Lecture 3 (FW)
January 28, 2009
Cloning of DNA; PCR amplification
Reading assignment: Cloning, 240-245; 286-87; 330 PCR, 270-274; 329.

Take Home Lesson(s) from Lecture 2:

1. DNA is a double helix of complementary strands held together by A-T and G-C hdrogen bonds.
2. Cellular DNA is located in chromatin (which become chromosomes during mitosis) in the nucleus, and is faithfully replicated and distributed in the cell cycle and mitosis.

Lecture 3. Cloning and PCR. The primary goal: understanding how knowledge of DNA structure allows two powerful new technologies, molecular cloning and PCR.

I. Cloning; What is a **clone**?

A. Webster. 1) genetically identical cells descended from a single common ancestor, or 2) one or more organisms descended asexually from a single common ancestor, or 3) an exact replica.

B. Cloning , now used loosely in popular press to mean "making a clone". The term clone is used to cover DNA molecules, cells, stem cells, or even an individual organism.

II. Molecular Clones

A. Viruses are clones of DNA (or sometimes RNA) molecules that use cellular machinery for asexual reproduction of multiple copies.

1. A virus-like DNA called a **plasmid** can "infect " bacteria. They have very few genes, but sometimes confer interesting properties on the host bacterium, especially drug resistance.

B. Viruses and plasmids can be used as carriers (**vectors**) of exogenous DNA sequences of interest.

Genetic engineering uses plasmids (or viruses) recombined with other DNA sequences to produce clones of DNA molecules, which are then replicated in bacteria (or other kinds of host cells). This is the basis for the biotech industry.

1. Many copies of DNA can be produced. This can be used for gene therapy, or production of useful pharmaceuticals.

When you want to deliver DNA to an organism you have to take the cloned DNA you want and put it in a carrier , or vector, that can infect the cells of interest , yet not cause a disease or other unintended consequences.

2. For commercial purposes, you can use the bacteria (or other types of cells) that have been transformed with the recombinant plasmid or virus to produce gobs of the protein encoded by the recombinant DNA sequence.

a. For instance, the first commercial application of this technology led to production of insulin.

C. The way DNA is cloned is:

1. Produce a double stranded DNA copy of the sequence you want to clone.

a. It helps if the ends of the sequence have features that make it easy to attach to the vector DNA. One way to due this is to somehow utilize (or attach) the recognition sequence for a **restriction enzyme**.

b. A restriction enzyme is an enzyme that recognizes a specific base sequence (usually 6 base pairs long), and cuts both strands at a specific place. For example, the sequence CTTAAG is cut between the G and A in both strands.
GAATTC

2. Prepare vector DNA, a bacterial plasmid for example. Open the circular DNA so that it has ends" compatible" with the insert DNA. This could be done using the same restriction enzyme. Then mix Vector and Insert, and the compatible ends will form double stranded bridges, and these can be permanently sealed by using an enzyme called "ligase". Do you understand why the ends of the vector and insert are "compatible", i.e., why they stick to one another?

[A problem: A piece of DNA was cut by a restriction enzyme to produce a piece of DNA with the following sequence:

GGTTAACC.....(98 nucleotides)....TATACCCTTAA (watson)
AATTCCAATTGG.....(98 nucleotides)....ATATGGG (crick)

What would be the "sticky" ends that would have to exist in the cut vector to permit easy cloning of this piece of DNA?]

3. Use this "recombinant" DNA to transform bacteria. If the plasmid confers drug resistance, then bacteria containing the recombinant DNA can be grown in the presence of antibiotic. The drug is a used for genetic **selection**.

4. It's even possible for the host organism to produce protein from this recombinant DNA. You have used the host as a "factory" to produce a protein of interest.

II. Polymerase Chain Reaction (**PCR**)

A. There is one more thing you need to know about the double helix structure of DNA. Each DNA strand has a front and a back end, called 5' and 3'. Each strand has a polarity. Each strand runs opposite to its **complementary strand**.

