

# Flow Cytometry Core Facility – Canto II V4. Send completed form to [fccf@salk.edu](mailto:fccf@salk.edu) for access to Canto Conditions and Acknowledgement form for use of instrument

- FCCF has analyzers in 2 places: “Parker Room” (LSRII-2/MACSQuant VYB/Canto) or “STEM” (LSRII-1) that can be booked concurrently (are more than 6 feet apart).
- Buffer time between users: 30min between users to allow for clean up and unexpected issues
- Parker Room has Max Occupancy = 3 with priority given to instrument operators.
- Only one person at an instrument unless pre-approved by core. Any time you bring a co-worker**, please indicate their attendance/name on the Log sheet (for contact tracing).
- Always check the calendar before going to the lab: be 100% aware of who is working especially if you are jumping in on short notice ahead of a user – **obtain their permission first** if there is any chance you are running late and could be present when they come to use the instrument.
- Changing to another instrument: Make a new calendar booking on the instrument you are using for **contact tracing** (inform the core if you need cancellation help on the original booking).
- Analyzers are running autoclaved MQ water (instead of PBS) as sheath at this time.
- Instrument operation is subject to new usage instructions uploaded in Infinity.
- You may be asked to execute an extended cleaning protocol if the post use QC shows it is needed.
- No drop ins, bookings/appointments only:** You must reserve a session on Infinity BEFORE using the machine. *Your first autonomous session:* Plan to come M-Th during the day, and book extra time to learn how run the QC; ask staff when you should book your session in case you need help.
- COMMUNICATE with each other by email, especially if you are delayed on an instrument.
- Enable wifi calling to use cell phone in the basement areas (FCCF or STEM).
- Access requires protocol review by FCCF (for inclusion of DNase I: which steps/how much): **Submit your protocol for review along with this form.**

