

Sorter setup and shutdown (And common problems and solutions)

Start Up

1. Turn on **in the following order**:
 - a. Sorter (left side of unit near bottom)
 - b. Computer and monitor
 - c. Laser, located under the monitor (switch is on front near right)
 - d. Vacuum trap located in the fume hood to the right of the sorter
 - i. align the red handle with the red tape and the blue handle with the blue tape.
 - e. Plug in pump and turn on pump located under the sorter (switch is on a black box in the back of the unit)
 - f. Vacuum pump to the left of the sorter (on the bench top, between two wooden shelves on the wall)



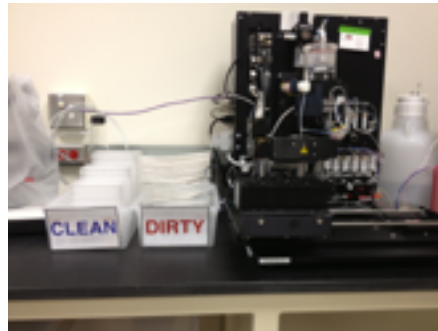
vacuum off



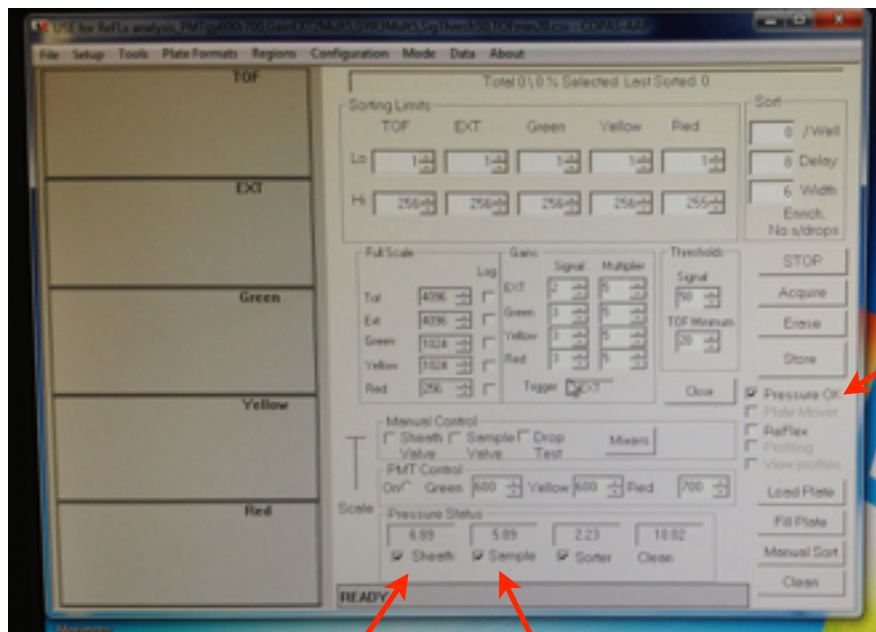
vacuum on

2. Open sorter software (located on the Desktop).
3. In software, select the appropriate template (depending on what you are doing that day (sorting, scoring, etc)) from the Open -> COPAS templates folder.

4. Click “Start”.
5. When the laser control dialog box comes on, click “RUN”. Wait for the 90 seconds for the laser to power on.
6. While the laser is starting:
 - a. Take a clean 96-well plate (from the “CLEAN” box) (i.e. - not a brand new plate)
 - b. Fill one row as: 4 wells with ddH₂O, 4 wells with 50% bleach, and 4 wells with cleaning solution. You can use the squirt bottle to fill the water and bleach wells. Pipette 250 μ L of cleaning solution.
 - c. Fill the next row (all 12 wells) with ddH₂O.



7. When the laser is powered on, click “Done”.
 - a. Laser will be approximately 13.4 mW
8. The pressure comes on while the laser is warming up:
 - a. “Sheath” pressure should be in the range of 4.8-5.1.
 - b. “Sample” pressure should be \sim 5.6.
 - c. If the sheath pressure seems low, make sure that the sheath bottle cap is on **tightly** and the sheath liquid is at the correct level.



