



Recombinant DNA Technology and its Applications

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Introduction

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- ▶ A series of procedures used to recombine DNA segments.. Under certain conditions, a recombinant DNA molecule can enter a cell and replicate.
- ▶ Recombinant DNA technology is genetic engineering which effects artificial modification of the genetic constitution of a living cell by introduction of foreign DNA through experimental techniques.
- ▶ The technique involves the splicing of DNA by restriction endonucleases, preparation of chimeric DNA molecule, followed by cloning for the production of large numbers of identical target DNA molecules

Recombinant DNA (rDNA)

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- ▶ rDNA molecule are DNA sequences that results from the use of laboratory methods (molecular cloning) to bring together genetic materials from the multiple sources, creating sequences that would not otherwise be found in biological organisms.
- ▶ rDNA is possible because DNA molecules from all organisms share the same chemical structure; they differ in the sequence of nucleotides within that identical overall structure.
- ▶ Consequently when DNA from a foreign source is linked to host sequences that can drive DNA replication and then introduced in a host organisms the foreign DNA is replicated along with the host DNA.
- ▶ rDNA are sometimes called as chimeric DNA

History of recombinant DNA technology

- ▶ Recombinant DNA technology is one of the recent advances in biotechnology, which was developed by scientists named Paul Berg, Herbert Boyer and Stanley Cohen in 1973.
- ▶ This led to the development of recombinant DNA technology which permitted biology to move from an exclusively analytical science to a synthetic one.

Tools of Recombinant DNA technology

The various “**biological tools**” used to bring about genetic manipulations are:-

- ▶ Enzymes
- ▶ Passenger DNA/foreign DNA/ insert DNA fragment
- ▶ Vector or vehicle DNA

Enzymes used in rDNA technology

- ▶ Restriction endonucleases
- ▶ Exonucleases
- ▶ Endonucleases
- ▶ Reverse Transcriptase
- ▶ DNA polymerase
- ▶ DNA ligase (T4 ligase)
- ▶ S1 nuclease
- ▶ Alkaline phosphatase

Enzymes with their functions

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Some Enzymes Used in Recombinant DNA Technology

Enzyme(s)	Function
Type II restriction endonucleases	Cleave DNAs at specific base sequences
DNA ligase	Joins two DNA molecules or fragments
DNA polymerase I (<i>E. coli</i>)	Fills gaps in duplexes by stepwise addition of nucleotides to 3' ends
Reverse transcriptase	Makes a DNA copy of an RNA molecule
Polynucleotide kinase	Adds a phosphate to the 5'-OH end of a polynucleotide to label it or permit ligation
Terminal transferase	Adds homopolymer tails to the 3'-OH ends of a linear duplex
Exonuclease III	Removes nucleotide residues from the 3' ends of a DNA strand
Bacteriophage λ exonuclease	Removes nucleotides from the 5' ends of a duplex to expose single-stranded 3' ends
Alkaline phosphatase	Removes terminal phosphates from either the 5' or 3' end (or both)

Passenger DNA / Foreign DNA

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- ▶ cDNA (complementary DNA)
- ▶ Synthetic DNA
- ▶ Random DNA

Vector or Vehicle DNA

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- ▶ Bacterial Plasmids
- ▶ Bacteriophages
- ▶ Cosmids.
- ▶ Bacterial Artificial Chromosomes (BAC)
- ▶ Yeast Artificial Chromosomes (YAC)

Bacterial Plasmid

- ▶ Small circular duplex DNA molecule whose natural function is to confer antibiotic resistance to the host cell and replicate independently from the bacterial chromosomal DNA.
- ▶ Plasmids are the most commonly used vectors and can accept short DNA pieces about 6 to 10 kb long.
- ▶ Some useful plasmids for cloning are:
 - pBR322
 - pUC 8/18, pUC19.

Types of Plasmid

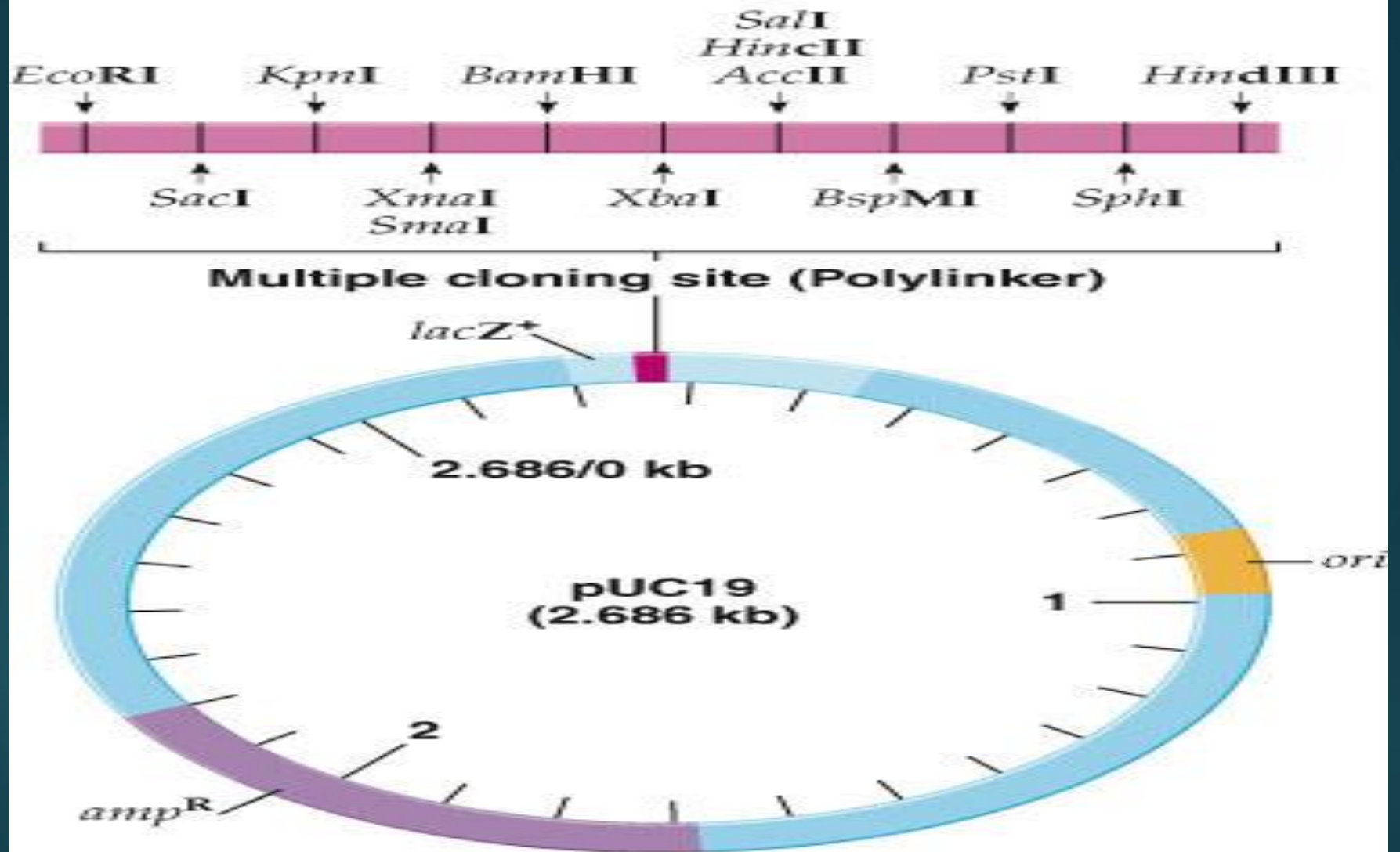
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- ▶ F Plasmid (Sex Plasmid)
- ▶ R Plasmid (Drug Resistance Plasmids)
- ▶ Col Plasmid (Colicinogenic factor Plasmids)
- ▶ Ent Plasmid

Special features of a plasmid cloning vector

- ▶ An E. coli plasmid cloning vector must have three features:
 - An ori (origin of DNA replication) sequence.
 - A dominant selectable marker which enables E. coli cells that carry the plasmid to be distinguished easily from the cells that lack the plasmid.
 - amp^R
 - tet^R
 - unique restriction enzyme cleavage sites.

pUC19 cloning vector



ori = Origin of replication sequence
amp^R = Ampicillin resistance gene
lacZ⁺ = Part of β -galactosidase gene

Bacteriophages

- ▶ Viral DNA capable of replicating in bacterial cell.
- ▶ They usually have linear DNA molecules into which foreign DNA can be inserted at several restriction enzyme sites.
- ▶ The chimeric or hybrid DNA is collected after the phage proceeds through its lytic cycle and produces mature, infective phage particles.
- ▶ They can accept DNA fragment 10 to 20 kb long.
- ▶ Some useful phages for cloning are:
 - Lambda (λ) phage
 - M13 phage.

