



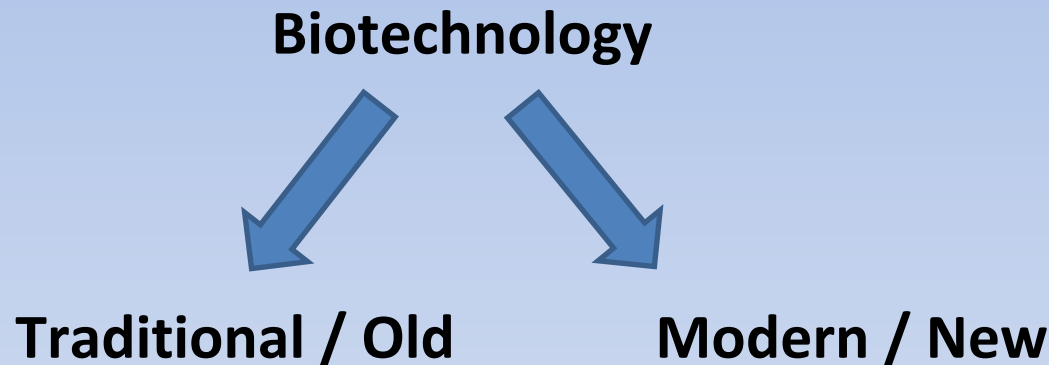
BIOTECHNOLOGY

---- The Basic Principles

Pratim Chaudhuri,
Allen Career Institute

WHAT IS

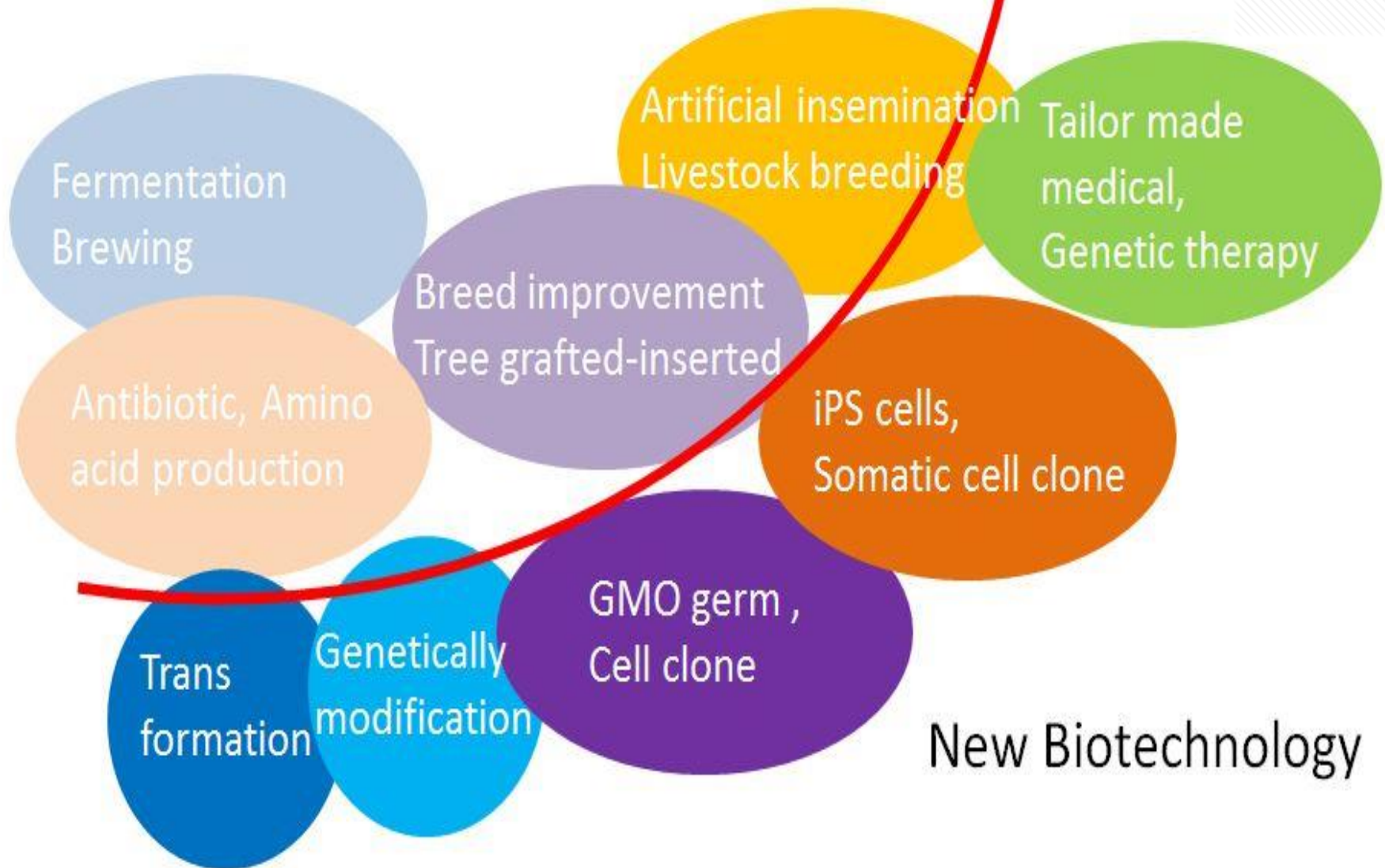
According to **European Federation of Biotechnology**,
"Biotechnology is the integration of natural science and organisms, cells, parts thereof and molecular analogues for products and services."



Principles of Biotechnology:

- (i) Genetic engineering or Recombinant DNA Technology (RDT).
- (ii) Maintenance of Microbial contamination free (sterile) surrounding in chemical engineering

Old Biotechnology



TRADITIONAL BIOTECHNOLOGY



MODERN BIOTECHNOLOGY

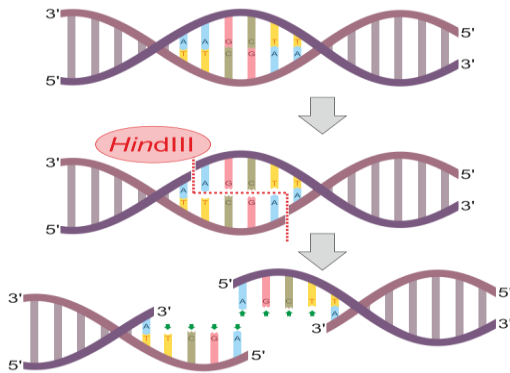
ENZYMES IN RECOMBINANT DNA TECHNOLOGY

I. Nucleases

a) Restriction Endonucleases

b) Restriction Exonucleases

c) Ribonuclease H



II. DNA Modifiers

a) DNA polymerase

b) Reverse transcriptase

c) Alkaline phosphatase

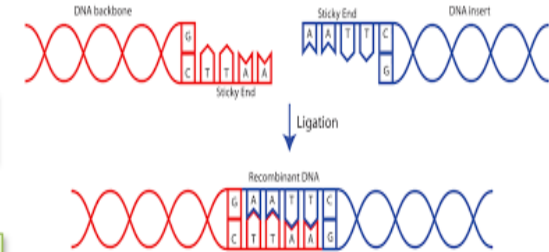
d) Polynucleotide kinase

e) Terminal nucleotidyl transferase

f) Methyl transferase

III. DNA Ligases

a) DNA ligase



Action of Different Nucleases

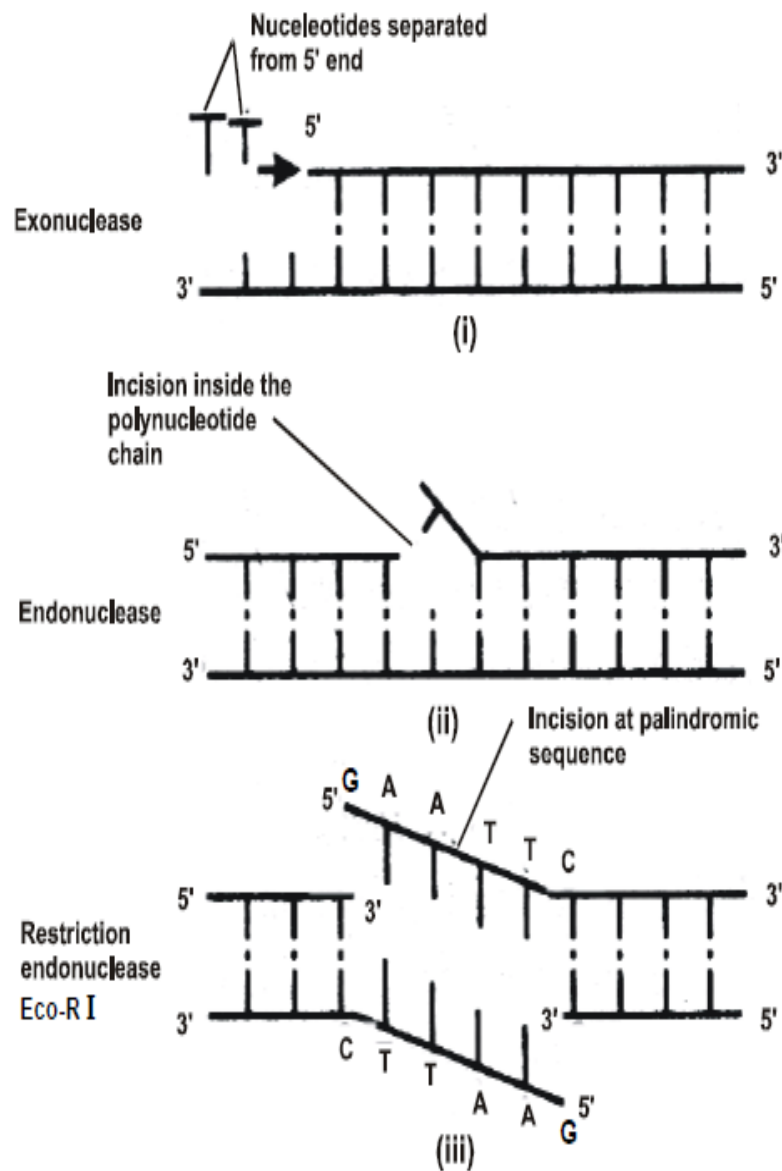


Fig:- (i), Action of exonuclease (ii), Action of endo nuclease (iii), Action of restriction enzyme.

Different R.E. Used in RDT

Some restriction enzymes, their origin and recognition sites

Enzyme	Organism	Recognition sequence (one strand mentioned)
<i>EcoRI</i>	<i>Escherichia coli</i> Ry13	5'G↓AATTC3'
<i>BamHI</i>	<i>Bacillus amyloliquefaciens</i>	5'G↓GATCC3'
<i>BglII</i>	<i>Bacillus globigii</i>	5'A↓GATCT3'
<i>HindIII</i>	<i>Haemophilus influenzae</i> R _d	5'A↓AGCTT3'
<i>HinfI</i>	<i>Haemophilus influenzae</i> R _i	5'G↓ANTC3'
<i>Sau3A</i>	<i>Staphylococcus aureus</i>	5'↓GATC3'
<i>AluI</i>	<i>Arthobacter luteus</i>	5'AG↓CT3'
<i>HaeIII</i>	<i>Haemophilus aegyptius</i>	5'GG↓CC3'
<i>TaqI</i>	<i>Thermus aquaticus</i>	5'T↓CGA3'
<i>NotI</i>	<i>Nocardia otitidis-caviarum</i>	5'GC↓GGCCGC3'
<i>HpaII</i>	<i>Haemophilus parainfluenzae</i>	5'C↓CGG3'
<i>PstI</i>	<i>Providentia stuartii</i>	5'CTGCAG3'
<i>SmaI</i>	<i>Serratia marcescens</i>	5'CCC↓GGG3'
<i>XmaI</i>	<i>Xanthomonas malvacearum</i>	5'C↓CCGGG3'

TYPES OF RESTRICTION ENDONUCLEASES

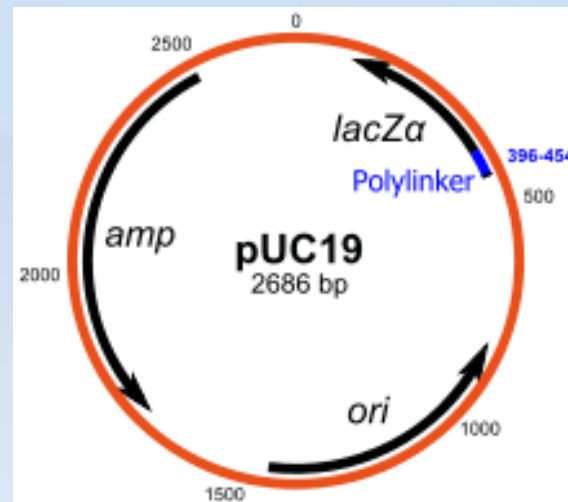
PROPERTY	TYPE I	TYPE II	TYPE III
Enzyme activities and structure	Single multisubunit, having both endonuclease and methylase functions, Large size	Separate endonuclease and methylase enzymes, Small size	Separate subunits for restriction and methylation
Recognition Sequence	Assymetric Bipartite	Palindromic with dyad symmetry	Assymetric Unipartite
Recognition site length	Long, 8-16bp	Short, 4-9bp	Short, 5-7bp
Methylation site	Recognition Site	Recognition Site	Recognition Site
Requirements for Restriction	ATP , Mg^{2+} , SAM	Mg^{2+}	ATP , Mg^{2+} , SAM (optional)
Cleavage Site	Random 1000bp away from Recognition Site	At or Near Recognition Site	≈25bp downstream of Recognition Site
Examples	EcoK , EcoB	EcoRI , EcoRV	EcoPI , EcoP15

Only Type II Restriction Endonucleases are used in RDT for their accuracy

Cloning Vector



- The molecular analysis of DNA has been made possible by the cloning of DNA. The two molecules that are required for cloning are the **DNA to be cloned** and a **cloning vector**.
- A **cloning vector** is a **small piece of DNA** taken from a **virus**, a **plasmid** or the **cell of a higher organism**, that can be **stably maintained** in an organism and into which a foreign DNA fragment can be inserted for cloning purposes.
- Most vectors are **genetically engineered**.
- The cloning vector is chosen according to the **size and type** of DNA to be cloned.



