MALLA REDDY
College of Pharmacy

ADVANCED DRUG DELIVERY SYSTEM

SEMINAR:
Recombinant DNA Technology

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Introduction:

Based on the concept of gene recombination.

Definition:

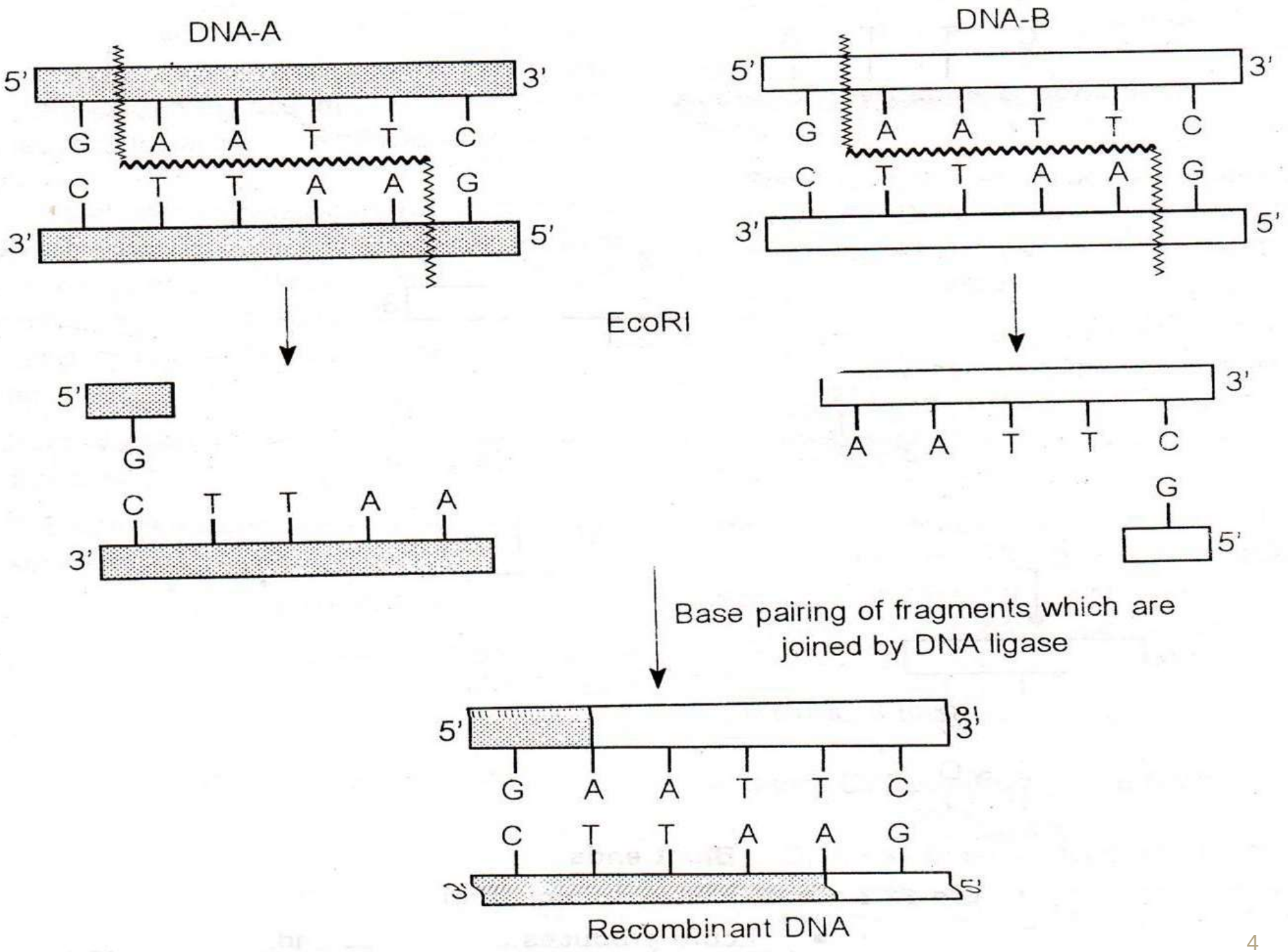
Encompasses a number of experimental protocols

leading to the transfer of genetic information(DNA)

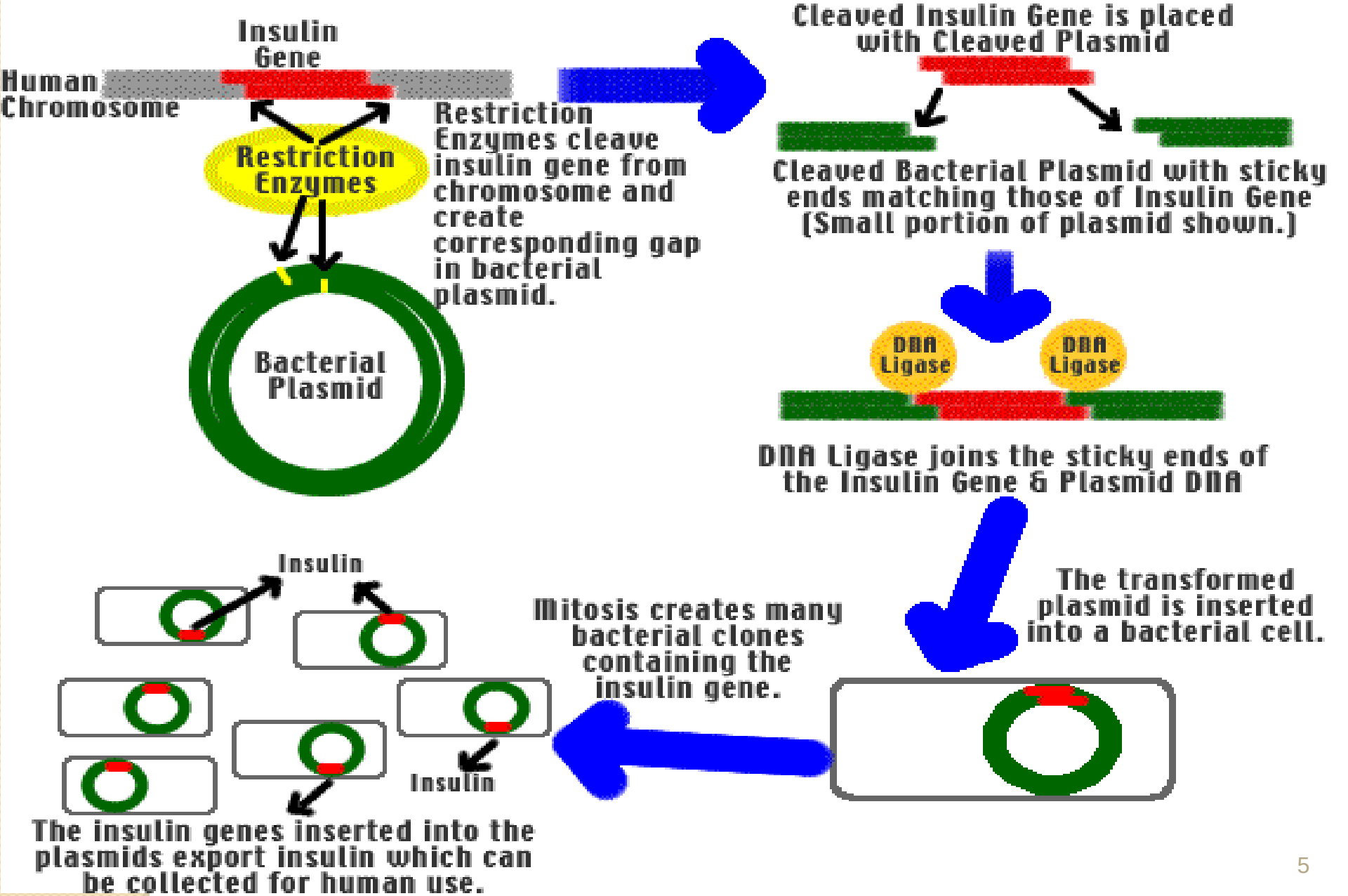
from one organism to another. Involves the manipulation of genetic material(DNA) to achieve the desired goal in a pre-determined way. The present day rDNA technology has its roots in the experiments performed by Boyer & Cohen

