BASIC PRINCIPLES OF GENETIC ENGINEERING

Suman Kumar Mekap

Asst. Professor (Pharmacology)

CUTM, Bhubaneswar

WHAT IS A GENE ?

- A Gene is a **fundamental**, **physical** and **functional** unit of heredity.

- It is responsible for the **physical** and **inheritable** characteristics of an organism.

DEFINITION

- Genetic Engineering is **manipulation/alteration** of structure of a gene to create a desired characteristic in an organism.
- Genetic recombination technology consists of the breakage and joining of DNA molecules.
- Genetically engineered DNA prepared by transplanting or splicing genes from one species into the cells of a host organism of a different species. Such DNA becomes part of the host's genetic makeup and is replicated.
- Genetic engineering primarily involves the manipulation of genetic material (DNA) to achieve the desire goal in pre determined way.

- If genetic material from another species is added to the host, the resulting organism is called transgenic.

- Genetic engineering can also be used to remove genetic material from the target organism, creating a knock out organism.

HISTORY

- In 1973 **Herbert Boyer and Stanley Cohen** created the first transgenic organism by inserting antibiotic resistance genes into the plasmid of an *E.coli* bacterium.

- The first trials of genetically engineered plants occurred in France and the USA in 1986, tobacco plants were engineered to be resistant to herbicides.

TRANSGENIC PLANTS

- The Flavr Savr tomato was a tomato engineered to have a longer shelf life.

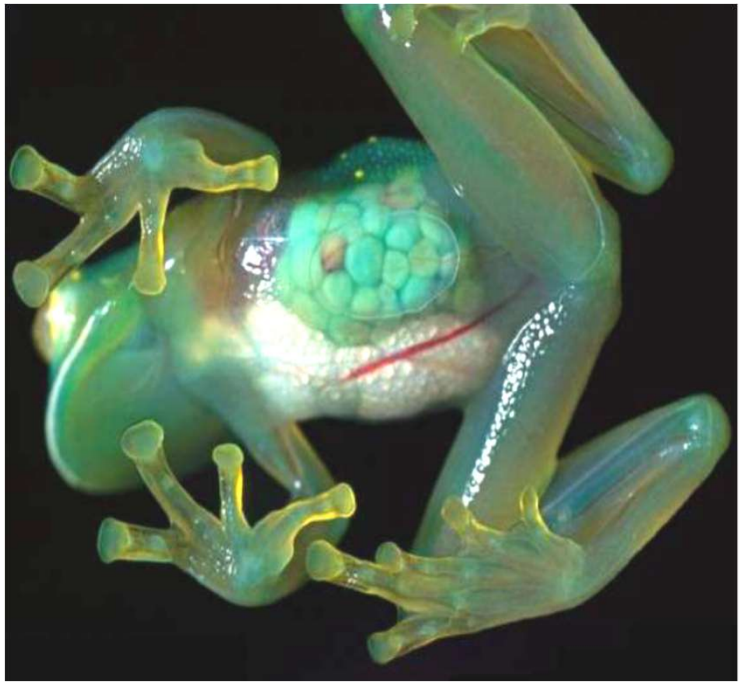
- Bt-Cotton is a genetically modified cotton which is resistant to pests.

- Golden Rice genetically modified to contain beta-carotene (a source of Vitamin A).

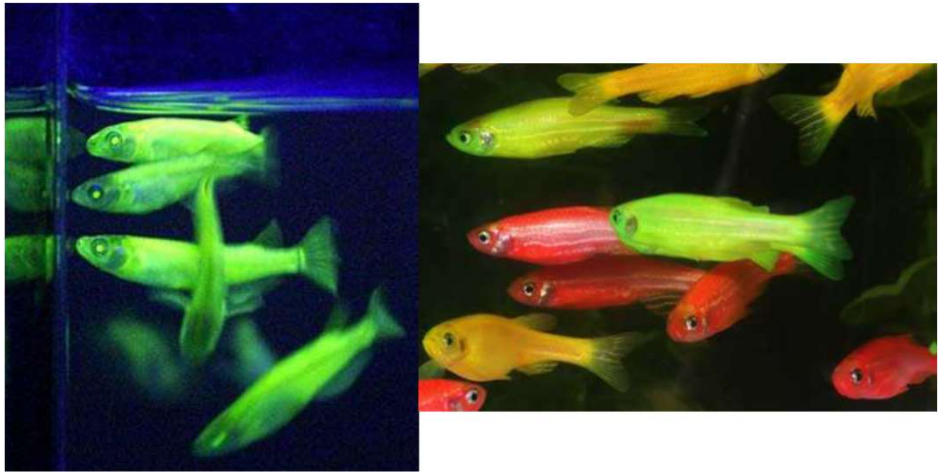
- A Blue Rose is a genetically modified Rose.

TRANSGENIC ANIMALS

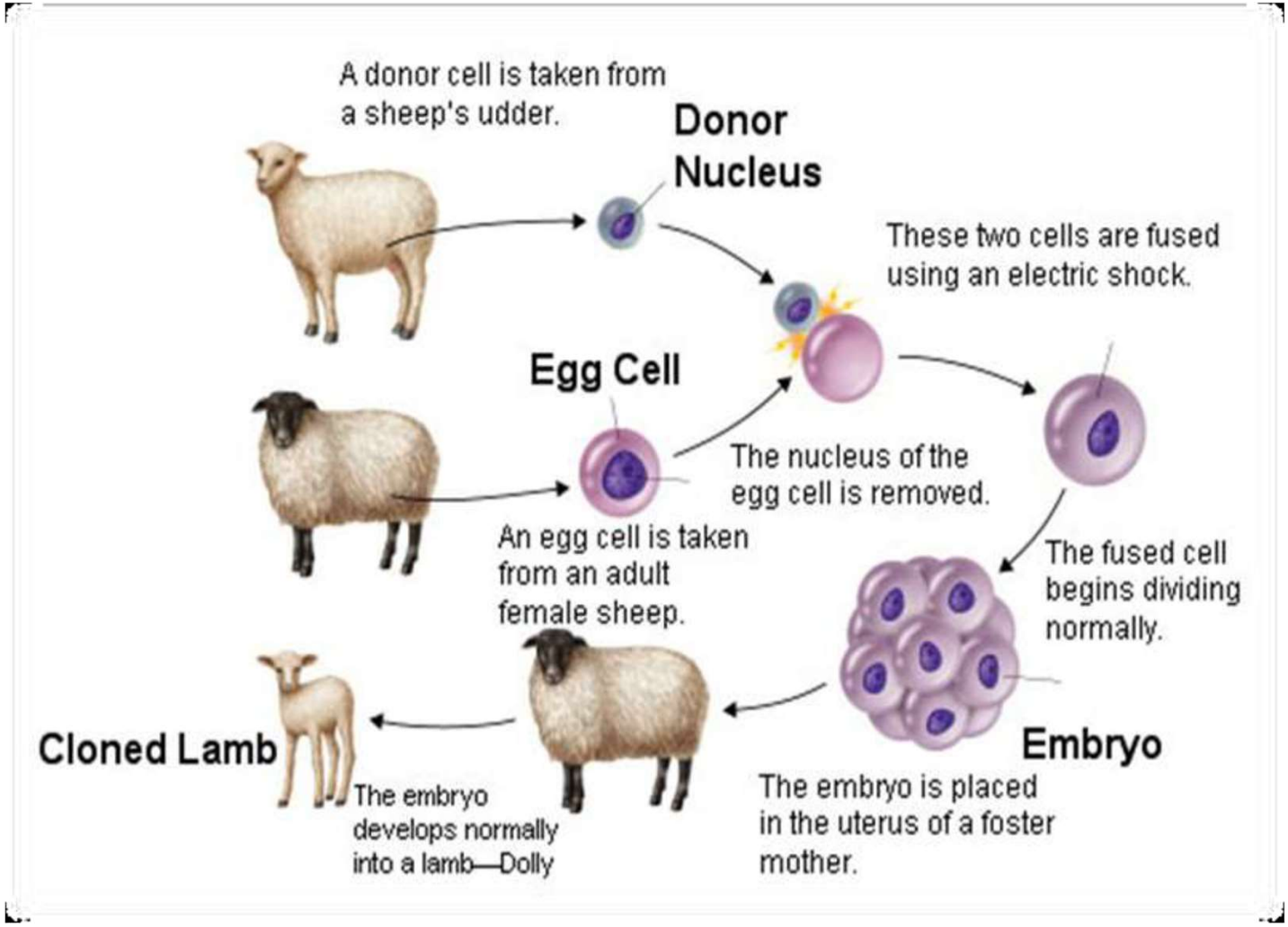
- It's a miracle of genetic engineering. You can see through the skin how organs grow, how cancer starts and develops without dissecting the Frog.



- The Glow Fish was the first genetically modified animal to become available as a pet. It is a natural Zebrafish which has genetic information from bioluminescent jellyfish added to its DNA.



DOLLY THE SHIP



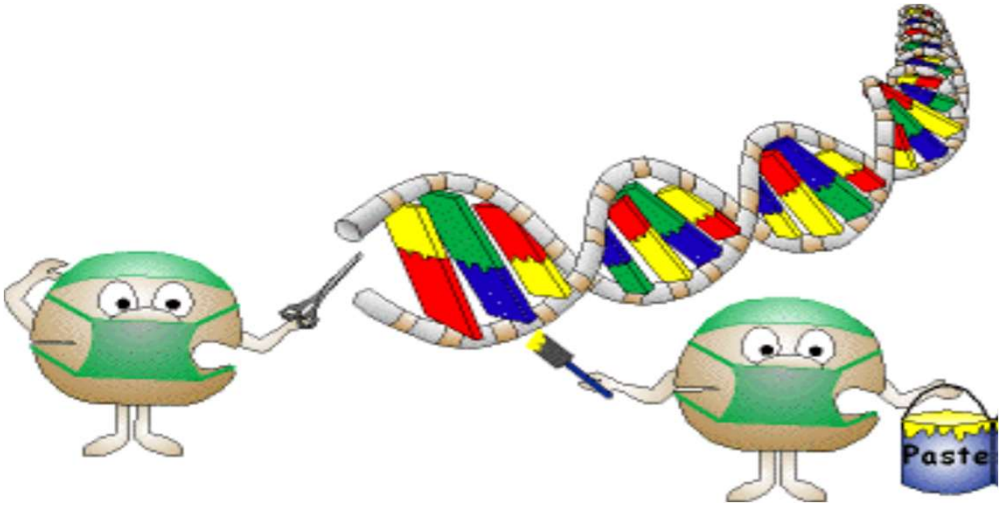
- Dolly the sheep is the world's most famous clone.
- Dolly was born 5 July 1996 to three mothers (one provided the egg, another the DNA and a third carried the cloned embryo to term).

