From Gene to Protein

(an overview)

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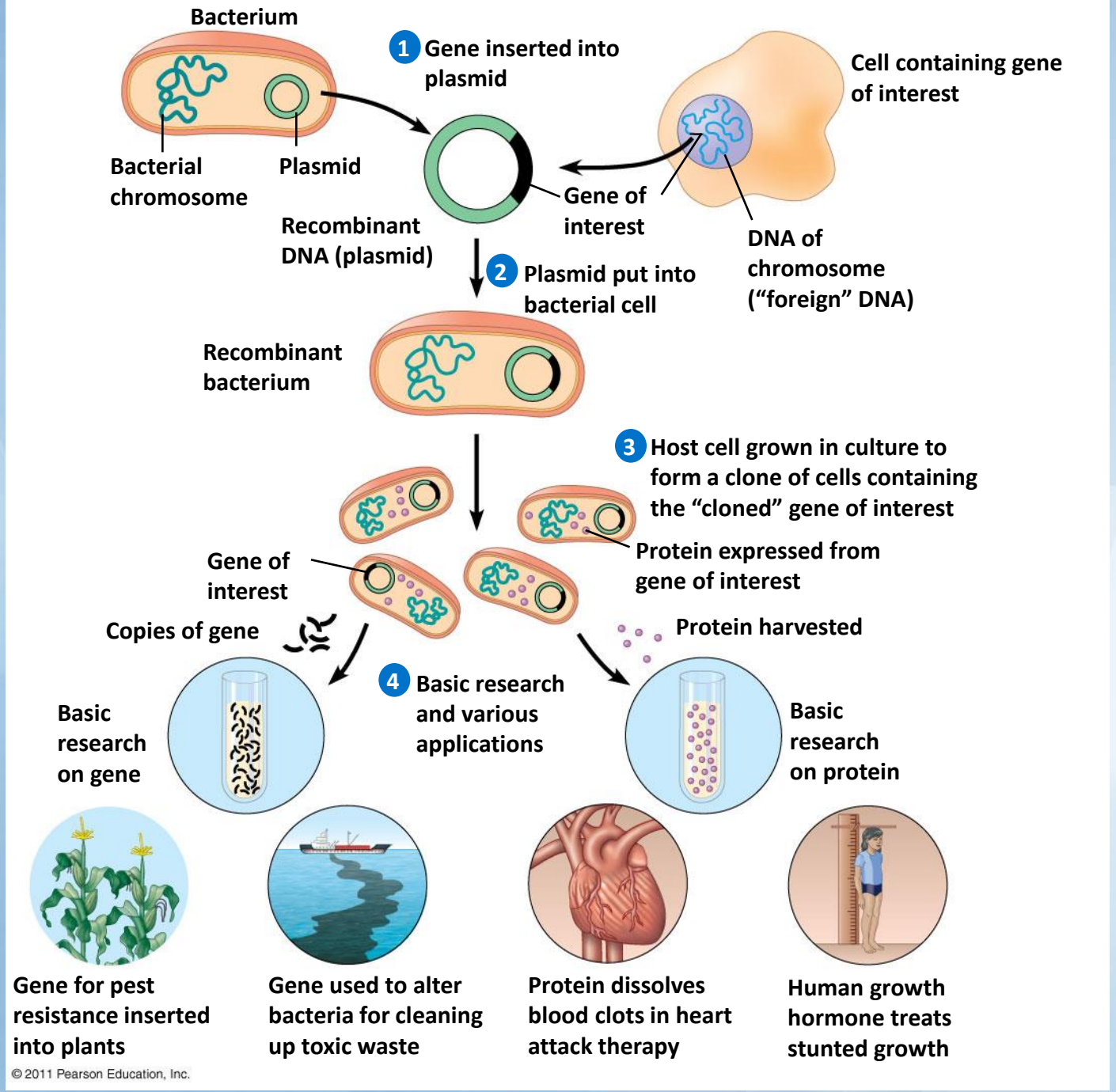
DEFINITION

Gene cloning is a set of experimental methods in molecular biology that are used to assemble recombinant DNA molecules and to direct their replication within host organisms.

The use of the word ***cloning*** refers to the fact that the method involves the replication of a single DNA molecule starting from a single living cell to generate a large population of cells containing identical DNA molecules.


Bacterium
1 Gene inserted into plasmid
Cell containing gene of interest
Bacterial chromosome
Plasmid
Gene of interest
Recombinant DNA (plasmid)
DNA of chromosome ("foreign" DNA)
2 Plasmid put into bacterial cell
Recombinant bacterium
3 Host cell grown in culture to form a clone of cells containing the "cloned" gene of interest
Protein expressed from gene of interest
Gene of interest
Copies of gene
Protein harvested
4 Basic research and various applications
Basic research on gene
Basic research on protein
Gene for pest resistance inserted into plants
Gene used to alter bacteria for cleaning up toxic waste
Protein dissolves blood clots in heart attack therapy
Human growth hormone treats stunted growth
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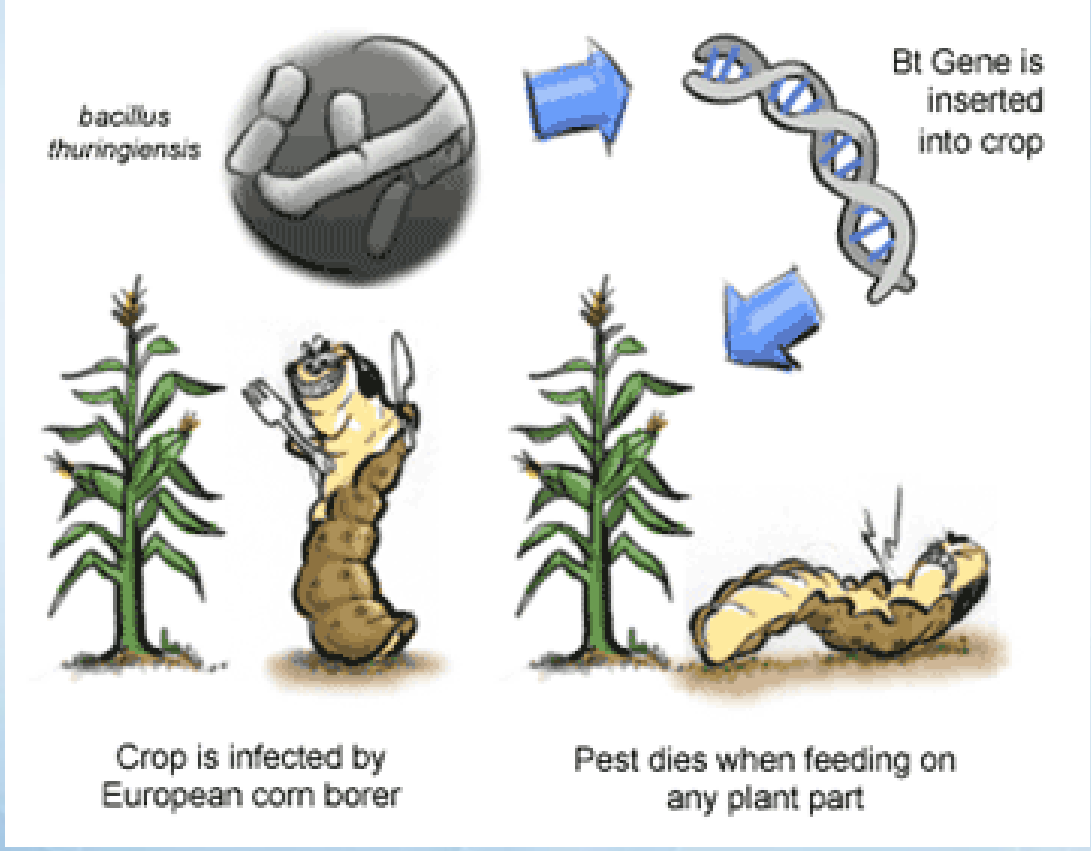
What is transformation used for?

