



## TFS Autoloaders. NCCAT specific procedures.

### Safety

#### General Precautions:

- Use the Personal Protective Equipment (PPE) provided by NCCAT. Nitrile gloves, goggles (or eyeglasses), and additionally cryo-gloves and a face shield while dispensing LN<sub>2</sub> and a face shield while dispensing ethane.
- The dehumidified room is equipped with an oxygen monitor. If the monitor alarm sounds, you **MUST** stop working, leave the room and leave the door propped open for air replacement. You must wait until air has recirculated and the alarm turns off before reentering the room (this generally takes only a minute or two).
  - If you continue working in the room while the alarm is sounding, you will lose access to the lab.
- Inspect equipment and accessories for weaknesses, cracks or damage before beginning work. If you suspect any damage, or encounter any problems during use, please fix immediately and ask for help from staff if needed.
- Report all accidents, no matter how minor.

#### Liquid Nitrogen:

- Ask for assistance to fill 4 L LN<sub>2</sub> dewars from the large tanks. Use cryo-gloves for LN<sub>2</sub> tank operation.
- Do not discard of LN<sub>2</sub> on the floor. Empty any used nitrogen into LD10 collection dewars.
- Be careful with any metal or tweezers in vacuum sealed dewars. If a dewar breaks, the release of vacuum can cause glass to shatter around the room.

#### Mandatory PPE for LN<sub>2</sub> dispensing





#### 4.1 and 4.2 Setup

- NanoCabs & empty cassettes are stored in the ovens on the bench in the NCCAT dehumidified room (Fig 1A). Loading stations are stored on the left side of the bench (Fig 1B). NanoCabs are stored upside down.

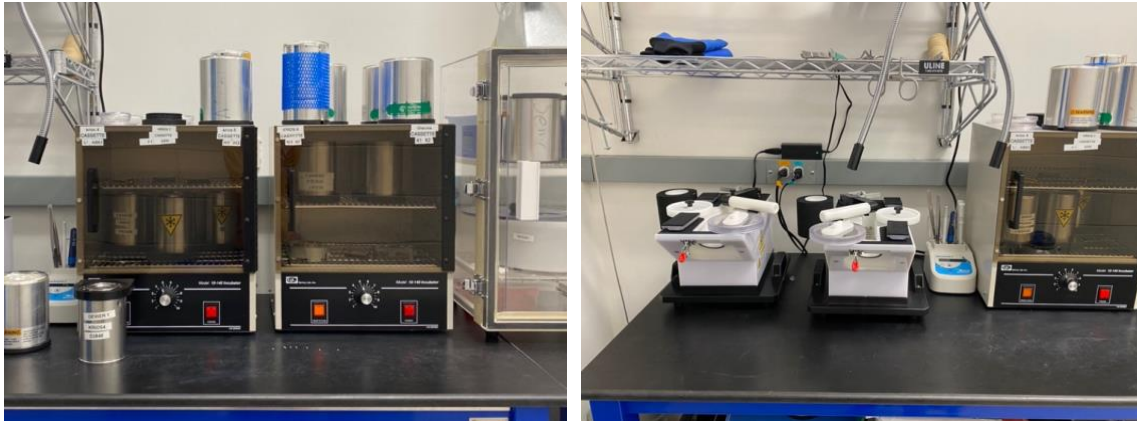


Figure 1. NCCAT dehumidified room bench. A) Two ovens are used to dry and store NanoCabs and empty cassettes. B) Two loading stations are kept on the left end of the bench.

- Each microscope has two designated NanoCabs, clearly labeled (Fig 2A). Each microscope also has designated cassettes, with labels etched onto the bottom (Fig 2B). NanoCabs and cassettes should be stored in the proper oven (labels show in Fig 2C).



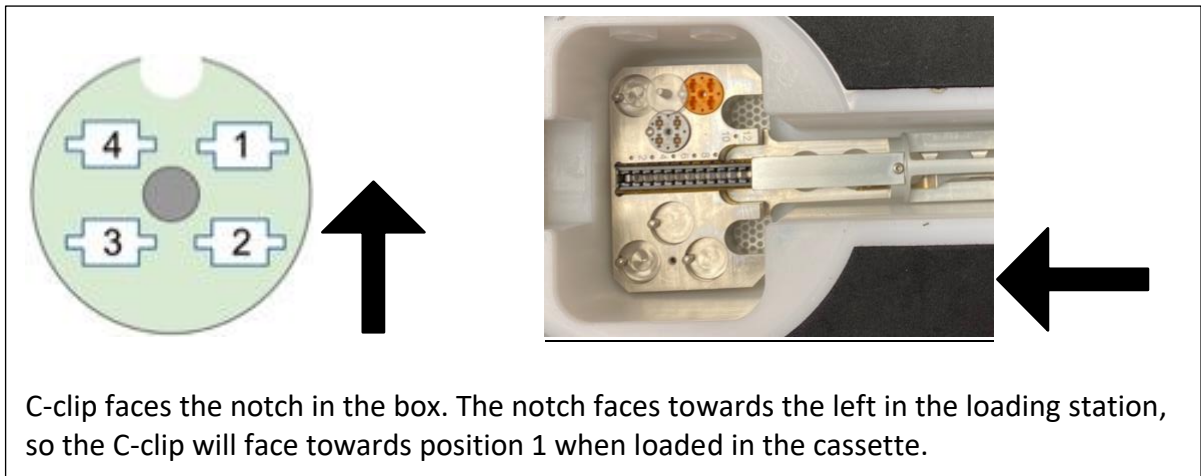
Figure 2. Labels clearly indicate which NanoCab and cassette are to be used for each microscope. A) Each NanoCab is labeled (here the NanoCab for Krios6 is shown). B) The silver end of the cassette has an etched label (here labels "H2:" and "AB63" are shown). C) Each oven is labeled with which NanoCabs and cassettes should be placed inside.

- Forceps and grid box opening tools are in the drawers below the lab bench.



#### 4.6 Add autogrids to cassette

- **4.6.5 Orientation of autogrids in the cassette:** Our expectation is that grids have been placed into autogrid boxes with the C-clip facing the notch (Fig. 3) Autogrids should be loaded into the cassette with the C-clip facing the first position – if grids are transferred without rotation, they will be in the correct orientation.



- **4.6.7. Leave an empty position in the cassette:** A cross-grating grid should have been left on the stage. This grid will go into position 1, so do not load a grid into position 1.

#### 4.9 Cleaning up

- Place all cold tools into a heat block to warm and dry (see image on the right).
- Empty remaining LN<sub>2</sub> in the NanoCab into and LD10. Store NanoCab upside down in the oven in the designated spot.
- If someone needs the loading station after you, leave cold and fully covered.
- If no one else is using the station, remove covers and leave the loading station upright on the bench for the LN<sub>2</sub> to evaporate and dry.
- Empty any unused LN<sub>2</sub> in LD4s into the LD10 collection dewar and invert on the rack to dry.

