TRANSGENIC ANIMALS

1. What are Transgenic Animals?

Trangenic animals are the animals with modified genome. A foreign gene is inserted into the genome of the animal to alter its DNA. This method is done to improve the genetic traits of the target animal. Initially, the improvement of genetic traits was done by selective breeding methods. Since this technique was time-consuming and expensive, it was later replaced by recombinant DNA technology.

Transgenesis is the phenomenon in which a foreign gene with desired characteristics is introduced into the genome of the target animal. The foreign gene that is introduced is known as the transgene, and the animal whose genome is altered is known as transgenic. These genes are passed on to the successive generations. The transgenic animals are genetically engineered and are also known as genetically modified organisms. The first genetically modified organism was engineered in the year 1980.

2. Methods for Creating Transgenic Animals

The three principal methods used for the creation of transgenic animals are

a) DNA microinjection
b) Embryonic stem cell-mediated gene transfer
c) Retrovirus-mediated gene transfer

a) DNA microinjection.

This method involves the direct microinjection of a chosen gene construct (a single gene or a combination of genes) from another member of the same species or from a different species, into the pronucleus of a fertilized ovum. It is one of the first methods that proved to be effective in mammals (Gordon and Ruddle, 1981). The introduced DNA may lead to the over- or under-expression of certain genes or to the expression of genes entirely new to the animal species. The insertion of DNA is, however, a random process, and there is a high probability that the introduced gene will not insert itself into a site on the host DNA that will permit its expression. The manipulated fertilized ovum is transferred into the oviduct of a recipient female, or foster mother that has been induced to act as a recipient by mating with a vasectomized male. A major advantage of this method is its applicability to a wide variety of species.

b) Embryonic stem cell-mediated gene transfer.

This method involves prior insertion of the desired DNA sequence by homologous recombination into an in vitro culture of embryonic stem (ES) cells. Stem cells are undifferentiated cells that have the potential to differentiate into any type of cell (somatic and germ cells) and therefore to give rise to a complete organism. These cells are then incorporated into an embryo at the blastocyst stage of development. The result is a chimeric animal. ES cell-mediated gene transfer is the method of choice for gene inactivation, the so-called knock-out method.

This technique is of particular importance for the study of the genetic control of developmental processes. This technique works particularly well in mice. It has the advantage of allowing precise targeting of defined mutations in the gene via homologous recombination.

c) Retrovirus-mediated gene transfer.

To increase the probability of expression, gene transfer is mediated by means of a carrier or vector, generally a virus or a plasmid. Retroviruses are commonly used as vectors to transfer genetic material into the cell, taking advantage of their ability to infect host cells in this way. Offspring derived from this method are chimeric, i.e., not all cells carry the retrovirus. Transmission of the transgene is possible only if the retrovirus integrates into some of the germ cells.

For any of these techniques the success rate in terms of live birth of animals containing the transgene is extremely low. Providing that the genetic manipulation does not lead to abortion, the result is a first generation (F1) of animals that need to be tested for the expression of the transgene. Depending on the technique used, the F1 generation may result in chimeras. When the transgene has integrated into the germ cells, the so-called germ line chimeras are then inbred for 10 to 20 generations until homozygous transgenic animals are obtained and the transgene is present in every cell. At these stage embryos carrying the transgene can be frozen and stored for subsequent implantation.

3. Transgenic mice

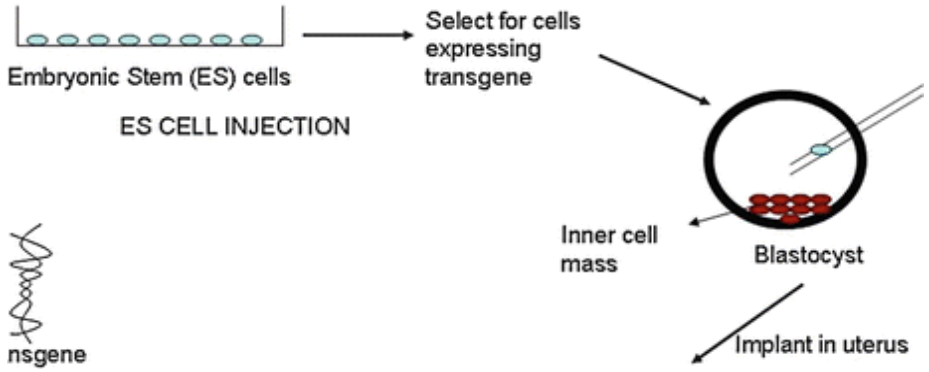
Two methods of producing transgenic mice are widely used:

a) Transforming embryonic stem cells (ES cells) growing in tissue culture with the desired DNA

b) Injecting the desired gene into the pro-nucleus of a fertilized mouse egg.

a) The Embryonic Stem Cell Method:

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.

