The Mouse That Prompted a Roar

Restrictions on sharing mice engineered with DuPont's patented technology have drawn protests from prominent researchers; some organizations, including HHMI, have accepted the conditions

Jamey Marth doesn't like to ask permission to send a colleague a research tool refined in his own lab. But the University of California, San Diego, geneticist no longer freely gives away a type of mouse that's prized for its power to reveal gene function. He waits until he gets approval from E. I. du Pont de Nemours and Co. of Wilmington, Delaware. As a result, he says, he has been forced to make "heart-wrenching" decisions to withhold the animals from some researchers.

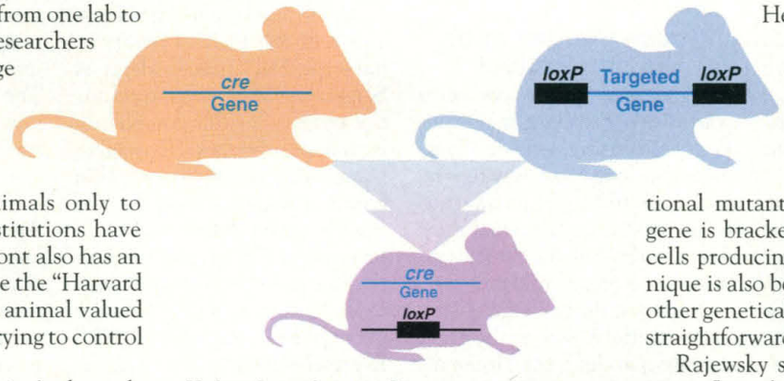
Marth finds himself in this predicament because DuPont holds a patent on a powerful method of manipulating genes in the mouse. The company doesn't want transgenic mice created with this technology, called Cre-*loxP*, handed around loosely from one lab to another. So it insists that researchers using the mice acknowledge the company's rights to the animals, share any money that may be made on discoveries from the technology, and distribute the animals only to other researchers whose institutions have agreed to these terms. (DuPont also has an exclusive license to distribute the "Harvard oncomouse," a tumor-prone animal valued in cancer research, and it is trying to control its use as well.)

The Cre-*loxP* technique isn't the only basic research tool whose use is restricted by patent rights (see sidebar). But it has become a lightning rod for scientists chafing at restrictions on the free flow of research materials. Harold Varmus, director of the National Institutes of Health (NIH), has sent a letter to DuPont protesting the company's policy. It can be "an incredible burden for the individual investigator" to comply with the administrative requirements, Varmus believes, and he worries that the legal fallout will "slow things down, make research unattractive, and turn people off." Varmus has established a panel to look into this and other restrictions on sharing materials. The issue is also coming to a head because the largest breeder and distributor of lab animals in the United States—The Jackson Laboratory of Bar Harbor, Maine—has declined to sign an agreement with DuPont and is not distributing any Cre-*loxP* mice, making the animals hard to come by.

DuPont licensing executive Robert Gruetzmacher says the company has no desire to hamstring basic researchers. "Our philosophy is: Let's make it as easy as practical for the researchers to use [DuPont's patented technology] for research, but gosh, if they go beyond the research and get into a commercial mode, let's see if we can't capture some of that fairly."

Inventing a better mouse

The technology at the center of this battle was not always so popular. In fact, the inventor of record—geneticist Brian Sauer, a former DuPont employee who is now a staffer at NIH's National Institute of Diabetes and Digestive and Kidney Diseases—says he got little response when he first presented his Cre-*loxP* system in public at a biotech poster session in 1985 in San Francisco. "It was the kind of thing where you stand around for a long time. ... Not many even stopped by."



Molecular scissors. One mouse expresses the Cre enzyme in selected tissues; the mate carries a targeted gene flanked by *loxP* markers. In offspring, cells expressing Cre delete the targeted gene.

Sauer's technique adapts a natural gene-splicing system from a bacteriophage—a virus that infects bacteria—for use in cells of complex organisms (eukaryotes). It is based on two genetic elements of the P1 bacteriophage: a gene called *cre* that expresses an enzyme not normally seen in higher organisms, and a stretch of DNA called *loxP*. They work together like a powerful editing machine. When Cre encounters two *loxP* sites in a stretch of genetic code, it clips out the intervening DNA, along with one of the *loxP* sites, reattaching the ends to make a seamless strand.

