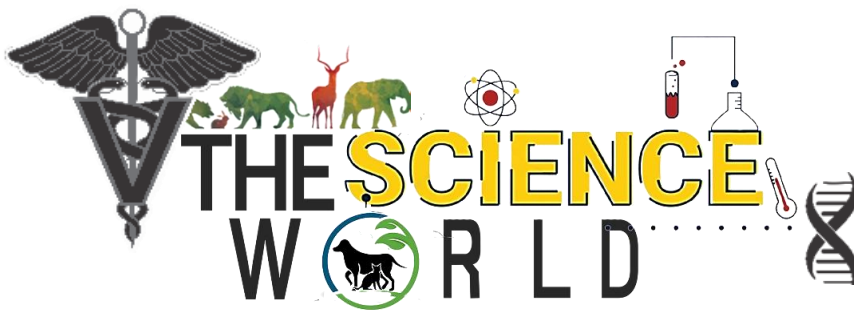




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Transgenic Animals: Methods and Protocols

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An organism obtained by the addition of a foreign DNA sequence to its genome is called a transgenic. The process of insertion of a transgene into a living organism so that the modified organism shows new traits and transmits it to its progeny is called as transgenesis. A transgene is a segment of recombinant DNA that has been isolated, engineered, and introduced into a host organism's genome. Transgenesis may be done by retroviruses, stem cells, DNA microinjection, and cloning methods using the sperm, fertilized eggs, gonads and embryo. Green fluorescent protein is the most useful transgenic marker. Addition of antibiotic resistance gene, western and southern blot, PCR and ELISA can be used to check success of transgenesis. Around 15-20 % of injected mice will contain transfected sequence. Multiple copies can be integrated in a tandem array into a single chromosomal site. Copy number varies from 1 to 150, and progeny level gene expression leads to their inheritance. It is highly variable due to both copy number and integration site. If it integrates within an active chromatin domain, a gene may be highly expressed, but if it integrates in or near a silenced region of chromosome, it will not. Transgenic technology has been developed in lab mice and hundreds of genes have been integrated to produce new strains of mice.

Methodology:

The principle is foreign gene or genes are inserted into animal genome and these must be transmitted through the germinal line so that all cells including the germ cell would contain the modified sequence. Mice were the first transgenic animals to be produced in 1980.



TECHNIQUES	REMARKS
Pronuclear micro- injection	Technical simplicity; low success rate; most widely used; unpredictable effects
Embryonic stem (ES) cell manipulation	Substitution of a functional gene with an inactive gene
Cre-lox technique	Preferable method; control over resulting phenotype; time-consuming
Viral vectors	Efficient delivery into non-dividing or dividing cells; high transduction rates; historically associated with insertional mutagenesis and gene silencing
Cytoplasmic injection	Less efficient than PMI
Primordial germ cells	Chimeric animals
Nuclear transfer	Large potential for genetically modifying livestock
Spermatogonial manipulation	Recipient testes in situ transplantation

DNA Microinjection:

Gordon et al first described the pronucleus injection. After the sperm entry into the oocyte, pronuclei can be microscopically visualised for several hours, to which the transgene may be microinjected. Pronucleus microinjection method has been used to produce Big Blue animals and Muta Mouse. Out of all the microinjected embryos, only 0.5–3% actually form transgenic offspring because the integration of the transgene into the genome of parental generation animals is less. While technically demanding and limited to processing individual zygotes one by one, pronuclear microinjection remains one of the primary historical and practical standards for generating random-integration transgenic founder lines. First, the transgene construct (containing the promoter, cDNA/gene of interest, and polyadenylation signal) is excised from the plasmid vector and thoroughly gel-purified away from the prokaryotic backbone, as bacterial vector sequences significantly impair embryonic survival and promote transgene silencing. Pronucleus is the sperm/ovum nucleus before their fusion to form zygote. Then, the isolated DNA construct is loaded into a fine glass micropipette and microinjected into the male pronucleus using a micromanipulator. Following pronuclear fusion, the surviving zygotes or dividing two-cell embryos are surgically transferred into the oviduct or uterus of a pseudopregnant surrogate female mouse. Since transgene DNA integrates in a random process, the progeny may or may not contain the transgene and express



the traits. This could happen if the gene inserts itself in an area of DNA that is not normally expressed.

COMPLETE PROTOCOL FOR DNA MICROINJECTION:

<https://doi.org/10.1002/0471143030.cb1911s42> or

https://dx.doi.org/10.1007%2F978-1-60327-378-7_22

Retroviral mediated gene transfer method:

Retroviral transduction of male germ cell lines is used to produce transgenic mice. The male germ cells can self-renew. For studying the biology of the transgenic animals and to generate wide range of strains through genetic modifications these germ cells are used. Retroviruses possess an RNA genome that reverse-transcribes into double-stranded DNA upon infection, making them effective viral vectors to transfer genetic material into the cells, that leads to the formation of chimera. These chimeras are inbred for 20-25 generations till homozygosity is found in the transgenic offspring. This method was used in 1976 to insert a simian virus into mice embryos that produced offspring containing the DNA.

Disadvantages of this method:

- Lower copy number variants are produced
- Process is cumbersome and additional retroviral production steps are required
- There is limitation on the size of foreign DNA insert (9-15 kb) transferred
- Undesired genetic recombination that may cause alteration of the retrovirus
- Higher frequency of mosaicism
- Possible interference by integrated retroviral sequences in transgene expression.
- The genome of the retroviral strain may be integrated into the identical nucleus as the transgene. Thus virus itself might be produced by using the transgenic organism
- Provirus draws methylation which in all likelihood in conjugation with other mechanisms disables its expression when it passes through the germ line.

COMPLETE PROTOCOL FOR RETROVIRAL METHOD:

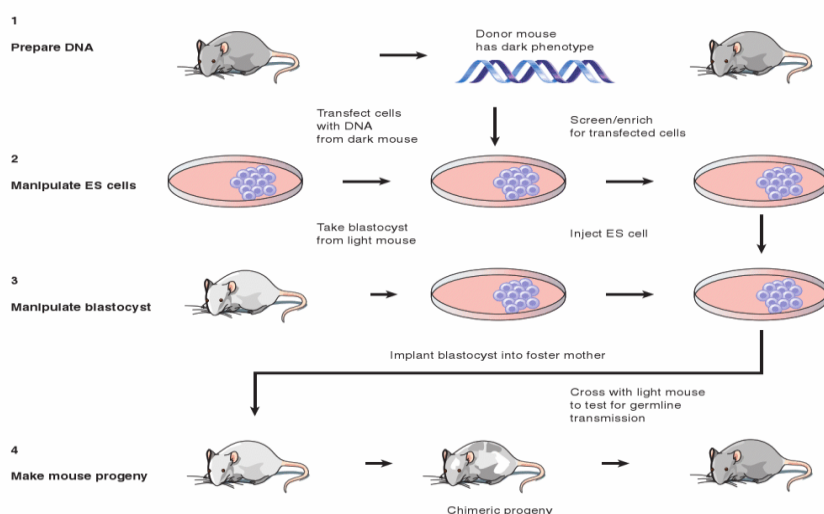
<https://doi.org/10.1073/pnas.231473498>

Embryonic Stem Cell-Mediated Gene Transfer:

The inner cell mass (ICM) of mouse blastocysts is the origin of Embryonic stem cells (ES cells) which are grown in culture and/or stored. These cells retain their pluripotency to produce all the cells of the animal, inclusive of its gametes.



- DNA molecules containing the desired gene, vector DNA to carry and insert into host DNA and promoter and enhancer sequences to enable the gene to be expressed by host cells are prepared using recombinant DNA technology.
- Transformation of ES cells in culture is done to expose cultured cells to the DNA so that some will incorporate it. Markers are added and selection is done for successfully transformed cells.
- These cells are injected into the inner cell mass (ICM) of mouse blastocysts that will form the embryos. These are transferred into the uterus of a pseudopregnant mouse. The F1 generation offspring are called chimeras whose somatic cells are composed of both transgenic embryonic stem cells and host cells. The gene for coat shade is used in transgene construct as a visual marker that helps in quicker detection. The chimeric mouse is crossed with a mouse lacking the donor trait to ascertain whether the ES cells contributed to progeny. During in vitro electroporation of cultured ES cells, homologous recombination occurs at low frequency (<1%), necessitating dual positive–negative selection (e.g., G418 and ganciclovir) to isolate correctly targeted colonies. Once verified clones are injected into blastocysts, chimeric founder males transmit the modified ES cell genome through the germline to F1 progeny at variable rates (typically 10–50% depending on chimeric contribution), producing heterozygous offspring.
- Establishing a transgenic strain: two heterozygous mice are mated and their offspring are screened for the 1:4 that will be homozygous for the transgene. Mating the F1 Generation will form the F2 generation which will be the transgenic strain.



Gene knockout: in this technique, one or few genes of an organism are deleted or made inoperative. These knocked out animals are used to study gene function and alterations.



Gene knock-in: involves substitution of DNA sequence information in a genetic locus or the insertion of sequence information not found within the locus, may also involve the substitution of a functional gene locus for a mutant phenotype that results in some loss of function.

PROTOCOL FOR ES CELL MEDIATED CONDITIONAL TRANSGENESIS:

<https://link.springer.com/protocol/10.1385/1-59259-241-4:285>

Testis cell transplantation method:

Steps are as follows:

- (A) From a fertile donor testis a single-cell suspension is generated.
- (B) Germ cells are cultured
- (C) In an infertile recipient mouse, the cultured cells are microinjected (in situ injection) in the testes (i.e., in the lumen seminiferous tubule)
- (D) Spermatogenic colonies are generated exclusively by spermatogonial stem cells within the recipient testes. When these donor cells carry a reporter transgene (such as lacZ), colonies are identified by distinct blue-stained segments within the seminiferous tubules upon X-gal staining.
- (E) Recipient male is allowed to mate with female of wild type.
- (F) After mating, donor genes are transmitted and produces progenies.

FOR PROTOCOL FOR SPERM MEDIATED GENE TRANSFER:

https://link.springer.com/protocol/10.1007/978-3-642-20792-1_12

nuclear Transfer Method:

In this method, nuclear transfer of foetal somatic cells was used to produce transgenic mice, in which donor karyoplasts were derived from somatic cells of female foetus produced by artificial inseminating a non-transgenic adult female with transgenic male semen. Two nuclear transfer procedures are followed. In the first one, oocytes arrested in metaphase II stage (which contain high levels of M-phase-promoting factor (MPF)) are enucleated and electrofused with somatic cells of donor and were activated simultaneously. Blunt micropipettes that were attached to a piezo impact-driving micromanipulation device was used to increase the success rate of mouse cloning. There is a significant reduction of time that is required for the micromanipulation of oocytes and hence reduces the in vitro loss of viability of oocyte. In the second one, activated oocytes (in-vivo) at the telophase II stage were enucleated and then were electrofused with somatic cells of donor, and once again activated for induction of reactivation of genome. Generation of healthy offspring and consequent



genome analyses confirming that all offspring were derived from donor cell line, proved the efficacy of this Somatic Cell Nuclear Transfer method.

Transfection of Gametes:

In the 1960s, the first transfection procedures were done, and different cell type and tissues experiments have now been introduced and have become widespread in use. Various types of transfection methods are used viz. in vitro procedure of introducing foreign genes into cultured cells and tissues, in vivo procedure of direct introduction of genes into tissue through, injection, gene gun, aerosol etc. and the ex-vivo procedure where transfection of cells is done in vitro and further introduced into host. Incubation of gametes is done for short period of time in solution that contains gene constructions and then they are screened for transfection positives. These can be used for inseminating females, or otherwise for in vitro fertilization processes. Although naked DNA can be transfected successfully, DNA-liposome complexes and electroporation can also be used in lieu of naked DNA. In vitro transfection was successful in female gametes using retroviruses or liposomes. Along with these methods, microprojectiles/nanoparticles with high velocity or gene guns have also been used for transfection purposes. Autoradiography, immunocytochemistry or in situ hybridisation are employed for localisation of the foreign gene of interest in the host spermatozoa. In any case, both in vivo and in vitro techniques for transfection of spermatozoa have high success rate (80%), and most of the spermatozoa are transfected to be fertilised with the female ovum. The foreign gene can directly be placed in the spermatozoal nucleus and gene sequencing/southern blotting/PCR revealed the same (presence of transgene segment in DNA of gametes).