Types of Cloning Vectors

Maximum DNA insert possible
with different cloning vectors

Vector	Host	Insert size
λ phage	<i>E. coli</i>	5–25 kb
λ cosmids	<i>E. coli</i>	35–45 kb
P1 phage	<i>E. coli</i>	70–100 kb
BACs	<i>E. coli</i>	≤ 300 kb
YACs	<i>Saccharomyces urocerevisae</i>	200–2000 kb

- **Plasmid** - an extrachromosomal circular DNA molecule that autonomously replicates inside the bacterial cell; cloning limit: 100 to 10,000 base pairs or 0.1-10 kilobases (kb)
- **Phage** - derivatives of bacteriophage lambda; linear DNA molecules, whose region can be replaced with foreign DNA without disrupting its life cycle; cloning limit: 8-20 kb
- **Cosmids** - an extrachromosomal circular DNA molecule that combines features of plasmids and phage; cloning limit - 35-50 kb
- **Bacterial Artificial Chromosomes (BAC)** - based on bacterial mini-F plasmids. cloning limit: 75-300 kb
- **Yeast Artificial Chromosomes (YAC)** - an artificial chromosome that contains telomeres, origin of replication, a yeast centromere, and a selectable marker for identification in yeast cells; cloning limit: 100-1000 kb



Features of a Cloning vector

(i) Origin of replication (ori):

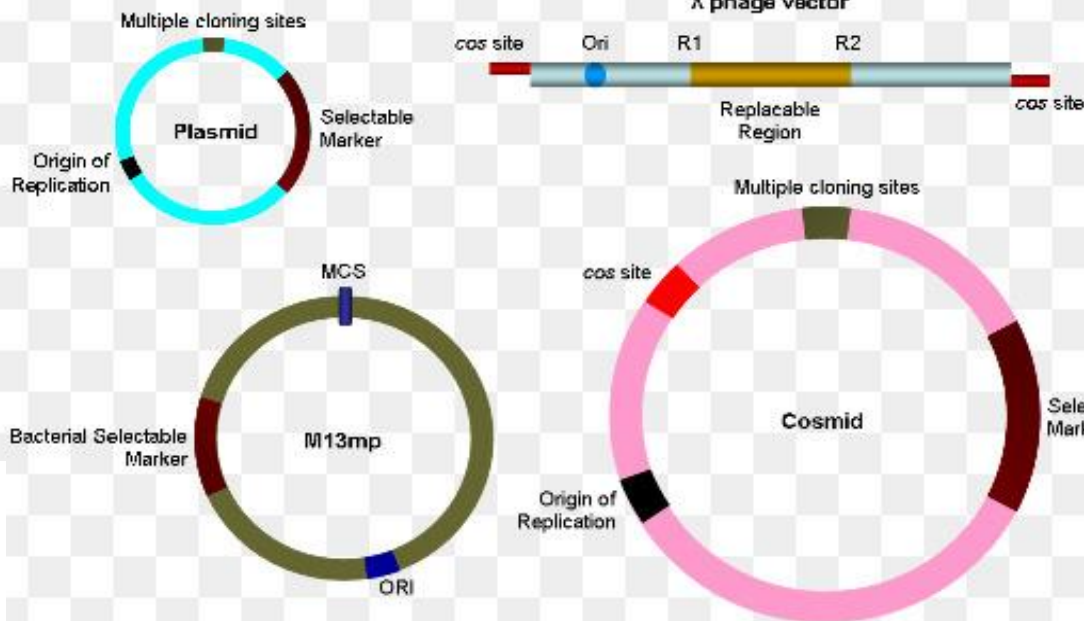
It has specific sequence from where replication starts if any piece of foreign DNA is linked to this sequence.

(ii) Selectable marker:

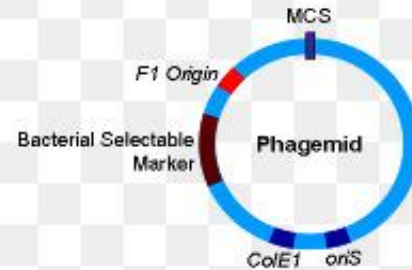
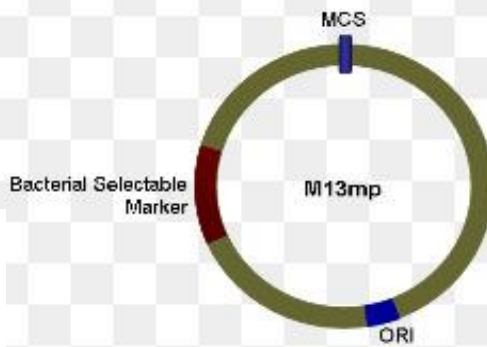
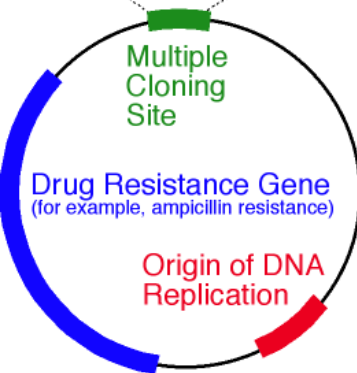
The vector also requires **to identify and eliminate non-transformants and selectively permit the growth of the transformants.** Genes encoding resistance to antibiotics such as Tetracycline, ampicillin, kanamycin or chloramphenicol are useful in selectable markers for *E.coli*.

(iii) Recognition Sites (Multiple Cloning site):

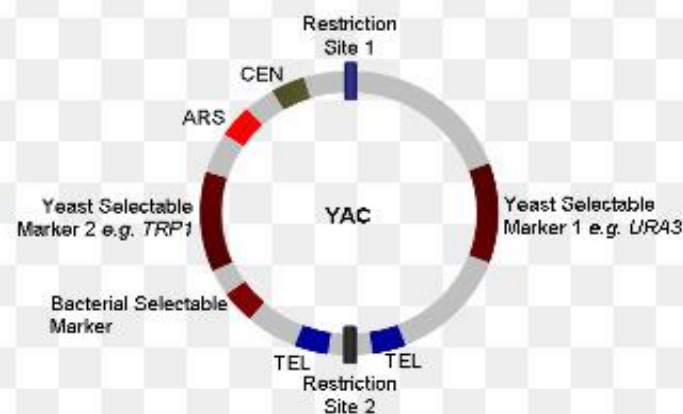
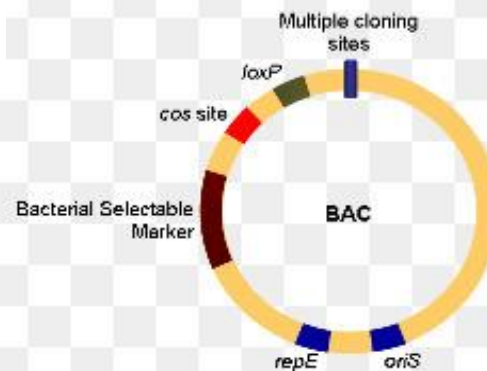
Vector bears one or more unique restriction endonuclease recognition sites which enable foreign DNA to be inserted into the vector during the formation of recombinant DNA molecule.



EcoRI Sall KpnI BamHI PstI
HindIII XhoI PvuII EcoRV BglII



**Schematic diagram
of different vectors
used in RDT**



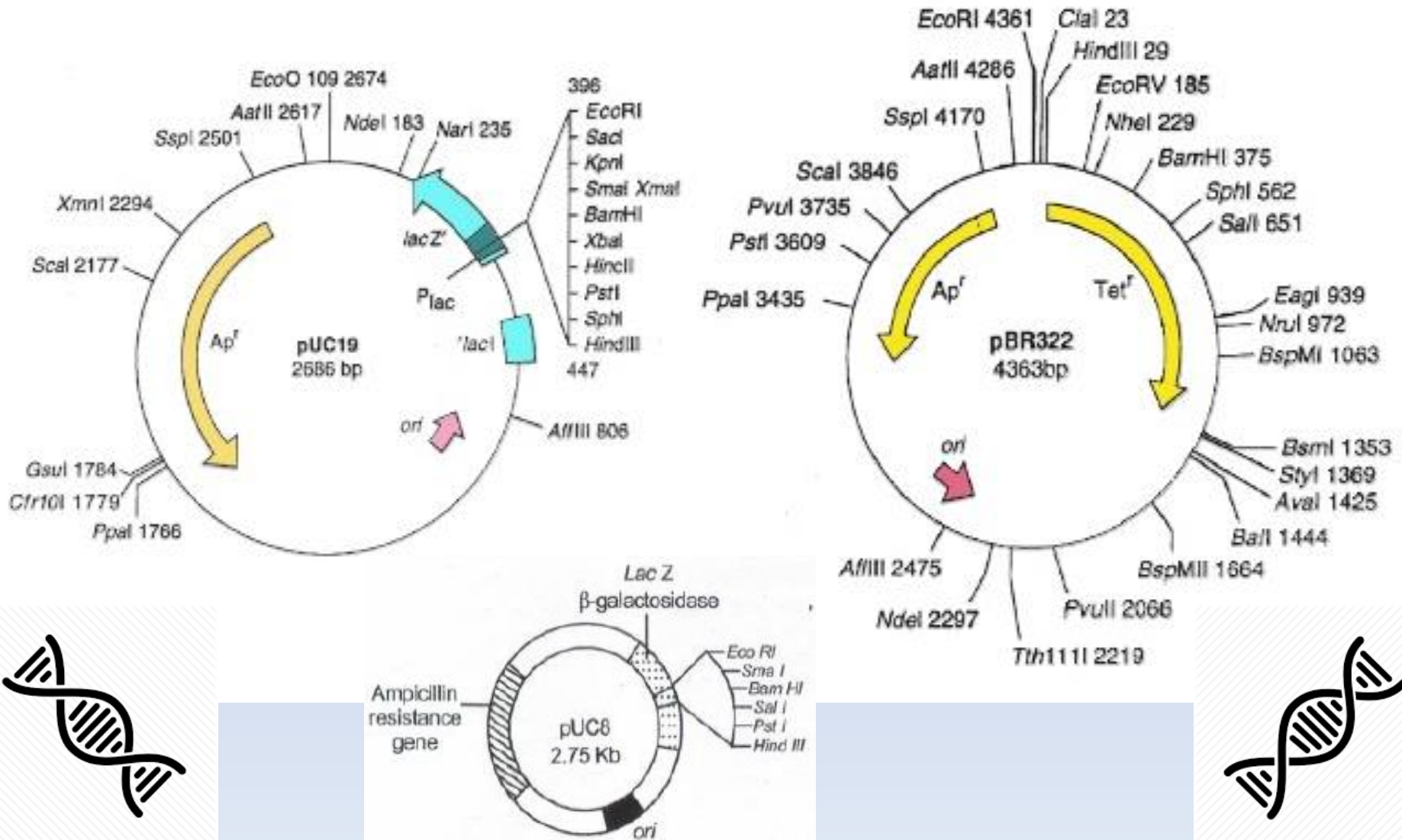
Types of Vectors



Plasmid vectors

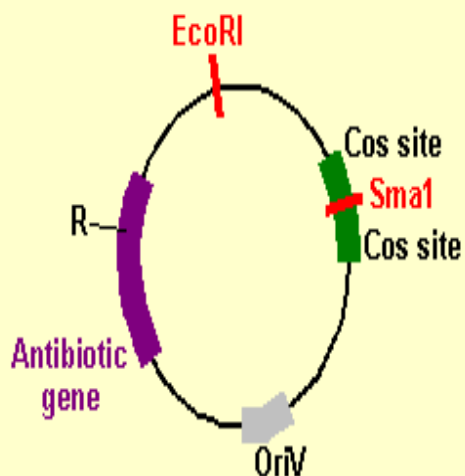


Examples of some important plasmid vectors used in RDT



Features	Cloning Vector	Expression Vector
Definition	A cloning vector is a small piece of DNA, which is used to introduce the foreign gene of interest into the host cell. It can be stably maintained within the host organism.	An expression vector is a plasmid that not only introduces a gene of interest into host organism but also aids in the analysis of the gene of interest via relevant protein product expression.
Role	It is used to obtain multiple copies of the foreign gene of interest.	It is used to obtain or analyse the gene product, which may be RNA or protein, of the inserted gene of interest.
Types	They can be plasmids, cosmids, and phages, BACs, YACs or MACs.	Only a plasmid can be an expression vector.
Features	A typical cloning vector consists of an origin of replication, unique restriction sites, reporter gene and an antibiotic resistance site.	An expression vector consists of regulatory elements like enhancers, promoters, termination sequences, transcription initiation site and translation initiation sites, in addition to having all the features of a typical cloning vector.

