

Fast cryo-plunge freezing

General Information

This protocol describes sample preparation with the Cryo freezing apparatus. **Once the apparatus is setup, the freezing is automatic and does not require user control.**

For specimen preparation, it is necessary to align the instrument for handling of glass capillaries. Please notice glass capillaries of Hilgenberg design version 3.0 and higher is required. In the case of other capillaries this manual is not sufficient. Once the instrument is setup for handling of glass capillaries, it should be cooled with liquid nitrogen. For controlled cooling of the specimen, the apparatus is equipped with dry nitrogen flow controls and a set of heating elements. The specimen is frozen by controlled (speed and acceleration) plunging into liquid propane. The propane is cooled via liquid nitrogen. All glass capillaries are stored in the storage pucks specifically designed for this instrument. **ALWAYS WEAR SAFETY GLASSES WHEN USING THIS INSTRUMENT. Before operating you must receive in person training!**

Required materials

Liquid nitrogen tank 100L for dry gas and 50L for liquid supply

Propane, liquified for specimen plunging

Safety glasses

Gloves for cryo handling

Glass capillaries (Hilgenberg 3.0)

Magnetic holder for glass capillaries

Metal plate for inserting capillary into a holder

Plastic tube to remove residual cold gas

Thermally isolated box for loading liquid nitrogen

Steal pack for specimen storage

Plastic pack for puck storage

Thorlabs tool for transporting the pucks inside freezing apparatus

Grabber to transporting pack to long term storage

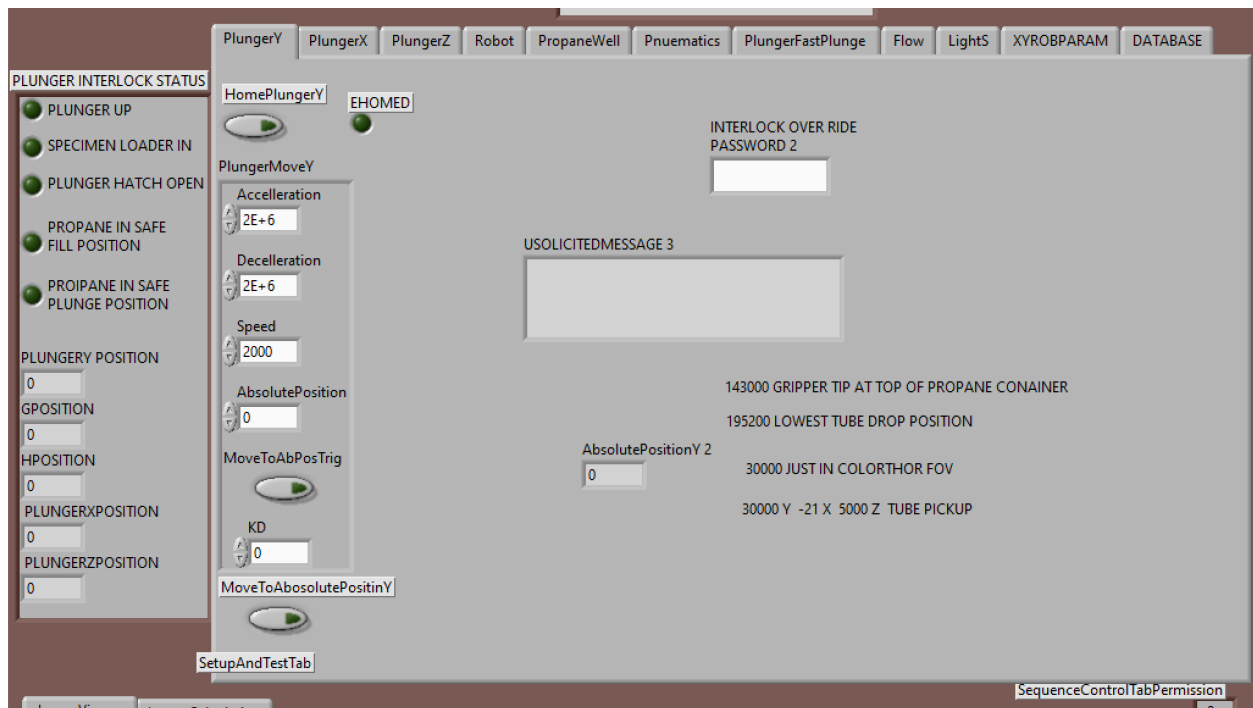
Tubing to fill in propane

Estimated time For 60 specimen freeze down

3 hours

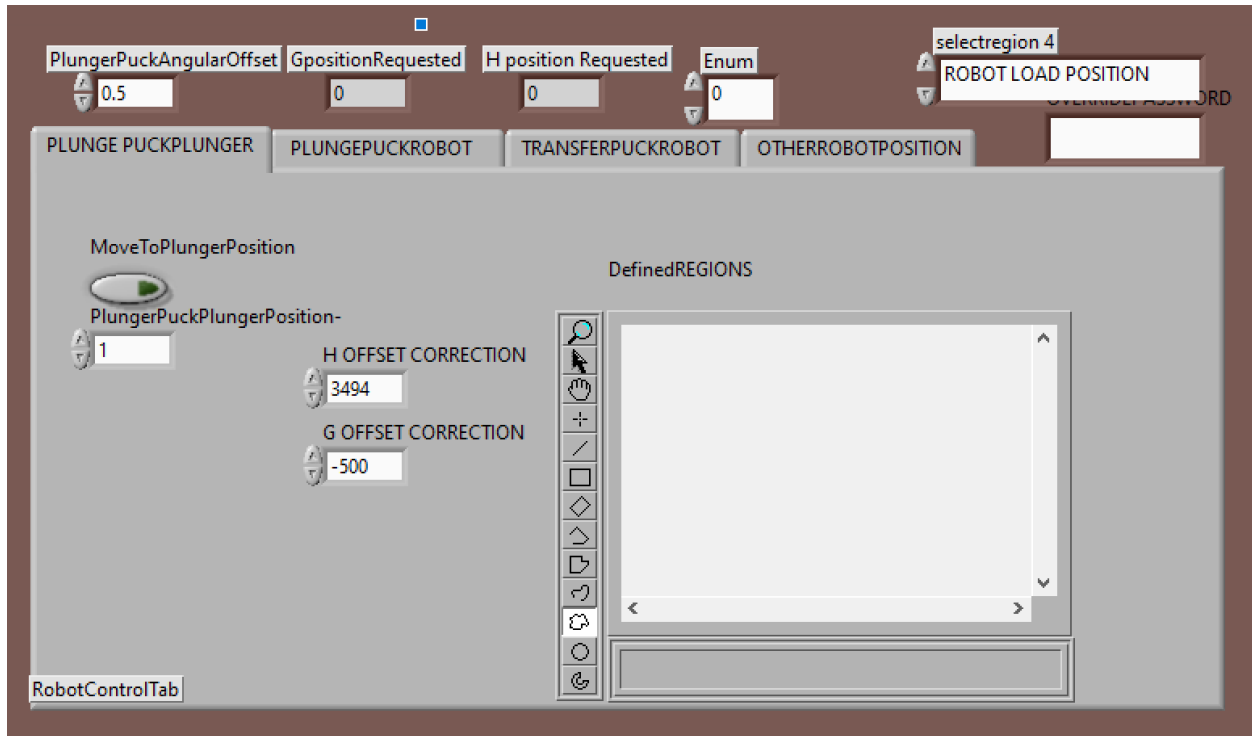
Start-up

1. Ensure that there is enough Liquid nitrogen in 100L tank and 50L tank. Check propane. Check holders and storage capacity for capillaries.