- Agricultural
  - Genes coding for **traits** such as frost, pest or drought resistance can be genetically transformed into plants

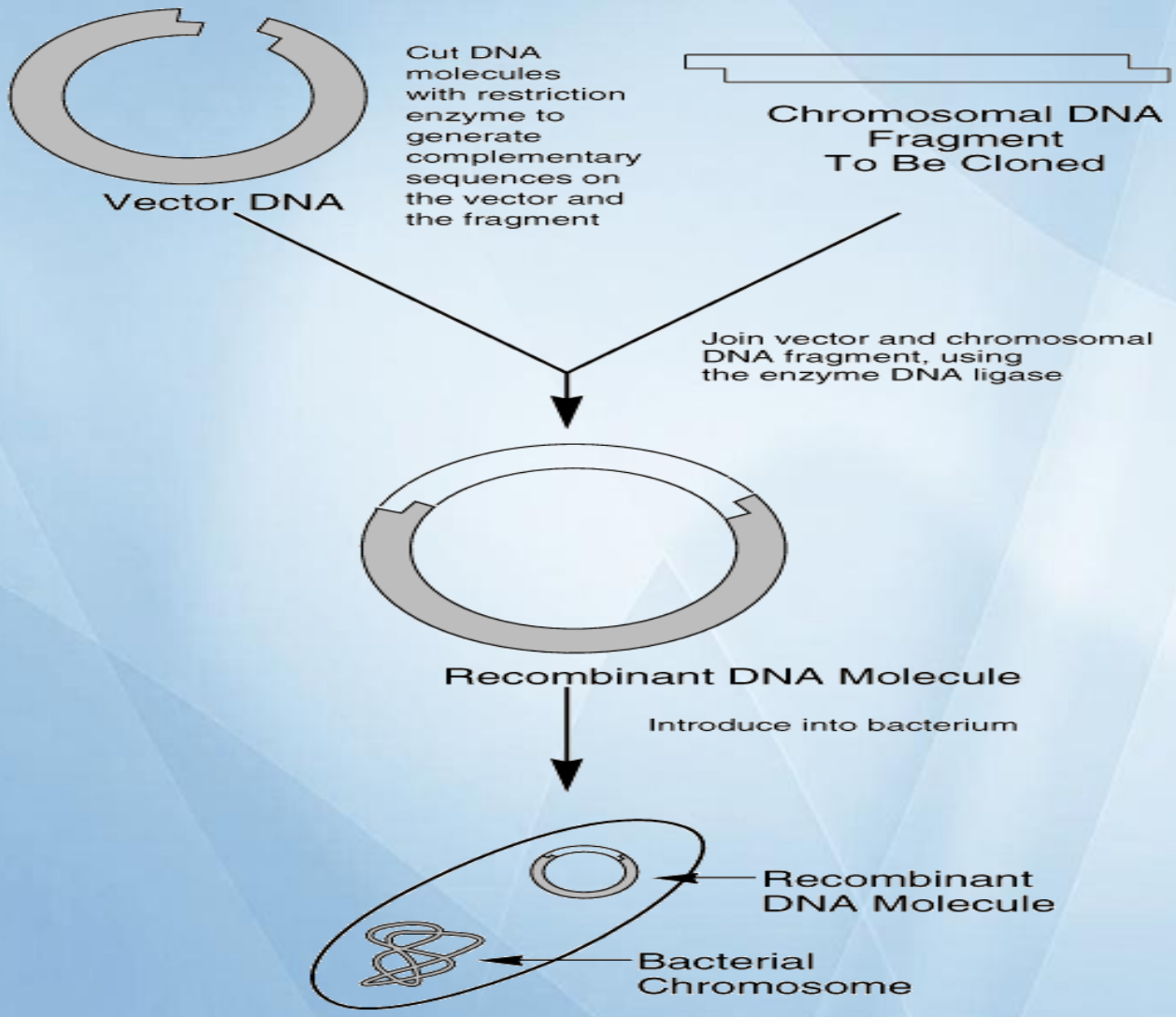


- Medical
  - Production of human **proteins** to treat genetic diseases

Protein	Disease/Disorder
Human insulin	Diabetes mellitus
Human Growth Hormone	Deficiency in children
Erythropoietin	Anemia
DNase I	Cystic fibrosis
Human antibody blocker	Asthma

- Environmental
  - Bacteria can be genetically transformed with genes enabling them to **digest oil spills** or remove **pollutants** from the environment

