7.03 Final Exam Review
12/19/2006

1. You have been studying eye color mutations in Drosophila, which normally have red eyes. White eyes is a recessive mutant trait that is caused by **w**, a mutant allele found on the X chromosome. You have isolated a new mutation, **ap**, that causes the recessive phenotype of apricot colored eyes. The location of the **ap** locus in the fly genome is unknown.

(a) A female from a true-breeding **ap** strain is crossed to a male from a true-breeding **w** strain. The females in the F_1 progeny have very pale peach colored eyes. Explain what this result tells you about the relationship between the **w** and **ap** mutations and why.

(b) What would you expect the phenotype of the male F_1 progeny to be and why?

(c) F_1 females are crossed to wild-type males, and 10,000 male progeny are examined. Most of these males have either white eyes or apricot eyes, however, one of the males have red eyes. What is the origin of this red-eyed male?

(d) What is the distance between the **w** and **ap** loci in cM?

(e) Crossveinless is a recessive phenotype caused by the **cv** mutation. The **cv** locus maps about 10 cM away from the **w** locus. A female from a true-breeding **cv**, **w** strain is crossed to a male from a true-breeding **ap** strain. The females from this cross are then crossed to wild-type males, and a very large number of the resulting male progeny are examined. Eight males with red eyes are found; seven of these have normal wings and one has crossveinless wings. Draw a map showing the relative positions of the **cv**, **w**, and **ap** loci.

(f) Why don't the distances between the farthest genes on a map equal the sum of the distance in between them?

2. **(16 pts)** You are studying a recessive trait in a diploid rodent species in which XX organisms are female and XY organisms are male. This trait is determined by a single gene, but you have no idea where in the genome this gene is located. This rodent has 20,000 distinct genes in its genome, 400 of which are found on the X chromosome.

(a, 4pts) Given this information, give your best estimate of the probability that the trait you are studying is X-linked.

(b, 12pts) You mate a female rodent displaying the trait to a wild-type male. You then mate an F1 female to a wild-type male to produce F2 offspring, and analyze only the F2 males. The first three F2 male offspring display the wild-type phenotype. Given that the first three male F2 offspring show the wild-type phenotype, determine the probability that the trait you are studying is X-linked. Show **all** steps of your work, using clear labels.

3. For the following merodiploid E. coli strains, determine the level ß-galactosidase expression in either the presence or absence of IPTG. Assume that, when no repressor is bound to DNA, 100 units of ß-galactosidase activity are produced from each functional copy of the **LacZ** gene. Assume that, when repressor is fully bound to DNA, only 1 unit of enzyme is produced for each functional copy of **LacZ**. Finally, assume that the presence of **Lac I^{-d}** protein will fully prevent all other forms of the repressor in the same cell from binding to DNA.

lac I^{-d} Z^{+} / F' lac I^{+} Z^{-}

lac O^{+} Z^{-} / F' lac O^{c} Z^{+}

lac I^{+} Z^{-} Y^{-} / F' lac I^{s} Z^{+} Y^{+}

lac I^{+} O^{c} Z^{+} / F' lac I^{-d} O^{+} Z^{+}

lac I^{+} O^{c} Z^{+} / F' lac I^{s} O^{+} Z^{+}

lac I^{-d} O^{+} Z^{+} / F' lac I^{s} O^{+} Z^{+}

lac I^{-d} O^{c} Z^{+} / F' lac I^{s} O^{+} Z^{+}

lac I^{-d} O^{c} Z^{-} / F' lac I^{s} O^{+} Z^{+}

4. You are given a double mutant E. coli strain that you know contains an F' plasmid that carries the Lac genes, but you don't know precisely which alleles of these Lac genes are on the chromosome of the strain, or on the F' contained in the strain.

(a) First you test the Lac phenotype of the strain and find that it expresses ßgalactosidase constitutively. Next you set up a mating of your strain to an F– strain that has a chromosomal deletion of the Lac genes -- you find that the strains that received the F' Lac expresses ß-galactosidase in a normally-regulated fashion. Given these observations, propose what the two lac mutations in this original strain were, and whether they were on the chromosome or on the F' plasmid. List all possibilities.

