Question 1

Unlike bacterial RNA polymerase, eukaryotic RNA polymerases need additional assistance in finding a promoter and transcription start site. You wish to reconstitute accurate transcription by RNA polymerase II *in vitro*. You start with a HeLa cell nuclear extract and you want to identify what is needed for promoter recognition.

A) Describe an assay to measure accurate transcription initiation. Be specific about what is in the assay, what the read out looks like, and what the controls would be. (2 points)

B) Using the assay in part A, how would you isolate the protein factors needed specifically for initiation? (2 points)

C) Based on your knowledge of transcription, what factors do you expect to ultimately identify? (1 point)

D) Based on your knowledge of transcription, what functions/activities do these proteins have? (2 points)

E) You want to obtain a cDNA clone for one of these purified factors. Explain how you would do so without using the polymerase chain reaction (PCR). (3 points)

Question 2

Two novel recessive mouse mutants not yet mapped share a phenotype characterized by motor neuron defects and progressive paralysis in the hind limbs. You hypothesize that these two mutated genes likely encode for proteins that form a complex. However, the possibility exists that the two mice have mutations in the same gene.

A) What kind of a genetic experiment should be used to discriminate whether both mutations are in the same gene? Explain how to perform this experiment with these two mice. (2 points)

B) You have mapped these two mutants to two different loci. The mutants turn out to be in one gene that is completely novel and one gene that has been studied in another process. You are ready to perform biochemical experiments to test whether the two proteins are in the same complex. Unfortunately, only one of the two proteins has an antibody available and you CANNOT raise an antibody to the other.

Ba) Describe **two** experimental approaches that you could use to test whether these two proteins are in the same protein complex but that require only one antibody or no antibodies. Describe each of these approaches briefly but clearly in no more than 4-6 sentences. For each approach, be sure to describe the experimental design (including proper controls) and the results expected if the two proteins indeed interact. (4 points)

Bb) Propose a **third** experimental approach that you could use if you were to have any reagent needed (including antibodies to both proteins). As requested in "Ba)" above, describe this approach briefly but clearly including the experimental design, proper controls, and expected results. (2 points)

C) Could you use genetics to determine if the proteins are in the same complex? Briefly state why your answer is positive or negative. (2 points)

Question 3

You have cloned and expressed a monomeric bacterial methyltransferase protein [molecular weight 24.5 kDa, isoelectric point (pI) 8.3] with an N-terminal 6xHis-tag. Additional tags cannot be added to the C-terminus as these inactivate the enzyme. You plan to lyse the cells in sodium phosphate buffer containing 200 mM NaCl and, after initial purification using Ni^{2+}-NTA affinity chromatography, to use **two additional** chromatographic steps to obtain this protein at a purity suitable for crystallization and other *in vitro* experiments.

(A) From the information provided, outline two possible additional chromatographic steps you could use to purify the protein. Your answer should include which columns you will use, specific conditions that are necessary (e.g. buffer and pH) or any additional reagents required to allow protein elution from the column. Be sure to provide a clear rationale for your choices including the order in which the steps are performed. (***4 points***)

(B) Your purification strategy was a success and you are able to crystallize and solve the structure of your enzyme bound to the methyl group donor S-adenosyl-L-methionine (SAM; Fig. 1A).You mutate each of the residues highlighted and find that enzyme with a W47F change is partially active while those with S130A or W133F exhibit no detectable activity in your assay. To further understand the roles of these residues in enzyme function, two additional experiments are performed: measurements of SAM binding (using a fluorescent SAM analog; Fig. 1B) and ^{19}F nuclear magnetic resonance (NMR) using proteins expressed in media containing ^{19}F-tryptophan (Fig. 1C). Using these data, explain the results of the enzyme activity assays and suggest roles for each of the three mutated residues and also the structural motif containing S130 and W133 in methyltransferase enzyme function. (***6 points***)

