TOPIC : RECOMBINANT DNA TECHNOLOGY- I

(HISTORY, TOOLS, TECHNIQUES AND STEPS INVOLVED)

COURSE : M.SC. BOTANY PART II

PAPER : XVI (BIOTECHNOLOGY AND BIOINFORMATICS)

PREPARED BY : DR. RASHMI KOMAL

COORDINATED BY : DR. SHYAM NANDAN PRASAD

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RECOMBINANT DNA TECNOLOGY AND GENETIC ENGINEERING

- **Recombinant DNA Technology (RDT)** is the technique of joining together distinct/different DNA molecules to produce new genetic combinations as Recombinant DNA(rDNA).
- **Genetic engineering** is defined as the direct manipulation of an organism's genes including heritable and nonheritable recombinant DNA constructs. It is the process of using recombinant DNA (rDNA) technology to alter the genetic makeup of an organism.
- **Paul Berg** is the "father of genetic engineering".
- The first production of recombinant DNA molecules, using restriction enzymes, occurred in the early 1970s.
- Stanley Cohen and Herbert Boyer were the first scientists to transplant genes from one living organism to another, a fundamental discovery for genetical engineering.

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Paul Berg

Herbert Boyer

Stanley Cohen

The pioneering work of Paul Berg, Herbert Boyer, and Stanley Cohen in the early 1970s led to the development of **recombinant DNA technology**, which has permitted biology to move from an exclusively analytical science to a synthetic one.

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HISTORY

- ✓ First breakthrough in 1960s : in 1969, Herbert Boyer isolated restriction enzyme EcoRI from E. coli that cleaves the DNA between G and A in the base sequence GAATTC.
- ✓ In 1970, **Howard Temin and Davin Baltimore** independently discovered the enzyme reverse transcriptase from retroviruses. This enzyme was used to construct a DNA called **complementary DNA** (cDNA) from any mRNA.
- ✓ In, 1972 **David Jackson**, **Robert Symons and Paul Berg** successfully generated rDNA molecules. They ligated the sticky ends of complementary DNA by using an enzyme DNA ligase.
- ✓ In 1973 for the first time S.Cohen and H.Boyer developed a Recombinant plasmid (pSC101) which on using as vector replicated well within a bacterial host.
- ✓ In 1975, Edwin Southern developed a method for detection of specific DNA fragments for isolation of a gene from complex mixture of DNA. This method is known as the **southern blotting technique.**

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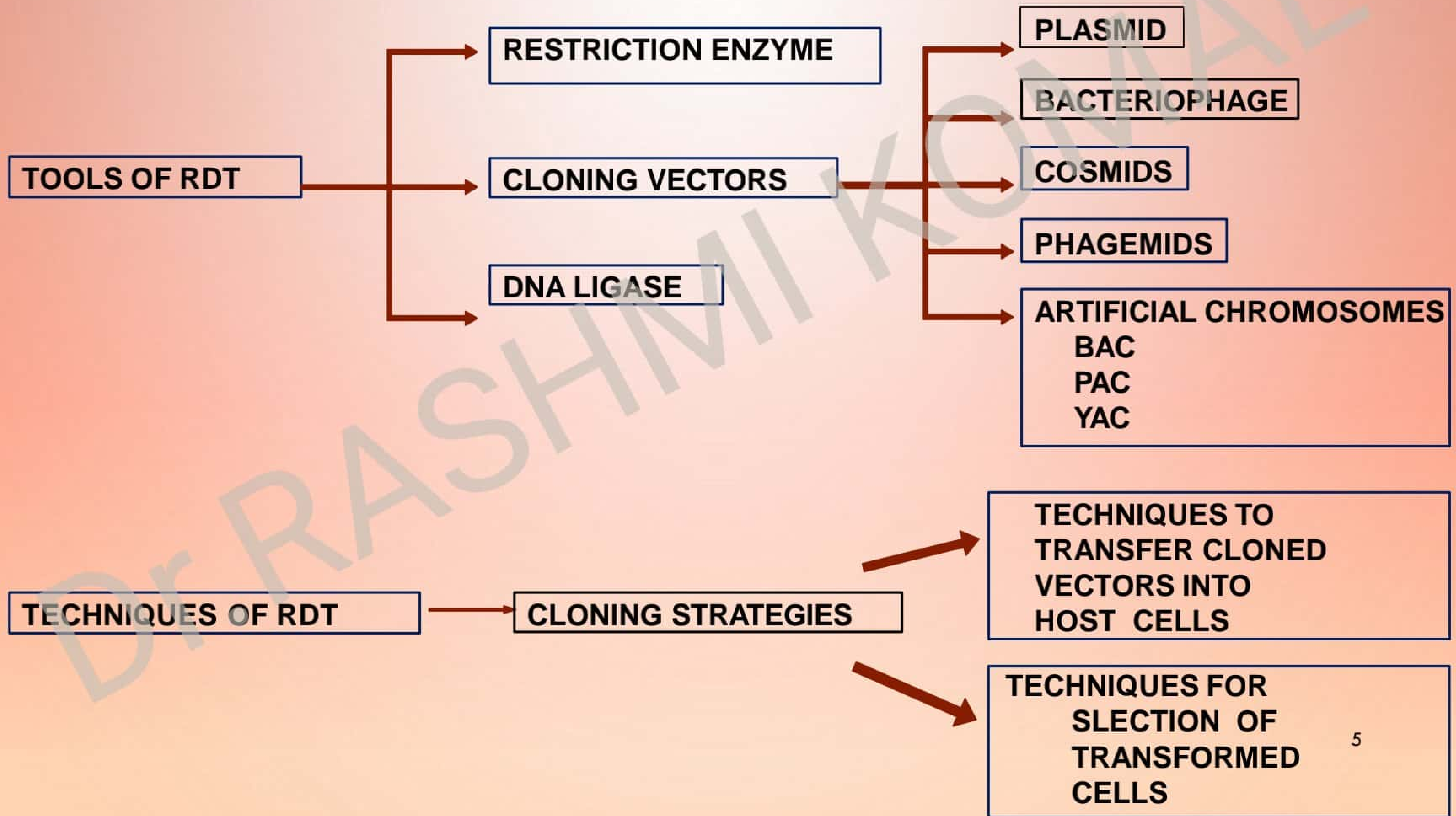
TOOLS AND TECHNIQUES OF RECOMBINANT DNA TECHNOLOGY (RDT)



