

Administrative/Experimental Basics Information

Email: mghcrmflowcore@mgb.org

Website(s): <https://researchcores.partners.org/crmfcc/about>

Phone: 617-643-0790

Staff: Maris Handley and Sara Shanbrom

Facility Hours: Monday - Friday: 8:30am-5:00pm

Required forms:

Biosafety form – To be filled out by all new users; submitted once

Human sort form - submitted with each unfixed BSL1 human sort

BSL2 sort form - submitted for BSL2 samples; submitted once

Rates/Billing:

You are charged for scheduled time, or logged/used time, whichever is greater

With staff

MGB:

FACSArias: \$100/hr

Bigfoot: \$100/hr

Training (any instrument): \$100/hr

Self-run:

FACSArias: \$70/hr

Bigfoot: \$80/hr

Cytpix/VYB: \$70/hour

Non-MGB Academic:

FACSArias: \$144/hr

Bigfoot: \$144/hr

Training (any instrument): \$144/hr

FACSArias: \$100.08/hr

Bigfoot: \$155/hr

Cytpix/VYB \$100.08/hour

External For-Profit:

FACSArias: \$159.00/hr

Bigfoot: \$159.00/hr

Training (any instrument): \$159.00/hr

FACSArias: \$90/hr

Bigfoot: \$100/hr

Cytpix/VYB: \$90/hour

Cancellation policies:

	<u>CytPix</u>	<u>Arias/Bigfoot/Training</u>
Time frame:	24hrs	7 days
No one else books the time:		Full charge
Someone books all of the time:		No charge
Someone books part of the time:		Charged for remaining time

Scheduling:

<https://researchcores.partners.org/>

You will need to create an account before scheduling any time. If you have a Partners username and password you can simply log in. If you don't have a Partners account, you'll need to create an account; click "Sign-up". You will be asked for basic information and an account will be created.

The next step is to link a fund number with your account. To do that, go to Users->Funds->New Fund Number. Here you can type in your fund number and click "Look-up". This will find your fund number in the system and fill in the associated information. You can then hit "Assign Fund" to link it to your account.

A completed Biosafety Agreement needs to be submitted before booking any appointments. If you will be working with live human cells on A1 with hood, A3 in biosafety cabinet, or the Bigfoot, you will need to provide your PIBC number. If you are not working with live human cells, you can write "NA" for this number when booking time. If you are working with primary human cells, or any BSL2 cells, your PI will need to complete a BSL2+ form.

Each day of the schedule opens 2 weeks in advance, at 9am

The last 10 minutes of each appointment is for cleaning; for BSL2+, it is the last 30 minutes of each appointment

Samples:

Minimum volume is 250ul

For sorting, concentration should be around 40×10^6 /ml

Include a viability dye!

Make sure samples are filtered using appropriate mesh

Mesh cap tubes: BD 352235; mesh sheets: Small parts Inc B-CMN-35

Sample buffer should not contain more than 4% serum (FBS, BSA, etc...)

Bring extra dilution buffer with you

Bring samples covered to protect them from room lighting

Collection tubes/plates should have media in them; media can be whatever you want (PBS, culture media, lysis buffer, etc...)

Controls:

Instrument QC is run by facility staff each morning

Always bring compensation controls (EVERY time). Compensation controls are single color only, and can be beads or cells; these must include both positive and negative populations, or a separate unstained control(s)

Negative cell control(s), FMO controls, and experiment controls should also be included

Negative controls are unstained **cells** or cells stained with isotypes (not beads)

FMO stands for Fluorescence Minus One. These samples (always cells, not beads) contain all of your fluorochromes **except** one. These are used for accurate negative/positive gating during your analysis

BSL2+ Sorting:

All of these appointments must be booked on A3 in the hood or the Bigfoot; these instruments are housed in separate BSL2+ level containment rooms.

Prior to being able to sort BSL2 samples, we will need your PI to fill out our “BSL2+ PI Questionnaire”, which can be found on the “About” page of our website. Once this is submitted and reviewed by us, you will receive a Flow Approval Number (FAN) that grants permission to sort your cells in our facility. **Only users with approved FANs may do BSL2+ sorting. There are no exceptions to this.**

In addition, the following guidelines are in place during BSL2+ sorting or analysis for the safety of our users and staff:

- During BSL2+ sorting, entrance to and exit from the room must be limited. Sorting must be stopped before exiting or entering.
- *ALL* samples *MUST* be filtered immediately before sorting to minimize the risk of clogging.
- Personal items should not be used in the room (i.e. laptops, cell phones, etc...). Anything that is used in the room must be able to be sprayed with bleach or thrown away in the case of a biohazard emergency.
- All waste must be returned to the lab from which it came for disposal (i.e. empty sample tubes, unused samples, tips, etc...).
- The last **30 minutes** of every appointment are reserved for cleaning and decontaminating the machine and benchtops.

