MOLECULAR MARKERS

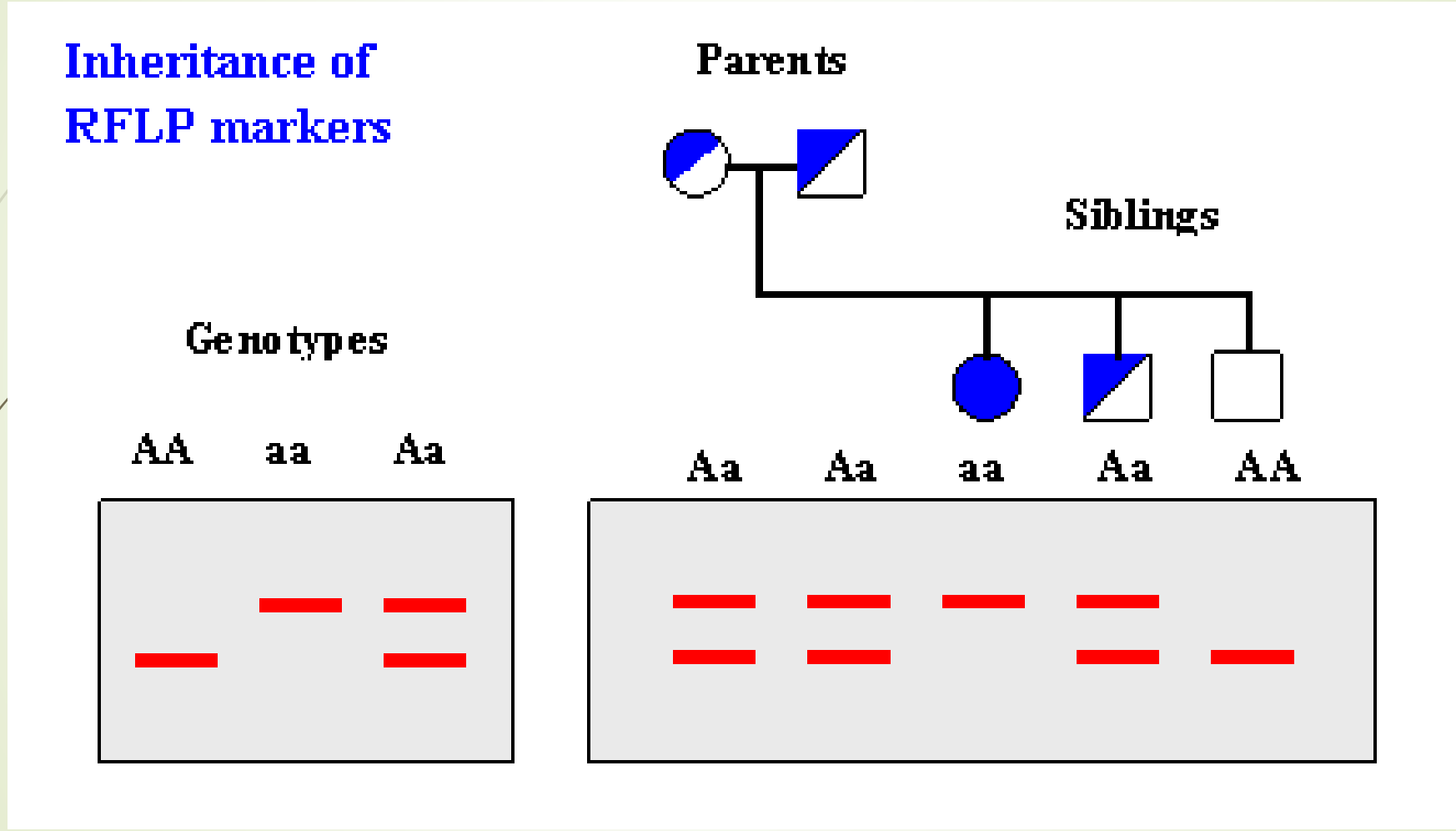
RAPD

- Random amplified polymorphic DNA.
- Use single primer and proceeds with PCR
- Assumes somewhere it will bind with genomic DNA

RACE

- Rapid amplification of cDNA ends
- To obtain the full length sequence of an RNA transcript found within a cell
- Production of a cDNA copy of the RNA sequence of interest
- Followed by PCR amplification of the cDNA copies (see RT-PCR)

