**Sampling Event 1: Initial Provision Microbiome**

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
* Razor blade - sharp

Post-collection processing

**Materials**

* DNase spray
* 70% ethanol
* Sharpie
* Beakers (2)
* Razor blade
* Forceps
* Laboratory spatula
* Sterile microcentrifuge tubes
* Nesting tubes
* Scale (must measure in milligrams)

**Methods**

1. Sanitize the biosafety fume hood with DNeasy spray and 70% ethanol.
2. Sterilize a razor blade and forceps by submerging them into 70% ethanol and then 5% bleach.
3. Using the sterile razor blade, cut the nesting tube longitudinally.
4. Label sterile microcentrifuge tubes.
5. Weigh the microcentrifuge tubes in milligrams.
6. Using sterile forceps, pick up a provision and place into the correspondingly labeled sterile microcentrifuge tube.
7. Repeat steps 3-6 for every nesting tube, sterilizing the razor blade and forceps between every sampling effort.
8. Weigh each microcentrifuge tube containing the sampled provisions.
9. Subtract the sample weight from step 8 from step 5 to determine the weight of each provision.

Culturing the provision microbiome

**Materials**

* DNase spray
* 70% ethanol
* P1000 micropipette
* P100 micropipette
* Sterile 1000 µL micropipette tips
* Sterile 100 µL micropipette tips
* Sterile 1x PBS-0.15% Tween 20
* Sterile glass beads
* Beaker
* Vortexer
* YM agar plates (100 mg/mL chloramphenicol)
* R2A agar plates (100 mg/mL cycloheximide)
* Parafilm
* Incubator (27°C)

**Methods**

1. Sanitize the biosafety fume hood with DNeasy spray and 70% ethanol.
2. Pipette 1000 µL of 1x PBS-0.15% Tween 20 into each microcentrifuge tube.  
   ***NOTE:*** *For every new bottle of PBS-Tween solution, pipette 1000 µL into a sterile microcentrifuge tube as a control for sequencing. This will help you identify potential contaminants.*
3. Label a YM and R2A media plate for each provision sample.
4. Vortex each sample immediately before plating.
5. Pipette 100 µL of the sample onto corresponding labeled R2A and YM plates.
6. Using ~5 sterile glass beads, spread the sample over the plate.
7. Empty used glass beads into a beaker.
8. Once all samples have been plated, seal the plates with parafilm.
9. Place the sealed plates into an incubator set at 27°C.
10. Store the samples into the 4°C fridge until you are satisfied with your plated samples (i.e., no replating is needed).
11. After 5 days, count the total number of colonies on each plate.
12. Record the data in your lab notebook and Excel spreadsheet.