Homework 5:

Problem 1: 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.

Problem 2: Functional genomic screens

You are interested in identifying genes involved in the process of DNA repair. In general terms, describe two approaches (forward genetic and reverse genetic) you can use to identify the genes that function in this process.

Forward genetic:

Reverse genetic:

Problem 3: RNAi

a) Feeding *C. elegans E. coli* that express double stranded RNA from the unc-54 gene, which encodes the major form of muscle specific heavy chain myosin, causes the animals to be paralyzed because they lack this myosin. You mutagenize wild-type worms with EMS and screen the F2 progeny for worms that are no longer paralyzed when fed these bacteria. The mutations that cause this resistance are recessive and are also resistant to RNAi for other genes. From what you know about RNAi, what could be the normal functions of the genes that are mutated?

b) Why doesn't homologous recombination work in higher eukaryotes? You may need to do some research online.

c) What is the main pathway behind RNAi, what is thought to be its original purpose in the cell?

d) Aside from Homologous Recombination not working well in higher eukaryotes, why might you want to perform an RNAi knockdown over a knockout?

Problem 4: ChIP-seq

a) Write out a general protocol to perform ChIP-seq, from identifying interactions to sequencing (include necessary reagents, and steps you would take)

b) A main issue with traditional ChIP-seq is the generation of primary antibodies against the transcription factor of interest. How would you modify ChIP-seq to allow for ChIP-seq without primary antibodies?

Problem 5: Yeast 2 Hybrid (Y2H) interaction mapping

a) You are gearing up to perform a large 1000 x 1000 (bait x prey) Y2H interaction screen. How might you detect and control for baits that cause auto-activation?

b) How might you detect bridging interactions? (this is harder, just give us your reasoning)

Extra Credit: How might you use a magnetic field to separate peptides by M/Z (mass/charge)? Draw your plan here in as much detail as necessary. Use text and pictures to describe the approach, which can be an existing apparatus you research or a novel invention.