

Exam Questions from Exam 3 – Eukaryotic Gene Regulation, Genome Modifications in Eukaryotes, Population Genetics

1. Consider an autosomal recessive trait that occurs at a frequency of 10^{-6} in a specific human population that is at Hardy-Weinberg equilibrium (ie. random mating is occurring). When answering the following parts, show all of your calculations.

(a) Draw a pedigree below that shows a mating between two relatives that would correspond to an inbreeding coefficient that equals 0.007813. Denote the mating between relatives with a double-bar connecting the two related parents. Start your pedigree with the common pair of ancestors and end your pedigree with the two related parents who are mating.

(b) Now say that all matings in Generation X of the given population are either between unrelated individuals, or have the same inbreeding coefficient as the mating described in part **(a)**. If the incidence of the trait in Generation “X+1” increases to a frequency of 2×10^{-6} , what percentage of matings in Generation X must have been between **unrelated** individuals?

(c) Now assume that this autosomal recessive trait causes lethality in childhood. If a constant percentage of matings are between related parents for many generations, would you predict that q would increase OR decrease?

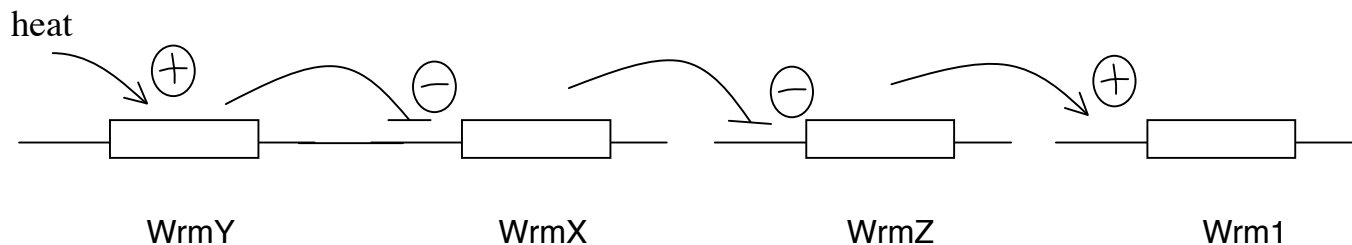
(d) A constant percentage of matings has occurred between related parents for many generations, and yet you find experimentally that q has not changed. Of the three choices below, circle **ALL** that could potentially act against the effect you chose in part **(c)** in order to keep q constant. Explain in one sentence why you chose or did not choose each option.

Choice A: migration

Choice B: heterozygote advantage (Aa over AA)

Choice C: mutation

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

-
-
-
-

3. The scenario in this question asks a biological question that can be addressed by creating genetically engineered mice. When creating engineered mice, the following 8 steps need to be considered. **For the 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

“Non-homologous end joining” is the process by which a DNA sequence gets inserted into a chromosomal region to which it is not homologous. Having a functional copy of the gene “NheJ” is necessary for this process to occur in mice. A mouse with no copies of the NheJ gene is sensitive to irradiation as an adult, but a heterozygote is not sensitive.

You decide to test whether one copy of the *Drosophila* “d-Nhe” gene could fully compensate for the absence of the mouse NheJ gene. You have wild-type homozygous mice (NheJ⁺/NheJ⁺), heterozygous mice (NheJ⁺/NheJ⁻), and homozygous mutant mice (NheJ⁻/NheJ⁻) readily available to you.

4. You are studying how yeast cells grow on the sugar maltose as a carbon source. You find that the sugars maltose and glucose both affect the regulation of the principal enzyme for maltose utilization, which is called maltase. In yeast cells grown without maltose, maltase is not expressed, but maltase is induced when maltose is added to the growth medium. In cells grown in medium that contains both maltose and glucose, maltase is not expressed. You have isolated mutations in three different genes that alter maltase regulation, called **A⁻**, **B⁻** and **C⁻**. All three mutations give recessive phenotypes, and none of the three loci are linked either to maltase or to each other. The maltase expression of wild-type and each of the three mutants are shown below.

| | <u>Maltase activity</u> | | |
|-----------------------|-------------------------|----------|--------------------|
| | - maltose | +maltose | +maltose & glucose |
| Wild type | - | + | - |
| A ⁻ | + | + | - |
| B ⁻ | - | - | - |
| C ⁻ | - | + | + |

(a) For each of the three wild-type genes: state whether it encodes a positive regulator or a negative regulator of maltase, and state whether it affects maltase regulation by maltose or glucose.