B. In the late 1980's, in Berkeley, Mullis and his colleagues devised the PCR technique. The technique consists in repeating, over and over again, the same three steps.

1. Heat the DNA, thereby separating the two strands

2. Allow short (~ 20 nucleotides) chemically synthesized "primers" to bind to complementary sequences of DNA. Also add precursors of DNA, i.e., deoxynucleotides.

3. Add **DNA polymerase** so that the complement of each strand is synthesized. Heat stable DNA polymerase is now used so that new enzyme does not have to be added for each round.

C. The DNA sequence between the **primers** is thus amplified. After 10 rounds it is amplified 1000 times, after 20 rounds a million.

Very, very small samples of DNA can then be amplified by PCR, and the amplified material can be used to characterize the DNA.

For example, DNA from crime scenes, from small tissue samples for diagnosis, and from historical samples can be used to identify the origin of the DNA

1. Is there an issue of genetic privacy here? Sometimes whole communities have been asked to donate their DNA to help identify a miscreant.

Words to know: clone, plasmid, vector, selection, PCR, complementary strand of DNA, DNA polymerase, primer.

Take Home Message: The structure of DNA provides a technological foot-hold to encourage unlimited synthesis by recombinant DNA technology or PCR.

Next Reading Assignment: pp.115, 123, 129-133 , 138, 140,
Or, easier, just read chapters 8 and 9 for the next two lectures.

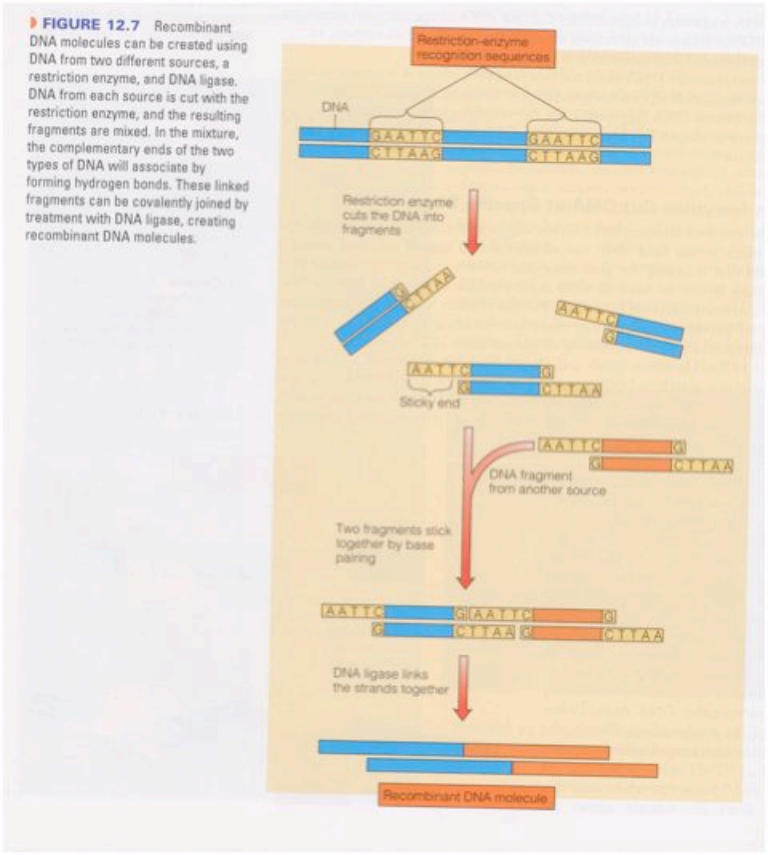

FIGURE 12.7 Recombinant DNA molecules can be created using DNA from two different sources, a restriction enzyme, and DNA ligase. DNA from each source is cut with the restriction enzyme, and the resulting fragments are mixed. In the mixture, the complementary ends of the two types of DNA will associate by forming hydrogen bonds. These linked fragments can be covalently joined by treatment with DNA ligase, creating recombinant DNA molecules.