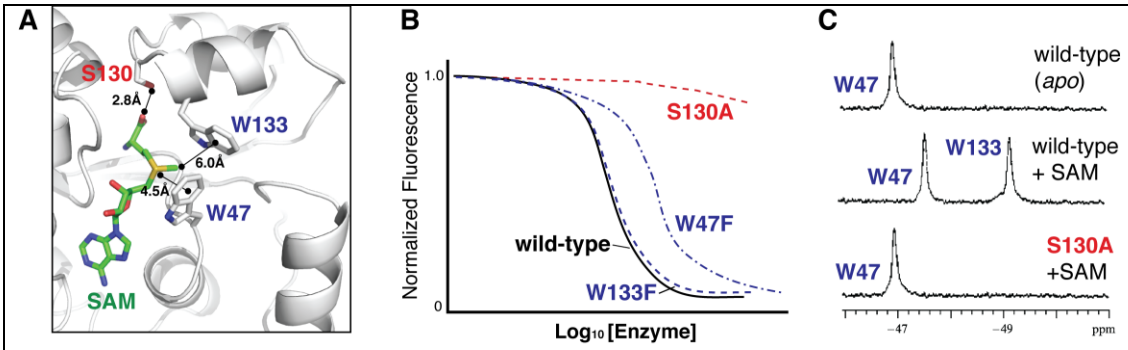


Figure 1.A) Structure of the enzyme surrounding the bound SAM molecule (atoms are shown with standard atomic coloring: N, blue; O, red; S, yellow; C adopts the color of the shown molecule, green in SAM and white in the enzyme). **B)** Fluorescent SAM binding assays. **C)** One dimensional ^{19}F NMR spectra for the samples indicated. [**Note**: ^{19}F nuclei are highly active in NMR. Each will produce one distinct peak per conformationally restrained ^{19}F-derived tryptophan at a chemical shift (ppm) that is very sensitive to the local protein environment. Your protein contains only the two tryptophans shown in Panel A.]

Question 4

(A) If an E. coli cell is a cylinder 1 micron (10^{-6} meters) wide and 3 microns long, what is its volume in liters? (hint: 1 mL of water has a volume of 1 cm^3) (2 points)

(B) You express a protein in E. coli and purify 5 mg from one liter of culture. If the protein has a molecular weight of 14.4 kD, what is your yield in moles? (hint: one Dalton equals one atomic mass unit, or 1/12 the mass of a single atom of the most abundant carbon isotope) (2 points)

(C) Assume that you managed to purify 100% of the protein expressed in that E. coli culture, and that it contained 10^9 cells/mL. What was the *in vivo* molar concentration of the protein before you lysed the cells? (2 points)

(D) How many molecules of protein did the average cell contain before lysis? (hint: 6.02×10^{23} carbon atoms weigh 12 grams) (2 points)

(E) If the protein had a binding partner with which it formed a complex (equilibrium dissociation constant = 0.15 mM), and this binding partner is expressed at an intracellular concentration of 10 micromolar, what fractions of the protein and binding partner would be bound? (2 points)

Question 5

You have just cloned a novel cDNA fragment. You sequenced the cDNA and mapped it to gene X. You found that gene X has never been characterized although expressed sequence tags (EST) sequence of cDNA from gene X is highly conserved among vertebrates. Because the genomic locus of gene X in humans is linked to a familial form of intellectual disability, you are funded by the disease foundation to fully investigate gene X. Assuming you can generate all reagents needed, please answer the following questions with careful experimental design and appropriate controls.

