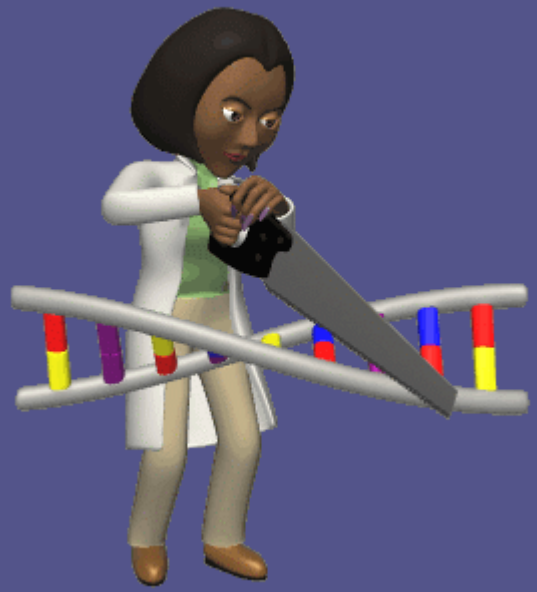
Mutagenic and transgenic



Arya Adiningrat

Department of Oral Biology and Biomedical Sciences

Dental School of Medicine and Health Sciences Faculty

Universitas Muhammadiyah Yogyakarta

Mutagen or mutagenic agent → is something which may induces mutation

Mutation in some certain gene
→ may lead to the malignant transformation of the cells

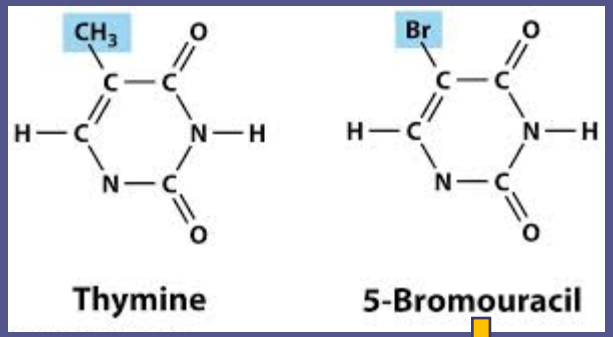
Some mutagenic agents are carcinogenic agent

Spontaneous mutation usually grow slowly, however the existence of mutagen will greatly increase the rate of mutation.

