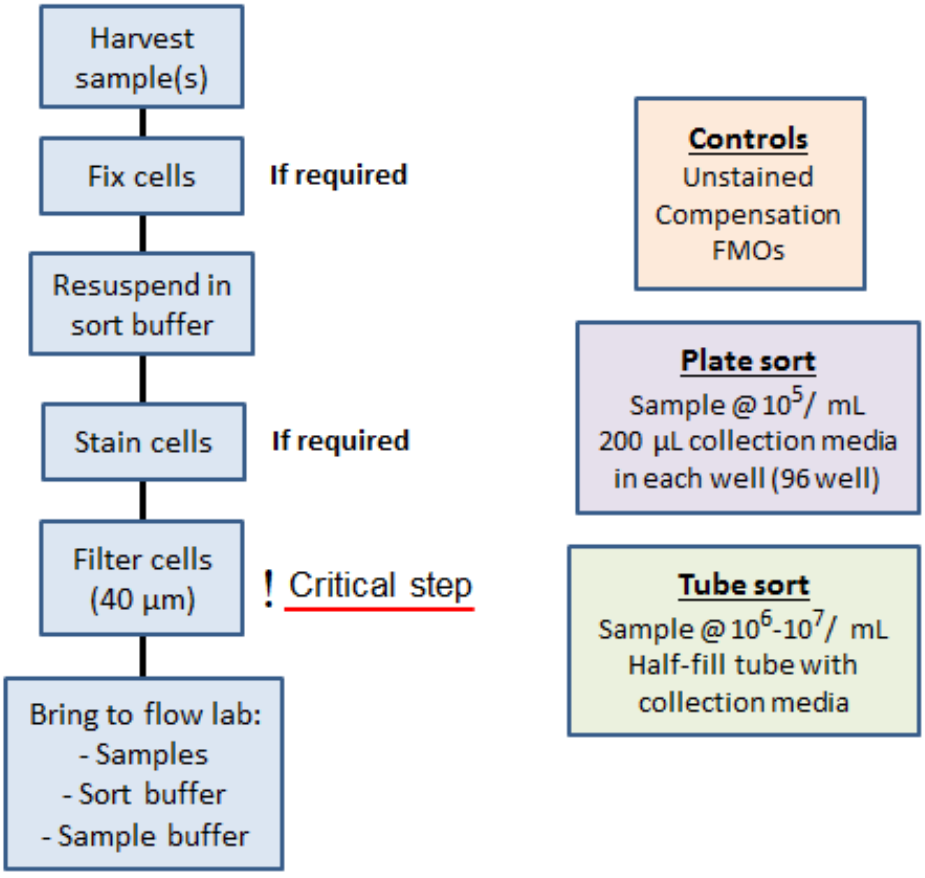
Cell Sorting

How it works?
You bring us sort sample(s) and appropriate controls and we sort the desired population within your sample. FACSAriaII: 3-lasers 405,488,633 nm; sort into tubes or plates Astrios: 5-lasers 355,405,488,561,630 nm; sort into tubes or plates; sort microparticles.
Remember, book your sort and submit a sort-form at least 2 days before sort-day https://dfcf.acls.analytical.unsw.edu.au/

Flow chart



Sort buffers

Basic	Sticky cell
1x PBS 1 mM EDTA 25 mM HEPES, pH 7 up to 3% serum	1x Hanks BSS 5 mM EDTA 25 mM HEPES, pH 7 up to 3% serum

Next page, gotchas

Gotchas /!\

There are a number of critical steps for successful cell sorts

- **Cells MUST be filtered**
  - Even if your cells don't need it.
  - **Consequence**: Machine clogs, you take a big hit to your yield. Lose sort time for the rest of your samples.
- **Bring correct controls**
  - **Action required**: Common controls: unstained, compensation, FMO
  - **Consequence**: We will have to guess which population to sort if we can even proceed with the sort.
- **Over confluent cells**
  - **Action required**: Keep your cells <70% confluent (adhesive), $8x10^5$ / mL (suspension)
  - **Consequence**: single-cell suspension will form aggregates faster, causing machine blockages even if filtered. Cells will be starving and not behave as expected (unless you always starve your cells?).
- **Low viability samples**
  - Action required: Add DNAse to sample
  - Consequence: DNA released by dead cells causes clumping
- **Low yield**
  - **Main Causes**:
    - Population of interest was not as represented as expected
    - Total cell count was lower than expected
    - Clumped cells – which those events were aborted
    - Machine capability (up to 40% loss (aria), 25% loss (astrios). This includes cells exploding on impact in the tube
  - **Solutions**:
    - Low % of desired cells; optimise transfection – don't just follow someone else's protocol, establish the best conditions for your work.
    - Cell loss; try to identify cause of loss (during washes, column), or increase input amount if possible
    - Clumped cells; avoid over-confluence, we can filter the sample just before the sort to reduce cells re-adhering, use DNAse to break up low-viability samples
    - Exploding cells; Astrios delivers higher yields for plate sorting

Sort time; as predictable as pachinko.

The sweet spot for a reasonable sort time is to sort a population with greater than 1% sample representation.

0.1	1.0	10.0	30.0	% cells of interest in sample
2,000				event rate / sec
1666.7	166.7	16.7	5.6	minutes for 200k
5,000				
666.7	66.7	6.7	2.2	
9,000				
370.4	37.0	3.7	1.2	

- Well, sort at 9000/s *all* the time!
- This is only possible if your cells are pristine. We usually run at 5000 events / s.