OTHER TERMS USED FOR GENETIC ENGINEERING:

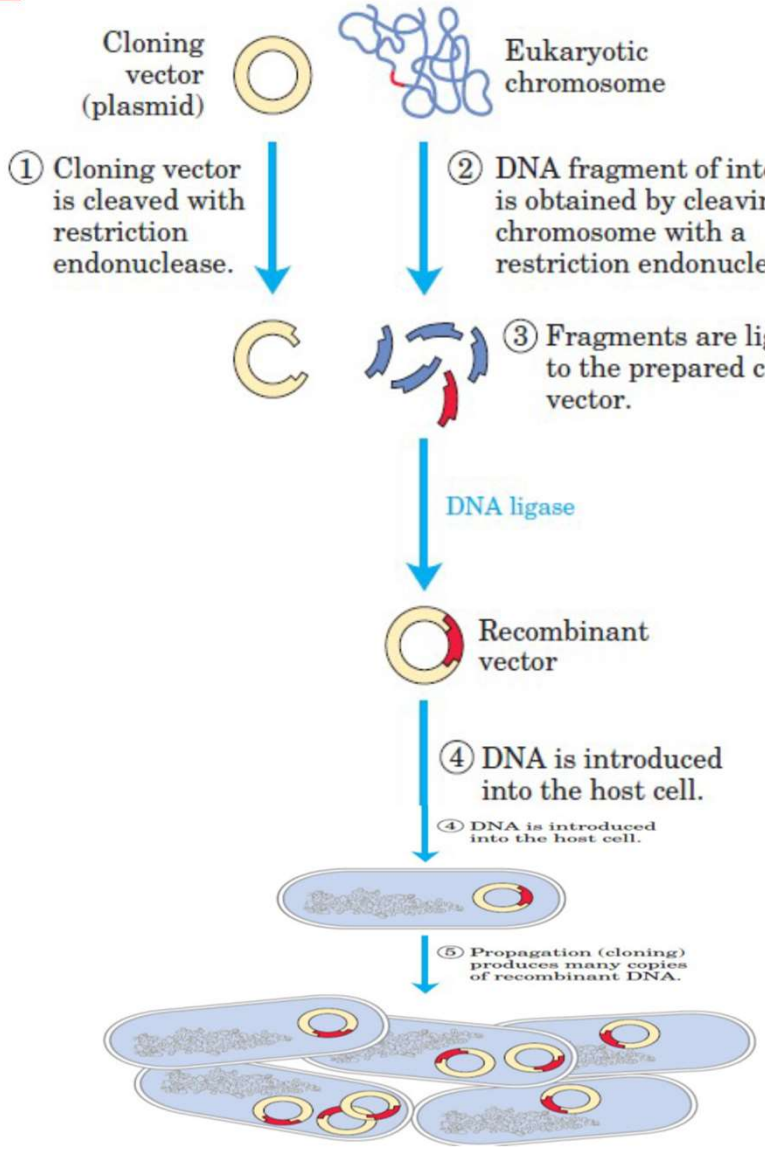
- Recombinant DNA technology
- Gene manipulation
- Gene cloning
- Genetic modifications
- New genetics

Basic principle of recombinant DNA technology

- Manipulation and alteration of genes
- Artificially copying a piece of DNA from one organism and joining this copy of DNA into the DNA of another organism



Basic principle of rDNA technology



5. Propagation (cloning) produces many copies of recombinant DNA

Molecular tools of genetic engineering

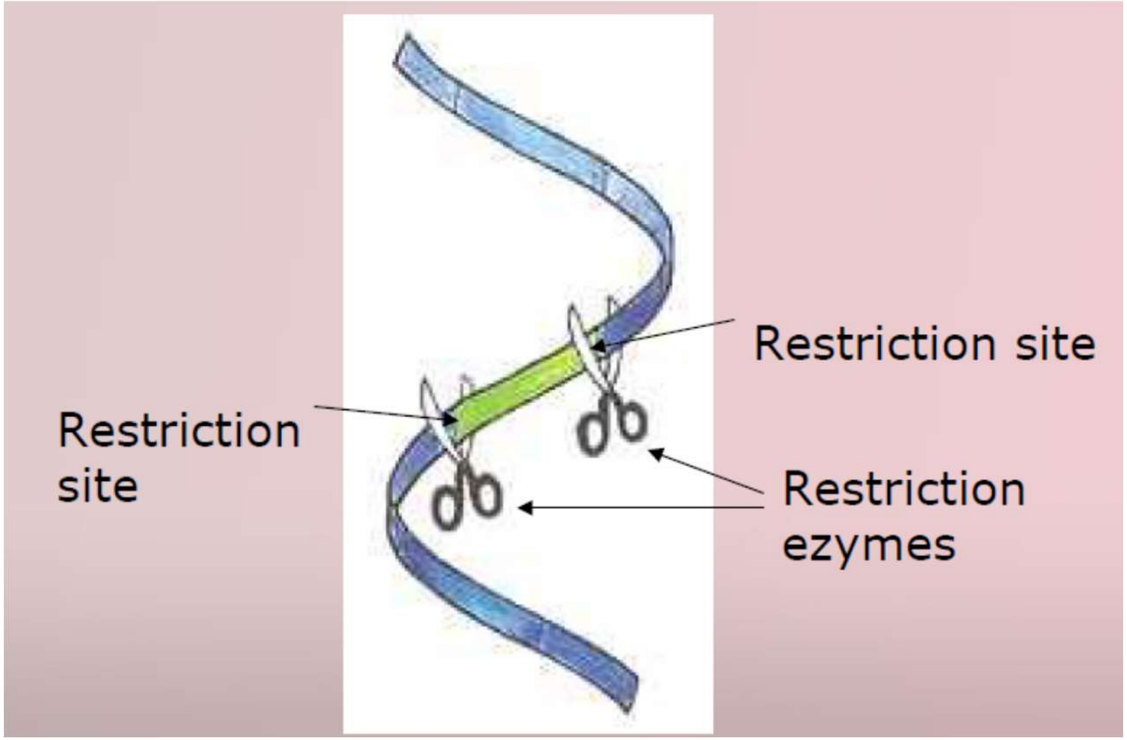
• The genetic engineer's tool kit or molecular tool namely the enzymes are most commonly used in recombinant DNA experiments

These are:

- **Restriction endonucleases -**DNA cutting Enzyme.
- **DNA Ligases-** DNA joining Enzyme.

