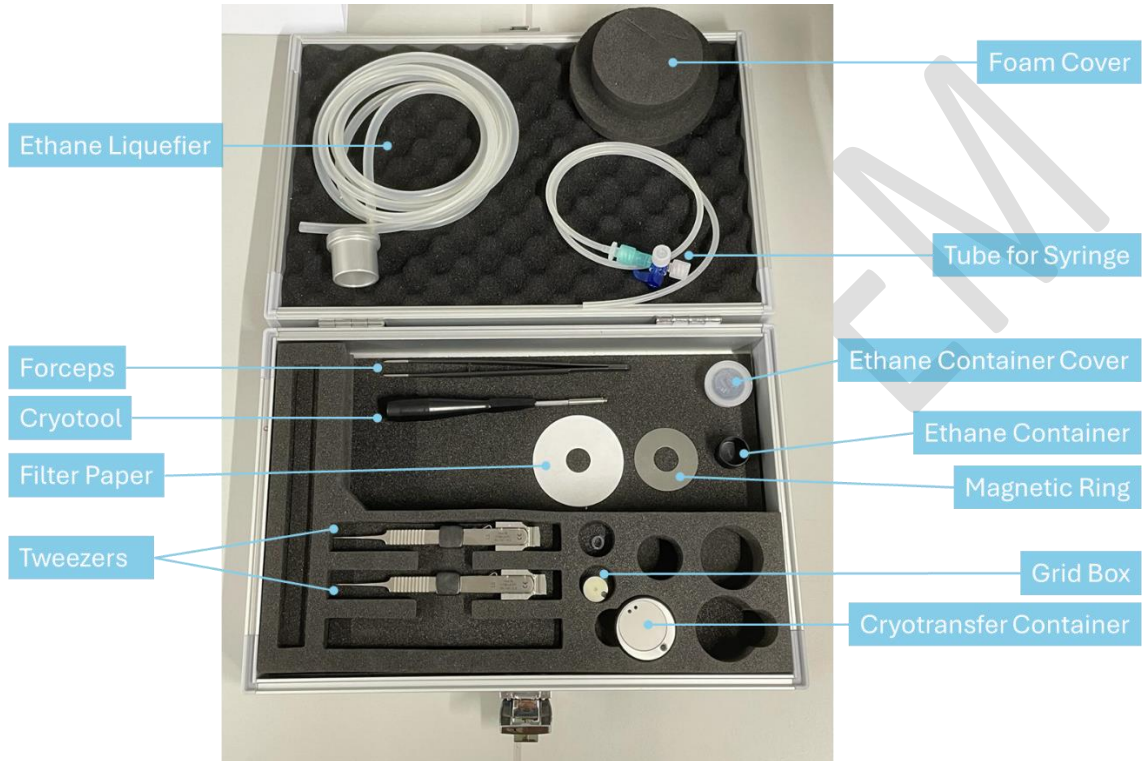


Leica EM GP2 Standard Operation Protocol

A. Preparation

- 1) Sign in the logbook.
- 2) Take out the toolbox from the drawer.



- 3) Switch on the GP2.

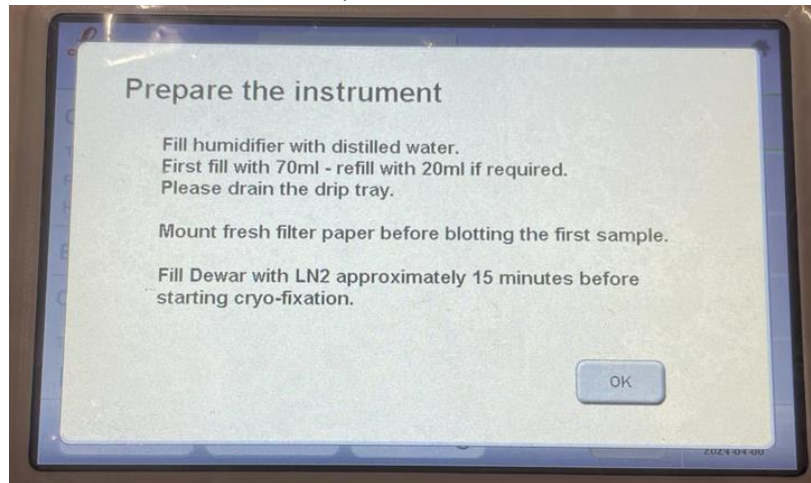
Side



Back



- 4) Turn on the Touch screen to initialize the system.

