

## Vitrobot MARKIV protocol

### Location:

DE-797 (EM sample prep room)

### General rules:

- Do not use the Vitrobot unless you are accompanied by an EM core staff member or have been cleared as an independent user
- Use tools only for their intended purposes
- Clean up after yourself in DE-797
- Log use in the physical logbook next to the VB
- Report any tool or instrument issues to staff ASAP

### Sign-up rules:

- Use iLab (base rate, \$16/hr) to reserve VB for the entire time you will need use it (including cooling and cleanup)
- Staff services (grid freezing) can also be booked through iLab
- Door access can be requested from Caleigh ([cazumaya@fredhutch.org](mailto:cazumaya@fredhutch.org))

### Training plan:

- Please contact Caleigh at [cazumaya@fredhutch.org](mailto:cazumaya@fredhutch.org) to schedule training
  - Three 2-hour training sessions and qualification test required before independent use approval can be granted
  - If returning >30 days after last freezing session, please contact Caleigh (phone or email) for refresher walk-through and quiz

### Training objectives

- Protect Vitrobot tools (from bending or melting) and yourself (from cryogenics)
- Maintain vitrified ice on grids with minimal contamination
- Determine blotting conditions from initial 4 grids

### User provided materials:

- Cryo-EM grids (after initial 2 training sessions)
- Sample
- Pipettes and tips larger than P10, if needed
- Frozen grid button storage tube/puck (please see Caleigh to discuss if you need help)

**Shared resource tools list:** if anything is missing/out of the ordinary, please contact Caleigh ASAP

- 12" tweezers, 6" tweezers, sharp tweezers, VB spider, foil covers, screwdriver, kim wipes, logbook and pen, checklists and protocol, blotting papers, hole punch, P10 pipette and tips, grid buttons, permanent marker, 1.5mL eppendorfs, safety glasses, 15 and 50 mL tubes, transport dewars, glow discharge slides, string
- Hood: VB freezing container
- VB: VB tweezer

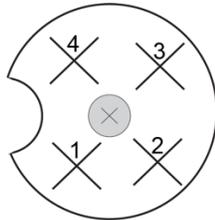
**Startup checklist:**

- Inventory tools and make sure you have everything needed for freezing
- Dry and assemble freezing container (**only dry with low heat setting on hair dryer**)
- Attach the humidifier (check O-ring) and fill with 40-50mL distilled water
- Turn on the VB with the back switch and wait for it to boot up
- Set "Console" to your preferences (humidifier will turn on once it reaches the set temp)
  - Recommended: humidifier off during process, skip grid transfer, auto raise ethane, 100% humidity
- Fill 4L LN2 dewar with nitrogen
- Punch new blot papers and affix to the VB, close chamber door
- Click "Reset Blot Papers"
- Check the small knob on the ethane tank is closed and open main valve
- Glow discharge grids you will freeze – easiGLOW protocol
- Set "Options" tab with the blotting conditions you want to use

**Freezing protocol:**

- Fill freezing container with liquid nitrogen, cover, and wait for it to stop boiling
- Fill up LN2 in outer foam ring
- Condense ethane into center cup to bottom of spider (**remove cap from ethan and wear safety goggles**)
- Cool ethane cup until solid ethane remains by leaving spider attached or flooding around ethane cup
- **Remove spider**
- Run test blot without freezing container to confirm you have all of the options the way you want them
- Top off ethane in center cup (even with top of cup, recommended if you will freeze >8 grids)
- Fill freezing container to bottom lip with liquid nitrogen as starting point and make sure that your first button is located where you want it

- **\*\* Place freezing container on VB and freeze\*\***
  - “Place New Grid” (allows room to attach tweezers)
  - Pick up grid with VB tweezers and lower bottom of black clamp to highest ridge, place on VB with carbon side facing your dominate hand
  - “Continue” (raises plunger and freezing container)
  - “Start Process” (lowers tweezers for sample application)
  - Add sample to grid
  - “Continue” (wait, blot, drain, plunge, lowers container and tweezers)
  - Fill LN2 to bottom lip
  - Transfer grid out of ethane, pull back black clamp, and deposit into grid button (load grid button counterclockwise from below notch)



- Repeat \*\*\*\* until finished freezing all grids
- Secure tops of grid buttons
- Safely store your frozen grid buttons in a tube or puck

### Shutdown checklist:

- Move freezing container with LN2 and ethane to the hood (designated area)
- **Close the ethane tank at the main valve, cap ethane line, and vent the line INTO the hood**
- “Exit” VB software, wait for shutdown, and turn off VB with switch on back
- Wipe out any pooled water in the environmental chamber
- Remove and toss blotting papers
- **Carefully store the VB tweezers** in the environmental chamber with tips protected (close tweezers before capping) and leave door slightly open
- Empty, detach, and store humidifier on its side perpendicular to the edge of the bench
- Return all tools to their storage places
- Sign logbook with all session details
- Turn desk light off
- Take anything you brought into the room out
- Report anything wrong to Caleigh via email

Finished! 😊