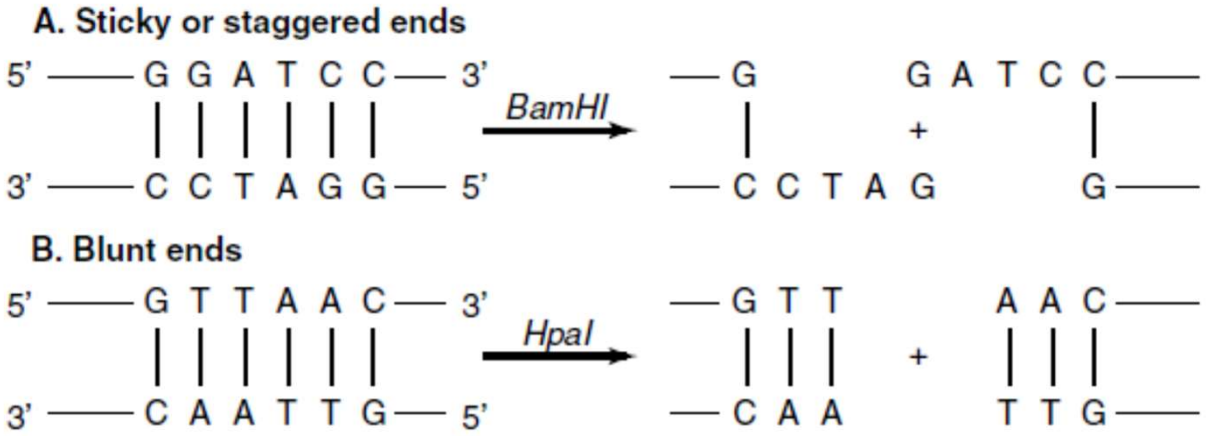
Restriction endonucleases

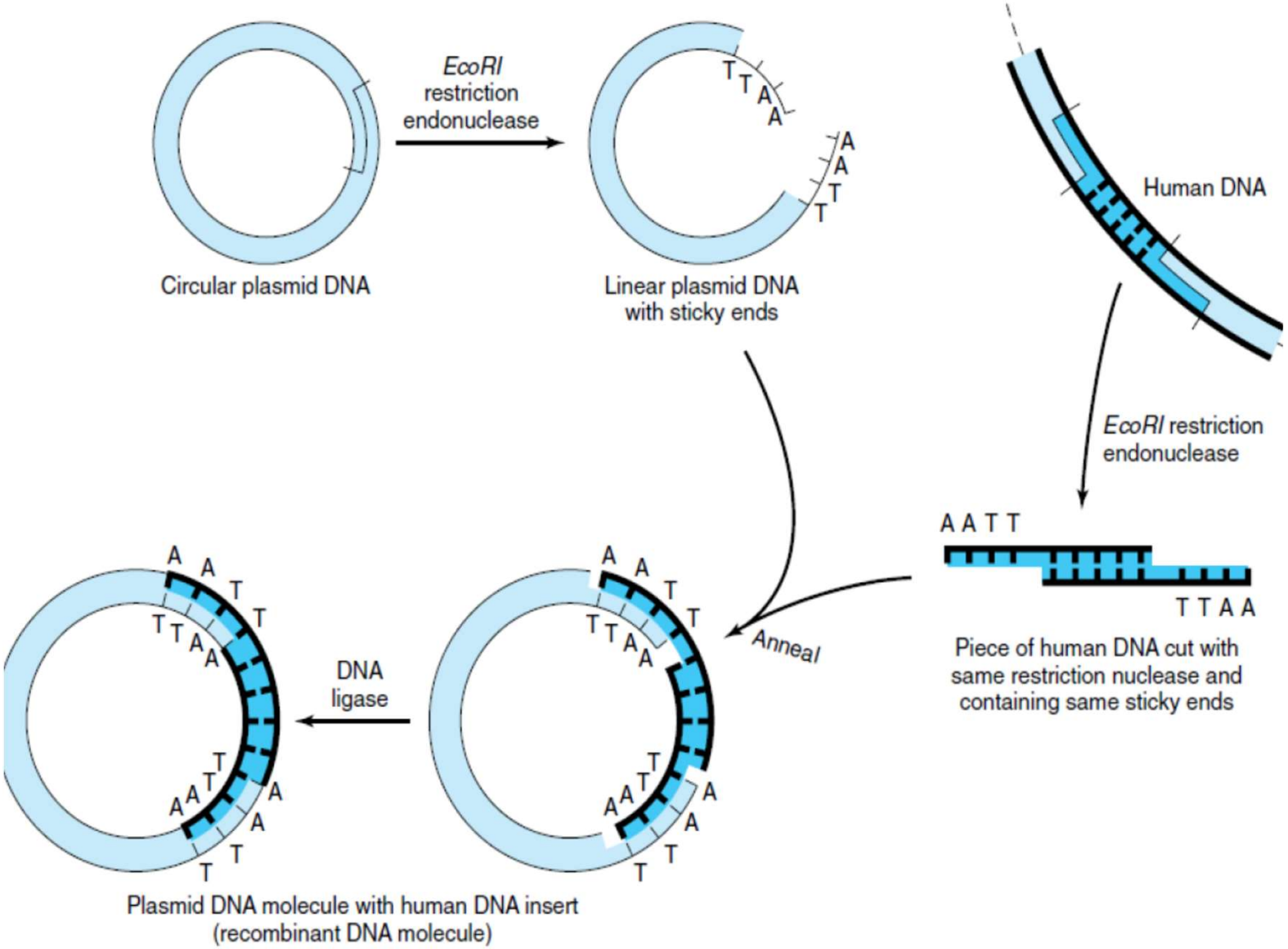
- Restriction enzymes act as molecular scissors and cut DNA at specific sites called restriction sites



Restriction endonucleases

- Named with particular reference to the bacteria from which they are isolated.
- Eg. EcoRI: Types 3

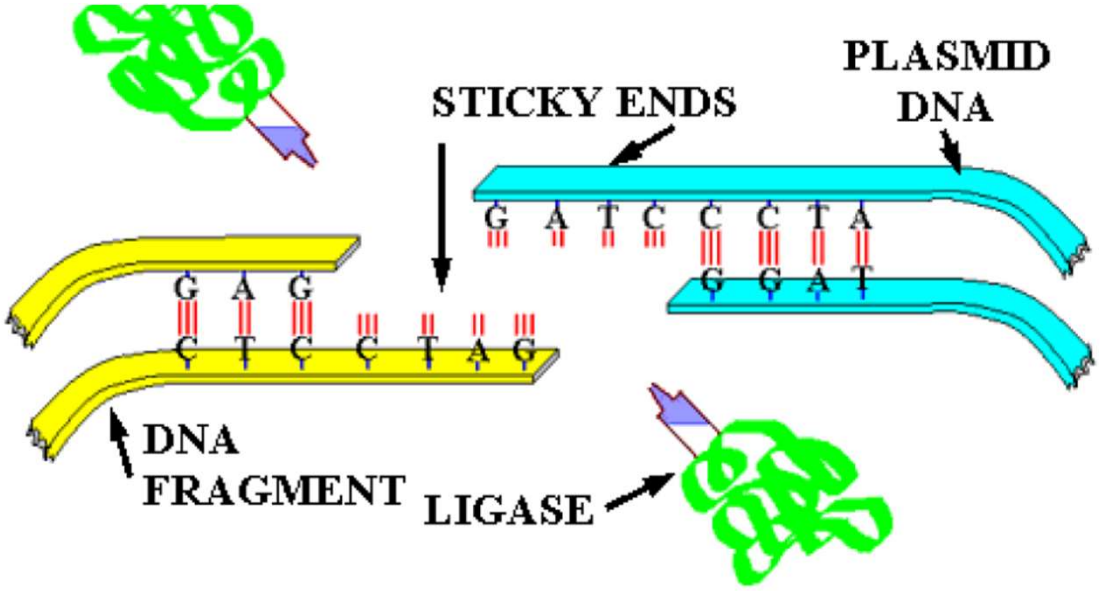



EcoRI restriction endonuclease
Circular plasmid DNA
Linear plasmid DNA with sticky ends
Human DNA
EcoRI restriction endonuclease
A A T T
T T A A
Piece of human DNA cut with same restriction nuclease and containing same sticky ends
Anneal
DNA ligase
Plasmid DNA molecule with human DNA insert (recombinant DNA molecule)

Endonucleases and their Source

Endonuclease	Sequence Recognized Cleavage Sites Shown	Bacterial Source
BamHI	↓ GGATCC CCTAGG ↑	*Bacillus amylo-liquefaciens* H
BglII	↓ AGATCT TCTAGA ↑	*Bacillus glolbigii*
EcoRI	↓ GAATTC CTTAAG ↑	*Escherichia coli* RY13
EcoRII	↓ CCTGG GGACC ↑	*Escherichia coli* R245
HindIII	↓ AAGCTT TTCGAA ↑	*Haemophilus influenzae* R_d
HhaI	↓ GCGC CGCG ↑	*Haemophilus haemolyticus*
HpaI	↓ GTTAAC CAATTG ↑	*Haemophilus parainfluenzae*
MstII	↓ CCTNAGG GGANTCC ↑	Microcoleus strain
PstI	↓ CTGCAG GACGTC ↑	*Providencia stuartii* 164
TaqI	↓ TCGA AGCT ↑	*Thermus aquaticus* YTI

DNA ligase



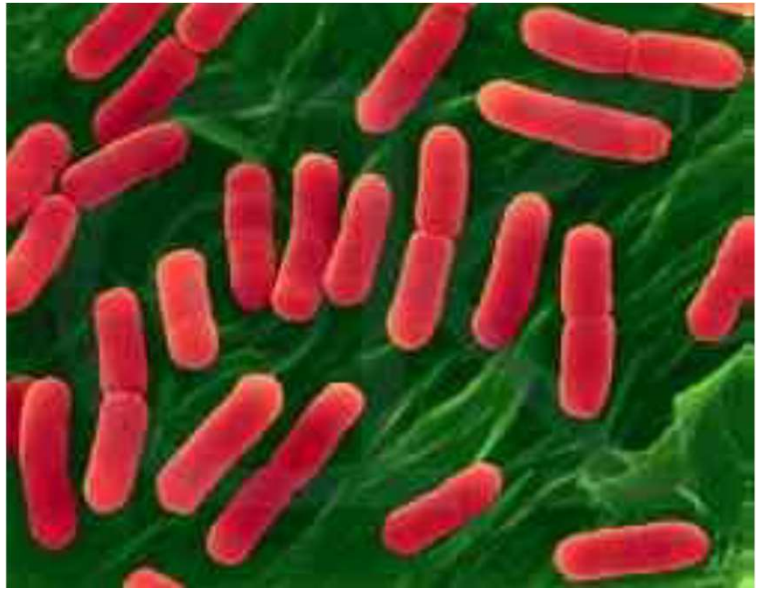
Host cells: the factor of cloning

- The hosts are the living system or cell in which the carrier of recombinant DNA molecule or vector can be propagated.

Prokaryotic

- **Bacteria**

Escherichia coli
Bacillus subtilis
Streptomyces sp.