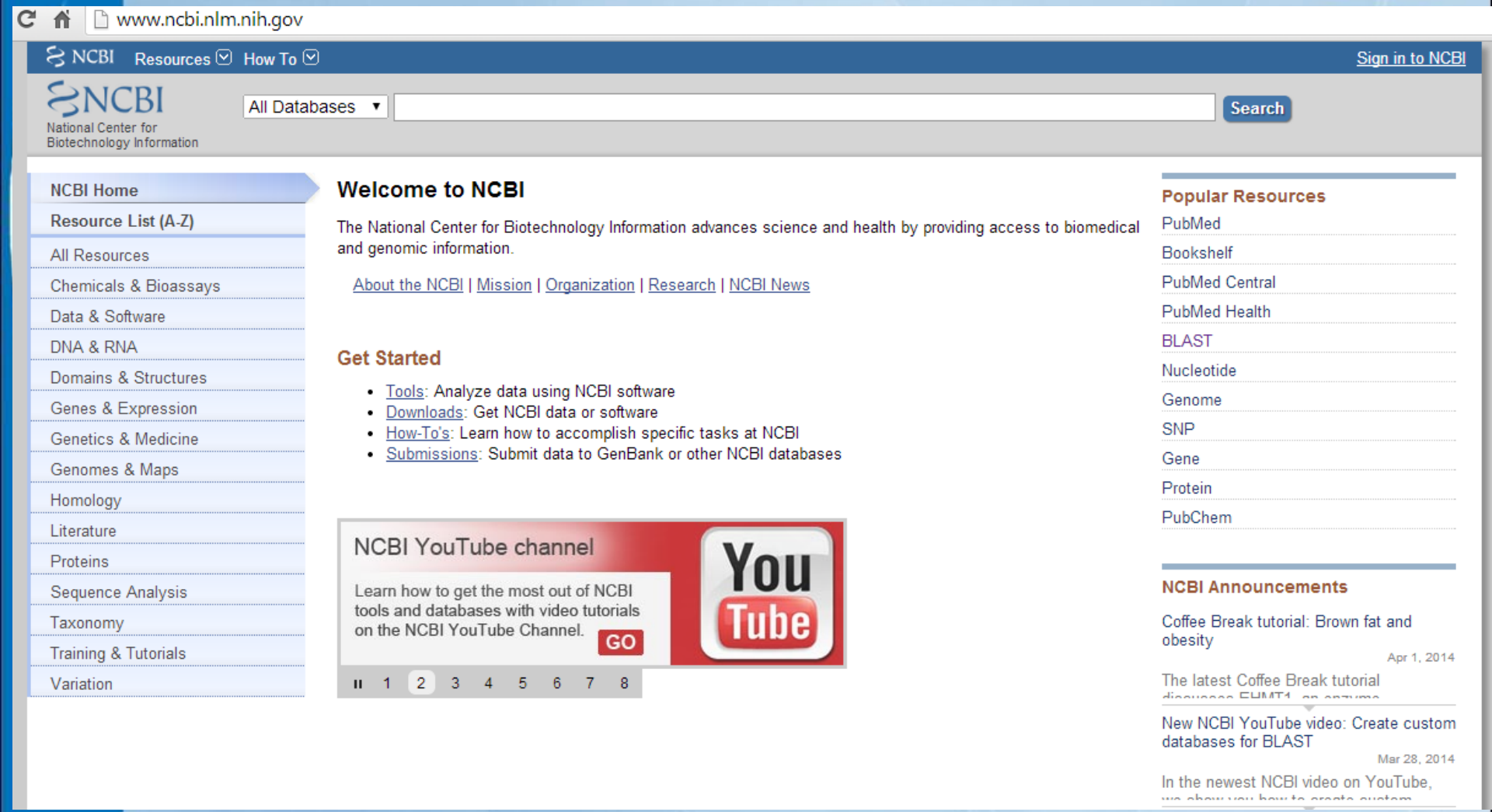
CLONING PROCESS



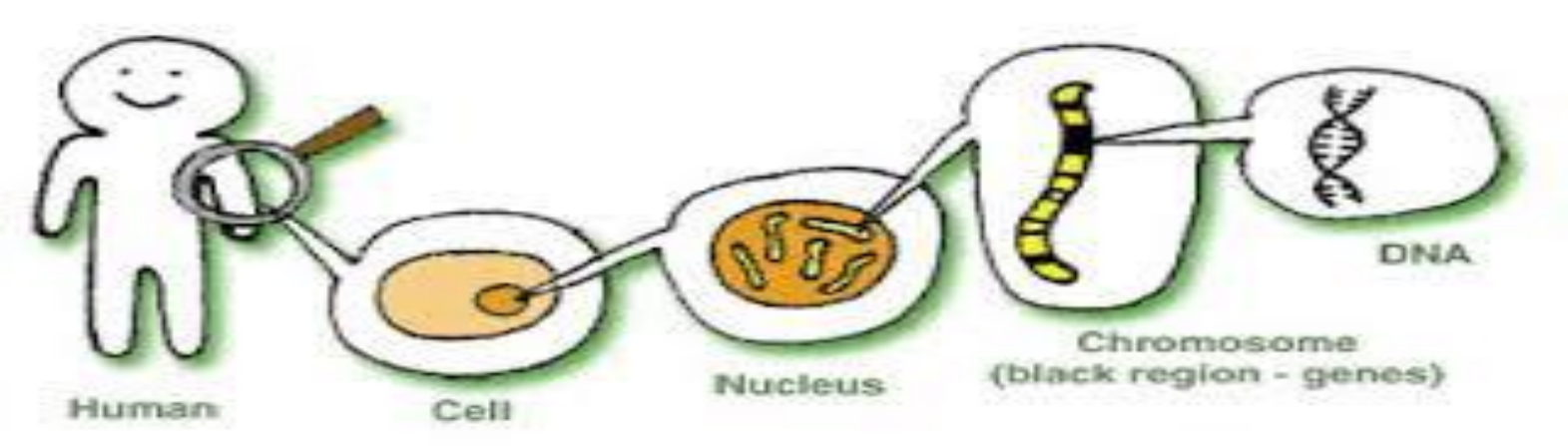
CLONING PROCESS

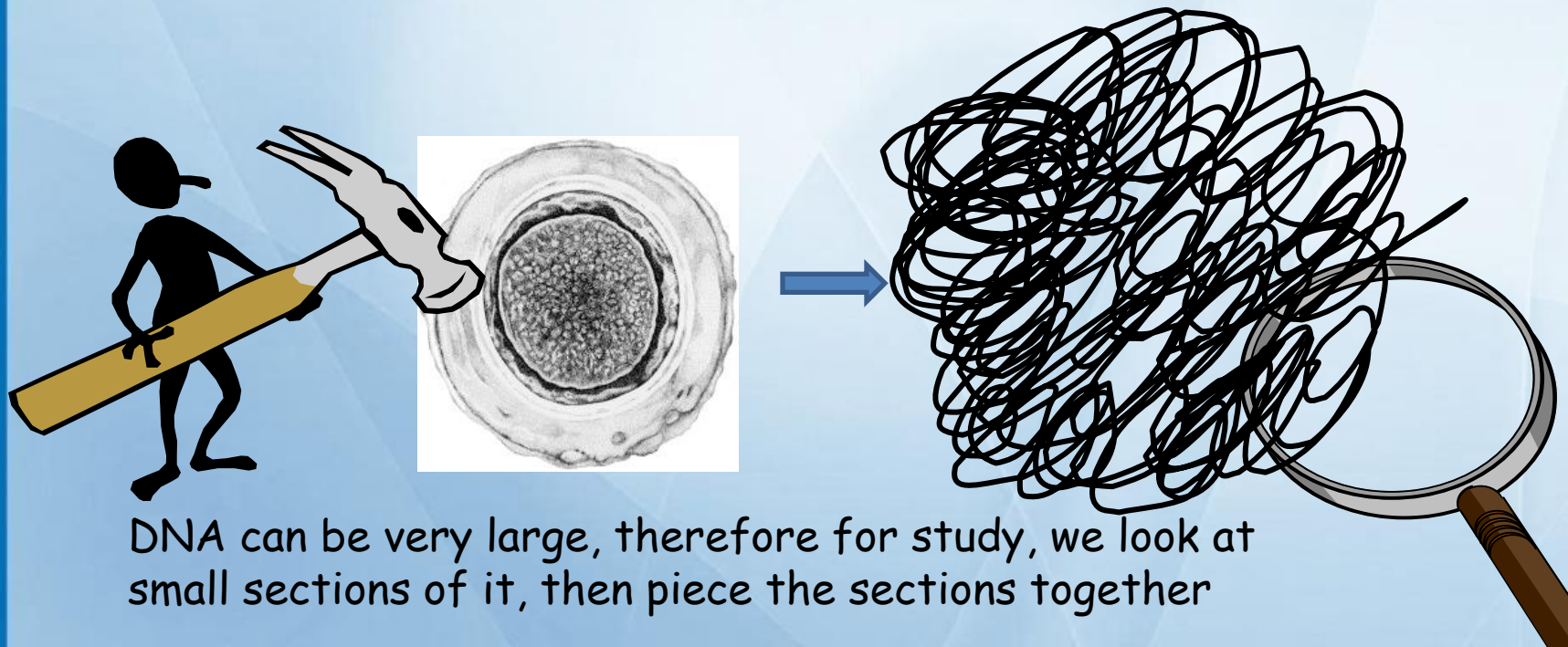
- Amplify Target Gene
- Cut Target Gene and Plasmid
- Ligation
- Transformation
- Cellular Screening
- Protein Expression

Study the gene of interest



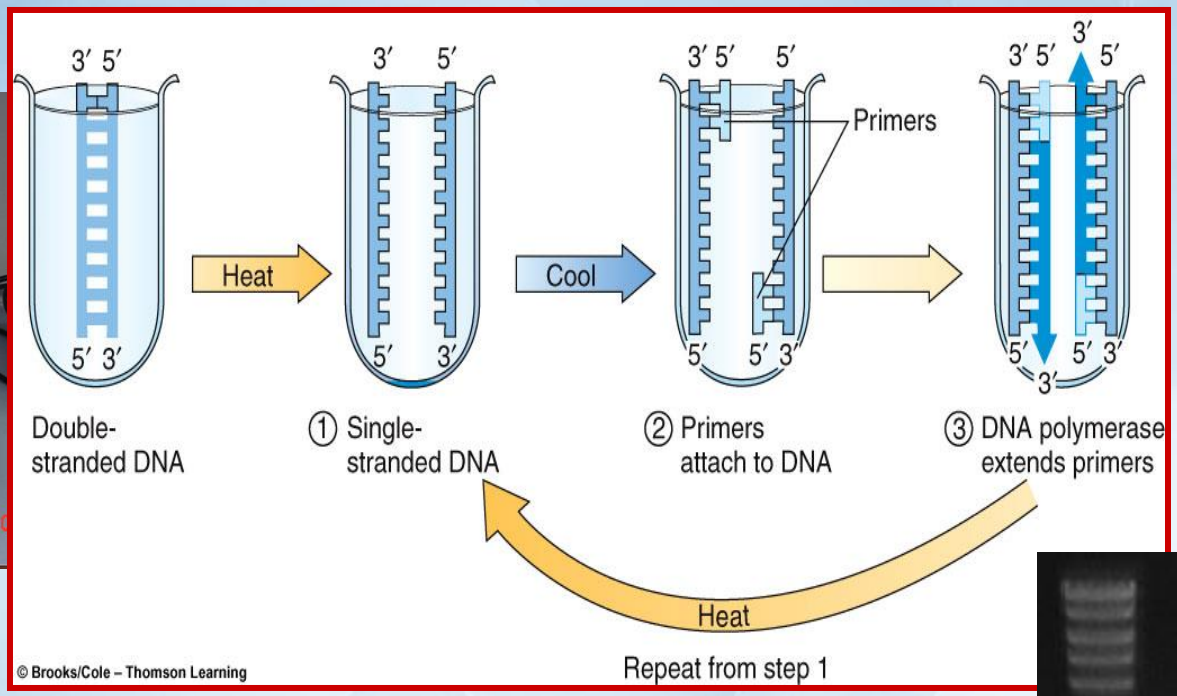
STEP 1. DNA isolation and PCR

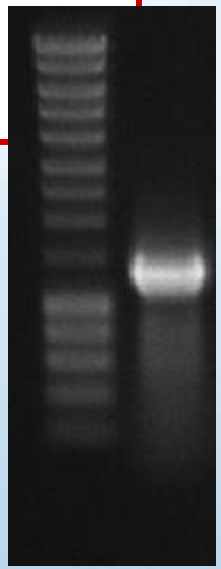




DNA can be very large, therefore for study, we look at small sections of it, then piece the sections together

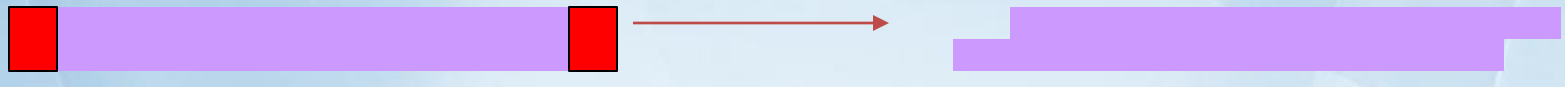
Polymerase Chain Reaction (PCR)





- PCR is used to:
  - Specifically amplify the target gene
  - Introduce the recognition site of the Restriction enzyme

