VUMC Flow Cytometry Shared Resource
Cell Sorting

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Cell Sorting Guidelines

Lab policies

The VUMC Flow Cytometry Shared Resource (Flow Core) has three FACS (fluorescence-activated cell sorting) instruments. One is located in a BSL2+ facility, but all three are housed in biosafety enclosures (BSL2). The BSL2+ sorter is prioritized for sorts requiring a higher level of containment (such as Sars2/Covid 19+, HIV+, etc) but can be reserved when the other two sorters are already booked. You will need to email the flow core (vmcflowThis higher biosafety level refers to higher containment precautions for operator safety. The 3 sorters are not identical. This means that the same panel might not work on all 3 instruments, so please refer to the provided configuration pdfs or ask core staff about your panel before booking.

Sorters are typically run by core personnel; no training is required to have samples sorted by the Flow Core. This means all sorts must be booked with assistance. We have provided pdfs that walk through how to view available assistance alongside instrument calendars and how to book a sort per request. Note that it is lab policy to delete any appointments that are not made correctly.

Sorting services are available Monday-Friday from 10:00am-5:30pm, by appointment only. We are closed for VUMC holidays. If the sorters are unavailable for other reasons such as service/maintenance, the instrument calendar may be blocked. We do have more instruments than personnel, so there is always a chance that a sorter will be available when no core personnel are available to run it. Please keep in mind that our cancellation policy for no charge is 24-hours. Once we are within 24 hours of an appointment, sorts can only be cancelled by core personnel. Please send a cancellation request to our lab email so it goes to all core personnel. **The lab email is vmcflow@vumc.org. The lab phone number is (615)343-8323.**

The email address that is associated to your iLab account is the one we will default to using if we have any questions/issues about a reservation, including sending a heads up that your appointment has been deleted. Please be sure the email associated to your iLab account is your preferred contact email. Since the VU/VUMC split, not all email addresses have been updated to the new vumc.org address, please update your profile if you have not already done so.

BSL2+ considerations

Because of the aerosolizing nature of the cell sorters, some BSL2 reagents require BSL2+ sorting procedures; any and all questionable reagents / treatments / materials / infections should be approved by the Office of Clinical Research and Safety (OCRS) prior to scheduling the sort, and must be documented in the event notes.

This sorter is located outside of our main lab space, so users cannot accompany core personnel. Gating can be confirmed either via email or zoom and a shared screen.

Current price (subject to change)

Sorting: $153/hour (setup fee of $76.50 added to each sort)

Booking time

All sorts are booked in iLab, so all users require an active iLab account with access to the appropriate center numbers. All center numbers are added by CORES/OOR, and then designated to users by the PI or lab manager. If you need assistance getting new center numbers added to iLab, email vumccores@vumc.org.

Users make their own reservations, including choosing billing information. Please be sure you are picking the correct center number. If a lab has scholarships/vouchers, these are assigned the same as other cost centers in iLab. When using a scholarship, please be aware that the current total iLab shows does not consider the current appointments that haven't been billed. This means that as long as there is enough to cover a single appointment, it can be used for multiple separate appointments in that billing cycle; effectively over billing the scholarship. If core staff notices this, appointments may be deleted, and the user will need to rebook with a new center number. If center number changes are needed, a "journal entry" may be required by your administrative team. Please double check the correct billing number prior to making your reservation.

When booking sorts, a nozzle size must be left in the comments so core staff can setup the sorter properly. Be sure these comments are viewable to all users. The nozzle size used depends on a.) what type of cells you have and b.) what your sorting goals are. Generalized recommendations for nozzle size:

70um: T cells, samples being taken to Vantage for 10X
100um: B cells, isolated nuclei
130um: iPSCs

Please keep in mind these are *only* recommendations to maintain cell viability without shearing.

If there is already a sort booked before or after your time of interest, be sure to check the nozzle size. If it's different than then nozzle you wish to use, you must leave a 30-minute block of time (*that is available with assistance*) so we can book time for a switch over. If you do not leave the necessary time for a switch over, it is lab policy that the appointment be deleted.

This comments section is also where you can leave any additional instructions (for example, if a sample/collection tube should not be vortexed, or if collection into plates will be required).

Nozzle size/collection

Each nozzle size also has its own range of recommended sample concentration. The recommendation for each nozzle size depends on sample prep. If a sample has been pre-sorted,

then the sort sample concentration can be higher. On the other hand, if the cells in the sort fall out of suspension easily or like to stick, then a lower concentration may be necessary despite the nozzle size.

70um: min: 20x10^6 cells/ mL, max: 40x10^6 cells/mL
100um: min: 5x10^6 cells/mL, max: 14x10^6 cells/ mL
130um: 2.5x10^6 (or up to 5x10^6 for pre-sorted samples) / mL

Maximum (max) recommendations are based on ideal samples (no clumping, cells stay in suspension). If cells are sticky because of cell death (DNA is sticky), some DNAse at room temperature may help. No matter the recommended concentration, please always be sure to bring extra buffer in case a sample needs to be diluted. *Personnel can refuse to sort if the sample clogs the instrument.*

<u>It takes approximately 1 hour to get through 1mL of volume</u>.

For collection we can do bulking sorting into individual PCR or micro-tubes, 1.7mL Eppendorf tubes, 5ml round-bottom tube, or 15-mL conical tubes. We can do 4-way sorting (sort 4 different populations from a single sample) into either 1.7mL eppendorfs or 5mL round-bottoms. When using 15mL conicals or PCR tubes for collection, only 2 populations can be sorted from a single sample.

All cells are sorted encased in a droplet of sheath (we use Beckman Coulter Isoflow Sheath Fluid). The volume added to the collection tube is different for different nozzles.

70um: 1x10^6 cells collected = 1.060mL volume added with the highest concentration being 1000 cells/uL
100um: 1x10^6 cells collected = 3.070mL volume added with the highest concentration being 325.7 cells/uL
130um: 1x10^6 cells collected = 5mL with the highest concentration being 200 cells/uL

Because of the amount of volume sorted, samples being sorted to run directly on the 10X (genomics stuff here) should be done on the 70um nozzle, assuming shearing isn't an issue. For larger cells, it may be required that cells be sorted on a larger nozzle and then spinning; however, there will likely be cell loss in this process.

We can also sort into plates. This includes 4-well chamber slides, single 6cm dish, all tissue-culture plates (12, 24, 48, 96, 384-wells). This also includes single-cell sorting into 96 and 384-well tissue culture and PCR plates. Media should already be loaded into plates when they arrive in the core. We have an incubator kept at $37°C$ in which plates can be kept in pre and post sort. (separate incubators for mammalian vs bacteria). If the cells of interest are frequent enough the instrument can sort with no pauses, it takes 2-3 minutes to single-cell sort into a 96-well plate and 7-8 minutes for a 384-well plate. All plate sorting is a single population at a time. When planning your experiment, please account for an additional 3 min / plate for washing between samples.

