Genetics Lab Instructions Sheet

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

be sure responses are all **typed** and **paraphrased** in your own words to receive credit.

1. You are sequencing the *white* gene alleles from a phenotypically wildtype female fly using the procedures carried out in the Molecular Module of our lab course this semester to determine what alleles may be present in your fly population.
   a. This is a collaborative effort among members of your research team. You have been assigned two of the procedural steps performed (noted as A and B below) to contribute to the overall work.
      - **Step A assigned**: **Bacterial transformation**. Record on your answer sheet.
        - Describe the purpose of this procedural step. What specifically did this step do and how is the step important to the overall sequencing analysis? (2.5pt)
        - Is the material you are interested in obtaining by performing this step genomic DNA or plasmid DNA? (1pt)
      - **Step B assigned**: **Restriction digest**. Record on your answer sheet.
        - Describe the purpose of this procedural step. What specifically did this step do and how is the step important to the overall sequencing analysis? (2.5pt)
        - Describe how the result from this step is analyzed/visualized? (2pt)
   b. While the female fly population analyzed is phenotypically wildtype, your sequencing analysis identified the presence of a mutant allele (in addition to identification of a wildtype allele). Use the cDNA sequences provided below to analyze the mutant allele identified.

White gene database cDNA sequence:

ATGGGCCAAGAGGATCAGGAGCTATTAATTCGCGGAGGCAGCAAACACCCATCTGCCGAGCATCTGAACAATGGTGACAGCGGAGCG
GCTTCGCAGAGCTGCATTAACCAGGGCTTCGGGCAGGCCAAAAACTACGGCACGCTCCGGCCACCCAGTCCGCCGGAGGACTCCGGT
TCAGGGAGCGGCCAACTAGCCGAGAACCTCACCTATGCCTGGCACAATATGGACATCTTTGGGGCGGTCAATCAGCCGGGCTCCGGAT
GGCGGCAGCTGGTCAACCGGACACGCGGACTATTCTGCAACGAGCGACACATACCGGCGCCCAGGAAACATTTGCTCAAGAACGTTT
GCGGCGTGGCCTATCCGGGCGAACTTTTGGCCGTGATGGGCAGTTCCGGTGCCGGAAAGACGACCCTGCTGAATGCCCTTGCCTTTCG
ATCGCCGCAGGGCATCCAAGTATCGCCATCCGGGATGCGACTGCTCAATGGCCAACCTGTGGACGCCAAGGAGATGCAGGCCAGGTG
CGCCTATGTCCAGCAGGATGACCTCTTTATCGGCTCCCTAACGGCCAGGGAACACCTGATTTTCCAAGCCATGGTGCGGATGCCACGAC
ATCTGACCTATCGGCAGCGAGTGGCCCGCGTGGATCAGGTGATCCAGGAGCTTTCGCTCAGCAAATGTCAGCACACGATCATCGGTGT
GCCCGGCAGGGTGAAAGGTCTGTCCGGCGGAGAAAGGAAGCGTCTGGCATTCGCCTCCGAGGCTCTAACCGATCCGCCGCTTCTGAT
CTGCGATGAGCCCACCTCCGGACTGGACTCCTTTACCGCCCACAGCGTCGTCCAGGTGCTGAAGAAGCTGTCGCAGAAGGGCAAGACC
GTCATCCTGACCATTCATCAGCCGTCTTCCGAGCTGTTTGAGCTCTTTGACAAGATCCTTCTGATGGCCGAGGGCAGGGTAGCTTTCTTG
GGCACTCCCAGCGAAGCCGTCGACTTCTTTTCCTACGTGGGTGCCCAGTGTCCTACCAACTACAATCCGGCGGACTTTTACGTACAGGT
GTTGGCCGTTGTGCCCGGACGGGAGATCGAGTCCCGTGATCGGATCGCCAAGATATGCGACAATTTTGCCATTAGCAAAGTAGCCCGG
GATATGGAGCAGTTGTTGGCCACCAAAAATCTGGAGAAGCCACTGGAGCAGCCGGAGAATGGGTACACCTACAAGGCCACCTGGTTC
ATGCAGTTCCGGGCGGTCCTGTGGCGATCCTGGCTGTCGGTGCTCAAGGAACCACTCCTCGTAAAAGTGCGACTTATTCAGACAACGA
TGGTTGCCATCTTGATTGGCCTCATCTTTTTGGGCCAACAACTCACGCAAGTGGGTGTGATGAATATCAACGGAGCCATCTTCCTCTTCC

