Gene cloning

(an overview)

Ameer Effat M. Elfarash

Dept. of Genetics

Fac. of Agriculture, Assiut Univ.

amir_effat@yahoo.com

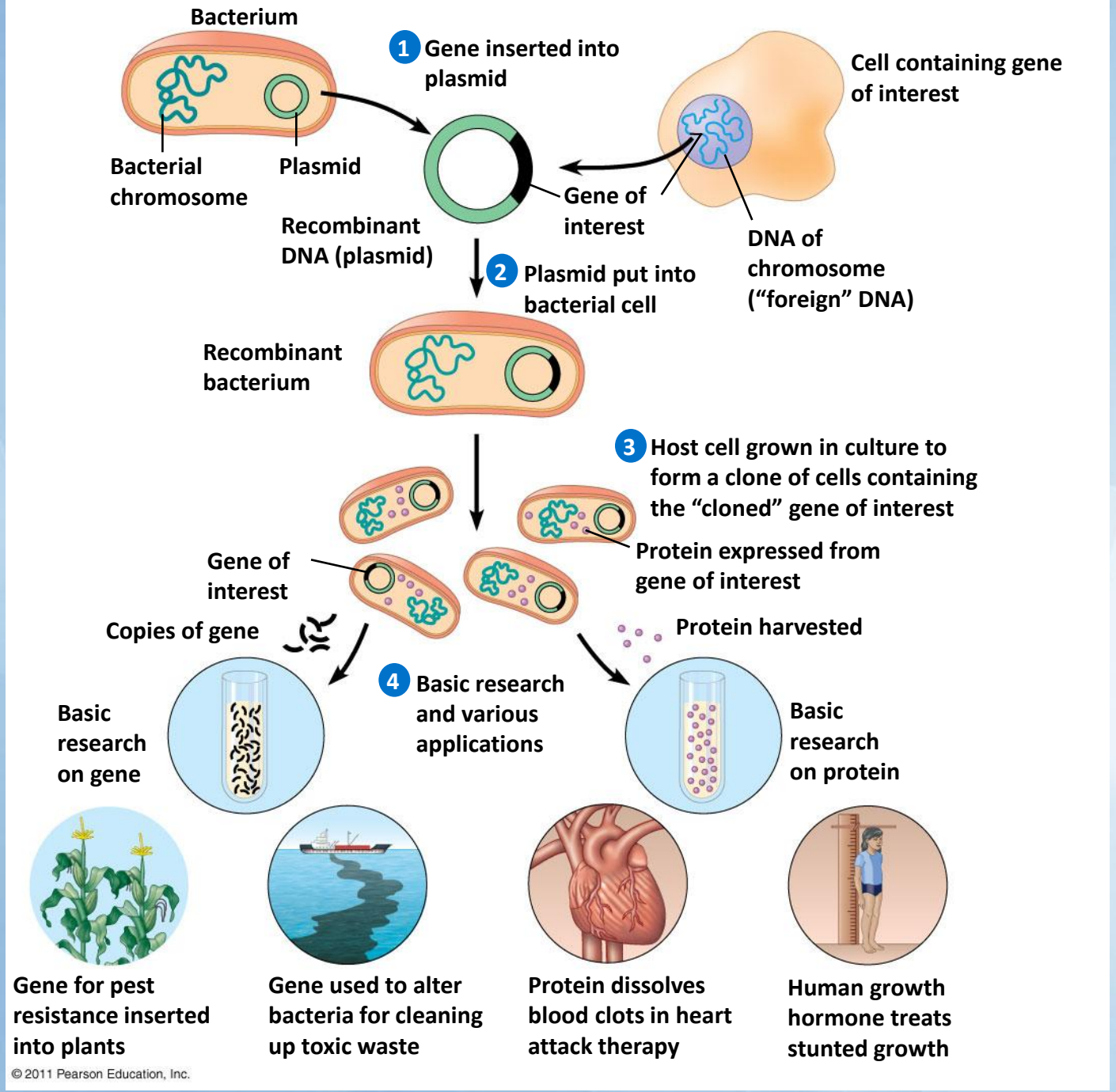
DEFINITION

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.

Goals of the DNA Technology:

- Isolation of a particular gene, part of a gene or region of a genome
- Production of a desired RNA or protein molecule in large quantities
- Increased production efficiency for commercially made enzymes and drugs
- Modification of existing organisms so that they express a particularly desirable trait not previously encoded in the genome.
- Correction of genetic defects in complex organisms, including humans.
- etc.


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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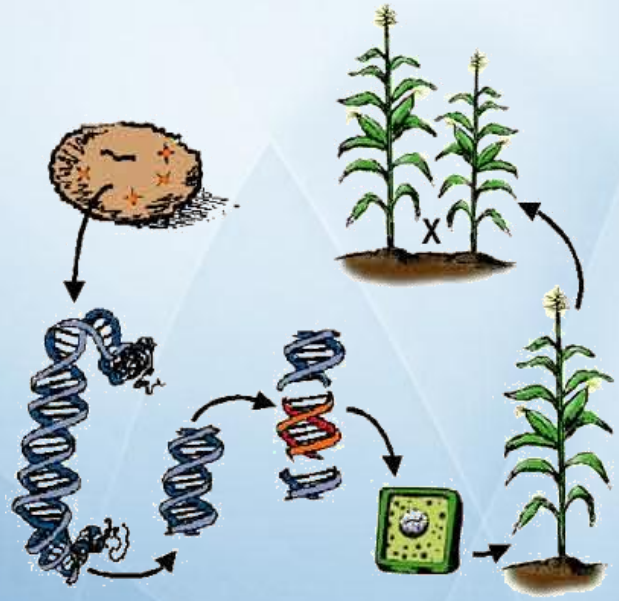
What is transformation used for?

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



What is transformation used for?