Eukaryotic

1.Fungi

Saccharomyces cerevisiae

Aspergillus nidulans

2.animals Insect cells

- Oocytes
- Mammalian cells
- Whole organisms

3.plants Protoplast

- Intact cell
- Whole plants

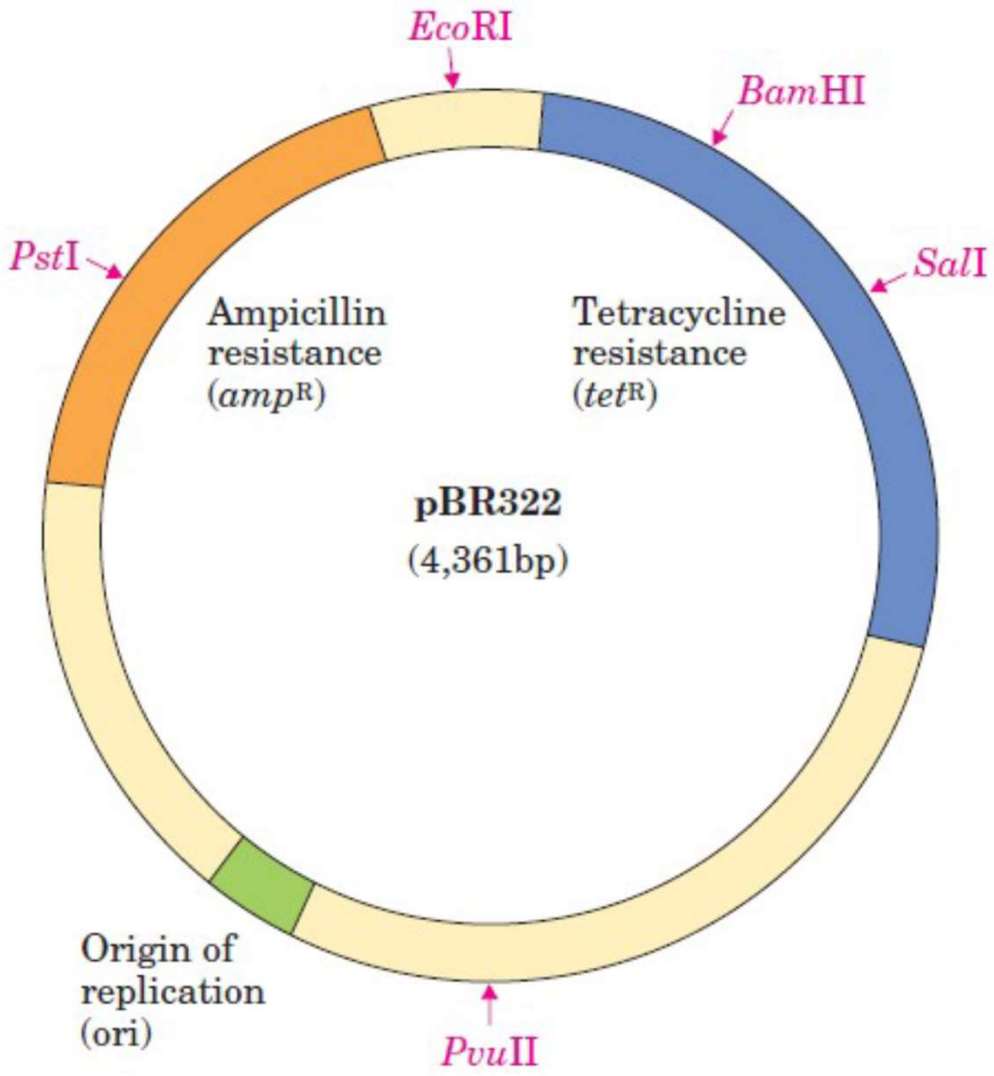
Cloning vectors

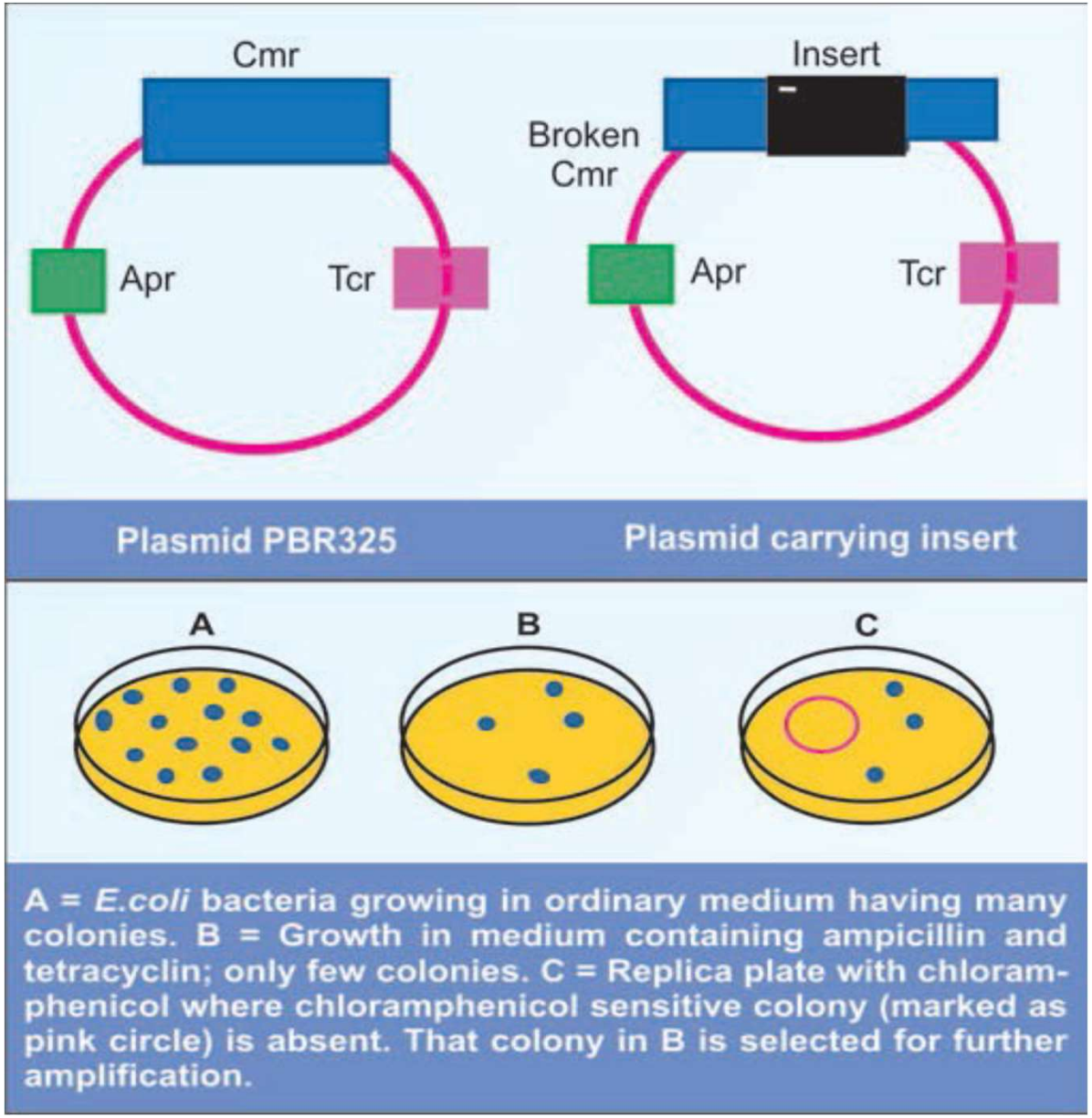
- Vectors are the DNA molecule, which can carry A foreign DNA fragment to be cloned. They are self replicating in an appropriate host cell.

- The most important vectors are Plasmids, Bacteriophages, cosmids.

- An ideal characteristics of an vector is should be small in size with single endonuclease site.

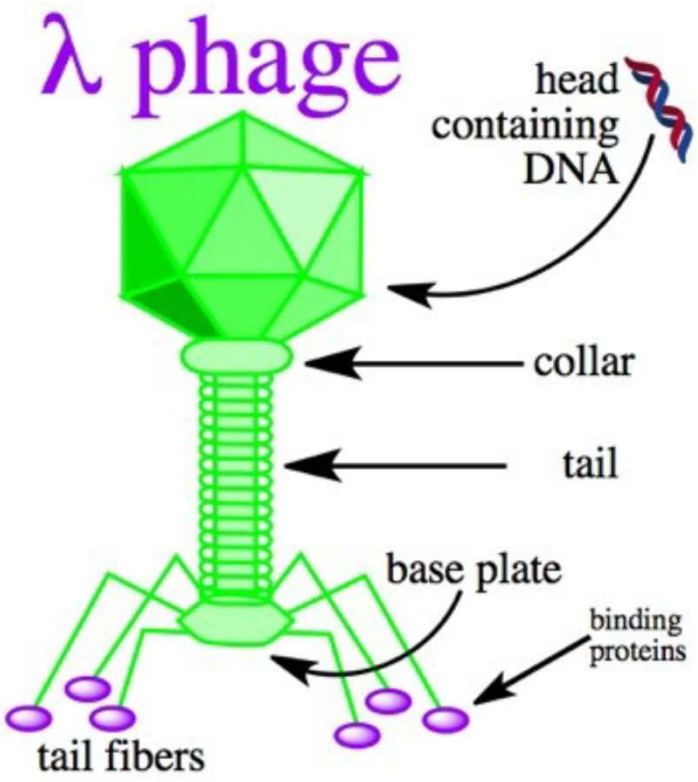
- But natural occurring rarely posses this characteristics.


EcoRI
BamHI
PstI
SalI
Ampicillin
resistance
(ampR)
Tetracycline
resistance
(tetR)
pBR322
(4,361bp)
Origin of
replication
(ori)
PvuII



A = *E.coli* bacteria growing in ordinary medium having many colonies. B = Growth in medium containing ampicillin and tetracyclin; only few colonies. C = Replica plate with chloramphenicol where chloramphenicol sensitive colony (marked as pink circle) is absent. That colony in B is selected for further amplification.

Bacteriophage λ (Lambda)



Cosmid

•Cosmids are vectors posses the characteristic of both plasmid and bacteriophage.

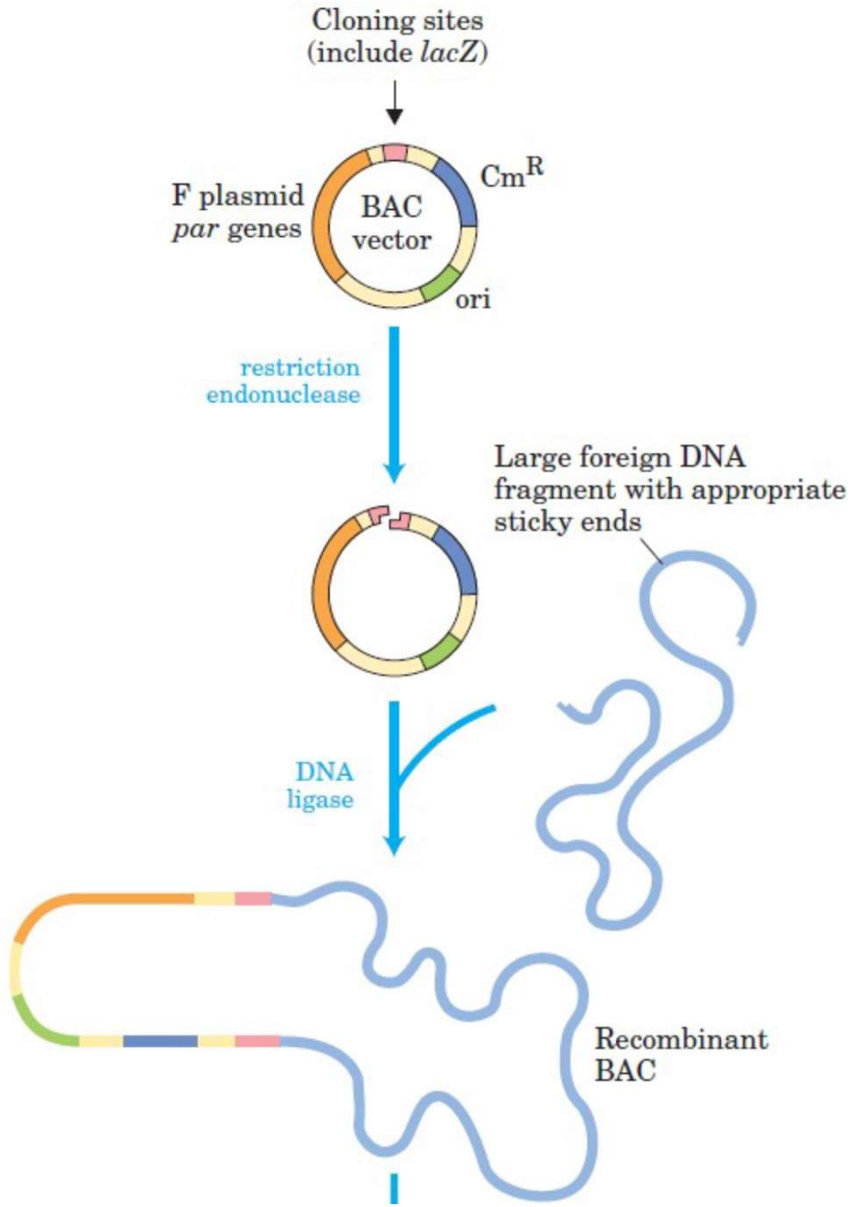
•Cosmids can be constructed by adding a fragment of phage DNA to plasmid.