RFLP


Inheritance of
RFLP markers
Parents
Siblings
Genotypes
AA
aa
Aa
Aa
Aa
aa
Aa
AA

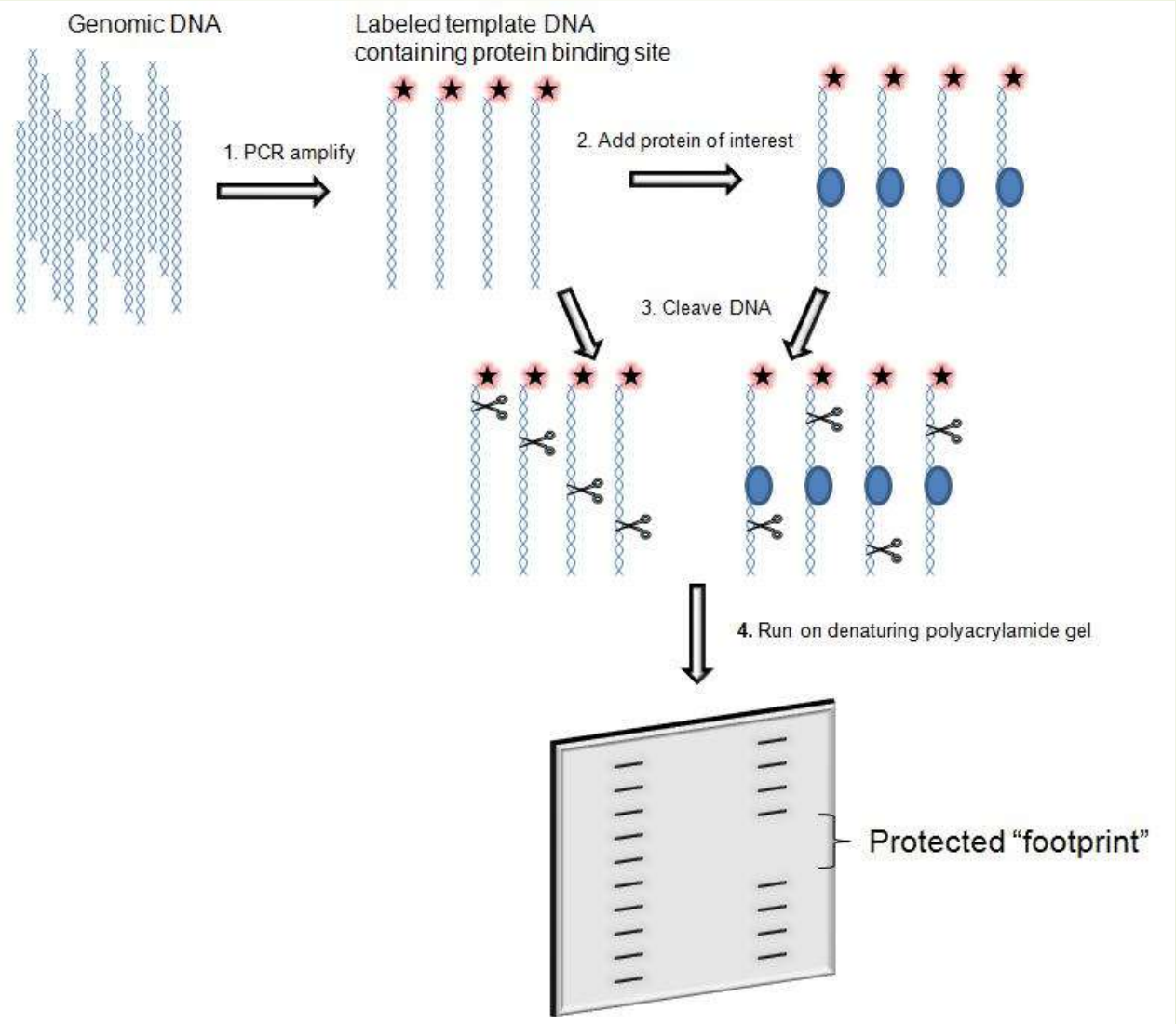
AFLP

- Uses restriction enzymes to digest genomic DNA.
- Followed by ligation of adaptors to the sticky ends of the restriction fragments.
- Primers complementary to the adaptor sequence
- Amplified fragments are separated and visualized on denaturing polyacrylamide gels, either through autoradiography or fluorescence_methodologies or autosequencing.

Applications

- Forensic science , pedigree analysis, biodiversity, genetic counseling and germplasm maintenance
- DNA footprinting.
- Investigating the sequence specificity of DNA-binding proteins in vitro.
- Used to study protein-DNA interactions both outside and within cells

