

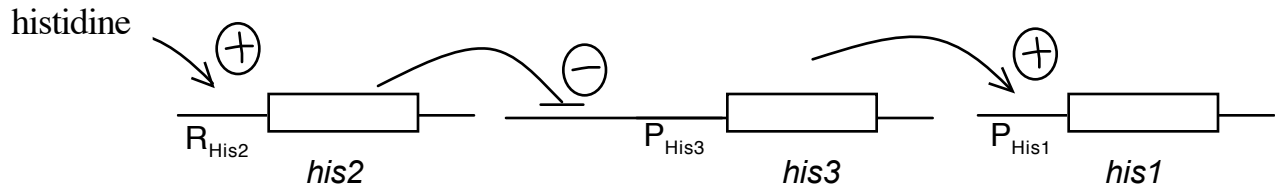
2005 7.03 Problem Set 5

Due before 5 PM on WEDNESDAY, November 16, 2005.

Turn answers in to the box outside of 68-120.

PLEASE WRITE YOUR ANSWERS ON THIS PRINTOUT.

1. You are studying the regulation of a yeast gene (*His1*), which is necessary for synthesis of the amino acid histidine. To begin your analysis of the regulation of *His1*, you fuse the *cis* regulatory region (“ P_{His1} ”) that lies upstream of the *His1* open reading frame to the *LacZ* coding sequence. You then place this hybrid gene on a yeast plasmid. This reporter gene construct behaves the way you expected based on the pathway for the regulation of *His1*, which is as follows:



Keep in mind that this model is a genetic pathway that should not be interpreted as a molecular model.

You monitor the expression of the *his1* gene using your reporter gene construct in order to perform a genetic screen looking for mutants that do not properly regulate expression of *his1*. In your screen, you isolate a series of haploid mutant strains that either show constitutive or uninducible expression of *his1*. You identify the genes that are mutated in the mutants you find, and discover that you have identified new alleles of two genes, *his2* (which lies on chromosome #1), and *his3* (which lies on chromosome #5).

In your screen, you isolate five strains, each of which contains one of the following single mutations:

his2a, which is in the **coding region** of *his2*. This mutation gives a recessive phenotype.

his2b, which is in the **coding region** of *his2*. This mutation gives a constitutive phenotype.

his3c, which is in the **coding region** of *his3*. This mutation gives a constitutive phenotype.

his3d, which is in the **coding region** of *his3*. This mutation gives a recessive phenotype.

R_{His2}^- , which is a deletion in the **cis regulatory region** in front of *his2*.

(a) Is *his2a* cis-acting or trans-acting with respect to *his1*?

Trans. *His1* and *his2* are 2 different coding regions that exist in separate parts of the genome. Thus *His2* acts in trans on *His1*. You can also see from the diagram that *His2* acts in trans on *His1*, because *His2* is drawn as a protein-encoding gene (indicated by an open box). If *his2* were in cis to *His1*, *His2* would have been drawn as a non-coding DNA sequence that was directly upstream of the *His1* open reading frame.

(b) Is R_{His2} cis-acting or trans-acting with respect to *his1*?

Trans. Although R_{His2} acts in cis to *his2*, *his2* then acts in trans with *his1*, so R_{His2} is also trans-acting with respect to *his1*.

(c) What is the phenotype of a *his2a his3d* double mutant with respect to expression of *his1*?

Uninducible. This is an epistasis test. The phenotype you see is the phenotype of the single mutation in the gene that acts closer to the reporter in the regulation pathway. Therefore you will see the *his3d* phenotype. Since you know that *his3d* is recessive, you know that it is a loss of function mutation in an activator gene, which gives you an uninducible phenotype.

(d) Would the *his3c* mutation give a dominant or recessive phenotype with respect to expression of *his1*?

Dominant. Because this mutation is in the coding region of an activator and gives a constitutive phenotype, it must be a gain of function mutation and therefore will be dominant. Also, if *His3c* is always activating *His1*, even if you add a regulated form of the *His3* gene, it cannot overpower the constant activation of *his1* by *his3c*. Note that *His3c* is a superactivator allele of the *His3* gene.

(e) What type(s) of mutation might *his2b* be with respect to *his1*? (Your choices are: repressor⁻, activator⁻, promoter⁻, UAS⁻, URS⁻, dominant negative repressor, dominant negative activator, super-repressor, super-activator.)