With the exception of plates and sorting into lysis buffer, collection tubes are kept chilled at $4°C$ during sorting unless told otherwise by the user. Post sort collection is placed back on ice if provided by user. We do recommend everything (samples, collection, extra buffer) be brought down on ice.

Other sort sample considerations

While we can sort into whatever media in which cells are happiest, there are a couple considerations for the sort sample buffer. Ideally, media containing phenol-red should be avoided as it can add to the background. Protein can be added, but no more than 5% unless absolutely necessary. EDTA can also be used if cells are sticky, but optimization for proper concentration is recommended.

Samples should be filtered. If you can see something floating in the sample, then it will clog our instrument. We recommend FACS tubes that have the filter built into the cap (Falcon 352235). Any samples that continue to clog the instrument will be stopped without completing the sort.

We recommend using a viability dye; however, it's ultimately up to the user. If your sample has a lot of dead cells or when integrity of target cells is a concern, then a viability dye should be used. Ask core personnel if you would like any recommendations based on needs or panel design.

Sorting efficiency (potential target cells vs actual sorted target cells) will never be 100%. A cleaner sample at the optimal concentration for a nozzle is the best path forward to higher recovery. The only way to know the efficiency of a sample is once it's on the sorter.

Users can stay for the sort setup and gating but not for the entire sort. If a user prefers to drop off samples, a sheet of paper or email with sample details and gating strategy is required. We will also need an email address or phone number in case we need to contact the user. We can also verify gates via zoom or text if desired. If a user chooses to drop off their samples, they should be readily available via the contact information provided throughout the duration of the sort.

If sorting bacteria or a sample that is cultured with bacteria, please be sure to alert core personnel when booking as extra time may be required for cleaning.

Users should always provide a negative (WT/parental) control. Single-color controls are also highly recommended, even necessary most of the time. Please be sure core personnel is aware of everything in a sample. Just because the user "isn't interested in sorting a signal", doesn't mean it can't cause issues with other parameters. It's always going to be easier to setup for a signal and then sort around it, then it will be to potentially waste time trying to figure out where a mystery signal is coming from.

If a user wishes to optimize a protocol or panel prior to sorting (as analysis time is cheaper than sorter time), we recommend the 4-laser Fortessa. The 4 laser Fortessa has the closest configuration to the sorters (namely the 561nm laser for PE, PE tandem dyes, and certain fluorescent proteins such as mCherry).

If you have issues with your collection after the sort, please alert core personnel immediately. This includes issues with post-sort viability, presence of cell debris, and possible

contamination. We do daily cleaning each morning and deeper system cleans every other week, but unfortunately nothing is 100%. We also rely on user feedback.

Data analysis

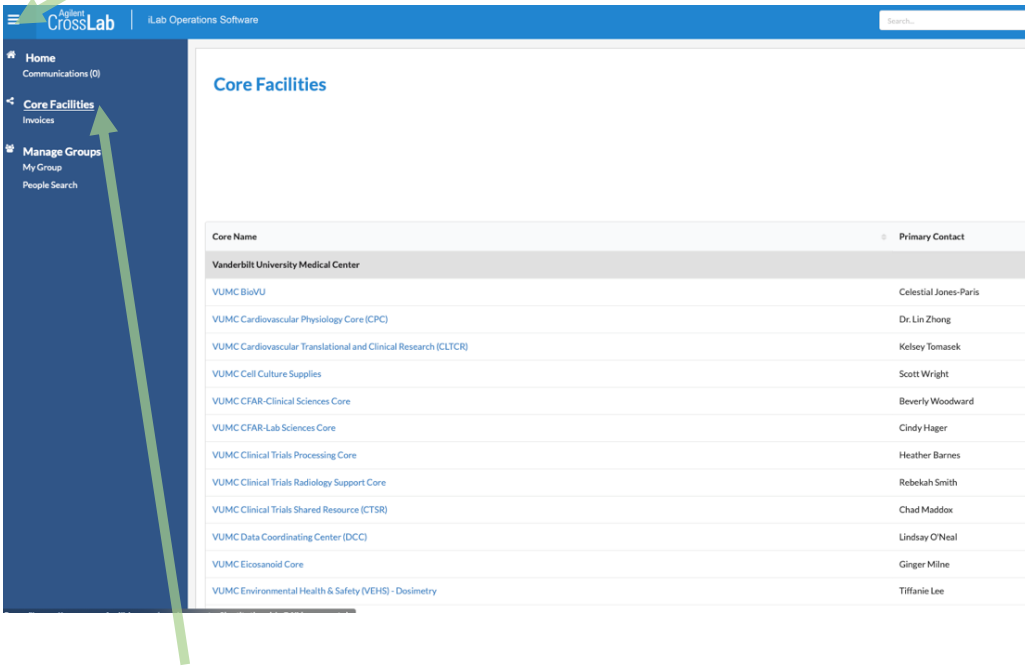
We offer access to Flow Jo on a monthly basis. The current cost is $25.00, and the signup is through iLab. Vouchers and scholarships cannot be used. Email request must be sent to stop access, otherwise billing will continue. Access is for all Flow Jo plugins on their exchange.

