Hollings Cancer Center Flow Cytometry and Cell Sorting Shared Resource
Rules and Regulations

Objective:

The Flow Cytometry & Cell Sorting (FCCS) Shared Resource Facility is co-sponsored as a Hollings Cancer Center (HCC) Core Facility and a University Research Resources Facility. The goal of the FCCS Shared Resource is to provide consultation, comprehensive analytic flow cytometry and high speed cell sorting, and assay development to facilitate and expand the flow cytometry-based research of HCC investigators.

Services:

Analytical Use: LSRFortessa, LSRFortessa X-20, FACSVerse

The analytical cytometers are available for use by investigators or their staff. Facility staff will also run samples at your request at an assisted user fee rate. Independent operation is provided at a reduced rate but comes with the expectation that the users are properly trained. Facility staff provides individual and laboratory training free of charge. Please note, all new FCCS users must be trained through the facility regardless of prior experience before independently operating facility cytometers. Failure of any user to conform to FCCS regulations outlined below may result in suspension of facility privileges.

- **Reserved time:** Please be courteous to your fellow investigators. If you do not cancel your reservation for independent usage within 24 hours of your scheduled time, you will be charged for up to the first hour of time you booked. If you exceed your previously scheduled reservation it is up to the discretion of the following investigator to allow you extended time.
- **Sample preparation:** Samples known to contain particulate matter or that are prone to clumping must be filtered prior to analysis.
- **Cleaning:** Following every use of the LSRFortessa or LSRFortessa X-20, investigators must clean the instrument by running a solution of 10% bleach for 5 minutes followed by H_20 for 2 minutes on high speed. Note, it is the responsibility of the user to incorporate this time into their scheduled experiment. Users should log out of FACSDiva and the iLabs tracker screen to avoid charges during the mandatory cleaning process. When cleaning the FACSVerse, initiate the Daily Cleaning mode and follow instructions to run 10% bleach for up to 2 minutes then H_20 for up to 2 minutes.

- **Fluidics:** Following every use of the cytometer, sheath and waste containers must be checked. If the sheath container is less than half full, investigators must replenish sheath fluid by filling the tank up to the top line. If the waste container is greater than half full, empty the waste container and add 200ml bleach when finished. Please be courteous to your fellow investigators by maintaining cytometer fluidics.
- **Shut Down:** If you are the final user for a cytometer for the day, turn off cytometer and fluidics cart if available.

- **Reagents:** The FCCS shared resource will provide sheath fluid, bleach, and all reagents necessary for the maintenance and operation of flow cytometers. All addition supplies are the responsibility of the investigator.

Cell Sorting: FACSAria, Astrios EQ, MoFlo

- Sorters are only operated by Facility staff. For all sorts, we will conduct a post-sort analysis to verify the sort purity unless instructed otherwise by the investigator.
- Before a sort is started we will ask you to dictate gating strategies and verify appropriate sort logic.
- Samples must be filtered. Use 35-60um mesh from one of the following products: Falcon 2235 tubes with integrated filter cap (VWR or Fisher); CellTrics sterile filter caps from Partec; SpectraMesh nylon filters (VWR) is sold by sq. feet.

- Samples can be accepted in capped and labeled 1 ml (BioRad Microtiter), 5 ml (any 12x75mm), 15 ml (conical) tubes, or 96-well plates. Please prepare an appropriate collection tube or plate for each sample to be sorted.
- Make sure to include the proper controls to set up the instrument. You should include at least one negative (unstained and/or isotype) for each cell type and one compensation sample for every fluorochome or dye that you use in your staining procedure. Contact Facility staff if you require advice on planning/generating proper controls for your experiments.
- For a cell sorting experiment to be successful, you will need to know beforehand the scattering profile and fluorescent patterns of your target cells. Therefore, it might be necessary to first perform flow cytometric analysis of your cells before the actual sort can be performed.

Creating Reservations and Project Requests:

The FCCS uses the iLabs system allowing investigators to reserve time and request assisted-use time online at their convenience. Arranging for the FCCS facility to assist you through cell sorting or analytic cytometry can be initiated by providing experimental details and goals via the "Schedule Use" tab, then requesting a reservation to process your samples. Details and instructions to get started can be found at http://www.hollingscancercenter.org/research/shared-resources/cell-evaluation-therapy/flow.html under the "Schedule Use" tab. Please note that if a requested reservation cannot be confirmed due to conflicts, the FCCS staff will contact the investigator to reschedule the experiment at the soonest available time.

