A global initiative to knock out every mouse gene struggles to get its act together

A Mouse for Every Gene

IN ADRIANO AGUZZI'S EXPERIENCE, getting hold of a new mouse strain can be nothing but trouble. A neuropathologist at the University Hospital of Zurich in Switzerland, he is one of thousands of researchers who study mutant mice for clues to what particular genes do. "Once I requested a mouse, and the guy wanted everyone from himself to his grandmother to be a co-author on everything we published with that mouse," says Aguzzi. "It was like scientific prostitution." Another time, he says, a researcher promised him a mouse but took more than a year to deliver: "[The investigator] should have just said his cat ate it; it would have saved us a lot of trouble."

Most mouse researchers can tell similar horror stories. But help is on the way. Several large-scale projects plan to disable every gene in the mouse genome and make the resulting mice readily available to the research public. In January, Europe and Canada embarked on ambitious efforts that together will produce more than 30,000 knockouts. And this summer, the U.S. National Institutes of Health (NIH) will announce the Knockout Mouse Project (KOMP), which will add another 10,000 to the list. China, too, is gearing up to make 100,000 mutants, with the goal of making 20,000 lines of mice, each with a different gene knocked out. (see sidebar, p. 1864). All told, these efforts will cost almost $100 million. Although separate entities, "the plan is to have every center work together, much like [what] was done with the Human Genome Project," says Allan Bradley, director of the Wellcome Trust Sanger Institute in Cambridge, U.K., which is part of the European effort.

Indeed, overall, the knockout effort is arguably the largest international biological research endeavor since the Human Genome Project. And it is the next major step in figuring out what makes us tick. The human and mouse genome projects each identified some 25,000 genes, most quite similar between the two species. But researchers have no idea what more than half of these genes do. Because the mouse is so amenable to genetic manipulation, and so well studied, mass-produced mutant mice offer a window into these unknown genes. "The Human Genome Project wasn't done just to get the sequence," says Christopher Austin, director of the NIH Chemical Genomics Center and KOMP's founding father. "It was a prerequisite for figuring out what our genes do."

How the individual mass-knockout projects will work together is still being ironed out. Each project is embarking on a different—and not necessarily compatible—approach to making its mutant mice, and the logistics of keeping track of all the mutants made are daunting. In addition, each effort will need to work out an efficient way to catalog and distribute the mice it creates. They will also have to deal with intellectual-property claims when one of the new mutants turns out to be a previously patented mouse strain. "The mouse project could open up huge areas of science, just like the Human Genome Project did," says Marina Picciotto, a molecular neurobiologist at Yale University, "but there are likely to be hiccups along the way."

Holy Grail? Marina Picciotto would love to find a mouse that caves to peer pressure, but chances are it's hidden away or hasn't been made yet.

Although Picciotto and most of her colleagues are optimistic about mass-produced knockouts, some wonder whether the efforts are the best use of public resources. Knocking out genes is really just the beginning. Those tens of thousands of mutant mice won't do many researchers much good until the behavior, morphology, and physiology of these knockouts have been described. Characterizing each mouse will not be easy. "You can knock out every gene, but if you don't have assays to evaluate them, it's hard to figure out what the

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Buyer beware. Deactivating the same gene in Black 6 (*left*) and 129 mice may yield widely different phenotypes.

gene is doing," says Marnie Halpern, a zebrafish geneticist at the Carnegie Institution of Washington in Baltimore, Maryland.

Hiding out

As a group, the knockout projects are trying to create something akin to the international superstore IKEA, where, in a single trip, customers can buy a houseful of easy-to-assemble furniture at reasonable prices. In this case, however, researchers wouldn't even have to make a trip to the store. Ideally, they would simply go to a central database and click their own computer mouse to order the knockout mouse of their choice. Within weeks, frozen embryos would arrive at their door. Like IKEA, some assembly would be required: turning those frozen embryos into live mice. But that requirement is minimal compared to the tens of thousands of dollars and a year or more of work involved in creating an average knockout mouse.