(b) An F' plasmid carrying a segment of chromosomal DNA can occasionally recombine
with the homologous chromosomal sequences to produce an Hfr strain. Starting with the F' strain that you were given originally in the introduction to this problem, you isolate a number of derivatives that have become Hfrs. You can deduce the structure of these different Hfrs by mating each Hfr strain to an F– strain that has a chromosomal deletion of the Lac genes, and then testing the resulting strains for the properties of the Lac operon that are transferred at early times after mating. By performing this test, you find that you have three isolated different Hfr strains from your original F' strain:

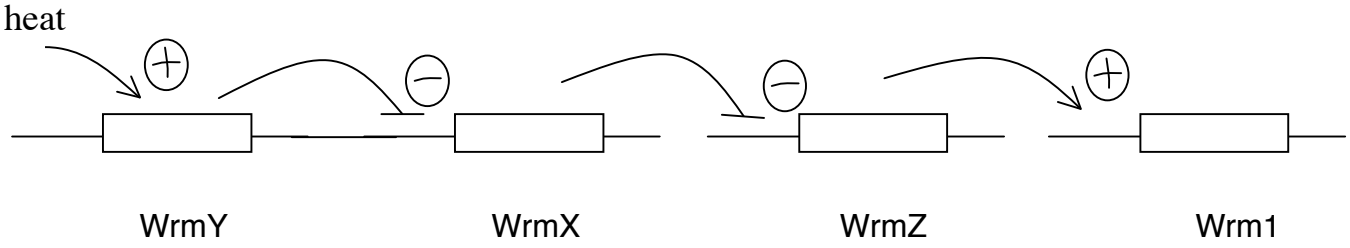
-- one Hfr strain transfers a Lac operon that expresses ß-galactosidase constitutively early
-- another Hfr strain transfers a Lac operon that gives uninducible expression early
-- and another Hfr strain transfers a Lac operon that shows normal regulation early.

Use this information to narrow down any ambiguities about the lac mutations you proposed to be in the original strain from part **(a)**.

(c) On the basis of these results, draw a map of the F' plasmid that existed in our original F' strain. On your diagram, show the direction of the origin of transfer relative to the arrangement of Lac genes.

(d) For each of the three Hfrs, show the F' factor recombining with the chromosome in the way that it must have done to create each Hfr strain. (Hint: to solve this tricky problem, it will help to draw out both possible orientations and then determine the behavior of all possible recombination events between the F' plasmid and the chromosome.)

2. You are studying regulation of the Wrm1 gene, a yeast gene that is expressed in response to heat. You isolate a wrm1::lacZ strain that expresses β-galactosidase when Wrm1 is normally expressed (which is at 36°C but not at 24°C). You use this wrm1::lacZ strain to perform a genetic screen looking for mutants that do not properly regulate expression of Wrm1. In your screen, you isolate a series of mutant strains that either show constitutive or uninducible expression of wrm1::lacZ.
Your results indicate that the following is the correct pathway for regulation of Wrm1 expression. Note that WrmY and WrmX are on the same chromosome, and that WrmX, WrmZ, and Wrm1 are all on different chromosomes.



One of the mutant strains you isolate contains a mutation called WrmX⁻, which is in the **coding region** of WrmX. You mate a WrmX⁻ wrm1::lacZ haploid strain to a wrm1::lacZ haploid strain. The resulting diploids are white on X-gal plates that are incubated at 24°C, and are blue on X-gal plates that are incubated at 36°C.

(a) Classify the WrmX⁻ mutation as constitutive OR uninducible.

(b) Classify the WrmX⁻ mutation as dominant OR recessive.

(c) Classify the WrmX locus as cis-acting OR trans-acting with respect to Wrm1.