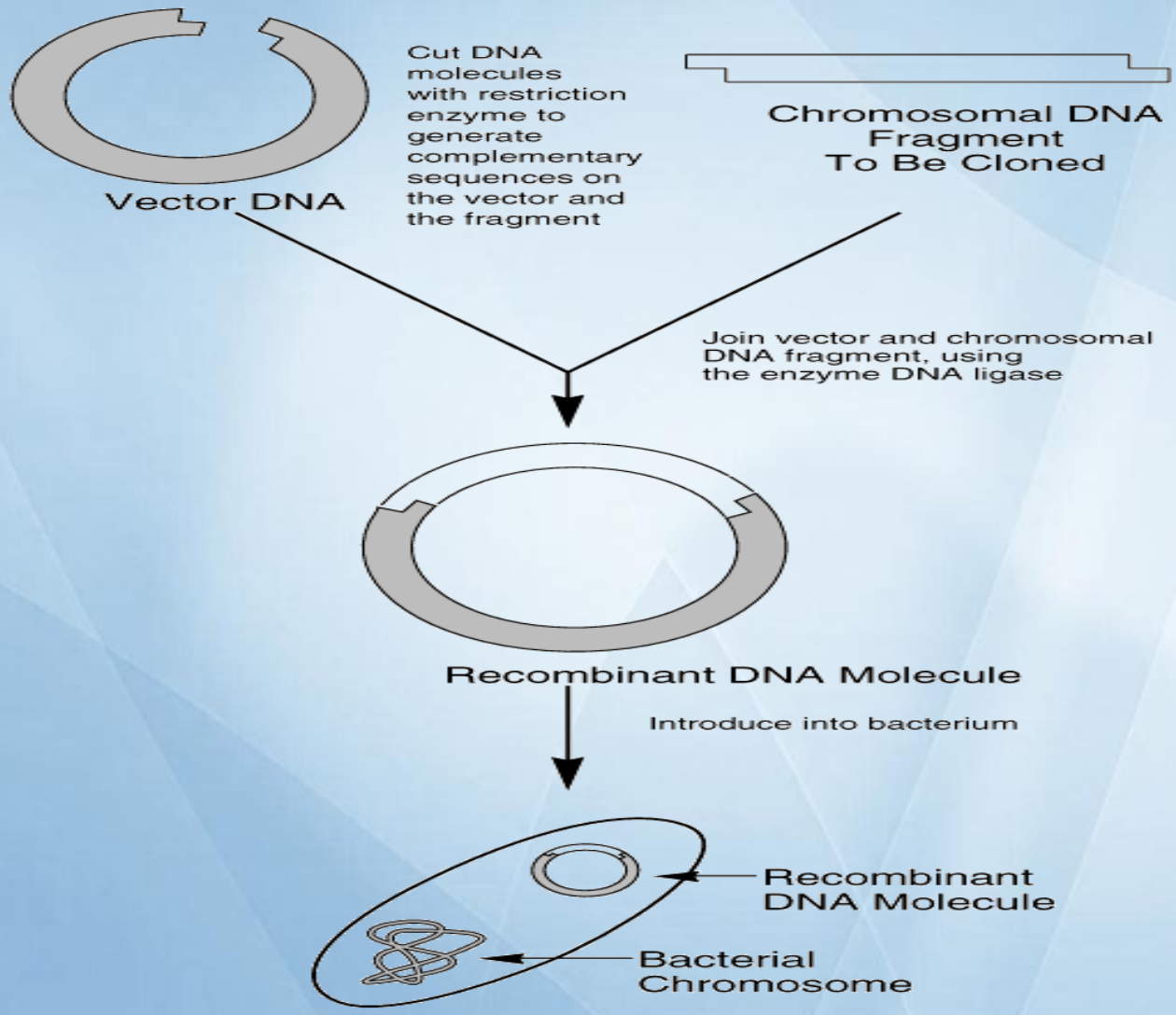
- Environmental
  - Bacteria can be genetically transformed with genes enabling them to **digest oil spills** or **remove pollutants** from the environment

What is transformation used for?

