Lecture 9 animal biotechnology

A transgenic animal is one that carries a foreign (exogenous) gene that has been deliberately inserted into its genome. Another definition: are animals that have been genetically modified for a variety of purposes including producing drugs, enhancing yields, increase resistance to disease

The foreign gene is constructed using recombinant DNA methodology.In **1974 Rudolf Jaenisch** created a transgenic mouse by introducing foreign DNA into its embryo, making it the world 's first transgenic animal.

The introduced DNA is called a **transgene** and the overall process is called **transgenic technology** or **transgenesis.**

He injected retrovirus DNA into early mouse embryos and showed that leukemia DNA sequences had integrated into the mouse genome and also to its offspring.

The vast majority of genetically modified animals are at the research stage with the number close to entering the market remains small.

Genetic modification of an animal involves altering its genetic material by adding, changing or removing certain DNA sequences in a way that does not occur naturally.

Three basic methods of producing transgenic animals

DNA microinjection: microinjection into the enlarged sperm nucleus (male pronucleus) of a fertilized egg.

Retrovirus-mediated gene transfer. Retroviral vectors that infect the cells of an early stage embryo prior to implantation into a receptive female.

Embryonic stem cell-mediated gene transfer. introduction of genetically engineered embryonic stem cells into an early-stage developing embryo before implantation into a receptive female.

Changes introduced in an animal 's genetic make -up can therefore be transmitted to the next generation.

While this technology has so far been used in plants for agriculture and in microorganisms to produce enzymes, the potential application of genetic modification techniques to animals is also being researched.

Several international organizations, including FAO/WHO and the United States Food and Drug Administration, have already published guidelines for the safety assessment of these animals and their derived products.

Process of GM animals

The process of genetically engineering mammals is a slow, tedious, and expensive process.

As with other genetically modified organisms (GMOs), first genetic engineers must isolate the gene they wish to insert into the host organism.

This can be taken from a cell containing the gene or artificially synthesised.

If the chosen gene or the donor organism's genome has been well studied it may already be accessible from a genetic library.

The gene is then combined with other genetic elements, including a promoter and terminator region and usually a selectable marker.

A number of techniques are available for inserting the isolated gene into the host genome.

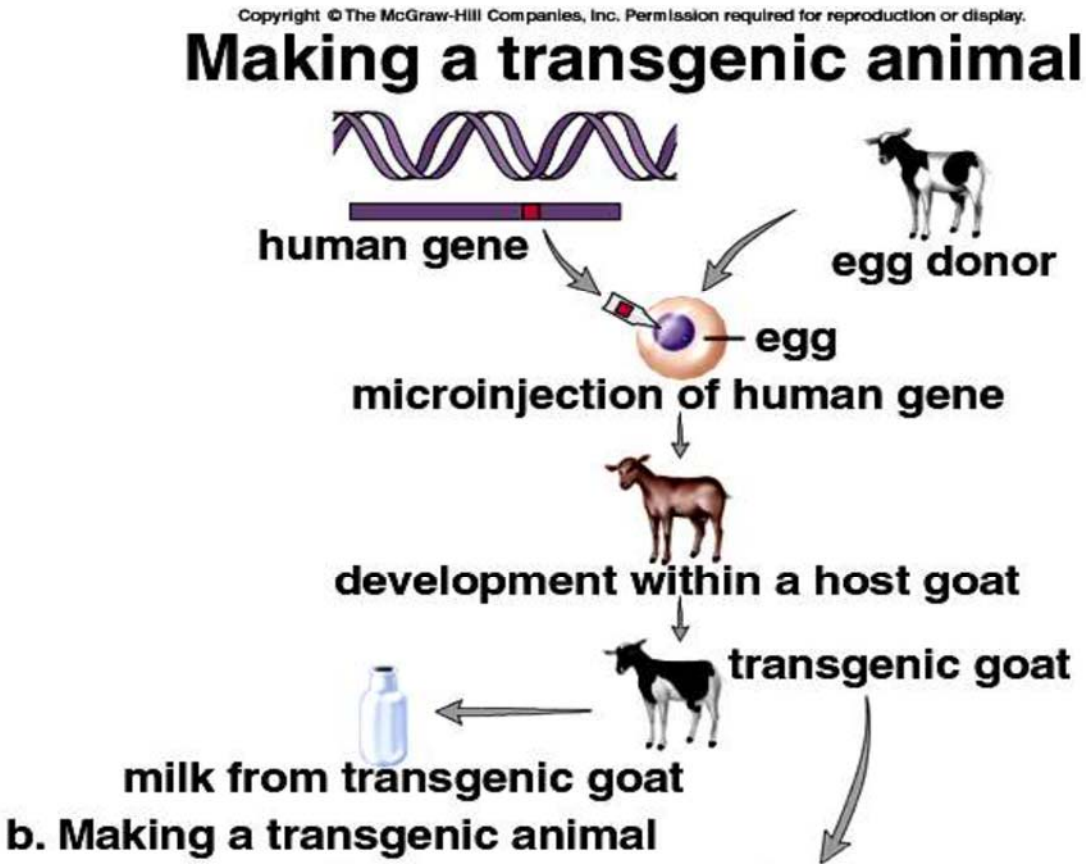
With animal's DNA is generally inserted into using microinjection, where it can be injected through the cell's nuclear envelope directly into the nucleus, or through the use of viral vectors.

The first transgenic animals were produced by injecting viral DNA into embryos and then implanting the embryos in females.

It is necessary to ensure that the inserted DNA is present in the embryonic stem cells.

The embryo would develop and it would be hoped that some of the genetic material would be incorporated into the reproductive cells.

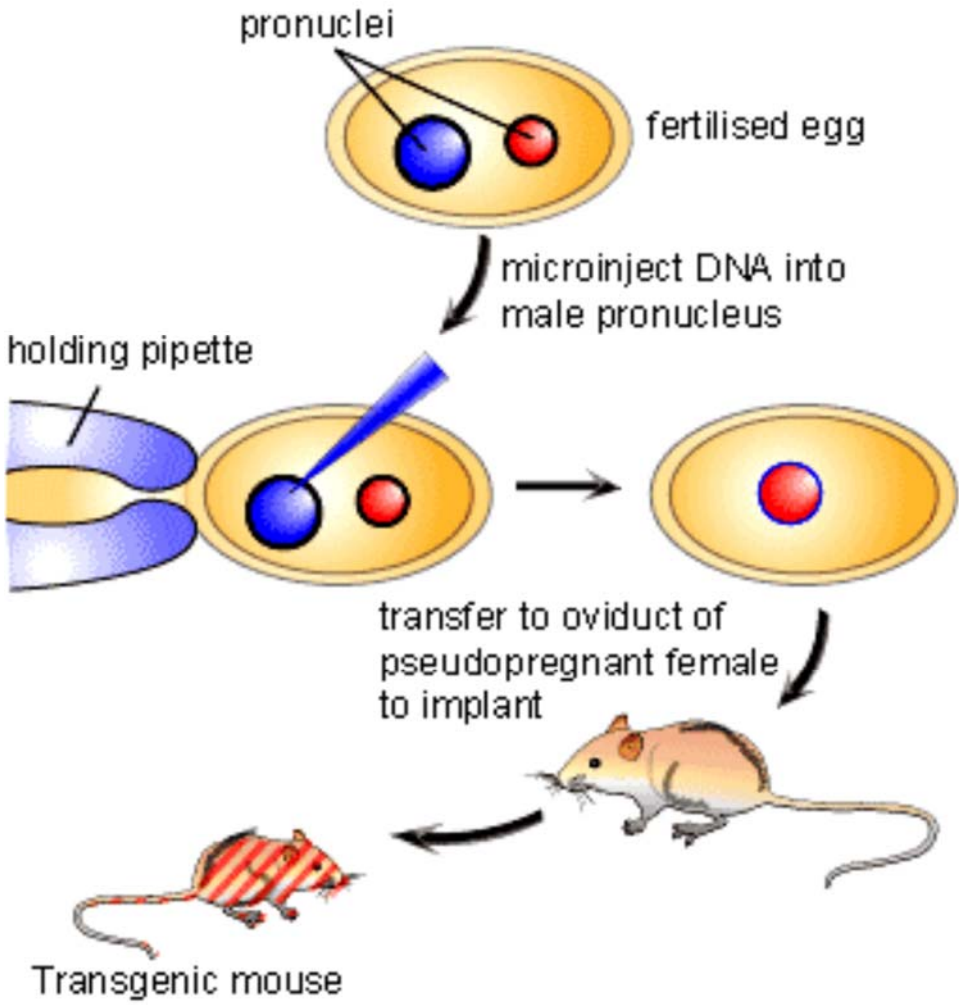
Then researchers would have to wait until the animal reached breeding age and then offspring would be screened for presence of the gene in every cell, using PCR, Southern hybridization, and DNA sequencing.



b. Making a transgenic animal

1(a). DNA Microinjection (Nuclear Microinjection)

1. Transgene injected in fertilized egg cells. Pronuclei will fuse after DNA is injected into male pronucleus (5 -40% success rate).
2. Eggs kept inculture during first divisions of embryonic development
3. Embryos are implanted into foster mother. (mouse has been treated with hormones so that she accepts the embryo and carries on with the pregnancy)
4. Transgenes stably maintained in founder animal. Single copy of transgene on one chromosome. Heterozygous for the transgene.



Creation of Transgenic Animals by Nuclear Injection

In vitro fertilization is used to start a transgenic animal. Harvested eggs and sperm are fertilized, and before the pronuclei fuse, the transgene is injected into the male pronucleus. The embryo continues to divide in culture and is then implanted into a mouse. The “foster mother & rdquor; mouse has been treated with hormones so that she accepts the embryo and carries on with the pregnancy. The offspring are screened for stable integration of the transgene. Founder mice have one copy of the transgene.

1(b). Electroporation

Electroporation is a physical transfection method that uses an electrical pulse to create temporary pores in cell membranes through which substances like nucleic acids can pass into cells.

It is a highly efficient strategy for the introduction of foreign nucleic acids into many cell types, including bacteria and mammalian cells.

Advantages:

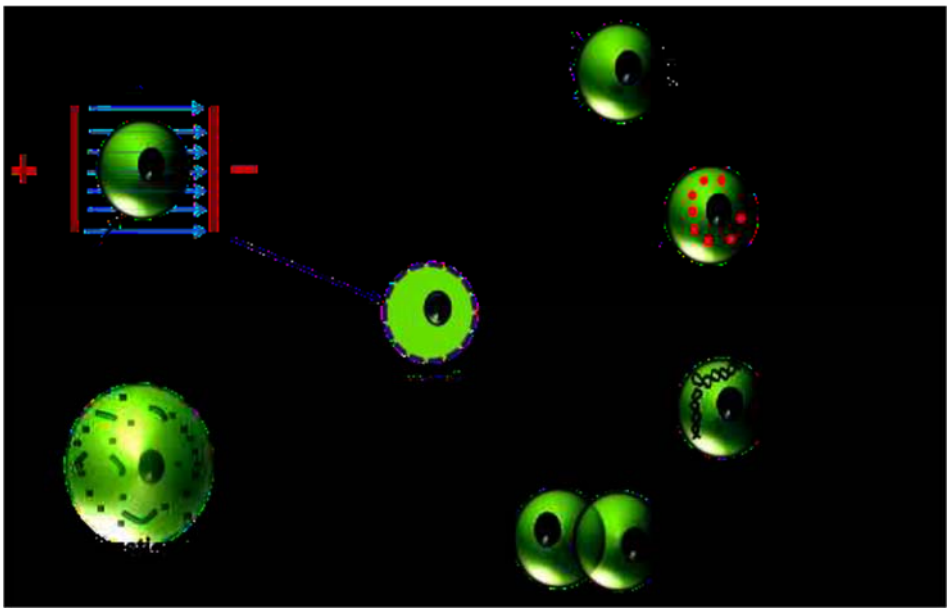
Most common technique for transferring the transgene.

Most reliable technique for transgenesis.

Process is inexpensive.

Drawbacks:

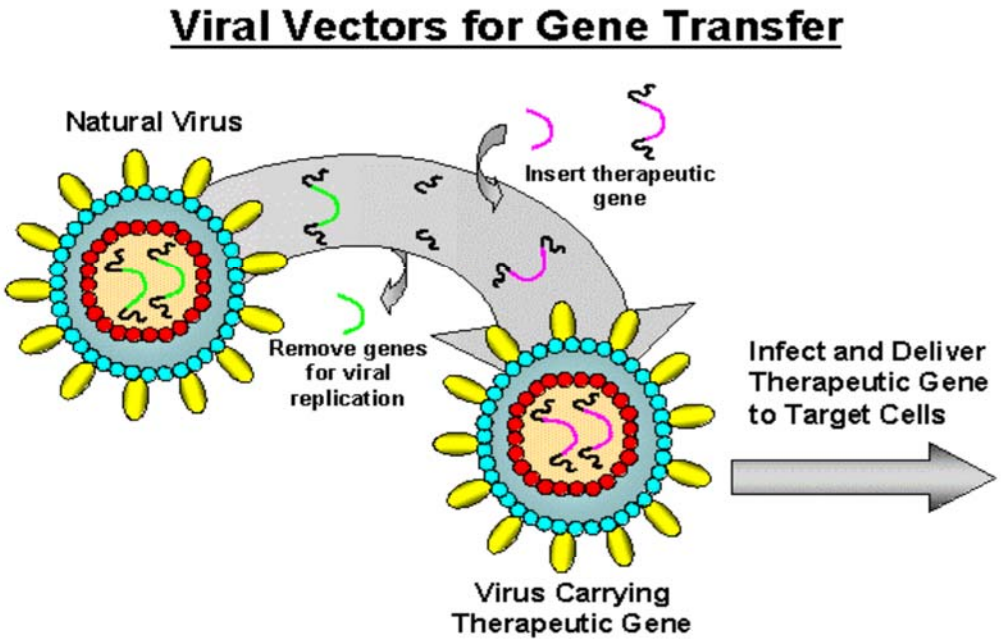
- Requires the coordination of a number of experimental steps and highly trained practitioner.
- Often multiple copies of the injected DNA are incorporated at one site which disrupts the normal physiology of the animal.
- Only 5% of the inoculated eggs to develop into live transgenic animals.

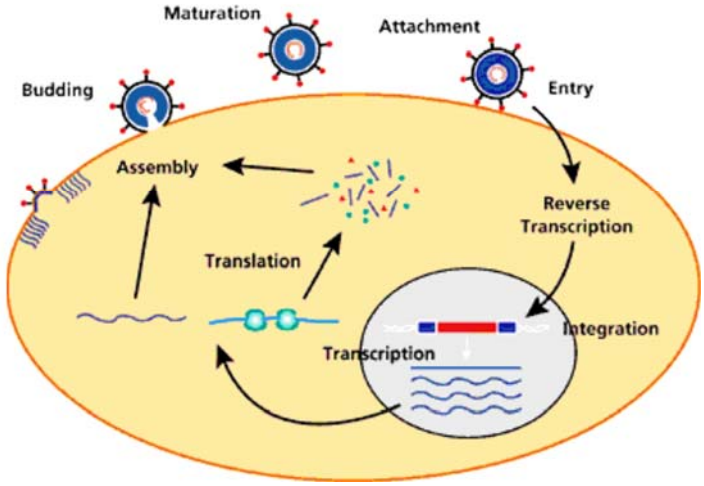


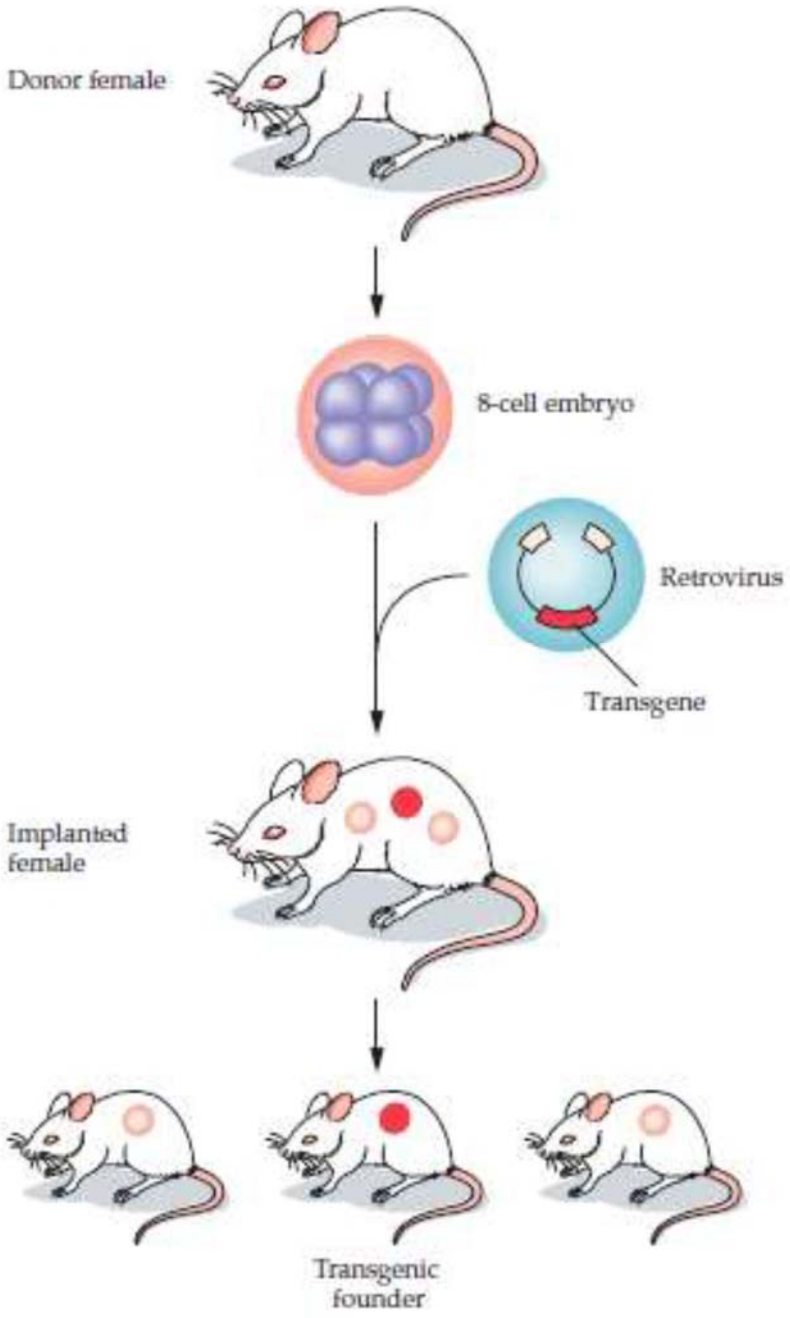
2. Virus-Mediated Gene Transfer

- Four classes of viral vector:

1. Retrovirus- commonly used (Offspring's derived from this method are chimeric; not all cell carries the retrovirus i.e. diverse genetic constitution)
2. Adenovirus
3. Herpesvirus
4. Adeno-associated Virus(AAV) Vectors







Retrovirus Injection

Advantage:

An effective means of integrating the transgene into the genome of a recipient cell.

Weaknesses:

Vectors derived from these viruses can transfer only small pieces (~8 kilobases).

The genome of the retroviral strain can be integrated into the same nucleus as the transgene.

3. Embryonic Stem Cell- Mediated Gene Transfer

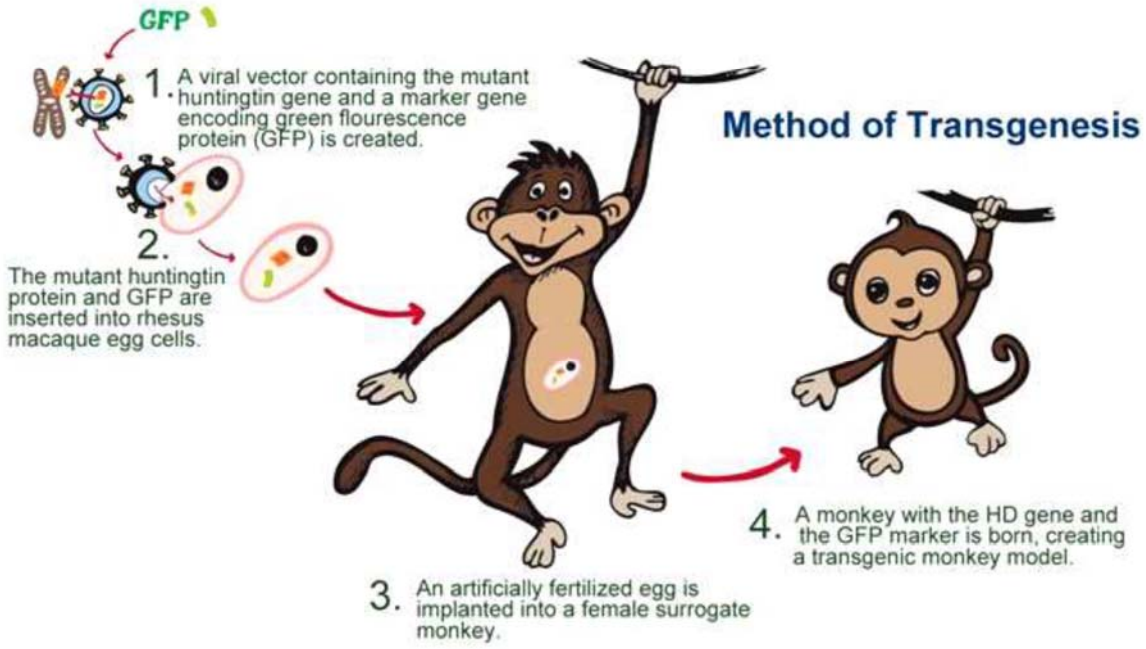
A. Embryonic stem cells
B. Formation of gametocytes
C. Injection into blastocysts
D. Injection into foster mother
E. Formation of new individual

- Strategy used for this method:

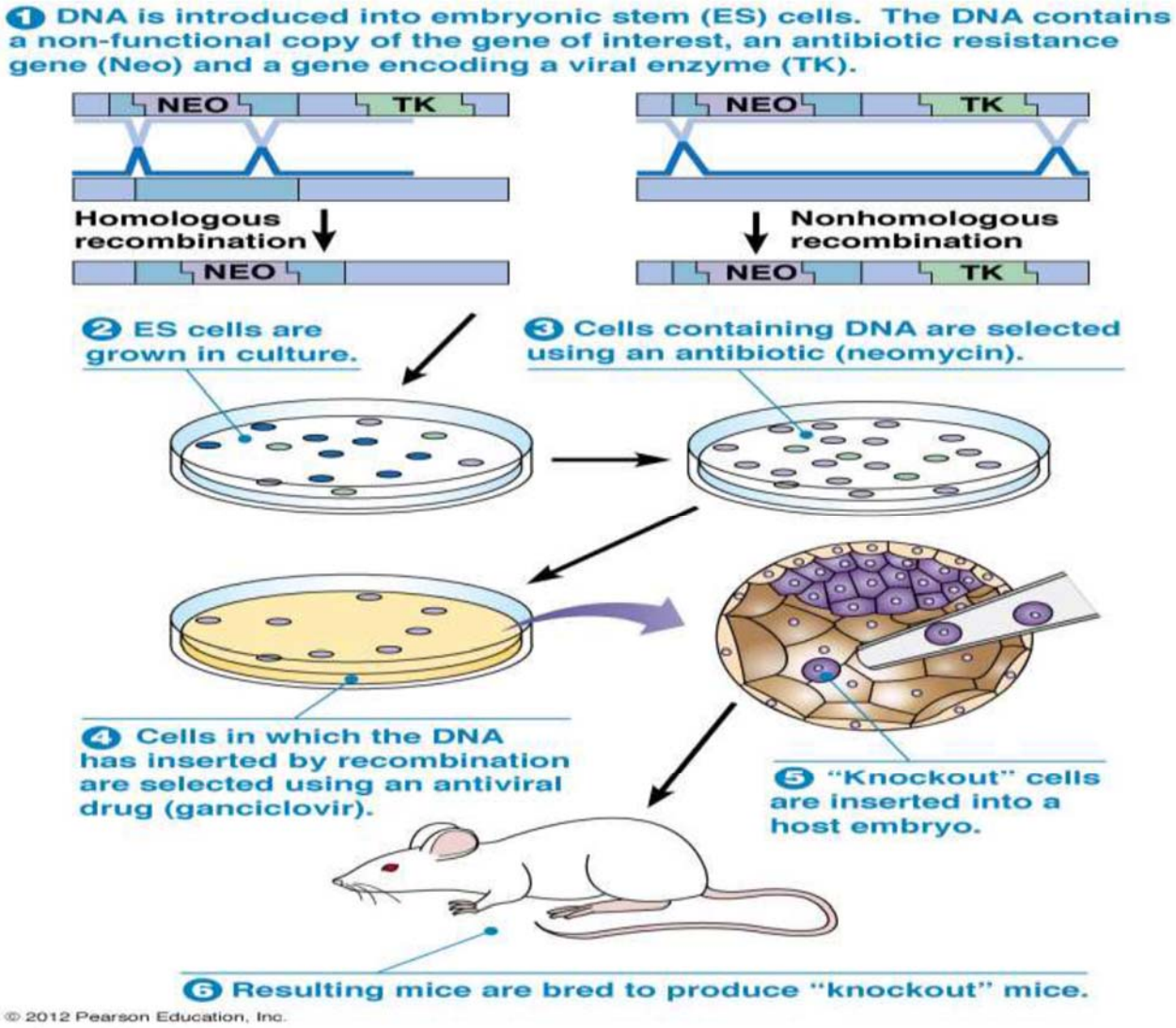
A cloned gene is injected into the nucleus of a fertilized egg.