Basic Features of a Cosmid



KEY

OriV - origin of replication.
 Cos sites - provide blunt ends.
 R - recombinant site
 EcoRI } - Restriction endonuclease
 SmaI } recognition sequence.

Bacteriophage vectors

Advantages:

- Useful for cloning large DNA fragments (10 - 23 kb)
- Inherent size selection for large inserts

Disadvantages:

- Less easy to handle

Uses of Bacteriophages:

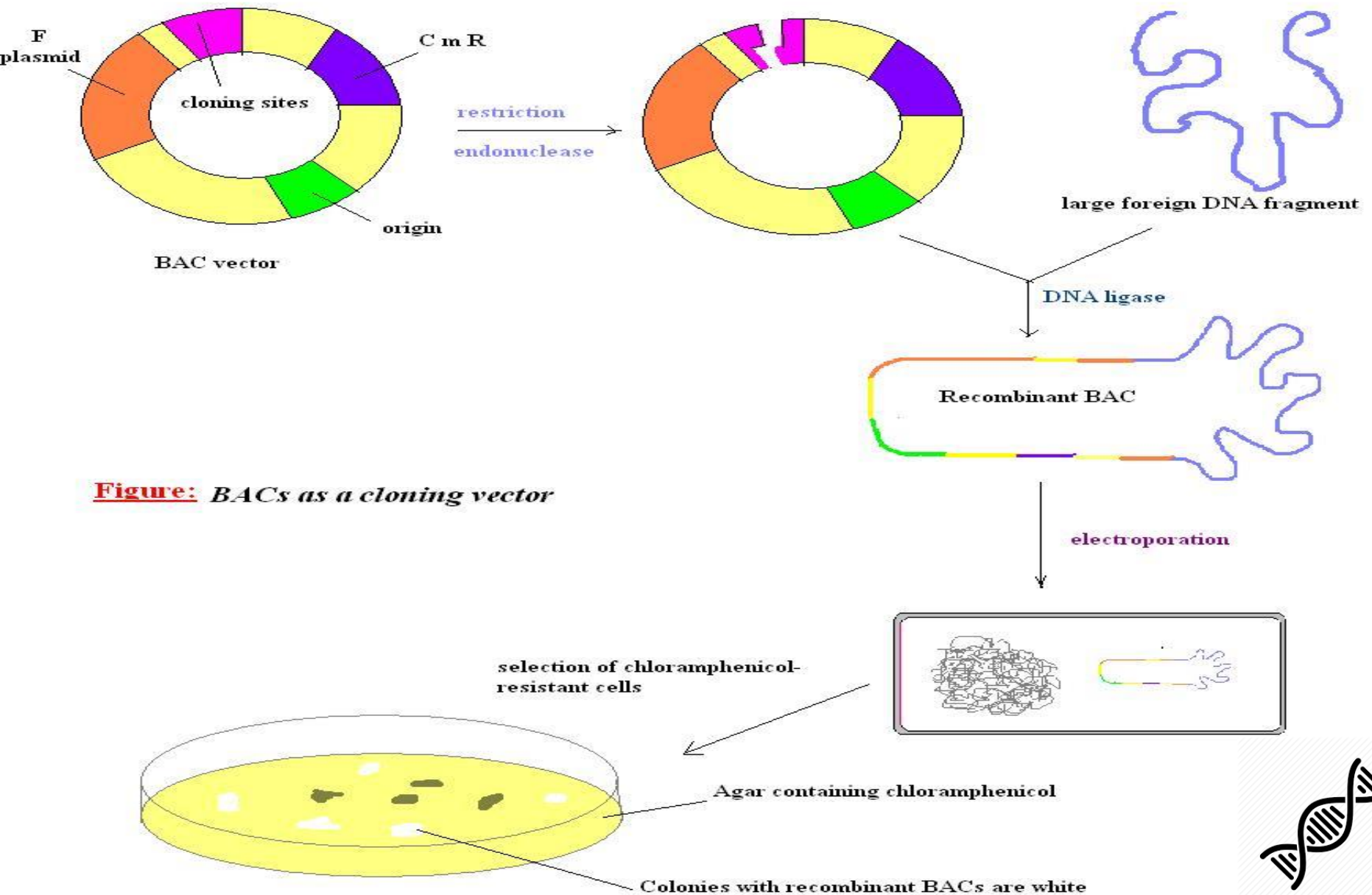
Lambda phage cloning vectors:

- For gene cloning of large DNA fragments (eukaryotic genes)
- Excellent selection capability (stuffer stuff)
- Clone lots of precisely-sized DNA fragments for library construction

M13 based cloning vectors:

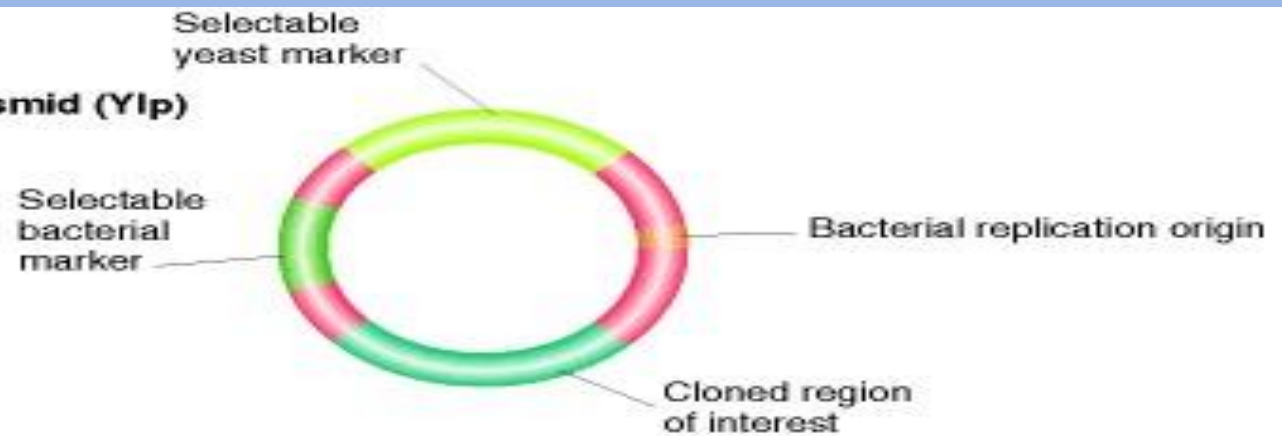
- Single-stranded DNA
- Sequencing
- Site-directed mutagenesis

Bacterial Artificial Chromosomes (BACs)



Yeast Artificial Chromosomes

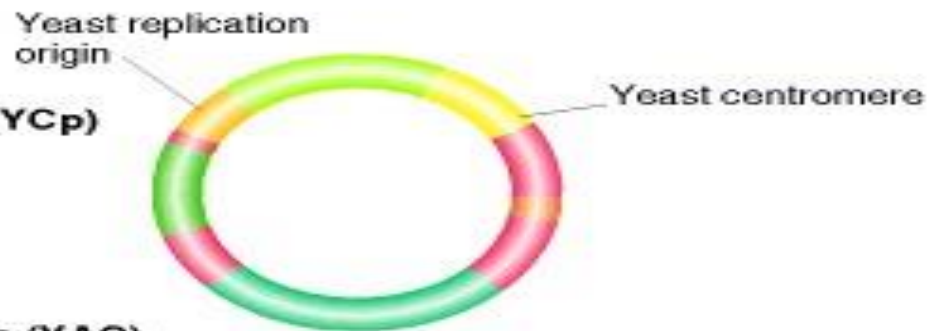
(a) Yeast integrative plasmid (YIp)



(b) Yeast episomal plasmid (YEp)



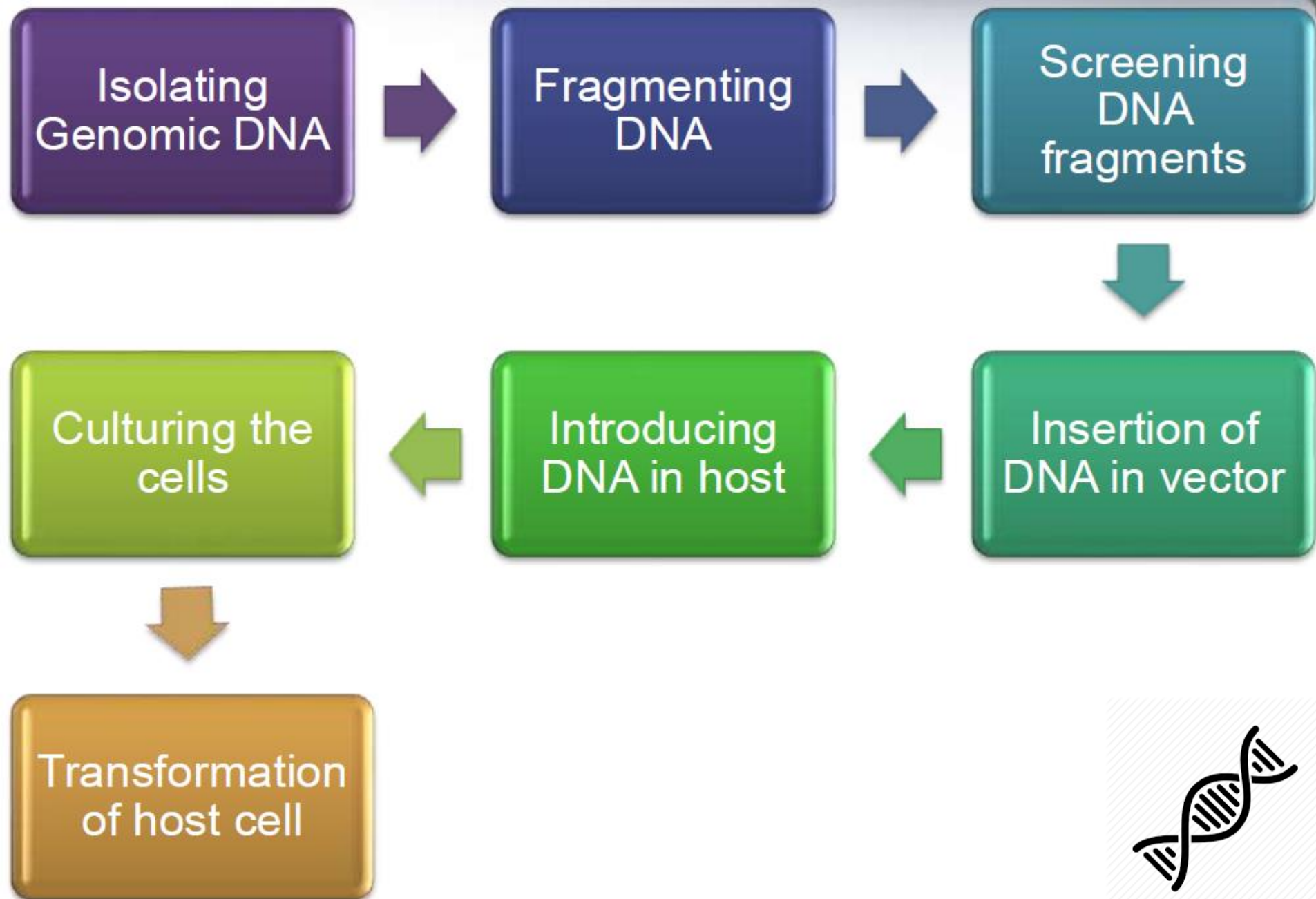
(c) Yeast centromere plasmid (YCp)



