Part A: Genetics Experiment

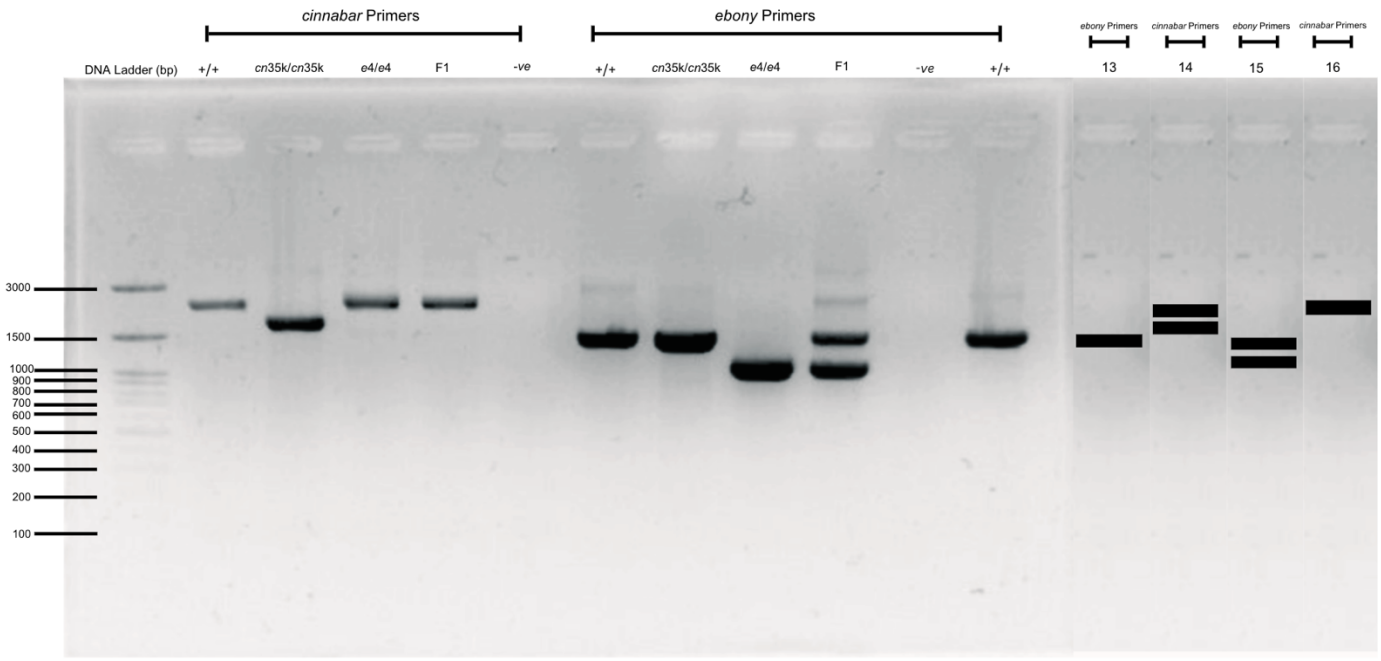

Figure 1: Gel electrophoresis image of PCR (polymerase chain reaction) products from Pod B in LIFESCI 3L03, Lab L03, using a 1.5% (w/v) agarose gel. PCR results for the *ebony* and *cinnabar* genes of *Drosophila melanogaster* are presented via gel electrophoresis through the GelDoc EZ Imaging System. The lanes consist of the following: DNA ladder, wildtype (+/+), mutant strains ($cn^{35}k/cn^{35}k$, e^4/e^4), F1 offspring (heterozygous), and negative controls (-ve). Predicted genotypes for F1 offspring from both crossings are displayed in lanes 13–16. In the gel, the *cinnabar* primers are related to lanes 2-6, 14, and 16, while the *ebony* primers are related to lanes 7-12, 13, and 15. The clear bands aid in displaying that the PCR worked well.