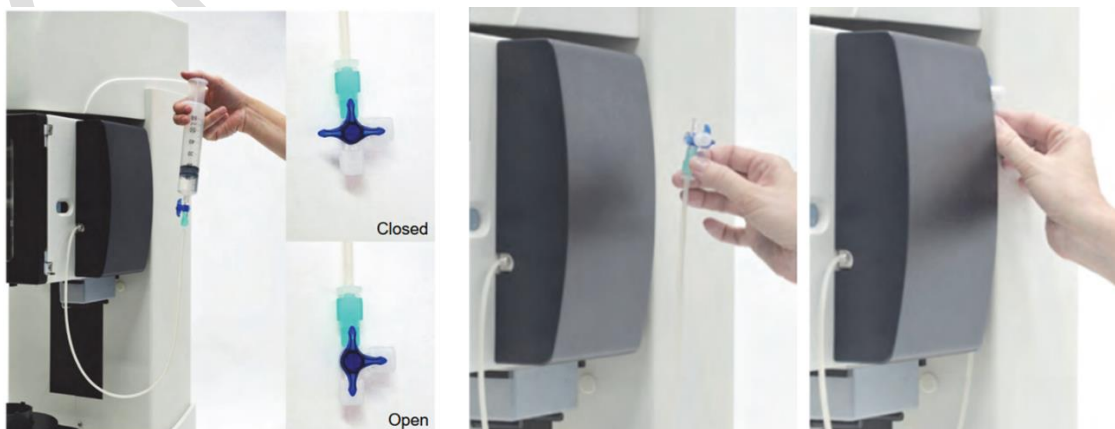


- 5) Connect syringe (with 90mL distilled water) to the tube with the Luer™ lock and open the valve. Add water into the humidifier tank mounted on the right-hand side of the environmental chamber by pressing the syringe piston slowly but steadily to fill in the water.



When the humidifier tank is filled to its maximum, a warning will be displayed together with a warning beep asking to stop the filling.

- 6) After filling, close the valve, remove the syringe, and fix the tube on the clip on the rear side of the humidifier cover.

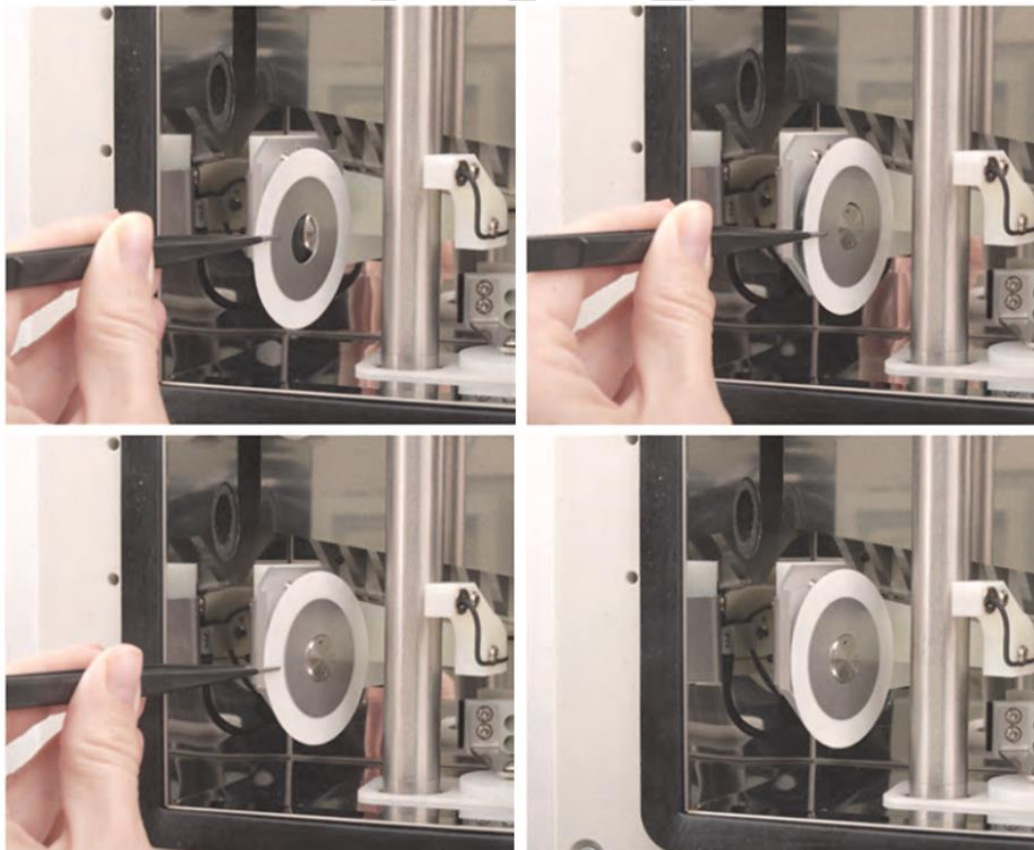



When the filling of the humidifier tank falls below its minimum level during operation, the system will beep and indicate that refilling is needed. Re-filling requires only 20 ml distilled water.


- 7) Pull out the drip tray to the right, discard of the condensate, and re-insert it to the original position.