A

B

C

Next you cross an **A**⁻ haploid mutant to a **B**⁻ haploid mutant, and induce sporulation of the resulting diploid. After the resulting tetrads are dissected and evaluated for maltase expression in either the presence or absence of maltose, the following tetrad types are observed:

| <u>Type 1</u> | <u>Type 2</u> | <u>Type3</u> |
|---------------|---------------|--------------|
| constitutive | constitutive | constitutive |
| constitutive | constitutive | constitutive |
| regulated | regulated | uninducible |
| uninducible | regulated | uninducible |

(b) What is the phenotype of the **A**⁻ **B**⁻ double mutant haploid yeast strain? Explain how you arrived at your answer.

(c) Draw a genetic pathway showing the interactions between the different regulatory factors encoded by the wild-type **A**, **B**, and **C** genes. Be sure to include the maltase gene and to indicate where and how the sugars glucose and maltose act.

Next, you construct a set of 50 base-pair deletions within the cis regulatory region of the maltase gene. The ability of each of these deletions to express maltase in cells grown on different sugars is shown below.

| | -300 | -250 | -200 | -150 | -100 | -50 | +1 | - maltose | +maltose | +maltose and glucose |
|----|-------|-------|-------|-------|-------|-------|-------|-----------|----------|----------------------------|
| | | | | | | | | | | |
| 1) | _____ | _____ | _____ | _____ | _____ | _____ | _____ | - | + | - |
| 2) | _____ | _____ | _____ | _____ | _____ | _____ | _____ | - | + | + |
| 3) | _____ | _____ | _____ | _____ | _____ | _____ | _____ | - | - | - |
| 4) | _____ | _____ | _____ | _____ | _____ | _____ | _____ | - | + | - |
| 5) | _____ | _____ | _____ | _____ | _____ | _____ | _____ | - | + | - |
| 6) | _____ | _____ | _____ | _____ | _____ | _____ | _____ | - | - | - |

(d) The **C** gene encodes a DNA-binding protein. Assuming that the product of gene **C** binds to the cis regulatory region of the maltase gene, to which 50 basepair long region is it most likely to bind?

(e) In general, upstream activation sequences function normally regardless of their spacing relative to the TATA box sequence that binds RNA polymerase. Which of the deletion mutants shown above show this to be true for the upstream activation sequence that responds to the sugar maltose?

5. You have a mutant mouse that displays the phenotype of white fur (instead of the wild-type color for this strain, which is brown fur). You have found that the white mouse is homozygous for a specific mutant allele in the “whfr” gene. You want to know if being homozygous for this allele is enough to cause the white fur phenotype, or if this mouse is white because of a combination of mutations that it contains. You decide to address this issue using a genetically engineered mouse.

For the mouse you make, please indicate:

1. The method you would use (transgene or gene targeting)
2. What DNA would be introduced into mouse cells
3. The cell type you would introduce the DNA into (fertilized egg or ES cells)
4. The genotype of the cell you would introduce the DNA into
5. The site of integration (i.e. which genomic locus the DNA would enter into)
6. Which additional breeding steps are required to make the mouse you want
7. The possible results of your experiment and how you would interpret each possible result

6. An allele that causes the recessive phenotype of microcephaly has a frequency $q = 0.0001$ in a randomly-mating population in Hardy-Weinberg equilibrium.

(a) What is the expected frequency of microcephalic individuals in this population?

(b) Mutations to produce new alleles for microcephaly occur at frequency of $\mu = 10^{-9}$. If the current frequency of microcephaly was established during human evolution at a time when the fitness of microcephalic individuals was zero but there was a selective advantage for individuals heterozygous for microcephaly, then what was the value of the heterozygous advantage h ?