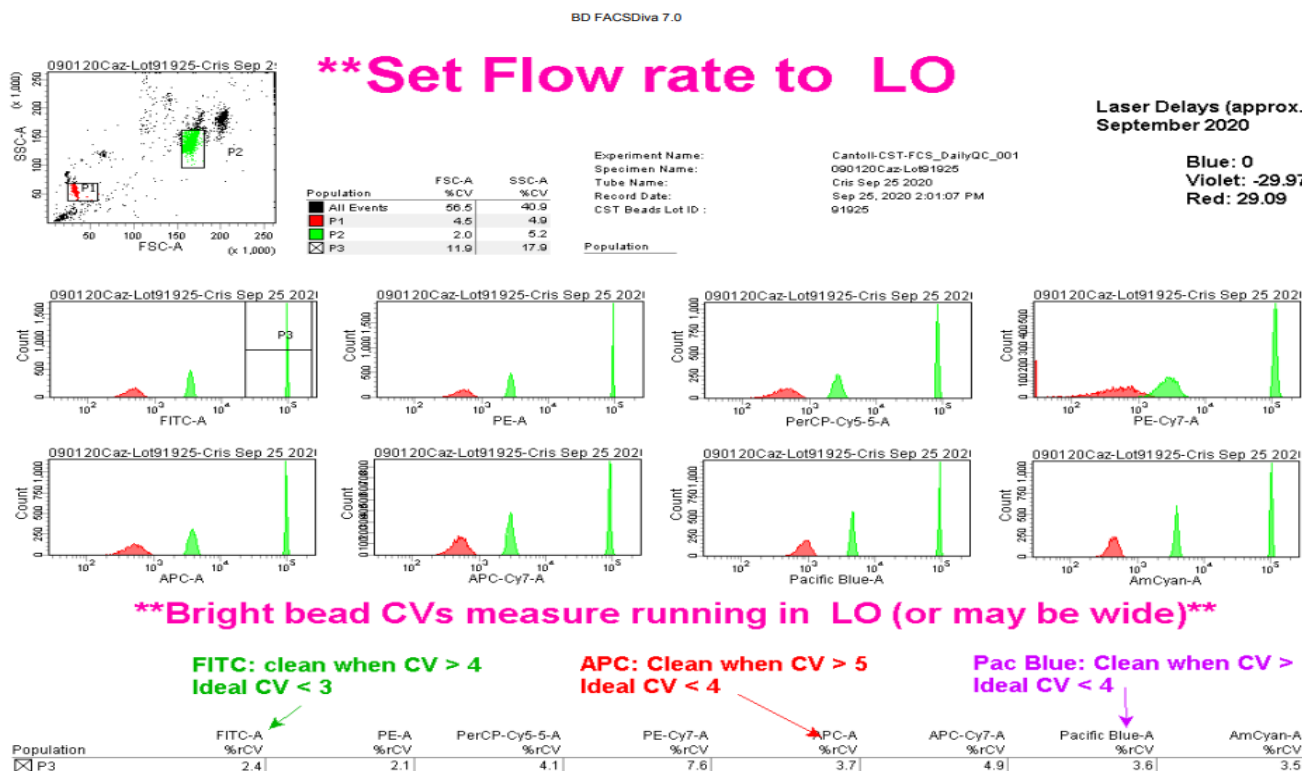
|                                                                                                                                                                                                                                                                                                |      |             |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|-------------|
| Name:                                                                                                                                                                                                                                                                                          | Lab: | Cell phone: |
| My protocol has been approved by FCCF (steps indicating use of DNase I: e.g./how much)                                                                                                                                                                                                         |      |             |
| I understand that the instrument is running MQ water as sheath fluid instead of PBS                                                                                                                                                                                                            |      |             |
| I have already located all the instrument Help Files in Salkland or Infinity and read them                                                                                                                                                                                                     |      |             |
| I know where to locate more: <b>Bleach</b> (by door in FCCF), <b>CS&amp;T beads</b> in STEM, Parker Room (or in FCCF), and <b>MQ water sheath carboys</b> (if not nearby, autoclaved carboys are delivered by Salk Glass-wash staff to the corridor area immediately outside FCCF’s main door) |      |             |
| I will put <b>rinsed</b> empty bleach bottles into <b>recycling</b> , and <b>change the red bio trash bag</b> if full                                                                                                                                                                          |      |             |
| I will log into Diva under <b>Calibration</b> (pw = <b>orangechst</b> ) to look at CST bead profiles and make PDFs                                                                                                                                                                             |      |             |
| I will check instrument cleanliness first, before running beads, looking for <b>low event rate (fluctuating as low as 0-20evt/s when very clean, less than 50-100 evts/s) with freshly filtered water.</b>                                                                                     |      |             |
| I will <b>change the syringe filter</b> if I see high event rate for water, and will <b>re-check before using to make up beads</b>                                                                                                                                                             |      |             |
| If I am <b>the first user of the day</b> , I will execute the automated calibration and export a PDF to send to <a href="mailto:fccf@salk.edu">fccf@salk.edu</a>                                                                                                                               |      |             |
| I will produce a “before” bead profile PDF if (1) I’m the first user of the day or (2) a user immediately running before me on the same day has not left an acceptable “after” file in the Calibration login (that will suffice as “before” data for me).                                      |      |             |
| I have accessed Help Files in Salkland or Infinity and read the Usage Guide; I will comply with providing:                                                                                                                                                                                     |      |             |
| (i) “5min cleaning” as described every 30min use (if samples prove difficult to clean up after)                                                                                                                                                                                                |      |             |
| (ii) Recording cleaning files from the 5min cleaning for (a) bleach, (b) rinse and (c) water                                                                                                                                                                                                   |      |             |
| (iii) Checking after use for cleanliness according to instructions, or staff directions                                                                                                                                                                                                        |      |             |
| (iv) Submitting screengrab proof (eg Diva Browser with time stamps)                                                                                                                                                                                                                            |      |             |
| I will read and will perform every step and sign off the clipboard after use:                                                                                                                                                                                                                  |      |             |
| (i) Tank (Sheath & Waste) maintenance                                                                                                                                                                                                                                                          |      |             |
| (ii) Data backup→ I will <b>delete my previous session’s data</b>                                                                                                                                                                                                                              |      |             |
| (iv) Instrument OFF → I will take a photo showing the <b>GREEN POWER BUTTON (OFF)</b>                                                                                                                                                                                                          |      |             |
| (v) I understand that <b>ONLY A PHOTO OF THE POWER BUTTON IS ACCEPTABLE</b> for (iv)                                                                                                                                                                                                           |      |             |
| (vi) I will take a photo of the completed/signed clipboard                                                                                                                                                                                                                                     |      |             |

