FACSARIA FUSION CHECKLIST **16.3.2018 Laura Kummola**

BEFORE TURNING ON THE INSTRUMENT

1. **Check air pressure lock on the wall, should be closed.**
2. Check last logbook entry for anything that could affect your run.
3. Check sample line and clean anything that is dirty **(deflector plates especially)**.
4. Replenish solutions.
5. Switch air and fluid tubing from EtOH tank to sheath tank.
6. Open the air pressure lock on the wall.

BASIC STARTUP PROCEDURE

1. Turn on computer -> Admin account -> password is BDIS#1. Let computer finish loading.
2. Turn on FACSAria Fusion, open up FACSDiva software. Allow systems to connect.
3. Whenever a CST mismatch window appears, click *use CST settings*.
4. Perform Fluidics startup (*Cytometer* from toolbar). Check sheath filter: there should be no air.
5. Check that configuration matches the current filters and lasers. If not, go to *Cytometer* -> *View configuration*.
6. Turn on stream. In case of funky stream, clean the nozzle.

CST check (once in 24 hours)

7. Perform CST check when lasers are warm (takes 20-30 min). Select *Cytometer* -> *CST*. Keep the cytometer (upper) door closed during the check!
8. CST-beads: one drop of beads (shake the bottle well, **do not vortex the bottle**) + 350 µl of PBS. Vortex the beads **in the tube**.
9. Bead bottle number must match the lot number on the screen. Choose *Check performance*. Click *Run*.
10. If CST fails or passes with warnings, cleaning the flow cell usually helps.
11. For sorters: check if the stream is now stable. If not, adjust amplitude and frequency (**Gap to ~11 value with 100 µm nozzle)**. Wait for 5-10 minutes after adjustments. Put *Sweet spot* on when stream is stable.
12. Create experiment. You can begin it while the stream is stabilizing.

BASIC SHUTDOWN PROCEDURE

1. (After sorting: remove tube holder, put back splash shield. Clean the holder if dirty!)
2. Run on acquire, 10 min each, flow rate 11: **a**. FACSClean **b.** FACSRinse **c.** mqH_2O.
3. *Optional: clean flow cell. Stop the stream. Insert the broken 100 µm nozzle. Select* Cytometer -> Cleaning modes. *Use FACSClean. Perform 3x, let soak 5-10 min on last time.*
4. *Clean flow cell 3x with mqH_2O. No soaking is needed.*
5. Perform Fluidics shutdown (toolbar, *Cytometer)*. Mind the pressure during tube switch!
6. When prompted during fluidics shutdown, use mqH_2O as the final cleaning solution.
7. Turn off the FACSAria Fusion.
8. Clean all spatter and do not forget to also clean the nozzle!
9. Close the air pressure lock on the wall.
10. Empty the waste tank. Pour some FACSClean on the bottom.
11. Remember database management! Close FACSDiva and turn off the computer.
12. Fill in the logbook (both of them).

SETTING UP A NEW EXPERIMENT, SAVING TEMPLATES AND APPLICATION SETTINGS

1. In *Browser* menu, create a new experiment from *brown folder icon*.
2. Create specimens *(syringe icon)* and tubes under specimens *(tube icon).*
3. Activate any tube (arrow turns green) in the *Browser,* select *Cytometer window* (*red laser icon*) if not yet open. In Parameters tab, delete any detectors you are not using.
4. Open your plots, histograms and statistical views the way you want them in the *Global worksheet*.
5. From toolbar, select *Experiment -> Experiment layout*. Add labels to your fluorochromes. Set the amount of cells you want to collect (Acquisition tab).
6. Set the detector voltages: activate any tube and run an unstained sample on acquire. Flow rate can be low **(1 to 2)**. Set the negative population with unstained sample, and with a stained sample check that the positive population is on scale. Adjust voltages in Parameters tab (*Cytometer window)*.
7. Draw your gates and set gating hierarchy, if gates are finished. Unload sample.
8. If you want to set a stopping gate, go to *Experiment -> Experiment layout and* click the Global Worksheet column. Choose "Global sheet 1" from the drop-down menu. Set the stopping gate. Do this when you have decided on final gating. You can always come back after compensation to finish it.
9. Give correct labels for your plots in the *Global Worksheet* by clicking the axis labels.
10. Save application settings and experiment template if setup is finished. Right-click experiment name in *Browser*. *Select Export -> Experiment Template*. Right-click *Cytometer settings* under experiment name in *Browser.* Select *Application Settings -> Save*. Name the settings.
11. Proceed to running compensation, sort setup, or just recording your sample data.

