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Review of Mouse Gene Targeting

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Abstract
The mouse has become an extremely useful experimental model in both basic and clinical research because to the ease with which new DNA sequences of interest may be introduced into the germline genome. Homologous recombination facilitates the random or targeted integration of DNA sequences into a host cell's genome for a wide range of applications, such as: modifying genes of interest by introducing deletions or additions to test their function; using mice with human genes inserted into their genomes as experimental subjects for research on human illnesses; increasing mouse susceptibility to human-specific infections; and introducing indels (such as those of viruses). There are recombination systems like Cre/loxP and FRT/FLP that may be used to conditionally activate or inhibit gene expression of interest within a given period, in a small subset of cells or organelles region of the mouse. We will discuss numerous considerations while developing gene-targeted mice and present a brief history of gene targeting technology.

Keywords: MultiSite Gateway Cloning, Targeting Vector, ES Cell Lines, Reporter Mice, Knockout Mice, Transgenic Mice

1. Antiviral Immunity and Pathogenesis in Mice Using Transgenic Models

We have learned a lot more about the processes of viral pathogenesis and antiviral immune responses thanks to the generation of mice with germline genetic changes during virus-host interactions. It has been common practice, for instance, to research virus-specific T cell responses using transgenic mice models expressing antigen-specific, major histocompatibility complex (MHC)-restricted T cell receptor (TCR) transgenes. These transgenic mice include those that express a TCR transgene specific for influenza virus hemagglutinin (HA) in the context of major histocompatibility complex (MHC) class I or II molecules. Moreover, individuals who have a T helper cell epitope derived from the glycoprotein of express a T cell receptor for lymphocytic choriomeningitis virus (LCMV), also known as receptor (TCR) that is MHC-restricted to recognize this epitope.

Transgenic mice human receptors tailored to detect and stop the spread of these viruses have been successfully produced since Mice do not have any kind of inherent resistance to the vast majority of human viruses, including measles, hepatitis viruses, papillomaviruses, polioviruses, and human immunodeficiency virus-1 (HIV-1).

It is possible to use transgenic mice instead of animals transfected with human virus receptors because transgenic mice may express (conditionally) certain genes or full viral genomes. Scientists may research the pathophysiology and immunological responses elicited by viruses of interest in humans by (conditionally) expressing viral genes in mice, so imitating infection.

Several cytokine reporter mice have been developed to permit monitoring of cytokine production during, for instance, an infection using a cytokine reporter mouse as a model. This is necessary for elucidating the cellular sources of cytokines, essential soluble mediators of innate and adaptive immune responses. IFN-Luciferase-knocking reporter mice, are used to examine the induction production by mouse cytomegalovirus of type I IFN (MCMV).

2. Methods for Creating GM Mice

The Although genetically modified mice have been used in studies related to viral pathogenesis and immune response, it is important to note that it was the use of viruses that first allowed researchers to edit the mouse genome and lead to the creation of the first transgenic mice in 1976. The Moloney murine leukemia virus was used by Rudolf Jaenisch to infect mouse embryos before they were implanted (5-9 cell stage) to test the hypothesis that exogenous viruses (which are carried from person to person via casual contact as opposed to being handed down from generation to generation) have the potential to mutate into endogenous viruses (of which DNA sequences are present in all somatic and germ cells of an individual and are passed on to the offspring) (M-MuLV). Mice born from contaminated preimplantation embryos had an increased risk of developing leukemia caused by M-MuLV, and the leukemia was passed on to subsequent generations through the germ line.

Microinjecting target DNA entering the nucleus of mature murine eggs that have been fertilized is a common method for creating transgenic mice.

Mice may be genetically edited by injecting foreign DNA into fertilized murine eggs or by infecting embryonic mice with retroviruses; in both cases, the foreign DNA is randomly integrated into the mouse genome. Therefore, the transgene may be expressed inconsistently, and other genes may be disrupted by accident at the insertion site. When compared to what may be predicted from the random integration of a transgene into the genome, the frequency of abnormalities resulting from insertion site modification by a transgene (which is about 9%) is much greater, and this is largely due to the significant erasures and intricate rearrangements at the point of integration when transgenes are introduced via pronuclear injection. However, since its inception in the late 1980s, homologous recombination in murine ESCs has made it possible to integrate foreign DNA of interest at a precise position in the mouse genome, allowing for precise and targeted genetic modification.

3. Animals with Embryonic Stem Cell-Induced Mutations

Extensive research into whether cultured mammalian cells can mediate homologous recombination between their indigenous DNA and exogenously inserted DNA molecules made it possible to comprehend that genes of interest could be specifically edited in a whole mouse. This revelation was made possible as a result of the findings of the research. Concurrently, mouse embryonic stem cells (ESCs) maintained their pluripotency after *in vitro* culture. Therefore, they have the potential to add to the germ line if put into a preimplantation embryo. As a consequence of this, embryonic stem cells (ESCs) have seen considerable usage as vectors for the purpose of delivering mice with targeted genetic changes in their germ cells.

