September, 2010
Volume 1, Issue 2

The Stream

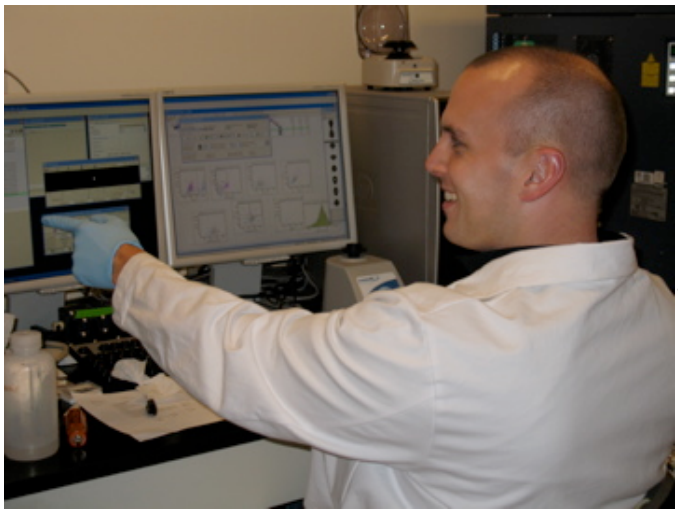
Monthly Newsletter of the Stanford Stem Cell Institute Flow Cytometry Core

Special Interest Areas this month:

- Do you sort cells from solid tumor?
- Is your solid tissue sample difficult to dissociate
- Are you trying to design a complex multicolor flow cytometry panel to analyze or sort your cells?

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

Individual Highlights:



Stephen Willingham,flow user of the month

Flow User of the Month: Tissue Dissociation Key to Stephen Willingham's CD47 Antitumor Therapy Studies

Many at the Stem Cell Institute are dissociating tumors into single cell suspension for downstream applications. Flow user of the month Stephen Willingham is no exception. His studies require single cell suspension of tumor cells both for an initial step of transducing the cells and for later steps of sorting the transduced cells. If you routinely receive tumor samples and are having difficulty getting them into single cell suspension, he is the expert to turn to.

Stephen's initial human tumor samples come from surgery. While he receives many different types of samples, he is often presented with ovarian cancer tumors, which are particularly difficult to dissociate. Getting these cells into single cell suspension without killing them is a very real obstacle to his downstream research so he perfected a dissociation cocktail and system to help (make sure you check out page 2 for the "Magic Brew" reagents and protocol.) Once he obtains a single cell suspension, he transduces the cells overnight so they express GFP and luciferase, making them easy to track when they are injected into mice and develop into tumors. As the tumor grows, Stephen can image the whole mice for tumor luciferase expression.

Once the tumors are identified, they are removed and the cells dissociated again. At this point, some may be frozen for future assays. Others are sorted for GFP expression and lack of mouse markers and injected again into mice. The tumors in these mice can be monitored via imaging to study the response to CD47 antitumor therapy. Stephen is currently using this method to determine the efficacy of CD47 therapy in many different solid tumors: ovarian, breast, colon, bladder, prostate, lung, and head and neck. Thanks very much Stephen for sharing your dissociation protocol.

Stephen's Tumor Digestion Magic Brew

Magic Brew:

20ml 199 Media

285μl TM Enzyme (Roche)

285μl TH Enzyme (Roche)

200μl DNAse (Worthington, Stock solution=12,500 units/ml)

200μl Pluronic-68 (Sigma, Stock solution=126 mg/ml)

Protocol:

1. In standard sized tissue culture plate, mechanically dissociate tumor by chopping into tiny pieces with razor blades.
2. Resuspend tumor pieces in Magic Brew.
3. Place in 37 degree incubator.
4. Pipette up and down every 15-20 minutes until single cell suspension is achieved.

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 am desperate! I would like to identify my tumor stem cells, but am having a hard time deciding which fluorochrome combination to use. Also, when I decide on my colors, how do I decide which color to use with each marker?

A Dear Flower,

Hopefully you attend the Multicolor Panel Design Seminar August 31,2010 at 2pm. Mervi Reunanen, Ph.D., from BD Biosciences will be speaking and answering questions on this topic to all that are interested.

As a general rule, you will want to design a panel with the bright fluorochromes directly conjugated to your dimly expressed surface marker antibodies and the dim fluorochromes directly conjugated to antibodies against highly expressed markers. With our instruments, the brightest commonly used fluorochromes are PE, APC, PE-Cy5, PE-Cy7, and PerCP-Cy5.5. The dimmest commonly used fluorochromes are Pacific Orange, Am Cyan, V500, APC-Cy7, and PerCP. Fluorochromes such as FITC, Pacific Blue, and Alexa 700 have intermediate intensity. Qdots can also be used, their intensity is generally intermediate, but this can depend on the laser configuration of a particular instrument.

It is also important to consider fluorescence spillover. If one color spills over a lot into another detector, you may lose the ability to resolve dim positive cells on the spilled-into channel. You can use the spectral viewer on the BD website to determine spectral overlap of the fluorophores you want to use. Remember that spectral overlap depends on instrument configuration. For example, on Optimus Prime and Megatron, FITC spills over quite a bit into PE. On Mystique and Rogue, this is not the case.

Confusing? Come to the seminar or catch me for a chat. 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 July 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:

James Krings is in the Sunwoo lab. He will be using the flow cytometers to analyze response of head and neck tumors to a small molecule drug. He will be doing mostly analysis without sorting.

Kipp Weisskopf in the Weissman lab will be using the sorters to study macrophages in cancer.

Erica Anderson, working with the Reijo-Pera / Longaker lab, will be using the sorters in a study of Marfan syndrome.

New User Photos

James Krings

Erica Anderson

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

Kipp Weisskopf