

Genetic Manipulations of Animal Cells

Genetically Modified Organisms:

Genetic material altered by any means including conventional breeding

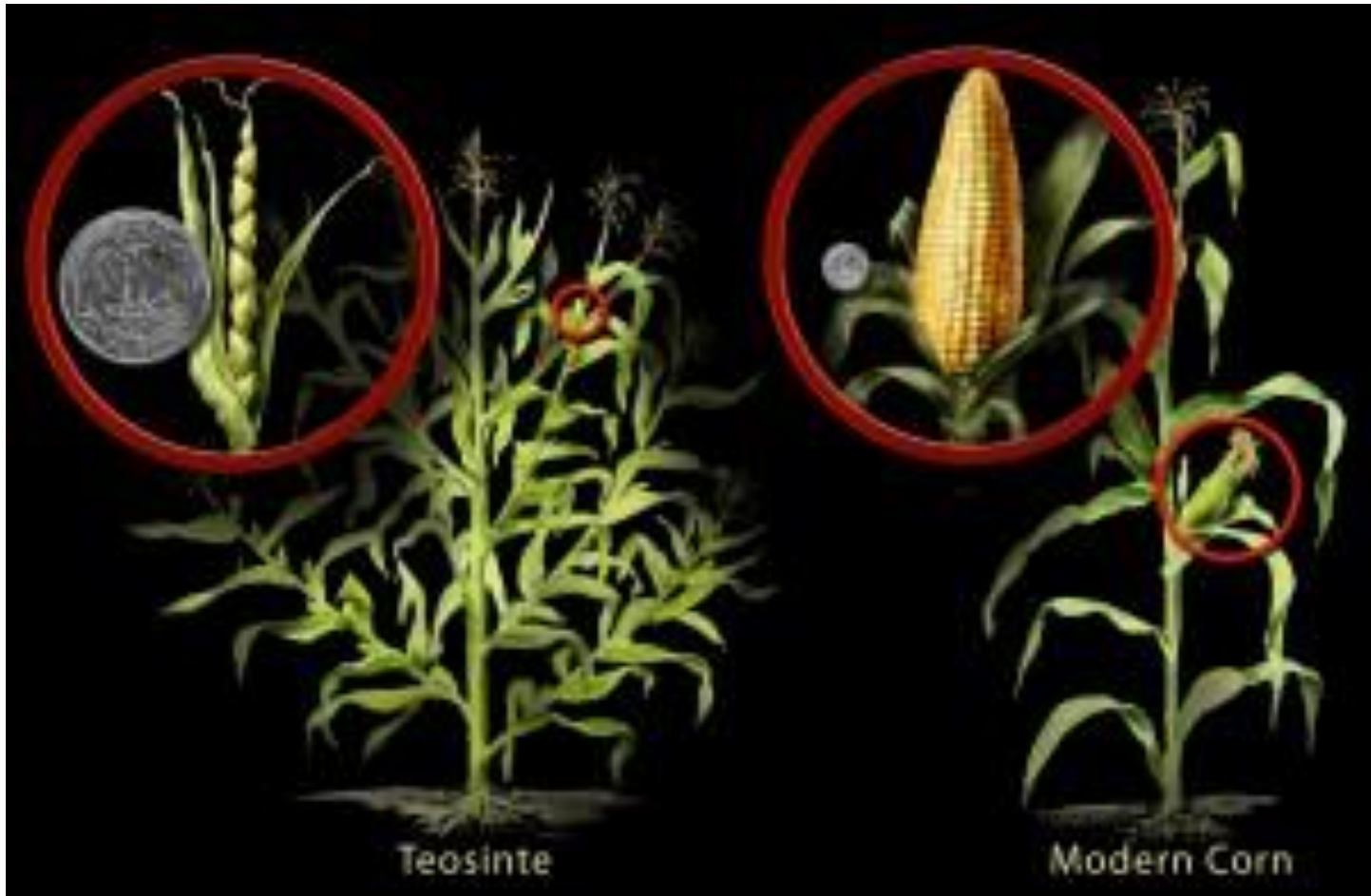
Genetically Engineered Organisms:

Genetically Modified Organisms made by using recombinant DNA technology

Transgenic Organisms:

Genetically Engineered Organisms made by introducing a foreign gene, from another species

Most scientists would say that almost all the food we eat has been “genetically modified” by man and that genetic modification includes not only conventional breeding, but simple selections man has made over millennia.



