Flow Facility Tips & Tricks

Back Gating

By Patti McDermott

Back gating is a tool to use if you are unsure of your population of interest’s location in the FSC/SSC plot when acquiring your unstained control, as depicted in the scenario in Plot 1 below. To back gate in Diva, acquire one of your positively stained controls => draw a gate on the positive population (Plot 2) => right click on the FSC/SSC plot => Show Population => select the gate around your stained population => look for where the population falls in the FSC/SSC plot (Plot 3) => adjust your FSC/SSC voltages accordingly to put your population of Top left (Plot 1): Unstained control. Top right (Plot 2): Stained control. Draw a gate around the population of interest. Bottom left (Plot 3): Gated population in FSC/SSC plot. Note that the gated population falls off-scale, meaning that you must decrease the voltage for both forward and side scatter. Bottom right (Plot 4): Gated population at adjusted FSC/SSC voltages.

Note: So that you don’t waste your stained control, when your population of interest is in view you can delete the gate and put your unstained control tube back on to finish the FSC/SSC voltage adjustments.

Sort Efficiency

By Steve Slater

Whether sorting by yourself or with an operator, you may have noticed that the flow cytometer measures many things, but one of the most crucial things measured during sorting is something known as sort efficiency.

Simply put, sort efficiency is the ratio of target cells sorted vs. the total number of target cells detected. For example, if the cytometer sees 1,100,000 cells but only sorts 1,056,000 cells, our recovery rate is about 96% (arrow above). So far so good, right? So, what are machine factors that impact sort efficiency? The most critical machine factor that affects efficiency is sort rate (red circle above), which can be controlled by adjusting the flow rate. The faster the machine sorts, the less efficient the sort will be. So, let’s see what happens if we increase the flow rate from 1 to 2.

By increasing the flow rate, we have tripled the sort rate (circle above) but decreased efficiency from 96% to 85% (arrow above). So, what does this really mean? If there were 1.1 million cells that could potentially be sorted from this sample, we would only recover 935 thousand. The increase in speed caused a loss of 11% of the target population.
Importantly, sort efficiency is a measure of yield, not purity! The sample purity will remain the same regardless of sort efficiency.

The importance of sort efficiency depends on your sorting needs. If the desired cells are rare, then a lower and slower flow rate will result in a better yield. However, if your target population is in excess, then an increased flow rate will reduce the duration of your sort.

**BenchSci: A Quick Review**

*By Derek Cain*

In this short article, we profile an online tool for biomedical researchers called BenchSci (benchsci.com). The company’s profile states that, “BenchSci is a free online platform designed to help scientists find antibodies from publications. Their proprietary machine-learning algorithm was trained by PhD-level scientists to identify and understand the usage of commercial antibodies in the research literature.”

We took BenchSci for a test run. After generating an account (free for academics), we logged in and were welcomed by a slick but simple search bar. Since we are always on the look out for anti-CXCR5 antibodies for flow cytometry, we entered “CXCR5” into the search bar.

Our search returned an array of thumbnails depicting figures from publications in which anti-CXCR5 antibodies were used. Each thumbnail contained the figure, the journal name and year, and the application for which the antibody was used (i.e., FACS, IHC, etc.). Clicking on a thumbnail took us to a new page containing the full-size figure, where we could see the data as well as the figure legend. Conveniently, BenchSci provided links in a side panel to the antibody reagents reportedly used in the figure.

At the top of the search page, we saw that our original search on CXCR5 yielded 530 figures and 465 products. With a quick click, we could cycle between publication figures and lists of commercial reagents for CXCR5 antibodies. Benchsci also features filters to narrow down searches. For example, we could narrow our search to focus on reagents used in certain techniques (i.e., flow cytometry, western blot, ELISA, etc.), organisms tested (i.e., mouse, human, turtle, etc.), tissue used (i.e., spleen, lymph node, etc.), cell type used (i.e., B cells, T cells, etc.), cell line used (i.e., HeLa, 293t, etc.), and disease (i.e., tumor, osteoarthritis, “normal”).

There is a point in almost every project when the question arises, “Is there a good antibody for that protein?” BenchSci may reduce the time in finding the optimal antibody for your experimental needs.

Visit BenchSci at www.benchsci.com for more information.