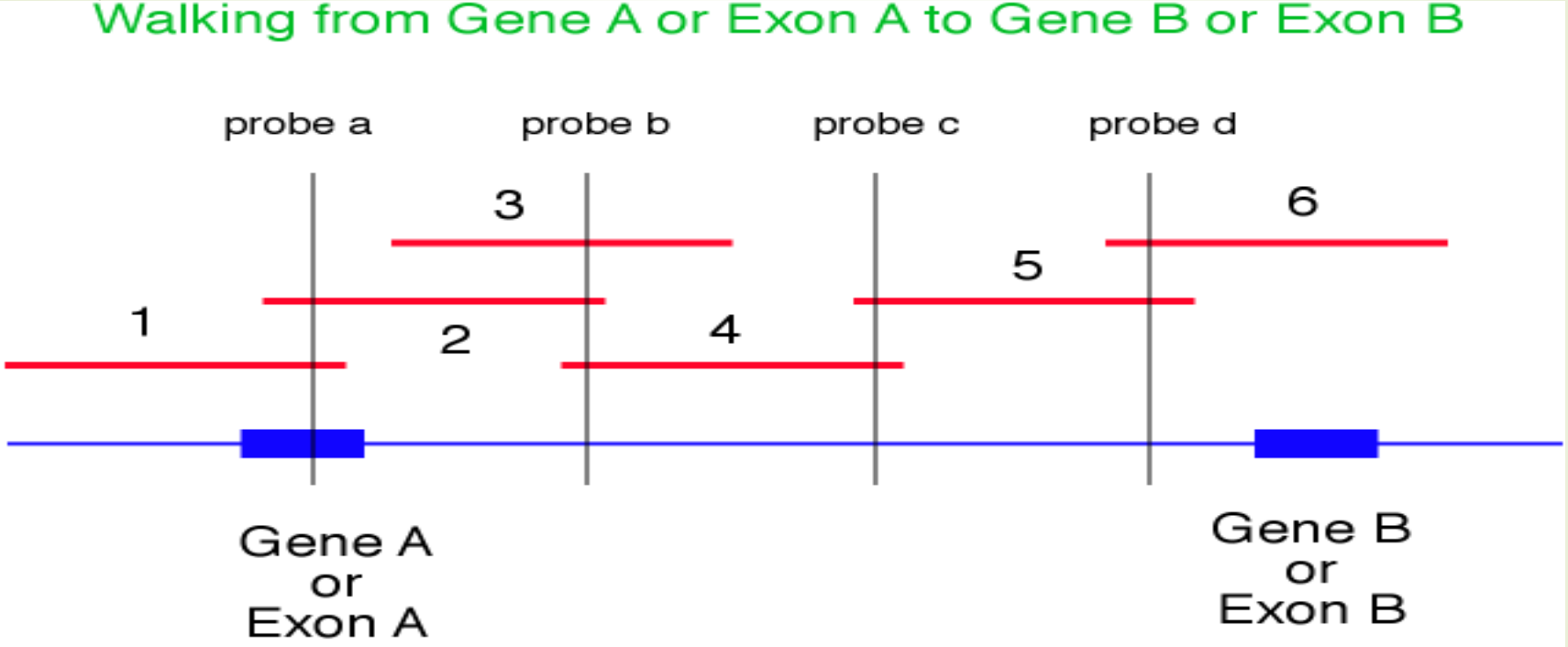
DNA foot printing



Chromosome walking

- Is a sequencing method of choice for sequencing DNA fragments between 1.3 and 7 kilobases.
- which is not possible in maxim gilbert sequencing.
- Identification of fragments with overlapping sequences may be a key to the reconstruction or characterization of large chromosome regions.
- From the genomic library a clone of interest identified by a probe is selected.

- One end of the clone is subcloned.
- Subcloned fragment hybridized with other clones in the library.
- Second clone hybridizing with the subclone of the first clone is identified due to presence of overlapping region.
- End of the second is then subcloned and used for hybridization with other clones.


Walking from Gene A or Exon A to Gene B or Exon B
probe a
probe b
probe c
probe d
1
2
3
4
5
6
Gene A
or
Exon A
Gene B
or
Exon B

Chromosome jumping

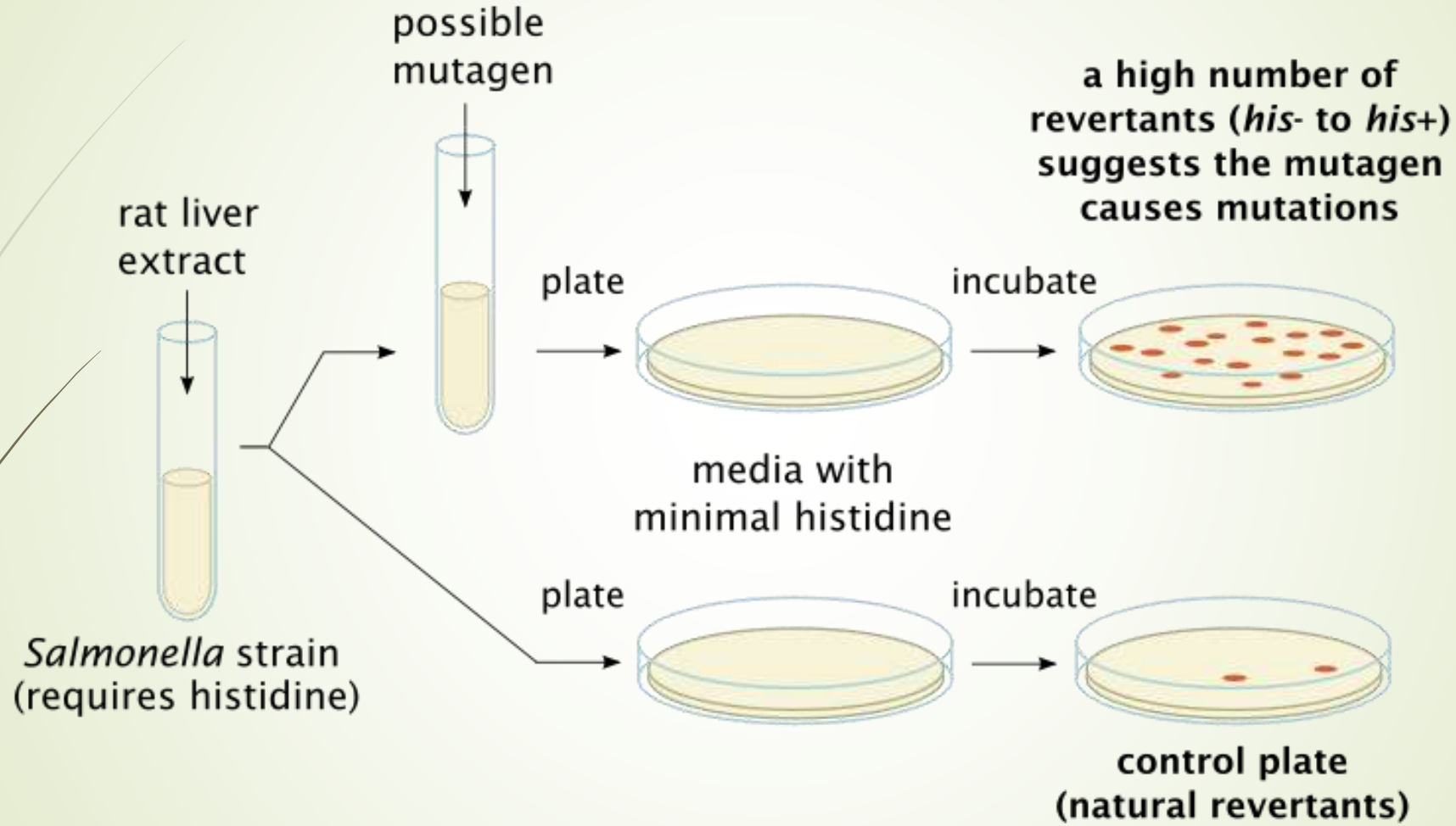
- Chromosome jumping allows the use of one point on a chromosome as a starting point for exploring another potentially distant point on the same chromosome without cloning the intervening sequences as in chromosome walking.