For practical reasons, i.e., their small size and low cost of housing in comparison to that for larger vertebrates, their short generation time, and their fairly well defined genetics, mice have become the main species used in the field of transgenics.



Stem cells are cells that can divide into multiple kinds of other cells.

Stem cells are formed and differentiate during early embryonic development, and are also a natural part of the tissue renewal processes throughout life.

Totipotent, Pluripotent, and Multipotent Stem cells

The key differences between these cells are:

1. When each appears during the process of human development.
2. Their degree of “flexibility” to become other cell types.

The key similarity is that all three of these cells types are characterized by self-renewal and the capacity to turn into into mature cell types. Together, they form the 200+ cell types that create a human being.

Hierarchy of cell potency

Totipotent stem cells

Totipotent (omnipotent) stem cells can give rise to any of the 220 cell types found in an embryo as well as extra-embryonic cells (placenta).

Totipotent Stem Cells

Totipotent stem cells are the most versatile stem cell type, because they are formed shortly after fertilization of an egg cell by a sperm cell. They can become all of the cells of the human body, as well as the cells of the embryo and developing fetus.

At about four days into development, these totipotent cells specialize slightly, becoming pluripotent stem cells.

Pluripotent stem cells

Pluripotent stem cells can give rise to all cell types of the body (but not the placenta).

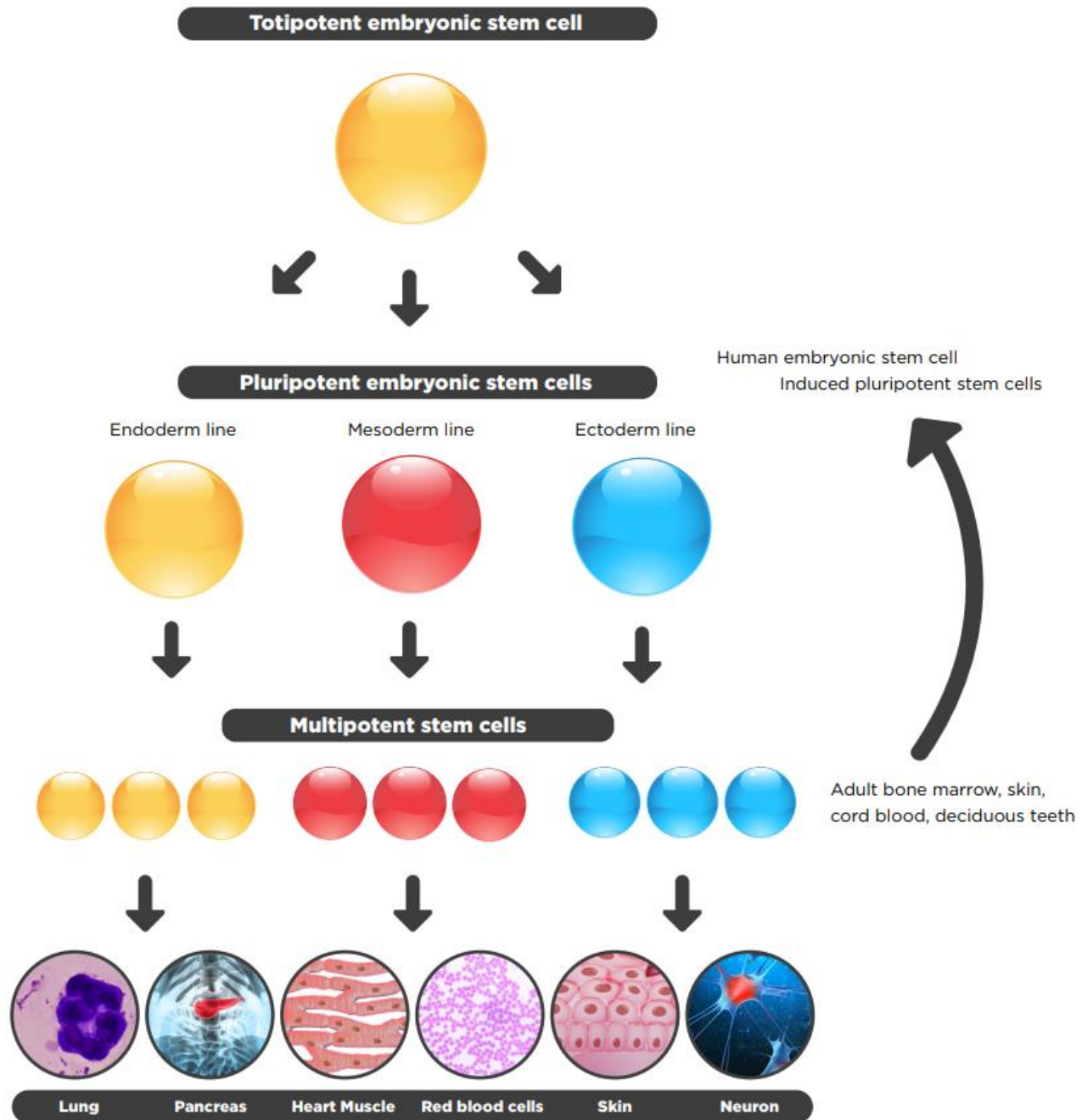
- **Embryonic stem cells** are controversial, because they are collected from early-stage embryos.
- **iPS cells** are not controversial, because they are made from adult cells. iPS cells are differentiated cells that are reprogrammed back into an embryonic-like state. They are usually created from skin or blood cells.