Basic principles of rDNA technology:

- Generation of DNA fragments & selection of the desired piece of DNA.
- Insertion of the selected DNA into a cloning vector to create a rDNA or chimeric DNA.
- Introduction of the recombinant vectors into host cells.
- Multiplication & selection of clones containing the recombinant molecules.
- Expression of the gene to produce the desired product.


DNA-A
5'
3'
G A A T T C
C T T A A G
3'
5'
DNA-B
5'
3'
G A A T T C
C T T A A G
3'
5'
EcoRI
5'
G
C T T A A
3'
3'
A A T T C
G
5'
Base pairing of fragments which are joined by DNA ligase
5'
3'
G A A T T C
C T T A A G
3'
5'
Recombinant DNA

Production of Recombinant DNA Using a Human Gene & Bacterial Plasmids



Restriction Endonucleases

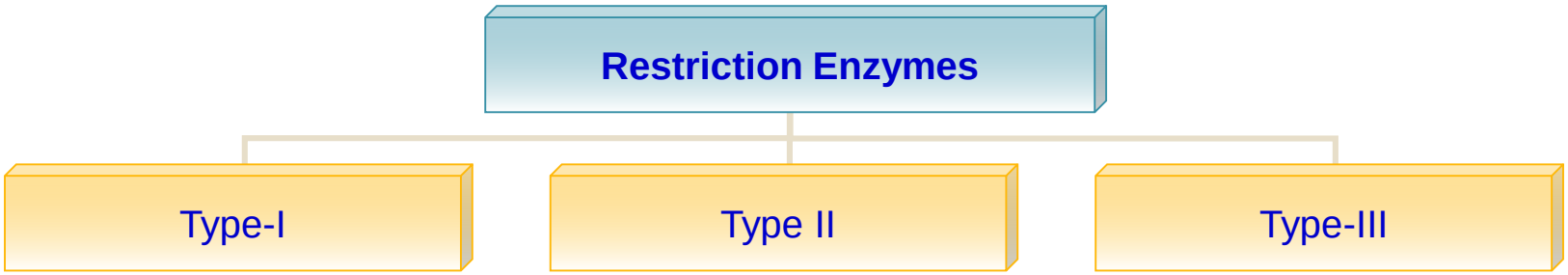
- Enzymes for the manipulation of DNA.
- Are bacterial enzymes that can cut/split DNA at specific sites.
- These were first discovered in E.coli restricting the replication of bacteriophages,by cutting the viral DNA(The host E.coli DNA is protected from cleavage by addition of methyl groups).Thus,the enzymes that restrict the viral replication are known as restriction enzymes or restriction endonucleases.

Recognition sequences:

- Recognition sequence is the site where the DNA is cut by a restriction endonuclease.
- Restriction endonucleases can specifically recognise DNA with a particular sequence of 4-8 nucleotides & cleave.

Cleavage patterns:

- The cut DNA fragments by restriction endonucleases may have mostly sticky ends or blunt ends.
- DNA fragments with sticky ends are particularly useful for rDNA experiments,since single stranded sticky DNA ends can easily pair with any other DNA fragment having complementary



Type I

Don’t Generate specific fragments

Require the presence of Mg+2,ATP and S-adenoyl methionane;the latter activates the enzyme.

Tracks along the DNA for a variable distance before breaking.