Classification of mutagenic agent
- Chemical
- Physical
- Biological

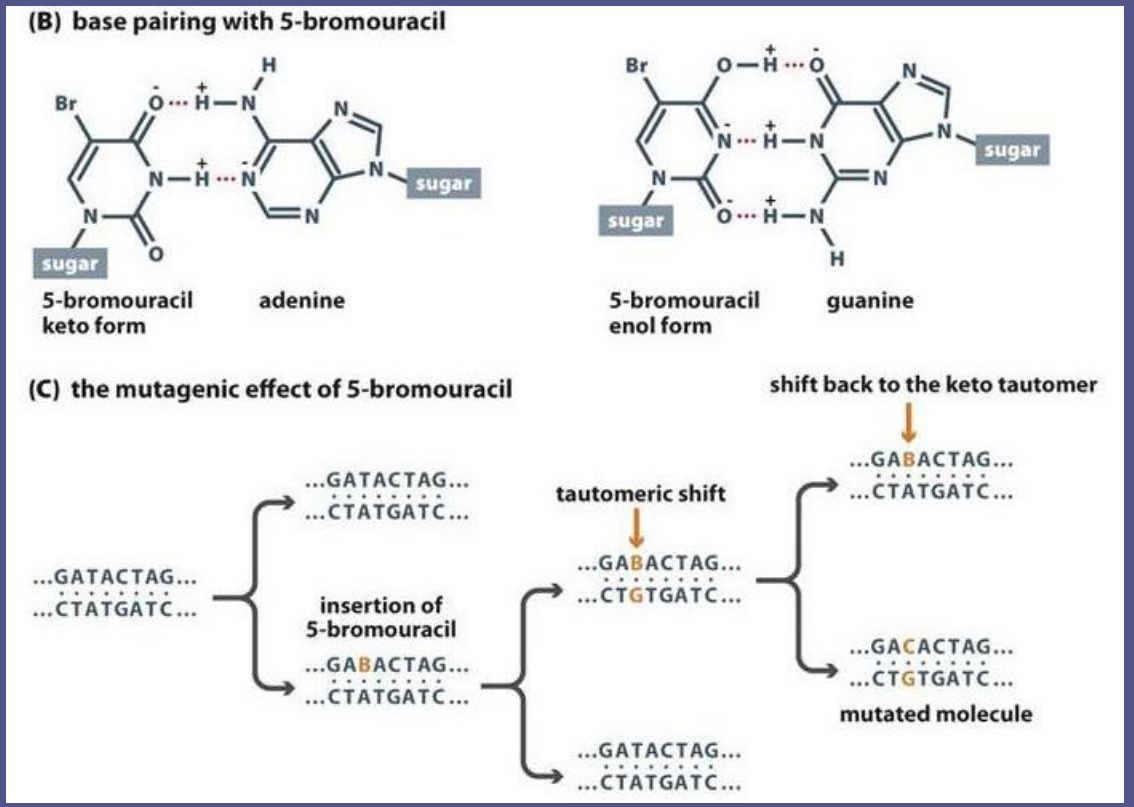
Chemical mutagenic agents

1. Base analogs



Shape-shifter

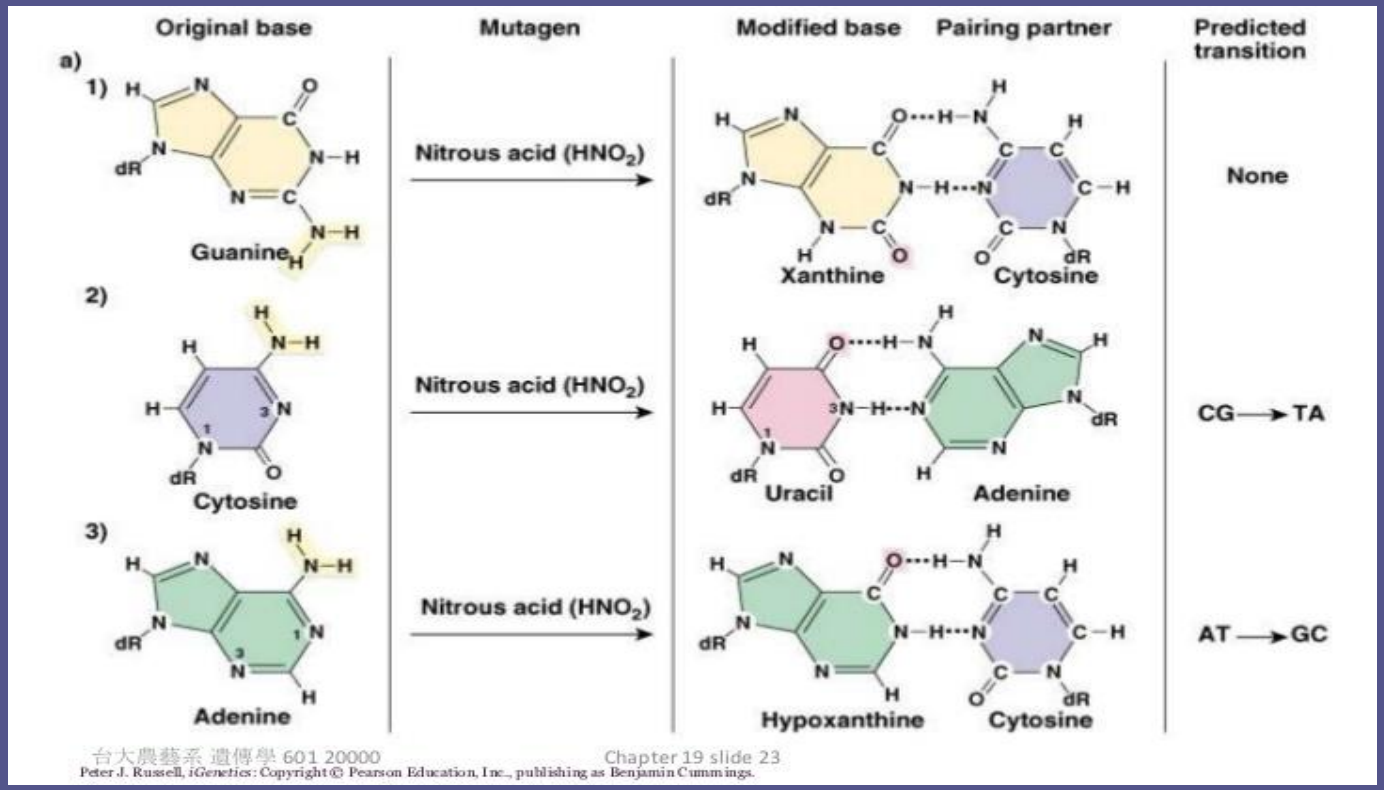
they are chemicals that are very similar to the normal nucleotides that make up DNA. In their name, 'base' refers to the nitrogenous base in the nucleotide, and 'analog' means 'analogous,' or similar to.



Chemical mutagenic agents

2. Base modifying agents

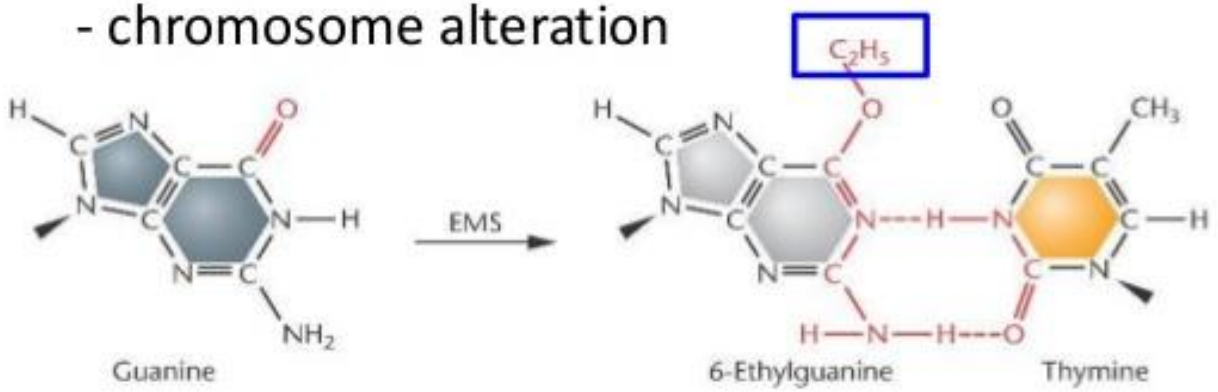
These are chemicals that modify or change the structure of bases in the DNA, causing mispairing.



Chemical mutagenic agents

3. Alkylating agents

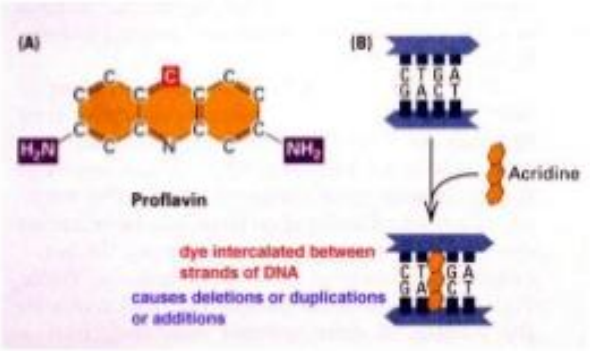
- nitrogen, sulfur mastard, methyl & ethyl methane sulfonate (MMS & EMS)
- Adding methyl or ethyl group → "alkylation"
- transition, transversion or frameshift mutations
- chromosome alteration

