Name: _____key_____

7.03 Exam Three -- 2005 KEY

Name: _____

Exam starts at 11:05 am and ends at 11:55 am.

There are 8 pages including this cover page.

Please write your name on each page.

Only writing on the **FRONT** of every page will be graded.
(You may use the backs, but only as scratch paper.)

Question 1	**17 pts_____**
Question 2	**45 pts_____**
Question 3	**20 pts_____**
Question 4	**18 pts_____**
TOTAL	**out of 100_____**

Name: _____key_____

1. (17 pts) You are studying the expression of the yeast gene ProA that is necessary for the synthesis of the amino acid proline. ProA is normally expressed only when the cell is lacking supplemental proline in the growth medium. You isolate two haploid yeast strains (ProB$^-$ and ProC$^-$) that misregulate ProA expression.

You mate a ProB$^-$ haploid strain to a wild-type haploid strain. The resulting diploid expresses ProA properly.

You mate a ProB$^-$ haploid strain to a ProA$^-$ haploid strain. The resulting diploid expresses ProA properly.

You mate a ProA$^-$ ProC$^-$ haploid strain to a ProC$^-$ haploid strain. The resulting diploid expresses ProA when proline is present in the growth medium.

You mate a ProC$^-$ haploid strain to a ProA$^-$ haploid strain. The resulting diploid expresses ProA properly.

You mate a ProB$^-$ ProC$^-$ haploid strain to a wild-type haploid strain. The resulting diploid expresses ProA properly. You induce sporulation of this diploid, and examine 40 tetrads. 30 (of those 40) each contain: two spores that do not express ProA when proline is absent from the growth medium, one spore that expresses ProA when proline is present in the growth medium, and one spore that expresses ProA properly.

(a, 6pts) Classify the ProB$^-$ mutation by its genetic properties (cis vs. trans, constitutive vs. uninducible, dominant vs. recessive).

Trans, recessive, uninducible
Trans – You mate a ProB$^-$ haploid strain to a ProA$^-$ haploid strain. The resulting diploid expresses ProA properly. This means that the A and B mutations complement each other and are thus in different genes. This means that B must act in trans to A.
Recessive -- You mate a ProB$^-$ haploid strain to a wild-type haploid strain. The resulting diploid expresses ProA properly. This means B– is recessive.
Uninducible – The only mutant phenotypes you see when you sporulate a diploid that contains both the B– and C– mutations are constitutive and uninducible. C– gives constitutive, so B– must give uninducible.

(b, 6pts) Classify the ProC$^-$ mutation by its genetic properties (cis vs. trans, constitutive vs. uninducible, dominant vs. recessive).

Trans, recessive, constitutive

Trans – You mate a ProC$^-$ haploid strain to a ProA$^-$ haploid strain. The resulting diploid expresses ProA properly. This means that the A and C mutations

complement each other and are thus in different genes. This means that C must act in trans to A.
Recessive -- You mate a ProC⁻ haploid strain to a ProA⁻ haploid strain. The resulting diploid expresses ProA properly. This means C– is recessive.
Constitutive – You mate a ProA⁻ ProC⁻ haploid strain to a ProC⁻ haploid strain. The resulting diploid expresses ProA when proline is present in the growth medium. This means that a cell that has a functional copy of A but has no functional copies of C expreses ProA even when it is not supposed to (i.e. when the cell already has proline available to it).

(c, 5pts) If you a drew a linear pathway showing the regulation of ProA, which function would you place closer to ProA: ProB or ProC?

ProB

When you sporulate a diploid that was produced from a mating of B– C+ to B+ C–, you see mostly tetratypes. You know they are tetratypes because the types of spores do not come in pairs. (There are three types of spores.)
Each tetratype contains: two spores that do not express ProA when proline is absent from the growth medium (uninducible), one spore that expresses ProA when proline is present in the growth medium (constitutive), and one spore that expresses ProA properly (inducible). A tetratype resulting from this mating would contain the spores:

GENOTYPE	**PHENOTYPE**
B– C+	**uninducible**
B+ C–	**constitutive**
B+ C+	**inducible**
B– C–	**NOT KNOWN PREVIOUSLY**

The spore of unknown phenotype must be the double mutant, and it shows the single mutant phenotype of B (uninducible), so B must be more downstream in the pathway (i.e. closer to the reporter gene).