Lambda Phage

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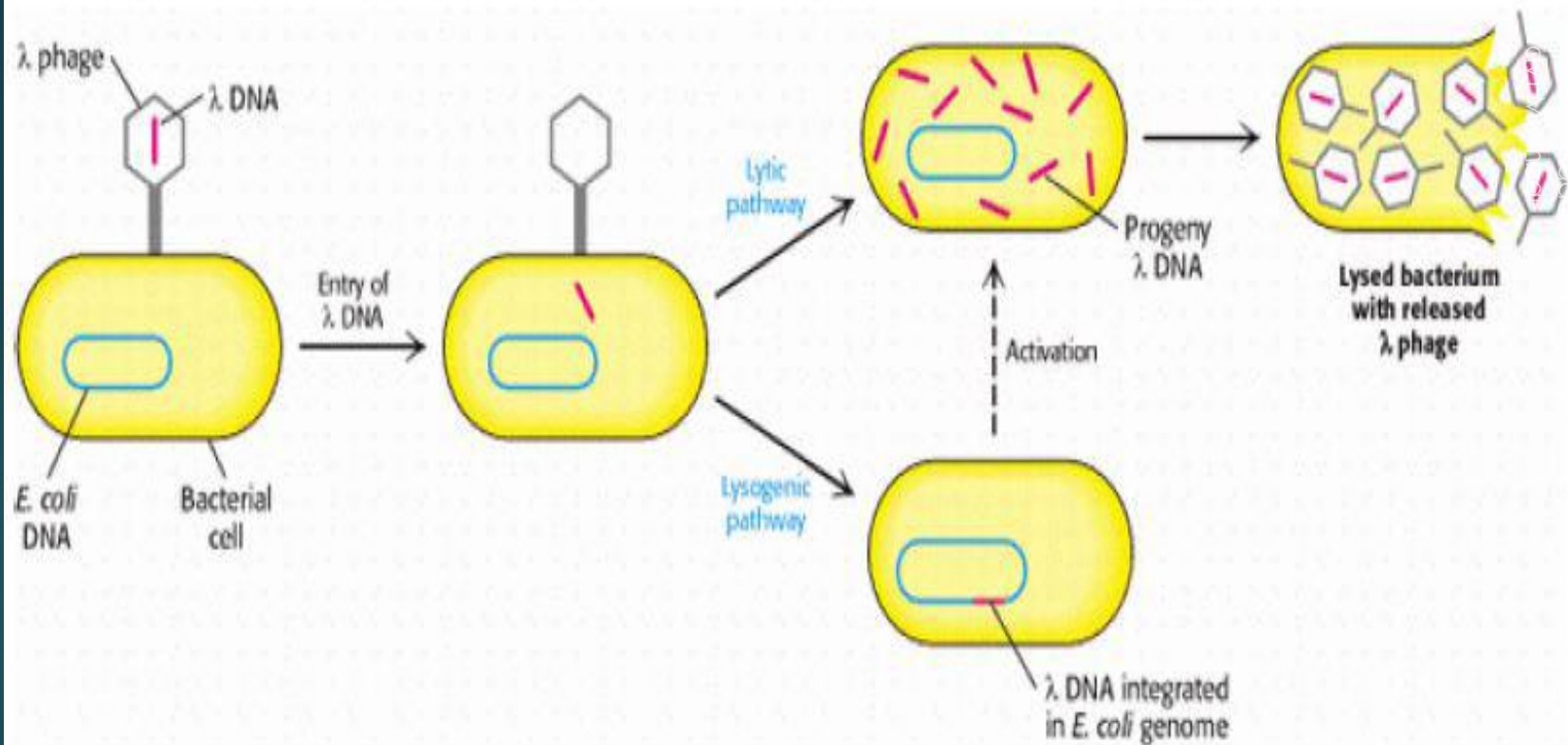
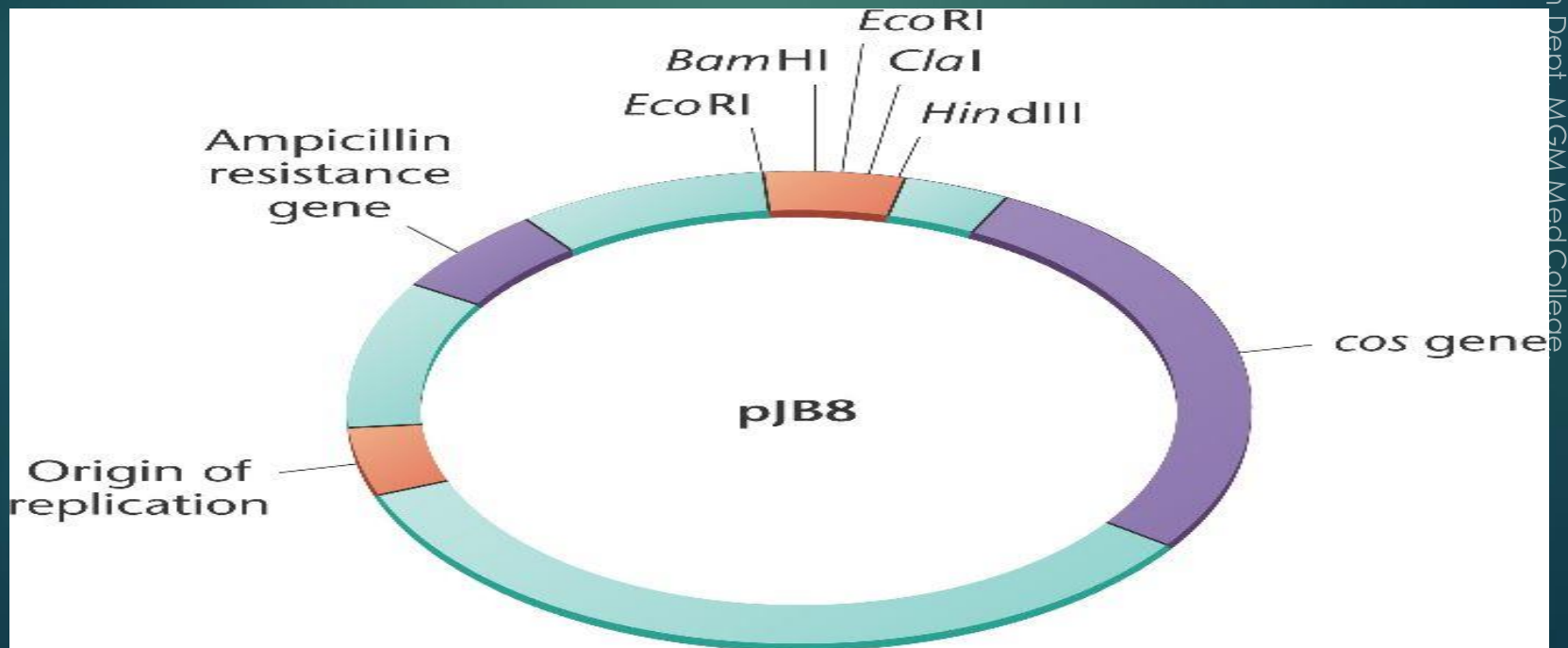


Figure . Alternative Infection Modes For λ phage. Lambda phage can multiply within a host and lyse it (lytic pathway), or its DNA can become integrated into the host genome (lysogenic pathway), where it is dormant until activated.