Scheduling Sorts

Viewing core-availability calendars

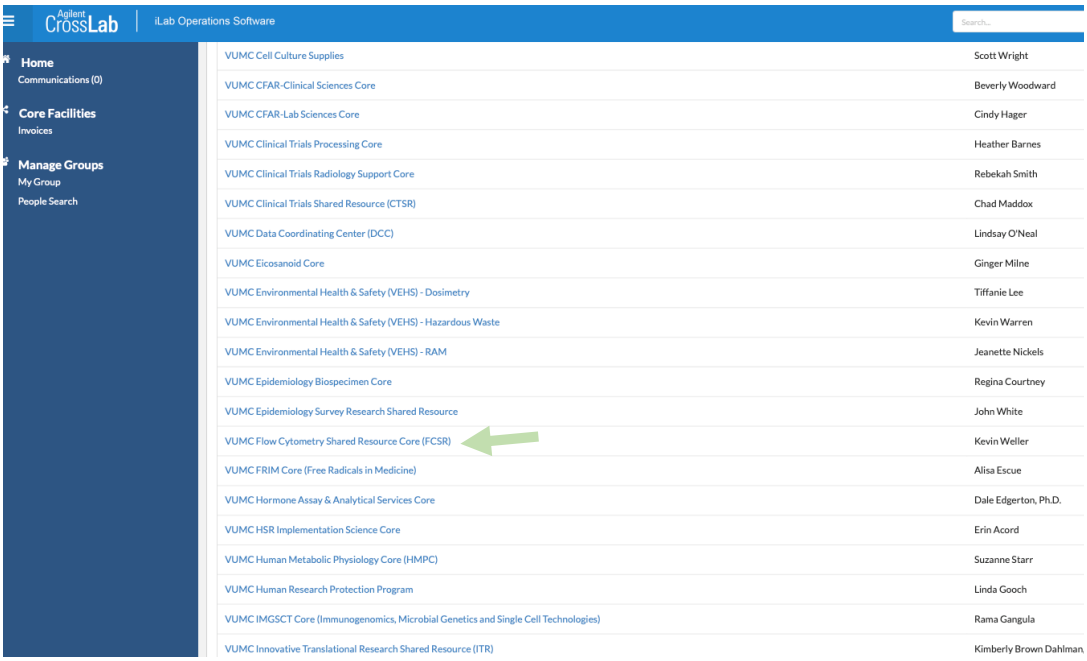
Viewing Core Assistants Calendars

1. Log into iLab
2. Click the ☰ icon in the top left

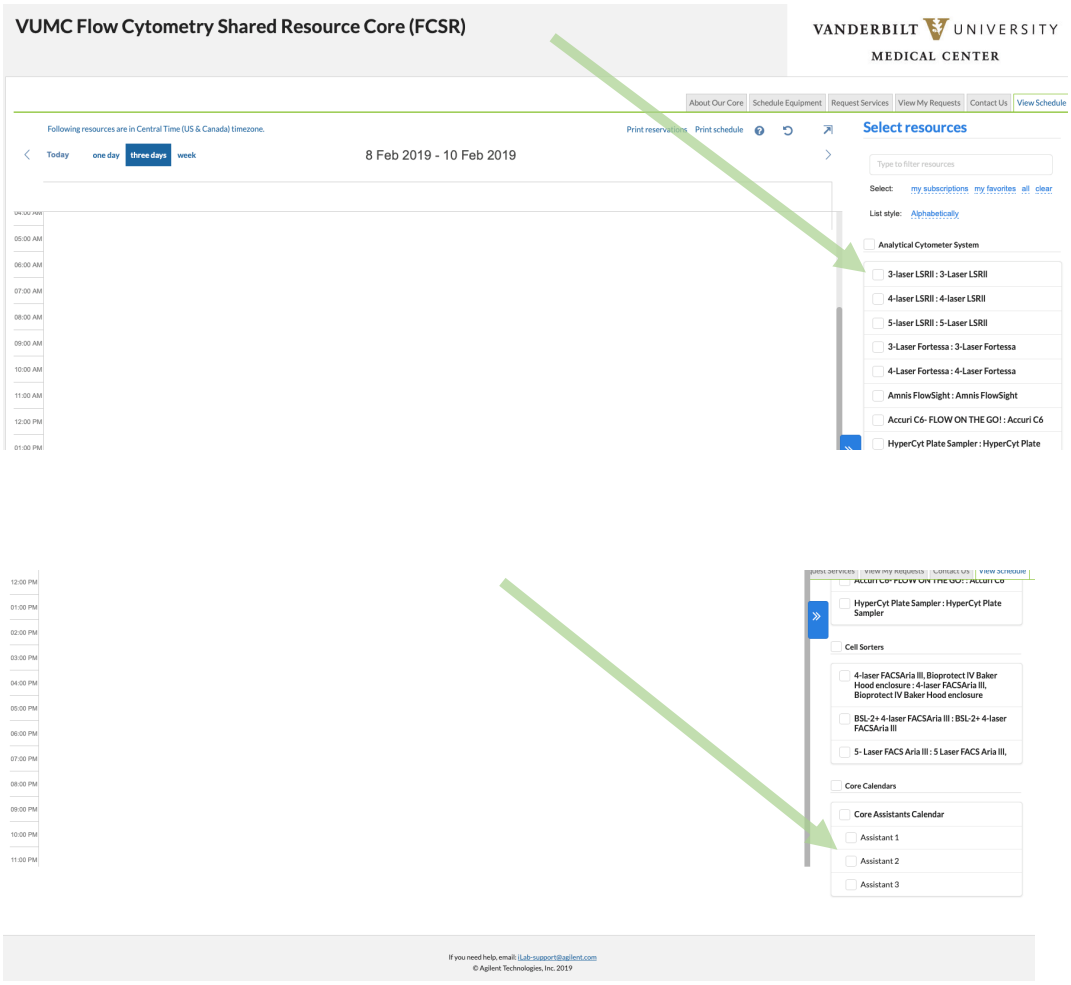


3. When the menu appears, click “Core Facilites”

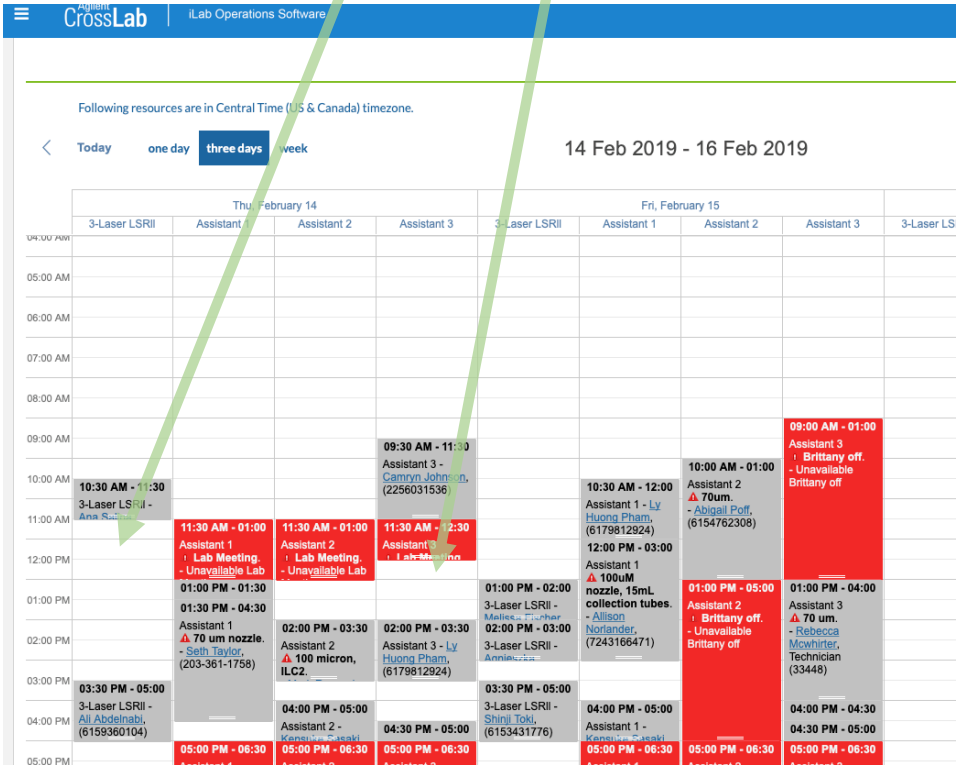
4. From the Core Facilities list, scroll to find “VUMC Flow Cytometry Shared Resource (FCSR)” and click the link.