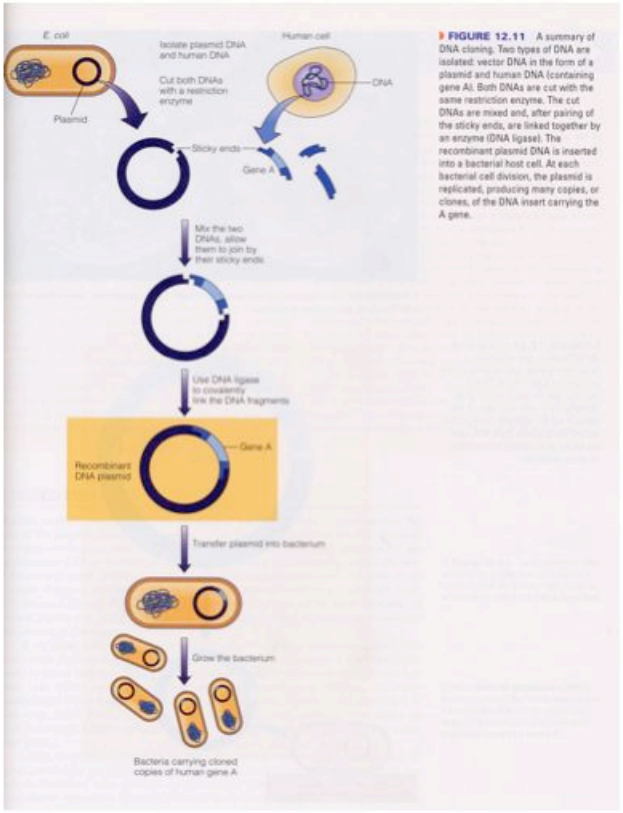

FIGURE 12.11 A summary of DNA cloning. Two types of DNA are isolated: vector DNA in the form of a plasmid and human DNA (containing gene A). Both DNAs are cut with the same restriction enzyme. The cut DNAs are mixed and, after pairing of the sticky ends, are linked together by an enzyme (DNA ligase). The recombinant plasmid DNA is inserted into a bacterial host cell. At each bacterial cell division, the plasmid is replicated, producing many copies, or clones, of the DNA insert carrying the A gene.

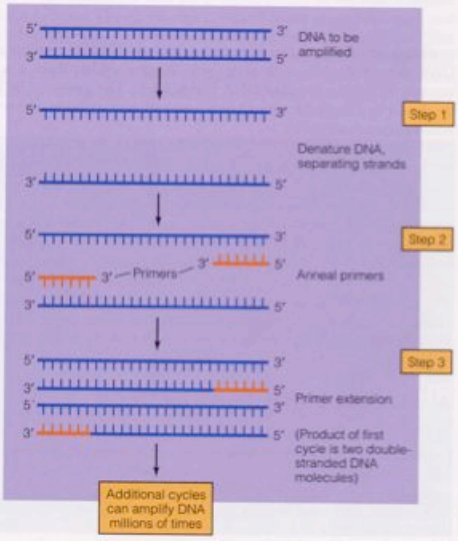

FIGURE 12.15 The polymerase chain reaction (PCR). In step 1, the DNA to be amplified is converted into single strands. In step 2, short single-stranded primers pair with the nucleotides adjacent to the region to be amplified. These primers are made synthetically and are complementary to the nucleotide sequence that flanks the region to be amplified. In step 3, enzymes and nucleotides for DNA synthesis are added, and the primers are extended, forming a double-stranded DNA molecule. The primers are incorporated into the newly synthesized strand. This series of three steps is called a cycle. In the second cycle, the double-stranded molecules produced in the first round of PCR are denatured to form single strands, which are templates for primer binding and another round of DNA synthesis. Repeated cycles can amplify the original DNA sequence by more than a million times.