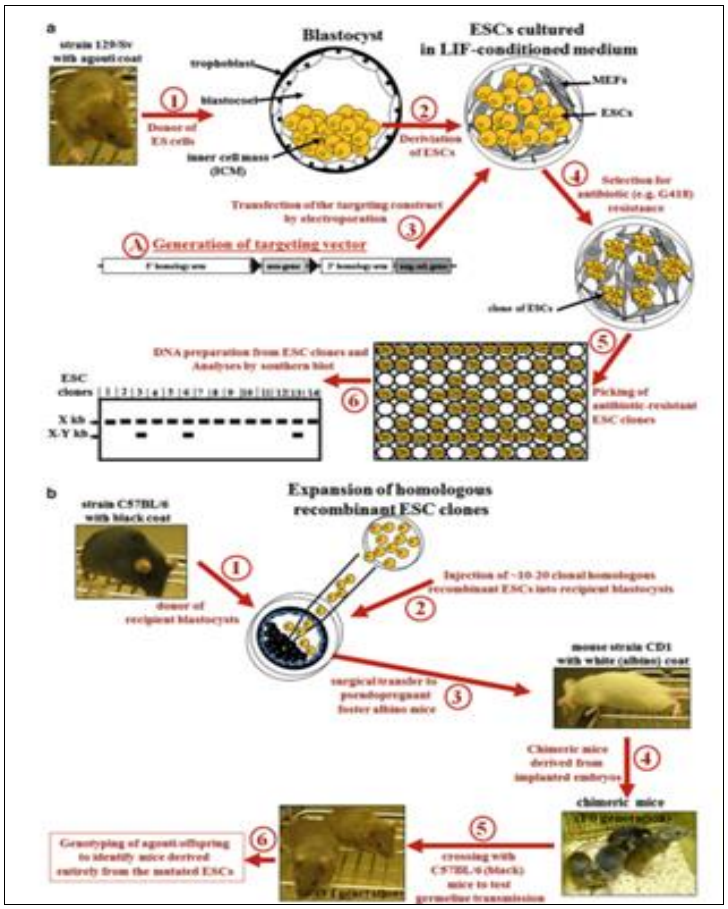

Fig 1: (a) Mouse ESC isolation and homologous recombinant ESC cloning procedures. Super ovulated mouse females, such as those with an agouti fur coat (129/Sv strain), generate embryos (blastocysts) at 3.5 days of development and remove them from the uterine horn. (2) ESCs are isolated from blastocysts' inner cell mass and cultured on mitotically inactivated MEF feeder layers in ESC media with LIF. After electroporation with the targeted vector, ESCs are selected by adding an appropriate selection agent to the ESC medium, and ESC clones are generated. chosen (step 5). (step 5). Southern blotting can find ESC clones. To insert a gene into a precise region on a chromosome, genomic DNA obtained from ESC clones must be digested with a restriction enzyme to create one cut within the targeting vector and one cut slightly outside (downstream or upstream) the vector. If a probe is used that isn't part of the targeting construct, the band's size will correspond to the unmodified wild-type allele(s), which we'll call X kb, and the targeted allele, which we'll name X-Y or X+Y kb. Creating mice with a particular genetic alteration by cloning embryonic stem cells from the same source. ESCs from agouti-coated mice like 129/Sv must be derived female black-coated mice (C57BL/6). Homologous recombinant ESC clones are generated also put into mice embryos before they were born (blastocysts) by a woman black-coated mice (C57BL/6) (see Fig. 1a). The third and last stage is to transfer the implanted blastocysts to a pseudo-pregnant a woman who takes in foster children. CD1 mouse strain females are foster moms because of their excellent parenting skills. Coat-color chimeras may be used to identify the intended chimeric offspring as both the embryonic stem cells (ESCs) and the recipient blastocysts (BCs) were from mouse strains with distinct coat colors (the black-agouti hair proportion on a mouse). Step five involves mating a chimera with a C57BL/6 mouse to create an F1 progeny as most ESC lines are male. Southern blotting or polymerase chain reaction on F1 agouti (not black) mouse tail DNA may confirm germ line transfer

Mice ESCs may contribute to all embryonic tissues, including germ cells, since they are produced from blastocysts' pluripotent inner cell mass (ICM) (Fig.1.). The pluripotency and ability of isolated ESCs to divide (self-renew) need certain culture conditions. It is usual practice to culture ESCs mouse embryonic fibroblasts that have been bombarded with gamma rays or treated with mytomicin C serve as a feeder layer, which allows for the cells to maintain their self-renewal and pluripotency (MEFs). The status and pluripotency of fi broblast-fed mice ESCs were shown to be influenced by factors such as Signaling pathways such as leukemia inhibitory factor (LIF), wingless/integrated (Wnt), and transforming growth factor beta (TGF)/basic fibroblast growth factor (BMP) ligands.

Recombinant LIF added to ESC culture media aids in the maintenance of pluripotency. Preservation of pluripotency has been proven to be a key.

Several well-tested ESC strains are now in widespread usage. When ES cells were first employed, they were almost exclusively isolated from the 129-mouse strain. The E14 cell line, the D3 cell line, the J1 cultured cells, the Cell line R1 the Cell line AB2.1 are just a few of the many that have been developed and investigated. ESC lines produced from the 129-mouse strain function better in cell culture and have a higher rate of germ line transfer than those from the C57BL/6N and C57BL/6J strains of mice.

Among mouse strains, C57BL/6 is the most common. most often used for immunological, neurological, physiological, and behavioral studies in mice, and its reference library is the backbone of the mouse genome-sequencing effort. A C57BL/6 background mouse requires at least two years of backcrossing mice from 129 ESC lines.

Direct creation of genetically altered C57BL/6 mice is now possible because to the development of ESC lines from this strain that efficiently colonize the germ line. C57BL/6 ESC-derived mice have a reduced (but not abolished) need for backcrossing due to the possibility of mutagenic effects of prolonged culture on a petri dish.

