1.) DNA

A. The *Magnetococcus MC-1* [this genus of bacteria can migrate in response to an external magnetic field!!] chromosome is closed (no nicks) and circular. It contains 4,720,000 base pairs. **In the relaxed state (no writhes), what is the linking number of the *Magnetococcus MC-1* chromosome?**

To be able to molecularly manipulate the gene for a protein involved in the abiotic stress response, you will need to increase the number of copies of the specific DNA encoding the information for the gene using PCR. The following is the DNA sequence of the gene that contains 846 bases of which 159 are adenosines and 188 are thymidines:

```
ATGAACACCATGAAGACAGCCATGTTGTTGGCGGCATTAACGGCGCTGTTTATGATGATTGGGTTTGCCT
TGGGTGGCCAGGGTGGCATGCTCATCGCCCTGCTGTTTGCGGGGGGTATGAACCTCTATGCCTACTGGAA
CTCGGATCAGATGGTGCTGCGCATGCACAATGCCCAGGAGGTAGGCCCCCGAGAGGCTCCCGAACTCTAC
GGGTTGGTCCAATCCTTGGCAAAACGGGGCAAGATGCCCATGCCCAAGGTCTATGTCATCCATGATCCTT
CGCCCAACGCTTTTGCCACGGGGCGGGATCCTGAACATGCGGCGGTGGCGGCTACCACCGGTTTGATGCA
GATACTCACCCGCGAGGAGCTGGCCGGGGTGATGGCCCATGAGTTGGGCCATGTGATGAACCGAGATACC
CTGATTAGCACCATCTCGGCCACCTTTGCCGGGGCCATTACCGCCATCGCCAACATGGCGCAGTTTGCGG
CCATTTTTGGCAACCGGGATGAAGAGGAGGGTGGTGGTGGCCCGATGGGCTTGATTATGATGATCCTAGC
ACCCATTGCGGCGGCACTGATCCAGATGGCCATCTCCCGTACCCGTGAGTATAAGGCGGATCGTGTGGGT
GCGGAGCTGTGCGGCAATCCCCTGTGGTTAGCCAGCGCTTTGCATAAGCTGGAGCGTGGGGTGCAACAGA
TTCCCAGCCCGGTGGCTCAGGCGCATCCTGAGGCGGCGCACTTGTACATTTGCAACCCTTTGGCGGGGGG
TTTGGGTTCGTTGTTCTCCACCCATCCACCCATCCCGGAGCGCATTCGTAAACTGCAACGGATGACCGGT
CGCTAA
```

B. List the sequences of primers (5' to 3') that are 25 bases in length, which would amplify the above sequence.

C. How many angstroms in length is each PCR DNA product of the above gene?

D. How many cytosines are contained in each of the <u>double-stranded</u> PCR DNA products of the above gene?

2.) DNA Repair

A. **Draw the oxidative deamination product of adenine (replace the primary amine at C6 of adenine with a carbonyl group… make C6 a ketone).**

B. **Is the resulting product a pyrimidine or purine based structure?**

C. **Label the hydrogen bond donors (D) and acceptors (A) on your drawing.**

D. **If this mutated adenine base had to pair with another base in the normal Watson-Crick face, with which base is it most likely to pair?**

This base mutation can be corrected in the cell by the process of *nucleotide excision repair* (NER). The UvrA:UvrB complex binds to the DNA lesion. UvrA leaves while UvrC binds to UvrB. UvrB and UvrC are DNA endonuclease which introduce DNA nicks at the 5^{th} phosphodiester backbone bond in the 3' direction from the mutation and the 8^{th} phosphodiester backbone bond in the 5' direction from the mutation, respectively.

E. **What is the nucleophile during the chemical reaction catalyzed by UvrB (be very explicit)?**

F. **What is the leaving-group during the chemical reaction catalyzed by UvrB (be very explicit)?**

G. **What is the nucleophile during the chemical reaction catalyzed by UvrC (be very explicit)?**

H. **What is the leaving-group during the chemical reaction catalyzed by UvrB (be very explicit)?**

UvrD, a DNA helicase, moves 5' to 3' on the non-nicked DNA strand to create a single-stranded gap that spans 14 nucleotides. This gap is filled in by the action of DNA polymerase I.