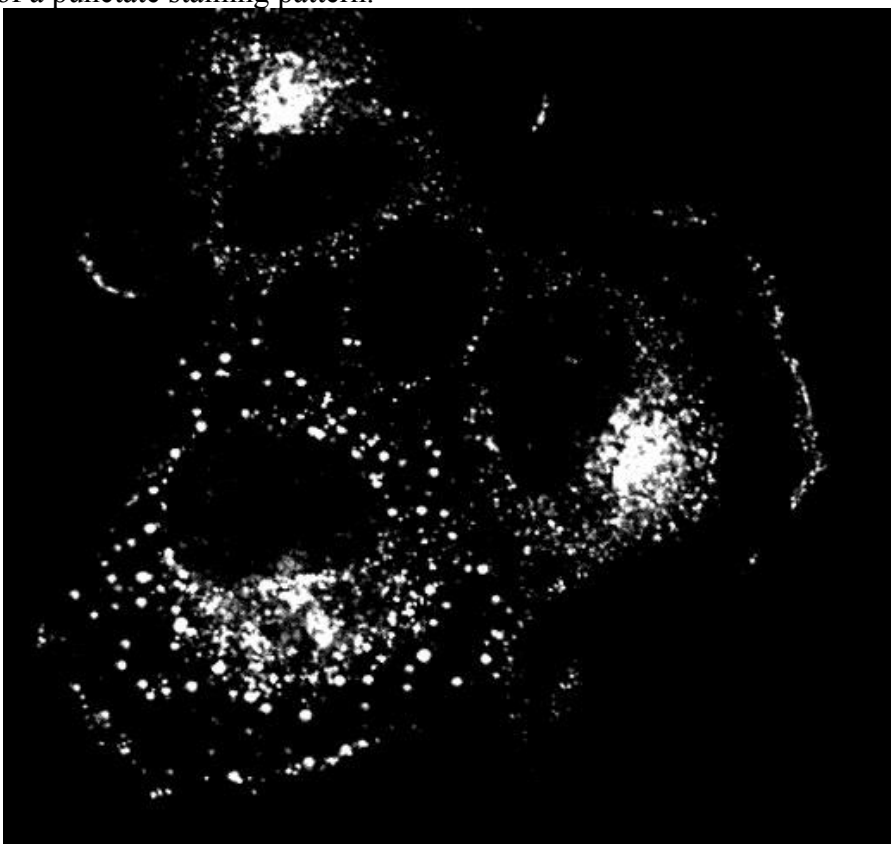
A) Assuming you have accurate cDNA and genomic sequences, how would you experimentally determine what size transcripts are generated from gene X and define the transcription start site? How would you predict the size of the protein isoforms encoded by gene X? How could you make predictions about intron-exon boundaries? Specifically, what positive and negative controls would you use to ensure specificity? (3 points)

B) You found that you could detect two mRNA transcripts of 2 kb and 4 kb in human brain and cultured mouse brain neurons encoded by gene X. Please describe two alternative mechanisms that could explain the presence of two distinct species of mRNA produced by the same gene. Describe an experimental approach to distinguish between the two possibilities you suggested. (3 points)

C) Interestingly, you found that glia cells only express the 2 kb mRNA from gene X. You identified two protein products from gene X with different sizes in neurons but only one in glia. Please state a hypothesis to explain the mechanism that underlies the differential neuron-glia expression of gene X and describe an approach to test your hypothesis. It is critical that you provide sufficient specificity controls for all experimental approaches. (4 points)

Question 6

BOSS is a membrane protein, that when activated, results in the transcription-dependent differentiation of neural stem cells. In a series of preliminary screenings, you have identified a protein called WORKER that might be involved in the signal transduction of BOSS. You hypothesize that WORKER is associated with BOSS at the resting state. Upon activation of BOSS, WORKER translocates to the nucleus. After taking the imaging module of BCDB Foundations, you decide to use imaging techniques to test your hypothesis. Assume that both BOSS and WORKER display a punctate pattern in the cell. The following image provides an example of a punctate staining pattern.