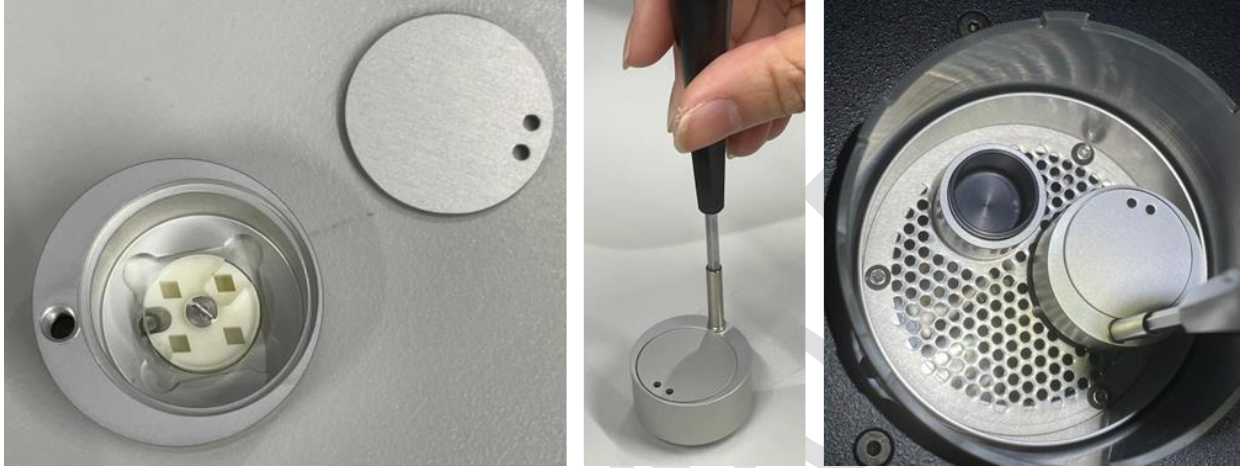
- 8) Mount fresh filter paper (Whatman™ #1) with clean gloves or forceps and place the metal ring over it. Place them well centered onto the magnetic mount in the environmental chamber.



- 9) After setting in the fresh filter paper, press the  button on the main screen to reset the counter. The instrument will rotate one ring of filter paper 10 times before the paper needs to be replaced.

The number of blots remaining is displayed on the main screen and can be reset by pressing the  button.

- 10) Load grid box into the cryotransfer container and position the container on its platform at the front right in the freezing chamber. Position the black ethane container in the freezing chamber.



- 11) Cover the black ethane container.



- 12) Add LN₂ to the freezing chamber until just above the metal mesh. It will take around 1.8 litres LN₂ to cool the Dewar and fill it to 100%.

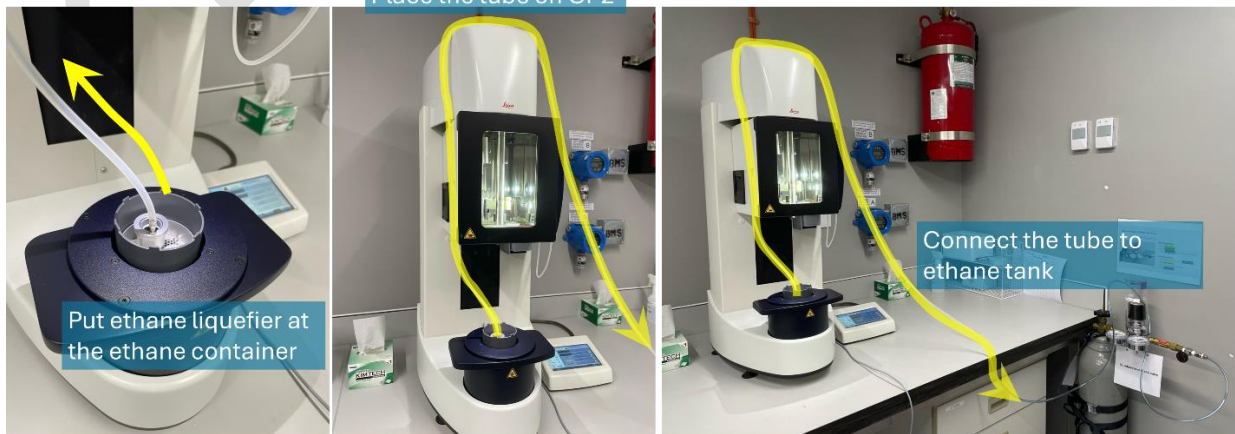


A warning will be displayed when the LN₂ flow cannot function anymore below 25% LN₂ level. Always use the ethane container cover during refill of LN₂.

- 13) Connect ethane liquefier to the ethane tube. Guide the tube on the instrument. When the temperature of the cryogen container drops to the set value, insert the liquefier in the chamber over the ethane container.



Place the tube on GP2



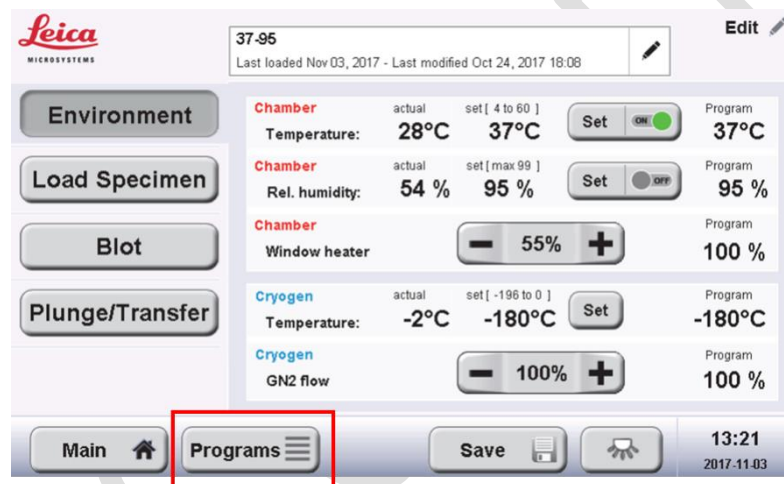
14) Open the Main valve 1 on the ethane tank. Slowly adjust the flow rate via turning the Flow valve 2. Check the ethane level continuously looking through the window of the liquefier.