(c) Say that, in modern times, the fitness of microcephalics increased to 0.5 and the heterozygous advantage has become zero. Assuming that these conditions hold for many centuries, what will the steady state allele frequency for the allele causing microcephaly eventually become?

7. You are studying a yeast strain that will grow using the sugar raffinose as a carbon source. The gene encoding the raffinase enzyme (Raf1) is expressed when raffinose is present, but it is not expressed when raffinose is absent. To study the regulation of Raf1, you construct a fusion of the Raf1 upstream regulatory sequences to the *E. coli* LacZ reporter gene and place this gene fusion (designated Raf1-LacZ) on an extrachromosomal plasmid. Yeast cells carrying this plasmid express β -galactosidase only in the presence of raffinose. You use this inducible reporter gene construct to identify two new regulatory mutants designated Raf2⁻ and Raf3⁻. The effect of these mutants on expression of the Raf1-LacZ reporter is shown below:

| | β -galactosidase activity | |
|-------------------------------|---------------------------------|--------------------|
| | <u>+ raffinose</u> | <u>- raffinose</u> |
| Wild type (Raf1-LacZ) | + | - |
| Raf2 ⁻ (Raf1-LacZ) | + | + |
| Raf3 ⁻ (Raf1-LacZ) | - | - |

You extend your analysis of these mutants by constructing three diploids strains (each also carrying the Raf1-LacZ reporter plasmid) with phenotypes shown below:

| | β -galactosidase activity | |
|---|---------------------------------|--------------------|
| | <u>+ raffinose</u> | <u>- raffinose</u> |
| Raf2 ⁻ / Raf2 ⁺ (Raf1-LacZ) | + | - |
| Raf3 ⁻ / Raf3 ⁺ (Raf1-LacZ) | + | - |
| Raf2 ⁻ / Raf3 ⁻ (Raf1-LacZ) | + | - |

(a) What do these results tell you about the relationship between the Raf2⁻ and Raf3⁻ mutations? Be as specific as you can about the conclusions that you can draw.

(b) Next, you induce sporulation of a diploid strain produced by crossing a $Raf2^-$ ($Raf1-LacZ$) haploid mutant to a $Raf3^-$ ($Raf1-LacZ$) haploid mutant. Out of a total of 100 resulting tetrads, 70 are Type One, 19 are Type Two, and 11 are Type Three.

| <u>Type One</u> | <u>Type Two</u> | <u>Type Three</u> |
|-----------------|-----------------|-------------------|
| constitutive | uninducible | regulated |
| uninducible | uninducible | regulated |
| uninducible | constitutive | uninducible |
| regulated | constitutive | uninducible |

Does a $Raf2^- Raf3^-$ double mutant haploid yeast strain show regulated, constitutive, or uninducible expression of the $Raf1-LacZ$ reporter?

(c) On the basis of your answer for part **(b)** and from the rest of the information given in this problem, diagram a linear genetic pathway to explain the regulation of $Raf1$. In your model, include the wild-type $Raf1$, $Raf2$ and $Raf3$ genes. Also show how the sugar raffinose itself might act.

(d) Say that you isolate an allele of the $Raf2$ gene that gives the dominant phenotype of uninducible expression of $Raf1-LacZ$ expression. This allele (designated $Raf2^U$) lies within the coding sequence of the $Raf2$ gene. You do a cross between a $Raf2^U$ ($Raf1-LacZ$) haploid mutant and $Raf2^-$ ($Raf1-LacZ$) haploid mutant strain. You then induce sporulation of this diploid. What kind(s) of tetrads would you expect to get and in what frequencies? (Specify the tetrad types in terms of the spore phenotypes with respect to $Raf1-LacZ$ expression, following the format of the list of tetrads shown in part **(b)**.)

8. Suppose that, in an isolated population, there exists a very rare inherited anemia which is autosomal recessive. Assume that selection and mutation rates are negligible.

(a) Given that the frequency of the allele for the anemia is q , calculate the probability that a child will be born with the anemia assuming random mating. Express your answer as a function of q .

(b) What is the probability (as a function of q) that a given individual in the population is a heterozygote? Use the approximation that is valid for a very small q .