- No need to clone the repeated units as like chromosome walking

Mutagenecity test

Ames test:

- Method that uses bacteria to test whether a given chemical can cause mutations in the DNA of the test organism
- Standard mutation assays on mice and rats are time-consuming
- Use the bacterium *Salmonella typhimurium* that carry mutations in genes involved in histidine synthesis
- These strains are auxotrophic mutants
- The tested substance in creating mutations that result in a return to a "prototrophic" state


possible
mutagen
rat liver
extract
plate
incubate
a high number of
revertants (*his*- to *his*+)
suggests the mutagen
causes mutations
media with
minimal histidine
plate
incubate
Salmonella strain
(requires histidine)
control plate
(natural revertants)

COMET assay

- Single cell gel electrophoresis assay.
- Sensitive technique for the detection of DNA damage
- Cells embedded in agarose on a microscope slide
- Lysed with detergent and high salt to form nucleoids
- Electrophoresis at high pH results in structures resembling comets
- Observed by fluorescence microscopy

Comet Assay Overview

Bacterial Culture

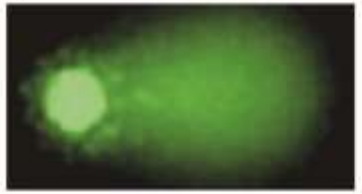
Blood/Serum Sample

Tissue Sample

DNA Damage (Chemicals or radiation)

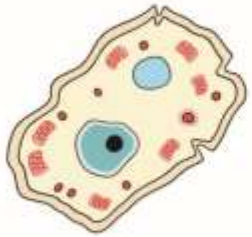
Analysis of a DNA Comet

"Head" = intact DNA



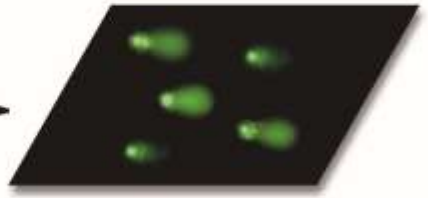
"Tail" = damaged DNA

^{Fluor}Tail: ^{Fluor}Head $\propto$ extent of DNA damage



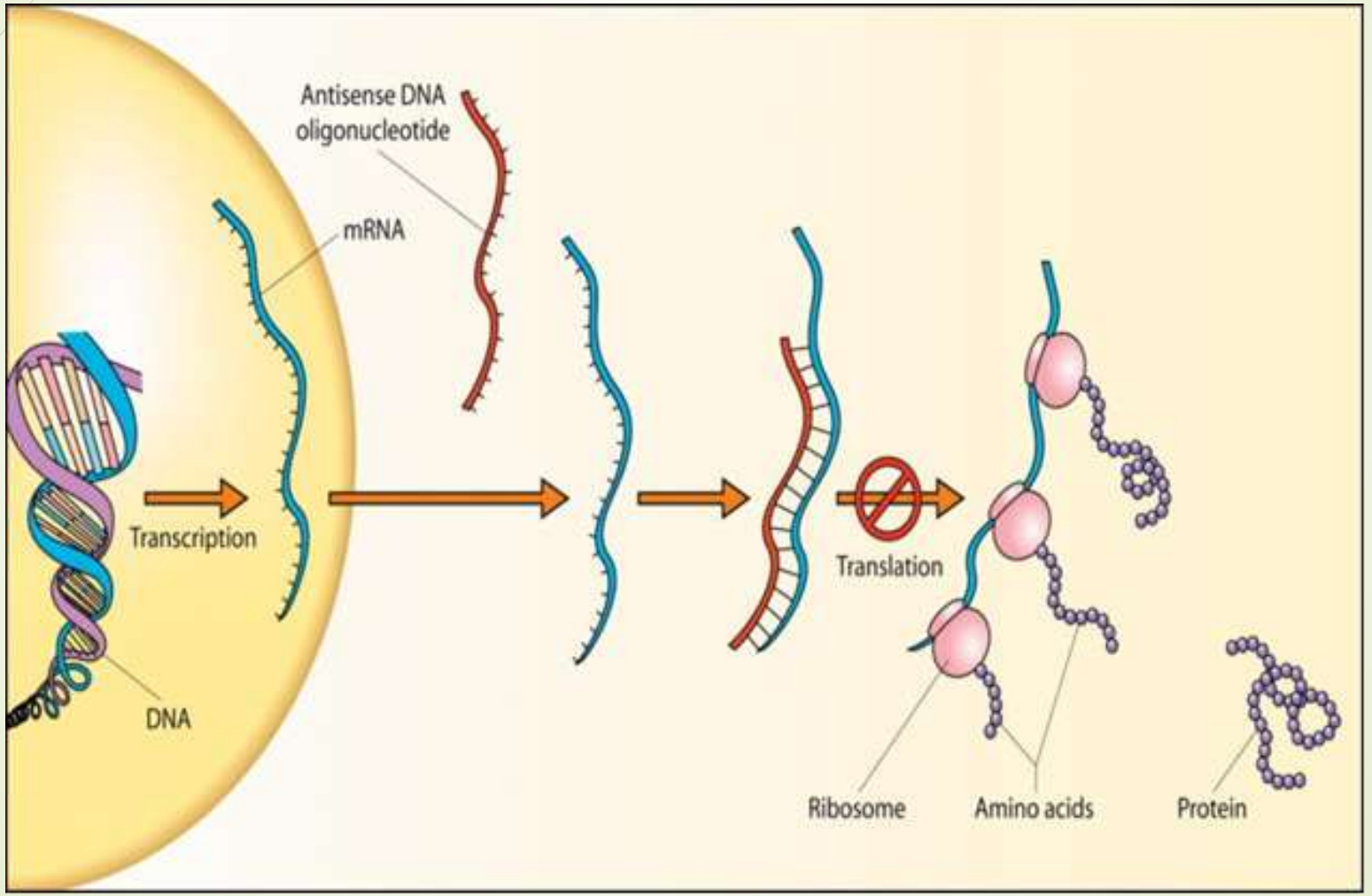
Damaged (relaxed) DNA in cells occurs from single-strand and double-strand breaks

