TRIzol extraction of ITN RNALater tissue biopsies

1. Remove 24 samples from the -80°C and allow them to thaw. One "batch" equals 12 samples.
2. Remove 1 tube of 5mg/uL Glycogen from the -20°C.
3. For each sample you will need:
    a. 2- 1.5 mL tubes (Tubes A and B, for extraction)
    b. 1- 0.5 mL tube (bioanalyzer)
    c. 1- 0.5 mL ITN stock tube (with insert)
    d. 1 – conical pestle grinder
4. Label each tube with the corresponding toughspot. Adhere the corresponding barcode label to the ITN stock tube. Make sure both labels match!
5. Add 400 uL of TRIzol to Tube A (24).
    a. Dispose of ALL TRIzol contaminated tips, gloves, paper in the TRIzol waste bucket.
6. Partially remove each ITN sample label so you can visualize the tissue. Look for:
    a. Tissue size (the ITN liver biopsies range in size from thick, long pieces to skinny long pieces or small chunks.
    b. Crystallization of the RNALater. If you see crystallization, is the tissue embedded in it? If so, the crystal+tissue will need to be rinsed with 1mL of PBS to release the tissue. Carefully transfer the entire contents of the sample onto a 15 mL tube. Add 1mL PBS 1X. Stir until dissolved. Then continue with Step 6.
7. Using a 200 uL pipette, CAREFULLY transfer all the tissue to Tube A. Maintain the plunger depressed so you don't pick up any RNALater. Close the cap.
8. Dispose of the RNALater from all samples in a common 50 mL tube (when done, add bleach to this waste tube before disposing down the sink). Recap sample tube and throw away in biohazards box.
9. Repeat step 6-7 for 12 or 24 samples – up to you.
10. By the time you are done transferring all samples, the tissue should be softer and easier to homogenize.
11. Use a clean conical pestle grinder to homogenize each sample. Crush as much tissue as you can as quickly as you can. Spend only about 2 minutes homogenizing the tissue per sample. Too much time spent at this step will not generate better RNA.
12. Rinse the pestle with 600 uL of TRIzol to make sure that all the homogenized tissue is in the tube. Close Cap.
13. Vortex tube for 10 seconds.
14. Repeat Steps 10-12 for all samples.
15. Incubate the homogenized sample for 5 minutes at room temp
16. Proceed with Step II of RNA-TC-1 (TRIzol RNA Tissue Extraction SOP) using volumes for a 1.0 mL extraction.
17. Step III.1 comment: For very small tissues, add 1uL of glycogen.
18. Step V.1 comment: Hydrate all samples with 40 uL of NF water. For very small tissues, use 20 uL.
19. Freeze at -80°C.
20. Spec and Bioanalyze. When 72 RNA samples have been accumulated:
    a. Spec. Using a UV 96 well plate, add 80 uL of NF water to Rows A to G. Add 1 uL of RNA sample to each well (document well ID in bench sheet). Rows A-F have RNA samples, Row G is the blank well. Cover plate and spin down for 1 min. Read plate in the Genios Pro using ITN program. Export results excel file and save in Study Directory under Specs.
    b. Make bioanalyzer aliquots as described on the table:
    c. Name each Nano chip "ShakedB1_Batch-#"
    d. Export files per ITN SOP (xmL, jpg, csv, pdf) and print the pdf file as well.
21. Transfer RNA samples to the corresponding ITN stock tube and record the volume.
22. Freeze RNA samples at -80°C.

Bioanalyzer Aliquots:
<400 ng/uL = 2 uL RNA
400-2000 ng/uL = 1.0 uL + 3.0 uL H_2O
2000 ng/uL = 1.0 uL + 5.0 uL H_2O