|                                                                                                                                                                                                                   |  |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|
| I will restart the PC before I leave (restart, NOT shut down)                                                                                                                                                     |  |
| I will submit all files and photos to <a href="mailto:fccf@salk.edu">fccf@salk.edu</a>                                                                                                                            |  |
| (i) Beads before/after, CST report in PDF format as appropriate                                                                                                                                                   |  |
| (ii) Photo/screengrab of Diva browser showing cleaning files with time stamps                                                                                                                                     |  |
| (iii) Photo of signed clipboard with instrument power button to <b>show how I left it</b>                                                                                                                         |  |
| I will perform corrective maintenance (long cleaning) under staff directions if needed                                                                                                                            |  |
| I have looked at the examples <b>on the pages following this form</b> , and understand exactly which files/photos to send <b>immediately after my session</b> to <a href="mailto:fccf@salk.edu">fccf@salk.edu</a> |  |
| I understand that "Duplicate without Data" is NOT TO BE USED as it can break the DIVA database; I will ask FCCF staff how to correctly re-use instrument settings                                                 |  |
| Troubleshooting instrument issues: I understand how to use the Cytometer Status desktop icon to open a window that will allow me to copy computer code if needed (such as error codes)                            |  |
| New instructions may require another form to acknowledge my reading and understanding                                                                                                                             |  |

## Good Examples of What You Are REQUIRED to Send to [FCCF@salk.edu](mailto:FCCF@salk.edu) DIRECTLY AFTER Using an Instrument.

Emailing **items 3, 4, and 5** (unless specifically directed otherwise) is a condition of your access to the core's autonomous use instrumentation. **Item 1** is required if the user before you does not have an acceptable "before" beads in the Calibration account. **Item 2** is required if you are the first user of the day.

### 1. CST Beads Profile PDF (Pre-running samples): required if the user before you does not have an acceptable "before" beads in the Calibration account



## 2. CS&T Report PDF (2 pages): required if you are the first user of the day

### Cytometer Performance Report

|                          |                                 |                     |                     |
|--------------------------|---------------------------------|---------------------|---------------------|
| Cytometer:               | FACSCantoII                     | User:               | CristineC           |
| Cytometer Name:          | FACSCantoII                     | Institution:        | Salk Institute      |
| Serial Number:           | V33896201783                    | Software:           | BD FACSDiva 7.0     |
| Input Device:            | Manual                          | Date:               | 09/25/2020 02:06 PM |
| Cytometer Configuration: | Master 3-laser, 8-color (4-2-2) | Cytometer Baseline: | 08/31/2020 09:27 PM |
|                          |                                 | P/F:                | Pass                |

#### Setup Beads

|                       |                 |                  |            |
|-----------------------|-----------------|------------------|------------|
| Bead Product:         | CST Setup Beads | Part #:          | 642412     |
| Lot ID:               | 91925           | Expiration Date: | 07/31/2021 |
| Bead Lot Information: | Not available   |                  |            |

#### Detector Settings

| Laser  | Detector | Parameter    | Target Value | Actual Target Value | % Difference Target Value | Bright Bead % Robust CV | Mid Bead Median Channel | Mid Bead % Robust CV |
|--------|----------|--------------|--------------|---------------------|---------------------------|-------------------------|-------------------------|----------------------|
| Blue   | FSC      | FSC          | 125000       | 123462              | -2                        | 1.69                    | 123309                  | 1.80                 |
| Blue   | F        | SSC          | 125000       | 124073              | -1                        | 2.44                    | 125799                  | 3.16                 |
| Blue   | E        | FITC         | 57349        | 56867               | -1                        | 1.87                    | 1972                    | 8.22                 |
| Blue   | D        | PE           | 49188        | 47784               | -3                        | 1.61                    | 1363                    | 8.68                 |
| Blue   | B        | PerCP-Cy5-5  | 52834        | 52177               | -2                        | 3.16                    | 1609                    | 15.25                |
| Blue   | A        | PE-Cy7       | 71029        | 69900               | -2                        | 5.95                    | 1663                    | 32.81                |
| Red    | C        | APC          | 44537        | 43776               | -2                        | 2.97                    | 1663                    | 12.26                |
| Red    | A        | APC-Cy7      | 41771        | 39920               | -5                        | 3.78                    | 1274                    | 10.62                |
| Violet | B        | Pacific Blue | 25318        | 25407               | 0                         | 2.34                    | 1245                    | 6.85                 |
| Violet | A        | AmCyan       | 51924        | 52188               | 0                         | 2.16                    | 1938                    | 6.01                 |