Part A Q1:

The data in our gel regarding the genotype of the F1 progeny suggests that the progeny is heterozygous for both the *cinnabar* and *ebony* genes. The fact that two separate bands are noticeable in the lanes that relate to the F1 offspring serves as evidence for both genes being heterozygous (Refer to **Figure 1**). The F1 progeny received one copy of each allele from their parents. One of the bands denotes the wild-type allele and the other band denotes the mutant allele. The data in our gel regarding the father of the F1 progeny suggests that the father

possessed the mutant *ebony* allele. The lower band in lane 10, which represents the F1 progeny for the *ebony* gene helps confirm this (Refer to **Figure 1**). The visibility of this band indicates that the father provided the mutant ebony allele (e^4), as it relates to the size of the mutant allele. Also, the fact that lane 5 displays no mutant *cinnabar* allele also helps suggest that the father did not carry the mutant *cinnabar* allele. This would mean that the father passed on the wild-type version of the gene to the F1 progeny. The gel that we created in LIFESCI 3L03, Lab L03 does contain all the data needed to conclusively determine the genotype of the father of the F1 progeny (Refer to **Figure 1**). The gel has two separate bands that are noticeable in the lanes that relate to the F1 offspring which help indicate that both genes are heterozygous. The presence of both the wild-type and mutant alleles in the *ebony* gene's PCR results indicates that the father passed on a mutant *ebony* allele to the F1 offspring. The F1 offspring inherited only the wild-type *cinnabar* allele. This is confirmed by the lack of the mutant allele in the *cinnabar* gene. If data such as the bands appearing in the male 2 (+ / Y; cn+/cn+; e^4/e^4) lanes for either set of genes (*e*) or (*cn*) was missing, it would restrict the ability to identify the father's genotype. If there were no bands or a lack of bands in these lanes, it would be difficult to determine if the father has the mutant allele. Due to this, it would be difficult to confirm with certainty which alleles were given to the F1 offspring. Thus, the paternal genotype can only be deduced from inferences. It would be very hard to confirm if the father is heterozygous or homozygous. **[408 words]**

Part A Q2:

It is important to run PCR for both *the cinnabar (cn)* and *ebony (e)* genes when determining the genotype of the father of the F1 progeny. Because both genes are required for accurate results, testing only one would lead to inferences being made about the other gene. **[47 words]**

Part A Q3:

We were successful in extracting gDNA. A noticeable band in lane 12 serves as evidence for this. The gDNA can be quantified by utilizing fluorescence measurement through nucleic acid-

binding dyes or spectrophotometry which helps measure DNA concentration at 260 nm. (*DNA and RNA*, 2024). **[44 words]**