Multipotent stem cells

Multipotent stem cells can develop into a limited number of cell types in a particular lineage.

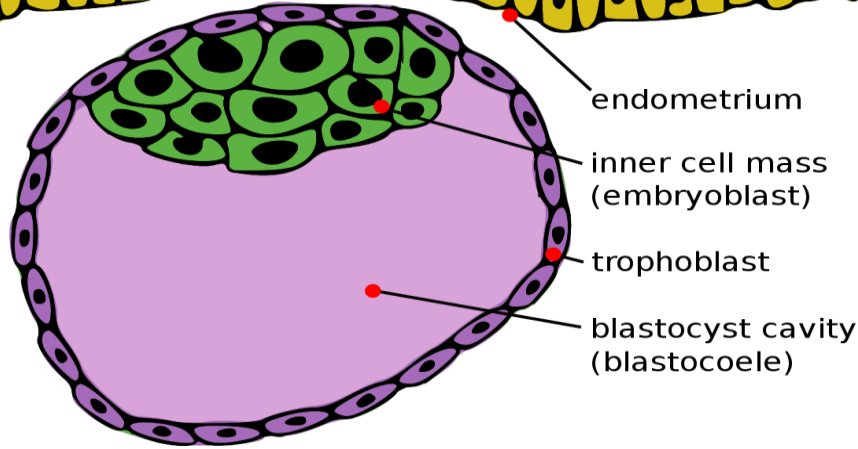
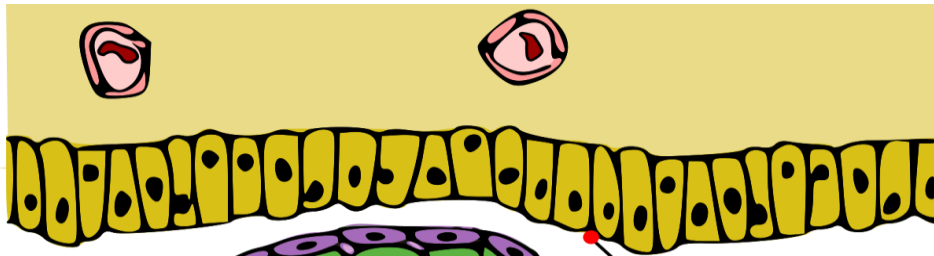
For example:

- **Hematopoietic stem cells (HSCs)** are a type of [multipotent stem cell](#) that prefer to become cells of the blood and immune system, although it is possible to induce them to become other cell types.
- **Mesenchymal stem cells (MSCs)** are a type of multipotent stem cell found in bone marrow and fat, among other tissues. These cells tend to turn into bone, fat, muscle and cartilage cells.
- **Neural stem cells** are a type of multipotent stem cells found in the brain. Unsurprisingly, they like to differentiate into neurons, glia, and other similar cells.