2. (45 pts) You are studying the transcriptional regulation of a mouse gene called *Stringy*. This gene is normally only expressed in tail cells due to the presence of a tail-specific inducer molecule in these cells. You have isolated two true-breeding mutant strains of mice that do not spatially regulate the expression of the *Stringy* gene properly. The strains of mice that you have, and their corresponding phenotypes, are listed in the table below.

Genotype of mouse	Phenotype of mouse
Wild-type	*Stringy* expressed only in tail

A^{-} / A^{-}	*Stringy* not expressed anywhere
B^{-} / B^{-}	*Stringy* expressed in all cells in the body

When you cross mice that are B^{-} / B^{-} to mice that are deficient in *Stringy*, the resulting mice only have *Stringy* expressed in the tail.
When you cross mice that are B^{-} / B^{-} to mice that are A^{-} / A^{-}, and then cross the resulting F1 mice to each other, you get a genotypic ratio in the F_2 that indicates that the A and B loci segregate independently of each other.

You inject a piece of DNA containing the A^{-} allele of the A gene into a fertilized egg produced by the mating of two true-breeding B^{-} mice. You then transfer this injected fertilized egg into a pseudopregnant mouse. The mouse that is born does not express *Stringy* in any cells in its body.

(a, 6pts) Classify the A^{-} mutation by its genetic properties (cis vs. trans, constitutive vs. uninducible, dominant vs. recessive).

Trans, dominant, uninducible

Trans, dominant, and epistatic to B -- You inject a piece of DNA containing the A^{-} allele of the A gene into a fertilized egg produced by the mating of two true-breeding B^{-} mice. You then transfer this injected fertilized egg into a pseudopregnant mouse. The mouse that is born does not express *Stringy* in any cells in its body. The phenotype you are seeing in this A+/A+/A– B–/B– mouse is the phenotype of A–, not the phenotype of B– (which is constitutive). This tells you three things:
1) A– is dominant. This mouse has 3 copies of A and two of them are wild-type, and yet you see the mutant phenotype. Thus A– must be dominant.
2) A– can affect Stringy in trans. A– must have randomly integrated into the mouse genome, and yet it can influence Stringy expression. This means that A is capable of acting on Stringy from a distance.
3) A is downstream of B. This mouse is a double mutant : A– and B–/B–. The phenotype shown is that of A–. Thus A must be downstream of B in the pathway.

Uninducible – Line #2 of the chart

(b, 6pts) Classify the B^{-} mutation by its genetic properties (cis vs. trans, constitutive vs. uninducible, dominant vs. recessive).

Trans, recessive, constitutive

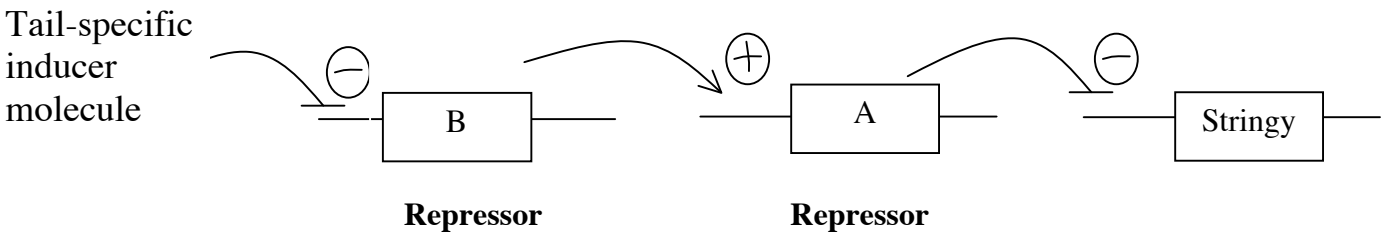
Name: _____key_____

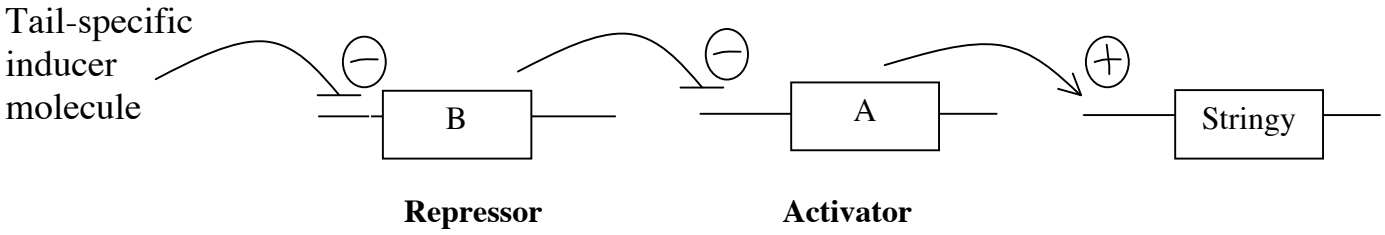
Trans -- When you cross mice that are B^{-} / B^{-} to mice that are deficient in *Stringy*, the resulting mice only have *Stringy* expressed in the tail. This means that B and Stringy complement each other, so B and Stringy must be in different genes. Thus B must act in trans on Stringy.

