

Name: _____

Section: _____

Solutions to 7.012 Problem Set 6

Question 1

The hematopoietic system of mammals is a convenient model to use to study development and differentiation. The bone marrow transplantation experiment discussed in lecture indicates that stem cells are more than a figment of some biologist's fertile imagination.

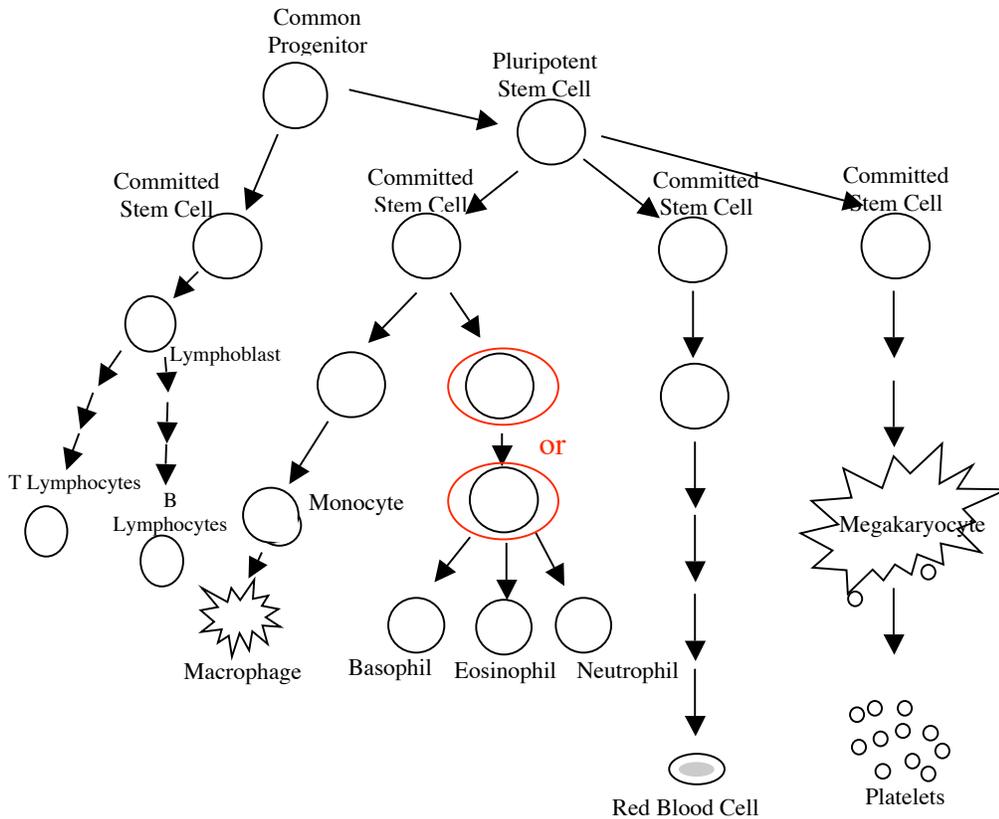
a) Briefly outline the experiment.

A mouse is lethally irradiated, killing all bone marrow cells. Bone marrow cells are obtained from a non-irradiated donor mouse. The donor mouse marrow is injected into the irradiated mouse. The injected cells save the irradiated mouse from death.

b) Explain how the experiment proves that stem cells exist in the bone marrow.

The number of injected marrow cells is small, and not all the blood cell types are represented. The injected cells divide and replace all the cells and cell types that were killed by irradiation.

Use the figure below as an aid for the following questions.



Question 1, continued

c) Treating a population of normal stem cells with a light dose of X-rays can “mark” each cell with a distinctive tag. This light dose will not kill the stem cells but instead will create random breaks and rearrangements of chromosomes that can be seen under the light microscope. As a consequence, each of the cells in such a lightly irradiated population will have a distinctive, unique chromosomal abnormality that serves to distinguish it from its sister cells in the population. You create a population of “marked” stem cells to be used as donor cells in the rescue of lethally irradiated mice.

i) When you examine the blood cells of the first rescued mouse you find that all Basophils, Neutrophils, and Eosinophils have the same chromosomal marker. In the diagram, circle the type of donor cell where chromosomal rearrangement first occurred?

ii) When you examine the blood cells of the second rescued mouse you find that all Basophils, Neutrophils, Eosinophils, and Monocytes have the same chromosomal marker and all megakaryocytes have a different marker. How many different kinds of stem cells from the donor population had a rearrangement?

One

iii) You examine the blood cells of the last rescued mouse. You realize that this mouse shows the existence of a pluripotent bone marrow stem cell. What did you find in this mouse?

The Macrophages, Basophils, Neutrophils, Eosinophils, Monocytes, Red Blood Cells, and Megakaryocytes all have the same marker.