You next isolate a mutant strain containing a mutation called WrmY⁻, which is in the **coding region** of WrmY. You mate a WrmY⁻ wrm1::lacZ haploid to a wrm1::lacZ haploid. The resulting diploids are white on X-gal plates, regardless of the temperature at which the plates are incubated.

(d) Classify WrmY⁻ by the type(s) of mutation it could be **with respect to Wrm1**. (Your choices are: repressor –, activator –, UAS–, URS–, super activator, super repressor, dominant negative repressor, dominant negative activator.)

You create diploid yeast by mating WrmX$^-$ WrmY$^-$ wrm1::lacZ haploid yeast to wrm1::lacZ haploid yeast. Sporulation of these diploids yields two types of tetrads, and you correctly conclude (given the number of each type of tetrad) that the WrmX and WrmY loci are linked at a distance of 2.22 cM.

(e) Depicted below are the two types of tetrads that resulted when you sporulated the above diploids. For each type of tetrad, state **how many** you found of that tetrad (out of a total of 90 tetrads), **classify** the tetrad as PD, NPD, or TT, and **color in** all of the spores that would be blue on each of the following Petri plates.

Tetrad Type A
Number of these tetrads out of a total of 90: _____
Classification of these tetrads (PD, NPD, or TT): _____

Color in the spores that would be blue in color when growing on the following plates:

X-gal, 24°C	X-gal, 36°C
○	○
○	○
○	○
○	○

NOTE that the two plates are replicas, so the top spore on the left plate has the same genotype as the top spore on the right plate.

Tetrad Type B
Number of these tetrads out of a total of 90: _____
Classification of these tetrads (PD, NPD, or TT): _____

Color in the spores that would be blue in color when growing on the following plates:

X-gal, 24°C	X-gal, 36°C
○	○
○	○
○	○
○	○

21. You are studying a recessive eye-color mutant phenotype (called *pinkeye*) in the mouse. You have mapped the pinkeye locus down to a small interval that contains two genes, gene A and gene B. The *pinkeye* gene has not yet been defined at a molecular level, but you are confident that either gene A or gene B must be the site of the *pinkeye* mutation. You have pieces of genomic DNA that contain either wild-type gene A or wild-type gene B. In this problem, you will be asked to create two genetically engineered mice. When creating engineered mice, the following 8 steps need to be considered.
For each mouse you make, please state:

i) whether you are using pronuclear injection or gene targeting techniques
ii) what DNA you would introduce into the mouse cells (also draw the DNA)
iii) whether you would put the DNA into a fertilized egg or ES cells
iv) what is the genotype of the fertilized egg or the ES cells you would start with
v) where in the mouse genome the DNA you introduced would integrate
vi) whether creating the mouse should involve the generation of a chimera or not
vii) which additional breeding steps you would do to make the mouse you wanted
viii) two possible phenotypic results you could get from the newly made mice, and the corresponding conclusions you would make based on each result

(a) Propose an experiment involving one or more gene-targeting constructs (but no transgenes) that would test whether the **phenotypically defined** *pinkeye* mutation is in the **molecularly defined** gene A.

(b) Propose an experiment involving one or more transgenes (but no gene-targeting constructs) that would test whether the **phenotypically defined** *pinkeye* mutation is in the **molecularly defined** gene A.

22. An autosomal recessive inherited disease with a selective disadvantage of 0.1 occurs at a frequency of 10^{-4} in a randomly mating population.

(a) Say that the allele frequency for the disease was set by a balance between new mutations and selection against the homozygote. In any given generation, what fraction of the disease alleles within the population are new mutations? (You can use approximations that are accurate to within 10%).

(b) Now consider the effect of consanguious matings among members the Aztec royal family. For an autosomal recessive trait that is present in the population at a frequency of 10^{-2} , give the probability that a child with the trait will be produced by the following types of matings. Assume that selection and mutation rates are negligible.

