GENETIC ENGINEERING

Presentation by

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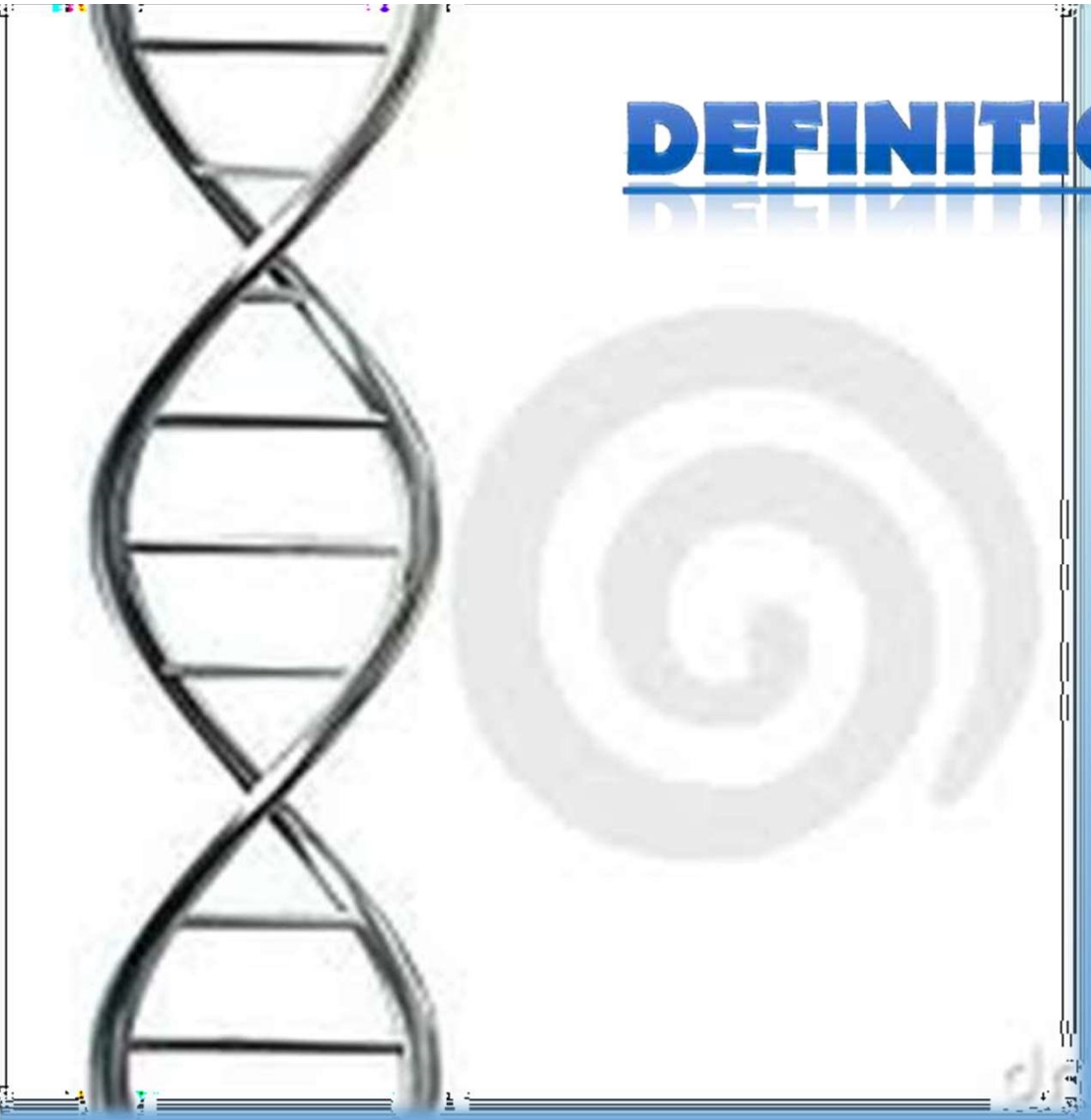
WHAT IS A GENE ?

- **A Gene is a fundamental, physical and functional unit of heredity.**
- **It is responsible for the physical and inheritable characteristics of an organism.**

Genetic Engineering:

- 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.



DEFINITION:

Genetic Engineering is manipulation/alteration of structure of a gene to create a desired characteristic in an organism.

GENETIC ENGINEERING

- DEFINITION: ABILITY TO PRECISELY MANIPULATE DNA SEQUENCES FROM WIDELY DIFFERENT ORGANISMS.
- PROCESS REQUIRES
  - ABILITY TO CUT DNA
  - TO INSERT FOREIGN DNA SEGMENT
  - "GLUE" DNA SEQUENCES TOGETHER

History:

- The term “Genetic Engineering" was first coined by Jack Williamson in his science fiction novel.
- James Watson and Francis Crick showed that the DNA molecule has a double-helix structure.
- In 1972, Paul berg created the first recombinant DNA molecules by combining DNA from the monkey virus SV40 with that of the lambda virus.

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.

- Other terms – Recombinant DNA technology
  Gene manipulation
  Gene cloning
  Genetic modifications
  New genetics

DISCOVERY OF RECOMBINANT DNA TECHNOLOGY

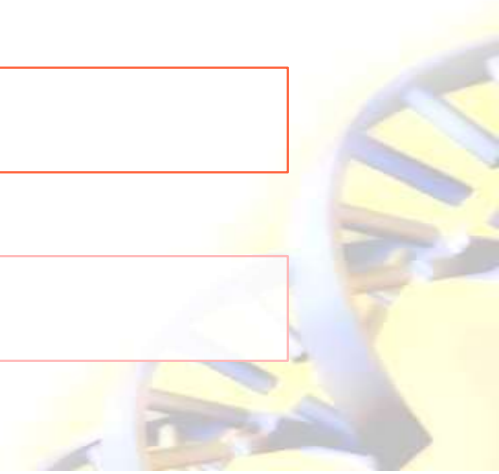
- Discovery of DNA structure Watson & Crick in 1953
- Isolation of DNA ligase in 1967
- Isolation of REase in 1970
- Paul Berg generated rDNA technology in 1972
- Cohen & Boyer in 1973 produced first plasmid vector capable of being replicated within a bacterial host

GOALS OF RECOMBINANT DNA TECHNOLOGY

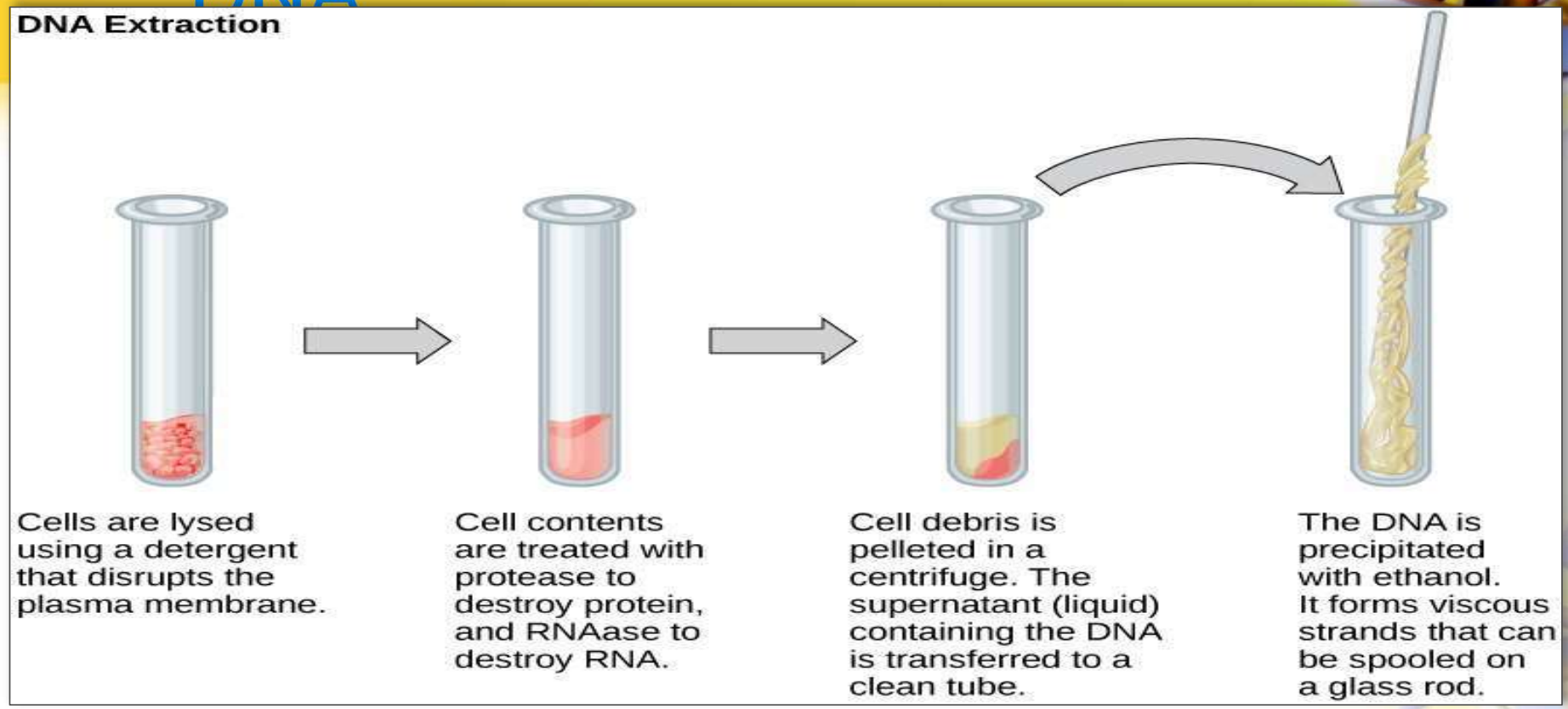
- To isolate and characterize a gene
- To make desired alterations in one or more isolated genes
- To return altered genes to living cells
- Artificially synthesize new gene
- Alternating the genome of an organism
- Understanding the hereditary diseases and their cure
- Improving human genome