Recessive -- When you cross mice that are B^{-} / B^{-} to mice that are deficient in *Stringy*, the resulting mice only have *Stringy* expressed in the tail. This means that B– is recessive.

Constitutive – Line #3 of the chart

(c, 12pts) Draw TWO different linear genetic pathways that are consistent with your answers to parts **(a)** and **(b)**. Be sure to indicate the wild-type A, B, and *Stringy* genes in your model, and also include the tail-specific inducer molecule.





B must have a net negative effect because B is trans and, when you take B function away, you get constitutive expression of Stringy.

A's net effect is unknown, because the only mutation you have in A is a dominant mutation, and you cannot determine wild-type function from a dominant mutation.

The tail-specific molecule is the signal to start the pathway, and it must have a net positive effect because it is an inducer molecule.

In any gene regulation pathway, the reporter gene is always at the end (because it is the output), and the signal is always at the beginning (because it is the input).

We did an epistasis test when we made a transgenic mouse that was a B–/B– mouse with an A– transgene. A is dominant, so this mouse is a double mutant mouse that is mutant both in the A function and in the B function. Whichever phenotype this mouse displays is the phenotype of the single mutation in the more downstream gene in the pathway. This mouse shows uninducible expression of Stringy, so A is the most downstream gene.

(d, 6pts) Clearly state which one piece of information you would need to know in order to determine which of the models you drew in part **(c)** was correct.

You would need to know the wild-type function of the A gene. The A locus operates in trans, and thus it must either encode an activator or a repressor. However you do not know which one it encodes because the only mutation you have in A gives a dominant phenotype, and dominant mutations cannot be used to determine wild-type function. Given that you do not know the wild-type function of A, you do not know whether the mutant allele A– is a superrepressor allele or a dominant negative activator allele.

Most people wrote here that you needed an epistasis test, but in fact we already gave you an epistasis test when we made a transgenic mouse that was a B–/B– mouse with an A– transgene. A is dominant, so this mouse is a double mutant mouse that is mutant both in the A function and in the B function. Whichever phenotype this mouse displays is the phenotype of the single mutation in the more downstream gene in the pathway.

(e, 15pts) You want to distinguish between the two models listed in part **(c)**. You could do this by creating a genetically engineered mouse. **For the mouse you make**, please state:

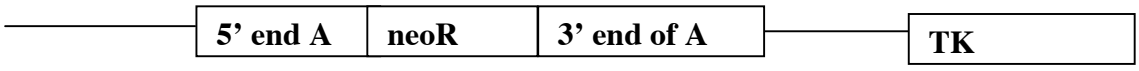
i) whether you are using pronuclear injection **or** gene targeting
ii) what **DNA** you would introduce into the mouse cells (also draw the DNA)
iii) what is the **genotype** of the fertilized egg or the ES cells you would start with
iv) which **additional breeding** steps you would do to make the mouse you wanted
v) **two possible** phenotypic results you could get from the newly made mice,
and the corresponding conclusion you would make for each result

Describe a way to create a genetically modified mouse that would allow you to gain the piece of information you stated in part **(d)** (and thereby distinguish between your models).

IF YOU GOT PART d) RIGHT, YOU NEED TO DETERMINE THE WILD-TYPE FUNCTION OF A:

i) gene targeting

ii)



iii) wild-type ES cells

iv) Mate the resulting chimera to wild-type to get out non-chimeric heterozygotes. Mate two heterozygotes together and 1/4 of them will be homozygous for the A gene knockout.

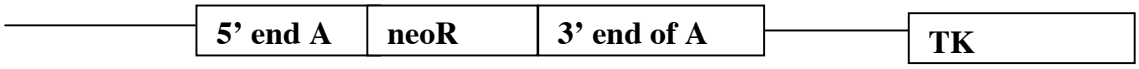
v) If the mice express Stringy everywhere, then the wild-type function of A is to be a repressor (in which case the A– allele was a superrepressor). If the mice express Stringy nowhere, then the wild-type function of A is to be an activator (in which case the A– allele was a dominant negative activator).