2. Open the lid of the freezing instrument. Make visual inspection of the reservoir, propane well and heating elements to propane well. Make sure the propane is safe fill position. Install steal holder for capillaries on the left (marks on the side of it towards the corridor), install plastic storage box on the right from it.
3. Make visual inspection of the robot below the main table (white isolated box). Ensure that there are no obstacles, screw drivers or anything else left to obstruct the motion.
4. Switch on freezing apparatus (main power plug below the table) and computer. Make sure that the GPAs on the table are working as well (blinking green light-DC ok).
5. Startup labview library “**CURRENTPLUNGERGUI - Shortcut**”. In the library startup program “**cameradriv**”. Press run, wait until it shows it is in operation. **If code is left open from previous day; close and reload code to reinitialize!!!! Left over surprises cause trouble with startup protocole.**
6. Start to home in the order from left to right: home Plunger Y, THEN remove the safety pad, home Plunger X (if it makes sudden move to the side, home it again), home Plunger Z, home robot (If the Labview gives error on time out press continue)



Panel for homing each of the motion controlled components, follow in sequence from left to right

7. Go To “RobotControlTab:OTHERROBOTPOSITION:PropaneMotionSafe” and “MoveToOtherPositions”, check position of the robot with respect to the red arrow on the dewar **(!!!!IMPORTANT)**, **home propane well, home pneumatics.**



Control for moving to selected robot positions, choose other robot position Propanesafe setting

8. Make a test run with a capillary. Put capillary in the steal holder and mount the holder into specimen loader arm. Activate step 1 of specimen preparation. Adjust x and z, (y if necessary) according to the position of the capillary. Setup these new values in the automatic routine. Activate step 2. If required, find the tip position.