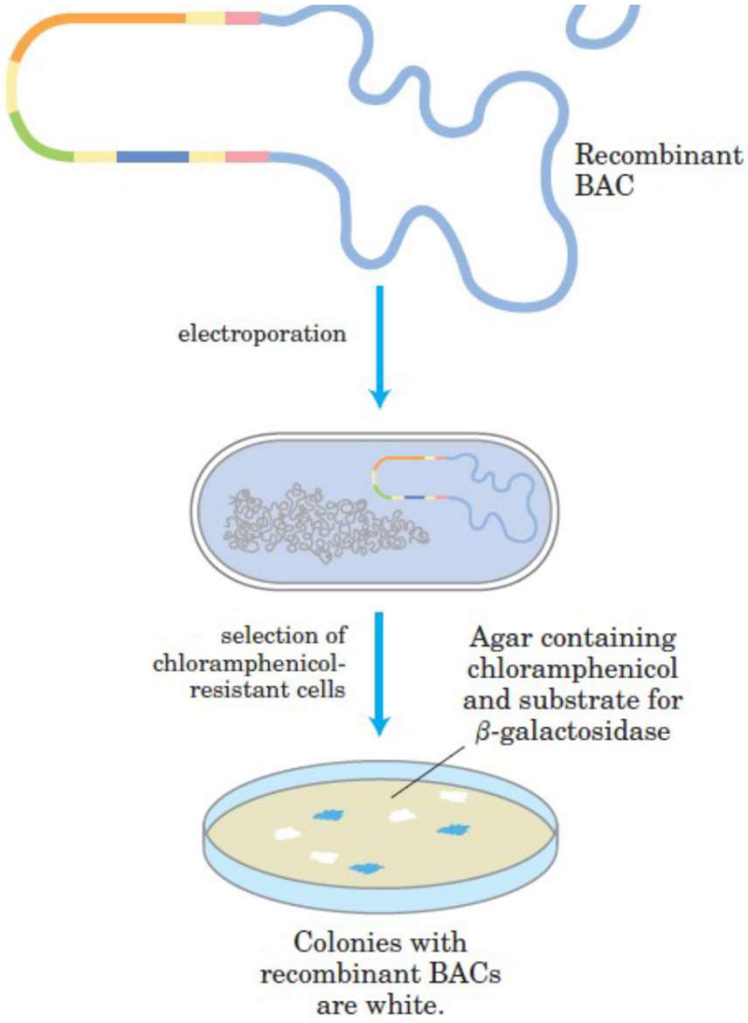
•A foreign DNA 40 Kb can be inserted into cosmid DNA

BACs: Bacterial Artificial Chromosomes

•The construction BAC is based on F plasmid which is large than other plasmid used as cloning vector.

•BACs can accept DNA inserts of around 300kb


Cloning sites
(include *lacZ*)
F plasmid
par genes
BAC
vector
Cm^R
ori
restriction
endonuclease
Large foreign DNA
fragment with appropriate
sticky ends
DNA
ligase
Recombinant
BAC


Recombinant
BAC
electroporation
selection of
chloramphenicol-
resistant cells
Agar containing
chloramphenicol
and substrate for
β-galactosidase
Colonies with
recombinant BACs
are white.

YACs: Yeast Artificial Chromosomes

- Yeast Artificial Chromosomes (YAC) is a synthetic DNA that can accept large fragment (particular human DNA).

- It is possible to clone large DNA pieces by using YAC.

Human Artificial Chromosomes

- Synthetically produced vector DNA, possessing the characteristic of human chromosome

- Size range from 1/10th to 1/5th of human chromosome

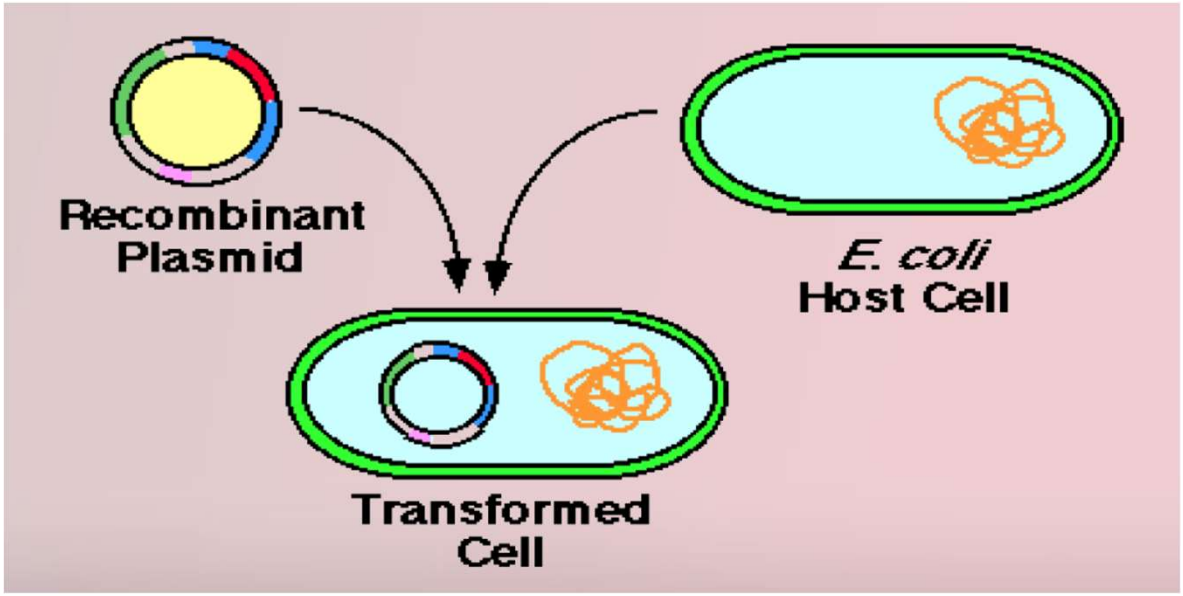
Vector	Basis	Size limits of insert	Major application
Plasmid	Naturally occuring multicopy plasmids	≤ 10 kb	Subcloning and downstream manipulation, cDNA cloning and expression assays
Phage	Bacteriophage λ	5–20 kb	Genomic DNA cloning, cDNA cloning, and expression libraries
Cosmid	Plasmid containing a bacteriophage λ *cos* site	35–45 kb	Genomic library construction
BAC (bacterial artificial chromosome)	*Escherichia coli* F factor plasmid	75–300 kb	Analysis of large genomes
YAC (yeast artificial chromosome)	*Saccharomyces cerevisiae* centromere, telomere, and autonomously replicating sequence	100–1000 kb (1 Mb)	Analysis of large genomes, YAC transgenic mice
MAC (mammalian artificial chromosome)	Mammalian centromere, telomere, and origin of replication	100 kb to > 1 Mb	Under development for use in animal biotechnology and human gene therapy

Methods of gene Transfer

- Transformation
- Electroporation
- Conjugation
- Liposome-mediated gene transfer.
- Transduction
- Microinjection

Transformation

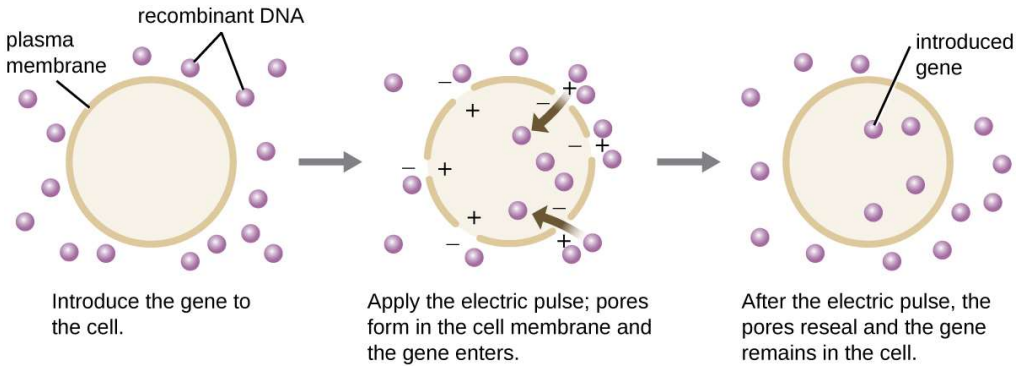
Transformation is the genetic alteration of a cell resulting from the direct uptake and incorporation of exogenous genetic material from its surroundings through the cell membrane.



