Modified: Plant Extraction Standard Protocol

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Using Qiagen RNeasy mini kit

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PREPARING SPECIMENS AND HOMOGENIZING TO CREATE CRUDE EXTRACTIONS:

1. Using disinfected forceps, weigh out 1.5 g of flower material (use fresh looking flowers). Transfer to labeled extraction bag. Place bag into the canister of liquid nitrogen.
2. Note sample name in lab notebook and mark the top of the falcon tube with the lab ID. Return rest of plant material back into the cooler with liquid nitrogen.
3. After flash freezing, use a pestle on outside of extraction bag to crush plant sample into powder (~30 sec.).
4. Add 3 mL of GITC buffer to the extraction bag and homogenize using pestle on outside of bag for 2.5 minutes.
5. Use pippette to extract as much liquid as possible and place into 1.4 ml vial.
6. Put vial on ice and throw away extraction bag.
7. Repeat for all specimens.
8. Centrifuge for 3 minutes to push specimen to bottom of tube. Before continuing, sample should be brought to room temperature.

BEGINNING RNAISOLATION (Making Crude Extraction)

1. Label spin columns. Fill second set of 1.5 vial with 500 ul of Buffer RLT- B-mercaptoethanol (crude extraction).
2. Add 200 ul of plant homogenite/GITC buffer to corresponding 1.5 vial containing RLT buffer buffer (crude extraction).
3. Using a single pipette tip per sample, add 700ul of 70% ethanol to each crude extraction. Use pipette to to mix 5-6 times before drawing up 700ul and moving intocorresponding spin column.
4. Create a negative control here (200 ul GITC, 500 ul RLT buffer + 700 ul ethanol).
5. Put crude extraction back on ice and set aside to be placed in freezer.

RNA ISOLATION (from Qiagen)

1. Centrifuge spin columns for 1 minute at high speed then discard flow-through into small beaker.
2. Add 700ul of Buffer RW1 to spin column, centrifuge for 1 min at high speed and discard flow-through into small beaker.
3. Add 500ul Buffer RPE to spin column, centrifuge for 1 min at high speed and discard flow-through into small beaker.

4. Add 500ul Buffer RPE to spin column, centrifuge for 2 min at high speed and discard flow-through into small beaker. Discardbeaker contents down sink.
5. Transfer spin columns into new 2ml tubes and spin at high speed for 1 min. Discard old 2ml tubes.
6. Assemble new 1.5ml tubes and cut off lids.Transfer spin columns to 1.5ml tubes.
7. Add 50ul of RNase-free water directly to the spin column membrane without touching the membrane. Centrifuge for 1 min. Do NOT discard flow-through.
8. Label 0.5ml tubes with corresponding lab ID #s.
9. Pipette flow through from 1.5ml tubes into corresponding 0.5ml tubes and immediately place on ice. Discard spin columns and their tubes.
10. Take 0.5ml tubes up to nanodrop.