to buffer RLT before use, 10 $\mu$ l  $\beta$ -ME to 1ml RLT) to the tissue.
3. Homogenize sample (use Fisher Scientific PowerGen125)
4. Centrifuge the tissue lysate for 3 min at 14,200 RPM; carefully transfer the supernatant to a new microcentrifuge tube by pipetting. Use only the supernatant (lysate) in the subsequent steps
5. add equal volume(300-600 $\mu$ l) of 70% EtOH to above lysate.
6. Mix by pipetting and apply up to 700 $\mu$ l to RNeasy column
7. Spin 15s at 11,500 RPM, save flow through in clean 2ml or 5ml tube for DNA extraction.
8. Apply all remaining sample to column, spin and save flow-through for DNA extraction.
9. Apply 700 $\mu$ l buffer RW1 to column.
10. Spin 15s at 11,500 RPM, save flow-through for DNA extraction.
11. Add 500 $\mu$ l buffer RPE to column.
12. Spin 15s at 11,500 RPM and discard flow-through.
13. Add another 500 $\mu$ l buffer RPE to column.
14. Spin 2min at 11,500 RPM and discard flow-through and collection tube
15. Optional: place column on a new 2ml tube to dry column at max speed for 1 minutes

16. Transfer the column to a new 1.5ml tube, add 30-50ul RNase-free water to the column membrane, and spin 1min at 11,500 RPM to elute the RNA. Store RNA at -80°C.

### **Pat2 DNA Extraction**

Kit: Qiagen DNeasy tissue kit Cat# 69504

1. Split saved flow-through (From RNA extraction steps 7, 8 and 9) into two 2ml tubes.
2. Add 250µl 100% EtOH to both tubes.
3. Freeze for 1-2 hours at -20°C or -80°C.
4. Spin flow-through for 15' at 10,000 RPM in cold centrifuge (4°C).
5. Discard supernatant and re-suspend pellet in 180µl buffer ATL(in DNAeasy Kit, from here begin to use DNA kit) with 20µl Proteinase-K.
6. Heat in 56°C water bath for 3 hours or until pellet is dissolved – vortex Occasionally
7. Vortex for 15s and add 200µl buffer AL
8. Vortex and add 200µl 100% EtOH
9. Apply to DNeasy mini-column and spin 1' at 9,750 RPM
10. Discard flow-through and repeat with remaining sample
11. Discard flow-through and collection tube
12. Place column in new tube and wash with 500µl AW1
13. Spin column 1' at 9,750 RPM and discard collection tube
14. Place column in new tube and wash with 500µl AW2
15. Spin column 1' at 9,750 RPM and discard collection tube

16. Place column in new tube and spin for 3' at max speed to dry the Column membrane
17. Place column in clean 1.5ml centrifuge tube and add 100µl buffer AE
18. Incubate 1' and spin 1' at 9,750 RPM to elute
19. Repeat elution with another 100µl AE
20. Store DNA at -80°C.