Several Electronic Signalling Circuits generated in the C57BL/6 strain strain are available for use, including Bruce4JM8, LK1, BL/6-III. Targeted mutant C57BL/6 mice were created using JM8 cell lines as part of the huge mouse knockout effort. The JM8 cell line may be easily grown *in vitro* under standard ESC culture conditions, with or without feeder cells. Using targeted correction of the C57BL/6 no agouti mutation, JM8 cells have been genetically modified to produce animals with a dominant agouti coat color, enabling an examination of the role of this gene in the production of the coat color and its effect on the animal's appearance germ line transfer, which would significantly improve breeding operations.

Before settling on an ESC line, think about the following. (3) The targeting vector's homology The ESCs need to have artificial limbs built from isogenic DNA. Analyzing the differences between the C57BL/6 and 129 embryonic stem cell lines (1) for their advantages and disadvantages. To target genes effectiveness in mice, and phenotypic generated ESC line-by-line may be affected by, among other things, (2) the genetic diversity and consistency seen in embryonic stem cell lines derived from a number of different C57BL/6 and 129 substrains. What's more, the cell culture circumstances and knowledge of individual labs determine which ESC line is the best to utilize.

In vitro generated genetically modified ESCs have the potential to be re-implanted either into the blastocoel (cavity) of blastocysts (a pre-implantation mouse embryo that is 4 days old) (Fig. 1) or into morale (early mouse embryo) (3 days old pre-implantation mouse embryo). It is common procedure to inject anywhere from 15 to 25 ESCs into a blastocyst. An alternative method for creating chimeric embryos is to combine ESCs with morale in a single aggregate. It then involves surgically implanting five to ten chimera embryos into the wombs of supposedly pregnant foster women. As long as the women are suitable surrogate moms, it does not matter what their genotype is. That is, they take in pups from other mothers and care for them until they are old enough to wean themselves. Because of this, you should utilize BALB/c F1 females x CD1 females or C57BL/6. Injecting genetically modified ESCs into a mouse embryo result in a small percentage to a large percentage of the resulting pups being with chimera mice, embryonic stem cells were instrumental in the development of most or all tissues (Fig. 1). For example, if ESCs that have been genetically modified have played a role in the development of of germ cells, then the offspring of chimera mice have a chance of inheriting the implanted genetic alterations.

The ESCs and the recipient blastocysts (both of which are of the same mice that are genetically identical to one another in the appropriate allele for hair color) are of readily distinguishable coat-color strains (Fig. 1b). This means that although the ESCs may originate origin: a mouse strain named 129 (which has brown coats / agouti), the recipient blastocysts might come from a C57BL/6 strain (which has black coats). As a straightforward sign of the ESCs' role in molding the chimeric mouse, coat-color chimeras (How many agouti hairs the mouse has compared to how many black hairs) are readily detectable (Fig.1b).

4. Electrophoretic cassettes target embryonic stem cell genes

It is now possible to introduce a wide variety of changes, including new genes, point mutations, small and large deletions, inversions, and even a mix of these. Inserting loxP or FRT sites flanking certain exons allows for conditional knockouts or knockins to be accomplished.

Gene targeting, in which site-specific modifications are introduced into an organism's genome by homologous recombination, is accomplished by electroporation into a target-specific vector embryonic stem cell (ESCs).

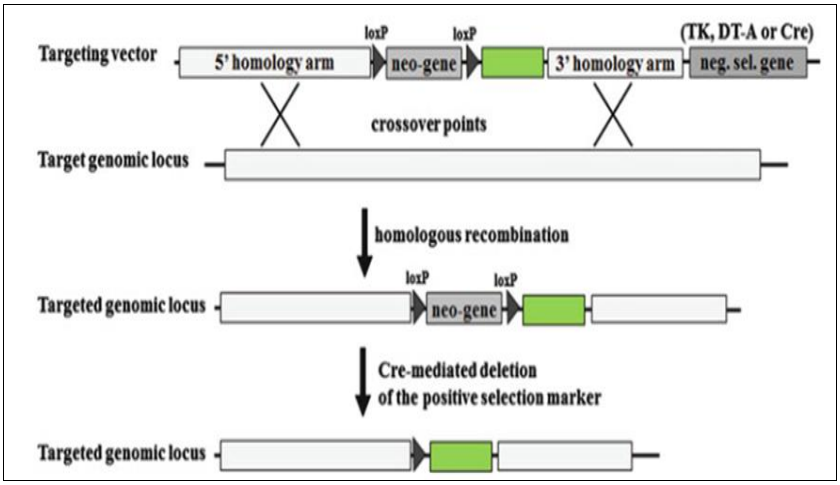

Fig 2: This is your run-of-the-mill gene targeting plan. To facilitate positive selection, a targeting vector often includes a gene marker (such neomycin resistance gene (neo)) and two homology arms, one at the 5' end and one at the 3' end. In addition, a thymidine kinase (TK) or diphtheria toxin fragment A (DT-A) gene, or a Cre recombinase gene Negative selection markers (neg. sel. Markers) may be placed beyond the homology arms of a positive selection marker loxP sites flanking the positive selection marker (Cre). Any DNA sequence of interest may be placed between the targeting vector's homology arms for homologous recombination into the target genome. This can be done in a number of different ways. The homologous recombination that occurs, the negative selection marker gene in the vector is lost, and a copy of the targeted genomic locus is disrupted when there is a mismatch between the targeting vector and the relevant chromosomal region. Within the context of this story, the letter "X" stands for a meeting of two different groups. It is feasible to get rid of either by producing Cre recombinase in the recombinant embryonic stem cells (ESCs) or by mating chimera mice with transgenic mice that express Cre in order to get the fl oxed (loxP sites fl anked) positive selection marker gene