Single cells are embedded on agarose-coated slides and lysed

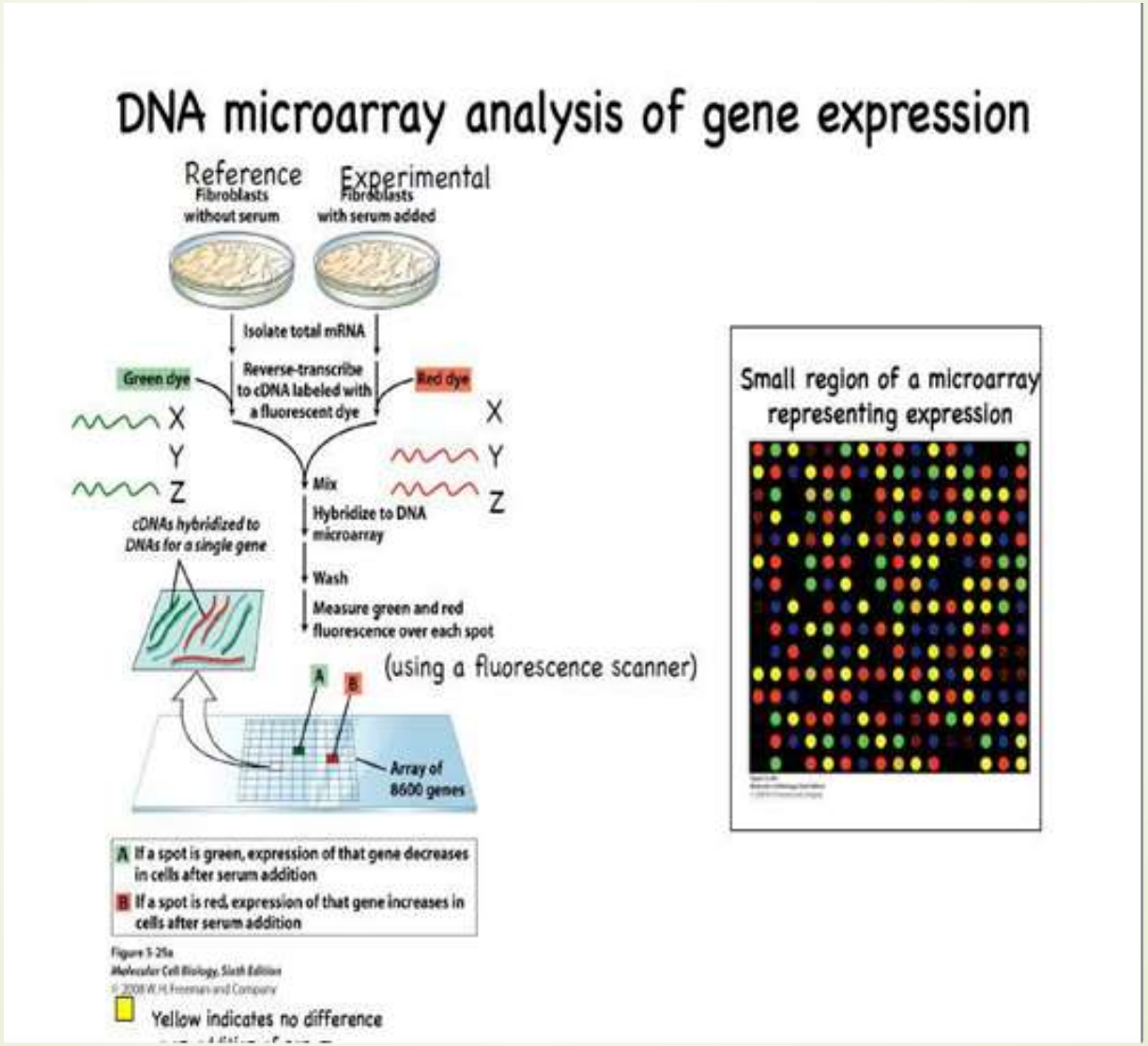


Electrophoresis and fluorescent staining generate DNA "comets"