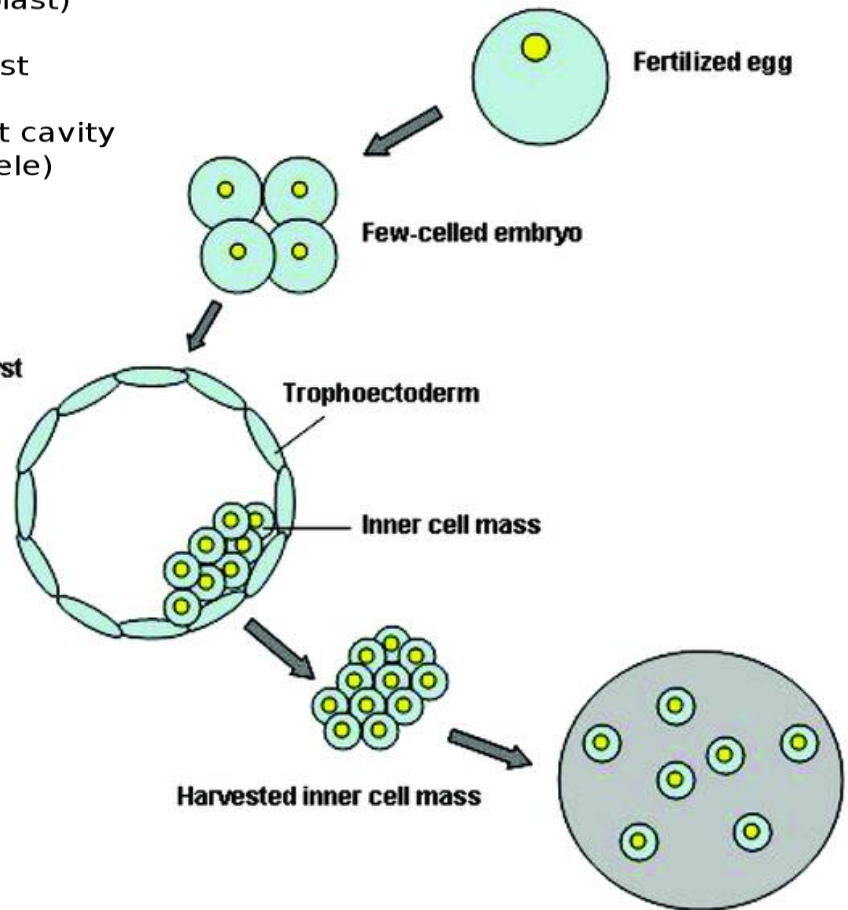


	Totipotent	Pluripotent	Multipotent
Relative potency	High	Medium	Low
Cell types capable of generating	Differentiate into any cell type	Differentiate into cells from any of the three germ layers	Differentiate into a limited range of cell types
Terminology	Toti = Whole	Pluri = Many	Multi = Several
Examples	Zygote, early morula	Embryonic stem cells, Induced pluripotent stem cells	Hematopoietic stem cells , neural stem cells , mesenchymal stem cells
Found	Early cells of fertilized egg	Inner mass cells of the blastocyst	In many tissues
Expression of pluripotency genes	+++	++	+
Expression of lineage-specific genes	+	++	+++
Pros of use in research	Easy to isolate and grow	Easy to isolate and grow	Less ethical issues, less chance of immune rejection if taken from same patient
Cons of use in research	Ethical issues	Ethical issues, teratoma formation	Hard to isolate, limited differentiation, scarce

