Cryo EM - Grid Preparation Protocol Using Vitrobot Mark IV

Potential Hazards/Toxicity

- Liquid ethane and liquid nitrogen may cause frostbite.
- Ethane is a flammable gas. The ethane cylinder contains gas under pressure and should be stored in a chemical fume hood or gas cabinet.

Procedures

A. Vitrobot Setup

1. Turn on the instrument (via the switch in the back).
2. Add 1 blotting paper to each side of the blotting pads and reset the instrument. (To set the optimal blotting force, check to see a thin line between 2 blotting papers.)
3. Add 60 mL of Milli-Q water to the humidifier by using the 60 mL syringe that comes with the instrument and remove any air bubbles from the container.
4. Set the desired temperature and humidity settings in the chamber.
5. Place cryo boxes into their slots and add the spider and dry ethane cup to the bowl apparatus.
6. Pour liquid nitrogen into the cryo bowl and wait for the bowl to cool down, adding liquid nitrogen as needed. (Make sure bubbling has stopped before moving to the next step.)
7. Once the ethane cup is cooled, slowly start dispensing liquid ethane into the cup using a Pasteur pipette. Wait for a fizzing sound to know that the ethane is cool enough to condense. Fill the ethane cup with liquid ethane, and once it begins to solidify, remove the spider.

B. Grid Preparation

1. Place grids on a glass slide, making sure that the sample side is facing up.
2. Use a glow discharger or plasma cleaner to plasma-clean the grids.

C. Vitrification

1. Pick up the glow-discharged grid by using Vitrobot tweezers and attach the tweezers onto the plunge rod.
2. On the touch screen, press “Place Grid” and then “Continue.”
3. Make sure the liquid ethane is 10 seconds away from a perfect solid/liquid interface.
4. Place the cryo bowl on the Vitrobot and hit the “Process” button on the control screen.
5. Quickly but carefully deposit the sample onto the grid (2.5 µL) using a side slot, press “Continue,” and wait for the bowl to be lowered.
6. After plunging the grids, carefully remove the tweezers from the plunge rod and quickly transfer the grid from the ethane cup to liquid nitrogen, and then to its position in the grid box.
7. Ensure that the liquid ethane is not solid before repeating the process for the next grid. In addition, ensure the secondary ring on the cryo bowl is frequently replaced to avoid ice contamination.

Final Considerations

After the experiment, make sure that:

- The humidifier is emptied and detached.
- The ethane gas cylinder is closed.
- All used instruments are turned off.
- All components are dry (especially before restarting the procedure).

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