

# From Gene to Protein

(an overview)

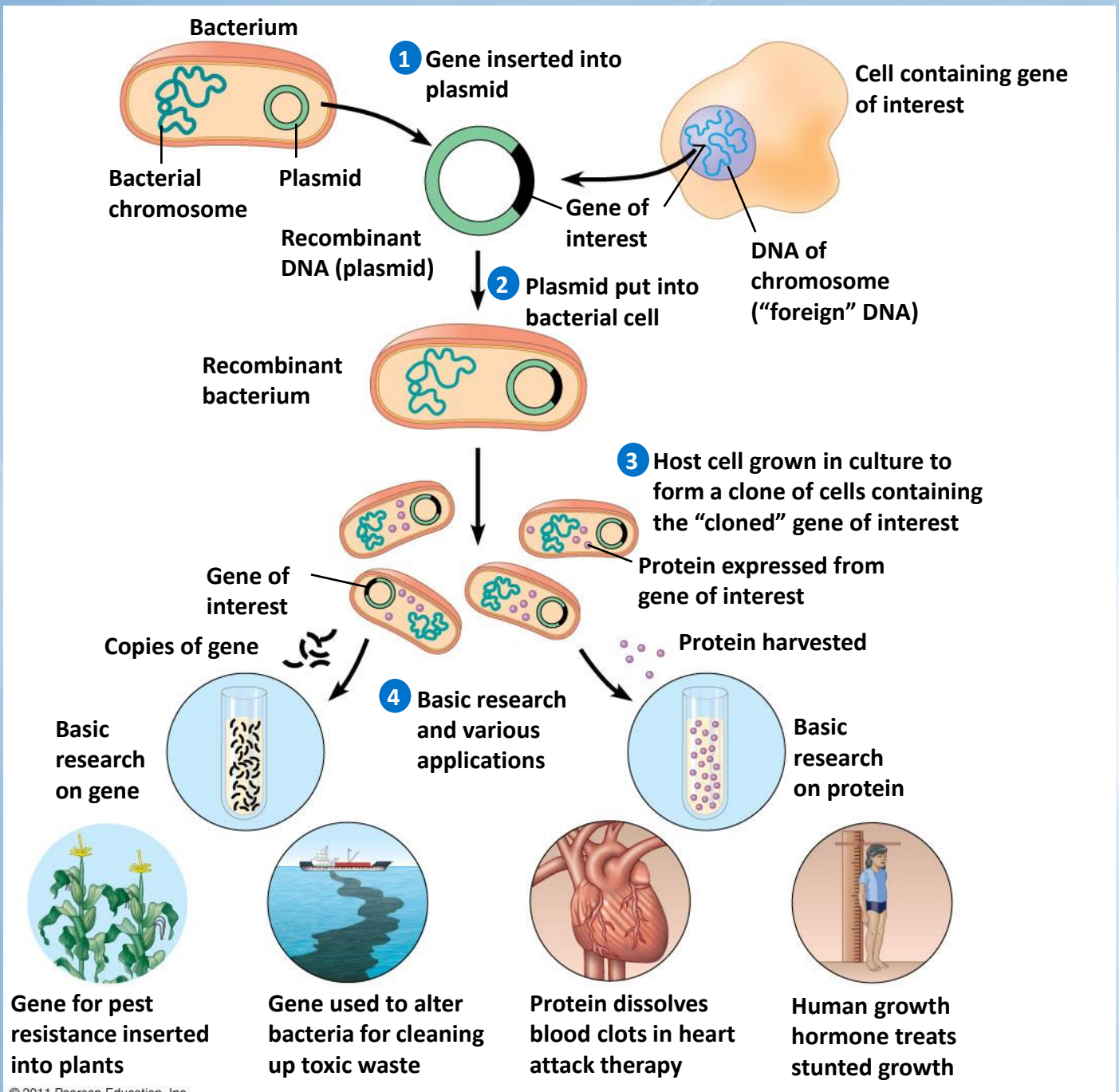
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# DEFINITION

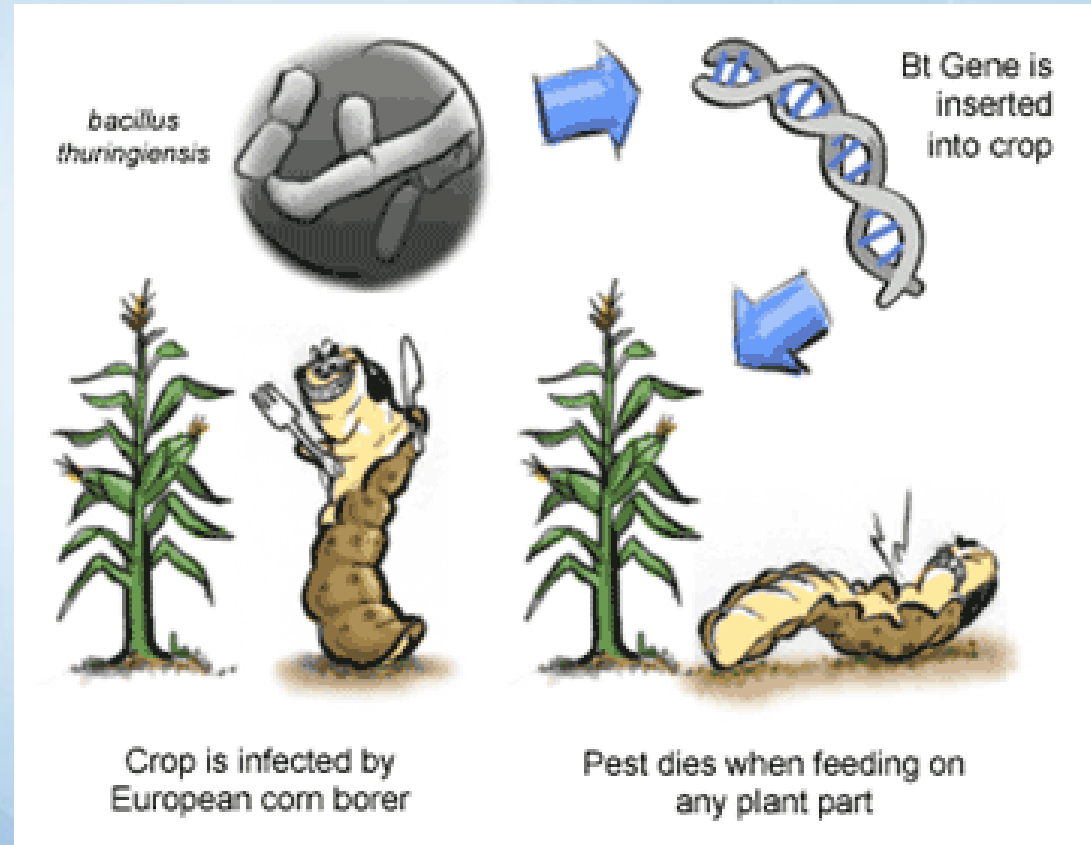
**Gene cloning** is a set of experimental methods in molecular biology that are used to assemble recombinant DNA molecules and to direct their replication within host organisms.

