

Attune NxT standard operating procedure

Start up

If the computer is off:

- The correct startup order is:
 1. Computer
 2. Auto-sampler
 3. Flow machine
- Open Attune software and login into your account

If the computer is on:

- Close the Attune software if it's still open
- Switch off and back on the Flow machine and the Auto-sampler
- Open the Attune software and check whether the performance test has been completed on the day.
 - If yes, you can log-in and proceed with your experiment.
 - If no log-in and carry out the performance test.

Performance test

If the performance test has not already been performed:

1. Log-in
2. Click on "performance test", follow the suggested checks, and click on "run startup".
3. Follow instructions to remove shutdown plate from the autosampler and lower the tube-lifter.
4. If prompted, refill fluid the necessary fluid bottles.
 - If you are using the autosampler it is wise to empty the waste bottle and to the other bottles have plenty of fluid in them (particularly the focusing fluid). This is to prevent the machine halting during the run.
5. Take the "Performance tracking Beads" from the shelf in the fridge door.
 - Double check that you're **NOT** using the "Rainbow beads"
 - Check the batch number against the one displayed on the screen
6. Prepare Performance Tracking Beads solution as instructed on the screen
 - 2 mL focusing fluid + 3 drops of the beads.
 - Vortex the beads before adding them to the solution and vortex the final solution
7. When the machine is done with the startup procedure you can click on "Run performance test".

IMPORTANT: if the performance test fails repeat it. Often the second attempt will work.

Please report any fails to luca.rosa@ucl.ac.uk.

Shut down

When you are finished with your experiment check on the calendar whether you are the last person of the day to use the machine.

- If no one else is scheduled:
 - In the top ribbon go to "instrument" -> "shutdown" and select "thorough"
 - Follow instructions on screen:

- Prepare 3mL of 10% bleach solution
- Load the shutdown plate in the autosampler.
 - If the wells on the top row of the shutdown plate look dirty, wash with some 70% ethanol.

Consumables

- This includes: bleach, focusing fluid, shutdown solution, wash solution, debubble solution, pipette tips and FACS tubes.
- The focusing fluid is either in the bottles on top of the machine or in the big cardboard container under desk. All other consumables are kept in the drawer.
- Please let Luca know if we are running out of any solution (luca.rosa@ucl.ac.uk)

Removing fluid bottles

Sometimes you need to remove a fluid bottle from the machine to fill or empty it.

- The correct way to do this is:
 1. Disconnect the sensor (the cable that looks like a 3.5mm jack)
 2. Disconnect the fluid line (some involve pressing a release catch)
 3. Take bottle out.
- Replace the bottle in the reverse order.

Flow rates

It is our experience that flow rates above 100 μ L/min significantly degrade the quality of the data (although admittedly this is working with bacteria).

Troubleshooting

Occasionally there are problems with the machine. Please follow any on-screen instructions in order to rectify the problem. If you are unsure then contact luca.rosa@ucl.ac.uk or christopher.barnes@ucl.ac.uk.

Calendar

It is **essential that you use the calendar** as it can take days to plan an experiment and so that people know if they are the last on the machine for the day