Production of transgenic animals:

A transgenic animal is one whose genome has been altered by the transfer of a gene or genes from another species or breed. 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.

This method is done to improve the genetic traits of the target animal either for

- production purposes e.g. cattle
- control diseases e.g. transgenic mosquitos
- research purposes e.g. GFP mice and humanized pigs

Initially, the improvement of genetic traits was done by selective breeding methods. In this, the animals with desired genetic characteristics were mated to produce an individual with improved genetic characteristics. Since this technique was time-consuming and expensive, it was later replaced by recombinant DNA technology.

Thus, transgenic animals are genetically engineered and are also known as genetically modified organisms. The first genetically modified organism was engineered in the year 1980.

Methods used in transgenesis: Following methods can be used for introduction of transgene in the genome of target organism.

Physical Transfection

In this method, the gene of interest is directly microinjected into the pronucleus of a fertilized ovum. It is the very first method that proved to be effective in mammals. This method is applicable to a wide variety of species.

Other methods of physical transfection include particle bombardment, ultrasound and electroporation.

Chemical Transfection

One of the chemical methods of gene transfection includes transformation. In this method, the target DNA is taken up in the presence of calcium phosphate. The DNA and calcium phosphate co-precipitates, which facilitates DNA uptake. It is possible because the mammalian cells possess the ability to take up foreign DNA from the culture medium.

Retrovirus-Mediated Gene Transfer

To increase the chances of expression, the gene is transferred by means of a vector. Since retroviruses have the ability to infect the host cell, they are used as vectors to transfect the gene of interest into the target genome.

Viral Vectors

Viruses are used to transfect rDNA into the animal cell. The viruses possess the ability to infect the host cell, express well and replicate efficiently.

Figure 1 showing principal for production of transgenic mice.