Cosmids

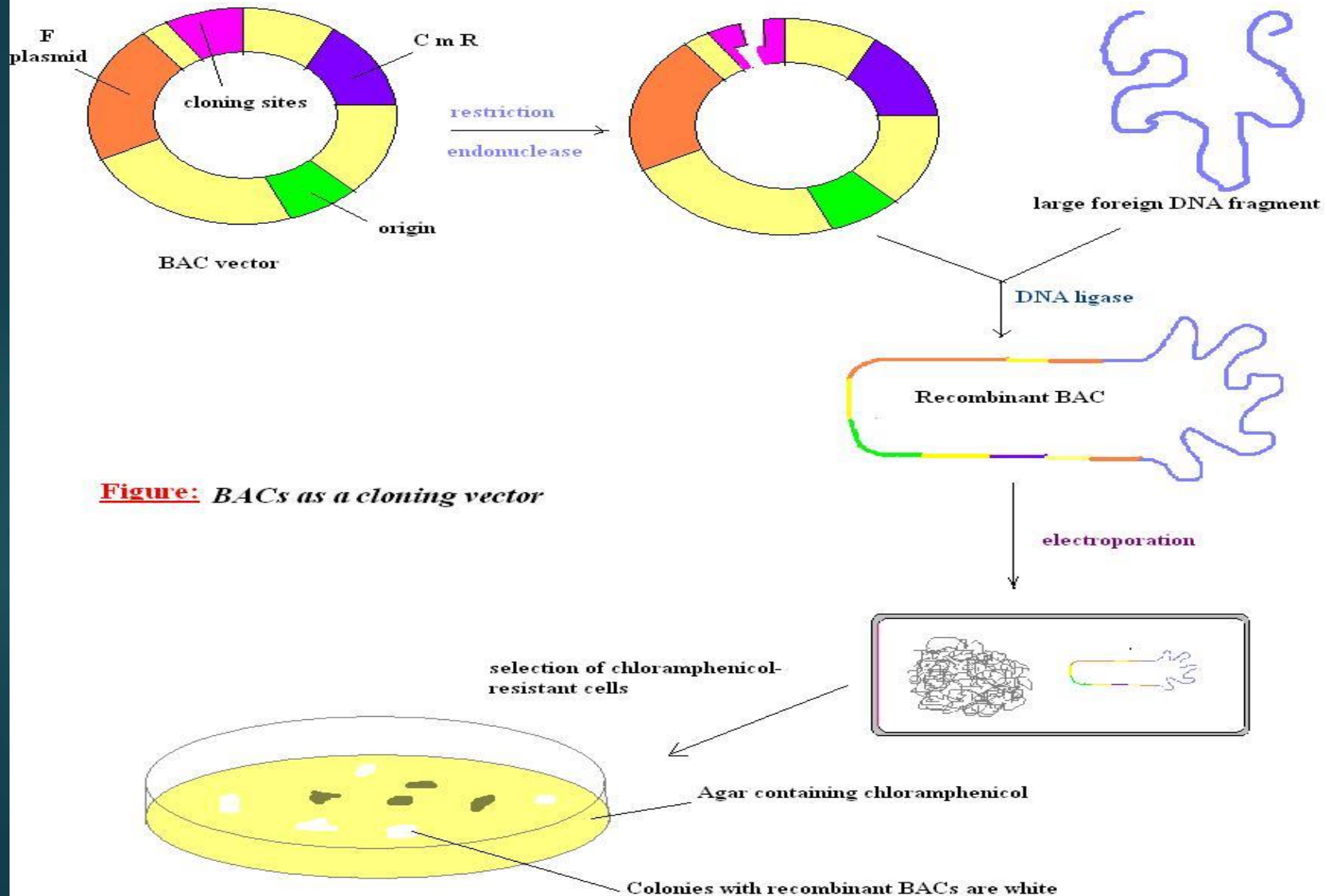
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- ▶ Specialized plasmids that contain DNA sequences, so called “COS Sites” required for packaging lambda DNA into the phage particle.
- ▶ They are recombinant plasmids and bacteriophage.
- ▶ Larger fragments of DNA can be inserted in it which combine with best features of plasmid and phages.
- ▶ It can accept very large DNA fragments 35 to 52 kb.



Bacterial Artificial Chromosomes

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Yeast Artificial Chromosomes (YAC)

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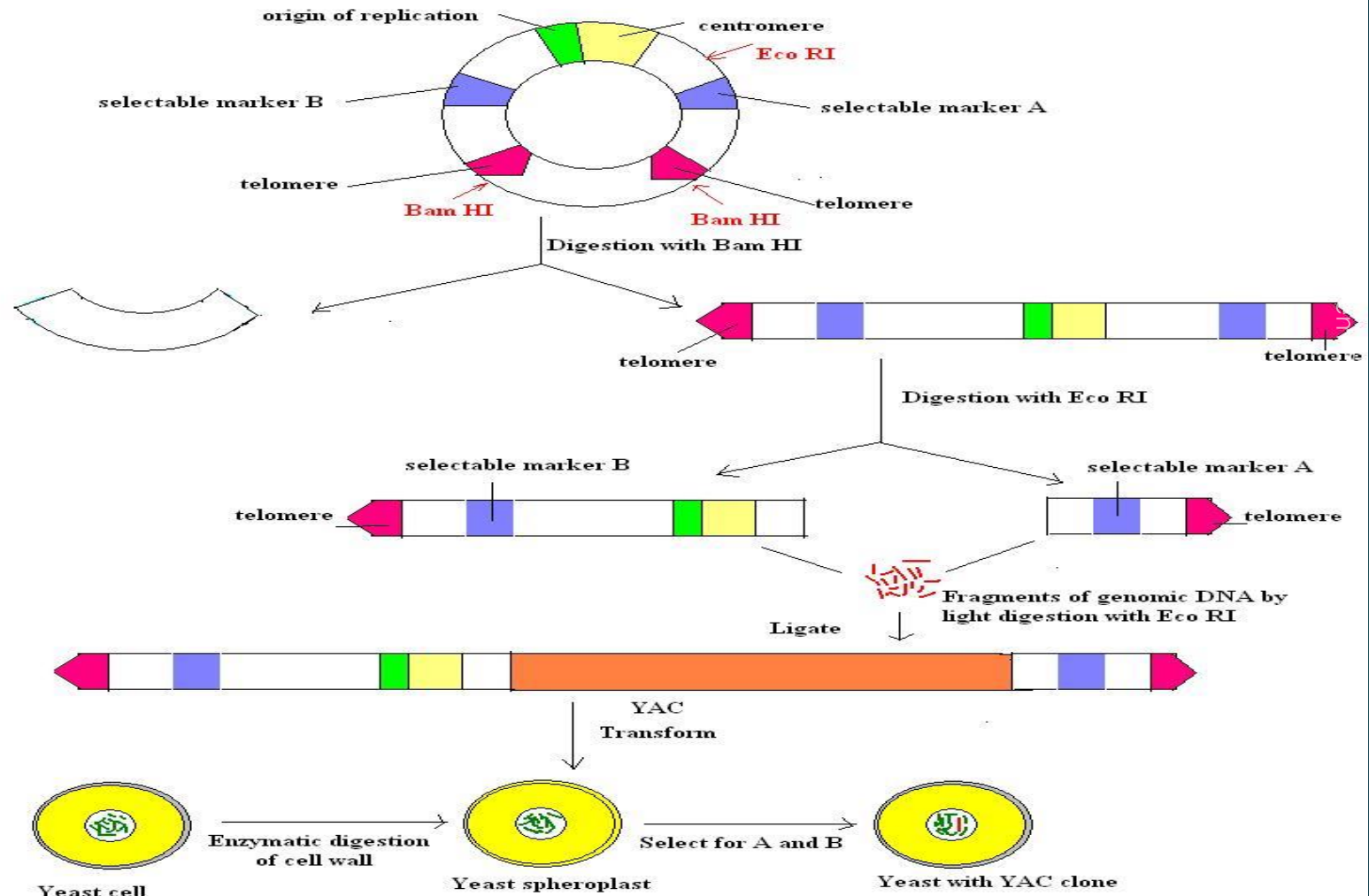