PROTOCOL FOR TRANSFECTION- ICSF: <https://doi.org/10.1101/pdb.prot094482>

Artificial Chromosome Mediated Gene Transfer:

Due to the extreme shear sensitivity of high-molecular-weight DNA (100–1000 kb), Yeast Artificial Chromosomes (YACs) and Bacterial Artificial Chromosomes (BACs) require specialized purification and are introduced into oocytes either by low-pressure pronuclear microinjection using wide-bore pipettes or via Intracytoplasmic Sperm Injection (ICSI). A new transgenic founder lineage originates after receiving the transgene DNA by an individual lab animal. Also, by the method of pronuclear microinjection YACs (Yeast Artificial Chromosomes) transgenic mice are produced and have significant advantage in insert size, which is newest generation of vector. It is observed that this methods is successfully used in rabbits and mice.



PROTOCOL FOR YAC GENERATION: <https://doi.org/10.1385/1-59745-158-4:151>

Recent methods for production of Transgenic Animals:

Lentiviral Transfer of Oocytes and Zygotes:

The limitations of using retroviral mediated transgene transfer that may lead to transgenic locus silencing, and thus lower expression levels, has been overcome by usage of lentivirus. In this method, the lentivirus that contains the transgene to be incorporated in its genome, is microinjected into the oocytes. As per the newer study by from Roslin Institute, U.K., instead of using retroviral methods, using lentiviral based vectors has proven more useful. They offer distinct advantages over conventional oncoretroviral vectors: they can transduce non-dividing cells, accommodate larger inserts, and provide more stable long-term expression.

PROTOCOL FOR LENTIVIRUS MEDIATED TRANSGENESIS:

<https://link.springer.com/protocol/10.1385%2F1-59745-037-5%3A273>

CRISPR/Cas9-Mediated Genome Editing:

Direct zygote injection or electroporation of CRISPR/Cas9 components (Cas9 protein or mRNA along with single-guide RNAs and optional repair templates) has largely superseded classical microinjection and ES cell targeting for generating knockouts, site-specific knock-ins, and point mutations. This approach enables targeted double-strand breaks repaired via non-homologous end joining (NHEJ) or homology-directed repair (HDR), drastically reducing the generation time of mutant lines.

Chimera Generation by injecting the Pluripotent Cells:

Chimeras can be generated from pluripotent cell injections. For example, after blastocyst injection Pluripotent cells (Embryonic stem cells) can lead to generation of organ and gametocyte production. Knock-ins can be created, and ES cells are important for this procedure. Another type of cells that can be used for this purpose are primordial cells, which are currently being used for generation of transgenic farm animals, and also chimeras that don't transmit the transgene to offspring have also been reported.

LINES OF TRANSGENIC MICE:

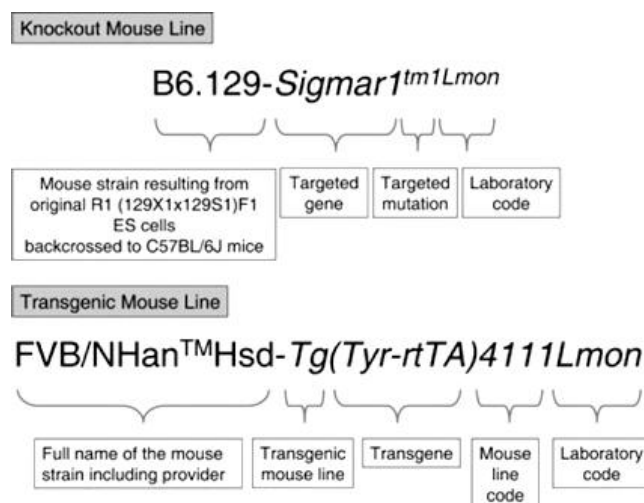
Nomenclature:

According to the International Committee on Standardized Genetic Nomenclature for Mice:

1. Random Transgenic Insertions: Designated as Tg(Promoter-Transgene)
LineNumberLabCode (e.g., Tg(ACTB-EGFP)10sb).



2. Targeted Mutations (Knockouts / Knock-ins): Designated by the affected gene locus followed by <tm#LabCode> (e.g., Trp53<tm1Tyj>).



