NAME: _____

STUDENT ID #: _____

MCB 140 FINAL EXAM
Spring 2009

NAME (Please print):_____

STUDENT ID #:_____

REMINDERS

You have 180 minutes for the 225 point exam.

Print your name and ID# on each page of the exam. You will lose points if you forget to do this.

There are 13 pages total, including this cover page. The back of each page is work space only (no problems). All pages must be turned in.

Only the front of each page will be graded. If you use the back of a page, transcribe your answer to the space provided on the front of the page.

Use a non-programmable calculator.

----------(Do not write below this line)----------

2 (25)	_____	11 (20)	_____
3-6 (25)	_____	12 (20)	_____
7-8 (30)	_____	13 (45)	_____
9 (35)	_____	**TOTAL**	_____ / 225
10 (25)	_____		

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Questions on Prof Urnov's section (single-sentence answer to each is fine)

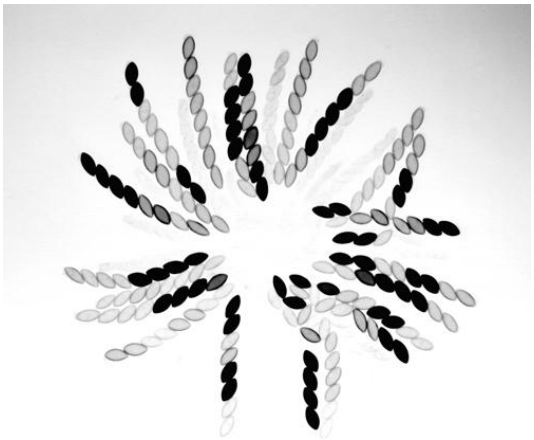
Neurospora grows naturally as a haploid. This proved uniquely useful to Beadle and Tatum in their pioneering forward genetic screen on this organism. Why? (5 pts)

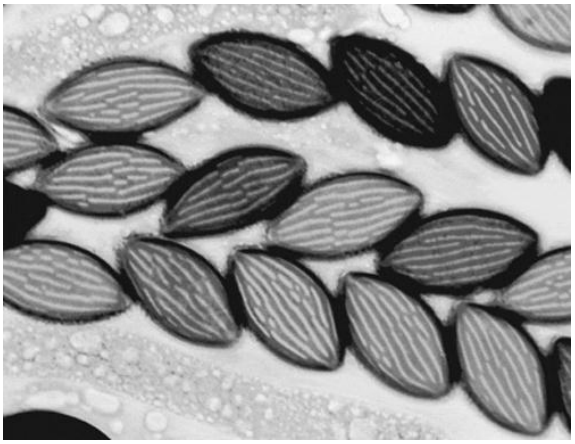
Beadle and Tatum mutagenized *Neurospora* with X-rays. Mendel, in contrast, did not have access to an X-ray source, nor did Morgan – both relied on naturally occurring mutants of peas and *Drosophila*, respectively. Why did Beadle and Tatum feel the need to do the mutagenesis – why not rely on natural *Neurospora* auxotrophic mutants? (5 pts)

As your textbook shows, following the cross of mutagenized *Neurospora* to wild-type and sporulation, each product of this meiosis (called an ascospore), *before* being tested for auxotrophy on minimal medium, was *first* grown in complete medium. Why was this done? (there are two reasons, actually, but one will do) (5 pts)

Two haploid *Neurospora*, each auxotrophic for vitamin B6, are crossed to each other, the diploid is sporulated, and the progeny are grown on medium lacking vitamin B6. One quarter are wild-type, the remaining 75% fail to grow. Propose a simple explanation for this somewhat unexpected progeny ratio. (10 pts)

And now, a short break for a bit of trivia. In 1927, B.O. Dodge named this filamentous fungus *Neurospora* because of the "nerve-like ornamentation on developing ascospore walls" (below right). Individual asci are shown below left.