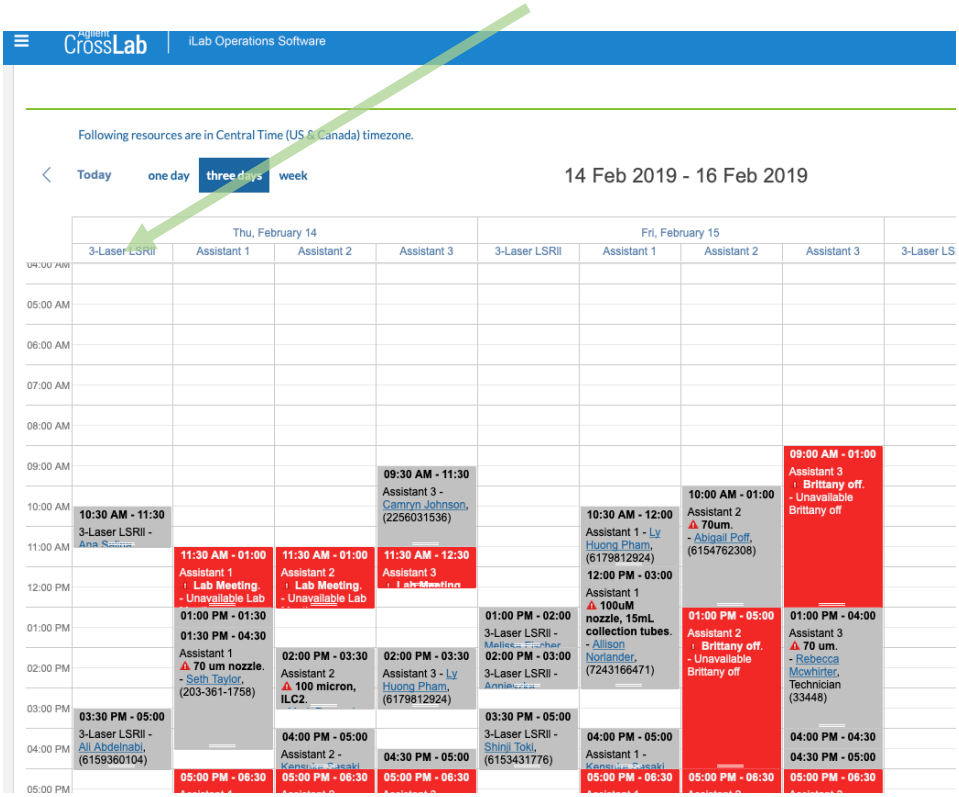
5. The calendar view might be empty. On the right of the screen is a list of all available calendars. Click the box next to the instrument calendar(s) you want to see. Scroll down to find the Core Assistants Calendars, and check Assistant 1, Assistant 2 and Assistant 3. You can view multiple calendars at a time.



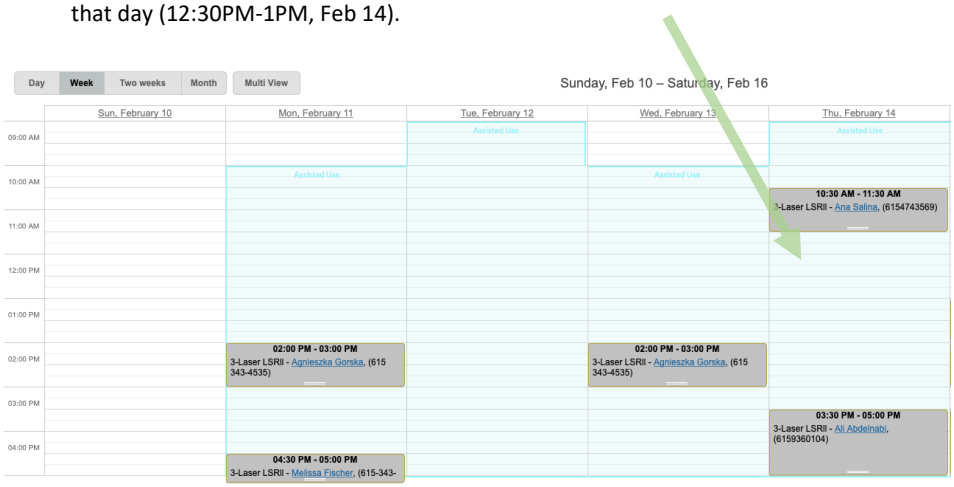
6. Find a time when both the instrument and an assistant are available. For example, on February 14, both the instrument and Assistant 3 are available from 12:30PM-1PM.



7. To book the 12:30PM-1PM spot, click the instrument calendar's name for that day



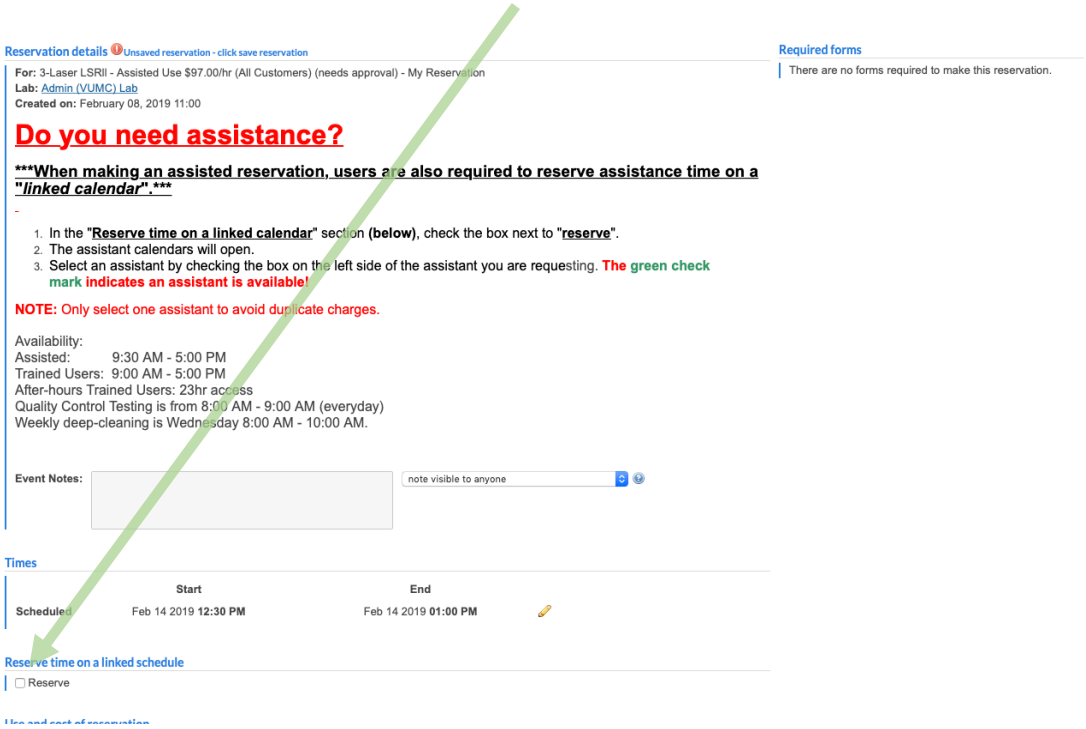
8. When the instrument calendar only comes up, left click and drag the time you want for that day (12:30PM-1PM, Feb 14).



