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

HOMOGENIZATION AND PHASE SEPARATION (unhomogenized samples in TRIzol® only)

1. Stored in TRIzol® @ -80°C for days-weeks on ship followed by transport for 2-3 d @ RT.
2. Decanted sample+TRizol into mortar and added 0.5-1.5 ml additional TRIzol.
3. Homogenized, added 1 ml slurry+TRizol to new tube, and incubated @ \_\_\_\_ for \_\_\_\_\_. Optional: added backup sample slurry+TRIzol to original tube and re-froze @ -20°C. \_\_\_
4. Added 200 ul chloroform, vortexed, and incubated @ RT for \_\_\_\_\_\_\_min.
5. Spun @ 12,000 xg 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 1% \_\_\_-agarose gel @ 100 V for 30-45 min and stained w/EtBr for 15-30 min.
6. Diluted to 20 ng/ul.

**DNA EXTRACTION**

PHASE SEPARATION

1. Added organic phase from step 5 to BEB & incubated on shaker table for \_\_\_\_\_\_\_ min.
2. Spun @ 12,000 xg for 10 min @ 4°C & transferred aqueous phase to new tube.

PRECIPITATION

1. Precipitated DNA w/ \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ & 600 ul isopropanol @ \_\_\_\_\_ for \_\_\_\_min.
	1. w/: 1.5 ul Pellet Paint™\_\_\_\_\_.
2. Spun @ 12,000 x g for 10 min @ 4°C.

PURIFICATION

1. Re-suspended pellet in \_\_\_\_ ul \_\_\_\_\_ buffer from Protech’s PCR clean-up kit.
2. Carried out spins and washes as recommended by manufacturer.
3. After evaporating residual ethanol in 60°C oven, eluted DNA into 50 ul pre-warmed (to 60°C) “eluent.”
4. Electrophoresed 6 ul on 1% \_\_\_-agarose gel @ 100V for 30-45 min and stained w/EtBr for 15-30 min.
5. Diluted to 10 ng/ul.

**PROTEIN EXTRACTION**

1. Precipitated protein in 1.4 ml acetone @ -20°C 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.