PROCEDURE OF MAKING RDNA

- Isolating of DNA
- Cutting of DNA
- Joining of DNA
- Amplifying of DNA



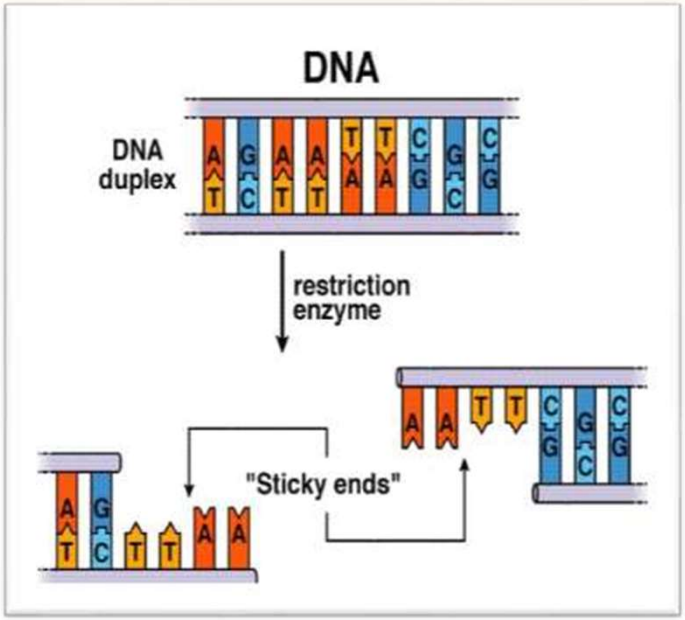
ISOLATING OF DNA



CUTTING OF DNA

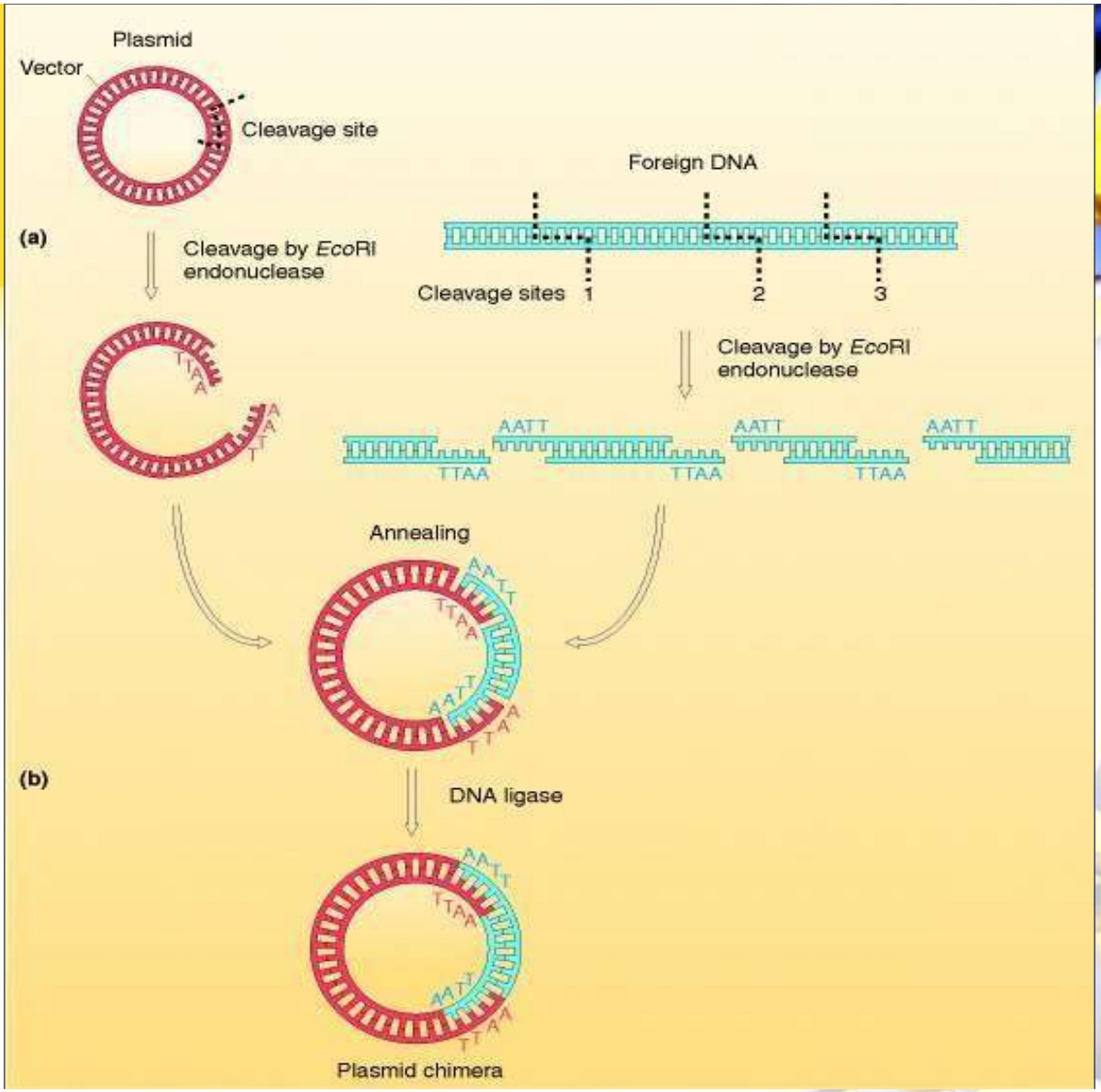
- DNA can be cut into large fragments by mechanical shearing.
- Restriction enzymes are the scissors of molecular genetics.

RESTRICTION ENZYME



- A special class of sequence-specific enzyme
- Found in bacteria
- Site-specific-cleave DNA molecules only at specific nucleotide sequence
- REases recognize DNA base sequence that are palindrome (MADAM)
- REase make staggered cuts with complementary base sequences for easy circulization

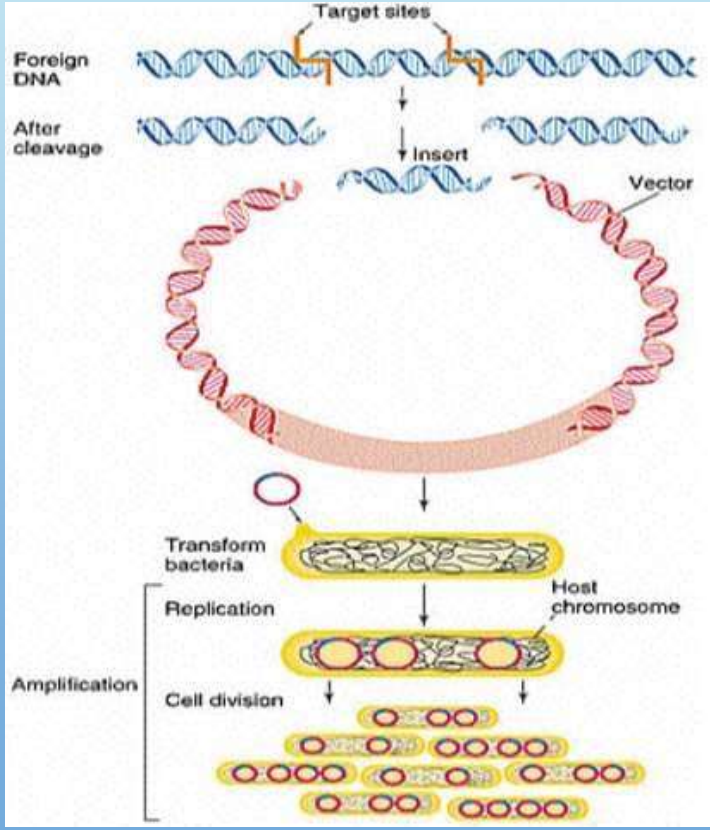
JOINING DNA


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

AMPLIFYING THE RECOMBINANT DNA

- Transforming the recombinant DNA into a bacterial host strain.
- The cells are treated with CaCl2
- DNA is added
- Cells are heat shocked at 42 C
- DNA goes into cell by a somewhat unknown mechanism.
- Once in a cell, the recombinant DNA will be replicated.
- When the cell divides, the replicated recombinant molecules go to both daughter cells which themselves will divide later. Thus, the DNA is amplified
- POLYMERASE CHAIN REACTION PCR

AMPLIFYING THE RECOMBINANT DNA



ENZYMES USED IN RECOMBINANT DNA TECHNOLOGY

DNA ligase	• Bind to DNA molecules
Type II restriction endonuclease	• Cleaves DNA at specific sites
Reverse transcriptase	• Make a DNA copy of RNA molecule
DNA polymerase I	• Fill single stranded gaps of DNA duplex
Polynucleotide Kinase	• Adds a phosphate to the 5'-OH end of a polynucleotide
Terminal transferase	• Adds homopolymer tails to the 3'-OH ends
Exonuclease III	• Removes nucleotide residues from the 3' ends
Bacteriophage {lamda} exonuclease	• removes nucleotides from the 5' ends
Alkaline phosphatase	• Removes terminal phosphates

GENETIC ENGINEERING may be described as the introduction of manipulated genetic material in to a cell in such a way as to replicate and be passed on to the progeny cell.