Question 2

Keratinocytes are the predominant cell type found in the epidermal layer and are named for their role as producers of the filamentous protein keratin, which helps to strengthen the skin. Knowing that many of these cells die each day while an equal number are produced, you are interested in understanding where they come from and how the population is maintained.

a) You collect a sample of keratinocytes from a mouse epidermal layer and spread them on a plate in the proper media to allow them to grow. Though the cells are visibly indistinguishable from each other, you find that some fail to divide while others have formed large colonies. What might be different about the two types of cells?

b) You take all the cells from one of the large colonies, and grow them on a fresh plate. You see some of the cells again form large colonies, but most fail to divide. Explain these results.

c) You're curious about what distinguishes the large-colony forming cells from those that fail to divide. You suspect there might be a genetic difference. First, you amplify many of the genes known to be involved in keratinocyte development from both populations and then you compare restriction digests of the DNA from large-colony forming cells to the DNA from non-dividing ones.

i) Do you expect the digests to be identical or different? Why?

ii) To check for differences in what the cell populations are expressing, you build a cDNA library for each cell type. Do you expect these libraries to be identical or different? Why?

Question 2, continued

Your friend, Dr. Tantivy frequently works with mice, but unfortunately is both clumsy and forgetful and the mice often escape and hide in the dark corners of the room. To make them easier to find, he has engineered a retrovirus that carries a gene that when expressed, glows softly green. The virus efficiently infects keratinocytes in the epidermis, and can, on occasion, stably integrate itself into the host cell genome. By infecting adult mice with the virus, he can make them glow green.

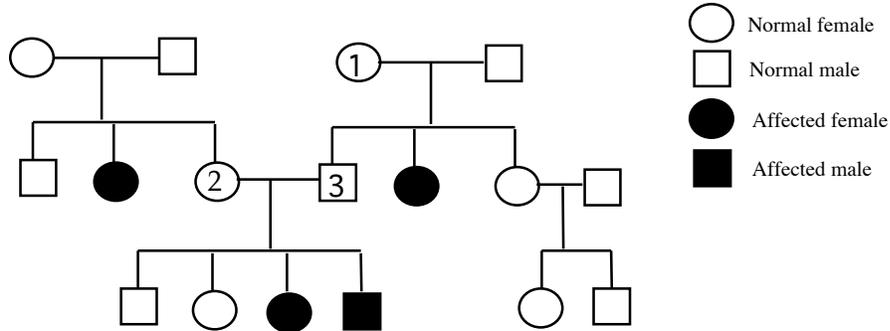
d) The new "glow-mice" are indeed much easier to catch, but they are also beginning to look tired and unhappy and Dr. Tantivy realizes that their glow is keeping them up at night. Luckily, he designed the retrovirus to only infect terminally differentiated keratinocytes. By treating the mice with a drug that inhibits retroviral replication, he believes he can rid the mice of virus even though it may have stably integrated itself into the host cell genome. What's his reasoning behind this strategy?

e) After the treatment, the mice become refreshingly dim for a few days, but then, much to everyone's dismay, one of the mice relapses and lights up again. When Dr. Tantivy compares this recurrent virus strain to the original one, he finds that it is additionally infecting a new population of epidermal cells. How might this explain why the treatment failed?

Question 3

Geneticists studying humans rely upon the analysis of pedigrees to determine the cause of certain traits such as genetically heritable diseases. In the following pedigrees, shaded symbols represent affected individuals.

a)



i) What is the mode of inheritance? Assume that all individuals from outside these families are homozygous for the wild-type alleles.

ii) What is the genotype of the following individuals?

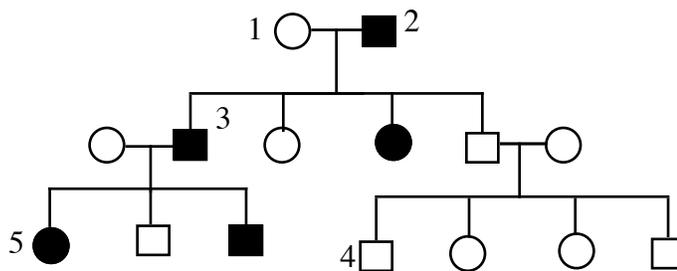
Individual 1

Individual 2

Individual 3

iii) Suppose individuals 2 and 3 have another child. What is the chance that he/she will be affected?

b)



i) What is the mode of inheritance here? Assume that all individuals from outside these families are homozygous for the wild-type alleles.

Question 3, continued

ii) What is the genotype of the following individuals?