Such a resource would be a far cry from today's mouse trade, which is more like buying furniture from neighbors. Selection is limited, quality varies, and some items just aren't for sale. Part of the problem, says Francis Collins, director of NIH's National Human Genome Research Institute in Bethesda, Maryland, is that until recently, researchers often didn't know what the lab down the street—let alone one in another country—was doing. Investigators aren't required to place their mice in public repositories, and some never write up knockouts they don't find useful.

To remedy this situation, NIH went on a mouse hunt. It started its inquiry at the Jackson Laboratory (JAX) in Bar Harbor, Maine. JAX stores more than 800 varieties of mutants and maintains a database of every published mouse knockout. Then NIH went door-to-door, publishing a request asking investigators go to a JAX Web site and list any knockouts they had created and were willing to share with the research public.

The findings were dispiriting. All told, the mouse community had knocked out about 11,000 genes, but many labs were repeating work done elsewhere. More than 700 knockouts had been created three times or more; in one case, a single mouse had been duplicated 11 times. And of the 4000 unique knockouts that have been published, more than 3000 are not in public repositories, meaning most are either unknown or unavailable to the wider community. "It's embarrassing," says Collins. "A graduate student shouldn't spend a year making a knockout that's already been made. It's not a good use of resources."

Yale's Picciotto is a case in point. As a researcher who studies the genetics of addiction, she would love to find a mouse that caves to peer pressure. So far, she's managed to make a few handy knockouts. Some shun nicotine; others dig opiates. One even seems to be operating on a natural antidepressant. But for a complete picture of the mouse social psyche, Picciotto

CREDIT: LEXICON GENETICS INCORPORATED

Out cold. Lexicon is making thousands of mouse knockouts in embryonic stem cells. These frozen lines will become part of the TIGM resource.

NIH Knocks Out Key Mouse House

When the Texas Institute for Genomic Medicine (TIGM) applied to be part of a new $50 million U.S. National Institutes of Health (NIH) program to knock out as many mouse genes as possible, it seemed to be a shoo-in. Thanks to a partnership with Lexicon Genetics in The Woodlands, Texas, TIGM already has in its freezers knockouts for nearly a third of all mouse genes—twice what global knockout projects have achieved so far (see main text). "Taking us on would have made it easy for [NIH] to fulfill its mission," says TIGM President Richard Finnell.

Instead, he says, NIH has rejected his institute's application, potentially forcing NIH's Knockout Mouse Project (KOMP) to start from scratch and positioning TIGM as a possible competitor. NIH won't comment on the move until it announces the winners of the competition later this summer, but some in the mouse community feel that Lexicon's reputation for tough intellectual property (IP) restrictions may have hurt TIGM's chances. Finnell insists, however, that TIGM will place no IP restrictions on its knockouts.

Founded as a nonprofit organization last summer with a $50 million award from the Texas Enterprise Fund—a $295 million pot set up by the state to create jobs—TIGM's mission is essentially identical to that of the global knockout effort: Establish a massive mouse-mutant resource in embryonic stem cells and distribute these lines to academic scientists at cost. But while the global program's players are just beginning to churn out knockouts, TIGM, which is based in Houston and College Station, has lept ahead.

It has used $30 million of its $50 million to purchase Lexicon's growing library of knockouts in the coveted Black 6 strain of mice; starting this month, researchers can order any of 7500 unique knockouts—representing about a third of the mouse genome—and they'll have access to knockouts covering more than two-thirds of the genome by late 2007, says Finnell.

Becoming part of KOMP would not only have helped NIH achieve its goals more quickly and cheaply, says Finnell, but it would have also made TIGM's mouse lines more economical for researchers. Without NIH support, TIGM will still be supplying knockouts years before KOMP, says Finnell, although researchers may have to pay more for them. (Pricing details are still being worked out.)

Lexicon CEO Arthur Sands is confounded by NIH's decision. "It just doesn't make sense," he says. "[Our] resource is already on the ground." Neither Sands nor Finnell would speculate on why NIH decided not to collaborate with the institute. And outside scientists were hesitant to speak on the record. But some researchers *Science* spoke to said IP restrictions Lexicon has imposed in the past—such as requiring labs and universities to sign away certain rights related to discoveries made using its mice—have been problematic. Under the TIGM deal, however, those restrictions are lifted, says Finnell, "so that wouldn't have been an issue."

