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Q&A OVERVIEW

Controls for a Flow Cytometry Experiment

What controls do you need to run in a flow cytometry experiment? Choices:

1. **Single Stain**
2. **Biological**
3. **Unstained / Vehicle**
4. **Fluorescence Minus One**

SINGLE-STAIN CONTROL

Question: You have an x-color experiment on a fancy new cytometer. You inspect the instrument configuration and your dye choices and realize that some of the dyes will have emission spectra overlap in several detectors. What kind of control do you need to measure, and correct for, spectral overlap?

Answer: Single-stain controls. Single-stain controls tell us the spectrum of any given dye on the day we run our experiment. By subjecting the single stains to the same treatment as our fully stained sample, we ensure that we capture the same emission spectra for any given dye in the experiment and thus can correct for any spillover that occurs.

BIOLOGICAL CONTROL

Question: You are designing an experiment to monitor the expression of 7 antigens in an x- color assay from mice that are deficient in certain functional immune populations. You are unsure what the expression patterns will be but wish to compare the expression to a "normal" unengineered mouse of the same genetic background. What type of control will permit you to observe the normal expression of the antigens of interest?

Answer: Biological control. Any experiment where a meaningful biological conclusion is to be made needs a biological negative control. (Biological positive controls are also useful but only sometimes known or available). This control helps us understand the homeostatic gene expression profile versus a perturbed or pathogenic expression profile.

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UNSTAINED CONTROL

Question: You run an x-color experiment on a spectrally-enabled cytometer (more detectors on the machine than the number of colors you have in your panel) probing tumor-infiltrating macrophages obtained from the pancreas in mice. The samples were hard to come by, and there wasn't much material to spare. After running compensation and applying it to your samples, you observe three things in the data:

1. You cannot see the difference between a negative and positive population for a given antigen, especially with your macrophages of interest
2. The background fluorescence of "negative" populations appears higher than normal
3. You notice that some of your lymphocyte populations are over or under-compensated. What type of control would help you resolve these issues? Hint you must ALWAYS have this type of control.

Answer: Unstained control. We always need an unstained control tube to 1) have a reference point for the autofluorescence of our cells,
and 2) subtract the autofluorescence if possible, when compensating or troubleshooting our analyses.

FLUORESCENCE MINUS ONE (FMO):

Question: The new cytometer in the lab can run up to 30+ colors. This is a great windfall because you were restricted to only 12 colors before you could not observe cytokine secretion or functional profiling without running multiple panels. The prospect of the new and previously unexplored markers is too strong to resist. You design a new 30-color panel but at least 10 antigens are new to you, and 2 of those have never been observed using flow cytometry. What type of control would help you ensure your gating was correct with respect to the positive/negative boundaries for the antigens that are new and/or unknown to you

Answer: FMOs are extremely useful for determining the +/- boundaries especially when working with new panels, new antigens, or antigens whose expression is unknown or smeared (e.g., CD14, IFNg). FMOs include all the fluorophores except the one(s)of interest; thus they account for the dye spillover (think the width of the negative population) and tell you where the true positive signal will lie. You can then easily set a gate between positive and negative boundaries.