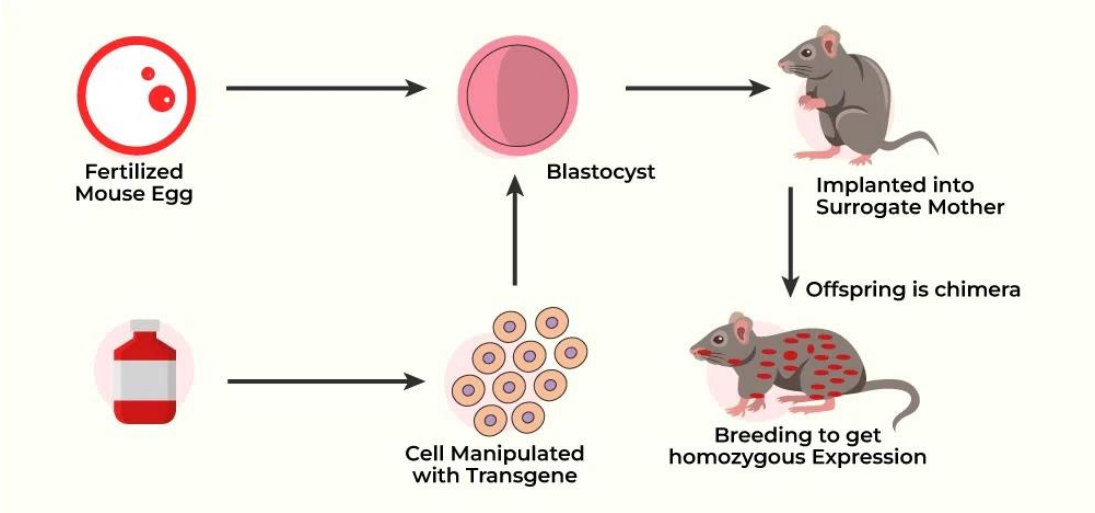
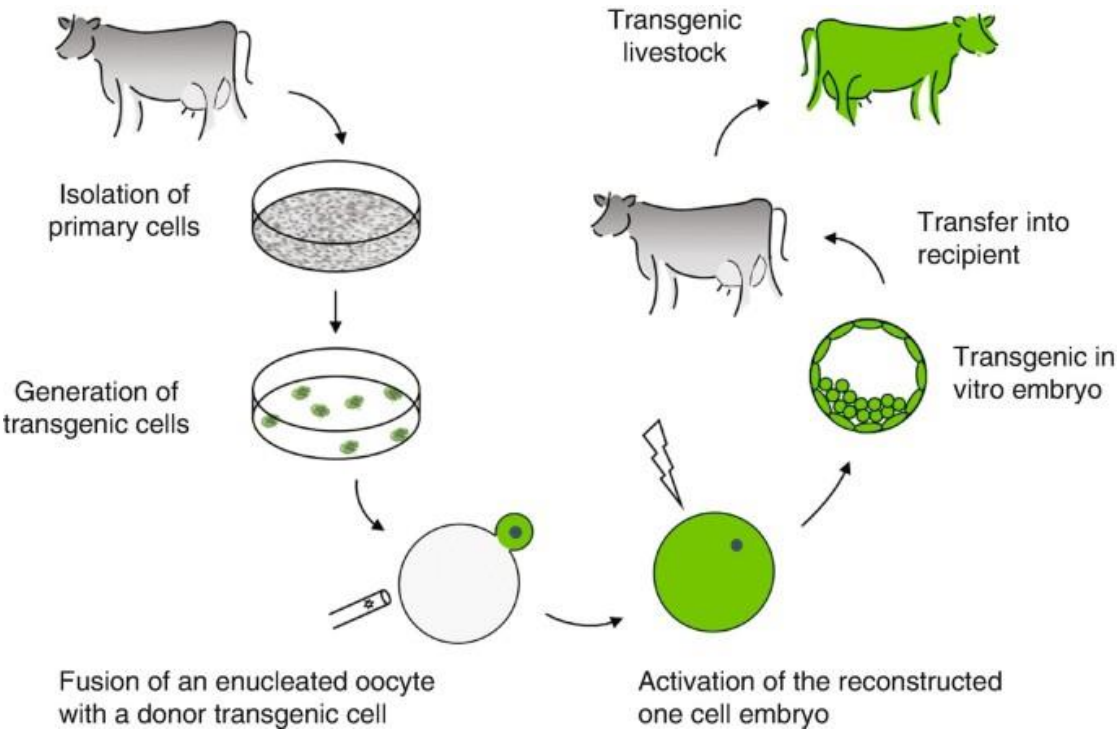


Figure 2 showing principal for production of transgenic cattle.



Examples of Transgenic Animals

Following are the examples of transgenic animals:

Dolly Sheep

Dolly the sheep was the first mammal to be cloned from an adult cell. In this, the udder cells from a 6-year-old Finn Dorset white sheep were injected into an unfertilized egg from a Scottish Blackface ewe, which had its nucleus removed.

The cell was made to fuse by electrical pulses. After the fusion of the nucleus of the cell with the egg, the resultant embryo was cultured for six to seven days. It was then implanted into another Scottish Blackface ewe which gave birth to the transgenic sheep, Dolly.

This was done by a team led by Sir Ian Wilmut.

Transgenic Mice

Transgenic mice are developed by injecting DNA into the oocytes or 1-2 celled embryos taken from female mice. After injecting the DNA, the embryo is implanted into the uterus of receptive females.

Applications of Transgenic Animals

The transgenic animals are created because of the benefits they provide to the man. Let us discuss a few of them here.

Normal Physiology and Development: In transgenic animals, a foreign gene is introduced due to which the growth factor is altered. Hence, these animals facilitate the study of gene regulation and their effect on the everyday functions of the body.

Study of Diseases: Transgenic animals are specially designed to study the role of genes in the development of certain diseases. Moreover, in order to devise a cure for these diseases, the transgenic animals are used as model organisms.

These transgenic models are used in research for the development of medicines. For example, we have transgenic models for diseases such as Alzheimer's and cancer.