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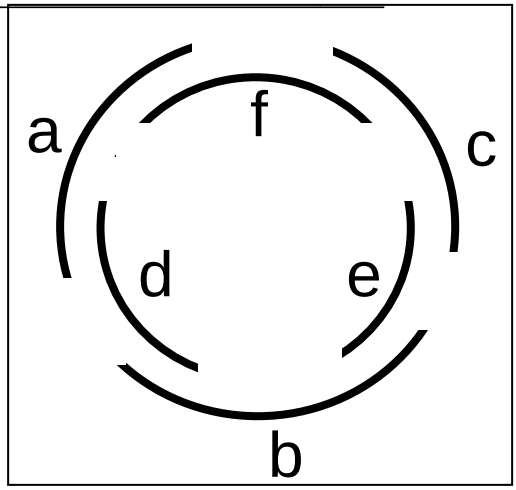
Questions on Prof. Cline's section (25 points total)



C1 (10 points total).
The various parts of this question are all with reference to the following complex (circular) complementation map to the right. It was generated from studies of the behavior of a large number of recessive point mutants in a diploid organism:

C1a (3 points) Based on this complementation map, would you predict that the phenotype of an a^-/b^- mutant individual would be mutant or wildtype. Explain briefly (note that we're using the "a" and "b" designation to refer to classes of mutant alleles on the complementation map without implying that a and b alleles are necessarily in different genes).

C1b (2 points) Do you think the group of alleles that were used to identify the "a" complementation group included any neomorphic alleles? Explain briefly.

C1c (2 points) Based on this complementation data can you predict whether, on a map based on meiotic recombination frequencies, alleles in the "a" complementation group are likely to be farther from alleles in the "c" group than they are from alleles in the "f" group? Explain briefly.

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C1d (3 points) What is the minimum number of complementation groups one would have to remove from this map to convert it into a linear map?

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C2 (3 points) Recall that nonsense suppressor tRNAs are allele-specific, gene nonspecific suppressors that we refer to as "informational suppressors" because they change how the genetic code is read. Although best studied in prokaryotes, nonsense suppressor tRNAs have been generated and used in model diploids as well (such as nematodes). If a particular wildtype tRNA gene (lets call it "*a*") were mutated to become such a suppressor (a^{sup}), into which of Muller's mutant categories would a^{sup} appropriately fall? (consider the possibility that it might fall into more than one at the same time).

C3 (2 points) Recall that mutations in the "*suppressor of Hairy-wing*" gene of Drosophila can be recessive, allele-specific, gene nonspecific suppressors. We could recognize a rare antimorphic mutant allele of *suHw* based on its dominant ability to suppress the same mutant alleles of other genes that the recessive *suHw* mutants suppress. The $yellow^2$ mutant allele is supressable by *suHw* (y^+ flies are brown while unsuppressed y^2 flies are yellow). Imagine that we "reverted" the dominance of an antimorphic *suHw* allele, then determined the phenotype of an animal that carried the resulting revertant allele in trans to a garden variety recessive *suHw* allele. What color would you expect this animal to be if it were also homozygous for y^2? Explain briefly.

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C4 (2 points) Recall that XXY human aneuploids are sterile males, while XXY fruit fly aneuploids are fertile females. If you were told that for species Q, a ZZW aneuploid is a fertile male, could you say whether the sex-determination mechanism of species Q would be more likely to resemble that of the human or of the fly? Explain briefly.

C6 (4 points total) In developing the FRT/FLP site-specific recombination system for use in fruit flies, the FRTs were brought in on engineered P elements and randomly inserted into fly chromosomes. Then the position of each independent insertion was determined, the goal being to keep the insertions that were closest to centromeres.

C6a (2 points) Why was proximity to a centromere a criterion for keeping the FRT insertion lines?

C6b (2 points) Certainly some of these FRT insertions would have disrupted vital genes. If an FRT insertion in this category had been the closest one to a particular centromere, would it have been worth keeping that insertion line for use in the future to induce site-specific mitotic recombination? Explain briefly.