Figure: **Construction of a yeast artificial chromosome (YAC)**

Steps in the construction of Recombinant DNA

- ▶ Fragmentation of DNA (restriction fragments) by restriction endonuclease enzyme.
- ▶ Isolation of specific human DNA
- ▶ Insertion of isolated human DNA into vector to form chimeric or hybrid DNA molecule
- ▶ Joining of two different cut DNA fragments by DNA ligase.

circular
double-stranded
plasmid DNA
(cloning vector)



CLEAVAGE WITH
RESTRICTION
NUCLEASE

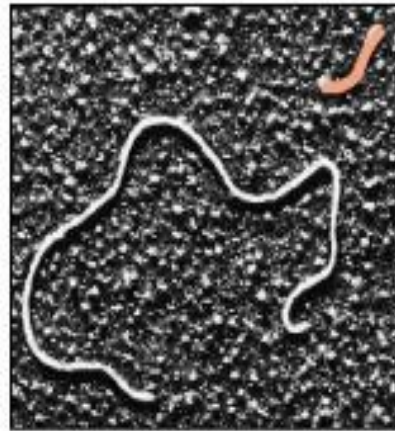


DNA fragment
to be cloned

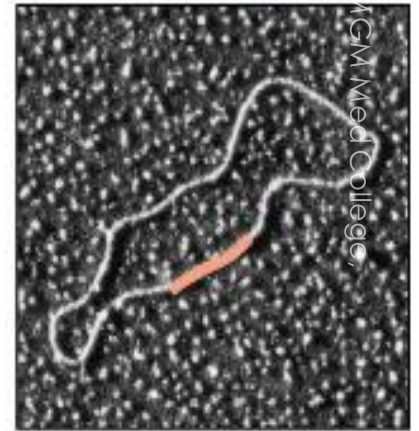


COVALENT
LINKAGE
BY DNA LIGASE

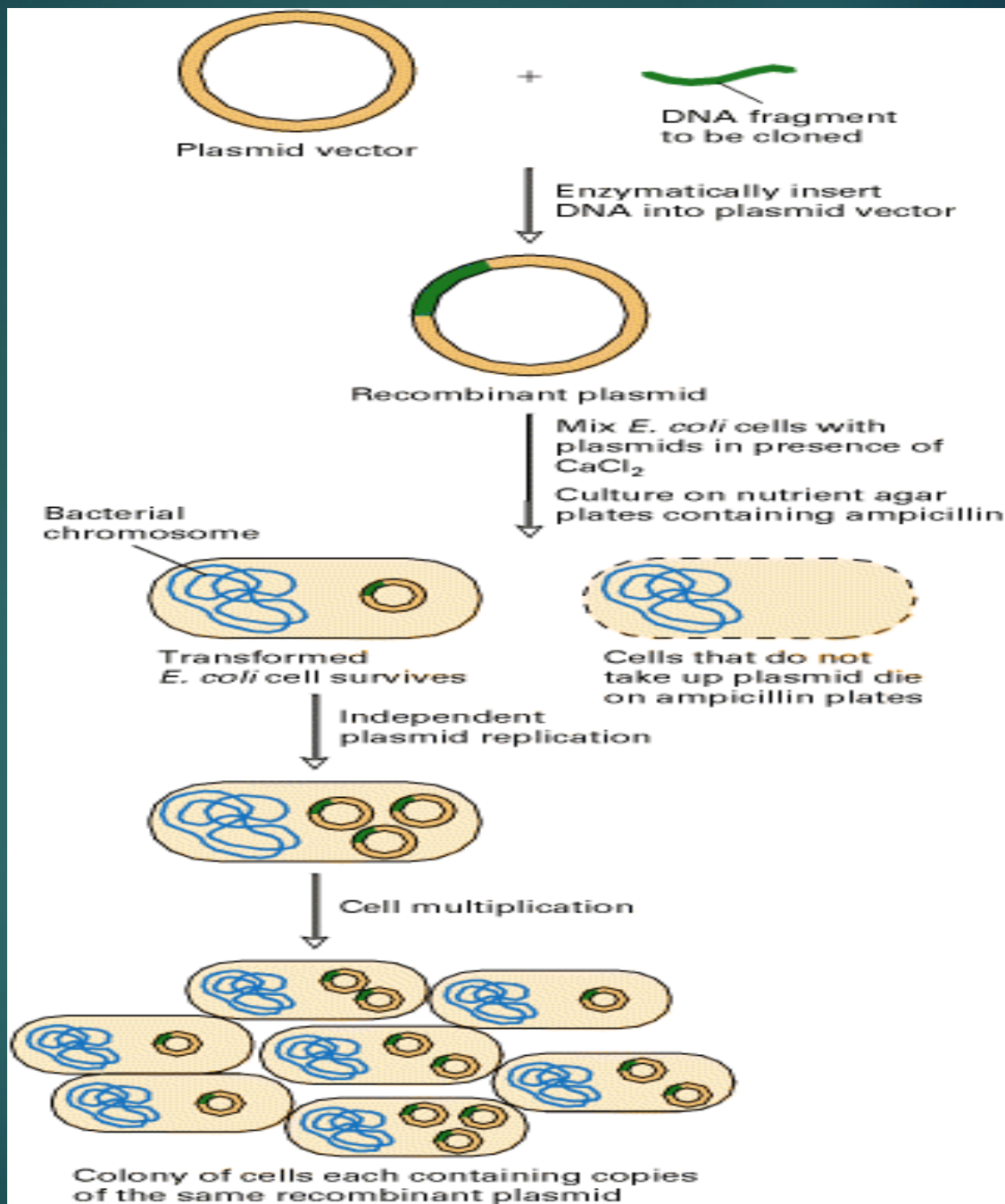
recombinant DNA



200 nm



200 nm



Step I- Fragmentation of DNA

- ▶ Its achieved by various restriction endonuclease enzymes. They are found in wide variety of prokaryotes.