Biological Products: A number of biological products such as medicines and nutritional supplements are obtained from transgenic animals. Research for the manufacture of medicines to treat diseases such as phenylketonuria (PKU) and hereditary emphysema is going on.

The first transgenic cow, Rosie (1997), produced milk containing human protein (2.4 grams per litre). This milk contains the human gene alpha-lactalbumin and could be given to babies as an alternative to natural cow milk.

Vaccine Safety: Transgenic animals are used as model organisms for testing the safety of vaccines before they are injected into humans. This was conventionally done on monkeys.

GFP mice as specific example of transgenic animal:

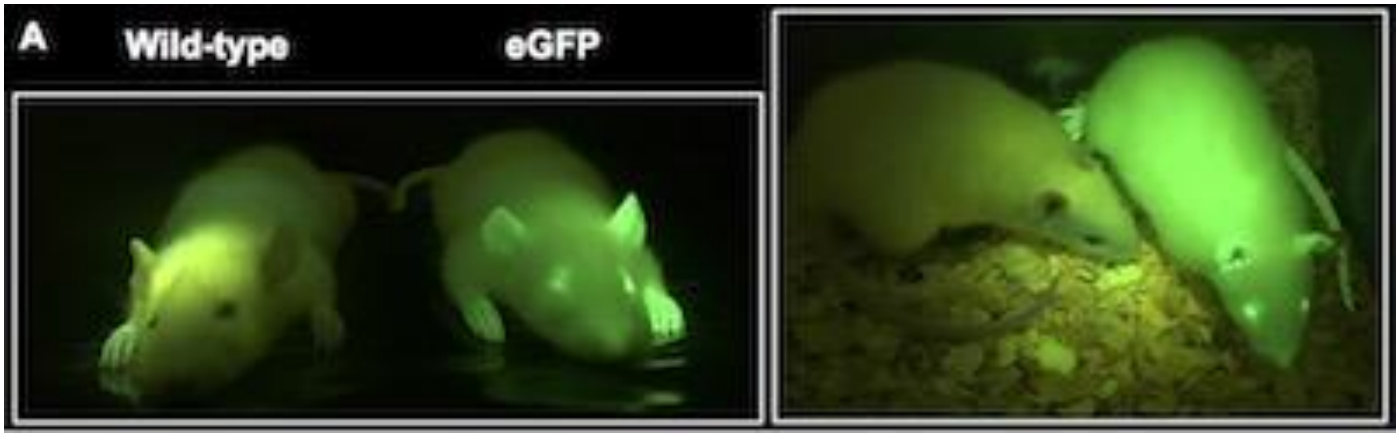
GFP mice are transgenic mice that express the green fluorescent protein (GFP) gene in their entire bodies. The GFP gene comes from the jellyfish *Aequorea Victoria*. GFP mice are used as a marker in transgenic studies as they can be detected without treatment. This property also makes them useful for cell sorting and quantitative assays.

Mice were chosen as model to develop the fluorescent organisms because their genome is similar to humans and the physiological and behavioural tests on mice can be applied to human diseases with less lag.

To visualize the GFP mice, we use blue LED lamps (465 -470 nm) and a green acetate filter in a dark environment.

There are many strains of GFP mice which have been developed but the most common on is called as CRH – GFP. These mice are viable, fertile and appear normal on physical inspection.

Figure showing GFP – mice.



Microinjection: It is a method that is used for injecting substances into single cells using a very thin needle. The substances injected can be cells, genetic material, peptides, antibodies, drugs or other exogenous agents.

This process is performed under an inverted microscope while using magnification of 200X. It requires a lot of pressure to force the solution out of the very minute tip as it has a diameter of less than one micron.

Applications of microinjection are:

- Direct injection of nucleic acids into the cytoplasm or nucleus.

- Introducing DNA by microscopic examination of the targeted specimen.
- Implanting microinjected eggs into the oviducts during IVF procedures.

Figure showing microinjection under process: