Transgenic animals

Transgenic animals

Animals which have been genetically engineered to contain one or more genes from an exogenous source.

Transgenes are integrated into the genome.

A large number of transgenic animals have been created.
Mice Cows Pigs Sheep Goats Fish Frogs Insects

Introduction of foreign genes into intact organisms

There are three main ways to produce transgenic animals:

- Microinjection
- Embryonic stem cell (ES) based transgenesis,
  ES cell injection into blastocyst
- Using retrovirus

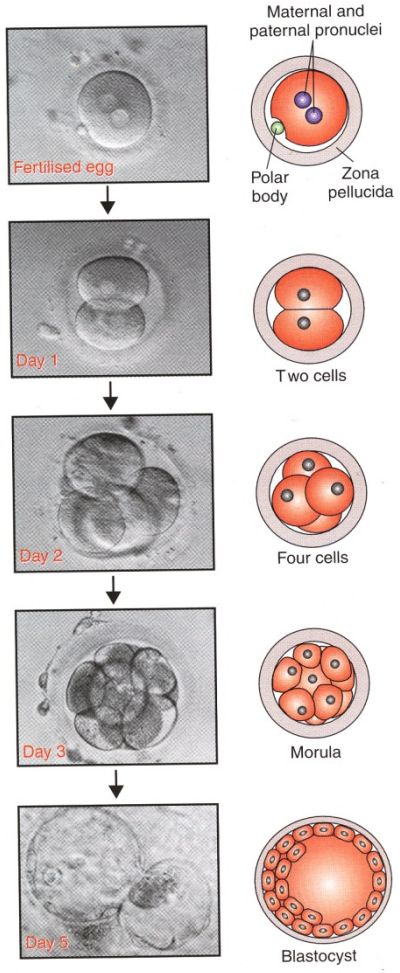
Early embryonic development

When sperm enters the egg, the fertilized cell is called ZYGOTE, it contains two nuclei – PRONUCLEI

Maternal and paternal pronuclei fuse with each other to form a single fertilized nucleus.

Zygote then starts to divide into 2, then 4, 8, and more forming a ball of cells – MORULA

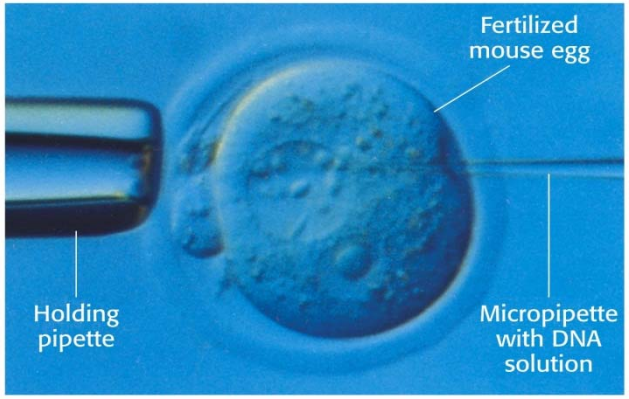
Morula continues to divide and the cavity is filled with the fluid from uterus. It is called BLASTOCYT in this stage.



Pronuclear Injection

The injection into the nuclei of newly fertilized eggs

- The eggs are harvested from mice (superovulated or natural matings).
- Following fertilization, large male and small female pronuclei are visible under the microscope.
- DNA injections are usually made into the larger male pronucleus and the while egg is held using pipette.



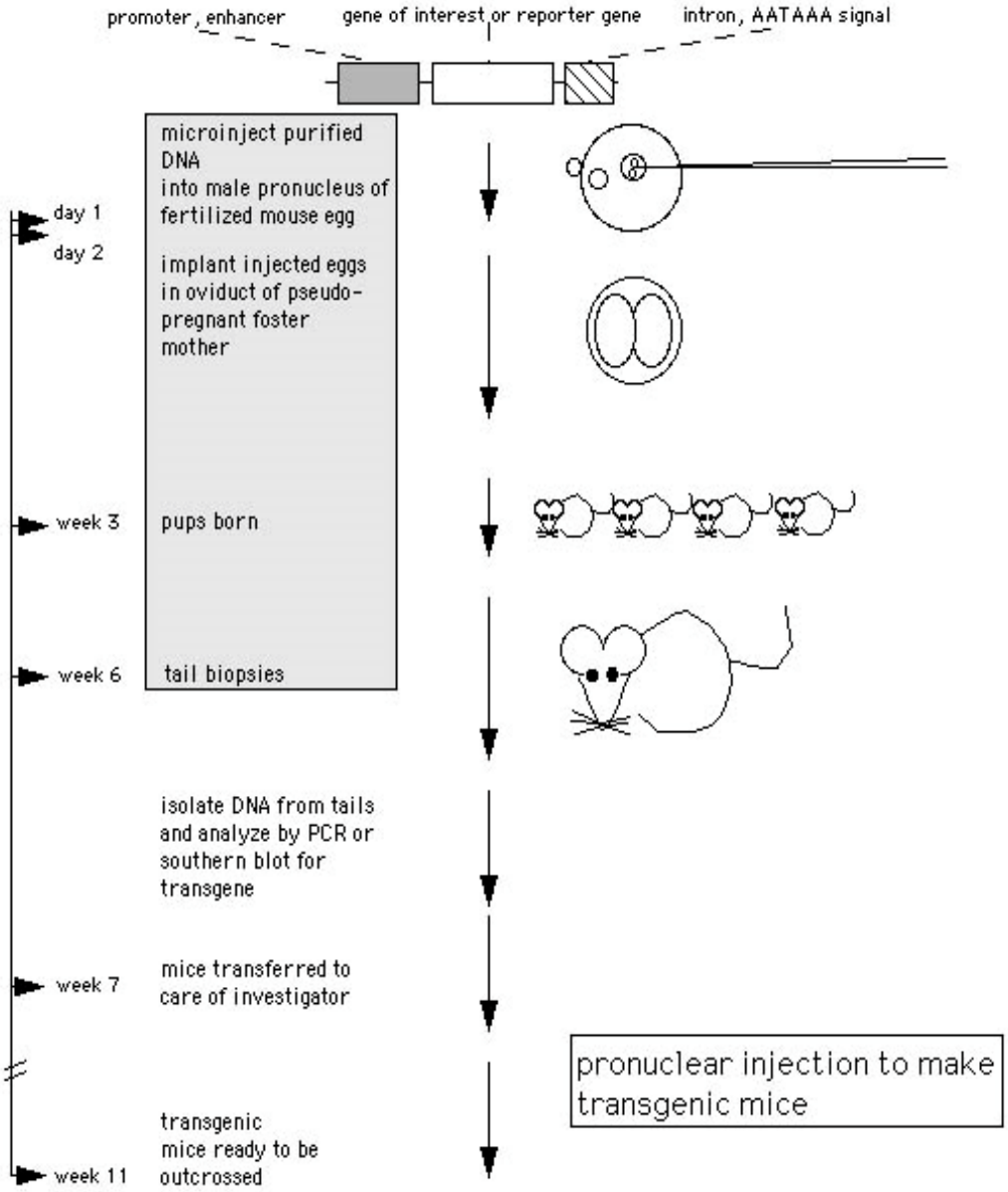
Gene is injected into the male pronuclei

