Mighty mouse

Coordination and integration of the results of animal research are an ever-increasing challenge. Jane Qiu finds out what happens when big biology meets a small rodent.

If mice could build a high-rise supercity, it might look something like this. Stack upon stack of clean, shiny plastic cages gleam inside the air-conditioned suites at the Wellcome Trust Sanger Institute in Cambridge, UK. With a capacity to house thousands of mouse lines, the institute has the ambition of becoming the world's largest warehouse for knockout mice, in which individual genes have been selectively deleted, or 'knocked out', from the genome. There are about 25,000 mouse genes, so the scale of the project is enormous and the cost is astronomical. "No single institute will be able to accomplish this alone," acknowledges Allan Bradley, director of the institute.

The Sanger Institute is part of an international effort to accomplish this feat, which promises to change the way mouse genetics is done for ever. "Biology is not a cottage industry anymore. We have to move away from thinking about one gene and one project at a time," says Steve Brown, director of the Medical Research Council's Mammalian Genetics Unit in Harwell, Oxford. "To face the enormous challenge of understanding the genome, we should be thinking about projects that will allow us to access every single gene."

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It sounds ambitious, but such projects are very much a sign of the times. To crack the big questions about how something as complex as a mammalian genome works, biologists are increasingly turning to multi-million dollar projects that aim to capitalize on all the information coming out of genome-sequencing projects. Although researchers are certain that the mouse projects will yield a wealth of valuable new knowledge, questions remain about whether this approach is really the best one. "Although there is no doubt useful models will result, would the outcome of these knockout projects justify the cost and colossal number of animals involved?" asks David Threadgill, an expert on mouse models of human disease at the University of North Carolina at Chapel Hill. "That's the million-dollar question."

Certainly, such large-scale approaches can pay off. Last week, the Allen Institute for Brain Science in Seattle, Washington, published the results of its three-year endeavour[1] that looked at the activity in the mouse brain of almost every gene in the genome — at a cost of US$40 million. "This is a relatively small cost for the huge amount of information we have gained," says Ed Lein, a director of the neuroscience division at the Allen Institute. The resulting atlas provides significant insight into the brain's function and lets scientists measure the differences in the level of a gene's activity in different parts of the brain.

Big ambitions

The mouse mutagenesis schemes, however, are the most ambitious projects yet. Two research initiatives — the European Conditional Knockout Mouse Mutagenesis (EUCOMM) and the Canada-based North American Conditional Knockout Mouse Mutagenesis (NorCOMM) programmes — were launched in October 2005. They were joined a few months ago by the Knockout Mouse Project (KOMP), which was spearheaded by the US National Institutes of Health (NIH). At a total cost of more than US$70 million, the immediate goal of these projects is to turn the first steps to making knockout mice into a high-throughput enterprise by disabling genes in embryonic stem cells. Japan and China have also indicated an interest in developing similar projects. The teams plan to establish centralized archiving systems to distribute reagents and mice to research communities around the world. This is only a prelude to the ultimate goal of generating and characterizing the entire cohort of 25,000 knockout mouse lines, which would cost at least another US$600 million.

A further project, the European Mouse Disease Clinic (EUMODIC) will be launched next February with a handsome sum of €12 million (US$16 million) for creating another 300 knockout mice and analysing 600 mouse lines in total, including those that will be generated by EUCOMM. Meanwhile, the Mouse Genetics Programme at the Sanger Institute is planning to create and characterize 250–500 knockout mice per year in the following decade. "Ultimately, we would like to have comprehensive genotype and phenotype databases for every single knockout," says Brown, who heads EUMODIC.

Geneticists are no strangers to attacking genomes with high-throughput, production-line approaches — they have been doing it for years with yeast and fruitflies, and even vertebrates, such as the zebrafish[2]. But trying to pull off a similar trick in a mammal will be much more costly. Mutants of yeast and fruitflies are easy to produce and cheap to maintain. The generation of mouse knockouts is more involved and expensive, yet these costs are dwarfed by the price tag of maintaining the animals and characterizing their physical attributes, or phenotype.