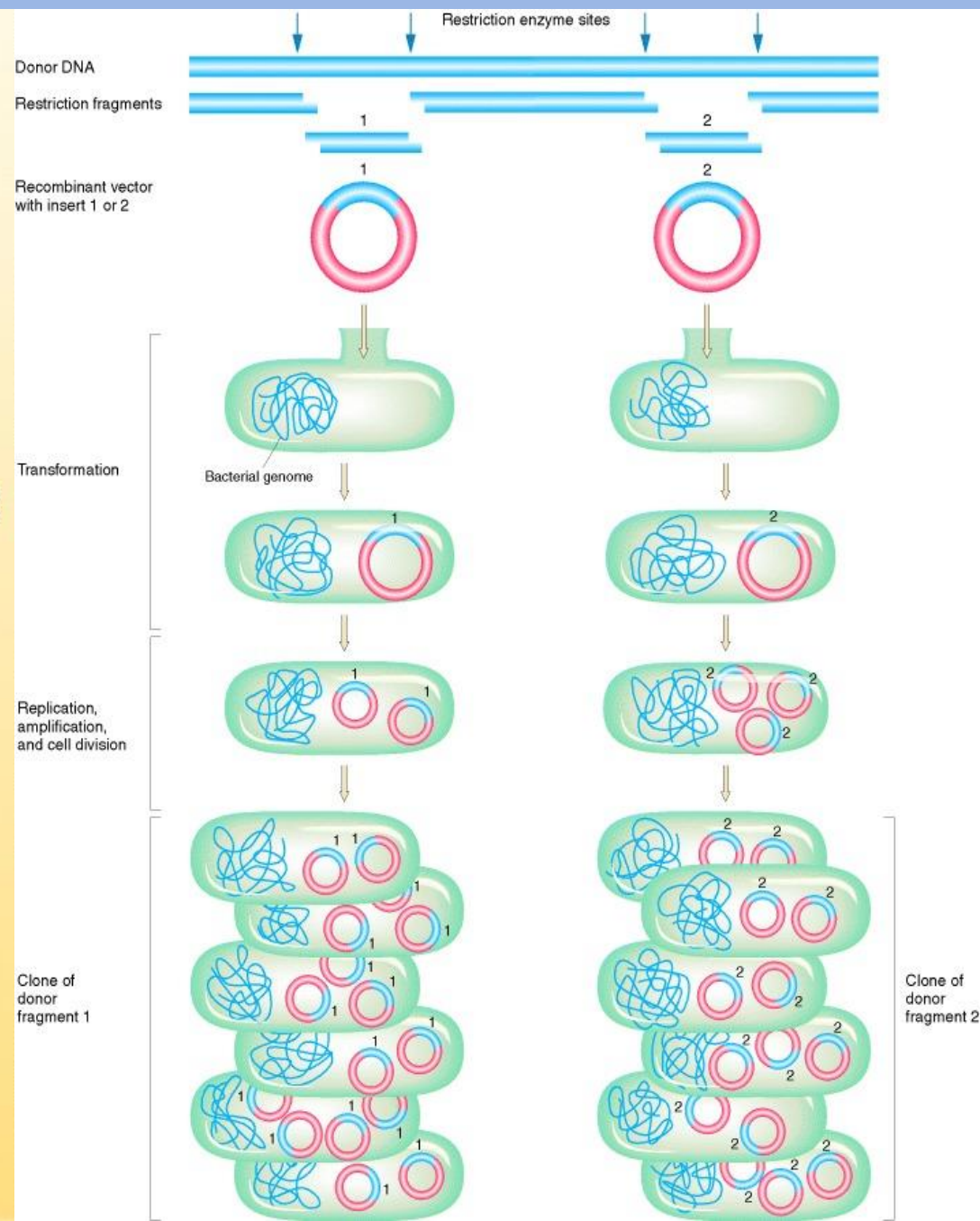
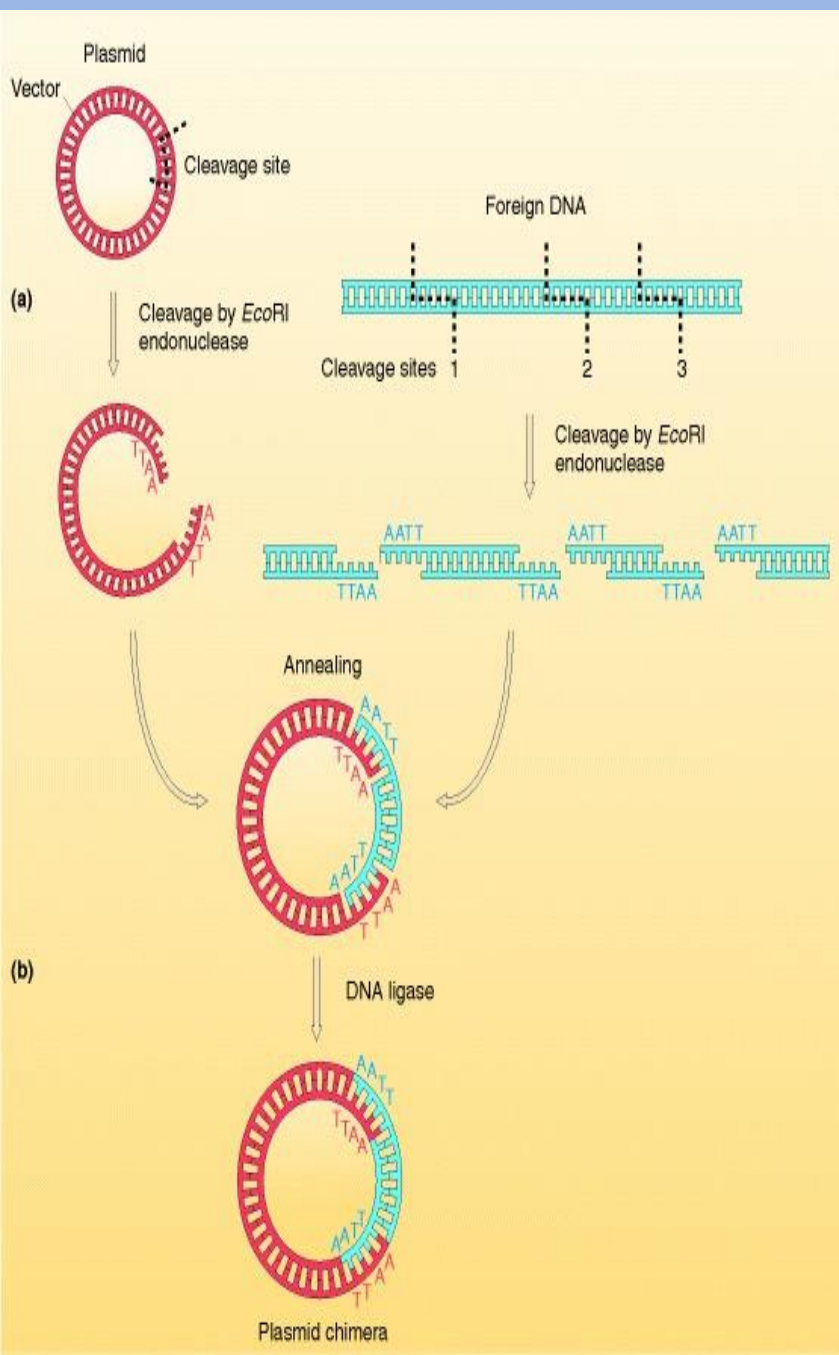
(d) Yeast artificial chromosome (YAC)



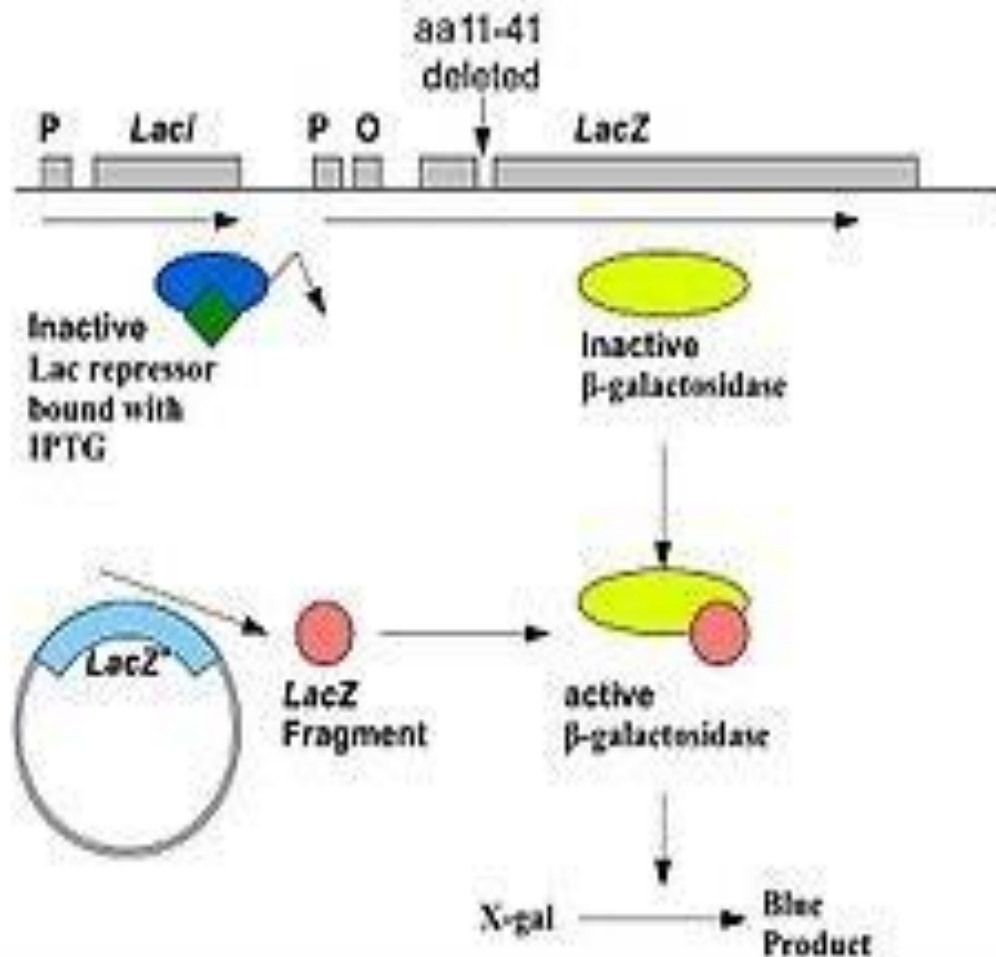
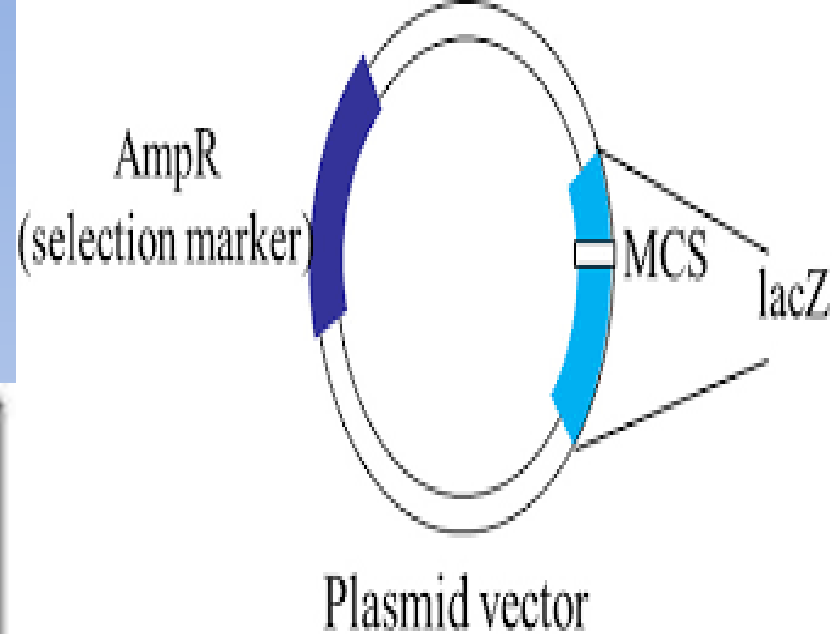
Mechanism of Molecular Cloning in RDT



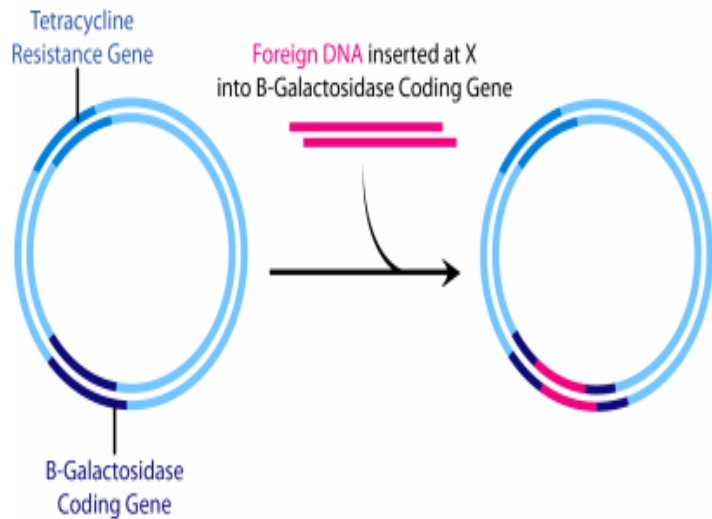
Schematic diagram of the basic concept of Molecular cloning



