https://www.sonybiotechnology.com/us/instruments/sh800s-cell-sorter/software

FACS: Do things in the below order

* Fluids
* Air
* Cytometer
* Software

**Fluids**

1. Empty waste container (bottom left with the red line) into a sink, then fill up to the yellow tape with bleach
2. Check sheath fluid level. If low, make more sheath fluid if none available:
   * Dispense 1L of from 4L 10x Preservative-free ClearSort Sheath Fluid into the larger bottles and then dispense 9L of DI water into it using the automatic dispensing from our MilliQ

\*This usually leaves it below the fill line. I’m not sure if it’s because our milliq is not actually dispensing the right amount of water, or if the bottle is inaccurate

* + Two options:
    1. Pour into sheath fluid container to fill up to 10L
    2. Pour old sheath fluid into the sink and then fill container. To remove, click the buttons that attach the container to the green and blue tubes to detach. To open sheath container, pull up the pressure valve and turn so it’s open. Then pull up the handle, then turn 90 degrees and pull out Once full and back on the tray MAKE SURE TO REATTACH THESE TO THE FLUID LINES (should hear a click. If not, try again. AND CLOSE THE PRESSURE VALVE. Otherwise, pressure will release from the instrument and you’ll have to start over.

1. Clean up any spills on the tray that holds the fluids.
2. Refill DI water bottle on the back left side of the machine (says pull open on the panel). The DI bottle is the bottom left bottle. Remove it from its compartment and then click the button to detach from the green tube. Pour out bottle into sink and then fill up to marked fill line on the bottle. Place back into the machine and reconnect the tube, making sure to hear a click.

**Air**

1. Turn on the air pressure machine (switch is on the back right, top)
   * Make sure the pressure stabilizes around 80 (black lines on the machine).
   * If it keeps bouncing back and forth at this point, check to ensure that your tubes are properly clasped to their corresponding containers and that the sheath fluid pressure valve is closed.

**Cytometer**

1. Open up the bottom left panel.
2. Splash a kimwipe with 70% ethanol. Clean the deflector plates (top, black), the sensors (black ‘windows’ on each side and in the back).
3. Turn on cytometer
4. Make sure the pressure is stabilized at 4.3 for both the sheath and the sample fluids and that the tanks for each of your fluids looks reasonable (assuming you filled them up and emptied waste, that should show up on the screen)

**Software**

1. Login to the computer with your NetID and password and open Cell Sorter Software
2. Login using the lab username and password:

Username: SenesLab

Password: Slarba4211!

1. It should then prompt you to show your chip QR code. First, write your name, the date, and the time on the white part. Scan the QR code

\*It can be used anytime within 24 hours, so if you start a run earlier the next day you can use it again

1. The machine should then prompt you to replace the current chip. Open up the top panel and then wait for the chip to be ejected from the left side. Remove the chip by touching only the sides and put it into the last used sleeve. Remove your chip from its sleeve by only touching the sides and insert into the slot. The machine should take in the chip.
2. Make sure that the laser settings are 488nm and 561nm and continue.
3. The machine should then start a fluidics check. During fluidics check, there will be prompts for you to follow:
   * **Sheath Filter Debubble**
     + - Follow the prompts. When asked to, open the back left side panel to access the top left container (blue tubing).
       - Remove the sheath filter from the clamps by gently pulling it towards you
       - Tap the sheath filter with your hand to release air bubbles trapped on the inside of the filter.
       - Use a kimwipe to slowly open the TOP valve of the filter until you hear air escaping and eventually sheath fluid on the kimwipe
       - Tighten the valve and place the sheath filter back into the machine
   * **Clean sample lines**
     + Skip bleach run
     + For DI water, run 12mL of 70% ethanol INSTEAD of DI water until the bar is ~25% complete
     + Abort the cleaning

\*If you get stuck on the cleaning and the prompts don’t allow you to proceed, it is possible to bypass it by just running DI water cleaning using DI water until 100%.

