Process samples in the order in which they were collected.

Before you go down to the wet lab, book time on the HPL calendar.

Remember that we are working with DNA, so it is very important to keep your environment sanitized in order to avoid contamination.

**Sample Transfer**

* Wear appropriate PPE:
  + Gloves
  + Lab coat
  + Safety glasses
  + Surgical Mask
  + Closed-toe shoes
  + Long pants
  + Hair tied back
* Prepare your station and ensure that you have the following:
  + Paper towels
  + Incubator with thermometer
  + Vortex machine
  + Plastic pipette
  + 2.0mL cryogenic vials (with O rings, sterile)
  + Saliva samples in room temperature plastic box
  + Test tube rack
  + Transport box with divider
  + Industrial Sharpie for labeling
  + Notebook for sample logs with pen

**Steps for incubation:**

* Retrieve the box of saliva samples at room temperature in BAB Lab Cabinet 1 and carry them to HPL.
* Plug in and turn on the incubator, turn the dial to level 1 and ensure the red “heating light” is on.
* Continuously check the incubator to see if temperature has reached 50C.
* While waiting for the incubator to hit 50 C, clean workspace using ethynol and paper towels (dispose these in the regular trash bin)
* Open the saliva sample box in HPL and note down the list of samples to be processed today, and ensure the lid on each of these samples is shut very tight. If the lid is slightly opened, the sample may risk evaporation during incubation.
* Ensure you are wearing gloves when grabbing the 2.0mL cryovials. Label two cryovials (**legibly)** per sample for those that will be processed today (use the Industrial Sharpie; example: “Saliva\_001 (1)” & “Saliva\_001 (2)”). Place them on a sanitized surface.
* When the temperature reaches 50C, open the incubator door and place all saliva tubes standing upright on the incubator tray.
* **IMPORTANT NOTE:** the incubator is extremely sensitive to movement - the saliva samples are likely to fall over with any large tap and/or placement of saliva samples on the tray inside. Be very careful placing samples onto the tray, closing the incubator door, and opening the incubator door once the incubation has completed.
* Close the incubator door *very* carefully and turn the knob as needed to regulate the temperature towards 50C. More extreme temperatures can cause the incubator temperature to fluctuate more, so be gentle.
* Stay in the room to babysit the incubator. You can take this time to prepare equipment for vortexing and storage. You can also put in 2 batches of samples staggered by about 15-20 minutes, so that the second batch of samples can finish incubating while you are vortexing the first batch and transferring them to the freezer.
* Note down the current time and set an alarm for 2 hours to continue processing. Continue to check the incubator regularly to ensure temperature level is still at 50C and heat level does not need adjusting.

**Steps for processing:**

* Once the tubes have incubated for 2 hours, retrieve the cryovials, test tube rack, pipettes, pipette tips, and sample log notebook with pen.
* **Very carefully**, open the incubator door and remove samples to place in the test tube rack. Close the incubator door. (NOTE: the samples are not very hot, so no additional safety protection is needed beyond gloves. If extremely sensitive to heat touch, use a paper towel to grab the samples out of the incubator.)
* Carry the five samples in the test tube rack to the vortex machine. Place one sample on the vortex machine and press down, holding for 20 seconds while the sample is shaken.
* After each sample is vortexed, turn the sample upside down and back up to see if any is still too viscous. If so, vortex for an additional 20 seconds.
* Carry the samples back to the Pipettes in the BAB area of HPL.
* **PIPETTING SAMPLE:** Locate the labeled cryovials for the sample you will process. Open the sample tube and place back down into the test tube holder. Open the cryovial and hold tube with one hand. Pipette the sample into the cryovial, pressing down until second stop. Place pipette down on its side. Close the cryovial and place back into transfer box. Open the second cryovial, pipette what is left from the saliva sample into the second cryovial before placing into the transfer box. When there is no saliva left in the sample tube, close it and dispose in the biohazard bin. Release the pipette tip by pressing on the button located on the back of the pipette into the biohazard bin. **Write down the sample quality and location in the sample log notebook**.
* Wipe down the table with ethynol before replacing gloves, disposing in biohazard bin.
* **Make sure to sanitize your work area and change gloves in between each sample!**
* Return to the incubator for the next five samples, repeating the **PIPETTING SAMPLE** step above for the rest of the samples.
* Once the samples have been processed, transfer the samples to the -80 freezer in the appropriate box. **Make sure the freezer is locked when you leave**.
* Turn the knob on the incubator back to zero, and turn the incubator off. If researcher would like to return the incubator to room temperature quick, the incubator door may be opened to release heat faster.
* **Clean your workspace, take out the biohazard trash if it is full or almost full, and wash your hands before you leave**
* **Take a picture of the sample log notebook and transfer notes to the sample log on Box.** ALL information that is in the notebook must be transcribed into the sample log.