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.



# 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

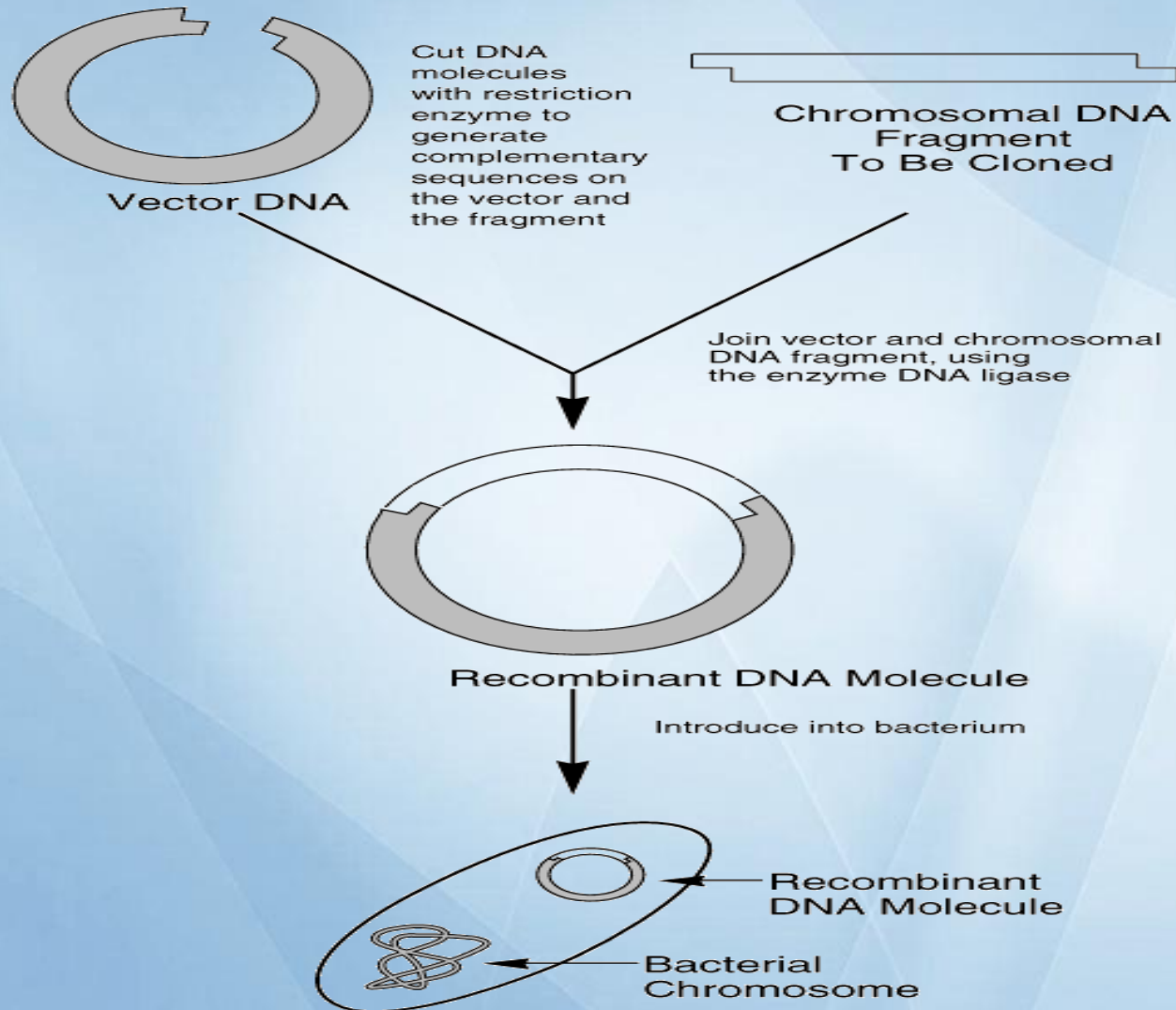
Protein	Disease/Disorder
Human insulin	Diabetes mellitus
Human Growth Hormone	Deficiency in children
Erythropoietin	Anemia
DNase I	Cystic fibrosis
Human antibody blocker	Asthma



