Chapter 20 Reading Guide

Try to read 20.1 and 20.2 for the general sense of it. Here's what you need to know:

Genetic engineering and Synthetic Biology are based on two principles:

- complimentary base pairing
- universality of the genetic code--the same three bases code for the same amino acid in any organism

Think of this as our engineering workbench

Various enzymes that come from living organisms are just as happy to work in a test tube as they are in a cell. These are our tools:

- **restriction enzymes**
- **DNA polymerase**
- **reverse transcriptase***
- **DNA ligase**

*This is an enzyme taken from particular viruses that use RNA as their genetic material. When the virus infects a host cell, the host cell's ribosomes translate the RNA to make the reverse transcriptase enzyme. The enzyme then *reverse transcribes* the viral RNA into DNA. Now the virus genes are in a form that the host cell cannot distinguish from its own. The host goes to work and transcribes and translates the *viral* DNA. In most cases this results in serious disruption of the normal function of the host cell (causing symptoms, possibly fatal) and reproduction of the virus.

Recombinant DNA

This term refers to synthetically cutting and pasting DNA from two different organisms. See Fig. 20.2 and Fig. 20.4.

In the Color My World lab, you will be using two recombinant plasmids: one contains genes to make a purple pigment, one contains genes to make a green pigment. These two plasmids are named pPRL and pGRN, respectively.

RFLP analysis

This is related to our sickle cell lab. Please review the animation. Remember, we simulated protein electrophoresis. This is DNA electrophoresis.
http://bcs.whfreeman.com/thelifewire/content/chp17/1702001.html

Fig 20.11 gives you an explanation of the use of a "probe". You might wonder, "Where does the probe come from?" The probe is synthetic. There are a couple of way to make them:

1) You can use **reverse transcriptase**. Take some cells that you know are making a ton of the gene product you are interested in. They will have a ton of mRNA for that gene accumulated inside. Break up the cells, purify the mRNA , add a primer, lots of nucleotides and reverse transcriptase to the mix. The enzyme will go to work and make DNA for you. Make sure some of the nucleotides have a fluorescent or radioactive tag, and now you have a probe! See Fig. 20.6

Sometimes the mRNA itself can be used as a probe, but you still have to tag it and due to the fact that the DNA still contains introns, the mRNA might not be a good enough match.

2) You can use **PCR** (fig 20.8). You have to know something about your gene to make the primers. Sometimes it's enough to know the amino acid sequence of the protein and work backwards to the genetic code. There will be some mistakes due to "wobble," but you might get lucky. Anyway, once you decide on the sequence of your primers, you go on line, order them and wait for the FedEx delivery. Seriously.

If you're underwater right now, that's okay. Just know that probes are synthetic pieces of DNA that are used to make visible the DNA fragments in a gel that contain the gene you are interested in.

- Take a look at Fig 20.10. This assumes that you are loading only DNA that contains your gene, not DNA fragments cut up from the whole genome. That is why there is no probe used. The DNA just needs to be stained to be made visible like the glowing bands you see in Fig. 20.9
- You can get DNA that contains only the gene you are studying by using **PCR** (see fig 20.8)

If you're hooked and you're going for a 4 or 5 on the AP, here's more...

DNA sequencing

We take for granted now that the sequence of the human genome is known. When this work was being done in the 1980's and 1990's it was done by human hands. (I was a lab tech in one of these labs at Washington University during one of my summer jobs in college. My job was to wash the beakers and make reagent solutions and boring stuff like that, but I had lunch with the people who were doing the cool stuff!)

Fig 20.12 shows the chemistry behind automated DNA sequencing. It is important to understand that this method only works for moderately long pieces of DNA. You cannot sequence the whole genome of an organism by this method. The genomic DNA has to be cut into fragments (with restriction enzymes) and sequenced piece by piece. There is still quite a bit of work to do to reconstruct how the pieces fit together to make up the chromosomes of the organism. Today this is largely done by computer software, not lab scientists. There is at least as much work for programmers in the field of biotechnology today as there is for lab scientists.

DNA microarrays
This is one of the most powerful tools used in molecular biology today. Take a look at Fig 20.15.

This technique can be used to answer very deep questions like: is this cancer cell acting like a stem cell?

- prepare a microarray with fragments of genomic DNA in each spot (this is a highly automated process)
- apply cDNA from the cancer cell and cDNA from a stem cell (see **reverse transcriptase,** above)
- label the cDNA with flourescent tags
    - green--cancer
    - red--stem cell
- yellow spots will identify DNA that is being actively expressed in both cell types

If this were a course in biotechnology, I would also present some of the techniques desribed in chapter 21. Read it for fun, if you wish. Most of these topics are beyond the reach of our curriculum.