Another big issue concerns the implications for animal welfare. Depending on individual genes and the method used to produce them, the number of mice needed to establish a line stretches from 50 to several hundred. On top of this, another couple of hundred animals are needed for basic analyses of genetic make-up and phenotype. So, for the 25,000 genes in the mouse genome, more than 7 million animals would be needed to generate and characterize all the knockout lines.

And the consequences of knockout mutations vary greatly, from small effects that are barely noticeable to debilitating conditions. "To closely monitor potential and actual suffering and distress in those animals on a large scale is very challenging," says David Mor-

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ton, a biomedical ethicist at the University of Birmingham,UK. In Europe, bodies that regulate animal research are committed to the principle of the Three Rs — refinement (to minimize suffering and distress), reduction (to minimize the number of animals used) and replacement (to avoid the use of living animals). Is the spirit of the knockout projects in line with this principle? "That is the issue researchers, funding agencies and regulatory bodies have to consider very carefully," says Morton.

A balancing act

Proponents would argue that the benefits in terms of making large advances in biomedical knowledge balance these costs. And many, such as Karen Steel, director of the Mouse Genetics Programme at the Sanger Institute, say that even though a huge number of mice is needed, the coordination and technological efficiency of these projects mean that, in the long run, fewer mice would be used than if individual labs were left to their own devices. "This kind of high-throughput project will, in fact, reduce the number of animals used in research," she says. "It will be a lot more cost-effective than what can be done by individual laboratories."

The idea of an international mouse knockout project sprang from the difficulties that researchers had in generating or obtaining mutant mouse lines. Not all laboratories have the capacity to create knockout mice. And finding out which mutant lines are available is not always easy, let alone getting hold of them. A recent survey conducted by the NIH[3] shows that researchers have knocked out 11,000 mouse genes, most of which are not reported either because they belong to the private sector or because they do not have obvious phenotypes. Of the 4,000 unique knockout mouse lines that have been published, only 1,000 are in public repositories. As a result, there is a large duplication of effort: more than 700 knockouts are generated three times or more; in an extreme case, a mouse line is replicated 11 times.

"There isn't a common mechanism in place to enforce deposition of knockout mouse lines into public repositories," says Wolfgang Wurst, coordinator of EUCOMM and director of the Institute of Developmental Genetics at the German National Research Centre for Environment and Health (GSF) in Munich. Many knockouts were not generated in a standardized way or properly characterized, he adds. So, rather than trying to 'recover' mouse lines scattered around various laboratories, the researchers have come to the conclusion that the way forward is to generate a comprehensive and public resource of mutant embryonic stem cells — almost from scratch — by capitalizing on efficiencies of scale and a centralized production effort.

The enormous value of the mouse in biomedical research is not in dispute. Mice provide useful models for human disease and have helped researchers to make important breakthroughs in basic research in fields ranging from molecular biology to behavioural science. "Nobody would work with animals unless it were the only way to address biological questions," says Steel. "It's much easier and cheaper to work with cells in a Petri dish, but that doesn't necessarily tell you how genes behave in living organisms." Instead, much of the debate about the mutagenesis projects centres around their cost-effectiveness and the validity of their scientific approaches.

To many researchers, it is one thing to generate a useful resource for a wider scientific community, and yet another to create and characterize 25,000 knockout mouse lines as the starting point to understand gene function. Some researchers doubt whether this is the most efficient way to obtain useful information about how the genome works.

The complexities of function

A key issue is that the function of genes is context-dependent — it depends on the genetic make-up of individual animals. "So it is not a matter of which genes do what, but how they behave as part of a complex genetic network," says Allan Balmain, a cancer biologist at the University of California, San Francisco. Researchers often come across the problem that inactivating the same gene can yield very different phenotypes depending on the breed or strain of mouse. The strain used in research is therefore often crucial for the specific biological questions being addressed. So much so, in fact, that the Jackson Laboratory in Bar Harbor, Maine, launched the Mouse Phenome

Project in 2000 to collect baseline physiological and behavioural information for 40 different inbred strains of mouse, so that researchers could choose the most appropriate strain for their area of interest.