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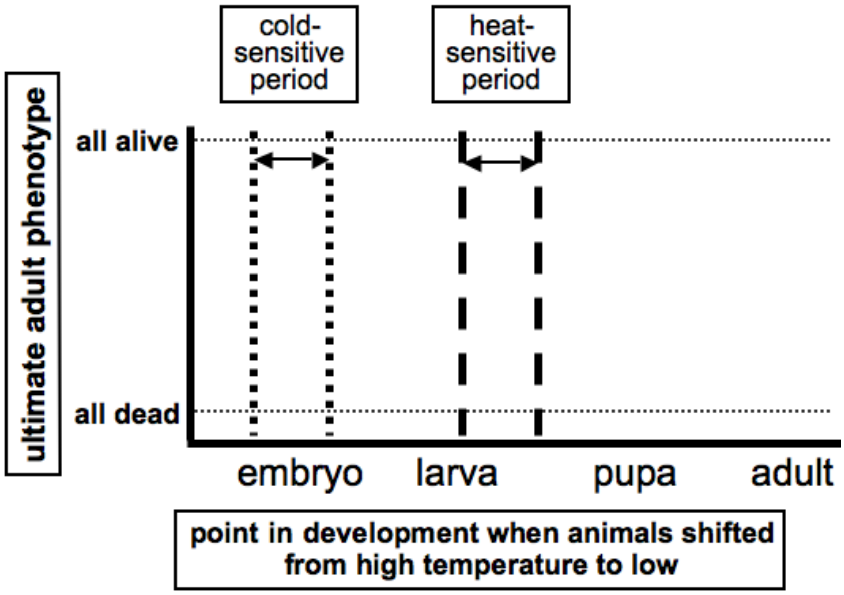
C7 (4 points) Consider a temperature-sensitive recessive lethal mutant allele that is both heat-sensitive AND cold sensitive, but is fine at intermediate growth temperatures. As shown on the graph below, the TSP for this allele's cold sensitivity was found to be distinct from that for its heat sensitivity. On this graph draw the (single) line you would expect for the results of a single-shift experiment in which groups of animals were shifted FROM high temperature TO low temperature at various points in development.



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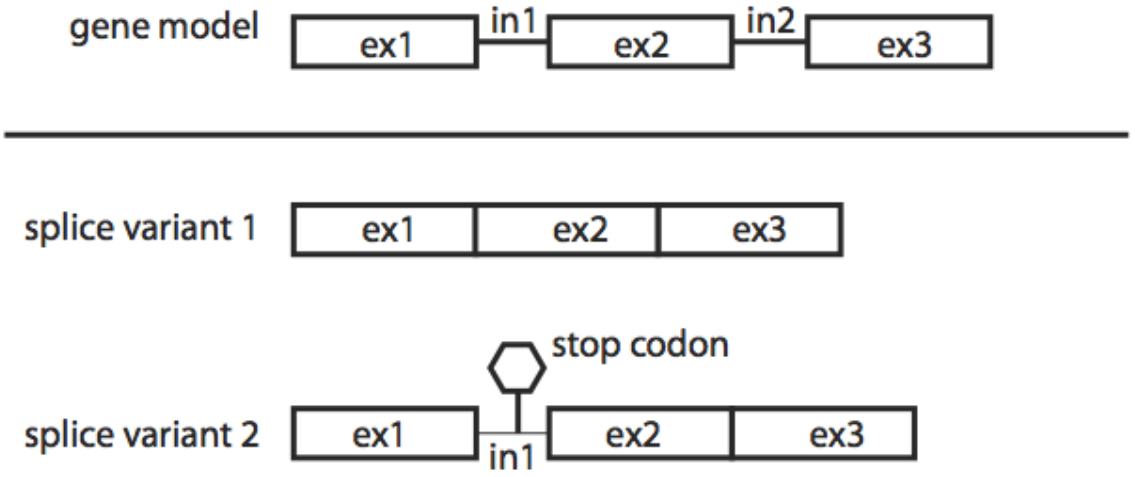
Questions on Prof. King's section (175 points total)



A putative tumor suppressor gene (Gene X, above) contains three exons and two introns. You have been collecting EST sequences from discarded umbilical cord tissue (same genotype as newborn). In most samples you observe only a single splice isoform, one in which both introns have been spliced out (variant 1). However, in some umbilical cord samples, a second isoform is observed in which the first intron is retained (variant 2). A stop codon in the retained intron renders proteins encoded by variant 2 truncated and non-functional.

After following the long-term health of infants from which the umbilical cord samples were collected, you observe that those expressing variant 2 are at significantly higher risk of developing a brain tumor in their early teens.

