University of California San Francisco (UCSF) – (Sarwal Lab)

Abstract: Total RNA isolated from bulk tissue or cells isolated from kidney tissue is an important entity for KPMP. This will be useful in comparing sequence coverage of bulk RNA Seq vs isolated single cells. Also it will provide a picture of how sensitive or insensitive single cell RNA Seq is compared when the cells are used to extract RNA followed by sequencing as a bulk RNA Seq.

The method described here is based on the method being used at the Sarwal lab.

Materials and Reagents:

1. Qiagen RNeasy minikit: Qiagen #74136 (for 5-30mg tissue)
2. TRIzol: Thermo Fisher Scientific, #15596-026
3. 2.0 mL microcentrifuge tube: USA Scientific, Cat no. 1620-2700
4. TissueLyser II: Qiagen, Cat no. 85300

Total RNA Isolation from Biopsy Tissue

1. Place biopsy in a 1.8 mL microcentrifuge tube in **1.5 mL** of RNAlater solution.
    - 1.5 mL is required to ensure total immersion of tissue.
2. Store biopsy at **2 to 8°C** for one day and then transfer to **-20°C** until ready for RNA isolation
3. Prepare, on ice, a master mix of **790 µL** TRIzol and **10 µL** glycogen in a clean 2.0 mL microcentrifuge tube (USA Scientific, Cat no. 1620-2700).
    - This master mix has been optimized for kidney biopsy
4. Transfer tissue section into **800 µL** of TRIzoL/glycogen master mix.
5. Homogenize tissue sample using TissueLyser II (Qiagen, Cat no. 85300) with **one** sterile autoclaved stainless steel bead (**5 mm** diameter) in the tube.
    - Sample volume should not exceed 10% of the volume of TRIzol Reagent used for homogenization

Note*: Sample can now be stored frozen at -80°C for ~3 months or until ready for extraction*

6. Incubate (or thaw) sample at **15 to 25°C** for **5 min**.
7. Add **160 µL** of chloroform for phase separation
8. Vortex sample vigourously **~15** seconds and incubate at **15 to 25°C** for **2 to 3 min**.
9. Centrifuge sample at **12,000 x g** for **15 min** at **4°C**.
10. Transfer the aqueous phase to a new clean 1.8 mL microcentrifuge tube
    - The organic phase can be saved if isolation of DNA or protein is desired.

Note*: Aqueous Phase can be taken to RNeasy Micro Kit (page 55) or continue to step #11.*

11. Precipitate RNA in **400 µL** of isopropyl alcohol. Invert 20 times to mix.
12. Incubate sample at **-20°C** for a minimum of **1 hr**.
    - Do not incubate longer than **8 hours** at **-20°C**

13. Centrifuge sample at **12,000 x g** for **10 min** at **4°C**.
14. Remove the supernatant carefully without disturbing the pellet.
15. Wash the RNA pellet once with **800 µL** of 75% ethanol.
16. Mix sample by vortexing and centrifuge at no more than **7,500 x g** for **5 min** at **4°C**.
17. Decant ethanol wash and carefully pipette off remaining ethanol without disturbing the RNA pellet.
18. Air dry the pellet in a clean hood for **5 to 10 min**.
    - Do not use centrifugation under vacuum to dry pellet
    - Do not dry completely so as to avoid decreased solubility
19. Dissolve RNA pellet in **30 µL** of RNAse-free water or 10mM Tris-HCl (pH 7.5).
20. Measure RNA concentration and quality with spectrophotometer and Bioanalyzer.

***Note**: For tissue less than <5 mg please use RNeasy Micro Kit (Qiagen, Cat no. 74004) and follow the manufacturer's instructions.*