Type II

Cut within or immediately adjacent to target sequence

Generates specific fragments

Mg2+ essential

No ATP, No adenosyl methionine

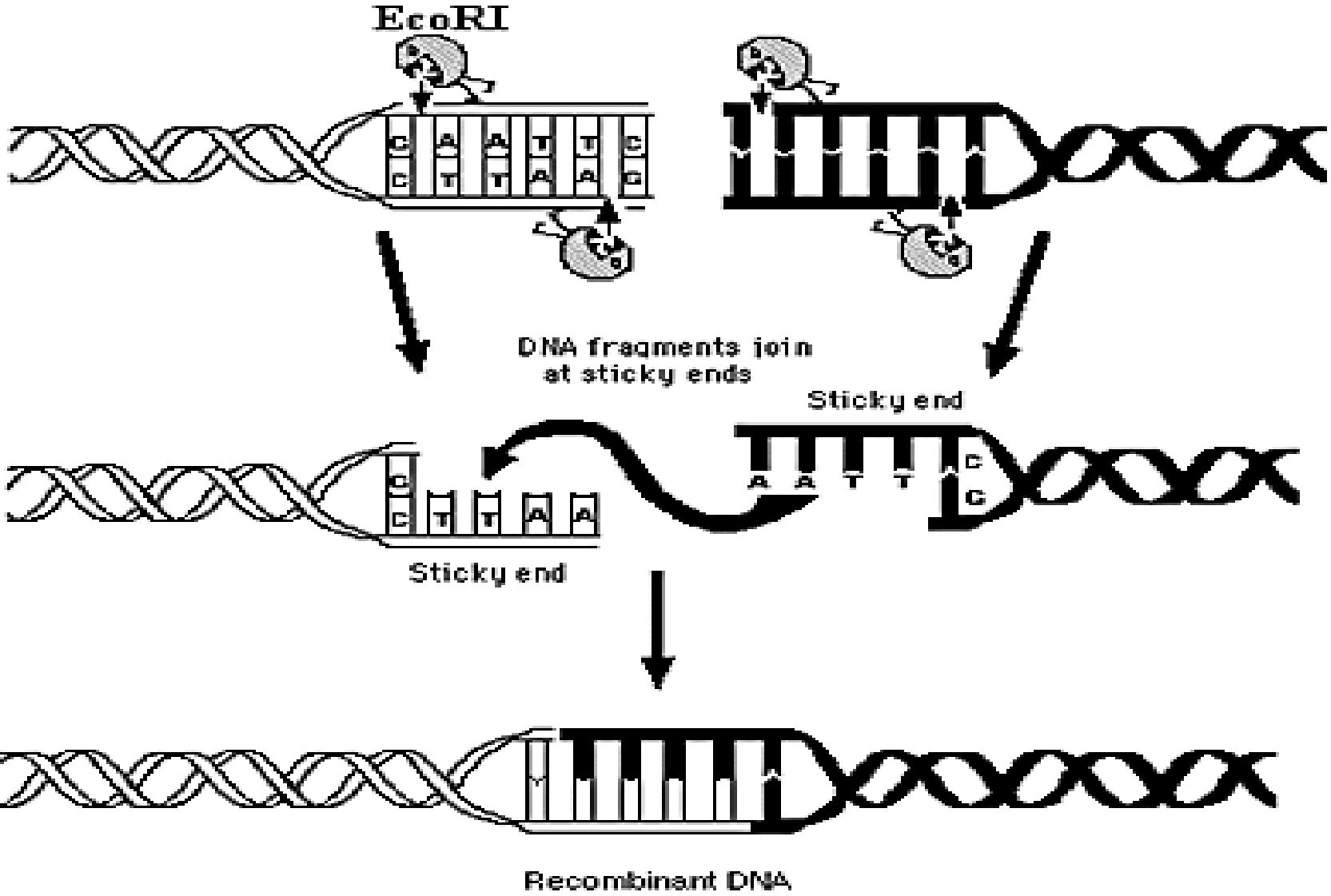
Type III

Requires ATP and S-adenosyl methionine for Cleavage.

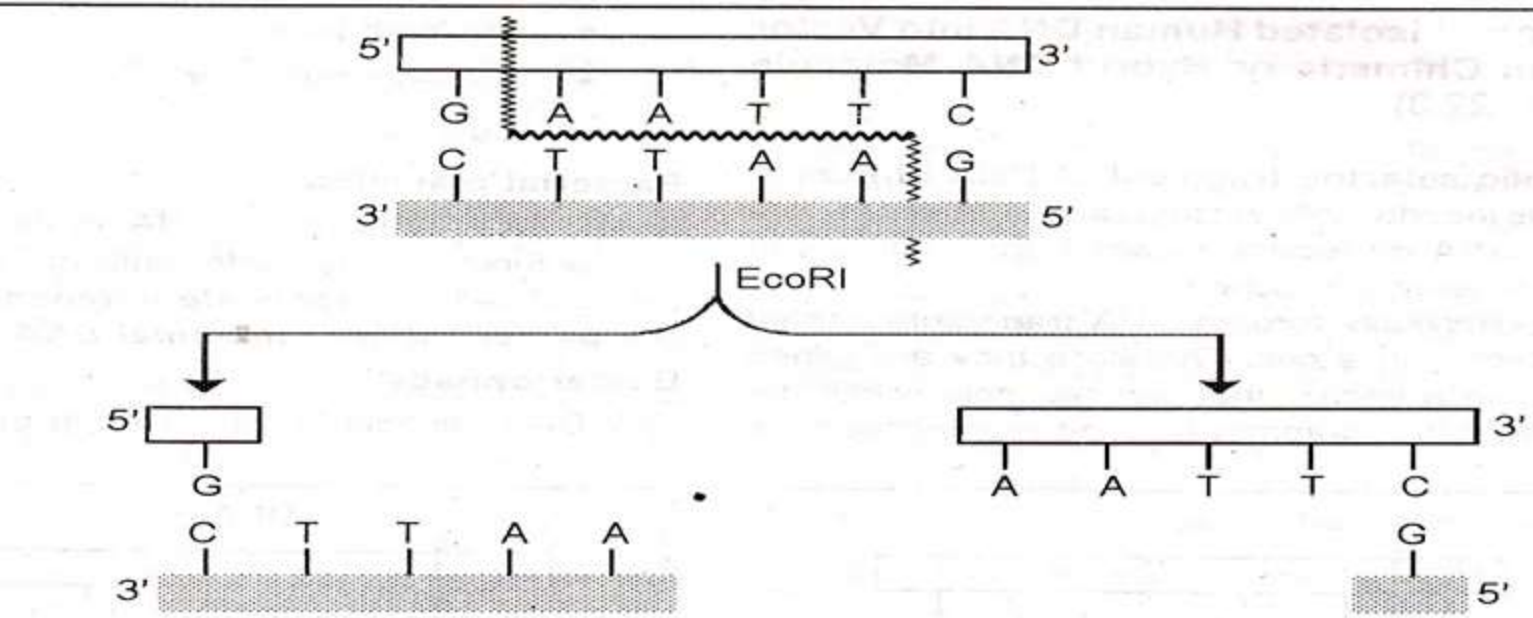
Make breaks in DNA 25 bp away from recognition site.

