Rieder - Genetics
Worksheet 1
Due: 9/12/17 at the beginning of class

Name: _____

About how long did this homework take you? _____

I consulted/worked with: _____

POINTS: / 50

1. In 2011, researchers claimed they had found a bacterium that could use arsenic (As) to build its DNA. FYI, many scientists were skeptical of the arsenic DNA, and this finding was quickly debunked.

A. (2pts) Which element in DNA would As replace (hint: look at a periodic table)? Where would As be located in the DNA molecule?

B. (3pts) Imagine researchers found a bacterium that they think uses Selenium (Se) instead of Sulfur (S). In what kind of molecule would the Se be found and why (hint: think like Hershey and Chase)?

2. When we write the nucleotide sequence of a DNA molecule, it is difficult to remember that DNA is directional:

ATGCCCTAGACGGTT

However, you will learn that the directionality of a DNA strand is of critical importance, so scientists have adopted the convention of writing DNA sequences from 5' (on the left) to 3' (on the right). FYI: This is the same direction as both DNA polymerase and the ribosome:

5'- ATGCCCTAGACGGTT -3'

This directionality is still implied, even if the 5'/3' notation is not marked on the molecule. Write the **complementary** (also called "**antiparallel**") DNA sequence, from 5' to 3' (4 pts):

3. Scientists sometimes refer to the "**G/C content**" of a region of DNA. The average piece of double-stranded DNA will have 50% G/C content, suggesting it has equal proportions A, T, G, and C nucleotides.

A. (3 pts) If a piece of double stranded DNA has 40% G/C content what is the %G, %C, %A, %T? How did you arrive at these numbers?

B. (4 pts) To separate the two single strands of a DNA helix, scientists apply heat to break **hydrogen bonds** and "melt" the strands apart, but this is more difficult than average, and requires a higher temperature, when a piece of double stranded DNA is high in G/C content. Why?

4. In 1993, the Nobel committee awarded Kary Mullis the Nobel Prize in Chemistry "for his invention of the **polymerase chain reaction** (**PCR**) method." PCR is now a staple of all molecular biology labs in the world. PCR requires the activity of **DNA polymerase**, an enzyme which, when given a single-stranded piece of DNA, will synthesize a new, complementary DNA strand in the 5' to 3' direction. All organisms with DNA-based genomes (some viruses have RNA-based genomes!) require DNA polymerase for DNA replication. Some of the first DNA polymerases used in PCR was derived from the thermophilic ("heat-loving") bacterium ***Thermus aquaticus*** (*T. aquaticus*), which grows in hot springs. The DNA polymerase from *T. aquaticus* is often called "Taq" polymerase. Although most DNA polymerases are only stable at the body

temperature of the organism (human DNA polymerases work best at 37 degrees C, for example) or environment, Taq polymerase is stable at very high temperatures.

Important components of a PCR reaction include:

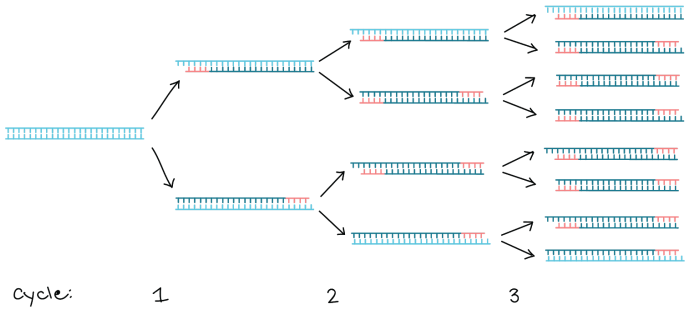
- Taq DNA polymerase
- Nucleotides (A, T, G, C -- the raw materials for DNA polymerase)
- Double-stranded template DNA (for example, extracted from cells)
- Forward primer (short, single stranded DNA)
- Reverse primer (short, single stranded DNA)

A. (2 pts) Are there both **leading strands** and **lagging strands** in our PCR reaction (are there **Okazaki fragments**)? How did you arrive at this conclusion?

These components are mixed together and they are taken through a series of three temperatures:

~95 degrees C ("**melts**" the double-stranded DNA template into single strands)
55-65 degrees C ("**anneals**" the primers to the template DNA strand)
~72 degrees C (DNA polymerase "**extends**" off the primer--this is about the temperature of a hot spring, BTW)

After extension, the cycle starts again with melting. PCR **exponentially increases** the number of DNA copies you have. For example, if you start with one DNA copy, after one cycle you have two copies, after two cycles you have four copies, then eight… After 30 cycles you have ~500 billion copies (2^29).