- 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



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## NCBI Announcements

Coffee Break tutorial: Brown fat and obesity

Apr 1, 2014

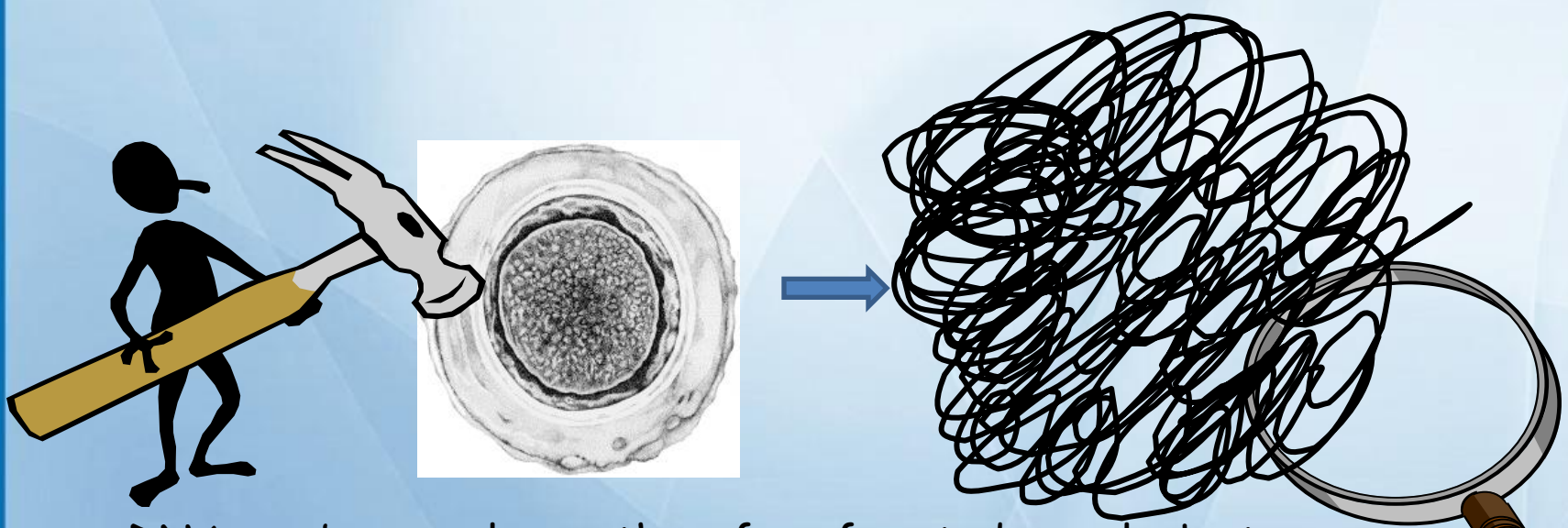
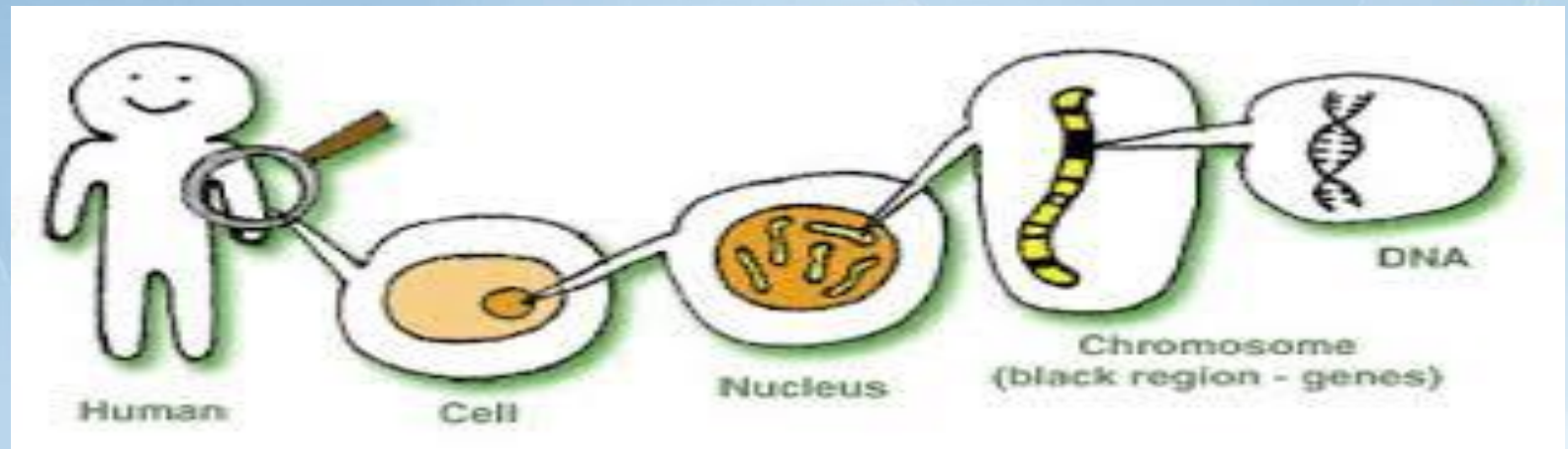
The latest Coffee Break tutorial discusses EUMT1 as a...