Part B: *Drosophila* Morphology and Sexual Dimorphism

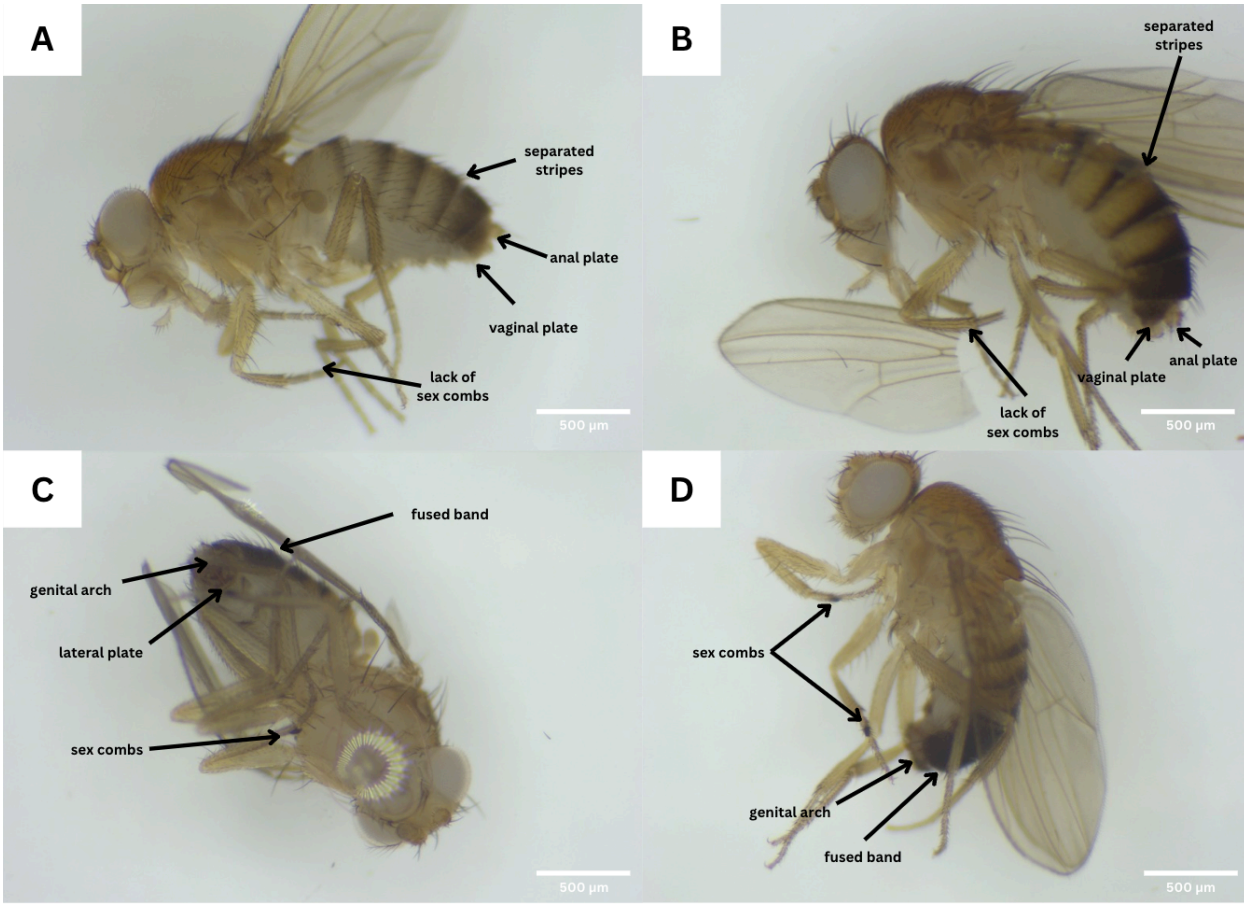

Figure 2: Sexual Dimorphic Traits in *Drosophila melanogaster* displayed in Females (Panels A, B) and Males (Panels C, D). Panels A and B show female Drosophila melanogaster, while panels C and D show male Drosophila melanogaster. In Panel A, the female shows separated abdominal stripes, a visible anal and vaginal plate, and a lack of sex combs. Panel B shows a female with separated stripes, a vaginal and anal plate, and a lack of sex combs. In Panel C, the male displays a fused abdominal band, a genital arch, a lateral plate, and visible sex combs. Panel D shows a male with a fused abdominal band, a genital arch and visible sex combs. All 4 panels display specific sexual dimorphic traits. A Stemi305 stereomicroscope with a 30x magnification was used to take the images.

Table I: Thorax Lengths Measurements of Male and Female *Drosophila melanogaster* Using Research Data and LIFESCI 3L03 Lab Data. Both male and female Drosophila melanogaster thorax length measurements (µm) were analyzed and displayed drawn from the LIFESCI 3L03 Lab Data and the Research Data. Different photos represented by different file names were analyzed to measure numerous thorax lengths.

Source of Data	File Name	Length (µm)
Research data – Male	SM_L2S2_M_Plate10_02.tif	845.398
Research data – Male	SM_L2S2_M_Plate10_08.tif	887.193
Research data – Male	SM_L2S2_M_Plate10_09.tif	840.278
Research data – Male	SM_L2S2_M_Plate10_11.tif	888.164
Research data – Male	SM_L2S2_M_Plate10_15.tif	835.911
Research data – Male	SM_L2S2_M_Plate10_19.tif	835.767
Research data – Male	SM_L2S2_M_Plate10_21.tif	871.670
Research data – Male	SM_L2S2_M_Plate10_27.tif	913.664
Research data – Male	SM_L2S2_M_Plate10_30.tif	841.234
Research data – Male	SM_L2S2_M_Plate10_45.tif	892.847
Research data – Female	EP_E1C1_F_Plate_10_86.tif	1019.865
Research data – Female	EP_E1C1_F_Plate_10_87.tif	985.350
Research data – Female	EP_E1C1_F_Plate_10_88.tif	1003.100
Research data – Female	EP_E1C1_F_Plate_10_89.tif	998.647
Research data – Female	EP_E1C1_F_Plate_10_90.tif	1024.456
Research data – Female	EP_E1C1_F_Plate_10_91.tif	996.361

