**RNA EXTRACTIONS-Tonga Date: \_\_\_\_\_\_\_**

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| Sample | Transport temp. | Storage temp. | Homogenized on ship? | Notes |
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HOMOGENIZATION AND PHASE SEPARATION

1. Transferred RNALater-tissue+skeleton slurry from cryotube to 2-ml tube or homogenized in TRIzol on \_\_\_\_\_\_\_.
2. Spun @ 12,000 xg for \_\_\_\_ min @ \_\_\_\_and siphoned off RNALater with 1-ml pipet (except for samples homogenized in TRIzol).
3. Added 1 ml TRIzol, vortexed, and incubated @ \_\_\_\_ for\_\_\_\_\_\_\_\_\_.
4. Added 200 ul chloroform, vortexed, and incubated @ RT for \_\_\_min (phase separation).
5. Spun @ 12,000 x g for 15 min @ 4°C and transferred aqueous phase to new tube.

PRECIPITATION

1. Precipitated w/ 250 ul isopropanol and 250 ul high salt solution @ \_\_\_\_\_\_ for \_\_\_\_\_

and spun @ 12,000 x g for 10 min @ 4°C.

PURIFICATION

1. Re-suspended pellet in 350 ul Planet Lysis buffer A and 350 ul 100% ethanol.
2. Added 700 ul to GeneMark Plant Total RNA kit spin column.
3. Followed manufacturer’s recommendations and incubated columns in 60°C oven.
   1. On-column DNase digestion\_\_\_\_\_\_
4. Eluted into 30 ul DEPC-treated water.
5. Electrophoresed 6 ul on \_\_\_\_\_\_% \_\_\_-agarose gel @ \_\_\_\_V for \_\_\_\_ min and stained w/EtBr for \_\_\_\_\_min.
6. Diluted to 20 ng/ul.

**DNA EXTRACTION**

PHASE SEPARATION

1. Added 500 ul BEB to organic phase (step 7) & incubated on shaker table for \_\_\_\_\_\_\_ min.
2. Spun @ 12,000 x g for 15 min @ 4°C & transferred aqueous phase to new tube.

PRECIPITATION

1. Precipitated DNA w/ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ & \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ @ \_\_\_\_\_ for \_\_\_\_\_\_.
   1. w/: 1.5 ul Pellet Paint™\_\_\_\_\_.
2. Spun @ 12,000 x g for 10 min @ 4°C.

PURIFICATION

1. Re-suspended pellet in 500 ul Buffer DF from Yeastern kit\_\_\_\_\_\_\_.

OR: dissolved in Buffer Wl\_\_\_\_\_\_.

OR: 75% ethanol wash \_\_\_\_\_\_\_.

1. Carried out spins and washes as recommended by manufacturer except with two alcohol washes.
2. After evaporating residual ethanol in 60°C oven, eluted DNA into 50 ul pre-warmed (to 60°C) “eluent” (Tris buffer, pH 8.5)
3. Electrophoresed 6 ul on \_\_\_\_% \_\_\_-agarose gel @ \_\_\_\_V for \_\_\_\_ min and stained w/EtBr for \_\_\_\_\_min.
4. Diluted to 10 ng/ul.

**PROTEIN EXTRACTION**

1. Precipitated protein in \_\_\_\_\_\_ ml acetone @ \_\_\_\_\_\_\_ indefinitely.
2. Spun @ 12,000 x g for 10 min @ 4°C.
3. Washed pellet 3x w/ PWI @ 8,000 x g for 5 min @ 4°C.
4. Added 1 ml PWII, vortexed, and split proteins in PWII into two tubes (500 ul per tube), one for later re-suspension in sample buffer and the second for later re-suspension in rehydration buffer.
5. At a later date, spun tubes @ 8,000 x g for 5 min @ 4°C, decanted supernatant, and dried on bench for \_\_\_\_\_min.
6. Re-suspended protein in \_\_\_\_\_ul “sample” buffer, sonicated for \_\_\_\_\_ min, boiled for \_\_\_\_ min @ 100°C, and transferred \_\_\_\_\_ul to each of \_\_\_\_tubes.
7. Or re-suspended in \_\_\_\_\_ul rehydration buffer containing\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_, and dissolved by\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_.
8. Quantified \_\_\_\_\_ul with the 2-D Quant kit.