IF YOU GOT PART d) WRONG, YOU PROBABLY TRIED TO DO AN EPISTASIS TEST HERE, in which case you could have tried 4 different experiments:

<u>POSSIBILITY ONE</u> (best option of 4 b/c it also tells you the wt function of A)

i) gene targeting

ii)



iii) B–/B– ES cells

iv) Mate the resulting chimera to B–/B– mice to get out non-chimeric mice that are B–/B– A+/A^{KO}. Mate two of these mice together and 1/4 of them will be homozygous for the A gene knockout and will be B–/B–.

v) If the mice express Stringy nowhere, then the wild-type function of A is to be an activator, AND A is downstream in the pathway.

If the mice express Stringy everywhere, you can't really conclude anything because you don't yet know the loss-of-function phenotype of A. A could be a repressor, and if it were, this result would not give you order in the pathway because both single mutant phenotypes would be constitutive.

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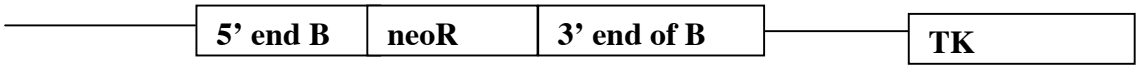
POSSIBILITY TWO

i) gene targeting

ii)



iii) A–/A– ES cells

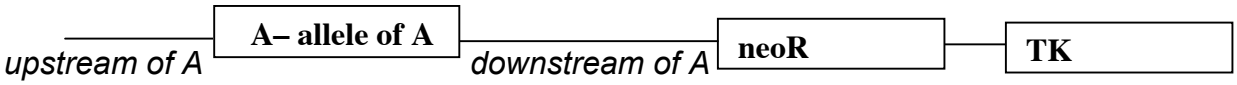
iv) Mate the resulting chimera to A–/A– mice to get out non-chimeric mice that are A–/A– B+/B–. Mate two of these mice together and 1/4 of them will be homozygous for the B gene knockout and will be A–/A–.

v) A–/A– mice are going to have uninducible expression of Stringy no matter what, because we basically made this mouse for you already in the introduction to this question. The only difference between your experiment and ours was that you ended up with an A–/A– mouse, and we ended up with an A–/A+/A+ mouse. However the result of the experiment would be the same. Thus your conclusion should have been that you know you would get the uninducible result (because that is what we told you happened in the introduction).

POSSIBILITY THREE

i) gene targeting

ii)



iii) B–/B– ES cells

iv) Mate the resulting chimera to B–/B– mice to get out non-chimeric mice that are B–/B– A+/A–.

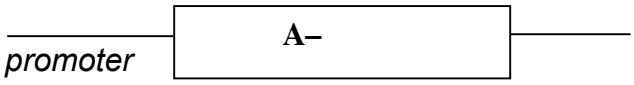
v) A– mice are going to have uninducible expression of Stringy no matter what. We basically did this experiment for you in the introduction to this question (except we added a copy of A– using pronuclear injection instead of gene targeting). Thus your conclusion should have been that you know you would get the uninducible result (because that is what we told you happened in the introduction).

Name: _____key_____

POSSIBILITY FOUR

i) pronuclear injection

ii)



iii) B–/B– egg

iv) no breeding is necessary but you could have bred the transgene to homozygosity if you wanted to

v) We actually did this experiment for you in the introduction to this question. Thus your conclusion should have been that you know you would get the uninducible result (because that is what we told you happened in the introduction).

3. (20 pts) For each situation below, predict whether the frequency of the ALLELE ("q") associated with the trait/disorder in consideration will **stay the same**, **rise**, or **fall**. If you cannot conclude, choose "***inconclusive***."

For parts **(a)**, **(b)** and **(c)**, assume that the mutation rate is zero.

(a, 5pts) There is a population in which a rare autosomal recessive trait (with S = 0, h = 0) exists. This population always mates randomly. All of a sudden, heterozygotes obtain a selective advantage.

The allele frequency "q" will: stay the same (rise) inconclusive fall
(CIRCLE ONE OF THE FOUR)

Heterozygotes are being selected for, and this is the only force acting upon q. The allele frequency q would therefore rise.

(b, 5pts) There is a population in which a rare autosomal recessive disorder (with **S = 1**, h = 0) exists. All of a sudden, this population goes from mating randomly to participating in some amount of inbreeding.

