DEAR Labby

Repeating an Expensive Experiment

Dear Labby,

I am a fourth-year graduate student looking forward to completing my degree. I recently did a combined microarray analysis for both microRNAs and messenger RNAs that go up or down (or don't change) in my experimental system when I give the cells phorbol ester (this is a cancer project). My lab head loves the results, and they will be part of my thesis and a paper I am starting to write. But last week at my penultimate thesis committee meeting a member stopped me short with a question: Will I repeat this analysis? My lab is underfunded and the first microarrays cost a lot. I know experiments should be repeated, but does this apply to very expensive ones?

— *N = 1*

Dear N = 1,

This is an issue that has arisen widely in biomedical research as certain very costly methods have come upon the scene. Projects involving microarray analysis or the construction of a gene knock-out mouse are expensive, and concerns about costs have been aggravated by the decline in National Institutes of Health funding. It is a genuine dilemma (that word coming from the Greek for "two premises").

The "pure" answer is that your microarray analysis must be repeated. And (are you ready for this?) more than once, at least in principle. Biostatisticians have often commented that N = 2 is the most dangerous number. First, it encourages the investigator to believe (falsely) that the observation has been sufficiently replicated. (But try flipping a coin several times and often you will get a run of four or five heads or tails.) Moreover, these statistical gurus remind us that when N = 2 the ability to apply standard or advanced tests for the analysis of variance is severely compromised. This is about "precision," a term often misunderstood or misused that means reproducibility (not "accuracy," "robustness," or anything else).

However, there may be situations where common sense forces a compromise. To posit an extreme case, just to make the point, let us say a lab decided to determine the genome sequence of a certain spirochete, one that has been postulated to have contributed flagella to eukaryotic cells by an endosymbiotic step in evolution. The lab spends $50,000 to do so and then its National Science Foundation funding runs out. Meanwhile, the genome sequence (done once) tells, or at least very plausibly presents, the evolutionary story. The lab goes forward with a new hypothesis, and the community of eukaryotic evolution is thus enriched. Is there anything wrong with this story? In this case an "experiment" with N = 1 would be enabling, especially if the solitary genome analysis itself has multiple reads of contigs in the library.

Your case is obviously less straightforward. The first question is whether there was anything in your RNA isolation that might have been worrisome. Second, was there anything in either the microRNA or mRNA profiles that struck you as suspicious or implausible based on the known biology of your system? Such anomalous results can be huge clues that there are experimental errors (or that previous hypotheses were ill-founded), and if you see such problems you might want to prevail on your cash-strapped PI to run a repeat. Absent a repeat, you of course need to be above board and convey in your publication that the analysis was done only once. The guild will accept that as appropriate and properly weigh it in their assessment of your work. If you are not making an extreme claim, e.g., that Oswald Avery was wrong and that the gene is actually a carbohydrate, your thesis research publication(s) will not be tarnished by an experiment done only once, if that fact is fully revealed. ■

—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.