Please do not open and begin the exam until instructed to do so. There will be plenty of time to finish.

Relax, read each question carefully and think about each question before you begin to answer. Be succinct but be complete in your answers. Partial credit may be given in some cases if the answers are logical and well thought out.

Write only on the exam itself and use the back of the pages if you need more space.

Please take a moment to ensure that you have all 7 pages. There are 13 questions that total 70 points.

Good luck!

Please follow these guidelines:

Please place all extraneous materials (including pagers and cellular telephones) into your backpack, close it and slide it under your seat as far as it will go. Please do not open your backpack during the exam for any reason.

Confirmed incidents of academic dishonesty will be dealt with swiftly and harshly. There will be no exceptions.
1. (5 points) You have just identified a novel nuclear body and a protein, “X”, that resides at that site in the cell. You wish to determine its function in the cell. You have labeled the bodies in cells by microinjecting plasmid DNA encoding X-GFP. Using the fluorescent bodies as a target for a laser, you have delivered several pulses of energy to each of the bodies. Wow, you look at the bodies and see that they now look like doughnuts. First, is it possible that you didn’t really ablate anything at all?
   a. explain.

   Yes, you may have only photobleached the GFP fluorophore.

   b. Second, how can you prove that you really ablated the center of the body?

   Use an antibody to protein X.
   Immunostain.
   If you still see “doughnuts”, then you really did ablate. If not, then only photobleached.
   Ultimately, need to look at the ultrastructural level with TEM.

2. (4 points) Define LASER and the fundamental properties of laser light.

   LASER – Light Amplification by Stimulated Emission of Radiation
   Fundamental properties of laser light:
   1) Temporal coherence (discrete wavelength of light)
   2) Spatial coherence (photons travel in phase with each other)
   3) Low divergence (photons travel parallel to each other)
   4) Intensity (enormous photon density)
3. (5 points) What are five important issues to consider when selecting a reporter gene to use for gene expression assays?

- What is the required sensitivity
- What dynamic range is needed
- Speed and ease of assay
- Expense of required reagents
- Availability of equipment
- Requirement for in vitro or in vivo throughput requirements
- Local expertise
- What reagents are already available vis a vis the number of assays, etc.

4. (3 points) If you were doing a large scale analysis of gene expression in living animals, what would be the reporter gene of choice? Justify your selection.

GFP or a related fluorescent protein would be the method of choice in living animals since these need only minimal equipment and allow analysis in the living animal without adding substrates. B-lactamase might be used but would be cost prohibitive. B-galactosidase would not be a good choice since you must fix the animals before staining.

5. (3 points) What is a common feature of nearly all transfection methods?

A small, dense DNA complex of between 75 and 100 nm is taken up by cells via endocytosis.
6. (6 points) What are the essential differences between standard transgenesis and gene targeting using mice? Be sure to mention which types of experiments each is best suited for.

Std transgenesis involves microinjecting DNA into one pronucleus of the fertilized egg. No control of copy number or integration site is possible. It is a gain of function technique that is good for promoter analysis, gene overexpression, mutant rescue and other techniques that do not require precise control over copy number or integration site.

Gene targeting involves the replacement of an endogenous gene by a usually nonfunctional variant. It can be a loss-of-function method and you have precise control over the integration site and copy number. Gene targeting is best used to analyze gene function and to engineer specific mutations into the animal.

7. (6 points) What are three important things we need to know about a founder transgenic mouse before proceeding with large scale breeding and other time consuming and expensive analyses? How would you check each of these?

How many copies of the transgene are present?
   Prepare DNA from tails, do Southern analysis and compare with DNA standards
Is the transgene expressed?
   Northern or Western analysis
Is transgene expression as predicted?
   Northern or in situ hybridization
Is the transgene transmitted faithfully?
   Southern analysis to be sure copy number does not change with breeding
8. (7 points) Many people are unlucky and develop skin cancer as a result of too many years out in the sun. You decide to study skin cancer and figure out the genetic defect(s) that allowed a cancer to form. You discover one mRNA that is expressed at high levels in normal cells but not in tumor cells.