Data:

Experiment naming format: FirstnameLastname_Date-machine used

Data backup

Export both .fcs files AND the Experiment

.FCS: data files that can be analyzed in FlowJo (or other flow software)

Experiment: contains all experiment information including instrument settings, plots, gates, and collected data

Data is moved to your home drive or our shared drive (from the shared drive through dropbox or our OneDrive

The facility archives experiment folders (but not .fcs files) to an MGH server so data is accessible in the future

Rules:

- Name your experiments with your FirstnameLastname_Date-instrument
- All human samples run on the analyzers need to be fixed
- All live human cell lines must test negative for HIV, Hep B & C, and you must provide testing documentation. All primary human tissue, NHP cells, BSL2 cell lines, and any infected samples are considered BSL2, and must be done on A3 or Bigfoot under BSL2+ conditions. (If doing human cells, please ask us for more details.)
- All samples must be filtered.
- Don't change any of the emission filters unless you have been taught how by the core staff.

- **No USB drives in the instrument computers; don't access the internet**
- If there is an issue with the equipment, please let us know
- If you are running more than 10 minutes late, please let us know

Equipment:

Computers

FlowJo dongles are available to borrow.

2 MGB PCs for general use

1PC for data collection

FACSAria1

488nm, UV, 405nm, 532nm, 633nm lasers, total of 18 colors

488nm: 4 colors

UV: 2colors

405nm: 5 colors

633nm: 3 colors

Sorts into plates or tubes

DIVA software

Containment appropriate for BSL1 sorting

FACSAria3

488nm, UV, 405nm, 561nm lasers, total of 15 colors

488nm: 3 colors

UV: 2colors

405nm: 6 colors

561nm: 4 colors

Forward scatter PMT

Sorts into plates or tubes

DIVA software

Containment appropriate for BSL2+ sorting

Bigfoot

UV, 405nm, 455nm, 488nm, 561nm, 640nm, 785nm lasers, total of 50 colors

UV: 12 colors

405nm: 11 colors

455nm: 4 colors

488nm: 7 colors

561nm: 12 colors

640nm: 5 colors

785nm: 3 colors

Sorts into plates or tubes

Sasquatch software

Containment appropriate for BSL2+ sorting

Attune CytPix

488nm, 405nm, 633nm lasers, total of 14 colors

488nm: 6 colors

405nm: 2 colors

633nm: 3 colors

633nm: 3 colors

Image collection/processing

Read from 5mL tubes or 96-well plates

Can detect red fluorescent proteins (RFP, TdTomato, dsRed, mCherry, etc...)

Attune software

Sample tubes:

Aria and Bigfoot sorters:

- Microtiter tubes
- 5mL (12x75) tubes [with 35um mesh filter cap (part no 352235)]
- 15mL Falcon tubes

CytPix:

- 5mL Polystyrene or Polyethylene tubes
- Various plate sizes and formats

Collection devices for sorting:

Various small tubes	Eppendorf tubes	12x75 tubes (5ml)
15ml tubes	50ml tubes	Various plates

Things to remember:

1. Come on time. If you will be more than 10 minutes late, please call or email to let us know.
2. Bring samples either wrapped in foil or in a container with a lid. This protects your staining. If your cells like to be cold, bring them on ice.
3. There should be a minimum of 250ul in every control and sample.
4. Bring appropriate controls **every** time you bring samples. These should be prepared at the same time as your samples, under the same conditions, and brought together with your samples. These may include compensation, FMOs, and experiment controls.
5. All samples need to be filtered before being run on the cytometers. You can bring filter capped tubes if you would like us to do this for you. **Bring extra filter tubes** in case we need to re-filter.
6. Do not do any time course experiments without standards and the appropriate controls. Laser powers, alignment, detector sensitivity, and fluidic pressures change with time (even throughout a single day); staining intensities will also change with antibody lots, staining volume, pipet calibration, and how many times you've taken an antibody out of the refrigerator and into the light. If interested, let us know so we can discuss the best approach.
7. If sorting, bring collection tubes or plates with media (culture medium, lysis buffer, etc.) in them. Label your collection tubes.

8. Plan for a maximum of 20 samples/hr unless you have spoken with us about it before you book the appointment.

As the complexity of the panel/gating goes up, the number of samples/time goes down. As the number of events you wish to collect goes up, the number of samples/time goes down.

Compensation on any panels over 5 colors will average 30 minutes. A less complex compensation, in a protocol we've already run, may take less than that; more complex and/or new protocol set up will probably take longer.

9. Plan for a maximum of 4 sorts/hr unless you have spoken with us about it before you book the appointment. As the complexity of the panel/gating goes up, the number of sorts/time goes down.

10. You are responsible for setting your sorting gates and understanding why you are setting them that way. It is best if you are present while gates are being set, and please be available during your sorting appointment in case we need to contact you to clarify or confirm sample or sort details.