USING TEMPLATES AND APPLICATION SETTINGS

If you already have set up your experiment previously and want to reuse its settings, these are the best options.

- *If you want your experiment to be updated by the latest CST to ensure data comparability, you must use application settings – not just copies and templates!*
- *Application settings include parameters, area scaling and PMTV. Templates and copies include all these, and also gates and compensation.* ***Saved compensation matrices can never be updated so compensation must be run anew!***

1. Either copy an old experiment (right-click-> *Duplicate without data*), or open a template: from toolbar, *Experiment -> New experiment*. Choose your template from the list.
2. In *Browser* menu, right-click *Cytometer settings* below experiment name. Make sure to have run CST at this point. Select *Unlink from (the name compensation data)*, if applicable.
3. Right-click again *Cytometer settings* below experiment name. Choose *Application settings -> Apply*, and choose previously saved settings from the list. If asked about **compensation**, ***Set to zero***. If eg. Area scaling has changed, choose *Overwrite*.
4. If compensation is not needed, proceed to either sort setup or just recording your sample data.
5. Otherwise check that compensation in your samples is now zero: activate a sample, open the *Cytometer window* and see Compensation tab. Diva is sometimes tricky here!
6. Run new compensation controls. Check that the new compensation is now applied to your samples.

COMPENSATION

1. From toolbar, select *Experiment -> Compensation setup -> Create compensation controls*. Delete marker-specific samples if not needed. If you use beads, check the box that includes a separate unstained tube. If compensating with cells, leave it unchecked. If you need an empty channel, delete it here but leave it in the *Cytometer window*, Parameters tab.
2. Switch to *Normal Worksheet* (icon on the worksheet corner). Activate the first compensation sample in the *Browser* menu.
3. Load the correct single-stained sample. Set the gate to singlets and press *Record*. Collect preferably at least 10 000 events. Unload sample.
4. Right-click the P1 gate and select *Apply to all*. Activate the next sample, load and record. Repeat until you have run all the compensation tubes.
5. Verify correct positioning of the P2 gates. If you did not include a universal negative, create P3 gate on the negative peak by using the *Snap-to-gate icon*.
6. In the toolbar menu, select *Experiment -> Compensation setup -> Calculate compensation*. In the pop-up window, choose *link and save*. Change back to *Global Worksheet*.
7. Now you can verify and fine tune correct gating and set gating hierarchy by running (on acquire) an appropriate sample, if necessary. You must not change any voltage other than FSC or SSC from now on – it would make the compensation invalid.
8. Proceed to setting up sorting or recording your data.

RECORDING YOUR SAMPLE DATA AND EXPORTING FSC-FILES

- ***Filter your samples before sorting.***

1. Activate your first sample in *Browser* and place the tube into the loading chamber, load.
2. Set the flow rate so that you get enough events per second.
3. Press *Record*. You can change the event number to be recorded in the *Experiment layout* or in the *Acquisition dashboard,* if needed.
4. Move to the next sample by activating it in the *Browser* or by clicking *Next tube* in the *Acquisition dashboard*. Load and record, repeat for the rest of the samples.
5. After the last sample, insert a USB storage device and right-click your experiment in the *Browser*. Select *Export -> FSC Files*.
6. A window opens, click OK after checking that file format is FSC 3.0.
7. Choose where to save your files and click OK.

SORTING

- ***Use polypropylene tubes as recipient tubes to avoid cells adhering to tube walls****.*

Setup of side streams

1. Remove the splash shield under the sort block. Stream should now be stable and the *Sweet spot* on!
2. Insert tube holder with four tubes in it. *Side stream window* should be in Four tube mode. This can be changed from the icon in the lower taskbar of the computer.
3. Activate any sample and open a *Sort layout* (*Blue plate icon*). Choose four tubes or two tubes from the Device drop-down menu in the sort layout.
4. Perform test sort: in the *Side stream window*, turn on *Voltage*, close *Waste drawer* (icon now shows stream going into tubes), press *Test sort*.
5. Check that the streams enter the tubes. If needed, adjust the streams with sliders in the *Side stream window.*
6. Turn off *Voltage* and open *Waste drawer* (icon now shows blocked stream and the stream enters the waste drawer).