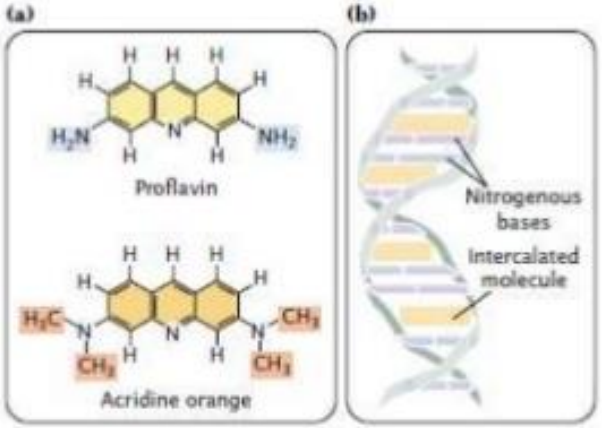


Chemical mutagenic agents

4. Intercalating agents

- Intercalating agents produce mutations by sandwiching themselves (intercalating) between adjacent bases in DNA .
- They distorts the three-dimensional structure of the helix and causing single-nucleotide insertions and deletions in replication.
- These insertions and deletions frequently produce frameshift mutations.
- And so the mutagenic effects of intercalating agents are often severe.
- Because intercalating agents generate both additions and deletions, they can reverse the effects of their own mutations.
- E.g. : proflavin, acridine orange, ethidium bromide, and dioxin





Physical mutagenic agents

1. Ionizing radiation

X-ray and Gamma-ray → short wave length and high energy.

Can cause:

- Base deletion
- Single nick in DNA strand
- Cross-linking
- Chromosomal breaks

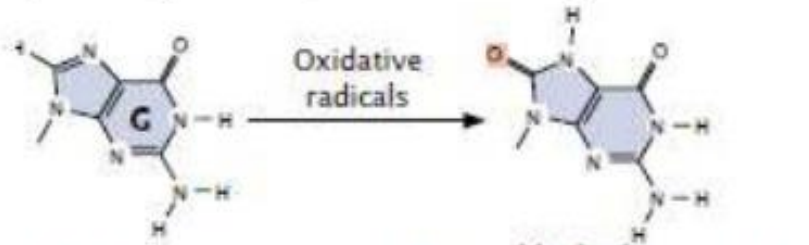
2. Non-ionizing radiation

Electromagnetic radiation
→ UV radiation (around 260 nm wave-length)
Can react with DNA and other biological molecules
Leads to:
Cyclobutane pyrimidine dimers

Physical mutagenic agents

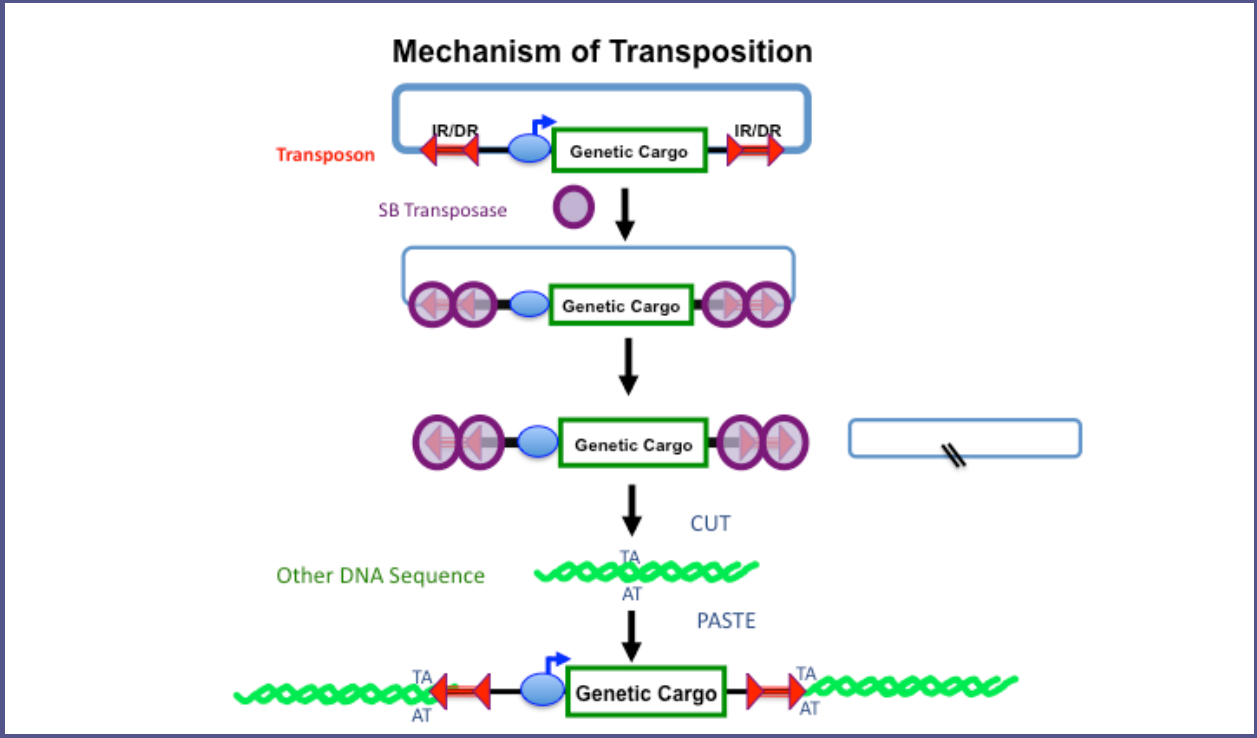
Reactive oxygen species as one of physical mutagenic product

- Reactive forms of oxygen damage DNA and induce mutations by bringing about chemical changes to DNA.
- Reactive forms of oxygen includes:
- Superoxide radicals
- Hydrogen peroxide
- Hydroxyl radicals
- They are produced in the course of normal aerobic metabolism, as well as by radiation, ozone, peroxides, and certain drugs.