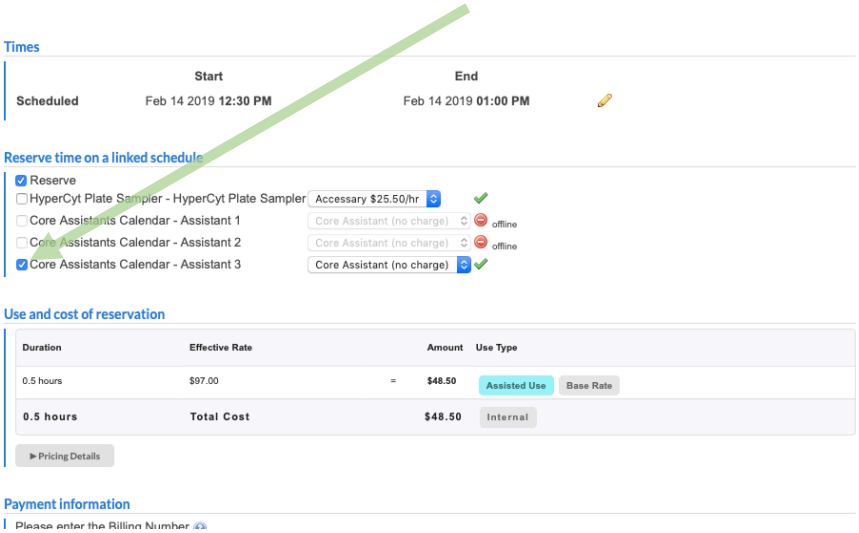
9. After you click and drag, you may be prompted to select which PI this appointment needs to be associate to. Note that scholarships are VUMC, so if you are part of a VU lab, then you'll need to select the VUMC option for your PI here.

10. The "Reservation details" page is where you will include any Event Notes (information the user feels is pertinent to Core Personnel), BOOK THE ACTUAL ASSISTANCE, and confirm payment.