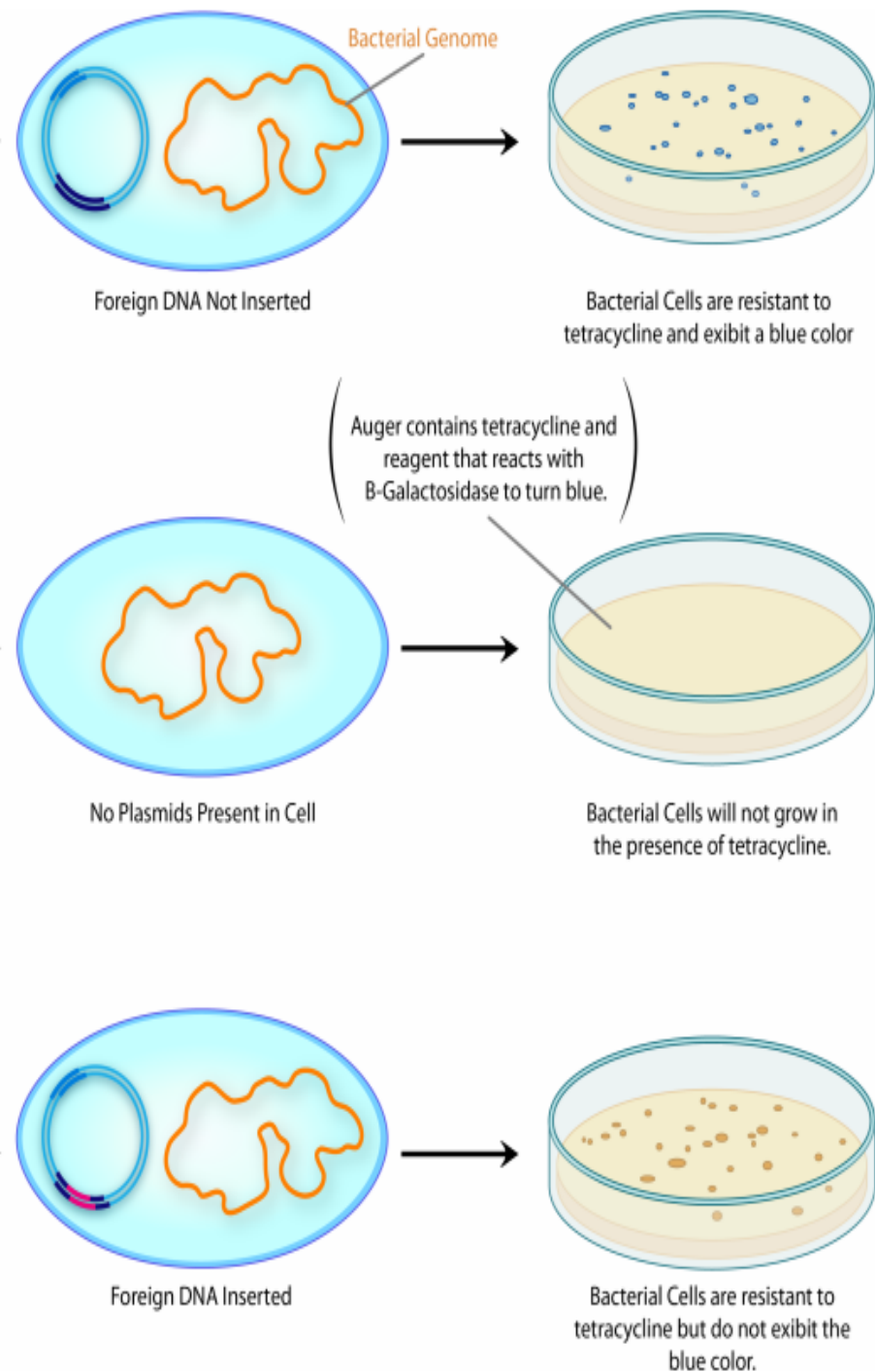
Selection of Transformants – Concept of BLUE-WHITE SELECTION by insertional inactivation of *lacZ* gene

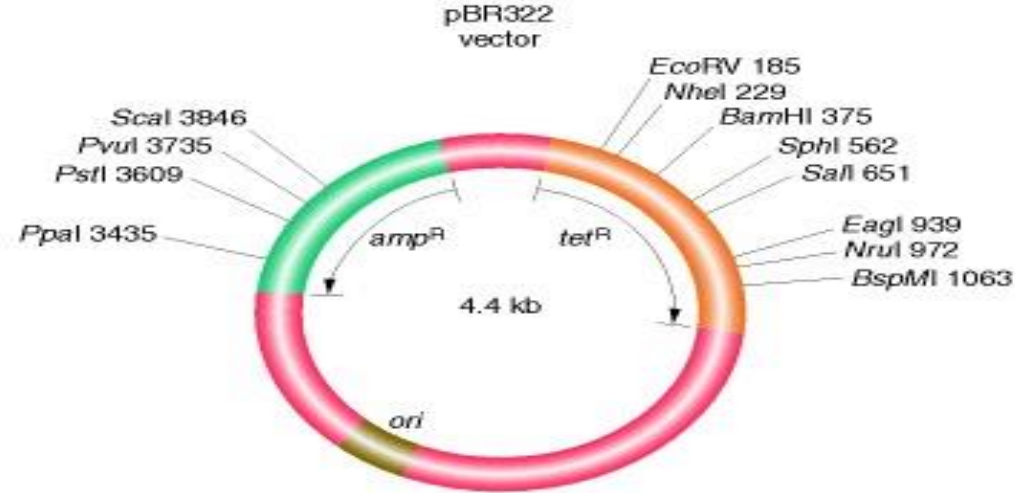


Procedure of screening by Blue-White Selection



Mix Plasmids into Bacterial Cells





Cut foreign DNA and vector with, say, *SalI*

Transform bacteria

Plate on ampicillin

Amp^R Tet^R

Amp^R Tet^S

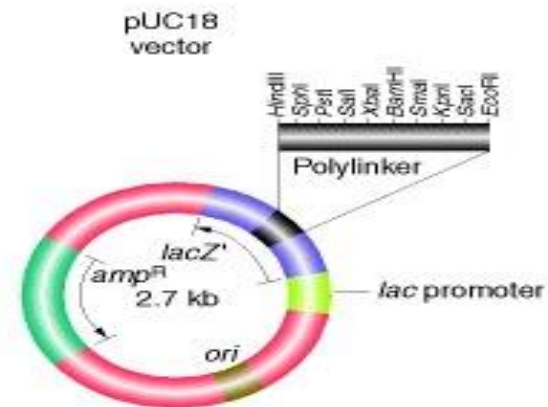


No insert



Insert

Cloning in pBR322 and pUC18 and selection of clones by Blue- White Selection



Cut foreign DNA and vector with, say, *XbaI*

Transform bacteria

Plate on ampicillin and X-Gal

Blue

White

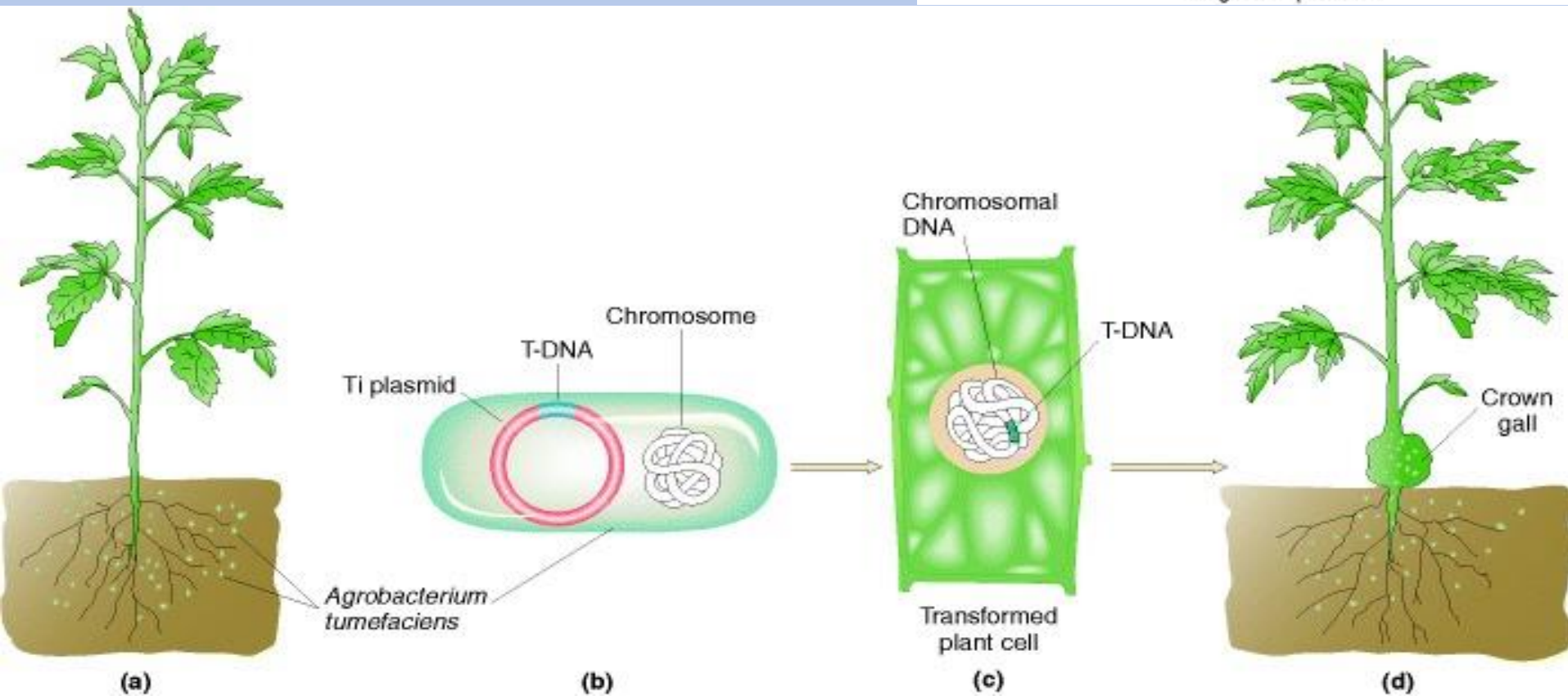
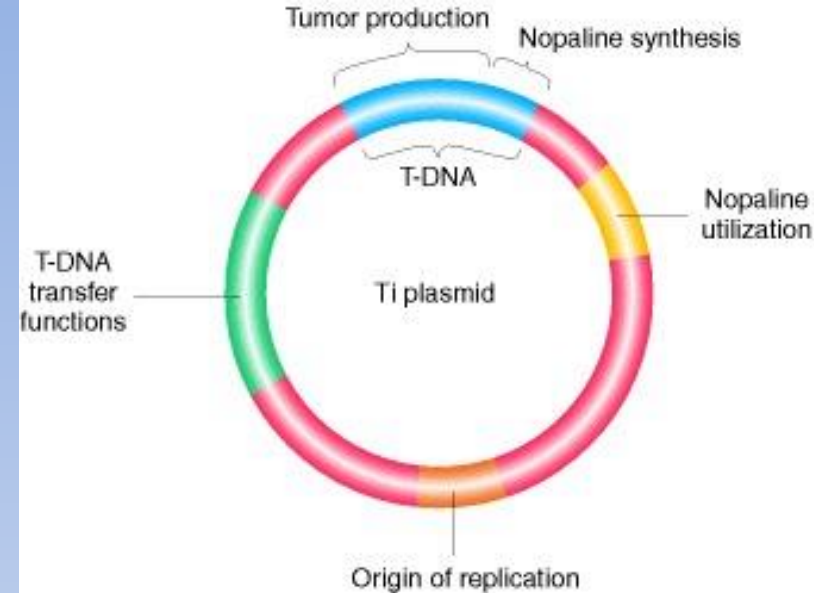


