Sorting at the Flow Cytometry Facility

At the present time, the assistance of a trained cell sorter operator is needed for all sorting applications. As such, if you are planning a first time sort you will need to contact the flow lab jmarvin@cores.utah.edu in order to schedule a sort time. The sorter schedule fills up quickly, the earlier you reserve the instrument the better chances you will get your first choice. We will do our best to accommodate "last minute" sorts, however there are times when this will not be feasible. Also, since the sorter schedule is typically booked everyday, it is imperative that you show up promptly at your scheduled time. Extending your time past what has been scheduled is difficult. Many of our users have been incredibly accommodating when this happens but if it continues there will most likely be problems. It's not always the case, but improper scheduling on the users side is the main culprit for when things get backed up in the lab. We are more than happy to book extra time for you if you're not sure how many cells your going to have. But please keep us updated if things change.

Below is a list of things you should be aware of before you schedule your sort.

85um tip

-45psi
-most cell lines
-30-40million cells per hour. (Plus some time to set up gates and switch samples)
-1ml of sheath fluid put into your collection tube for every 420,000cells collected.

100um tip

30psi
-very large, sticky, or fragile cells
-20-30million cells per hour. (Plus some time to set up gates and switch samples)
-1ml of sheath fluid put into your collection tube for every 300,000cells collected.

70um tip

-70psi
-mouse phenotyping
-90million cells per hour. (Plus 15min to run controls and set up gates)
-1ml of sheath fluid put into your collection tube for every 1,00,000cells collected.

Speed*:* The difference between a high speed sorter and a regular sorter has little to do with the sample volume flow rate (i.e. how much liquid the instrument takes in per unit time). The event rate (i.e. Number of cells detected/second) is determined by the concentration of cells in your sample, and is limited by the system's electronics. A general rule of thumb is that for every million cells/mL concentration you have your

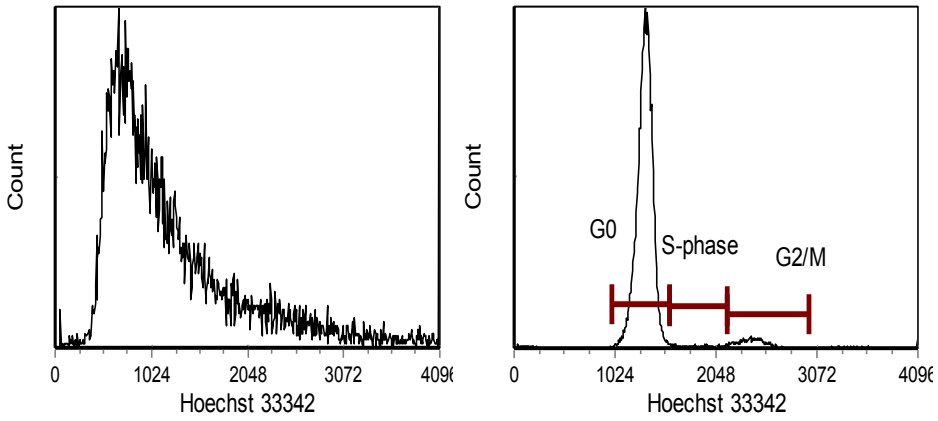
sample at, the instrument will detect an equal number of thousand cells/second (for example, if your cells are concentrated to 10 million/mL, then the maximum throughput rate will be 10 thousand/second). The maximum sample volume flow rate is about 60uL/min.

Cell characteristics: The type and size of cells dictate in part how a cell sorter is configured. The orifice the cells pass through (commonly referred to as the tip) ranges in size from 50um up to 250um in diameter. Tips can be changed on the cell sorter depending on the size of the cells being sorted. (Changing tips does take about 1hour.) Typically, a sorter with a tip diameter of 'X'um can run cells no bigger than 'X'um/5 (for example, a sorter with a 70um tip can run cells up to 14um in diameter). Also, adherent cell lines and fragile cells (e.g. dendritic cells) may require the use of a larger tip size as well as lower pressure and sort rates.

Sample and Collection buffer*:* Generally, the buffer you want to resuspend your cells in, or sort into is the buffer your cells are "happiest" in. This typically means "FACS" Buffer (PBS/BSA) or Culture Media (DMEM, RPMI, etc…). There is a fair amount of data suggesting that collection material can greatly affect downstream viability and functionality. *For example, cells collected into an empty tube will suffer greatly compared to cells collected into media.* The presence of phenol red in culture media does affect the background fluorescence of the cells slightly, but generally is not a problem. There are instances where additives may be beneficial or necessary. For example, with particularly "sticky" cells, the addition of EDTA will help avoid clumps and clogs, or the addition of Hepes may help buffer the pH of the solution and aid in cell viability. Sorters also have the ability to control the temperature of the sample (warm or cold). It may be important to note whether or not your cells or staining methods are temperature sensitive.

