Cyagen Biosciences – Helping you choose the right animal model for your research

While many animal models are available “ off the shelf ” through various repositories and collaborations, generation of novel animal models has allowed for more effective studies, not limited by previously derived lines/ strains. Historically, researchers have generated custom animal models for their research by a combination of (1) components generated “in-house;” (2) partnering with a core facility, typically academic; (3) outsourcing portions of the model generation to a contract research organization (CRO). However, some of these approaches can fall short in terms of reliability, guarantees in the event of failure, and cost effectiveness. Cyagen Biosciences has become the world leading provider of custom animal models, delivering hundreds of novel lines each year since its founding in 2006. With novel technologies such as TurboKnockout®, enabling conditional animal model generation in 6-8 months, to a full money back guarantee, Cyagen’s services allow researchers to generate novel animal models for their research more reliably, faster, and cost effectively than ever before, with increased peace of mind. Understanding the key differences between different types of animal models however, is crucial to developing the most relevant model for a given area of research.

Key Differences in Models

The term "transgenic" often refers to any animal model that has its DNA permanently reengineered either by gene insertion, gene deletion, or gene replacement. This broad term thus includes standard transgenic, knockin, conventional knockout, and conditional knockout animals.

The key difference between standard transgenic and gene targeted (knockout or knockin) animals is that in knockout and knockin models the genomic DNA sequence is altered at a specific locus in the target genome via homologous recombination, with an engineered segment of DNA replacing a homologous endogenous segment. By contrast, in transgenic animals an engineered segment of DNA is randomly integrated, such that the inserted DNA could end up almost anywhere in the host genome, and does not normally disrupt the homologous endogenous sequence.

Knockouts (KO) and knockins (KI) are both types of gene targeting, and differ from one another in the functional outcome for the targeted gene. Knockouts are changes that result in the inactivation of the targeted gene. Knockins are targeted insertions into the genome that result in an altered gene product, such as a point mutation or the addition of a fluorescent tag. Knockout and Knockins are usually made using homologous recombination in embryonic stem cells (ES cells), but nuclease-mediated genome editing using TALEN and CRISPR/Cas9 can also be used to generate knockouts or small sequence changes without the use of ES cells.

Standard Transgenic Models

In this type of genetically modified animal, a foreign piece of DNA (transgene) is introduced into the genome via random integration. Depending on the location of integration, the expression level of this transgene will vary. Furthermore, each transgenic founder animal will have different site(s) of integration and a varied number of transgene copies. Thus, each transgenic founder should be treated as a separate line, and should be bred independently of other founders.

Advantages:

The general advantages of this approach include a shorter timeline for development and easier molecular biology work required, when compared to other animal models.

Disadvantages:

Integration is random, and can interfere with expression of endogenous genes or may occur at genomic sites that are transcriptionally silent resulting in poor or inconsistent expression.

Key considerations:

Concerns regarding insertion site and copy number can be mitigated by using an alternative transgenic method: PiggyBAC. These transgenics are transposon based; whereby the piggyBAC transposase acts to insert the target DNA into specific sites in the genome (TTAA regions) resulting in improved integration (one copy per integration site) and more consistent expression.

Conventional Knockout/Knockin & Conditional Knockout/Knockin Models

In this approach, the desired genomic changes are introduced into the genome by homologous recombination at a selected locus in ES cells. The conventional knockout approach requires that the gene of interest be completely inactivated in all cell and tissue types. Inactivation of a gene can typically be achieved through random mutation, gene trap approach, or through gene targeting. Embryonic lethality is not uncommon for this model. The conditional knockout approach, on the other hand, inactivates the gene of interest either in a subset of tissues or at a particular time in development. Conditional knockouts are usually achieved through the Cre-lox technology. While

both models are complex and require significant time to construct, the conditional knockout approach has the advantage of avoiding potential embryonic lethality. In both conventional and conditional knockin approaches, a single copy of the transgene is introduced into a specific locus; as a result, expression of the transgene in ES cell based models is more consistent than standard transgenic models.

Advantages:

This method allows control over the site of integration and the number of transgene copies integrated. It also allows for large fragments to be knocked in, or knocked out, both in conventional and conditional manners with high efficiency.

Disadvantages:

ES cell based approaches are significantly slower than standard transgenic methods (~12 months as opposed to 3-5 months). Knockin models can require more time to assemble the DNA targeting construct and screen for correctly integrated embryonic stem cells.

Key considerations:

Cyagen utilizes a proprietary ES cell based approach called Turboknockout® which significantly reduces the time it takes to generate animals. By utilizing a super competent ES cell line and a self-deleting targeting cassette, TurboKnockout® can deliver germ line transmitting animals in only 6-9 months, on par with CRISPR based approaches.

Genome Editing Knockout & Knockin Models

Several types of artificially constructed nucleases (e.g., TALEN, CRISPR/Cas9) can be engineered to recognize and cleave arbitrary sequences. When such nucleases (or their DNA or mRNA precursors) designed to target a specific site in the genome are microinjected into fertilized eggs, cleavage at the target site followed by imperfect repair can result in small deletions (and insertions, more rarely) of one or more base pairs. If the cut site is in the coding region of a gene, this can generate a knockout. If a repair template is present during the repair process, specific point mutations can be introduced at the cleavage site, generating a knockin.

Advantages:

CRISPR base methods are faster than traditional ES cell based approaches and are typically less expensive.

Disadvantages:

Historically, compared to ES cell targeting, the efficiency of knockins of large pieces of DNA using CRISPR is very low and is one major limitation of the CRISPR approach. Additionally, CRISPR can introduce changes to off-target sites which must be screened for to prevent undesired changes and secondary phenotypes.

Key Considerations:

Cyagen has performed extensive R&D and offers CRISPR based knockouts and knockins with guaranteed delivery for fragments up to 6kb in length (2kb for rat). Cyagen also performs a thorough bioinformatics analysis for potential off-target effects and subsequently screens founders for potential off-target genomic modifications.

Humanization Models

Humanization of mouse and rat alleles is a powerful approach to make mice and rats better suited for human biomedical research. A humanized allele consists of a rodent gene which is eliminated and replaced by the corresponding human orthologous gene sequence. Essentially, this allows experiments on human genes to be performed *in vivo*, within a rodent. Humanized rodent models have been used in immunology, cancer research, drug discovery, transplantation research, infectious disease research, and other fields[1-10].

Approaches to humanization

There are two distinct approaches to humanization. In so-called "knockout-plus-transgenic humanization", a knockout animal is crossed with one carrying a randomly integrated human transgene, while "*in situ* humanization" consists of the direct replacement (i.e. knockin) of a rodent gene with its human counterpart at the same genetic location. In both cases, the human allele may be either a human bacterial artificial chromosome (BAC) or a smaller human gene construct.

In situ humanization is often more laborious, due to the need for ES cell manipulation. Since many mouse knockouts have already been made, the knockout-plus-transgenic approach often requires only the making of a new transgenic mouse and crossing with an available knockout strain. However, random integration in the transgenic allele may have unexpected consequences in the humanized animal.

Humanization by CRIPSR/Cas9 or TALENs

The recent advances in nuclease-mediated genome editing now allow rapid and inexpensive mouse and rat knockouts and specific point mutations without the use of ES cells. For humanization studies, this can be used to quickly mutate specific bases to match a human allele of interest or to efficiently knockout a rodent gene for us in knockout-plus-transgenic humanization. Rat knockouts have previously been very difficult due to the lack of robust rat ES cell lines, and

humanized rats are now likely to become a powerful tool for human biomedical research.

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