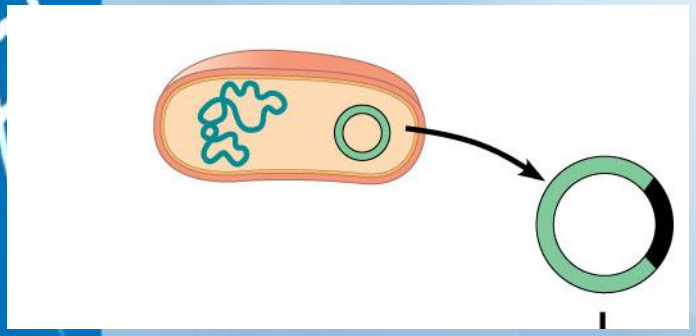
PCR

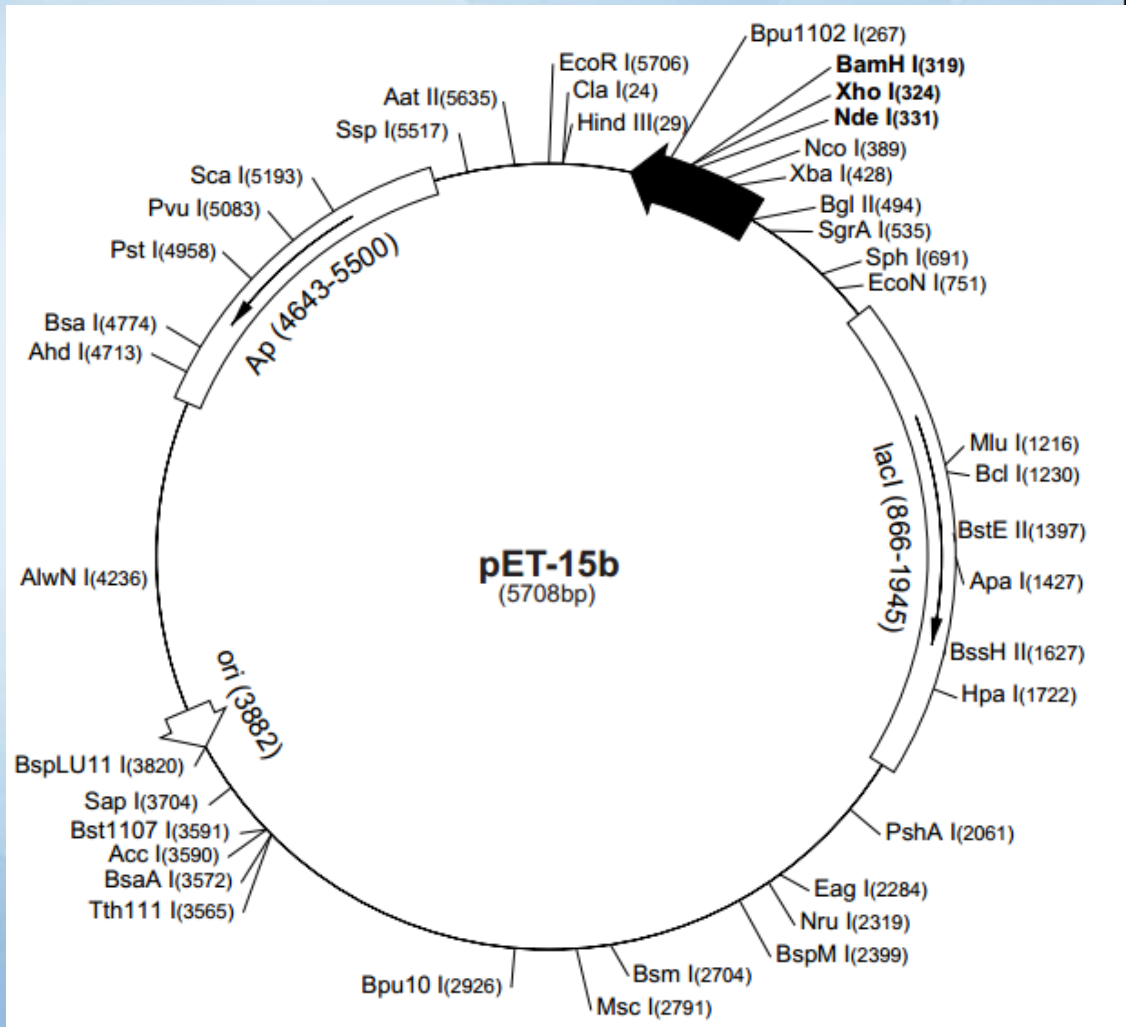


	oligo sequence	% cleavage 2h	20h
BamHI	CGGATCCG	10	25
	CGGGATCCCG	>90	>90
EcoRI	GGAATTCC	>90	>90
	CGGAATTCCG	>90	>90
HindIII	CAAGCTTG	0	0
	CCAAGCTTGG	0	0
NdeI	GGGTTTCATATGAAACCC	0	0
	GGAATTCCATATGGAATTCC	75	>90

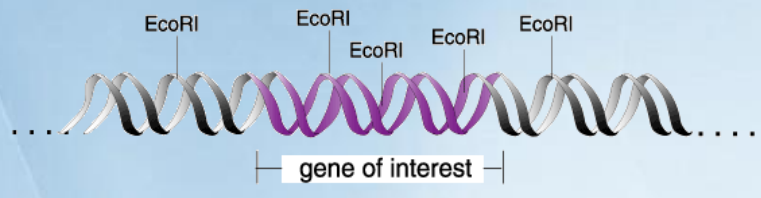
Plasmid DNA isolation

- **To introduce a gene of interest into bacteria.**
- **Hallmarks:**
  - Multi cloning site.
  - Selection marker.
  - Promoter.