Research data – Female	EP_E1C1_F_Plate_10_92.tif	1005.237
Research data – Female	EP_E1C1_F_Plate_10_93.tif	996.896
Research data – Female	EP_E1C1_F_Plate_10_94.tif	1023.831
Research data – Female	EP_E1C1_F_Plate_10_95.tif	993.718
Research data – Female	EP_L1S1_F_Plate11_06.tif	998.629
Research data – Female	EP_L1S1_F_Plate11_07.tif	970.273
3L03 data – Male	20241008_stemi_3X_DMM1.jpg	767.293
3L03 data – Male	20241008_stemi_3X_DMM2.jpg	810.390
3L03 data – Male	20241008_stemi_3X_DMM3.jpg	713.464
3L03 data – Male	20241008_stemi_3X_DMM4.jpg	880.985
3L03 data – Male	20241008_stemi_3X_DMM5.jpg	867.987
3L03 data – Male	20241008_stemi_3X_DMM6.jpg	975.234
3L03 data – Male	20241008_stemi_3X_DMM7.jpg	710.160
3L03 data – Male	20241008_stemi_3X_DMM8.jpg	784.431
3L03 data – Male	20241008_stemi_3X_DMM9.jpg	796.777
3L03 data – Male	20241008_stemi_3X_DMM10.jpg	849.398
3L03 data – Female	20241008_stemi_3X_DMF1.jpg	954.238
3L03 data – Female	20241008_stemi_3X_DMF2.jpg	890.990
3L03 data – Female	20241008_stemi_3X_DMF3.jpg	943.294
3L03 data – Female	20241008_stemi_3X_DMF4.jpg	900.873
3L03 data – Female	20241008_stemi_3X_DMF5.jpg	941.443

3L03 data – Female	20241008_stemi_3X_DMF6.jpg	866.213
3L03 data – Female	20241008_stemi_3X_DMF7.jpg	984.724
3L03 data – Female	20241008_stemi_3X_DMF8.jpg	960.911
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3L03 data – Female	20241008_stemi_3X_DMF10.jpg	916.665