Pressure OK
box

Sheath Pressure

Sample Pressure

9. When the pressure is correct, check “Pressure OK” box.
 - a. Wait for 70 seconds while the ReFlex module primes.
10. After the ReFlex module primes, check the “Reflex” box (located two below the “Pressure OK” box. When prompted, select “Run without bubble trap.”
11. Pop off the tubing coming from the top of the sample cup, and unscrew the lid. Aspirate out any liquid, and fill with ~25% filtered bleach (roughly half the sample cup with 50% filtered bleach (located on the benchtop to the left of the sorter), and half with autoclaved water (same place as the bleach)). Put the lid back on, put the tubing back on, and click the check box by “Sample” under the manual control area. This will prompt a warning that you will contaminate the flow cell. Select “OK”. This step will run bleach through the flow cell. Do not allow the sample cup to completely empty. Set a timer for three to four minutes so that the sample cup does not empty completely. Once almost empty, click the “Sample” check box again to stop the flow. Remove the tubing and lid, aspirate out the bleach, fill the sample cup halfway with cleaning solution, and run until almost empty (use at timer). Once almost empty, click the “Sample” check box again to stop the flow. Remove the tubing and lid, aspirate out the cleaning solution, and fill with with autoclaved H₂O, and aspirate out the water. Repeat twice for a total of three rinses. Fill with autoclaved H₂O again, replace the top and tubing, and run the water in the same manner as the bleach. Repeat for a total of two washes. On the final wash, don’t run the liquid down all the way; 1/2 or 3/4 of the way down is fine.
12. Click on the bottom right “Close” button to close the Parameters screen and you will see a screen that looks like a 96-well plate.
13. Go to Plate Formats, and in the drop down menu select “New 96 well template”. A box will say “Current polygon will be reset. Keep the current one?” Select “Yes”. Now select the same rows that you filled the 96-well plate with ddH₂O and bleach. This action will allow the ReFlex to run those rows on the sorter, to clean out the ReFlex tubing. On whichever rows have the water and bleach, select those rows in this orientation.

1	2	3	4	5	6	7	8	9	10	11	12
24	23	22	21	20	19	18	17	16	15	14	13

14. Click “Sample” (near the bottom right corner of the COPAS program window) and you will be asked to title the file. Create a folder for the day’s data (the date(yyyymmdd), assay, sorting or scoring, etc) and title the file “clean1”. Save, and the sorter will run the two rows you selected.
15. Load a different template in the program (file -> open). Do not save any changes. Click “Yes” to the question about keeping current polygon, if it asks. Loading the new template without saving changes to the old one allows you to re-open the old one, which you want to use, without having to re-program the template after doing the two clean rows. Now re-open the template you want to use.

16. Make sure the sheath fluid level is within the width of the piece of tape on the sheath bottle. This keeps the air pressure relatively constant, leading to constant flow rate through the flow cell. If the sheath level is too low, fill with M9 to an appropriate level and be sure the put the lid back on tightly.
17. Check the flow rate. First, place a paper towel beneath the flow cell/waste tray. Then manually open the sheath valve to start flow. Have a 15 mL conical tube ready, and then under “Pressure Status” uncheck the sorter pressure box. The diverter valve will shut off, so sheath fluid flowing through the flow cell will come out the bottom of the waste tray, onto the paper towel. Use a clock or stopwatch to time the amount of flow in one minute. Flow rate should be in the 9.7 to 10.2 mL/min range. If the flow is too high or low, get Erik. Be sure to note the sheath pressure and flow rate after one minute, and enter those data into the log notebook we keep on top of the sorter computer.
18. Now you are ready to run your plates. Place the first plate on the tray labeled “Sample”
 a. If you are sorting to a new plate, put your target plate on the tray labeled “Dispense”.



19. Click “Sample”.
20. A dialog box will appear to name the file. Go into the same folder as the clean1 file you made and write a new file name. You must make a new file EVERY time you run the sorter. There are four components to naming a file on the sorter for the current data analysis pipeline. The general outline looks like: **pXX_strains_condition_control**. **pXX** corresponds to the plate number. Plate 1 should be p01. Plate 21 should be p21. **strains** corresponds to the strains that you set up in the deep-well plate (and subsequent 96-well plates). This area will be titled whatever this assay is called. Example: McGrathRILs1a, NILsTest4b, etc. **condition** corresponds to the condition in each individual plate. abamectin, DMSO, copper, and tunicmycin would all be examples of conditions. **control** is the appropriate control for the condition. DMSO, None, water, and DMSO would be the associated controls for the conditions listed above. We normally title these files the plate number in the format pXX_treatment-extra, where XX is the number of the plate (if the plate number is less

than 10, number the plate 01, 02, etc), treatment is whatever you are testing (drug, food source, etc), and extra is any additional information (temperature or concentration).

