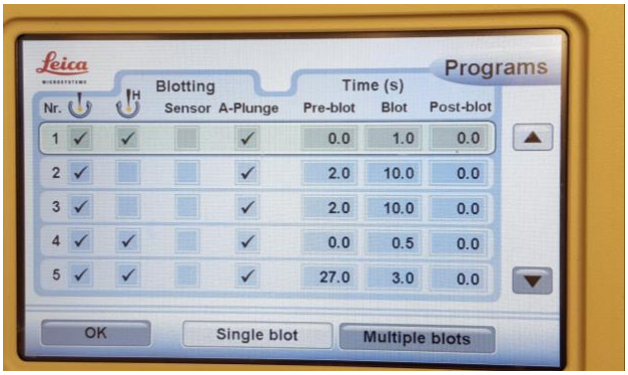
Leica EM-GP & GP2 User Guide

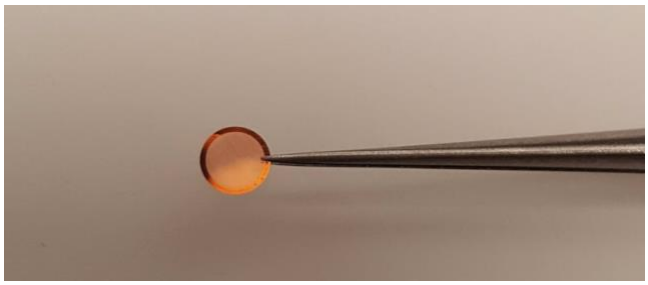
- Users must wear protective gloves and goggles when handling liquid nitrogen and ethane
- Setup of the EM-GP takes 30 - 60 minutes. Please plan in advance.
- Shutdown of the EM-GP takes 1 - 2 hours. Please check if you are the last user of the day. If you are not, please coordinate with the next user.

1. Turn the machine on (power switch at back side of machine).
2. Place the black ethane cup and the silver transfer container into the dewar chamber (usually stored inside of fume hood).
3. Fill the humidifier with 60 mL distilled water.
4. Fill dewar chamber with LN_2 until 100% full.
    a. refill every ~20 minutes
5. Once the cryogen container temperature < -175℃, liquefy ethane gas until black container full
    a. refill 2 - 3 times as ethane will warm up container temp no longer liquefy
6. Fill silver transfer container with liquid nitrogen.
    a. refill every ~5 minutes
7. Gently transfer cryo gridbox into silverver transfer container. Refill liquid nitrogen.
8. Setup environmental chamber settings
    a. T_C = 4℃ - 25℃ (will only go as low as 7℃)
    b. H_R = 90 - 99% (~15 minutes); NOTE- humidifier will only run when T_C is reached
    c. Setup temperature of cryogen = -175℃
9. Setup blotting parameters
    a. On main screen, click the long rectangular grey box in the upper left side (P1 ___/1.0/___).
    b. Select option Nr. 1 (see image →). This is the standard recipe used at the NYSBC. You are welcome to make your own recipe, **but DO NOT change Nr. 1 recipe.**
    c. Select “OK” to exit screen



10. Setup tweezer/blotter position parameters. The 2 main settings that need to be adjusted are “blotter settings” and “grid blot position”.
    a. On main screen, select “Setup” button
    b. Blotter settings
    c. Click “Adjust”
    d. Lower chamber? OK
    e. Select “Blot Position”
        i. Using binoculars and adjust buttons, move blotter closer or further from grid to optimize contact (**218** works well for us)
        ii. Select “Back” when done
    f. Grid Blot Position
        i. Click “Blot Position”
        ii. Using microscope and adjust buttons, move tweezer up or down from blotter to optimize contact (**3.6mm** works well for us)
        iii. Select “Back” when done
    g. Select “Prepare” to exit screen
11. Freeze grid (practice first with a dummy grid and water)

a. Add new blotting paper and secure using magnetic ring. Mark blotting paper with pencil to track rotation of paper. Switch blotter paper every 4 grids.
b. Load forceps with carbon side facing the blotting papers (tweezer needs to grab a significant portion of the grid to ensure good blotting; see image →)



c. Select “Lower chamber/R”
d. Add 3 uL sample to right side of grid
e. Incubate 30 seconds
f. Select “Rotate home, Blot/A-Plunge”
g. Transfer grid to box
h. Repeat a - f until done.

SHUTDOWN

If you are the last user for the day, then continue to steps below. If not, then top off nitrogen in dewar, cover nitrogen dewar with a kimwipe (to prevent frosting) and let next user know you are done.

- Remove forceps, wipe them down and put in storage
- Empty water from humidifier
- Open environmental chamber door
- Discard blotting paper and store magnetic ring
- Remove both cryogen containers and transfer to fume hood
- Discard H2O from chamber for **GP2** before initiating Backout
- Click “Bake Out” for 1 hour (default)
- This takes ~ 2 hours total
- When bakeout done, lower chamber and turn machine off

- **Above photo is H2O holding Chamber, H2O must be discarded during bakeout or for next user when not baking out.**

- **LEAVE THE LEICA EM-GP BENCH LOOKING LIKE THIS:**