Chapter 10 (Part II)
Gene Isolation and Manipulation

Practice Questions:

Answer the following questions with one or two sentences.

1. What does PCR stand for?
2. What does the term “template” mean in PCR?
3. What does the term “target sequence” mean in PCR?
4. What is the name of the machine you do PCR in?
5. What type of DNA Polymerase is used in PCR?
6. From which type of organism was the DNA Polymerase used in PCR originally isolated?
7. What is a PCR Primer?
8. What is a “20mer” PCR Primer? What is a 15mer?
9. What are the two main applications of PCR?
10. What is a “PCR cycle?”
11. Approximately how many cycles are normally used in a standard PCR reaction?
12. What are the three steps involved in a PCR cycle?
13. What are the components of a PCR reaction? (List the things you have to mix together.)
14. What does the term PCR “melting” or “denaturation” step mean?
15. What is the term PCR “annealing” step mean?
16. What does the term PCR “extension” step mean?
17. What is normally the temperature of the denaturation step in PCR?
18. What is the approximate temperature of the annealing step in PCR? (Note, I say “approximate” because it will vary depending on the length of the matching sequence between the primers and the template.)
19. Approximately how many “cycles” are used in a typical PCR reaction?
20. In PCR, what does the term “ramping” refer to?
21. What is the approximate “processivity” rate for Taq Polymerase? (How many nucleotides will it add per minute?) Hint: the answer is listed below.
22. In PCR primers, what is a “hook,” or a “hooked” PCR primer?
23. Which solid matrix is used in DNA gel electrophoresis?
24. When you are running a DNA gel, do you use agar or agarose to make the gel?
25. When you are making bacterial plates (ie-to do a blue/white screen) do you use agar or agarose?
26. When you are running a DNA gel, which electrical pole will the DNA run to?
27. (Electrophoresis question) What is an anion, and what is an anode?
28. (Electrophoresis question) What is a cation, and what is a cathode?
29. Is DNA positively charged or negatively charged at neutral pH?
30. Is DNA a cation or an anion at neutral pH?
31. What is “neutral pH?”
32. What kind of a macromolecule is agarose?
33. What is Ethidium Bromide (EtBr) used for?
34. In DNA agarose gel electrophoresis, what does it mean when you say you are going to “excise” certain “bands” from a gel?

Be able to answer the following questions in one or two paragraphs:
1. What is “DNA Fingerprinting”?
2. What is “DNA Barcoding”?
3. What does the term “stringency” mean when you are talking about the annealing step of a PCR reaction?
4. What would happen in a PCR reaction if you used an annealing temperature that was too high?
5. What would happen in a PCR reaction if you used an annealing temperature that was too low?
6. In PCR, what is a “hook” or a “hooked primer,” and what is it usually used for?
7. What is a “DNA Ladder” and how is it used in DNA gel electrophoresis?
8. What are “molecular weight standards,” and how are they used in DNA gel electrophoresis?
9. In DNA agarose gel electrophoresis, what are “bands,” and what is the purpose of “excising” certain bands from a gel?
10. What is the approximate resolution limit of an DNA agarose gel?
11. Which type of gel electrophoresis system would you use to separate very large DNA fragments (100kb or larger)?
12. Which type of gel electrophoresis system would you use to separate very small DNA fragments (50bp to 200bp)?
13. Which type of gel electrophoresis system would you use to separate DNA fragments between about 0.5kb and 15kb in size?
14. What concentration of agarose would you use in a DNA gel if you were interested in separating or analyzing fragments that are between about 0.5kb and 2kb in size? (0.1, 0.5, or 2.5%)
15. What concentration of agarose would you use to separate or analyze DNA fragments that are between about 10kb and 20kb in size? (0.1, 0.5, or 2.5%)
16. Explain how varying the concentration of agarose in a DNA agarose gel can help you to separate (but not resolve) DNA fragments of different sizes.
17. Explain how PFGE works.