a) (1 point) does this mean that the gene encoding the mRNA is a tumor suppressor gene?
b) (2 points) why or why not

c) (4 points) name two classes of tumor suppressor genes and describe how a loss-of-function mutation in each contributes to the formation of a cancer

a) Not necessarily
b) because the gene in question must be required for preventing the proliferation of cells in order to be a tumor suppressor gene when it is knocked out.

c) Classes of tumor suppressor genes

1) intracellular proteins that regulate or inhibit progression through the cell cycle – mutation leads to inappropriate division
2) receptors for secreted hormones that should inhibit cell proliferation (e.g. TGF-beta) – mutation prevents cells from responding to negative signals
3) checkpoint control proteins that arrest the cell cycle if DNA is damaged or chromosomes are abnormal – mutation leads to loss of checkpoint function and propagation of damaged DNA
4) proteins that promote apoptosis (programmed cell death) – loss-of-function leads to cells not dying when they otherwise should.
5) enzymes that participate in DNA repair – loss-of-function blocks repair of DNA and propagates damage at next replication cycle

9. (5 points) Assume that you have isolated genomic clones containing the gene identified in question 8 and mapped the introns and exons. Describe a strategy to identify the important regions of the promoter. Be sure to describe the key controls and what reporter constructs you would make and why.

Map the transcription start site
Clone the putative promoter into a suitable promoterless reporter construct

Key control: Test this promoter for activity in cultured cells, microinjected embryos or transgenic animals

If regulation is appropriate create a series of deletions to map the regions required for correct expression

If regulation is not appropriate, get more promoter

When regions are found, narrow these down to specific elements

Test elements in minimal promoter reporter vector for their ability to confer same regulation as when they were in full promoter
10. (10 points) Your work in question 9 has led to the identification of a regulatory element that appears to be required for expression of the gene in skin cells. The element has the following sequence AGGTCATCAGATGACCT.

a) (2 points) What can you deduce about the protein(s) from the sequence of the binding site?

b) (2 points) Describe how you could show that skin cancer cells, but not normal skin cells contain a protein that binds to this site.

c) (6 points) How would you identify cDNAs encoding the protein that binds to this element? Be sure to explain any potential limitations of the method you chose and how they could be overcome.

   a) The element has symmetry therefore a multimer binds here
   b) EMSA using nuclear extracts from both normal skin cells and tumor cells
   c) You don’t know whether a homodimer or heterodimer binds here so biochemical fractionation is best. Purify protein, microsequence, design and oligo or PCR primers and screen a skin cDNA library. Other approaches are possible if you mention that homodimers will be detected but probably not heterodimers.
11. (6 points) Describe the best approach to make a mouse model for the type of skin cancer you have been studying, i.e., which method you would utilize and why?

Best approach is to create a mouse deficient in the gene using targeted disruption. Screen a mouse genomic library and isolate the corresponding gene. Make a targeting construct that will block the expression of the gene after it is homologously recombined into ES cells. Perform selection experiments to identify ES cells that have undergone homologous recombination with your insert. Microinject into blastocysts and identify chimeric progeny. Breed these to establish homozygous mice. Test whether these mice have a higher incidence of skin cancer.

Alternatively, you could insert loxP sites flanking the mouse gene and cross the resulting mice with an appropriate Cre line to establish whether cancers develop in the absence of the gene.

12. (5 points) Using the mouse model described above, what experiments could you do to show that skin cancers form in the absence of the gene identified in question 8 but not when it is present?

Some possibilities are:
1) Rescue the knockout by creating a transgenic line expressing the cDNA under the control of an inducible promoter. Ecdysone or reverse tet (tet-ON) are preferred. Show that the mice get tumors when the gene is absent but not when it is present.
2) Using standard transgenesis, show that adding back the gene product rescues the cancer phenotype.
3) If you made a conditional knockout in 11 above, cross with a line expressing cre recombinase under the control of a skin-specific promoter. Cancers should only form when the gene is removed from the skin.
13. (5 points) Name five ways that the activity of transcription factors can be regulated.

- Factor is not generally present but synthesized only where it is needed
- The factor is present but must be modified to be active
  - phosphorylated
  - dephosphorylated
- A ligand is required for activity (or inactivity)
- The factor is localized to an inactive compartment (e.g. cell membrane) and required cleavage for activity
- The factor may be bound to an inhibitory factor in the cytoplasm
- A dimeric factor can have multiple partners. Which partner is present determines activity