ingenious targeting laboratory

Building Better Humanized Mouse Models

INTRODUCTION

Humanized animal models revolutionized biological research and there has been a steady pace of method development to expand the options for this type of animal model.

Multiple complementary strategies are now routinely used to create mouse models that mimic different aspects of human biology. Below we'll briefly discuss some of the most popular options with a focus on genetically humanized mouse models, a particular specialty of **ingenious targeting laboratory**.

Two Pathways towards Humanization:

Commonly used methods to recapitulate relevant parts of human biology:

(1) Transferring cells into a host animal to duplicate a specific feature of human biology, for example to recapitulate the human gut microbiome or to make the animal's immune system function more like a human one.

(2) Engineering human genetic features into an animal genome, from a single nucleotide change to the insertion of potentially kilobases or even megabases of human genomic sequence.

Humanization Of The Mouse - A Historic View

Comparative medicine is founded on the ability of animal models to mimic human physiology, for example by reacting similarly to a specific infectious disease. A constraint on this kind of research in the past was the limited number of methods available to modify potential animal models. Often the best animal model for a specific condition, the one which most closely matches the course of human disease, would not be the easiest to work with. For example, if the most faithful animal model was the pig or dog then the associated costs would restrict which experiments could be performed. Techniques for genetic manipulation that became available in the 1980s radically changed the decision-making process for selecting an animal model. Mice could now be genetically manipulated and made into viable models for specific diseases, where previously they would have been unsuitable.

Today more kinds of mouse models are available that are humanized in different ways, from changing a single nucleotide in the genome to creating a partial human immune system within a mouse. The methods to create humanized mouse models are constantly improved and people are finding new applications for these models every day.

HUMANIZATION OPTION 1

Transplanting Cells or Tissues into an Animal Host:

Humanization via transplanting cells or tissues creates a platform to study human biology in the context of a living animal model.

This circumvents many of the experimental limitations that can interfere with interpreting the results of an experiment. No cell culture system can duplicate the conditions within a live animal, and no non-human cell is a perfect model of its human equivalent. These differences between human and non-human cells are compounded at the tissue level, such as in the heterogeneous mixture of cells found in a tumor or in the gut microbiome.

In the past the idea of transplanting human tissues into an animal host was generally unfeasible due to rejection by the animal's immune system. After immune-compromised mice were identified it was quickly realized that they could offer new options for humanization at the level of cells and tissues. The innovative use of immunodeficient mice allows human cells to proliferate and differentiate after transplantation, without interference from the immune system. One example is tumors derived from human tissue which are much more likely to successfully engraft and grow when transplanted into such a host.

It is also possible to partially reconstitute the human immune system by transplanting human stem cells, and the resulting mice with humanized immune systems are fundamental to studies of immune system/tumor interaction. There's also increasing awareness that the bacteria living in the human gut are an important aspect of human physiology and play a role in human health. Mice are now available with a mixture of gut bacteria that mimics what would be found in a human gut.

The methods for generating humanized mice using various transplantation protocols are under constant development, including the creation of new genetically modified mice that are more receptive to transplantation. The major obstacle to using mice which are humanized by cell transplantation is that every single mouse must undergo a potentially complex procedure. Reconstituting a human immune system by this method requires transplantation of human stem cells into a mouse with no immune system of its own. The great utility of these models is balanced by the costs and complexity of using them.

HUMANIZATION OPTION 2

Genetic Humanization:

The term genetic humanization covers a broad array of strategies for introducing and expressing human genetic sequences in an animal model.

An animal model that's genetically modified to contain human genetic sequence is said to be humanized with regard to that sequence. The genetic change may be as small as a single altered nucleotide (to humanize a single crucial site in a protein) or as large as kilobases or megabases (potentially an entire gene or gene cluster including promoter regions). When looking at the best strategy to create a model of this type it's vital to consider as many factors as possible – each of the approaches described below (and shown in **Figure 1**) has its own advantages and disadvantages.

Figure 1 The most common genetic manipulations used to create humanized mouse models.