I. **What is the nucleophile during the chemical reaction catalyzed by DNA polymerase I (be very explicit)?**

J. **What is the leaving-group during the chemical reaction catalyzed by DNA polymerase I (be very explicit)?**

The resulting DNA nick is sealed by DNA ligase.

K. **What are the nucleophiles during the chemical reactions catalyzed by DNA ligase (be very explicit)?**
   - **a.**
   - **b.**
   - **c.**

L. **What are the leaving-groups during the chemical reactions catalyzed by DNA ligase (be very explicit)?**
   - **a.**
   - **b.**
   - **c.**

3.) Peptides

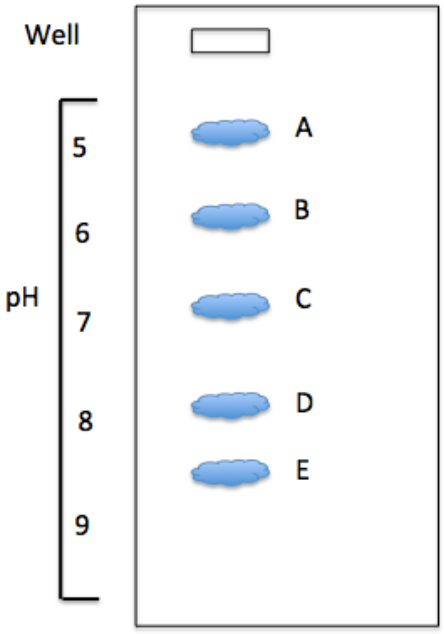
A. **Draw the peptide CHER.**

Not to be confused with:

B. **Circle <u>all</u> the peptide bonds within the peptide drawing.**

C. **Draw a square or rectangle around <u>each</u> backbone bond that is free to rotate.**

D. **The image below could be the result of an isoelectric focusing gel, which contains a pH gradient (as indicated). If a mixture of five peptides including the peptide CHER were loaded into the well, which band (A, B, C, D, or E) contains the peptide CHER?**



E. **Indicate the sign (positive or negative) of the applied electrical potential on <u>each</u> end of the gel, which would separate the peptides as drawn.**

4.) The Central Dogma of Molecular Biology

Utilize the following duplex DNA sequence to answer these questions:

```
5′-CGATCGATCGAGATCTCTAGAAAGCTCGTAGCA-3′
3′-GCTAGCTAGCTCTAGAGATCTTTCGAGCATCGT-5′
```

A. If the above sequence was part of a bacterial chromosome, write out the expected product of DNA replication.

B. If the above sequence was part of a bacterial chromosome contained within a gene, write out the expected final product of transcription.

C. If the above sequence was part of a bacterial chromosome contained within a gene, write out the expected final product of translation.

5.) Describe how to make Solution A from the stock materials.

Solution A (250 mL)	Stock Materials
100 mM Tris	1.0 M Tris
0.25 M NaCl	solid NaCl (58.44 g mol^{-1})
1X SDS	5X SDS

6.) Enzyme Kinetics!
You propose that enzyme E utilizes the following mechanism:

$$E + S \overset{K_1}{\Leftrightarrow} ES \overset{k_2}{\rightarrow} E + P$$

where the substrate binding step completely equilibrates before any ES is turned over to product (this is the rapid equilibrium limit).

A. Write a rate equation for the appearance of product in terms of the concentration of ES and rate constant(s).

$$v_0 = \frac{d[P]}{dt} =$$

B. Define the equilibrium <u>association</u> constant K_1 in terms of the concentrations of E, S, and ES.

$$K_1 =$$

C. Rearrange your expression for K_1 to isolate the concentration of ES.

$$[ES] =$$

D. Write a mass balance equation for the total concentration of enzyme.

$$[E]_T =$$

E. Rearrange your mass balance equation to isolate the concentration of free enzyme.

$$[E] =$$

F. Substitute your expression for the concentration of free enzyme into your expression for the concentration of ES (part C).

$$[ES] =$$

G. Rearrange your expression to isolate the concentration of ES.

$$[ES] =$$

H. Substitute your expression for the concentration of ES into your original rate equation (part A).

$$v_0 = \frac{d[P]}{dt} =$$

I. What is the limit of your rate equation as substrate concentration becomes very high?

$$V_{max} =$$

J. Incorporate V_{max} into your rate equation.

$$v_0 = \frac{d[P]}{dt} =$$

K. Divide the numerator and detonator by K_1. This should look similar to the MM equation result.

$$v_0 = \frac{d[P]}{dt} =$$

(K.) If you got stuck during this derivation, write down the general MM equation and continue.