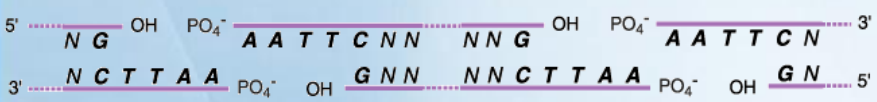




STEP 2. DIGESTION

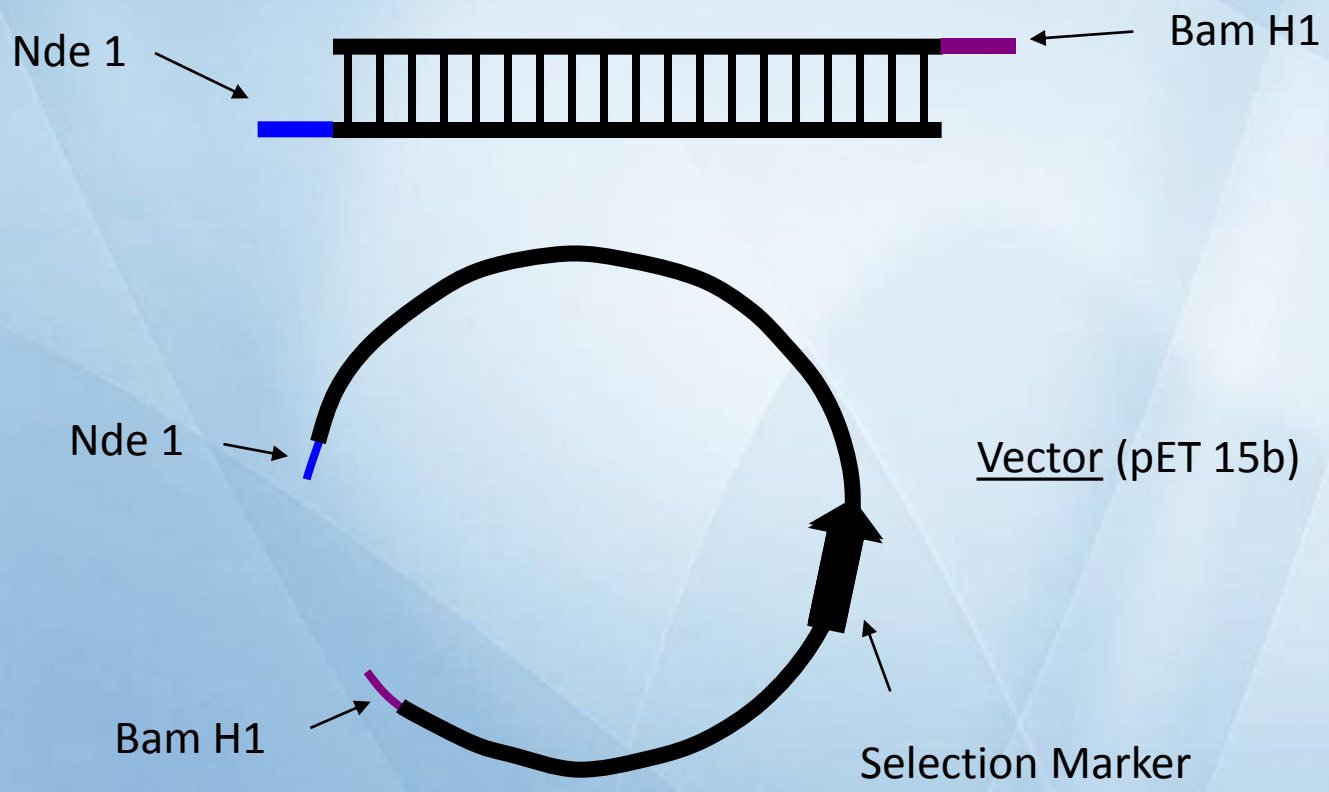


Digest DNA sample with EcoRI enzyme

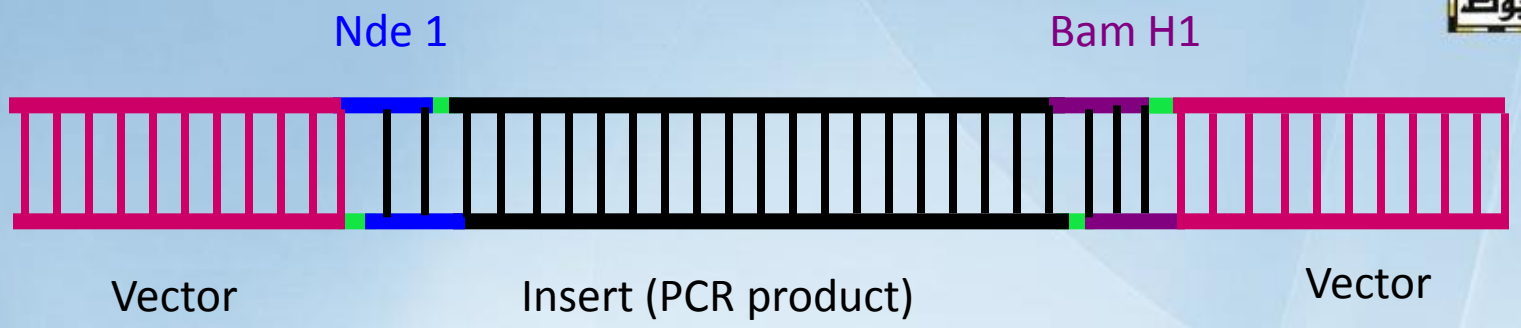


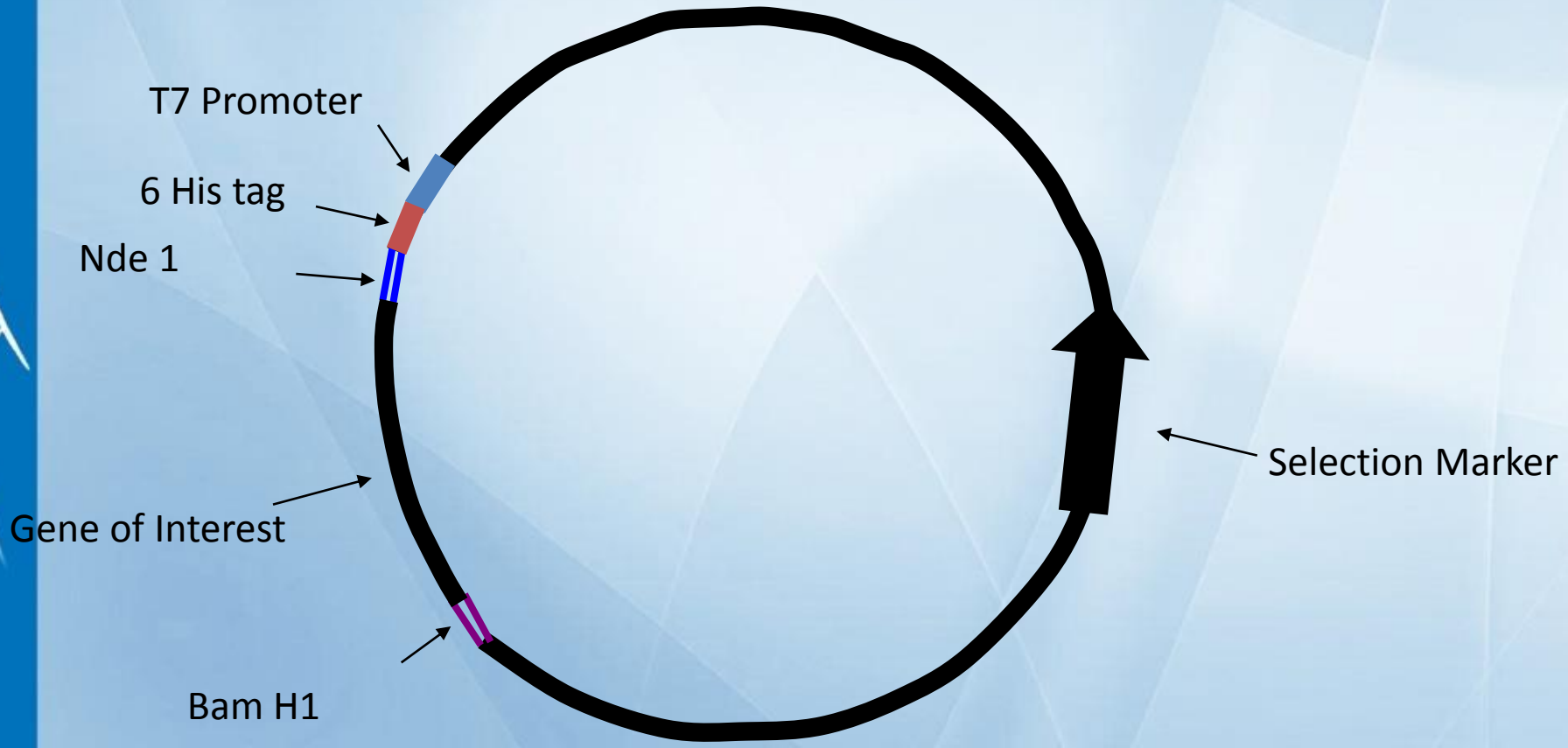
Digest plasmid vector with EcoRI enzyme

Restriction Digestion



STEP 3. LIGATION