Dominant negative repressor or repressor⁻. We know that *his2* is a net repressor, so all alleles of the *his2* gene must be alleles of a gene that encodes a repressor. The only options listed that are alleles of a repressor gene are the two correct answers and superrepressor. The two repressor mutations that give constitutive phenotypes are dominant negative repressor and repressor⁻. (Superrepressor alleles cause uninducible phenotypes.) Since we don't know whether the *his2b* mutation is dominant

or recessive, we cannot determine whether it is a dominant negative repressor or repressor⁻ allele.

(f) You cross a *his3d* haploid mutant strain to a *his2a* haploid mutant strain. What is the phenotype of the resulting diploid with respect to expression of *his1*?

Inducible. Since *his3d* and *his2a* are both recessive mutations and are on separate genes, there will be complementation and you will get a wild-type phenotype. The cross you did is *his3d his2+* X *his3+ his2a*. The result from crossing two haploid yeast strains to each other is a diploid yeast, which in this case has the genotype: *his3d his2+ / his3+ his2a*. Note that there are wild-type alleles of both *his3* and *his2* in this diploid.

(g) You induce sporulation of the diploid from part (f). You analyze 90 tetrads. Three distinct tetrad types are obtained. Below, fill in each blank with the phenotype of each of the spores that is not provided to you.

Type 1: TT	Type 2: NPD	Type 3: PD
regulated (wt)	<u> </u> regulated <u> </u>	constitutive
uninducible <u> </u>	<u> </u> regulated <u> </u>	<u> </u> constitutive <u> </u>
uninducible <u> </u>	<u> </u> uninducible <u> </u>	<u> </u> uninducible <u> </u>
constitutive	<u> </u> uninducible <u> </u>	<u> </u> uninducible <u> </u>

If you induce sporulation of a diploid yeast that has the genotype *his3d his2+ / his3+ his2a*, the three types of tetrads you can get out are:

Type 1: TT	Type 2: NPD	Type 3: PD
3+ 2+	3+ 2+	3+ 2a
3d 2+	3+ 2+	3+ 2a
3d 2a	3d 2a	3d 2+
3+ 2a	3d 2a	3d 2+

Your parental spores will be *his2+3d* (which gives an uninducible phenotype) and *his2a3+* (which gives a constitutive phenotype because it is a loss of function mutation in a repressor). Therefore the PD will have 2 constitutive and 2 uninducible spores.

The non-parental spores will be his2+3+ (which will be regulated because it is a wild-type spore) and his2a3d (which is uninducible (see part c)). Therefore the NPDs will have 2 regulated and 2 uninducible spores.

The tetratypes will have 2 parental and 2 non-parental spores so there will be a regulated spore, 2 uninducible spores and 1 constitutive spore. Since this is the only type that has both regulated and constitutive spores, it must be Type 1, so we know that the two blanks are uninducible.

Type 3 must then be the PD because NPD does not have any constitutive spores. We can then fill in that there must be one more constitutive spore and two uninducible spores.

By process of elimination, Type 2 must be the NPD and have two regulated and two uninducible spores.

(h) How many "Type 3" tetrads would you have most likely observed?

15. Type 3 tetrads are the parental ditypes. The ratio of PD:TT:NPD is 1:4:1 when two genes are unlinked, and you know that his2 and his3 are unlinked because they lie on different chromosomes (see the introduction to this question). Therefore the PDs will be 1/6 of the total or 15/90.

2. You are studying the metabolism of a sugar called struliose by yeast cells. (Note that yeast will use struliose even when glucose is present.) You have already isolated one gene that is necessary for the use of struliose as a carbon source. This gene is induced whenever struliose is present. You want to do a genetic procedure (i.e. a screen or selection) to look for more genes involved in struliose metabolism, and you have two reagents that could help you do this. One reagent is a reporter gene that you have created by attaching the promoter region of the known struliose-utilization gene to the open reading frame for *E.coli lacZ*. The other reagent is a form of struliose (called toxo-struliose) that can be metabolized in the same way as struliose, but when it is metabolized, it creates a byproduct that is toxic to yeast cells. You have a collection of thousands of haploid yeast, and each yeast is mutant in a different gene. However, you don't know which of these yeast are mutant in "struliose metabolism" genes (versus which yeast are mutant in any of the other genes in the yeast genome that have nothing to do with struliose metabolism).