(Image available to see online and print in full color)

The typical DNA targeting vector (shown in Fig. 2) has three parts: (1) a 4′ homology arm, (2) a positive selectable gene marker (such as the neomycin resistance gene (neo) or hygromycin (hyg) resistance gene), and (3) a 3′ homology arm. Whether the transfected targeting vector integrates at random or the length of an organism's 4' and 2' homology arms is what determines whether or not it may undergo homologous recombination. Positive selection of Growing embryonic stem cells (ESCs) in a medium containing neomycin (G418) or other suitable antibiotics, such as hygromycin or puromycin, is the method that leads to the formation of transfected cells (Fig. 1). Whether in chimeric mice or recombinant ESCs transfected with Cre- or FLP-expressing vectors (FLP-expressing transgenic mice or through crossing with Cre), the positive selection marker gene may be deleted from specific loci. It's important to remember that inserting selection marker genes like neo into a genome might drastically neighboring endogenous genes' expression may be influenced.

Very seldom (at a frequency of 10-3 to 10-4 compared to no homologous recombinants), a targeted vector will undergo homologous recombination into a genomic location of interest. In general, the targeting frequency improves with increasing lengths of the 5' and 3' homology arms. Additionally, it is possible that the fact that Since the ESC DNA is isogenic with the targeted vector's homology arms, would increase the probability of homologous recombination. The genomic DNA utilized to produce the homologous arms should ideally come from the same mouse strain as the ESCs themselves. The probability of homologous recom- bination may also be increased by linearizing the targeted construct prior to transfection into ESCs. Nonetheless, since homologous recombination is so uncommon, for the small number of the process of creating hybrid clones by combining DNA from two different sources screening of at least 250 and frequently up to 1,500 is required.

To improve the odds of selecting Markers for negative selection are employed to isolate homologous recombinant clones. Examples include herpes simplex virus thymidine kinase (TK), as well as fragment A of the diphtheria toxin (DT-A) from Corynebacterium diphtheriae. The gene encoding the negative selection marker is not inside the homology arms. Many times, while repairing a genomic region by homologous recombination, non-homologous sequences are lost. On the other hand, the negative selection will not work if the gene-targeting vector integrates randomly in the genome. signal is usually preserved. Thymidine analogues like 6-iodo-3′-fluoro-3′-deoxy-1-β-D-ganciclovir or arabino-furanosyluracil (FIAU) are added to cultural transmission medium of ESCs in order to eliminate clones that have randomly inserted targeting vectors. This is done in order to prevent the spread of the randomly inserted targeting vectors. These thymidine analogues are activated by the TK enzyme, which results in their incorporation into DNA replicating at an unusually high pace. This, in turn, finally results in the chain being terminated prematurely, which leads to cell death. The capacity of DT-A to catalyze via nicotinamide adenine dinucleotide (NAD)-dependent ADP-ribosylation of an elongation factor 2-histidine residue with a point mutation. is what causes the toxin effects that DT-A is responsible for (eEF2).

The use of an "auto-selecting targeting vector" in a straightforward negative selection technique has recently been devised (Fig. 2). The targeting vector was modified by inserting an extra Cre cyclization recombination gene homology region subject to the directives of the herpes simplex virus (HSV) promoter as a marker for negative selection. To facilitate the deletion of the neomycin resistance gene (neo), two loxP sites (fl oxed) were placed on either side of the gene. With the use of Cre recombinase, the positive selection marker flanking a recombining loxP site may be removed. is then maintained and expressed by the targeting vector. To put it another way, ESCs cannot expand in G418-containing media (neomycin). Even while

the Cre gene may be removed after positive clones have been identified by homologous recombination, in most instances, in other words, an indicator of positive selection that preserved without any integration taking place.

5. Genome-selective mutagenesis by means of the Cre/loxP recombination system

The bacteriophage P1 generated a 38-kDa protein termed cre recombinase, which acted as a catalyst for two-loxP site-specific recombination (locus of X-over of P1). loxP which consists of two inverted repetitions of 13 base pairs each and is separable 9-base-pair sequence that is not palindromic and asymmetric, is what determines the loxP site's general orientation. The loxP sequence has a length of 34 base pairs and is composed of two inverted repetitions of 13 base pairs each (Fig. 3). When there are two loxP sequences that are aligned in the opposite direction, Cre recombinase is able to facilitate the inversion of the DNA in between, rather than cutting it out of it. On the other hand, when there are two sites aligned in the same direction, Cre recombinase is able to facilitate the excision of the DNA that is located in between the sites, leaving behind only a single loxP site. When the target Cre recombinase may recombine DNA between loxP sites even if they are on distinct chromosomes. has the potential to make it easier for translocations to occur across chromosomes (Fig. 4).

The Cre/loxP system has become an important resource for scientists working with genetic modification. In most cases, loxP sites may be used to remove any desired DNA sequence. After the targeting vector has been successfully integrated into the ESC or mouse genome, for instance, the selection marker may be deleted with the use of Cre/loxP. Through the process of homologous recombination, it is possible to insert loxP sites into the target genetic area. This was previously statedThe elimination of the DNA sequence that has been fl oxed may also be timed and targeted by utilizing conditional expression of Cre recombinase, which can be temporally or spatially controlled. Because of this, one is able to exercise exact control over the time and location at which the deletion takes place (Fig. 4a). In the majority of instances, this will need the use of two different lines of mice (Fig. 4a) in order to perform conditional site-specific genome alteration.