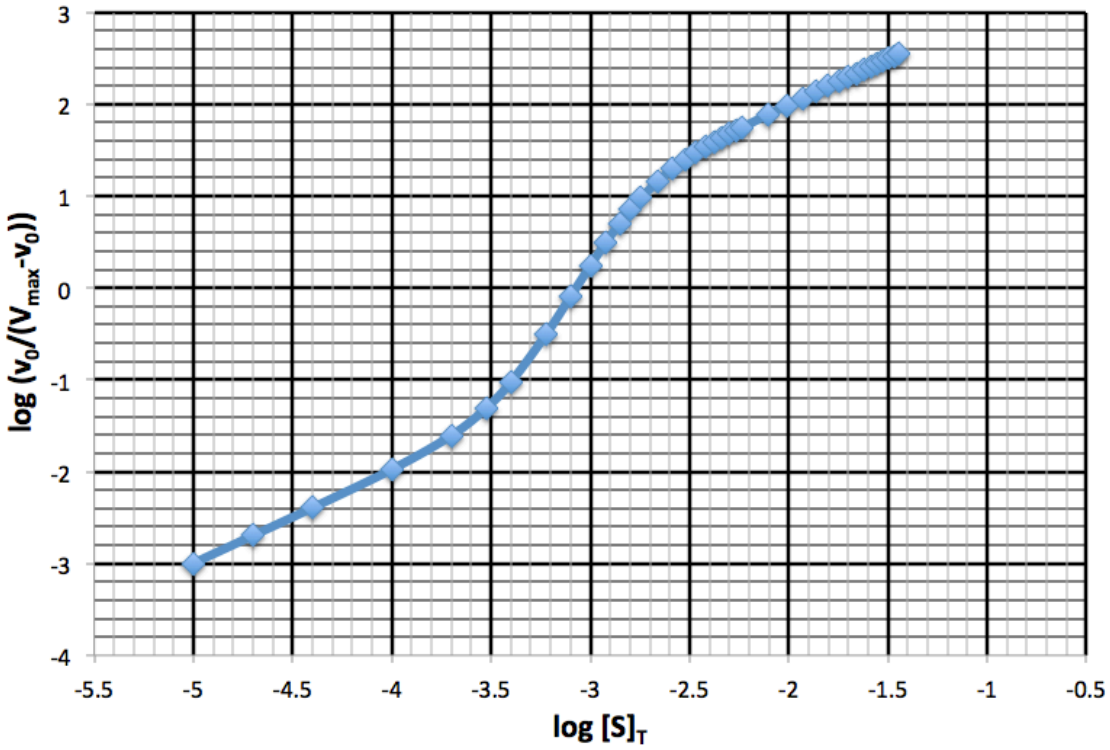
L. Substitute your rate equation (part K) into the following expression:

$$V_{max} - v_0 =$$

M. Using your expressions from parts K and L, write a simplified expression for $\frac{v_0}{V_{max} - v_0}$.

N. Write an expression for the $\log\left(\frac{v_0}{V_{max} - v_0}\right)$.

Imagine that you collect the following kinetic data for the reaction catalyzed by enzyme E:



O. Does this experimental data support your original equation?

P. Explain your answer to part O.

Q. When $v_0 = \frac{1}{2} V_{max}$, what does the y-axis $\left(\log \frac{v_0}{V_{max} - v_0} \right)$ of the data plot equal?

R. When $v_0 = \frac{1}{2} V_{max}$, what does your rate equation (part K) equal?

S. Determine the equilibrium association constant(s) for substrate binding to enzyme E [or K_M if the derivation didn't go so well].

T. Rewrite the original mechanism to include a competitive inhibitor.

U. How would you expect the apparent K_1 (or K_M) to be effected by the presence of the competitive inhibitor?

V. How would you expect the apparent V_{max} to be effected by the presence of the competitive inhibitor?

W. Draw a representative cure on the above data plot for the expected result of the kinetic assays in the presence of the competitive inhibitor.