Others say NIH is interested in more cutting-edge science than Lexicon is using to make its lines. Ideally, for example, KOMP centers will use gene-specific targeting technology in addition to random gene-trapping technology. According to Finnell, Lexicon's library is being made almost exclusively by means of gene trapping (see figures, p. 1865), although he says that—with NIH funding—TIGM would have tried to complete the remaining third of the resource using gene targeting.

Despite the NIH setback, TIGM is planning to make its mark in the mouse world. "It will cost more now, but we're going to get these lines out to researchers," says Finnell. "When people think about knockout mice, they'll think about TIGM." **–D.G.**

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China Takes Aim at Comprehensive Mouse Knockout Program

SHANGHAI—Geneticist Xiaohui Wu looks through a window into a clean room on the campus of Fudan University here and proudly points to a growing collection of mutant mice. To a visitor, the 4000 cages and 20,000 mice representing 400 mutant strains look pretty impressive. To Wu, the scale of the operation is a frustrating limitation.

"We plan to mutate 70% of the mouse genome over the next 5 years," he says. Yet, their current facilities are filled to capacity. A new building will provide space for 10,000 more cages. But Wu needs 50,000 more, enough for about 100,000 mutant mice. Those cages, he says, require a lot more space and "a lot of money."

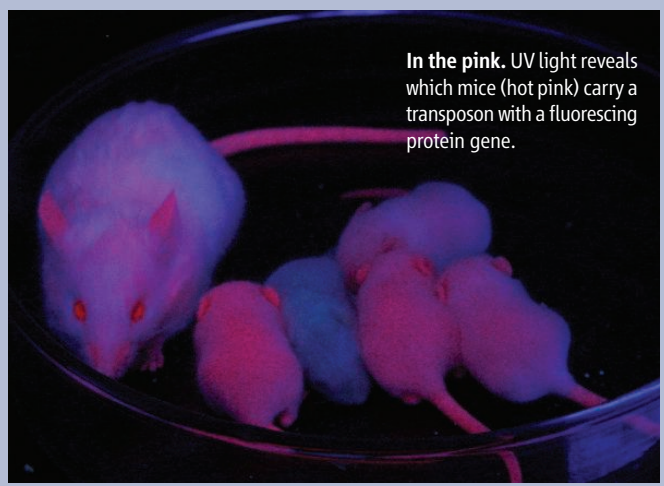
Throughout the world, researchers are setting up programs to shut down the mouse genome gene by gene to learn what each gene does (see main text). The Fudan University mouse facility—a joint effort with Yale University—is shooting to be a key player and hopes to team up with the U.S. National Institutes of Health (NIH) Knockout Mouse Project.

The driving force behind the tentatively named Mammalian Functional Genome Project is Tian Xu, a geneticist at Yale University School of Medicine who is also an adjunct professor at Fudan. The Fudan-Yale group, along with colleagues at the University of Colorado, Boulder, and Duke University in Durham, North Carolina, has come up with an efficient way to knock out mouse genes. They use a transposon, a short segment of DNA that invades genomes, sometimes inserting itself into a gene and deactivating it.

Developmental biologists have used transposons to disable genes in plants, worms, and fruit flies for years, but they had not found one that worked well in mammals. After 8 years of searching, Xu and his colleagues found "piggyBac," which was first identified in the cabbage looper moth by molecular virologist Malcolm Fraser of the University of Notre Dame in Indiana. "We don't know why it works," says Xu. But it does. The group reported its finding in the 12 August 2005 issue of *Cell*.

Loyal alum. Yale's Tian Xu and his alma mater are making mutant mice.

The technique is similar to gene trapping in that it randomly disables genes. But using a transposon avoids the laborious manipulation of embryonic stem cells required by other knockout techniques. The researchers made a line of mice that carry both the transposon and DNA that causes the transposon to move. When they mate these mice with wildtype mice, the transposon hops to a new place, preferably to a gene. "All you need to do is just breed mice, and each has different genes mutated," Xu says. This approach can hit genes other knockout approaches tend to miss, he adds.