ES CELLS



Blastocyst



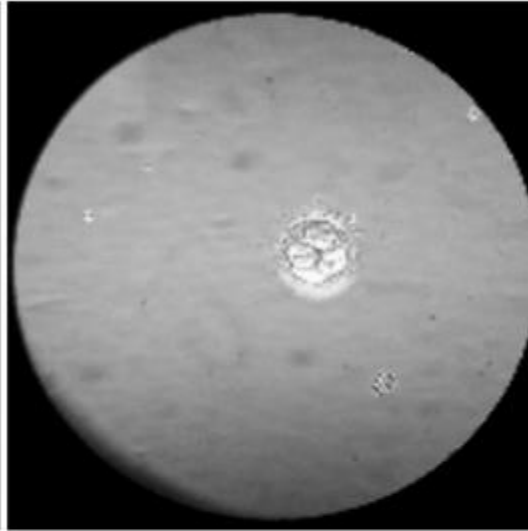
Embryonic stem cells in culture

Very early human embryos

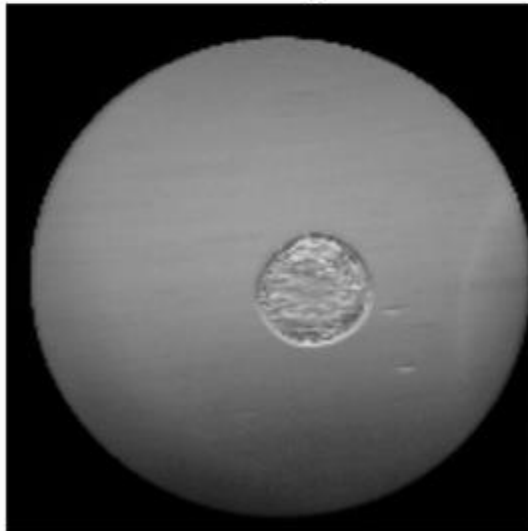
2-cell



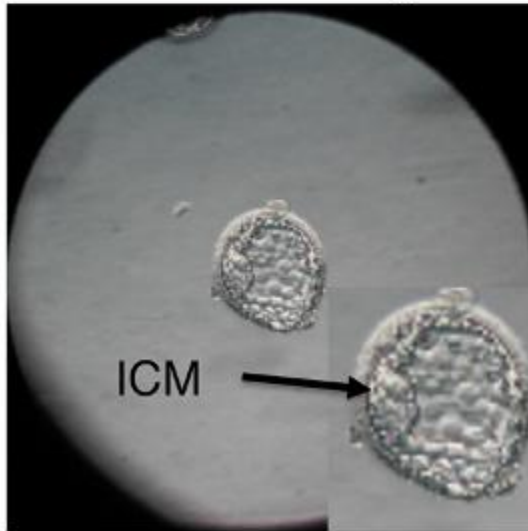
4-cell



