**Microbiome Extractions**

Safety Considerations

*Required PPE*

* Gloves
* Glasses
* Lab coat

*Materials of concern*

* Ethanol – flammable, toxic, health hazard, irritant
* Bleach – corrosive and irritant to eyes and skin

Microbiome Extractions

**Materials**

* DNase spray
* 70% Ethanol
* Sharpie
* Qiagen DNeasy Blood & Tissue Kit
* Samples
* 96-100% Ethanol
* Incubator
* Sterile 0.1 mm glass beads
* Fisher Bead Mill
* P1000 Micropipette
* 1000 µL Pipettes Tips
* P200 Micropipette
* 200 µL Pipette Tips
* Sterile Microcentrifuge Tubes

**Methods**

1. Set an incubator to 56°C.
2. Place buffers into the incubator to ensure that they have not precipitated.
3. Record kit lot number and samples IDs you are processing.
4. Wipe down hood, sharpies and micropipettes with DNase spray and 70% ethanol.
5. Let samples reach room temperature.
6. In a sterile 2 mL screw cap microcentrifuge tube, add 100 uL of 0.1 mm glass beads.
7. Secure tubes in the Fisher Bead Mill.
8. Set program to run for 3 cycles of: 1:15 min beating with 45 sec rest.
9. Add 180 µL Buffer ATL.
10. Add 25 µL proteinase K.
11. Pulse vortex for 5-10 sec.
12. Incubate at 56°C overnight.

*NOTE:* Lysate should be viscous, but not gelatinous. If the lysate is gelatinous, add another 1 hr

of incubation. Record any alterations to the protocol.

1. Pulse vortex for 15 sec.
2. Centrifuge the samples for 2 min at 6,000 rpm to force lysates to the bottom of the tube.
3. Pipette the supernatant into a sterile, labeled microcentrifuge tube.
4. Add 200 µL Buffer AL.
5. Pulse vortex for 5-10 sec.
6. Add 200 µL 96-100% ethanol.
7. Pulse vortex for 5-10 sec.

*NOTE:* If there is a gelatinous lysate, vigorously vortex the sample.

1. Pipet the mixture into a DNeasy Mini spin column placed in a 2 mL collection tube.
2. Centrifuge for 1 min at ≥ 6,000 x g (8,000 rpm).
3. Discard the flow-through and collection tube.
4. Repeat steps 20-22 until all of the sample has been processed.
5. Place the spin column in a new 2 mL collection tube.
6. Add 500 µL Buffer AW1.

*NOTE:* Make sure the spin column stays dry, as ethanol can prevent certain reactions from

occurring.

1. Centrifuge for 1 min at ≥ 6,000 x g (8,000 rpm).
2. Discard the flow-through and collection tube.
3. Add 500 µL Buffer AW2.

*NOTE:* Make sure the spin column stays dry, as ethanol can prevent certain reactions from

occurring.

1. Centrifuge for 3 min at 20,000 x g (14,000 rpm).
2. Discard the flow-through and collection tube.
3. Transfer the spin column to a new 1.5 mL or 2 mL microcentrifuge tube.
4. Elude the DNA by adding 200 µL Buffer AE to the center of the spin column membrane.
5. Incubate for 1 min at room temperature (15-25°C).
6. Centrifuge for 1 min at ≥ 6,000 x g.

Source(s):

Dew, RM, McFrederick, QS & Rehan, SM (2020). Diverse diets with consistent core microbiome in wild bee pollen provisions. *Insects, 11*: 499.