- 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

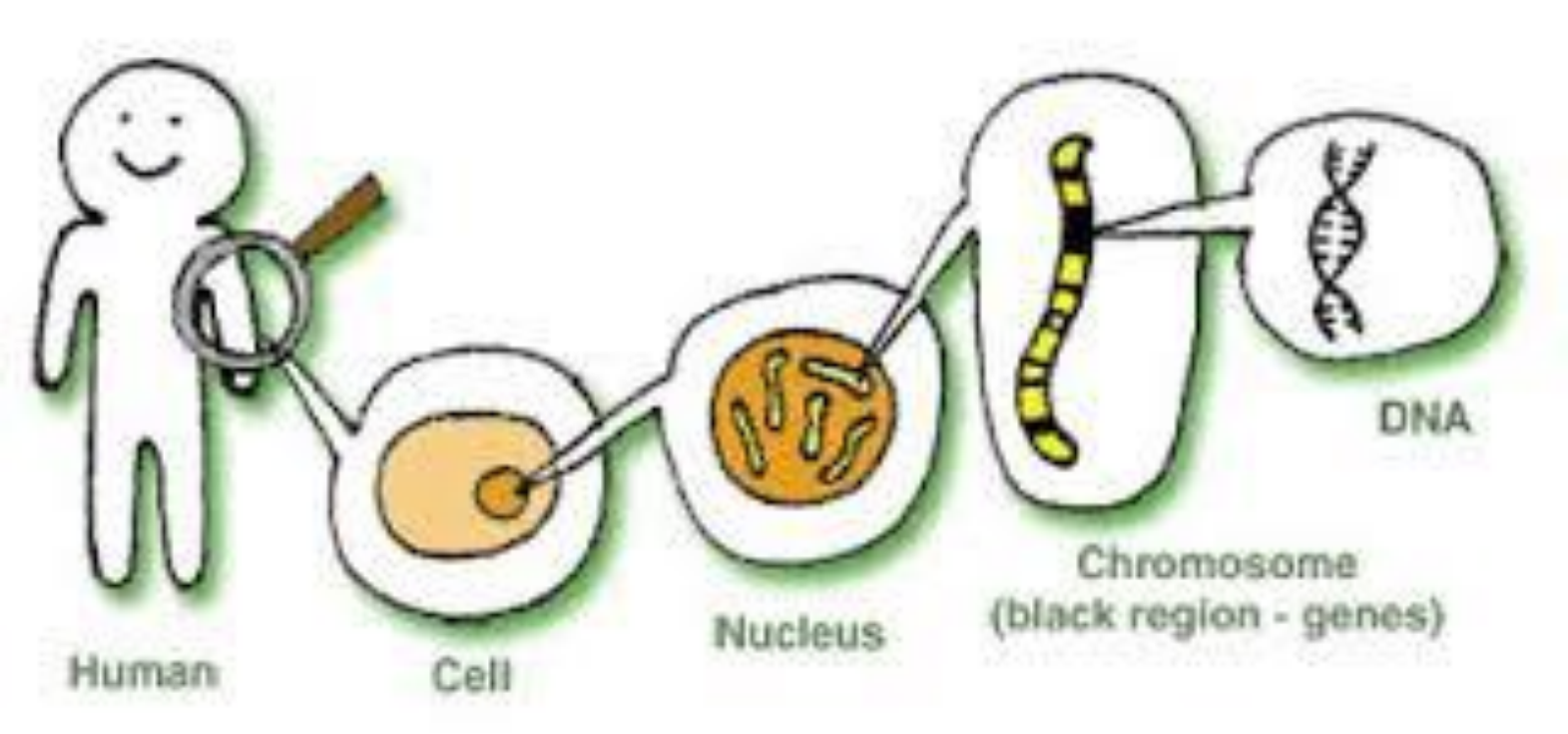
CLONING PROCESS



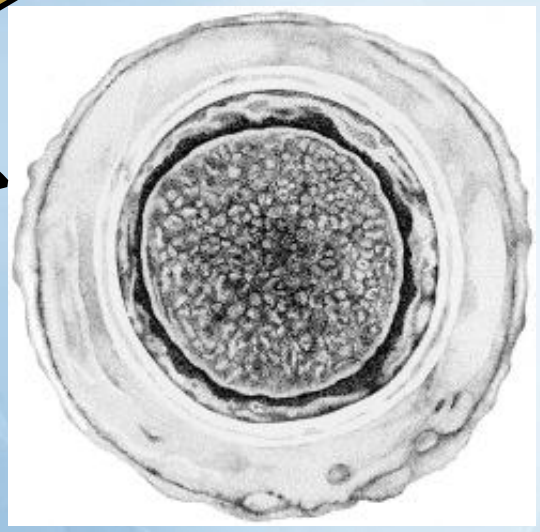
CLONING PROCESS

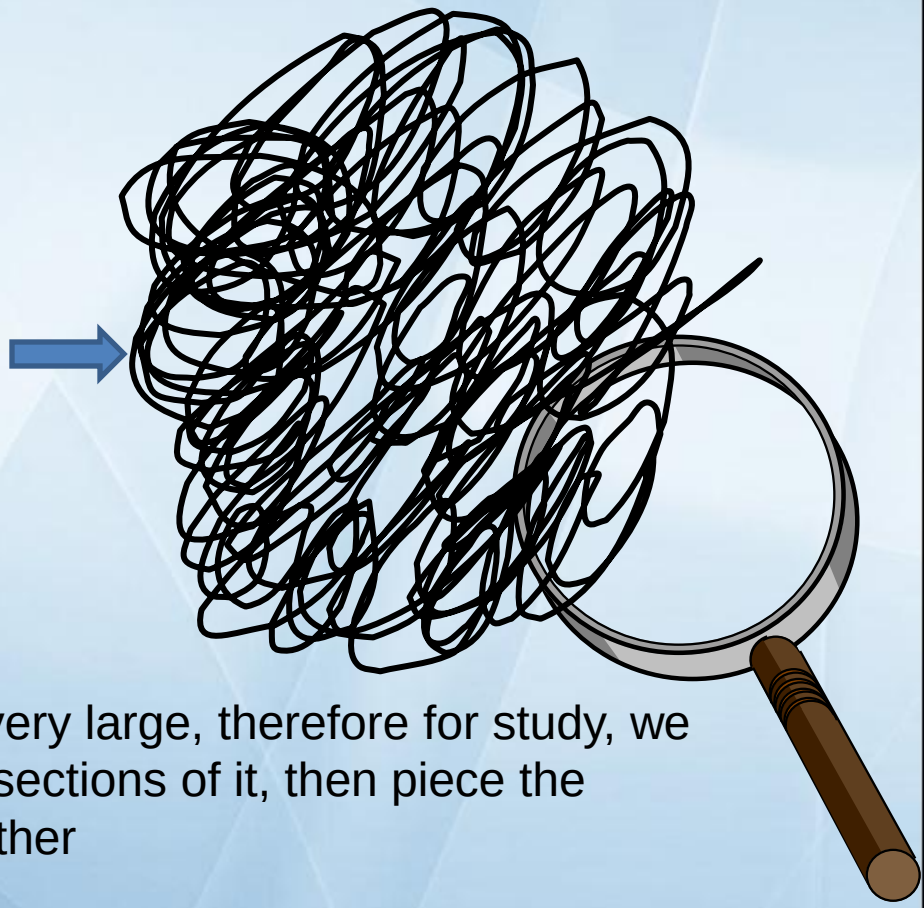
- Amplify Target Gene
- Cut Target Gene and Plasmid
- Ligation
- Transformation
- Cellular Screening
- Protein Expression

STEP 1. DNA isolation and PCR



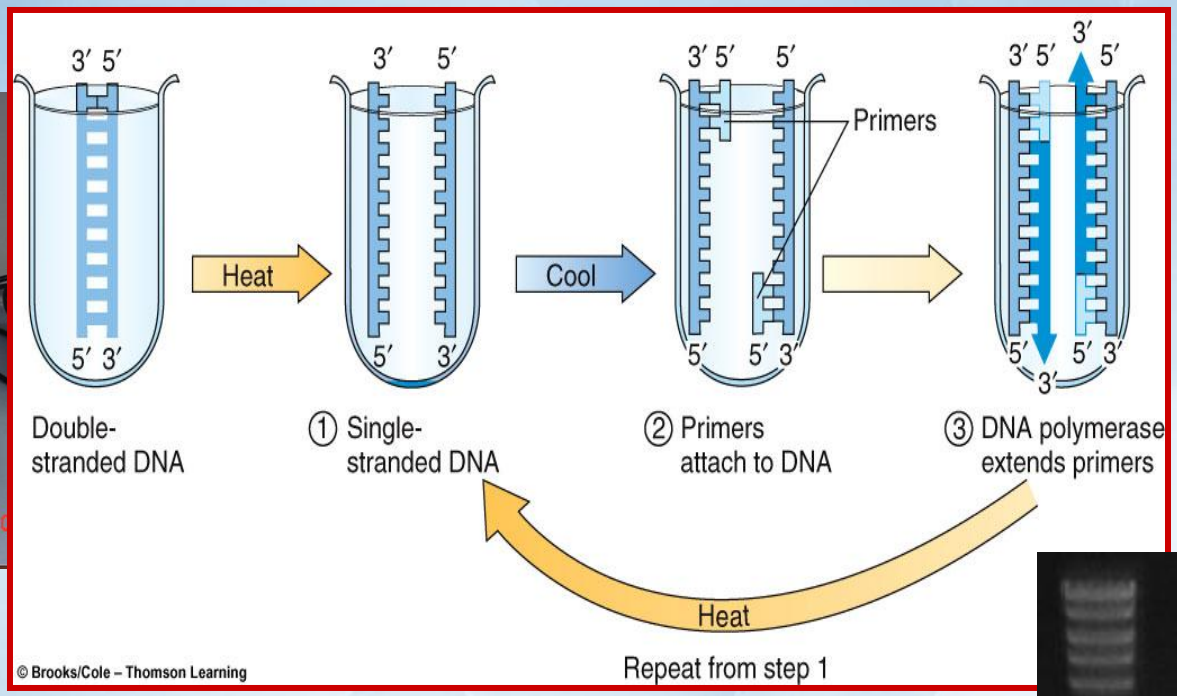
Extracting DNA from Cells

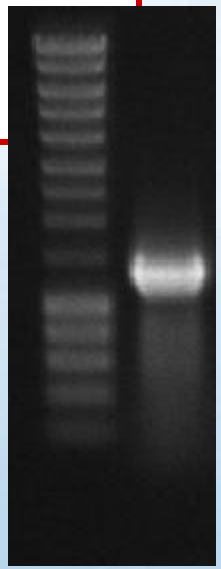




DNA can be very large, therefore for study, we look at small sections of it, then piece the sections together