21. Sorter will start automatically once you save your new file.
22. Watch the sorter vigilantly while it runs for the day. The more you pay attention, the faster you can catch any problems that might arise, and the faster they get caught, the less data gets ruined.

Shut-Down

1. Clean the Reflex module
 - a. Take a clean 96-well plate (from the "CLEAN" box) (i.e. - not a brand new plate)
 - b. Fill one row as: 4 wells with ddH₂O, 4 wells with 50% bleach, and 4 wells with cleaning solution. You can use the squirt bottle to fill the water and bleach wells. Pipette 250 µL of cleaning solution.
 - c. Fill the next row (all 12 wells) with ddH₂O.
2. Under the Plate Format drop down menu, select new 96-well template. Click "No" to remove old formats. Do not save over the format file.
3. Select the wells for cleaning and run the Reflex by clicking Sample and saving the file as "clean2" (same manner as the way you did when starting the sorter).
4. While the clean plate is running, power down the laser.
 - a. Select the "Tools" menu
 - b. Click on Run external laser
 - c. Click "Stop" and then "Done"
5. Turn off the laser power (on the box below the monitor).
6. Once the Reflex is cleaned, remove the lid of the sample cup (first pop out the tubing) and aspirate out any remaining fluid.
7. Clean the sample cup in the same manner as the start up procedure. Once with 25% bleach, once with cleaning solution, then rinse out with autoclaved water, and then run three sample cups filled of autoclaved water.
8. At the end, make sure that the sample cup is roughly half full with autoclaved water.
9. Click "STOP" two times to shut off any valves and then to shut off pressure to the system.
10. Close software window.
 - a. When the software asks to shut down with purging save, select "Shutdown without purging".
 - b. When the software asks to "Save changes to (whatever file you were using)", select "No".
11. Turn off the sorter power (located on the left face of the sorter, if looking at the front of the machine).

12. Turn off the pump power and unplug.
13. Turn off the computer (Start -> Shutdown).
14. Turn off the monitor.
15. Make sure the purple ethernet cable is not plugged in.
16. Turn off the vacuum flask in the flow hood and empty the liquid. If you have been running toxic compounds, empty the flask into the five gallon drum that is on the floor near the sorter (large funnel in the top). If we have only been sorting, or running bacterial food plates, dump down the sink).
17. Fill the Clean bottle and ReFlex bottle (the smaller bottles with red mesh on them) with autoclaved H₂O to the fill line. Fill the sheath bottle to the tape line with M9. If we are bleaching the bottles the next day (without sorting or scoring anything beforehand), then there is no need to fill any bottles.
18. Pop off the thick black tubing from the air compressor that connects to the pressure gauge at the top front of the sorter. There is a small white connector in the small bin on top of the laser box. Connect this to the end of the black tubing, which allows the air to bleed out of the system. This should take 2-3 minutes, or until the hissing is much quieter than it was to start.



Sterilizing the sorter (complete with replacement of parts)

This procedure includes how to remove all of the parts and tubes connected to the sheath fluid and replace them. If you do not intend to replace these parts, start this process at Step 4 and ignore the steps on the final page of this document (after the image of the waste tray).