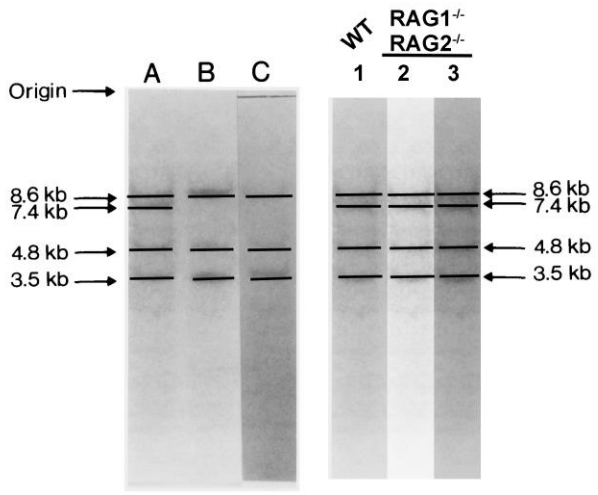
Design three **imaging-based experiments** to test the following model. Design one experiment that tests each part of the model.

(A) BOSS is a membrane protein that stays on the membrane following activation. (3 points)
(B) WORKER binds to BOSS at the resting state. However upon activation, the WORKER-BOSS complex dissociates. (4 points)
(C) Following BOSS activation, WORKER translocates into the nucleus. (3 points)

You can choose any imaging approach for each experiment but have to provide the rationale for choosing this technique. Discuss how the experiments will test each part of the model, how to quantify, the proper controls, and caveats of the approach.

Question 7

Fig.1



The elucidation of the mechanisms generating antibody diversity in mammals led to Susumu Tonegawa's Nobel Prize in 1987. Fig. 1 is a Southern blot published in Cell 15:1-14(1978). The left panel of this figure presents the seminal finding providing the foundations for this award. In this experiment genomic DNA from a BALB/c mouse embryo at age 13 days was digested with EcoR1, DNA fragments were resolved by electrophoresis transferred to a membrane and probed with a radioactive probe specific for the genomic region encoding Fc fragments of immunoglobulins (lane A). Note the presence of four bands. In contrast, lanes B and C were loaded with EcoR1-digested DNA from two BALB/c immunoglobulin secreting cell lines (monoclonal myelomas) that each secretes a different immunoglobulin. Based on these results:

A) Describe the findings on the left panel (2 points) and provide **two** hypotheses (2 points) that could account for these observations.

B) Describe briefly **one** experimental strategy that would allow you to discriminate between your two hypotheses. Discuss what experimental predictions of your hypotheses are discriminated by your experimental approach. (2 points)

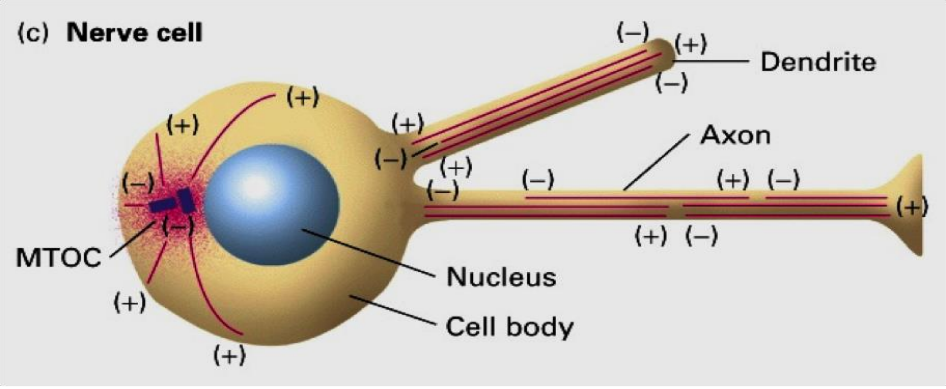
C) Lane 1 of the right panel of Fig. 1 depicts an experiment identical to Lane A of Fig. 1. Lane 2 contains genomic DNA from a RAG1$^{-/-}$,RAG2$^{-/-}$ BALB/c mouse embryo at age 13 days digested with EcoR1. The RAG1$^{-/-}$,RAG2$^{-/-}$ double mutation prevents the generation of immunoglobulins in these mice. Lane 3 depicts genomic DNA isolated from a monoclonal myeloma generated from BALB/c RAG1$^{-/-}$,RAG2$^{-/-}$ mutant mice. All lanes were digested and probed as in the left panel.