15) When the ethane container is filled, close all valves on the ethane bottle.

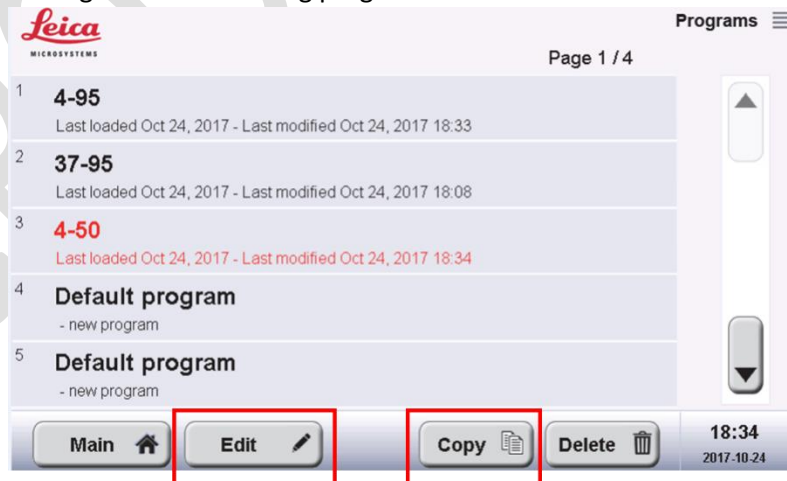
16) Slowly lift the liquefier and make sure to not drop excess ethane around.

B. Programs loading

1) Programs are used to save and recall all experiment settings. Up to 20 programs can be saved in the instrument's memory. The program list can be accessed via the **"Programs"** button on the main screen.

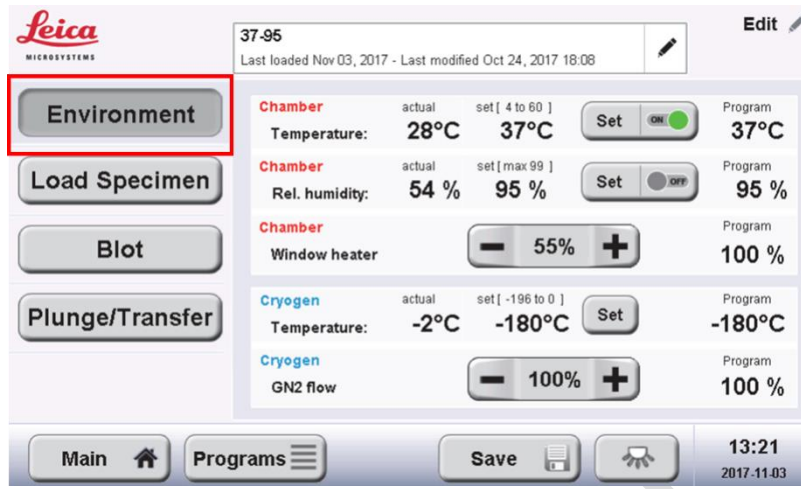


2) You can **Copy** the settings from the existing program and **Edit**.



C. Program settings

1) Environment Parameters



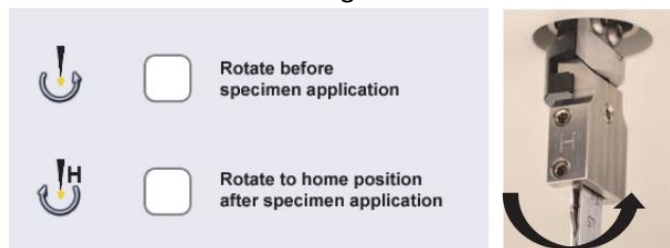
- a) Press “Set” to change the current value of chamber temperature, chamber relative humidity, window heater, cryogen temperature and GN₂ flow on the keypad and “OK” to confirm.



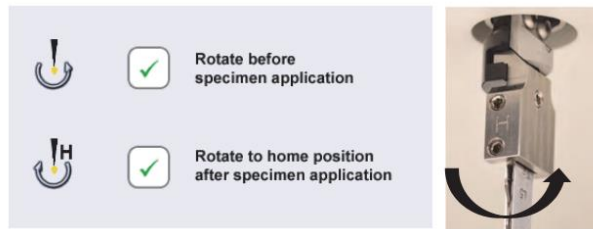
2) Load Specimen Parameters

The “**Load Specimen**” screen controls the vertical 180° rotation of the forceps holding the grid before and after applying the specimen through the ports on the left or right side. This rotation allows a flexible workflow for both left- and right-handed operators.

