IN this section

Free tumor profiling from Strata Oncology p895

First insulin/GLP-1 pens p897

Setbacks rattle allergy vaccine frontrunner Circassia p901

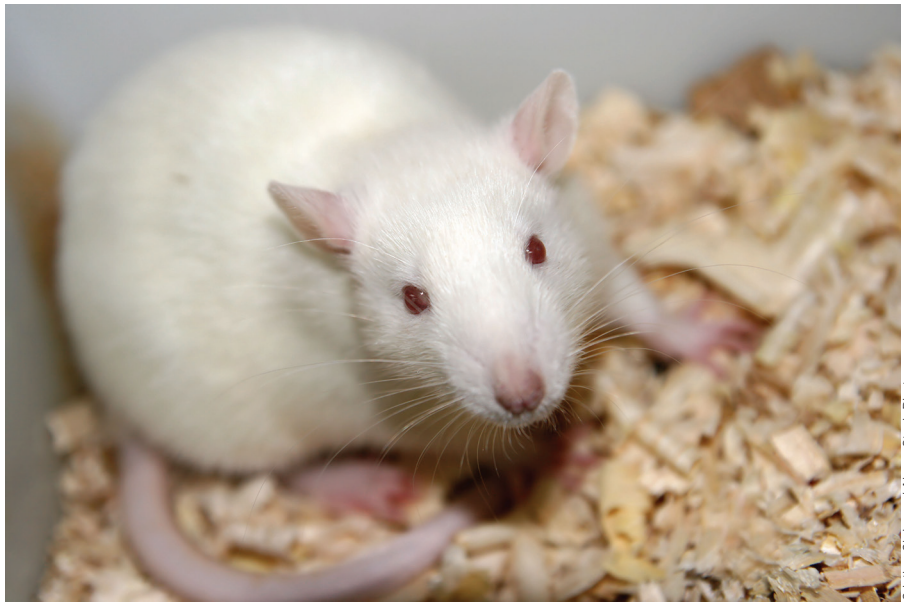
CRISPR mouse model boom, rat model renaissance

The US National Institutes of Health awarded The Jackson Laboratory $28.3 million in August to produce 1,000 lines of genetically modified mice. The task, which would have seemed intractable a few short years ago, has been made feasible by the emergence of the CRISPR–Cas9 gene editing system. Animal model providers and contract research organizations are witnessing a boom in demand as academic, biotech and pharma clients rush to embrace CRISPR-customized animal models. CRISPR is not only enabling more complex combinations of traits to be tested in mouse models but also opening up new possibilities for modeling disease in rats. Even so, the technology remains a work in progress, particularly for large DNA insertions.

Reagent and materials suppliers have been quick to capitalize on burgeoning demand from the research community for CRISPR products. Offerings include design tools for synthesizing guide RNAs (gRNAs) against custom targets, predesigned plasmids, RNAs or purified components for targeting coding regions in human, mouse and rat genomes, engineered Cas9 endonucleases (e.g., paired nickases), viral delivery systems for use in knockout and knock-in screens, and mutation detection and verification kits (using, e.g., T7 endonuclease).

At the same time, contract research organizations are also embracing the CRISPR–Cas9 system to generate custom transgenic and knockout animal models. Mice make up the majority of modified animals, but rats in particular are making a comeback because of their greater relevance to human disease. What's more, CRISPR-engineered rabbits and zebrafish are also becoming increasingly popular. Dozens of universities, research institutes and national laboratories in North America, Europe and Asia also provide CRISPR–Cas9-modified animals for outside researchers.

CRISPR can cut the time and cost of making a new animal model by a third, putting tailor-made models within reach of a wide range of researchers. For example, a mouse model made using embryonic stem cells (ESCs) typically takes 12–18 months and costs ~$100,000, owing to the need to extract, purify and modify ESCs and then implant them into blastocysts. In contrast, CRISPR–Cas9 methods simply involve injecting a fertilized egg with Cas9 protein and gRNA, which introduce double-strand breaks in DNA; cellular DNA repair by non-homologous end joining (NHEJ) can then result in the addition or deletion of nucleotides from a targeted gene, producing frameshift mutations downstream of the DNA break and creating knockouts of gene function. Alternatively, a donor DNA plasmid or oligo can be added to the mix, in which case the less-efficient cellular homology-directed repair (HDR) pathway results in insertion either of a single nucleotide or an entire sequence/gene (from the plasmid/oligo) into the desired sequence. Using these approaches, a typical CRISPR–Cas9 knock-in mouse can cost about $35,000 and take just eight months to generate (**Table 1**).



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CRISPR-Cas9 genome editing has rekindled interest in the rat as a model of human disease.

Iva Morse, corporate vice president and CSO at Charles River Laboratories of Wilmington, Massachusetts, says, "So now, you're more likely to create a novel model than settle for something that already exists."

Craig Ferris, director of the Center for Translational NeuroImaging at Northeastern University in Boston, uses custom CRISPR–Cas9-modified rats provided by Cambridge, UK–based Horizon Discovery. The center studies neurodegenerative diseases and collaborates with biopharma and traditional pharma companies to assess their therapies by imaging the brain activity of animal models.

CRISPR-modified animal models are shrinking the time it takes to arrive at a go/no-go decision, says Michael Wiles, senior director of technology evaluation and development at The Jackson Laboratory of Bar Harbor, Maine. "They'll have the animal in front of them rapidly," he says. As a result, within months a group can say, "Okay, the project's alive, let's pump resources into it, let's develop it," or "'The idea was wrong', and they kill it off," says Wiles.