Random Transgenic Humanization

The first major decision when creating any genetically modified mouse model is whether to use a random or a targeted approach. Several of the first transgenic mouse lines created in the early 1980s used the strategy of randomly inserting human genomic sequence into the mouse genome, which could include an entire gene and its promoter. An evolution of this strategy is the insertion of human genetic sequence into a mouse line where the homologous mouse gene is already knocked out.

Random transgenic lines can be made for a relatively low cost and potential founder animals are generated quickly. The method is versatile and it's a straightforward way to address a specific research question such as the effects of overexpression. However the random insertion of new sequence into the genome is not the best way to get consistent results. Different lines created this way can have widely varying expression levels for example. Expression of the transgene will depend on the location where it's inserted. Ideally it will land in a region of the genome without genes or regulatory sequences and the promoter driving the transgene will function as desired. However it's possible for a transgene's expression to be affected by the surrounding genetic landscape, altering its expression pattern, and the transgene may also disrupt expression of nearby mouse genes. Identifying the location of transgene insertion is part of characterizing a new line and skipping this step adds an element of uncertainty to all experiments using the line. Additionally, when combining a transgenic knockin of human sequence with knockout of the corresponding mouse gene the two independent alleles must be managed. This can complicate breeding necessary for experiments. The additional time and cost necessary for the creation of a targeted transgenic line may outweigh the uncertainty of a random transgenic.

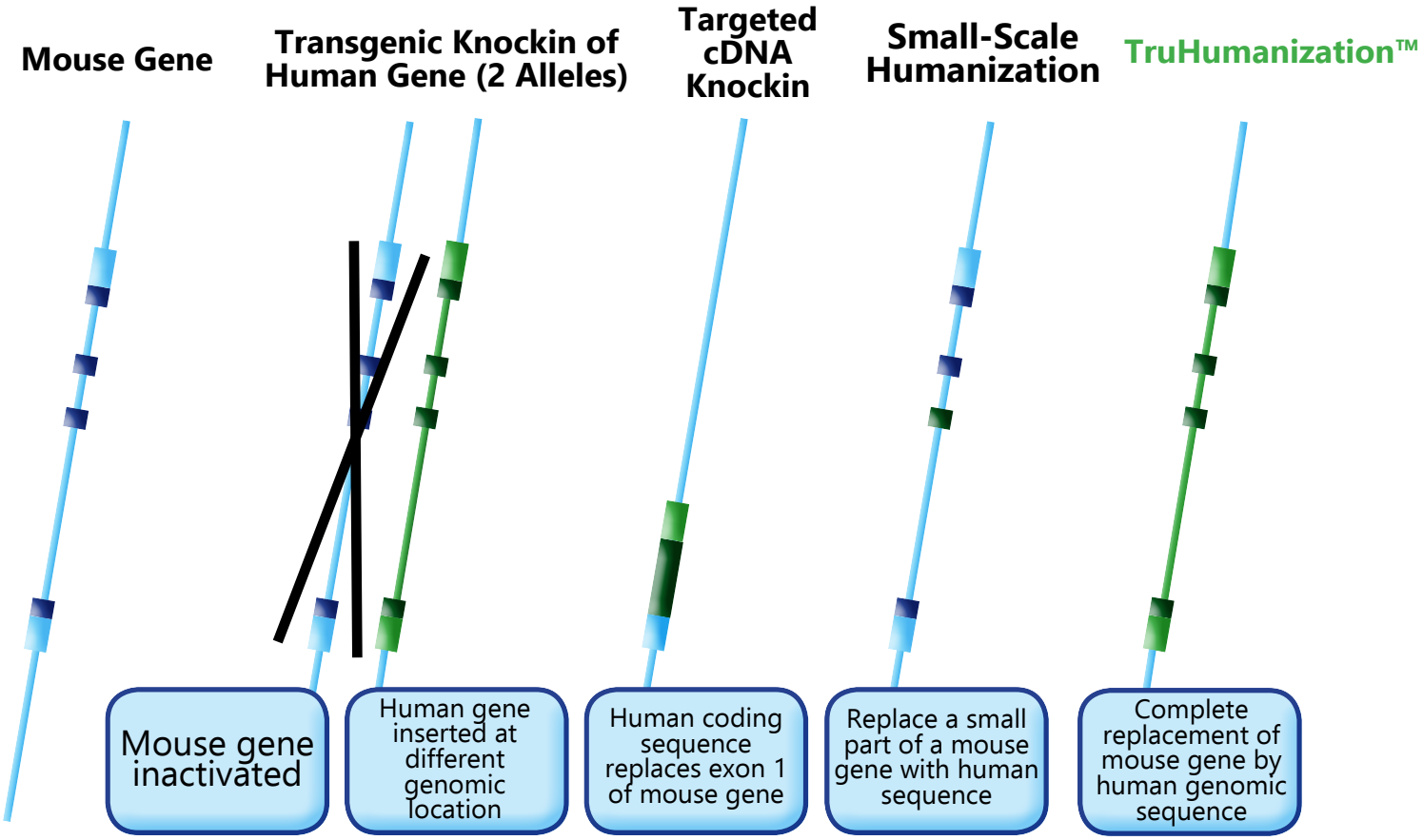
Targeted Genomic Humanization: ingenious' specialty