1. Automatic setup beads for cell sorter SH800
   * In the top left of the brown refrigerator to the left of the FACs is a box that says Automatic Setup Beads. Take out one of the droppers and be sure to shake it very well (you’ll be able to tell if you don’t shake it well if the color of the liquid that comes out doesn’t have a purple-ish tint to it)
   * Place 7-10 drops of the beads into a 1.5mL microcentrifuge tube with the top and most of the side part of the lid cut off.
   * Place the microcentrifuge tube into the FACS with the appropriate sample adapter (snug fits only)
   * Make sure the tube is placed into the sample adapter vertically and that it touches the bottom of the adapter, and that the tube is able to 360 spin in the adapter without getting caught (otherwise, cutoff more of the lid attachment)
   * Follow instructions on the screen for the bead setup

Attach image of end result here

Look for first satellite drop distinct from stream

Last drop in stream is well formed

Satellite drops aligned with stream and major drops

Thin waste stream (infrared image on the right, middle stream)

Brightest drop is the last on in the stream (final center image)

If calibration fails in the middle of the run (pop-up on the screen); abort and restart after adding more beads to the microcentrifuge tube

**Starting the experiment**

1. Select a template or use a blank experiment

\*The only bad part about picking a template experiment from a past run is that you can’t reorganize the tubes in the experiment window (at least as far as I know)

1. Set up the controls to ensure that the instrument is reading properly (GpA, G83I, No TM)
   * 300uL PBS + 2uL of overnight into separate Eppendorf tubes, vortex a couple times very quickly, then allow to sit for 10 minutes.
2. Under recording, set the stop condition to event count and set the stop value to 100,000.
3. After 10 minutes, vortex one control sample a couple of times, remove cap, and place into the appropriate tube holder. Again, check if it can freely spin 360 degrees.
4. Setup the tube holder in the cytometer (bottom right) and then click start.
5. Adjust the sample pressure so that the events per second (eps) is below 10,000.
6. Click record. It should stop recording as soon as it reaches 100,000 events.
7. Remove tube from sample adapter. Repeat with the other two controls.

**Gating**

1. If the controls seem reasonable (No TM with low fluorescence, G83I slightly higher, GpA higher), then you can measure a mix of your sample that will be used to define your bins/gates. Take 300uL of PBS and add 2uL of your overnight sample. Vortex and wait 10 minutes, vortex again, then put in the sample holder and press start. Adjust the sample pressure so that the eps is below 10,000 and then record 100,000 events.
2. …

**Test Sort**

To ensure that your gates are reasonable and that the bins can be distinguished from each other, we run test sorts

Important:

* Eps < 10000
* Start around 3 sample pressure
* Don’t sort adjacent bins simultaneously
* The later bins with less percent population take longest to sort, so it’s best to try to sort those along with some of the faster bins
  + This is a good time to estimate how long it might take for a long sort: if you’re sorting 500000 events and the rate of events per second is 10, you’ll be here all night. Make sure that the final bin is large enough so that it takes closer to 4-6 hours (between 0.7-1%)
* The screen can be quickly overpopulated with data from each run (sorts, controls, flows, etc.) and having too many open can slow the computer down. Make sure to close some of the windows as the run gets going.
* Record as well! This way you’ll be able to look at the distribution in the future (I didn’t notice that we both record and sort until looking back at some data on 3/30/2022, so I only have some recordings for those)
* MAKE SURE THAT THE SORT MODE IS “Ultra Purity”. Since our dynamic range is very low, this is important to ensure that the sorter is aiming to separate bins as best as it can. This sort mode effects the overall sort efficiency and time of the run. I haven’t tried at this point, but it may be possible to optimize this for your run, depending on how much time your have and how important it is that you get pure bins…

1. Prepare x tubes with 150uL of PBS per tube, where x is the number of bins. Label each of them with their bin number
2. Add the diluted sample from your 50mL falcon tube to a 15mL falcon tube (< 13mL or it will splash back and make a mess)
3. Make sure it is well mixed by inverting a couple of times.
4. Remove the cap of the 15mL falcon tube, put it in the correct sample adapter, and put the sample into the FACS.
5. At the bottom of the screen is the sort control. Choose which bins you would like to sort (remembering not to sort adjacent bins) and set the stop count to 20,000 for each.
6. Add the tubes of the corresponding bin numbers into their respective positions on the loading adapter (image).
7. Place the loading adapter into the machine on the bottom left, ensuring that it is properly attached. Hit the load collection button on the sort control menu to prepare the tubes for sorting.
8. On the left, change the sample stop condition to Sorting, then hit start.
9. Wait about 15 seconds for the system to stabilize, then change the sample pressure so that the eps is consistently below 10,000.
10. Once it is consistently below 10,000, hit sort start on the sort control menu. It will sort until both are finished.
11. Continue with the rest of your samples. If you have an odd number of samples, you can leave one of the bins next to “To Sort:” blank and you can just sort into the given bin.

