SOP: RNA Extraction From Tissue

Total Time: ~2.5 - 3 hours

- Place mortar and pestle on dry ice and allow to cool.
- Add Trizol to 10mL tubes (the amount of Trizol is based on the amount of tissue. For small amounts of tissue, 2mL of Trizol should be plenty, fatty tissues will require more Trizol).
- Place tissue (approximate size of pencil eraser) and a small amount of dry ice in the mortar. Crush the tissue until it is a fine powder and place in Trizol.
- Homogenize and split the sample into 2 - 1.5mL tubes (1mL/tube).
- Centrifuge for 5 minutes @ 14,000g and 4C.
- Avoiding any fat layer that may have formed at the top of the liquid, transfer the clear Trizol solution to a new 1.5mL tube.
- Add 200uL chloroform to each tube (200uL of chloroform for each 1mL of Trizol).
- Shake vigorously.
- Incubate 15 minutes @ room temperature.
- Centrifuge for 15 minutes @ 14,000g and 4C.
- Transfer the top clear layer to a new 1.5mL tube (another fat layer may have formed…avoid transferring this).
- Add 500uL isopropanol and invert to mix.
- Incubate for 10 minutes @ room temperature.
- Centrifuge for 30 minutes @ 14,000g and 4C.
- Pull off the supernatant and discard.

Written By: William Thompson USDA – ARS (Clay Center) Date: 26th June 2018

- Wash the pellet with 1mL 75% EtOH. Try not to disturb the pellet.
- Centrifuge for 5 minutes @ 14,000g and 4C.
- Pull of the supernatant, quick spin, and pull of remaining EtOH.
- Allow to air dry 5-10 minutes (pellet should change from white in color to opaque or almost clear).
- Resuspend in Mo. Bio. Grade water (51uL/sample—pipet gently to dissolve the RNA) and pool the resuspended RNA.
- QC RNA:
  Spec. with the Nanodrop.
  Run on the Fragment Analyzer or run 1uL on a 1% agarose gel.
- Store extracted RNA at -80C.

DNase Treatment

Perform DNase treatment with DNase I from NEB followed by a cleanup with a Qiagen RNeasy MinElute kit.

1. Resuspend 10 µg RNA in 1X DNase I Reaction Buffer to a final volume of 100 µl.
2. Add 2 units of DNase I, mix thoroughly and incubate at 37°C for 10 minutes.
3. Add 1 µl of 0.5 M EDTA (to a final concentration of 5 mM).
4. Heat inactivate at 75°C for 10 minutes.