Objectives for Exam 4

Genetics, Spring 2012

Chapter on Recombinant DNA – Chapter 17

- *Be able to define the term recombinant DNA.*
  - **recombinant DNA-** joining together DNA sequences from different organisms (sources) in a test tube
    - not found in nature- usually produced by artificial means
    - How it works:
    - 1. DNA to be cloned is purified from cells or tissues
    - 2. Proteins called **restriction enzymes** are used to generate specific DNA fragments. These molecules recognize and cut DNA molecules at specific nucleotides
    - 3. Fragments produced by the restriction enzymes are joined to other DNA molecules that serve as **vectors (plasmids)**, or carrier molecules. A vector joined to a DNA fragment is a recombinant DNA molecule.
    - 4. The recombinant DNA molecule is transferred to a host cell. Within the host cell, the recombinant molecule replicates, producing identical copies (clones) of the recombinant molecule.
    - 5. As the host cell replicates, the recombinant molecules within them are passed on to all their progeny, creating a population of host cells, each of which carries copies of the cloned DNA sequence
    - 6. The cloned DNA can be recovered from host cells, purified, and analyzed
    - 7. The cloned DNA can also be transcribed, its mRNA translated, and the encoded gene product isolated and used for research or commercial purposes.
- *Know what restriction enzymes do, what organisms have them, and what a palindromic DNA sequence is.* **(Lect. 30, Slides 5-6)**
  - **restriction enzymes (endonucleases)-** cut the DNA in a sequence specific manner; recognize and bind to specific nucleotide sequences
  - the 'recognition sites' are **Palindromic sequences**

        - can cleave the specific sequence with the restriction enzyme on the organism DNA and the vector DNA.
            - now you have fragments with complementary single stranded sequences.
            - These single stranded sequences can now anneal (or stick together via complementary base pairing) to form a recombinant DNA
            - DNA ligase seals the gaps
    - restriction enzymes are produced by bacteria as a defense mechanism against viral infection by degrading the DNA of invading viruses
- *Be able to do restriction enzyme mapping problems like the ones in the homework assignment.*
    - I found this link, which helped me better understand how to do the restriction mapping problems, it has some practice problems as well:
    - http://people.rit.edu/rhrsbi/GEPages/LabManualPDF5ed/16%20mapping.pdf
- *Be able to describe how restriction enzymes are used in cloning DNA.*
    - ex:
        - 1. The DNA to be cloned is isolated and treated with a restriction enzyme to create fragments ending in specific single-stranded tails.
        - 2. The fragments are then linked to plasmid molecules that have been cut with the same restriction enzyme, creating a collection of recombinant vectors.
        - 3. The recombinant vectors are transferred into E. coli host cells. Inside of the host cell, a vector replicates to form many clones, or clones.
        - 4. The bacteria are plated on nutrient medium, where they form colonies.
        - 5. The colonies are screened to identify those that have taken up recombinant plasmids.
- *Know what a cloning "vector" is, and why different vectors (plasmid, lambda, and BAC vectors) are used in different situations.*
    - **Vectors** (carrier DNA molecule) transfer and help replicated inserted DNA fragments.
        - the fragments produced by restriction enzymes must be joined to a vector before they can be inserted in a host cell

    - The first vectors were modified plasmids (dbl. stranded, extrachromosomal DNA found in certain bacterial strains)
- Many different vectors are used for cloning--they differ in which host cells they are able to enter, the size of the inserts they can carry, and other properties
- Vectors must:
    - replicated independently once inside the host cell (along with the DNA fragment it carriers)
    - contain several restriction-enzyme cleavage sites that allow insertion of the DNA fragments that are to be cloned
    - carry a selectable marker gene to identify host cells that contain recombinant vectors (usually an antibiotic resistance gene)
    - Types:
        - **standard plasmids-** inserts less than 10 kbp
        - **Bacteriophage vectors-** inserts 10-50 kbp
        - **BACs-** inserts 100 kbp
        - **YACs-** inserts 1000 kbp
- *Know the difference between a genomic library and a cDNA library, what each of these libraries are used for.*
    - **library-** collection of clones
    - **genomic libraries-** cloned fragments of DNA isolated from chromosomes
        - used for cloning regulatory sequences (promoters), studying genome organization, sequencing of whole genomes
    - **cDNA libraries-** cloned DNA copies of mRNA isolated from cells or tissues
        - used for isolating expressed genes
        - cDNA = 'complementary DNA' made from mRNA
            - synthesis of cDNA uses reverse transcriptase to make a DNA copy of mRNA
- *Be able to explain how mRNA is separated from bulk RNA in the cell, and how double stranded cDNA is made from mRNA.*