Antisense technology

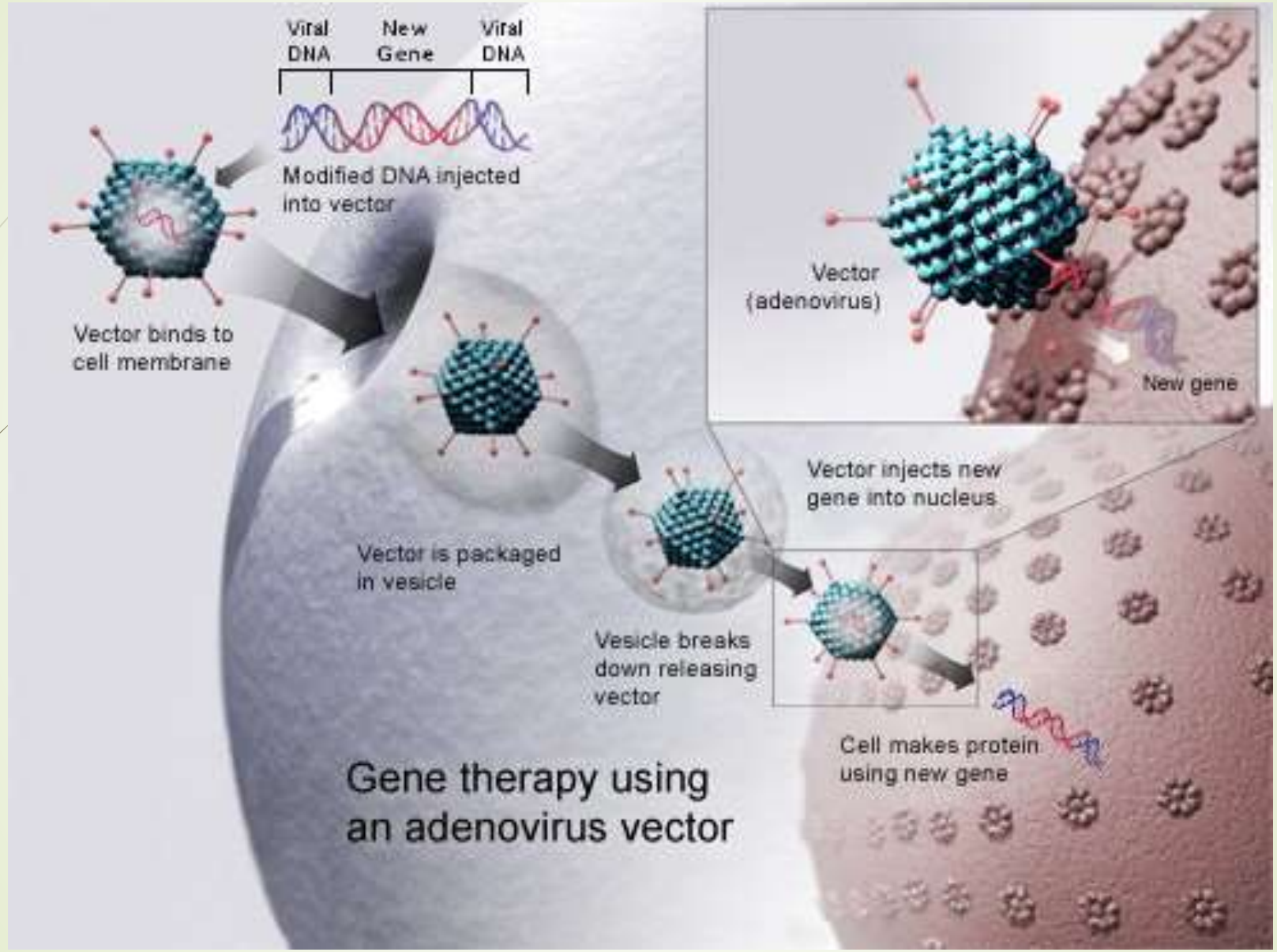


Microarray technology



Gene therapy

- Therapeutic delivery of nucleic acid polymers into a patient's cells as a drug to treat disease.
- The polymers are either expressed as proteins, interfere with protein expression, or possibly correct genetic mutations.
- Uses DNA that encodes a functional, therapeutic gene to replace a mutated gene
- Molecule is packaged within a "vector", which carries the molecule inside cells


Viral DNA
New Gene
Viral DNA
Modified DNA injected into vector
Vector binds to cell membrane
Vector (adenovirus)
New gene
Vector injects new gene into nucleus
Vector is packaged in vesicle
Vesicle breaks down releasing vector
Cell makes protein using new gene
Gene therapy using an adenovirus vector

Gene therapy may be classified into two types

Germline:

- Germ cells (sperm or eggs) are modified by the introduction of functional genes into their genomes.
- Modifying a germ cell causes all the organism's cells to contain the modified gene.
- The change is therefore heritable and passed on to later generations.

Somatic cell:

- The therapeutic genes are transferred into any of any cell other than germ cells.

- Any such modifications affect the individual patient only, and are not inherited by offspring.

Somatic gene therapy can be broadly split into two categories:

- ex vivo, which means exterior (where cells are modified outside the body and then transplanted back in again).
- in vivo, which means interior (where genes are changed in cells still in the body).
- Insitu introducing the vector directly to the tissue

Gene Augmentation Therapy (GAT):

- Gene augmentation therapy (GAT), where DNA is added to the genome inorder to replace a missing gene product.
- Gene targeting to correct mutant alleles.
- Gene inhibition therapy, using antisense RNA expression or expression of intracellular antibodies to treat diseases.

ADA Deficiency

- First gene therapy trial for an inherited disorder was initiated on 14 September 1990 against inherited disorder, adenosine deaminase (ADA) deficiency.
- ADA is involved in the purine salvage pathway of nucleic acid degradation.
- Inherited deficiency of this enzyme suffer from severe combined immunodeficiency

ADA gene therapy approach involved essentially four steps:

(i) Cloning a normal ADA gene into a retroviral vector.

(ii) Transfecting the ADA recombinant into cultured ADA^- T lymphocytes from the patient.

(iii) Identifying the resulting ADA^+ T cells and expanding them in culture.

(iv) Re-implanting these cells in the patient.

CFTR

- Cystic fibrosis (CF) transmembrane conductance regulator (CFTR) gene.