#### Detector Settings (Continued)

| Laser  | Detector | Parameter    | Dim Bead Median Channel | Dim Bead % Robust CV | PMTV | Δ PMTV | Qr     | Br  | P/F  |
|--------|----------|--------------|-------------------------|----------------------|------|--------|--------|-----|------|
| Blue   | FSC      | FSC          | 22011                   | 2.30                 | 521  | -5     | N/A    | N/A | Pass |
| Blue   | F        | SSC          | 57190                   | 2.05                 | 429  | -3     | N/A    | N/A | Pass |
| Blue   | E        | FITC         | 266                     | 26.36                | 522  | -1     | 0.0818 | 78  | Pass |
| Blue   | D        | PE           | 260                     | 29.48                | 511  | -3     | 0.1391 | 460 | Pass |
| Blue   | B        | PerCP-Cy5-5  | 260                     | 38.75                | 592  | -2     | 0.0274 | 0   | Pass |
| Blue   | A        | PE-Cy7       | 267                     | 82.73                | 613  | -4     | 0.0057 | 5   | Pass |
| Red    | C        | APC          | 220                     | 35.38                | 567  | -2     | 0.0417 | 11  | Pass |
| Red    | A        | APC-Cy7      | 218                     | 28.80                | 517  | -4     | 0.0840 | 68  | Pass |
| Violet | B        | Pacific Blue | 235                     | 23.06                | 372  | -1     | 0.2703 | 416 | Pass |
| Violet | A        | AmCyan       | 215                     | 18.87                | 402  | -1     | 0.1624 | 0   | Pass |

#### Specifications

|                                                      |                    |
|------------------------------------------------------|--------------------|
| PMTV Delta from baseline:                            | 50 (BD)            |
| Violet Laser Primary Channel Bright Bead %Robust CV: | 6.00 (Recommended) |
| Blue Laser Primary Channel Bright Bead %Robust CV:   | 6.00 (Recommended) |
| Red Laser Primary Channel Bright Bead %Robust CV:    | 6.00 (Recommended) |

## Cytometer Performance Report

|                          |                                 |                     |                     |
|--------------------------|---------------------------------|---------------------|---------------------|
| Cytometer:               | FACSCantoII                     | User:               | CristineC           |
| Cytometer Name:          | FACSCantoII                     | Institution:        | Salk Institute      |
| Serial Number:           | V33896201783                    | Software:           | BD FACSDiva 7.0     |
| Input Device:            | Manual                          | Date:               | 09/25/2020 02:06 PM |
| Cytometer Configuration: | Master 3-laser, 8-color (4-2-2) | Cytometer Baseline: | 08/31/2020 09:27 PM |
|                          |                                 | P/F:                | Pass                |

### Laser Settings

| Laser  | Power (mW) | Power Spec. (mW) | Current (mA) | Current Guidelines (mA) | Delay (Trigger on FSC) | Delay (Trigger on Fluorescence) | Area Scaling Factor |
|--------|------------|------------------|--------------|-------------------------|------------------------|---------------------------------|---------------------|
| Violet | 59.80      | 47.84 - 71.76    | N/A          | N/A                     | -29.07                 | -28.70                          | 1.31                |
| Blue   | 20.42      | 16.34 - 24.52    | 1.47         | 1.18 - 1.78             | 0.00                   | 0.00                            | 1.48                |
| Red    | 16.41      | 12.92 - 19.38    | N/A          | N/A                     | 30.20                  | 30.59                           | 1.25                |