No insert

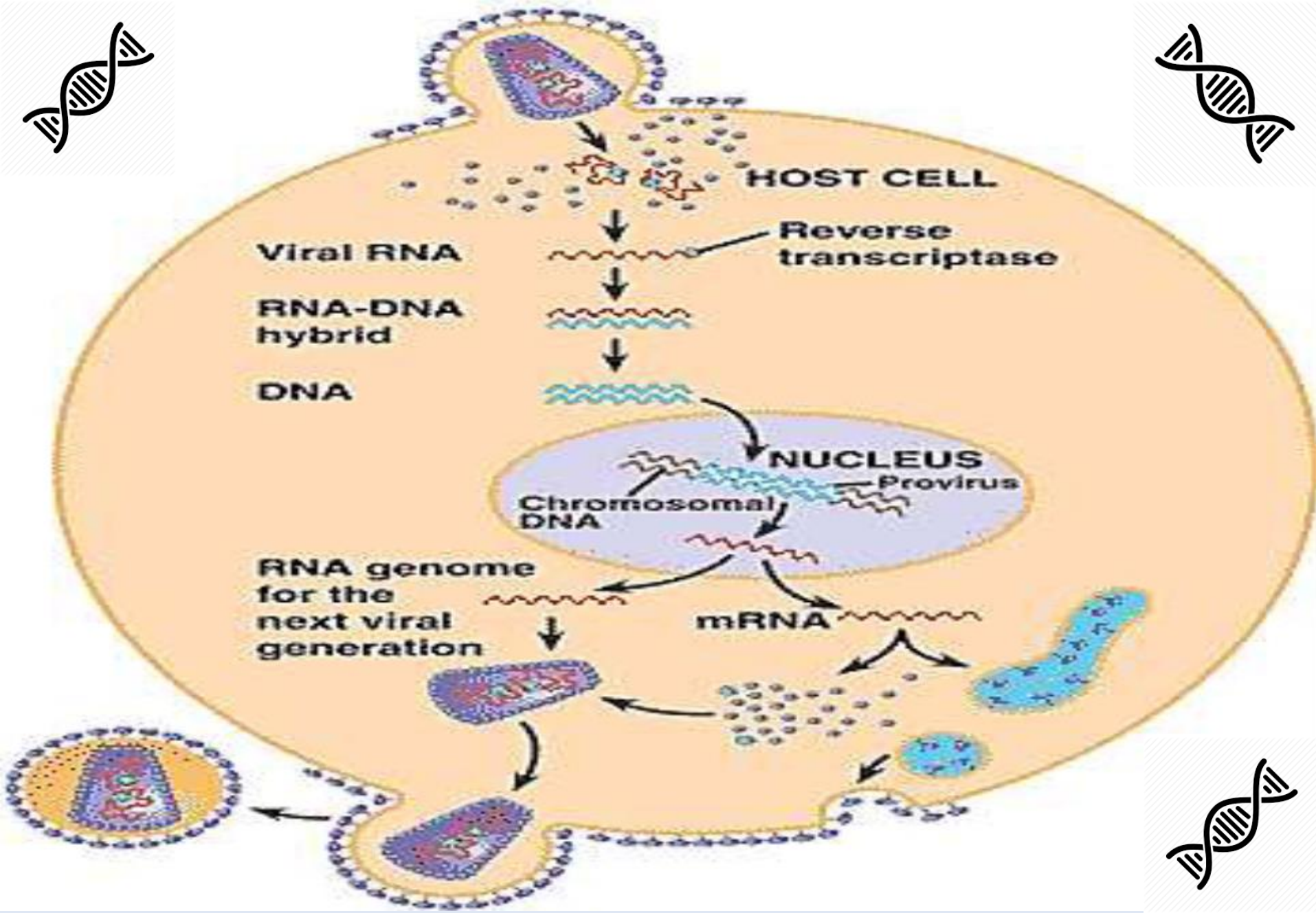


Insert

Vectors for Cloning genes in plants – **Ti plasmid**

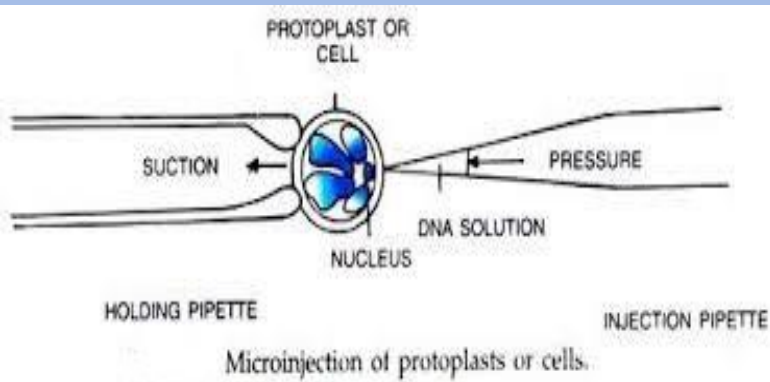


Retroviral vector for transferring genes to animal cells

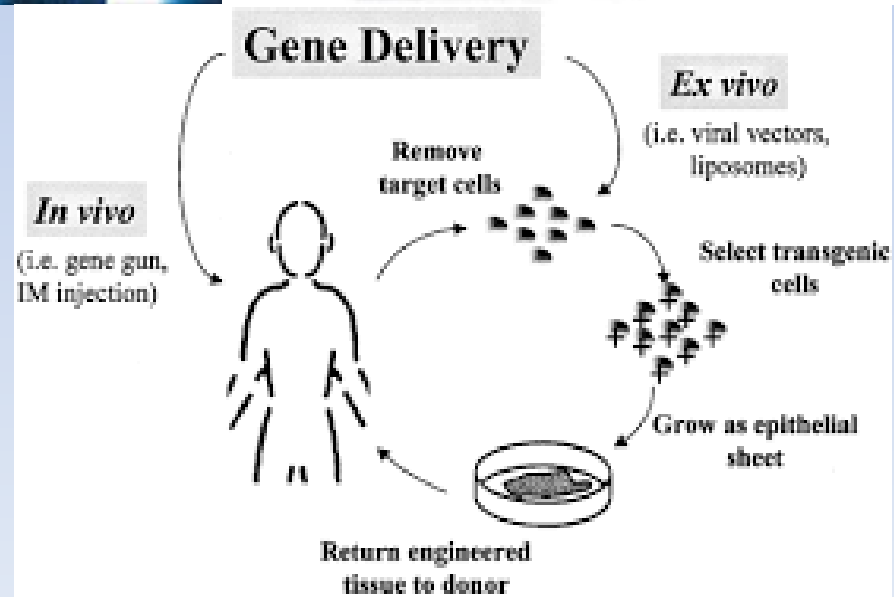
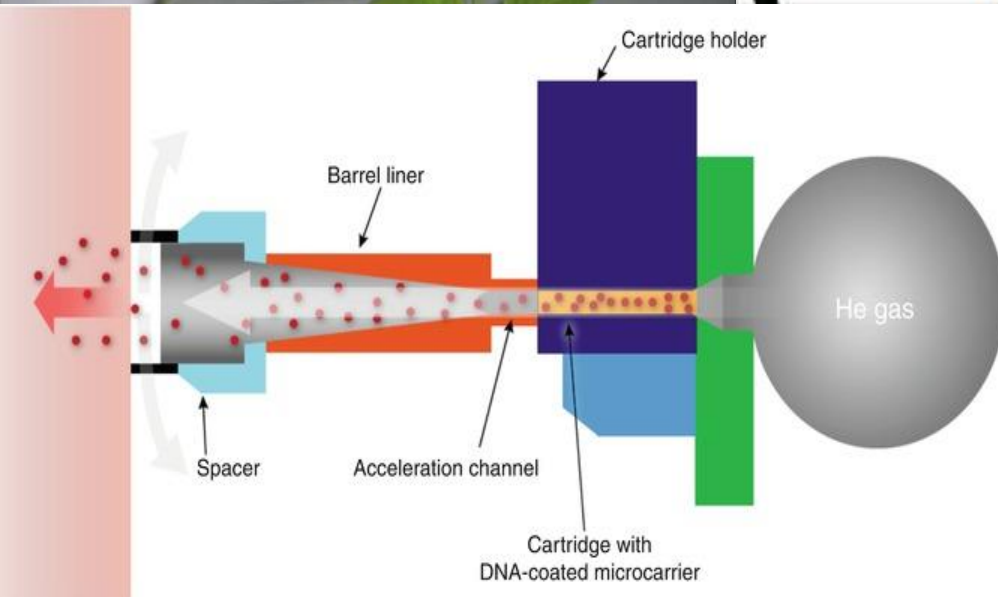
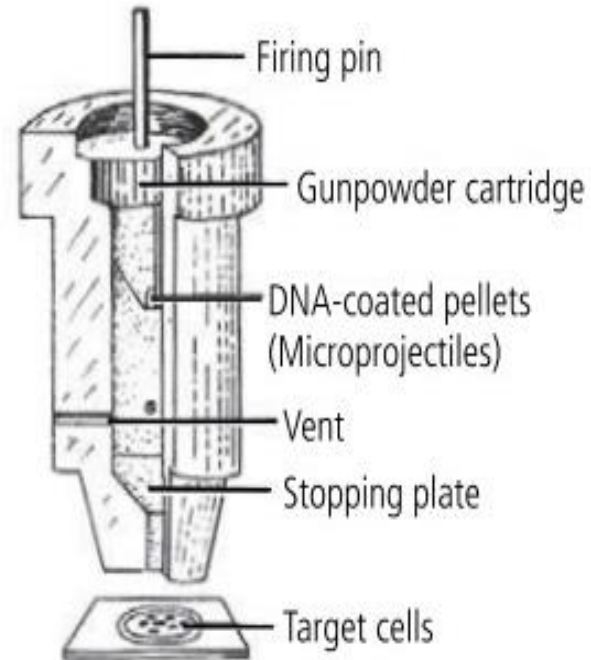


DIRECT GENE TRANSFER TO CELLS

- **MICROINJECTION** -- Jeffrey S. Chamberlain et al (1993)



➤ GENE-GUN (PARTICLE GUN OR BIOLISTIC)

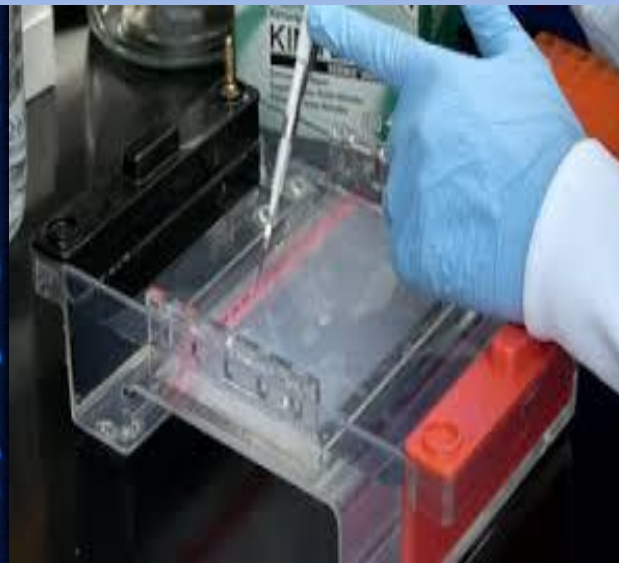


Other important tools and techniques used in RDT

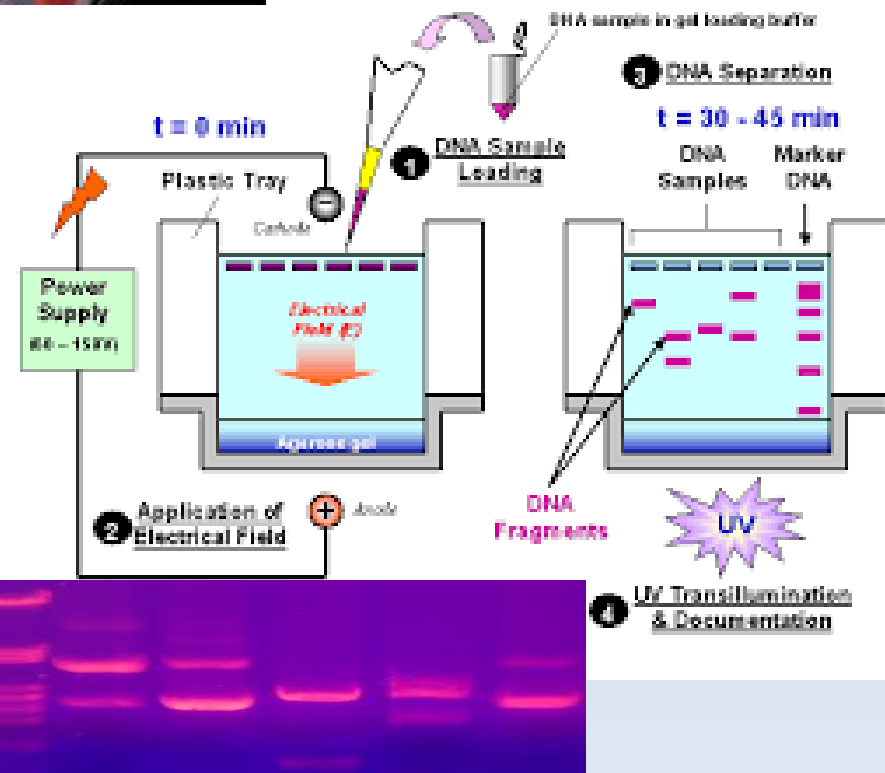
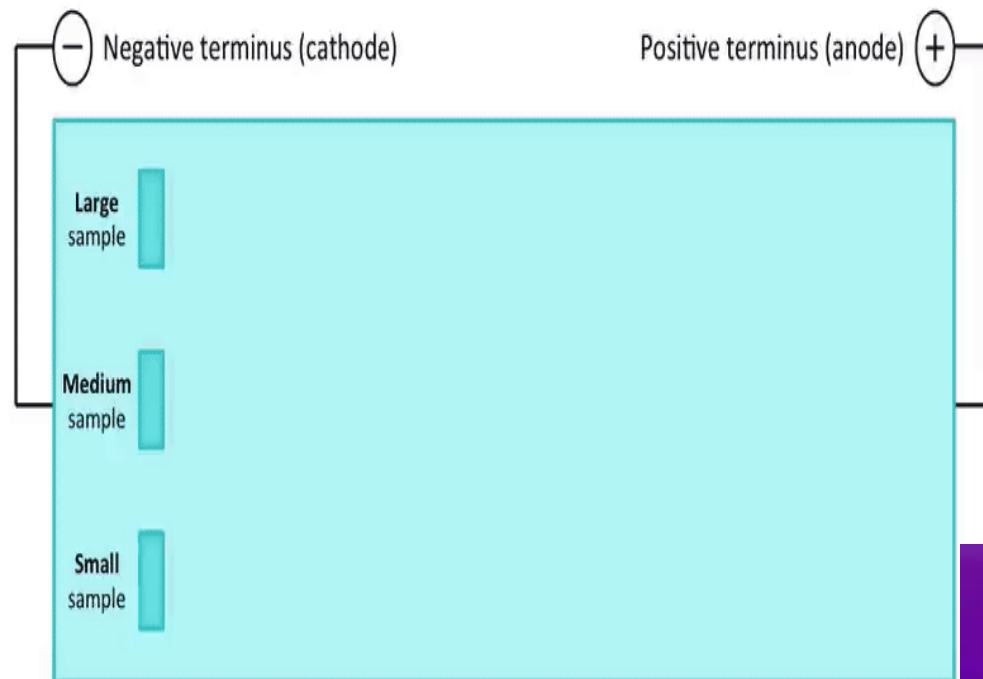
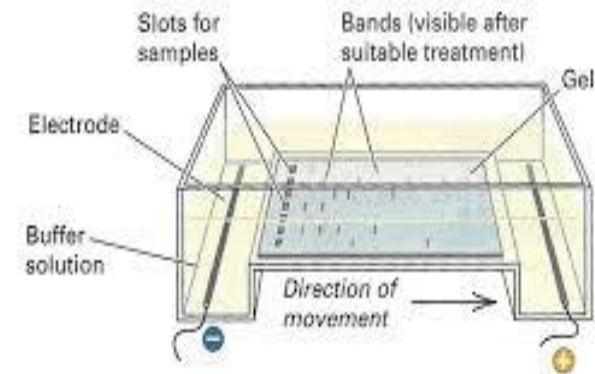
- 1) Agarose gel electrophoresis
- 2) SDS Gel Electrophoresis
- 3) Southern Blotting
- 4) Northern Blotting
- 5) Western Blotting
- 6) Enzyme Linked Immunosorbent Assay
- 7) Polymerase Chain Reaction
- 8) Flow cytometry
- 9) DNA Sequencing
- 10) DNA Microarray
- 11) cDNA formation