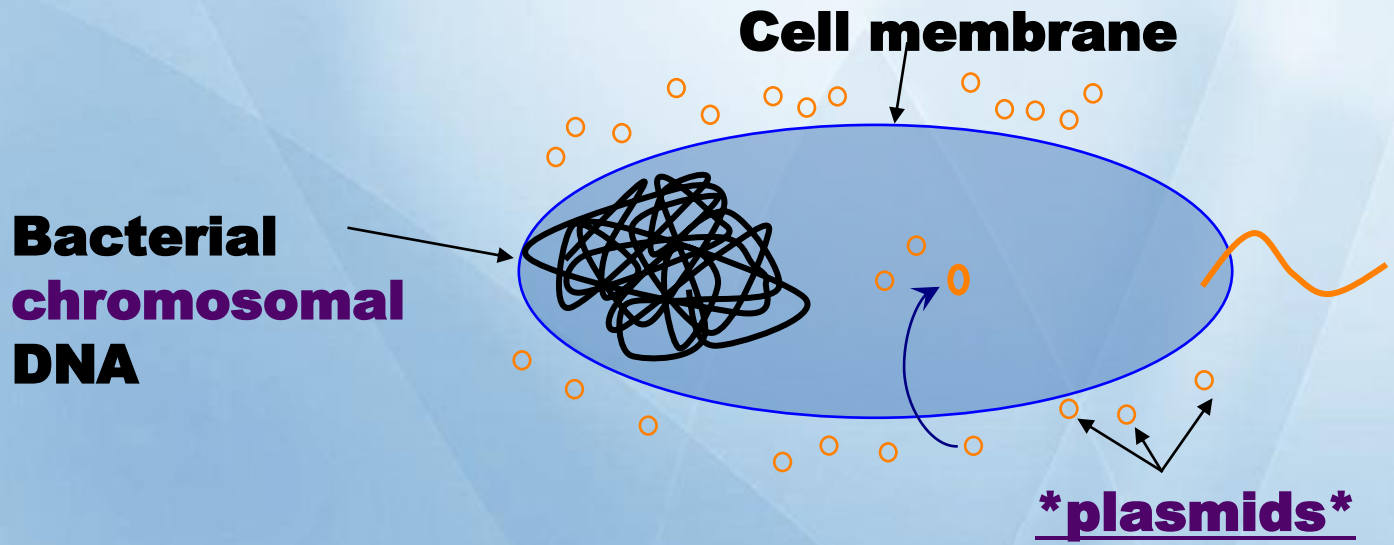




STEP 4. TRANSFORMATION

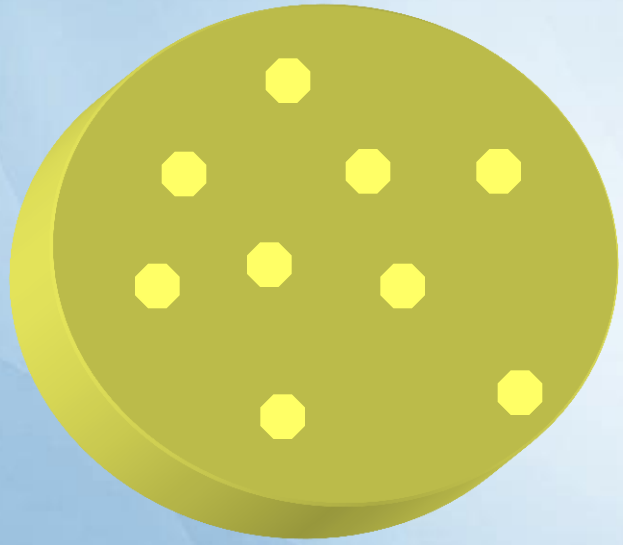
- The process of transferring exogenous DNA into cells is call "transformation"

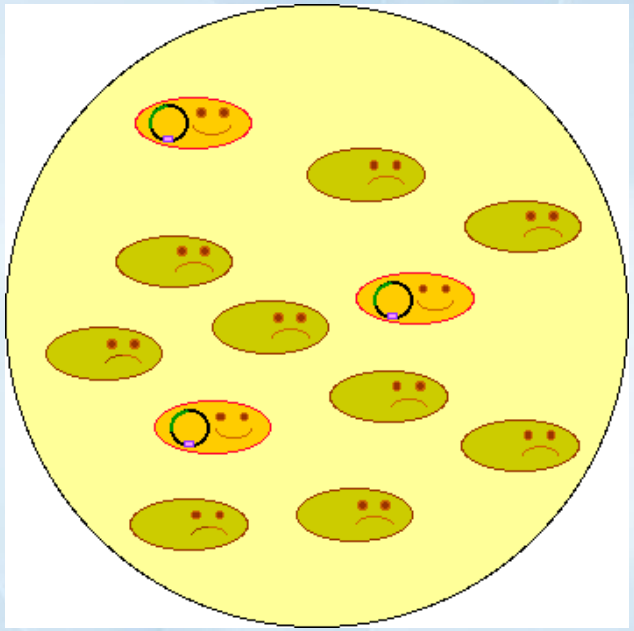
- There are basically two general methods:
  - chemical method utilizing CaCl2
  - electroporation