(c) In this population, marriages between a niece and her biological uncle occur sometimes. Given that the niece in one such marriage is heterozygous for the allele for the anemia, what is the probability that her child will have the anemia?

(d) Given that uncle-niece marriages occur at a frequency of **0.008**, use the answers derived above to calculate the chance that a child born into the population will have the anemia and will have been produced by an uncle-niece marriage. Express your answer as a function of q .

(e) If half of the children with the anemia come from uncle-niece marriages and half come from marriages with no obvious inbreeding, what is q ? If helpful, you may use the approximation that the frequency of random marriages is about one.

9. You are studying the *sihZ* gene in mice, and you isolate a mutation called “regX” that disrupts proper transcriptional regulation of the *sihZ* gene. This mutation causes the dominant phenotype of constitutive expression of *sihZ*. The mutation maps to within a thousand base pairs of the coding sequence of the *sihZ* gene. You want to know whether regX is a mutation in a non-coding DNA sequence that controls *sihZ* expression, or whether regX is a mutation in a gene near to *sihZ* that encodes a regulatory protein for *sihZ*. You decide to address this issue by creating a genetically engineered mouse.

(a) Would you choose to utilize transgenic or gene-targeting technologies to make your genetically modified mouse?

(b) What exact type of modification to the mouse genome would you make to test your hypothesis?

(c) Draw the DNA construct that you would use to modify the mouse genome, and explain how your construct would integrate into the mouse genome.

(d) What cell type would you put your DNA construct into? Include your choice of fertilized egg or ES cell, and what the genotype of the cells you would use should be.

(e) Explain what (if any) steps you will need to do to proceed from part (d) above to obtaining the final modified mouse you actually want.

(f) Name the two possible phenotypic results you could get from the newly made mice, and the corresponding conclusions you would make based on each result.

10. A early nonsense mutation in the yeast **URA9** gene gives an intermediate level of growth without the nucleotide uracil being provided in the growth medium (Ura^{+/-}). From the **ura9⁻** strain, you isolate a robust Ura⁺ derivative (strain 1) which you then cross to a haploid wild-type (**URA9⁺**) strain. You induce sporulation of the resulting diploids, and the tetrads you observe are as follows:

| | | |
|--------------------|---|---|
| 4 Ura ⁺ | 2 Ura ⁺ : 2 Ura ^{+/-} | 3 Ura ⁺ : 1 Ura ^{+/-} |
| 101 | 98 | 414 |

(a) What genetic event occurred to give robust growth without uracil in strain 1?

Next, starting with the **ura9⁻** strain, you isolate a completely Ura⁻ derivative (strain 2) which does not grow without uracil. You cross a haploid strain 2 with a haploid wild-type (**URA9⁺**) yeast strain. When you induce sporulation of the resulting diploids, the tetrads you observe are as follows:

| | | |
|---|----------------------|--|
| 2 Ura ⁻ : 2 Ura ⁺ | 4 Ura ^{+/-} | 2 Ura ^{+/-} : 1 Ura ⁻ : 1 Ura ⁺ |
| 94 | 97 | 385 |

(b) What genetic event occurred to give the Ura⁻ phenotype in strain 2?

(c) Next, you cross a haploid strain 1 to a haploid strain 2, and induce sporulation of the resulting diploids. The first 30 tetrads all show 2 Ura⁺ : 2 Ura⁻ segregation. Explain these data with respect to what they tell you about the mutations in strains 1 and 2.

(d) Finally, you look at 10 more tetrads from the cross in part (c) and find two that consist of 2 Ura⁺ spores: 1 Ura^{+/−} spore: 1 Ura⁻ spore. Explain these data with respect to what they tell you about the mutations in strains 1 and 2.

11. Trekking in the Himalayas, you discover a “founder generation” of 1000 goats barricaded on all sides by high peaks and massive glaciers. This founder generation consists of 200 **AA** goats, 200 **Aa** goats, and 600 **aa** goats.

(a) What are the frequencies of alleles **A** and **a** in the founder generation?