Sauer says he already had the idea of turning this editing technique into a kind of molecular scissors when he arrived at DuPont in 1984. He reasoned that by inserting *loxP* signals on either side of a target gene and exposing the DNA to Cre enzyme, the target gene would be snipped out. The system worked in eukaryotic cells and even in mice. Sauer and DuPont filed for a patent on using Cre-*loxP* to modify DNA in eukaryotic cells. It was granted in 1990.

Since then, Cre-*loxP* mouse technology has taken off and—according to independent scientists—been significantly improved in taxpayer-funded labs. Among those who have used and improved the system are Marth, Klaus Rajewsky and Werner Müller at the University of Cologne in Germany, Heiner Westphal at NIH, Susumu Tonegawa and colleagues at the Massachusetts Institute of Technology, and others (*Science*, 1 July 1994, p. 26). The system's main value is in creating "conditional mutants," mice in which a specific gene is bracketed for deletion in particular cells producing the Cre enzyme. The technique is also being used to create a variety of other genetically engineered mice, including straightforward "knockouts."

Rajewsky says that published papers do not reflect the growing importance of the technology. "It takes a long time to breed and to analyze the conditional mutants," he says, and results are just coming out. Rajewsky reports that the Volkswagen Foundation is sponsoring a new program on conditional mutagenesis in Germany. At its inaugural scientific meeting last week in Cologne, 15 of 30 attending groups said they are using Cre-*loxP* technology, according to Rajewsky. "The interest is huge," says Arthur Beaudet of the Baylor College of Medicine in Houston, who chairs NIH's mammalian genetics peer-review section.

Thomas Caskey, genetics chief at Merck & Co. in Whitehouse Station, New Jersey, says, "I think this concept is going to have extremely broad applications in trying to understand gene function." He foresees a system in which researchers could draw from a shared library of mice with *cre* expressed in a variety of cells. Investigators could develop their own animals with *loxP* targeted genes and breed the two lines to get Cre-*loxP* offspring with genes inactivated in specific tissues. According to Caskey,

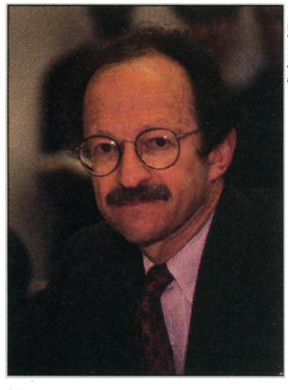
Merck is entertaining proposals right now to create a library of *cre* mice for distribution to researchers. But, of course, anyone who wanted to participate would have to come to an agreement with DuPont—and that may take some negotiating.

Resistance at NIH

DuPont is trying to control the technology through no-cost "research licenses." Institutions that sign up agree that their researchers will share the mouse only with other licensees, and they may be asked to pay unspecified royalties on commercial discoveries enabled by the Cre-*loxP* system. Commercial outfits must negotiate their own, expensive licenses, which can run to more than $100,000.

DuPont says some 70 institutions have agreed to sign research licenses. They include the Howard Hughes Medical Institute (HHMI) of Chevy Chase, Maryland. All HHMI investigators—who are employees of the institute—must therefore abide by DuPont's rules. This includes Marth, an HHMI investigator since 1995. Maxwell Cowan, HHMI's chief scientific officer, explains, "We felt [signing the agreement] was the right thing to do. ... We had a number of investigators who were using the technology and had obtained animals prepared with that technology from others." DuPont holds a valid patent, Cowan says, and has the right to enforce it. The only other option was to instruct investigators not to use Cre-*loxP* mice—calling a halt to many research projects. Accepting DuPont's terms seemed the wise thing to do, says Cowan, adding: "We have a difference of opinion with Harold Varmus on that."