- Genetic engineering- isolate & introduce only one or set of desirable genes without introducing undesirable genes in target organism
- Techniques of genetic engineering- creation of **recombinant DNA**, use of **gene cloning** & **gene transfer** to *host*
- Recombinant DNA (rDNA)/ alien DNA- cannot multiply itself until integrated in host genome
- When inherited in host DNA- ability to replicate due to **origin of replication** (host DNA)- initiates replication
- Alien DNA- linked with host DNA replicates & multiply itself along with host DNA- **Cloning**

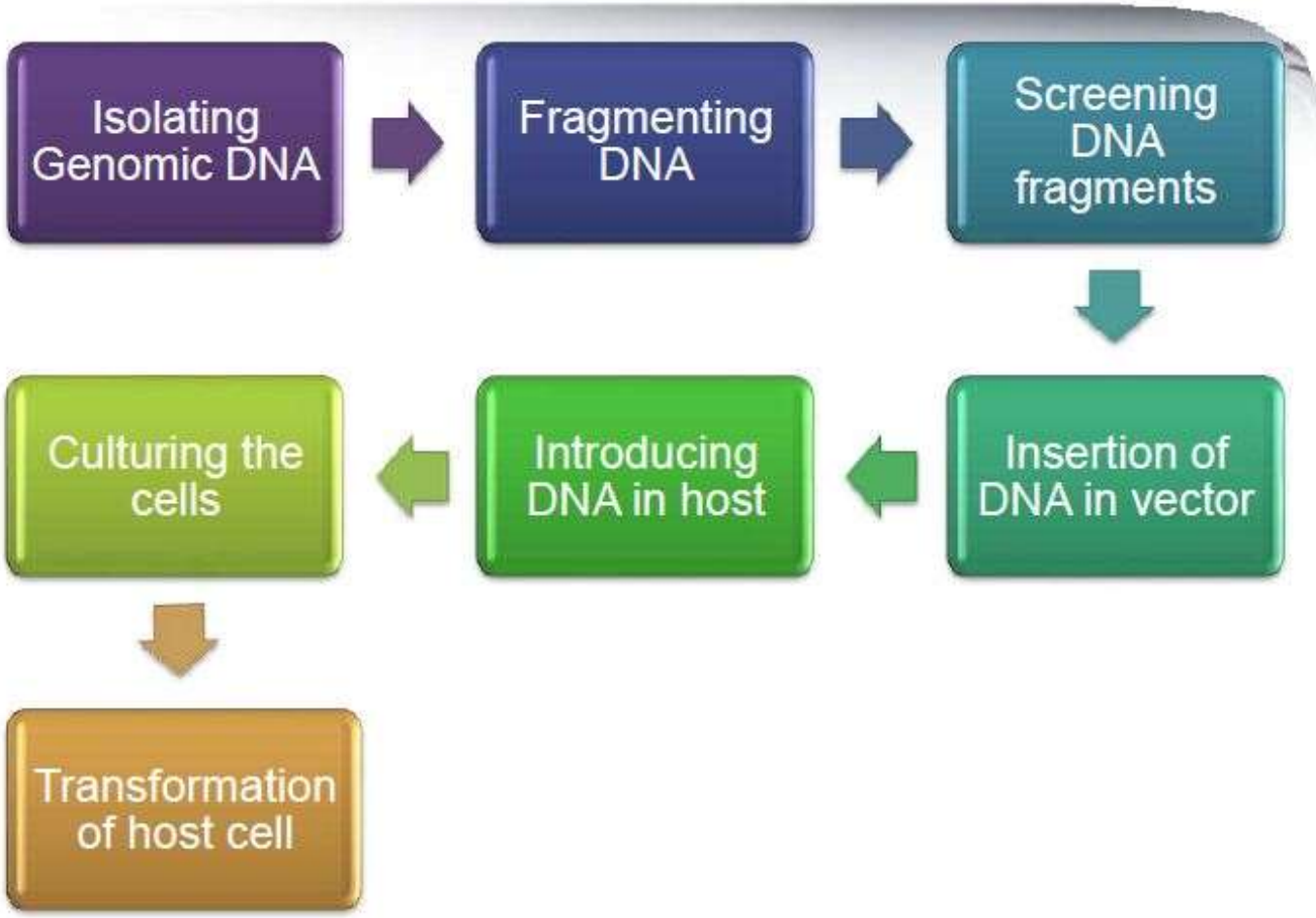
- Gene transfer to host require **Vector**
- Commonly used vector- **Plasmid** (small, circular, double stranded, self replicating extra chromosomal material of bacteria)
- First recombinant DNA was constructed- Stanley Cohen & Herbert Boyer (1972) by linking gene encoding for antibiotic resistance with plasmid of *Salmonella typhimurium*
- Isolation of desirable gene (antibiotic resistant)- cutting out piece of DNA from a plasmid responsible of antibiotic resistance which involve 'molecular scissors'- **restriction enzymes**
- Desirable gene/ alien DNA – linked with plasmid (vector) to transfer into host organism
- Linking of DNA involves **DNA ligase**- acts on cut DNA molecules & join their ends- new combination circular autonomously replicating DNA created *in vitro*- **Recombinant DNA**

Process of recombinant DNA technology

- Recombinant DNA technology involves following steps:

1. Isolation of genetic material DNA
2. Fragmentation of DNA by restriction endonuclease
3. Isolation of desired DNA fragments
4. Ligation of the DNA fragments into a vector
5. Transferring rDNA into host
6. Culturing host cells in a medium at large scale
7. Extraction of desired product

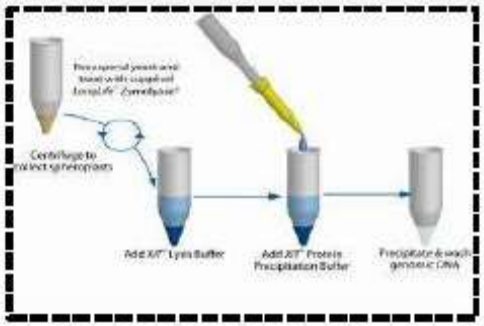
Basic steps involved in process



Basic steps involved in process

1. Isolating genomic DNA

Isolating genomic DNA from the donor.



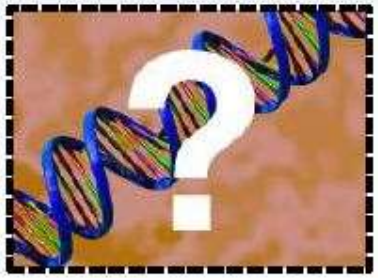
2. Fragmenting this DNA

Fragmenting this DNA using molecular scissors.

Basic steps involved in process

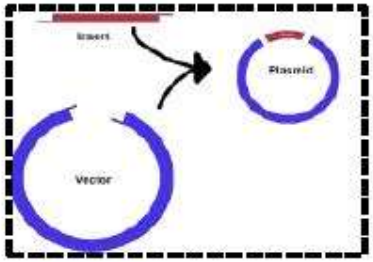
3. Screening the fragments

Screening the fragments for a "desired gene".



4. Insertion of DNA in a vector

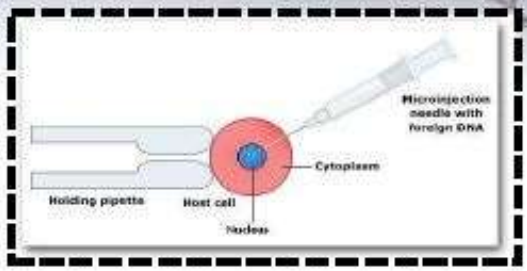
Inserting the fragments with the desired gene in a 'cloning vector'.