Pronuclear Injection

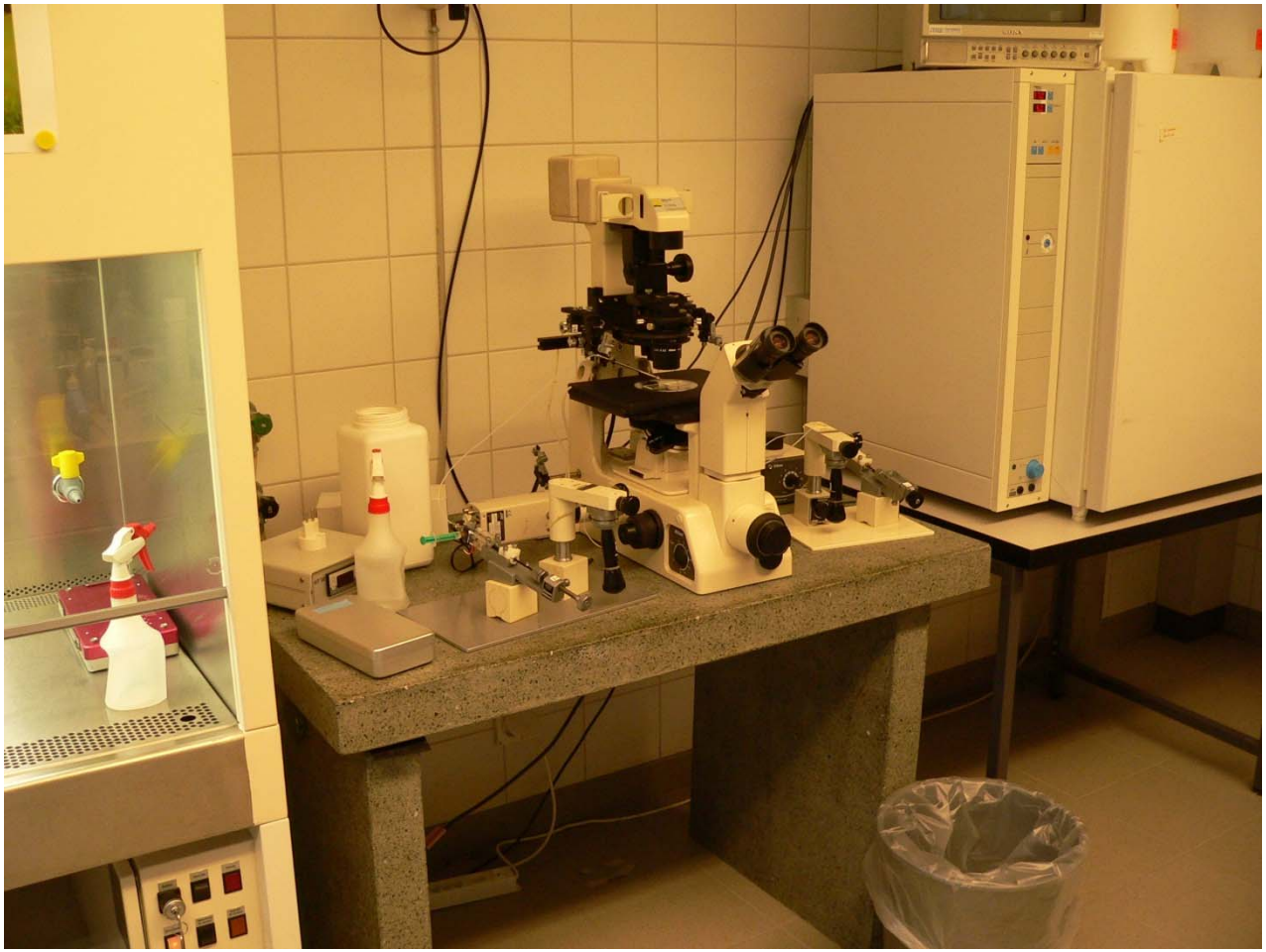
The injected DNA may be integrated into the pronuclear DNA and upon fusion with the female pronucleus, is incorporated into zygote.

The injected embryos are cultured *in vitro* until the morula stage and then implanted into the pseudo-pregnant female mouse **that has been previously mated with a vasectomized male, which stimulated the appropriate hormonal changes needed to make her uterus receptive.**

The implanted embryo is then allowed to develop into a mouse pup.



Microinjection station



Mechanisms of DNA Integration

- Linear molecules integrate more efficiently than circular molecules (~5x).
- Once in the oocyte, the linear molecules circularize.
- Usually all of the molecules that integrate are on the same chromosome and at the same site.
- Multiple copies are usually arranged in a tandem, head-to-tail array.
- The size of the DNA molecule (0.7 – 50Kb) is not an important parameter.
- The concentration and purity of the injected DNA is critical (1-3 μg/ml maximum).

Possible Reasons for Lack of Transgene Expression

- Integration in cis-acting silencer sequences (the negative counterpart of enhancer elements) might be sites for covalent modification of DNA (e.g. methylation) which might initiate condensation into an inactive chromatin configuration, or they might phase nucleosomes in an inappropriate manner.

- Use of cDNA rather than genomic DNA. (Introns thought to contribute to stability of mRNA and may even contain enhancer sequences essential for tissue-specific expression. Flanking DNA may also contain regulatory sequences.)

Application of Pronuclear Injection

Variety of foreign DNA were introduced into mice:

This technique has been used to attempt to produce therapeutic proteins, e.g. human α-antitrypsin (AAT) for treatment of cystic fibrosis.

A DNA fragment containing the human ATT gene, whose promoter was replaced by that from the sheep β -lactoglobulin milk promoter was injected into the pronucleus. The transgenic Mouse expressed ATT in the mammary gland secreted into its milk.

The transient sheep expressing ATT in their milk have been produced in the same way.