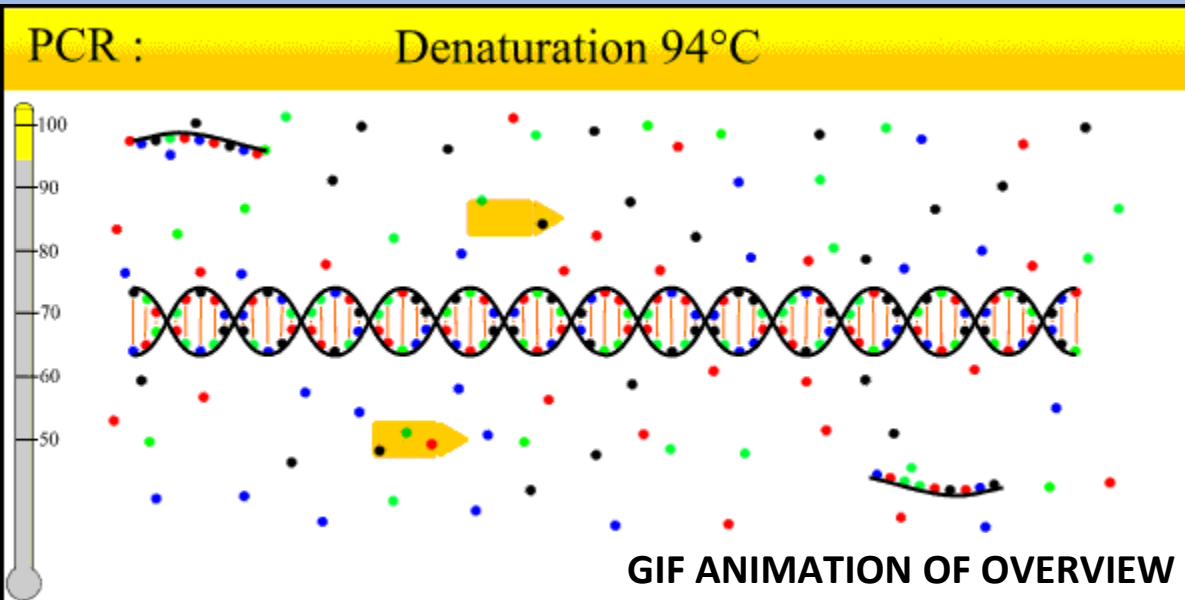
Agarose Gel Electrophoresis



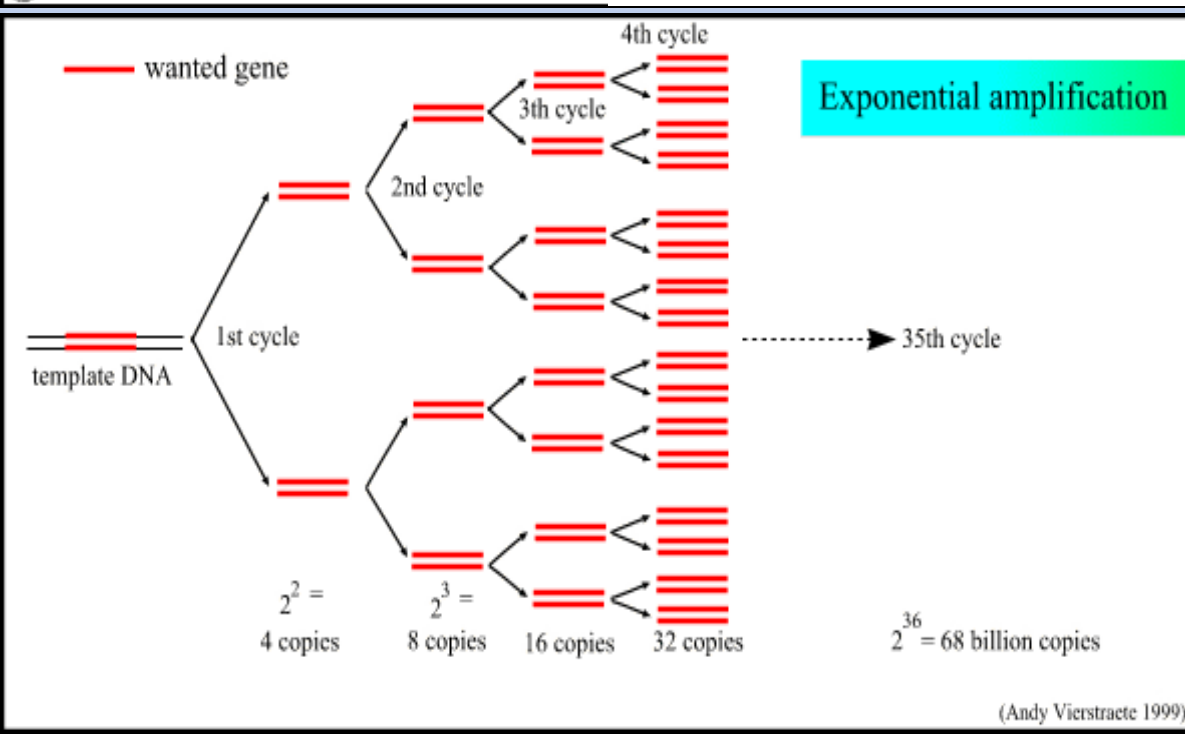
Agarose gel electrophoresis of DNA



POLYMERASE CHAIN REACTION

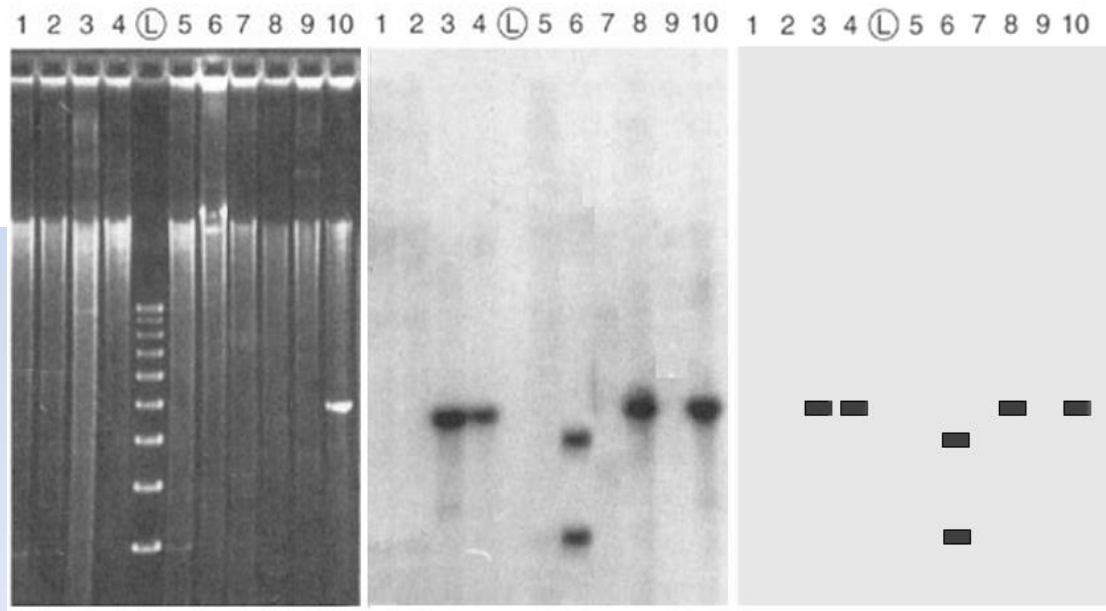
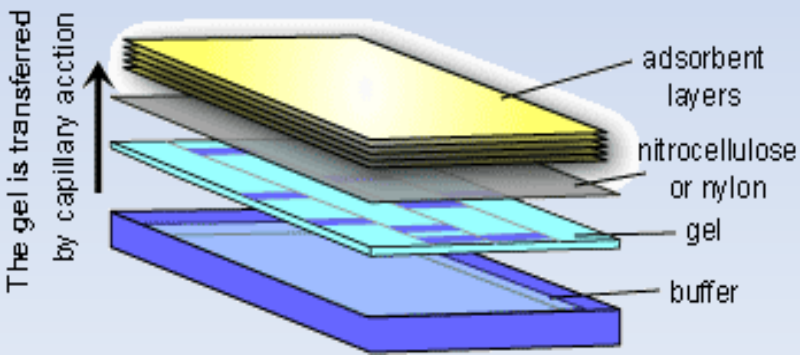
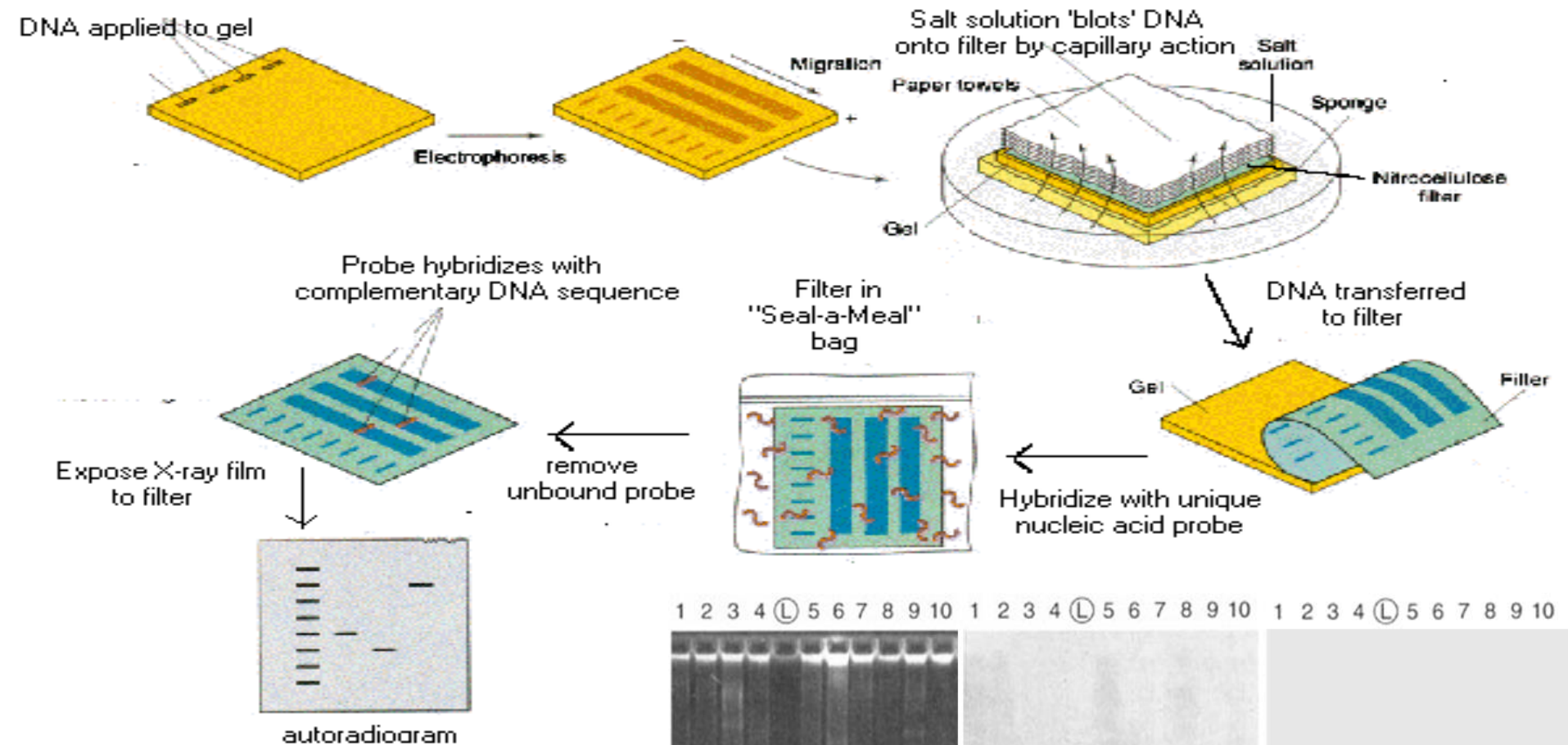