Training:

All users must be trained by HCC FCCS staff for unassisted use on any machine. The FCCS staff provides training for independent use of the LSRFortessa, LSRFortessa X-20, and FACSVerse cell analyzers free of charge. Training sessions are 1.5 hours and can be repeated if necessary until the user is comfortable operating the cytometer. We recommend that the investigator brings practice cells to analyze to become acclimated to the settings they will use in future experiments. Note that no data will be collected during training. Please contact the FCCS staff for a consultation of your needs and to schedule training.

Service Fees:

- Charges are based on the time a user is logged in to iLabs tracking software. This represents time a user is occupying the equipment and thus, prohibiting others from utilizing the resource.
- Billable time accrues in 1 minute increments.
- If a user repeatedly reserves time in iLabs in excess (greater than one hour) of that necessary to complete their experiment, the user may be subject to billing for total time scheduled.
- There is a 15% hourly usage subsidy for HCC Research and Associate Research Members for all services provided.
- After hours and weekend access will be granted by Facility Manager and PI (see Dr. Soloff for access).
- Revised service fees are listed at: http://www.hollingscancercenter.org/research/shared-resources/cell-evaluation-therapy/flow.html under the "Pricing" tab.

Cancellation Policy:

- Unassisted cytometry: There is no penalty when a user cancels a reservation for independent operation of cytometers given they cancel their reservation in iLabs up to one hour beforehand allowing other investigators to utilize this time. If an investigator fails to cancel their reservation less than 1hr prior to its start time, the user will be charged for the full time reserved. The FCCS Staff may waive charges if user has a reasonable excuse (i.e., you are sick, kid is sick, etc.).
- Assisted Cytometry: There is no penalty when a user cancels a reservation for assisted cytometry, consisting of cell sorting and analytic cytometry requiring a FCCS staff member, if at least 24 hours' notice is provided. If an investigator cancels a reservation for assisted cell analysis less than 24 hours prior to the appointment, the investigator will be charged for the time reserved. Please note, if we can use some or all

of your previously reserved time for another investigator you will not be charged for the portion of your scheduled time that they use.

- Sorting: If an investigator cancels a reservation for cell sorting less than 24 hours prior to the appointment the investigator will be charged for only one hour regardless of the duration of their reservation. The sort setup fee will also be waived. Again, if we can use some or all of your previously reserved time for another investigator you will not be charged for the portion of your scheduled time that they use.
- No Shows: Failure to keep an appointment without contacting the FCCS Staff will result in in a charge for the full amount of time scheduled. If a user repeatedly makes appointments and does not use the time, the FCCS management will contact the PI of the laboratory to discuss billing issues.

Biosafety Regulations:

The use of analytic flow cytometers and, especially, aerosol-generating sorters with samples that contain viable infectious agents presents a serious risk to the FCCS Facility users and staff.

- **Analyzers:** The use of the LSRFortessa and FACSVerse with un-fixed biohazardous samples is strictly prohibited. All such samples must be sterilized with the use of an approved agent - usually 1-2% paraformaldehyde. Per MUSC biosafety regulations, materials that are not to be used in a viable state on these instruments include human cells of any type (includes human cell lines as well as primary cells) whether it is known or unknown if the cells contain infectious agents (HIV, Hepatitis, etc.) and cells with recombinant DNA vectors (e.g. lentivirus, adenovirus, plasmids). If you are in doubt of if your samples meet these requirements please discuss this with the Facility manager (Dr. Adam Soloff) soloff@musc.edu and or MUSC biosafety officer (Dr. Chris Voelkel-Johnson, johnsocv@musc.edu) if you would like to analyze un-fixed biohazardous samples, a member of the FCCS staff will be happy to process these samples for you using the biocontained FACSAria at standard assisted analysis charge.

- **Sorters:** All BSL-2 designated samples must be sorted on the FACSAria. The sorting of viable biohazardous materials (including any human cell lines and primary cells) may NOT be performed on the MoFlo, used strictly for BSL-1 sorting or analysis.

Data Management:

All user data must be deleted from cytometers within 30 days of collection. Storage of user data is solely the responsibility of the investigator. User generated data will be backed-up and then deleted from machines on the last day of every month. Facility data backup is provided as a courtesy, and will be overwritten each month due to space requirements.

Data Analysis:

An independent Dell workstation is available for use with FlowJo, Modfit and FACSDiva in the facility HO324 free of charge. In addition, users may sign out a FlowJo security key for periods of 24 hours at a time (no fee associated). The key is located in HO308 and can be reserved under the equipment section of iLabs.