# Transgenic Animals

## METHODS OF PRODUCING TRANSGENIC MICE:

A transgenic animal is one that carries a foreign gene that has been deliberately inserted into its genome. The foreign gene is constructed using recombinant DNA methodology. In addition to the gene itself, the DNA usually includes other sequences to enable it

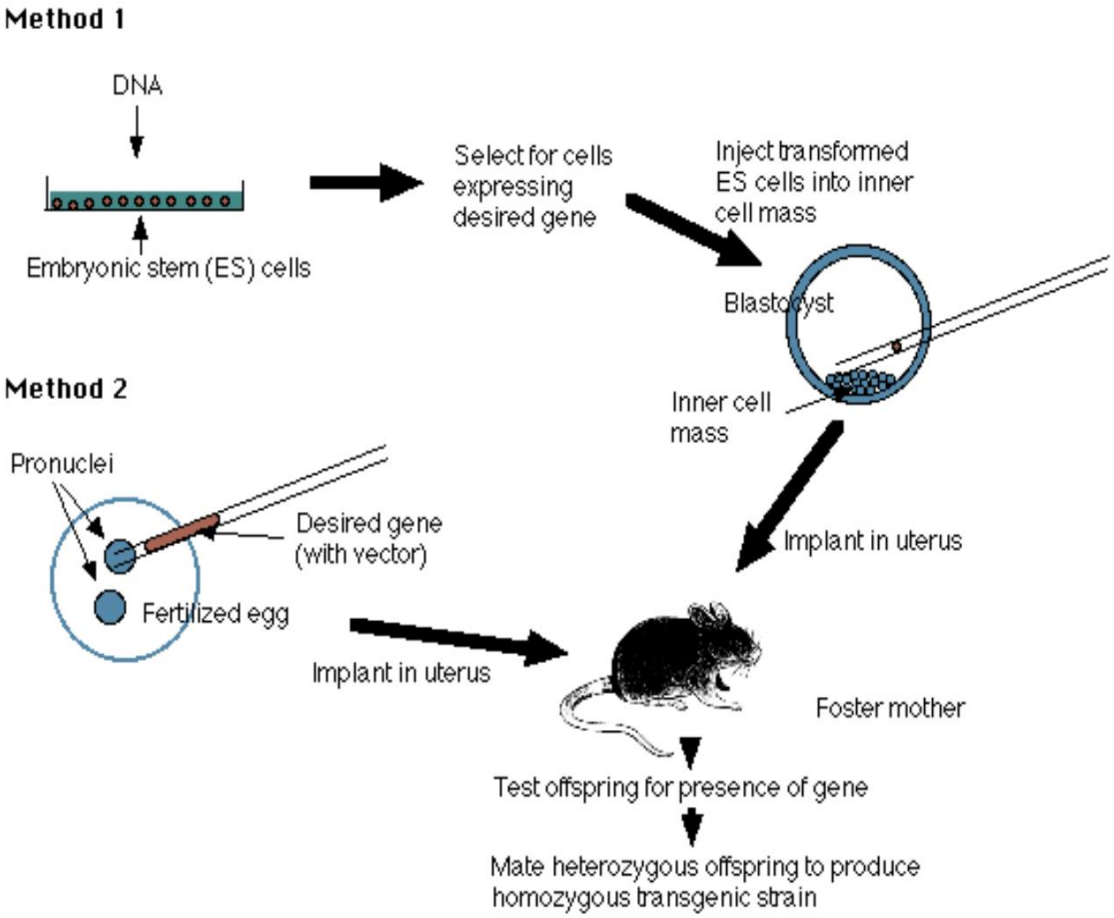
- to be incorporated into the DNA of the host and
- to be expressed correctly by the cells of the host.
- Transgenic sheep and goats have been produced that express foreign proteins in their milk.
- Transgenic chickens are now able to synthesize human proteins in the "white" of their eggs.

**Two methods of producing transgenic mice are widely used:**

- **Embryonic stem cells** (**ES** cells) growing in tissue culture with the desired DNA;
- injecting the desired gene into the **pronucleus** of a fertilized mouse egg.

## THE EMBRYONIC STEM CELL METHOD : (METHOD "1")

Embryonic stem cells (**ES** cells) are harvested from the **inner cell mass** (ICM) of mouse blastocysts. They can be grown in culture and retain their full potential to produce all the cells of the mature animal,**including its gametes**.