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The allele frequency "q" will: stay the same rise
(CIRCLE ONE OF THE FOUR)

inconclusive (fall)

Inbreeding increases the proportion of homozygotes in the population. Everytime a homozygote "aa" is created, those two alleles are removed from the population because the "aa" organism dies (S = 100%). Thus "a" alleles are constantly being removed from the population.

(c, 5pts) There is a population in which a rare autosomal **dominant** trait (with S = 0, h = 0) exists. All of a sudden, this population goes from mating randomly to participating in some amount of inbreeding.

The allele frequency "q" will: (stay the same) rise
(CIRCLE ONE OF THE FOUR)

inconclusive fall

Inbreeding does not affect autosomal dominant or X-linked traits in any way. This is because dominant traits only need to be inherited from one parent, and rare X-linked traits only affect males and are thus only inherited from the mother.

(d, 5pts) There is a population in which a rare autosomal recessive disorder (with **S = 1**, h = 0) exists. All of a sudden, this population goes from mating randomly to participating in some amount of inbreeding. Simultaneously, the mutation rate rises from being negligible to a rate that would now affect allele frequency.

The allele frequency "q" will: stay the same rise
(CIRCLE ONE OF THE FOUR)

(inconclusive) fall

Part "b" tells you that the combination of S=100% and inbreeding makes q go down, but mutation rates make q go up. It is unclear how large mu is, and how many people are inbreeding (and what relation these people are to each other), so you cannot conclude what the relative strength of these two forces are with respect to each other.

Name: _____key_____

4. (18 pts) Consider a gene in which mutations occur at a rate of 10^{-6}. Mutations in this gene will cause an autosomal recessive disease. Homozygotes for the allele associated with the disease have a fitness which is 10% that of those not carrying that allele. SHOW ALL OF YOUR WORK, indicate all equations you use, and use clear labels.

Note: If you need the quadratic formula, it is: $[-b +/- \sqrt{(b^2 - 4ac)}] / 2a$

(a, 6pts) Assume that, for many generations, this population has been at steady state because of a balance between mutation, selection for heterozygotes, and selection against affected individuals. Assume heterozygotes have a fitness which is 103% that of those not carrying the allele associated with the trait. Assume random mating. Calculate the steady-state value of q.

For this population to be at steady state:

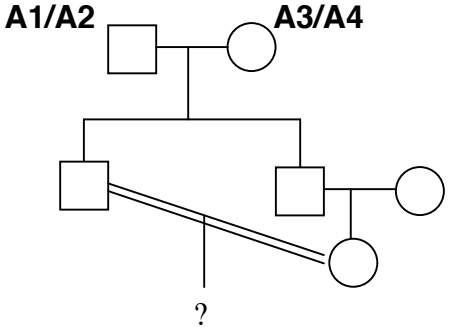
$$\Delta q_{\text{sel against homozygotes}} + \Delta q_{\text{sel for heterozygotes}} + \Delta q_{\text{mut}} = 0$$

$$-Sq^2 + hq + \mu = 0$$

S = 90%
h = 3%
mu = 10^{-6}
Solve for q using the quadratic equation

q = 0.033 (the other answer from the quadratic is negative)

(b, 5pts) Now assume that, for many generations, this population has been at steady state because of a balance between mutation, inbreeding, and selection against affected individuals (i.e. there is NO heterozygote advantage in this population). For a very long time, 15% of all children have been products of uncle-niece matings (and the remaining 85% have been products of random matings).
What is F equal to for an uncle-niece mating?



Chance (child is A1/A1) = (1/2) * (1/2) * (1/2) * (1/2) * (1/2) = (1/32)

F = chance (child is A1/A1) + chance (child is A2/A2) + chance (child is A3/A3) + chance (child is A4/A4)

F = 4 * (1/32)

F = 1/8

(c, 7pts) Calculate the steady-state value of q for the situation described in part **(b)**.

For this population to be at steady state:

$$\Delta q_{\text{sel against homozygs from random mating}} + \Delta q_{\text{sel against homozygs from inbreeding}} + \Delta q_{\text{mut}} = 0$$

$$(-Sq^2) * 85\% \quad + \quad (-SFq) * 15\% \quad + \mu = 0$$

S = 90%
mu = 10^{-6}
F = 1/8
Solve for q using the quadratic equation

q = 5.9 X 10^{-5} (the other answer from the quadratic is negative)