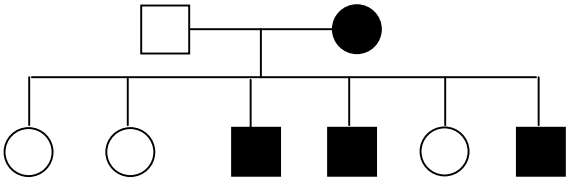
Mating between two unrelated individuals:

Brother-sister mating:

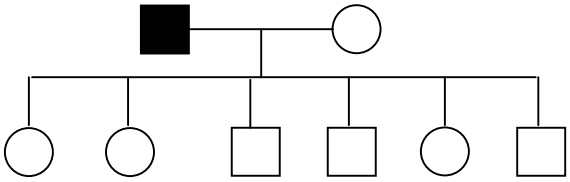
(c) Now consider an X-linked recessive trait that is present at a frequency of 10^{-2}. Give the probability that a child with the trait will be produced by a mating between two unrelated individuals. (You can use approximations that are accurate to within 10%, and you can assume that that the fitness of individuals with the trait is 1.0, the mutation rate is zero, and that the sex of the child is not known.)

21. The following three crosses involve mice from either true-breeding mutant strains or true-breeding wild-type strains. For this problem, you can assume that a mouse is true-breeding if its parents are not shown in the pedigree. In each case, mice exhibiting the rare mutant traits are indicated by solid symbols. Square symbols designate males and circles designate females. In each case, describe the mode(s) of inheritance that are consistent with the data (your choices are: autosomal recessive, autosomal dominant, X-linked recessive, X-linked dominant). Assume that traits are completely penetrant, and that no new mutations have arisen.

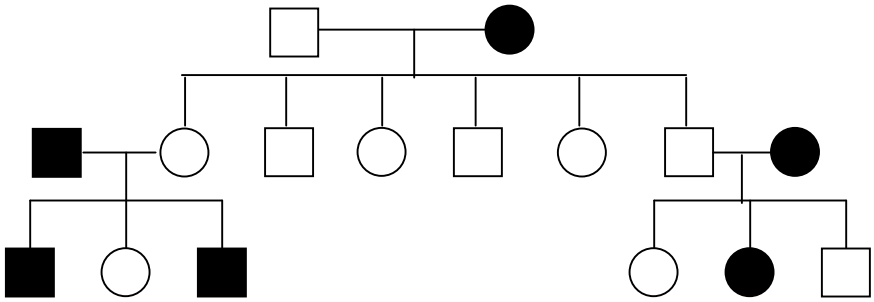
(a)



(b)

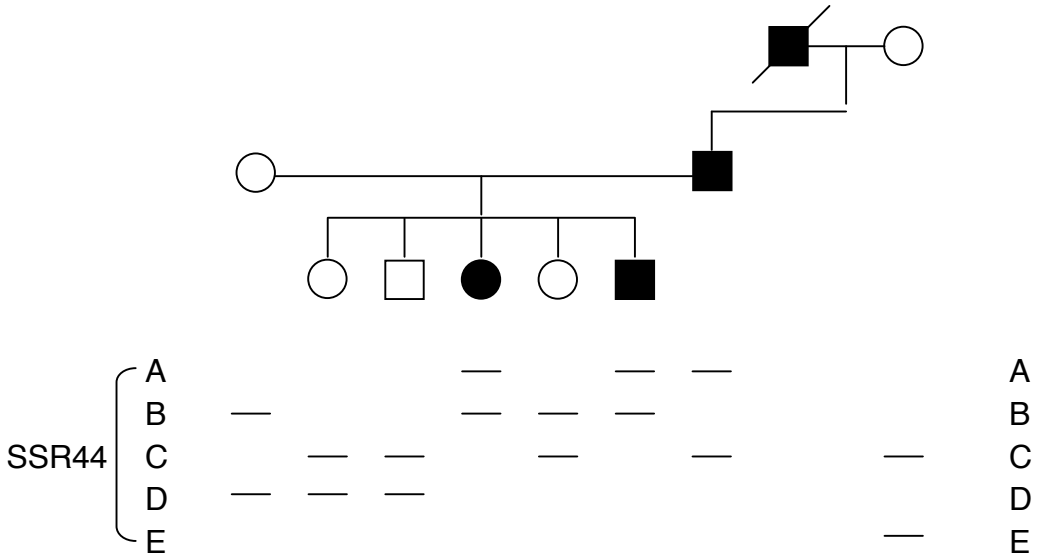


(c)