Ca) What would you propose is the likely function of the RAG1 and RAG2 gene products? (2 points)

Cb) You have identified 10 genomic loci in humans that when mutated generate a phenotype that resembles the BALB/c RAG1$^{-/-}$,RAG2$^{-/-}$mutant mouse. How would you determine if those loci encode products that belong to the RAG1, RAG2 pathway? Briefly describe **two** strategies (2 points)

Question 8

Polarized kinesin-mediated and dynein-mediated transport of cargo is required for nearly every phase of development and function of neurons and other highly polarized cells. For example, in axons, microtubules are oriented with uniform polarity (see Figure).



In contrast to the axon, microtubules in dendrites are mixed in orientation: some of the microtubules are oriented with plus ends away from the cell body and some of the microtubules are oriented with the minus ends away from the cell body. In this case, one unsolved problem is how the oppositely oriented microtubules are established and maintained. In addition, another problem is whether plus end directed motors, such as kinesin, or minus end directed motors, such as dynein, move dendritic cargo away from the cell body and toward the post-synaptic area. Another interesting problem is that different members of the kinesin super family are targeted, along with cargo, to different compartments in the same cell. For example, kinesin 1 is targeted to the axon where it moves mRNA's and organelles. In the same cell, kinesin 17 is directed to the dendrite or, in some cases, to the cilium.

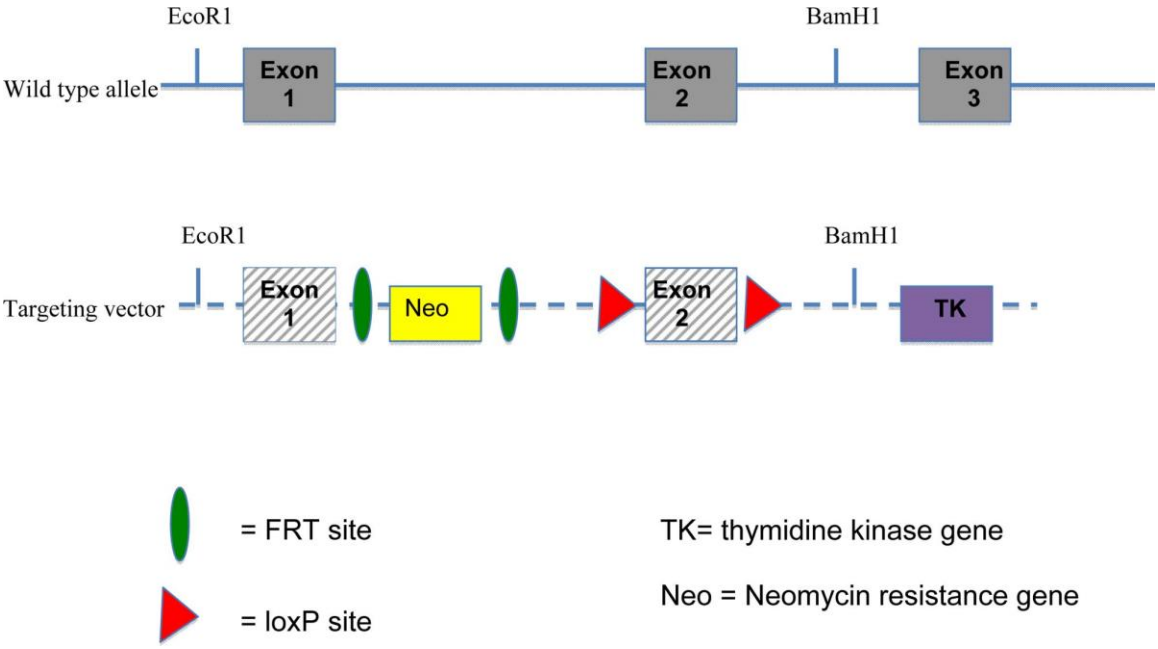
A) Kinesins, such as kinesin 1 or kinesin 17, are referred to as plus end motors. What is the definition of a microtubule plus end? (2 points)

B) Assuming you can purify the kinesin, describe an in vitro experiment to determine whether the kinesin is a plus end directed motor. Describe the assay and the way that microtubule polarity will be established (3 points).