Basic steps involved in process

5. **Introducing in Host**

Introducing the recombinant vector into a competent host cell

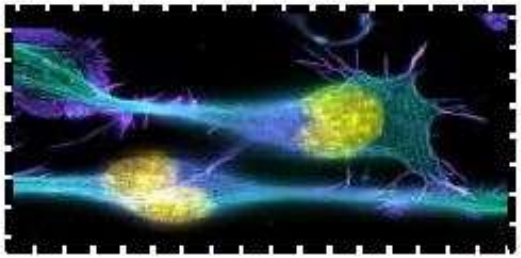


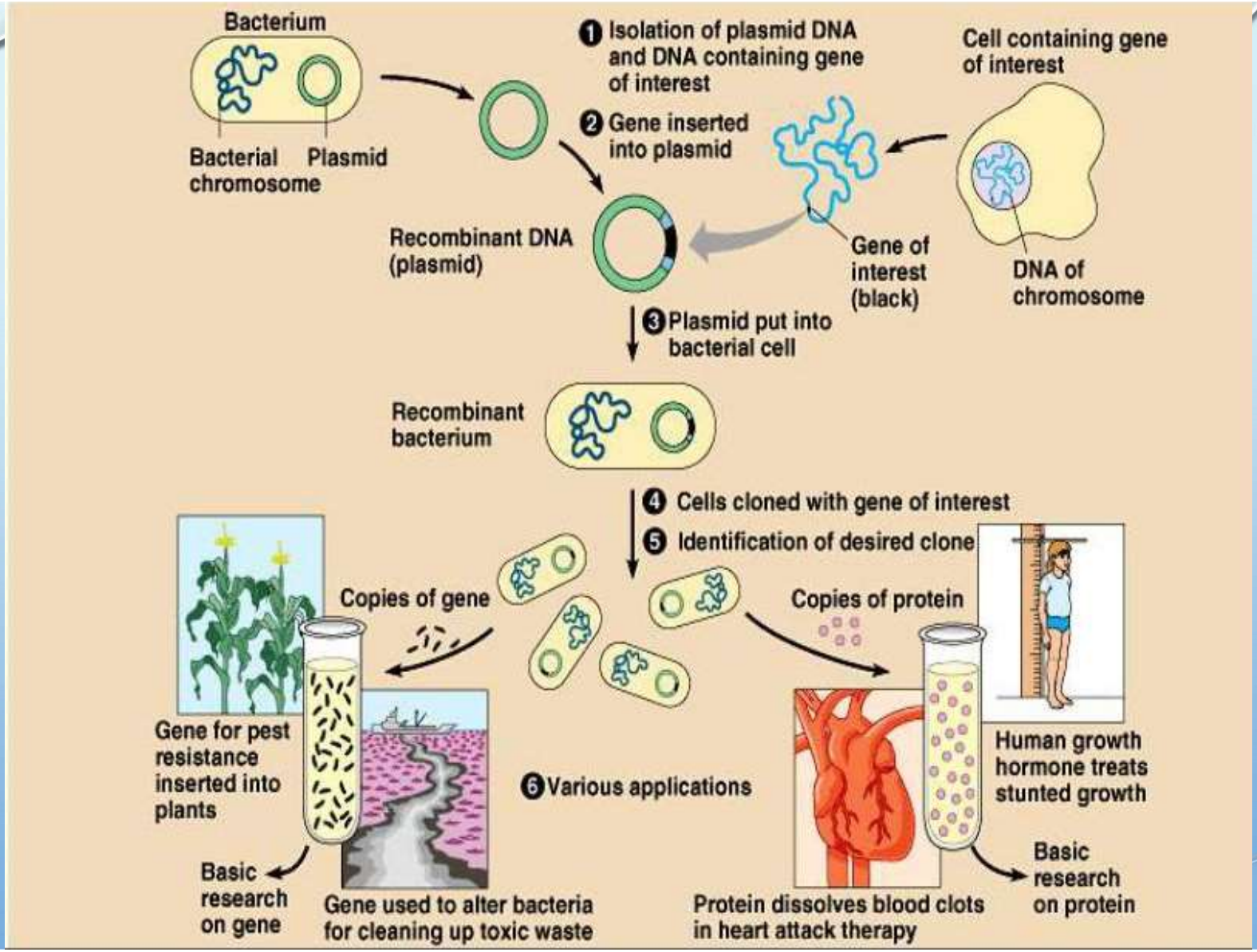
6. **Culturing the cells**

Culturing these cells to obtain multiple copies or clones of desired DNA fragments

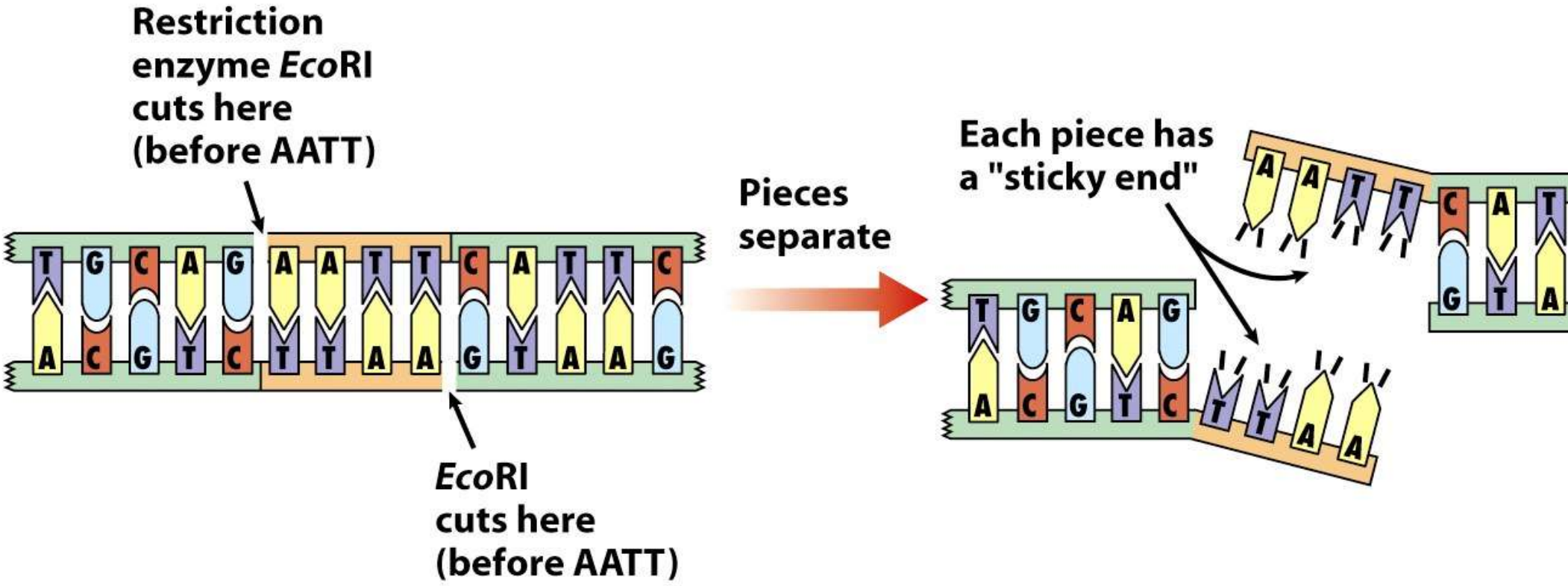
7. **Transformation of host cell**

Using these copies to transform suitable host cells so as to express the desired gene.

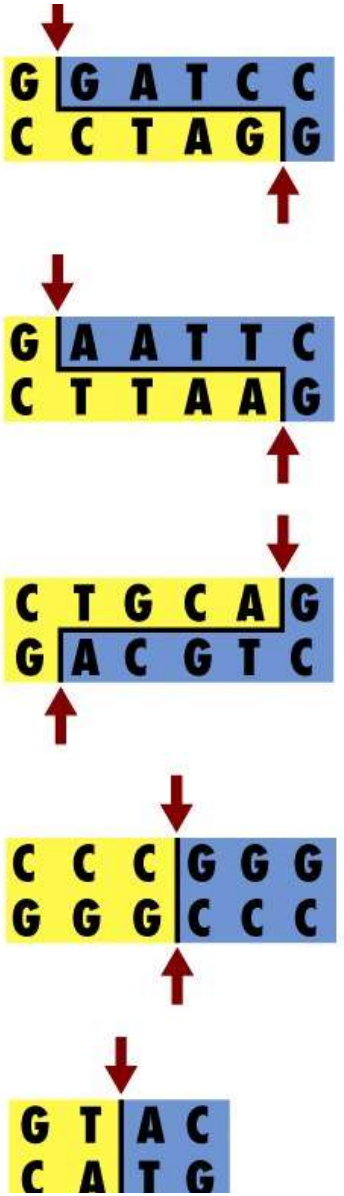



Bacterium
Bacterial chromosome
Plasmid
1 Isolation of plasmid DNA and DNA containing gene of interest
2 Gene inserted into plasmid
Cell containing gene of interest
Recombinant DNA (plasmid)
Gene of interest (black)
DNA of chromosome
3 Plasmid put into bacterial cell
Recombinant bacterium
4 Cells cloned with gene of interest
5 Identification of desired clone
Copies of gene
Copies of protein
Gene for pest resistance inserted into plants
Basic research on gene
Gene used to alter bacteria for cleaning up toxic waste
6 Various applications
Protein dissolves blood clots in heart attack therapy
Basic research on protein
Human growth hormone treats stunted growth

MOLECULAR SCISSORS/ RESTRICTION ENZYMES



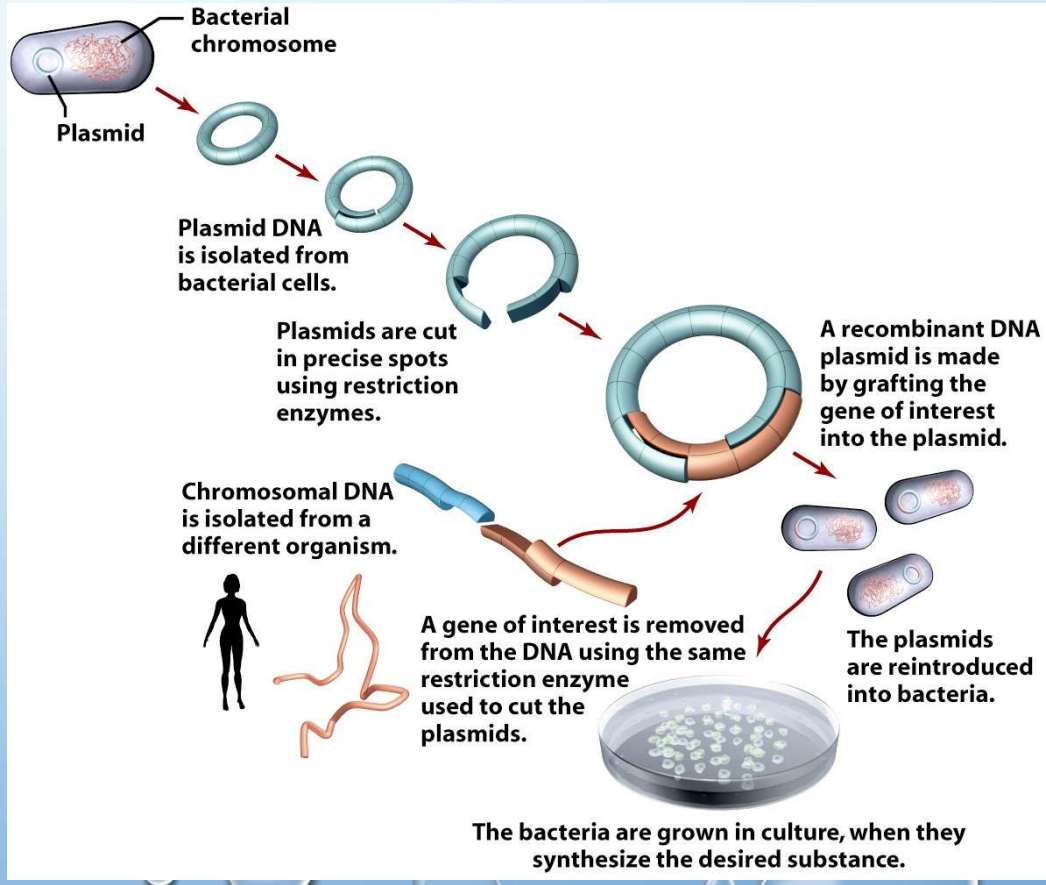
Bacillus amyloliquefaciens H	***Bam* H1**
Escherichia coli Ry13	***Eco* R1**
Providencia stuartii 164	***Pst* 1**
Serratia marcescens SB	***Sma* H1**
Rhodopseudomonas sphaeroides	***Rsa* 1**