STEP 5. GROWTH ON AGAR PLATES

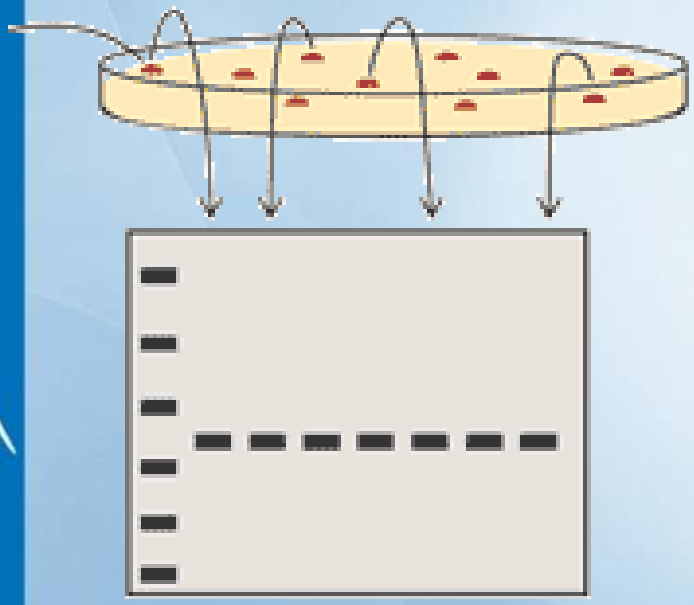
Growing Culture



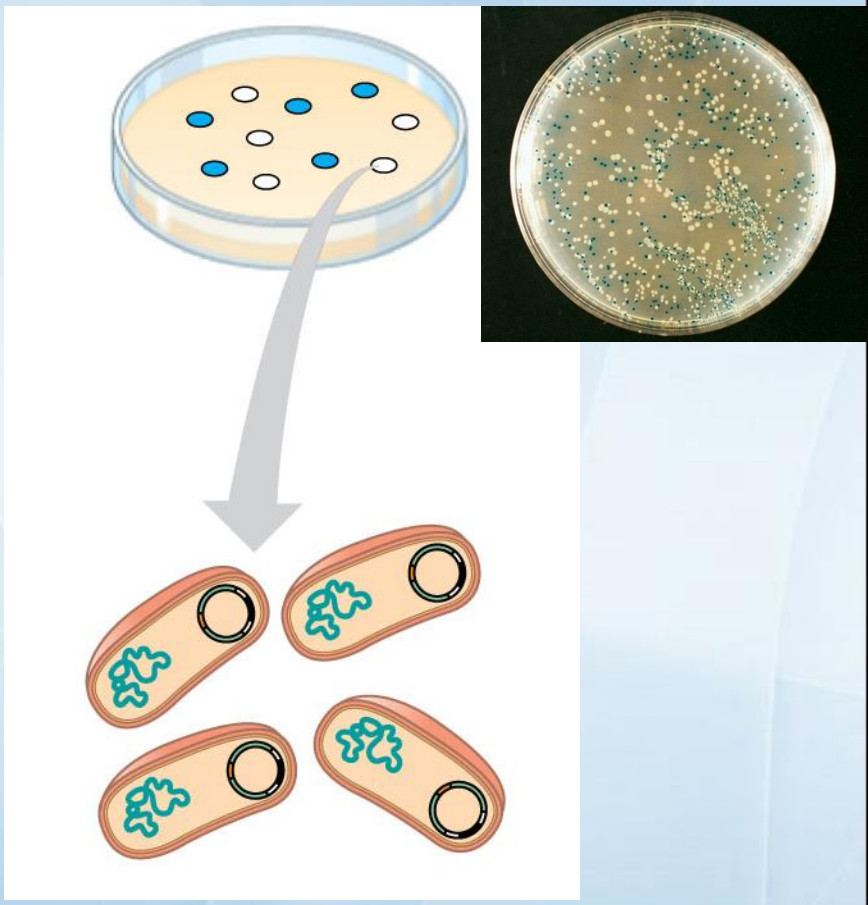


Spread transformed bacterial cells on the LB plate with selection drug and grow overnight.

Detection of the right cloning

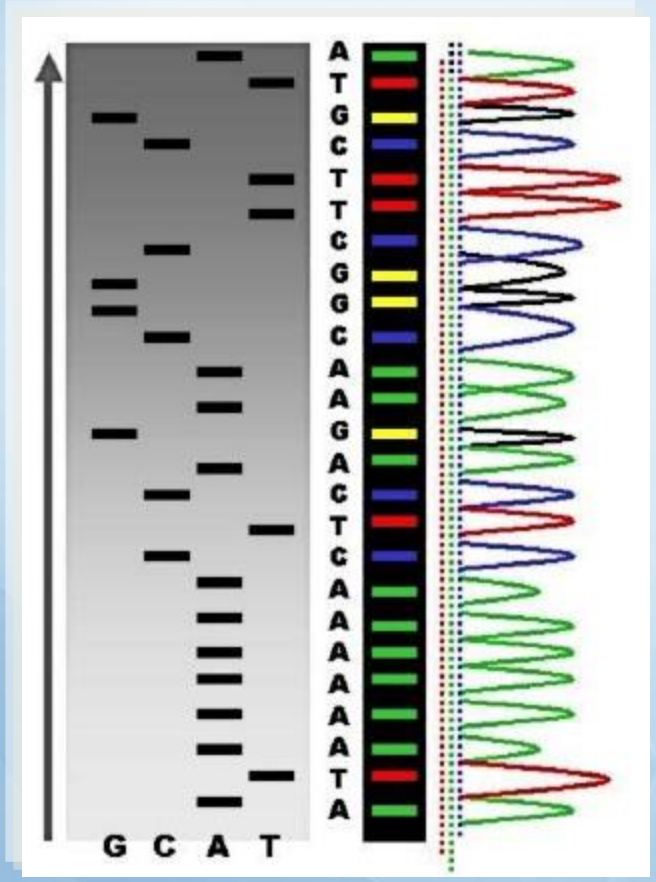


Screening with PCR



Blue white screening

Conformation with DNA Sequencing



Sequence alignment