Disadvantages

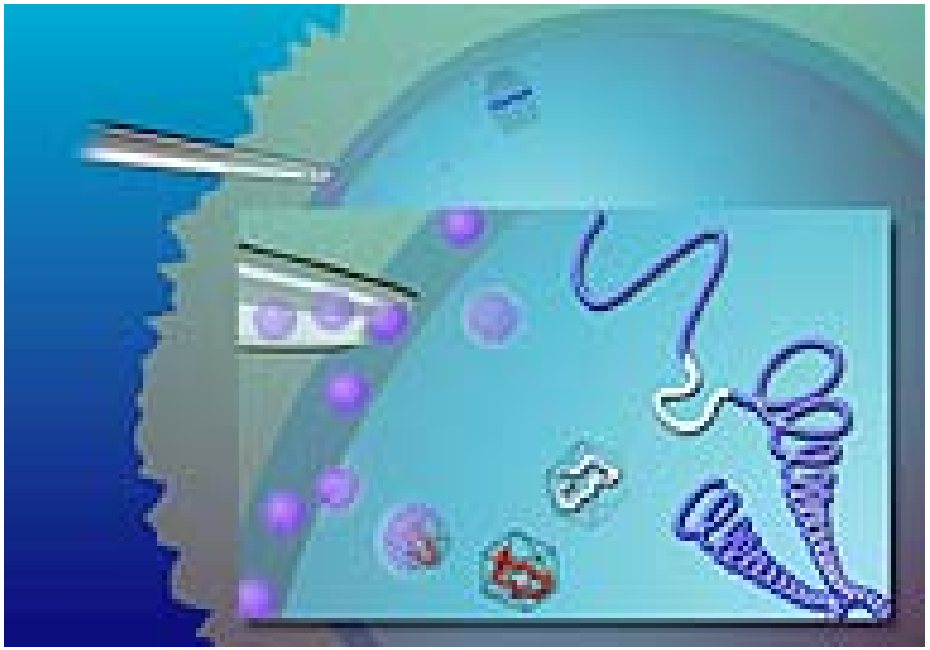
- Pronuclear injection cannot be used for knock-out genes or to alter existing genes.

- Randomness of the insertion can dramatically effect the expression of the foreign gene. The expression of the transgene cannot be readily controlled.

  Random insertion (foreign gene may disrupt an endogenous gene important for normal development, and the chance is about 10%.)
- The transgene can be present in a limited set of tissue and organs, when integration of the transgene is delayed after the first cell division.
- More than one copy of the gene may get into the genome.

Lentiviral infection

Lentiviral based vector

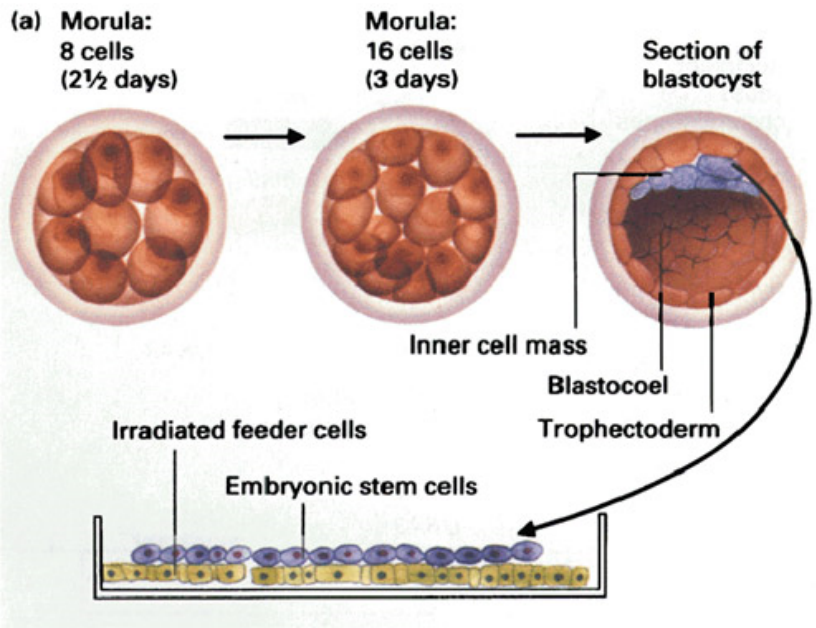


Eggs are infected prior to fertilization

Virus integrates into one of the chromosomes

Blastocyst Injection and Embryonic STEM Cells

- This involves placing the transgene into an embryonic stem cells (ES cells).
- ES cell can be cultured *in vitro* in a dish coated with mouse embryonic skin cells that do not divide (feeder layer).
- Feeder layer provides a surface for attaching ES cells and releases nutrients in the medium.
- ES cells in the culture are not differentiated during cultivation, they grow separately.
- When clump together, they begin spontaneously differentiate.
- ES cells have the potential to form all the cell types (muscle, nerve, skin, gametes= are pluripotent.



Blastocyst Injection

The recombinant embryonic stem cells cells are introduced into the fresh blastocyst where they mix with the inner cell mass.

