GENE CLONING (AN OVERVIEW)

BY

PROF. DR. ASMAA HUSSEIN

DIRECTOR OF THE MOECLULAR BIOLOGY RESEARCH UNIT (MBRU)

ASSIUT UNIVERSITY

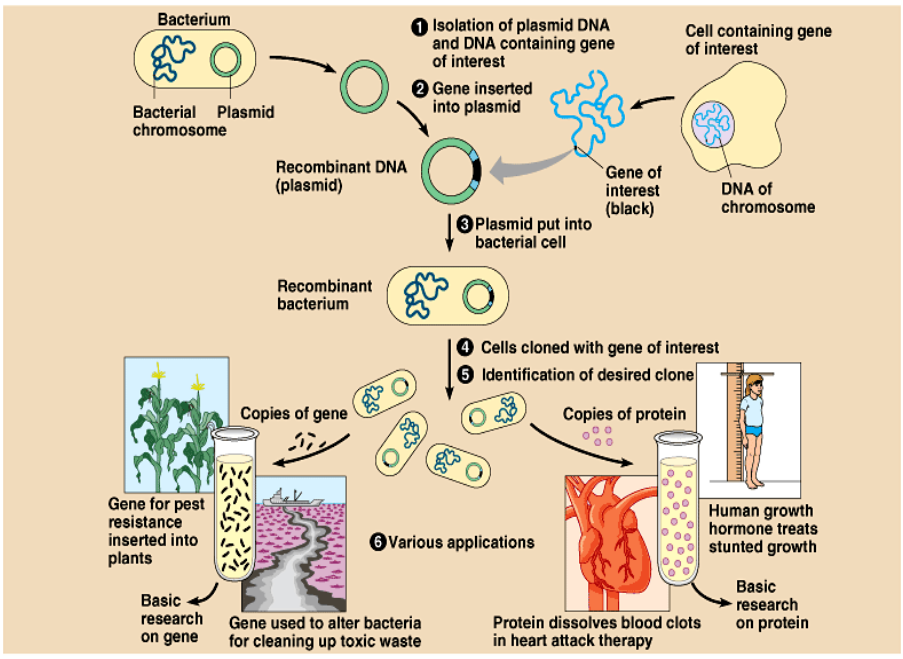
DEFINITION

Gene cloning is a set of experimental methods in molecular biology that are used to assemble recombinant DNA & Direct their replication within host organisms

Word "cloning" refers to the method involves replication of a single DNA molecules starting from a single living cell to generate large population of cells containing identical DNA

DNA Technology Goals:

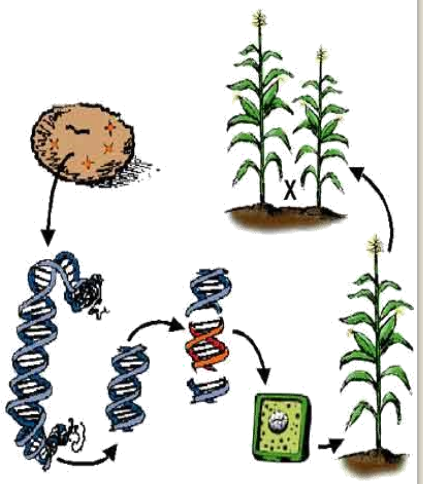
- ☑ Isolation of particular gene, part of gene or region of a genome
- ☑ Production of desired RNA or protein molecule in large quantities
- ☑ Increased production efficiency for commercially made enzymes & drugs
- ☑ Modification of existing organisms so that they express a particularly desirable trait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



WHAT IS TRANSFORMATION USED FOR?

