Name with Last Name, First: **Student ID:**

BIOE111: Functional Biomaterial Development and Characterization
MIDTERM EXAM (October 7, 2010)
93 TOTAL POINTS

Question 0: Fill in your name and student ID on each page. (1)

Question 1: What is the role of puromycin in mRNA display (4)?

Question 2: Which process utilizes DNA libraries such as the one in the figure below? What is the role of the promoter? What is the role of the constant sequences (6)?

Question 3: Briefly define the following terms:

(A) Error-prone PCR (4)

(B) FMOC group (4)

(C) Yeast two-hybrid assay (5)

Question 4: Of the following library based methods, which can possibly benefit from an automated fluorescence based sorting machine? What prevents the methods you didn't choose from working with the sorting machines? (5)

A. One-bead one-compound
B. Phage Display
C. Bacterial Surface Display
D. mRNA display

Question 5: You perform phage display to identify gold binding peptide sequences. For each round you place pieces of gold into a polystyrene tube then add the phage library solution, wash, then elute, using harsher conditions each round. As a control, you run the exact same procedure but without adding any gold to the tubes. When you look at the sequencing results, the exact same binding motif emerges for your experiment and your control. What is the consensus motif likely binding to? How can you alter your protocol to improve your results? (7)

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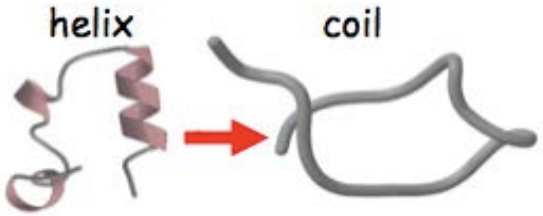
Question 6.

Circular dichroism (CD) spectroscopy measures differences in the absorption of left-handed versus right-handed polarized light which arises due to structural asymmetry. CD is conventionally used to determine the presence of protein secondary structures such as alpha helices, beta-sheets, and random coils. There is a protein with an alpha helix conformation at low temperature. By increasing the temperature of the system, we can observe the loss of alpha-helical character as the molecules transform to a random coil. We can observe this transition using the CD. The variation of signal with temperature is shown below



T (Celsius)	Ellipticity
10	-34
20	-34
30	-30
35	-22
38	-12
40	-1
42	10
45	20
50	26
55	30
65	32
75	32

(A) Define the equilibrium constant for the helix to random coil transition in terms of the fraction in coil form and the fraction in helix form(3)

(B) What is the value of the equilibrium constant at 38 ^{o}C? (3)

(C) What is the value of the equilibrium constant at 50 ^{o}C? (3)

(D) What quantities could be plotted to obtain an estimate of ΔH for the helix to coil transition? (4)

(E) How is ΔG defined in terms of ΔH and ΔS? (3)

(F) What equation describes the relationship between equilibrium constant K_{eq} and ΔG? (3)

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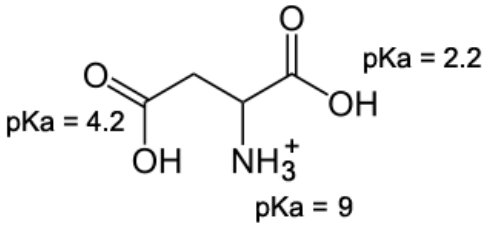
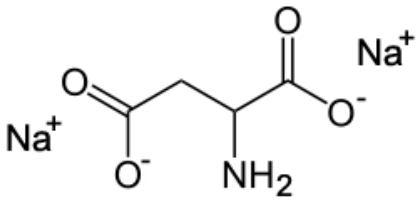
Question 7: Assume aspartic acid, shown in the figure on the left has the associated three pKa values





(A) Define isoelectric point (pI) Calculate aspartic acid's isoelectric point. (3)

(B) If you create a 0.1M solution of the disodium salt of aspartate seen in the figure on the right what would the pH of the solution be? (5)

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Question 8: Suppose we created a peptide library by insertion of random nucleotides into a protein's gene sequence. The coding strand is shown below with an insertion of "n" random nucleotides. The protein's sequence flanking the insertion is shown above the sequence. The methionine in bold corresponds to the n-terminus of the protein

```
     M  N  R  E          Y  T
5` - ATGAACAGGGAATT (X)n TATACG – 3`
```

Suppose that we perform DNA sequencing on one member of the library starting upstream of the region of interest. We determine the coding strand sequence to be the following:

```
5` - GCTGTTGGATGAACAGGGAATTGAACAGGATTCATTGTTATACGCGC - 3`
                          L  N  R  I  H  C
```

Genetic Code

First letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third letter
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

(A) How many random nucleotides were inserted? What is the theoretical number of unique equal length peptide sequences this library could generate? (6)

(B) What is the amino acid sequence of the library member starting from the protein's N-terminus? (The flanking amino acid sequences should not change) (4)

(C) What is the probability that the library would generate the peptide sequence you listed in part B? (4)

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Question 9 (10): Bombyx mori (Chinese silkworm) silk is known to possess strong mechanical properties (E-modulus: 10–50 GPa). It is produced in a specialized silkworm gland. What methods could you use to identify the silk's gene and protein sequences. You do not have to describe how the methods work (7)

Question 10: Are the following statements TRUE or FALSE. **If False explain why.**

(A) mRNA display does not require bacteria for library amplification between rounds. (3)

(B) During solid phase peptide synthesis peptides are synthesized starting from the N-terminus, lengtheinging in the N-terminal to C-terminal direction (3)

(C) Of the methods we learned, mRNA display would be the one best suited to create a library of peptides made of both L and D amino acid optical isomers (3).