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

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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

A549 cells or using calf thymus DNA, they found that cells provide about 100-fold protection to their DNA. They also applied the method to demonstrate that eukaryotic cells turn on inducible DNA repair in response to low-level irradiation. A549 cells treated with 0.25 Gy 4 hours before a 2-Gy irradiation showed an enhanced rate of initial thymine glycol removal compared to A549 cells that were not pretreated.

Armed with such a specific and sensitive assay, one can only begin to imagine some of the possibilities. For instance, scientists should more easily detect the type and frequency of DNA lesions in living tissues after exposure to environmental radiation or chemical carcinogens. Monoclonal antibodies targeted at other base lesions could be used to look at a series of DNA or RNA lesions. The system could also be used to monitor protein adducts or drug metabolites, as long as monoclonal antibodies are raised to specific moieties. There is little doubt that, over the forseeable future, we will see an explosion in the number of reports making use of this seminal technique in the various fields of life sciences, from toxicology to molecular biology.

–Richard Peters and Robert Sikorski

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Internet Connectivity

NET TIPS

Over the past few months, we have looked into the details behind a variety of Internet topics, ranging from opening files transferred via the Internet to finding sites on specific topics. Although it is obvious that the Net will find an ever increasing role in the day-to-day activities of scientists, it is not obvious today how any given scientist will actually connect to the Net in the future, particularly when the scientist accesses the Net from home.

For discussion purposes, all of the hardware and software involved in a typical user's access to the Internet can be grouped into a client (your PC), a connection (hardware and software for access), and a server. This month, we look into competing connection technologies that are likely to dramatically speed the way information is piped to your desktop. We organized the technologies in order of increasing speed, which is measured in Kbps (1000 bits per second).

1. *POTS*. The standard "plain old telephone service" connection to the Internet is the slowest, but most ubiquitous. When you dial up to an Internet service provider (ISP) or your university, you are using POTS. Currently, modems that run at 28.8 Kbps are the most common POTS gateways to the Internet. The top speed of modems today is 56 Kbps, but they will only work at that speed if your ISP has special hardware on their end, so you should check into this before you make the investment. 56K modems cost between $100 to 250 and work on a regular phone line. Several include ISPs include 56K modem support in their basic pricing; others will charge about $10 more per month to provide 56K access.

2. *ISDN*. An integrated services digital network (ISDN) connection to the Internet can usually be obtained from a local telephone company. ISDN coverage varies greatly around the country and you should contact your local phone company to check for availability and pricing. Once in service, an ISDN connection can be treated like an additional phone line and used for making ordinary calls. For the Internet, the use of a special modem connects a computer to an ISDN line and the Internet at maximum speeds of 128 Kbps. A nice feature of ISDN is that you can split it into different channels, allowing you to access the Net and make a telephone call at the same time. ISDN modems can be difficult to set up and configure, and the monthly service fees can be expensive. An ISDN adapter costs between $400 to $800. The set-up fee that your local phone company will charge is about $100, then you will have to pay them $10 to $20 per month. Your ISP also has to support ISDN and will charge you between $20 to $40 above their basic service rate.

3. *Satellite*. Next on our list of connection options is the use of a small, 21-inch satellite dish. Although this may sound high-tech, the service does exist as a commercial product and is accessible throughout most of the United States. The advantage of satellite-based Internet connections (400 Kbps maximum speed) is that there are few geographic limitations to their use. You simply point the dish at a defined angle and direction in order to target the service provider's orbiting satellite. Then, you install a card in your PC that connects the dish to the computer. Satellite Internet connections won't be cheap, since you need a POTS connection as well. A phone connection is also needed because the outgoing request is actually sent via a POTS line. In fact, all outgoing messages—including file transfers—must be done through the slower phone connection. Depending on quality of access, service can cost from $100 to $200 per month.

4. *Cable*. The same cable that delivers TV channels to your home can also deliver high-speed Internet connectivity. Speeds obtained by a cable modem connection are impressive and can theoretically reach 10 Mbps. Cable modems use co-axial cables to transmit data. The effective download speed is between 1.5 to 3 Mbps, rivaling the speed form T1 lines. The speed for uploading documents is usually less than that. The major drawback of cable modems is their lack of availability. Many areas are not yet wired with the fiberoptic network required to run cable modems. While your speed will be less depending on how many multiple users are configured in your neighborhood, cable modems provide the fastest access in the home setting. A cable modem may even be faster than the access a scientist has in their university lab. Another bonus is that you don't have to dial up to connect a cable modem to the net. It is always on. Given their rather inexpensive price, less than $50 per month (with the cable TV channels), this method is hard to beat.

5. *DSL*. The digital subscriber line is a service that is provided by your telephone company. With maximum speeds at about 8 Mbps, a DSL line is similar to a cable modem. DSL uses conventional copper phone lines. There are several DSL varariations: high-bit-rate DSL (HDSL), symmetric or single-line high-bit-rate DSL (SDSL), very high-bit-rate DSL (VDSL), dedicated ISDL DSL (IDSL), and asymmetric DSL (ADSL). Set-up fees and modem charges can total $1000 and monthly charges are about $200 per month at the moment. Like cable connections, a DSL line is always live on the Net. DSL service is limited and can be expensive to set up, but this technology has the potential for widespread use.

6. *Internet2*. The Internet is being redesigned by a group of more than 120 U.S. universities with the goal of providing high bandwidth connections that can be used for research purposes. Internet2 will not replace the current Internet, but it will serve as a platform to achieve communications speeds that are 100 times faster than currently possible. For more information, check out the Internet2 Web site at www.Internet2.edu. Here you can find out about current demonstration projects that have applied Internet2 technology to topics such as three-dimensional brain mapping, molecular modeling, microscopy, and virtual reality anatomy.

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