Finno "Tendon modifications" of the RNeasy Lipid Tissue Kit (Qiagen)

Modified by Erin Burns for FAANG Isolations

(http://www.qiagen.com/products/rnastabilizationpurification/rneasysystem/rneasylipidtissuemini.aspx)

- Have liquid nitrogen ready
- Have tendon frozen in liquid N_2
- Have pulverizer ready "Cat. No. 59013N, BioPulverizer, capacity 0.1-1g"
- Have centrifuge at $4^{O}C$
- Have RNeasy Micro columns (The cleanup kit is cheaper than the isolation kit and works just as well and has the necessary reagents.)
- Don't forget to gather ice and clean instruments

1. Pulverize the tissue with BioPulverizer to create a fine powder. Complete powder gives better result. Keep the mortar and pestle frozen with liquid N2.
2. Take the powder directly into the 2ml tube and freeze until ready to use. **Fill two 2 mL tubes with 100 mg of tissue** for each sample. In total, you will use 200 mg of tissue for each sample.

Homogenization:

3. Add **700 µl Trizol** to pulverized tissue
4. Homogenize for 30 seconds 3 times, 1 min on ice in between.
5. Add **300 µl Trizol** and mix by pipetting. Rest sample on ice while remaining samples are homogenized.
6. Cut the end off a 1000 µl tip and use it to move the homogenized sample into 15 ml conical. Add **2 ml Trizol** to homogenization tube and wash. Then transfer this additional Trizol to the 15 ml conical tube. Put both 2 mL tubes of homogenate into one 15 ml tube.
7. **Incubate** at room temperature for 15 minutes on a rotisserie.
8. **Split** the cartilage-Trizol® homogenate into 1.5 ml microcentrifuge tubes with 750 µl in each microcentrifuge tube.
9. Add **300 µl chloroform** to each tube and Vortex 15 seconds until the solution looks like Pepto-Bismol®.
10. Incubate at room temperature 3 minutes.
11. Centrifuge 12,000 x *g* for 15 minutes at $4^{O}C$

RNA Collection:

12. **Transfer** aqueous phase (top layer) to a new 1.5 ml tube 200 µl at a time. It is important not to contaminate the aqueous supernatant with the white interphase layer or lower pink layer. If necessary, it is better to be cautious and clean, rather than try and pipette up every bit of the supernatant.
    - Use the 200 µl transfers to measure 600 µl in each tube. One tube can serve as the overflow and the amount in this tube is approximated using the markings on the side.
13. **Add** one volume of **70% ethanol** (600 µl for most tubes and lower for the overflow tube) – mix thoroughly by inverting 3 times.

14. Pipette **700 µl** of this RNA/ethanol **mixture** onto the **RNeasy Micro Spin Column** with supplied waste collection tube. Centrifuge at **8,000 x *g* for 30 seconds** at 4^{O}C. Discard the flow through into labeled waste beaker.
15. **Repeat** step 12 until finished loading all volume of mixture from 1.5ml tubes. (Should be approximately 6 tubes worth of mixture.)

DNA Digestion:

16. Add **350 µl Buffer RW1**, centrifuge at **8,000 x *g* for 20 seconds** at 4^{O}C. Discard flow through.
17. Add **70 µl Buffer RDD** to a **10 µl Qiagen DNase aliquot**. Mix by pipetting and add the **80 µl Reaction mixture** to the column.
18. Incubate at room temp for 15 minutes.

RNA Clean up and Collection:

19. Add **350 µl Buffer RW1,** centrifuge at 8,000 x *g* for 20 seconds at 4^{O}C. Discard flow through.
20. Add **500 µl Buffer RPE**, centrifuge at 8,000 x *g* for 20 seconds at 4^{O}C. Discard flow through.
21. Add **500 µl Buffer RPE again** (for micro kit, this step is 80% ethanol), centrifuge at 8,000 x *g* for **2 minutes** at 4^{O}C. Discard flow through and the collection tube.
22. Place Spin Column in new collection tube. Open lid to dry; centrifuge for **5 minutes at full speed**. Discard flow through and the collection tube.
23. Place spin column in a new, labeled tube 1.5 ml microcentrifuge tube, and add **20 µl RNase-free water.** Spin at **8,000 x *g* for 3 minutes** at 4^{O}C.
24. Nanodrop the sample and **store at -80^{O}C**. Run Bioanalyzer when enough samples have been isolated.

Protocol adapted from Annette Marie of the McCoy lab January 2017