BIO 130 – Introduction to Biotechnology

Cloning Genes and Producing Genetically Engineered Proteins
Study Outline

You have learned some basic tools of recombinant DNA technology. In this unit, you will learn how two of those tools, restriction enzyme digestion and gel electrophoresis, can be used to find one gene within an entire chromosome. You will also see how restriction enzymes are used to combine DNA from a bacterium with genes from any other organism. The hybrid DNA, called a recombinant clone, can then be produced in large quantities inside bacterial "factories". In some cases, the cloned gene is produced so that a large amount of it is available for further study. Often, however, the bacteria are used to synthesize large amounts of the protein encoded by the cloned gene. Human insulin is the first example of a protein to be produced by this type of genetic engineering.

I. Finding a Gene – Detecting Specific DNA Sequences

A. How do you locate one gene in an entire genome?
1. *DNA Hybridization:* a technique to find a specific sequence in a DNA molecule that involves "fishing" for it with a small piece of DNA containing its complementary sequence
    a. Complementary DNA is called a *probe*
    b. It is the same idea as a PCR primer – it finds a specific sequence of bases on a large DNA molecule, and pairs
    c. Probe is tagged with a fluorescent dye so it can be seen.
    d. Probe sequence corresponds to a gene of interest, such as an allele that is present in a certain disorder
2. Steps in Process
    a. Target DNA (eg, DNA from a patient) is cut with a restriction enzyme
    b. DNA fragments are separated by gel electrophoresis
    c. A solution containing the labeled probe is added
    d. The probe base pairs with its target in the patient's genome
    e. Probe fluorescence shows the band on the gel that contains the gene you are looking for

II. Cloning a Gene – Making Recombinant DNA

A. A gene can be cut out of the DNA of one organism, and "spliced" into the DNA of another organism
1. *Recombinant DNA:* a hybrid DNA molecule containing DNA from ≥2 organisms

3. Purpose: to produce many copies of a specific gene
    f. many copies are needed to study the gene – determine its DNA sequence, learn about the protein that it codes for, etc
    g. many copies are needed to produce large amounts of the encoded protein

i. use protein for commercial purposes, if it is a useful enzyme or other product
ii. study the protein
iii.

B. Most recombinant DNA involves splicing "foreign" genes into *bacteria*, such as specialized lab varieties of *E. coli*
1. Bacteria are easy and inexpensive to manipulate in a lab or factory
2. Bacteria grow quickly, so they produce many copies of the desired gene in a short time

C. Bacteria cannot take in foreign genes directly – the DNA would be destroyed when it entered the bacterial cell

D. Foreign DNA is carried into a bacterial cell by a DNA transporter called a *plasmid vector*

1. *plasmid vector:* a small loop of DNA naturally found in bacterial cells
   i. bacteria use DNA replication to make many copies of these plasmids

2. genetic engineers have modified these natural plasmids so that they will work as DNA "ferries" for carrying foreign DNA into bacteria
   i. can be purchased from molecular biology supply companies
   ii. contain a gene that encodes a protein to make bacteria survive in the presence of an antibiotic, such as ampicillin
      a. bacteria are not killed by the antibiotic – become *antibiotic resistant*
      b. only bacteria containing this plasmid will survive in the presence of antibiotic

E. Process of making a recombinant plasmid

1. Use a restriction enzyme (eg, EcoR I) to cut the gene that you want to clone out of its chromosome
   example: a banana gene that encodes an interesting protein called "yummase"

2. Use the same restriction enzyme to cut the plasmid, so that the DNA loop opens up into a linear chain

   iii. Mix the cut up banana DNA with the linear plasmid
   a. The "sticky ends" of the EcoR I cut banana DNA and plasmid join together
   b. Add an enzyme called *DNA ligase* to glue together the DNA fragments
      i. now you have a larger loop of DNA
      ii. it contains the original plasmid DNA with the banana gene spliced in
      iii.

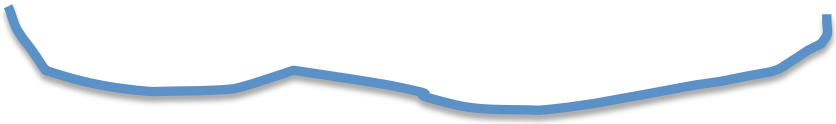
banana DNA **AATTC**TAATAGTTAAACTTTT**G**
GATTATCAATTTGAAAA**CTTAA**

+

plasmid DNA ACTTTT**G** **AATTC**TAATAG
TGAAAA**CTTAA** **G**ATTATC

=

recombinant DNA ACTTTT**G** **AATTC**TAATAGTTAAACTTTT**G** **AATTC**TAATAG
TGAAAA**CTTAA** **G**ATTATCAATTTGAAAA**CTTAA** **G**ATTATC



Please check out the diagram of cloning a gene into a plasmid vector using a restriction enzyme.

F. Process of transferring a recombinant plasmid into *E. coli*
(called *bacterial transformation)*

1. Mix recombinant plasmid with *E. coli* cells

2. Treat the mixture (using heat and calcium) so that the cell membrane allows the plasmid to pass into the cell

3. Allow the bacteria to grow in solid medium(food) containing an antibiotic
   i. bacteria that took in the plasmid will survive and grow– all others will die, because the antibiotic will kill them (they are not antibiotic resistant)
   ii. As bacteria grow, they make many copies of the plasmid
   ii. as individual bacteria duplicate themselves, they grow in piles called colonies
   iii. each colony consists of millions of bacterial cells, each containing hundreds of recombinant plasmids
4. Purify recombinant plasmids from the bacterial colonies for further use

II. Genetically Engineered Proteins

A. When a plasmid containing a foreign gene is introduced into bacteria, the bacterial protein synthesis machinery (called transcription and translation, remember?) will often synthesize the protein encoded by the foreign gene

   (eg, the banana protein "yummase" will be produced in *E. coli*)

B. The foreign protein accumulates in the bacterial cells, and can be purified for further use

C. The details involved in getting a functional protein produced can be complicated, but the idea is simple – bacteria act as a factory to produce large amounts of a protein that they normally do not synthesize

D. Genetic engineers sometimes change the foreign gene (by mutating its DNA sequence) in order to change the function of the protein produced
   1. example: changing the DNA sequence to make the resulting enzyme work better at higher temperature

E. *Insulin* was the first major human protein to be genetically engineered into bacteria, and then produced for commercial use
   1. Diabetics now use human insulin engineered in microbes
   2. Prior to genetic engineering, insulin for diabetics had to be purified from the pancreases of cows or pigs

Please check out the animations and video interviews about genetically-engineered insulin, including a tour of a facility that produces insulin commercially