MOLECULAR PASTE

- DNA LIGASE:
  - FORM BONDS BETWEEN THE SUGAR AND PHOSPHATE BACKBONE OF THE DNA MOLECULE.
- RESTRICTION ENZYMES AND DNA LIGASE MAKE POSSIBLE THE COMBINATION OF DNA FROM DIFFERENT ORGANISMS INTO ONE DNA MOLECULE
  - CALLED RECOMBINANT DNA

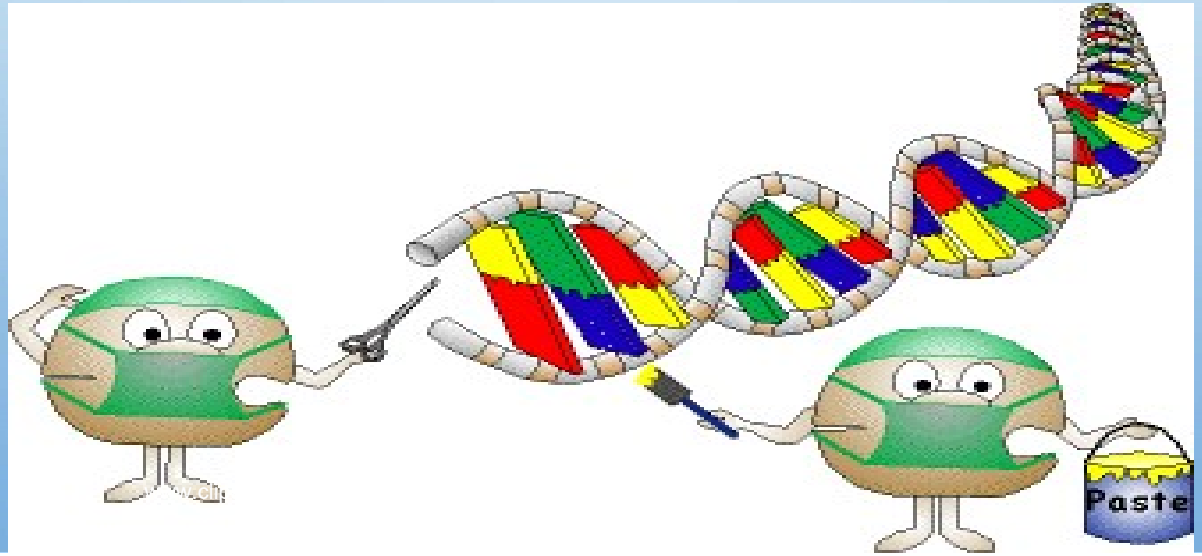
MAKING RECOMBINANT DNA



GENETIC ENGINEERING

Is:

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



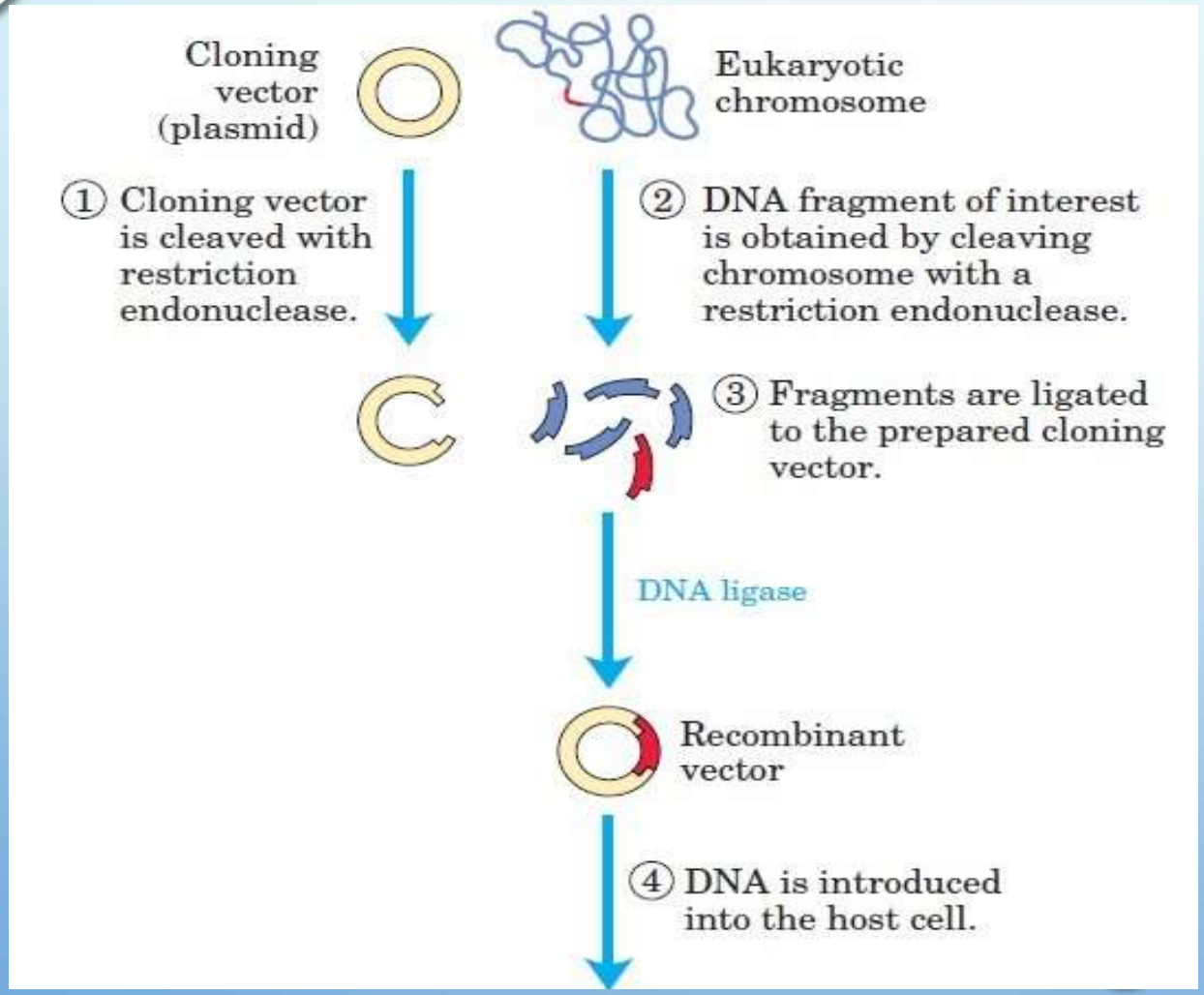
GENETIC ENGINEERING

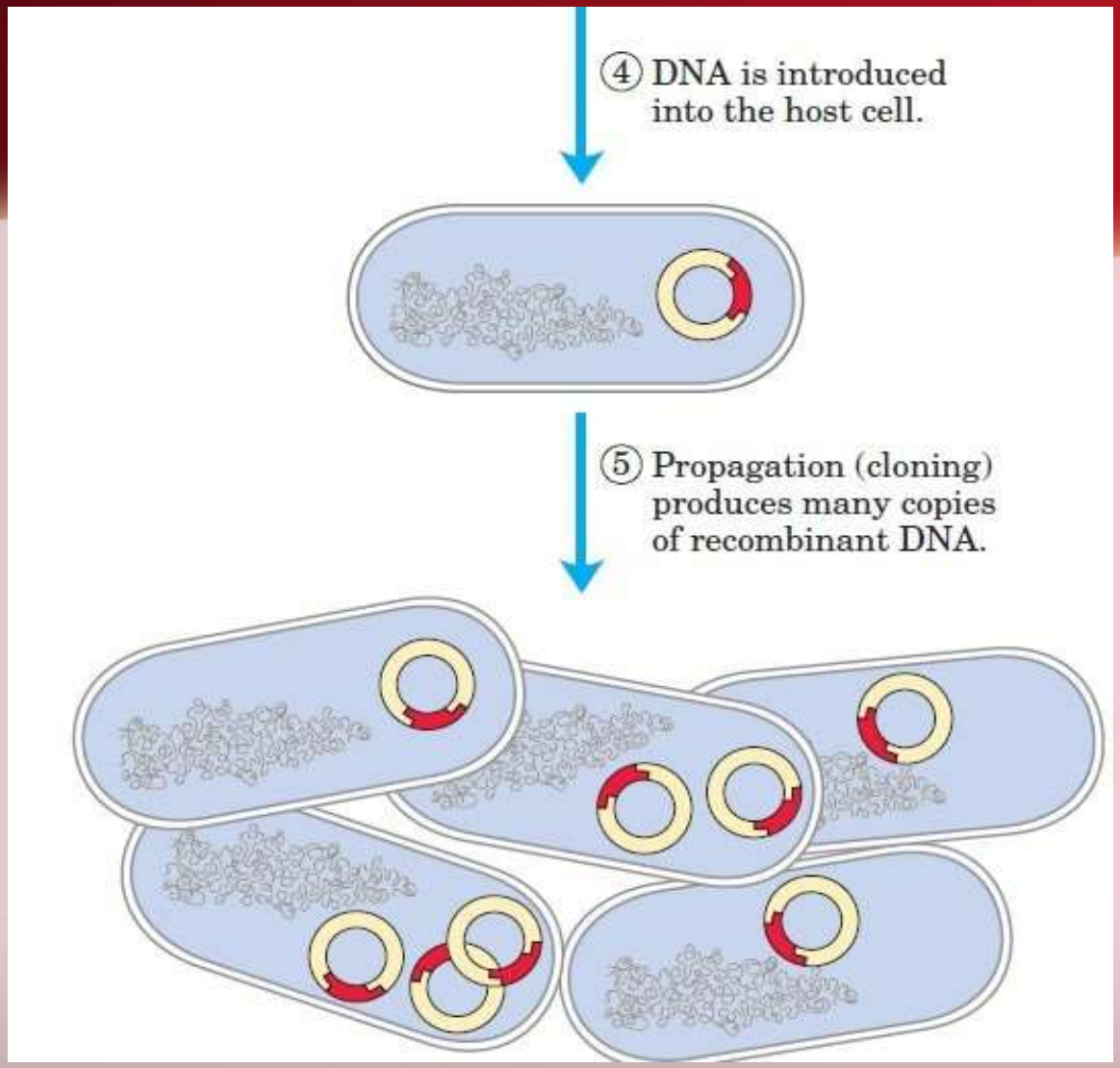
- GENETIC ENGINEERING MEANS THAT DNA FROM DIFFERENT ORGANISMS CAN BE COMBINED
- BACTERIA CAN BE ENGINEERED TO PRODUCE HUMAN PROTEINS
- HUMAN GENES CAN BE INSERTED INTO OTHER ANIMALS