Setting drop delay

7. Open the Accudrop template and add a *Sort layout*.
8. Accudrop beads: shake the **Accudrop bottle** and add one drop into 500 µl of PBS. Vortex **the tube** and acquire on **flow rate 1**. Dilute If event number over **1500/s on 100 µm nozzle**.
9. Add P1 population to the left tube in the *Sort layout* (Right-click -> *Add*).
10. Turn on *Voltage.* Turn on *Optical filter*. Press *Sort* in the *Sort layout.* Click *Cancel*.
11. Beads (≥98%) should go to the **left** box. If not, press *Auto delay* and *Start run.* A few rounds might be needed. If several attempts fail, disable the *Optical filter* and adjust the camera screw until center stream is sharp and side streams visible.
12. Stop sorting, close *Optic filter*, turn off *Voltage* and unload tube. Amplitude and frequency should not be adjusted after the drop delay is set – otherwise you must set it again.

Sorting sample to tubes (OR jump to "Sorting to plate")

13. Return to your experiment.
14. Activate the sample you want to sort and open *Sort layout*. Verify correct amount of tubes (two or four) and correct precision. Set target events to the number you wish or continuous.
15. Add your target gates to the wells in the *Sort layout*. Double check gating and sorting logic!
16. Run FACSClean at least 10 min, check for debris in gates. Optional: run 70% ethanol to sterilize fluidic lines. Run PBS 5 min.
17. Place the polypropylene tubes to the tube holder. There should be some medium on the bottom. Wet also the tube walls!
18. Vortex your (filtered!) sample and load it. Press *Sort*.
19. Record some data if necessary by pressing *Record*.
20. Save only the sort reports you need.

Sorting to plate

- *Falcon well plates fit the ACDU best!.*

Side stream adjustment in ACDU mode for plate

1. Remove the tube holder under the sort block, put back in the splash shield.
2. Return to your experiment.
3. Switch *Side stream window* to ACDU mode.
4. Adjust the far left side stream to the same value as the proximal left side stream.
5. From toolbar, select *Sort -> Home Device*. Select you well plate from the list.
6. Activate sample, open *Sort layout*. Choose your well plate from the drop-down menu. Press "eject" button. Place your test well plate (lid on) into the holder, A1 at the front left corner.
7. In the arrow window, press *Go to home*. The ACDU moves into A1 sorting position.
8. Turn on *Voltage*, close *Waste drawer*. Press test sort two times quickly, to sort a droplet onto directly on A1. Side stream should fall between the lines – you can check this by keeping the test sort on longer (remove plate lid!).
9. Continue fine-tuning of the ACDU position. When the ACDU is in home (A1) position, move it with the arrows and repeat test sorting until droplet is in the middle of the A1 well. Double arrow moves the ACDU five steps and single arrow moves it one step. Press Set home in the arrow window to save the new A1 coordinates. Use the "eject" button to bring it back to the front for inspection between adjustments, if needed. Press set home only when the plate is in A1 position – not in the front unless you want extra work!

Final adjustment of sorting in rows and columns (with beads)

10. Put plate into home position and click *Apply* to link the position coordinates to *Sort layout*.
11. Load and run some beads (Accudrop or CST, or use cells if you have spare).
12. Create a gate for the beads and add it to horizontal and vertical well rows. **Choose 10-50 events** (**Target Events to: 10-50)** for sorting. Press *Sort*.
13. If droplets are not in a satisfactory row on the lid, click *Go to home* and do the needed adjustments with arrows. Click *Apply* and *Set home*. Acquire sample, sort. Repeat this whole step until position is good.
14. Unload bead tube, check that *Voltage* is off and *Waste drawer* open. Delete the bead gate.

Sorting sample to plate

15. Replace test plate with your sorting plate. There should be some medium on the well bottom.
16. In the *Sort layout*, select the desired precision. **Set target events to the number you wish.**
17. Add your target populations (gates) to the wells in the Sort layout. Double check gating and sorting logic!
18. Run some FACSClean before sorting (at least 10 min), check for debris in gates. You can also run 70% ethanol to sterilize fluidic lines. Run PBS 5 min.
19. Vortex your (**filtered!**) sample and load it. Press *Sort*. Record some data if necessary.
20. Save only the sort reports you need!