1. Disconnect (using LT Quick Connectors) the tubes from the sheath bottle and the two clean bottles. Empty all liquids from the three bottles.
2. Completely disconnect and disassemble the current sheath bottle set up.
 - a. Remove 1/2 inch Tygon tube from sheath bottle and from the 1/2-to-3/8 connector. Throw away.
 - b. Remove 1/2-to-3/8 connector from the 3/8 inch tube. Set aside.
 - c. Remove the 3/8 inch Tygon tube from the male end of the 3/8-to-1/16 inch LT Quick Connector. Throw away.
 - d. Disconnect the male and female parts of the 3/8-to-1/16 inch LT Quick Connector. Set aside.
 - e. Remove the 1/16 inch Tygon tube from the female end of the 3/8-to-1/16 inch LT Quick Connector. Set aside.
 - f. Follow the 1/16 inch Tygon tube all the way to where it connects to the sorter (second valve from the left, right-hand attachment site on that valve, orange tape is closest) and remove the tube from that connection. Set aside.
 - g. Remove sheath bottle from the sorter area, set aside with other pieces to be bleached and autoclaved.
3. Get an autoclaved sheath bottle, and reassemble a new sheath bottle set up. All pieces (besides new bottle) should be located in the metal cabinet underneath the fume hood closest to the sorter.
 - a. Attach a new length of 1/16 inch Tygon tube to the sorter (second valve from the left, right hand attachment site on that valve, orange tape is closest).
 - b. Attach female end of the 3/8-to-1/16 inch LT Quick Connector to the 1/16 inch Tygon tube.
 - c. Attach male end of the 3/8-to-1/16 inch LT Quick Connector to the female end.
 - d. Attach 3/8 inch Tygon tube to male end of the 3/8-to-1/16 inch LT Quick Connector.
 - e. Attach 1/2-to-3/8 connector to 3/8 inch Tygon tube
 - f. Attach 1/2 inch Tygon tube to 1/2-to-3/8 connector.
 - g. Attach 1/2 inch Tygon tube to boss connection onto sheath bottle.
4. Disconnect the sheath bottle and two clean bottles (at LT Quick Connector) rinse them each one time with 25% bleach (just use plain Clorox).
5. Fill the sheath bottle with a ~liter of 25% filtered bleach.
6. Fill the clean bottles with ~200 mL of ~25% filtered bleach.
7. Fill the sample cup with ~25% filtered bleach
8. Reconnect all bottles.

9. Disconnect the vacuum flask connected to the waste tray, empty it into the sink, then reconnect the flask
10. Power on the sorter. Start all pressures.
11. Open the sample valve, run almost the liquid through the sample cup, and then close the sample valve.
12. Open the sheath valve and leave it open for half an hour.
13. During that time, press the clean button 25-30 times.
14. Take a clean 96-well plate, fill every well with 50% bleach.
15. After half an hour with the sheath valve open, run the Reflex for the entire plate.
16. Once the bleach steps are complete, stop the flow of any liquid.
17. Disconnect and remove all bottles and rinse three times with dH₂O in the sink.

NOTE: Before proceeding to steps with cleaning solution (1% unscented Tide), make sure the vacuum flask connected to the waste tray is **NO MORE** than a quarter full. The cleaning solution makes lots of bubbles that should not be allowed to back up into the vacuum system.
18. Fill the sheath bottle with ~500 mL of cleaning solution. **NOTE:** Due to the smaller volume used, you will likely have to tilt the bottle at an angle for fluid to flow into the tube. Use a styrofoam block to hold the bottle at an angle.
19. Fill the clean bottles with ~100mL of cleaning solution. **NOTE:** Due to the smaller volume used, you will likely have to tilt the bottle at an angle for fluid to flow into the tube. Use a styrofoam block to hold the bottle at an angle.
20. Reconnect all bottles.
21. Restart all sorter pressures and repeat steps 11 through 15. Step 14 should be carried out with cleaning solution
22. Once the cleaning solution steps are complete, stop the flow of any liquid.
23. Disconnect all bottles and rinse three times with dH₂O in the sink.
24. Fill the sheath bottle with ~1 liter of 70% ethanol.
25. Fill the clean bottles with ~200 mL of 70% ethanol.
26. Fill the sample cup with 70% ethanol.
27. Reconnect all bottles.

28. Restart all sorter pressures and repeat steps 11 through 15. Step 14 should be carried out with 70% ethanol.
29. Once the ethanol steps are complete, stop the flow of any liquid.
30. Disconnect all bottles and rinse three times with dH₂O in the sink. While bottles are not connected, spray the tube connections with 70% ethanol and dry with a Kimwipe.
31. Fill the sheath bottle with ~ 2 liters of dH₂O.
32. Fill the clean bottles with ~500 mL of dH₂O.
33. Fill the sample cup with dH₂O.
34. Reconnect all bottles.
35. Restart all sorter pressures and repeat steps 8 through 12. Step 11 should be carried out with dH₂O.
36. Once the water steps are complete, stop the flow of any liquid.
37. Disconnect and remove all bottles.
38. Rinse the the clean bottles once with with dH₂O in the sink, and then fill with dH₂O up to the fill lines
39. Rinse the sheath bottle once with dH₂O in the sink and then fill the sheath bottle with ~8 liters of M9 (to the green tape fill line on the sheath bottle).
40. Reconnect all of the bottles.
41. Power on the sorter. Start all pressures.
42. Take a clean 96-well plate, fill with dH₂O.
43. Run the Reflex for the entire plate.
44. Run a bead test
 - a. Use bead solution supplied by Union Biometrica at a 1:10 dilution (~1mL beads + 9mL water in a 15mL conical)
 - b. Aspirate out any remaining liquid in the sample cup and add the bead solution to the sample cup.
 - c. You MUST use the file 42UControlBeads (File -> Open -> Desktop -> 42UControlBeads)
 - d. Click Tool -> Run Control Beads.
 - e. In the program window, click Acquire (just below Start/Stop button)
 - f. Wait for the sorter to run 500 control beads
 - g. After the operation has finished, go to File -> save screen image. Save the image as a .bmp and in the folder Control Bead Images on the Desktop.

