Cornell Institute of Biotechnology

Biotechnology Resource Center
Flow Cytometry Facility

Panel Design
Things to Do and The Don'ts

Things to Do:

- Preparation, preparation, preparation.
- Know your instrument configuration (determines fluorochromes and dyes you can use).
- Understand fluorochrome brightness.
- Know the antigen density as it pertains to your application (activation, etc).
- Know your gating strategy ahead of time – what we are now referencing as a "Biological Tree" – to determine co-expression.
- Pair Antigen with fluorochrome in a way that maximizes resolution (Dim Ag on moderate to bright fluorochromes and bright Ag on moderate to dim fluorochrome). Be mindful of co-expressing markers.
- If possible, fix your cells if not analyzing soon after the sample prep (fixatives need to be checked against fresh samples to see if possible.)
- Know the fluorochrome/dyes excitation and emission spectras (FITC, AF488, GFP, CFSE, SybrGreen…. Are all measured in the same channel)
- To minimize compensation spillover try to choose the primary detector off each laser first then pay attention to similar spectra emissions.
- Re-suspend cells in proper buffers.
- Optimize staining by titrating the antibodies before use.
- Check you are using the correct reagent species and clone.
- Include viability dyes – Fixable viability stains for cells you are fixing for IHC or to analyze at a later time.
- Be sure you have the appropriate controls: Cells only, single stained controls for spillover, biological/experimental controls, FMO's.
- Use appropriate blocking reagents to mitigate unspecific binding.
- Be sure the instrument has been properly QC'ed before use.
- Run samples on appropriate flow rate (ex – Cell Cycle should be run on low to minimize CV.)
- Choose your PMT voltages optimized for the best resolution of signal.
- Try to cover the samples from light.
- Keep samples at the appropriate temperature.
- Understand you may need to change the panel once you see the first set of data.
- Contact your flow core or BD support if you have questions sooner than later.

The Don’ts:

- Add more antibody to stain – more isn’t better.
- Assume every flow experiment is the same as your lab member – IHC is different than surface labeling, which is different than cell cycle, etc.
- Don’t measure the spillover for a reagent with a different one – ex: don’t use a AF488 single stained control for a FITC sample.
- Don’t assign co-expressed markers in channels that have large spread.
- Don’t take shortcuts.