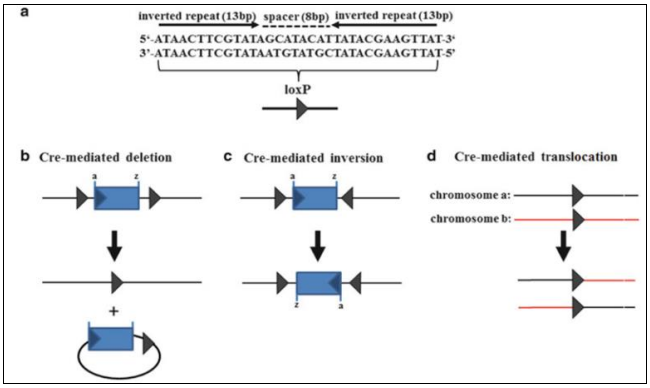

Fig 3: Recombinations mediated by the Cre recombinase and the structure of the LoxP protein. At a given loxP site, there are two sets of 13 base-pair repeats that are mirror images of one another, segregated by an uneven distance 9-base-pair series (a). The direction and placement of the loxP sites determine the kind about recombination mediated by the Cre element When Cre is used to induce the process of genetic crossover involving two separate strands of linear DNA, both of which include a loxP site, the flanking DNA sequences are swapped where loxP sites are located (b) by removing between two loxP sites, a cyclic molecule that are positioned parallel to one another; (c) by switching which direction two loxP sites are placed in DNA and what order their flanking sequences are in. The original figure by Torres and Kuehn was altered

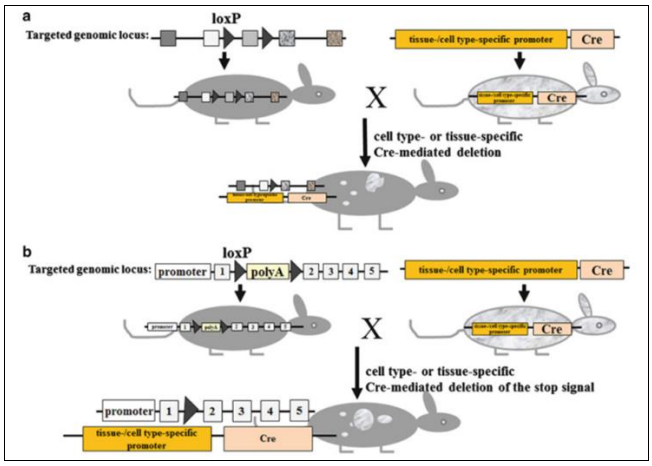

Fig 4: Gene targeting depending on conditions determined by the Cre/loxP recombination mechanism. An important gene is silenced with the use of the Cre recombinase. Left mouse: ESC-based homologous recombination for the insertion in a genomic region of interest by inserting loxP sites in a controlled manner (see also Fig. 1). To avoid disrupting the target gene's function, LoxP sites are inserted in a certain way. To get the desired results, you will need a transgenic mouse strain that expresses Cre-recombinase from a tissue- or cell-specific promoter. Inbreeding of Cre transgenic mice with fl oxed mice causes gene knocked out because Cre facilitates the removal of the fl oxed genomic region. The expression of Cre (white fl ecks) defines the "region" in which genes may be deleted. Activating a gene of interest using the Cre-Lox system (b). To impede proper transcription of the intended gene, a fl oxed intervening region (such as a polyadenylation signal sequence) is introduced into the left mouse genome. When a Cre transgenic mouse is bred with a fl oxed mouse, the intervening region loses its existence, reactivating the targeted gene. The context in which Cre is expressed (in white fl ecks), expression of genes is restricted to that specific "region" (cell type or tissue)

First, loxP-fl oxed (floxed) mice in which the sequence of interest in their DNA has been fl anked. Cre recombinase transgenic mice are the second kind, with Cre being produced either transiently regulated by a promoter only present at a certain phase of a cell's or tissue's development, or permanently under the control of the ability of a promoter to activate only in certain kinds of cells or tissues. Flax-background mice hybridized with Cre transgenic mice had their fl oxed DNA sequence erased in organs and cell types where Cre is active. The existence of There are a large number of Cre transgenic mice lines that express Cre recombinase in various tissues. cell types or tissues. have been collected and are searchable via databases (e.g., http://creline.org/; http://bioit.fleming.gr/crezoo/; http://www.creportal.org/; http://www.ics-mci.fr/mousecre/).

As well as allowing for the deletion (shutoff) of target genes, the Cre/loxP system has been shown to successfully allow for the specific activation (switched on) on the level of activity of any transgenic gene. This is achieved by silencing the target gene in question, often by the introduction of fl oxed the presence of polyadenylation signal sequences in a core region of the target gene causes

transcription to halt prematurely. Gene expression in certain cell types or tissues may be activated by intercrossing with a Cre-transgenic mouse or by introducing Cre into fl oxed transgenic mice using, for example, Cre-expressing adenoviruses. This is accomplished by the removal of fl oxed polyade- nylation signal sequences.

In order to prevent undesirable side effects of transgenic expression during mouse embryogenesis or to induce immunological tolerance to the transgene product Using Cre/loxP (as is common with viral genes) to activate genes has been shown to be efficient. For instance, Transgenic mice harboring human hepatitis C virus transgenes were generated using Cre/loxP technology by injecting Cre-expressing adenoviruses into the veins of the animals. The pathophysiology of HCV infection and the immune response to it have been studied in these animals.