11. To actually book with assistance, go to “Reserve time on a linked schedule” and click the box next to Reserve.

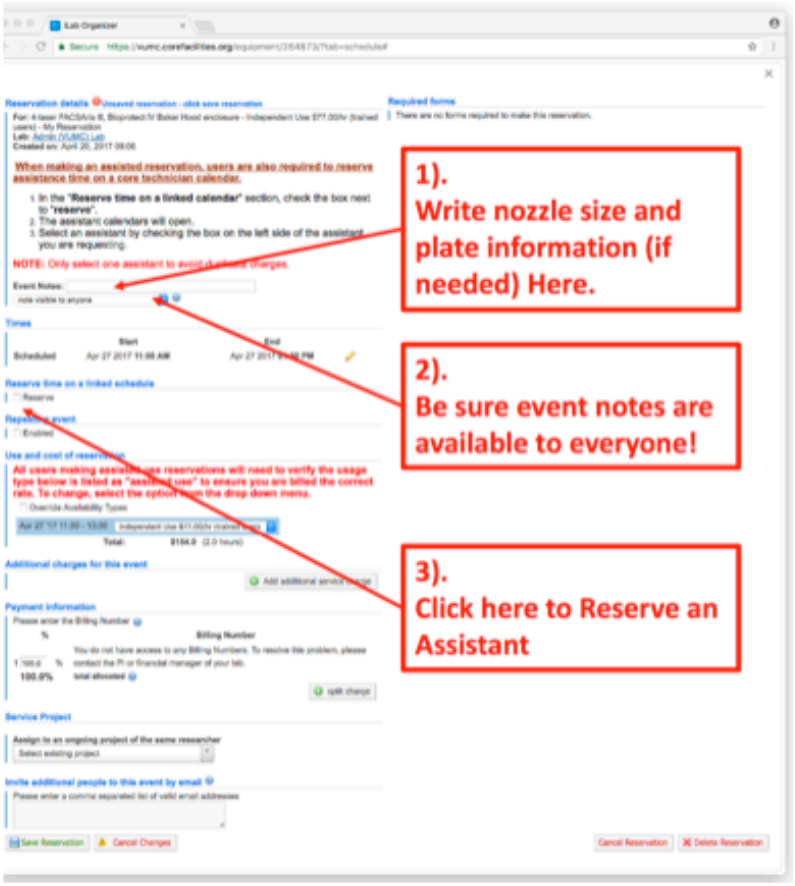


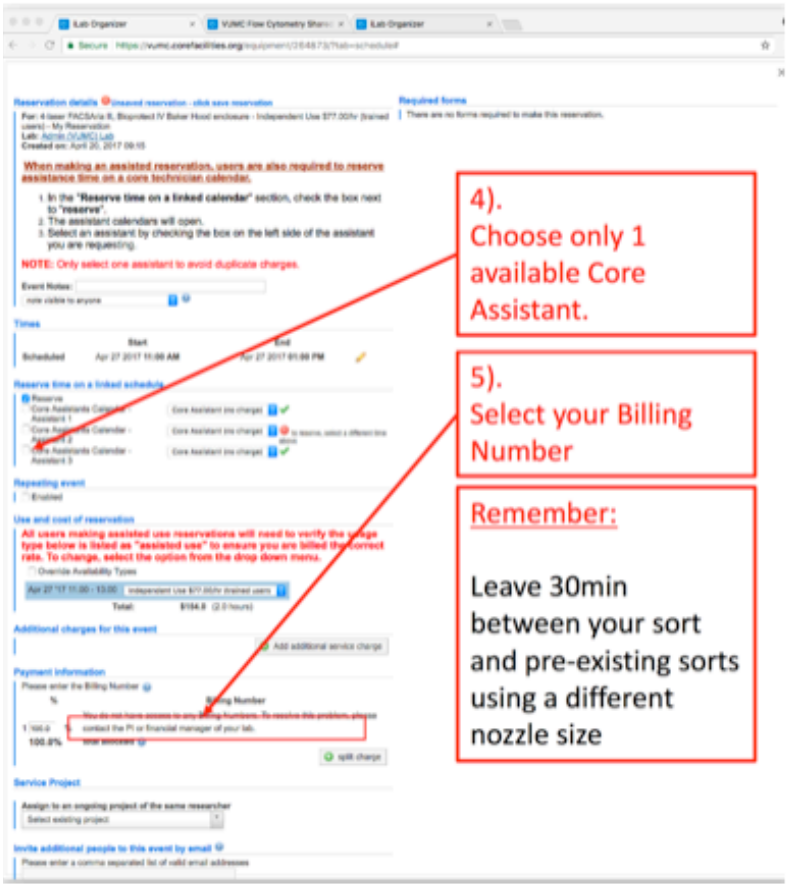
12. A list of individual assistants (1, 2 and 3) will appear. Only assistants with a green check next to it, are available for booking during the time chosen. If a red circle (with the words “offline”) is next to it instead, then that assistant is not available during that time. Note that assistants are not assigned to any individuals in the Core. **Hypercyt is NOT an assistant so don’t check it.*



13. After the available assistant is picked, scroll down to confirm payment and submit reservation.

Details of booking a sort



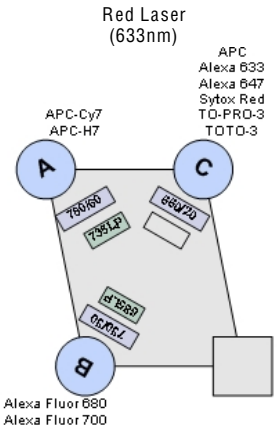

4).
Choose only 1 available Core Assistant.
5).
Select your Billing Number
Remember:
Leave 30min between your sort and pre-existing sorts using a different nozzle size

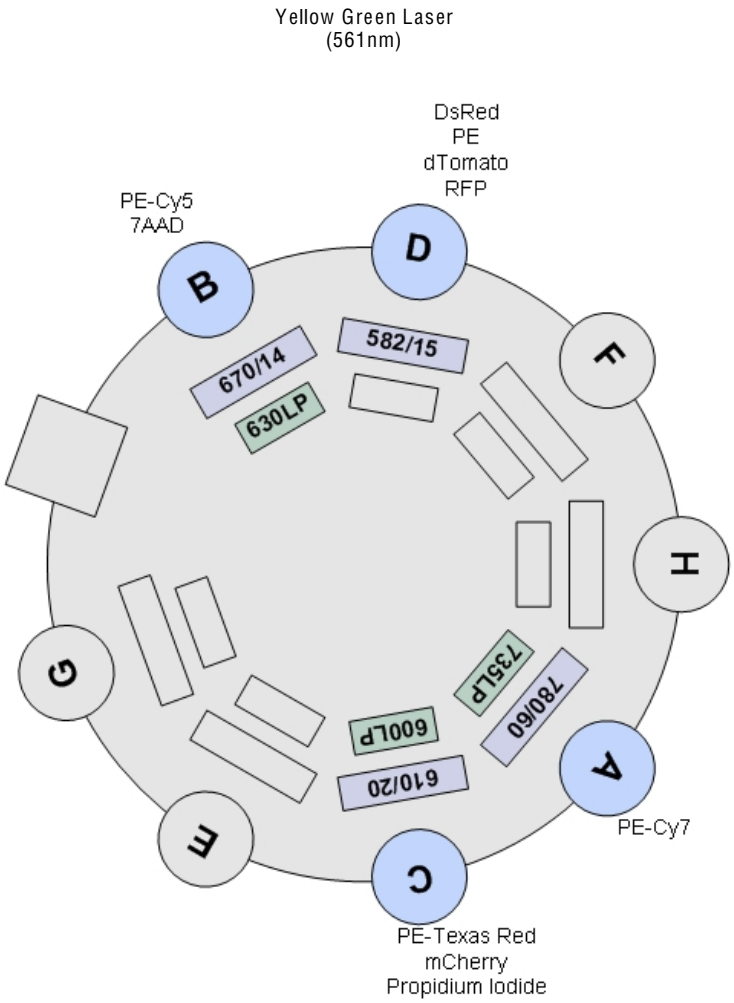
Instrument Configurations

Bakerhood Aria

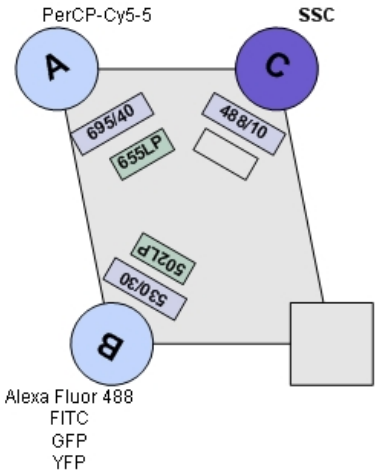
New 100 488,561,633,405

Cytometer:	FACSAriaII	User:	BDService
Cytometer Name:	FACSAriaII	Institution:	
Serial Number:	1	Software:	BD FACSDiva 6.1.3
Input Device:	Manual	Date:	7/31/2013 10:36:47 AM
Sheath Pressure:	17.00		
Nozzle Size:	100		
Window Extension:	2.00		