Restriction Endonucleases:

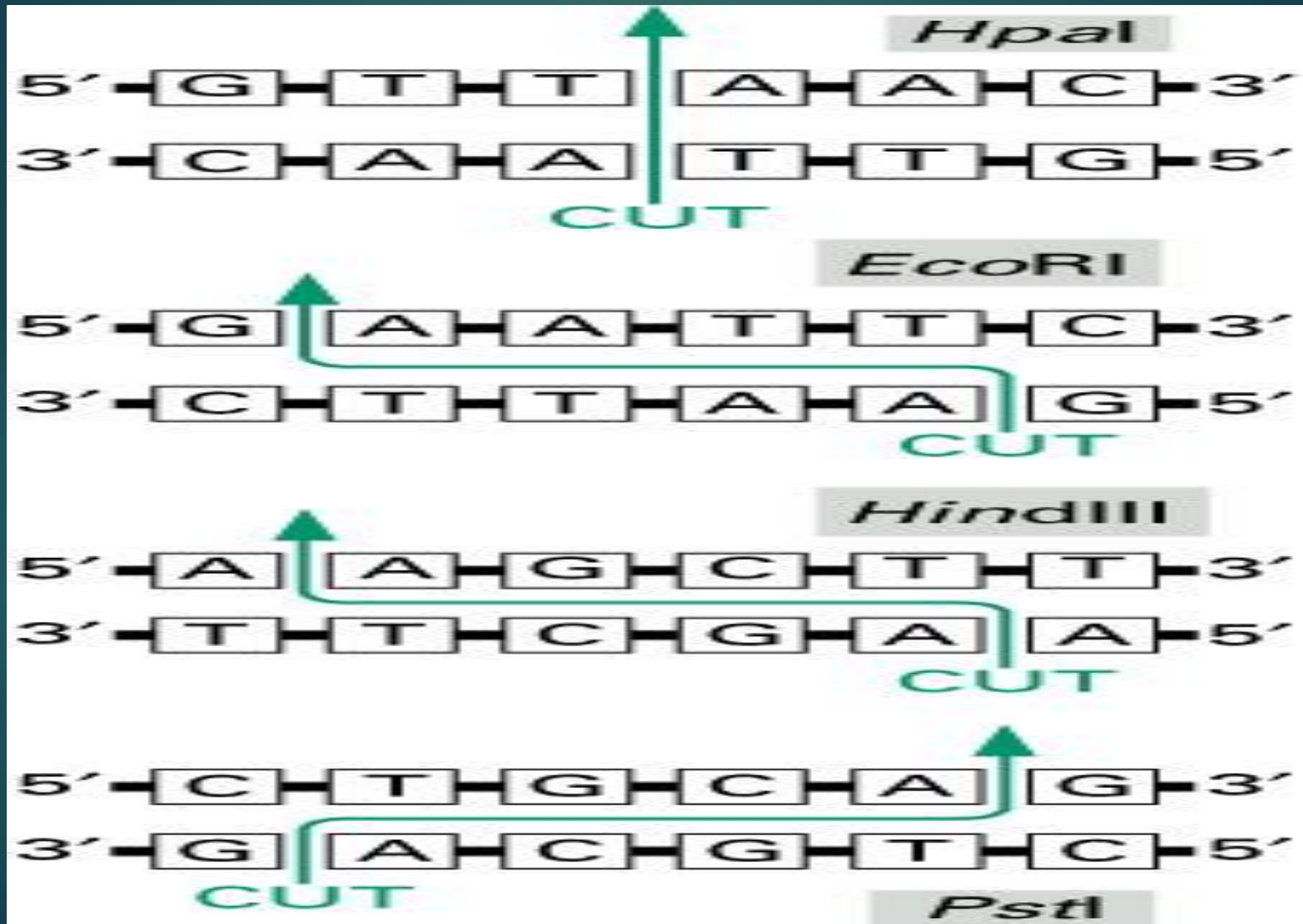
- ▶ These enzymes were originally called restriction enzymes.
- ▶ The cells own DNA is not degraded because the sequences recognized by its own restriction enzymes are methylated.
- ▶ Restricted enzymes cut DNA of any source in a sequence specific manner in contrast to action of most other endonuclease which break DNA randomly.

- ▶ The restricted enzymes are named according to the bacteria from which they are isolated. For example EcoRI, BamHI
- ▶ Restriction endonuclease recognizes specific base sequences of double helical DNA, usually 4-6 basepairs in length and cleave both strands within this sequence.
- ▶ Most of the DNA sequence recognized by restriction endonuclease are palindromic.

Table 8.1 Characteristics of Some Restriction Enzymes

	Enzyme Name	Pronunciation	Organism in Which Enzyme Is Found	Recognition Sequence and Position of Cut
Enzymes with 6-bp Recognition Sequences	BamHI	"bam-H-one"	<i>Bacillus amyloliquefaciens</i> H	$ \begin{array}{c} 5'-G \downarrow GATCC-3' \\ 3'-CCTAG \uparrow G-5' \end{array} $
	BglII	"bagel-two"	<i>Bacillus globigi</i>	$ \begin{array}{c} \downarrow AGATCT \\ TCTAG \uparrow A \end{array} $
	EcoRI	"echo-R-one"	<i>E. coli</i> RY13	$ \begin{array}{c} \downarrow GAATTC \\ CTTAA \uparrow G \end{array} $
	HaeII	"hay-two"	<i>Haemophilus aegypticus</i>	$ \begin{array}{c} \downarrow RGC G C Y \\ Y C G C G R \uparrow \end{array} $
	HindIII	"hin-D-three"	<i>Haemophilus influenzae</i> R ₄	$ \begin{array}{c} \downarrow AAGCTT \\ TTCGA \uparrow A \end{array} $
	PstI	"P-S-T-one"	<i>Providencia stuartii</i>	$ \begin{array}{c} \downarrow CTG C A G \\ G A C G T C \uparrow \end{array} $
	SalI	"sal-one"	<i>Streptomyces albus</i>	$ \begin{array}{c} \downarrow GTC G A C \\ C A G C T \uparrow G \end{array} $
	SmaI	"sma-one"	<i>Serratia marcescens</i>	$ \begin{array}{c} \downarrow CCC G G G \\ G G G C C C \uparrow \end{array} $
Enzymes with 4-bp Recognition Sequences	HaeIII	"hay-three"	<i>Haemophilus aegypticus</i>	$ \begin{array}{c} \downarrow GGCC \\ CCGG \uparrow \end{array} $
	HhaI	"ha-ha-one"	<i>Haemophilus haemolyticus</i>	$ \begin{array}{c} \downarrow GCGC \\ CGCG \uparrow \end{array} $
	HpaII	"hepa-two"	<i>Haemophilus parainfluenzae</i>	$ \begin{array}{c} \downarrow CCGC \\ GGCC \uparrow \end{array} $
	Sau3A	"sow-three-A"	<i>Staphylococcus aureus</i> 3A	$ \begin{array}{c} \downarrow GATC \\ CTAG \uparrow \end{array} $
Enzyme with 8-bp Recognition Sequence	NotI	"not-one"	<i>Nocardia otitidis-caviarum</i>	$ \begin{array}{c} \downarrow GCGGCCGC \\ CGCCGGCG \uparrow \end{array} $
Enzyme with Recognition Sequence That is Not Symmetrical	BstXI	"b-s-t-x-one"	<i>Bacillus stearothermophilus</i>	$ \begin{array}{c} \downarrow CCANNNNNNTGG \\ GGTNNNNNNNACC \uparrow \end{array} $

*In this column the two strands of DNA are shown with the sites of cleavage indicated by arrows. Since there is an axis of twofold rotational symmetry in each recognition sequence, the DNA molecules resulting from the cleavage are symmetrical. Key: R = purine; Y = pyrimidine; N = any base.



Action of Restriction endonuclease

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- ▶ Some enzymes cleaves both strands of DNA, so as to leave no unpaired bases on either end. These ends are often called blunt ends.
- ▶ Others make staggered cuts on the two strands, leaving two to four nucleotides of one strand unpaired at each resulting ends. These are referred to as cohesive ends or sticky ends.
- ▶ Different restriction fragments of DNA can base pair with each other. If they have sticky ends which are complementary.
- ▶ Therefore two unrelated DNA fragments can base pair with each other, if they were cleaved by the same restriction endonuclease enzyme.