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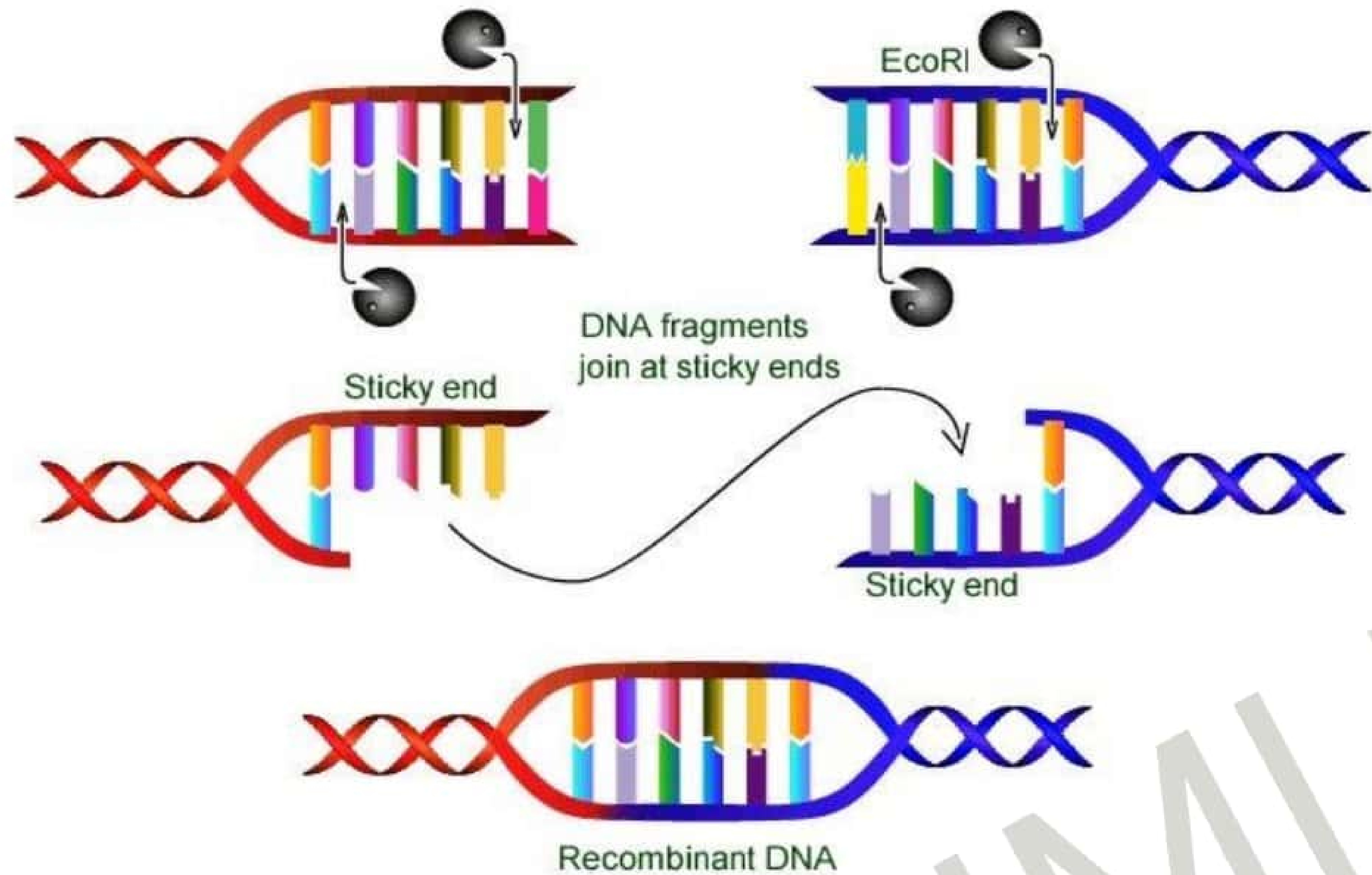