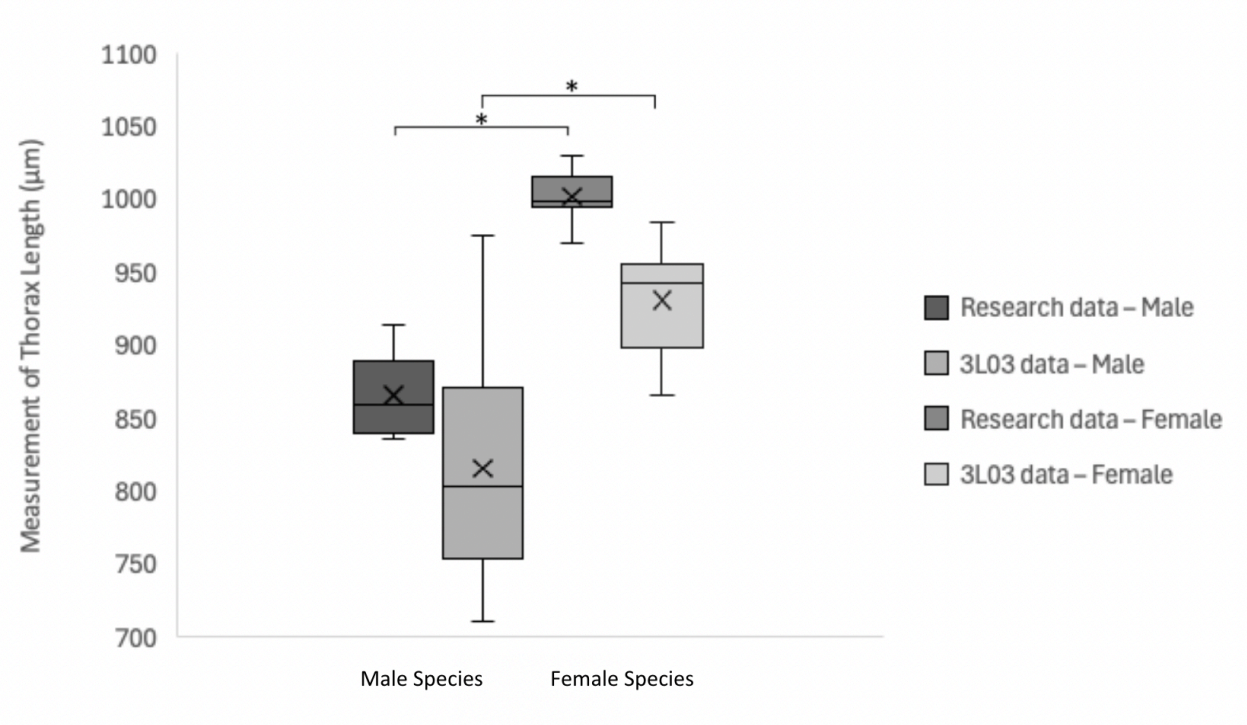

Figure 3: A Box and Whisker Plot Utilized to Examine the Thorax Measurements of Male and Female *Drosophila melanogasters*. This graph compares thorax lengths (µm) of male and female Drosophila melanogaster from Research Data and 3L03 Lab Data. The y-axis is labelled "Measurement of Thorax Length (µm)," and the x-axis is named "Male Species Female Species." A legend is used to help indicate which shade represents which group. Asterisks are used to

help differentiate the differences in thorax lengths between the groups. An "X" is used in each plot to mark the average. Excel was used to compose all the data.

Part B Q1:

The groups being compared are the "Research Data - Male" and "Research Data - Female" groups. Female fruit flies are expected to have a longer overall thorax compared to male fruit flies, according to data. An F-test was conducted, and it revealed a p-value of 0.014 ($p < 0.05$) which shows a significant difference in thorax lengths between male and female *Drosophila melanogaster*, thus supporting the hypothesis.

Part B Q2:

The groups being compared are the "3L03 Data - Male" and "3L03 Data - Female" groups. Female fruit flies are expected to have a longer overall thorax compared to male fruit flies, according to data. An F-test was conducted, and it revealed a p-value of 0.037 ($p < 0.05$) which shows a significant difference in thorax lengths between male and female *Drosophila melanogaster*, thus supporting the hypothesis.

Part B Q3:

Two potential confounding variables in this experiment include the temperature and exposure to light. Temperature is a confounding variable because it influences the way fruit flies develop. This can impact growth which can cause changes in their appearance such as the appearance of the thorax size. To account for this confounding variable, I would place all fruit flies being used for an experiment in a moderated area where the temperature remains the same throughout their development. An example would be 21°C. Another confounding variable is light exposure. Sunlight or artificial light can impact the development of fruit flies. To account for this confounding variable, I would make sure that all flies are housed in a moderated area

where the same amount of light is being administered. I would cycle through different intensities, such as 6 hours of light and 6 hours of darkness to create a consistent environment. **[148 words]**

Part C - Primer Design Activity

Part C Q1:

The name of the gene that contains the sequence I was assigned for the *Drosophila melanogaster* is the hippo (hpo) gene.

Part C Q2A:

The gene summary from http://flybase.org:

"hippo (hpo) encodes a kinase in the Salvador-Warts-Hippo pathway. It controls tissue growth by controlling cell growth, proliferation and apoptosis. It has several roles…including fate specification of photoreceptors and tiling of dendritic neurons."

Part C Q2B:

The name of a human orthologue assigned to this gene is STK3 (MST2). This has the greatest orthology score which is 13/14.

Part C Q2C:

The human orthologue STK3 (MST2) is associated with cancer. Cancer is classified as a proliferative disease because it involves irregular development and division of cells. This is managed by the STK3 (MST2) gene. The STK3 (MST2) gene is also associated with

various infections and metabolic problems. This can negatively impact numerous body parts and processes including the central nervous system.