Blastocyst



Hatched blastocyst



Methods of creation of transgenic animals

A. Retrovirus-mediated gene transfer

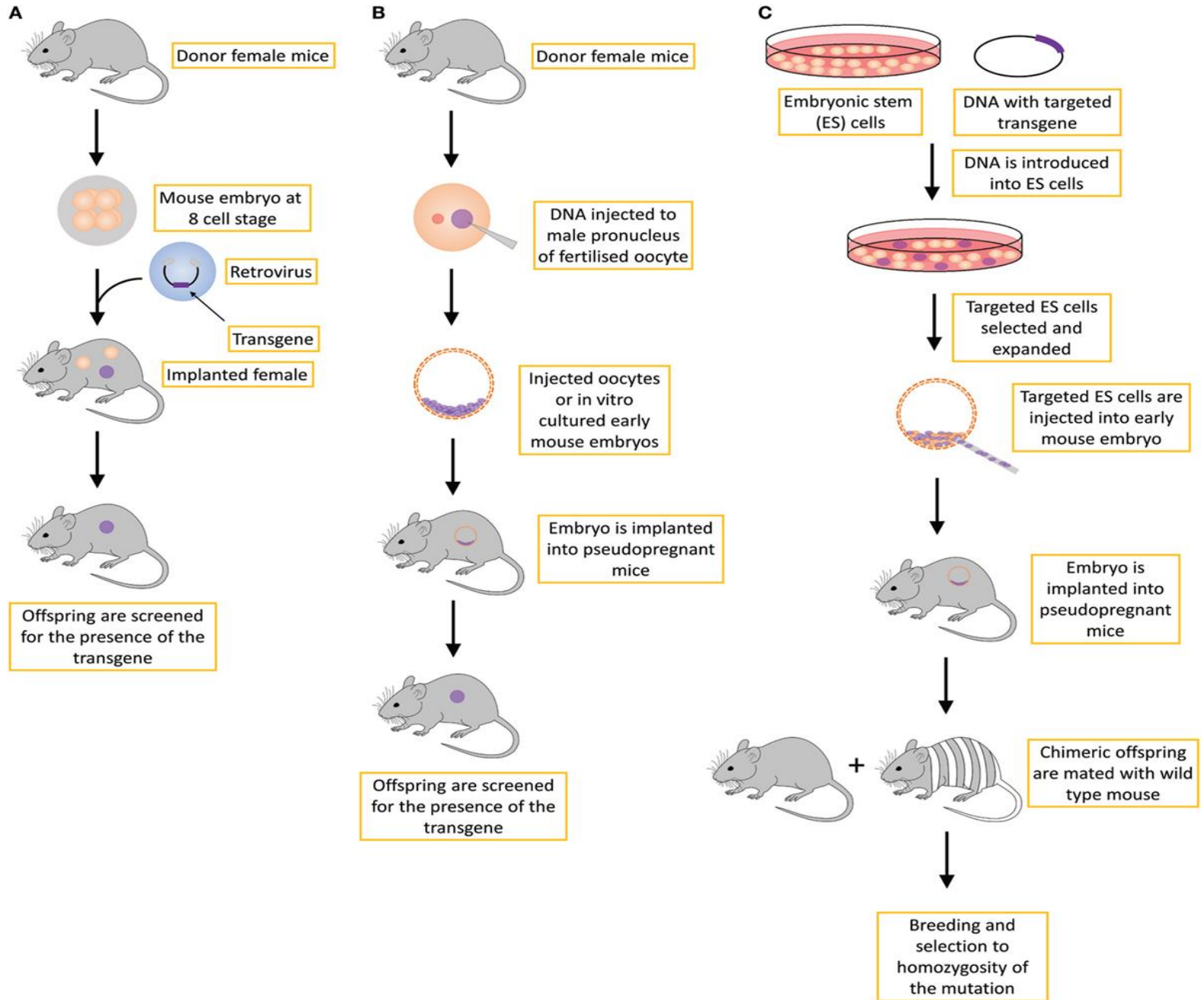
(Introduction of retroviral vectors in the cells of an early stage embryo)