33. You are genetically mapping the locus that determines a rare skin disease that shows autosomal dominant inheritance.
Alleles: + (normal) SD (associated with skin disease)

Here is a family in which some individuals are affected. Assume complete penetrance and no new mutations.



(a) Which parent(s) is/are informative with respect to linkage between the skin disease gene and SSR44?

(b) What allele at SSR44 did the affected father inherit from his (deceased) father?

(c) Diagram the phase relationship between the skin disease locus alleles and the SSR44 alleles in the affected father.

(d) Calculate the LOD score for linkage at $\theta = 0.1$ between the skin disease gene and SSR44 in this family.

(e) How many families of this exact type would be needed to achieve a publishable LOD score at $\theta = 0.1$?

	TRANSGENIC TECHNIQUES	GENE TARGETING TECHNIQUES
What types of modifications can you make?	Addition of new genes	Deletion or replacement of existing genes
What stage of developing embryos do the donor mothers donate?	Fertilized Egg Cells (in the male pronuceus)	Embryonic Stem Cells from blastocyst inner cell mass
At what site does the construct integrate?	Randomly	At the the specific gene you are targeting
By what type of recombination does the construct insert?	Non-homologous	Homologous
How do you select for the integration event?	No selection- event occurs so frequently that it is not necessary	Select for a resistance gene and against the TK^{hsv} gene
Which part of the mouse DNA has been deleted?	No deletion	The targeted gene
Does every cell in the newly born mouse have the construct?	Yes	No, only those derived from the embryonic stem cells that the gene was added to
Have you created a chimeric mouse?	No	Yes
What additional breeding is now required?	None	Breed chimeras to wildtype and select for heterozygotes. Breed heterozygotes and select for homozygotes.
Can your constructs lead to visible dominant **and** recessive phenotypes?	Dominant only (gain of function)	Dominant and recessive

Autosomal Recessive (q = recessive allele):

Genotype	frequency	after selection	Δ frequency
A/A	p^2	p^2	0
A/a	$2pq$	$2pq$	0
a/a	q^2	$q^2 (1 - S)$	$-Sq^2$

$$\Delta q_{sel} = -Sq^2$$

Autosomal Dominant (q = dominant allele)

Genotype	frequency	after selection	Δ frequency
A/A	–	–	–
A/a	$2pq \approx 2q$	$(1 - S)\, 2q$	$-2Sq$
a/a	p^2	p^2	0

$$\Delta q_{sel} = {}^1/_2\, [\Delta f(\mathbf{A/a})] = {}^1/_2\, (-2Sq)$$
$$= -Sq$$

X-linked recessive (q = recessive allele)

Genotype	frequency	after selection	Δ frequency
X^A y	p	p	0
X^a y	q	$(1 - S)q$	$-Sq$

$$\Delta q_{sel} = {}^1/_3\, [\Delta f(\mathbf{X^a\ y})] = {}^1/_3\, (-Sq)$$
$$= -Sq/3$$

Autosomal Recessive, Heterozygote Advantage (q = recessive allele)

Genotype	frequency	after selection	Δ frequency
A/A	p^2	p^2	0
A/a	$2pq \approx 2q$	$(1 + h)\, 2q$	$2hq$
a/a	q^2	$(1 - S)q^2$	$- Sq^2$

$$\Delta q = \Delta f(\mathbf{a/a}) + {}^1/_2\ \Delta f(\mathbf{A/a}) = -Sq^2 + {}^1/_2(2hq)$$

$$= -Sq^2 + hq$$