Electroporation

OR

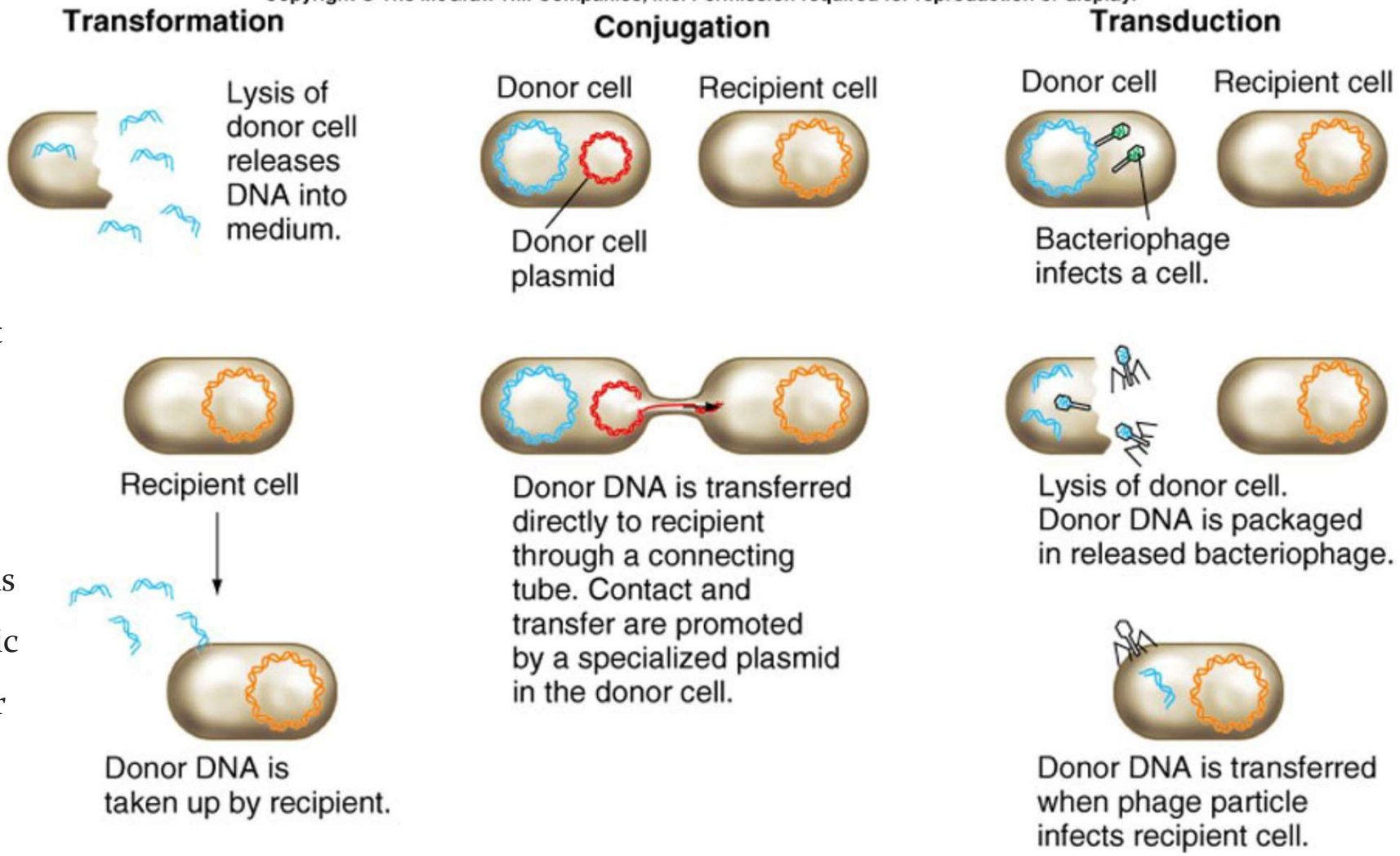
Electropermeabilization, is a microbiology technique in which an high voltage electrical field is applied to cells in order to increase the permeability of the cell membrane, allowing chemicals, drugs, or DNA to be introduced into the cell



CONJUGATION

Conjugation is the process by which one bacterium transfers genetic material to another through direct contact.

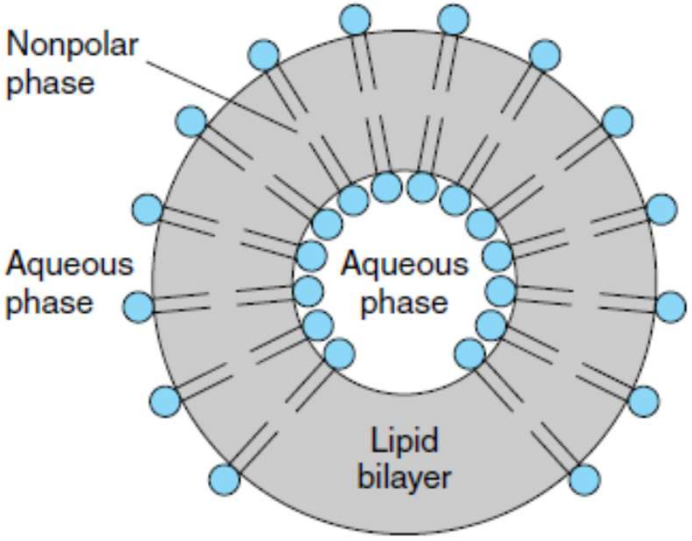
During **conjugation**, one bacterium serves as the donor of the genetic material, and the other serves as the recipient.



Liposome mediated gene transfer

- **Liposomes** are artificial phospholipid vesicles used for the **delivery**.

- They can be preloaded with DNA by two common methods- membrane membrane fusion and endocytosis thus forming DNA- **liposome** complex.

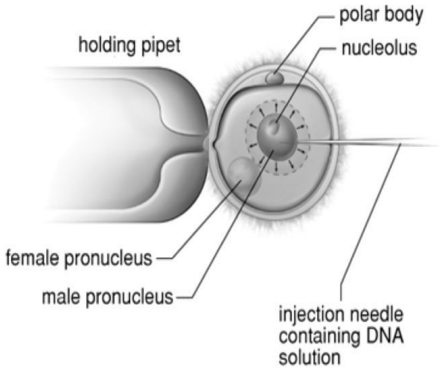


LIPOSOME

Direct transfer

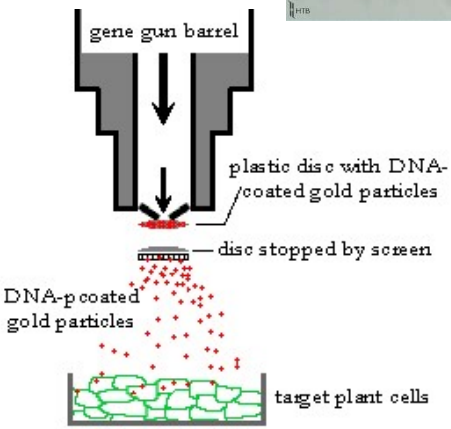
Microinjection

- Microinjection is the use of a glass micropipette to inject a liquid substance at a microscopic or borderline macroscopic level. The target is often a living cell but may also include intercellular space.



Particle bombardment

- The **Particle bombardment** device, also known as the gene gun, was developed to enable penetration of the cell wall so that genetic material containing a gene of interest can be transferred into the cell.



Applications of Recombinant DNA Technology

1)Large-scale production of human proteins by genetically engineered bacteria.

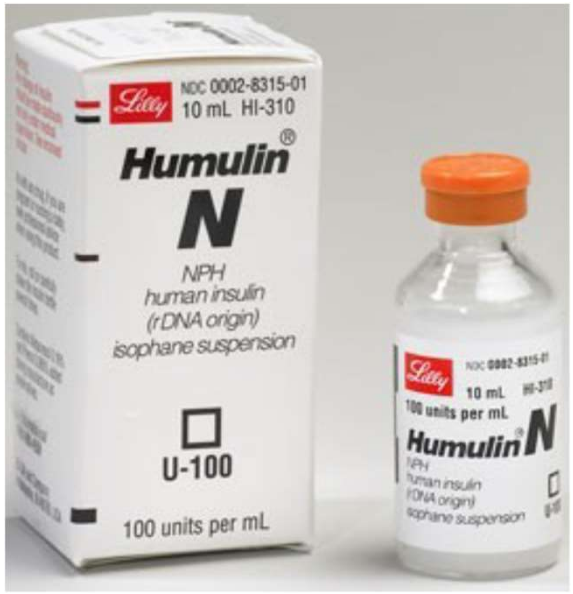
- Recombinant human insulin
- Recombinant human growth hormone
- Recombinant blood clotting factors (VIII, IX, tPA)
- Recombinant hepatitis B vaccine, HPV vaccine
- Cytokines and growth factors (IF, IL)
- Monoclonal antibodies
- Recombinant enzymes
- Recombinant HIV protein for ELISA testing
- Albumin, fibrinolytic and thrombolytic agents

Applications of Recombinant DNA Technology

2) Gene therapy for genetic diseases

3) Food production

4) Plant: genetically modified corn



Gene library

- A DNA library is a collection of cloned restriction fragments of the DNA of an organism

2 types

1. Genomic library

2. cDNA library

Genomic DNA libraries

- Collection of DNA fragments from a particular species.

- Constructed by isolating the entire DNA from a cell which is cut into fragments and cloned in suitable vector.

Gene library for humans

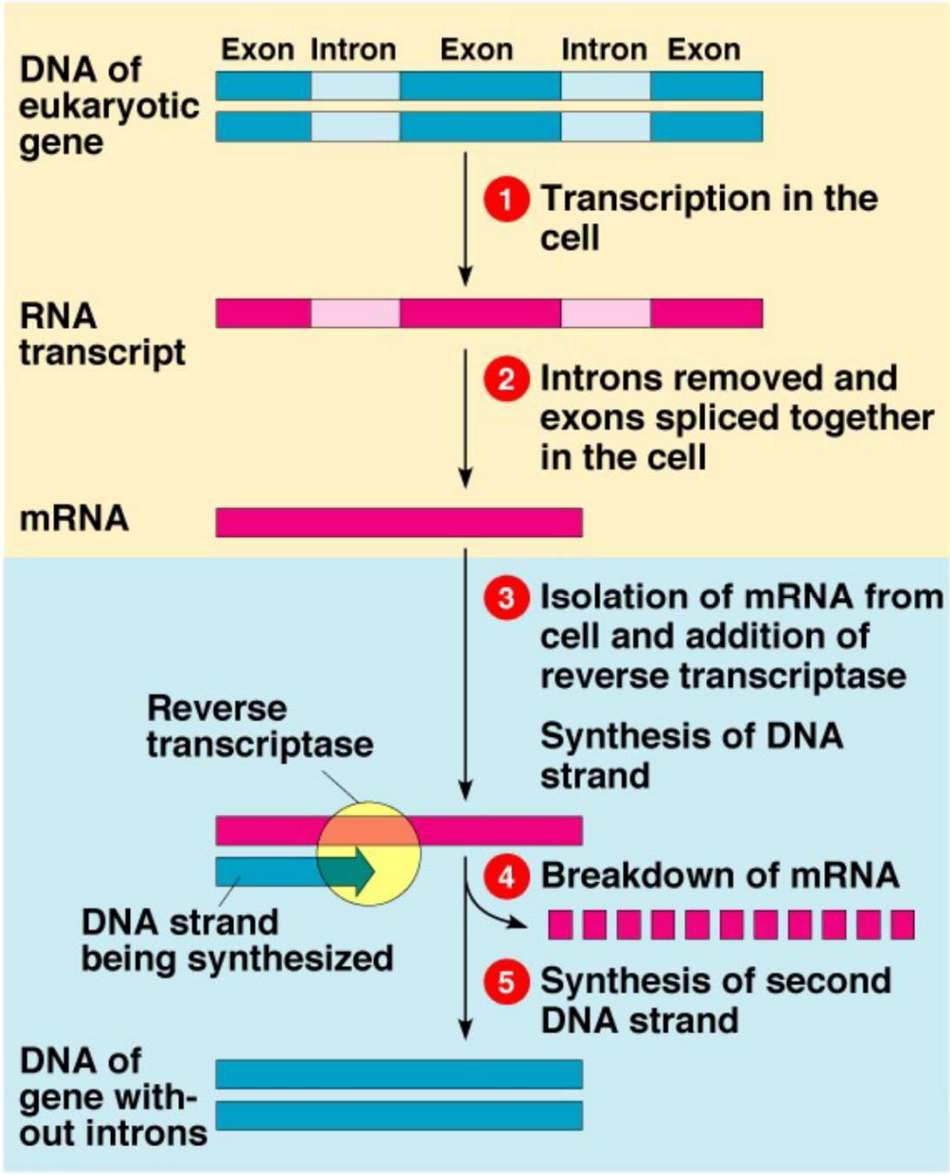
- Each human chromosome, containing approximately 100000 kb can be cut into about 25000 DNA fragments of average size of 4 kb.