Polymerase Chain Reaction (PCR)



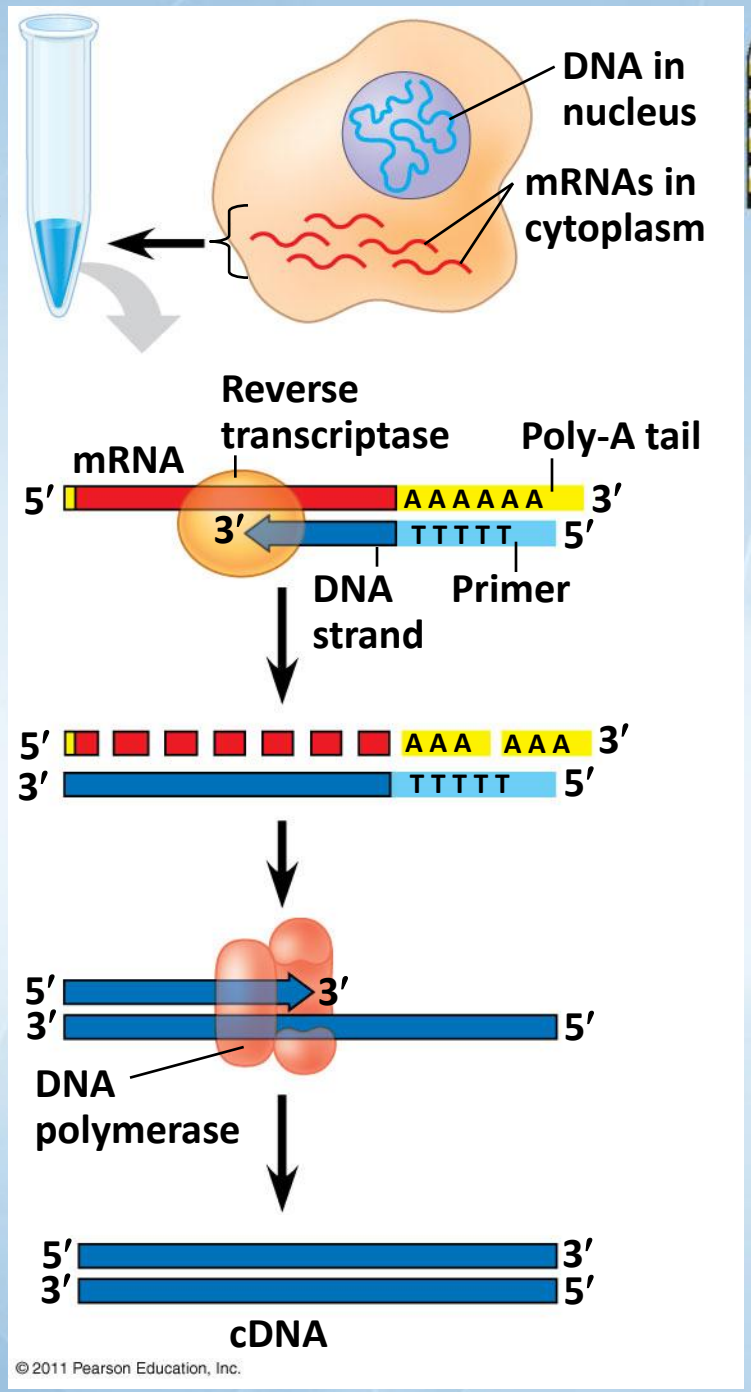


- PCR is used to:
  - Specifically amplify the target gene
  - Introduce the recognition site of the Restriction enzyme


5’
3’
PCR
RE

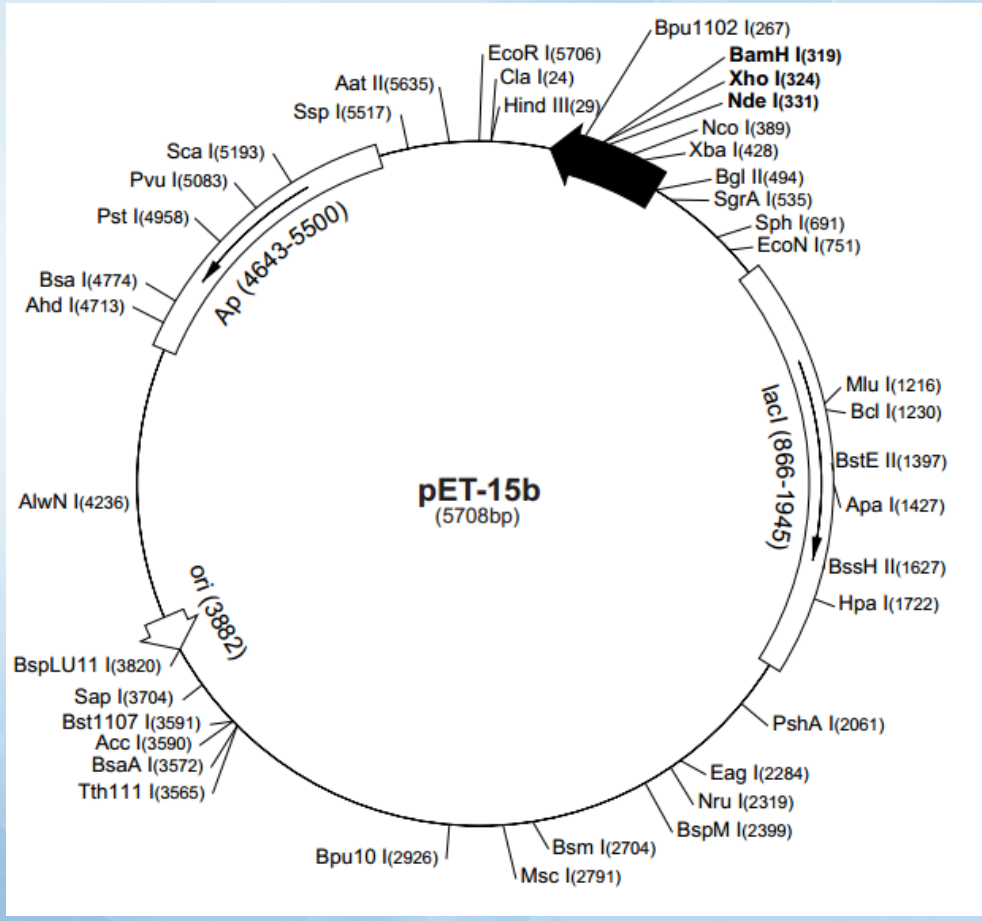
Reverse transcriptase

Produce complementary DNA (cDNA) from an RNA template.

