10 good reasons why our custom animal models are made using **CRISPR/Cas9**

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CRISPR/Cas9 technology is revolutionizing animal model generation. Horizon Discovery utilizes CRISPR/Cas9 for the generation of genetically modified rats, mice and rabbits. Here's why you should consider making your next animal model with CRISPR/Cas9:

1. More efficient production

Conventional technology to create genetic modifications in mice has been reliant on homologous recombination (HR) in embryonic stem (ES) cells. The rate of HR in ES cells is remarkably low and requires selection. Generation of a double-stranded break markedly increases the rate of HR by orders of magnitude. Gene editing tools, such as CRISPR/Cas9 and zinc finger nucleases (ZFNs) are able to produce double-stranded breaks at predefined, highly specific and targeted loci. These technologies greatly increase the frequency and efficiency of gene targeting events.

2. More cost-effective overall

While the "retail price" of model generation using ES cells is typically lower than for CRISPR/Cas9 and ZFNs, taking the costs of the whole project into consideration, CRISPR/Cas9 and ZFNs are often more cost-effective. Additional breeding expense can happen with the additional backcrossing, chimera segregation, and cassette removal required by ES cell-based techniques, not to mention the added cost of long generation and breeding times. With ES cell-based techniques, there's always the real possibility that a model may lack germline transmission, which financially impacts the researcher along with delays of several months to a year, all leading to a possible loss of competitive advantage over another lab or company. These risks are reduced with CRISPR/Cas9 and ZFN technologies; not only is the model generation process faster, the researcher can also skip all of the additional breeding required to overcome the shortcomings of ES cell technology. CRISPR/Cas9 and ZFN technologies have a 100% germline transmission, there's no risk of catastrophic loss of the line.

3. Faster generation time

Traditional ES cell technology used to create genetically modified mice can take a year or more. By contrast, the effectiveness of the CRISPR/Cas9 system speeds up model generation time and allows the creation of a founder animal with even a complicated conditional knock-in or humanization, in as little as 2 months.

4. Any strain can be produced

Mouse background strain has received much attention recently for its profound effect on phenotypes, therefore it must be taken into consideration when developing a new model organism. Unfortunately, only a few mouse strains have been permissible for gene targeting via ES cells. CRISPR/Cas9 removes this background strain limitation allowing the researcher to go directly in their strain of choice. In addition, rabbits can also be used. At Horizon, we've successfully modified a number of mouse strains including C57Bl6, FVB, Balb/C, and CBA CA mice and Sprague-Dawley, Long Evans Hooded, Wistar, Wistar Han, Brown Norway, Fisher 344 rats among many more.

5. Any species can be created

As mentioned, conventional gene editing technologies have relied on ES cells, and only mouse ES cells have been amenable to modification. As a result, genetically modified models have been limited to the mouse. However, most in vivo assays, particularly for behavioral and cardiovascular research, were originally developed and validated in the rat and other model organisms and only recently adapted to the mouse. Next generation gene editing technologies such as CRISPR/Cas9 and ZFNs do not require ES cells removing this species limitation. Researchers have now successfully modified many other model organisms including rabbits, silkworms, cows, zebrafish, mosquitoes, and many more. For the first time in 25 years, the researcher can now choose the best model organism for their research rather than being forced to adapt to the mouse model.

6. No chimeras created

In ES cell-based gene editing, modified ES cells are injected into blastocysts. The resulting mouse is therefore a chimera of modified and unmodified cells. The generation of chimeras then requires additional breeding to segregate the genotypes. The extreme efficiency of the CRISPR/Cas9 system, facilitates direct injection into embryos at the single-cell stage, eliminating the generation of chimeras.

7. Knockouts generated together with knock-ins

CRISPR/Cas9 and ZFNs both create double stranded breaks at targeted loci, and these breaks are then repaired using either non-homologous end joining (NHEJ) or HR. Researchers take advantage of these pathways for genetic engineering, namely NHEJ for the production of knockouts and HR for knock-ins. However, it's important to note that both processes occur simultaneously, and NHEJ events still occur even though a researcher is attempting to insert a donor plasmid via HR. Indeed, during founder selection and screening, we routinely observe the generation of knockout animals as a by-product of knock-in generation. This offers the researcher a unique opportunity to create two models (knockout and knock-in) with little to no additional cost or effort.

8. No molecular scar left behind

Due to the efficiency of gene editing in ES cells being so low, selection for the modified allele is required, and this necessitates the insertion of a selection cassette. The introduction of a selection cassette has the potential to alter expression of the targeted gene, which is of particular importance in knock-in projects. Techniques using cre-lox technology have been developed for selection cassette removal, however, the removal is often incomplete, leaving behind a loxP as a molecular scar. As mentioned, the efficiency of CRISPR/Cas9 and ZFN mediated genomic editing is far superior to ES cells, and sufficiently high to eliminate the need for selection.

9. 100% germline transmission rates

One of the biggest risks in the production of modified mice using ES cells is to spend nearly one year in generation time (and associated costs and labor) only to find out that the modified allele does not get transmitted to offspring. While advancements in technique have reduced this risk, no ES cell-based generation method can produce modified mice with 100% germline transmission, so this gamble must always be considered. Conversely, CRISPR/Cas9 (and ZFNs) deliver modified alleles with 100% germline transmission.

10. No backcrossing necessary

Only a few mouse strains are amenable for gene targeting in ES cells, therefore the researcher must often backcross their line onto their strain of interest, typically requiring at least 10 generations and an additional year or longer after generation of a model. Even when ES cell-based modification can be done directly in the desired strain, some backcrossing is still required to eliminate chimeras as well as selection cassettes. Using CRISPR/Cas9, the researcher is able to avoid these issues by generating their model in their strain of choice, without the production of chimeras or need for selection.

Why Choose Horizon Discovery?

At Horizon, we've pioneered nuclease-based genetic engineering, publishing the world's first knockout rat[1], the world's fastest knockout mouse generation[2], as well as the first knock-in[3] and conditional knockout rats[4]. And, we've even created the first targeted knockout in the rabbit[5]. Our expertise, combined with the latest, most cutting-edge genomic tools, and our dedicated project management teams ensure that you'll get the exact model you want, when you want it. We have the broadest gene-editing license coverage (The Broad Institute, ERS Genomics, Harvard, and Sigma-Aldrich) in the in-vivo model generation market for CRISPR/Cas9 and ZFN.

[1] Science 2009 325(5939): 433

[2] Genetics 2010 186(2): 451-2

[3] Nat Biotechnol 2011 29(1): 64-7

[4] Nat Methods 2013 10(7): 638-40

[5] Under review

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Horizon has licenses from The Broad Institute, ERS Genomics, and Harvard University for use and commercialization of CRISPR-Cas9 technology.

Horizon has a license for commercial use of Zinc Finger Nuclease technology from Sigma-Aldrich.
This Transgenic Model and its use are the subject of one or more of the following patents controlled by Sangamo BioSciences, Inc.: U.S. Patent Nos. 6,534,261,6,607,882, 6,746,838, 6,794,136, 6,824,978, 6,866,997, 6,933,113, 6,979,539, 7,013,219,7,030,215, 7,220,719, 7,241,573, and 7,241,574, and corresponding foreign patent applications and patents. The Broad Institute: U.S. Patent Nos. 8,697,359, 8,771,945, 8,795,965, 8,865,406, 8,871,445, 8,889,356, 8,889,418, 8,895,308, 8,906,616 and corresponding foreign patent applications and patents.