- As we have 23 different chromosomes, there are total of 575000 fragments of 4 kb formed.

cDNA library

• cDNA libraries contain those DNA sequences that appear as mRNA molecules, and these differ from one cell type to another.

• This mRNA can be used as a template to make a complementary cDNA molecule using the enzyme reverse transcriptase.


DNA of eukaryotic gene
Exon
Intron
Exon
Intron
Exon
1 Transcription in the cell
RNA transcript
2 Introns removed and exons spliced together in the cell
mRNA
3 Isolation of mRNA from cell and addition of reverse transcriptase
Reverse transcriptase
Synthesis of DNA strand
4 Breakdown of mRNA
DNA strand being synthesized
5 Synthesis of second DNA strand
DNA of gene without introns
©Addison Wesley Longman, Inc.

BLOTTING TECHNIQUES

• Blots are techniques for transferring DNA , RNA and proteins onto a carrier so they can be separated, and often follows the use of a gel electrophoresis.

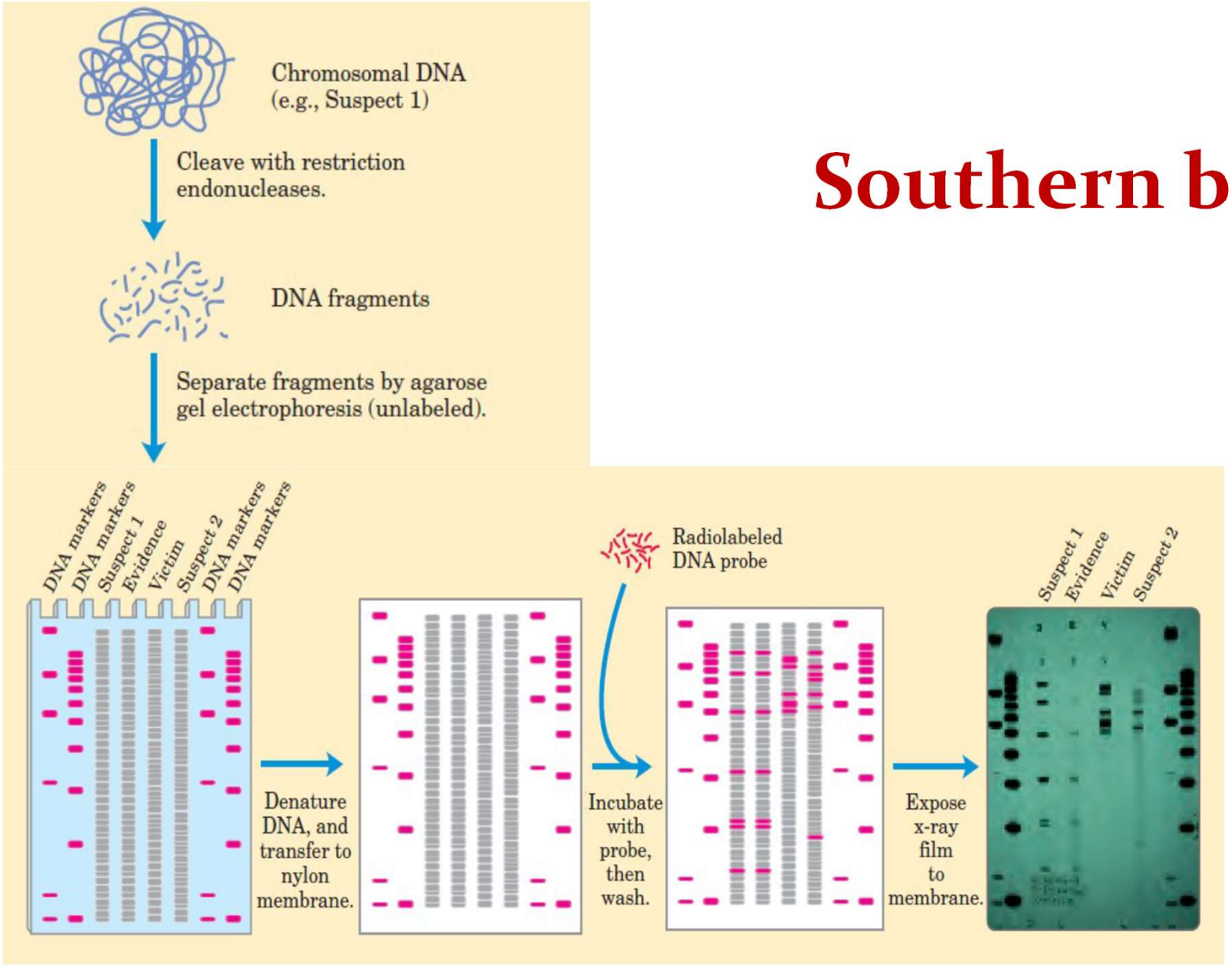
• The Southern blot is used for transferring DNA, the Northern blot for RNA and the western blot for PROTEIN.

Southern blotting

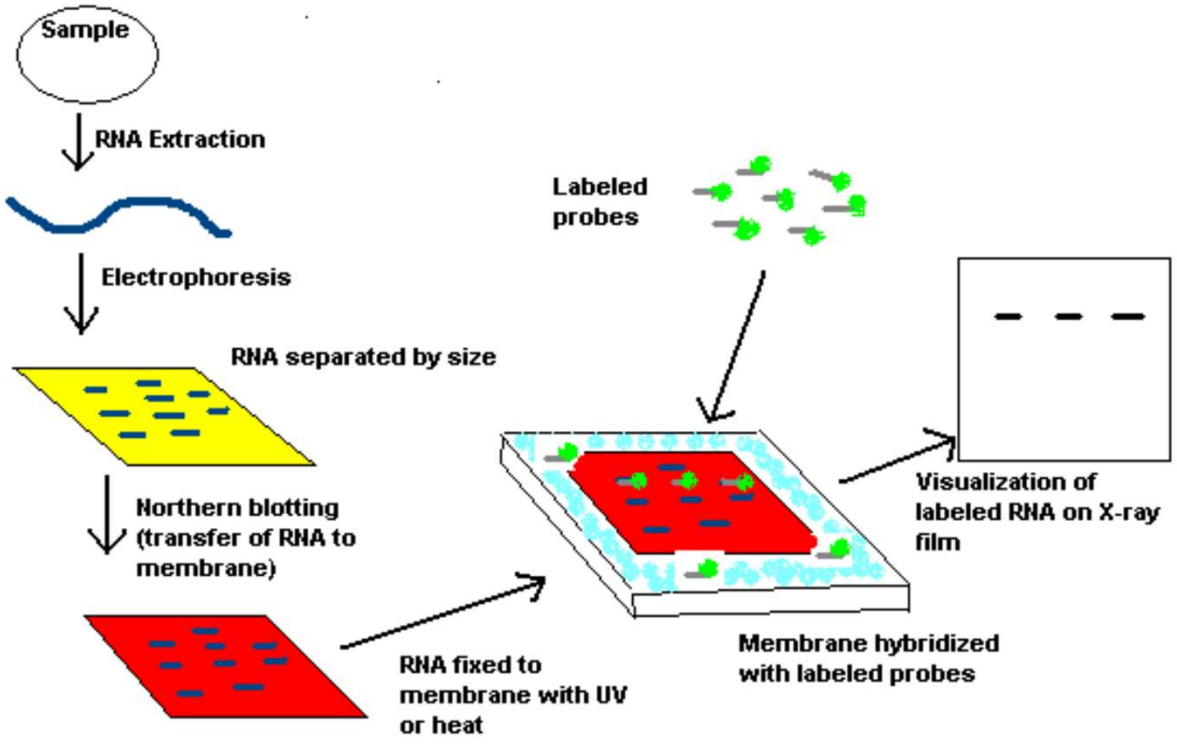
- Named after scientist Edward Southern who developed it in 1975