B. (5 pts) **Primers** are short pieces of DNA that are **complementary** to the ends of the region you want to amplify. You need both a "forward" and "reverse" primer for a PCR reaction. They are red in the above diagram. Think about how DNA is directional. What kind of amplification (not exponential) would you get if you used only one primer (either forward or reverse)? You can draw it out, if that helps.

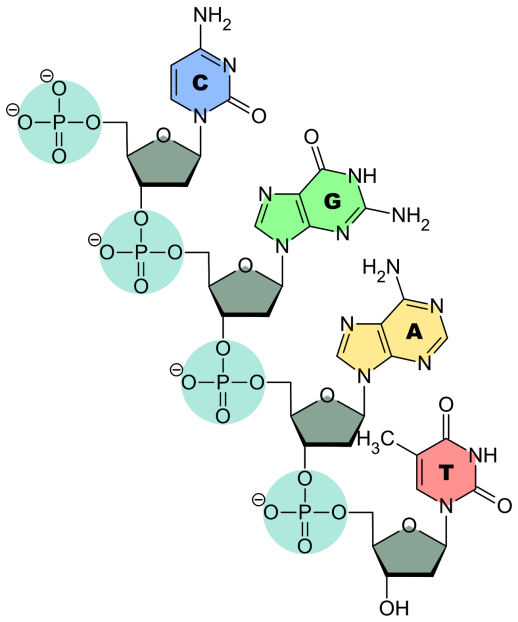
C. (1 pt) Approximately how many cycles would it take to get ~500 billion copies of DNA if you add only one primer?

D. Different kinds of DNA polymerase work at different paces. Let's say the kind you're using in your PCR can extend 500 base pairs per minute (this is relatively slow for DNA polymerases). If the region between your primers is 750 base pairs, how long should you set your extension time (2 pts)? What happens if you calculate incorrectly and you set your extension time to 1 minute (2 pts)? What do you think happens if you set your extension time to 2 minutes (2 pts)?

E. DNA polymerase has **3' to 5' proofreading** activity. That is, if it accidentally incorporates the incorrect nucleotide, it can fix it by backtracking (DNA polymerase synthesizes **5' to 3'**), excising the offending nucleotide and reincorporating the correct nucleotide. However, every now and then DNA polymerase will leave an error in the new DNA sequence, leading to a **mutation**.

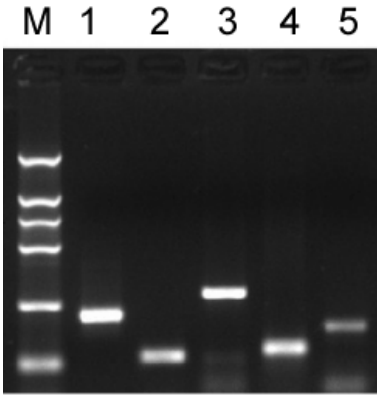
Mutations aren't always "bad" for the cell/organism. Why might a mutation be beneficial (3 pts)?

5. In class we learned that DNA has a net negative charge, due to its **phosphate backbone**:



A. (5 pts) Note the negative charges on all the phosphate groups. Which end (C or T) is 5'? Which end is 3'? How do you know?

Scientists take advantage of DNA's negative charge in a technique called **gel electrophoresis**, usually after a **PCR** reaction like you learned about above. In this situation, a scientist pours a gel slab made out of agarose (a polymer molecule found in seaweed), which forms a porous matrix for the DNA to run through. She puts the DNA (from the PCR reaction) into openings in the gel (you can hopefully see these dark "wells" at the top of the gel picture below), and applies an electrical current so that the DNA is pulled into and through the gel:



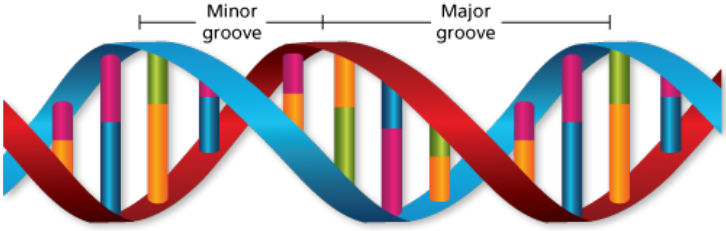
In the above picture, "M" refers to a marker **ladder** of known DNA sizes, while 1-5 are products from different PCR reactions. Using the ladder, the scientist can interpret the sizes of the DNA.

B. (2 pts) The DNA has run down this gel (from top of the picture to bottom). During gel electrophoresis, to which side of the gel, top or bottom, should you apply the negative electrode and why?