Step II- Isolation of Specific DNA

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- ▶ Once DNA has been cleaved into fragments by restriction endonuclease, a particular fragment of interest can be separated by various techniques like electrophoresis, HPLC etc.

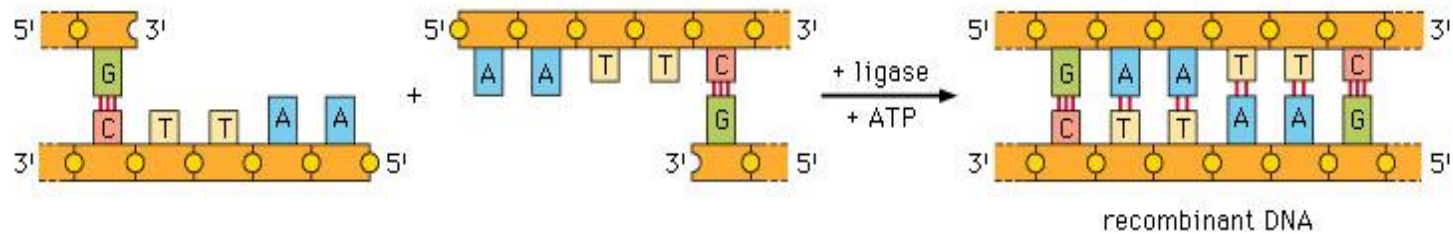
Step III: Insertion of isolated DNA into Vector to form Chimeric or Hybrid DNA

- ▶ A specific selected fragment of DNA of human genome is inserted into vector (carrier). A vector is a carrier DNA molecule, to which the fragment of DNA of interest is attached.
- ▶ A segment of the foreign DNA and the DNA vector are usually cleave with the same restriction enzyme to produce complementary sticky ends in both the foreign DNA and the DNA vector. These complementary sticky ends can base pairs.

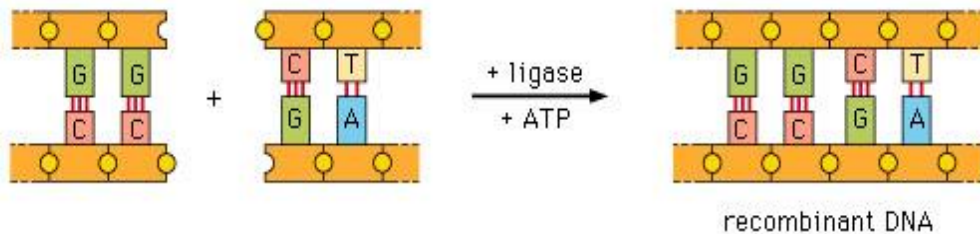
Step IV: Joining of Different cut DNA fragment by DNA Ligase:

- ▶ After the fragments have base paired , the ends are covalently joined by the action of DNA ligase. Restriction enzymes in conjunction with DNA ligase, therefore, can produce vector containing recombination or hybrid or chimeric DNA

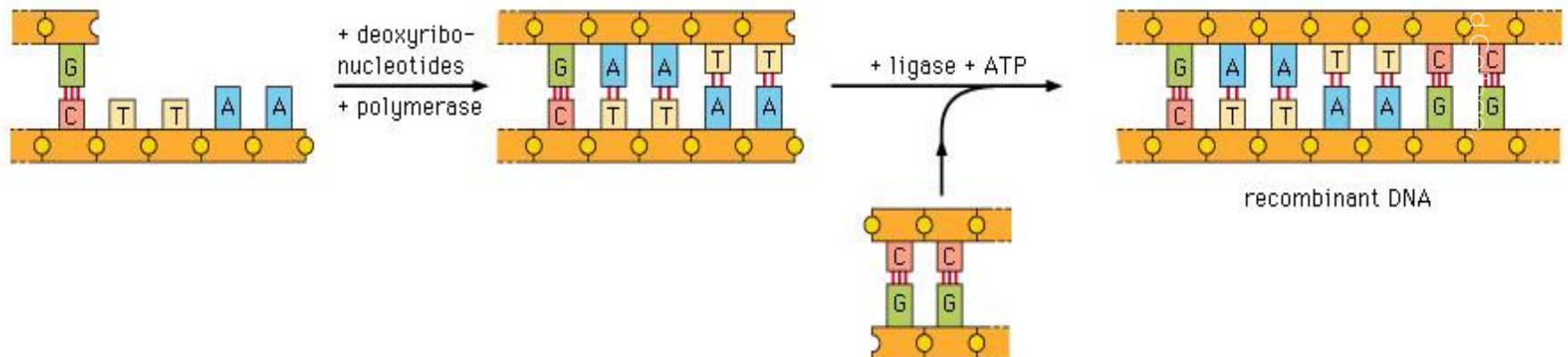
(A) JOINING TWO COMPLEMENTARY STAGGERED ENDS



(B) JOINING TWO BLUNT ENDS



(C) JOINING A BLUNT END WITH A STAGGERED END



Molecular Cloning

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- ▶ Molecular cloning is a technique developed for amplifying the quantity of DNA, in vivo.
- ▶ A clone is a large population of identical molecules, bacteria, or cells that arise from a common ancestor.
- ▶ Cloning allows for the production of a large number of identical DNA molecules, which can be produced as follows:
 - introduction of recombinant DNA into appropriate host cell. (transformation)
 - Amplification of recombinant DNA

Application of Recombinant DNA Technology:

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- ▶ **Molecular basis of disease.**
 - familial hypercholesterolemia -cystic fibrosis
 - sickle cell disease -muscular dystrophy
 - thalassaemias
- ▶ **Diagnosis of disease**
- ▶ **Production of proteins/hormones:**
 - anticoagulants
 - human growth hormone
 - growth factors
 - colony stimulating factors
 - Erythropoietin
 - Blood factors
 - insulin
 - interferons
 - interleukins
 - monoclonal antibodies
 - superoxide dismutase
 - proteins for diagnosis
 - vaccines

Application of Recombinant DNA Technology

- ▶ Gene therapy
- ▶ Gene isolation/purification/synthesis
- ▶ Transgenic animals
- ▶ Cell/tissue culture
- ▶ Genetic counseling
- ▶ In forensic medicine
- ▶ Commercial application
 - Industrial application
 - Agricultural application