(b) Is the founder generation in Hardy-Weinberg equilibrium? Show your work.

(c) What is the frequency of the **A** allele in the second generation (that is, in the generation after the founder generation)? (Mating of the founder generation goats is random, fitness does not differ among the three genotypes, and mutation occurs at a negligible rate.)

(d) What are the frequencies of the **AA**, **Aa**, and **aa** genotypes in the second generation?

12. The scenario in this question asks a biological question that can be addressed by creating genetically engineered mice. When creating engineered mice, the following 8 steps need to be considered. **For the 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

You have a colony of mutant mice that are unable to smell perfume because they are homozygous for a loss-of-function mutation in the PrfM gene (a gene that you have recently identified and cloned). In that colony, you isolate one mouse with one copy of an unmapped suppressor mutation, SupR; that mouse can now smell perfume. You want to determine whether SupR is an extragenic suppressor mutation or an intragenic suppressor mutation. Do this by creating one type of genetically engineered mouse. (You have wild-type mice, and the mice mentioned above, available to you.)

13. You are studying the regulation of Gln1, a yeast gene involved in glutamine synthesis. Gln1 is not expressed when glutamine is present in the growth medium and is expressed when glutamine is absent. To begin your analysis of regulation, you fuse the promoter region of the Gln1 gene to the LacZ coding sequence and then place this hybrid gene on a yeast plasmid. You find that yeast cells carrying this plasmid (P_{Gln1} -LacZ) only express β -galactosidase activity when glutamine is absent. You next identify two different mutants that show abnormal regulation of your reporter. You call these mutants Gln7⁻ and Gln8⁻. The table below shows the phenotypes of a variety of haploid and diploid yeast strains containing the P_{Gln1} -LacZ reporter. A filled circle indicates a yeast colony that expresses β -galactosidase activity.

| | β -galactosidase activity | |
|---|---------------------------------|--------------------|
| | <u>- glutamine</u> | <u>+ glutamine</u> |
| wild type (P_{Gln1} -LacZ) | ● | ○ |
| Gln7 ⁻ (P_{Gln1} -LacZ) | ● | ● |
| Gln7 ⁻ / Gln7 ⁺ (P_{Gln1} -LacZ) | ● | ○ |
| Gln8 ⁻ (P_{Gln1} -LacZ) | ○ | ○ |
| Gln8 ⁻ / Gln8 ⁺ (P_{Gln1} -LacZ) | ● | ○ |

When you mate a Gln7⁻ (P_{Gln1} -LacZ) mutant to a Gln8⁻ mutant, the resulting Gln7⁻ / Gln8⁻ (P_{Gln1} -LacZ) diploid shows normal β -galactosidase expression and regulation. After sporulation, this diploid produces three different tetrad types. Out of a total of 50 tetrads, five are Type 1, thirty-eight are Type 2, and seven are Type 3.

| <u>Type 1</u> | | <u>Type 2</u> | | <u>Type 3</u> | |
|---------------------------------|--------------------|---------------------------------|--------------------|---------------------------------|--------------------|
| β -galactosidase activity | | β -galactosidase activity | | β -galactosidase activity | |
| <u>- glutamine</u> | <u>+ glutamine</u> | <u>- glutamine</u> | <u>+ glutamine</u> | <u>- glutamine</u> | <u>+ glutamine</u> |
| ● | ○ | ● | ● | ● | ○ |
| ● | ● | ● | ● | ● | ● |
| ● | ● | ○ | ○ | ○ | ○ |
| ● | ○ | ○ | ○ | ● | ● |

(a) Is a Gln7⁻ / Gln8⁻ double mutant regulated, constitutive, or uninducible?

(b) Are the Gln7⁻ and Gln8⁻ mutations linked? If so, how far apart are they in cM?

(c) In the 50 tetrads you analyze, there are a total of 200 spores. Out of those 200 spores, 17 are Gln7⁻ Gln8⁻ double mutant spores. How many of those 17 came from NPDs?

(d) On the basis of your answer for part (a) and from the rest of the information given in this problem, diagram a linear genetic pathway to explain the regulation of the Gln1 gene. Your model should include the wild-type Gln7, Gln1, and Gln8 gene products, as well as glutamine.

