ASSIGNMENT # 8

DEADLINE: RETURN AT THE END OF THE LABORATORY SESSION

OPTION 1: Deposit a physical copy in the cart (front of the lab)

OPTION 2: Submit an electronic copy (i.e., PDF file) through the LMS platform (Canvas)

NAME: _____ **STUDENT #:** _____ **DATE:** _____

IN-PERSON LABORATORY ANALYSES: DNA EXTRACTION AND PCR METHOD
WORTH 30% OF ASSIGNMENT GRADE (Q#1-2)

1. After you perform the DNA extraction, please estimate the concentration of your sample. Please follow the instruction of the TAs

- Sample concentration #: _____ ng/ul
- 260/280 _____
- 260/230 _____

2. Please perform this task before you start the PCR reaction. In groups of four students, **find one** of the three available PCR cyclers in the lab. Browse to the "pcr-white" program and answer the following questions:

- PCR cycler #: _____
- What is the **annealing temperature** in the program? _____
- What is the **temperature** of the step # 1? _____
- What is the **purpose** of step # 1? _____
- What is the **extension time** in the program? _____
- How many **cycles** are included in the program? _____
- What is the **annealing time** in the program? _____
- Is there a **final extension** step included in the program? _____

- Please discuss the different programs with your lab partners (temperatures, time conditions, cycles, etc.). Which PCR cycler contains the most suitable PCR program to genotype your samples?

PCR cycler #: _____

VIRTUAL PCR

Access here: www.ampossot.com/pcr

First, download the **five DNA sequence files** available at the Lab's Canvas website (Lab#8 folder).

MALE_1 **MALE_2** **FEMALE_1** **FEMALE_2** **FEMALE_3**

PRIMERS SEQUENCES

PF1: GTGCAAAGGTGGTCGAATTT
PF2: TCTGGGAGTTCATCTGGACA
PR3: GAGAGGAGTTTTGGCACAGC

3. Upload the five sequences in the app and input the primers. After running the virtual PCR, complete the following table using the PCR product table generated by the app.

Individual	P1 + P3 product present?	Size (bp)	P2 + P3 product present?	Size (bp)	Genotype (*Drosophila* notation)	Phenotype
Male 1						
Male 2						
Female 1						
Female 2						
Female 3						

Using the gel electrophoresis section of the app, drag each predicted PCR fragment into the correct sample lane. After placing the fragments, click Check gel.

Correct fragments placed: _____

INTERPRETATION

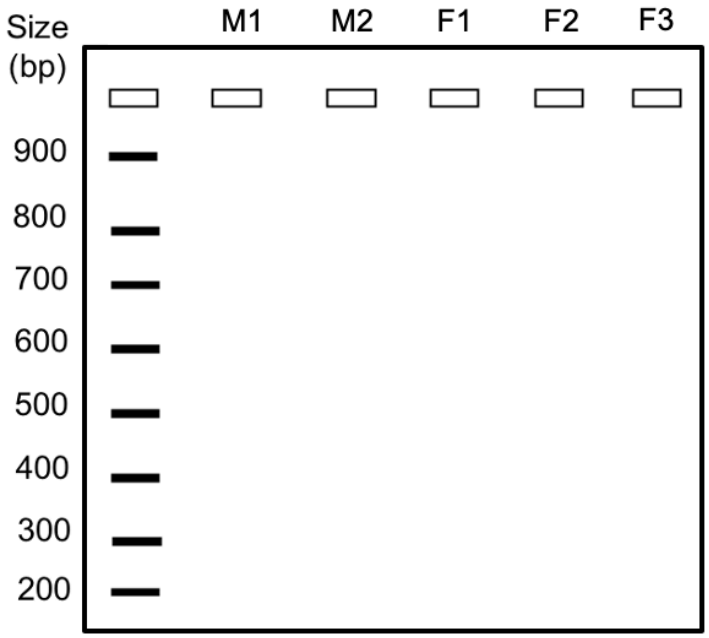
4. Which individuals carry the wild-type white allele (w+)?