Microorganism	Restriction Enzyme Name	Restriction Site
Bacillus amyloliquefaciens H	*Bam*HI	G ▽ G A T C C C C T A G △ G
Brevibacterium albidum	*Bal*I	T G G ▽ C C A A C C △ G G T
Escherichia coli RY13	*Eco*RI	G ▽ A A T T C C T T A A △ G
Haemophilus aegyptius	*Hae*II	Pu G C G C ◇ Py Py ◇ C G G C Pu
Haemophilus aegyptius	*Hae*III	G G ▽ C C C C △ G G
Haemophilus influenzae R_d	*Hind*II	G T Py ▽ Pu A C C A Pu △ Py T G
Haemophilus influenzae R_d	*Hind*III	A ▽ A G C T T T T C G A △ A
Haemophilus parainfluenzae	*Hpa*I	G T T ▽ A A C G A A △ T T G
Haemophilus parinfluenzae	*Hpa*II	C ▽ C G G G G C △ C
Providencia stuartii 164	*Pst*I	C T G C A ▽ G G ◇ A C G T C
Streptomyces albus G	*Sal*I	G ▽ T C G A C C A G C T △ G

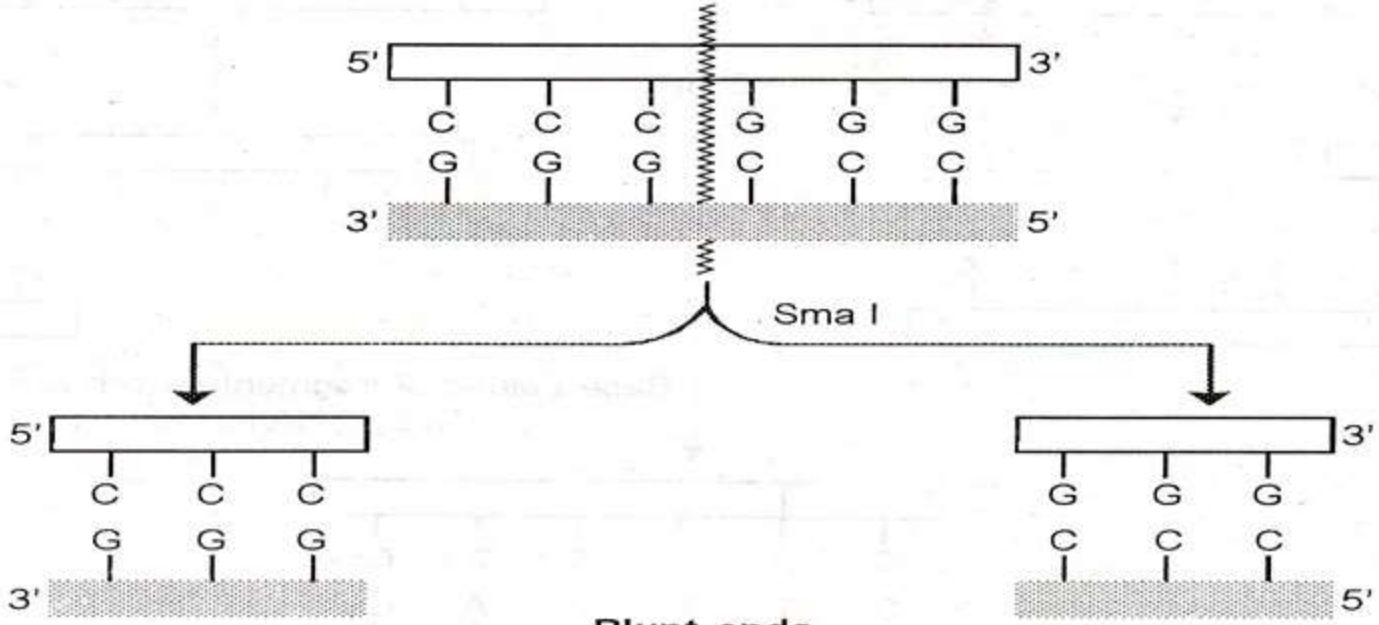
Table 2.1: *Restriction enzymes*



