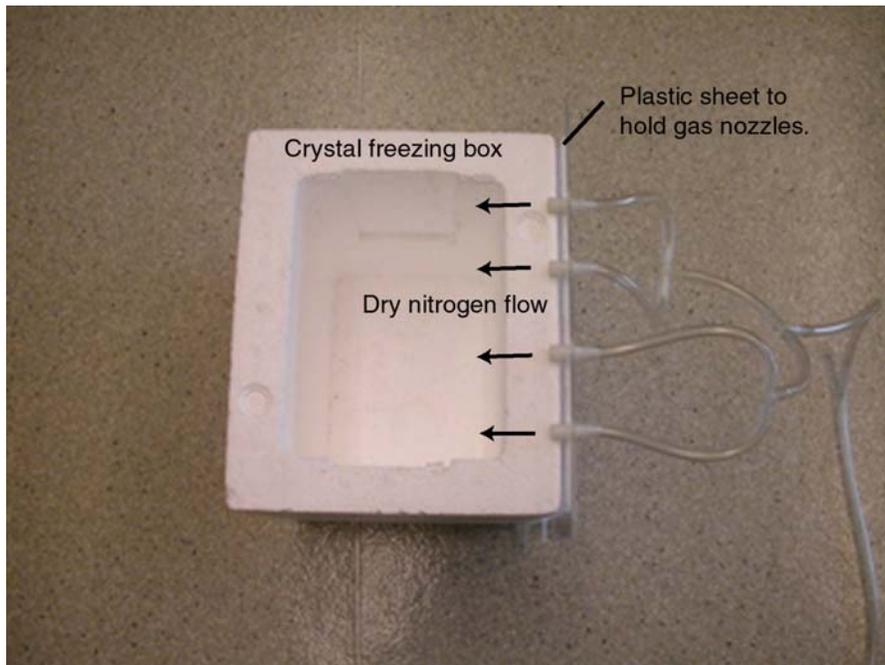


Recommendations for Cassette Loading

submitted by Nham Thi Nguyen of The Department Biochemistry and Molecular Biology, University of British Columbia

New ice free approach involves the following steps:

1. Initially crystals are mounted and flash frozen in liquid nitrogen - with the liquid nitrogen stored in a small styrofoam-type box (the sort of thing that one receives deliveries in), with a dry nitrogen gas stream blanketing the top of the liquid nitrogen. As Robert Thorne and his group have pointed out this seems to provide much nicer freezing and we believe it also serves to inhibit ice formation. As the photos below show the nitrogen gas nozzles are simply held on a plastic sheet next to the styrofoam-like box.



2. The first step in loading the SSRL cassette is to fill all the pin holes with blank pins before cooling in liquid nitrogen. This great idea was obtained from earlier discussion on the PX-SSRL mailing list. This seems to serve to prevent ice crystals from drifting into pin locations and then sticking to a crystal that is on a pin that is later loaded.

3. To prevent ice formation in the cassette loading box, we constructed a plastic device that surrounds it and is used to blow a steady laminar flow of dry nitrogen across the surface of the liquid nitrogen during crystal loading. Photos illustrating this device are below.

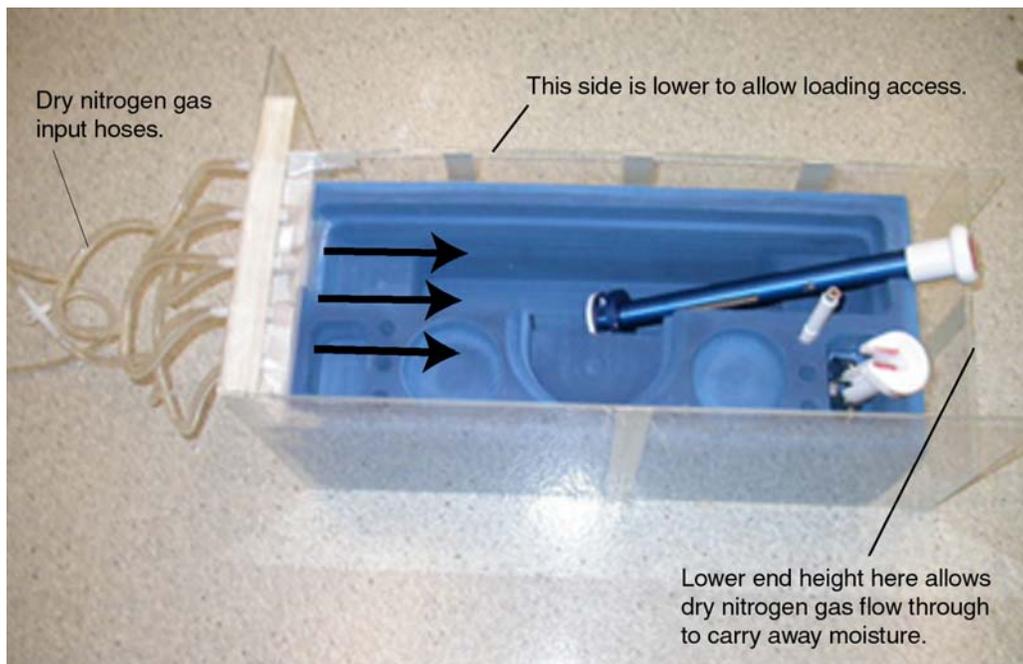
Some important points here are:

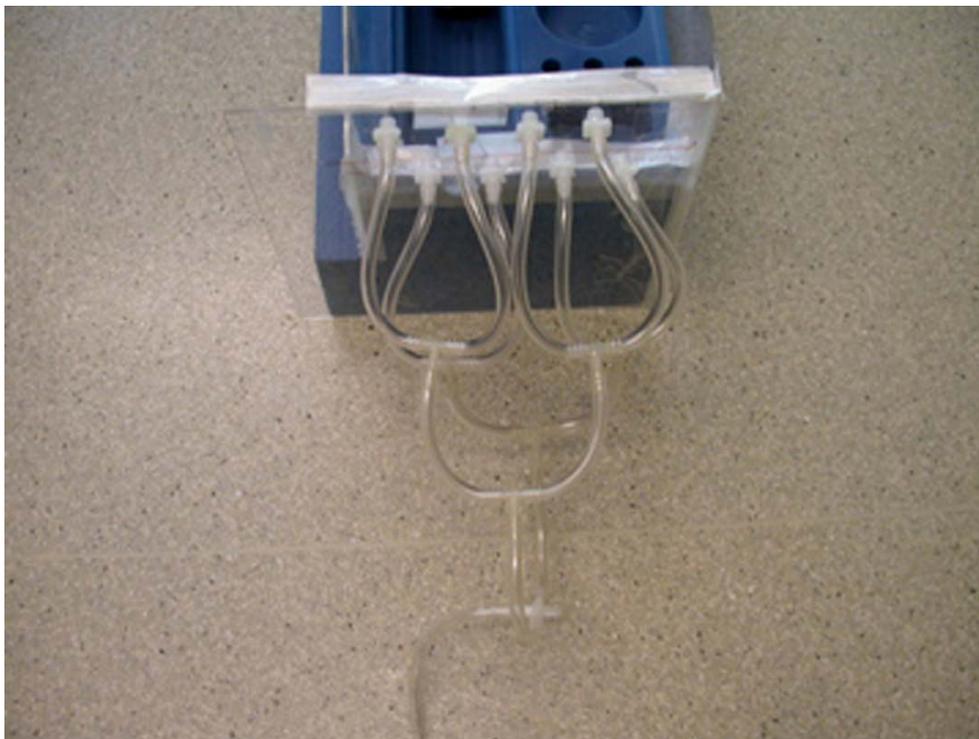
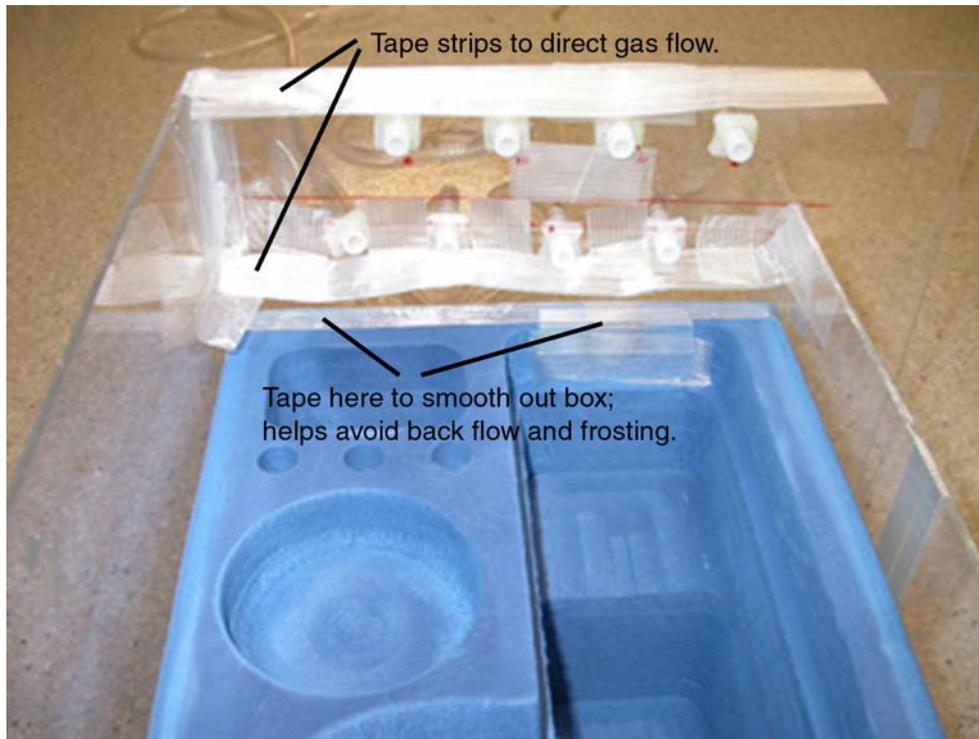
- A relatively slow dry nitrogen flow seems to work well - just enough to keep the moisture mist moving off to the far side.
- It is also a good idea to keep the nozzle end of the plastic frame tight against the cassette loading box, thereby avoiding a back gas flow eddy that tends to create frost at this end of the

box. Taping up depressions or holes in the cassette box at this end also helps, as does creating tape lips just below and above the nozzles to assist in directing the flow of gas - see photos below.

- Also, we keep the various loading tools in separate small styrofoam-like boxes containing liquid nitrogen to avoid having ice crystal transfer (these ice crystals form at the liquid nitrogen/air boundary of the cooled tool) from these tools into the main loading cassette box.

- Further, we occasionally brush away frost accumulation on the cassette transfer handle and tools during loading to make sure these ice crystals also do not end up in the loading box.





Using these approaches, we no longer see ice crystals in the liquid nitrogen in the cassette loading box even after extended crystal loading periods. **This is particularly remarkable given the temperate rain forest environment that we reside in!!**

Also device construction costs were minimal - a bit of plastic sheet, tape and rubber hose - most of which was recycled material that happened to be around the lab.