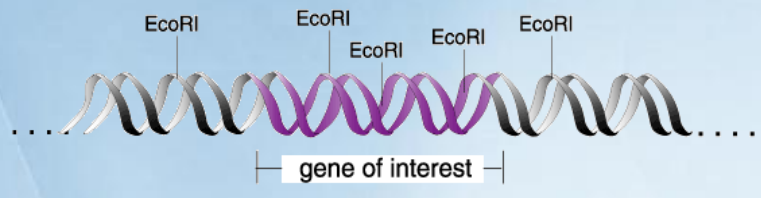


Plasmid DNA isolation

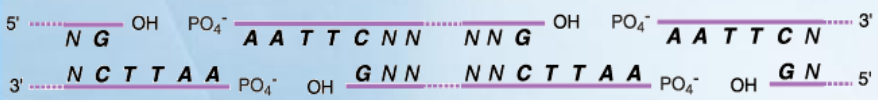
- To introduce a gene of interest into bacteria.
- Hallmarks:
  - Multi cloning site.
  - Selection marker.
  - Promoter.

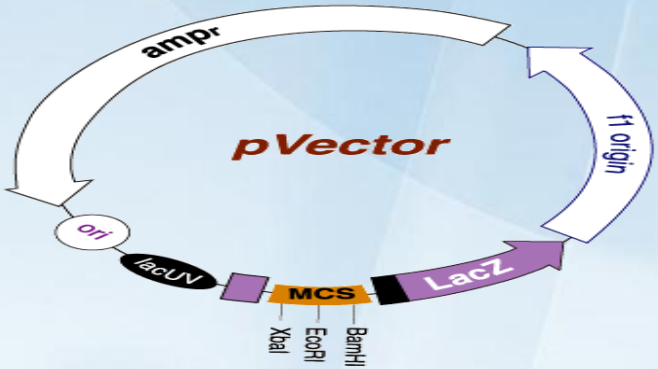


STEP 2. DIGESTION



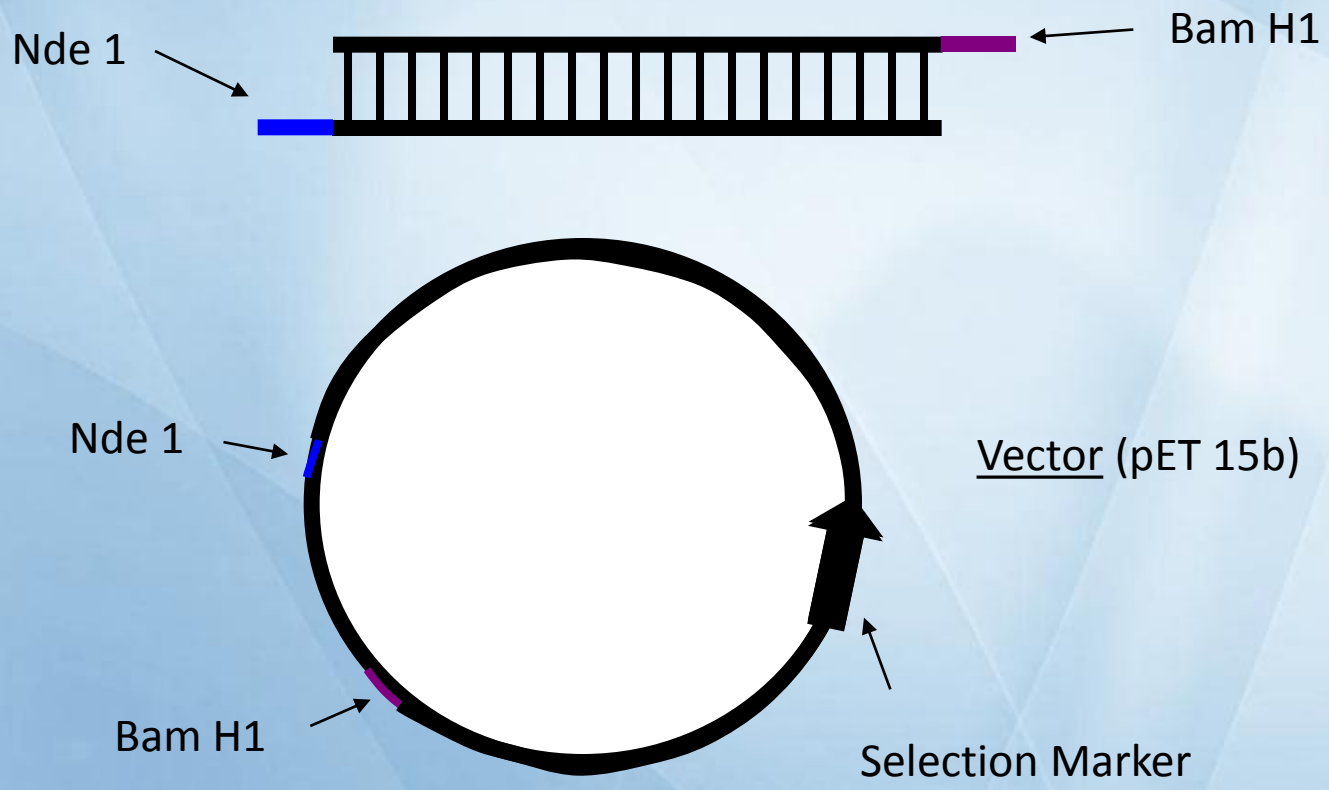
Digest DNA sample with EcoRI enzyme