BASIC STEPS OF GENETIC MODIFICATION:

1. Isolating a gene to be inserted
2. Inserting the gene in a Vector(Agent used to carry foreign gene)
3. Inserting Vector into the host.
4. Multiplication of host cells by cloning.
5. Extraction of desired product.

BASIC PRINCIPLE OF RDNA TECHNOLOGY




④ DNA is introduced into the host cell.
⑤ Propagation (cloning) produces many copies of recombinant DNA.

5 STAGES INVOLVED IN GE

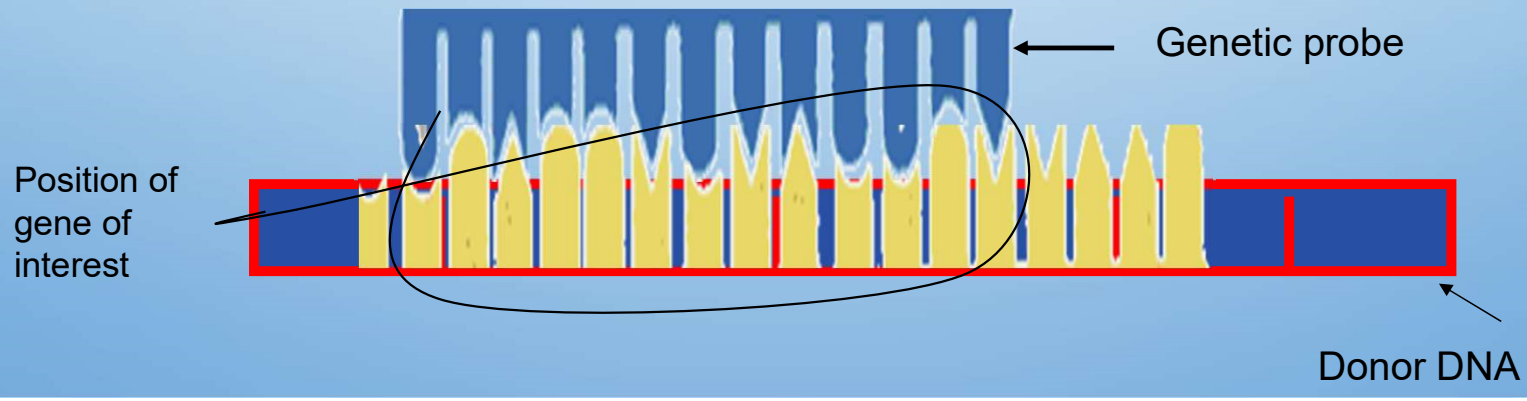
1. ISOLATION
2. CUTTING
3. LIGATION AND

   INSERTION
4. TRANSFORMATION
5. EXPRESSION

1. ISOLATION

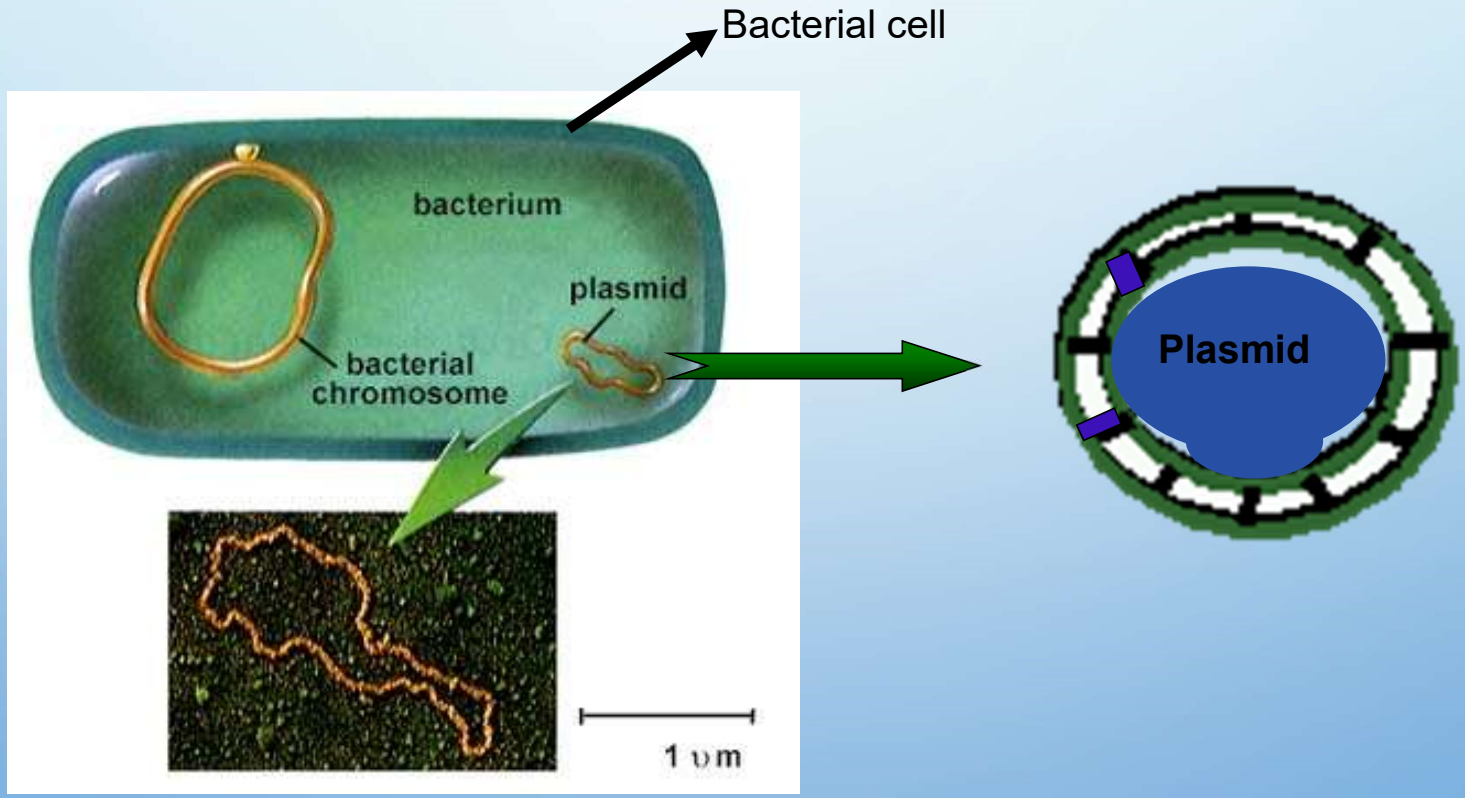
(A) **ISOLATION** OF A SPECIFIC GENE FROM DONOR E.G. HUMAN

- Cells broken open
- Genetic probe added
- Reveals position of the gene of interest