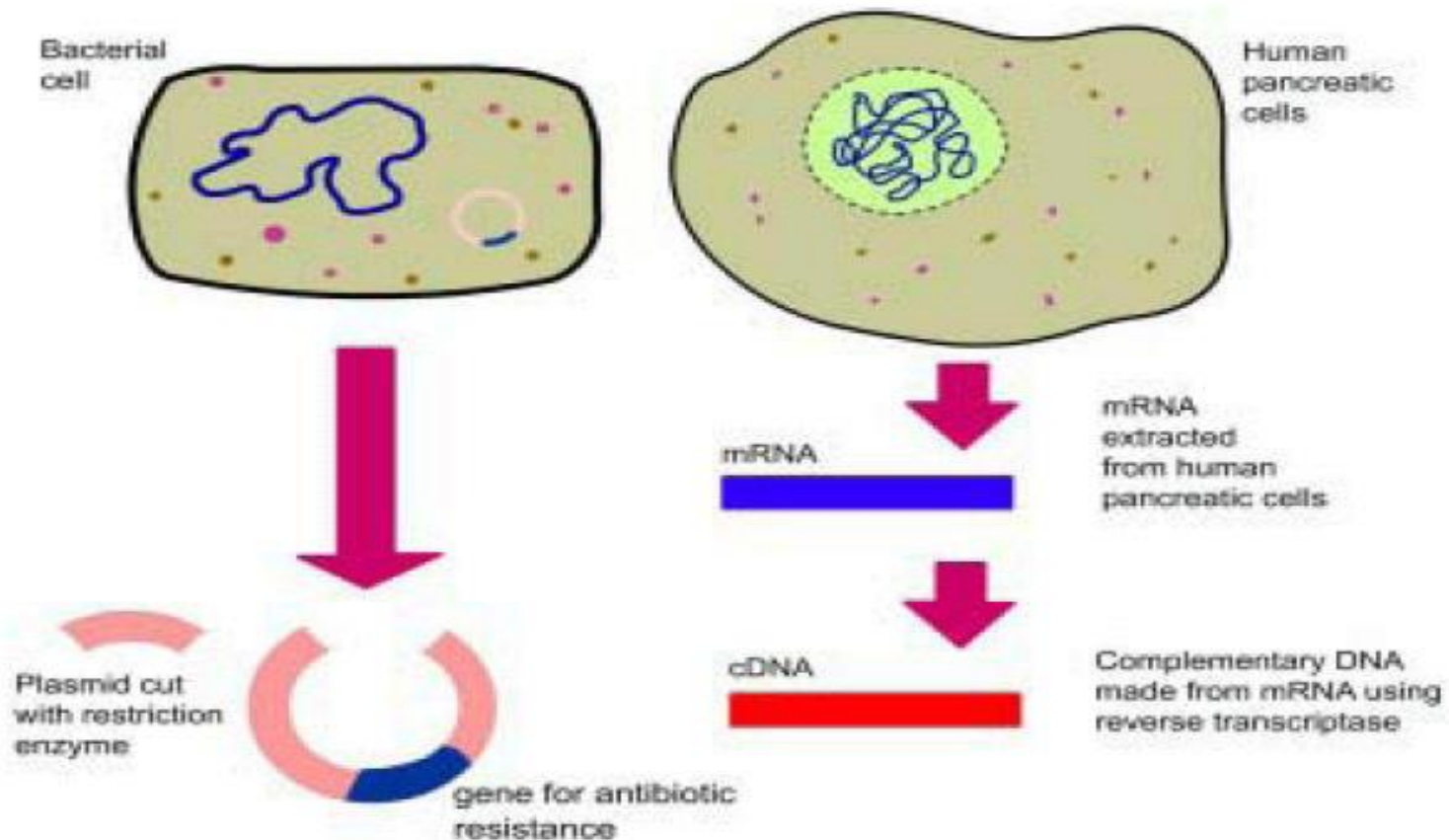
Production of Human Insulin

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1) Obtaining the human insulin gene

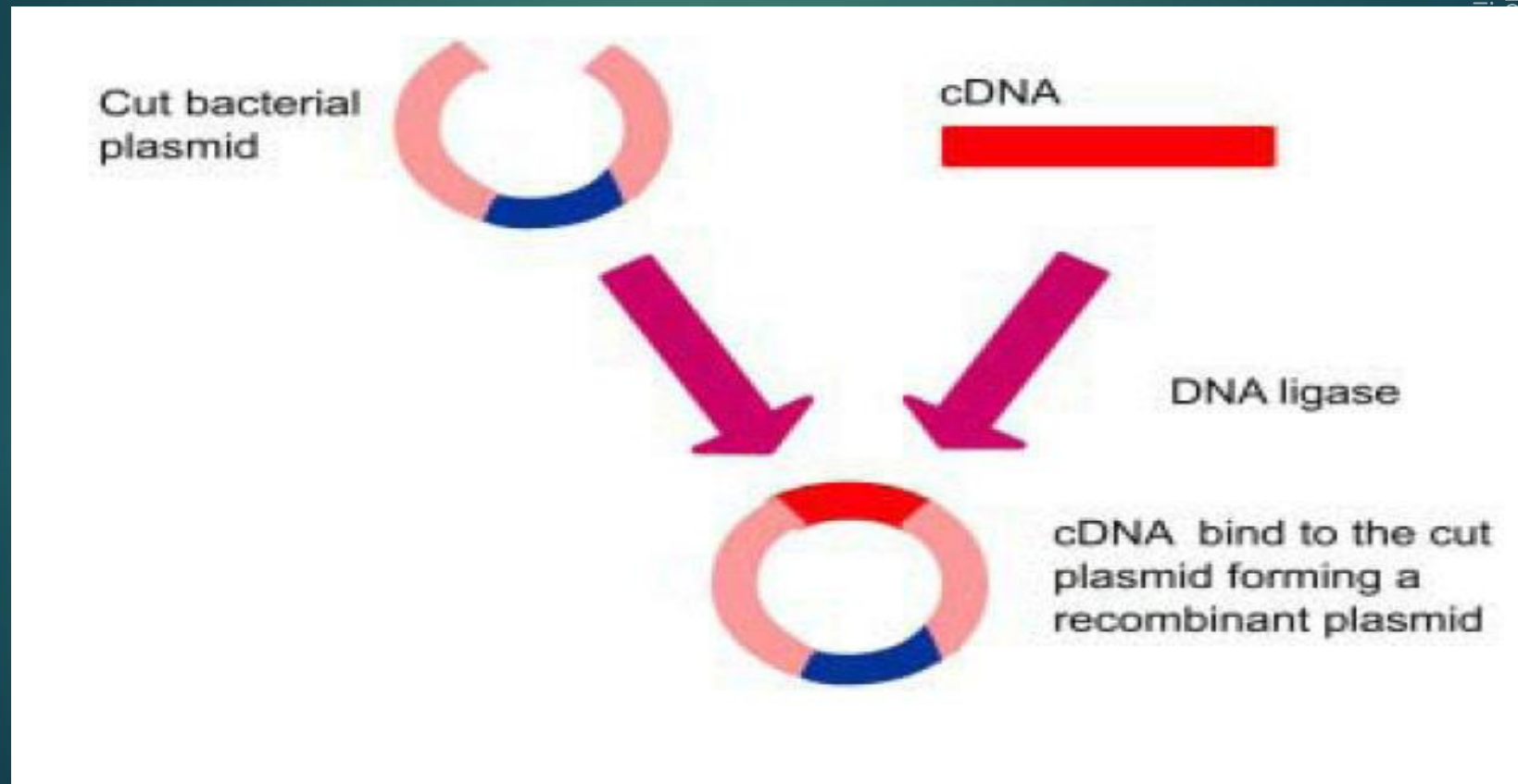
Human insulin gene can be obtained by making a complementary DNA (cDNA) copy of the messenger RNA (mRNA) for human insulin.

Prof. Dr. Z. C. M. V. M.



2)Joining the human insulin gene into a plasmid vector

- ▶ The bacterial plasmids and the cDNA are mixed together. The human insulin gene (cDNA) is inserted into the plasmid through complementary base pairing at sticky ends.