Finally, an alternate tool to Cre/loxP does the yeast Fli ppase Object of recognition (Flp)/(Flp) (FRT) recombination system, that does its tasks mechanically comparable manner. The possibility for conditional gene manipulation in mice is further expanded by combining both recombination mechanisms.

Vector Cloning for a Specific Purpose

One of the most important parts of making gene-targeted mice is designing and building the targeting vector. The creation of the aiming vector requires careful consideration of a wide range of factors.

Factors to be considered include, but are not limited to, the specifics Considering the intended genetic modification, and the accompanying scientific questions (such as knockout the if clause expression of a transgene of interest, reporter gene insertion for monitoring gene expression, point mutation insertion, and conditional or constitutive expression relevant transgene to study). In addition, before electroporation, the targeted vector must be linearized at a separate restriction site that is not located inside the homology arms, and the design must account for the possibility of removing the marker cassette used for selection later on. In addition, it is essential to create restriction sites and probes for spotting ESC homologous recombinant clones, in short. For Southern blotting to reliably differentiate between creation of clones of embryonic stem cells that are similar to one another it may be necessary to add new restriction sites. Long-range polymerase chain reaction (PCR) or quantitative polymerase chain reaction (qPCR) reactions are alternative methods for detecting homologous recombination that results from mutation caused by the absence of an endogenous allele. For the correct assembly relative to the vector used for aiming, information regarding restriction sites, promoter region, 5′UTR, exons, introns, intron-exon boundaries, splice donor and acceptor sequences, 3′UTR, and the overall structure of the target gene locus. is required. The whole mouse genome's worth of data have made this process much more manageable (http://www.informatics.jax.org/).

Cloning a gene of interest utilizing standard restriction enzymes, Recombi nation-mediated genetic, or MultiSite Gateway Technology engineering (Recombineering) are all viable options. Using MultiSite Gateway Cloning, we generate targeted vectors, and that process is covered extensive in the next section.

6. Transgenic Gene Insertion into the ROSA26 Promoter

Microinjecting DNA into fertilized mouse eggs to create the desired transgenic is a common method for inducing transgene expression. Nonetheless, this results in erratic transgenic expression and the unintended disruption of genes around the possible transgene insertion site.

If a transgene of interest is inserted into the Rosa26 region, then the transgene insertion and expression may be controlled. Analysis of embryonic stem cell pools that had been infected at a low multiplicity vector Gen-ROSAgeo (bifunctional lacZ/neomycin phosphotransferase gene cassette) for retroviral gene trapping enabled the Rosa26 locus to be located. Rosa26, which codes three transcripts, has incorporated the gene trap vector (only the third transcript, originating from the reverse strand, seems to encode for a protein).

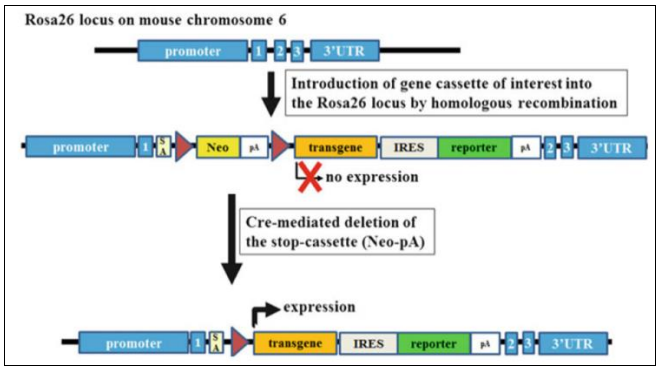

Fig 5: Aiming for the ROSA26 locus to insert transgenes. With the use of homologous recombination, there is the potential to introduce transcripts from relevant genes into the Rosa 26 place. In embryonic stem cells. The transcription of the transgene is inhibited by an artificial sequence called a protein sequence that directs polyadenylation of the neomycin (Neo) gene (pA). Expression of Cre then mediates removing the adjacent section that was fl oxed, allowing for a combination of transgenic expression and reporter gene expression. Monitoring transgenic expression is made easier with the help of the reporter gene. Blue rectangles with numbers (1-3) denote the exons that make up the Rosa26 locus. Acceptor of splice site SA. Drawings of DNA building blocks do not correspond in size (Color fi gure online)

Due to its strong transcriptional activity in all cells, the Rosa 26 locus is preferred to insert transgenes and express reporter genes systemically. Homologous recombination allows must be inserted into the Rosa 26 position, the desired genes. The Cre/loxP method allows for the conditional expression of genes that are Rosa26-targeted (Fig. 5 and Subheading 5).

7. Taking into Account the Form of Genetic Engineering Needed

Eliminating a gene's expression, either by germline deletion or by adding loxP sites around functionally relevant sections to allow conditional Cre-mediated deletion, is the easiest way to assess its function. Typically, remove the first exon from the gene to achieve this (s). However, it is important to take precautions in order to rule out the possibility of gene expression through alternative splicing of exons or internal transcriptional start sites. Instead of keeping the exons that control the protein's catalytic activity or its critical interactions with other proteins, RNA, or DNA, you may delete them. The challenge this being the case approach is that it raises the possibility of creating peptides or shortened proteins that are not neutral in terms of their ability to inhibit the activation of other proteins in the same family. Point

mutations that render the protein inactive in its enzymatic role or in its ability to interact with other proteins may be used to circumvent this problem. Point mutations in immunologically important genes include, but are not limited to, $Vav1^{R442G}$ (Guanine exchange activity has been lost) (interferes with the binding of lipids via the PH domain), $Vav1^{AA}$ (has no guanine exchange potential), $p110\delta^{DASA}$ (withdrawal from Ras interaction), $p110\delta^{KD}$ (unresponsive kinase), and $p110\delta^{D910A}$ (unresponsive kinase).