In the pink. UV light reveals which mice (hot pink) carry a transposon with a fluorescing protein gene.

Also, the Fudan-Yale group has put the gene for red fluorescent protein into the transposon. Mice that wind up with the transposon in their genomes are pink under ultraviolet light. "You just look at it, and you can tell" if the genome is carrying the transposon, Xu says.

The Fudan-Yale team opted to set up its large-scale mouse facility in China to save money. Xu estimates that this project could cost one-fifth to one-fourth what it would cost in the United States. But it is still not cheap, and international researchers are impressed by the $12.5 million already pledged from national and local government funding agencies. "I think it's great that [the Chinese] are doing this," says Phil Soriano, a developmental biologist at the Fred Hutchinson Cancer Research Center in Seattle, Washington. Wolfgang Wurst, director of the Institute of Developmental Genetics at the National Research Center for Environment and Health in Munich, Germany, thinks the project is a welcome indication of China's increasingly international orientation. "It is a sign that they are serious research partners," he says.

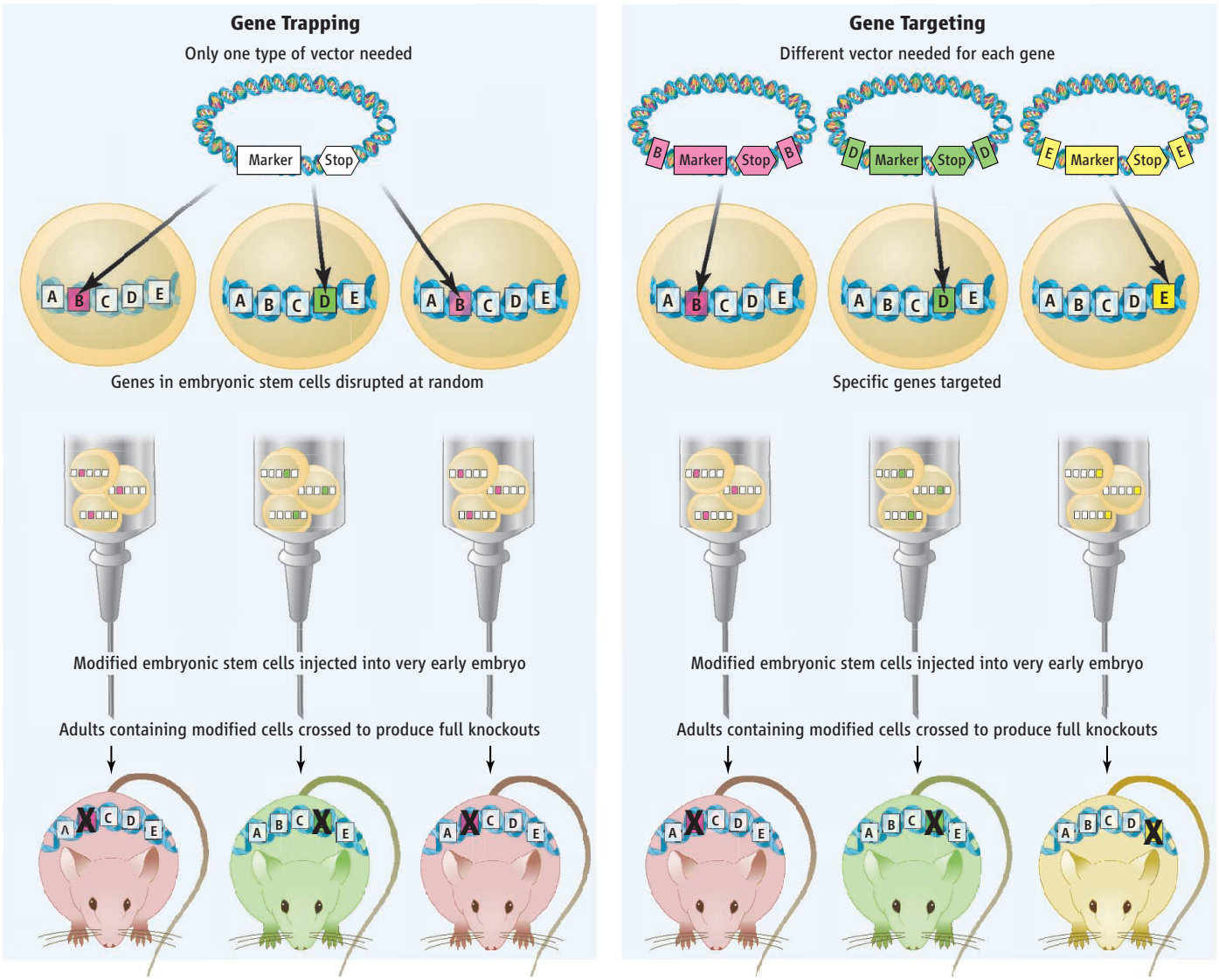
To leverage support from China itself, Xu and Wu are asking for $30 million from NIH to start mass-producing, preserving, and distributing mutant mice. For the cost of shipping and handling, researchers will receive frozen embryos or sperm, with no intellectual-property–rights restrictions attached. Also, the NIH money would go a long way toward producing the 100,000 strains of transposon-modified mice. Wu and Xu need that number of strains to be sure they have 20,000 genes covered, because the transposon also lands on non-coding regions. If they don't get NIH funding, they may have to recoup some costs by charging fees or placing restrictions on mutant mice, Wu says.

