RNA Extraction and Quantification

I. RNeasy Kit

Materials –

1. RLT
2. RNAse free tips
3. β- mercaptoethanol (amber bottle in the fridge)
4. RNA spin column
5. RH_1
6. RPE
7. RNAse free water
8. UP water

Methods –

1. Using RNAse free tips, prepare the buffer RLT using 1 mL RLT and 10 uL of β – mercaptoethanol
2. Take the fluid out of the microcentrifuge, leaving the pellet
3. Add buffer RLT with β – mercaptoethanol
    a. If you have $< 5 \times 10^6$ cells add 350 uL of RLT to the cells for lysis
    b. If you have $> 5 \times 10^6$ cells add 600 uL of RLT
4. Homogenize the cells using the pipettor and gently vortex them to make sure there are no clumps left
5. Add the volume (350 or 600 uL) of 70% ethanol to homogenize lysate and mix well
6. Transfer 700 uL of lysate to RNA easy spin column (microcentrifuge) placed in 2 mL microcentrifuge tube
7. Centrifuge for **15 sec** ≥10,000 RPM and discard the flow through
    a. Do not lose the pellet
8. Add 700 uL of buffer RW_1 for 15 seconds ≥10,000 RPM and discard the flow through
9. Add 500 uL of buffer RPE and centrifuge for 15 seconds at ≥10,000 RPM
10. Repeat step 9
11. Place RNAeasy column to new 1.5 mL microcentrifuge tube
12. Add 30 – 50 uL of RNAse free water to the spin column
13. Centrifuge for **2 minutes** at 10,000 RPM to elute RNA
    a. Make sure there is enough RNA!