This training is to ensure that the Vitrobot does not become damaged, samples are properly prepared, and that users remain safe.

Vitrobot Handout

i. Plan the number of samples, sample concentrations(s), and Vitrobot settings
ii. Reserve Vitrobot in iLab
iii. Alert Cryo-EM staff to reservation if any assistance or supervision will be needed
iv. Bring any supplies and/or tools you wish to use instead of what is already provided by the Core
v. (recommended) Bring a facemask to protect samples from your breath

- Set-up Vitrobot
  - Turn on back switch
  - Attach humidifier, syringe in 20-30mL of distilled H_2O, and syringe out the excess
  - Using gloves and flat forceps, remove old blot papers and replace with new – facing the lipped edge towards the blotting pads
- Set Vitrobot Settings: (recommended)

Temperature	22º or 4º
Humidity (Off/On/Manual)	On
Humidity (%)	95%
Blot Time	BT_i 2, 3, or 4
Wait Time	WT 0
Drain Time	DT 0
Blot Force	BF -10
Blot Total	BT_o 1
Skip Application	leave unchecked
Use Footpedal	check
Humidity off During Process	check
Skip Grid Transfer	check
Autoraise Ethanelift	leave unchecked

- Fill Metal Dewar with Liquid Nitrogen
- Assemble Vitrobot Pot:
  - Styrofoam pot, free of grids
  - Styrofoam ring, **PLACE WITH LIP AT THE TOP**
  - Ethane cup
  - Pedestal
  - Spider
- Cool Vitrobot Pot with Liquid Nitrogen
- Plasma Clean Grids
- Add Ethane-propane to Ethane Cup
  - Check that no LN2 is in the ethane cup, if so, soak up with a Kimwipe
  - Place ethane-propane needle nozzle in the ethane cup
  - Open ethane-propane needle valve, **DO NOT REMOVE YOUR HAND UNTIL DONE**
  - Fill ethane cup until touching under the spider, pulling out needle nozzle while topping it off
  - Close ethane-propane needle valve
  - Wait until the surface of the ethane-propane measures quickly to below -190ºC
  - **REMOVE SPIDER w/ tongs, don't touch w/ your hands, it is very cold**
- Label Grid Boxes (recommended: initials, date, alpha-numeric [e.g. CBL 040224 A])

- Dry Run Vitrobot Settings to Test that It's Working Properly (see steps below)
- Operate Vitrobot
    - Tap "Place New Grid"
    - Pick up grid with Vitrobot forceps **AND CLAMP TO THE FIRST MARK**
    - **PUT VITROBOT FORCEPS ON VITROBOT ROD AND CONFIRM THAT IT'S CENTERED**
    - Tap "Next" or footpedal to move arm up
    - Place Vitrobot pot
    - Tap "Next" or footpedal and wait for Vitrobot pot to move into place
    - Tap "Next" or footpedal to lower grid for applying sample
    - Pipet sample onto grid (recommended: 3µL)
    - Tap "Next" or footpedal for Vitrobot to apply WT, BT_i, BT_o, and DT settings and then plunge
    - Spin the Vitrobot pot as needed for ease of grid transfer
    - Confirm that the window is open over the grid box slot
    - Remove the forceps from Vitrobot arm, keeping grid under ethane-propane
    - Quickly dunk the grid into LN2 nearest to open cryo-box slot
    - Quickly place grid into cryo-box slot
    - Use forceps to spin lid so that the window to next grid slot is open
    - Place forceps onto heating blocks to warm up and melt
    - When melted, use Kimwipe to dry forceps or use the hair dryer
- Clean Up
    - Tighten grid box screws
    - Fill foam dewar with LN2 and quickly transfer grid boxes into it
    - Place Vitrobot pot under the snorkel to evaporate
    - **TAP "Exit" ON VITROBOT**
    - Wait for Vitrobot to shut down, and then turn off back switch
    - Detach humidifier, tip upside down into foam box, and syringe out H_2O
    - Return all tools to their original locations
- **FILL OUT VITROBOT NOTEBOOK**