New NCBI YouTube video: Create custom databases for BLAST

Mar 28, 2014

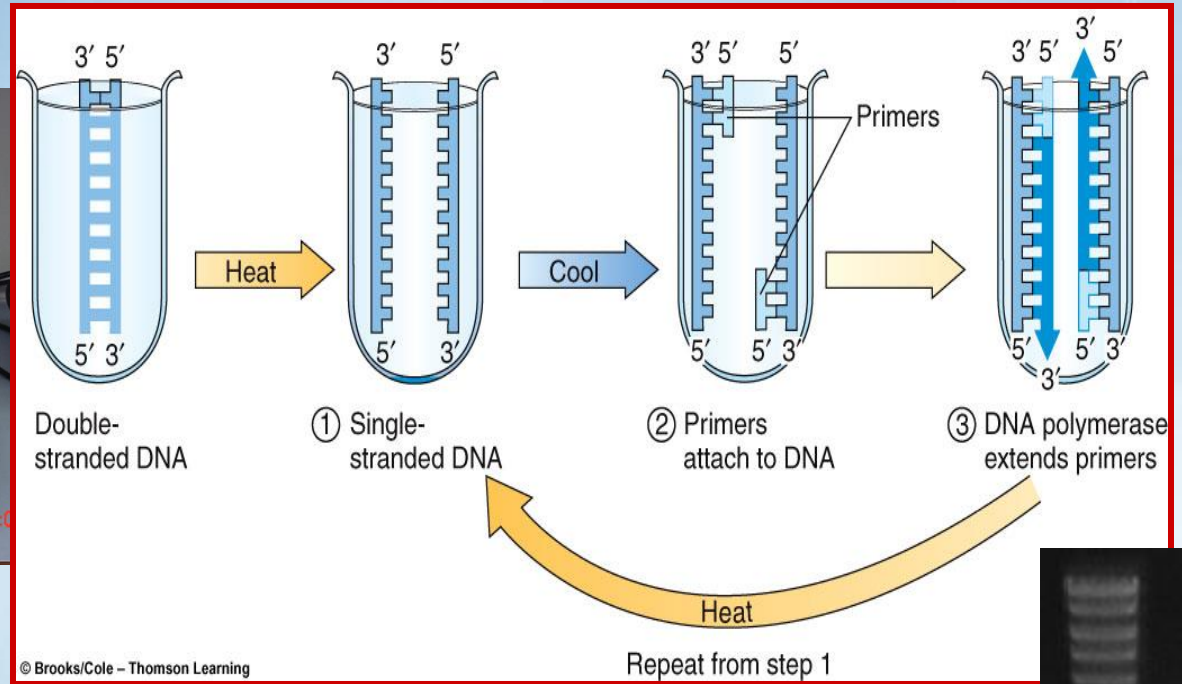
In the newest NCBI video on YouTube, we show you how to create custom...

# STEP 1. DNA isolation and PCR

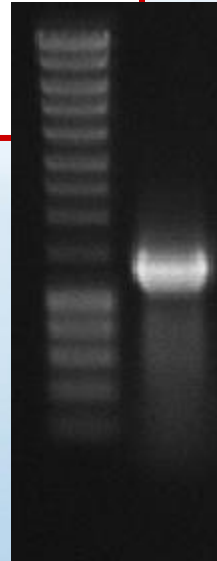


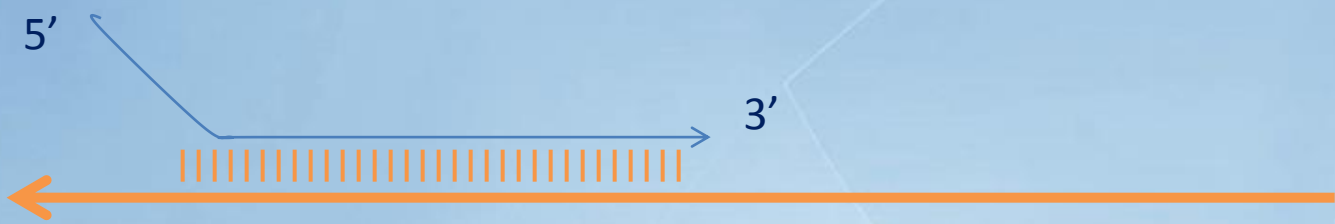
DNA can be very large, therefore for study, we look at small sections of it, then piece the sections together

# Polymerase Chain Reaction (PCR)

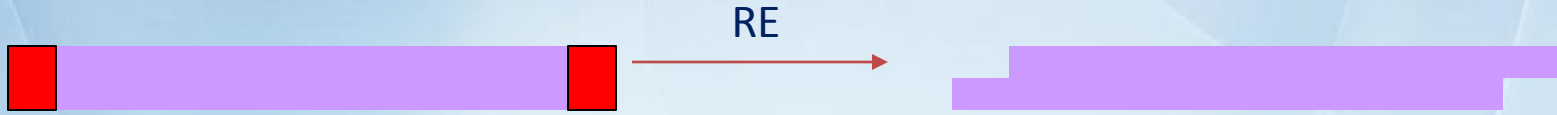


- PCR is used to:
  - Specifically amplify the target gene
  - Introduce the recognition site of the Restriction enzyme





