Bio 181 Final Exam Review

I dug up these questions from 2 years ago. This is not intended to be a thorough review for the exam, but rather a list of questions you should understand or be able to answer as you prepare for Monday.

1. Thermal cycling for PCR has several key variables, in particular annealing temperature and number of cycles of amplification. For our phage DNA PCR, we used only about 20 cycles; for RT-PCR, we used more than 30 cycles. What is the main advantage of using many cycles (why did we do it)? Answer: more cycles=more DNA molecules made, so you can get a large yield from very small starting material (as in RT-PCR, where any single specific mRNA species will be quite rare). What is a major disadvantage? (Taq fidelity is poor, more amplification = greater chance of sequence errors)

2. For reverse transcription of mRNA, name 3 kinds of primers you can use.

3. You wish to use sticky end ligation to clone a Taq-produced PCR product. What general approach to cohesive/sticky end ligation works for all such products, regardless of sequence?

4. You specifically wish to clone a PCR product into a BamHI site in your vector. There is no BamHI site in the sequence you will amplify. How can you do this?

5. Topoisomerase I can make single strand DNA breaks, and then ligate them back together. These two activities are used in the TOPO-TA cloning system.

A. Why *Vaccinia* topoisomerase?

B. Why is the ligation step in this system so much faster and more efficient than with regular DNA ligase?

C. Ligation products are transformed into bacteria and grown on ampicillin + x-gal plates. What color colonies should you pick to screen for your insert?

6. The following are steps in a standard cloning experiment. Put them in the proper order.

Ligate
Miniprep & restriction enzyme digest
Pick colonies for overnight growth in broth culture
Restriction digests of vector and DNA insert
Screen for insert by observing bands on agarose
Transform competent cells

7. Describe how Sanger DNA sequencing works (ddNTP method).

8. Describe how using fluorescently labeled ddNTPs of different colors allows automated reading of sequencing gels.

9. Describe how to label a probe using random hexamer oligonucleotides.

10. Shotgun Sequencing:

A. How can you use just 2 universal primers (forward and reverse) to sequence a HUGE amount of DNA (say, 1,000,000 bp) when sequence in only readable a few hundred basepairs out from the primer?

B. What is a contig? How do you fill gaps?

11. Southern Blotting:

A. Summarize key steps and purpose of each:

Digest. Run. Denature. Blot/transfer. Bake. Prehybridize. Hybridize. Wash. Detect.

B Restriction enzyme digestion of **genomic** DNA for a Southern blot looks like a bright smear in an agarose gel. Why?

Why denature the DNA in the gel before transfer?

Why is nylon membrane baked after DNA transfer?

Why re-block the membrane after washes before adding detection reagents?

Why wash at all? What would happen if you didn't wash the membrane after adding probe?

Describe how you can use PCR to make a labeled probe.

Why can't you directly blunt ligate a Taq PCR product?

How does topoisomerase become covalently linked to DNA?

12. Describe a direct and an indirect method of probe detection.

13. How can you detect a probe labeled with biotin?

14. How could you use a microarray to identify the specific mutations present in a cancer cell?

Be able to read DNA sequence from an old-fashioned autoradiogram (xray film) with 4 separate reactions run side by side on polyacrylamide; and also read a chromatogram from automated sequencing.

Given the predicted and actual results of a Southern blot, explain how to solve various problems with the experiment.

Given a probe, predict which bands will light up on Southern; given a Southern, indicate where the probe must be from.

Understand red/green/yellow type microarray data.