8. The Use of Reporter Genes in Breeding Mice: Some Reflections

Recently, "reporter mice" have shown to be valuable tools for studying gene expression both in and out of the body. This is notably the case in situations in which the gene products in issue are secreted proteins, such as cytokines, for example. A mouse that has had its expression of an endogenous gene of interest fused with that the expression level of a reporter protein that is found within the cell. referred to as a reporter mouse. This is often accomplished by the use (IRES) peptides or the internal ribosome entry site 2A. The option is to introduce the reporter gene around the endogenous gene's stop codon, rather than at its start. This will cause transcription factor gene used for studying the environment to be expressed rather than the allele that is being targeted.

AFPs like Because green fluorescent protein (GFP) reporters are the most popular, may be subject to level-specific monitoring of a single cell without the need for the therapy of cells via means that are intrusive or the use of substrates from outside the body. However, in order to see the fluorescence up against the background, a significant level of AFP expression is needed (about 10^5 molecules "0.1-1μM" per cell). This is a serious shortcoming when trying to keep tabs on the expression of low-key genes, including those responsible for producing numerous cytokines.

Recent research with IL-10 reporter mice has given evidence that enzymatic reporters are preferable than AFPs for assessing the expression levels of genes that are not very highly expressed. Examples include a mouse model for analyzing and quantifying IL-10 production that uses the Substrates for fluorescein-dependent enhanced fluorescence resonance energy transfer (FRET): coumarin, cephalosporin, fluoresc and the reporter enzyme -lactamase. While "normal" Mice transgenic with IL-10 that report changes in AFP allowed for finding out whether there's been any action with the reporter primarily, these animals had a far broader range of cells where their activity may be detected.

Table 1: The following sources were used to compile this article's bibliography

Molecular reporter	Enhancement of the signal	Substrate	FACS (at the level of a single live cell)	Cells must be lysed and permeabilized	*In vivo* imaging	Fluorometer	Luminometer	Expressions found in the body that are analogous to those seen in reporters	Fluorescence microscopy
β-lactamase (Bla)	True	CCF2-AM; CCF4-AM; nitrocefi n	True	Fault	Fault	True	True	Fault	True
β-galactosidase (LacZ)	True	ONPG; X-Gal; FDG; DDAOG	True/ Fault	Permeability of Cells	True	True	True	True (high)	True
Chloramphenicol acetyltransferase (CAT)	True	^{14}C-labeled chloramphenicol; fl uorescent chloramphenicol derivative	Fault	Death of cells	Fault	True	True	True (minimal)	Fault
Alkaline phosphatase enzyme that has been secreted (SEAP)	True	The PPQ, PNPP; CSPD; and FADP	Fault	Death of cells		True	True	Yes (in certain forms of cell)	Fault
Firefl y luciferase (Luc)	True	D-luciferin	Fault	Death of cells	True	Fault	True	Fault	Fault
Autofl uorescent proteins (GFP, RFP etc.)	Fault (a low threshold for detection)	No substrate is needed	True	Fault	Fault	True	Fault	Fault	True

9. The International Knockout Mouse Consortium

An effort is underway by Association for Research on Mouse Mutants (IKMC) (http://www.knockoutmouseorg/) the goal of which is to produce knockout alleles that are conditional on the presence of every gene on the genetic code of the mouse. This should be considered before starting work on a knockout or conditional knockout mouse (including also microRNA genes). They may provide a variety of targeting tools, including targeting vectors, targeted embryonic stem cells, and gene-targeted mice. In turn, this may lead to significant cost savings and a reduction in the time it takes to carry out tests with novel gene-targeted strains.

10. References

1. Okail, Hanan A, Ahmed S Ibrahim, Amira Badr H. The protective effect of propolis against aluminum chloride induced hepatorenal toxicity in albino rats. The Journal of Basic and Applied Zoology. 2020; 81(1):1-11.
2. Salama, Essam Eldin Abdelhady, *et al*. Protective effect of propolis against aluminum chloride-induced reproductive toxicity in male rats. GSC Advanced Research and Reviews. 2021; 9(2):83-88.
3. Sajjad S, *et al*. Ethanolic extract of propolis and vitamin E attenuates metal-induced testicular necrosis: Time related study on male reproductive system in albino mice. The European Zoological Journal. 2020; 87(1):138-147.
4. El_Roghy, Eman S, *et al*. Evaluation of Propolis Supplementation on Lung Tissue Toxicity Induced by Aluminum chloride in Adult Male Albino Rats: A Histological and Immunohistochemical Study. Egyptian Journal of Histology, 2021.
5. Gal AF, *et al*. Chemopreventive effects of propolis in