The inoculated fertilized eggs are implanted into a receptive female.

Some of the offspring derived from the implanted eggs carry the cloned gene



Engineered embryonic stem cells



▶ **Advantages**:

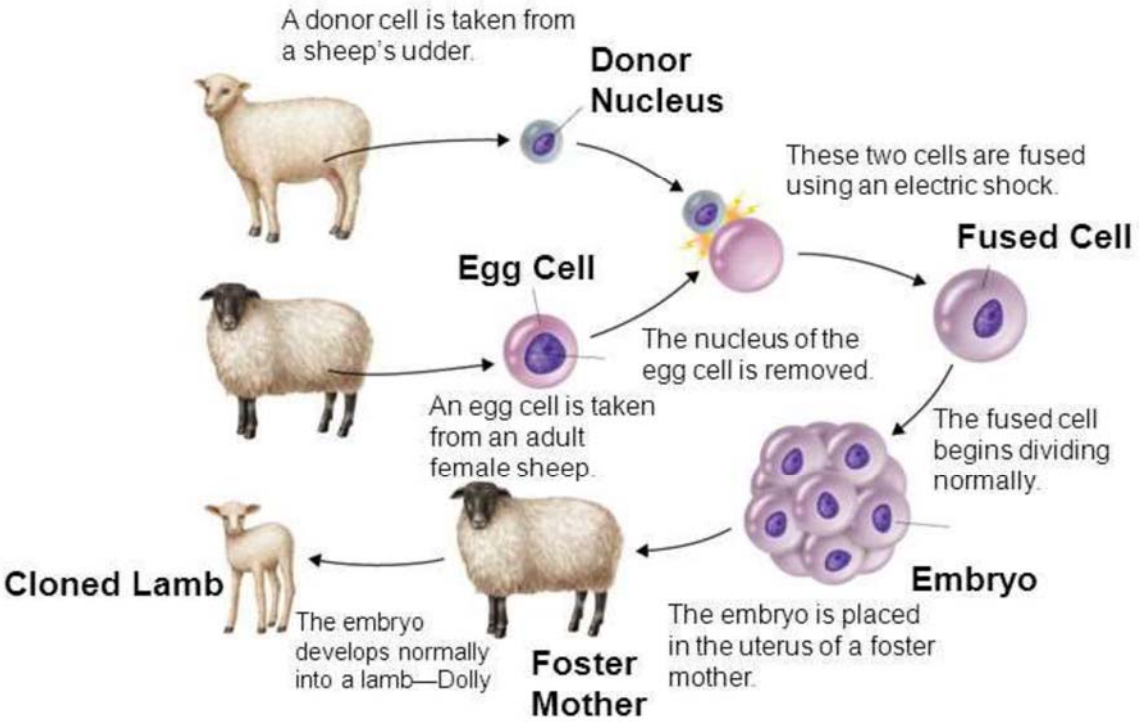
1. Reduction in the cost of producing a transgenic animal.
2. Randomness of integration is avoided.
3. ES cells enable the researcher to place new genes in advantageous places in the genome or to remove deleterious genes.

▶ **Weakness**:

1. Requires longer time.
2. Requires germline-competent ES cell lines.
3. Difficulties associated with the production, characterization and maintenance of pluripotent ES cell lines.

Cloning

Figure 13-13 Cloning of the First Mammal



Applications

The benefits of these animals to human welfare can be grouped into areas:
Agriculture
Medicine
Industry

1. Agricultural Applications
Breeding
Quality & quantity of (milk, meat, eggs & wool production.)
Disease Resistance

2. Medical Applications
Xenotransplantation
human gene therapy
nutritional supplements and pharmaceuticals
(Disease models)

3. Industrial importance
Toxicity sensitive transgenic animals to test chemicals.
Spider silk in milk of goat.