Examples:

- C57BL/6 mouse: This common inbred strain is often referred to as "B6", "C57 black 6", or "C57". It is the most widely utilized genetic background for genetically modified human disease models.
- FVB mouse: This inbred strain is suitable for most transgenic experiments and subsequent genetic analyses.
- Large mice, with a rat hormone gene that caused larger growth than normal mice size, and are used to study growth and development.
- OncoMouse, has an activated oncogene, and are predisposed to developing cancer. These mice have been vital to the understanding of many cancers and the development of technologies to treat them.
- Doogie mice show improved memory and capacity for learning. These mice have enhanced function at NMDA receptors, which are needed for the brain to store new information.

Common background and specialized lines include: C57BL/6, BALB/c, CD-1, CB17-SCID, A/J, ICR, C3H, MRL/MpJ-Fas, and cancer-susceptible stocks such as SENCAR.

Strain	Major Features	Advantages	Main Applications
C57BL/6	inbred, black	strain stability, easy breeding	In vivo physiological or pathological models, background strain in transgenics, oncogenics
BALB/c-	inbred, albino, immunodeficient	easy breeding, tumor-prone	hybridoma and Mab, cancer therapy and immunology research



CD-1	outbred, albino	genetic variability	positional cloning, genotypic selection, toxicology
CB17 SCID	inbred, albino	no T and B cells, tumor transplantation	immunodeficient animal, cancer study, as hosts for human immune system tissues.

For complete list visit:

IMSR: The IMSR is a searchable online database of mouse strains, stocks, and mutant ES cell lines available worldwide, including inbred, mutant, and genetically engineered strains. The goal of the IMSR is to assist the international scientific community in locating and obtaining mouse resources for research.

<http://www.findmice.org/>

Mouse Genome Informatics: MGI is the international database resource for the laboratory mouse, providing integrated genetic, genomic, and biological data to facilitate the study of human health and disease.

<http://www.informatics.jax.org/>

REFERENCES:

- Chapman K.M., Saidley-Alsaadi D., Syvyk A.E., Shirley J.R., Thompson L.M., Hamra F.K. (2011) *Rat Spermatogonial Stem Cell-Mediated Gene Transfer*. In: Pease S., Saunders T. (eds) *Advanced Protocols for Animal Transgenesis*. Springer Protocols Handbooks. Springer, Berlin, Heidelberg
- Cho, A., Haruyama, N., & Kulkarni, A. B. (2009). Generation of transgenic mice. *Current protocols in cell biology*, 42(1), 19-11.
- Faerman, A., & Shani, M. (1997). Transgenic mice: production and analysis of expression. *Methods in cell biology*, 373-404.
- Gertsenstein M., Lobe C., Nagy A. (2002) *ES Cell-Mediated Conditional Transgenesis*. In: Turksen K. (eds) *Embryonic Stem Cells*. Methods in Molecular Biology, vol 185. Springer, Totowa, NJ
- Houdebine, L. M. (2009). Methods to generate transgenic animals. In *Genetic Engineering in Livestock* (pp. 31-48). Springer, Berlin, Heidelberg.
- Kumar, T. R., Larson, M., Wang, H., McDermott, J., & Bronshteyn, I. (2009). Transgenic mouse technology: principles and methods. In *Molecular Endocrinology* (pp. 335-362). Humana Press, Totowa, NJ.
- McGrath, J., Solter, D., & Mann, J. (2017). Pronuclear transplantation in the mouse embryo. *Cold Spring Harbor Protocols*, 2017(8), pdb-prot094417.
- Moreira P.N., Pozueta J., Giraldo P., Gutiérrez-Adán A., Montoliu L. (2006) *Generation of Yeast Artificial Chromosome Transgenic Mice by Intracytoplasmic Sperm Injection*. In: MacKenzie A. (eds) *YAC Protocols*. Methods in Molecular Biology™, vol 349. Humana Press
- Nagano, M., Brinster, C. J., Orwig, K. E., Ryu, B. Y., Avarbock, M. R., & Brinster, R. L. (2001). Transgenic mice produced by retroviral transduction of male germ-line stem cells. *Proceedings of the National Academy of Sciences*, 98(23), 13090-13095.



- Oka M., Chang L.J., Costantini F., Terada N. (2006) *Lentiviral Vector-Mediated Gene Transfer in Embryonic Stem Cells*. In: Turksen K. (eds) *Embryonic Stem Cell Protocols*. Methods in Molecular Biology, vol 329. Humana Press
- Singhal, M., & Kansara, N. (2010). Transgenic animals: production and application. *INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH*, 1(9), 12-22.
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