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?

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

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.

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

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

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: of this fly:	Phenotype
_____ ; _____ ; _____	

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

3c. In the genotype you wrote above…

Why did you include/exclude CyO?

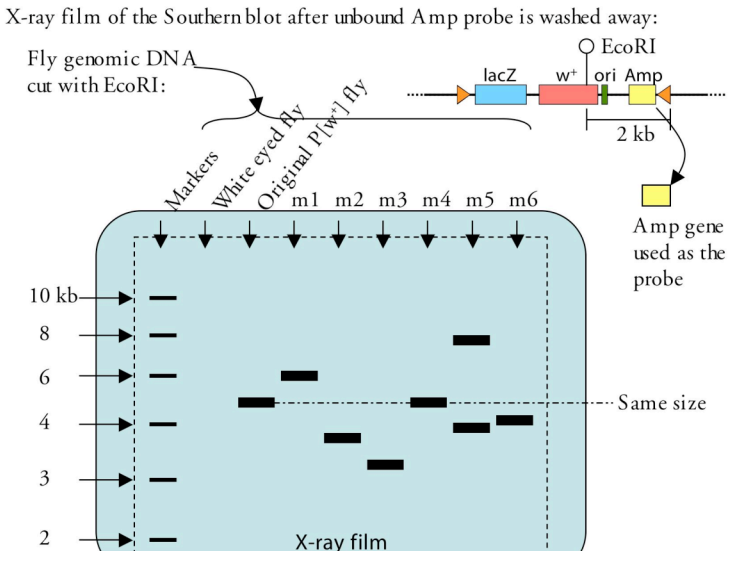
Why did you include/exclude Tb?

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:

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?

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?

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

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

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

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?

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?

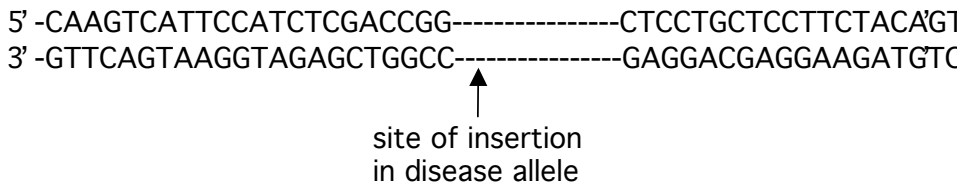
5b. What antibiotic-resistance pattern should be selected to obtain plasmids containing *Drosophila* inserts?

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

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?

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'

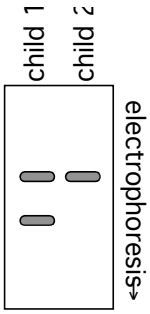
iii. 5'-GAGGACGAGGAAGATGTCA-3'

iv. 5'-CGCACTGTAGAAGGAGCAG-3'

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?

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

  Explanation 1:

  Explanation 2:

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