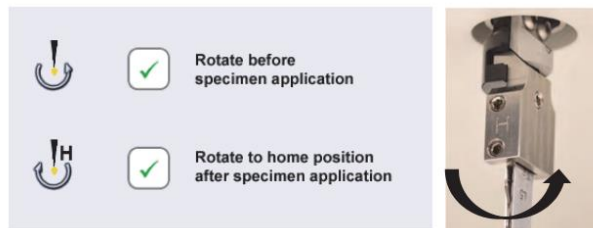
- a) For right-handed operators and back-side blotting:



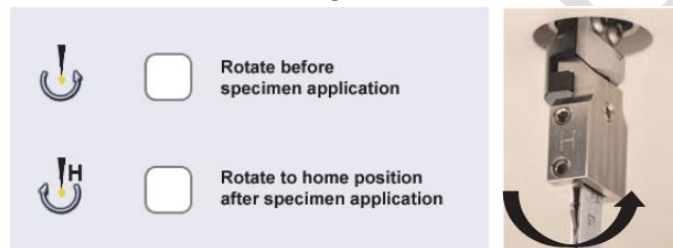
b) For right-handed operators and front-side blotting:



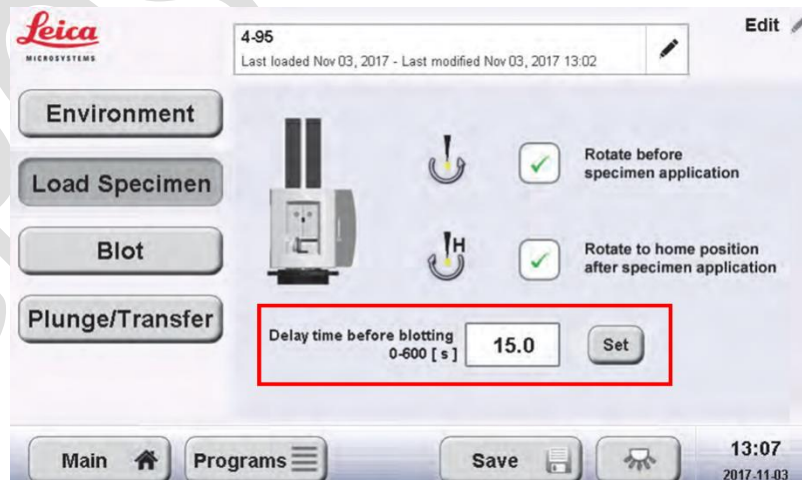
c) For left-handed operators and back-side blotting:



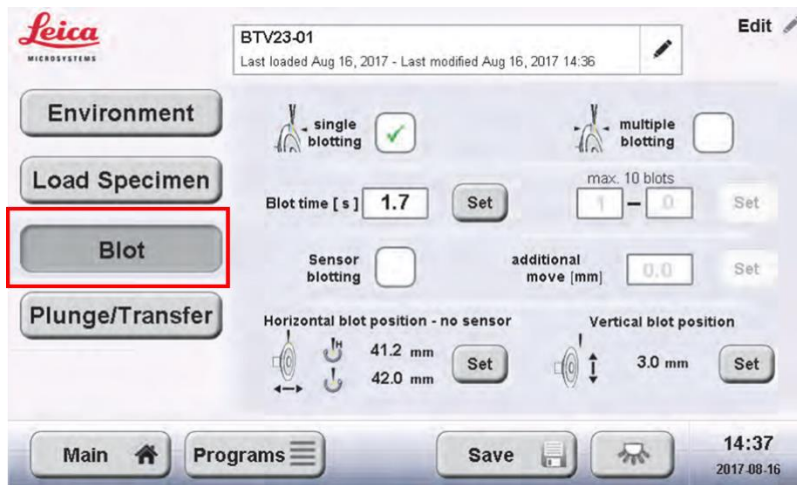
d) For left-handed operators and front-side blotting:



e) A delay between specimen application and the actual filter paper blotting (i.e. pre-blotting time) can be set with the **“Delay time before blotting – Set”** button. This waiting time can be useful to allow adsorption of the specimen to the support film in a controlled environment, typically 0 to 60 s are used.



3) Blot Parameters



a) Single Blotting without Sensor

In this mode, the filter paper will advance to the user calibrated position of the grid for blotting once, remain there for a defined time, retract, and then either proceed with the freezing process automatically or wait for the user to initiate freezing.

