Scientists

Building and Sharing Resources

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Bill Skarnes' development of gene trapping methods and his leadership of the International Knockout Mouse Consortium caught our attention. Curious to know more, *BioTechniques* contacted him to find out about the ambition, character, and motivation that led to his success.

What pivotal event would you say defined the focus of your research?

Actually, the experiment that most shaped my career was a failure. As a Ph.D. student in Alexandra Joyner's lab in Toronto, I was trying to knock out the transcription factor engrailed-2 (EN2) in mouse embryonic stem (ES) cells. At that time, nobody had succeeded in knocking out a gene for which there was no direct selection. My idea was to insert a *lacZ* reporter into the EN2 gene to form a *lacZ*-EN2 fusion protein. Then I planned to screen colonies for nuclear beta-gal staining in the hope of enriching for the correctly targeted event. But instead of knocking out EN2, I ended up knocking out everything else with that experiment, leaving me with a lot of *lacZ* positive clones. Those clones actually turned out to be cells with genes knocked out at random; with a single experiment, we had knocked out hundreds of genes.

So, we started thinking that if we did enough of these experiments, maybe we could knock out every gene in the genome using this approach. In the end, that turned out not to be the case, but it was the inspiration for our work in scaling this gene trapping method, improving its efficiency, and refining it so that we could characterize thousands of insertions.

These efforts led to a complete library of knockout ES cells for every gene in the mouse genome. We started to build this collection in the late 1990s using the high-throughput gene trapping method I just described, and then switched to systematic gene targeting to finish the job. The library is currently available as a public resource that I think is going to be a great benefit for the whole community for many years to come.

How are you and others using the collection now?

We are taking advantage of the knockouts to study certain developmental processes. Since I began my Ph.D. studies, my goal has been to understand the programmed events underlying early cell fate decisions in mammalian embryos. Life begins with an embryo that is totipotent and progresses through differentiation and specialization of tissues. What is happening in these cells at the genomic level? What series of events causes a cell to commit to one fate and specialize? These are very important questions in developmental biology and we have been chipping away at solving them by looking at the effects of single genes or a few genes in combination.

Now new technologies in transcriptomics, epigenomics, and proteomics allow researchers to look at what is happening in these cells as they differentiate at a global level. We expect that researchers in these fields will apply the resources and collections we have generated to a wide variety of other questions in basic cell biology and development as well.

How are researchers able to access the resources you have built?

For scientific progress, I think it's very important that reagents are shared freely, openly, and without restriction. We have generated this resource, but if no one uses it, it is worthless. An important part of all of this work has been to make sure that the resources are visible and available to the scientific community. We have put a lot

of effort into building websites to present this information and provide details for ordering the materials. We need to make sure that this information is available to the widest possible audience.

With the mouse knockout collection complete, what will be your next step in gene trapping?

Human ES and iPS cells have many properties similar to mouse cells, so perhaps we could develop technologies that would allow us to generate a resource of knockout human cells. The main challenge in working with human cells is that, in order to understand gene function, we have to knock out both gene copies. With mice, we can knock out one copy of the gene, put the resulting cells into mice, and obtain the homozygous mutation through breeding. With human cells, this is not possible.

I have always been interested in new technologies that could be applied to this problem, and it looks like zinc finger nucleases may provide the answer. If we can engineer site-specific endonucleases to cut the genome, we can mutate both copies of the gene simultaneously. We have begun preliminary experiments to adapt zinc finger technology so we can generate a resource of mutant human cells, and just as we did previously, we plan to take the best technology available and scale it up.

Interviewed by Kristie Nybo, Ph.D. Image courtesy of Antje Burger.

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