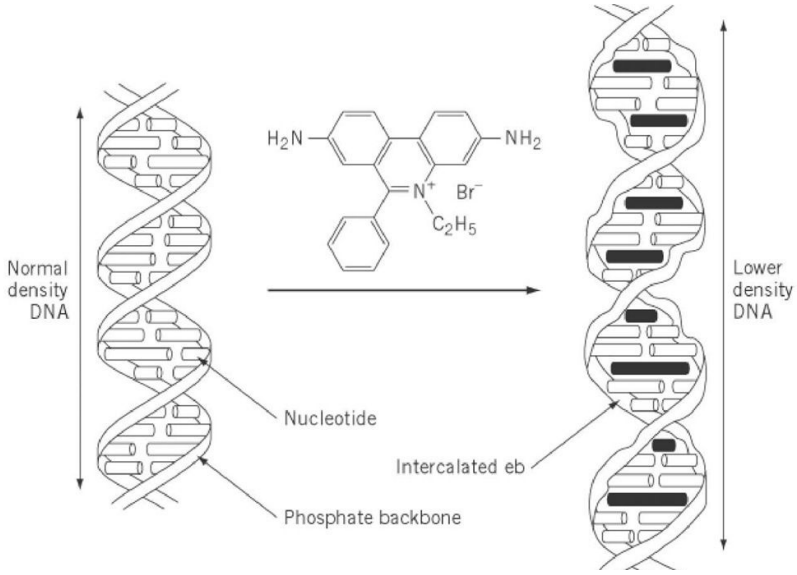
C. (1 pt) What happens if you reverse the electrodes by accident (I and so many other scientists have done this and it leads to much sadness…)?

D. (3 pts) Gel electrophoresis separates DNA pieces based on size, as you can see in the above image of a gel. Longer DNA pieces take longer to squeeze through the agarose matrix than do smaller pieces. I once heard this described as two people running through a dense forest, one carrying a matchstick and one carrying a long 2x4 piece of lumber. The person with the matchstick will be much quicker and will run farther in the same amount of time than the person encumbered by the 2x4. In the lane labeled "M" above, the DNA fragments are 2000 bp, 1000 bp, 750 bp, 500 bp, 250 bp and 100 bp. Please label them on the picture. How did you make these assignments?

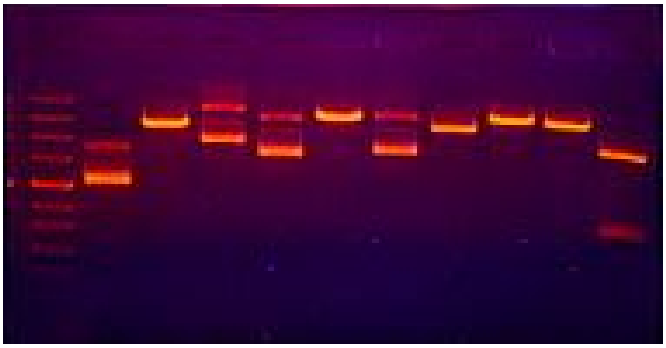
6. Most DNA is a "**right-handed**" helix with a major and minor groove. This is also sometimes called **B-DNA**:



We cannot "see" DNA in a gel (in the question above); we must stain it with a dye. Scientists often use the chemical dye **Ethidium Bromide** (EtBr) to stain gels. EtBr is an example of an **intercalator**, which inserts itself into the DNA double helix:



In the above picture you can see that EtBr molecules (the black bars) inserts into spaces between the nucleotide pairs and distorts the helix. Because of this property, EtBr is considered dangerous; if you spill some on your skin and it absorbs into your cells, it can intercalate into your cells' DNA and cause mutations. Once EtBr is intercalated, it fluoresces brightly under UV light (the picture below is similar to the gel in the question above, but the picture is in color this time):



(2 pts) DNA can also adopt a weird left-handed helix, called **Z-DNA**, although this has not yet been observed naturally. Why do you think you can/can't visualize Z-DNA using EtBr?

7. Eukaryotic DNA polymerase cannot replicate the ends (**telomeres**) of a **chromosome**, which is why **telomerase** is required. WHY can't DNA polymerase make it to the end of an eukaryotic chromosome (1 pt)? Why do bacteria (prokaryotes) not need telomerase (1 pt)?

8. (2 pts) Make up your own way (not my way that I shared in class) to remember which bases (A, T, G, or C) are **purines** and which are **pyrimidines**. It can be anything as long as it helps you remember. Share it below.