14. Albinism is a rare condition that is inherited as an autosomal recessive phenotype in many animals, including humans. This phenotype is caused by the body's inability to make melanin, the pigment responsible for most of the black and brown coloration in animals. In a particular population of wild hamsters, albinism occurs in about 1 out of 5500 animals. Assume selection and mutation rates are negligible.

(a) In this population, what is the frequency of the allele responsible for albinism? (Assume Hardy-Weinberg equilibrium for this part of the question.)

(b) What are the inbreeding coefficients for the following hamster matings?

Uncle-niece:

Grandparent-grandchild:

(c) In this population of hamsters, what is the probability that an animal resulting from a 1st cousin mating will be albino?

(d) In this population of hamsters, 1 in every 800 matings is between 1st cousins. (Assume that all other matings are random.) In this population, what fraction of all albino offspring will come from 1st cousin matings?

15. The genetics of the eye disease known as retinitis pigmentosa (RP) are complex in humans, with many dozens of genes implicated. You decide to model this hereditary disease in mice using transgene and/or gene targeting methods.

For both **(a)** & **(b)** indicate:

1. The method you would use (transgene or gene targeting)
2. What DNA would be introduced into mouse cells
3. The cell type you would introduce the DNA into (fertilized egg or ES cells)
4. The genotype of the cell you would introduce the DNA into
5. The site of integration (i.e. which genomic locus the DNA would enter into)
6. Which additional breeding steps are required

(a) You identify a human family in which RP displays autosomal dominant inheritance and is caused by a specific missense mutation in the RP5 gene, on human chromosome #5. Describe how you would create a mouse model of this family's disease. (You have access to a piece of DNA that contains the mutant RP5 gene from an affected member of the family, a piece of DNA that contains the wild-type RP5 gene, and wild-type mice.)

(b) In another human family, you find that RP displays autosomal recessive inheritance and is caused by a loss-of-function mutation in the RP11 gene, on human chromosome #11. Describe how you would create a mouse model of this family's disease. (You have access to a piece of DNA that contains the mutant RP11 gene from an affected member of the family, a piece of DNA that contains the wild-type RP11 gene, and wild-type mice.)

(c) You also obtain a true-breeding strain of mice, called Rpx, which has retinitis pigmentosa. You do not observe retinitis pigmentosa among offspring of Rpx mice mated with wild-type mice. The Rpx mutant has not yet been characterized molecularly, but you suspect that the Rpx strain is mutant in the RP11 gene. Propose an experiment you could do using the mouse model you created in part **(b)** to test this hypothesis, without doing any DNA sequencing or further genetic modifications. Describe the two possible results of your experiment, and how you would interpret each result.

16. To study the regulation of yeast genes that are necessary for the utilization of the sugar sucrose, you construct a fusion of **Suc1** (a gene encoding a sucrose-hydrolyzing enzyme) to the *E. coli* gene for β -galactosidase. The resulting gene fusion **Suc1-LacZ**, located on an extrachromosomal plasmid, is expressed only when sucrose is provided to the yeast cells. A screen for mutations that affect the regulation of **Suc1-LacZ** has yielded two different mutations that you call **Suc2⁻** and **Suc3⁻**. The table below shows the behavior of the original mutants as well as heterozygous diploids produced by mating the mutants to wild type.

| | β -galactosidase activity | |
|---|---------------------------------|-----------------|
| | <u>- sucrose</u> | <u>+sucrose</u> |
| Wild type (Suc1-LacZ) | - | + |
| Suc2⁻ (Suc1-LacZ) | - | - |
| Suc2⁻ / Suc2⁺ (Suc1-LacZ) | - | + |
| Suc3⁻ (Suc1-LacZ) | + | + |
| Suc3⁻ / Suc3⁺ (Suc1-LacZ) | - | + |

(a) When you mate a **Suc2⁻** haploid mutant to a **Suc3⁻** haploid mutant, the resulting **Suc2⁻ / Suc3⁻** diploid shows normal expression and regulation of **Suc1-LacZ**. What does this result tell you about the relationship between the **Suc2⁻** and **Suc3⁻** mutations?