**Reflow**

To ensure that your bins are spaced out well enough that the cytometer was able to distinctly separate them.

1. Take the tube for bin 1, vortex, cut the cap off, and place into the sample adapter.
2. Similar to what you did for the controls, you are going to flow the test sorted bins through the cytometer to look at the overall fluorescence. Set the sample stop condition to “Recording”. Under **Recording**, set the stop condition to “Event Count”, and set the “Stop Value” to 5000.
3. Click “Start” and once the sorting stabilizes (approximately 30s, once the Event Rate is pretty stable), hit Record. It will stop once it reaches 5000.
4. On the right, you will see on the graph of Events vs EGFP-A-Compensated that many of your cells were properly sorted, but that some are found in other bins. This is normal, so long as you aren’t sorting too much from the other bins (except between bins 1 and 2. Make sure that the difference in the median of the fluorescence is at least ~4000).
5. This will count 5000 events/cells from the 20000 sorted into each of your bins, and will give you an idea of how well the cytometer is able to distinctly characterize cells into your bins.
6. If you think your bins look good (not an overwhelming amount of events found from the early bins in your later bins and vice versa), then continue. Otherwise, you can troubleshoot a bit:
   1. Adjust the accepted events using the circle and triangle shape to take in a more stringent amount of data.
   2. Re-calibrate using the automatic setup beads.
   3. After any of the above, make sure to re-test sort and reflow
7. Write down the median values (or take a screenshot of your data) for each of the reflows, as this is what will be used to calculate reconstructed fluorescence values from each of your bins.

**Sorting**

To separate your sample population into distinct bins based on fluorescence. All of the information under the **Important** section in Test Sort are applicable here.

1. Prepare x tubes with 3mL LB + antibiotic, where x is the number of bins. Label each with their bin number.
   1. Before putting it in the collection area, vortex the tubes so that the LB coats the walls of the tube.
2. Under Sort Control, choose which bins you would like to sort (remembering not to sort adjacent bins, and that the final bin will take the longest) and set the stop count to 500,000 for each. Set the Sample Stop Condition to Sorting.
   1. It’s best to also consider the what the rest of your sorts will look like and to set those up on the instrument. I typically sort
3. Once you’re satisfied with your sorting strategy, refill your 15mL falcon tube with your sample + PBS mixture up to 13mL.
4. Make sure it is well mixed by inverting a couple of times.
5. Remove the cap of the 15mL falcon tube, put it in the correct sample adapter, and put the sample into the FACS.
6. Put the corresponding tubes for the sorting bins into the appropriate position in the collection area.
7. Click “load collection”, then click “Start”.
8. Because one sample is going to take longer than the other to collect, you have a couple of options:
   1. Keep collecting until both are finished
   2. End the run, subtract the number of events sorted so far for the unfinished run from 500,000, and then change the number from 500,000 to sort for this bin to the subtracted value

I have 5 bins, and I do the following: run 3 and 5, then 2 and 5 to completion. Put all those samples on ice, then start 1 and 4. Once 1 is finished, I take my samples off of ice to allow them to all reach RT by the time 4 finishes. This way, they’re all likely to grow at the same rate (the first time I grew them off of ice it took 3.5 hours, but after leaving them at RT it only took about 1.5-2 hours.

**Growth**

1. IF you have 3-4 hours, start growing them as soon as you can. Otherwise, leave them shaking at 20C overnight and then start growing them in the morning (should take around 1-2 hours)
2. Using the eye test, check and see if your samples look different than a normal tube of LB.
3. Once they do, go take the OD600. Aim for it to be around 0.1-0.3. Do not let them reach saturation. Spin these samples down and miniprep, or rid of all of the supernatant and freeze until ready to miniprep.