The NIH has not signed an agreement with DuPont, but has "friendly negotiations" under way, says Varmus. DuPont is allowing NIH researchers to continue using animals made available to them by Sauer years ago, before the company began trying to license all nonprofit institutions. But Varmus protested the company's license conditions in a 28 March letter to DuPont's president, John Krol. The restrictions, Varmus wrote, "will seriously impede further basic research and thwart the development of future technologies that will benefit the public." Varmus said in an interview that he is just as upset when university or NIH scientists try to patent research tools: "There are investigators here who would like to seek intellectual protections for everything they do, and I don't find it very appealing."



RICK KOZAK

Tough stand. Varmus is trying to persuade DuPont to change its rules.

Varmus, who uses transgenic mice in his own lab, has scheduled a meeting in late July with DuPont executives to discuss the issue. In addition, he's creating a small panel—including two experts in gene patenting, Rebecca Eisenberg of the University of Michigan, Ann Arbor, and John Barton of Stanford University—to advise him on how NIH should respond to the threat of "reach through" provisions in sharing agreements in which a company or researcher lays claim to discoveries not yet made.

Many U.S. and European researchers are also said to be quietly objecting to DuPont's terms. Bruce Alberts, president of the U.S. National Academy of Sciences, recently singled out restrictions on Cre-*loxP* technology in a statement on commercial barriers to basic research. Cologne's Rajewsky says he finds the strings attached to DuPont's license "much too strong for a basic technology." One mutinous researcher even admits to simply ignoring the rules and sharing mice with trusted peers. But the most important outsider is the Jackson Lab.

Jackson Lab and DuPont have been at a standoff in negotiations on Cre-*loxP* for 2 years. Kenneth Paigen, director of the Jackson Lab, says DuPont's terms have not been accepted because they would burden the lab and its clients with too many legal constraints. The result: The public distributor for the biomedical community neither accepts nor sends out Cre-*loxP* mice. Research is suffering, some researchers say. "The most serious practical problem we have at the moment," says Rajewsky, is that these mice "cannot be distributed by commercial mouse breeders like Jackson Lab," making it hard for the growing numbers of interested researchers to get animals. Beaudet, who says "NIH is making a huge investment in developing these mice," says the stalled talks between Jackson Lab and DuPont "could have broad implications" for biomedicine.

There are signs that DuPont may be willing to compromise. Gruetzmacher, DuPont's licensing chief, says, "We continue to modify our license to make it better. We are learning to make concessions to make it work." Many researchers are hoping the meeting between Varmus and DuPont later this summer will bring the stalemate to an end. Varmus is optimistic, but he has bigger objectives in mind. "The community really needs to rethink what a patent is for," Varmus says. He expects to explore that question in broad discussions this year.

–Eliot Marshall

Battling Over Basics

The clash over who may and may not use mice that have been genetically engineered with a patented technology (see main text) is just the latest skirmish in a decade-long battle over commercial controls on basic tools in biomedical research.

The most prominent case arose in the late 1980s, when biologists were steamed about controls on the polymerase chain reaction (PCR), a method of amplifying DNA sequences. It is now accessible to just about every lab doing DNA research, but basic scientists once feared that licensing fees might put it out of reach. The Cetus Corp., the original owner of the patents on PCR and its key reagent, Taq polymerase, initially tried to get all users to take out licenses. But many balked, some complained about the high fees, and a few threatened a boycott. When the furor was at its peak in 1991, Cetus sold its PCR rights to Hoffmann-La Roche, the Swiss pharmaceutical company. Roche set up a multicategory licensing system with special terms. Although Roche announced that it would not pursue people who were doing pure science, it has kept tabs on "infringers" who do not take out a license for use of Taq. Roche also claims in a lawsuit due to come to trial soon that researchers can be compelled to obtain such licenses.

Soon after the PCR flap died down, another battle flared up. The issue: who should own rights to fragments of human genes called expressed sequence tags (ESTs). Industrial DNA sequencing outfits are seeking patents on thousands of ESTs, even those with poorly understood biological function. One company—Human Genome Sciences of Rockville, Maryland—also offers researchers access to its proprietary EST database, provided they sign a restrictive license agreement and share rights to future discoveries. National Institutes of Health director Harold Varmus and Bruce Alberts, president of the National Academy of Sciences, recently asked the U.S. Patent and Trademark Office not to grant patents on ESTs (see p. 41). So far, only a handful of ESTs have been covered in patents, although thousands are awaiting review.

–E.M.