(a) Outline a genetic procedure that you would do to find more genes involved in struliose metabolism. In your procedure, use the reporter gene (but not toxo-struliose). To outline your procedure, include: **i)** the type(s) of growth medium you would plate your yeast mutants on (i.e. what would have to be added to a basic growth medium that contains

everything necessary for yeast to grow except a carbon source), **ii)** how you would identify the yeast mutants you are looking for (i.e. what would mutants and non-mutants look like on each type of growth medium), and **iii)** whether this method is a screen or a selection.

i) Plate cells on minimal medium + struliose + glucose + Xgal and also on plates with just minimal medium + glucose + Xgal. The X-gal will allow you to see whether or not the lacZ gene that is regulated by the struliose promoter is being expressed because the colonies will be blue when LacZ is expressed and white when it is not.

ii) Nonmutant colonies will be blue on struliose glucose Xgal plates and white on glucose Xgal plates. Mutants will be either blue on both plates (constitutive expression) or white on both plates (uninducible expression). You need to plate the cells on both struliose glucose Xgal plates and glucose Xgal plates in order to isolate both constitutive and uninducible mutants. You need glucose in both plates because you need to feed the cells with a carbon source so that all cells (even mutants that cannot metabolize struliose) will be able to grow.

iii) Screen – You have to look through all of the colonies to find the mutations you are looking for. Both mutants and non-mutants grow in a genetic screen.

(b) Outline a genetic procedure that you would do to find more genes involved in struliose metabolism. In your procedure, use toxo-struliose (but not the reporter gene).

i) Plate the cells on minimal medium containing toxo-struilose and glucose. (The glucose is necessary because, without glucose, the mutants you want will have no sugar to eat, because they cannot eat struliose.)

ii) Any cells that can grow must have a mutation that prevents them from metabolizing struliose. (All other cells cannot grow, because they WILL be able to metabolize toxo-struliose, and will thus produce the toxic byproduct that will kill them.)

iii) Selection – only the mutants that you are looking for will be able to grow on the toxo struilose plate. Everything you don't want will be killed. Only mutants grow in a genetic selection.

3. You have a true-breeding mouse that displays the phenotype of big feet. This phenotype is caused by a specific allele of the “FT1” gene called FT1*. You isolate the FT1 gene from this mutant mouse, and inject it into a fertilized egg produced by the mating of two wild-type mice. You then transfer this injected fertilized egg into a pseudopregnant mouse. The mouse that is born has big feet.

(a) What specific conclusion can you draw regarding FT1* from this experiment?

FT1* gene is dominant. There are two wildtype copies of the FT1+ allele in the egg in which FT1* gets inserted into the genome, but the egg that contains both FT1+ and FT1* shows the phenotype (big feet) that is associated with FT1*. This means that the big feet phenotype is dominant.

(b) Which breeding experiment could you have done to reach the same conclusion that you reached from part (a)?

Mate true-breeding mutants to true-breeding wild type. All of the offspring would be heterozygous for the FT1* mutation and you could therefore determine that it was dominant because all the children would have big feet.

You make a transgenic mouse that is transgenic for a gene that is involved in determining petal color in petunias. This mouse has no detectable mutant phenotype. You then mate two transgenic mice together to generate a mouse that has two copies of the same transgene. These TG+/TG+ mice now have a phenotype of slow movement. You hypothesize that this slow movement is caused either:

-- by the presence of two copies of the petunia transgene (for unknown reasons)

-- because each of the transgenes disrupted one copy of the “Dext” gene, a gene that is important for mouse motor skills

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 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

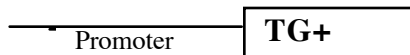
vii) which **additional breeding** steps you would do to make the mouse you wanted

viii) **two possible** phenotypic results you could get from the newly made mice, **and** the corresponding conclusions you would make for each result
Create a genetically modified mouse to distinguish between your two hypotheses if:

(c) You can use the TG+ DNA, but not the “Dext” gene.

i) **pronuclear injection** – you want to add the **TG+ gene** in. Therefore you need to use **pronuclear injection**, because **pronuclear injection** is the **easiest way to add DNA** into the genome of a mouse.