Biological mutagenic agents

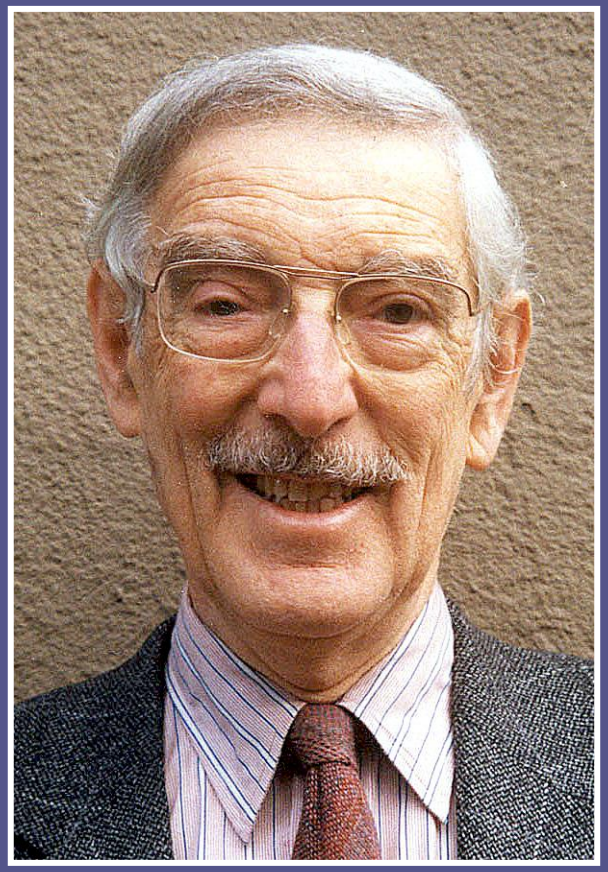
1. Transposon (transportable elements)
2. Virus → disrupting DNA (e.g. Raos sarcoma, HPV)
3. Bacteria (e.g. *Helicobacter pylori* → cause inflammation during oxidative stress occurs and reducing DNA repair efficiency)



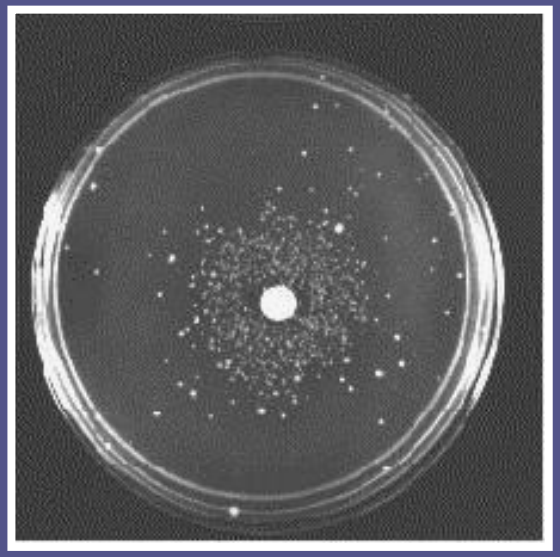
Variety of defenses to protect DNA molecules from the mutagens attack

- Physical shield
  - skin and the melanin pigment
- detoxifying enzymes
  - superoxide dismutase (SOD) & catalase
- free-radical scavengers
  - vitamin C, vitamin E, bilirubin
- glutathione-S-transferases (GSTs) reacting with electrophilc compounds

Ames test



Bruce Ames (born 1928)



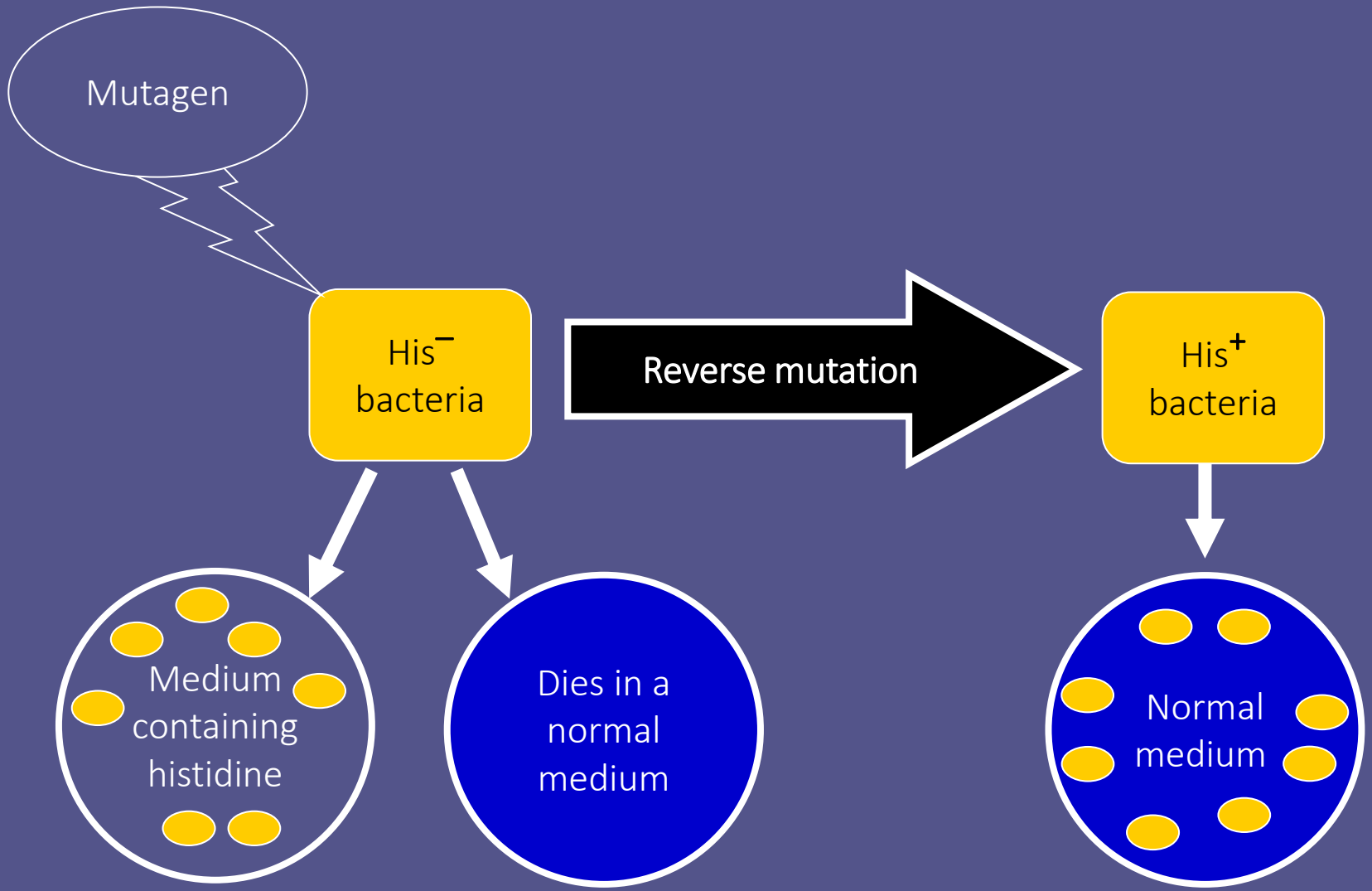
Ames test

Test system – auxotrophic strain of *Salmonella typhimurium* – survives only in medium with histidine (dies in normal medium without histidine)