**1. Make your DNA**

Using recombinant DNA methods, build molecules of DNA containing

- the gene you desire (e.g., the insulin gene);
- **vector** DNA to enable the molecules to be inserted into host DNA molecules;
- promoter and enhancer sequences to enable the gene to be expressed by host cells.

**2. Transform ES cells in culture:** Expose the cultured cells to the DNA so that some will incorporate it.

**3. Select for successfully transformed cells.**

**4. Inject these cells into the inner cell mass (ICM) of mouse blastocysts.**

**5. Embryo transfer**

- Prepare a **pseudopregnant** mouse (by mating a female mouse with a vasectomized male). The stimulus of mating elicits the hormonal changes needed to make her uterus receptive.
- Transfer the embryos into her uterus.
- Hope that they **implant** successfully and develop into healthy pups (no more than one-third will).

**6. Test her offspring**

- Remove a small piece of tissue from the tail and examine its DNA for the desired gene. No more than 10–20% will have it, and they will be heterozygous for the gene.

**7. Establish a transgenic strain**

- Mate two heterozygous mice and screen their offspring for the 1 in 4 that will be **homozygous** for the transgene.
- Mating these will found the transgenic strain.

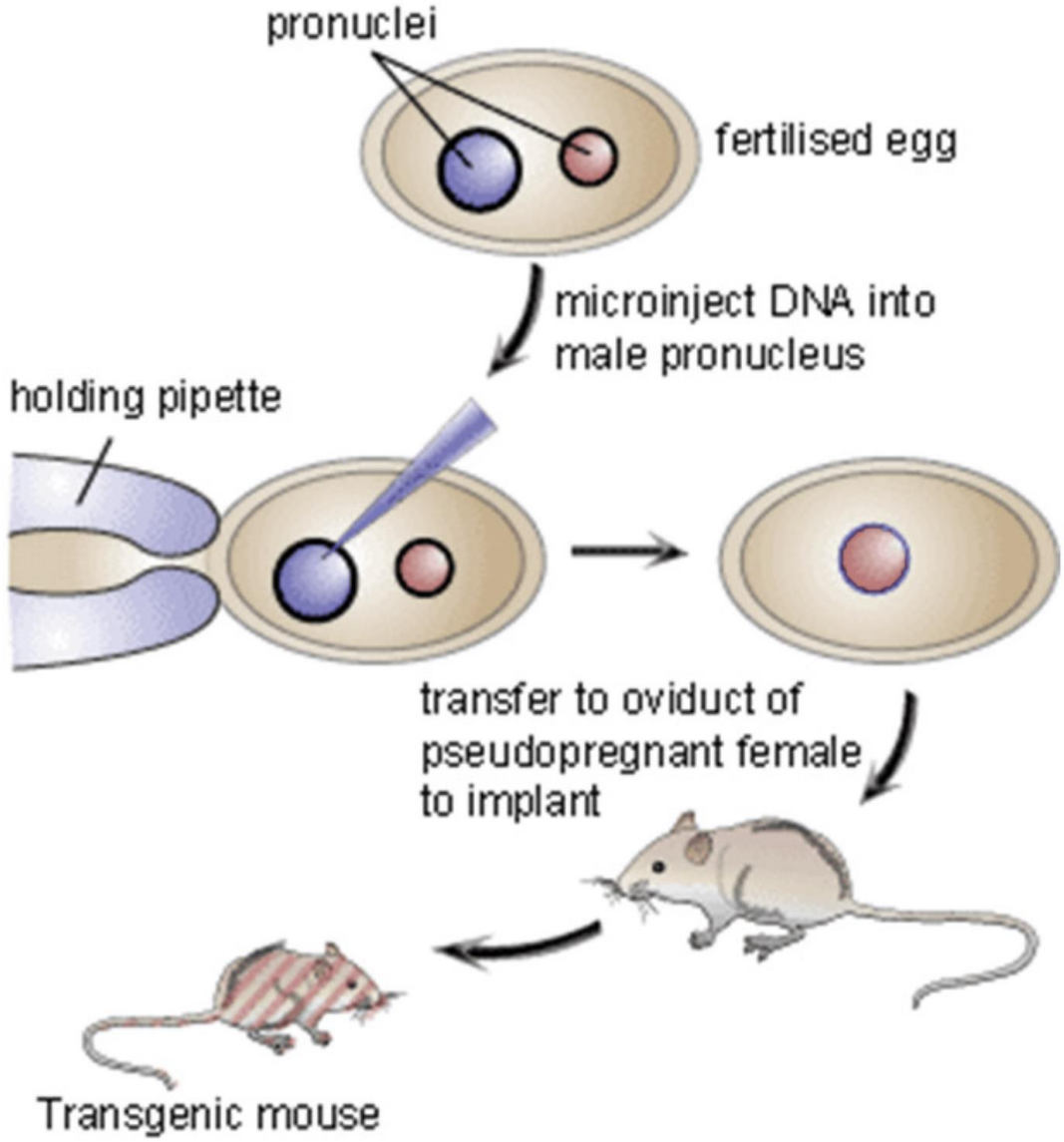
**THE PRONUCLEUS METHOD (METHOD "2")**

**1. Prepare your DNA as in Method 1**

**2. Transform fertilized eggs**

- Harvest freshly fertilized eggs before the sperm head has become a pronucleus.
- Inject the male pronucleus with your DNA.
- When the pronuclei have fused to form the diploid zygote nucleus, allow the zygote to divide by mitosis to form a 2-cell embryo.

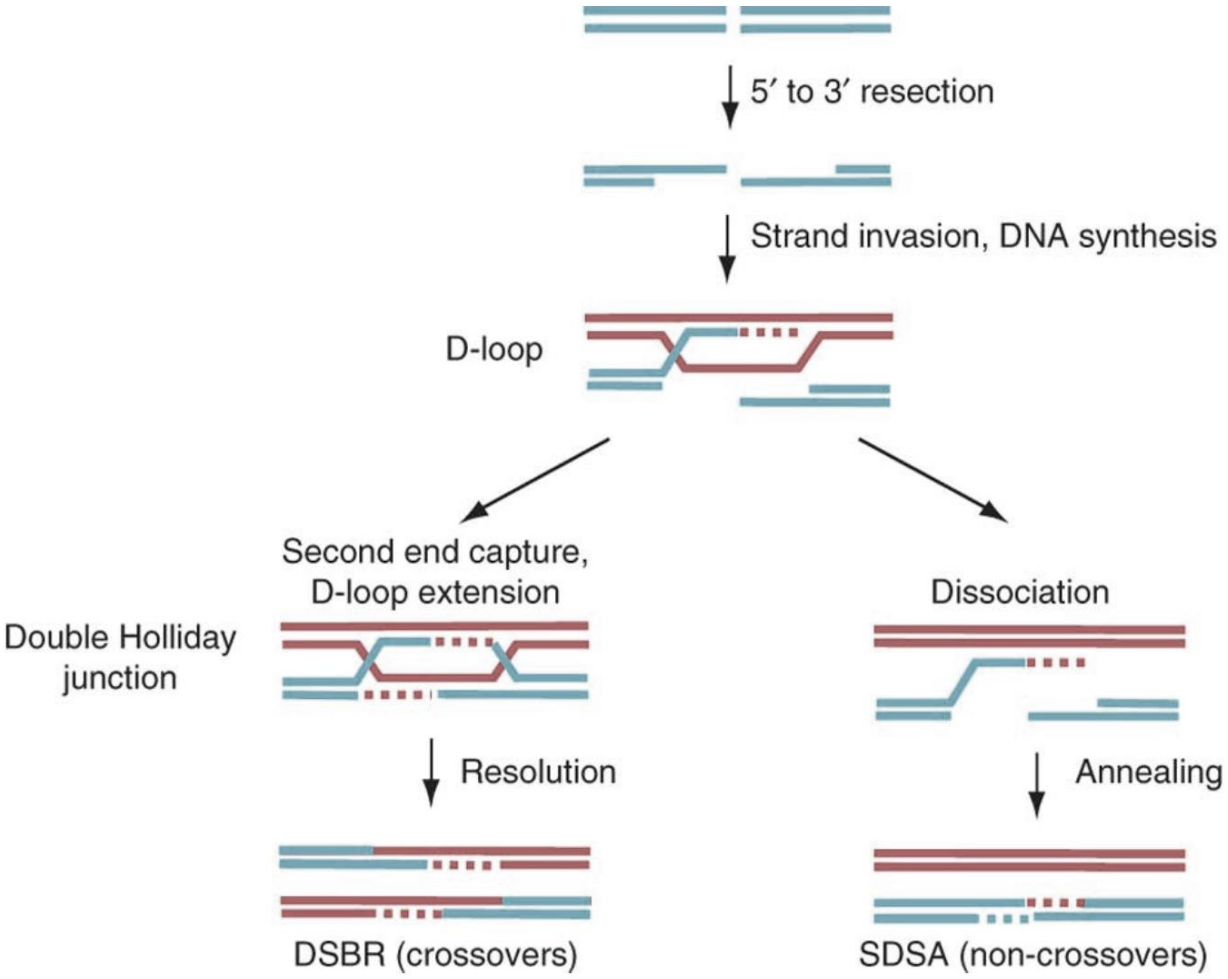
**3. Implant the embryos in a pseudopregnant foster mother and proceed as in Method 1.**