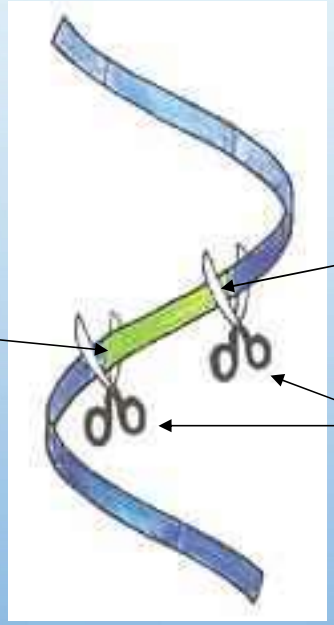
1. ISOLATION

(b) **Isolation** of plasmid from a bacterial cell



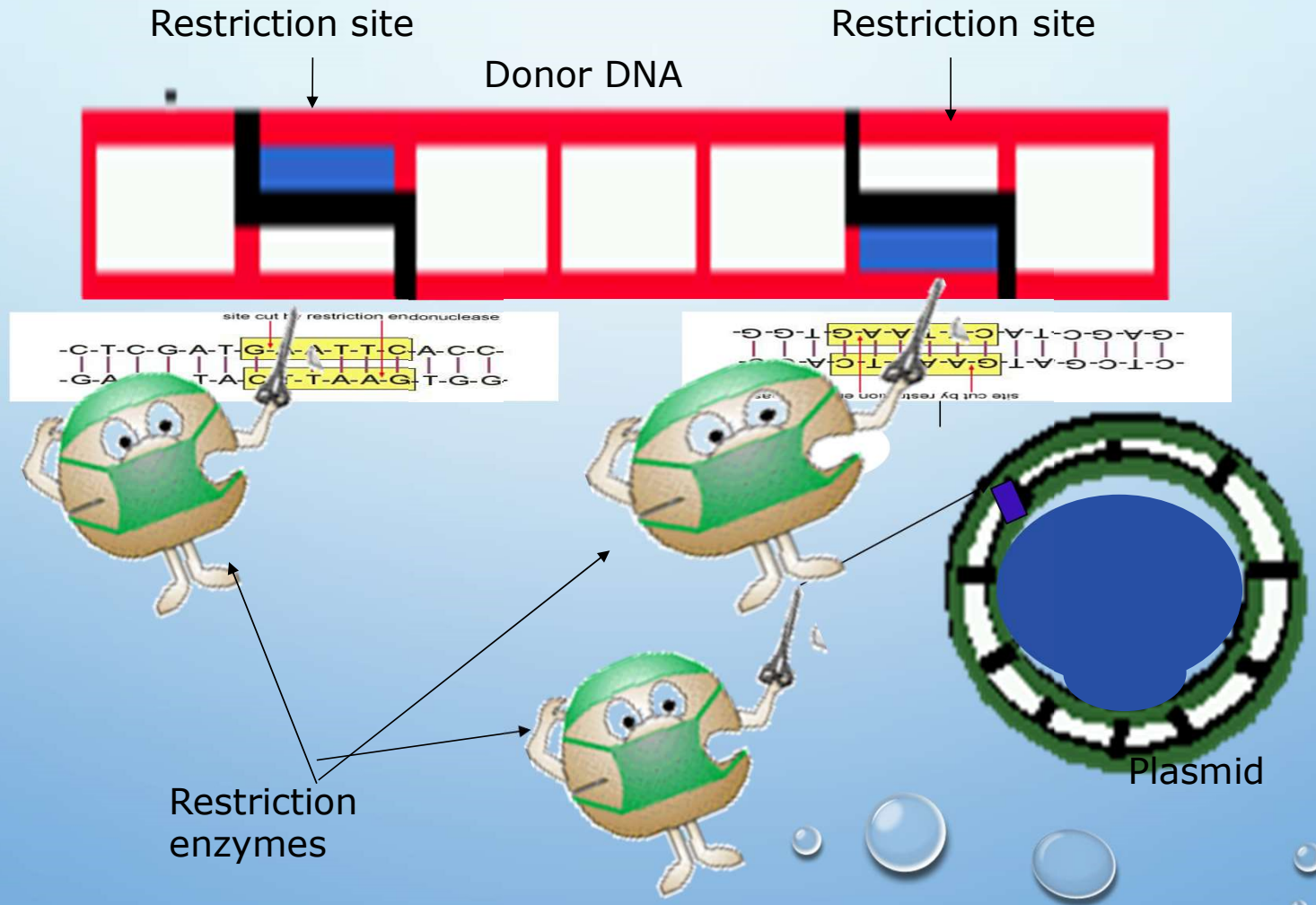
2. CUTTING

- RESTRICTION ENZYMES ACT AS MOLECULAR SCISSORS AND CUT DNA AT SPECIFIC SITES CALLED RESTRICTION SITES

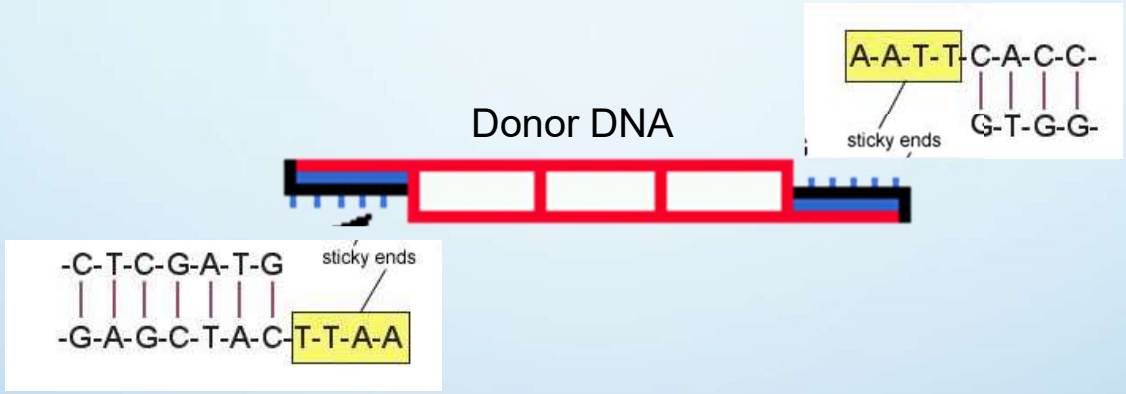


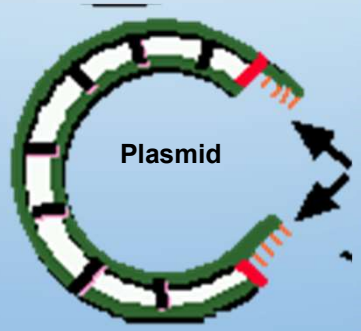
Clipartguide.com

2. CUTTING


Restriction site
Donor DNA
Restriction site
Restriction enzymes
Plasmid

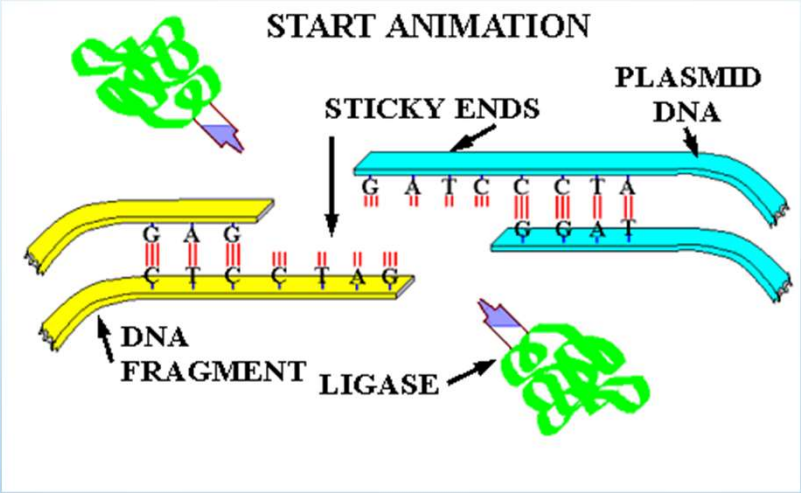
CUTTING





Sticky Ends

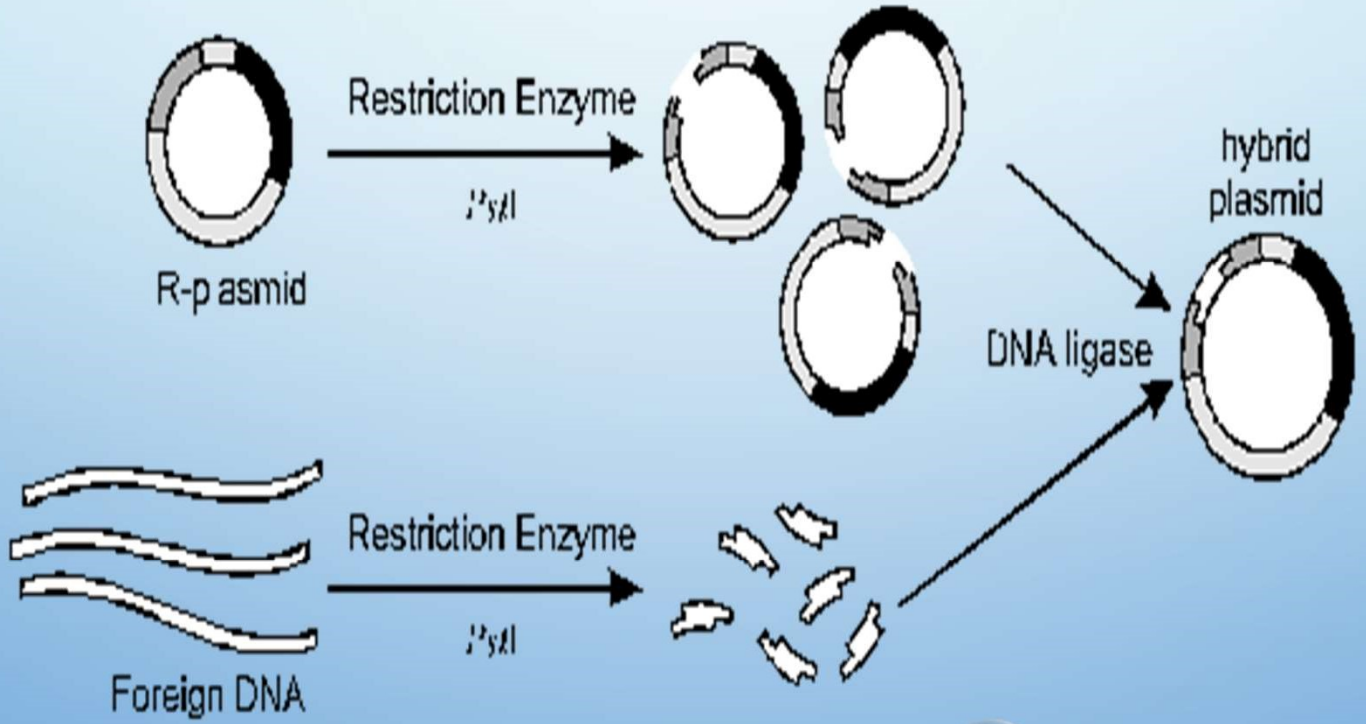
