Gene cloning
(an overview)

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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.
Bacterial chromosome

Gene inserted into plasmid

Gene of interest

Plasmid put into bacterial cell

DNA of chromosome ("foreign" DNA)

Recombinant bacterium

Host cell grown in culture to form a clone of cells containing the "cloned" gene of interest

Gene of interest

Protein expressed from gene of interest

Protein harvested

Basic research and various applications

Copies of gene

Basic research on gene

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

Basic research on protein
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

<table>
<thead>
<tr>
<th>Protein</th>
<th>Disease/Disorder</th>
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<tbody>
<tr>
<td>Human insulin</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Human Growth Hormone</td>
<td>Deficiency in children</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>Anemia</td>
</tr>
<tr>
<td>DNase I</td>
<td>Cystic fibrosis</td>
</tr>
<tr>
<td>Human antibody blocker</td>
<td>Asthma</td>
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CLONING PROCESS

1. Cut DNA molecules with restriction enzyme to generate complementary sequences on the vector and the fragment.

2. Join vector and chromosomal DNA fragment, using the enzyme DNA ligase.

3. Introduce recombinant DNA molecule into bacterium.

4. Recombinant DNA molecule and bacterial chromosome.
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.
• PCR is used to:
  • Specifically amplify the target gene
  • Introduce the recognition site of the Restriction enzyme
Reverse transcriptase

Produce complementary DNA (cDNA) from an RNA template.
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

Vector (pET 15b)

Selection Marker

Nde 1

Bam H1

Nde 1

Bam H1
STEP 3. LIGATION

Nde 1

Insert (PCR product)

Bam H1

Vector

T7 Promoter

6 His tag

Nde 1

Gene of Interest

Bam H1

Selection Marker
STEP 4. TRANSFORMATION

• The process of transferring exogenous DNA into cells is called "transformation".

• There are basically two general methods:
  • chemical method utilizing CaCl2
  • electroporation
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*
### Primers Amplifying Target DNA

**Cloning primers of Pyocin S5 gene**

<table>
<thead>
<tr>
<th>Primer Sequence</th>
<th>Type</th>
<th>Tm</th>
<th>Restriction Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>GGAATTCCATATGTCACAAGCTGAGTACCTGG</td>
<td>Fw</td>
<td>60</td>
<td>Nde1</td>
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<tr>
<td>CGGGATCCCTTGAAGCTTTAAATACATTTGGGC</td>
<td>Rv</td>
<td>54.8</td>
<td>BamH1</td>
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