Robotic freezing control panel, use this sequencer when testing warm and for specimen freezing

9. Activate steps 3-5. At step 5 make sure to check the storage position for 0.
10. Repeat with the adjusted procedure another capillary and set the storage to 60. If the capillaries are stored correctly, you are ready to cool down. If not adjust offset of G and H motor and their rotation.

Cooling down

1. Make sure there is at least one puck and the storage box inside -> or empty if you are going to use already loaded puck
2. Make sure that the robot is homed and there is space for the propane well to move around.
3. Put propane well to the position for plunging (y (absolute position) to 0, then rotation to 1800 and then back to -126000) -> **select "Propane to plunge position"**.
4. Attach tubes for dry nitrogen and tubes for gas to get out and liquid nitrogen fill space.
5. Start Heating code, start gas flow by clicking on "HighFlowOff" then check "on", you should hear a click. Set the flow to be 30 (Normal is checked in). 900 for freezing down. Check that the measured flow is 900, if not check that the valve for liquid nitrogen is open, if yes click the error up and down in the labview code. The flow should be measured as set.
6. Switch on heaters near plunger (**Press "ON + output purple"**) to 25 degrees:
7. "TOPHEATER", "LIDHEATER", "BIGEXCHANGEHEATER" **set to 60 degrees**
(change to 40 degrees during operation) → check the setting with the image on the control code.
8. Fill liquid nitrogen slowly to cool down slowly with gas first. Then start to fill freezing apparatus. Fill it until Liquid nitrogen is 1 cm below the top of the steal puck used for capillaries.
9. Turn on light (LED switch under plunger machine; should turn on automatically during warm test)
10. When cold remove the tube and put the cap on top of propane well. Also if you do not add anymore Liquid nitrogen remove the coupling box and replace with the cap.
11. Put propane well in Liquid nitrogen wait until boiling stops (heater value . 0.9V)
12. Fill propane and home the propane tank again.

Transfer of existing puck and storage box.

1. Make sure don't overfill liquid nitrogen. Open the cover and add the internal storage box with the puck. Make sure the propane well is in plunging position.
2. Close the lid, add some more liquid nitrogen on top of the storage puck
3. Other positions: move plunge puck to storage, open the robot hatch, take the thorlabs transfer stick and attach to the puck.
4. Fix the puck, go to other positions: Move plunge to "kinipick", insert the puck make sure of the right orientation and engage all 3 corners. Remove the transfer holder. Close the hatch and move robot to propane safe position "propanemotionsafe"
5. Home propane and fill with liquid propane.

Freezing

1. Setup plunger for acceleration/deacceleration. Set (Step 4-set AUGUSTA): 400000000 (**new: $5 \cdot 10^8$**), speed: 2000000 (**new: $3 \cdot 10^6$**) and TLE: 6 (1.13m/s).acc/deacc $4 \cdot 10^8$ 1.78. The same acc but speed $5 \cdot 10^6$ is 3.125 m/s. With speed $3 \cdot 10^6$ is 2.5m/s Set propane under plunge position

speed to 100 . Make sure the height of the propane well is set at -122000 for plunging specimens.

2. Make a test run with an empty tube. Check grabbing, speed of plunging and storage options.
3. Run through your samples.

Transfer

1. If you left box for filling liquid nitrogen, it is time to remove it.
2. Home propane well AND move to "propane plunge POSITION"
3. Move plunge puck to KINIPICK position
4. Open robot hatch with OPEN FROM PNEUMATICS button
5. Put in Thorlabs tool, screw it in the puck and lift the puck high enough for the storage box to pass by
6. Move plungepuck to storage PLACE IN (OTHER ROBOT POSITION). Put in your steal puck and unscrew the Thorlabs tool. Remove it
7. Close robot hatch
8. Move robot to propane motion safe
9. Move the box to storage. Remove the lid and move the box in the dewar.

Shut down

1. Move propane to fill position "PROPANE WELL FILL" Remove remaining propane
2. Switch off liquid nitrogen flow in Labview. Switch it off physically at the tank.
3. If the apparatus is still full with liquid nitrogen then leave the heater on for a few hours. Otherwise switch heaters off use button "EVERYTHING OFF AND CLOSE TOP".
4. Home propane well
5. Put in the safety cushion for the plunger
6. Switch off labview (ESC key)
7. Switch off main power.