Restriction Enzyme Action of EcoRI



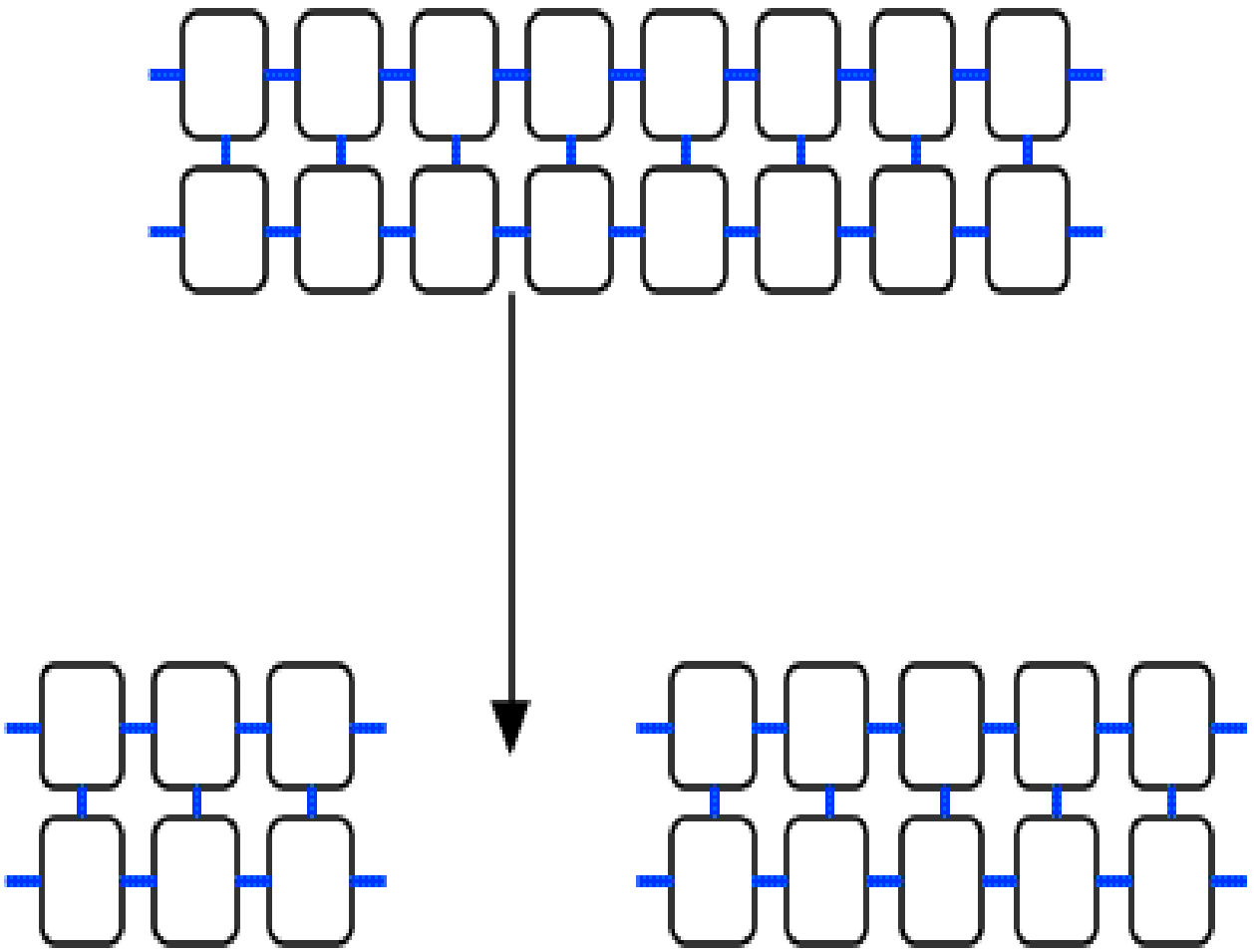
Sticky ends



Blunt ends

Figure 22.2 : Action of restriction endonuclease.

A restriction endonuclease producing blunt ends



No further cleavage (cutting)

DNA ligases

- These were originally isolated from viruses, also occur in E.coli & eukaryotic cells.
- The cut DNA fragments are covalently joined together by DNA ligases.
- DNA ligase joins the DNA fragments by forming a phosphodiester bond b/n the phosphate group of 5’-carbon of one deoxyribose with the hydroxyl group of 3’-carbon of another deoxyribose.

Host cells

The hosts are the living systems or cells in which the carrier of rDNA molecule or vector can be propagated.

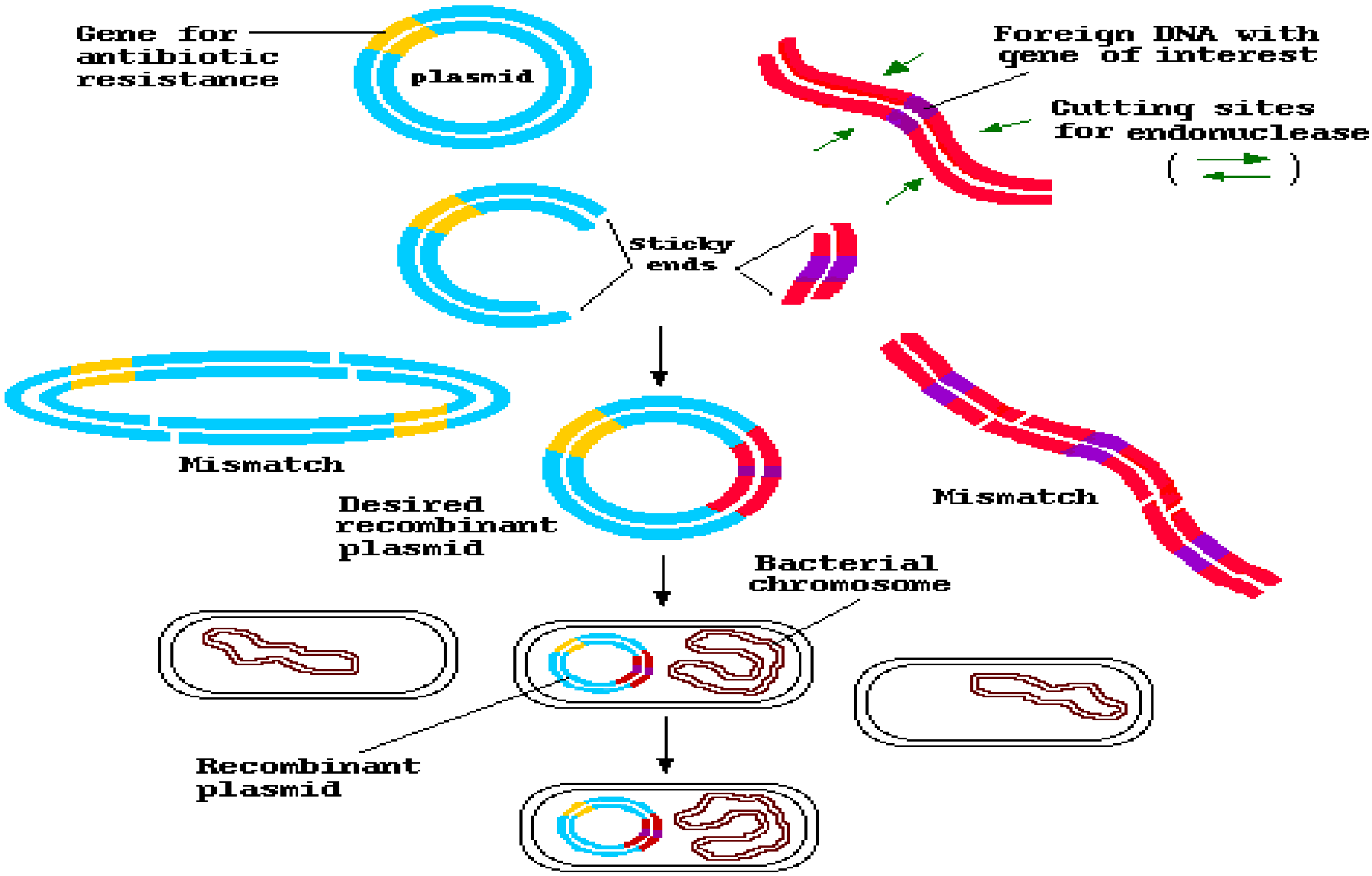
Host cells can be prokaryotic or eukaryotic.