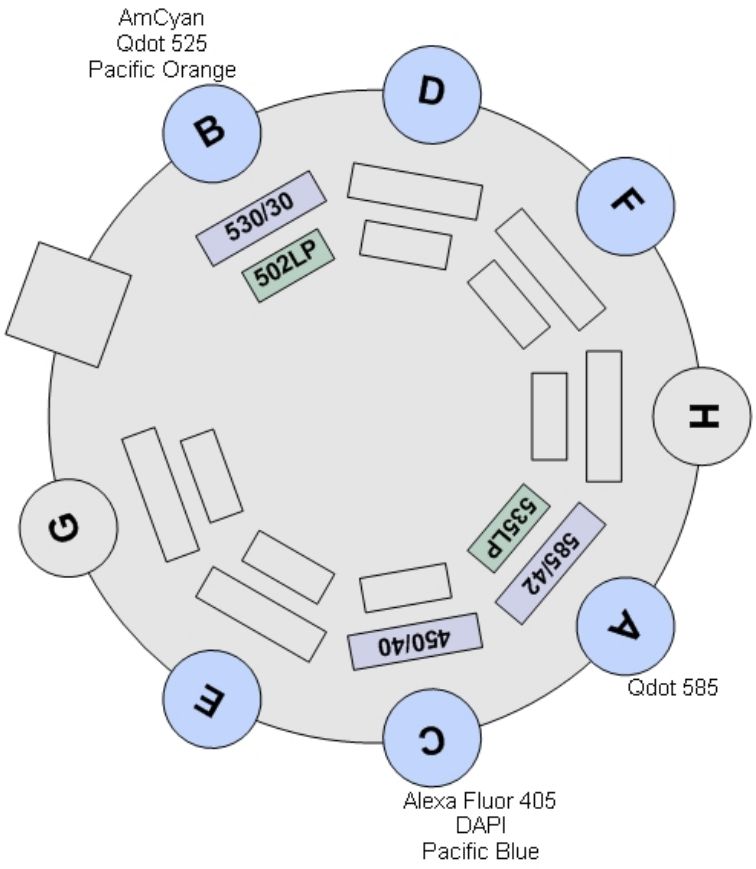



Yellow Green Laser
(561nm)
DsRed
PE
dTomato
RFP
PE-Cy5
7AAD
B
D
F
H
A
C
E
G
670/14
582/15
630LP
735LP
780/60
600LP
610/20
PE-Cy7
PE-Texas Red
mCherry
Propidium Iodide

Blue Laser
(488nm)
FSC



Violet Laser
(407nm)



Biobubble Aria

New 100 488,561,633,405

Cytometer:	FACSAriaII	User:	Administrator
Cytometer Name:	FACSAriaII	Institution:	
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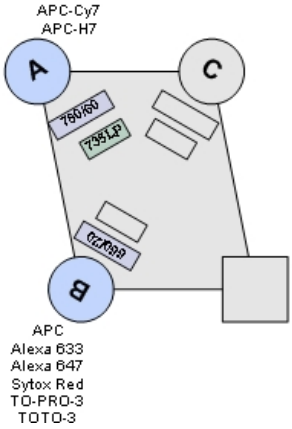
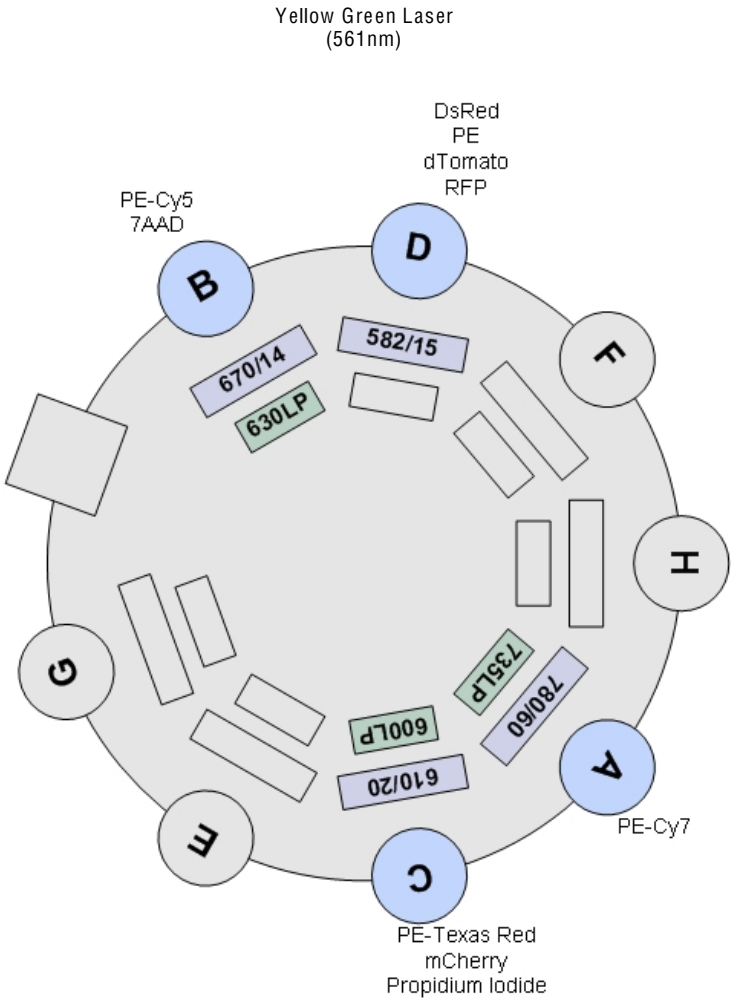
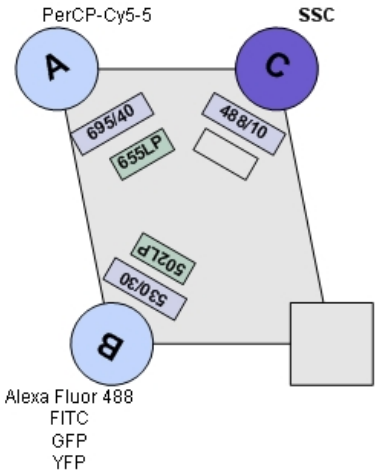
Input Device:	Manual	Date:	3/14/2013 1:56:14 PM
Sheath Pressure:	17.00		
Nozzle Size:	100		
Window Extension:	2.00		

Red Laser
(633nm)