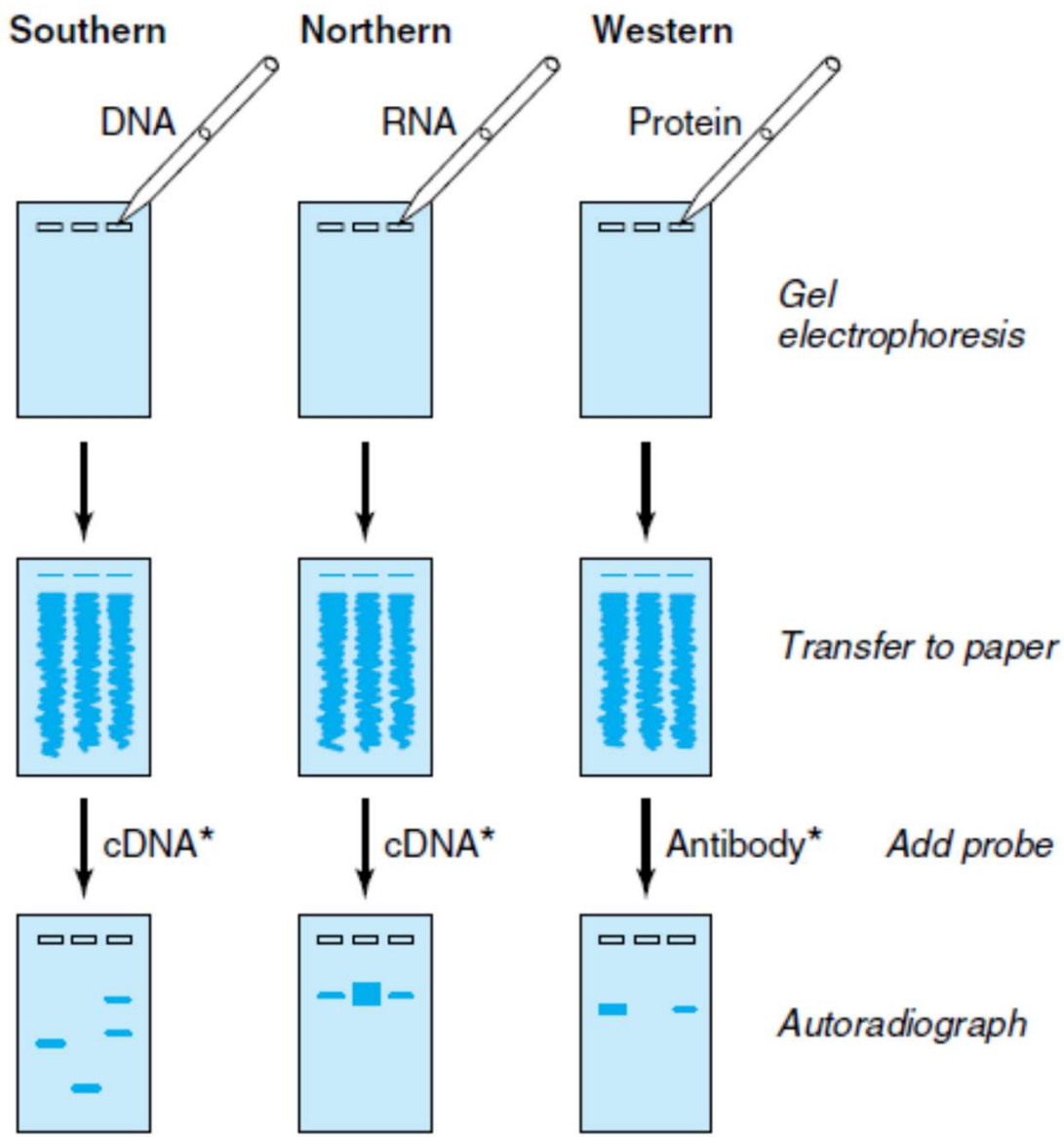
- Other names are laboratory jargons which are now accepted eg. Northern and western blotting

Southern blotting



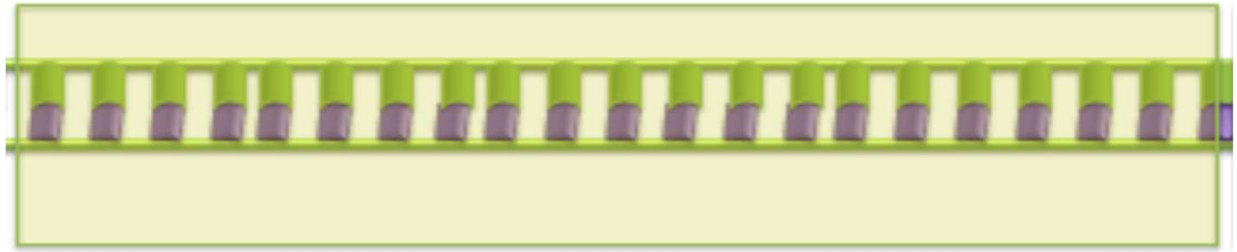
Northern Blotting




Southern
Northern
Western
DNA
RNA
Protein
Gel electrophoresis
Transfer to paper
cDNA*
cDNA*
Antibody*
Add probe
Autoradiograph

Polymerase Chain Reaction (PCR)

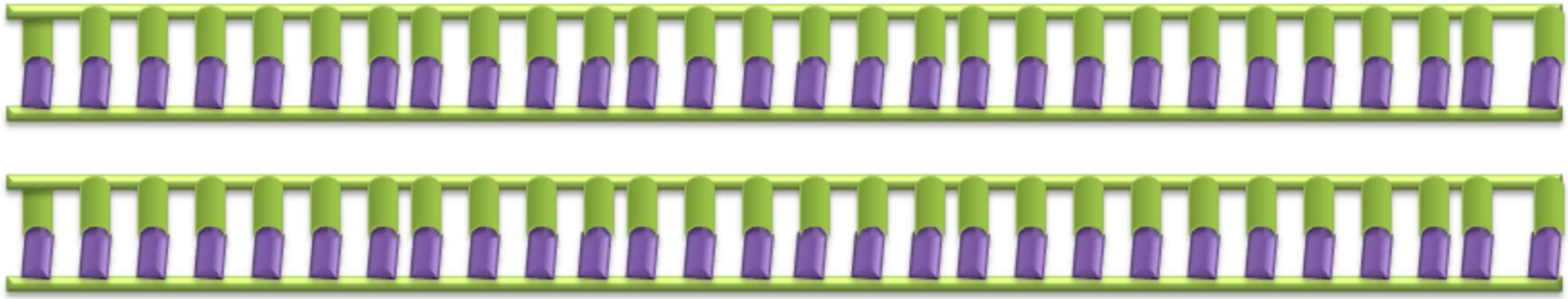
- PCR is an *in vitro* technique for the amplification of a region of DNA.
- Cell free amplification technique.
- Developed by Kary Mullis in the 1980s



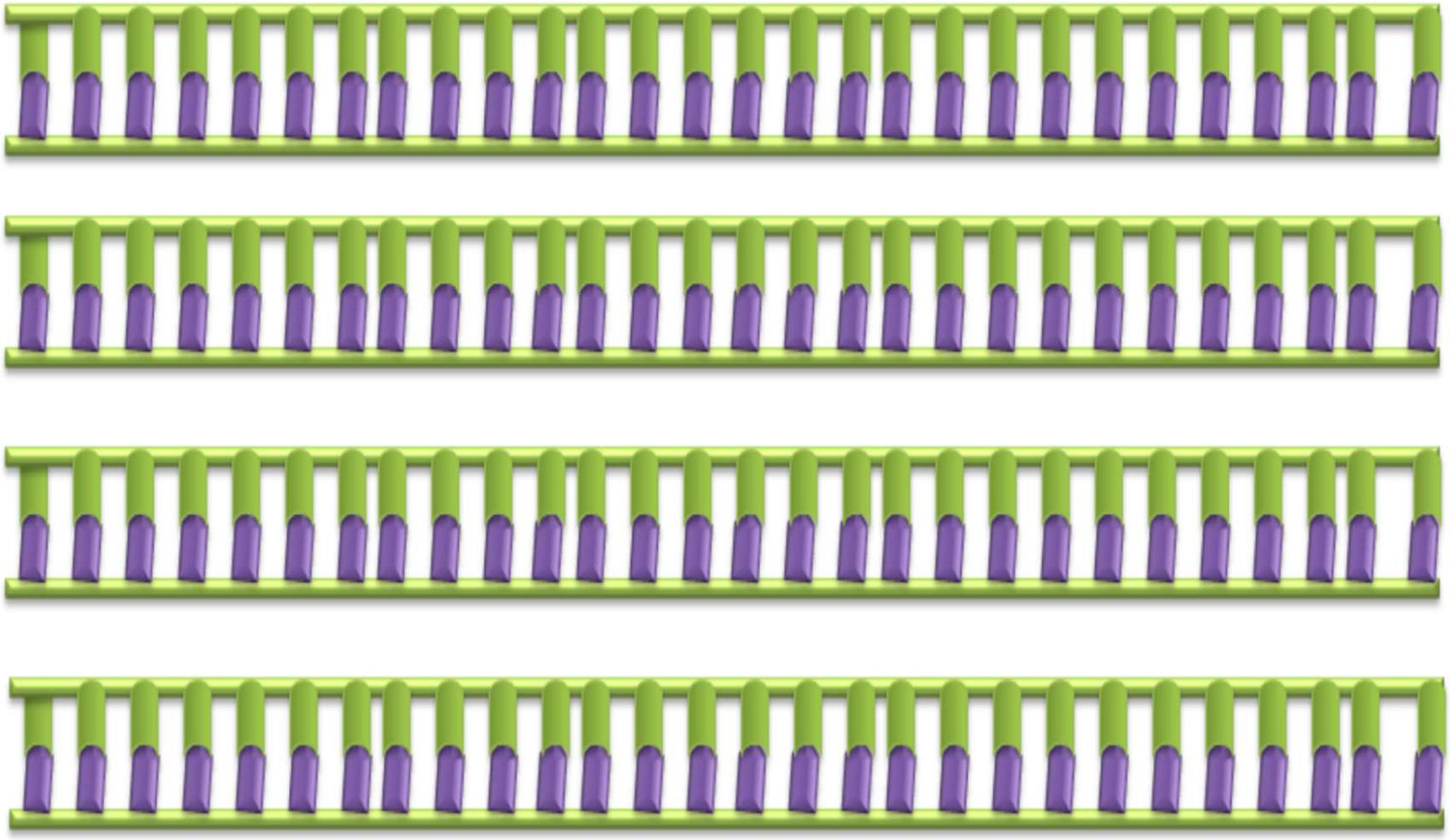
Principle

- Double stranded DNA of interest is denatured to separate strands.
- Each strand is then allowed to hybridize with a primer.
- The primer template duplex is used for synthesis.
- This three steps: **Denaturation, anneling and extension** repeated again and again to generate multiple forms of target DNA.
- The primer extension product synthesized in 1 cycle serve as template for next cycle.

1 cycle = 2 copies



2 cycle 4 copies



TYPES OF PCR

- REAL-TIME PCR
- NESTED PCR
- INVERSE PCR
- REVERSE TRANSCRIPTION PCR
- ASYMMETRIC PCR
- MULTIPLEX PCR

Applications of PCR

1. Diagnosis:

- Bacterial and viral diseases eg., TB, Hepatitis HIV etc.

2. Medicolegal cases:

DNA amplification from hair follicles and blood sample followed by RFLP

3. Diagnosis of genetic disorders:

Thalassemia, cystic fibrosis

4. Prenatal diagnosis of inherited disorders

5. Cancer detection:

- i. To monitor residual abnormal cells present in treated patients.
- ii. Identification of mutation in oncosuppressor genes eg., p53

6. Fossil studies:

To study evolution by comparing the sequences in the extinct and living organisms

THANK YOU

HAPPY TO ANSWER IF YOU HAVE ANY QUERIES.....?