A. Agricultural:

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



B. Environmental:

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

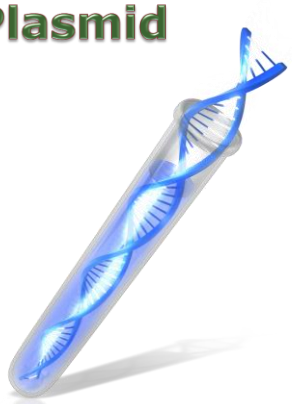
C. Medical

Production of human proteins to treat genetic diseases

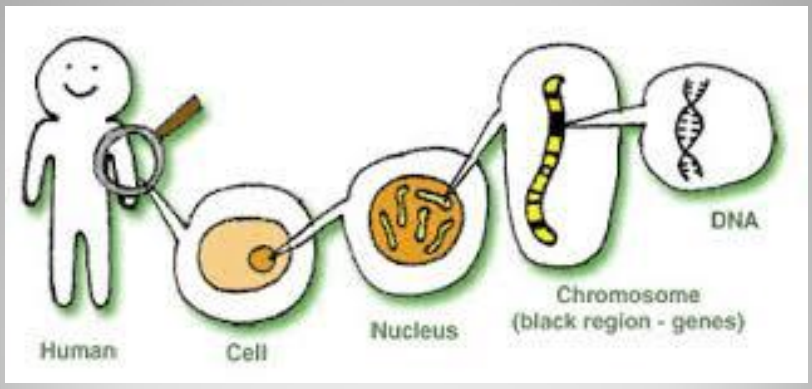
Protein	Disease/Disorder
HUMAN INSULIN	DIABETES MELLITUS
Human Growth Hormone	Deficiency in children
ERYTHROPOIETIN	ANEMIA
DNase I	Cystic fibrosis
HUMAN ANTIBODY BLOCKER	ASTHMA

CLONING PROCESS

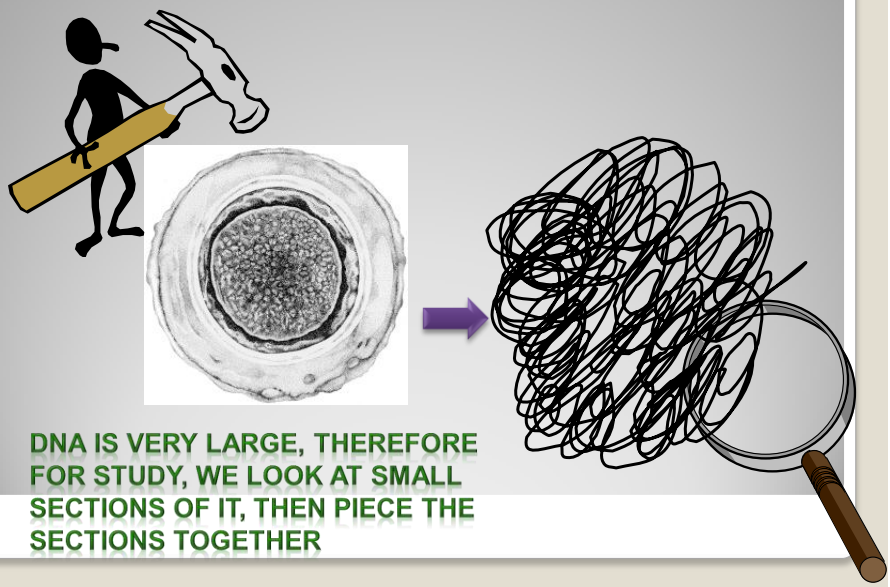
- Amplify Target Gene
- Cut Target Gene & Plasmid
- Ligation
- Transformation
- Cellular Screening
- Protein Expression



STEP 1. DNA isolation & PCR

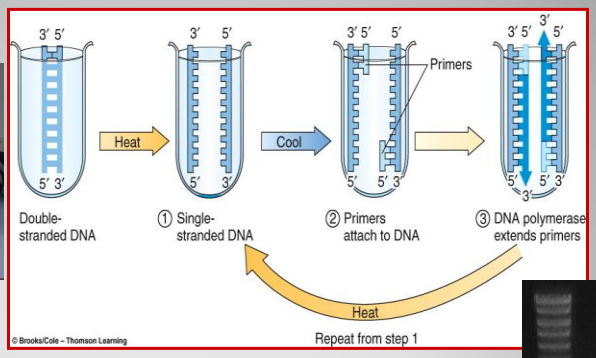


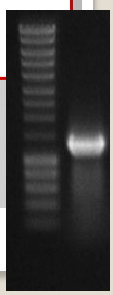
Extracting DNA from Cells:



DNA IS VERY LARGE, THEREFORE FOR STUDY, WE LOOK AT SMALL SECTIONS OF IT, THEN PIECE THE SECTIONS TOGETHER

Polymerase Chain Reaction (PCR)





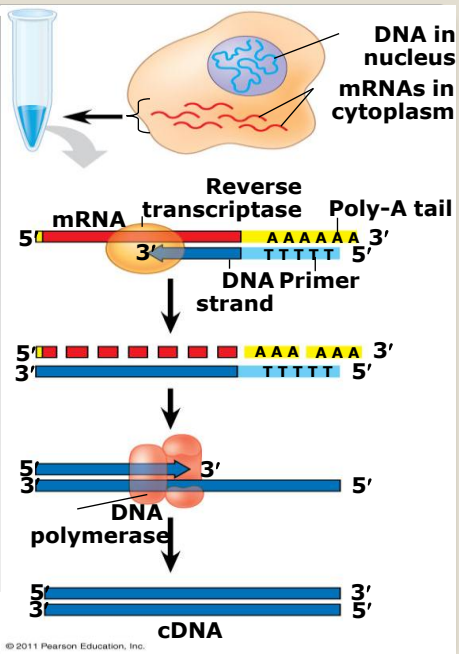
PCR is used to:

- **Specifically amplify target gene**
- **Introduce recognition site of the Restriction enzyme**

If we started with RNA

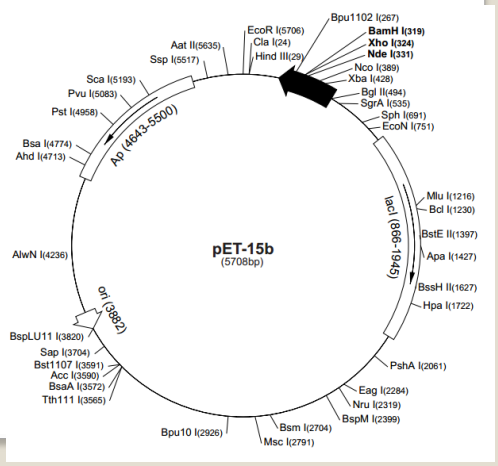
Reverse transcriptase

Produce complementary DNA (cDNA) from an RNA template

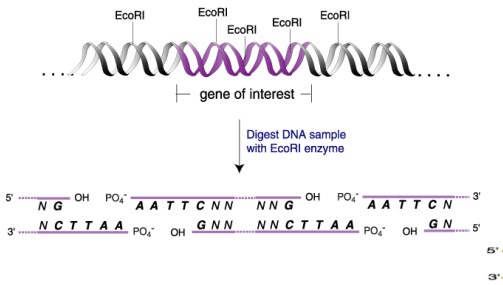


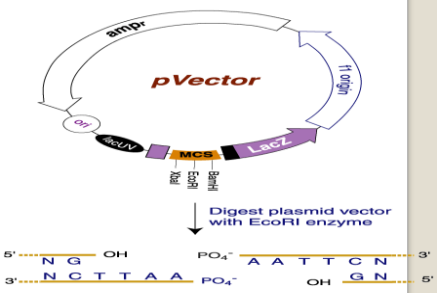
PLASMID DNA ISOLATION

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



STEP 2. DIGESTION:

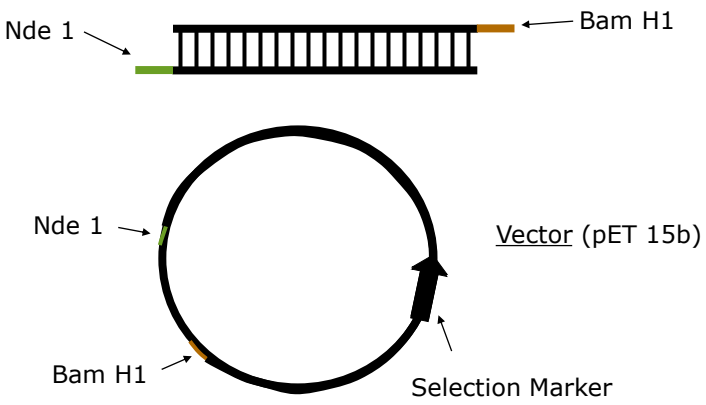




Application of restriction digestion to cut out the gene we want from the amplified product. We use the same enzymes to cut the vector so that the ends will be compatible (sticky end)

This is usually done by running the digest out on a gel & physically cutting out the fragment that you want with a scalpel