(b) Next, you induce sporulation of the **Suc2⁻ / Suc3⁻** diploid and dissect 50 tetrads. Among the tetrads, 10 are Type One, 5 are Type Two, and 35 are Type Three.

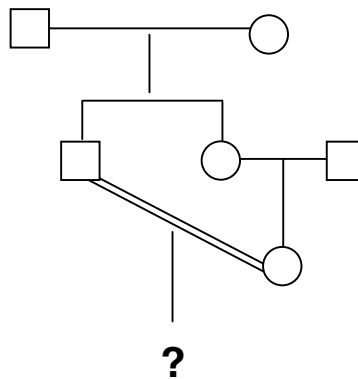
| <u>Type One</u> | <u>Type Two</u> | <u>Type Three</u> |
|-----------------|-----------------|-------------------|
| constitutive | uninducible | regulated |
| uninducible | uninducible | constitutive |
| uninducible | regulated | uninducible |
| constitutive | regulated | uninducible |

Are the **Suc2** and **Suc3** loci linked? Briefly explain your answer.

(c) Is a **Suc2⁻ Suc3⁻** double mutant regulated, constitutive, or uninducible? Briefly explain your answer.

(d) On the basis of your answer for part (c) and from the rest of the information given in this problem, diagram a linear genetic pathway to explain the regulation of the **Suc1** gene. For your model, include the wild-type **Suc1**, **Suc2** and **Suc3** genes. Also show how the sugar sucrose itself might act.

17. Diagrammed below is a consanguineous mating of an uncle and niece.



(a) Calculate the inbreeding coefficient for this mating.

(b) Calculate the expected number of genes at which the resulting child will be homozygous by descent. (Assume that there are 30,000 genes in the human genome.)

Now consider a rare disease (fatal in childhood) whose incidence in a random-breeding population is four per million births. In parts (c) and (d), calculate the incidence of the disease in the next generation assuming that 1% of all matings are between uncles and nieces (and all other matings are random), given that:

(c) The disease exhibits autosomal dominant inheritance.

(d) The disease exhibits autosomal recessive inheritance.

Let's now return to the purely random-breeding population in which this rare disease (fatal in childhood) has an incidence of four per million births. What rate of mutation (per generation) is required to maintain this incidence, given that:

(e) The disease exhibits autosomal dominant inheritance.

(f) The disease exhibits autosomal recessive inheritance.

Assume now that a new therapy allows many children with the disease to survive, such that affected individuals end up having 80% as many offspring as the population average. After many generations of random mating, a new steady-state balance between mutation and selection is achieved. At this new steady state, what would the incidence of the disease be, given that:

(g) The disease exhibits autosomal dominant inheritance.

(h) The disease exhibits autosomal recessive inheritance.

18. You generate genetically engineered mice that are homozygous for a P_{amylase^-} LacZ transgene insertion. These mice display a serious heart defect. A reasonable explanation for this observation is that the transgene had randomly inserted into a gene required for heart development or function. This transgene-induced heart defect reminds you of a recessive phenotype associated with a mutation in *small heart* (**sh**), a previously identified locus on mouse chromosome 12. Mice that are homozygous for the **sh** mutation also have serious heart defects.

Propose a breeding experiment to test the hypothesis that the **sh** mutation is in the same gene as the transgenic insertion mutation. Describe what two mice you would breed, the potential results of such a breeding, and how you would interpret each potential result.

19. Yeast cells have a set of enzymes that can synthesize the amino acid histidine. You select one of these enzymes, histidinol dehydrogenase, as a reporter gene to study the regulation of the histidine pathway. First, you learn that the **His4** gene is the structural gene for histidinol dehydrogenase, and that recessive **His4⁻** mutations cannot express histidinol dehydrogenase. You also find that the **His4** gene is regulated; it is expressed when histidine is absent from the medium, but is not expressed when histidine is present. These results are summarized below:

| | Histidinol dehydrogenase activity | |
|--|-----------------------------------|--------------------|
| | <u>+ histidine</u> | <u>- histidine</u> |
| Wild type | - | + |
| His4⁻ | - | - |
| His4⁻ / His4⁺ | - | + |

(a) You isolate a mutant, designated **His10⁻**, which shows constitutive histidinol dehydrogenase expression. A cross of a **His4⁻** haploid mutant to a **His10⁻** haploid mutant gives diploids that show wild-type expression of histidinol dehydrogenase. What does this result allow you to conclude about the **His10⁻** mutant?