ii) **DNA includes a promoter region and the TG + gene**. You need to the **TG+ gene to be expressed** in order to observe the phenotype, so you must attach it to a promoter region that will allow it to be expressed.



iii) **fertilized eggs**. For **pronuclear injection**, the DNA is injected into the **paternal pronucleus** before it fuses with the **maternal pronucleus**

iv) you would start with a **wildtype egg** – you want to see the effects that the **TG+ gene** has when inserted into the genome and whether or not it causes the mouse to move slowly, so you want all the DNA to start out being normal. Note that the mouse does **NOT** have endogenous copies of TG, because TG is a petal color gene from petunias, so a mouse would not have its own endogenous TG.

v) it would insert randomly. In **pronuclear injection**, the inserted DNA incorporates itself into the genome at a random location

vi) the mice would not be chimeric – you insert the DNA into a fertilized egg, which, once it begins dividing, creates all of the cells in that organism. Thus any DNA that inserts itself into the genome of a fertilized egg will be propagated into every cell of the mouse that grows up from that fertilized egg.

vii) You would then have to mate two of these TG+ mice together to get a **homozygous TG+/TG+** mouse – you are trying to determine the effects of having two copies of TG+, but **pronuclear injection** only gives you **heterozygotes** because only one copy of the DNA sequence inserts at a time. Thus, to get a homozygote, you need to mate 2 hets and take the 1/4 of their offspring that have 2 copies of the TG+ to be studied.

viii) Your homozygous mice could either move slowly, in which case you would know that the TG+ gene causes this phenotype, or they could move normally, in which case you could conclude that the phenotype of your original TG+/TG+ mouse was a result of the Dext gene being disrupted.

***Note that this method relies upon the fact that the second time you do this experiment, the TG insertion will not occur in the same place as the TG insertion did the first time you did this experiment (in the introduction to this question). TG insertion is random, so the chances of TG inserting into the same place in two different mouse eggs is essentially zero.

ALTERNATIVE ANSWER TO PART C:

i) *pronuclear injection*

ii) *DNA includes a promoter region and the TG + gene*



iii) *fertilized eggs*

iv) *TG+ egg that has only one copy of the transgene (from a mating of two mice that each had a single copy of the transgene that you made in the introduction to this question)*

v) *it would insert randomly*

vi) *the mice would not be chimeric*

vii) *no additional mating*

viii) *your mice with two copies of the TG could either move slowly, in which case you would know that the TG+ gene causes this phenotype, or they could move normally, in which case you could conclude that the phenotype you saw before was a result of the disrupted Dext gene. Note that this method relies upon the fact that the second TG insertion will not occur in the same place as the first TG insertion did, because TG insertion is random, so the chances of two TGs inserting in the same place is essentially zero.*

ANOTHER ALTERNATIVE ANSWER TO PART C:

i) pronuclear injection

ii) DNA includes a promoter region and two copies of the TG + gene



iii) fertilized eggs

iv) wild-type

v) it would insert randomly

vi) the mice would not be chimeric

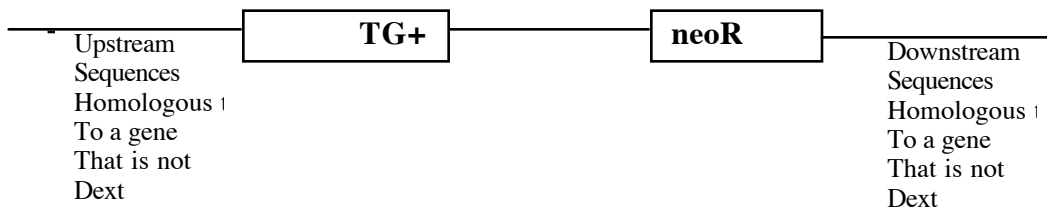
vii) no additional mating

viii) your mice with two copies of the TG could either move slowly, in which case you would know that the TG+ gene causes this phenotype, or they could move normally, in which case you could conclude that the phenotype you saw before was a result of the disrupted Dext gene.

