**Sampling Event 3 - Pre-wintering Adult**

Lab Safety

*Required PPE*

* Gloves
* Glasses
* Lab coat

*Materials of concern*

* Ethanol – flammable, toxic, health hazard, irritant
* Bleach – corrosive and irritant to eyes and skin
* Razor blade – sharp
* Microscissors - sharp

Post-collection preparation

*Materials*

* DNase spray
* 70% ethanol
* 95% ethanol
* 5% bleach
* Nesting tubes
* Razor blade
* Forceps (fine and wide tip)
* Microscissors
* Weigh boat
* Light microscope
* Sterile well-plate
* Kimwipes

*Methods*

1. Sanitize the biosafety fume hood with DNeasy spray and 70% ethanol.
2. Sterilize the razor blade, forceps and microscissors by submerging them into 70% ethanol and then 5% bleach.
3. Spray a weight boat with 70% ethanol and 5% bleach to sterilize it before use.
4. Using the sterile razor blade, cut the paper insert longitudinally.  
   *NOTE: Be sure to sterilize the razor blade between nesting tubes.*
5. With the sterile forceps (wide tip), carefully pick up a cocoon and place it into the sterilized weight boat.  
   *NOTE: Be sure to sterilize the forceps and weigh the boat in between bee samples. You can wipe the weight boats with kimwipes.*
6. Place the weight boat containing the cocoon under a light microscope.
7. Using sterile forceps (wide tip), hold the cocoon in place while using sterile microscissors to carefully cut a window out of the nesting material.  
   *NOTE: While using the microscissors, lay them flat (i.e., parallel to the sample) to avoid cutting the bee contained inside the cocoon.*
8. Using sterile forceps (thin tip), grasp one of the bee’s legs and gently pull it out of the cocoon.
9. Place the bee into a sterile, labeled well plate. Record which nesting tube and brood cell the bees came from.
10. Pour enough 95% ethanol into each well plate to submerge the bee. This will humanely euthanize the bee so that the body size and microbiomes can be determined in the protocols below.

Determining pre-wintering adult body mass and size

*Materials*

* Bees
* Forceps
* Weigh boat
* Digital calipers
* Scale (must measure in milligrams)
* Light microscope

*Methods*

1. Place a weight boat onto a scale. Tare the scale.
2. Using forceps, carefully pick up a bee and place it into a weight boat.
3. Weigh the pre-wintering adults using a scale
4. Under a microscope, use digital calipers to measure both the length and the intertegular distance of each bee in a weigh boat
5. Record the data in your lab notebook and Excel spreadsheet.

Isolating the pre-wintering adult gut microbiome

*Materials*

* DNase spray
* 70% ethanol
* 75% ethanol
* 5% bleach
* Sharpie
* Bees
* Beakers (6)
* Physiological saline solution (see ‘Stock Solution’ document)
* Small petri dishes
* Sterile microcentrifuge tubes
* P1000 micropipette
* Sterile 1000 µL micropipette tips
* Forceps (fine and wide tips)
* Microscissors
* Dissecting needle
* Laboratory spatula
* Light microscope

*Methods*

1. Sanitize the biosafety fume hood with DNeasy spray and 70% ethanol.
2. Sterilize forceps, microscissors, dissecting needle and a laboratory spatula by submerging them into 70% ethanol and then 5% bleach.
3. Label sterile microcentrifuge tubes for each sample
4. Pipette 100 µL physiological saline solution into each tube
5. Using the sterile forceps, surface sterilize the bee by submerging them in 75% ethanol three times for 5-10 sec per wash.
6. Wash the bee a final time in physiological saline for 5-10 sec to remove residual ethanol.

*NOTE: For every new bottle of physiological saline solution, pipette 1000 µL into a sterile microcentrifuge tube as a control for sequencing. This will help you identify potential contaminants.*

1. Place the surface sterilized bee into a sterile small petri dish
2. Submerge the bee with physiological saline solution
3. View bees under the microscope while dissecting the bee to isolate the gut
4. With a sterile dissecting needle, hold the bee down by apply moderate pressure to the second or third tegument on the ventral side of the abdomen
5. Using a sterile forceps (thin tip), grasp the last tegument and gently pull until it separates from the body. Once this happens, do not pull the tegument anymore.
6. At this point, the abdominal cavity should be more accessible. Carefully remove each tegument one at a time with sterile microscissors until the crop/foregut is exposed.
7. Snip the digestive tract with sterile microscissors along the esophagus (before the crop)
8. Transfer the dissected gut to a correspondingly labeled microcentrifuge tube
9. Repeat steps 5-14 for each bee, sterilizing the forceps between each sample.
10. With a sterile laboratory spatula, pulverize the bee. Sterilize the spatula between each sample. Plate the samples within 48 hrs.
11. Record notes on how the dissection went, noting any rupture of the digestive tract and whether all compartments (foregut, midgut and hindgut) were retrieved

**Sources**

*Isolating the pre-wintering adult gut microbiome*

Vojvodic, S., Rehan, S.M. & Anderson, K.E. (2013). Microbial gut diversity of Africanized and European honey bee larval instars. *PLoS ONE, 8*(8): e72106. <https://doi.org/10.1371/journal.pone.0072106/>

**(modified)**

Carrek, N.L., Andree, M., Brent, C.S., Cox-Foster, D., Dade, H.A., Ellis, J.D., Hatjina, F. & van

Englesdorp, D. (2013). Standard methods for *Apis mellifera* anatomy and dissection. *Journal of*

*Apicultural Research, 52*(4): 1-40. Doi: 10.3896/IBRA.1.52.4.03 <https://www.tandfonline.com/doi/pdf/10.3896/IBRA.1.52.4.03>

(**modified)**