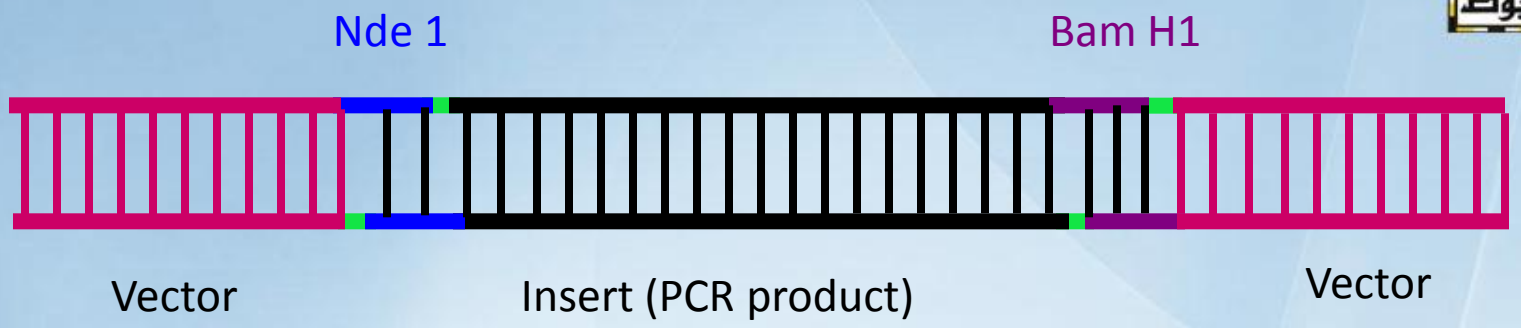


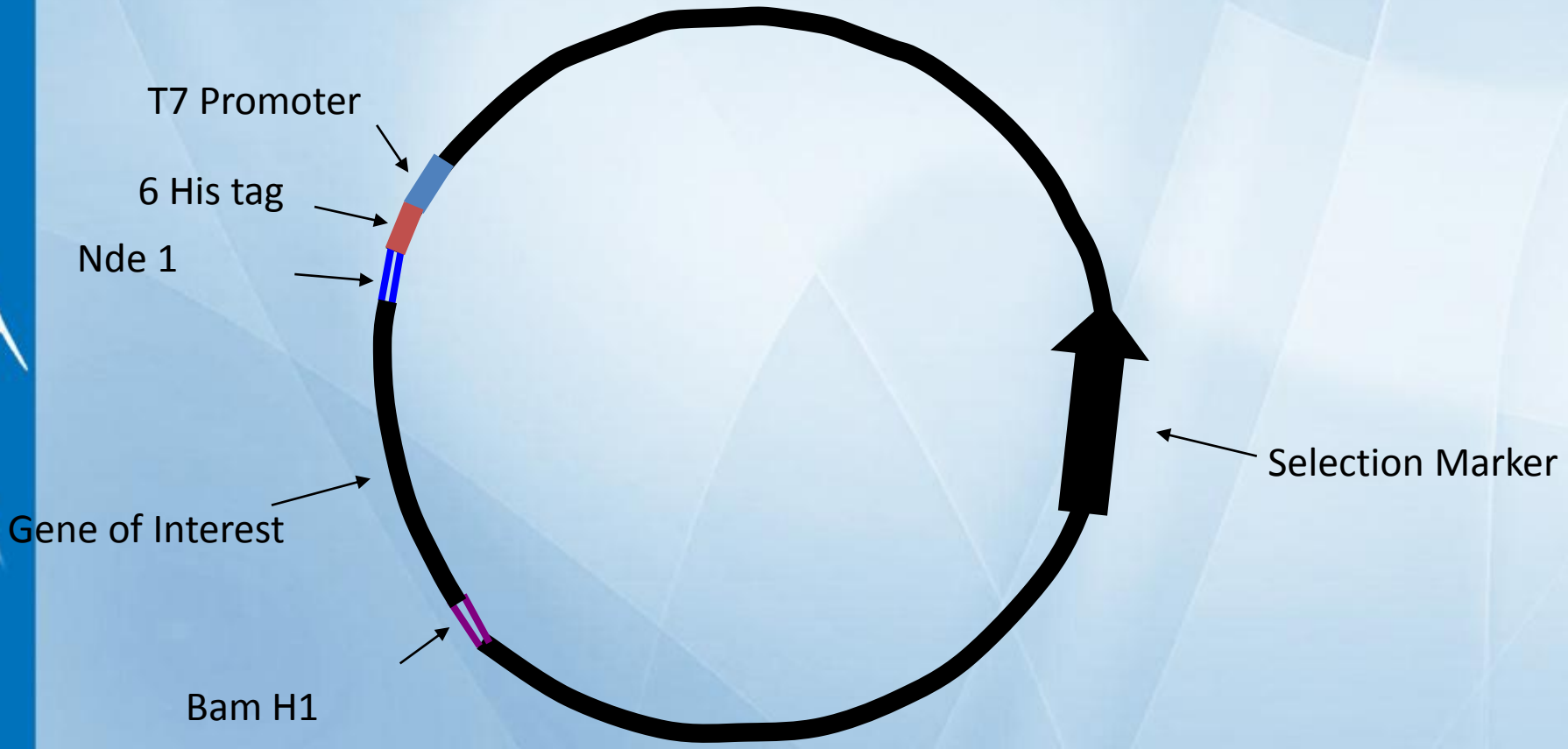
Digest plasmid vector with EcoRI enzyme

Restriction Digestion



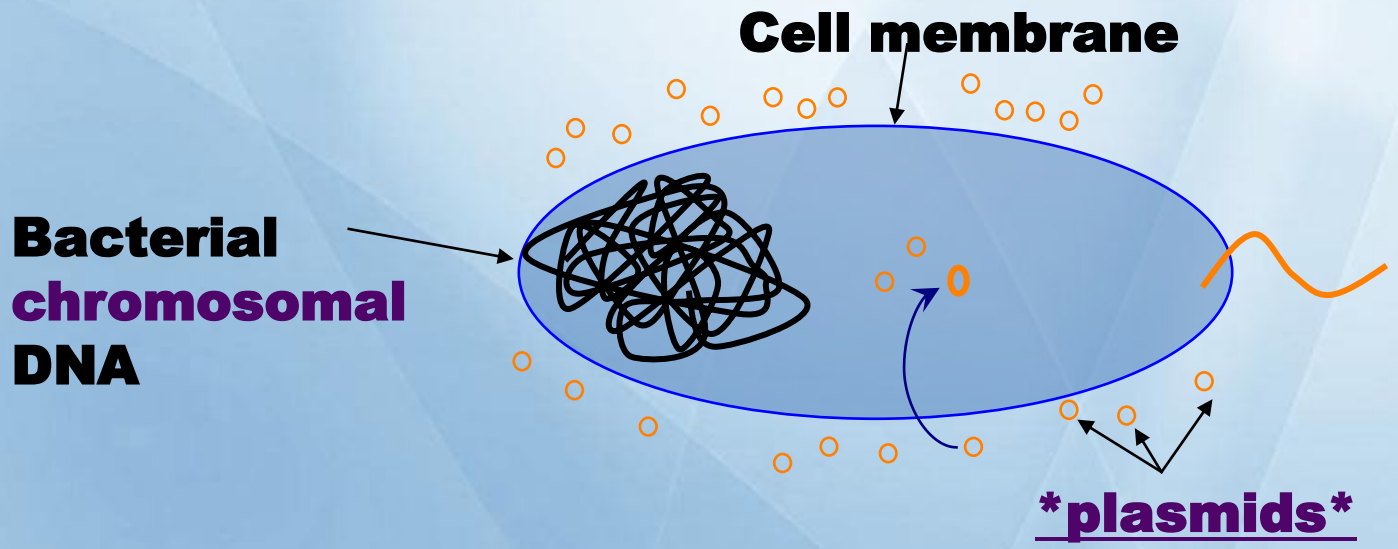
STEP 3. LIGATION





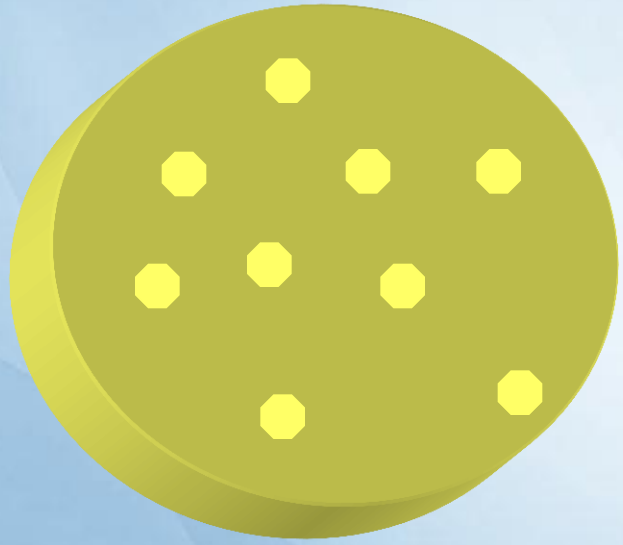
STEP 4. TRANSFORMATION

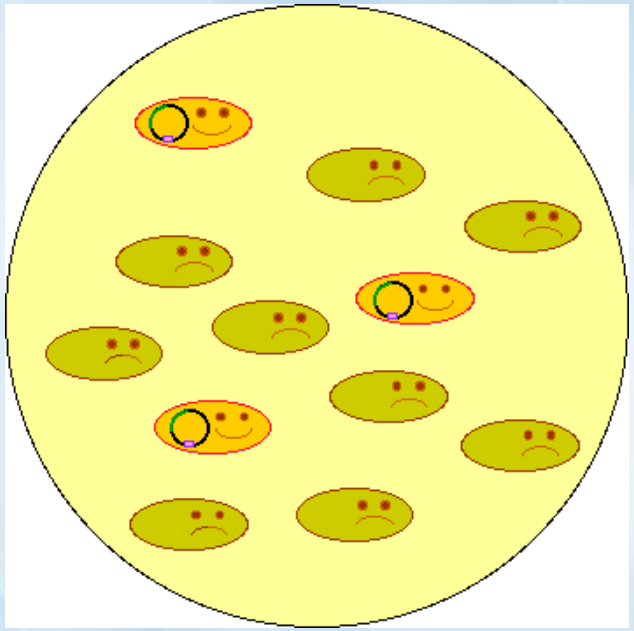
- The process of transferring exogenous DNA into cells is call "transformation"