(GIF ANIMATION OF STARTING CYCLE)



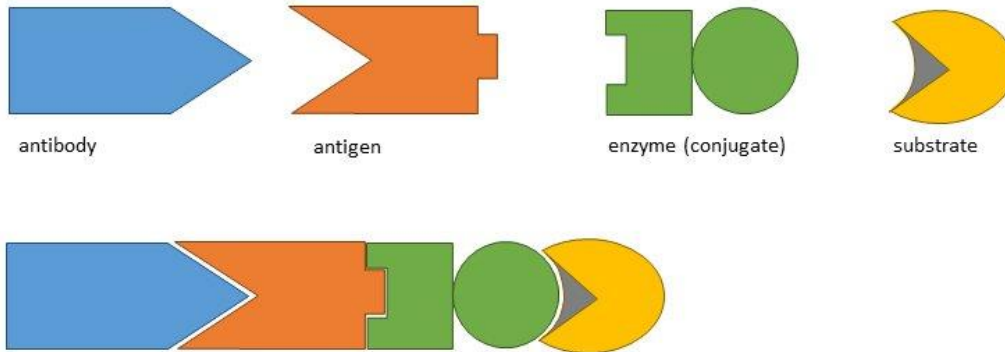
(GIF ANIMATION OF 3RD. CYCLE WHERE FIRST PURE PRODUCT IS OBTAINED)

SOUTHERN BLOTTING



ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

Principle

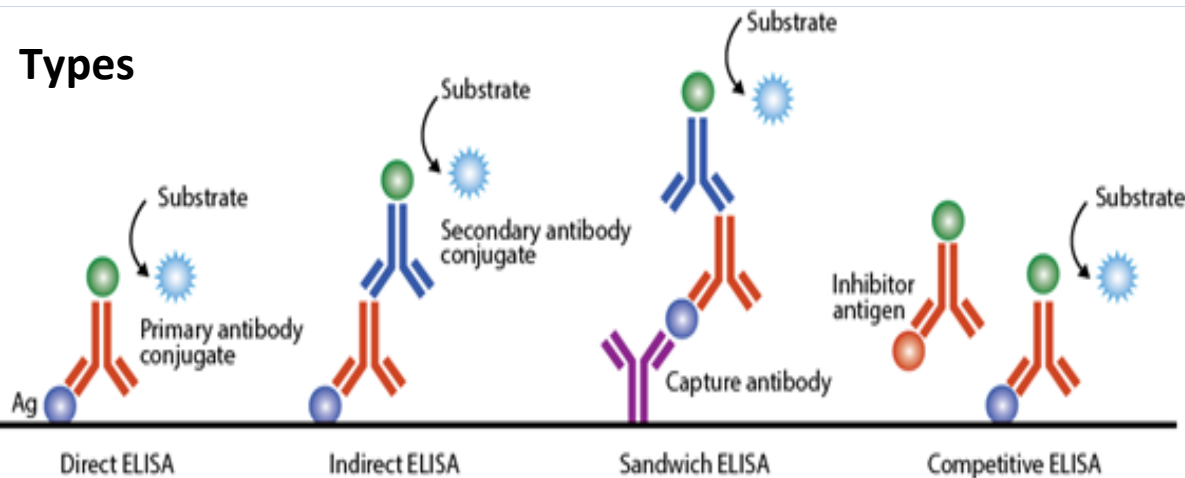


- Antibody bound to the microplate well
- Antigen added (from sample)
- Enzyme conjugate detects immune complex
- Substrate detects enzyme
 - Color is generated in the well

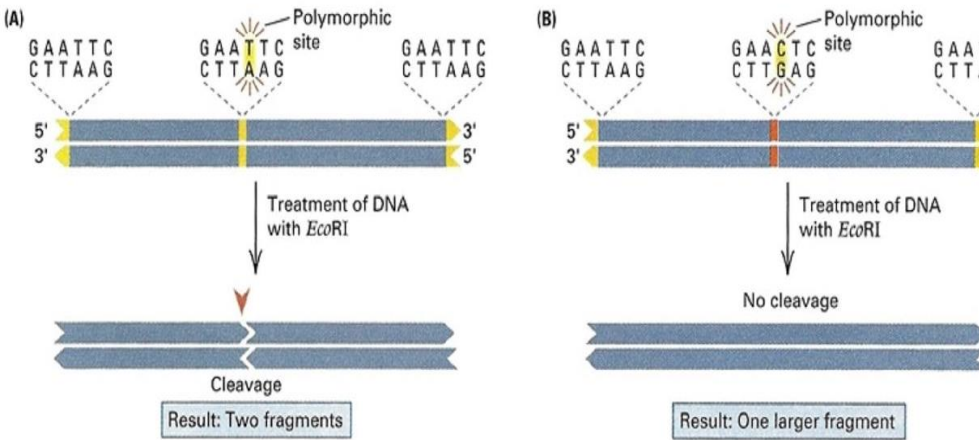
No color change or development indicates negative sample



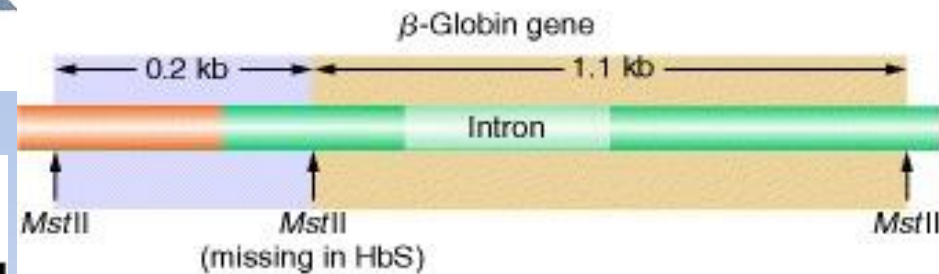
Types



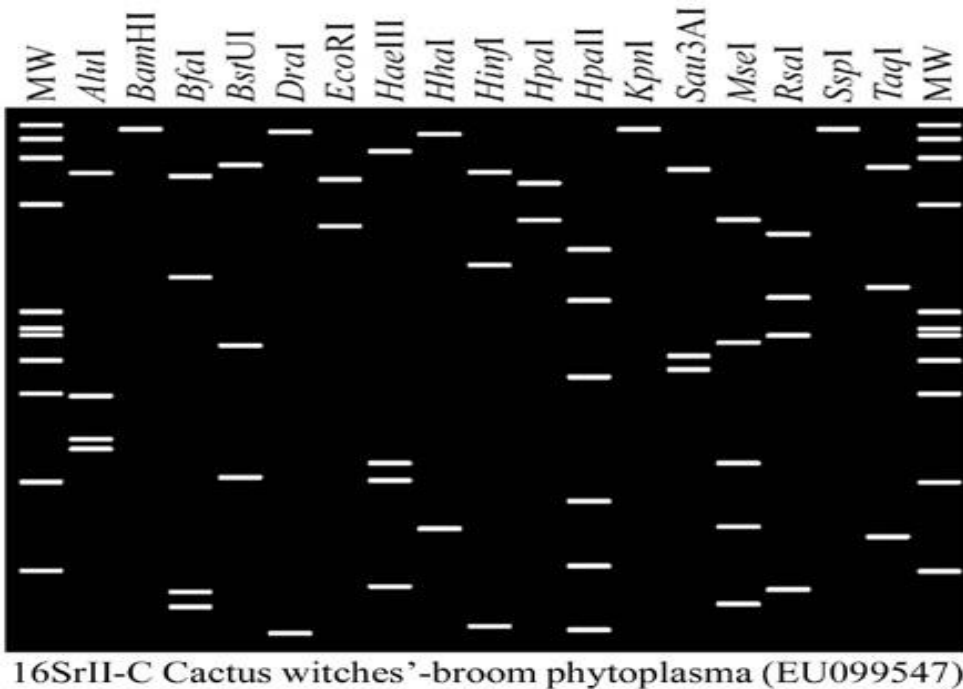
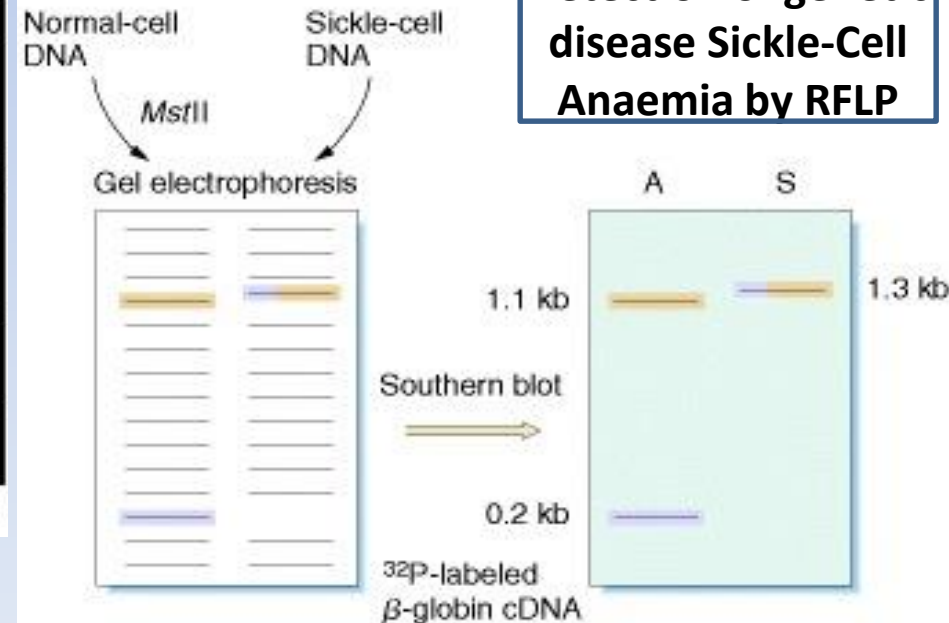
RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP)



Type of Hb	Amino acid sequence Nucleotide sequence
A	—Pro—Glu—Glu— —CCT—GAG—GAG— <i>MstII</i>
S	—Pro—Val—Glu— —CCT—GTG—GAG—



Detection of genetic disease Sickle-Cell Anaemia by RFLP



Genetic Profiling by RFLP

INDUSTRIAL PRODUCTION OF PROTEINS BY RDT

Isolation of gene from mother organism

Cloning of gene into competent bacteria

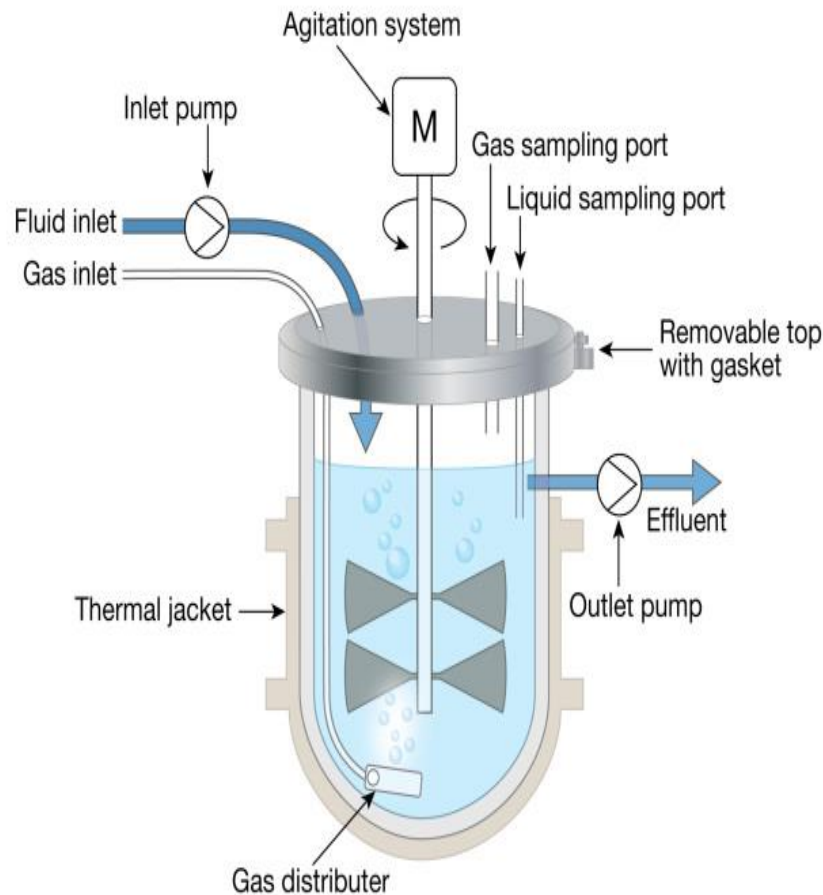
Selection of proper clones

Bulk culture of the cloned bacteria in Bioreactor

Lysis of Bacteria collected from culture

Downstream Processing, i.e. Isolation and purification of protein of interest (coded by the gene from mother organism)

Packaging of proteins for sale



Simple Stirred Bioreactor

Types of Fermentation in Bioreactor

- 1) Continuous culture (open)
- 2) Batch culture (closed)

CHALLENGES IN BIOTECHNOLOGY:

**How to apply the principles into
sustainable applications?**