Production of transgenic mice by ES cell gene transfer

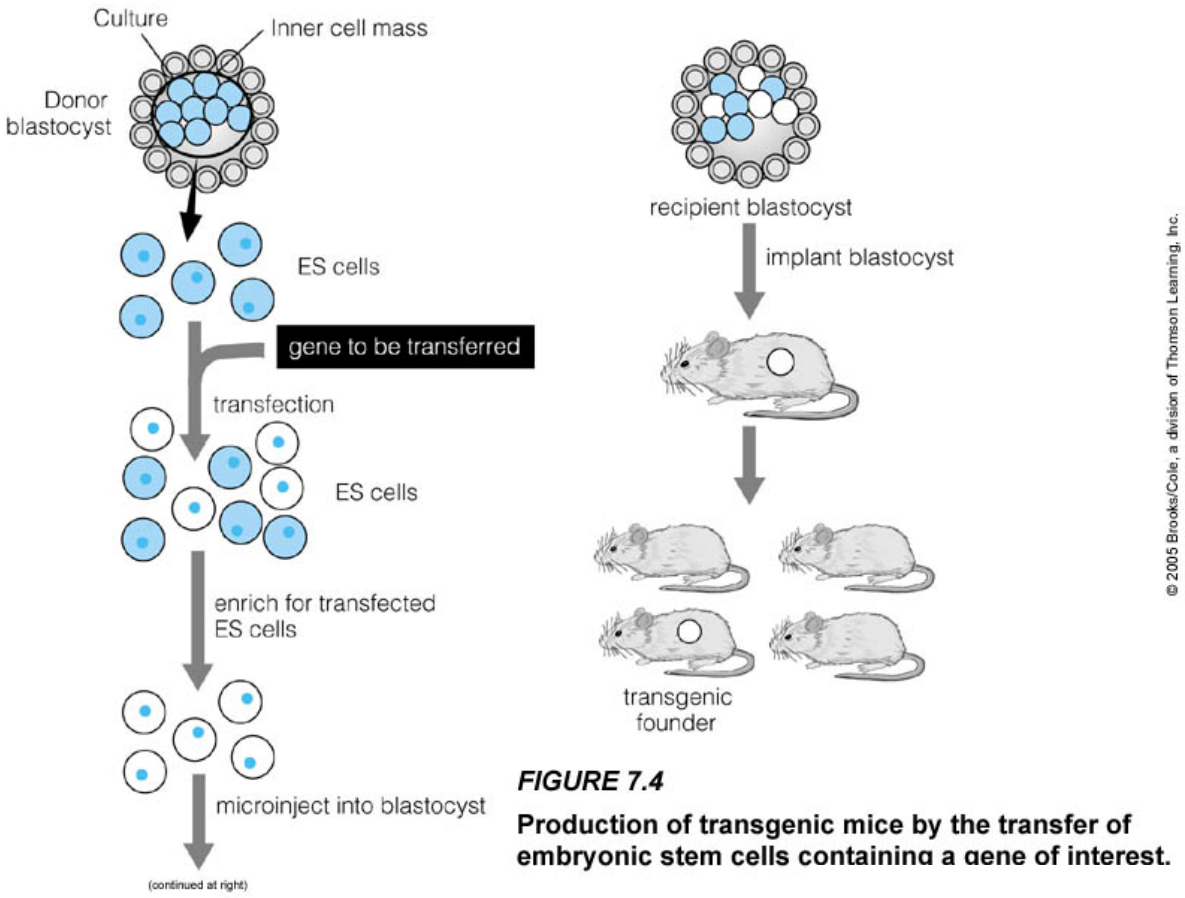


FIGURE 7.4

Production of transgenic mice by the transfer of embryonic stem cells containing a gene of interest.

The blastocyt is implanted into the uterus of a pseudopregnant female and pups are produced.

The implanted blastocyst contains two different ES cells (normal and recombinant).

The resulting offspring will be chimeric = some cells will contain transgene, other not.

The chimeric pups are crossed with the wild type animals to generate true heterozygotes, which can be subsequently inbred to create a homozygote.

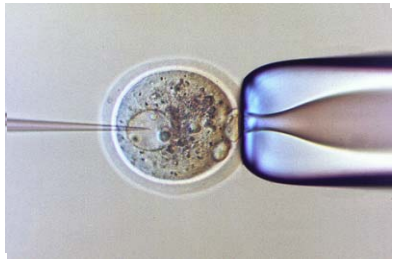
Advantages using ES Cells

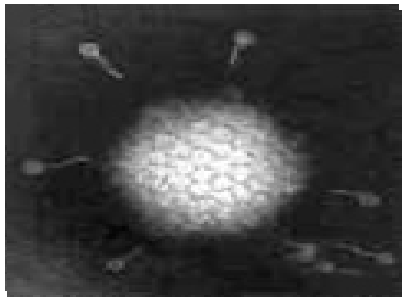
- Embryonic stem cells are relatively efficient at homologous recombination.
- Recombination between homologous sequence in the vector DNA and the genome is used to target the insertion of the foreign DNA to a specific sequence in the genome.
- Some levels of non-homologous recombination occur.
- It is necessary to separate these two types.

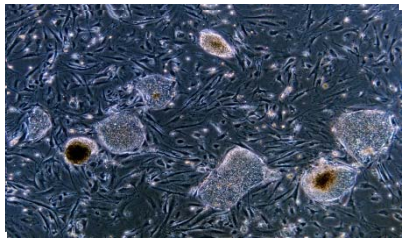
- This method allows also to delete (knockout) a gene.

Transgenesis Methods

	pros	cons
Pronuclear injection	Relatively simple and efficient Long transgenes possible Potentially all species	Random integration Multicopy insertions (Strain limitations)
Lentiviral infection	Very efficient Single copy insertions No technical equipment Works in many species	High embryo mortality 9.5 kb packaging limit Safety issues (?) Only random integration
ES based transgenesis	Long transgenes possible Gene targeting possible Single copy insertions	Technically difficult Time consuming Species / Strain limitations







Knockout animals why?

- To study effects of gene products, biochemical pathways, alternative (compensatory) pathways, and developmental pathways.

- To recreate human diseases in animals to establish models to test the beneficial effects of drugs or gene therapy.

Procedure for Generating Knockout Mouse

- Gene alteration in KO mice is targeted to very specific genes.
- DNA must integrate at precise positions in the genome.
- Integration of the altered gene takes place in embryonic stem cells ex vivo.
- Verification of exact location of integration occurs before the ESC is introduced into blastocysts to become part of the developing embryo.