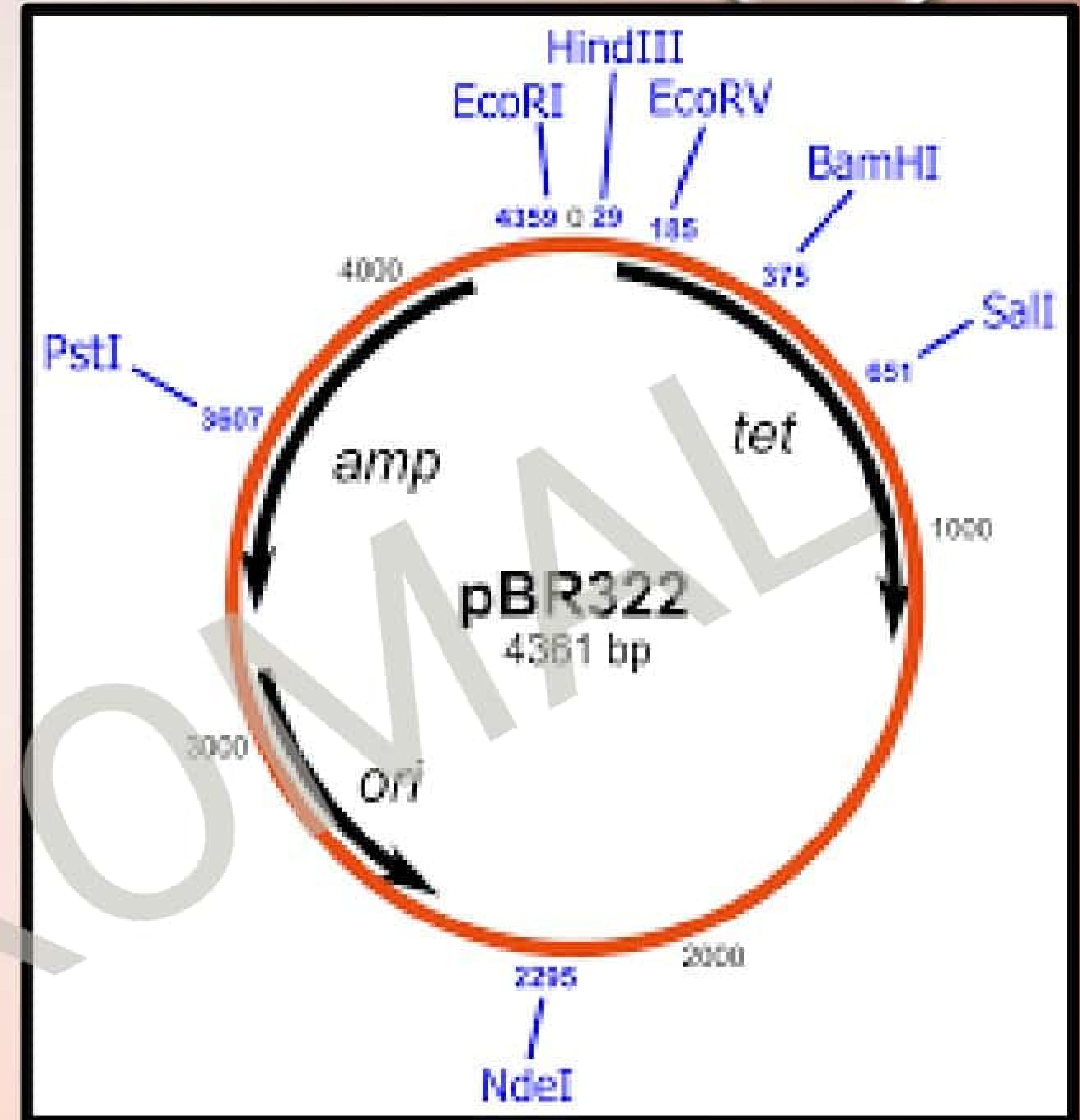

DNA backbone

Sticky End

DNA insert

G A A T T C

C T T A A G

Sticky End

Ligation

Recombinant DNA

G A A T T C

C T T A A G

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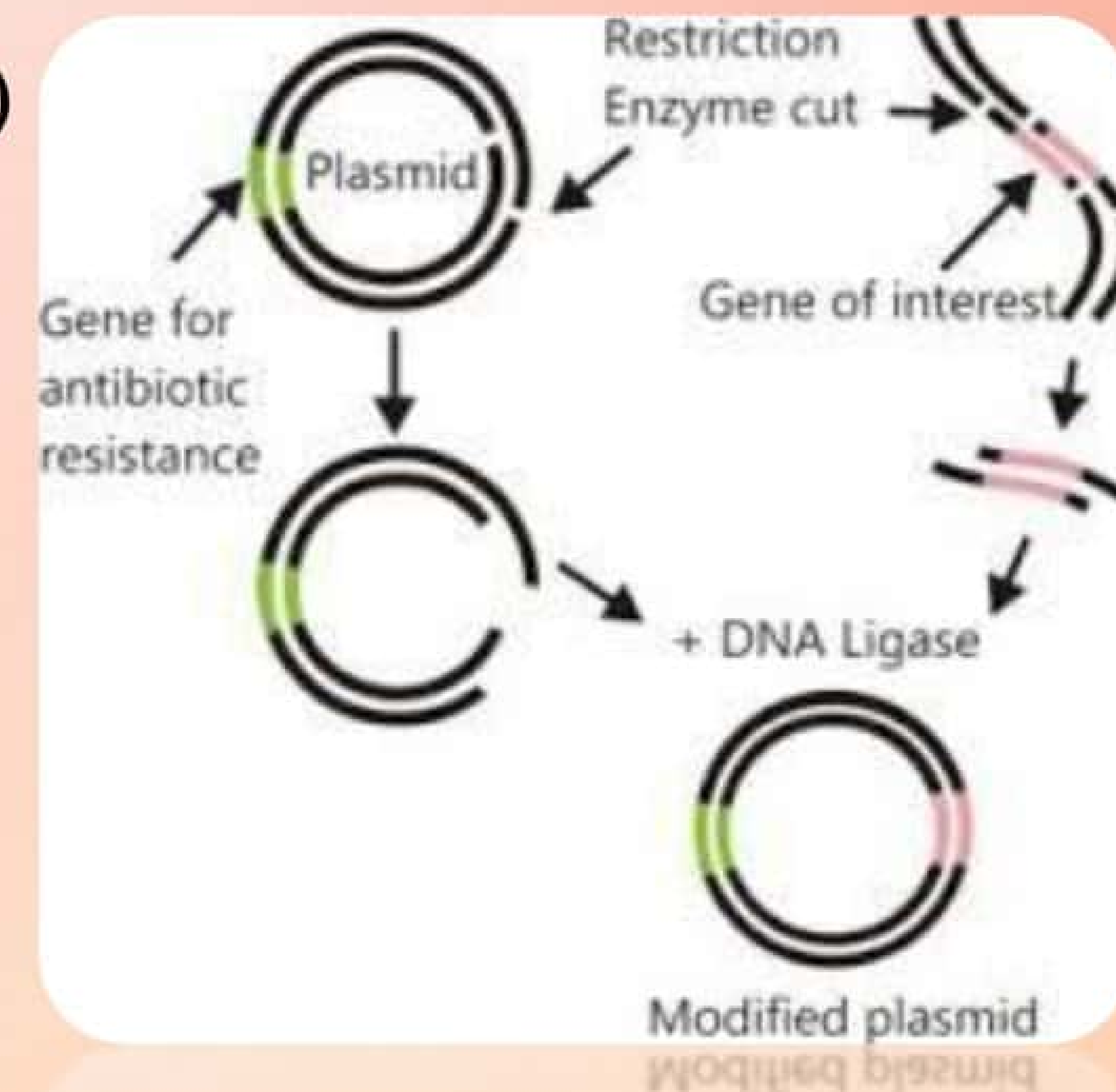
CLONING VECTORS

- ✓ Vectors are those, that transfer donar DNA fragment with gene of interest to host cell (recipient) and are capable of replicating in the host cell.
- ✓ Cloning vectors include plasmids, bacteriophages, cosmids, phasmids, Bacterial Artificial Chromosomes (BACs) & Yeast Artificial Chromosomes(YACs).
