



## COMMON QUESTIONS

### How can I use the facility's instruments?

New users of the facility should complete an [account creation request](#). All new users must go through training before using the instrument.

### Which flow cytometer analyzers do you have?

We have the following flow cytometers in the facility:

- BD FACSCanto RUO (3 lasers, 12 parameters)
- BD LSRFortessa SORP (4 lasers, 18 parameters)
- BD LSR Fortessa (4 lasers, 18 parameters)
- BD FACS Lyric (3 lasers, 14 parameters)

### Which cell sorters do you have?

We have the following cell sorters:

- BD FACSAria II SORP (4 lasers) in a class II biosafety cabinet
- BD FACSAria II SORP (5 lasers) in a class II biosafety cabinet
- BD FACSAria Fusion SORP (5 lasers) in a class II biosafety cabinet

### How do I book a sort?

If you are a new user, complete an [account creation request](#). After your account is validated, go to the [CRI PPMS](#) website calendar, log in and book the time you need on the Aria calendars. You can book sorts up to three weeks in advance.

### How do I need to prepare my samples for sorting?

See our [Sample preparation for sorting](#) section.

### How long will it take to sort all my cells on your sorter?

The answer to this question will vary greatly. However, you can make some rough estimates. For the typical low-pressure sort with the 100  $\mu\text{m}$  nozzle, you can estimate that we'll run a threshold rate of about 3000 cells/second. This translates to about 10 million cells in one hour. For the typical high-pressure sort with the 70  $\mu\text{m}$  nozzle, you can estimate about 10,000

cells/second, translating to around 35 million cells in one hour. These estimates are conservative and speculative. We'll make adjustments to the rate of cells/second based on the efficiency and quality of the sort in relation to each investigator's needs and the situation.

### Which pressure or nozzle settings are right for my sort?

This depends on the size and characteristics of the cells more than anything else. The nozzle size should be about four to five times that of the cells that are being sorted. For sorts targeting most lymphocytes, you should run the 70 µm nozzle. For larger cells (monocytes), you should choose the 100 µm nozzle. Increased pressure and the smaller orifice of the 70 µm nozzle will expose your cells to a slightly harsher environment, albeit very briefly. With the 100 µm nozzle, the threshold rate will decrease a lot compared to 70 µm, so the number of cells sorted in an hour will be 1/3 less with 100 µm nozzle compared to the 70 µm nozzle.

### How do I delete the doublets?

Use a pulse geometry gate (such as FSC-H x FSC-W & SSC-H x SSC-W) to delete doublets.

### Where do I put my gates?

The controls for the experiment are critical for ensuring the proper cells are identified. A Fluorescent Minus One (FMO) control, for example, is critical in identifying the proper placement of a gate in a multicolor experiment. You cannot use an isotype control (for example) to correct the spread of the data due to the fluorochromes in the panel.

### How many events should I record?

The number of events is critical. Be sure to record the right number for your statistic analysis. Here is a table of how many events you need to record depending of the frequency and the accuracy you want. For an accurate number of cells, you can use also TrueCount beads.

TABLE 1: Determination of database/sample size that will provide a given precision in rare event analysis.<sup>(a)</sup>

Desired CV (%) →		1	5	10	20	40
$r = \text{no. of events of interest} \rightarrow$		10000	400	100	25	6
When occurring at a frequency of (%)	1 : n	Total no. of events which must be collected <sup>(b)</sup>				
10	10	$10^5$	$4 \times 10^3$	$10^3$	$2.5 \times 10^2$	$6.3 \times 10^1$
1	100	$10^6$	$4 \times 10^4$	$10^4$	$2.5 \times 10^3$	$6.3 \times 10^2$
0.1	1000	$10^7$	$4 \times 10^5$	$10^5$	$2.5 \times 10^4$	$6.3 \times 10^3$
0.01	10,000	$10^8$	$4 \times 10^6$	$10^6$	$2.5 \times 10^5$	$6.3 \times 10^4$
0.001	100,000	$10^9$	$4 \times 10^7$	$10^7$	$2.5 \times 10^6$	$6.3 \times 10^5$
0.00001 <sup>(c)</sup>	10,000,000	$10^{11}$	$4 \times 10^9$	$10^9$	$2.5 \times 10^8$	$6.3 \times 10^7$