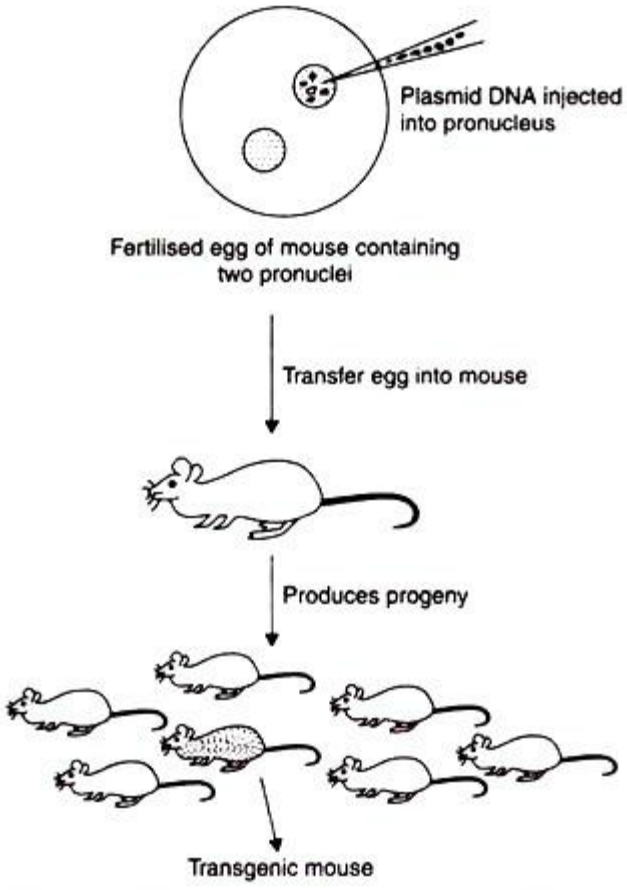


- Using recombinant DNA methods, build molecules of DNA containing the structural gene you desire (e.g., the insulin gene), vector DNA to enable the molecules to be inserted into host DNA molecules, promoter and enhancer sequences to enable the gene to be expressed by host cells.
- Transform ES cells in culture- Expose the cultured cells to the DNA so that some will incorporate it.
- Transformed cells were selected successfully.
- The transformed cells were injected into the inner cell mass (ICM) of mouse blastocysts.
- Embryo transfer- is done by preparing a pseudo pregnant 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.
- The offsprings were tested by removing 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.

- Transgenic strain are established by mating two heterozygous mice and screen their offspring for the 1:4 that will be homozygous for the transgene. Mating these will found the transgenic strain.

b) The Pro-nucleus Method:

- Cell divisions in the egg will pass on the foreign DNA to all cells of the embryo and subsequently to the adult animal.
- Mice that have been genetically engineered to carry such foreign genes in their chromosomes are called transgenic mice.
- Cloned DNA is microinjected into a pro-nucleus of the fertilised egg to generate transgenic mice.



- The eggs are then transferred to foster mothers and allowed to develop to term.
- Among the progeny mice, about 10% will have the foreign DNA integrated into the genome of the fertilised egg, and therefore, in all cells of the adult mice.
- Since the foreign DNA is also present in the germ line cells of the progeny mice, these mice are mated to breed new progeny mice which would inherit the foreign DNA.

4. Transgenic animals advantages and disadvantages

Advantages:

- ✓ Gene requires certain cellular mechanism to help for the production of protein. The animals used for transgenic purpose naturally carry the mechanism needed to produce complex protein. Theses mechanism is absent in cell culture.
- ✓ Expression through cell culture or bacterial culture requires constant monitoring and sampling.
- ✓ The isolation and purification of expressed protein in conventional method is more difficult than purifying proteins from an animal's milk or body fluid.
- ✓ It is more cost effective as the product is efficiently passed through milk with an average yield of 53% and with 99% purity.
- ✓ It has been estimated that transgenic animal can produce in its lifetime $100 to $200 million worth of pharmaceuticals.

Disadvantages:

- ✓ Transgenic animal project is extremely expensive.
- ✓ Generation of transgenic animals are also expensive, because of long gestation period, litter size and higher maintenance cost of the recipient animals.
- ✓ There may be high mortality rate and other deleterious effects on animals used by researchers to create transgenic breeds. It has been observed that transgenic pigs having enhanced growth rate and efficient feed conversion exhibit reduced reproductive performance and may suffer from arthritis and dermatitis etc.
- ✓ Similarly, transgenic sheep expressing growth horm6ne may show diabetic like conditions. Mayer argues that we do not understand the long term effects of genetic engineering on animals.
- ✓ Large number of recipients is required for embryo transfer because of low transgenesis rate.
- ✓ Transgenic foods have been produced and offer better productivity in terms of both yield and quantity. However, there are some apprehensions about the safety of transgenic foods.