July 1, 2010

Volume 1, Issue 1

The Stream

Monthly Newsletter of the Stanford Stem Cell Institute Flow Cytometry Core

Special Interest Areas this month:

- Do you want to sort using the 130 micron nozzle?
- Do you want higher efficiency when sorting into 96 well plates?
- Is bright GFP obscuring your dim PE or PE tandem signals?

Read about the user of the Month to find out where to get help.

Individual Highlights:

Piero Dalerba,flow user of the month

Flow User of the Month: Piero Dalerba Sorts Human Cancer Stem Cells

If you work late at night at Arastradero, you probably will find Piero Dalerba sitting in front of a sorter running a lengthy sort. Piero's work centers around the functional investigation of human cancer stem cells, primarily colon and breast. When you see him sorting late into the night, he is probably purifying cancer stem cells by their surface marker phenotype. Cancer stem cells exhibit well-defined characteristics following transplantation into immunodeficient mice: they are able to develop into a tumor which recapitulates the cellular heterogeneity of the original cancer tissue from which they have been purified (differentiation), and they can be serially transplanted (self-renewal). Once the cells are confirmed to be cancer stem cells, Piero performs functional studies using gene expression arrays, lentiviral vectors to interfere with pathways, and single cell pcr.

Having worked on FACSAria sorters for years, Piero has become an expert on several aspects of sorting. He typically identifies his stem cells using five colors. He takes advantage of Mystique's special optical layout which enables him to visualize mCherry or to see bright GFP without losing resolution in the PE or PE tandem channels, which sometimes occurs with our other instrument platforms. Since his cells are quite large and fragile, he uses the 130 micron nozzle at 10psi. Piero often sorts into 96-well plates and reports that he routinely recovers at least 80% of wells with cells. He typically gets even better. Thanks so much to Piero for volunteering to be the first Flow User of the Month!

What is "The Stream"

The purpose of this newsletter is to provide specialized information to the Stem Cell FACS Aria Users about Flow Cytometry. This first issue will introduce you to the format. Regular features will be the "Flow User of the Month," which will introduce one person's project and special areas of expertise. By introducing projects to everyone, people can have an idea of what others are doing and whom to go to for advice on special issues. This issue features Piero Dalerba, whose specialties include using the 130 micron nozzle, 96 well plate sorting with high efficiency for downstream pcr, and sorting with combinations of mCherry or bright GFP. Another user who is doing similar work could check with Piero for helpful hints and insights about any of these techniques (that is, if they stay up late enough!) Another feature of the newsletter is the "New Users" section. Here, the latest trainees are introduced and pictured so that everyone will know who they are and that they may need extra help as they start their flow cytometry experiments. Also, be sure to check out the advice column.

Flow Call- Advice for Flowers

Flow call is the advice column for "The Stream." Email your flow questions to lovelace@stanford .edu

Q Dear Flow Call, I do a purity check after my sort and sometimes the percent of cells that fall into the original sort gate is quite low. What does this mean? Are my cells impure? Do I have to resort?

A Dear Flower, an excellent question! And Flow Call is going to give you lots of different answers, but try not to muddy the sort even more. Several factors can cause a sort to "fall out" of the gate you originally set. Of course, the obvious reason is that the sort is not pure; in fact, the sort could be pure but not give 100% in your sort gate. For example, if your sort gate was extremely tight, some cells which were originally in the gate may fall outside the gate on the second pass through the instrument. You may have to extend the sort gate upon reanalysis to include these cells. Another explanation is that the sample line contains cells not flushed out completely from the previous sample. These cells show up as impurities when you run the purity check and they contaminate your sample as it runs through the line. Flushing the line with detergent and water prior to placing cells on for reanalysis will help with this problem. Occasionally at high speed sorts I have seen evidence that a fluorophore "broke off", making the cells fall outside the sort gate. This is hard to prove for certain, but after looking at the function of cells that appeared impure and verifying that they were pure funtionally, I believe that it does happen. If your sort does appear to be impure, verify that your accudrop drop delay was set correctly. Could there have been splashing inside the sort chamber from a very full tube or incorrectly aligned sidestreams? Check that your sidestreams are not fanning, which could happen if your cells are too big for your nozzle size. If you continue to have purity issues, let me know and I will help you to diagnose the problem. As always, sort well, sort pure, Flow Call.

New Users Section

Introducing the newest Aria users to the group. The following completed their training in June and are starting to run experiments for the first time on their own. Don't hesitate to ask if they need help or advice from more experienced users:

Jose Vicente Medrano Plaza from the Reijo-Pera lab will be sorting based on cell cycle profile

Debashis Sahoo will be sorting tumor cancer stem cells in conjunction with the Weissman lab.

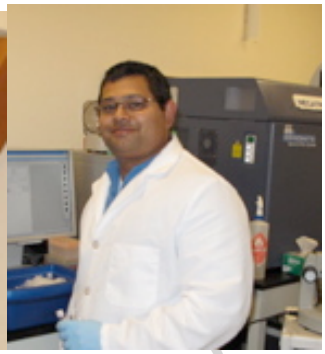
Jian Wang and Sid Mitra from the Weissman lab will be sorting tumor cancer stem cells.

Shaheen Sikandar from the Clarke lab will be sorting tumor cancer stem cells.

New User Photos

Shaheen Sikandar

Jose Vicente Medrano Plaza



Sid Mitra

"If you see these new users at the Aria, check in to see if they need some extra help."

Jian Wang

Debashis Sahoo

If you have suggestions for this newsletter, especially suggestions for a name, or articles send these suggestions to Patty Lovelace, lovelace@stanford.edu