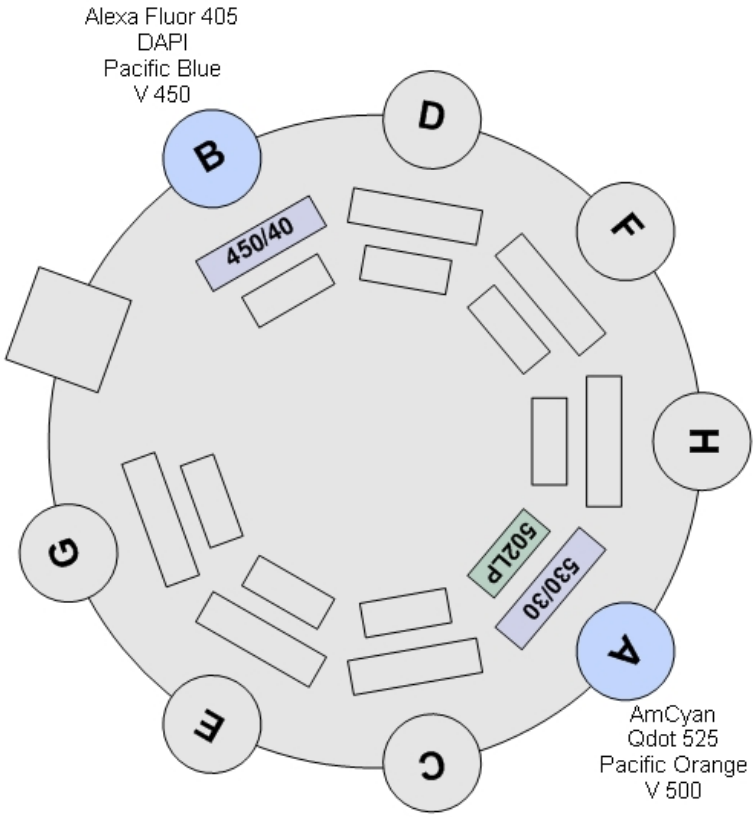



Yellow Green Laser
(561nm)
DsRed
PE
dTomato
RFP
PE-Cy5
7AAD
D
B
F
H
G
A
E
C
582/15
670/14
630LP
735LP
780/60
600LP
610/20
PE-Cy7
PE-Texas Red
mCherry
Propidium Iodide

Blue Laser
(488nm)
FSC



Violet Laser
(407nm)

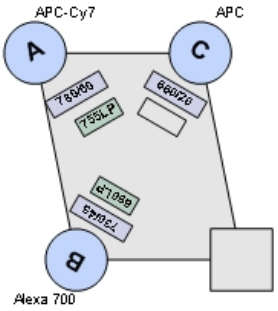


BSL2+ Aria (located outside of main lab space)

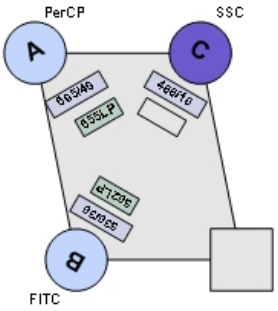
BSL 2 Aria III 70um

Cytometer:	FACSAriaII	User:	Administrator
Cytometer Name:	FACSAriaII	Institution:	N/A
Serial Number:	1	Software:	BD FACSDiva 8.0.1
Input Device:	Manual	Date:	8/30/2017 12:52:19 PM
Sheath Pressure:	70.00		
Nozzle Size:	70		
Window Extension:	2.00		

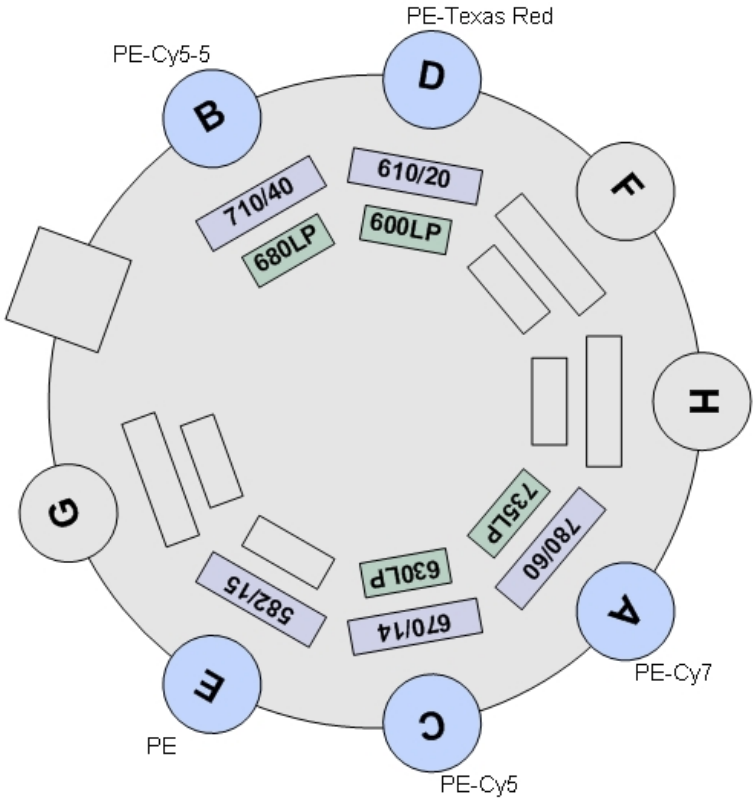
Red Laser (633nm)



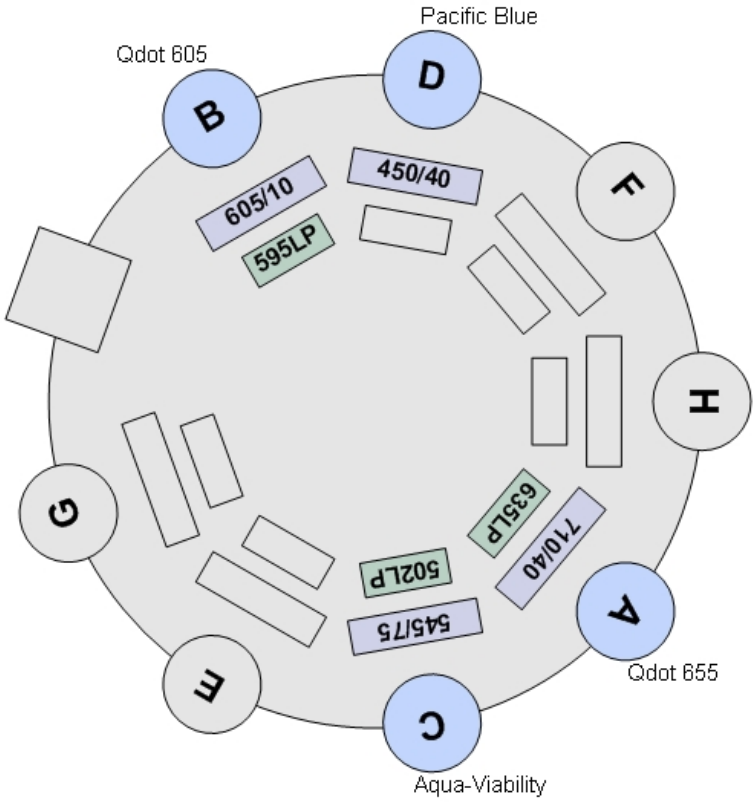
Blue Laser (488nm) FSC



Yellow Green Laser



Violet Laser
(407nm)



Acknowledging the Flow Cytometry Shared Resource:

Please remember to acknowledge our core with the following statement:

Flow Cytometry experiments were performed in the Vanderbilt Flow Cytometry Shared Resource. The Vanderbilt Flow Cytometry Shared Resource is supported by the Vanderbilt Ingram Cancer Center (P30 CA068485) and the Vanderbilt Digestive Disease Research Center (DK058404)