TGACCAACATGACCTTTCAAAACGTCTTTGCCACGATAAATGTGTTCACCTCAGAGCTGCCAGTTTTTATGAGGGAGGCCCGAAGTCGA
CTTTATCGCTGTGACACATACTTTCTGGGCAAAACGATTGCCGAATTGCCGCTTTTTCTCACAGTGCCACTGGTCTTCACGGCGATTGCC
TATCCGATGATCGGACTGCGGGCCGGAGTGCTGCACTTCTTCAACTGCCTGGCGCTGGTCACTCTGGTGGCCAATGTGTCAACGTCCTT
CGGATATCTAATATCCTGCGCCAGCTCCTCGACCTCGATGGCGCTGTCTGTGGGTCCGCCGGTTATCATACCATTCCTGCTCTTTGGCGG
CTTCTTCTTGAACTCGGGCTCGGTGCCAGTATACCTCAAATGGTTGTCGTACCTCTCATGGTTCCGTTACGCCAACGAGGGTCTGCTGAT
TAACCAATGGGCGGACGTGGAGCCGGGCGAAATTAGCTGCACATCGTCGAACACCACGTGCCCCAGTTCGGGCAAGGTCATCCTGGA
GACGCTTAACTTCTCCGCCGCCGATCTGCCGCTGGACTACGTGGGTCTGGCCATTCTCATCGTGAGCTTCCGGGTGCTCGCATATCTGG
CTCTAAGACTTCGGGCCCGACGCAAGGAGTAG

White gene mutant cDNA sequence identified:

ATGGGCCAAGAGGATCAGGAGCTATTAATTCGCGGAGGCAGCAAACACCCATCTGCCGAGCATCTGAACAATGGTGACAGCGGAGCG
GCTTCGCAGAGCTGCATTAACCAGGGCTTCGGGCAGGCCAAAAACTACGGCACGCTCCGGCCATGACCCAGTCCGCCGGAGGACTCC
GGTTCAGGGAGCGGCCAACTAGCCGAGAACCTCACCTATGCCTGGCACAATATGGACATCTTTGGGGCGGTCAATCAGCCGGGCTCC
GGATGGCGGCAGCTGGTCAACCGGACACGCGGACTATTCTGCAACGAGCGACACATACCGGCGCCCAGGAAACATTTGCTCAAGAAC
GTTTGCGGCGTGGCCTATCCGGGCGAACTTTTGGCCGTGATGGGCAGTTCCGGTGCCGGAAAGACGACCCTGCTGAATGCCCTTGCCT
TTCGATCGCCGCAGGGCATCCAAGTATCGCCATCCGGGATGCGACTGCTCAATGGCCAACCTGTGGACGCCAAGGAGATGCAGGCCA
GGTGCGCCTATGTCCAGCAGGATGACCTCTTTATCGGCTCCCTAACGGCCAGGGAACACCTGATTTTCCAAGCCATGGTGCGGATGCC
ACGACATCTGACCTATCGGCAGCGAGTGGCCCGCGTGGATCAGGTGATCCAGGAGCTTTCGCTCAGCAAATGTCAGCACACGATCATC
GGTGTGCCCGGCAGGGTGAAAGGTCTGTCCGGCGGAGAAAGGAAGCGTCTGGCATTCGCCTCCGAGGCTCTAACCGATCCGCCGCTT
CTGATCTGCGATGAGCCCACCTCCGGACTGGACTCCTTTACCGCCCACAGCGTCGTCCAGGTGCTGAAGAAGCTGTCGCAGAAGGGCA
AGACCGTCATCCTGACCATTCATCAGCCGTCTTCCGAGCTGTTTGAGCTCTTTGACAAGATCCTTCTGATGGCCGAGGGCAGGGTAGCT
TTCTTGGGCACTCCCAGCGAAGCCGTCGACTTCTTTTCCTACGTGGGTGCCCAGTGTCCTACCAACTACAATCCGGCGGACTTTTACGTA
CAGGTGTTGGCCGTTGTGCCCGGACGGGAGATCGAGTCCCGTGATCGGATCGCCAAGATATGCGACAATTTTGCCATTAGCAAAGTAG
CCCGGGATATGGAGCAGTTGTTGGCCACCAAAAATCTGGAGAAGCCACTGGAGCAGCCGGAGAATGGGTACACCTACAAGGCCACCT
GGTTCATGCAGTTCCGGGCGGTCCTGTGGCGATCCTGGCTGTCGGTGCTCAAGGAACCACTCCTCGTAAAAGTGCGACTTATTCAGAC
AACGATGGTTGCCATCTTGATTGGCCTCATCTTTTTGGGCCAACAACTCACGCAAGTGGGTGTGATGAATATCAACGGAGCCATCTTCC
TCTTCCTGACCAACATGACCTTTCAAAACGTCTTTGCCACGATAAATGTGTTCACCTCAGAGCTGCCAGTTTTTATGAGGGAGGCCCGAA
GTCGACTTTATCGCTGTGACACATACTTTCTGGGCAAAACGATTGCCGAATTGCCGCTTTTTCTCACAGTGCCACTGGTCTTCACGGCGA
TTGCCTATCCGATGATCGGACTGCGGGCCGGAGTGCTGCACTTCTTCAACTGCCTGGCGCTGGTCACTCTGGTGGCCAATGTGTCAACG
TCCTTCGGATATCTAATATCCTGCGCCAGCTCCTCGACCTCGATGGCGCTGTCTGTGGGTCCGCCGGTTATCATACCATTCCTGCTCTTT
GGCGGCTTCTTCTTGAACTCGGGCTCGGTGCCAGTATACCTCAAATGGTTGTCGTACCTCTCATGGTTCCGTTACGCCAACGAGGGTCT
GCTGATTAACCAATGGGCGGACGTGGAGCCGGGCGAAATTAGCTGCACATCGTCGAACACCACGTGCCCCAGTTCGGGCAAGGTCAT
CCTGGAGACGCTTAACTTCTCCGCCGCCGATCTGCCGCTGGACTACGTGGGTCTGGCCATTCTCATCGTGAGCTTCCGGGTGCTCGCAT
ATCTGGCTCTAAGACTTCGGGCCCGACGCAAGGAGTAG