Individual 1

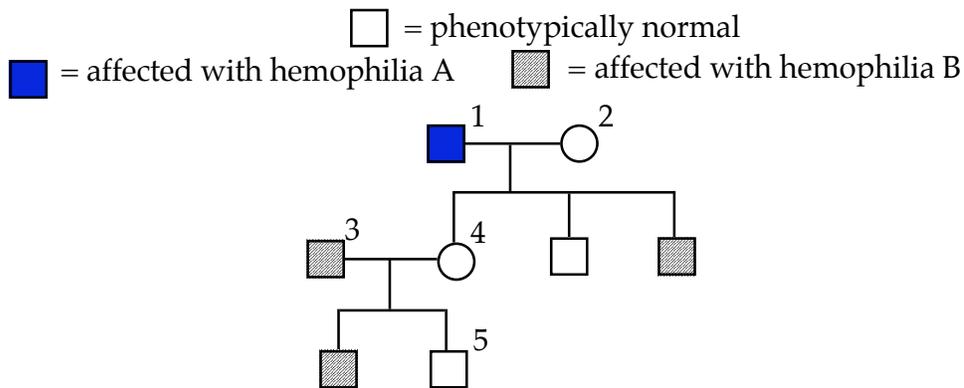
Individual 2

Individual 3

iii) Suppose individual 5's parents have another child. What are the chances that this child will be affected?

iv) This pedigree represents a disorder that is both lethal and relatively common in the population. Explain how this is possible?

c) Consider the pedigree below showing the inheritance of two X-linked diseases, hemophilia A (factor VIII deficiency) and hemophilia B (factor IX deficiency). Factor VIII and factor IX are encoded by two different genes, located at different positions on the X chromosome. Note that no individual shown in this pedigree is affected with both hemophilia A and hemophilia B.



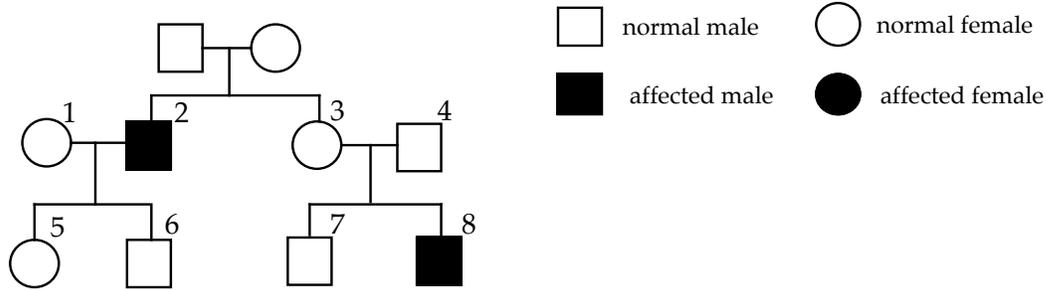
i) Write the genotypes for the following individuals at both the hemophilia A and hemophilia B disease loci. Clearly define your genotype symbols.

Individual	Genotype
1	
2	
3	
4	
5	

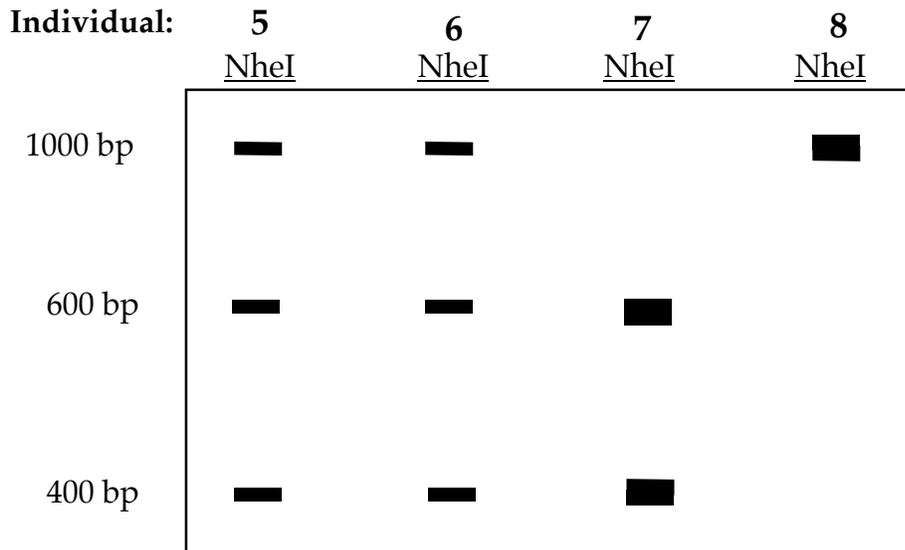
ii) How do you account for individual 5 not being affected with either hemophilia A or hemophilia B?

Question 4

You are studying a common genetic trait. The mutant allele associated with this trait differs from the wild-type allele by a single base-pair (bp) substitution. This substitution eliminates a *NheI* restriction site that is present in the wild-type allele. (The mutant allele is not cut by *NheI*.) A pedigree of a family exhibiting this trait is shown below:



You isolate DNA from four individuals in the pedigree. Using PCR techniques, you amplify a 1000 bp portion of their DNA which includes the site affected by the mutation. You digest the PCR products with *NheI* and analyze the resulting DNA fragments on a gel:



a) Based on these data, is this gene located on an autosome or the X-chromosome? Briefly justify your reasoning.

b) Based on these data, is the mutant phenotype dominant or recessive to wild-type and why?

c) If individuals 3 and 4 have a daughter, what is the probability that she will be affected? Justify your reasoning.