

## Vitrobot User Checklist

### General safety:

- No eating/drinking
- Door access is restricted to the person who made the equipment booking from 9am -5:30pm. Please note that there is only one main entrance near to subway.

Please note that the card will not work if present it on card reader together with Octopus card.

(Two Vitrobot are available for booking. Session of Vitrobot 10am-12pm; 1:30-3:30pm and 3:30-5:30pm.)

It is highly suggested to make booking at least 24hr in advance. Please note that the main entrance door may not be able to access if the booking is made in the same day.

It is not allowed to pass the student/staff card other users to access the lab.

- Familiarize yourself with the fire escape route.
- Locate and be aware of the emergency contact information and the first aid box in case of emergencies

### Protection:

- Proper clothing (cover the feet and leg properly)
- Laboratory Coat
- Nitrile Gloves
- Goggles / Safety Glasses
- Cryogenic Gloves
- Face Mask

### Operation related safety:

- Fire alarm
  - Stop the experiment immediately.
  - Follow the designated fire escape route or the instruction of CryoEM staff to safely exit the lab.
  - Do not re-enter the lab until it has been declared safe to do so by the appropriate authorities.
- Ethane, oxygen sensor alarm
  - Stop the experiment immediately
  - Add enough LN2 into the coolant container if you are in the middle of freezing
  - Must turn off ethane gas
  - Open the doors of the sample freezing room and wet lab as well as the main entrance door, leave the lab.
  - Immediately inform the CryoEM staff.
  - Wait until the alarm stops before re-entering the lab.

**Always remember human safety comes first over your samples!**

**Never leave the Cryo-EM lab unattended when the ethane tank or LN2 tank is in use!**

To prevent an alarm from sounding during operation, it is important to exercise caution:

- Avoid pointing the ethane gas tip directly at the ethane sensor.
  - LN2 handling safety guideline
    - Wear long trousers, closed-toe shoes, a long sleeve lab coat, and a face shield or protective goggles
    - Maintain a safe distance from nitrogen gas to prevent inhaling it
    - Ensure that the coolant container is not overfilled with LN2 to avoid spillage when freezing on the Vitrobot
    - Do not add LN2 into ethane liquid to avoid spillage
    - Always place the 4L Dewar on the floor near a corner to prevent it from falling off
    - Exercise caution while transferring the coolant container and the 500ml LN2 bottle, and use both hands for stability when transferring the coolant container
  - Ethane handling safety guideline
    - Always handle ethane slowly and with caution
    - When turning valve 1, only open it 0.5-1 full turn. Avoid turning it excessively.
    - Control the flow of ethane through valve 2 slowly and avoid setting it at too big gas flow.
    - Pay attention to the on/off direction of the valve before making any adjustments.
    - Never point the ethane gas tip to any person or ethane sensor.
    - Always wear protective goggles or a face shield when handling ethane.
    - Maintain a safe distance from the ethane cup when turning it on or off to prevent spillage in case of mishandling.
    - Follow the proper procedure for turning off the ethane tank and ensure it is done carefully.
- Must turn off the ethane tank after use!**
- Waste disposal (LN2, Water, Biological Waste)
    - Dispose of LN2 waste in the appropriate manner:  
LN2 in 500ml bottle - the designated LN2 waste container;  
LN2 in purple foam box - the designated LN2 waste container;  
LN2 in 4L dewar – pour into grid storage LN2 tank, be careful do not overfill the tank.
    - Contact staff if the waste water beaker is full.

- Dispose of biological waste as follows:  
Place the biological waste in the small container provided on the table.  
Transfer the contents of the small container into the designated larger biological waste container in the room.

#### Sharp handling

- Utilize a designated sharp box for the disposal of sharp objects such as needles or blades. Never dispose of sharp objects in regular waste bins to prevent accidental injuries
- Exercise caution when handling tweezers, as they can have sharp tips. Avoid any sudden or careless movements that could result in injury.
- Vitrobot tweezers is only for grid! Never use it for other purposes.

#### Operation reminders:

- 4L Dewar schedule
  - AM Dewar: Available from 9 AM to 1 PM.
  - PM Dewar: Available from 1:30 PM to 5:30 PM.
  - Night Dewar: Available from 6 PM to 11 PM
- Ethane container location (beside the Vitrobot)
- Purple foam box/clipping box (beside loading station)
- Tweezers (in the case beside the Vitrobot)
- Table lamp (beside Easiglow / loading station, avoid charging the lamp overnight and only charge it during the day time to prevent electrical hazards)
- 500ml bottle and its cover (in the oven)
- Humidifier (beside the Vitrobot)

#### Important notes for operating Vitrobot (refer to SOP)

- Ensure that the grid box and grid are ready before starting
- Familiarize yourself with opening different types of grid boxes
- Preparation of ethane container properly
- Installation of humidifier properly
- Add water into humidifier by syringe
- Perform the test run without grid/blank grid
- Install blotting paper properly
- Prepare LN2: sharing of 4L dewar with other users in the same time period and prepare the 500ml thermos bottle
- Never leave the cryo EM lab unattended when the ethane tank or LN2 tank is in use
- Pour LN2 into ethane cup
- The procedure of turn on/off the ethane gas

- Check the temperature and humidity before starting vitrification
- Install of tweezers in a proper way
- Pay attention of the level of the black clamping ring
- Ensure that the level of LN2 in the ethane container is appropriate to avoid overflow during vitrification
- Pay attention to the amount of ice inside the ethane cup to prevent damage to the grid and tweezers
- Know what to do if there is too much ice inside the ethane container
- Properly shut down the system, remove the filter paper, leave a small gap in the door
- Follow the two-step process for emptying the humidifier

Important notes for operating grid storage (refer to SOP):

- Make sure there is space in your puck for grid storage
- Ensure you have the proper tools before starting
- Operate on the desk beside the grid storage tank
- Please be cautious to avoid dropping the string of the puck rack into the LN2 tank. It is highly suggested to put the puck rack on the table beside the storage tank.

Important notes for operating of Easiglow (refer to SOP):

- Check the glow time before starting
- Handle the glass cover gently
- NEVER click "SAVE PROGRAM SETTINGS"

Checklist before leaving (checked by both operator and the second person)

- Turning off Vitrobot and Easiglow
- Turn off the ethane tank to prevent any potential leaks or accidents.
- Shut down the Vitrobot following the proper procedures.
- Remove the filter paper and dispose of it in the designated waste bin.
- Empty the humidifier of any remaining water.
- Place the Vitrobot tweezers back in the case.
- Place the coolant container on the table opposite to the Vitrobot to ensure proper ventilation and dry.
- Place the thermos bottle and its lid in the oven to dry.
- Tidy and clean the bench area.
- Sign the logbook.
- Remember to take all of your personal belongings with you when you leave.