Problem Set 5
BENG 183
Fall 2011
Due Monday November 14th 2011 before 11:59pm to Qi Ma (q1ma@ucsd.edu)

Problem 1. Microarrays.

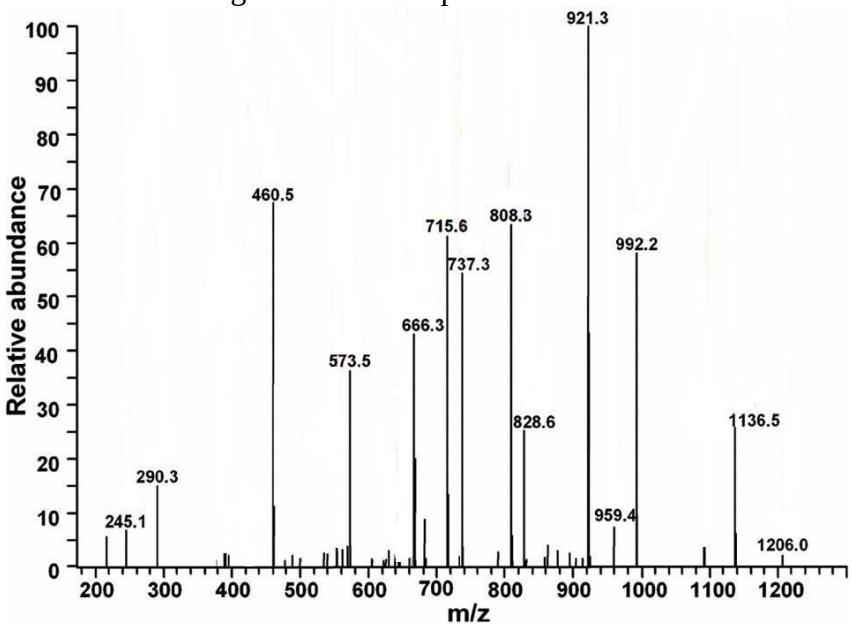
a) Both Agilent and Affymetrix are purveyors of synthesized microarrays of oligonucleotides. Explain the difference in printing technology between these arrays.

b) Affy chips tend to contain short oligos of ~20 bp long. Agilent arrays tend to be longer, ~65 bps. Why the difference in probe length?

c) As part of a quality control team you decide to analyze, via sequencing, the DNA sequence content of a spot on a synthesized array of oligos 25 bp long. You find that it is 88% pure, i.e. 88% is identical to the sequence that was originally designed for this location. What is the approximate per bp error rate of synthesis?

d) As we discussed in class, compare and list the characteristics, usage, advantages and disadvantages between microarrays and RNA sequencing technologies.

Problem 2. Consider the following tandem mass spectrum:



In this problem, you will compute the masses of b and y fragments. The mass of a b ion is equal to the sum of the prefix amino acid masses plus 1 (for a hydrogen). The mass of a y ion is equal to the sum of the suffix amino acid masses plus 19 (for water+hydrogen). For instance, if PANTS broke into b fragment PAN and y fragment TS, the masses would be 283 (97+71+114+1) and 207 (101+87+19). And the amino acid masses table is (http://www.ionsource.com/Card/aatable/aatable.htm)

a) Compute the b and y fragment masses for these two candidate peptides: Y*MIAALAVDX* (which Y* mass=244.1; X* mass=271.3) and Y*GPTAGHVDX*.
b) Which b and y fragments are present in the spectrum? Ignore low-intensity peaks. (Use the list of masses with value, for more accuracy than eyeballing the chart)
c) Which peptide looks like the best match?

Problem 3: You are sequencing a novel protein through Edman degradation. However, you can only carry out a few rounds of Edman degradation with high yield, so you first use peptidases to cut your protein into short pieces. This table shows the fragments produced; a question mark indicates an additional unsequenced C-terminal portion. Staphylococcal protease Trypsin Chymotrypsin.

Staphylococcal protease	Trypsin	Chymotrypsin
MAFSSLLRSAASYTVAAPRPD	MAFSSLLR	MAF
MAFSSLLRSAASYTVAAPRPDFFSSPASD	SAASYTVAAPR	SSLLRSAASY
HSKVLSSLGFSRNLKPSRFSSGISSSLQNGN	PDFFSSPASDHSK	TVAAPRPDF
PRPDFFSSPA	VLSSLGFSR	F
APRPDFFSSPA	PSR	SSPASDHSKVLSSLGF
	FSSGISSSLQNGN	SSGISSSLQNGN
	NLK	SRNLKPSRF

a) Find the full sequence of this peptide.
b) Use BLASTP to search for this sequence. What sort of protein is it?(No need for details, a couple of words is enough)

Problem 4: You are using ion exchange chromatography to separate a mixture of peptides. You start with a relatively acidic solution (pH 4), and shift toward more basic elution conditions (pH 8).
The peptides in question are: 1. RLKPIE 2. TRIEKY 3. GALAGQ
a) Which will elute first and last, if your column uses diethyl aminoethyl groups and you go from low to high pH? (Note: DEAE is basic, and positively charged at most pH values)
b) Which will elute first and last, if your column uses carboxyethyl (CM) groups, and you go from high to low pH? (Note: CM is acidic, and negatively charged at most pH values)
c) What would be wrong with using DEAE and going from high to low pH?

Problem 5: A biochemist is attempting to separate a DNA-binding protein (protein X) from other proteins in a solution. Only three other proteins (A, B, and C) are present. The proteins have the following properties:

	pI (isoelectric point)	Size (Mr)	Bind to DNA?
Protein A	7.4	82,000	yes
Protein B	3.8	21,500	yes
Protein C	7.9	23,000	no
Protein X	7.8	22,000	yes

What type of protein separation techniques might the biochemist use to separate (i.e. Gel-Filtration, Ion-Exchange, and Affinity):

(a) protein X from protein A?
(b) protein X from protein B?
(c) protein X from protein C?

Briefly justify your answers. How might you use a magnetic field to separate peptides by M/Z (mass/charge)?

Problem 6: RT-PCR – In molecular biology, real-time polymerase chain reaction, also called quantitative real time polymerase chain reaction (Q-PCR/qPCR) or kinetic polymerase chain reaction, is a laboratory technique based on the PCR, which is used to amplify and simultaneously quantify a targeted DNA molecule. It enables both detection and quantification (as absolute number of copies or relative amount when normalized to DNA input or additional normalizing genes) of one or more specific sequences in a DNA sample.

a) What is reverse transcriptase and why is it needed in a RT-PCR experiment?
b) What are fluorophores and why are they needed in a RT-PCR experiment?
c) What is a poly (A) tail and how is it used in a RT-PCR experiment? In what circumstances would one use the poly (T) tail as a primer? What other type of primer could be used?
d) Define Ct to be the cycle threshold in a RT-PCR experiment. A sample whose Ct is 3 cycles earlier than another's has how many times more initial template?
e) Now assume the efficiency of doubling template A is 2, and the efficiency of doubling template B is 1.96. Template B has a Ct that is 3 cycles earlier than template A. How many times more initial template of B was there?