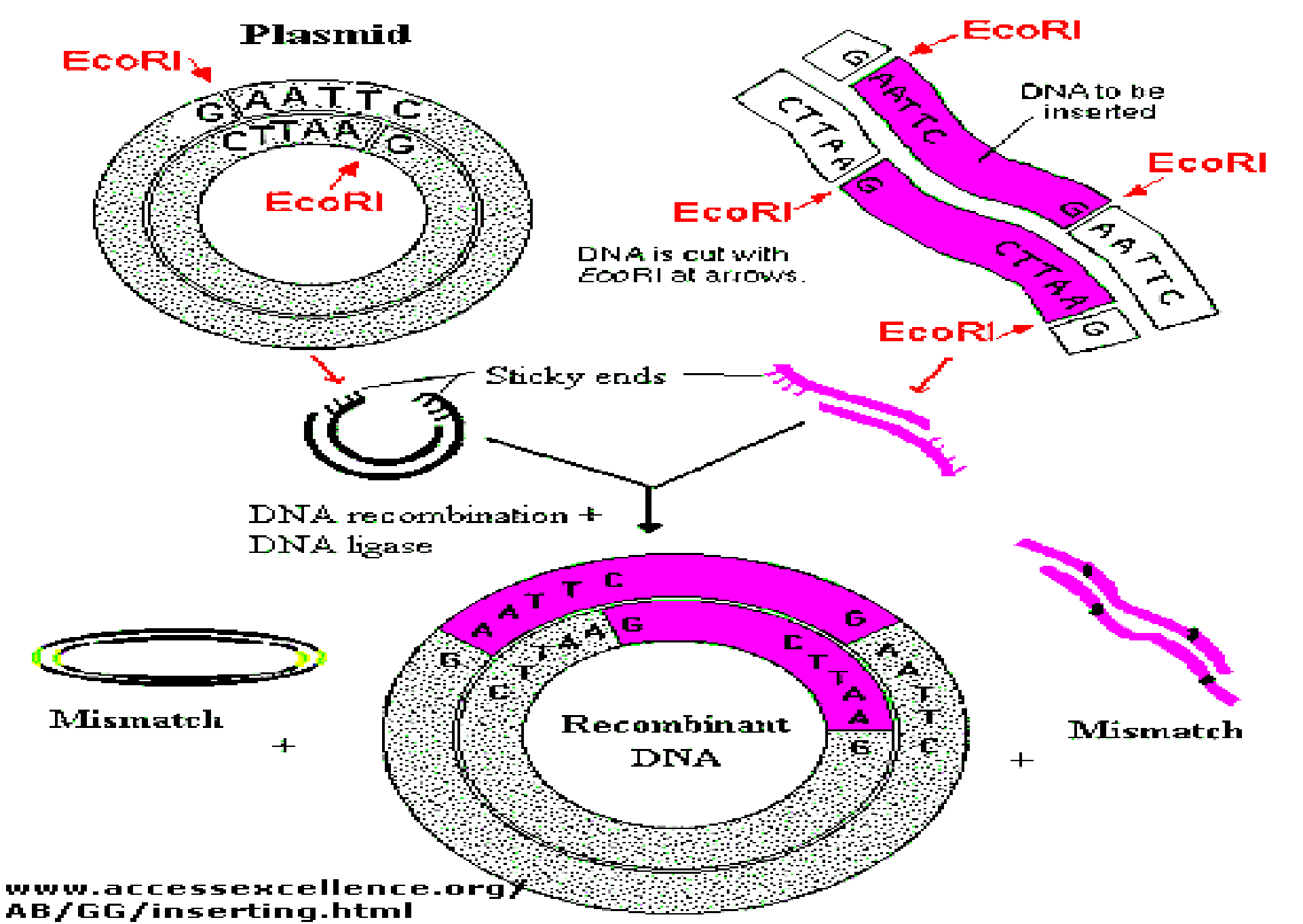
Microorganisms are preferred as host cells, since they multiply faster compared to cells of higher organisms.

E.coli

- This was the first organism used in the DNA technology experiments.
- The major drawback is that it cannot perform post translational modifications.