DNA LIGASE



http://www.slic2.wsu.edu:82/hurlbert/micro101/pages/Chap10.html#Sticky_ended_cut

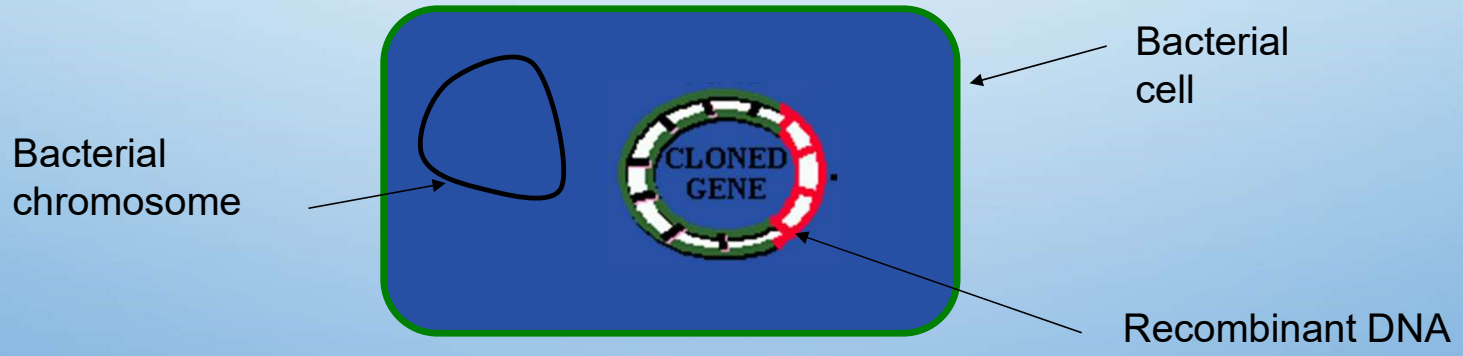
Ligation –rejoining cut fragments of DNA and forming artificial recombinant molecules

LIGATION AND INSERTION



4. TRANSFORMATION

RECOMBINANT DNA INTRODUCED INTO BACTERIAL CELL

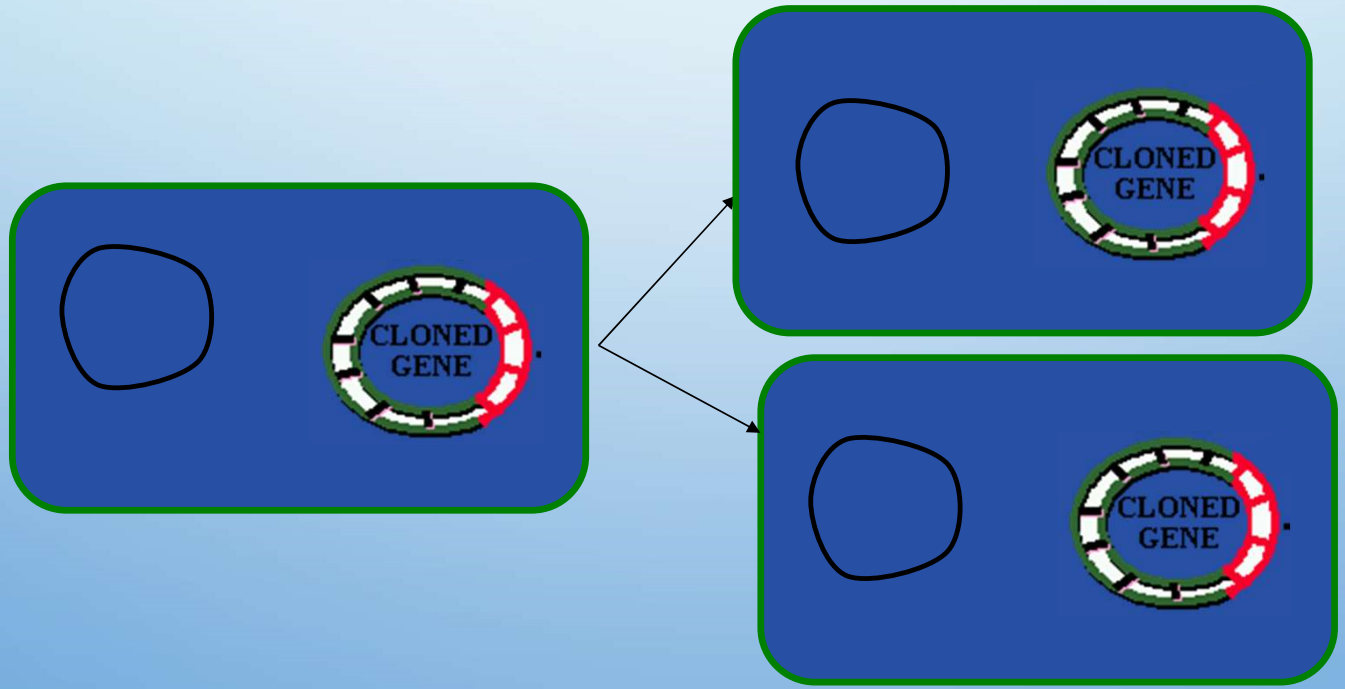


5. EXPRESSION

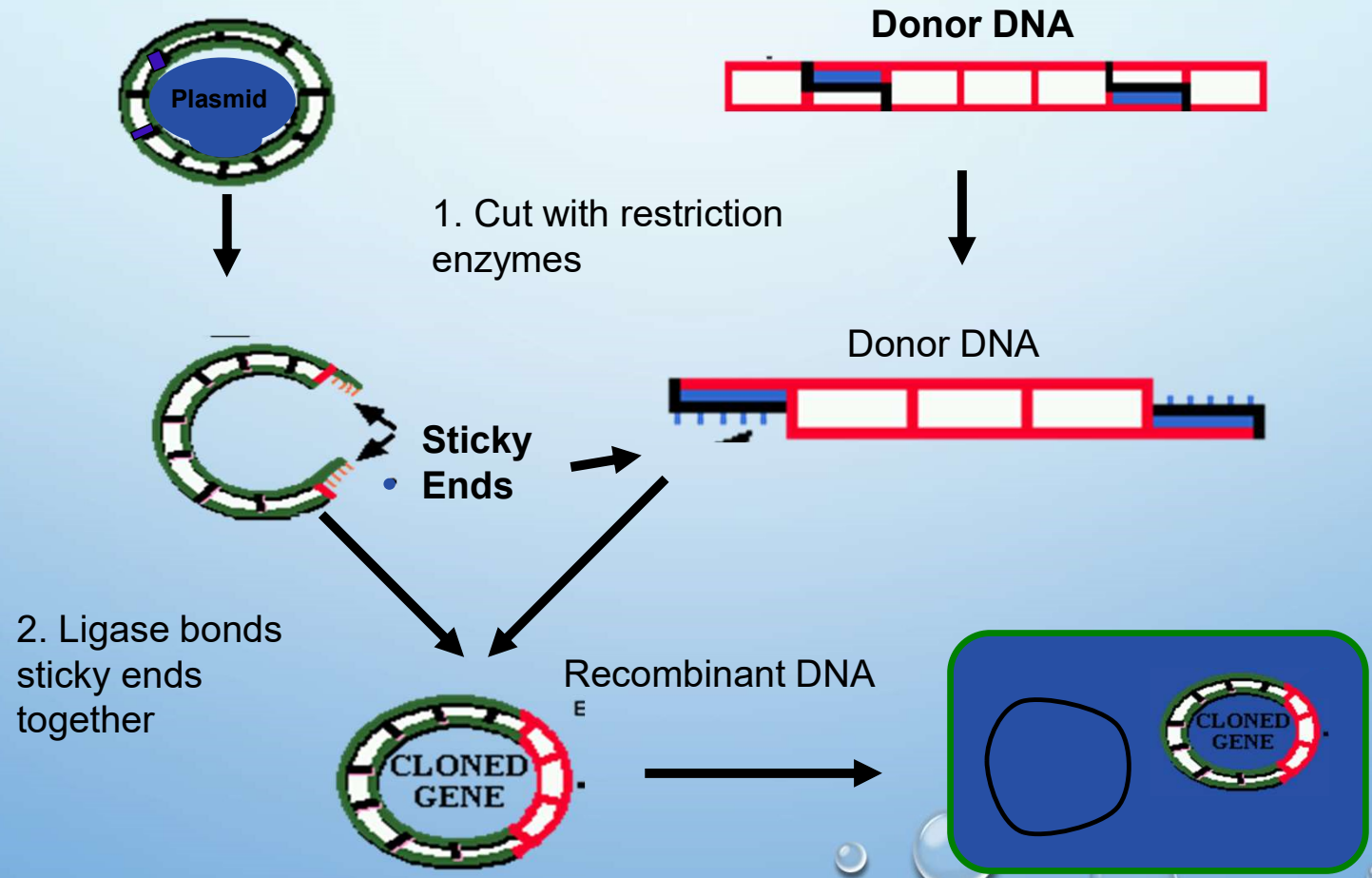
BACTERIAL CELL REPRODUCES BY BINARY FISSON

Bacterial cell produces the polypeptide

Coded for by the donor DNA



SUMMARY OF STEPS



THANK YOU