Is the long-term association of Gene X splice variant 2 with brain tumor development consistent with the hypothesis that Gene X is a tumor suppressor? Briefly, why or why not? (10 pts)

Describe a **simple** experiment to determine the relative abundance of variant 1 vs. variant 2 in normal tissue and brain tumors. Be specific about what probe(s) you will use. (15 pts)

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Which of the following observations would invalidate the hypothesis that Gene X is a tumor suppressor whose function is sufficient to prevent brain tumor development.

Scenario A: Observe only variant 1 in brain tumor biopsy.

Scenario B: Observe only variant 2 in brain tumor biopsy.

Scenario C: Observe an equal mix of variant 1 and variant 2 in brain tumor biopsy.

Briefly explain your answer in the space below. (5 pts)

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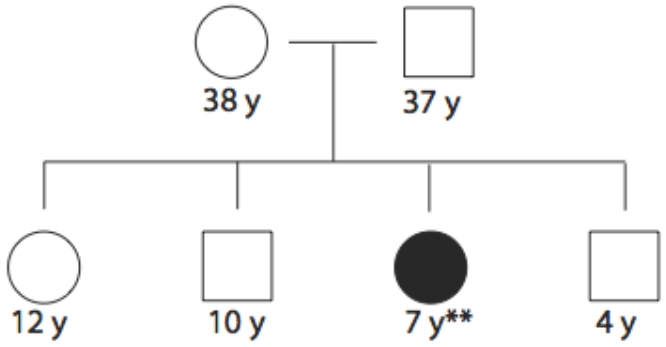
Young children with bilateral retinoblastoma have typically inherited a single active copy of the Rb gene. Inactivation of the single functional Rb allele during retina development subsequently triggers the onset of retinoblastoma.

Propose a hypothesis to explain why retinoblastoma cannot develop if the second hit to Rb occurs in the germline rather than the soma. (8 pts)

Describe an experiment to test your hypothesis (technical details are not necessary or desired). (10 pts)

In what organism will you do your experiment, and why? (7 pts)

Retinoblastoma afflicts 1 in 20,000 live babies worldwide. Bilateral cases are typically diagnosed within the first year and unilateral cases are typically diagnosed within the first 2.5 – 3 years of life.



** Afflicted with unilateral retinoblastoma. Diagnosed at age 2.5.

Given the above pedigree, what is the likelihood that the next child born to this couple will develop retinoblastoma? Explain briefly. (10 pts)

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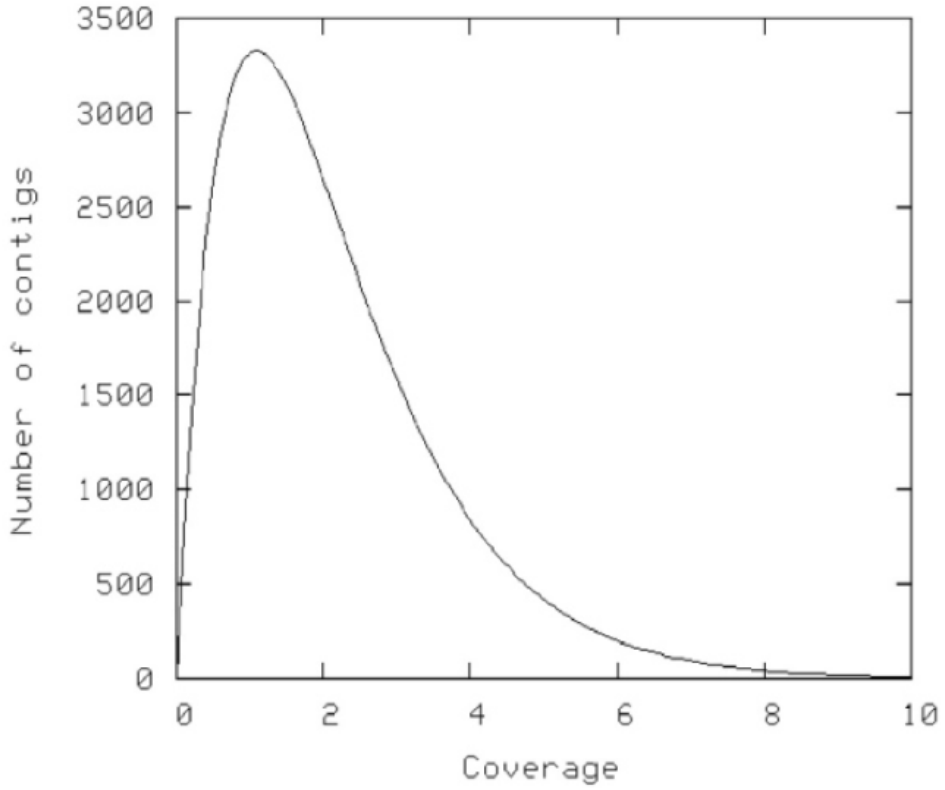
In class you were shown this graph of the relationship between contig number and sequencing coverage for a typical genome.