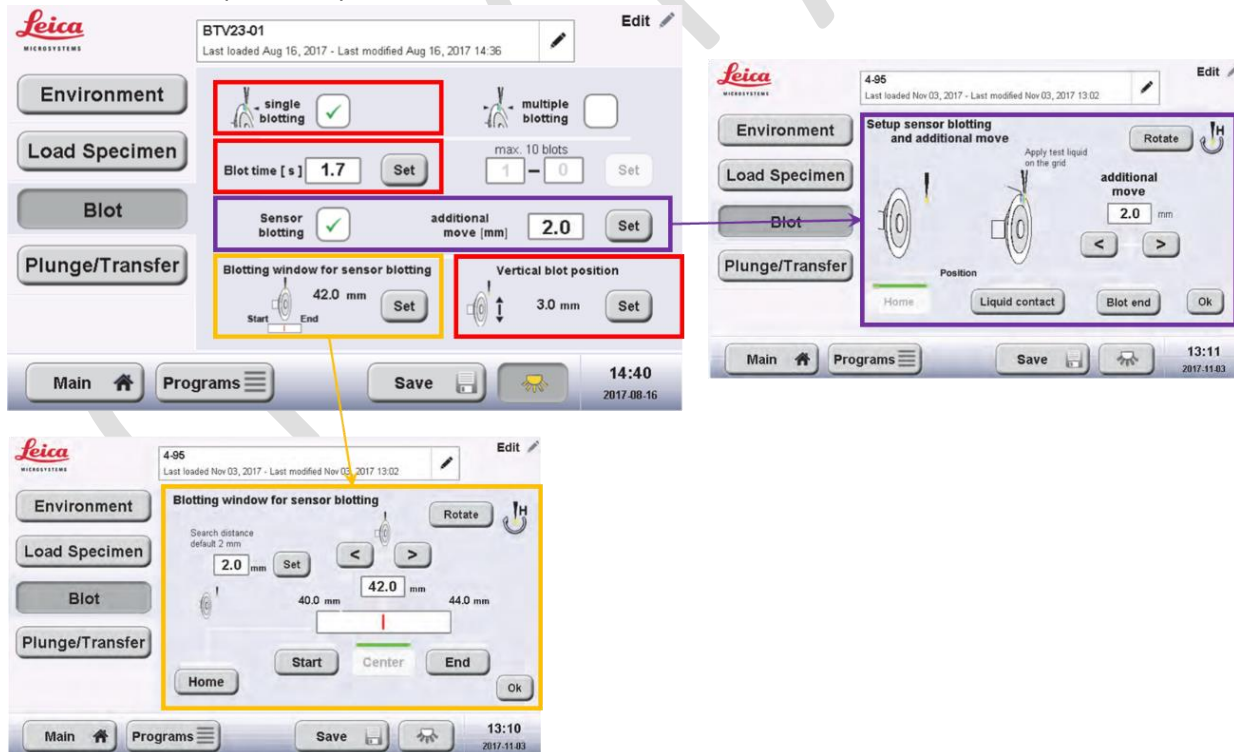
- To calibrate orientation, click “**Blot**” to move the filter paper to the blot position. Use “< / >” and “^ / v” to move the filter paper to the correct horizontal and move the blotter to correct vertical position. This is when the filter paper is touching all the grid and preferably the tips of the forceps too.



b) Single Blotting with Sensor

This is the most automatic setting, intended to increase reproducibility: around the expected position of the grid, the advancement of the filter paper will slow down, and a blotting sensor will constantly check for the point where the filter paper is getting wet and starts blotting the sample.

- To set up the blotting window, select “**Blotting window for sensor blotting – Set**” to get to the following screen with the chamber lowered.
- Go to the “**Center**” position and adjust the position of the filter paper with “< / >” buttons to touch the full area of the grid. The default size of the blotting window (search distance) does not require adjustment under normal conditions. It should be noted that the sensor scans through the search distance on both sides of the Center position (i.e. from “**Start**” to “**End**”).
- The “**additional move**” determines the distance through which the filter paper is moved after the sensor is triggered by the filter paper getting wet. This determines the blotting pressure: using a bigger additional movement, the filter paper will exert more pressure on the grid while blotting.
- Test the function of the sensor with the “**Liquid contact**” button, which will stop the filter paper right after the blotting sensor has been triggered. Then advance to the “**Blot end**” position and determine the desired pressure of filter paper vs grid with “< / >”.
- Go back to the home position, add more samples to the correct side of the grid, and repeat the test blot with “**Blot end**” until the desired result can be achieved reproducibly. Press “**OK**” to leave this screen.



c) Multiple blotting

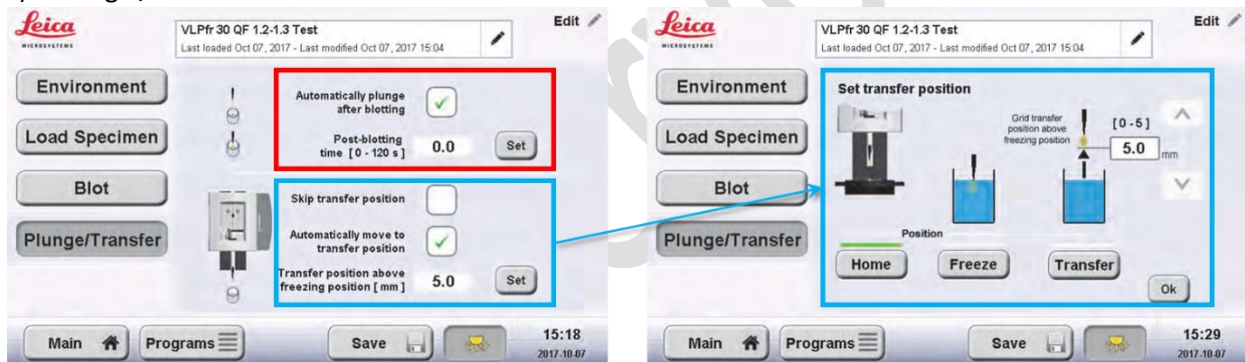
This mode of blotting is preferable for viscous specimens that cannot be thinned efficiently by single side blotting.

* The blotting sensor cannot be used in conjunction with multiple blotting

In this mode, it is essential to have the horizontal position of the filter paper for blotting calibrated accurately for both the home position and the 180° position in **“Horizontal blot position – no sensor – Set”**.