h. After saving the image, use a automatic pipetter to aspirate out the bead solution and save it in a conical wrapped in tin foil (beads are light-sensitive).

45. Power down the system.



46. Slide the waste tray out from under the flow cell chamber (WEAR GLOVES!). Spray out the accumulated salts from the waste tray with a spray bottle of water. Try not to move the teflon ring. If the teflon ring gets moved, simply place it back where it should be and press it down so it fits snugly. After washing out the waste tray, reattach the waste tray to the Tygon tubing and slide the waste tray back into position beneath the flow cell. Be sure to do this step in a way that you don't spray any water towards the sorter.

47. Clean the previously set aside pieces of the initial sheath bottle set up. The parts you should have left are a length of 1/16 inch Tygon tube, male and female ends of the LT Quick Connector, the 1/2-to-3/8 inch connector, and the initial sheath bottle.
48. Rinse out the sheath bottle with ~25% bleach
49. Rinse out the sheath bottle with ddH₂O three times.
50. Let dry, then cover boss connection and top of the bottle with tin foil and store until next use.
51. Cut new pieces of Tygon tubing; two inches of 3/8 inch and three inches of 1/2 inch tubing.
52. Soak 1/16 inch Tygon tube, male and female ends of the LT Quick Connector, the 1/2-to-3/8 inch connector, and the new pieces of 3/8 and 1/2 inch Tygon tubes in 25% bleach for 10min
53. Rinse 1/16 inch Tygon tube, male and female ends of the LT Quick Connector, the 1/2-to-3/8 inch connector, and the new pieces of 3/8 and 1/2 inch Tygon tubes with ddH₂O.
54. Wrap all pieces in tin foil and autoclave.

Common problems and solutions

Problem: Dripping from the waste tray

Solution 1: A. Make sure vacuum line is on with sufficient suction to remove liquid from Tygon tubing line.

Solution 2: Put towels down beneath the flow cell to prevent flooding. **With the sheath valve open and flowing, click on the sorter diverter.** A stream will flow straight down. The stream should flow straight down and constant. If it stops and starts and/or is not straight, or is flickering/wavering a lot, then the flow cell is clogged. Stop the sheath (and sample) flow. Remove the tubing from the T connection and run the stylus through the flow cell.

Solution 3: Remove the waste tray. Put towels down to the left of the flow cell to prevent flooding. Open the sheath valve. A stream should flow at a 45° angle. If it does not, call Erik.

Solution 4: Not a solution so much as a preventative measure: If you are running worms that are fully grown (grown with proper concentrations of cholesterol), be sure to watch for clogs, because more worms means more potential for clogs. Keep an eye on the objects being sorted/scored, and check the stream out of the waste tray every few plates to make sure it is not obstructed, and (if sorting) check the plate after it finishes to see if you see the correct number of animals swimming in the wells.

Problem: Clogs - you will know because the flow of objects will stop, tubes will be blown off and liquid will leak, and the sheath and reflex will backup into the sample cup. If liquid leaked onto the flow cell, call Erik. The flow cell will have to professionally serviced.

Solution: Remove the tubing from the T connection and run the stylus through the flow cell. Also, run steps 6-12 of the shut down procedure to bleach and clean the flow cell.

Problem: The Reflex stage is out of alignment. This problem happens if your hand or towels mess up the alignment.

Solution: Power down the system and power it back on. The stage should return to home. If not, please call Erik.

Problem: Many objects are recorded by the system.

Solution: Make sure the sheath and clean bottles are filled. Also, make sure that each well has the amount of liquid required to aspirate all worms and little air.