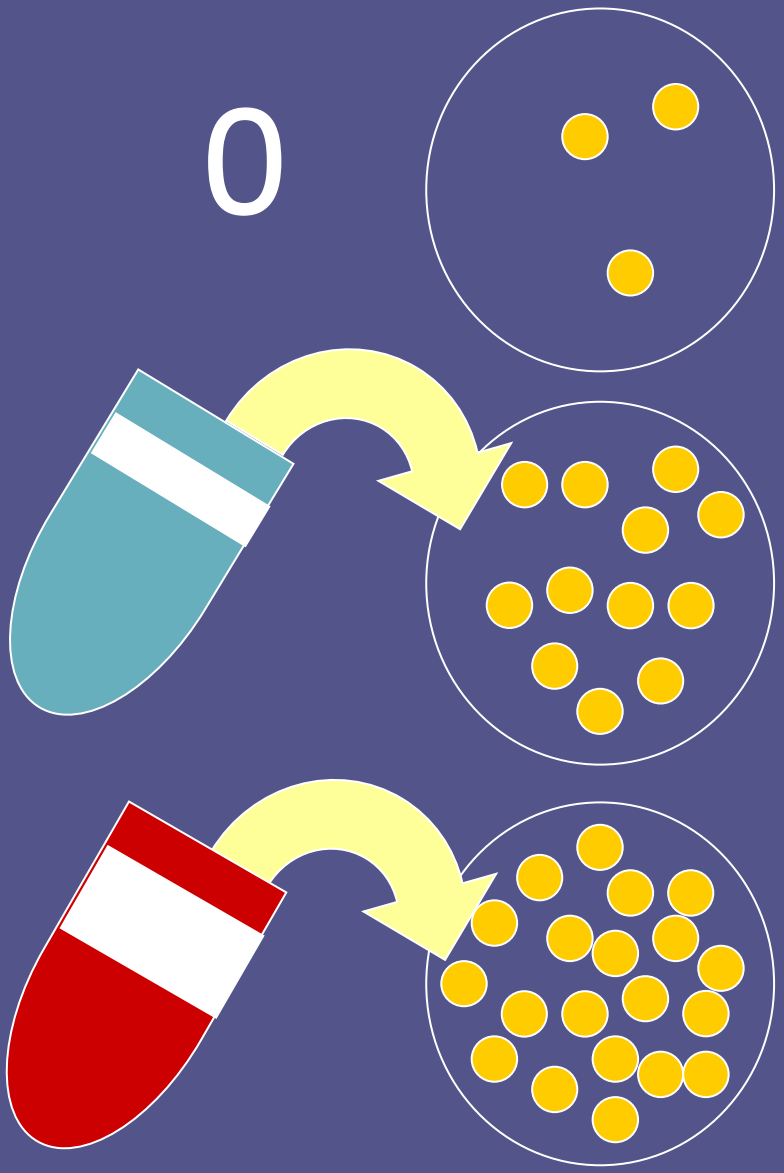
After treatment with mutagen some auxotrophic cells are turned into normal ones that synthesize histidine and survive in a normal medium.

These cells are called revertants (due to reverse mutation).

Ames test


Mutagen
His⁻ bacteria
Reverse mutation
His⁺ bacteria
Medium containing histidine
Dies in a normal medium
Normal medium

Ames test



Negative control

SPONTANEOUS REVERTANTS

A dish with a compound to be tested

GENOTOXICITY CONFIRMED

Positive control

IS USED FOR CONTROL OF THE TEST

Result of the Ames test

What is trans-genesis?

Definitions

Trans-genesis is the process of introducing an exogenous gene – called a transgene – into a living organism so that the organism will exhibit a new property and transmit that property to its offspring.

A **Transgene** is the name given to the introduced DNA

Why use trans-genesis instead of selective breeding?

More specific — scientists can choose with greater accuracy the trait they want to establish. The number of additional unwanted traits can be kept to a minimum.

Faster — establishing the trait takes only one generation compared with the many generations often needed for traditional selective breeding, where much is left to chance.

More flexible — traits that would otherwise be unavailable in some animals or plants may be achievable using transgenic methods.

Less costly — much of the cost and labour involved in administering feed supplements and chemical treatments to animals and crops could be avoided.

Biological Processes

First, the desired gene must be extracted from the donor organism.

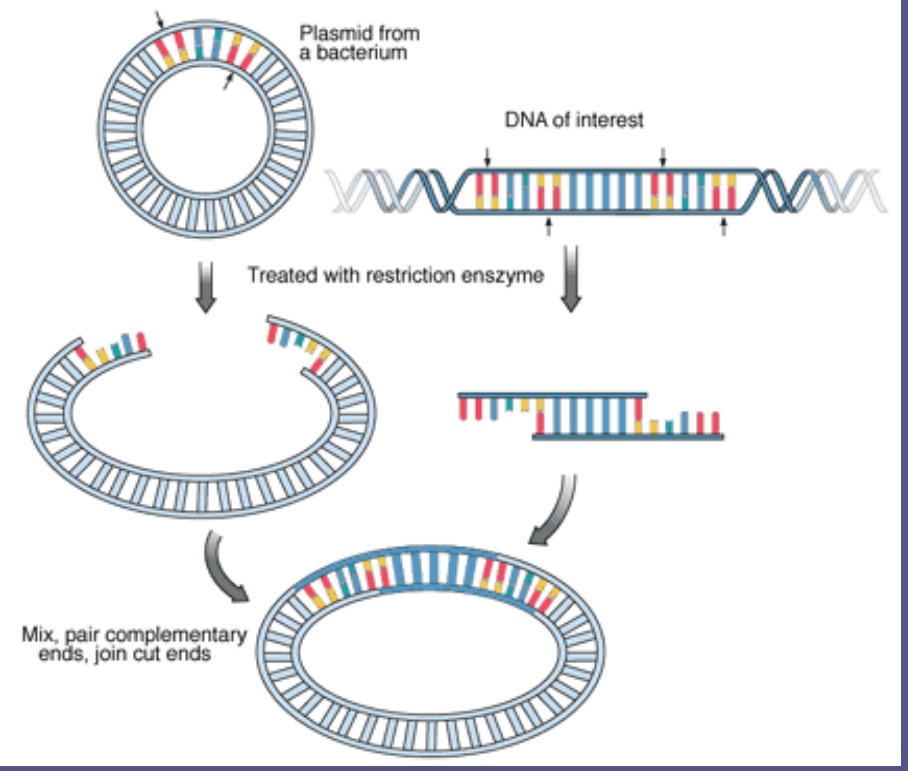
This is done using restriction enzymes (restriction endonucleases).