- ✓ **CHARACTERISTIC FEATURES OF CLONING VECTORS :**
  - An ori sequence (Origin of Replication)
  - A selectable marker
  - One or more restriction sites
  - Antibiotic resistant genes



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VARIOUS TYPES OF VECTORS AND THEIR INSERTSIZE	
CLONING VECTORS	**INSERT SIZE(kb)**
Plasmid	0.5-8
Bacteriophage	5-25
Cosmids	35-45
BAC	50-300
YAC	200-1000

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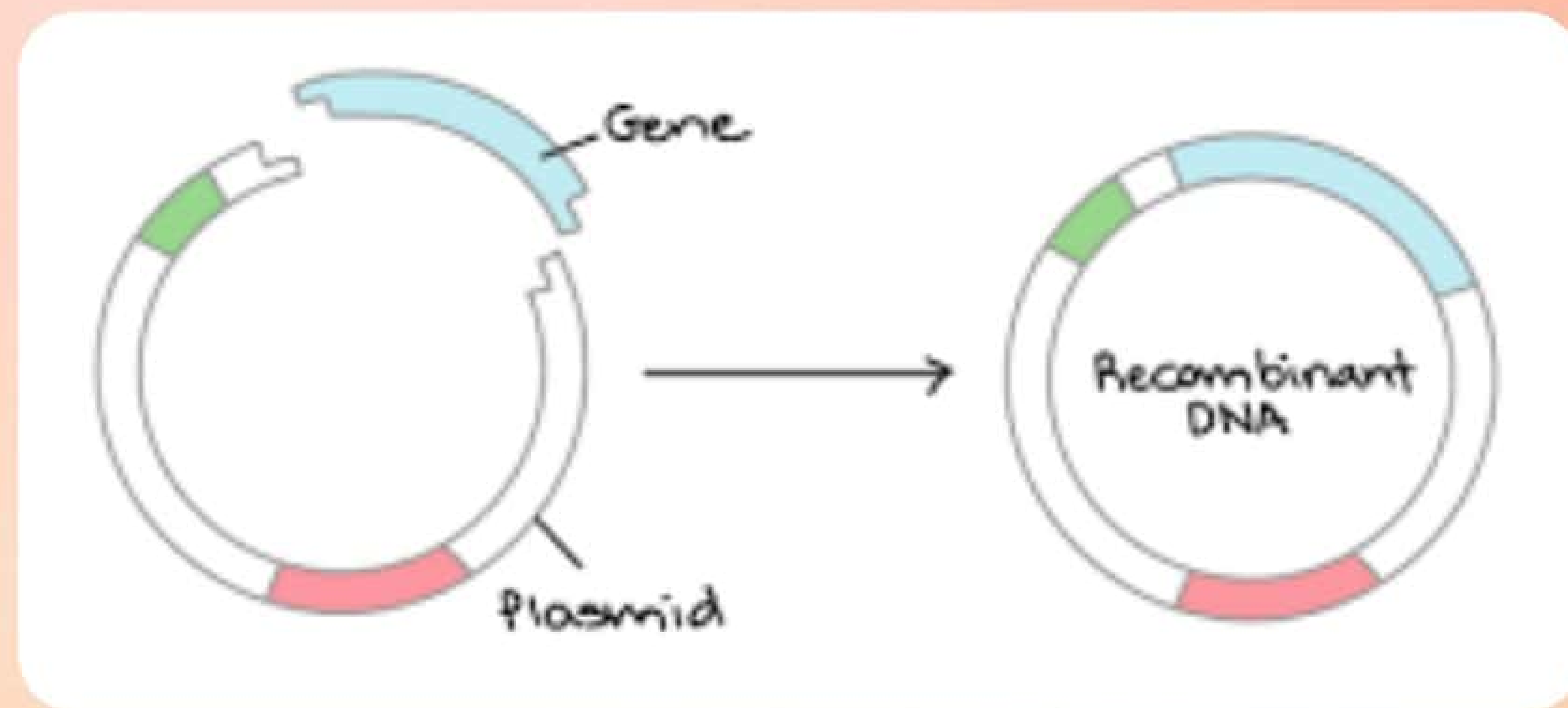
STEPS INVOLVED IN RDT :

