Protocol 604_FACSArray

Turn on FACSArray (large grey button on the front) and computer in any order

Login to the computer is:
Username: Administrator
Password: BDIS

Open the FACSArray software
Login is:
Username: eslack
Password:

Running an experiment
Click on the "new experiment" wizard button
This will open a dialogue box.
Select "default"
In the next window select
"4-color blue" for cells
"CBA flex blue array" for CBA flex
"CBA experiment" or "CBA Array" for all other CBA pre-configured kits (NB – important as fluorophores differ)

For cells and CBA flex you have some single-stained controls to set up the compensation which you will need to enter appropriately (should be obvious!)

enter how many samples you have (excluding compensation controls)
Do not change loading parameters for CBA settings. For cells, you will need to determine optimal parameters for your particular samples

Name the experiment and "finish"

Manually modifying the plate layout
At this point you can manually change the plate layout (samples can be deleted by right-clicking in the browser window and added by selecting wells and clicking on "add sample" or "add set-up" in "manual" mode. NB samples are added with default acquisition parameters (flow rate, volume, number collected) so you will need to change this for each well added)

For any set-up wells, ensure the sample volume is 20-50µl and flow-rate is set to 1 so that you can run the set-up several times until you are happy with your settings

The FACSArray has a dead volume of around 40µl so you will only get 3 x 50µl from a 200µl well.

Set-up wells for calibration must be set up in a single line in the following order:

1) unstained
2) FITC single stain
3) PE-single stain
4) APC-CY7 single stain

5) APC single stain

If fewer colours are being compensated, keep the order the same but omit the unused colours.

Running the experiment
Click to the "set-up" window and load the plate
Click "none" on the far right-hand side to deselect all wells. Then select the one you are interested in and click "set-up"
Under the parameters tab, alter the PMT voltages so the events fall in the desired areas (follow booklet instructions here for CBA kits on the Array!)
Chose the next well, until you are happy with the setup.

Then highlight all set-up wells and hit "Acquire"
This should calculate the compensation parameters

Change to the "Acquire" window and ensure your samples are selected
If not, go back to the browser and select the correct plate

Press Acquire and allow to run (empty flow and waste before starting avoids problems mid-run)

At the end of the run, export FCS files from the browser to the BDEXPORT/FCS folder.
Delete files from within the browser as soon as you have verified they have been successfully exported.

These files can be directly loaded into flowJo on a Mac, or to FCAP Array software on the PC.

Cleaning and shutting down the Array
Return to Array software and close the experiment (right click on the experiment name and select "close experiment")

If just beads have been run:
Fill the 1st column of a FACS plate with 4 wells of FACSclean then 4 wells of milliQ water
Select "clean" from the "instrument" pulldown menu
Load plate, and click "OK"

If bacteria or cells have been run:
Select "clean" from the "instrument" menu then click on "monthly clean". Fill the plate as on the screen, place it on the loader and click "OK"

Once the clean is complete, remove the plate

Select "Shut down fluidics" from the "Instrument" menu

Turn off the FACSArray with the grey button on the front (a fan will keep running – this is OK). Then shut down the program. This avoids power-surge issues during the night!