Window Extension: 7.00  
FSC Area Scaling Factor: 0.96

### Threshold

Threshold (FSC): 9922

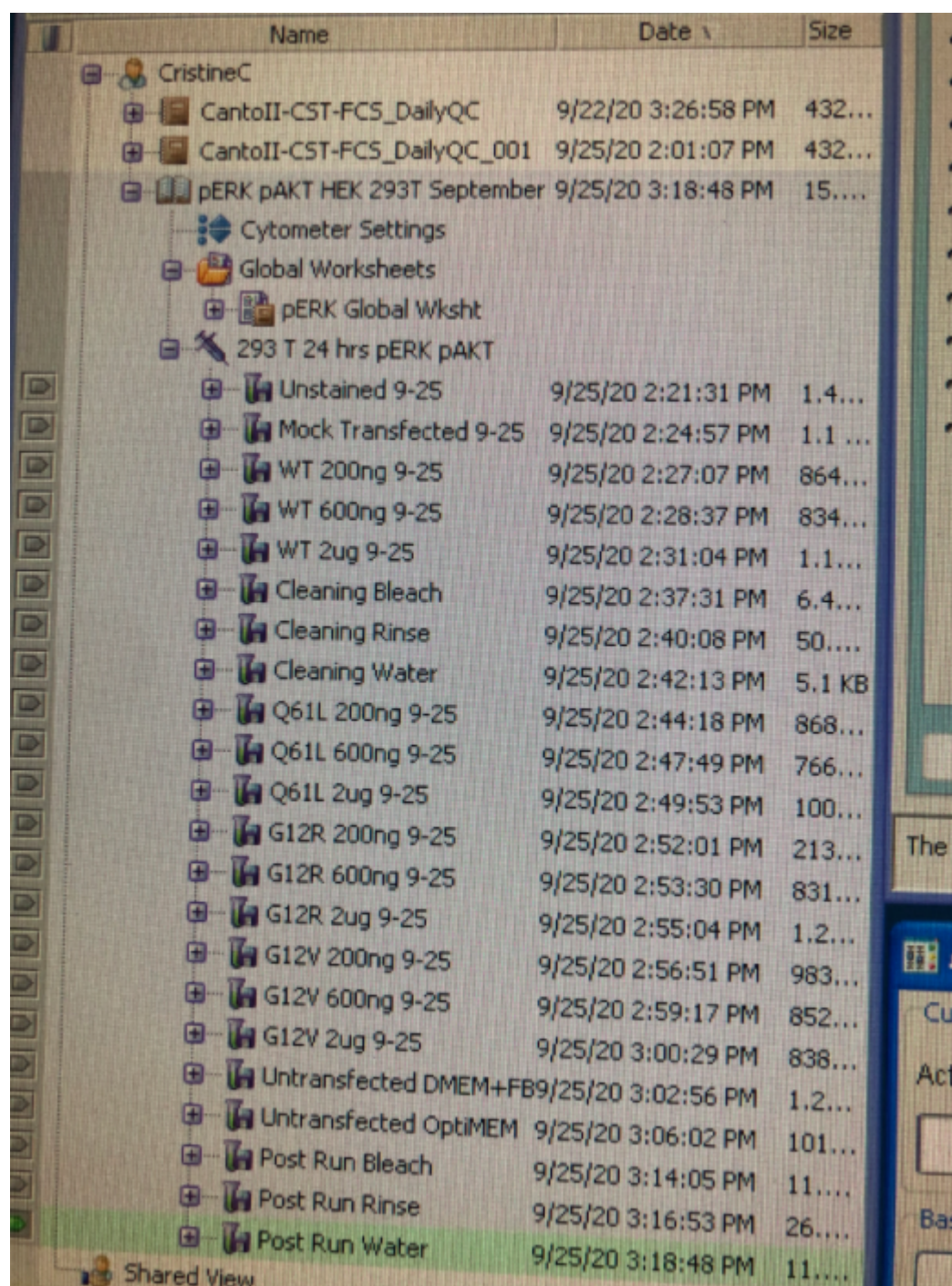
### Fluidics

FACSFlow Pressure: 4.00      Sample Pressure: 3.80  
Spec.: 3.90 - 4.10

Comments

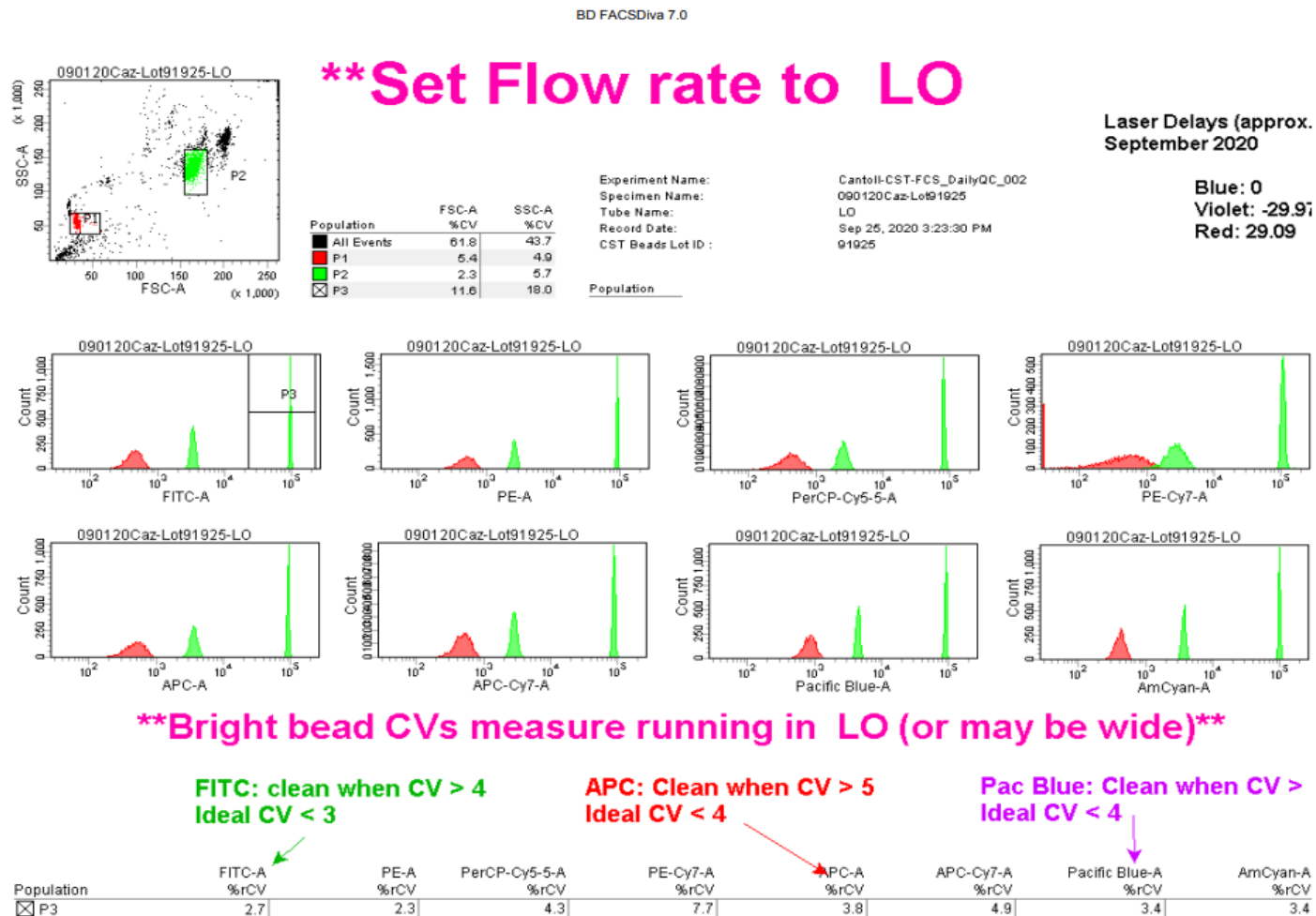


### 3. Cleaning Files: Screen grab with Time Stamps



| Name                         | Date               | Size    |
|------------------------------|--------------------|---------|
| CristineC                    |                    |         |
| CantoII-CST-FCS_DailyQC      | 9/22/20 3:26:58 PM | 432...  |
| CantoII-CST-FCS_DailyQC_001  | 9/25/20 2:01:07 PM | 432...  |
| pERK pAKT HEK 293T September | 9/25/20 3:18:48 PM | 15....  |
| Cytometer Settings           |                    |         |
| Global Worksheets            |                    |         |
| pERK Global Wksht            |                    |         |
| 293 T 24 hrs pERK pAKT       |                    |         |
| Unstained 9-25               | 9/25/20 2:21:31 PM | 1.4...  |
| Mock Transfected 9-25        | 9/25/20 2:24:57 PM | 1.1 ... |
| WT 200ng 9-25                | 9/25/20 2:27:07 PM | 864...  |
| WT 600ng 9-25                | 9/25/20 2:28:37 PM | 834...  |
| WT 2ug 9-25                  | 9/25/20 2:31:04 PM | 1.1...  |
| Cleaning Bleach              | 9/25/20 2:37:31 PM | 6.4...  |
| Cleaning Rinse               | 9/25/20 2:40:08 PM | 50....  |
| Cleaning Water               | 9/25/20 2:42:13 PM | 5.1 KB  |
| Q61L 200ng 9-25              | 9/25/20 2:44:18 PM | 868...  |
| Q61L 600ng 9-25              | 9/25/20 2:47:49 PM | 766...  |
| Q61L 2ug 9-25                | 9/25/20 2:49:53 PM | 100...  |
| G12R 200ng 9-25              | 9/25/20 2:52:01 PM | 213...  |
| G12R 600ng 9-25              | 9/25/20 2:53:30 PM | 831...  |
| G12R 2ug 9-25                | 9/25/20 2:55:04 PM | 1.2...  |
| G12V 200ng 9-25              | 9/25/20 2:56:51 PM | 983...  |
| G12V 600ng 9-25              | 9/25/20 2:59:17 PM | 852...  |
| G12V 2ug 9-25                | 9/25/20 3:00:29 PM | 838...  |
| Untransfected DMEM+FB9/25/20 | 9/25/20 3:02:56 PM | 1.2...  |
| Untransfected OptiMEM        | 9/25/20 3:06:02 PM | 101...  |
| Post Run Bleach              | 9/25/20 3:14:05 PM | 11....  |
| Post Run Rinse               | 9/25/20 3:16:53 PM | 26....  |
| Post Run Water               | 9/25/20 3:18:48 PM | 11....  |

#### 4. CST Beads PDF (post-running samples and post final round of cleaning):



#### 5. Clipboard checklist items, signed (legible), with power button OFF in the background:

System Power  