the MNU-induced rat mammary tumor model. Oxidative Medicine and Cellular Longevity, 2020.
6. Hawas, Asrar Mohamed Mourad, Laila Ahmed Rashed, Marwa Abd El Hameed Mohamed. Evaluation of glucosamine effect against heart and brain damage induced by Y-radiation or aluminium chloride in female rats. Brazilian Archives of Biology and Technology. 2020; 63.
7. Hosseini, Seyed Mohammad, *et al*. Geraniol.
8. Yokoi, Norihide, *et al*. A novel rat model of type 2 diabetes: the Zucker fatty diabetes mellitus ZFDM]. Fontana, Luis, *et al*. Bifidobacterium breve CNCM I-4035, Lactobacillus paracasei CNCM I-4034 and Lactobacillus rhamnosus CNCM I-4036 Modulate Macrophage Gene Expression and Ameliorate Damage Markers in the Liver of Zucker-Lepr fa/fa Rats. Nutrients. 2021; 13(1):202.
9. Wu-Peng, Sharon X, *et al*. Phenotype of the obese Koletsky (f) rat due to Tyr763Stop mutation in the extracellular domain of the leptin receptor (Lepr): evidence for deficient plasma-to-CSF transport of leptin in both the Zucker and Koletsky obese rat. Diabetes. 1997; 46(3):513-518.
10. Plaza-Díaz, Julio, *et al*. Gene expression profiling in the intestinal mucosa of obese rats administered probiotic bacteria. Scientific data. 2017; 4(1):1-10.
11. Seeley RJ, *et al*. Intraventricular leptin reduces food intake and body weight of lean rats but not obese Zucker rats. Hormone and Metabolic Research. 1996; 28(12):664-668.
12. Drotningsvik, Aslaug, *et al*. Dietary fish protein hydrolysates containing bioactive motifs affect serum and adipose tissue fatty acid compositions, serum lipids, postprandial glucose regulation and growth in obese Zucker fa/fa rats. British Journal of Nutrition. 2016; 116(8):1336-1345.
13. Li Hua, *et al*. MMP/TIMP expression in spontaneously hypertensive heart failure rats: the effect of ACE-and MMP-inhibition. Cardiovascular research. 2000; 46(2):298-306.
14. Chicco, Adam J, *et al*. Low-intensity exercise training delays heart failure and improves survival in female hypertensive heart failure rats. Hypertension. 2008; 51(4):1096-1102.
15. Geng Bin, *et al*. H2S generated by heart in rat and its effects on cardiac function. Biochemical and biophysical research communications. 2004; 313(2):362-368.
16. Guggilam, Anuradha, *et al*. TNF-α blockade decreases oxidative stress in the paraventricular nucleus and attenuates sympathoexcitation in heart failure rats. American Journal of Physiology-Heart and Circulatory Physiology. 2007; 293(1):H599-H609.
17. Sakata, Yasushi, *et al*. Activation of matrix metalloproteinases precedes left ventricular remodeling in hypertensive heart failure rats: its inhibition as a primary effect of angiotensin-converting enzyme inhibitor. Circulation. 2004; 109(17):2143-2149.
18. Nunes, Ramiro B, *et al*. Aerobic exercise improves the inflammatory profile correlated with cardiac remodeling and function in chronic heart failure rats. Clinics. 2013; 68:876-882.
19. Kolling, Janaína, *et al*. Homocysteine induces oxidative-nitrative stress in heart of rats: Prevention by folic acid. Cardiovascular toxicology. 2011; 11(1):67-73.
20. Khan, Haseeb A, *et al*. Effect of gold nanoparticles on glutathione and malondialdehyde levels in liver, lung and heart of rats. Saudi journal of biological sciences. 2012; 19(4):461-464.
21. Guo, Shuang, *et al*. Curcumin administration suppresses collagen synthesis in the hearts of rats with experimental diabetes. Acta Pharmacologica Sinica. 2018; 39(2):195-204.
22. Ono Keiji, Edward Lindsey S. Improved technique of heart transplantation in rats. Transplantation. 1969; 8(3):294.
23. Van Hees, Hieronymus WH, *et al*. Proteasome inhibition improves diaphragm function in congestive heart failure rats. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2008; 294(6):L1260-L1268.
24. Schultz, Rebecca L, *et al*. Effects of excessive long-term exercise on cardiac function and myocyte remodeling in hypertensive heart failure rats. Hypertension. 2007; 50(2):410-416.
25. Wang, Tianlun, *et al*. Responses of lean and obese Zucker rats to centrally administered leptin. Physiology & behavior. 1998; 65(2):333-341.
26. Drotningsvik, Aslaug, *et al*. A low dietary intake of cod protein is sufficient to increase growth, improve serum and tissue fatty acid compositions, and lower serum postprandial glucose and fasting non-esterified fatty acid concentrations in obese Zucker fa/fa rats. European Journal of Nutrition.
27. Korner, Judith, *et al*. Leptin regulation of Agrp and Npy mRNA in the rat hypothalamus. Journal of Neuroendocrinology. 2001; 13(11):959-966.
28. Grauballe MB, *et al*. Blockade of RAGE in Zucker obese rats with experimental periodontitis." Journal of periodontal research 52.1 (2017): 97-106.
29. Feresin, Rafaela G, *et al*. Effects of obesity on bone mass and quality in ovariectomized female Zucker rats. Journal of Obesity, 2014.
30. Misawa, Eriko, *et al*. Administration of phytosterols isolated from Aloe vera gel reduce visceral fat mass and improve hyperglycemia in Zucker diabetic fatty (ZDF) rats. Obesity research & clinical practice. 2008; 2(4):239-245.
31. Gidenne, Thierry, Sylvie Combes, Laurence Fortun-Lamothe. Feed intake limitation strategies for the growing rabbit: Effect on feeding behaviour, welfare, performance, digestive physiology and health: A review. Animal. 2012; 6(9):1407-1419.
32. Holman BWB, Malau-Aduli AEO. Spirulina as a livestock supplement and animal feed. Journal of animal physiology and animal nutrition. 2013; 97(4):615-623.
33. Davies, Ron Rees, Davies JAER. Rabbit gastrointestinal physiology. Veterinary Clinics: Exotic Animal Practice. 2003; 6(1):139-153.