10/18/2024

SCRC Flow and Mass Cytometry - FAQ

How should I prepare my sample for flow analysis and/or sorting?

Generally:

- ☐ Keep sample concentrations between 10^6 - 10^7 cells/mL, minimum volume of 200 uL in 5 mL FACS tube.
    - If using HTS plate reader on the Fortessa X-20, keep sample concentrations 10^6 - 10^7 cells/mL (optimization will be required) and do not fill it up to the maximum volume per well.
- ☐ Bring buffer solution (<2% serum) to dilute if necessary.
- ☐ Resuspend and filter samples before acquisition.
- ☐ Bring the proper single stained controls and unstained controls.
    - FMOs (fluorescence minus one) controls are highly recommended.

For example, in a 5 color panel consisting of FITC, APC, Pacific Blue, PerCpCy5.5, and RFP, proper compensation controls would be:

1) Unstained control (no RFP)
2) FITC single stained control
3) APC single stained control
4) Pacific Blue single stained control
5) PerCpCy5.5 single stained control
6) RFP+ only control

It is also recommended to have a viability dye to separate out the dead cells.

FMOs controls would look like this:

1) FITC FMO: all colors minus FITC
2) APC FMO: all colors minus APC
   And so on…

- ☐ Bring collection tubes/plates if sorting along with a collection buffer.
    - Sorters can accommodate 1.5 mL Eppendorf tubes, 5 mL FACS tubes, 15 mL conical tubes, and plates.

How long does sorting take?

It is dependent on the nozzle size, sample concentration, and population of interest %, and how many sorted cells needed. Smaller nozzle sizes = faster sort although cell shearing can occur. Choose the nozzle size that is >5x bigger than your cells.

Rough calculation below:
For a 1 mL cell suspension at 10^6 cells/mL running at flow rate 4.0, assume the flow rate 4.0 = 40 uL/min. This would take ~25 minutes to run. Likewise, if the flow rate is 10.0 = 10 minutes.

- Flow rate goes from 1.0 - 11 meaning ~10-110 uL/min
    - Flow rate depends on how rare the population of interest is. The rarer it is, the lower the flow rate to capture these rare events.
    - Some cells are sensitive to higher flow rate (because this slightly increases the sample pressure) so it's best to have it more concentrated to run it at a lower flow rate.

I want to get trained. How should I go about this?

Go to Stem Cell Research Center (agilent.com) (you will need to log in) and find the **Flow Cytometry Training Request (FLOW)** form. Fill it out and you will be notified once we have a training session available/confirmed.

Please note we are mostly offering flow training for analysis only, not sorting. Requests for sorting training will be approved depending on the user's projects and needs.

How do I book time on the sorter/analyzer/other equipment? How much time should I book?

Use the Stem Cell Research Center's iLabs system to schedule time on the calendar. Go to Schedule Equipment and open the folder for the corresponding equipment. Go to the calendar and reserve time by dragging the block or clicking the calendar and manually changing the times. You may also need to fill out forms. If you are trained, you will be given self-use privileges and can book for self-use.

If you don't have access, email vanessa.s@uci.edu or nguyenpu@uci.edu

Time-wise, see above for “How long does sorting take?” for a rough calculation. It depends on the number of events you’d like to attain as well as your sample quality.

Where is the SCRC Flow and Mass Cytometry Core located?

We are located in the Stem Cell Research Center/ Sue and Bill Gross Hall, Bay 1300. Badge access is required to access the building. If you do not have access, please call the lab phone (949) 824 - 0269 and we can let you in.

Which equipment should I book?

For sorting, we have two sorters:

- Aria II: Cell sorter,Sterile hood, 2Blue+SSC (488nm), 6 violet (405nm), 4 yellow/green (561), 3 red (640nm), 2UV 350nm, plate, slide, 15ml, 5ml, 1ml sorting collection
- Fusion: Cell sorter,Sterile hood, 2Blue+SSC (488nm), 6 violet (405nm), 4 yellow/green (561), 3 red (640nm), 2UV 350nm, plate, slide, 15ml, 5ml, 1ml sorting collection

For data acquisition, we have one analyzer:

- Fortessa X-20: Cell analyzer, 2Blue+SSC (488nm), 2 UV (355nm), 6 violet (405 nm), 5 yellow/green (561 nm), 3 red (640nm)

Recommendation to use fluorofinder panel design as this website also contains our configurations or contact us for exact configurations.

We also have CyTOF:

- Helios (cell suspension mass cytometry)
- Hyperion (imaging mass cytometry)

Other equipment (for enrichment, purification, dissociation):

- gentleMACS Octo Dissociator (must supply own C and M tubes)
- Mini & MidiMACS magnets and magnetic stand (must supply own columns)