PCR  
↓

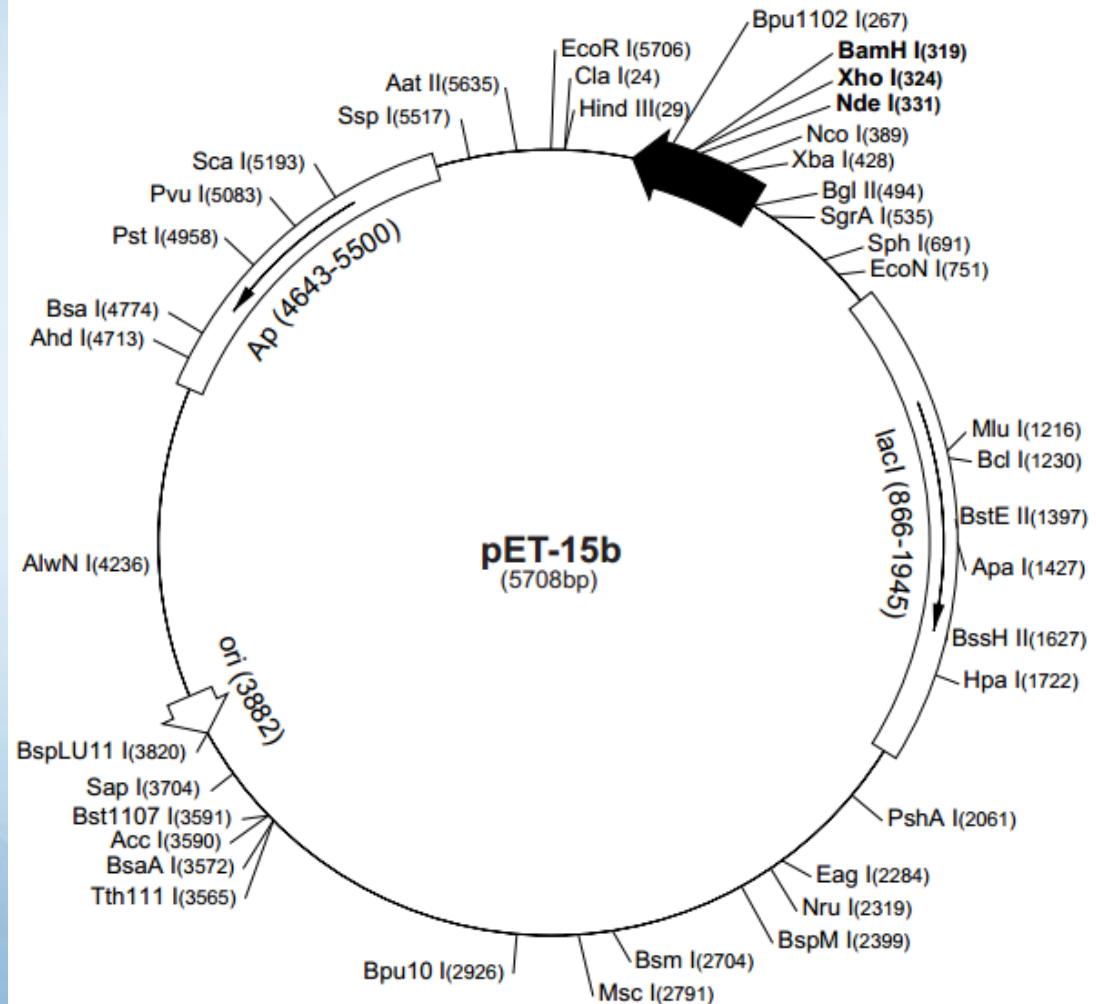
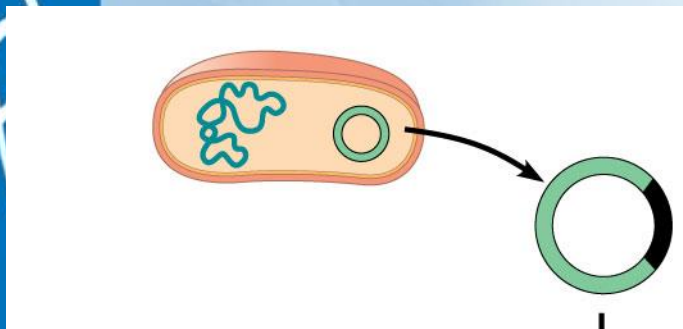


	oligo sequence	% cleavage	
		2h	20h
BamHI	<u>CGGATCCG</u> CG <u>GGATCC</u> CG	10 >90	25 >90
EcoRI	<u>GGAATTCC</u> CG <u>GAAATTC</u> CG	>90 >90	>90 >90
HindIII	<u>CAAGCTTG</u> CC <u>AAGCTT</u> GG	0 0	0 0
NdeI	GGGTTT <u>CATATG</u> AAACCC GGAATTCC <u>CATATG</u> GGAATTCC	0 75	0 >90

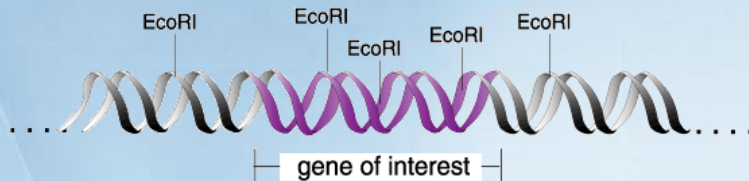


# Plasmid DNA isolation

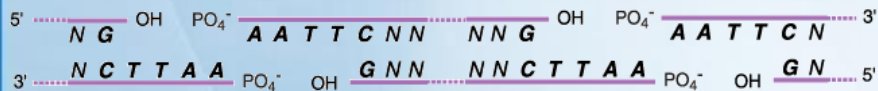
- To introduce a gene of interest into bacteria.
- Hallmarks:
  - Multi cloning site.
  - Selection marker.
  - Promoter.