(b) When the diploids from part (a) are sporulated, the resulting tetrads are of three types with respect to the regulation of histidinol dehydrogenase. Out of a total of 50 tetrads, 30 are Type One, 16 are Type Two, and 4 are Type Three.

| <u>Type One</u> | <u>Type Two</u> | <u>Type Three</u> |
|-----------------|-----------------|-------------------|
| constitutive | uninducible | uninducible |
| constitutive | uninducible | uninducible |
| uninducible | constitutive | regulated |
| uninducible | regulated | regulated |

Are the **His4** and **His10** loci linked? If so, how far apart are they in cM?

(c) Next, you isolate a mutation, designated **His11⁻**, which causes uninducible histidinol dehydrogenase expression. A cross of a **His4⁻** haploid mutant to a **His11⁻** haploid mutant gives diploids that show wild-type expression of histidinol dehydrogenase. On the basis of this result and the results for parts (a) and (b), diagram two different possible linear genetic pathways that can explain the functions of the **His10** and **His11** gene products in the regulation of **His4**. Be sure to include wild-type **His4**, **His 10**, **His11**, and the amino acid histidine in your answer.

(d) A cross of a **His10**⁻ haploid mutant to an **His11**⁻ haploid mutant gives diploids that you induce to sporulate. You get the following tetrad types as a result. Out of a total of 50 tetrads, 35 are Type Four, 8 are Type Five, and 7 are Type Six.

| <u>Type Four</u> | <u>Type Five</u> | <u>Type Six</u> |
|------------------|------------------|-----------------|
| uninducible | uninducible | constitutive |
| uninducible | uninducible | constitutive |
| constitutive | regulated | uninducible |
| regulated | regulated | uninducible |

Is a **His10**⁻ **His11**⁻ double mutant regulated, constitutive, or uninducible?

(e) On the basis of your answer for part (d) and from the rest of the information given in this problem, diagram a linear genetic pathway that can explain the functions of the **His10** and **His11** gene products in the regulation of **His4**. Be sure to include wild-type **His4**, **His 10**, **His11**, and the amino acid histidine in your answer.

20. Consider an autosomal recessive disease in humans that is caused by possessing a specific loss-of-function allele at a single gene locus. Assume complete penetrance and no selection or new mutations.

(a) In population I, the disease has an incidence of 4×10^{-8} . Assuming that mating in the population is random, what is the frequency of the disease allele (**q**)?

(b) What fraction of all matings in population I are between heterozygotes? Show your calculations.

(c) A second population (population II) is also characterized by random mating, but here the disease has an incidence of 10^{-6} . Now suppose that human migration produces a new, mixed population, with 90% of the members of the new population deriving (randomly) from population I and the remaining 10% deriving (randomly) from population II. One generation later, what would the incidence of the disease be in the new, mixed population if mating were random? Show your calculations.

(d) What would the incidence of the disease be in the new, mixed population (from part c) if mating were strictly assortative (that is, if individuals originating from population II mated only with other individuals originating from population II and vice versa for population I)? Show your calculations.

(e) Assume that the new, mixed population from part **(c)** has undergone at least one generation of random mating. What is the probability that a child whose parents are first cousins will have the disease?

(f) Now assume that the disease allele in population I differs from (but is in the same gene as) that in population II. Without doing calculations, answer yes or no to the following two questions: Would you modify your response ...to question **(c)**?
...to question **(d)**?

(g) Now assume that the disease allele in population I is in a different gene from that in population II. Without doing calculations, answer yes or no to the following two questions: Would you modify your response ...to question **(c)**?
...to question **(d)**?

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 consanguineous 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 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.)