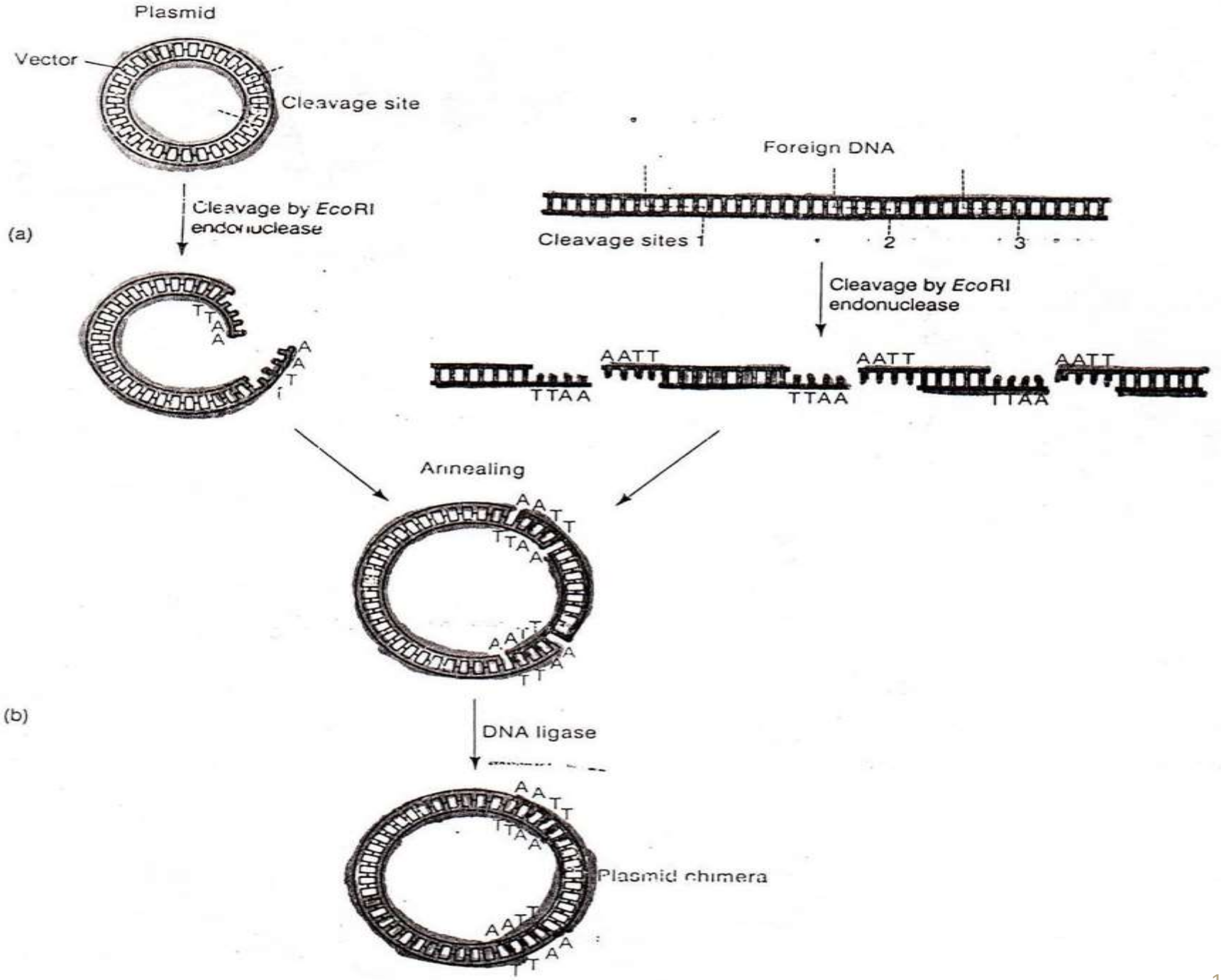
Plasmid Insertion





www.accessexcellence.org/AB/GG/inserting.html

Inserting a DNA Sample into a Plasmid


Plasmid
Vector
Cleavage site
Cleavage by EcoRI endonuclease
(a)
Foreign DNA
Cleavage sites 1
2
3
Cleavage by EcoRI endonuclease
TTAA
AATT
TTAA
AATT
TTAA
AATT
Annealing
(b)
DNA ligase
Plasmid chimera

Eukaryotic Hosts

- These are preferred to produce human proteins, since these have complex structure suitable to synthesise complex proteins.

- Mammalian cells possess the machinery to modify the protein to the active form.(post translational modifications)

  E.g., Tissue plasminogen activator

Vectors

- Are the DNA molecules, which can carry a foreign DNA fragment to be cloned.
- These are self replicating in an appropriate host cell.
- Most important vectors are plasmids, bacteriophages, cosmids & artificial chromosome vectors.

Plasmids

- Are extrachromosomal, double stranded, circular, self-replicating DNA molecules.
- Usually plasmids contribute to about 0.5%-5.0% of the total DNA of bacteria.
- A few bacteria contain linear plasmids

  E.g., streptomyces sp, Borelia burgdorferi.

  E.g., pBR322,pUC
- The plasmids carries genes resistance for ampicillin & tetracycline that serve as markers for the identification of clones carrying plasmids.

Bacteriophages

- Are the viruses that replicate within the bacteria.
- In case of certain phages, their DNA gets incorporated into the bacterial chromosome & remains there permanently.
- Can take up larger DNA segments than plasmids,hence preferred for working with genomes of human cells.

  E.g., phage λ, phageM13.

Cosmids

- Are the vectors possessing the characteristics of both plasmid & bacteriophage λ.
- These carry larger fragments of foreign DNA compared to plasmids.

Artificial chromosome vectors

- E.g.,Human artificial chromosome, Yeast artificial chromosomes, Bacterial artificial chromosome
- These can accept large fragments of foreign DNA

Methods of Gene transfer

1. Transformation

The uptake of plasmid DNA by E.coli is carried out in ice-cold CaCl2(0-5C) & a subsequent heat shock

(37-45C for about 90sec)

2. Conjugation

- Is a natural microbial recombination process.
- Plasmid-insert DNA is transferred from one cell to another.

3. Electroporation

- Is based on the principle that high voltage electric pulses can induce cell plasma membranes to fuse.
- Liposome-mediated gene transfer(Lipofection) are circular lipid molecules, having aqueous interior that can carry nucleic acids.

4. Direct transfer of DNA

- DNA is directly transferred into the nucleus by microinjection & particle bombardment.

Applications of rDNA technology

Manufacture of proteins/hormones Interferon, plasminogen activating factor, blood clotting factors, insulin, growth hormone.

- **AIDS test:** Has become simple & rapid
- **Diagnosis of molecular diseases:** sickle cell anaemia, thalassaemia, familial hypercholesterolaemia, cystic fibrosis
- **Prenatal diagnosis:** DNA from cells collected from amniotic fluid, chorionic villi

Gene Therapy:

- This is achieved by cloning a gene into a vector that will readily be taken up & incorporated into genome of a host cell.

ADA deficiency has been successfully treated

Application in Agriculture:

- Genetically engineered plants are developed to resist draught & diseases. Good quality of food & increased yield of crops is also possible.

- **Industrial Application:**

  Enzymes---use to produce sugars, cheese, detergents.

  Protein products---used as food additives, increases nutritive value, besides imparting flavour.

- **Application in forensic medicine:** The restriction analysis pattern of DNA of one individual will be very specific(DNA fingerprinting),but the pattern will be different from person to person. Helps to identify criminals & to settle disputes of parenthood of children.

- **Transgenesis:** Gene replacement therapy will not pass on to offspring. Therefore genes are transferred into fertilised ovum which will be found in

Gene cloning

- The recombinant DNA molecule is transferred to a host cell.Within the host cell it replicates producing dozens of identical copies i.e., it is cloned.
- The cloned DNA can be recovered from host cells purified, analysed & transcribed.
- It’s mRNA translated.
- Gene product isolated & used for research or sold commercially.