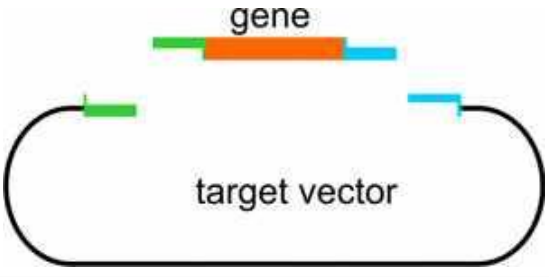
Restriction Digestion

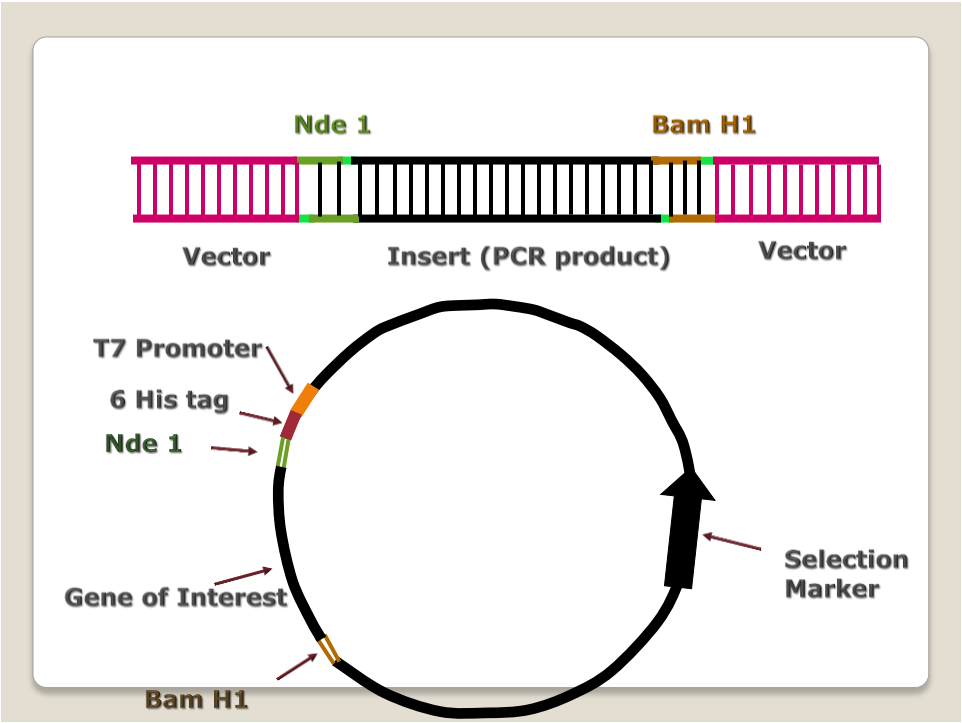


STEP 3. LIGATION

Once both the vector & the target DNA have been cut we mix them together & add the ligase enzyme

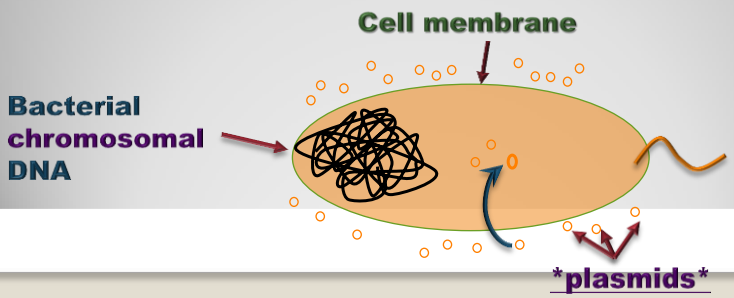
This enzyme ligates (connects) the phosphodiester backbone acting as a glue to stick the ends together





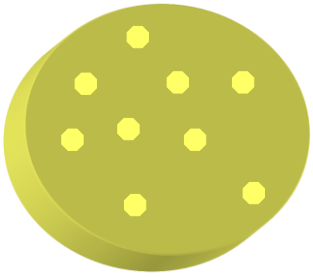
STEP 4: TRANSFORMATION

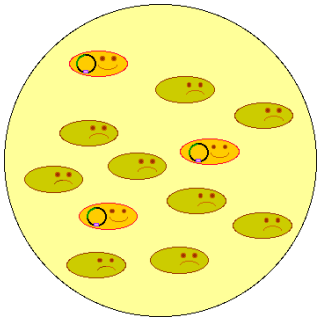
- The process of transferring exogenous DNA into cells is call "transformation"
- There are basically two general methods:
  - Chemical method utilizing CaCl2
  - Electroporation



STEP 5. GROWTH ON AGAR PLATES

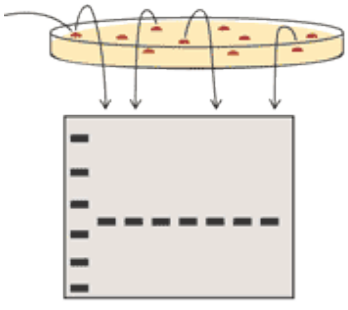
Growing Culture



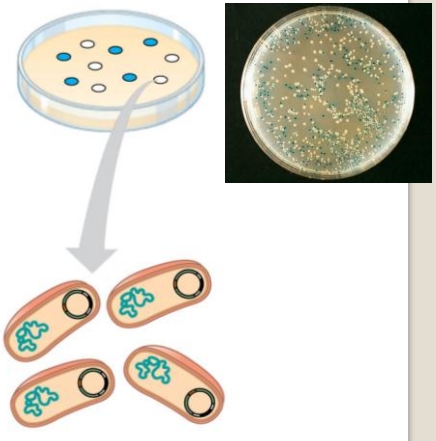


Spread transformed bacterial cells on the LB plate with selection drug & grow overnight

DETECTION OF THE RIGHT CLONING



Screen colonies on agarose gel



Screening with PCR

Blue white screening

Conformation with DNA Sequencing