4) Plunge / Transfer Parameters



- **Automatically plunge after blotting:** If the box is checked, **“Plunge”** button on the main screen will be disabled as the instrument plunges automatically after the post-blotting time. If **“Automatically plunge after blotting”** is disabled, the user is required to press either the **“Plunge”** button on the main screen/workflow list or the foot switch to trigger freezing of the sample at an arbitrary point after blotting.
- **Post-blotting time:** A delay time between blotting and freezing (i.e. post-blotting time) can be entered. The delay allows the water layer to even out over the surface of the grid. It requires the humidifier to be set up properly to avoid uncontrolled evaporation of the solvent. For typical applications, this delay is not required.

- Skip transfer position / Automatically move to transfer position: Entirely depending on the user's personal preference, the EM GP2 allows to:

	"Skip transfer position"	"Automatically move to transfer position"
Completely skip the transfer position	✓	✗
Automatically advance to the transfer position	✗	✓
Manually advance to the transfer position with the "Transfer" button on the Main Screen/workflow list or the foot switch	✗	✗

- Transfer position above freezing position: if transfer position is not skipped, the height of the transfer position as compared to the freezing position can be set at "**Transfer position above freezing position – Set**". Use the "**Freeze**" and "**Transfer**" buttons to switch between the individual positions. The transfer position can be changed with the adjust arrows only if "**Transfer**" is active/pressed.

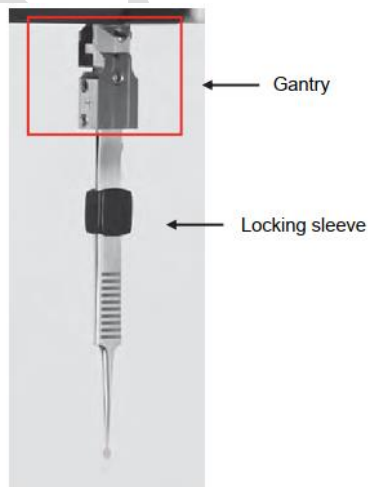
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D. Freezing a grid

- 1) Hold the grid with the GP2 tweezers. Fix the black locking sleeve to the first groove on the tweezers. Make sure to orientate the desired side of the grid on the side where you are going to load your sample (e.g. for application through the right-hand port, the carbon side should be facing to the right)
- 2) Click **“LOAD FORCEPS”**.



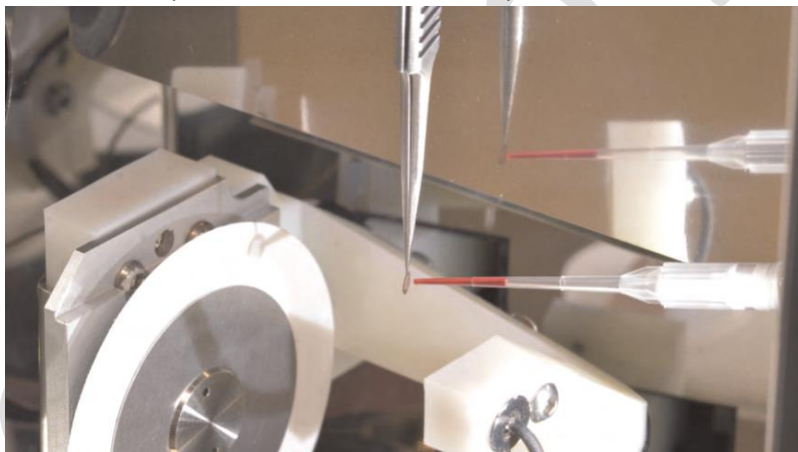
- 3) Slip the forceps into position on the gantry of GP2. The position during mounting is the **“home”** position for the forceps (**“H”** on adapter facing front).



- 4) Click “**LOAD SPECIMEN**”. The chamber will lower and if a 180° rotation for loading has been set up, the forceps will rotate by 180°.



- 5) Open the side port of the environmental chamber and dispense a 3 - 5 μ l specimen onto the grid. Close the side port immediately to maintain a stable atmosphere.



- 6) Click “**BLOT**”. Depending on program settings, the forceps will possibly rotate to the “*home*” position. If pre-blot or post-blot is programmed, the screen will show progress bar.

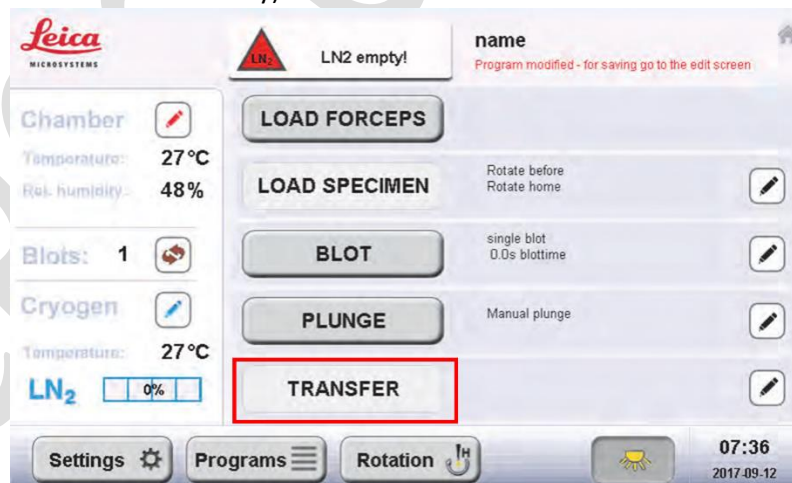


- 7) If “**automatically plunge after blotting**” is selected in the parameters, the GP2 will automatically plunge the grid into the secondary cryogen after blotting (and the elapse of the optional post-blot delay).

