

Instructions Sheet

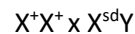
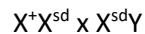
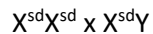
Please use the information in this **Instructions Sheet**, that you have been uniquely assigned, to complete your **Midterm Answer Sheet**.

Work on your own and be sure short answer written responses are all paraphrased in your own words to receive credit.

1. The *scalloped* gene on the X chromosome in the fly encodes for a protein required for proper wing development. Flies lacking a functional *scalloped* allele have scalloped shaped wings.
 - a. Match genotype to phenotype by filling out the chart below with the following terms: (2pt)
 - normal wings, scalloped wings, $X^{sd}X^{sd}$, $X^{+}X^{sd}$, $X^{+}X^{+}$, $X^{sd}Y$, $X^{+}Y$

Possible Genotypes	Corresponding Phenotype

- b. You are interested in studying if *scalloped* alleles follow the expected Mendelian inheritance patterns. Which of the following crosses will best allow you to study the inheritance pattern from a single generation? Why is your selection preferred over the other two options? (3pt)



- c. What is the expected phenotypic ratio of offspring from the cross you selected above in (1b)? (2pt)

2. You cross a female fly with normal wings with a male fly with scalloped wings and observe the following offspring: 150 female flies with normal wings, 122 female flies with scalloped wings, 123 male flies with normal wings, and 105 male flies with scalloped wings.

- a. What do you think the genotypes of the two parents are and why? (3pt)
 - b. Based on the parent genotypes proposed above in (2a), complete the following chart to determine if this result fits with the expected Mendelian Inheritance patterns (use as many rows as needed) (3pt)

Phenotype	Observed	Expected	(O-E)	(O-E) ²	(O-E) ² /E
Totals:			-----	-----	

- c. How many degrees of freedom are there? (1pt)

- d. What are the probability values associated with the χ^2 value? (2pt)
- e. Does the observed result fit with the expected Mendelian inheritance pattern? Yes or No? (2pt)
3. Put the following steps in the best order (#1-8) to illustrate the experimental design flow for our molecular module analysis of *yellow* gene sequences from wildtype and mutant flies. Each step should only be used once: (2pt)
- Synthesize insert from gDNA
 - Put plasmid into bacteria
 - Digest plasmid to verify presence of insert
 - Culture bacteria to copy plasmid
 - Isolate plasmid from bacteria
 - Extract gDNA
 - Sequence insert
 - Put insert into plasmid DNA
4. How do you expect the *yellow* gene sequences we obtain to compare between wildtype and mutant yellow body color flies and why? (3pt)
5. Complete the following to describe the PCR-mediated amplification of the *yellow* gene for your project in the lab this semester:
- a. The template is: single-stranded or double-stranded? (1pt)
 - b. The forward and reverse primers in a single PCR reaction bind to: the same template strand or opposite template strands? (1pt)
 - c. Taq polymerase uses a _____ (DNA or RNA) template to synthesize _____ (DNA or RNA). (1pt)
 - d. How many different size PCR products are we synthesizing from a single PCR reaction? (1pt)
 - e. How many different PCR reactions (ie. master mixes) did we use to amplify the coding region of our gene of interest? (1pt)
6. Why would you expect to encounter an issue when observing gel electrophoresis results if you forgot to include the 72C step when programming the thermocycler for your PCR analysis? (1pt)
7. The pCR4-TOPO vector for TOPO-TA cloning is designed specifically to mediate ligation of PCR products that were amplified by Taq polymerase. What does Taq polymerase add, non-template specific, to which ends (3' or 5') of PCR products, and what overhang is provided at which ends (3' or 5') of the vector to mediate ligation of these products? Briefly explain why these features facilitate ligation. (3pt)

8. We use a NanoDrop spectrophotometer to analyze plasmid DNA obtained from miniprep. What information about the plasmid does the 260nm reading alone provide and why? Include in your answer the specific information known about DNA absorption of UV light that allows for calculation of this information from the 260nm reading. (3pt)