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A review for transgenic mouse model creation and its use in drug discovery

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Abstract
Introduction of human disease trait genes into the mouse germ line is considered a major technical advancement in the fields of developmental biology and genetics. This technology now referred to as transgenic mouse technology has revolutionized virtually all fields of biology and provided new genetic approaches to model many human diseases. There were many transgenic lines with expression of foreign genes specifically targeted to desired organelles/cells/tissues have been characterized. In GLP standard *in vivo* laboratory facility housed knock out/in and transgenic mice. Right now, international institutions like Charles, Jackson labs are creating the new knock out/in and transgenic mice and supply those to worldwide. Moreover, cost of each model is very high and we paying in terms of dollars. More importantly, we have lagging of facilities to maintain and preserve the germ lines of existing transgenic mice. In this review we explain how to create transgenic and compared with different techniques of gene editing.

Keywords: Transgenic, mouse models and drug discovery

Introduction
For drug discovery labs or institutions require in-house transgenic facility for creation and maintaining and recreation of available transgenic models. That transgenic facility should comprise the gene manipulation technology as well as germ line preservation technology. Need the experts in the field of molecular genetics, and skilled in the microinjection, collection of mouse eggs and genetic manipulation of mouse embryonic stem cells to create transgenic, gene edition and gene knockout mice for understanding of regulation of gene expression and for delineating the function of mammalian genes.

Rationale of the study
Transgenic technology offers limitless opportunities to determine *in vivo* gene functions in numerous ways. In general, the transgenes are expressed in a tissue/cell-specific manner using either homologous (i.e. mouse) or heterologous (for example, rat, cow, sheep, human, pig, etc.,) gene regulatory sequences. To exploits the targeted manipulation of mouse embryonic stem (ES) cells at desired loci by introducing loss or gain of function mutations as small as a single base pair change to megabase range chromosomal alterations. ES cells are derived from the inner cell mass of E3.5 mouse blastocysts [1]. These cells are pluripotent and can contribute to all cell lineages of the embryo proper when injected into recipient blastocysts. Typically, the donor and recipient blastocysts are obtained from different coat color mice that enable the easy identification of the resulting offspring, called chimeras that display a characteristic patchy distribution of coat colors. The germline transmission of the mutant allele is achieved by breeding the chimeric male mice with normal control female mice [2]. The resulting heterozygous mice are intercrossed to obtain the homozygous mutant mice usually at 25 % frequency, if the mutation is not detrimental to embryo survival and development. In addition to the above standard gene targeting approach, gene inactivation can also be achieved both spatially and temporally and in a cell-specific conditionally restricted manner. The reader is referred to excellent reviews and various other sources for a detailed description of the principles and methods of gene targeting strategies in mice. In the following sections, we will describe the standard methodology used at our Transgenic and Gene-targeting Institutional Facility at the Kansas University medical Center [3].

Current status of research and development in transgenic technology

Transgenic technology offers limitless opportunities to determine *in vivo* gene functions in numerous ways. In general, the transgenes are expressed in a tissue/cell-specific manner using either homologous (i.e. mouse) or heterologous (for example, rat, cow, sheep, human, pig, etc.,) gene regulatory sequences. Mouse genes or cDNAs appropriately marked with random oligos or engineered with heterologous downstream polyadenylation DNA sequences are also used. The effects of overexpression of transgenes on organ development and/or physiology are then monitored. In some instances, ectopic expression of the transgene is also achieved by purposefully directing its expression to tissues/cells different from those in which the corresponding mouse gene is normally expressed [4].

In several instances, data obtained with cell transfection studies on mapping the regulatory regions of genes that confer tissue/cell specific expression most often are not very well correlated or difficult to interpret, compared to the corresponding *in vivo* scenario. In such cases, transgenic approach is often used to identify, map and define the minimal regulatory elements of a given promoter that dictate tissue/cell specific expression and hormonal regulation. This is usually achieved by first engineering a series of deletion constructs from a larger piece of the gene that is known to confer tissue/cell specific expression [2]. Subsequently, the truncated transgenes are microinjected to produce transgenic mice and their expression in the selected cell type is monitored at the RNA and/or protein level as an endpoint. Similar strategies have also been used in which a known transcription factor-binding site is mutated on a given promoter driving the expression of a transgene and its functional consequence tested *in vivo*. In case, the promoter elements of a given gene are already identified and characterized, these can be used to direct the expression of useful reporters that can be qauntitatively assayed. The commonly used reporters for quantifying the promoter activity include lacZ from *E. coli*, chloramphenicol acetyl transferase and the firefly luciferase [4].

