7.03 Final Exam Review Session Problems

Heritability

Problem 4

You are interested in the heritability of longevity.

1. You start by studying longevity in mice. Since lab strains appear to have little variation in lifespan, you decide to conduct a large scale breeding experiment in a wild population of mice captured in Boston. In your captured population, the mean life span is 2 years, and the standard deviation is 0.5 year. You choose as your truncation point 3 years, and obtain a population with a mean of 4 years. The offspring of the selected animals have a mean life span of 2.6 years. What is the narrow sense heritability of lifespan in your mice?

2. You decide to repeat the selection process, choosing a new truncation of ~~2.7~~ 3.5 years, obtaining a population (from the offspring) with a mean life span of ~~3~~ 4.2 years. What is the expected mean life span of their offspring?

3. You wish to compare your estimates to ones from human studies. You write your colleague, a human geneticist, who sends you data on longevity from a study of identical twins. The correlation coefficient of longevity between identical twins is 0.3. What is the estimated broad-sense heritability of longevity according to this data?

4. While you are preparing your study for publication, another study of longevity in humans is published, this one based on measures from full-siblings. The correlation coefficient reported by that study was 0.1. What is the broad-sense heritability of longevity based on your competitor's study? How can you reconcile your finding in (3) with this new one?

Problem 2

You study the genetic basis of Crohn's disease. Your colleague at MGH has identified 200 cases (with Crohn's) and 350 controls (without Crohn's). You perform a genome-wide study of 500,000 loci.

1. The best-scoring locus, in chromosome 5, had the following results:

Cases		Controls
11	**55**	**125**
10	**80**	**75**
00	**65**	**150**
Totals	**200**	**350**

Estimate whether the genotype at this locus is significantly associated with Chron's (chi-squared values are below) at a genome-wide significance level of 0.01.

2. What would have happened if you only worked with alleles, not genotypes? Would allele 1 be significantly associated with Crohn's and if so at which level? Assuming that you did NOT perform multiple tests, what could be the reason to the discrepancy between your result in 1 and 2?

p-value	df=1	df=2
0.1	2.705544	4.60517
0.01	6.634897	9.21034
10^{-3}	10.82757	13.81551
10^{-4}	15.13671	18.42068
10^{-5}	19.51142	23.02585
10^{-6}	23.92813	27.63102

Exam 2 Part of the Final Review Session

2nd Exam Review Session

I. Nonsense mutations and nonsense suppressor mutations
   A. Amber stop codon = 5' – UAG – 3'
   B. Ochre stop codon = 5' – UAA – 3'
   C. Mutagens – Example of one that changes G-C to A-T
      i. 5' – TA**G** – 3' → 5' – TA**A** – 3'
         3' – AT**C** – 5' 3' – AT**T** – 5'

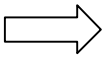
II. Transposons
   A. Features
      i. Carry antibiotic resistance gene
      ii. Transposase
      iii. Flanked by inverted repeats
   B. Are often used in conjunction with P1 phage experiments since they have a selectable marker.

III. Phage
   A. Phage Lambda
      i. Are not general transducing phage
      ii. Utilize att sequences to know exactly how much DNA to replicate and insert in the phage head
   B. P1 phage = general transducing phage
      i. Loads DNA into phage head until it's full
         a. Can take host DNA as well
      ii. Need multiples of two recombination events for markers on the P1 phage to be inserted into host chromosome

IV. F plasmids = extrachromosomal pieces of DNA
   A. Features of F plasmids:
      i. Origin of Transfer (OriT)

Direction of transfer

      ii. IS sequences



   B. F+ strains can transfer the F plasmid through the F pilus to an F- strain in a process called conjugation
   C. F plasmids can integrate into host chromosome, using homologous recombination between IS sequences
      i. Only one recombination event is necessary
      ii. This creates an Hfr
   D. Hfr can transfer some markers efficiently and others inefficiently
      i. This depends upon the position of the markers relative to the OriT

E. Utilize homologous recombination between IS sequences to recreate the F plasmid = F'.
    i. Can take host DNA as well
    ii. Select for this event by looking for markers that are now transferred efficiently.
    iii. Can be used to make merodiploids, which are diploid for the markers contained on the F'.

V. Regulatory pathways
A. For the cis/trans test always look if the dominant allele acts in trans (may or may not be the mutant allele).
B. In an epistasis test, whichever mutation's phenotype is seen in the double mutant is epistatic and the gene containing the mutation is downstream.
C. When working out a regulatory pathway, it is a good idea to start at the end and work backwards.
D. In a regulatory pathway, if a loss of function mutation leads to uninducible expression, the gene the mutation is in was originally an activator; if constitutive expression, the gene was originally a repressor.

VI. Lac Operon
A. Understanding how different mutations in the lac operon lead to phenotypes in the cell (as opposed to memorizing if a mutation is constitutive/uninducible, recessive/dominant, cis/trans) will make it easier to solve the problems
B. It is common to have a mutation that is caused by a loss of function of a big gene. It is more rare to have a gain of function mutation or a loss of function mutation in a small gene.

VII. Other Advice
A. It is more important to sleep the night before the exam then to cram!

Name:

1. (a 5 pts.) You are studying the gene for an enzyme in E. coli and you isolate a mutation that has no enzyme activity. When you introduce a gene for an amber suppressor tRNA into your mutant strain, enzyme activity is restored. Would you expect activity to be restored if a UGA suppressor tRNA had been introduced instead? Explain. (The sequences of the three stop codons are: 5'UAG3' (amber), 5'UAA3' (ochre), and 5'UGA3'.)