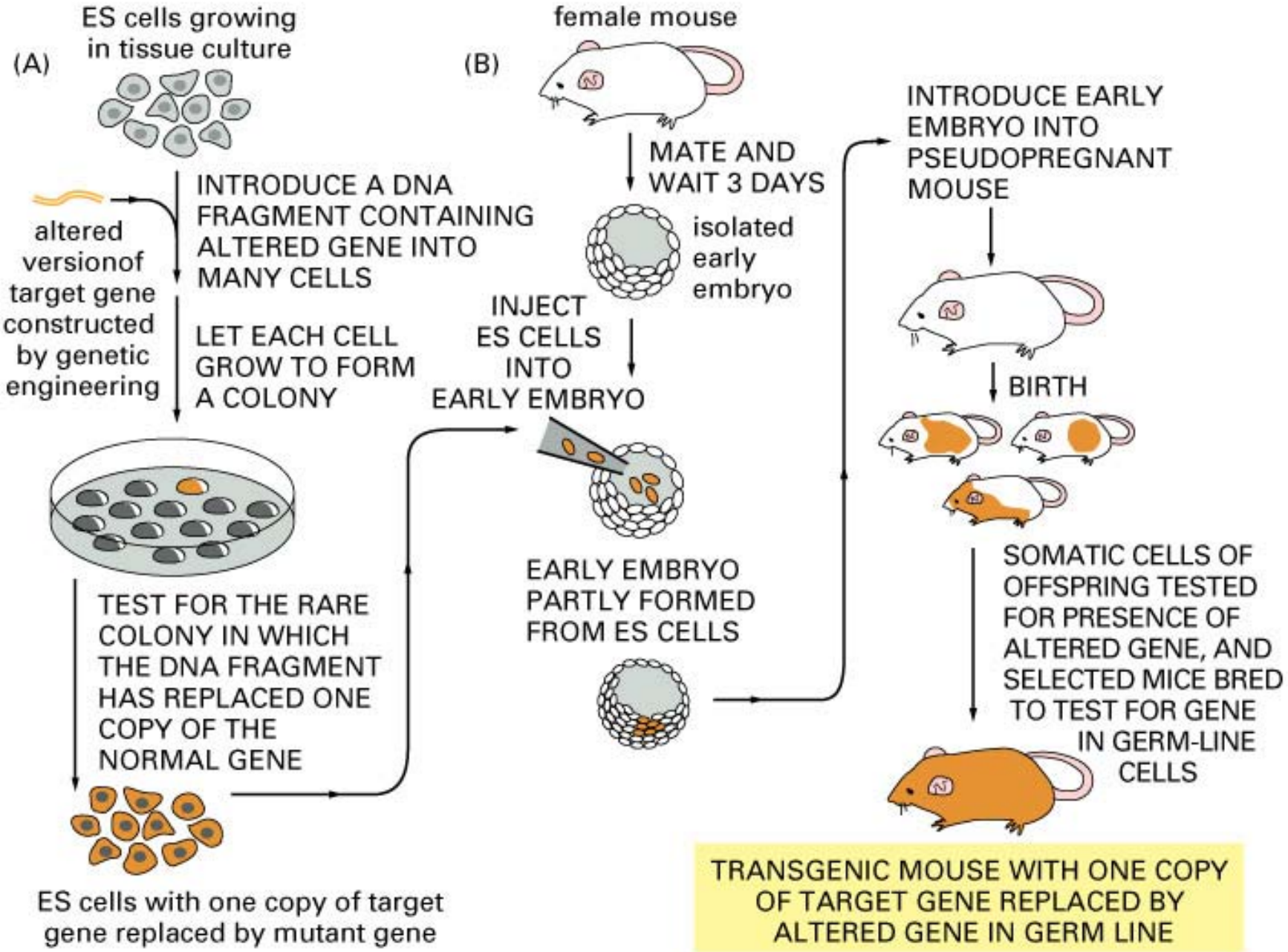
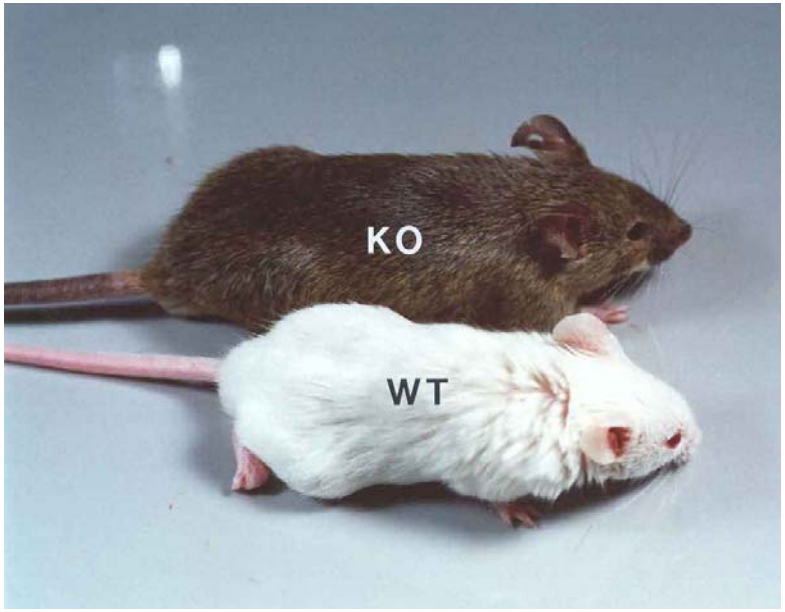


Figure 10-38 **Essential Cell Biology, 2/e. (© 2004 Garland Science)**

p27 knockout mouse

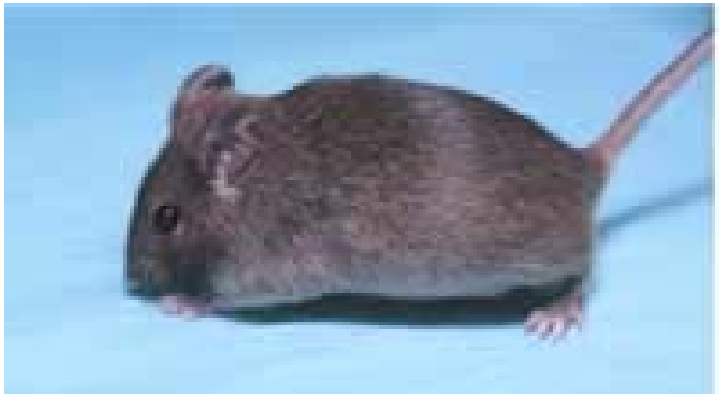


p27 knockout mouse is bigger than the control

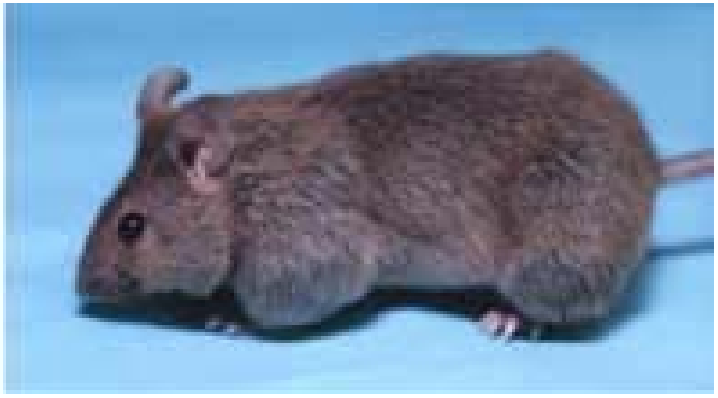
This is not due to obesity, but the skeletal structure is increased in size (everything about the mouse is larger)