C) State one hypothesis for how different kinesin family members become targeted to distinct compartments within the same cell. For convenience, you may use the example discussed above indicating kinesin 1 is targeted to the axon and kinesin 17 is targeted to the cilium. (2 points)

Ca) Describe an experiment to test your idea for targeting kinesins. Include a discussion of possible outcomes (3 points)

Question 9



(A) Draw the allele that results from homologous recombination of the targeting vector with the wild type allele (1 point). This allele is now known as the targeted allele.

(B) Draw the allele that results from treatment of the targeted allele with Flp recombinase (1 point).

(C) Why is it advantageous to treat the targeted allele with Flp recombinase (1 point)?

(D) What is the purpose of the TK gene in the targeting construct (1 point)?

(E) Draw the allele that results after treatment of the allele you created in (b) with Cre recombinase (1 point).

(F) How would you create a tissue-specific knockout mouse of exon 2? Describe your strategy (2 points).

(G) How would you modify your strategy to ensure that the knockout occurs only in adult animals (2 points)?

(H) Why might you want to create a knockout such as that described in (g) where the knockout occurs only in adult animals (1 point)?

Question 10

The interaction of a ligand with its receptor is the first step in initiating many types of cell signaling, cell-cell adhesion, and cell-matrix adhesion. Such interactions depend on both the specificity and affinity of the interactions.

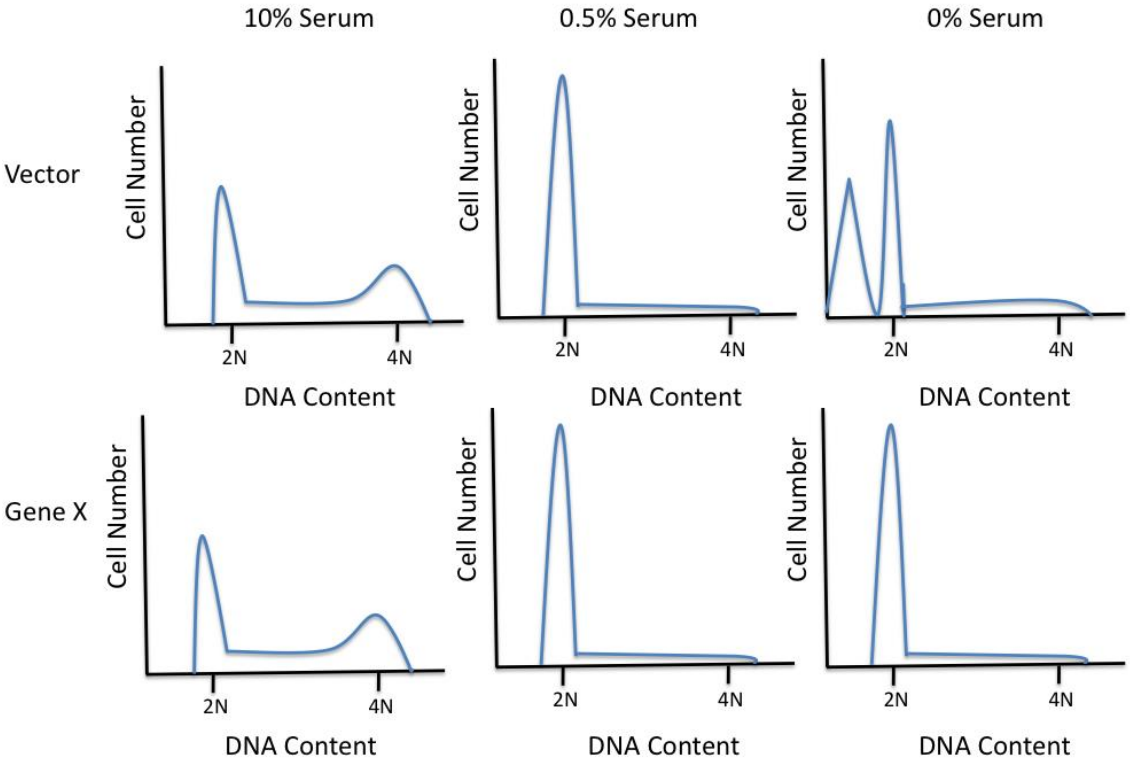
(A) Describe **two** approaches you could take to identify the ligand for a particular receptor you are studying. You may assume that the ligand and receptor are proteins and that the gene for the receptor and its amino acid sequence are known. Also assume the receptor is membrane-bound. In your description, discuss the strengths and limitations of each of your approaches. Specifically, if there are conditions under which your approach will not work, include them. (6 points)