1. Obtaining a copy of desired gene by cleaving the DNA with Restriction Endonuclease.
2. Inserting the gene in a suitable Vector.
3. Introducing the vector with desired gene in a host cell.
4. Selection of the transformed host cell.
5. Cloning of Gene.



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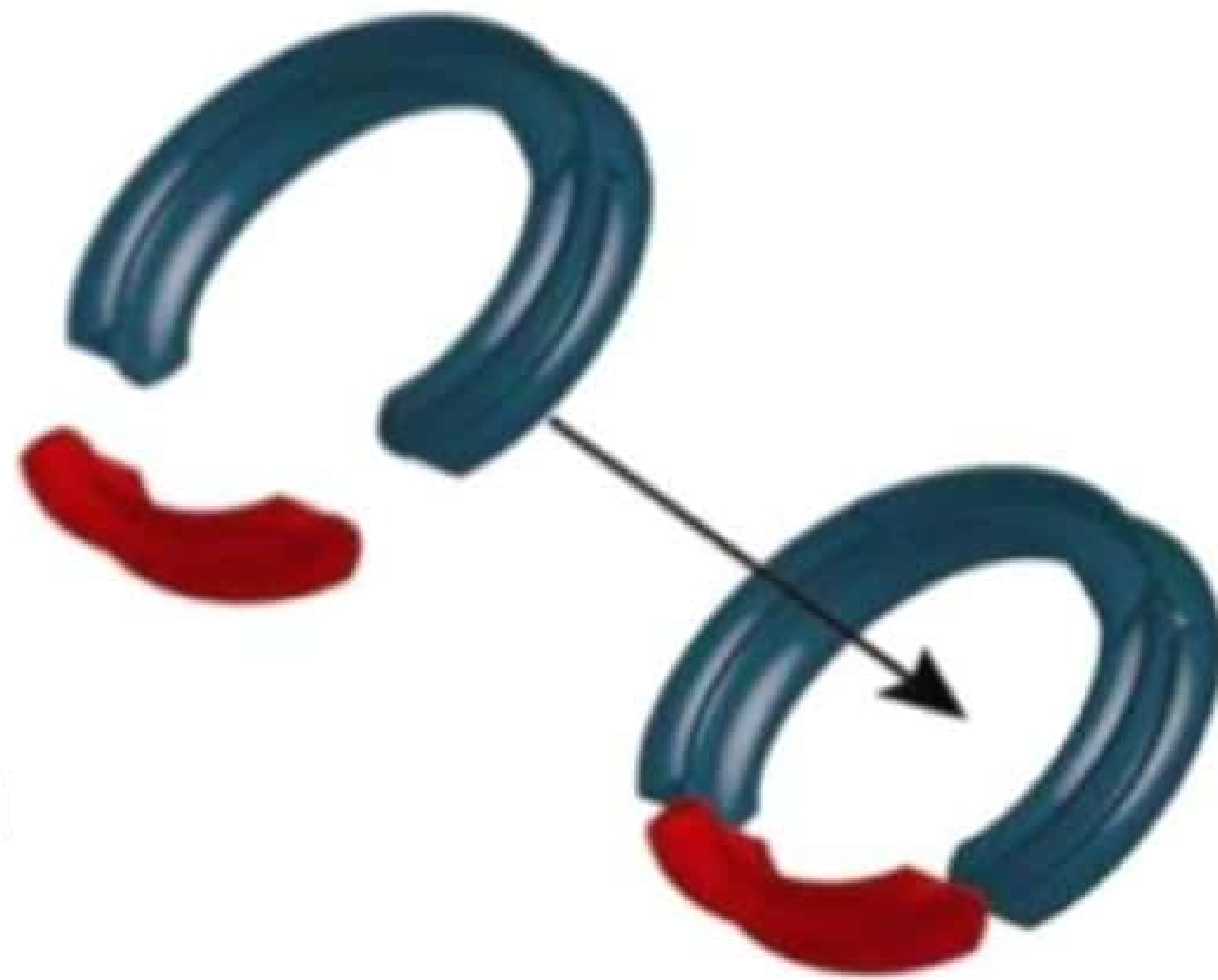
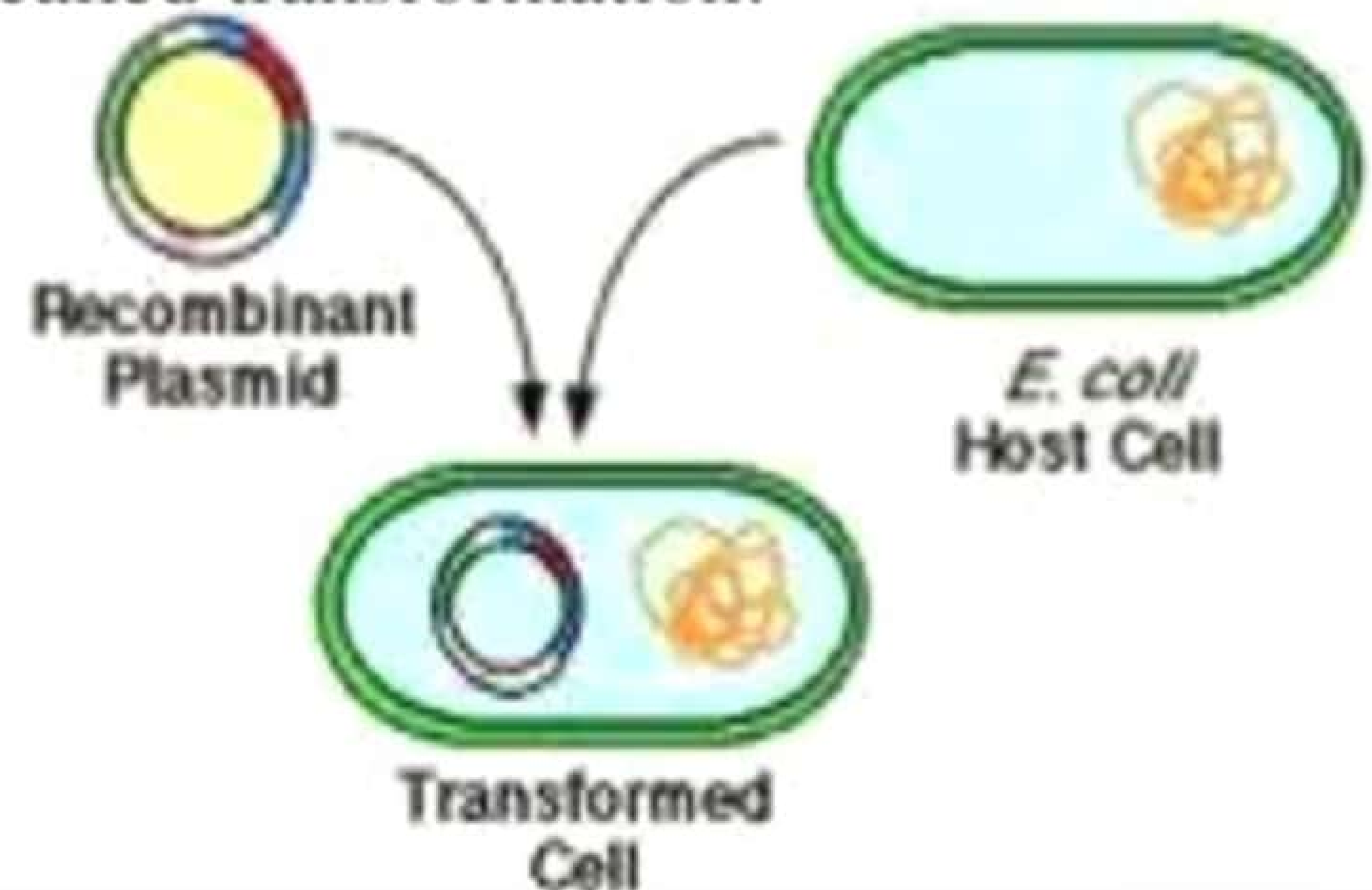
A fragment of DNA, containing the gene to be cloned, is inserted into a circular DNA molecule called a vector, to produce a chimera or recombinan DNA (rDNA) molecule.



STEP 1

STEP 2

The vector acts as a vehicle that transports the gene into a host cell, which is usually a bacterium although other types of living cell can be used. This process is called transformation.