http://www.bioreg.kyushu-u.ac.jp/saibouE.html

normal



knockout

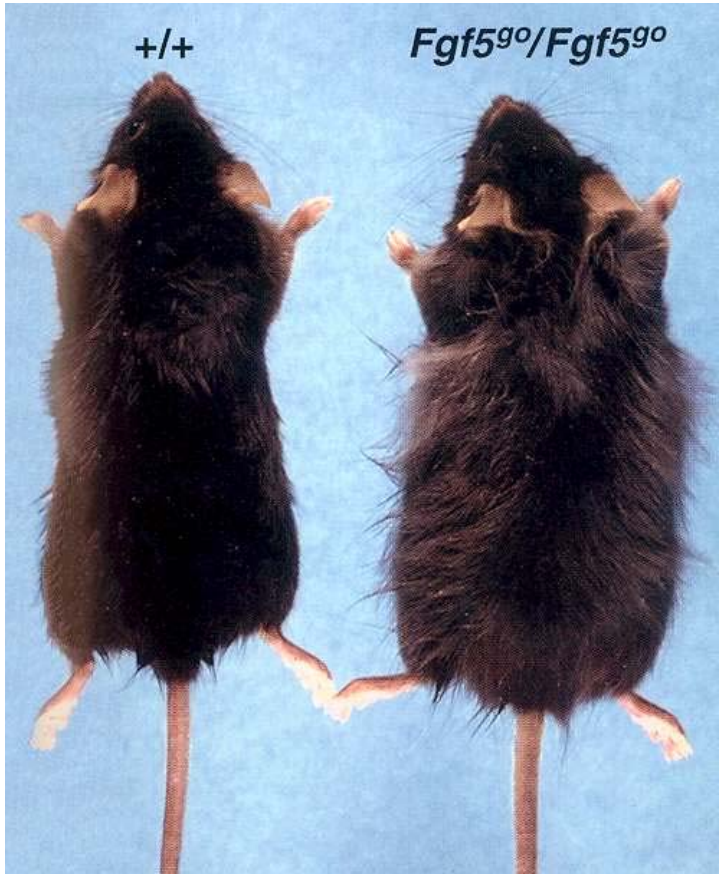


GDF8 (Myostatin) knockout mouse

Over twice the muscle mass of a wildtype mouse

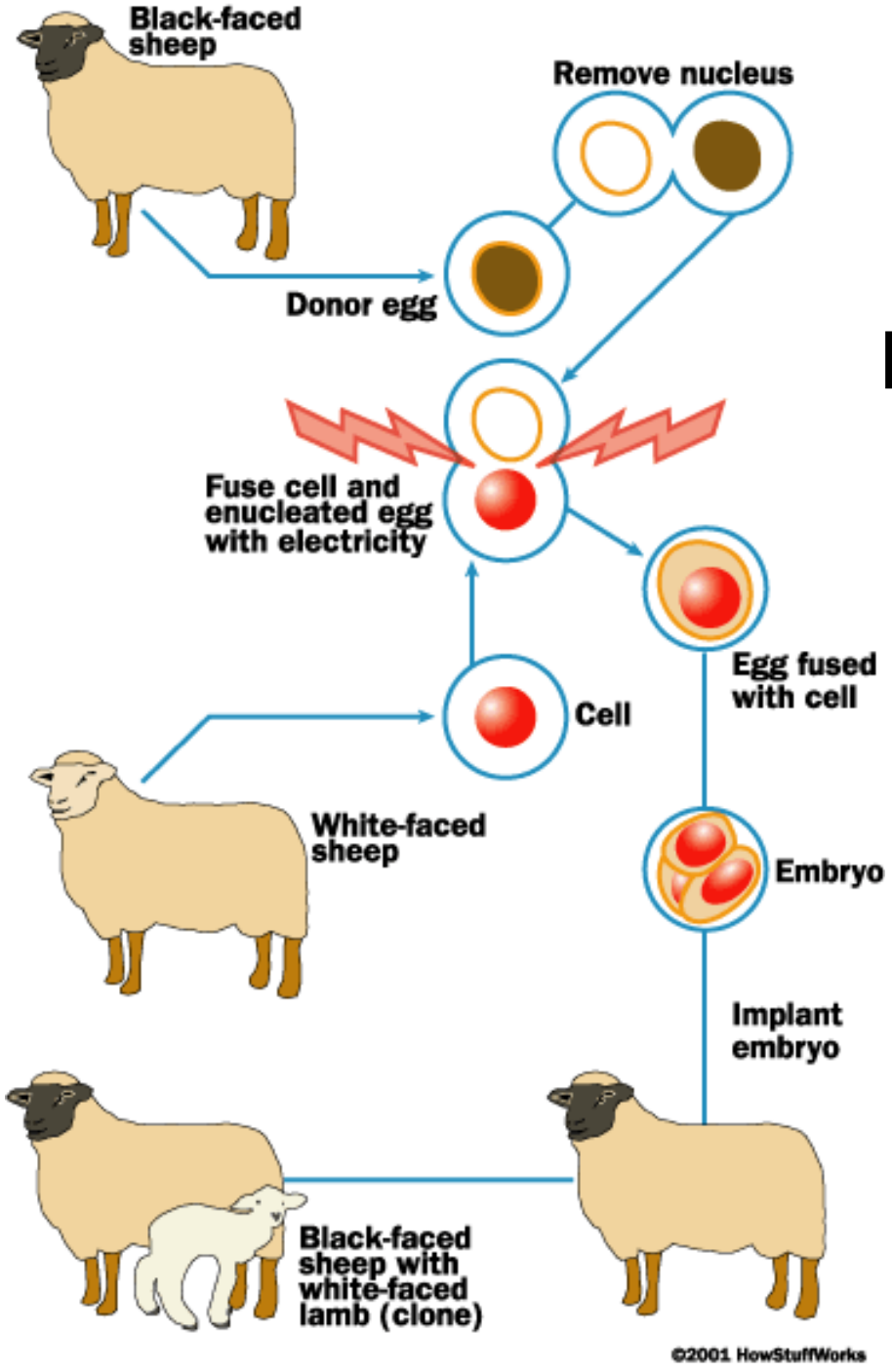
http://www.bbc.co.uk/science/genes/gene_safari/wild_west/bigger_and_better02.shtml

FGF5 knockout mouse has long, angora-like hair



http://www.med.uni-jena.de/ivm/deutsch/method/method_7.htm

Somatic Cell Nuclear Transfer



http://www.howstuffworks.com/cloning3.htm

What Has Been Cloned So Far?

Somatic Cell Nuclear Transfer

Sheep, Goat, Mouse, Rabbit, Cattle (domestic & wild),
Pig, Horse, Mule, Dog, Cat (domestic & wild), Deer

Embryo Splitting (Twinning)

Sheep, Cattle, Primate (Rhesus)

Applications of Transgenic Animals

Transgenic mice are often generated to

1. characterize the ability of a promoter to direct tissue-specific gene expression

   e.g. a promoter can be attached to a reporter gene such as LacZ or GFP

2. examine the effects of overexpressing and misexpressing endogenous or foreign genes at specific times and locations in the animals

3 Study gene function

   Many human diseases can be modeled by introducing the same mutation into the mouse. Intact animal provides a more complete and physiologically relevant picture of a transgene's function than *in vitro* testing.

4. Drug testing

Transgenic mice as tools

- Normal mice can't be infected with polio virus. They lack the cell-surface Polio virus receptor. But, human has Polio virus receptor.

- Transgenic mice expressing the human gene for the Polio receptor can be infected by polio virus and even develop paralysis and other pathological changes characteristic of the disease in humans.