(B) Design **the best** experiment to measure the affinity of the interaction. How would you determine if the binding in your assay is responsible for a biological response? (2 points)

(C) How would you show the interaction between a ligand and its receptor is specific, meaning the receptor binds selectively to the ligand in question, but does not bind significantly to other types of molecules ? (2 points)

Question 11

You have recently cloned a gene that is over-expressed in cancer as a result of a chromosomal translocation. As an initial test of function, you clone your gene into a eukaryotic expression vector and stably transfect mouse embryo fibroblasts with this vector as well as a control vector that does not express any gene. You then determine the effects of gene over-expression on cell cycle under 3 growth conditions: 10% serum, 0.5% serum and 0% serum. Cell cycle analysis is performed by flow cytometric determination of DNA content. Below are examples of the histograms generated from these experiments.



A) From the data above, what cellular process would you hypothesize is altered by overexpression of Gene X? (1 point)

B) Design one experiment to address each of the following (total of 3 experiments):

Ba) An experiment to test your hypothesis in A. (3 points)

Bb) An experiment to determine how the product of Gene X functions at the molecular/biochemical level. (3 points)

Bc) An experiment using a genetic approach to assess how the product of Gene X contributes to carcinogenesis. (3 points)

For molecular, cellular or biochemical experiments, be sure to describe the specific assays you would use. You have access to any cell line, vector, antibody for western blotting or immunoprecipitation, and any instrument needed to measure an outcome. For the final experiment, you may use any transgenic or knockout mouse you think would be useful.

Question 12

Proteins that fail to correctly fold or assemble into oligomeric complexes in the endoplasmic reticulum (ER) are degraded by a ubiquitin- and proteasome-dependent process known as ER-associated degradation (ERAD).

A) Assume that you are the first to discover the ERAD pathway. You must design an experiment to demonstrate that the pathway occurs in both a ubiquitin and proteosome-dependent manner. Assume that you have in hand a model substrate for the pathway that is degraded by ERAD. Design an experiment to demonstrate that degradation of your model substrate is: i) ubiquitin-dependent; and ii) proteosome-dependent. Be sure to include appropriate controls and describe the expected outcome for your experiments. (4 points)

B) You now want to identify cellular proteins that are degraded by the ERAD pathway. You are interested in those proteins that are subject to ERAD at high temperature so your goal is to identify substrates that are degraded at an elevated temperature. Design an approach (using your system of choice) to identify candidate ERAD substrates. Assume that you have already identified key proteins required for the ERAD pathway itself and that you have all tools available to carry out your desired experiments. Be sure to define the system that you have selected and provide controls to ensure that the candidate ERAD substrates you have identified are degraded by ERAD specifically. (4 points)

C) A major issue in describing the ERAD pathway in detail is a topological problem that occurs between recognition of the substrate for the pathway and the subsequent degradation. Briefly, describe the nature of this problem and how you might go about discovering the factor/protein/macromolecular machine that must exist in order for ERAD to proceed as hypothesized. (2 points)