LCM RNA preparation

Potter Group, GUDMAP Consortium

1. Make up RLT by adding 10 ul of pure beta mercaptoethanol per ml of RLT (in the hood).

2. **VERY IMPORTANT. ADD 1/2 UL OF EPICENTRE POLYINOSINE PER 100 UL OF RLT. THIS CARRIER IS REQUIRED TO PROTECT THE RNA SAMPLE AND PREVENT LOSS ON THE COLUMN.**

3. Take 0.5 ml tube with cap from –80ºC freezer and let thaw on ice. The tube is brittle if too cold, and will crack on handling, resulting in loss of sample.

4. Remove the cap, using a vise grip, and add the 100 ul of **RLT WITH CARRIER** to the tube.

5. Place the cap back on the tube, and invert, and tap the tube to get the RLT to drop onto the cap.

6. **Vortex vigorously for about a minute. Must vortex very strongly to remove the membrane from the CAP and expose the cells to RLT buffer to isolate RNA. Will get a very low yield if you do not vortex vigorously, and check for membrane removal with a dissecting scope after next step.**

7. Spin in centrifuge, with adaptor tube, for 30 sec to get sample back to the bottom of the tube. Remove and discard cap.

8. Add 100 ul of 70% ethanol to the tube and mix by pipeting, and place on RNeasy minelute column.

9. Spin 30 sec 2000 rpm, then 15 sec top speed. Discard flow through.

10. Add 700 ul of RW1 buffer, spin top speed room temp 15 sec and place in new collection tube.

11. Add 500 ul of complete RPE, spin top speed, and discard flow through.

12. Add 500 ul of 80% ethanol and spin top speed 2 min, with cap connection at bottom, and discard flow through and collection tube.

13. Place in fresh collection tube, rotate column 180 deg from previous spin, with cap connection now at the top, leave the cap open, and spin 5 min full speed, to dry column and remove any residual fluid.

14. Transfer column to a fresh collection tube, add 14 ul of RNase-free water directly to center of the column, wait 1-5 min then spin 1 min top speed to elute RNA.