It is important that the restriction enzymes cut out the whole gene required.

This gene can then be inserted into host cells (another enzyme, DNA ligase, is very important here)

Three basic methods of producing transgenic animals

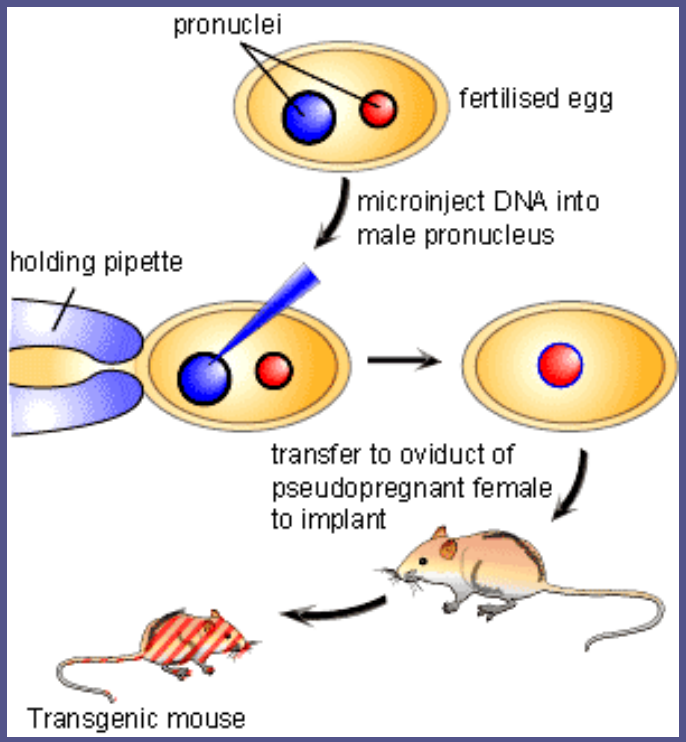
1. DNA microinjection
2. Retrovirus-mediated gene transfer
3. Embryonic stem cell-mediated gene transfer



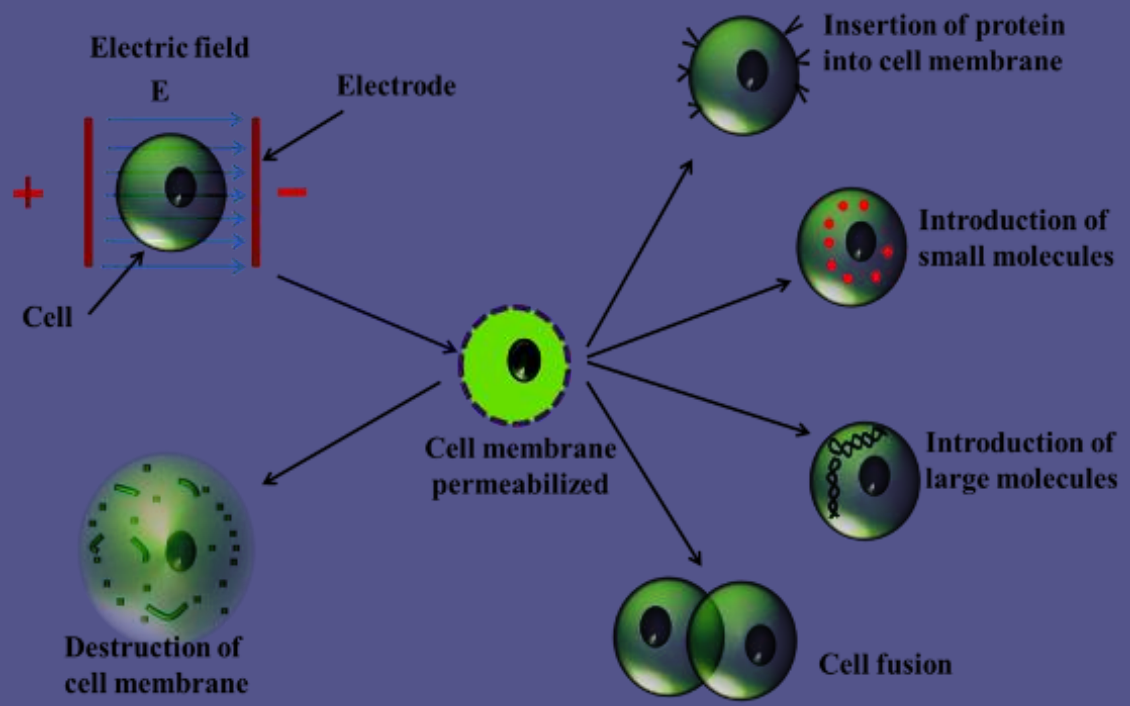
Genetic engineering: Recombinant DNA technology

Transgenic animals can be produced by injecting a cloned gene into the fertilized egg. If the gene becomes successfully integrated into a chromosome, it will be present in the germline of the resulting animal, and can be passed along from generation to generation. A giant mouse called “Supermouse” was produced in this way by injecting the gene for rat growth hormone into a fertilized mouse egg. In a similar way, transgenic goats and cows can now be designed that produce human proteins, such as blood clotting factors, in their milk. Sometimes, rather than introducing a functional gene into a mouse, a nonfunctional version is inserted. Such genetically engineered animals can be used to produce a colony of “knockout mice” that lack the product of the affected gene. Such animals can then serve as models for the study of a corresponding human disease. [Note: Knock-in mice result if the inserted gene expresses a mutated product or under (over)-expresses a product.]