# STEP 2. DIGESTION



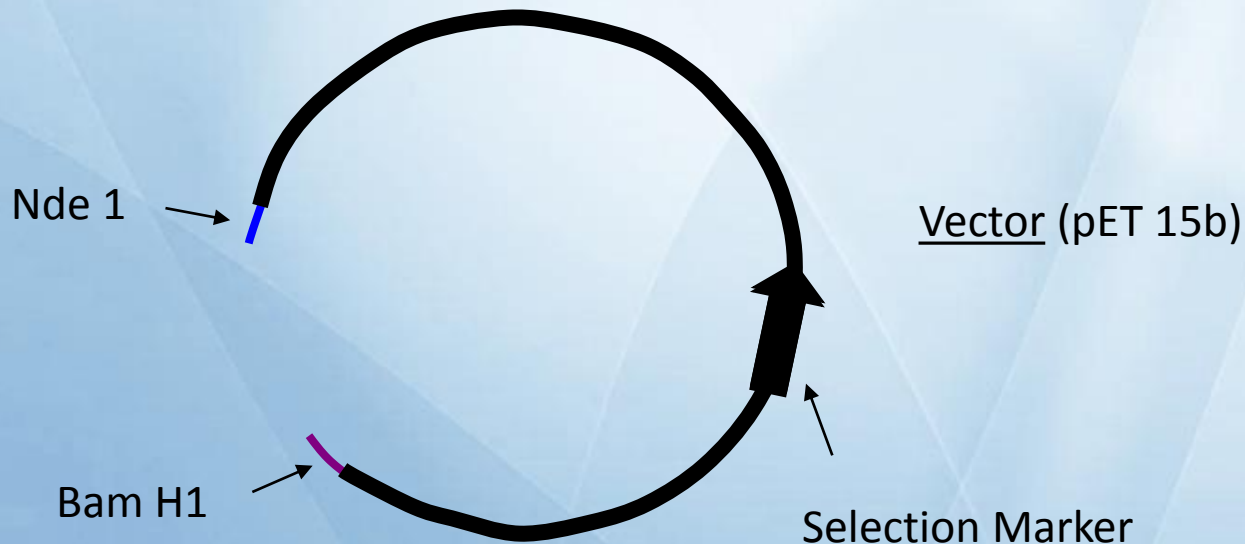
Digest DNA sample  
with EcoRI enzyme



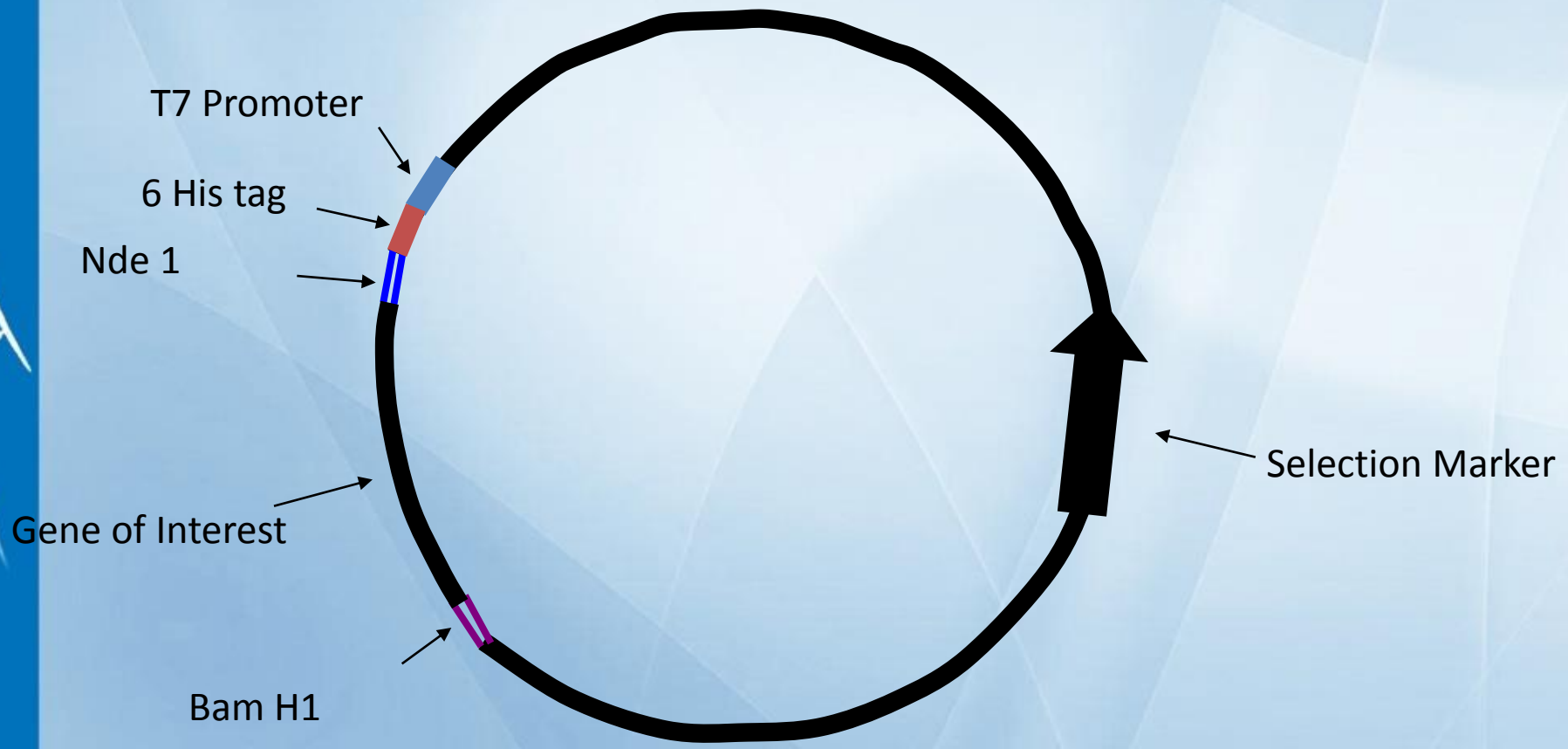
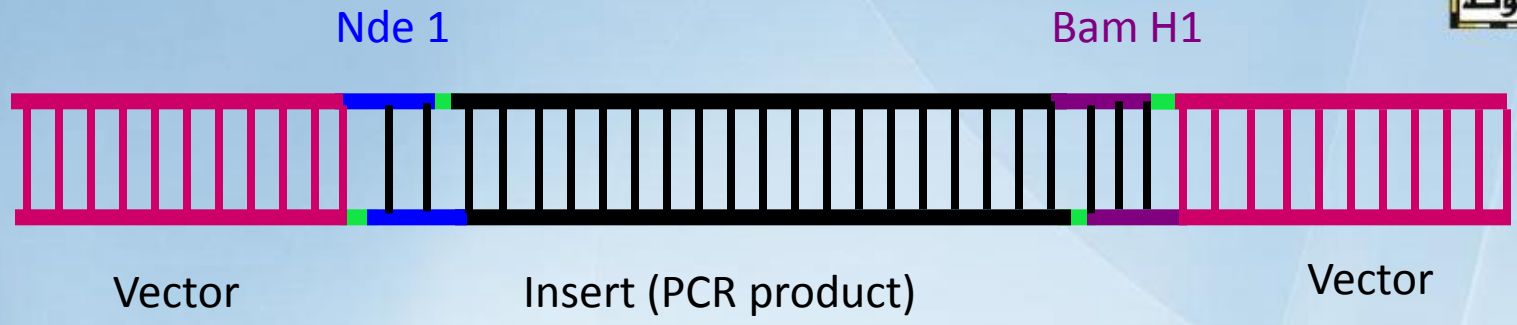
Digest plasmid vector  
with EcoRI enzyme