<u>MUT allele analysis</u>:

You find one location (position) in the **nucleotide** sequence with a change compared to that of the database sequence: Where and how is the change observed different from that of the database (nucleotide change(s) (e.g. GT -> AC, A -> T, etc.), addition (e.g. 4 nucleotide addition), or deletion (e.g. 10 nucleotide deletion)?

- The altered cDNA **nucleotide** position in the mutant sequence (1pt): _____
- The specific change observed at this **nucleotide** position (2pt):_____

Does this DNA mutation found alter the protein sequence? Yes or No? (1pt): _____

How does the encoded **protein** sequence from your MUT analysis compare to that of database? (Hint: **The normal wildtype White protein from the database sequence is 687 amino acids in length**) (2pt)

c. Is it possible that this mutation could disrupt the function of the White protein produced from this mutant allele? Yes or No? (1pt): _____

d. Design an experiment to determine if this mutant allele identified disrupts the function of the encoded White protein. You have heterozygous female flies $X^{+}X^{wnm}$ (where "nm" stands for the new white gene mutation you have identified), wildtype $X^{+}Y$ male flies, and white eye $X^{w}Y$ male flies available to you.
   - What cross will you set up to test the functionality of this newly identified allele? (1pt): _____
   - Briefly explain the rationale for this experimental design. (2pt)

e. Do you hypothesize the mutation identified results in a loss of function allele? Yes or No? **Briefly explain** your rationale. (2pt)

f. What result, from your experimental design (d.) would support your hypothesis (e.)? Please be specific for the expected result for male and female offspring phenotypes. (2pt)

2. We introduced you to several **experimental controls** in the molecular module procedural steps utilized to sequence the *yellow* gene from mutant and wildtype flies. One example was the use of the wildtype control fly sample. **Select one** of the other experimental controls utilized (other than the use of wildtype flies) to discuss in detail here:
   a. Specific experimental procedure/step involved (1pt): _____

   b. Control used and how it is different from the experimental sample(s) (1pt):

   c. Expected result for this control and technique used to visualize this result (2pt):

   d. Describe the purpose of this control. If the result of the control is as expected, what does it tell you about your experimental sample(s)? (2pt)

   e. Provide an example of an experimental sample result that could be misinterpreted without use of this control and why. What error/problem would this misinterpretation cause for a later step in the analysis? (2pt)

3. Here are expected results given the number of offspring analyzed from a genetic cross:
   - Mutant phenotype: 50 offspring
   - Wildtype phenotype: 0 offspring

   Is Chi Square Analysis appropriate in this case to determine if the mutant allele you are studying follows the predicted Mendelian frequencies? Yes or No **and provide rationale why**? (2pt)

4. Would any of the following errors likely explain the presence of an unexpected PCR product? Yes or No **and why** for each? (6pt)
   a) You forgot to add ethidium bromide to the gel
   b) You didn't add gDNA template to the PCR tube
   c) You added two primer sets to the master mix instead of one

5. Which Labster simulation (Mendelian Inheritance or Polymerase Chain Reaction) do you feel was most helpful to your understanding of our projects in the lab this semester **and why**? *For a reminder of the content and skills covered in each, you can visit the Simulation page summary for each in the Week 1 and 6 Canvas modules.* (2pt)