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

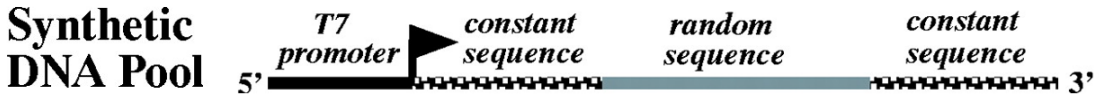
BIOE111: Functional Biomaterial Development and Characterization
MIDTERM EXAM (October 7, 2010)
93 TOTAL POINTS

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)?

Covalently links the translated polypeptide to its corresponding mRNA

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)?



SELEX. Promoter allows the sequence to be transcribed to RNA. The constant sequence is for amplification by RT-PCR and PCR

Question 3: Briefly define the following terms:

(A) Error-prone PCR (4)

A non-error checking polymerase is used during PCR to introduce random mutations into a gene for performing directed evolution

(B) FMOC group (4)

Base-labile amine protecting group used during solid phase peptide synthesis

(C) Yeast two-hybrid assay (5)

Method to determine protein-protein interactions. One protein, the bait, is linked to the binding domain of a transcriptional activator. The other protein, the prey, is linked to the transcriptional activator's activating domain. Physical interaction of the prey and bait brings the two domains in proximity allowing for transcriptional activation and expression of a reporter.

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

A. and C. B. and D. are too small for use in sorting machines

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)

Likely binding to the polystyrene of the tube. Alter the protocol by mixing the library with polystyrene tubes without gold and keeping the unbound fraction prior to mixing with the gold during each round of phage display.

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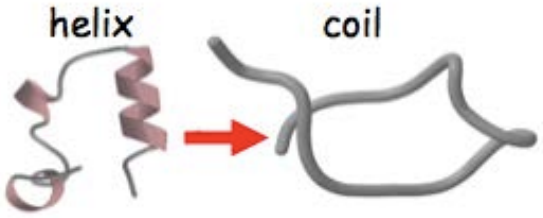
Question 6.

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)

K = f(coil) / f(helix)

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

f(coil) @ 75C = 1; f(coil) @ 10C = 0
f(coil) = [Ellipticity(T) – Ellipticity(10C)] / [Ellipticit(75C) – Ellipticity(10C)]
f(coil) @ 38C = [-12 – (-34)] / [32 – (-34)] = 22/66
f(helix) = 1 – f(coil) = 44/66
f(coil) / f(helix) = 0.5

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

f(coil) @ 50C = [26 – (-34)] / [32 – (-34)] = 60/66
f(helix) = 6/66
f(coil) / f(helix) = 10

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

Plot ln K versus 1/T where T is in Kelvin

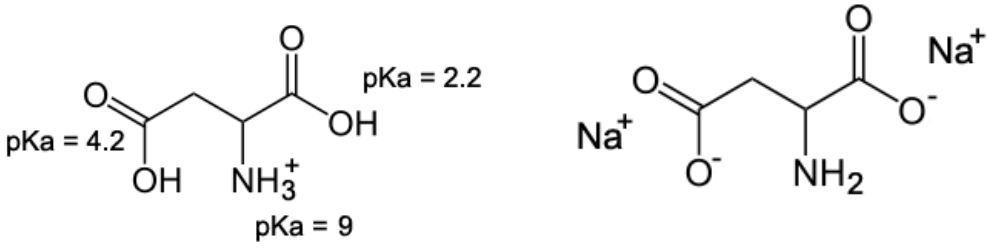
(E) How is ΔG defined in terms of ΔH and ΔS? (3)

$\Delta G = \Delta H - T\Delta S$

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

$\Delta G = -RT \ln K_{eq}$

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)

pH at which a molecule carries no electrical charge
Did not have to solve for isoelectric point but the answer is below for the curious

Ka1 Ka2 Ka3
AH_3^+ <--> AH_2 <--> AH^- <--> A^{2-}
Ka1 = 2.2 Ka2 = 4.2 Ka3 = 9
pI = (2.2 + 4.2) / 2 = 3.2

(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)

Equilibrium we are interested in is for the amine.

Kb
$Asp(NH2) + H_2O$ <-------> $Asp(NH3^+) + OH^-$

0.1	0	0
-x	+x	+x
(0.1-x)	x	x

$Kb = 10^{-14} / 10^{-9} = 10^{-5}$

$x^2 / (0.1 - x) = 10^{-5}$

Assuming x is small relative to 0.1

$x^2 = 10^{-6}$ $x = [OH^-] = 10^{-3}$

$pOH = -\log [OH^-] = 3$ $pH = 14 - pOH = 11$

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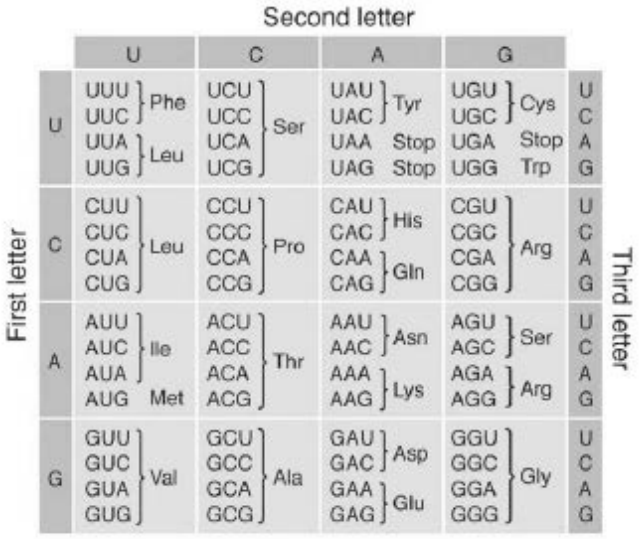
Question 8: Suppose we created a peptide library by insertion of random nucleotides into a protein's gene sequence. The coding strand is shown below with an insertion of "n" random nucleotides. The protein's sequence flanking the insertion is shown above the sequence. The methionine in bold corresponds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



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

```
                M  N  R  E  Z  X  X  X  X  X  Y  T  R
5` - GCTGTTGGATGAACAGGGAATTxxxxxxxxxxxxxxxxTATACGCGC - 3`
```

16 nucleotides.
Z can be Phe or Leu
X can be any of the 20 amino acids therefore $(2)(20)^5$

(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)
MNREZ**LNRIHC**TR

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

```
  L     R     I     H     C
(2/4) (6/64)(3/64)(2/64)(2/64)
```

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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)

Isolate cells and organic material from the silk gland.
SDS-PAGE – separate proteins by size, visualize highly expressed proteins
TANDEM MS/MS – determine protein sequence
Edman Degradation – determine protein sequence

RT-PCR for cDNA library synthesis of mRNA
DNA Sequencing for determining gene sequences

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)

TRUE

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

FALSE. Proceeds from C-terminal to N-terminal

(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).

FALSE. mRNA display uses natural amino acids