# Restriction Digestion



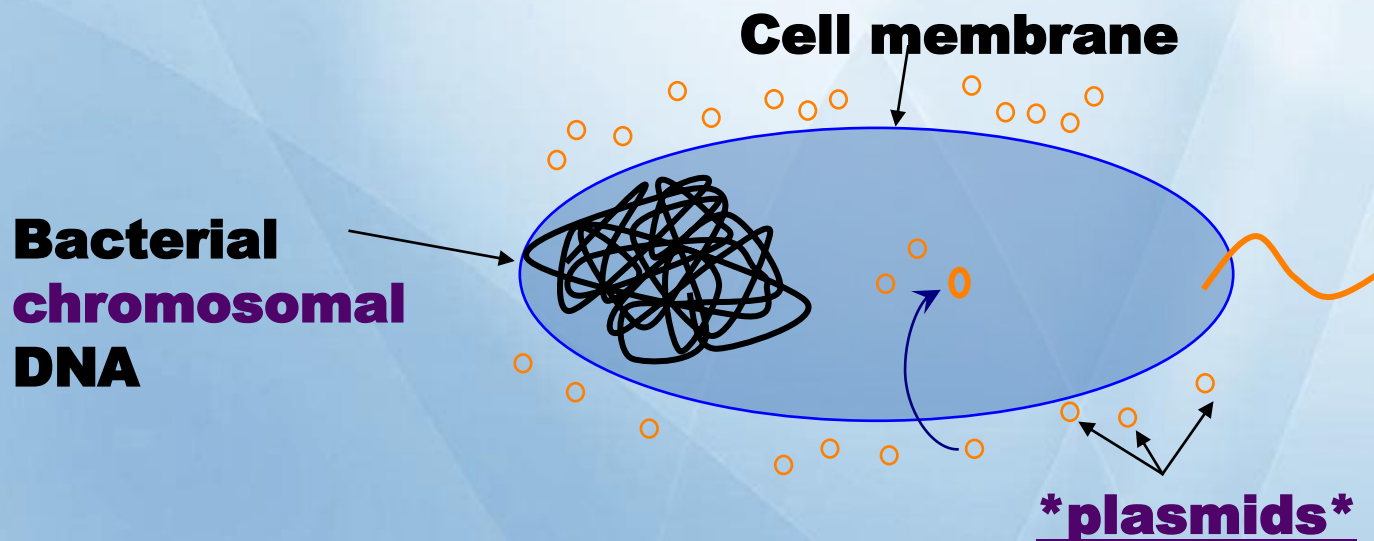
# STEP 3. LIGATION





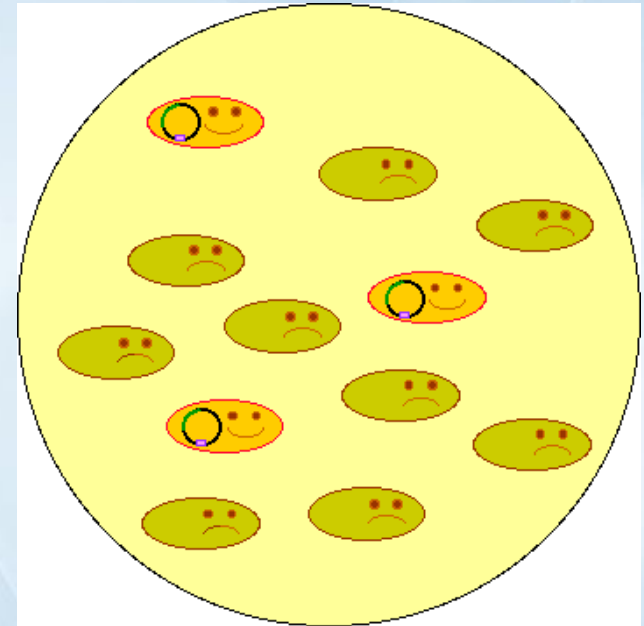
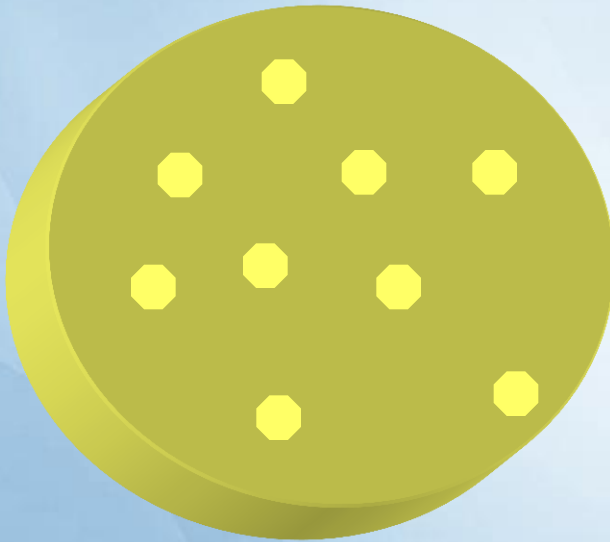
# STEP 4. TRANSFORMATION

- The process of transferring exogenous DNA into cells is call **"transformation"**
- There are basically two general methods:
  - chemical method utilizing  $\text{CaCl}_2$
  - electroporation