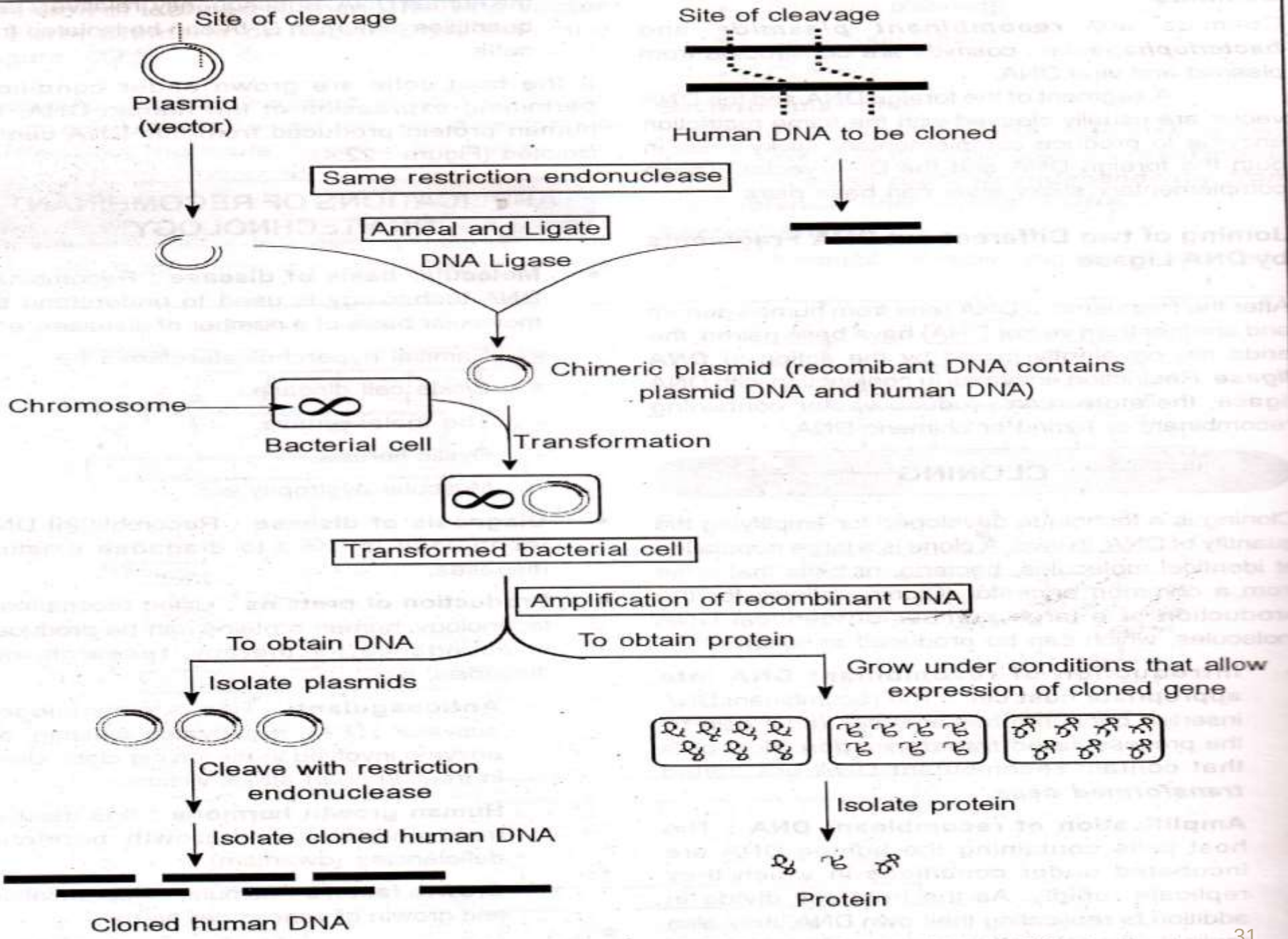
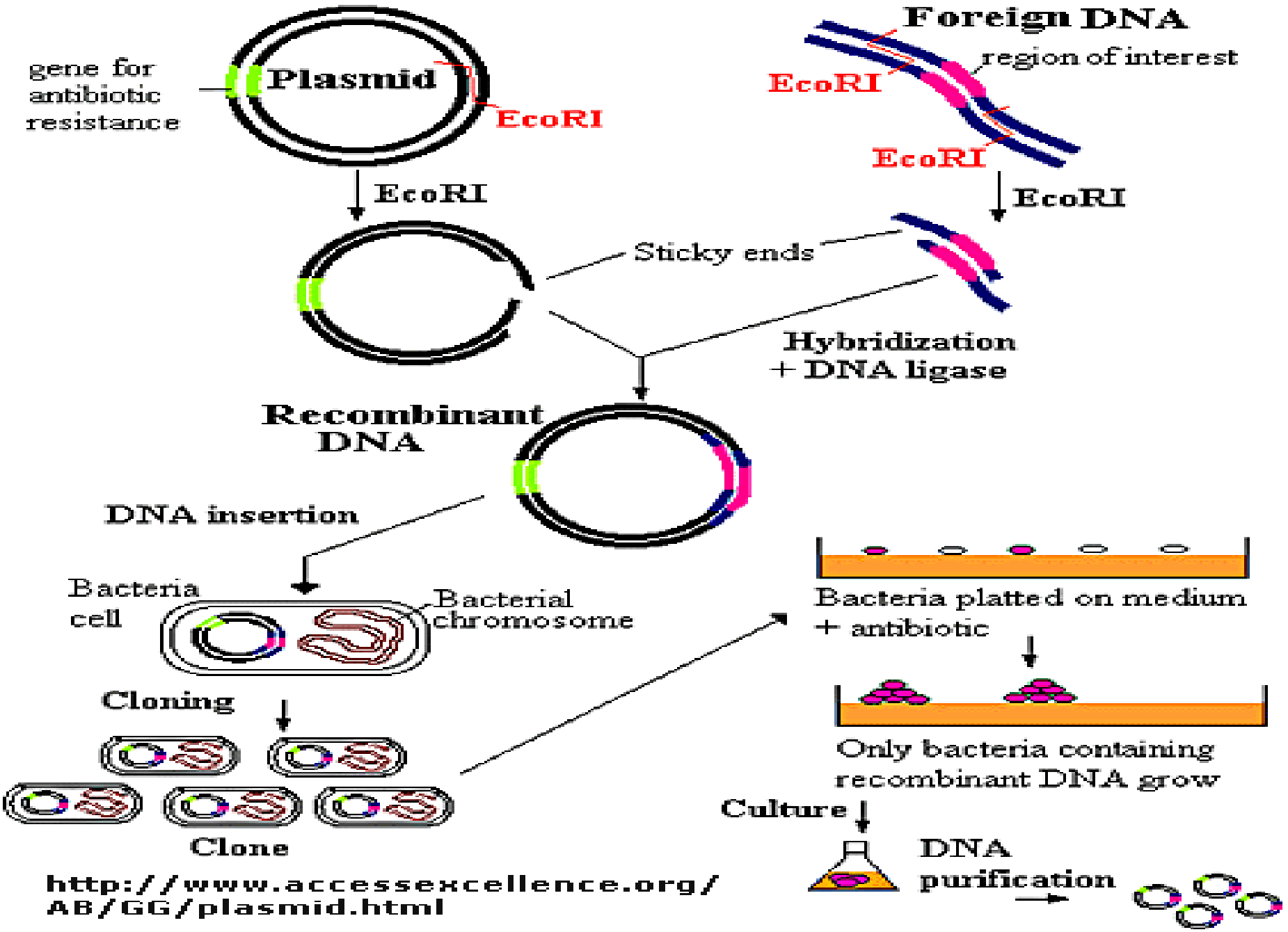


Figure 22.4 : Cloning of human DNA in bacteria using recombinant DNA technology



Cloning into a plasmid

Gene libraries

- Collections of cloned DNA sequences.
- Each cloned segment relatively small.
- Many separate clones are required.
- Libraries-----Genomic, chromosome specific c-DNA.
- IT is a resource that can be used to retrieve any of the genes from our starting material.

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THANK YOU