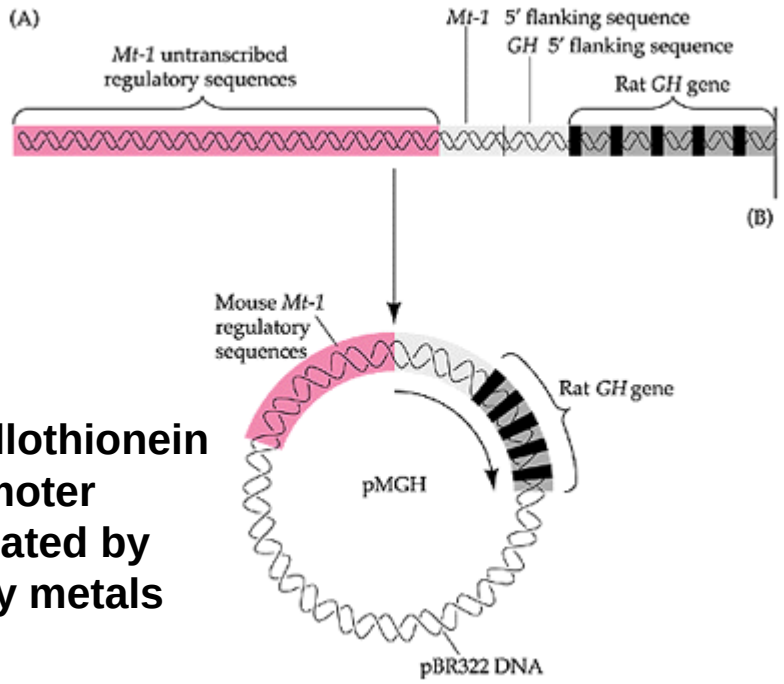
Transgenic Mouse

Transgenic mouse embryo in which the promoter for a gene expressed in neuronal progenitors (neurogenin 1) drives expression of a beta-galactosidase reporter gene. Neural structures expressing the reporter transgene are dark blue-green.

Transgenic Mouse

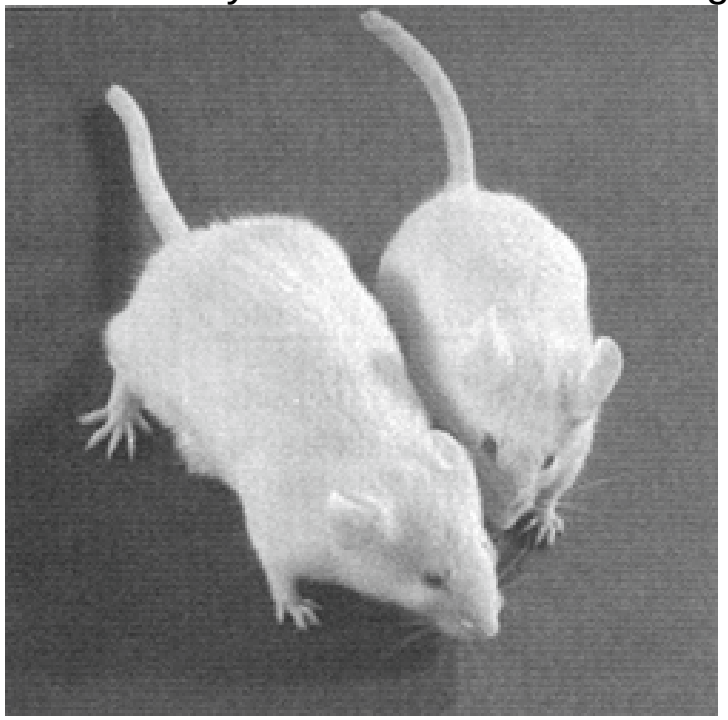
The growth hormone gene has been engineered to be expressed at high levels in animals.