But it turns out that, for technical reasons, the different groups of the knockout project have used different strains of mice as their starting points. EUCOMM and NorCOMM are using the 129 mouse — the strain in which knockout technology was first developed. But this strain has some conditions that mean it is not usually chosen for a number of disciplines, including immunology and behaviour. Most researchers work with a strain called C57BL/6, the strain that KOMP has chosen to make its knockouts, although the technology to do this is less efficient than that for the 129 mouse. Once the technology is better established, EUCOMM and NorCOMM will switch to the C57BL/6 strain. It is not clear how easy it will be to compare results from the different strains.

D. THREADGILL/UNC

Mouse supercity: warehouses of knockout mice could provide vital data about genes and their function.

A cog in the works

Another issue taxing mouse geneticists is whether these production-line systems will be sensitive enough to detect every aspect of a phenotype. In many cases, a knockout mouse does not show any obvious phenotypes, and researchers often have trouble knowing where to look for subtle phenotypes even when they have some idea what the gene does. This happens because genes often have overlapping functions, and can compensate for each other if one is lost.

To get around this problem, researchers frequently need to breed several knockout strains to generate double- and triple-knockouts, in which two or three genes are disabled simultaneously. "Using the knockout approach to study genes with unknown function on such a large scale may not be the most effective strategy," says Threadgill. William Richardson, a neurobiologist at the Wolfson Institute, University College London, agrees: "Phenotyping is not a straightforward business. We have moved a long way from the big dream that knockouts will explain everything."

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But not everybody agrees. Martin Hrabé de Angelis, director of the German Mouse Clinic at the GSF, argues that knocking out a gene because you think it might be involved in a particular process can mean that new leads are missed. "Many researchers got their working hypothesis wrong and therefore focused on the wrong pathways," he says. "And few would do comprehensive phenotype assays to study areas outside their specialities." In his clinic, established in 2001 and a partner of EUMODIC, 240 parameters in 14 different areas are measured routinely as part of a high-throughput phenotype screen of mutant mice. Of the 50 knockout mouse lines studied, 42% of them have new phenotypes not detected before.

Some researchers are not convinced. "Phenotype studies are more than just getting a bunch of numbers," says Richardson. Indeed, to uncover the mechanism for a particular characteristic, one needs to ask the right question. "It is a concern that we are going to end up with a lot of superficial phenotypes across many domains, but none of them is deep enough to answer difficult biological questions," cautions Threadgill.

At the end of the day, few would question the power of the knockout approach in deciphering gene function in the context of human disease. "The genome projects have generated a wealth of information in terms of gene sequence, but we have no clue what half of those genes do," says Colin Fletcher, programme director at KOMP. "We hope that the knockout projects will illuminate their function in the context of human disease."

But whether knockout mice can accurately mimic the complexities of human disease remains to be seen. The International HapMap Project has revealed more than 10 million polymorphisms — different versions of genes — in humans. And the Mouse Resequencing Project, which completed sequencing the genomes of 15 strains of mouse, has shown similar levels of complexity. These results are key to understanding individual variation in disease susceptibility and the effect of gene–environment interactions. "The main determinant of human genetic diversity is not which genes are or are not knocked out, but which genetic variants one carries in the genome. That is where the real interest of functionality lies," says Balmain.

Indeed, many researchers are trying to build such variation into the animal models of human disease and to design screening strategies by targeting specific genetic variants. To those who have a taste for big biology, genome-wide association studies focusing on variations in genes may well be the next big thing. ■

Jane Qiu is a science writer based in London.

1. Lein, E. S. *et al. Nature* doi:10.1038/nature05453 (2006).
2. Driever, W. *et al. Development* 123, 37–46 (1996).
3. Grimm, D. *Science* 312, 1862–1866 (2006).

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