YET ANOTHER ALTERNATIVE ANSWER TO PART C:

i) gene targeting

ii) DNA includes a TG gene and a neoR



iii) ES cells

iv) wild-type

v) *it would insert at the locus you chose*

vi) *the mice would be chimeric*

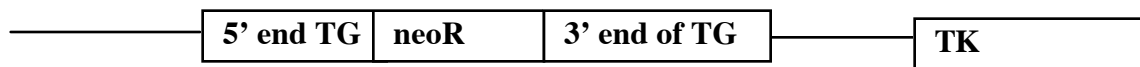
vii) *mate a chimera to wt to get non-chimeric heterozygotes and mate to hets together to get a homozygote*

viii) *your mice with two copies of the TG could either move slowly, in which case you would know that the TG+ gene causes this phenotype, or they could move normally, in which case you could conclude that the phenotype you saw before was a result of the disrupted Dext gene.*

STILL ANOTHER ALTERNATIVE ANSWER TO PART C:

i) *gene targeting*

ii) *DNA includes a TG gene disrupted by neoR*



iii) *ES cells*

iv) *TG+/TG+*

v) *it would insert at the locus where the TG is*

vi) *the mice would be chimeric*

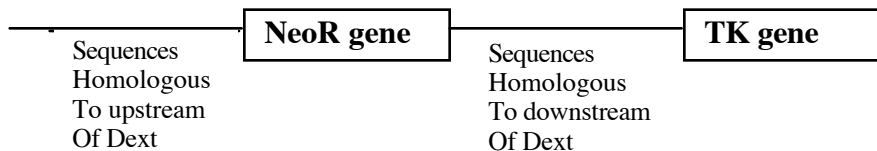
vii) *mate a chimera to wt to get non-chimeric heterozygotes (TG-KO) and mate those hets to TG+/TG+ homozygotes to get heterozygotes (TG+/TG-KO)*

viii) *your mouse could either move fast, in which case you would know that the TG+ gene causes this phenotype, or they could move slowly, in which case you could conclude that the phenotype you saw before was a result of the disrupted Dext gene.*

(d) You can use the “Dext” gene, but not the TG+ DNA.

i) **gene targeting.** You think that the slow phenotype could be caused by two inactive versions of the Dext gene. To test this, you need to knock out this gene, which can ONLY be done using gene targeting.

ii) The DNA would include sequences homologous to the DNA surrounding the Dext gene and then a drug resistance gene in between. The construct needs homologous sequences on either end so that it can recombine into the genome and replace the dext gene, therefore knocking it out. The construct also requires having a TK gene outside of the region of homology to dext. This is so that we can select against TK, thereby selecting against integration of the construct by nonhomologous recombination.



iii) you would put the DNA into ES cells – for gene targeting, you use embryonic stem cells which the DNA can incorporate in to

iv) you would start with wildtype cells or TG+/TG+ cells

v) the DNA would integrate into the place previously held by the Dext gene because the homologous sequences on either side of your construct would recombine with the endogenous Dext locus and integrate it in.

vi) the mice would be chimeric. You put the manipulated stem cells back together with other wild-type cells that already exist in the wild-type blastocyst into which you inject the manipulated cells. Thus, when the embryo grows up, parts of the embryo will have developed from your manipulated cells, but other parts of the embryo will have developed from the wild-type cells that were present in the blastocyst you began with.

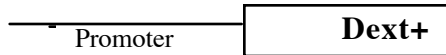
vii) You would have to mate the chimeric mice to wildtype mice in order to obtain non-chimeric heterozygous mice, and then you would have to mate two carriers so that 1/4 of their offspring will be homozygous knockout mice

viii) If the knockout mice move slowly, you know that the phenotype was caused by the disruption of the Dext gene. If they move normally, you can conclude that the phenotype you saw in your original TG+/TG+ mice was a result of the two copies of the TG + gene.

ALTERNATIVE ANSWER TO PART D:

i) pronuclear injection

ii) DNA includes a promoter region and the Dext + gene



iii) fertilized eggs

iv) you would start with a TG+/TG+ egg

v) it would insert randomly

vi) the mice would not be chimeric

vii) no additional mating

viii) your transgenic mice could either move slowly, in which case you would know that the TG+ gene causes this phenotype, OR they could move normally, in which case you could conclude that the phenotype was a result of the disrupted Dext gene (because adding in a wt copy of Dext rescued the mutant phenotype)