B. Pronuclear Microinjection

(Microinjection of DNA into the male pro-nucleus of one cell embryos)

C. Embryonic stem cell-mediated gene transfer

(Introduction of genetically engineered embryonic cells into early stage developing embryo)



1. A gene knockout (abbreviation: KO) is a genetic technique in which one of an organism's genes is made inoperative ("knocked out" of the organism).

Knockout organisms or simply knockouts are used to study gene function, usually by investigating the effect of gene loss.

Researchers draw inferences from the difference between the knockout organism and normal individuals.

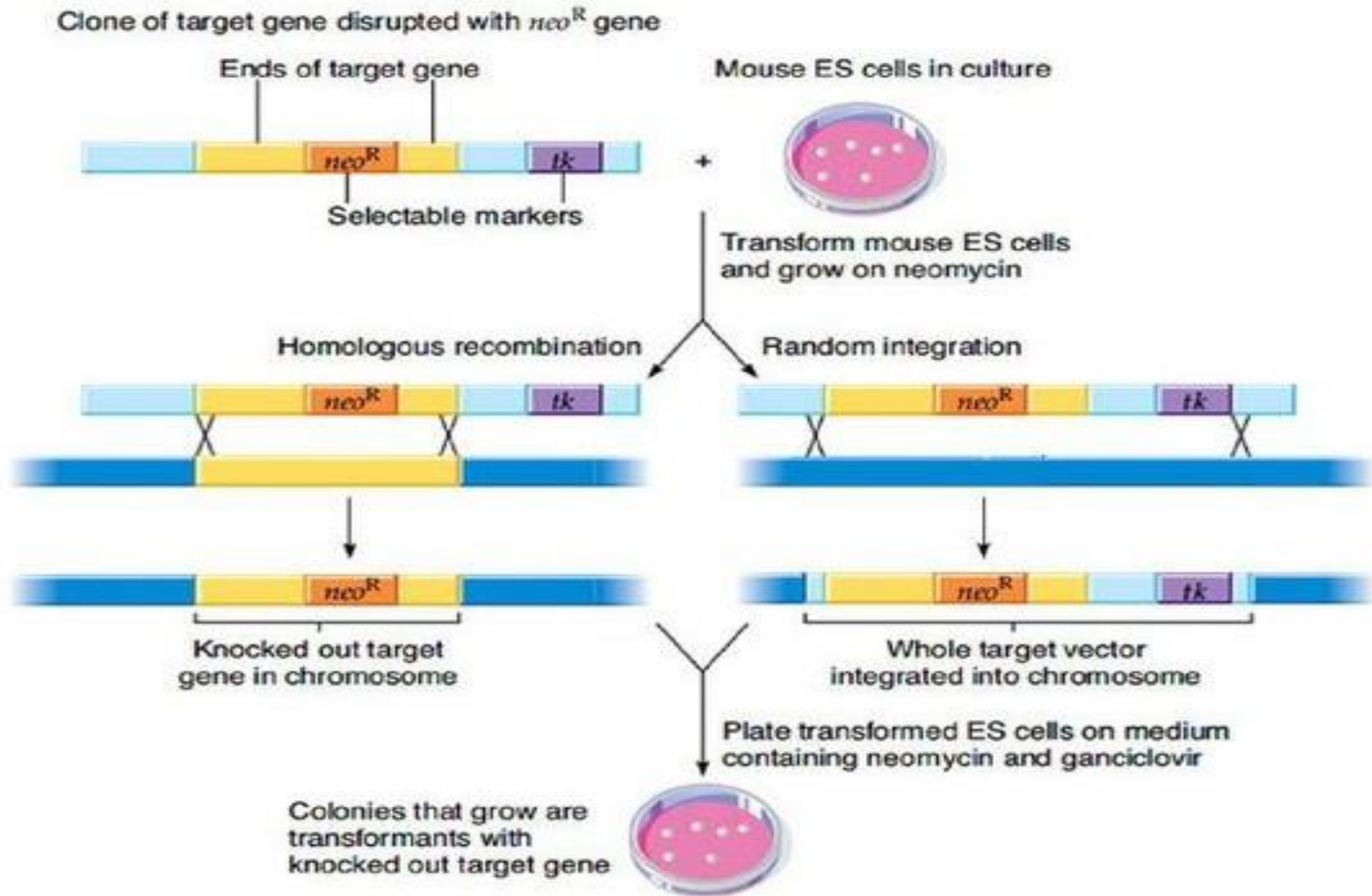
2. Gene knockin (KI) is the process of targeted insertion of an exogenous gene at a specific locus in the genome.

The difference between knock-in technology and traditional transgenic techniques is that a knock-in involves a gene inserted into a specific locus, and is thus a "targeted" insertion.

3. **Gene knockdown** - reduce the levels of expression of a gene in your cell. This is not as dramatic as a gene knockout, and there may still be some expression of your gene.

Process of developing KO Mouse

1.



b) Using the cells with the knocked out target gene to produce a knockout mouse strain

Knockout ES cells
from agouti mouse



Inject ES cells
into blastocyst



Blastocyst from
black mouse



Blastocyst develops into a chimeric mouse,
which may have agouti (knockout) germline cells



Mate chimeric mice
with black mice



Test agouti progeny for presence of knockout gene *ko* (knockout of target gene)
and mate siblings to establish homozygous knockout strain



The TP53 gene is located **on the long arm of chromosome 17** and contains 11 exons spanning some 20 000 bp of genomic sequence.

BRCA1/BRCA2 genes
p53 gene

Tumor suppressor genes

KO mice will develop tumor in between 3 and 6 months.

