***Bombus* Microcolony Protocol**

Constructing Microcolonies

For each microcolony arena you will need:
2 16oz deli cups
2 16oz deli cup lids
2 8 oz deli cups
1 small petri dish
1 3" length of tubing (½-1" inside diameter)
2 ½" lengths of tubing, cut in half (so that they form arcs)
Fine mesh screen (2mm)

Additional Supplies:

Glue gun
Glue sticks
Drill
Circular drill bit (the same size as your tubing – 1" is convenient)

Drill a hole into the side of each of the of the two 16oz deli cups, then cut the bottom out. Cut as close to the lip as possible, but leave some surface area for gluing. Cut a square of mesh screen to cover the bottom. Use a glue gun to glue the mesh to the bottom of the deli cup. Connect both cups with the tube. If your drill bit was the same size as the tubing you shouldn't need to glue it, the fit will be tight enough to hold. If you do glue this together, it will be more difficult to clean and/or replace. Drill small air holes through the lids in a circle around the outer edge making sure these holes are not big enough for a bee to squeeze out of. In the center, cut a hole the size of the petri dish. The petri dish should slide into the hole easily, but not fall through. Set the two 16oz deli cups into the 8oz deli cups, using the halved lengths of tubing to stabilize the 16oz cups above the bottom of the 8oz deli cups.

Creating Microcolonies

1st Day: For each source colony, remove the queen from the colony and vacuum all workers into a small canister. Put the canister in the fridge for several minutes to slow the bees, and then mark all the bees with a water-based paint marker, ideally white (as it is easily visible under red light). Return the queen to the colony.
2nd Day: Mark all callow bees (newly emerged, looking very fluffy and white, with no mark) with a different colour of water-based paint marker (easily distinguishable from the first), *e.g.* green.
3rd Day: Remove all bees painted with the 2nd color (*e.g.* green) and introduce them to a microcolony arena (up to 10 bees per arena). Mark all callow bees with the 2nd color water-based paint marker (*e.g.* green).
4th Day onward: Repeat the procedure for the 3rd day until you have filled all your microcolony arenas. **New callow bees emerge daily, so it is critical to follow this procedure every day, and remember to remove the painted bees first, then mark the newly eclosed callow bees.**

Caring for Microcolonies

Nectar and pollen should be provided ad libitum and colonies should be kept at 27°C and between 60-80% humidity. Pesticide-free bee-collected pollen should be crushed with a mortar and pestle, with 30% sucrose solution (1400mL DI water + 600g sugar = ~2L 30% sucrose solution) added until the pollen is paste-like in consistency. If you do not wish to quantify the pollen that bees are eating daily, create a 1g ball of pollen, then dip it in beeswax to prevent desiccation. The bees may lay eggs on this pollen ball. Alternatively, if you do wish to quantify the pollen that bees are eating daily, fill a small container with pollen and weigh it daily. In this case you will also need a control container (to account for natural mass lost to evaporation) outside the microcolony. You can make the pollen mixture in advance, but will need to freeze it at -20°C until use.

Sucrose solution should be provided in a ½oz deli cup. Cut a cross into the lid of the deli cup and insert a ½" piece of dental wick. Fill the deli cup with 30% sucrose solution, then dip the top of the wick into it, and close the deli cup. Weigh this daily to assess sucrose consumed, or record the date when it is replaced to get a rough estimate of how often the bees consume ½oz of sucrose solution.

Microcolonies should be rotated throughout the growth chamber on a daily basis. The 8oz deli cups should be cleaned periodically by rinsing with DI water. Additionally, any excrement build-up can be carefully cleaned using a small piece of tissue.

Monitoring Microcolonies

Each day, for each microcolony, record:
# of live bees
# of dead bees
# of nectar cells
# of egg clumps
# of drone clumps
# of ejected larva
weight of sucrose
weight of pollen
weight of desiccation control pollen

Wipe down all tools with ethanol between each microcolony manipulation. Sucrose should be replaced when it is under a threshold amount (the lip towards the bottom). Pollen should be replaced when it is under a threshold amount (approximately 0.01g per bee, with 0.02g extra) or no longer than 7 days. The desiccation control pollen should be replaced every 5-7 days or a separate experiment conducted to assess mass lost to evaporation over time for each treatment. **Microcolonies should be terminated at the first appearance of adult drones in the microcolony.** Once the assay has finished, all eggs and larvae should be counted and weighed. Adult bees should be weighed (inter-tegular distance and radial cell length can also be measured) to assess body size. Pseudoqueens can also be dissected to measure ovary growth.