Briefly explain why the contig number is expected to increase when sequencing coverage is increased from 0.3X – 0.6X. (5 pts)

Briefly explain why the contig number is expected to decrease when sequencing coverage is increased from 3X – 4X. (10 pts)

For a typical eukaryotic genome, do you expect the contig number to equal the chromosome number for a genome sequenced to 10X coverage? (10 pts)

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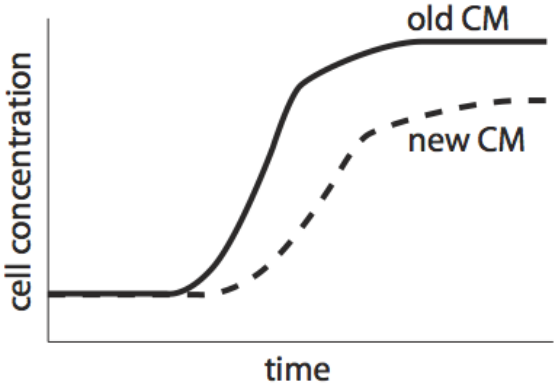
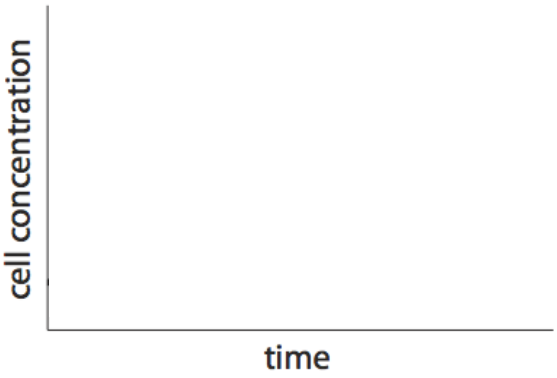
Ripped from the pages of King lab notebooks. For the past five years we have been growing *Flavobacteria* Mx1 in CM media, in which cells divide every 30 min. during exponential growth. The CM media supply company recently changed the formulation. In the new CM media, the cells only divide once every hour, and they don't reach the same cell density. The growth curves of the cells grown in old CM and new CM are depicted on the graph below:



With the remaining old CM, you grow up two clonal cultures of *Flavobacteria* (started from isolated colonies) and then freeze them for permanent storage. You then start passaging *Flavobacteria* regularly in the new CM. Every morning, you come into lab and transfer 100 ul of cell culture into 100 mL of new CM.

After a year has passed (~1500 generations), you decide to test whether the bacteria have evolved under the new growth conditions. On the graph below, draw two expected growth curves in new CM: one for the ancestral line (A) and one for the evolved line (E). (10 pts)



You have decided to sequence the genomes of the ancestral and evolved bacteria to identify point mutations that might contribute to relative fitness levels. Do you expect to be able to assemble longer sequence contigs from the ancestral bacteria or the evolved bacteria? Briefly explain why. (10 pts)

