Automating a Mouse

There is a powerful force sweeping through much of biological science: Automation. Spurred by the convergence of robotics, software, and molecular biology, automation is transforming entire fields.

SIGHTINGS

Given an automated solution to a problem in molecular biology, scientists can ask big questions: "What if we sequenced every gene in organism X? What if we made billions of compounds to look for drug targets for receptor Y? What if we isolated every Z?" Now a group at Lexicon Genetics (*1*) has asked another bold question: "What if we could systematically create mouse strains with knockouts of every gene?"

There is great interest in disrupting genes in the mouse genome and analyzing the resultant phenotypes. Often, these knockout mice yield clues for unraveling the mechanisms behind human diseases. Knockout mice, particularly in the field of neuroscience, provide clues to complex pathways that can only be studied in the intact organism. However, creating a knockout mouse can be laborious. The DNA constructs must be carefully designed to serve as targeting vectors. This step itself can require significant effort, involving gene mapping and multiple cloning steps. Next, embryonic stem cells must be manipulated to produce clones with individual gene targeting events. Positive clones are then introduced into mice where breeding must be done to look for introduction of the mutation into the germ line.

To begin to automate this process, Lexicon Genetics looked at the underlying problem differently. Instead of making just one knockout mouse for each gene, they decided to make a library of randomly mutagenized mouse cell lines from which individual mutant strains of mice could be generated.

They first designed a new vector system that could be randomly integrated into the mouse genome. A selectable drug marker, puromycin, was placed downstream of a strong and fairly ubiquitous promoter, that of the *PGK* gene. A consensus splice donor sequence was engineered at the end of the *PGK* gene. By itself, this plasmid will not confer puromycin resistance, because it lacks any 3′ polyadenylation sequences. However, upon integration into a mouse gene, the splice donor can serve as a way to link to a downstream acceptor and form a functional messenger RNA. The result is a puromycin-resistant clone.

In practice, the researchers employed both electroporation and retroviral delivery strategies to produce a bank of embryonic stem (ES) cell clones. They analyzed 3000 individual clones in detail, by isolating the 3′ insertion junctions with polymerase chain reaction (PCR) and performing sequence analysis on the DNA, which they termed Omnibank sequence tags (OSTs). Comparison of these OSTs to existing DNA databases gave interesting results. About 18% of the sequences seemed to match already known genes, 10% matched human or rodent expressed sequence tags (ESTs), 10% matched repetitive genomic elements, and 61% of the sequences were unique. The latter is important, as it suggests that random sequencing of OSTs can be a good way to expand databases of transcribed genes.

Zambrowicz *et al.* (*1*) described one targeting event that occurred in the Bruton's tyrosine kinase locus. Southern blots showed that the inserted DNA disrupted this gene by inserting into the first intron. In fact, they could show that 44% of all insertions in known genes occurred within 350 nucleotides of the 5′ end of the DNA. Thus, for creating gene inactivation mutations, the closer the insertion is to the 5′ end, the better.

Using a 96-well format, the authors claim they can process 500 mutant ES cells per week. But just how many clones would be needed for a complete knockout library? To get a clue, they looked for knockouts in a gene for which the null phenotype could be selected, *Hprt*. They estimate that it took 80,000 unique insertions to produce one *Hprt* disruption event.

With its high selectivity for identifying transcribed sequences, the Lexicon gene trap procedure can have broad applicability. For creating populations of knockout mice, the technique seems scalable. It will be a challenge now to finish a library's worth of cell lines. Once in hand, investigators should be able to use PCR-based strategies to identify clones with insertions in their favorite gene and move on to the process of creating new mouse strains.

–Robert Sikorski and Richard Peters

References

1. B. P. Zambrowicz *et al.*, *Nature* **392,** 608 (1998).

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Zeptomolar Damage

Ionizing radiation kills cells largely by its effect on DNA, inducing various lesions such as strand breaks, base modifications, and DNA-protein crosslinks. The current methods for measuring DNA lesions caused by ionizing radiation consist of assays that have limited detection capabilities. These techniques include gas chromatography–mass spectrometry, high-performance liquid chromatography (with electrochemical and mass spectrometry detection), postlabeling, and immunoassays. These techniques are not sensitive enough to measure the effect of low-level environmental radiation, and in some cases, can introduce oxidative DNA lesions through the sample manipulation itself. To date, scientists have circumvented sensitivity limitation of available techniques by exposing cells or whole organisms (that is, rodents) to radiation doses several orders of magnitude higher than clinically relevant doses. They then extrapolate back from the dose-response curves to postulate on the effect that low-level radiation may have on biological systems.

SIGHTINGS

A report in this issue of *Science* may change all of this and allow scientists to monitor the actual effect of low-level DNA-damaging agents (such as ionizing radiation or carcinogens) with a sensitivity that was not possible before (*1*). The system relies on the use of monoclonal antibodies which recognize specific DNA lesions. For instance, the authors used mouse antibodies to 5,6-dihydroxy-5,6-dihydrothymine (thymine glycol), a specific product of oxidative damage in DNA. They also used a secondary antibody labeled with tetramethylrhodamine, a fluorescent probe. For the separation of molecular entities, capillary electrophoresis was employed, because this technique allows fast sample resolution and requires little amount of sample material. Laser-induced fluorescence measurement was used for detection, because this technique provides selective excitation of the analyte to avoid interferences and, hence, provides a very sensitive way for making quantitative measurements. Altogether, the high degree of specificity provided by the monoclonal antibody to a single DNA lesion combined with the high sensitivity of the separation/detection system yielded detection limits in the 10^{-21} molar range (zeptomoles). Remarkably, sample manipulation is limited to DNA extraction, incubation with antibodies, and capillary electrophoresis; as a bonus, only nanogram amounts of DNA are needed. Although pulse-field gel electrophoresis and single-cell gel electrophoresis are also sensitive methods, their use is principally limited to the measurement of DNA strand breaks. So, the approach described by Le *et al.* (*1*) represents an improvement of 4 to 5 orders in magnitude compared to currently available techniques for detection of DNA base damage.

As proof of principle, the authors tested their new method with cellular DNA and naked DNA. They exposed A549 human lung carcinoma cells to 0.05 Gy and detected 4.3 thymine glycols per 10^9 bases, with a detection limit of 1 thymine glycol per 10^9 bases. When these results were compared with naked DNA, either extracted from