Problem Set 5 answers

1. Whether or not the shanks of chickens contains feathers is due to two independently assorting genes. Individuals have unfeathered shanks when they are homozygous for recessive genes at two loci; the presence of a single dominant gene at either locus causes feathers. Denote the dominant alleles at locus 1 and locus 2 as A and B, respectively. A cross between two chickens who are heterozygous at both loci is performed.

1a. What is the ratio of feathered to unfeathered shanks in the offspring of the above cross?

The expected ratio of feathered to unfeathered shanks for an AaBb x AaBb mating is 15:1.

1b. How does this ratio compare to what is observed in Mendel's dihybrid crosses?

This is an example of how epistasis can lead to modified dihybrid ratios, even for independently assorting loci. The 9:3:3:1 ratios that Mendel observed in his dihybrid crosses result from considering two traits, each of which are controlled by a single gene (i.e., in lecture 5 we considered dihybrid crosses of seed shape and color). In the case of feathered or unfeathered chicken shanks, the interaction of two genes determines the phenotype.

2. A plant that normally produces purple-colored flowers was mutagenized and the following mutant phenotypes were observed. Assume that the wild type alleles (upper case) are dominant to mutant alleles (lower case).

Genotype	**Flower color**
AABB (wild type)	Purple
aaBB	Red
AAbb	Yellow
aabb	Yellow

Propose a genetic pathway for flower color in this plant.

Based on the data above, it is clear that two genes are responsible for flower color. The following pathway is consistent with the mutant phenotypes:

Yellow intermediate ---(B protein)---→ Red intermediate ---(A protein)---→ Purple

3. Female flies are mated to males to set up a transposon mutagenesis experiment as follows:

$$\frac{X^{w,P[w^+]}}{X^{w,P[w^+]}} \; ; \; \frac{+}{+} \; ; \; \frac{+}{+} \quad \text{crossed to} \quad \frac{X^{w}}{Y} \; ; \; \frac{+}{\text{CyO P[TNase]}} \; ; \; \frac{+}{\text{Tb}}$$

CyO = curly wings, dominant;
Tb = tubby body, dominant
w^+ = red eyes, dominant
$X^{w,P[w+]} = X^w$ carrying $P[w^+]$ somewhere on that chromosome

3a. What genotype of offspring would you want to collect, in whose germ cells transposition could happen? What phenotype? (On an exam, you would lose points for using unnecessary markers.).

Genotype:	**Phenotype of this fly:**
$\frac{X^{w,P[w^+]}}{Y} \; ; \; \frac{\text{CyO P[TNase]}}{+} \; ; \; \frac{+}{+}$	Red-eyed male with curly wings, normal body

3b. What fraction of the offspring from the mating would you discard as unsuitable? Explain briefly.

7/8. Probability that a sperm will have a Y and have the copy of the 2nd chromosome with Jump-starter and get a wild type copy of the 3rd chromosome = 1/2 x/ 1/2 x 1/2 = 1/8. Everything else (7/8) is unsuitable.

3c. In the genotype you wrote above…

Why did you include/exclude CyO?

Included: Marker for presence of TNase; it's how we can pick out flies that have Jump-starter

Why did you include/exclude Tb?

Excluded: Don't need that marker for anything. (Alternative answer: included Tb because down the line, as you'll see in QS6, we will need a dominant marker on the 3rd chromosome to map the location of the jump. This answer is valid only if you did include Tb in the genotype above and explained your reasoning!)

3d. The flies whose genotype you wrote above were mated with white-eyed flies that were otherwise wild type. In the progeny of this new cross, what phenotype would you look for to indicate a stable new mutation?

Phenotype:

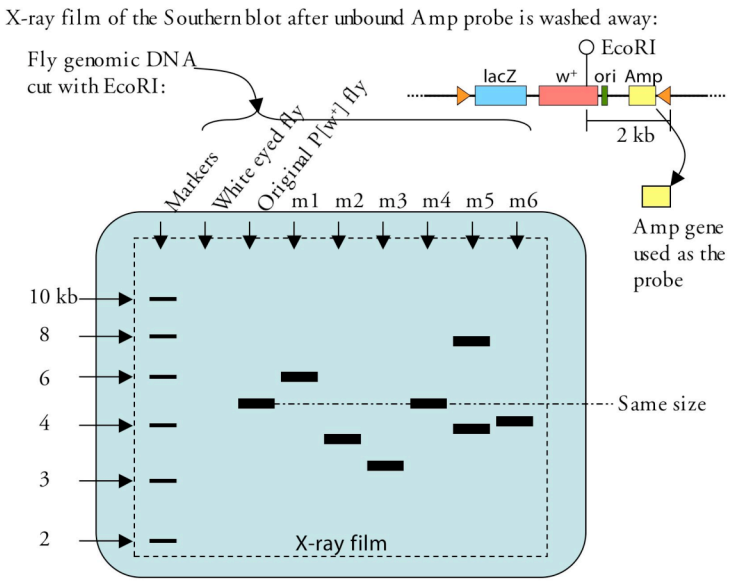
Red-eyed male with normal wings and normal body

3e. The mutant flies you found in 1(d) are mated to virgin females. Subsequent analysis shows that one of the new mutations is on the X chromosome. You know immediately that something is wrong with that fly line. Why are your suspicions being raised?

In this setup, the Y chromosome itself acts as the dominant marker to tell us when the transposon has moved**. To identify "hops" of the P-element, we had to have picked male progeny (i.e., receiving the Y chromosome) with red eyes. If the hop landed on the X, the P[w^+] marker would segregate away from the Y during meiosis, so if we only chose male progeny with red eyes, we shouldn't be picking up hops onto the X.