At this point, the other programs are simply making knockout strains, But here, researchers are busy screening the more than 400 mutant mice they have generated over the past year, looking for phenotypes from neuro-physiological, immunological, and disease angles, among others. That information will go up on the Web prior to publication, making it easier for potential users to see which mouse will best suit their needs, the duo point out. Four hundred mutants is about the limit until the team's new facility comes on line. After that, the view through these new clean-room windows will get even more interesting. **–DENNIS NORMILE**

needs an animal that wants drugs just because his companions have them.

Setting out to make her dream mouse is not really an option, however, because she has no clue what gene might influence peer-pressure sensitivity. Picciotto might be able to find the mouse in the community after an exhaustive search, but, if it exists, there's a good chance it's tucked away in a cage in a lab somewhere or frozen down as a clump of embryonic stem cells in a biotech company. Either way, it's as good as gone.

Even if Picciotto finds what she is looking for, that's hardly the end of the story. "I'm sorry to say that there are a few labs out there [that] won't share their mice even if they've published them in a journal [such as *Science* or *Nature*] that requires them to do so," says M. Celeste Simon, a developmental and cancer biologist at the University of Pennsylvania Cancer Center in Philadelphia. And as Aguzzi knows all too well, reticent mouse-makers can effectively quash efforts to use their mice by stalling delivery or making outrageous demands about co-authorship.



Different strokes. There's more than one way to knock out a mouse, but each has its pros and cons.

ILLUSTRATION: K. SUTLIFF/*SCIENCE*

Assuming the source of the mouse is cooperative, "transferring mice is an extremely difficult and time-consuming process," says Simon. Some of Simon's Penn colleagues lost 2 years of work when mice they ordered from a government facility turned out to be infected with an extremely contagious virus that can alter phenotypes. "It strikes fear into one's heart," she says. "Two years is a lifetime in the world of science." Other investigators complain about the cost and hassles of shipping or draconian material transfer agreements.

Over the past 6 years, several efforts have popped up to help address some of these problems. The International Gene Trap Consortium, for example, runs a database that enables researchers to track down about 20% of the existing unique mouse knockouts. And repositories themselves—most of which are publicly funded and store anywhere from 500 to 4000 mice—are beginning to work together under the Federation of International Mouse Resources to help make sure researchers around the world can get any mouse in any repository.

The big push

Realizing that these were just baby steps, mouse researchers from several countries decided in 2003 to take a giant leap. At a meeting at the Cold Spring Harbor Laboratory in New York, they called for a comprehensive international mouse knockout program. Besides shooting for an IKEA-like superstore, the participants agreed that it would be most economical to avoid trafficking in live mice and instead decided to maintain the knockouts as embryonic stem (ES) cells: clumps of tissue that can be frozen down and later grown up into full-fledged mice. Researchers could request ES cells or be provided with easier-to-use frozen embryos or sperm. They also proposed to use NIH's National Center for Biotechnology Information as their clearinghouse. Its Web site would act as a sort of Google to scan mouse repositories for the desired knockout. "The ultimate goal is to have one-stop shopping [for these mice]," says KOMP Program Director Colin Fletcher.