5. Which individuals carry the mutant white allele (w)?

6. Which individuals are heterozygous for the white locus?

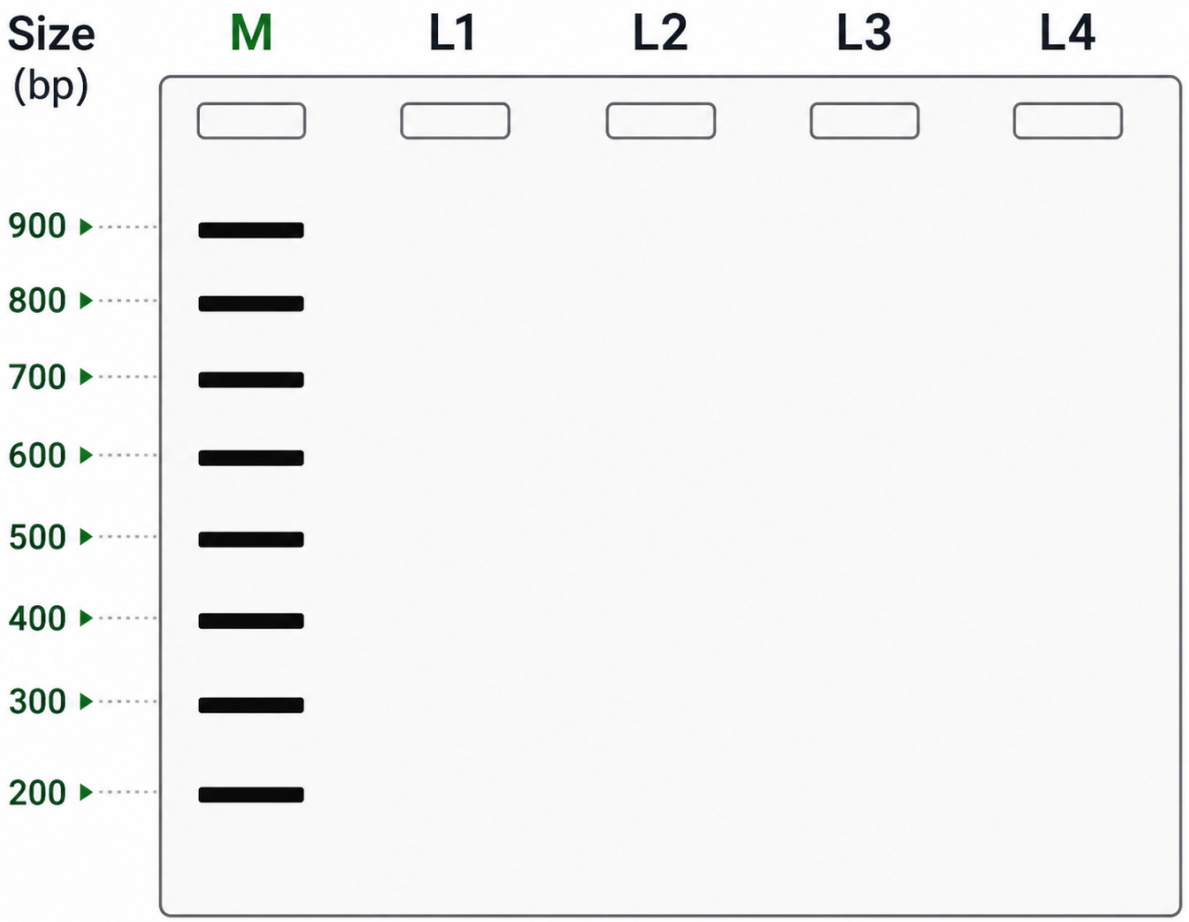
7. Explain how the PCR fragment pattern allowed you to infer the genotype of each individual.

8. Based on the PCR fragments obtained in question 3, please fill the following electrophoresis template. Please consider the DNA ladder (i.e., fragment sizes) loaded on lane 1.



9. Using the following schematic diagram, sketch the DNA fragments expected to be produced by the PCR method of the white locus in *Drosophila*.

L1: A heterozygous wild-type female
L2: A white-eyed male
L3: A white-eyed female
L4: A homozygous wild-type female



GENETICS PROBLEM: IMPROVE YOUR SOLVING SKILLS

10. A cross between a $\mathbf{P_1}$ white-eyed female (wild-type alleles for the *brown* gene) and a $\mathbf{P_2}$ brown-eyed male (both parents are homozygous) was performed. Based on your knowledge of the *white* (this lab) and brown genes (lab # 5), what is the expected phenotypic ratio of crossing $\mathbf{F_1}$ males and females? **Show your work**.

***IMPORTANT NOTE*:**
Remember to return your assignment at the END of the lab session or to submit an electronic copy (i.e., scanned PDF file) through CANVAS