Targeting the knockin of human sequence directly at the corresponding mouse gene removes the uncertainty inherent in random transgenic strategies and simplifies breeding for experiments. Although it is more complex to create targeted knockins, the results are predictable: insertion of a single copy of the desired sequence exactly at the desired location. A targeted knockin can be as small as a single nucleotide or much, much larger. Replacing a single nucleotide in the mouse genome may sound like too small of a change to fall under the umbrella of genetic humanization but of course a single nucleotide can make all the difference.

Another common strategy for a targeted knockin is to insert a cDNA for a human gene into the first coding exon of a mouse gene. Such a replacement achieves two goals: the inactivation of the mouse gene and the expression of the human sequence. Using such a line is simple as there is only a single allele to account for during breeding, and expression of the human sequence is driven by the mouse gene's promoter. The major limitation of this design is its artificiality. Any regulation of the gene at the level of pre-mRNA splicing is lost and it's common to omit the 3′ UTR when knocking in the sequence. Generally the effects of these changes will be subtle but researchers must be aware that some regulatory features are absent and this could affect experimental results.

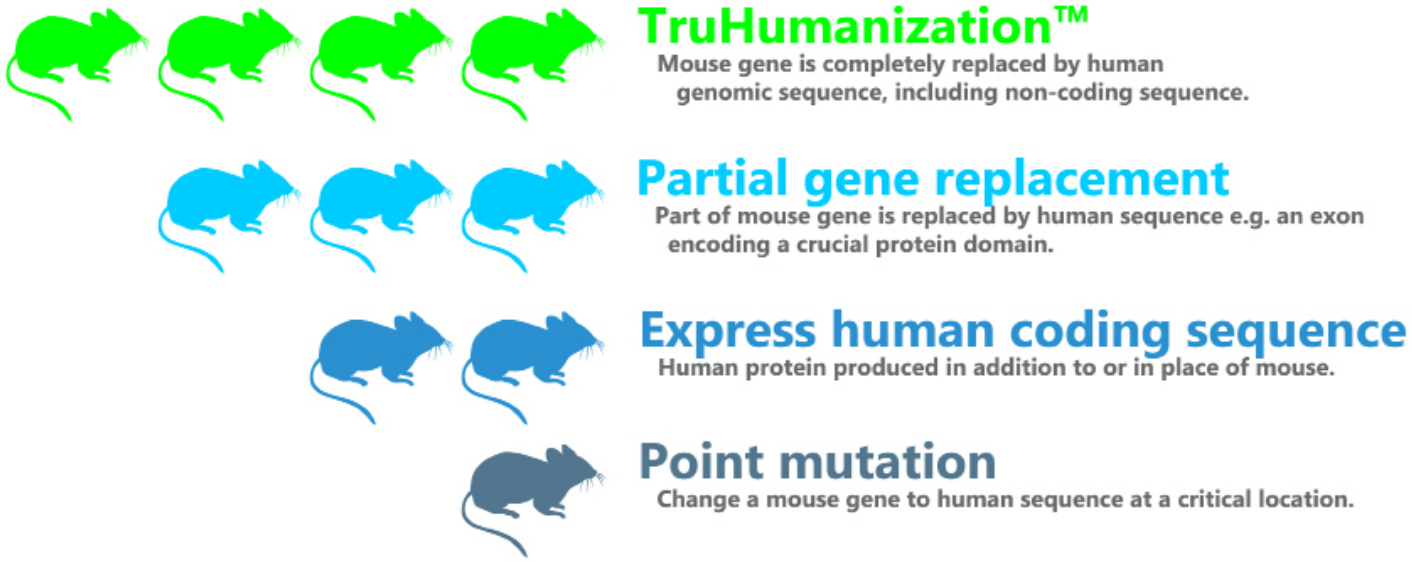
Partial gene replacement is another option and can be used to humanize a small, specific part of a mouse gene. This strategy is viable when a particular region of interest in the human protein, such as a substrate-binding domain, is encoded by a single exon. A one-for-one swap of human genomic sequence in place of mouse minimizes disruption of the gene while creating a model that's humanized specifically at the site of interest.