Cell population: The presence of dead cells or debris, greatly reduces the accuracy of sorting as well as the detection of subpopulations. Dead cells typically have higher autofluorescence and may hide dim populations resulting in a poor sort. Also, dead cells non-specifically bind antibodies and dyes and result in false positives. Finally, the presence of free DNA (from the dead cells) acts as a "glue" resulting in more cell aggregates and cell sorter clogs. To remedy this, samples with large amount of dead cells and debris should be cleaned up using a ficol gradient or equivalent or by the addition of DNAse. Also the use of a dead cell discriminator (e.g. Propidium Iodide, DAPI, etc…) can help in the resolution of dim populations.

***Below are two similar experiments performed on the Aria. This person was trying to purify cells in S-phase of the cell cycle. The figure on the left is taken from an experiment where the cells of interest made up about 20% of the total events (i.e. lots of debris and dead cells.) The figure on the right is the same experiment with a cleaner prep. There was no point in even sorting the sample on the left and the sample on the right gave great confidence in accurate sort gates and yield was maximized.



a. Poor sample prep and viability b. Good sample prep and viability

***Purity**:* Before each sort, the technologist will sort fluorescent beads with an accuracy of no less 99% purity. You can be assured that the instrument is functioning properly each and every time you sort. With that said, there are instances in which the sample will not allow for high purity. First of all, if the population to be sorted is not a separate population, that is, if the staining is so dim that the population does not extend far enough from the negative population, a gate drawn around the positives may include negative cells as well (see fluorochrome selection below). If the cells being sorted are too big for the tip being used (see above section of cell characteristics) fanning may occur and purity will decrease. If cells are non-specifically binding antibodies, you may be sorting false positives.

Yield: Not every positive cell that enters the instrument will be sorted. Cells that are not sorted are referred to as aborts, and there is an abort rate associated with every sort. The goal is to minimize the abort rate in order to maximize the yield. Cells can be aborted at three points during the sorting process. The first abort type is called the coincidence rate. If two cells enter the laser beam too close together, and the instrument cannot detect them independently, the instrument will abort them both. The percentage of cells aborted due to coincidence is typically around 5-10%, but no more than 15%. The coincidence abort rate will increase as throughput rates increase, that is, the faster your cells are going through the instrument, the more cells will be aborted. The second type of abort is referred to as the soft abort rate. This is the abort rate associated with the instrument attempting to sort the cells. In order to get high purity, if a negative cell is too close to the positive cell, the cell will not be sorted but aborted. The soft abort rate is typically less than 1%, but will increase as the sort rate increases, that is, if the instrument is sorting more cells per second, more cells will be aborted as well. The last type of abort is not intentional; this abort is called the fanning abort. Sometimes, a cell is sorted by the instrument, but misses the collection tube. The number of cells that are fanned varies greatly, but is due mostly to cells being too large for the tip size, the presence of dead cells, or cells sticking together. All of these aborts are NOT used in calculating the final number of cells that are put into your tube. *In a VAST majority of cases, the number of cells in your tube when you leave the lab is within 5-10% of what the instrument says is in there. If you are having issues with recovery when you get back to your lab. The most*

common solution is optimizing collection buffers and spinning the cells harder and or longer.

***Fluorochrome Selections**:* As with all flow cytometers, you want to use the "brightest" fluorochrome conjugated to the antibody that will bind the least amount of antigen on the cells. For instance, Phycoerythrin (PE) is typically thought of as a bright fluorochrome, and FITC is not, so you would use PE conjugated to an antibody that will bind few receptors on/in the cell (e.g. CD25), and FITC on an antibody that will bind many receptors on/in the cell (e.g. CD8). We can advise you on the best fluorochrome combination for your experiment. What we will need to know is the excitation and emission maxima for the fluorochromes you are using.

Sort Receptacle: The sorted cells can be collected into a variety of receptacles. From multi-well plates (6 well up to 1536 well), to 50mL conical tubes, any type of receptacle could be accommodated. Single cells can be deposited in individual wells on a plate, or millions of cells can be deposited into a 15mL tube. The cell will exit the sorter in a droplet of PBS. The volume of this droplet ranges from 0.001uL to 0.005uL. Under normal circumstances, the 70um tip will result in 1x10^6 cells/ml and the 100umtip will result in 3X10^5cells/ml. Any sort receptacle used should have some type of fluid in it. Whether it is PBS with 2% BSA or lysis buffer, your tube/well should contain an appropriate amount of fluid in it (be sure to leave enough space for your sorted cells, but not too much so the sorted cells get stuck to the side of the tube/well). Also, the sort receptacle can be kept cold if needed.

85um tip=1ml of sheath fluid put into your collection tube for every 420,000cells collected.

100um tip=1ml of sheath fluid put into your collection tube for every 300,000cells collected.

70um tip=1ml of sheath fluid put into your collection tube for every 1,000,000cells collected.

Sterility: Every sort is done aseptically regardless of what the user is doing with the cells. Due to the nature of sorting, no sort can be defined as "sterile." However, "sterility" checks are performed such that media sorted through the instrument can be cultured with no antibiotics without contamination. Many users have cultured their cells and injected them into laboratory animals without issue. The sheath fluid passes through a 0.22um filter before it goes into the instrument to ensure no "bugs" are present. Also, the sample tubing has bleach and ethanol run through it before and after each sort.

Quick review of Aria High Speed Sorter:
Laser lines: UV, 405nm, 488nm, 561nm, 640nm
Can detect up to 14 colors at once.

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