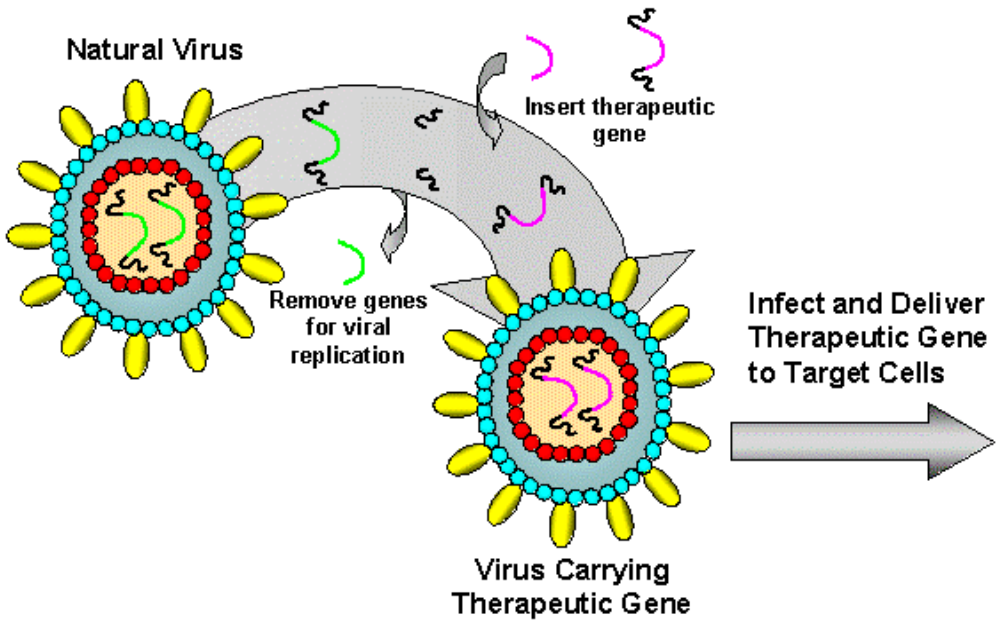
DNA Microinjection

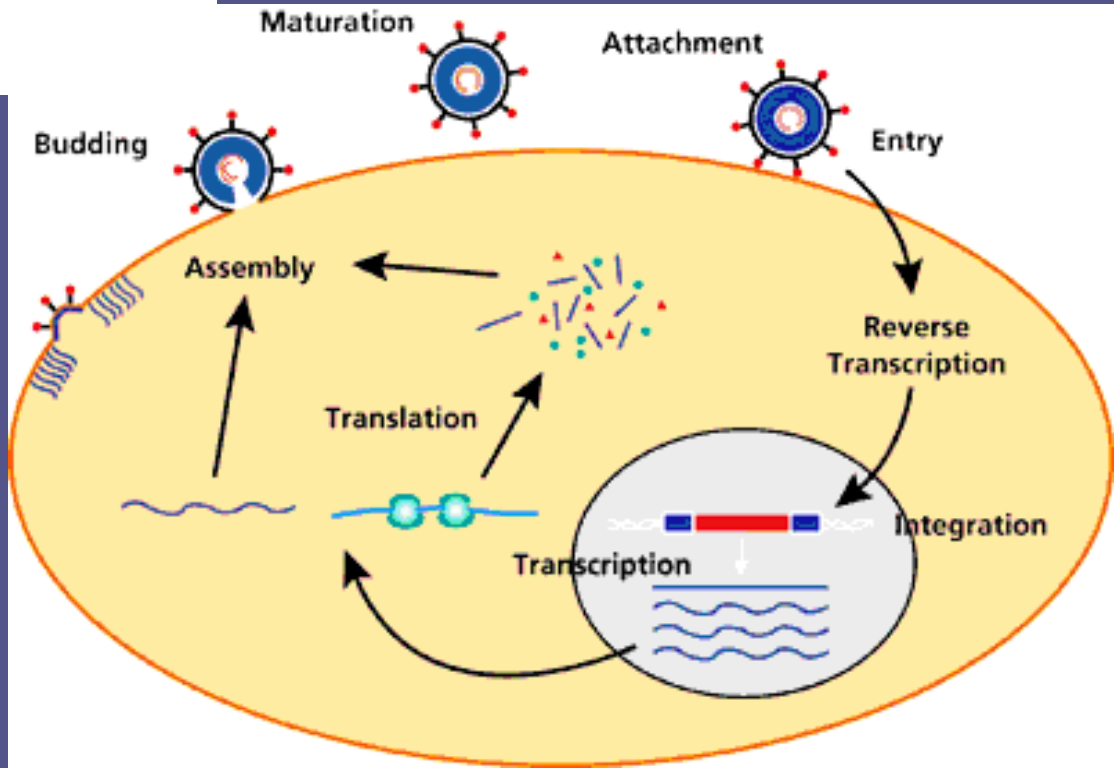


Electroporation



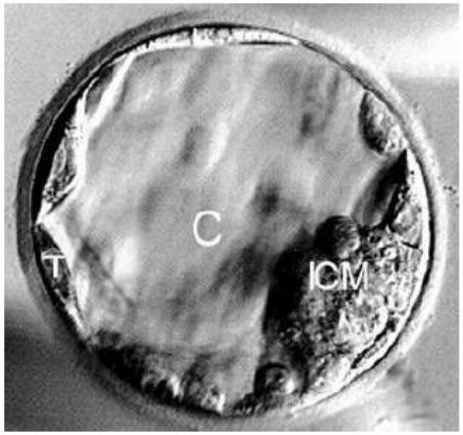
Viral Vectors for Gene Transfer

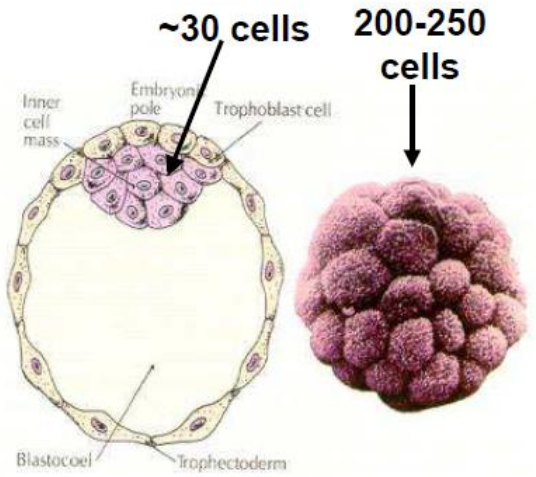


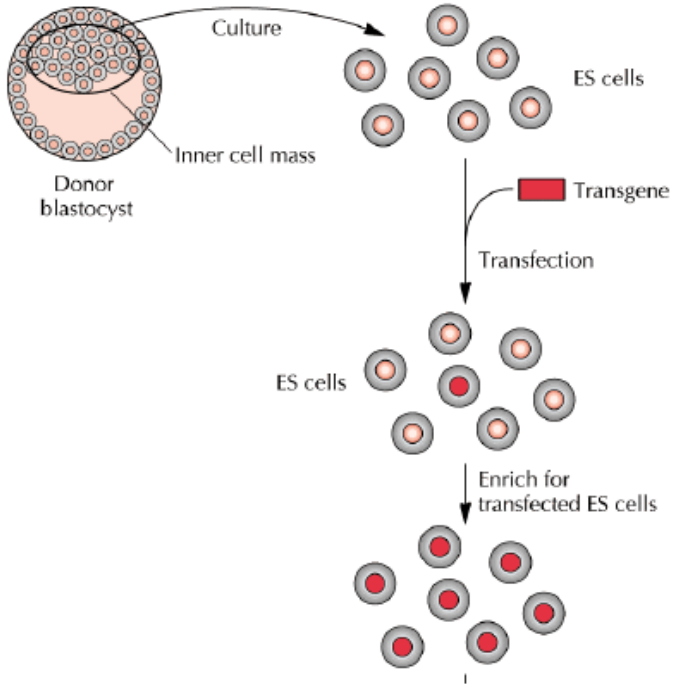


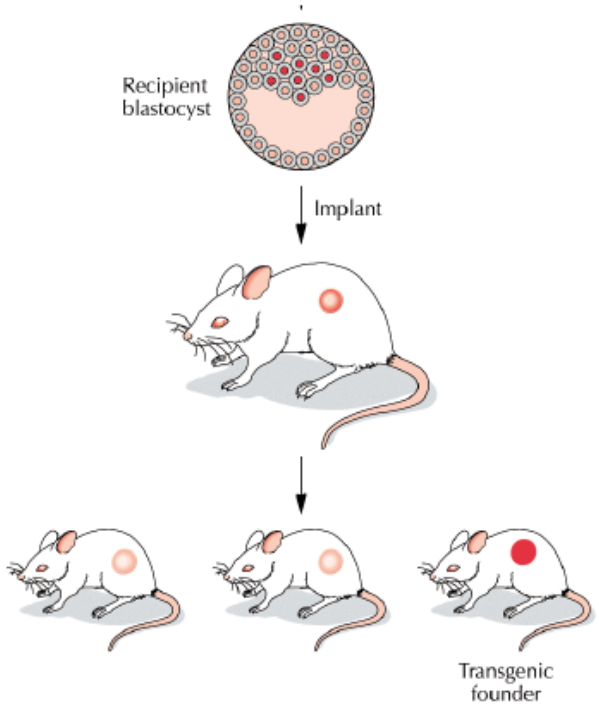
Life cycle of Retrovirus

Embryonic Stem cells





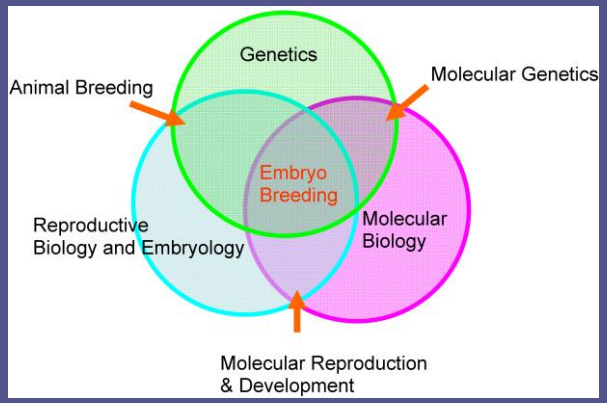