The result: BIG ANIMALS

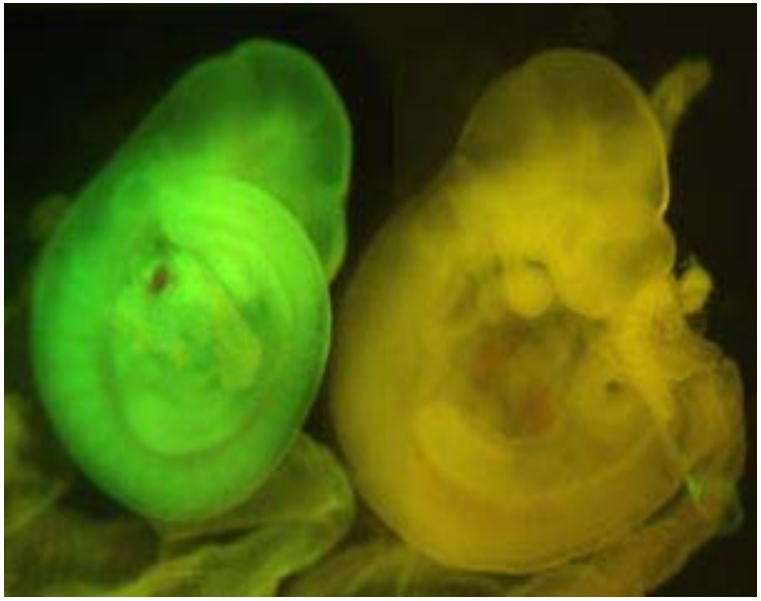


Metallothionein promoter regulated by heavy metals

Mice fed with heavy metals are 2-3 times larger

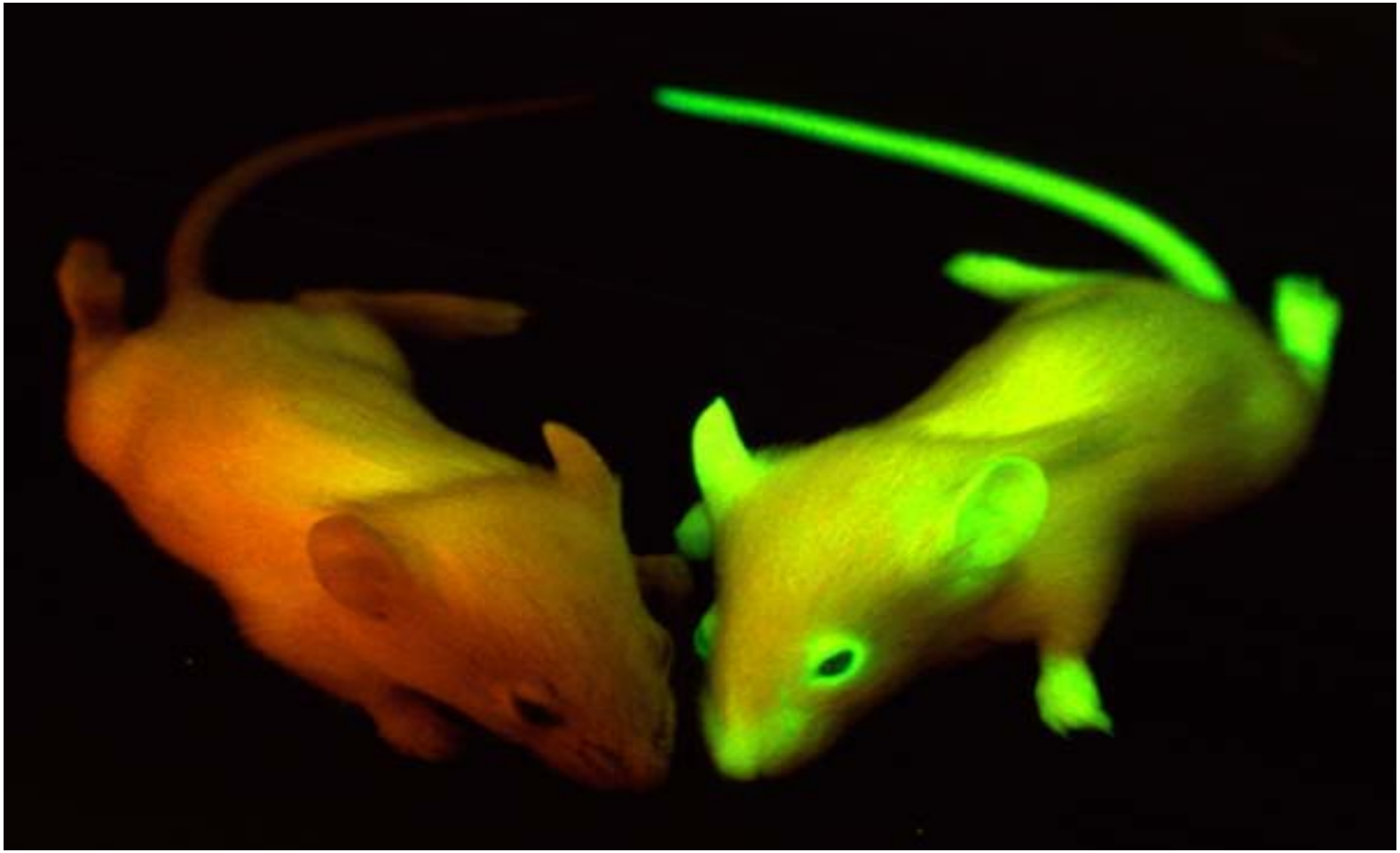


GFP transgenic mouse (Nagy)



9.5 day embryos -
GFP and wt

GFP transgenic mouse (Nagy)



Transgenic Cattle

Cloned transgenic cattle produces milk with higher levels of beta-casein and k-casein

Published in Nature, Jan, 2003
Dairy cows carrying extra copies of two types of casein genes produce 13% more milk protein

The milk is more nutritious, it allows to make more cheese

Currently the milk from these animals is under FDA review

DNA added is not foreign

EnviroPig ™

Transgenic pigs express phytase in their salivary glands

Phytic acid in the pig meal is degraded releasing phosphorus

The phosphorus is absorbed by the pig

Normally the phytic acid/phosphorus complex passes through the pig and is excreted as waste

Pig waste is a major pollutant & can cause eutrophication of lakes & streams

http://www.nature.com/news/2005/050221/images/nbt0305-283-I1.jpg

Transgenic Fish

Tilapia

Salmon/trout

Catfish

Can grow up to 6 times faster than wildtype fish

Most have extra copies of growth hormone (GH) gene

Transgenic

Wildtype

http://www.nature.com/nbt/journal/v19/n6/images/nbt0601_500a_I1.jpg

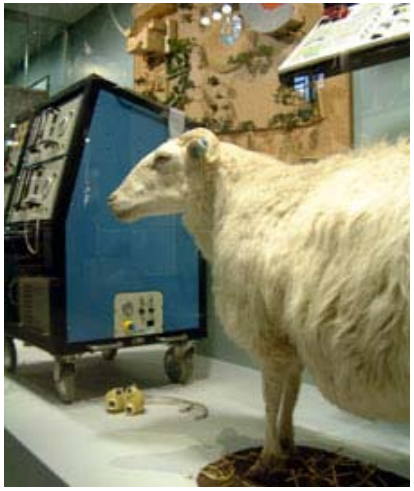
GloFish, originally developed in Singapore as a way to monitor water pollution

The normally black-and-silver zebrafish was turned green or red by inserting various versions of the GFP gene

Glofish are on sale throughout the US except in California

Glofish retail for about $5 per fish. Normal zebrafish cost around one tenth of the price

http://www.nus.edu.sg/corporate/research/gallery/research12.htm



1997, Tracy the sheep, the first transgenic animal to produce a recombinant protein drug in her milk

alpha-1-antitrypsin (AAT) treatment for emphysema & cystic fibrosis

Created by PPL Therapeutics & The Roslin Institute

http://nolswf.bbc.net.uk/science/genes/gene_safari/pharm/a_pharming.shtml

Nexia Biotechnologies transfered the silk gene from Orb spiders into goats.

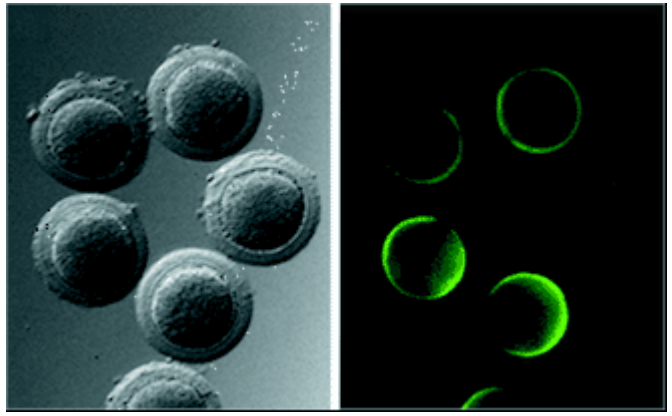
The resulting male goats were used to sire silk-producing female goats.

Each goat produces several grams of silk protein in her milk.

The silk is extracted, dried to a white powder, and spun into fibers.

The fibers are stronger and more flexible than steel.

Transgenic male kids carrying silk gene



ANDi, the first transgenic primate born in January, 2000

224 unfertilized rhesus eggs were infected with a GFP virus

~Half of the fertilized eggs grew and divided

40 were implanted into twenty surrogate mothers

five males were born,two were stillborn

ANDi was the only live monkey carrying the GFP gene

http://www.ohsu.edu/unparchive/2001/011001andi.shtml

Transgenic Animals: Potential Problems

- Technical problems to closely mimic a desired situation
- Underestimation of biological complexity
- Mouse – Human differences
- Inappropriate analysis
- Undefined genetic backgrounds