Shorter time frames are also allowing drug developers to validate research findings. Previously, budgets and timelines made it impossible to generate mouse models to corroborate linkage data identified in clinical situations, says Adriano Flora, associate director of product management of rodent model provider Taconic Biosciences of Hudson, New York. "This is a field that's completely opened up," he says.

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At Kallyope, a New York–based biotech company developing therapeutics that act on the gut–brain axis, the vast majority of the genetic models are engineered using CRISPR and other technologies. Weisheng Chen, the company's lead scientist for mouse genetics, says CRISPR cuts the time to make long-range deletions from several months to a few weeks. Chen says he has used the technology to delete a cluster of 12 genes of a gene family that spanned 200 kb. Similarly, CRISPR makes point mutations easy because there's no need to engineer a targeting vector, he says. Shortened time frames mean lower reagent and housing costs for animals than with other techniques, he adds.

For complex strains with stacked modifications, the time savings are even more dramatic. Previously, adding a modification to a strain that already had five modified genes took three years of breeding to put all the alleles back together, says Taconic Biosciences' Flora. Now, you can modify the strain directly and have the model in six months, he says.

Although CRISPR–Cas9 has proven useful for generating mutations by NHEJ and with lower-efficiency HDR of smaller (<5 kb) gene fragments, very often it is difficult to use the system to introduce genes >5 kb in length, which is often desired for human sequences. This means that current CRISPR–Cas9 technology is still often not competitive with ESC-based gene targeting when creating humanized animal models, which are important for validating human gene targets and for generating humanized monoclonal antibodies.

Even so, CRISPR–Cas9 greatly simplifies the process of introducing single-nucleotide changes into animal models that have already been humanized using ESC techniques. A key advantage of modifying humanized animal models is being able to test the effects of therapies on models with different variations in human genes. "You can introduce different variants to mimic different human populations," says Flora. The company has done this in several projects, including producing mouse models with high-, medium- and low-metabolizing variants of a human liver gene involved in metabolizing drugs, he says. For drug developers, being able to obtain highly predictive preclinical models can save them money by increasing the likelihood that therapies that reach expensive first-in-human clinical studies succeed there.

For animal models that lack robust ESCs or that have not been amenable to genetic targeting, CRISPR–Cas9 also offers advantages. For example, rat models are often favored over mice in neuroscience because they are higher up in the phylogenetic ladder and offer increased insights into neurobiology. For imaging, size really matters. "You're only as good as the number of protons you have," says Northeastern's Ferris. "So when you look at a rat with a head twice the size of a mouse, you're looking at that number cubed, so you're looking at eight times as many protons."

CRISPR–Cas9 could also drive renewed interest in the rat as a preclinical model. Until recently, the transgenic modifications available for mice weren't available for rats, says Ferris, because rat ESCs weren't widely adopted. With CRISPR–Cas9, "anything that was ever done in a mouse can be done now in a rat," says Ferris. "We're playing in a much different field." The technology is also enabling new applications as researchers have developed mice that express Cas9. These Cas9 mice can be injected with gRNAs to bring the endogenously expressed Cas9 to target genes in specific tissues to edit those genes. This makes it possible to manipulate animals rather than zygotes, says Flora. "You're mimicking somatic mutation," he says.

One drawback of CRISPR–Cas9 engineering is that it often produces mosaicism—a state arising from editing events that typically occur in the zygote after cell division has begun, at the two- and four-cell stage. According to Jackson Laboratory's Wiles, the result is a founder animal with a mixture of cell types. If the germ cells in that animal are chimeric, that can lead to problems. "We breed that animal and a zoo comes out of it," he says.

As a consequence, founder animals can't be used for genotyping or phenotyping. The animals have to be bred until the line is homozygous with the desired modification. If the CRISPR events could be made to occur at the single-cell stage, the result would be 100% modified founder animals. This would be a considerable advantage, especially with slower-breeding species, says Wiles.

CRISPR–Cas9's success has led to some unreasonable expectations, Wiles cautions. Some laboratories are looking for models with numerous and complicated modifications. But a salutary tale from the Jackson Laboratory is informative; after switching out a 900-base piece of mouse immunoglobulin for a piece of human immunoglobulin, in a mouse model, Wiles says they generated a model, "but it took 850 embryos microinjected with this to get out two females, and that was over a period of about 18 months." The resulting line has proven difficult to handle, Wiles notes. It doesn't breed well and it has bad habits, like eating its offspring, he says. The take-home message is: "We can do these techniques but it's not quite Lego bricks with DNA at this point."

Eric Smalley *Boston*

Table 1 Selected commercial providers of customized CRISPR/Cas9 animal models

Company (location)	Models and services offered	Turnaround time (months)
Applied StemCell (Milpitas, California)	Mouse and rat knock-ins and knock-outs, including conditional knockouts	3–5 (mouse) 4–7 (rat)
Creative Animodel (Shirley, New York)	Mouse and rat knock-ins and knockouts (<2.5-kb sequences), including conditional knockouts	5–7 (mouse) 6–8 (rat)
Cyagen Biosciences (Santa Clara, California)	Mouse (*Rosa26* large fragment knock-in of up to 8 kb); rat knockouts	3–6.75
genOway (Lyon, France)	Mouse and rat knock-ins and knock-outs, including conditional and tissue-specific knockouts	7 for knock-ins
The Jackson Laboratory	Mouse knockout with indels, knockout with insertion of premature stop codon, point mutation, tag insertion, conditional knockout, reporter insertion	3–5
Horizon Discovery	Mouse and rat knockout, conditional knockout, knock-in, humanized knock-in	3.75–6.5
Taconic Biosciences	Mouse and rat knock-ins and knockouts	3 (mouse) 4 (rat)
Ingenious Targeting Laboratory (Ronkonkoma, New York)	Mouse knock-ins and knockouts	7–10 months

Source: company websites

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