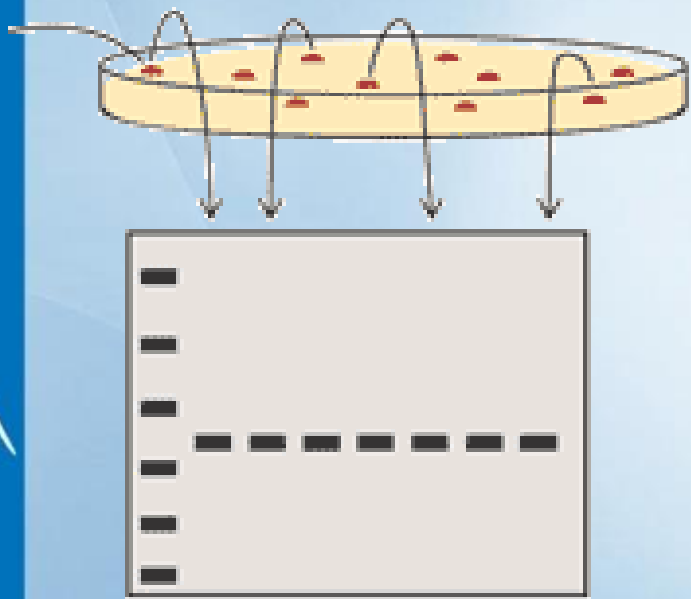
# STEP 5. GROWTH ON AGAR PLATES

Growing Culture



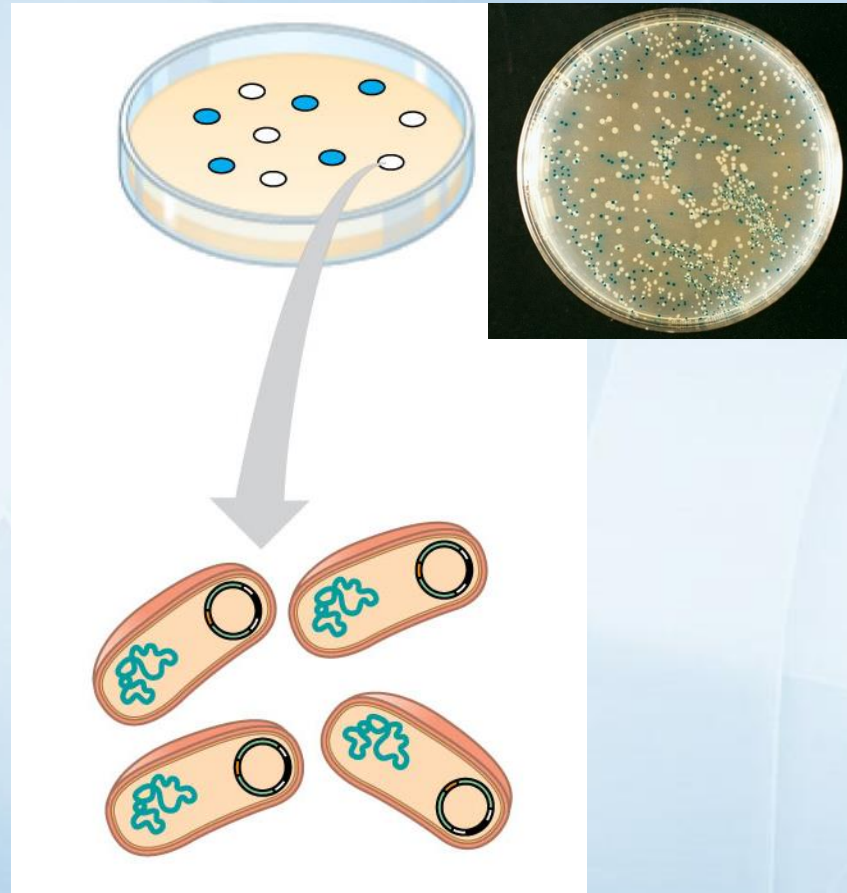
Spread transformed bacterial cells on the LB plate with selection drug and grow overnight.

# Detection of the right cloning



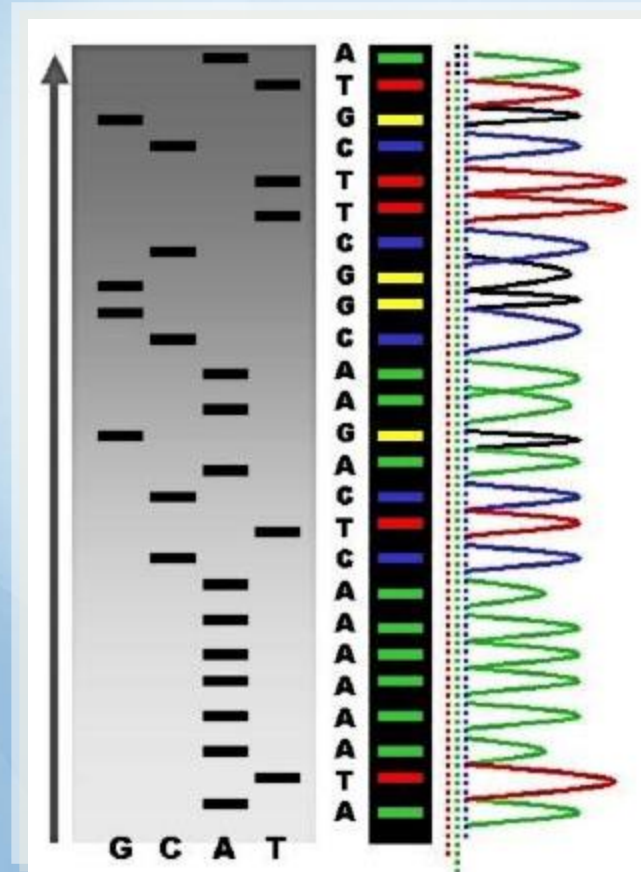
Screen colonies on agarose gel

**Screening with PCR**



**Blue white screening**

# Conformation with DNA Sequencing





# Sequence alignment

Sequence alignment - Wik x Multiple Sequence Alignm x COBALT:Multiple Alignm x

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