Double-stranded sequence:

5′ctagtgttggctagcgatcgagtgcgaataaagaacaaaagtgtgatatgtcgctgtgaatagccaattatctgtgtttt
3′gatcacaaccgatcgctagctcacgcttatttcttgttttcacactatacagcgacacttatcggttaatagacacaaaa

ctcgtgctaaatcagaaaatttttcgcaatgtctgagccagaggtgaccagcgttgtagatatgaaatcgcccaacatat
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cctcctcctgctccttcttcaagctgaagaagctgtcggaggagtcgcttctgcagccgccggaaaaggtcttcgacatt
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atgtacaagctgggcgagggcagctatggatcagtctacaaggcagtgcacaaggaaagcagctccattgtggccatcaa
tacatgttcgacccgctcccgtcgatacctagtcagatgttccgtcacgtgttcctttcgtcgaggtaacaccggtagtt

gctggtgccggtggagtccgacctgcacgagattattaaggagatatccattatgcaacaatgcgactcgccgtatgtgg
cgaccacggccacctcaggctggacgtgctctaataattcctctataggtaatacgttgttacgctgagcggcatacacc

tgcgctattacggctcctacttcaagcagtatgacctgtggatctgcatggagtactgtggcgccggcagcgtctccgac
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tctgcatctgcgtcgcaagatccatcgcgacatcaaggcggccaacatcctgctcaataccgaaggctatgctaagttgg
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gggaaagcccccatacggtgagatccatcccatgcgagccatctttatgattccgcagaagccaccaccatcgttccggg
ccctttcgggggtatgccactctaggtagggtacgctcggtagaaatactaaggcgtcttcggtggtggtagcaaggccc

aaccggatcgctggagcacagagttcattgacttcgtgagcaagtgcctggtaaaggagccagacgaccgggcaacggcc 3′
ttggcctagcgacctcgtgtctcaagtaactgaagcactcgttcacggaccatttcctcggtctgctggcccgttgccgg 5′

Figure 4: Double-Stranded DNA Sequence of *Drosophila melanogaster* Hippo (hpo) Gene. The hippo (hpo) gene's double-stranded DNA sequence is displayed, with the reverse primers highlighted in green and the forward primers highlighted in pink. The SNP is highlighted in yellow. This sequence represents only a section of the full hippo (hpo) gene. The forward primer has a melting temperature of 61.46°C and a GC content of 55%. The reverse primer has a melting temperature of 61.69°C and a GC content of 60%. The PCR product is 375 base pairs long. Both primers are 20 nucleotides in length. Both have similar melting temperatures (within 1°C of each other). The GC content is between 40-60%.

Table II: Summary of Primer Sequence and Key Details Regarding the Hippo (hpo) Gene.
The length of the PCR result is 375 base pairs. The forward and reverse primer sequences in the 5' to 3' direction are displayed. The length of the primer is 20 base pairs for the forward and reverse primers. The template strand and the melting temperatures (61.46°C for the forward primer and 61.69°C for the reverse primer) are also displayed. The GC content is 60% for the reverse primer and 55% for the forward primer.

Summary of primer pair details:

	Sequence (5’ -> 3’)	Template Strand	Length (bp)	T_m (ºC)	GC (%)
Forward	AGGCAGTGCACAAGGAAAGC	Plus	20	61.46	55.00
Reverse	CGACTCCGAAATCGGCCAAC	Minus	20	61.69	60.00
PCR Product Length	375				

Part C Q3:

The selected primers follow all the primer design criteria. The primers are correctly highlighted. The length is 375 base pairs, which is within the 300-700 base pair range. The forward and reverse primers are 20 nucleotides long. This is within the 20-25 nucleotide range. The melting temperatures are very similar. Both are within 1°C. The forward primer is 61.46°C and the reverse primer is 61.69°C. The GC concentration of the forward and reverse primers is 55% and 60%, respectively. This is within 40-60%.

References

DNA and RNA Quantification. Millipore Sigma. (2024). https://www.sigmaaldrich.com/CA/en/technical-documents/technical-article/genomics/dna-and-rna-purification/dna-rna-quantification