- There are basically two general methods:
  - chemical method utilizing CaCl2
  - electroporation



STEP 5. GROWTH ON AGAR PLATES

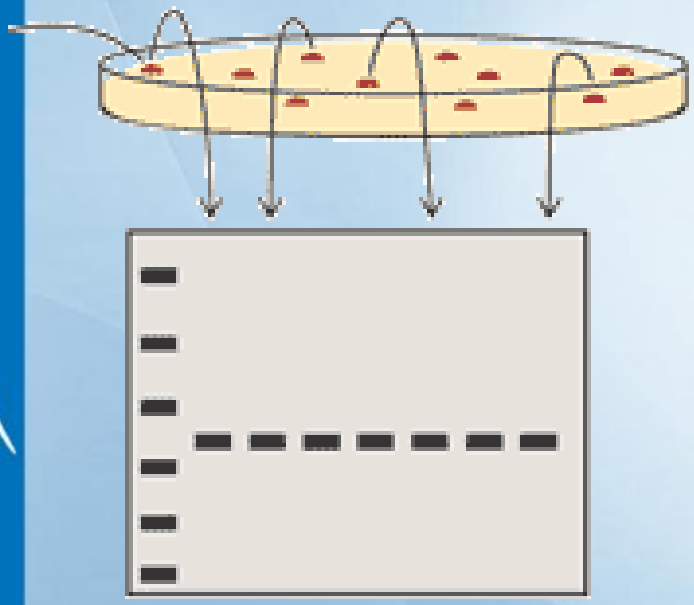
Growing Culture



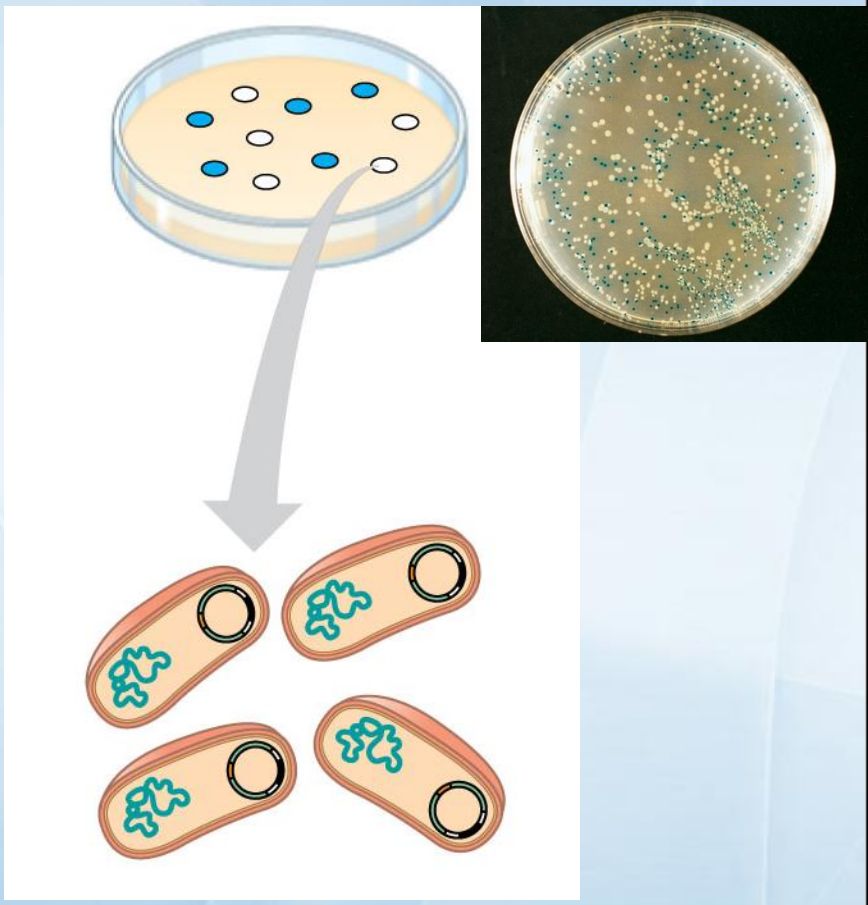


Spread transformed bacterial cells on the LB plate with selection drug and grow overnight.

