



Dear Labby,

I have a question about repeating experiments. In my case it isn't about how many times but whether to repeat at all. I know that sounds nonscientific, but here's the situation. I'm a second-year graduate student just getting going on my thesis research. Our lab studies a growth factor signal transduction pathway in mouse ascites tumor cells. My project began with me perfecting the immunoselection of a certain protein complex. Then we sent it to a campus core facility for proteomics by mass spec. The results came back and revealed the presence of several novel proteins I have now cloned and am expressing as GFP fusion proteins. This will allow me to study their localization and dynamic behavior in these cells, plus I am designing siRNAs to do knockdown experiments.

At a recent thesis advisory committee meeting one of the professors asked me about replication. I told her sure, I will repeat all my experiments, as is customary. But then she floored me by saying "No, I mean the isolation of the complex and the mass spec." My PI said this was "ridiculous," adding, "Plus it's too damned expensive." She later apologized to the committee member for calling her question "ridiculous." This got me wondering. Would Labby comment?

—A Nonrepeat Offender?

Dear Nonrepeat Offender,

Let's start by placing your question in the context of the three cornerstones of measurement: accuracy, sensitivity, and precision. Accuracy is the proximity of a measured parameter to the true value. This is unknown in many cell biology experiments and definitely unknown in your project (i.e., no Maxwell's demon dove into your cells and reported back exactly what proteins are or are not in the complex you are studying).

Sensitivity is the limit at which a given method can no longer detect the desired substance, event, or whatever. In your project, sensitivity is reasonably high given today's proteomics; your sensitivity for detecting the expressed proteins is also reasonably good (but not at the single molecule level, for example). In addition, your sensitivity for detecting effects in siRNA knockdowns cannot be predicted (because, assuming you actually get good knockdown, you don't know how dependent the observed phenomena are on the protein concentration, i.e., what is the shape of the concentration vs. effect curve?).

Finally, precision refers to the degree to which replicated measurements will give the same reading or finding. That is, the first measurement has no greater meaning than any other—precision refers to the degree to which the whole set of measurements vary from one to another. Since this is the focus of your query, let's look at the situation in detail.

In principle, all experimental work should be repeated enough times to support a negatable hypothesis at some community-accepted standard(s) of statistical confidence. In many studies where adequate amounts of data can be collected in repeated experiments, the analysis of variance is straightforward. In much of cell biology, however, observations are presented as typifying a certain percentage of cells in a population. Thus, findings are said to have been "consistently observed in multiple experiments." (This statement, of course, provides the reader with no information whatsoever regarding the precision involved.)

Nevertheless, there is room for common sense. For example, if an investigator makes an antibody (in a rabbit, or camel, or a monoclonal), and it displays adequate high affinity for the job at hand, and demonstrably high specificity, should the investigator drop everything and raise it again? No. (Of course, it might not be a "replicate" experiment anyway, in the narrow sense, as the immunized animal will not likely construct the very same antibody the second time, i.e., the same amino acid sequence.)

As to your PI's point about cost, it can certainly be a valid factor depending on the context. Does anyone really think that any of the sequenced genomes should be re-done *ab initio*, given the precision inherent in the overlapping coverage and multiple reads? (This is not to say that regions that stubbornly resist cloning or sequencing should not be worked over again and again, or that intraspecific genome sequence variation should not be pursued, as it is being intensely. The point is that those represent different issues than questioning the precision by which the genome sequences were determined.)

In your project, the question is whether the one immunoselection you did is sufficient. After considering questions of accuracy, sensitivity, precision, and cost, Labby would agree with your committee member that one immunoselection is insufficient. All sorts of variables may be at play. For one, you take your cells out of their only known, productive culture dish, the mouse's peritoneum. Perhaps on a repeat (or in the first experiment) maybe the needle inadvertently tore the intestine. Or maybe your conditions of cell lysis would not be quite the same. Might you go to lunch one time while the cells were in Triton (for a specified 20 minutes but actually 45) and not another time? And what about those proteomics results? You seem pleased by the presence of "novel" proteins, but is not this very attribute of the results all the more reason to conduct a repeat? Bear in mind that in your live cell experiments you will not be looking at the proteins' actual associations, unless you plan to use FRET or related methods. Moreover, how de-riched was your first mass spec-analyzed complex in various proteins? You want to end up arguing that you started your project from the foundation of a "specific complex." How enriched was it relative to, say, the ribosomal protein or cytokeratin content of your cells (assuming ribosomes and intermediate filaments are not suspected to be part of your complex)?

On balance, it seems best to do at least one additional "replicate" immunoselection and mass spec run. You will likely see some variation from your first results, but if the same proteins show up once again and in similar abundance, you will never be sentenced as a nonrepeat offender. ■

—Labby

Direct your questions to labby@ascb.org. Authors of questions chosen for publication may indicate whether or not they wish to be identified. Submissions may be edited for space and style.