Biol/Chem 475 Final Quiz Study Sheet

Final Quiz FAQs
When: TWR June 5, 6, 7 12pm in BI463
How much: 59 pts
What: The quiz will focus on PCR and primer design, but anything relating to gel electrophoresis (and other routine lab procedures) is fair game. There may be a computational section, where you will use a laptop to visit an internet site – from the primer design or sequence analysis exercises, for example. There will be no questions on ligations or the blue-white screen.

Review/practice questions: see PCR handouts and problems on this study sheet. Read the two (very) short articles posted on the web site. Come prepared to lecture on Monday June 4 with questions about the material. This will be our only review session. Be sure to hand in your lab notebook at the start of your last lab section.

Calculations Relating to PCR Reactions:
See also calculations on PCR handout

1. a. One sort-of “rule of thumb” is that an amplification reaction should start out with at least $10^4$ copies of target DNA. You are doing PCR reactions using 200ng of C. elegans genomic DNA as template. How many copies of the C. elegans genome is contained in 200ng of DNA? The genome size of C. elegans is $8 \times 10^7$ base pairs. The average molecular weight of a base pair is 660g/mole. Avogadro's number is $6 \times 10^{23}$.

b. If a 250bp target sequence is amplified by a factor of $10^6$, how many ng of product do you expect to get in one reaction using 200ng of template DNA?

2. Theoretically, after 30 PCR cycles, how many times is each starting template sequence amplified? (The observed amplification is will not as high as the theoretical amplification.)
3. A friend of yours is doing a series of PCR reactions and comes to you for advice. She has purchased, three sets of primers hoping that one set would amplify the template sequence shown below. Since she hadn’t taken Biol/Chem 475, she wasn’t exactly sure how to design the primers, so she tried three different strategies (as shown below for the 3 primer pairs). She is using DNA polymerase from *Thermus aquaticus*, which does not contain a 3’--3 exonuclease (proofreading) activity.

<table>
<thead>
<tr>
<th>Primer a</th>
<th>Primer b</th>
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<tbody>
<tr>
<td>Primer Pair #1 5’ GTCCAGC 3’ &amp; 5’ CCTGAAC 3’</td>
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<tr>
<td>Primer Pair #2 5’ GGACTTG 3’ &amp; 5’ GCTGGAC 3’</td>
<td></td>
</tr>
<tr>
<td>Primer Pair #3 5’ GTCCAGG 3’ &amp; 5’ CAAGTCC 3’</td>
<td></td>
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</tbody>
</table>

Template 5’ ... GTCCAGCTAGAGG....................ATTCCGGACTTG........3’
3’.....CAGGTCGATCTCC.........................TAAGCCTGAAC........5’

a. None of the three primer pairs produced any products. Carefully examine each primer pair and explain why it didn’t work. Be sure to indicate whether both of the primers are at fault or if just one primer is the problem.

b. Your friend doesn’t want to buy new primers. She asks you whether she can salvage this experiment. What do you tell her to do?

c. For reasons known only to her, she ignores your advice and decides to redo the PCR reactions with the same primer pairs and use *E. coli* DNA polymerase instead. At the annealing step in each cycle she adds a dollop of fresh polymerase. She gets product with one set of primers. Which primer pair gave her a product and why?

d. Why did she add fresh *E. coli* DNA polymerase at each cycle?
4. Shown below is the sequence of a pair of primers that we often use in Biol 322 for DNA fingerprinting. These experiments have been plagued with prime-dimer problems.

These primers amplify the VNTR (minisatellite) locus D1S80, which is located on the distal portion of the short arm of chromosome 1. This site is highly polymorphic with respect to the number of 16 base pair (bp) repeat units present between the priming sites. Although it is not currently in the FBI CODIS list of key fingerprinting sites, this locus has been used in population genetic studies, identification of extensively burned fire victims, identification of human remains and other forensic studies.

#1 5’ GAAACTGGCCTCCAAACACTGCCCCGCGCG 3’
#2 5’ GTCTTGTGGAGATGCACGTCGCCCTTGC 3’

a. Draw out a generic schematic of a primer-dimer.
b. Using Genewalker and/or Integrated DNA Technologies see if you can find a specific primer interaction (either homo or hetero) that would results in a primer-dimer (see gel). Be sure to label the 5’ and 3’ ends. The primer sequences can be copies from this link: http://fire.biol.wwu.edu/trent/trent/primersequence.htm
c. How would you reduce or eliminate the problem of primer-dimer?
5. There are many different slick molecular assays that can be used to directly test the genotype of a particular individual. One PCR-based approach is called ARMS, for amplification refractory mutation system.