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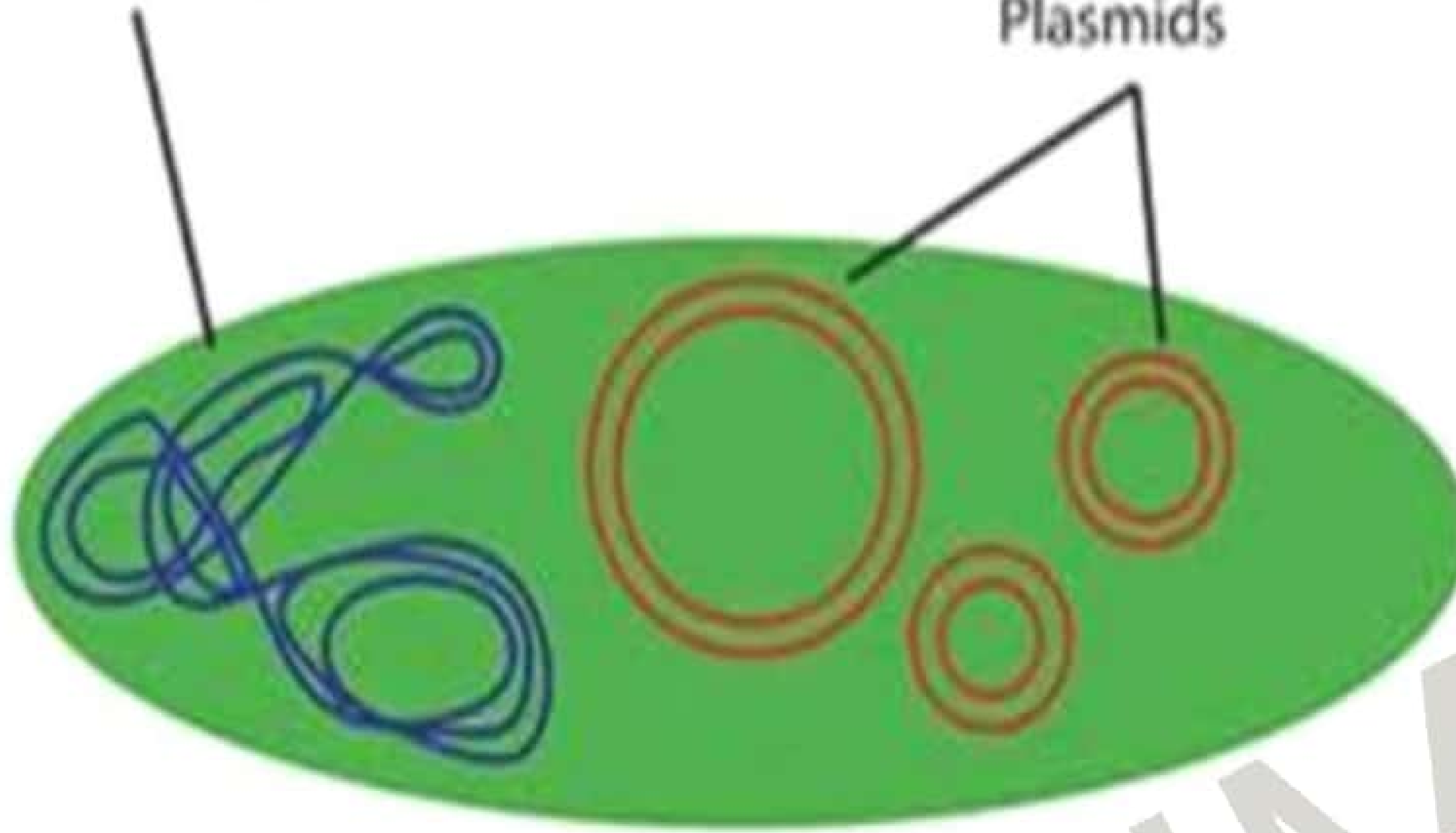
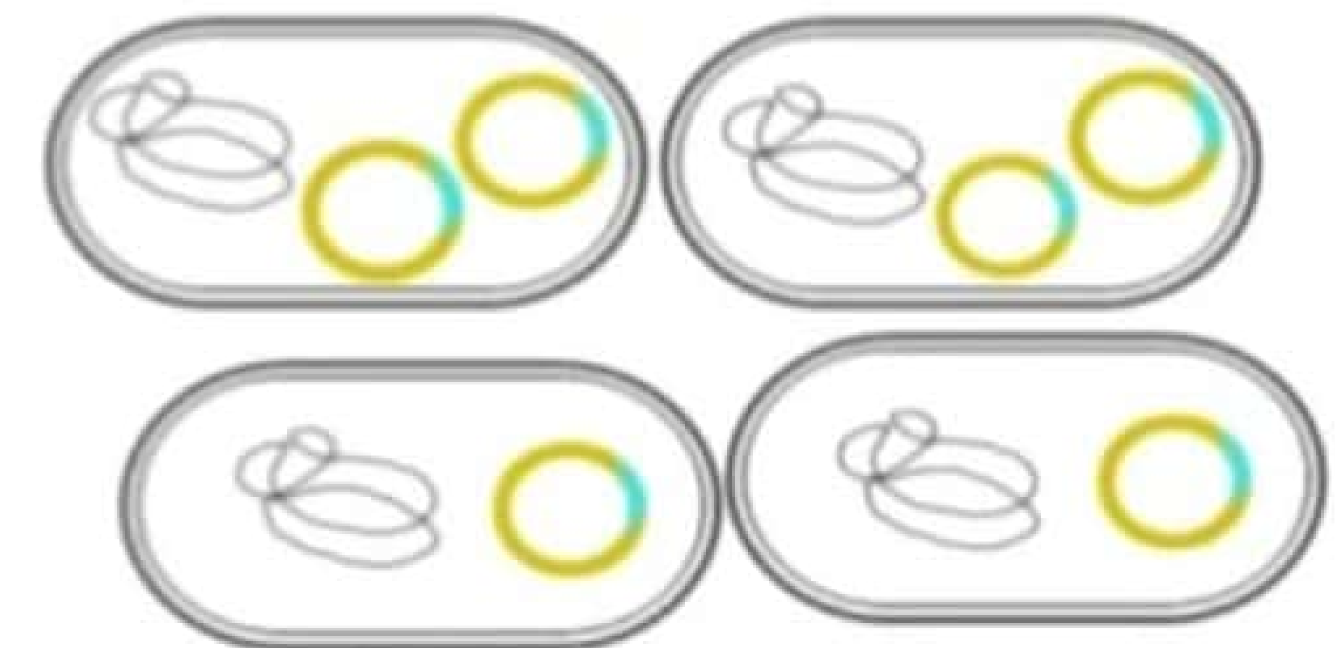
Within the host cell the vector multiplies producing numerous identical copies not only of itself but also of the gene that it carries.