Producing genetically living modified-organism

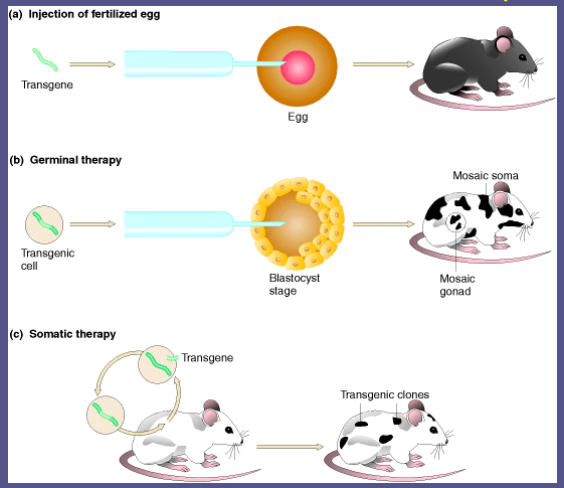
Traditional breeding

Crossing and selecting offspring



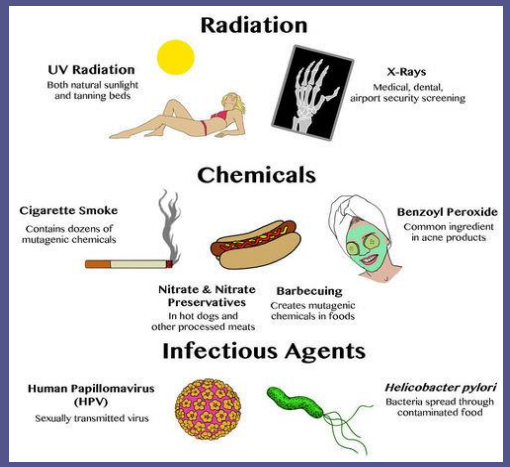
Transgenic

Introducing selected genes using DNA recombination technique



Mutagenesis

Chemical and physical mutagenic agent exposure



RNA interference

Switching off the selected genes