Detection of the right cloning

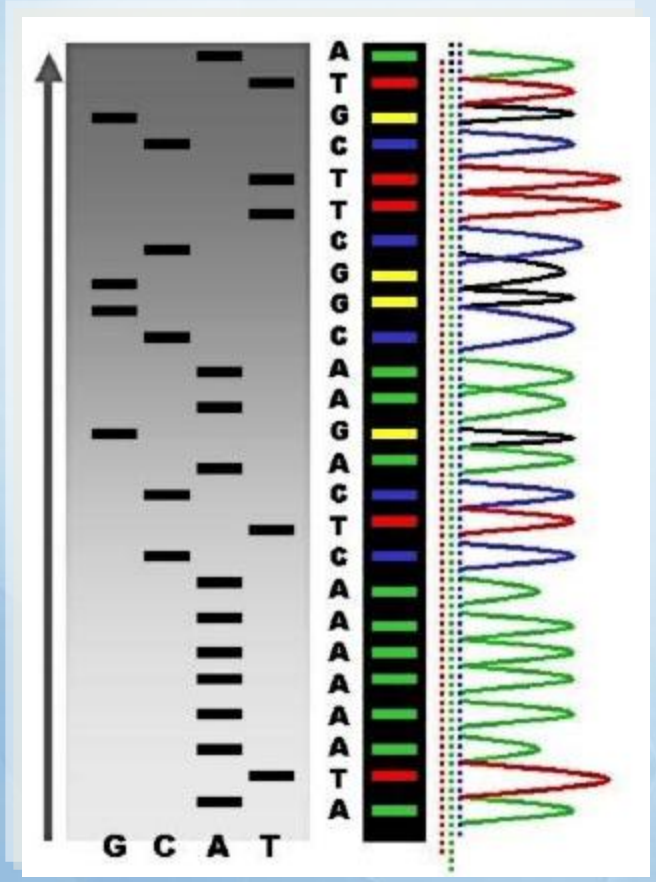


Screening with PCR



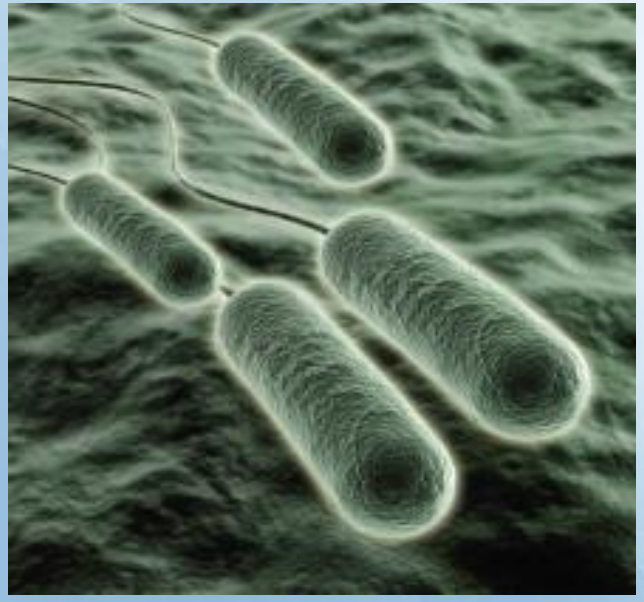
Blue white screening

Conformation with DNA Sequencing

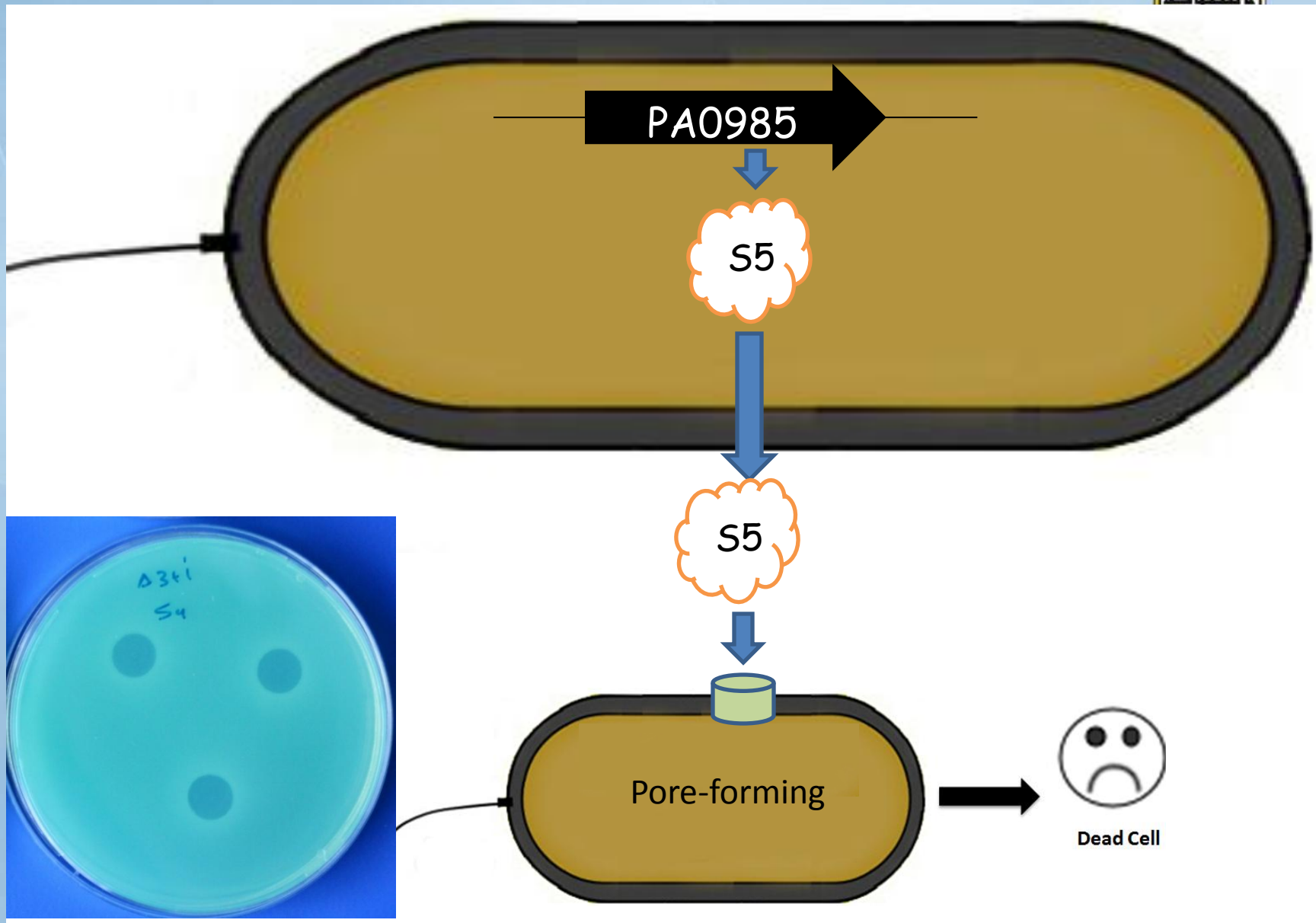


What are we doing?

- We will transform **bacteria** (***E. coli***), giving it the ability to make produce the Pyocin S5 protein from ***Pseudomonas aeruginosa***



Pyocin S5 of PAO1 strain



Primers Amplifying Target DNA

Cloning primers of Pyocin S5 gene				
GGAATTCCATATGTCCAATGACAACGAAGTACCTGG	Fw	60	1848	*Nde1*
CGGGATCCTTGAGCTTTAAATACTATTGGGC	Rv	54.8		*BamH1*