Two years after the meeting, Wolfgang Wurst, director of the Institute of Developmental Genetics at the German National Research Center for Environment and Health (GSF), and his colleagues set up the European Conditional Mouse Mutagenesis Program (EUCOMM). To get the program rolling, the European Union has promised $16.3 million over the next 3 years. The bulk of the EUCOMM effort is divided between two institutes: GSF and the Sanger Institute. GSF will use "gene trapping" (see diagram, above left) technology to randomly knock out 12,000 genes in ES cells. The Sanger Institute and GSF will use "gene targeting" technology to disable 8000 preselected genes (see diagram, above right).

"It's an ambitious program," says Bradley, who is leading the Sanger effort, "but we're fairly confident we can meet our goals." So far, GSF has produced about 3700 unique knockouts, which researchers can order for $631 apiece. Bradley expects Sanger's lines to start becoming available by late 2007.

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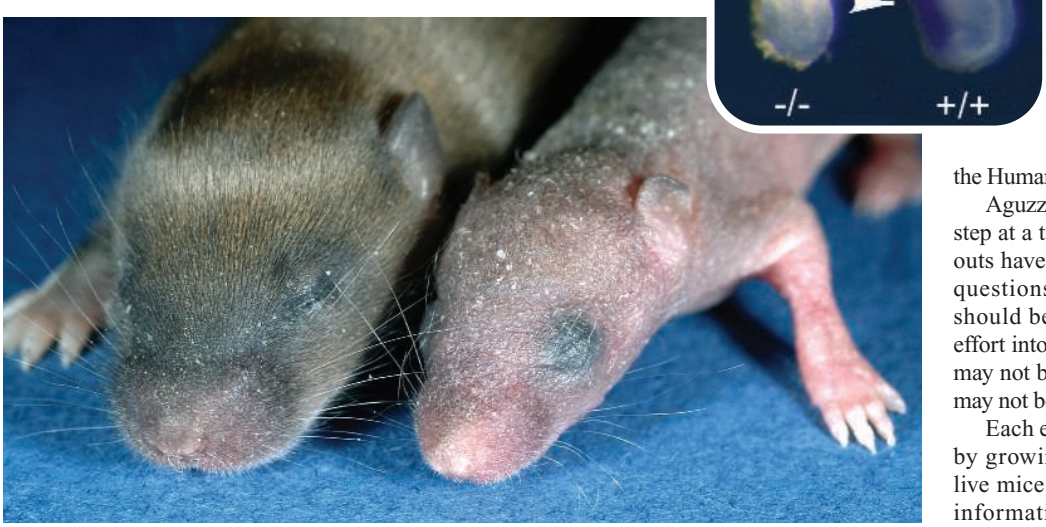
At the same time EUCOMM was getting started, Canada came out with the North American Conditional Mouse Mutagenesis Project (NorCOMM). Over the next 5 years, Genome Canada will spend $8 million for knockout work primarily at the University of Toronto and the University of Manitoba. The project has produced 3000 gene-trapped knockouts and hopes to make 9000 more over the next 18 months.

NIH's upcoming knockout effort is similar in scope and direction. KOMP expects to spend $50 million at up to four soon-to-be-named centers to build a library of 10,000 knockouts (see sidebar, p. 1863). Like EUCOMM, KOMP will likely use a combination of gene trapping and gene targeting to produce its knockouts. Targeting allows researchers to make precise mutations in their gene of choice, says Fletcher, and targeting will be easier to coordinate among KOMP centers and with the international partners because each group will know exactly what gene it's going after.

But there are important differences between KOMP and the other programs. EUCOMM and NorCOMM are making so-called conditional knockouts, in which the genes that are swapped into the genome have a self-destruct sequence.



