

### Gene cloning (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.



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### What is cloning used for?

### Agricultural

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



Crop is infected by European corn borer

Pest dies when feeding on any plant part



## Medical

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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







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- DNA isolation and Target Gene Amplification
- Cut Target Gene and Plasmid
- Ligation
- > Transformation
- Cellular Screening
- > Protein Expression

## STEP 1. DNA isolation and PCR

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# Polymerase Chain Reaction (PCR)





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#### PCR is used to:

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



# Plasmid DNA isolation



- Multi cloning site.
- Selection marker.
- Promoter.









oligo		% cleavage	
	sequence	2h	20h
BamHI	C <u>GGATCC</u> G	10	25
	CG <u>GGATCC</u> CG	>90	>90
	CGC <u>GGATCC</u> GCG	>90	>90
EcoRI	G <u>GAATTC</u> C	>90	>90
	CG <u>GAATTC</u> CG	>90	>90
	CCG <u>GAATTC</u> CGG	>90	>90
HindIII	CAAGCTTG	0	0
	CC <u>AAGCTT</u> GG	0	0
	CCC <u>AAGCTT</u> GGG	10	75
NcoI	C <u>CCATGG</u>	0	0
C	ATG <u>CCATGG</u> CATG	50	75
Ndel GGGT	TTT <u>CATATG</u> AAACCC	0	0
GGAAT	FTC <u>CATATG</u> GAATTCC	75	>90





# STEP 2. DIGESTION











# 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





Screen colonies on agarose gel

#### Screening with PCR



Blue white screening





## Conformation with DNA Sequencing







 We will transform bacteria (<u>E. coli</u>), giving it the ability to produce the Pyocin S5 protein from *Pseudomonas aeruginosa*



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## Primers Amplifying Target DNA

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