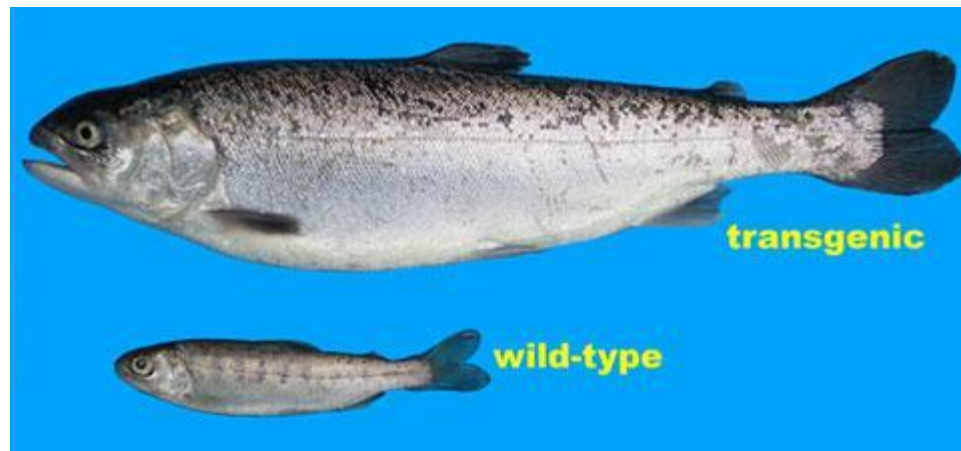
Transgenic animal

In 1974 [Rudolf Jaenisch](#) created a [transgenic mouse](#) by introducing foreign DNA into its embryo, making it the world's first transgenic animal.

Mice with genes [knocked out](#) ([knockout mouse](#)) were created in 1989.

The first genetically modified animal to be commercialised was the [GloFish](#), a [Zebra fish](#) with a [fluorescent gene](#) added that allows it to glow in the dark under [ultraviolet light](#). It was released to the US market in 2003.

The first genetically modified animal to be approved for food use was [AquAdvantage salmon](#) in 2015.



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The first genetically modified cow (in year 1997) or transgenic cow was Rosie. Rosie was able to produce milk enriched in the human protein. The milk was rich in human alpha-lactalbumin which was a more suitable product for human baby consumption than normal milk.



Tracy (1990 – 1997) was a [transgenically modified](#) sheep created by scientists at Scotland's [Roslin Institute](#) to produce the human protein [alpha 1-antitrypsin](#), a substance regarded in the 1990s as a potential pharmaceutical for the treatments of [cystic fibrosis](#) and [emphysema](#).

Notably, she is the first transgenic farm mammal ever created.^[2]



ANDi is the first genetically modified rhesus monkey, who was born at Oregon Health Sciences University on October 1, 2001. OHSU named the monkey ANDi because..... it stands for iDNA spelled backward.

The work demonstrates that a foreign gene can be delivered and inserted into a primate chromosome.

Gene = GFP



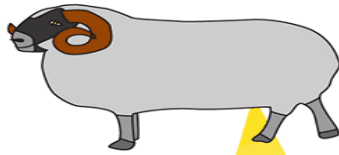
Animal cloning

Dolly was a female Finnish Dorset sheep and the first mammal cloned from an adult somatic cell. She was cloned by associates of the Roslin Institute in Scotland, using the process of nuclear transfer from a cell taken from a mammary gland. (1996 to 2003)

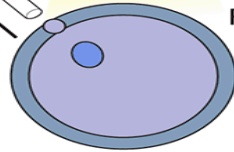
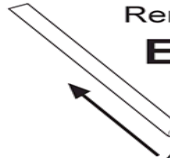


The Story of Dolly

Scottish Blackface
(Egg Donor)

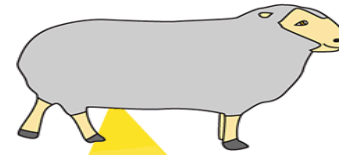


Remove a donor
Egg Cell

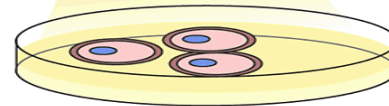


Remove the nucleus
from the egg
Enucleation

Finn-Dorset
(Nuclear Donor)

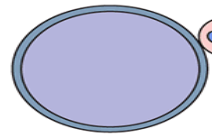


Remove cells from a sheep udder
Mammary Cell



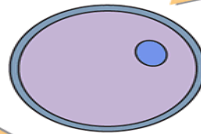
Select a
single cell

Combine Cells
using an electric shock.

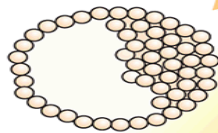


Blastocyst

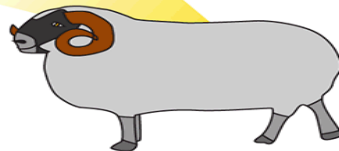
The combined cell begins
to divide normally to
become a blastocyst.



The combined cell now has
a single nucleus from
the nuclear donor.



The blastocyst is placed
in the uterus of a
Surrogate Ewe



The blastocyst develops into
a fetus and in about
five months a lamb is born -

Dolly