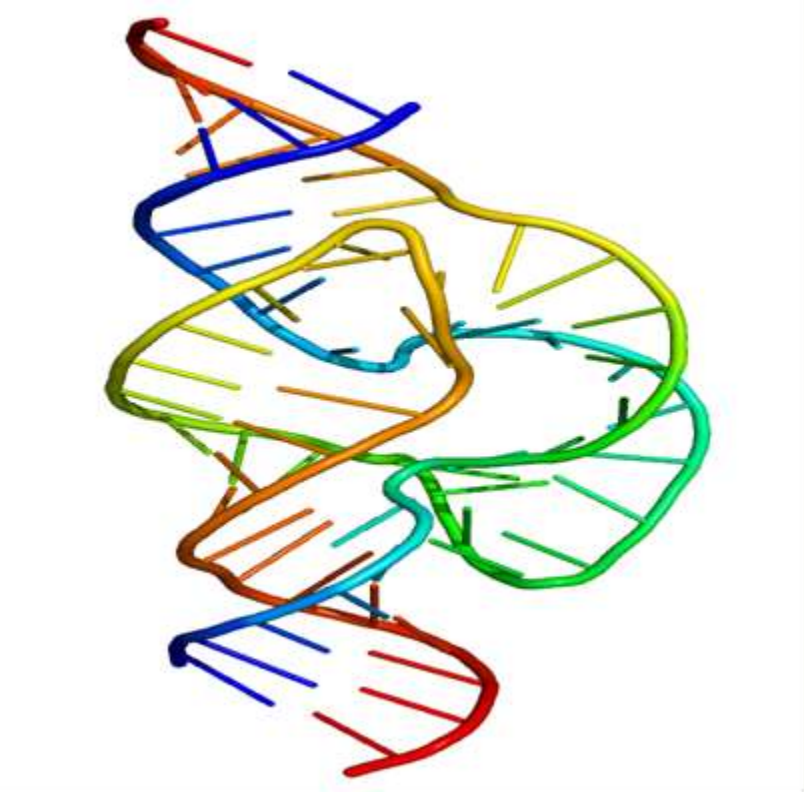
- Loss of CFTR function in cystic fibrosis patients

Antisense Therapy

Ribozymes

- RNA molecules that are capable of catalyzing specific biochemical reactions, similar to the action of enzymes.
- Most common activities of natural or in vitro-evolved ribozymes are the cleavage or ligation of RNA and DNA and peptide bond formation.
- Function to link amino acids during protein synthesis.
- They also participate in a variety of RNA processing reactions, including RNA splicing, viral replication, and transfer RNA biosynthesis.

- Examples of ribozymes include the hammerhead ribozyme, the VS ribozyme, Leadzyme and the hairpin ribozyme.



Protein Aptamers

- Peptide molecules that bind to a specific target molecule
- designed to interfere with other protein interactions inside cells.
- Consist of a variable peptide loop attached at both ends to a protein scaffold
- Greatly increases the binding affinity of the peptide aptamer to levels comparable to an antibody.

- variable loop length is typically composed of ten to twenty amino acids, and the scaffold may be any protein which has good solubility and compacity properties.

- Peptide aptamer selection can be made using different systems, but the most used is currently the yeast two-hybrid system

Intrabody

- Is an antibody that works within the cell to bind to an intracellular protein.

- Due to the lack of a reliable mechanism for bringing antibodies into the cell from the extracellular environment, this typically requires the expression of the antibody within the target cell, which can be accomplished by gene therapy

Stem Cell Therapy

- Use of stem cells to treat or prevent a disease or condition.
- Bone marrow transplant is the most widely used stem cell therapy,
- Therapies derived from umbilical cord blood are also in use.
- Research is underway to develop various sources for stem cells, and to apply stem cell treatments for neurodegenerative diseases and conditions, diabetes, heart disease, and other conditions.

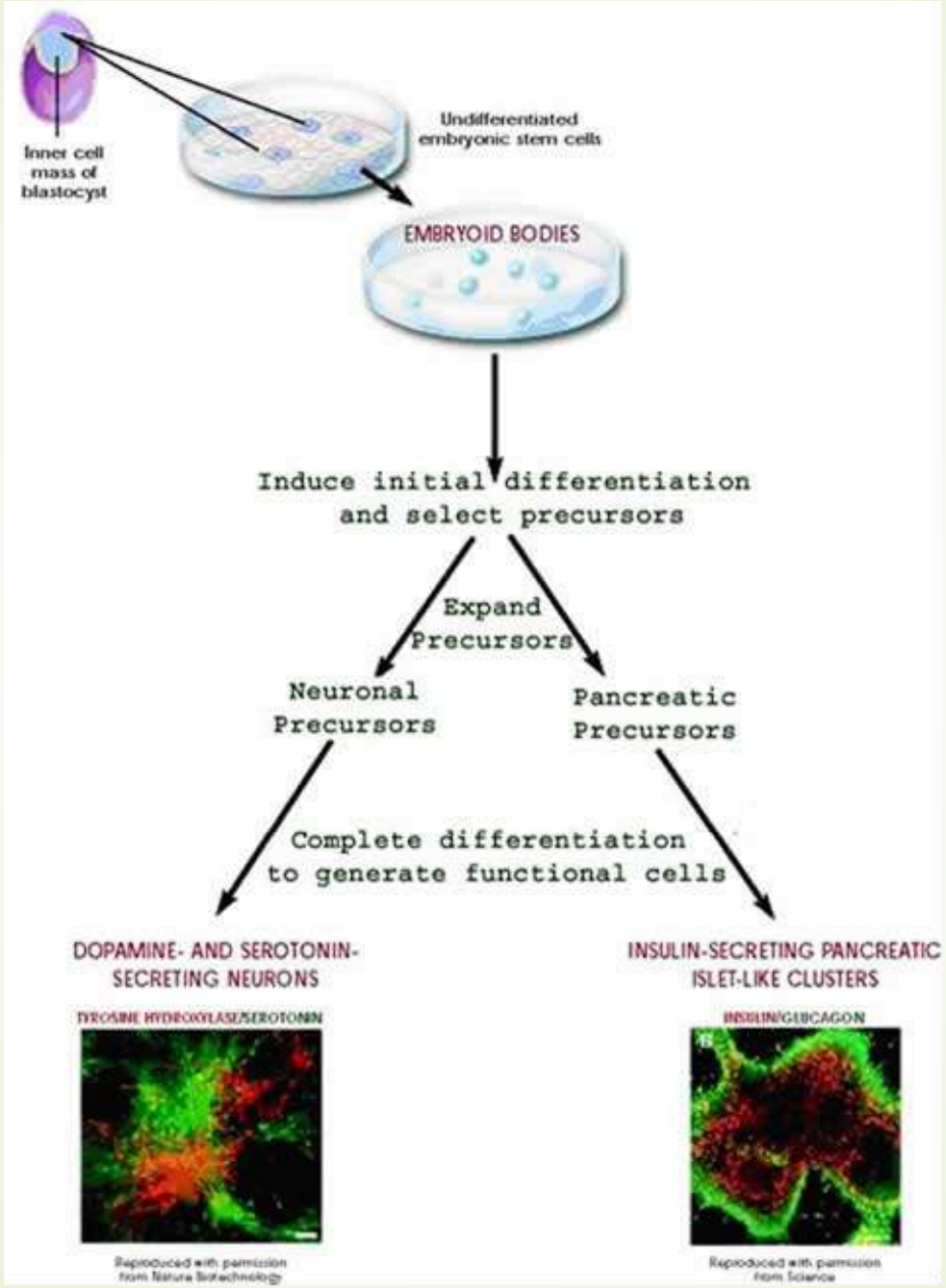
Embryonic stem cells and Adult stem cells