**In the absence of a hop, the red-eye marker (in P[w+] on the X) must segregate away from the Y. So without transposition, we should not get male (Y-chromosome) progeny with red eyes. If we find males with red eyes, we know that those males must be new mutants.

4. Let's assume that among the mutants defective for some trait like leg development that were identified in the *Drosophila* P-element mutagenesis screen performed in quiz section, there were 6 that all mapped to chromosome III. You isolated DNA from each of these flies, cut the DNA with EcoRI, ran it on a gel, and then transferred the genomic DNA to a membrane filter after denaturing it in place in the gel (i.e., you did a Southern blot). You then labeled the DNA from the **Amp** gene and hybridized it to the DNA on the filter. This is what you find (picture on the right):



4a. How does this use of the Amp gene differ from the use of the Amp gene in cloning?

In cloning experiments the amp ^{R} gene is used for selection--what is important is the product of the gene. In this experiment the amp^R gene is important for its DNA sequence only. Since it is a sequence only naturally found in bacterial cells, its presence in flies indicates they have received the engineered P-element.

4b. Does each of the flies represented on the gel contain a P-element? If no, which one(s) don't?

One fly does not contain a P element--the white eyed fly

4c. Do any of the flies contain more than one P element? If yes, which one(s) do?

Fly m5 has two P elements in its genome. The closest EcoRI site at one of these locations must be a different distance away than the closest EcoRI site at the other location.

4d. Mutant 4 has the same size band as the original P[w^+] fly. **Excluding experimental error,** propose one explanation.

When the P-element hopped it landed in a new place on chromosome III but at the same distance from an EcoRI site as the original P element had been. (The P-element also landed in the same orientation with respect to that site.)

4e. Based on this experiment, what can you conclude about the number of genes involved in leg development that have been interrupted by a P-element?

At least one gene, that's all we can say. It appears that all of the insertions are into new sites, but they could all have landed in the same gene, just at different places in that gene.

5. An ampicillin-resistant, tetracycline-resistant plasmid is cleaved with Eco RI, which cuts within the ampicillin gene. The cut plasmid is ligated with Eco RI digested *Drosophila* DNA to prepare a genomic library. The mixture is used to transform *E. coli*.

5a. Which antibiotic should be added to the medium to select cells that have incorporated a plasmid?

tetracycline

3b. What antibiotic-resistance pattern should be selected to obtain plasmids containing *Drosophila* inserts?

tetracycline resistant, ampicillin-sensitive

5c. How can you explain the presence of colonies that are resistant to both antibiotics?

The original plasmid failed to incorporate a fragment of the Drosophila genome and

ligated back shut.

6. You are working on tryptophan metabolism in E. coli and wish to conduct an experiment with the TrpE gene (which is required for growth on plates lacking tryptophan). Although this gene was cloned by complementation years ago, and could be obtained by asking any one of a number of laboratories to send it to you, you happen to have a trpE mutant strain (which fails to grow on plates lacking tryptophan) in your lab and decide to simply clone the gene using the same complementation cloning approach. To do this, you prepare a library from Bam HI digested *E. coli* genomic DNA. Although the original cloning of TrpE made use of a library composed of Bam HI partial digest products, you don't see any reason for this and proceed to make your library from complete Bam HI digest products. This library is then used to transform trpE mutant cells, and the desired colonies are selected by plating on media lacking tryptophan. Surprisingly, you find that none of the transformants are able to grow on plates lacking tryptophan. How can you account for these conflicting results?

The original digest was partial—your original clone happens to have a Bam HI site inside of the TrpE coding sequence that you disrupt when you are attempting to clone into a new plasmid by doing a complete digest of the plasmid.

7. A recessive form of adult night-blindness in humans is caused by a 200 bp insertion in a gene on the X chromosome. You are designing a PCR-based test to identify individuals who have the disease allele. DNA sequence flanking the site of insertion is shown:

7a. Which of the following primers would you use for the PCR reaction? Circle the primer(s) you choose:

i. 5'-CTCCTGCTCCTTCTACAGT-3'

ii. 5'-CAAGTCATTCCATCTCGAC-3' <-- this one

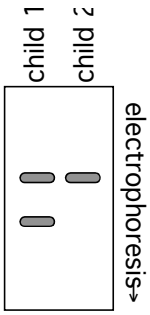
iii. 5'-GAGGACGAGGAAGATGTCA-3'

iv. 5'-CGCACTGTAGAAGGAGCAG-3' <-- and this one

v. 5'-GTTCAGTAAGGTAGAGCTG-3'

vi. 5'-GTCGAGATGGAATGACTTG-3'

7b. Using your night blindness test kit, PCR was performed on DNA samples obtained from two children. The PCR products were separated by gel electrophoresis and the DNA was visualized by staining; the results are depicted.



- Why are there two bands seen on the gel for child 1?

She is a heterozygote--one wild type allele (lower, faster-migrating band) and one insertion allele (the slower-migrating band). The presence of two copies of the gene indicates that it's a female.

- Suggest TWO explanations for why child 2 only shows one band on the gel.

  Explanation 1:

It's a girl who is homozygous for the insertion allele

  Explanation 2:

It's a male who has the insertion allele on his X.

- Which child will likely develop the disease? Explain in one sentence.

Child 2: whether it's a hemizygous male or a homozygous female, there are no wild type copies of the gene.