Be able to explain the following in one hand-written page or less:
1. Explain how PCR is done.
2. Explain how you could use PCR to put restriction sites onto a DNA fragment (restriction sites that weren’t there before), and then clone it into a vector?
3. You have identified and studied a very important gene in the model organism Drosophila melanogaster (fruit flies). Explain how you could use “low stringency” PCR to find out if any similar genes exist in humans.
4. Explain how DNA gel electrophoresis is done.
5. Explain how different types of gel electrophoresis can be used to separate DNA fragments of different sizes.
6. Explain how varying the concentration of agarose in an agarose gel can help you to better separate DNA fragments of different sizes.

**PCR Problems:**

**One PCR cycle:** 95°C melting, 55°C annealing, 72°C extension.

**Primer Annealing Temperature:** \[ T_m (°C) = 2(nA+nT) + 4(nG+nC) \]

(Yes, unfortunately you must memorize the annealing temp. formula. Sorry!)

**Problem 1:**

Part A) Calculate the annealing temperature of the two PCR primers that will anneal to this template.

5'-ACTTCGACTGGCG(5000bp)GCTTACGTAGCTTAGGCT-3’
3’-TGAAGCTGACCAC(5000bp)CGAATGCATCGAATCCGA-5

Part B) Assuming the region of DNA you wish to amplify is 5kb in size, calculate the time that one PCR cycle will take. Assume the Taq polymerase adds **3000 bp per minute**. Assume that the melting step takes 30s, the annealing step takes 30s, and the PCR thermal cycler takes 30s to change from one temperature to the next.

How long will ONE PCR cycle take?
How long will 35 PCR cycles take?

**Problem 2:**

The following is the secretion peptide from *C. botulinum*. You want to clone this into an expression vector, so that you can fuse other genes to it, and create recombinant proteins that will be secreted out of the expression host. Use PCR (with hooked primers) to incorporate an NcoI site onto the 5’ end of the secretion peptide, and a SpeI site on the 3’end. Design the primers, and calculate the annealing temperature of the primers. Each primer should have ten bases that actually hybridize to the template, and also incorporate the restriction site. In order to incorporate the NcoI site so that the ATG is “in frame” with the rest of the secretion peptide, you may have to change the first amino acid. Use the genetic code chart to determine what the amino terminus of the recombinant secretion peptide will look like compared to the Wild-Type sequence.

Sequence of secretion peptide:

5’-CGTATGACCGTACGCCG..(1000bp).....
..CCGTTAGCACCCTACAGUAACCT-3’

NcoI: CCATGG
SpeI: ACTAGT
**Gel Electrophoresis Problem:**

Observe the two plasmid maps labeled A and B. Plasmid A is an expression vector containing a secretion peptide. Plasmid B is a cDNA clone which contains the FGFR gene (Fibroblast Growth Factor Receptor) as well as some of its 3’UTR. The FGFR gene is contained in an 8kb EcoRI-HindIII fragment, and the 5kb UTR is contained between the two HindIII sites. Your job is to cut out the FGFR gene as an 8kb EcoRI-HindIII fragment, and insert it into the expression vector downstream of the secretion peptide. You can do this by doing a “double digest” of both plasmids with EcoRI-HindIII, and “excising” (cutting out) the correct fragments from an agarose gel, and then ligating them together.

The agarose DNA gel is shown below. Lanes 1 and 2 are what you see when you cut both plasmids with just HindIII. Lanes 3 and 4 are what you see when you cut both plasmids with just EcoRI. Lanes 5 and 6 are what you see when you do an EcoRI-HindIII double digest of both plasmids.

Notes: Note the total sizes of the vectors. Note that the EcoRI and HindIII sites in the expression plasmid are separated by a distance of 0.5kb. Note that the EcoRI-HindIII FGFR fragment that you want is 8kb, but the 5kb HindIII-HindIII fragment you do not want will also appear in the double digest.

**Exercise:**

1. Identify which plasmid is in each of lanes 1 through 6 based on the sizes of the fragments you see.
2. Identify which “bands” you will cut out of lanes 5 and 6 to ligate together.
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**Recommended questions from the textbook.**

3, 5, 6, 14, 15, 18, 20, 21, 32