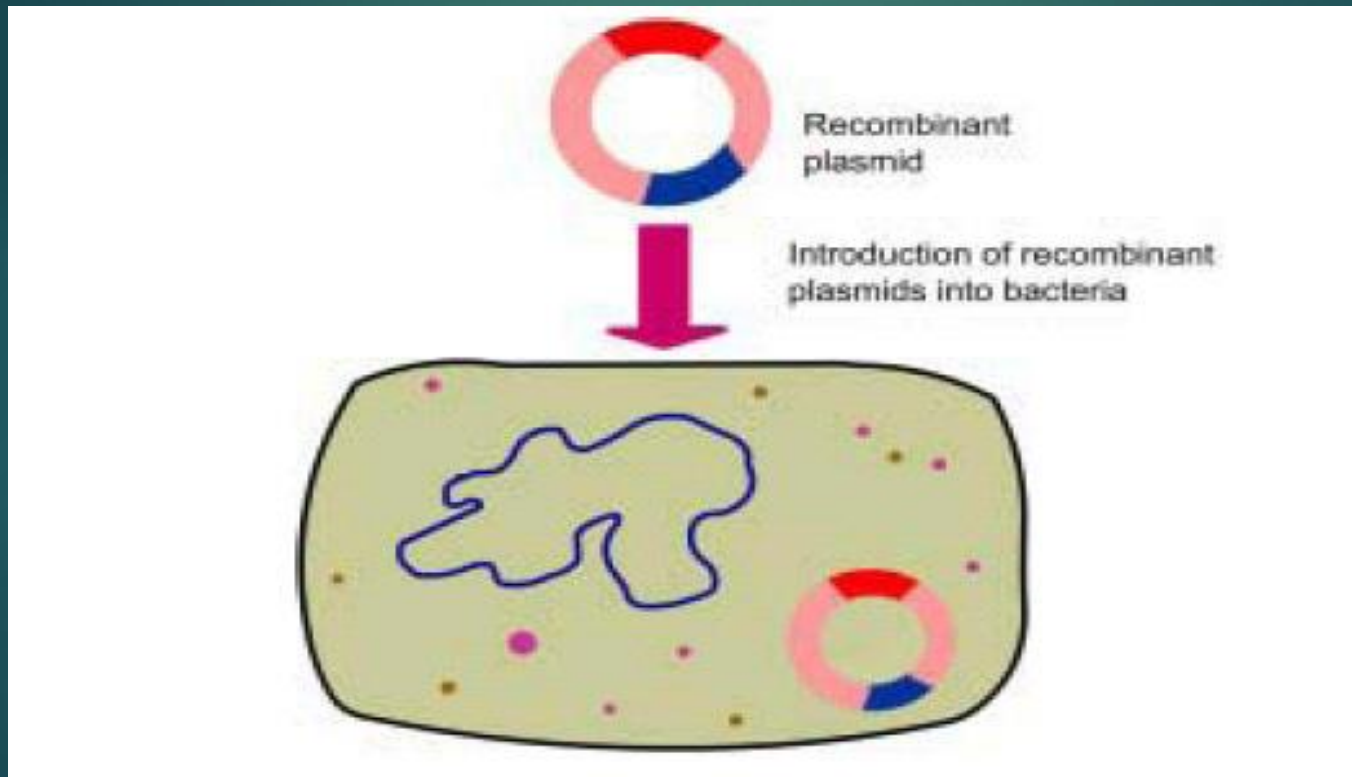


3)Introducing the recombinant DNA plasmids into bacteria

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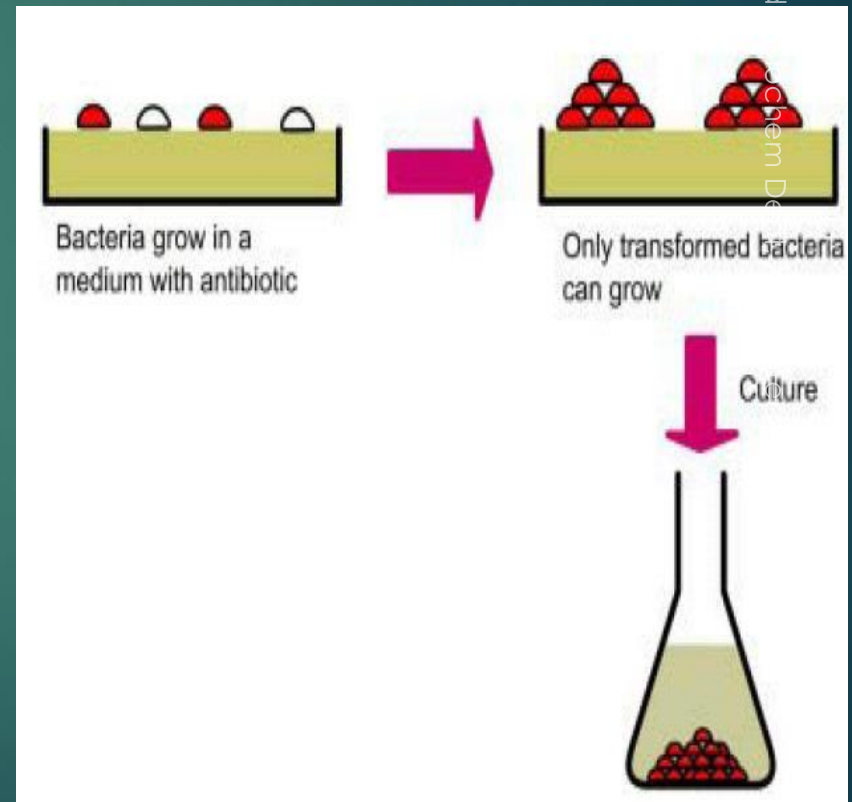
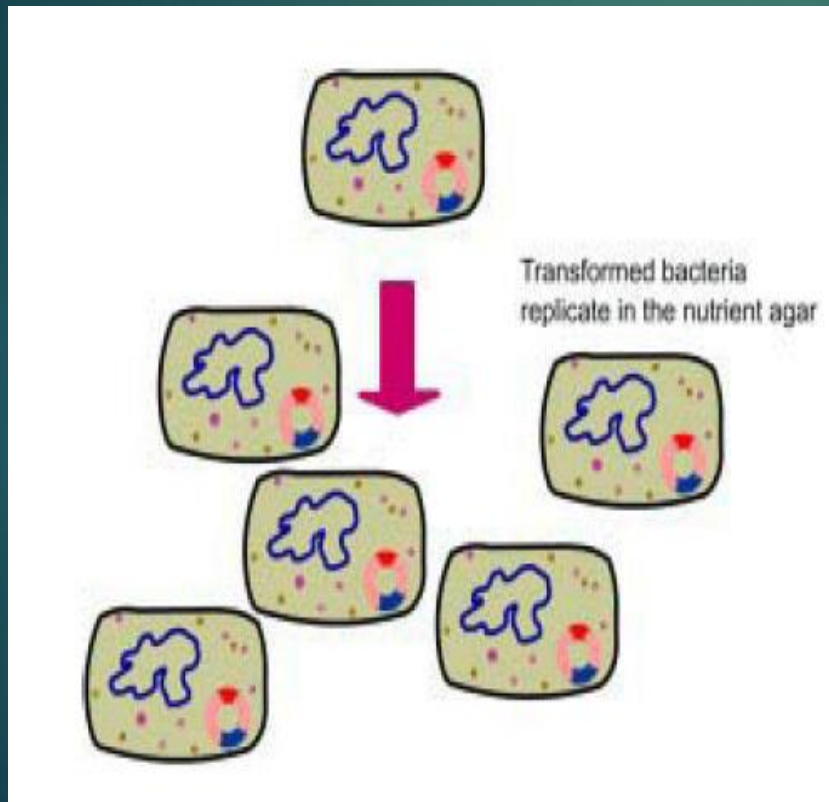
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- ▶ The bacteria *E.coli* is used as the host cell. If *E. coli* and the recombinant plasmids are mixed together in a test-tube.



4) Selecting the bacteria which have taken up the correct piece of DNA

- ▶ The bacteria are spread onto nutrient agar. The agar also contains substances such as an antibiotic which allows growth of only the transformed bacteria.



Safety Issues in relation to Recombinant DNA Technology

- ▶ As bacteria is commonly used in recombinant DNA work, there has always been a concern among scientists and a worry among people that there is a possibility that a clone of highly pathogenic recombinant bacteria were made by accident, then escaped from the laboratory and caused an epidemic for which no drugs were available.
- ▶ Recombinant DNA Advisory Committee (RAC) was established in 1974 in the United States, which responds to public concerns regarding the safety of manipulation of genetic material through the use of recombinant DNA techniques.

Danger of DNA recombinant technology

- ▶ It is always possible that an antibiotic-resistant plasmid could be accidentally incorporated into a dangerous pathogen with serious medical consequences.