

BERG/STRYER VI STUDY GUIDE
CHAPTER 5

1. HOMEWORK 1, 2, 3, 7. Look at the list under 5.1, page 135. We will briefly discuss each of these items except #4. **Restriction cleavage enzymes** are made by bacteria as a defense mechanism against viruses. Each enzyme has a "palindromic" target sequence. The most useful enzymes make "lap joint" cuts. See EcoRI, BamH1, etc. in Fig 5.1. Using a restriction enzyme to cleave DNA leads to fragments of various random lengths. After separation by size in an agarose gel, **Southern Blotting** (Fig 5.3) transfers and "glues" the fragments onto nitrocellulose sheets. This is useful in looking for a particular gene, and also for comparing DNA from different individuals. Read about **RFLP** (box, page 137), used in forensic analysis by law enforcement workers (Fig 5.9). The **Sanger Dideoxy method** of DNA sequencing is easy to understand if you know that you are growing random-length fragments of DNA. In a given test tube, all the growing fragments begin at exactly the same spot, and all end at the same nucleotide (say, "A"). Then a size separation shows how far from the beginning each "A" is. Repeating the process with C, T, and G gives the entire sequence. Note that we are skipping solid phase DNA synthesis – but you should know that it is now easy to synthesize any reasonably short sequence of DNA. This is how radioactive DNA probes are constructed. Finally, **PCR or Polymerase Chain Reaction** provides a powerful way to increase the amount of DNA available from some source. The use of DNA Pol from "**Taq**" or *Thermus Aquaticus* allows DNA to be polymerized at high temperatures. When the newly synthesized DNA is heated a bit more, the double stranded chains separate, and primers attach for another round of synthesis. Each round of PCR doubles the amount of DNA between the primers, so there is a million times more after 20 cycles and a billion times more after 30 cycles. Cycling is easy because the process can be run in a machine without human intervention. Obviously one must guard against contamination from any source of outside DNA.

2. The manipulation of DNA with modern techniques is conceptually very simple. Any two pieces of DNA that were cut with the same (lap-joint) restriction enzyme will have mutually "**sticky ends**" – see 143. Thus genes can easily be inserted into a "**vector**" which will carry them into cells. The vector can be a **plasmid** (know properties of **pBR322**, Fig 5.12) or a **virus** (see phage lambda). The *amp* and *tet* resistance genes in pBR322 allow for selection of host cells. Understand Fig 5.19 – if you wrote out 256 different DNA "**oligos**" you could cover every possible DNA sequence corresponding to the amino acid sequence shown. With modern techniques, synthesizing all of these (as radioactive probes) is a simple task. Thus if you know that sequence is in an enzyme you want to know about, and you cut up the DNA from the organism that makes that enzyme, and do a Southern Blot followed by a wash with your set of oligos, you will locate the correct sequence, and then you have the gene.

3. Knowledge of the genome allows scientists to construct "gene chips" which can sample the mRNA currently present in a cell. Oligos of cDNA are attached to chips, and if the mRNA is present it sticks to the DNA. To understand the diagram of these chips it helps to remember "Green means STOP and red means GO" – in other words genes being expressed show up red and genes which are repressed are green. Thus we can see what a given cell is doing in response to ambient conditions. The ability to synthesize any given DNA sequence also leads to gene knockout technology. A gene sequence is altered in some fatal way, so that the gene can not be expressed. The altered gene is put into the target genome using homologous recombination. Selective genes can be used just as with plasmids in bacterial cells, except here the target cells are mouse embryonic stem cells. When the mice grow up, they lack the affected gene.