If this option is not enabled, the user must press the “**PLUNGE**” button in the workflow list of the main screen or press the foot switch to trigger plunging.



- 8) Directly after plunging, the environmental chamber will rise to allow access to the frozen grid. The grid will remain either in the freezing position (lowest position in the secondary cryogen) or be automatically lifted to the “transfer” position a few mm higher if “**automatically move to transfer position**” is enabled.
- 9) After plunging, the forceps holding the sample can be lifted to a slightly higher position by pressing “**TRANSFER**” (if not done automatically).



- 10) Remove the lid of the grid box transfer container. Set the grid box to an open position. Remove the forceps from the gantry tilting it out forwards, do not let the frozen grid touch the wall of the ethane container.



- 11) Transfer the grid from ethane to the LN₂ in the grid box cryo transfer container and insert it into the assigned slot of the grid box.
- 12) Top up LN₂ in the container and cover the transfer container with the lid.
*** When refilling LN₂, always cover the ethane container to prevent spilling LN₂ into the ethane container.**
- 13) To commence another run, press "**LOAD FORCEPS**" in the workflow and the environmental chamber will rise for the next run.
- 14) After completing a cryo-grid box, set the grid box to a closed position and fasten it with a pre-cooled screwdriver.
- 15) Cover the grid box cryo transfer container with its lid, attach the pre-cooled cryotool and take the container out of the freezing chamber and put into a foam box filled with LN₂.

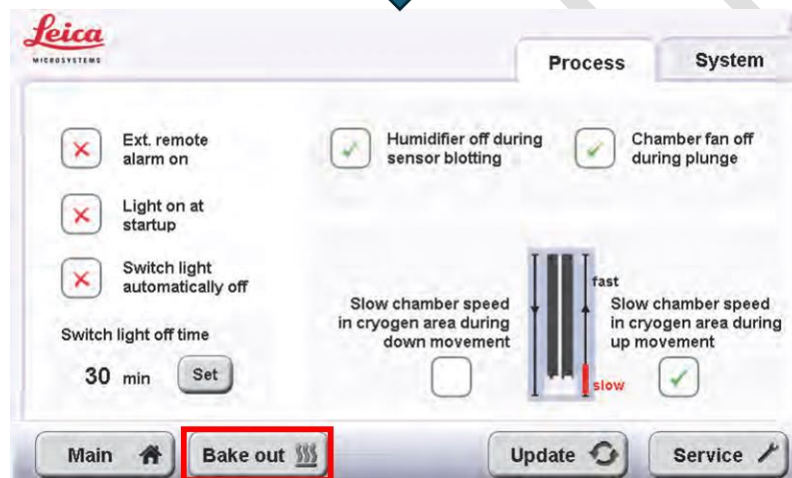
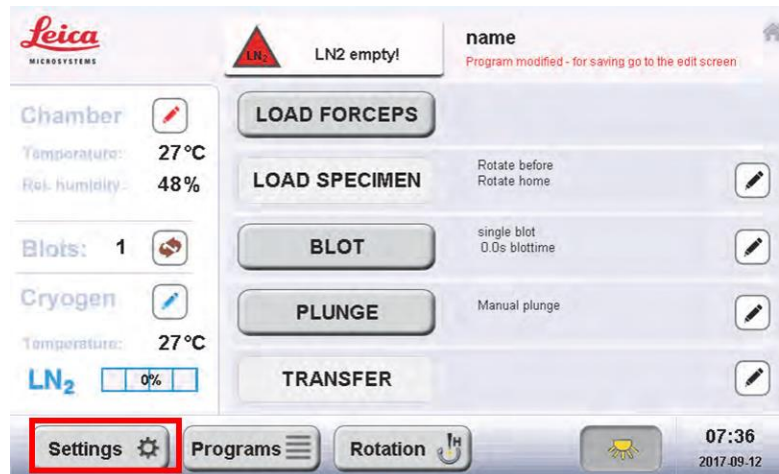


E. Bake out and shut down

- 1) Remove any remaining water from the humidifier tank by aspirating it with the syringe.
- 2) Remove filter paper and leave the door of the environmental chamber open.
- 3) Remove ethane container.
- 4) Click “**Settings**” and then “**Bake out**”. After confirming the warning messages, click “**Start**” to process the bake out.

* During the bake-out cycle, surfaces in the environmental chamber and the Dewar can become very hot. DO NOT touch those surfaces.

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- 5) After the bake out is complete, the environmental chamber will move automatically to the bottom position. Wait until the chamber arrives at the lowest position. Then, switch off the main switch at the back.

Checklist before leaving the lab:

- Turn off the ethane tank to prevent any potential leaks or accidents.
- Shut down the GP2 following the proper procedures.
- Remove the filter paper and dispose of it in the designated waste bin.
- Empty the humidifier of any remaining water.
- Empty the drip tray.
- Place the thermos bottle and its lid in the oven to dry.
- Tidy and clean the bench area.
- Sign the logbook.
- Remember to take all your personal belongings with you when you leave.

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