The most common mutation in the CFTR gene (mutated in cystic fibrosis) is the ΔF508 mutation shown below. In American populations of European descent, the ΔF508 mutation represents about 70% of the mutant copies of the gene. In an ARMS assay for this mutation, each template DNA is tested with two different sets of primers:

Primer combination a: F1 & R (with control primer pair that amplifies a non-polymorphic site elsewhere in the genome)

Primer combination b: F2 & R (with control primer pair that amplifies a non-polymorphic site elsewhere in the genome)

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a. Briefly explain the basis of this assay. Be sure to indicate what features (two) of DNA polymerase makes this assay possible.

b. This test must be performed with a DNA polymerase that does not have 3’-5’ exonuclease function. Why is this critically important?

c. Controls are important for all methods of genotype detection. But, why is a positive control especially critical for an ARMS test?

d. Indicate the genotypes of each of the three individuals tested. No explanation necessary. **Define allele symbols:**
6. **Stringency**: Reaction conditions, notably temperature, salt, and pH that dictate the annealing of single-stranded DNA/DNA, DNA/ rRNA, and RNA/RNA hybrids. At high stringency, duplexes form only between strands with perfect one-to-one complementarity, lower stringency allows annealing between strands with some degree of mismatch between bases.

**DNA-DNA hybrid**:  
\[ T_m = 81.5^\circ C + 16.6(\log_{10}[Na^+]) + 0.41(%G + C) - 0.63(% formamide) - (600/L) \]  
\( L = \) the length of the hybrid in base pairs

For mismatched hybrids, the \( T_m \) is reduced 1-1.5\(^\circ\)C for every 1% mismatch.  
*From a letter to the editor of Nature 367: 696-693 1994*

**Our nuclear DNA is 98-99% identical to that of chimpanzees.**

Although, the capture and trading of great apes has been banned in 112 countries since 1973, it is estimated that about 1,000 chimpanzees are removed annually from Africa and smuggled into Europe, the U.S. and Japan. This illegal trade is often disguised by private (such as zoo or circus) owners by simulating births in captivity. Until recently, genetic identity tests to uncover these illegal activities have not been used because of the lack of information on highly polymorphic chimp markers and the difficulties of obtaining chimp blood samples.

**a.** In 1994, a study was reported in which DNA samples were extracted from freshly plucked chimp hair roots and used as templates for the Polymerase Chain Reaction. The primers used in these studies are shown in Figure 2. The primer sequences are derived from **unique sequences in human DNA** that flank highly polymorphic VNTR sites in the human genome. Do you think the annealing of the human primers with the chimp genomic DNA template was done at higher or lower stringency? Consider the potential problems with a stringency that is either too high or too low and explain your answer in detail.

**b.** List two ways that the stringency of an annealing reaction can be adjusted. What is the simplest way to adjust stringency in a PCR reaction?

**c.** Examine the sequence of the VWF primers. Do you anticipate that primer dimers would be a problem with this primer pair (assuming a cold-start reaction)? **Explain your answer.**

![Figure 2](image-url)

<table>
<thead>
<tr>
<th>MBP1/MBP2 (coamplified):</th>
<th>5'-GGACCTCGTGAATTACAATC -3'</th>
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<tbody>
<tr>
<td></td>
<td>5'-ATTACCTACCTGTTGCTCCATCC -3'</td>
</tr>
<tr>
<td>COL2A1:</td>
<td>5'-CCAGGTTAAGGTTGACAGCCT -3'</td>
</tr>
<tr>
<td></td>
<td>5'-GTCATGAAGTAGCTGCTGTTG -3';</td>
</tr>
<tr>
<td>FESFPS:</td>
<td>5'-GGAAGATGGAGTGGCTGCTTA -3'</td>
</tr>
<tr>
<td></td>
<td>5'-CTCCAGCGTTGGCCGAAAGAAT -3'</td>
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<tr>
<td>VWF:</td>
<td>5'-CCCTAGTGATGATAAGAATAATC -3'</td>
</tr>
<tr>
<td></td>
<td>5'-GGACAGATGATGATAAATACATAGGATGGATGG -3'</td>
</tr>
<tr>
<td>SE33:</td>
<td>5'-ATCTGGGGCGACAGATGTA -3'</td>
</tr>
<tr>
<td></td>
<td>5'-ACACCTCCCTACCGCTATA -3'</td>
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<tr>
<td>HUMTH01:</td>
<td>5'-GTGGGCTGAAAGCGCTCCCGATTAT -3'</td>
</tr>
<tr>
<td></td>
<td>5'-ATTCAAGGCTATTCTGGCCTGG -3'</td>
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