(b 5 pts.) Write out the sequence of the anti-codon portion of the amber suppressing tRNA (be sure to label the 5' and 3' ends of the RNA).

(c 8 pts.) You sequence the gene for the enzyme in your mutant and by comparing the sequence to wild type you find a single C•G to T•A base-pair change. One strand of a short stretch of sequence containing the mutation is shown below.

Wild type: 5' … G T G T G A T C C A C A T C C … 3'

Mutant: 5' … G T G T G A T C T A C A T C C … 3'

Is the direction of transcrption left-to-right or right-to-left for this gene segment? Explain your reasoning.

d 10 pts.) Next, you treat your original mutant strain with a mutagen that causes T•A to C•G mutations and isolate revertants that have restored enzyme activity. When you assay enzyme activity you find that the mutants can be grouped into two types. Type 1 has exactly the same enzyme activity as a wild type strain, whereas Type 2 has somewhat less activity than wild type. Write out the DNA sequence of the gene for the enzyme that you would predict for revertants of each type. Write out the sequence of the same DNA strand as shown in the mutant sequence shown below and be sure to label the 5′ and 3′ ends)
Mutant: 5′ … G T G T G A T C T A C A T C C … 3′

2. (a 8 pts.) You have isolated a Tn5 insertion in a wild type E. coli strain that you think may be linked to the Lac operon. You grow phage P1 on the Tn5 insertion strain and use the resulting lysate to infect a LacI– mutant (i.e. a Lac repressor mutant) and select for kanamycin resistance (Kanr). You examine 12 Kanr transductants, and find that 9 exhibit normal Lac regulation whereas 3 show constitutive Lac expression. What is the distance between Tn5 and LacI– expressed as a cotransduction frequency?

(b 6 pts.) If the P1 lysate described in part a) were used to infect a LacOc mutant (i.e. an operator constitutive mutant) would you expect the phenotypes and the proportion of different phenotypes among Kanr transductants to be significantly different from that found for part a)? Explain your reasoning.

(c 10 pts.) You set up two reciprocal crosses with the Tn5 insertion described in part a). In the first cross you grow P1 phage on a strain with the Tn5 insertion and LacI- mutation. The resulting lysate is used to infect a LacOc mutant. Among 100 Kanr transductants, 5 show normally regulated Lac expression and 95 show constitutive expression. In the second cross you grow P1 phage on a strain with the Tn5 insertion and LacOc mutation. The resulting lysate is used to infect a LacI- mutant. For this cross, all 100 Kanr transductants show constitutive Lac expression. Draw a map showing the relative order of the Tn5, and the LacOc and LacI- mutations. Also include any relevant genetic distances that you can from the information in part a) and part c). (Warning, points may be deducted for inclusion of incorrect distances)

Name:

(d 10 pts.) To examine the results from the first cross in part c) in more detail, you mate an F' factor carrying the wild type Lac operon (F' Lac+) into each of the 95 Kanr transductants that show constitutive Lac expression. Among the 95 constitutive transductants, 25 are still constitutive when they carry F' Lac+, whereas 70 show normal Lac regulation when they carry F' Lac+. Based on these results revise your map for part c) putting in all of the relevant map distances.

3. You are studying the regulation of synthesis of the amino acid histidine in a new bacterial species and you find that the last enzyme in the pathway for histidine synthesis (the product of the HisC gene) is synthesized when there is no histidine in the medium, but is not synthesized when histidine is present.

(a 8 pts.) You mutagenize the bacteria by generating a collection of random insertions of the transposon Tn5 into the bacterial chromosome. You find a Tn5 insertion, designated His1−, which gives constitutive expression of HisC. Classify the His1− mutation in terms of its likely genetic properties taking into account the type of mutation usually caused by a transposon insertion (explain your reasoning). Propose the type of regulatory function probably encoded by the wild type His1 gene. Finally, diagram a model to explain the effects of histidine and the wild type His1 gene on HisC expression, assuming a linear pathway.

(b 8 pts.) Next, you isolate a second Tn10 insertion mutation, designated His2–, which also shows constitutive HisC expression (note that Tn10 confers tetracycline resistance, Tetr). In a transduction experiment you grow P1 phage on the His2– Tn10 (Tetr) strain and use the resulting lysate to infect the His1– Tn5 (Kanr) mutant strain, selecting for Tetr transductants. You find that all of the Tetr transductants are also Kanr. What does this result tell you about the relationship of the His1– and His2– mutations and what is the significance for understanding the regulatory pathway for HisC?

(c 8 pts.) Diagram the two possible models for linear regulatory pathways for HisC that account for the behavior of the His1 and His2 mutations. Include a role for histidine for each model.

Name:

(d 8 pts.) You mutagenize the bacteria with a chemical mutagen and find a new mutation that gives uninducible HisC expression. Transduction experiments show that this new mutation is closely linked to the Tn5 insertion of His1–. Sequencing of this region of DNA shows that the new mutation is a missense mutation in the same gene that has a Tn5 inserted into it in the His1– mutation. You therefore call the new mutation His1*. In a transduction experiment you grow P1 phage on the His2– Tn10 (Tetr) strain and use the resulting lysate to infect the His1* mutant strain, selecting for Tetr transductants. You find that all of the Tetr transductants give constitutive HisC expression. Draw out the model from part (c) that is consistent with this new observation? Explain your reasoning.

(e 6 pts.) Propose a molecular description of how the His1* mutation might affect the function of the corresponding gene product. Be as specific as possible as to what effect the His1* mutation might have. (For example, a good molecular description of the LacIs mutation would be: “a mutation in the repressor protein that prevents binding of the inducer lactose” – simply stating “super repressor” would not be adequate.)