- **Embryonic stem cells** can become all cell types of the body because they are pluripotent.
- **Adult stem cells** are thought to be limited to differentiating into different cell types of their tissue of origin.
- Totipotent, pluripotent and multipotent

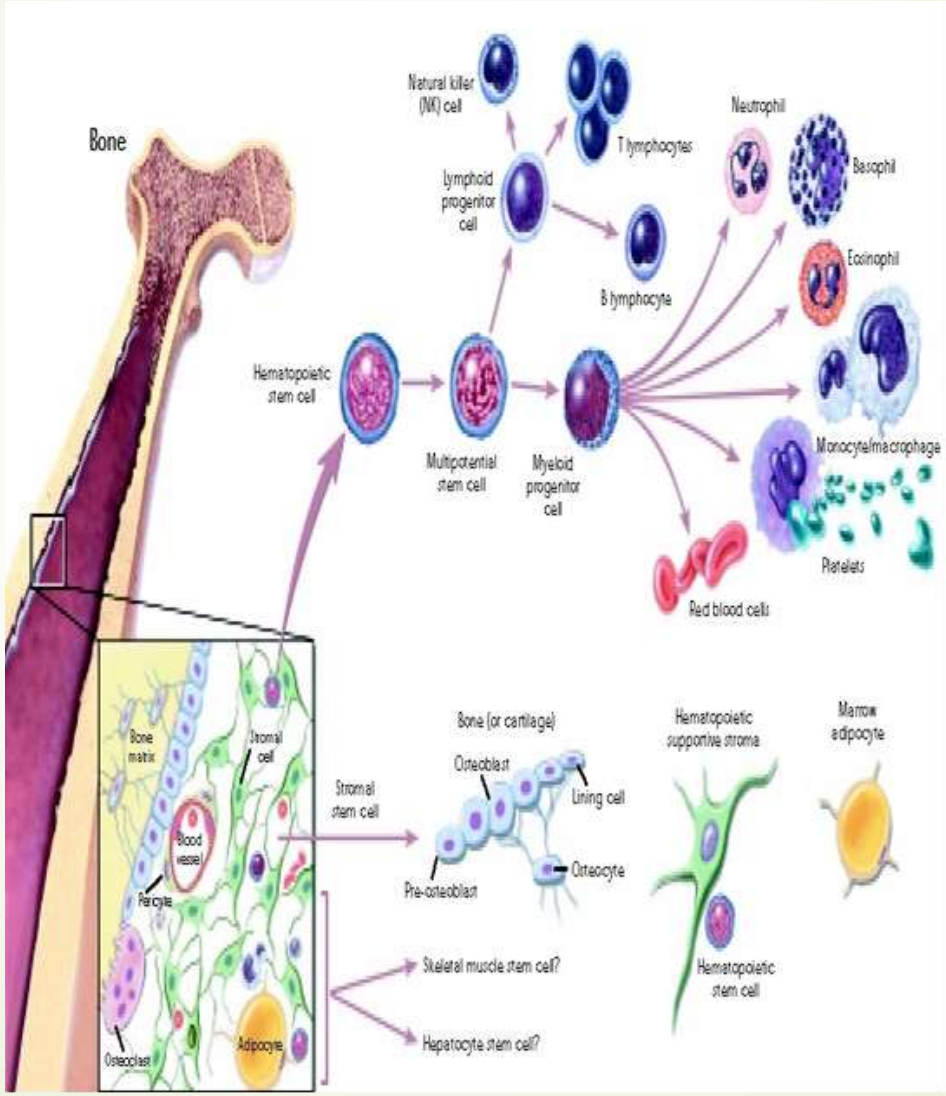
Testing and generation of embryonic stem cells

- Derived from embryos. Most embryonic stem cells are derived from embryos that develop from eggs that have been fertilized *in vitro.*

- In an *in vitro* fertilization clinic—and then donated for research purposes with informed consent of the donors. They are *not* derived from eggs fertilized in a woman's body.


Inner cell mass of blastocyst
Undifferentiated embryonic stem cells
EMBRYOID BODIES
Induce initial differentiation and select precursors
Expand Precursors
Neuronal Precursors
Pancreatic Precursors
Complete differentiation to generate functional cells
DOPAMINE- AND SEROTONIN-SECRETING NEURONS
TYROSINE HYDROXYLASE/SEROTONIN
Reproduced with permission from Nature Biotechnology
INSULIN-SECRETING PANCREATIC ISLET-LIKE CLUSTERS
INSULIN/GLUCAGON
Reproduced with permission from Science

Adult stem cell



Potential use of stem cells

- Nanotechnology
- Nanomedicine
- Nanoparticles
- Nanodevices
- Medical micro robotics
- Nano robotics
- Microbiovers
- Nanomedicine and nanosurgery for cancer and neurological disorders