Transformation

Amplification

When the host cell divides, copies of rDNA molecule are passed to the progeny and further vector replication takes place.



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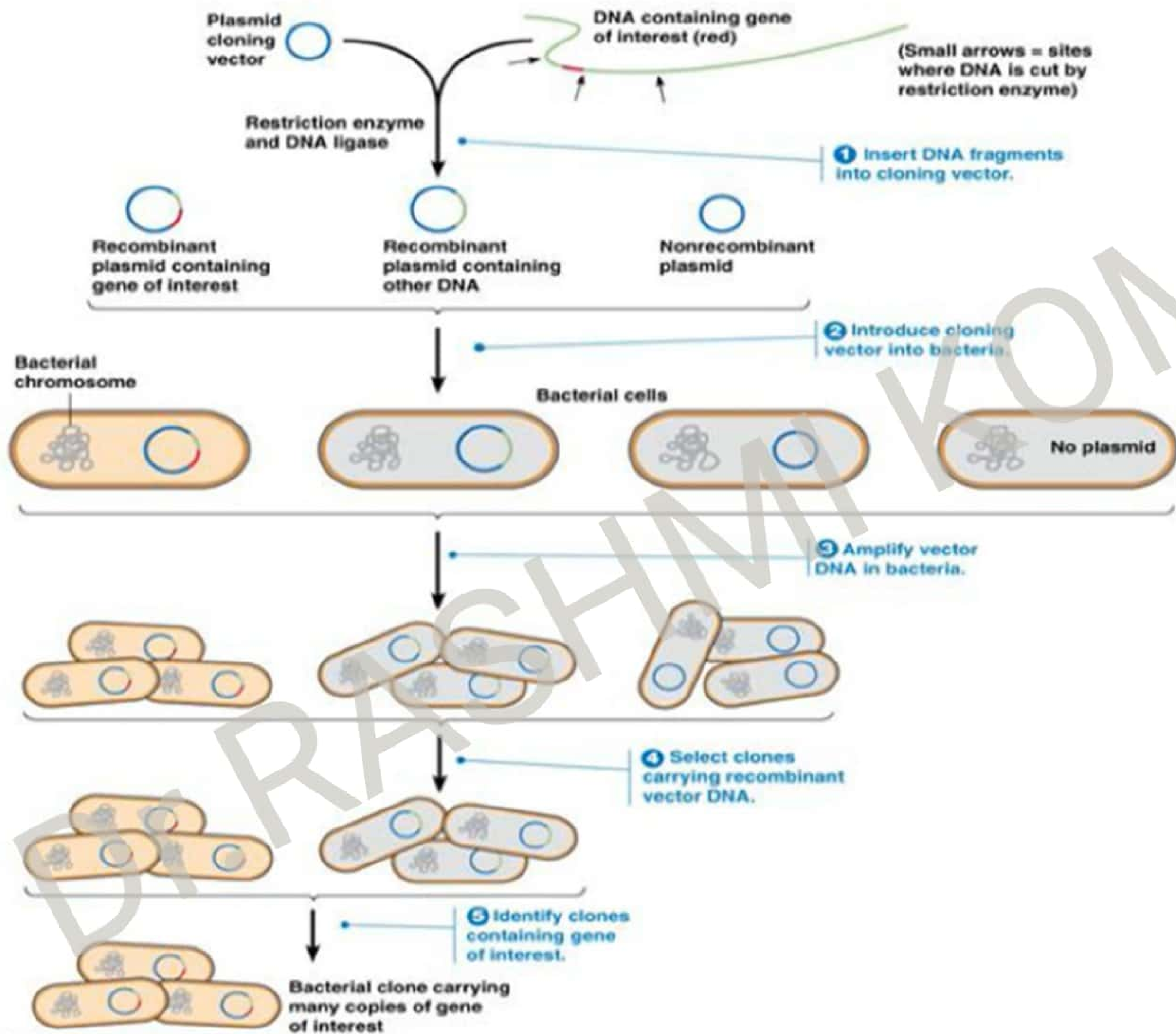
Steps in recombinant DNA Technology



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