## GENE TARGETING

**Homologous recombination** is a type of genetic recombination in which nucleotide sequences are exchanged between two similar or identical molecules of DNA. It is most widely used by cells to accurately repair harmful breaks that occur on both strands of DNA, known as double-strand breaks. Homologous recombination also produces new combinations of DNA sequences during meiosis, the process by which eukaryotes make gamete cells, like spermand egg cells in animals. These new combinations of DNA represent genetic variation in offspring, which in turn enables

populations to adapt during the course of evolution. Homologous recombination is also used in horizontal gene transfer to exchange genetic material between different strains and species of bacteria and viruses.




**Gene targeting** (also, replacement strategy based on homologous recombination) is a genetic technique that uses homologous recombination to change an endogenous gene. The method can be used to delete a gene, remove exons, add a gene, and introduce point mutations. Gene targeting can be permanent or conditional. Conditions can be a specific time during development / life of the organism or limitation to a specific tissue, for example. Gene targeting requires the creation of a specific vector for each gene of interest. However, it can be used for any gene, regardless of transcriptional activity or gene size.

The technology allowed unprecedented precision with which one could manipulate genes and study the effect of this manipulation on the central nervous system. With gene targeting, the uncertainty inherent in psychopharmacology regarding whether a particular compound would act only through a specific target was removed. Thus, gene targeting became highly popular. However, with this popularity came the realization that like other methods, gene targeting also suffered from some technical and principal problems. For example, two decades ago, issues about compensatory changes and about genetic linkage were raised.

Since then, the technology developed, and its utility has been better delineated. This review will discuss the pros and cons of the technique along with these advancements from the perspective of the neuroscientist user. It will also compare and contrast methods that may represent novel alternatives to the homologous recombination-based gene targeting approach, including the TALEN and the CRISPR/Cas9 systems. The goal of the review is not to provide detailed recipes, but to attempt to present a short summary of these approaches a behavioural geneticist or neuroscientist may consider for the analysis of brain function and behavior.

**Transgenic Mouse:** Generic term for an engineered mouse that has a normal DNA sequence for a gene replaced by an engineered sequence or a sequence from another organism.

**Knockout Mouse:** A transgenic mouse in which the normal gene is missing or engineered so that is not transcribed or translated. "Knocks out" that gene.

**Knockin Mouse:** A transgenic mouse in which the engineered "transgene" is subtly manipulated to: (A) alter the function of the gene (e.g., replace one amino acid with another in a site to determine if that site is essential for the protein's function); (B) change transcription rate to overproduce or underproduce the gene product; or (C) create a fluorescent gene product to map its distribution in tissue.

**Conditional Knockout (Knockin) Mouse:** A transgenic mouse in which the transgene is knocked out (or in) in specific tissues, at a specific developmental stage, or in response to an exogenous substance (e.g., an antibiotic).

**The First Knockout mouse was created by Mario R Capecchi,Martin Evans and Oliver Smithies in 1989 for which they were awarded Nobel Prize for Medicine in 2007.**

Reference: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4. Textbook of Animal Biotechnology, by B Singh (Author), S K Gautam (Author), M S Chauhan (Author)
5. Animal Biotechnology (3rd Edition), by M.M. Ranga (Author)