Gone, but not completely. Without the *Dicer* gene, a mouse embryo (*inset, left*) is small compared to a normal embryo (*inset, right*) and dies within a week. But when the gene is programmed to turn off just in skin cells, this conditional knockout mouse is born, but has very little hair (*above*).

The new gene encodes information that tells it at which point in development or in which tissue to disappear. The strategy is especially important for determining the function of essential genes, which, if shut off too early, can kill a mouse while it's still an embryo, short-circuiting studies of the gene's effects.

When KOMP knocks out a gene, however, it's dead from day one. More embryos may die than with conditional knockout technology, but these "frank null" knockouts are still very informative, says Fletcher. They tell researchers whether a gene is necessary for development.

Also, of all the mouse efforts, only KOMP will focus on "repatriation." Thanks to NIH's detective work, the agency has compiled a list of the "lost" mice in the community. Recently, in a sort of mouse version of *American Idol*, NIH posted a request asking researchers to vote for the top 20 mice on this list that they'd like to see in a public repository. "That helped us prioritize 500 to 600 mice to repatriate," says Fletcher.

Part of the KOMP effort will involve contacting the owners of these mice and asking them to put their animals in a globally accessible repository. NIH kicked off this program earlier this month, with $800,000 split between the University of California, Davis, and the University of Missouri, Columbia, to acquire 300 of these lines. KOMP leaders hope the repatriation effort will conserve resources by obviating the need to make these lines again.

Trouble ahead?

But before a global knockout mouse emporium opens it doors, the international effort must overcome a number of hurdles. Topping the list is figuring out how to avoid the knockout duplication already seen in the mouse community. That's going to be a challenge, especially once each effort is cranking out hundreds of knockouts a month, often in random genes. EUCOMM's Wurst admits it will be "hard to coordinate" his gene-trapping program with NorCOMM's, because neither can predict which genes it's going to knock out. And the American and European groups have yet to factor in the knockouts coming in from China.

Even if redundancy can be addressed, it will still be caveat emptor for researchers who need to compare mice made by different projects. KOMP plans to use a strain of mouse called Black 6, whereas EUCOMM and NorCOMM are making their mutants in strain 129. That could cause studies of behavioral genes, for example, to yield skewed results. "Some 129 strains are really stupid, while Black 6 has a reputation for being smarter," says Yale's Picciotto. "You can't compare the two."

Another unresolved issue is what to do about knockouts that are knockoffs of an already-patented mutant. Several biopharmaceutical companies, including Deltagen in San Carlos, California, make their money selling big-ticket knockout mice. Deltagen, which last year earned $6.7 million from its catalog of 900 knockouts, is seeking "broad patents" on the majority of its lines, says CEO Robert Driscoll. Driscoll would not comment on what steps, if any, the company would take if KOMP or another effort remade one of its patented mice.

On the academic side, some researchers question the way the global endeavor is taking shape. "I'm not totally convinced [this effort] is going about things the right way," says University Hospital of Zurich's Aguzzi. He worries that the variety of strains and technologies being used will lead to glitches in these high-throughput enterprises. The global effort is "layers of magnitude more complicated than the Human Genome Project," he warns.

Aguzzi also emphasizes the need to take one step at a time. He argues that plenty of knockouts have been made with specific biological questions in mind and that these questions should be answered first. "Putting so much effort into creating a bunch of lines that people may not be able to ask the right questions with may not be the best use of resources," he says.

Each effort will try to address this concern by growing a subset of its frozen lines into live mice and then characterizing them. This information will then be uploaded into the central database, so researchers such as Picciotto might find their dream mouse. But a massive phenotyping effort is still years away—the next big step after this big step.

Despite these caveats, the global project should have a dramatic impact on both basic and biomedical research, says Picciotto. "Ordering a mouse is never going to be as easy as ordering an antibody," she says. But as the global project matures and begins to characterize the knockout lines in its libraries, even researchers in small labs and those who are not mouse geneticists will be able to delve into the world of the knockout mouse. "Before, scientists were limited by their experience and their resources," she says. "Now they'll only be limited by their imagination."

–DAVID GRIMM

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