FIGURE 1



TRUHUMANIZATION™ BY INGENIOUS

The most faithful genetically humanized models duplicate as many features of a gene as possible including genomic location and regulatory regions.



The **TruHumanization™** strategy from **ingenious** represents the state of the art when it comes to genetic humanization. With this approach a region of the mouse genome is precisely replaced with human genomic sequence. Essentially this combines knockout of the mouse gene and knockin of the human into a single allele, but that description doesn't capture the power of this model type. **ingenious**' **TruHumanization™** process can place up to 200kb of human genomic sequence into a desired genomic location, in a single step. The method is precise, so you can design exactly the model you need with the exact amount of human sequence required for your experiments. For example, you may wish to humanize only and exactly the sequence that encodes an extracellular domain but leave part of the gene with mouse sequence. Such a region might span part of exon 1, all of exons 2 and 3, and part of exon 4. **ingenious**' **TruHumanization™** method can replace exactly this region with human exonic and intronic sequences.

Alternately you may want an entire gene humanized, from a promoter element 5kb upstream of the start site to the end of the 3' UTR. Every essential region of the gene can be included in the humanized region, including regulatory features you may not be aware of. This is the true power of a **TruHumanization™** model: by more faithfully recapitulating the human gene it can be used for any future study of that gene rather than being limited by what is known today. A **TruHumanization™** model created by **ingenious** will include all regulatory features in the replaced region such as promoters, splice sites and UTRs. The human sequence replaces the homologous mouse gene so it's more likely to have the same genomic landscape as the gene does in human cells.

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Whether you are looking to specifically replace a single nucleotide or an entire mouse gene with human, **ingenious targeting laboratory**'s capabilities and technologies can provide a solution that best meets your research and experimental needs.

ABOUT INGENIOUS TARGETING LABORATORY

ingenious targeting laboratory is a leading global provider in generating custom genetically modified mouse, rat, and rabbit models. As one of the very first mouse gene targeting companies, our trusted service is built on over two decades' worth of successful animal model creation for investigators, organizations, and companies worldwide. Our models have been published in hundreds of journals including *Science*, *Nature*, and *Cell*, making us one of the most validated and respected production companies in the industry.

In addition to our core capabilities, **ingenious** has recently started offering catalog mouse models, such as a large-scale humanized ACE2 model for COVID-19 studies. We've also partnered with Shanghai Model Organisms Center to distribute select single and double immune checkpoint humanized mice in North America and Europe.

ingenious is constantly developing genetically humanized models with greater potential usefulness. Our work to increase the genetic humanization capacity of the mouse and other animal models will broaden their experimental usage and translational potential for the research community.

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