Bumble Bee Extraction Protocol (2015 Survey protocols)

Preparing and Homogenizing Specimens : (homogenized bee samples contain: GITC buffer and bee tissue)

1. Using disinfected forceps, pull of legs of beesand pollen and place into the labeled small vials. Place in freezer.
2. Place the rest of bee into a 1.5 vial.
3. Use pestle to grind bee for 30 sec.
4. Add 600ul GITC buffer to vial
5. Homogenize with pestle for 1.5 minutes.
6. Put in ice
7. Repeat for all specimens
8. Centrifuge to push bee specimen to bottom of vial for 3 minutes

Creating the Crude Extractions (crude extractions contain: GITC mixed with bee tissue, RLT buffer with 10% beta mercaptoethanol, and 70% ethanol)

1. In a new 1.5 ml vial, add 100 ul of bee homogenite lysate to 600 ul of RLT buffer (10% Beta mercaptoethanol).
3. Create a negative control here (600 ul RLT buffer + 100 ul GITC buffer)
4. Add 1 volume (700 ul) of 70% ethanol to all vials

Proceed with Qiagen protocol for RNA extraction:

RNA ISOLATION (fromQiagen)

1. Use pipette to mix bee, buffer, and ethanol solution (crude extraction) 5-6 times before drawing up 700ul and moving into labeled spin column.
2. Place crude extraction back on ice to be placed in freezer.
3. Centrifuge spin columns for **1 minute** at high speed then discard flow-through into small beaker.
4. Add **700ul of Buffer RW1** to spin column, centrifuge for **1 min** at high speed and discard flow-through into small beaker.
5. Add **500ul Buffer RPE** to spin column, centrifuge for **1 min** at high speed and discard flow-through into small beaker.
6. 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.
7. Transfer spin columns into new 2ml tubes and spin at high speed for **1 min**. Discard old 2ml tubes.

8. Assemble new 1.5ml tubes and cut off lids.Transfer spin columns to 1.5ml tubes.
9. 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.
10. Label 0.5ml tubes with corresponding lab ID #s.
11. Pipette flow through from 1.5ml tubes into corresponding 0.5ml tubes and immediately place on ice. Discard spin columns and their tubes.
12. Take 0.5ml tubes up to nanodrop.

To put in freezer:

homogenized bees, crude extractions, RNA extractions