Fluidics Cart Connections

| Date | Name                | Time Off | CLEANING:                                                                                                                                       | WASTE:                                           | DATA:                                                                                               | BEFORE YOU LEAVE                                                                                                                                                                                                                                 |
|------|---------------------|----------|-------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------|-----------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|      |                     |          | Capped labelled tubes for CLEAN and RINSE can be reused; use fresh (DATED) tube for H2O daily/yr needed: 5min CLEAN, 3min RINSE, 1min water Y/N | TANK: Empty (added 1L bleach to empty tank?) Y/N | Previous session data deleted? Y/N<br>Instrument rate applies when core staff manages user data Y/N | (1) FLUIDICS SHUTDOWN, (2) LOG OFF Diva & PC, (3) on side: UNLESS next user arrives, (4) *200 SEC. REASONABLE, non-emergency rate changes implies 10 duration instrument is left on or you are logged in this offenders will have access revoked |
|      |                     |          | *\$100 penalty fee applies if requirements not met, repeat offenders will have access revoked.                                                  |                                                  |                                                                                                     |                                                                                                                                                                                                                                                  |
| 9/18 | Meredith Fric       | 2:55pm   | Y                                                                                                                                               | Y                                                | Y                                                                                                   | Y                                                                                                                                                                                                                                                |
| 9/22 | Tobias Schmidt      | 12:15pm  | Y                                                                                                                                               | N                                                | Y                                                                                                   | N                                                                                                                                                                                                                                                |
| 9/22 | Christine Christman | 3:45pm   | Y                                                                                                                                               | Y                                                | Y                                                                                                   | N                                                                                                                                                                                                                                                |
| 9/23 | Wade Timoney        | 8:50pm   | Y                                                                                                                                               | N                                                | Y                                                                                                   | N                                                                                                                                                                                                                                                |
| 9/25 | Chris Christman     | 3:30     | Y                                                                                                                                               | Y                                                | Y                                                                                                   | N                                                                                                                                                                                                                                                |

LEFT IN STANDBY (NO SHUTDOWN)? Y/N  
TURNED Y/N