Developmental expression of many genes that have important endcorine function can be tracked using lineage marking and cell fate mapping. Depending on specificity and expressivity of the gene regulatory sequences and the earliest time at which these are activated as early as during embryogenesis, expression of either lacZ, alkaline phosphatase or various fluorescent reporters (for example, GFP, CFP, YFP or dsRed) can be targeted to specific cell types [5]. Tissues/cells are harvested in such cases, and the activity of lacZ (formation of a blue product), alkaline phosphatase (formation of blue or red product) or the visualization of distinct colors under ultraviolet illumination is monitored starting from embryonic stages. Thus, tracking such "reporter-tagged" cells through distinct developmental stages will provide a novel way to study the linage specification and differentiation of desired cell types. Furthermore, cell-cell interactions during organogenesis can sometimes be visualized on a short-term basis in a Petri dish by live cell imaging using confocal microscopy. Because cells expressing fluorescent reporters can be sorted by fluorescence activated cell sorting, these transgenic mice will also provide novel resources to purify desired cell types from a tissue consisting of heterogeneous populations of various other cells. These can be further used for gene/protein expression profiling under normal physiological or pathological conditions [6].

Transgenic approaches that identify cell/specific regulatory elements have also been useful for selectively ablating cells at desired times and study the consequences of the loss of hormones secreted from these cells [7]. This has been achieved by expressing either diphtheria toxin, or herpes simplex virus thymidine kinase, or viral-specific ion channels. In the latter two cases, either an appropriate substrate (gancyclovir) or an ionophore (calcium or sodium channel activator or blocker) is used to produce either a cell-toxic product or changes in ion flux that affect hormone secretion, respectively [8]. These approaches have been used, for example, to the consequences of ablation of gonadotropes on gonadal development and reproduction. More recently, ablation of Sertoli cells has been achieved to study the consequences on germ cell development and function that consequently impact male reproduction.

Work Plan for creation of transgenic animal.

S. No	Techniques in the Work Plan	Trans-Genic	Gene Edition	Gene Addition	Knock-out	Knock-Down
1.	Sperm / Oocyte/ Embryo collection	✓	✓	✓	✓	
2.	Sperm / Oocyte/ Embryo Cryopreservation	✓	✓	✓	✓	
3.	Sperm / Oocyte/ Embryo Thawing	✓	✓	✓	✓	
4.	In vitro Sperm Capacitation	✓	✓	✓	✓	
5.	In vitro Oocyte maturation	✓	✓	✓	✓	
6.	In vitro Fertilization	✓	✓	✓	✓	
7.	In vitro Embryo Culture	✓	✓	✓	✓	
8.	Embryo splitting	✓	✓	✓	✓	
9.	Pluripotent Stem Cell Culture	✓	✓	✓	✓	✓
10.	Microinjection	✓	✓	✓	✓	✓
11.	Gene isolation, cloning / amplification	✓		✓		
12.	Gene knock-in (Transgenic)	✓		✓		
13.	Gene knock-out				✓	
14.	Gene Editing(CRISPR/Cas9)	✓	✓	✓	✓	
15.	Conditional knock-out		✓		✓	
16.	ROSA26 Knock-in		✓	✓		
17.	Reporter Knock-in		✓	✓		
18.	Construct Designing (miRNA/ siRNA /shRNA)					✓
19.	Gene Knock-Down / Silencing					✓
20.	Viral vector transmission	✓	✓	✓	✓	✓
21.	Non-Viral Vector transmission					✓
22.	ES Cell Transfer	✓	✓	✓	✓	
23.	Sperm mediated transfer	✓	✓	✓	✓	✓
24.	Electrophoration					✓

25.	Artificial insemination	✓	✓	✓	✓	
26.	Embryo transfer	✓	✓	✓	✓	
27.	Design and setup of SPF and Gnotobiotic lab	✓	✓	✓	✓	
28.	Delivery of transgenic, gene edition and gene knockout mice in Gnotobiotic lab	✓	✓	✓	✓	
29.	Nourishing of transgenic and knockout mice	✓	✓	✓	✓	
30.	Breeding of transgenic, gene edited & knockout mice	✓	✓	✓	✓	
31.	Clinical pathology and microbiology screening	✓	✓	✓	✓	✓
32.	Genetic and health quality control	✓	✓	✓	✓	✓

Conclusion

Transgenic strategies permit immortalization of rare cell types that are often difficult to obtain in large numbers and good purity for routine cell transfection analyses. This is usually achieved by targeted expression of viral oncogenes to immortalize desired cell types *in vivo*. Moreover, novel cell lines are derived from these tumors and established as useful *in vitro* tools for various studies. Since many cell types within the endocrine organs are post-mitotic, this approach has been particularly very useful for immortalizing these endocrine cell types and establishing novel cell lines. Many of these cell lines have been used to investigate specific signal transduction pathways, and transcriptional regulation. In some cases, these tumor-prone mouse models also phenocopy known human cancers and thus have tremendous potential to understand the pathobiology of the human disease. Furthermore, these models can also be a useful resource for identifying novel cancer biomarkers.

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