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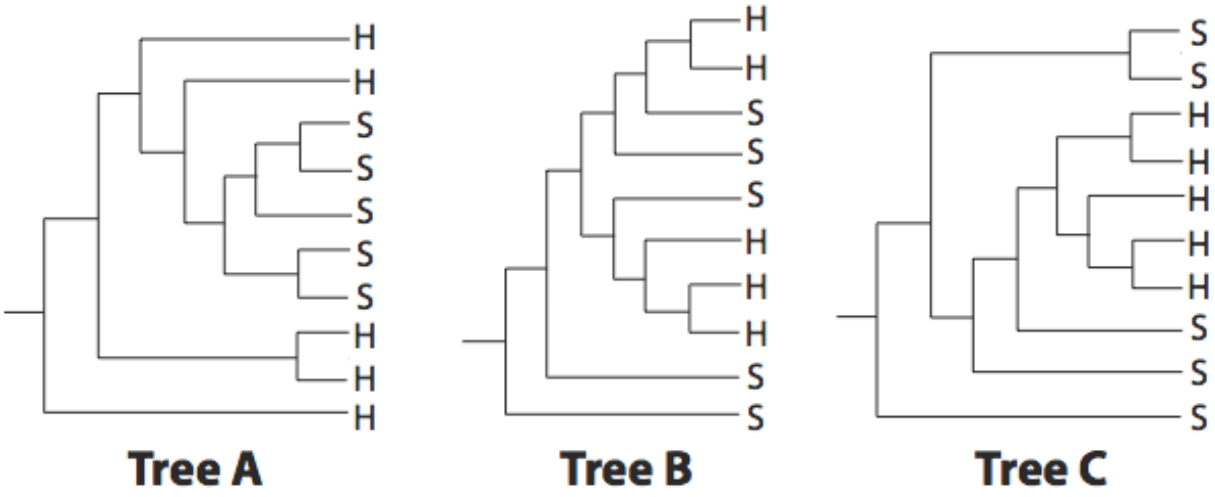

You are an epidemiologist studying the transmission of the swine flu virus from pigs to humans. As part of your study, you have sequenced ten viral genomes, five from infected pigs (S; each from a different farm) and five from infected humans (H; three from Mexico and two from the States). Using those sequences, you have generated a phylogenetic tree depicting the inferred evolutionary relationships among the different viral strains. Viral strains whose genomes are most similar are inferred to be most closely related.

1. Which of the trees above indicates that there have been multiple cross-species (i.e. pig --> human) transmission events? (5 pts)

2. Which of the trees above indicates that the virus has only crossed from pigs to humans once, and that it has subsequently been transmitted between humans? (5 pts)

3. For the remaining tree, briefly describe (<u>no more than two sentences</u>) what the tree indicates about the evolutionary history of the swine flu virus. (10 pts)

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You are studying a previously uncharacterized group of nematodes. You make the following seemingly incongruent observations:

#1: The genome lacks clear homologs of Dicer, Argonaut, and Piwi. (Assume that the genome has been fully sequenced and that there are no errors in gene prediction or annotation.)

#2: You inject 2-cell embryos with dsRNA against the endogenous gene *mcb140*, allow them to develop for 24 hours, and then grind them up to isolate total RNA. After running the total RNA on a high percentage acrylamide gel (good for separating very short RNAs), you perform a Northern analysis with a radiolabeled probe against *mcb140* and detect a faint band from a population of 21-23 nt small RNAs. Quantities of full-length *mcb140* are reduced relative to untreated nematodes.

a. Why is observation #2 surprising, given observation #1? (8 pts)

b. Please complete the following sentence to explain the presence of 21-23 nt RNAs in the absence of Dicer, Argonaut, and Piwi. (7 pts)

I hypothesize that the 21 – 23 nt RNAs result from ...

Back to *C. elegans*. As part of a RNAi screen you determine that feeding worms with bacteria expressing dsRNA from the gene *xyz* results in disruption of vulva development in their progeny. You then directly inject *xyz* dsRNA into early-stage embryos, and this also results in disruption of vulva development. Finally, to validate your results, you knock out *xyz* using homologous recombination. You perform a Southern and use PCR to confirm that the knock-out procedure was successful.

Much to your surprise, there is no mutant phenotype, even after following the mutant strain for multiple generations. Offer a hypothesis to explain how the introduction of *xyz* dsRNA can disrupt vulva development when a *xyz* deletion does not. (10 pts)

Describe an experiment to test your hypothesis. Be sure to include proper controls. (10 pts)

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Name two problems preventing RNAi from being used in therapeutics. (10 pts)