<sup>(a)</sup>For cell-based assays such as flow cytometry, a simple calculation can be used to determine the size of the database/sample that will provide a given precision:  $r = (100/CV)^2$ ; where  $r$  is the number of events meeting the required criterion, and  $CV$  is the coefficient of variation of a known positive control. Modified from <http://www.icms.qmul.ac.uk/flowcytometry/uses/rareeventanalysis/index.html>, Queen Mary, University of London.

<sup>(b)</sup>With a WBC count in the low-normal range ( $\sim 5 \times 10^9/L$ ), 10 mL of blood would contain  $\sim 5 \times 10^7$  events.

<sup>(c)</sup>Estimated frequency of CTCs in the peripheral blood of cancer patients.

(Allan and Keeney, J Oncol 2010)

### **How do I sort rare cell populations?**

- Watch the instrument to ensure success:
  - Keep the core stream tight – the tighter the core stream (low differential pressure), the tighter the CV on resulting data, therefore the easier it is to determine the best placement of gates for sorting. Tight core streams also reduce coincident events (two cells passing through the intercept at the same time). Because flow cytometry requires single cells for proper analysis, the more doublets, the fewer sorted cells.
  - Eliminate the aborts. Aborts arise from a cell arriving at the laser intercept while the electronics are processing the previous pulse. This new event is lost (aborted). This results in loss of cells as well as decreased purity. Optimizing the window extension for the cell type can have a dramatic effect in improving the quality of the sort (and reducing the abort rate).
  
- Avoid the artifacts:
  - Viability dyes – It cannot be stressed enough that a simple viability dye improves the quality of the sort for downstream applications by reducing the miss-sorted false-positive cells that are dead.
  - Avoid the aggregates – In establishing a gating strategy, consider adding a pulse gate (height vs. area, for example) to eliminate cells that are aggregated.
  - Gate for success – Use the appropriate controls for gate placement and avoid the use of the histogram. Histograms mask data and make it impossible to find rare events.
  
- Keep the threshold low:
  - When a threshold is set up, the cytometer is blinded to any signal that is below that threshold value. Although it is possible to eliminate debris from the data using a high threshold, if the cell sorter cannot see the debris, it will find its way into the sort tube and contaminate the cells for downstream applications.

When setting up a gating strategy on a cell sorter, either with an operator or alone, make sure you have addressed the steps above. This will help ensure a good sort, even for rare populations.

### **How can I get FlowJo on my personal or lab computer?**

The facility administers the CRI site license for FlowJo analysis software. This results in a large discount compared to individual purchase. To participate in this program, you must be affiliated with UT Southwestern and be able to provide a valid account number for billing for this license.

To obtain a serial number for your computer, download and complete [this form](#) and email it to [nicolas.loof@utsouthwestern.edu](mailto:nicolas.loof@utsouthwestern.edu). You can also request it on [CRI PPMS](#).

### **How do I transfer my data on my personal computer for analysis?**

Use [Cytobank](#) to transfer your data. Cytobank is a safe way to manage, search, back up, share and analyze your FCS files over the web.

### **What services can Core Personnel Support Services provide?**

- Experimental design.
- Protocol development.
- Instrument operation.
- Data analysis.
- Graphical production.
- SpaaS (Sample Processing as a Service).

The facility can provide full services for your study: experiment design, protocol development, samples staining, instrument operation and data analysis. This is particularly valuable for clinical trial in flow when all steps must be standardized and equipment or personnel need certifications.