NMSU-HHMI Brand

LABORATORY NOTEBOOK

Department _____

Subject _____

Name _____

Date _____

11-V02

15 Sheets, Wide Ruled, 8 1/2 " x 11"

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Activity 1: *Drosophila* phenotype

Pre-Lab Questions

1. How many possible phenotypes are there for the *eya*^{2} mutation of the *eyes absent (eya)* gene?

2. How many possible genotypes are there for the *eyes absent (eya)* gene?

3. What alleles could a fly that looks wild type (red eye) be carrying?

Analysis Questions

1. Record your phenotype and associated genotype of each of the flies you examined.

2. Using the Punnet Square determine the genotype and phenotypes of offspring from two flies mating that are (a) eyeless (***eya*^{2} / *eya*^{2}**) and wild type (***eya*$^+$ / *eya*$^+$**) and (b) eyeless (***eya*^{2} / *eya*^{2}**) and red eyed (***eya*$^+$ / *eya*^{2}**).

(a)

	***eya*^{2}**	***eya*^{2}**
***eya*$^+$**		
***eya*$^+$**		

genotype **phenotype**

(b)

	***eya*^{2}**	***eya*^{2}**
***eya*$^+$**		
***eya*^{2}**		

genotype **phenotype**

3. Using the Punnet Square (b).
What is the porportion (fraction) of offspring that are homozygous recessive for the *eya*^{2} mutation?

What is probability a fly will be born eyeless (homozygous recessive)?

4. Thought question. When might it be advantageous (good) to know if an organisim (human or otherwise) is carrying a mutated allele?

Activity 2: DNA Extraction

Pre-Lab Questions

1. Where is DNA located in the cell?

2. Why is a buffer solution used during DNA extraction?

3. What is the purpose of the Proteinase K in the squishing buffer?

Analysis Questions

1. Why were the tubes put into a hot water bath after the 30-minute incubation?

2. Form a hypothesis about an eyeless fly phenotype and its genotype.

3. Thought question: If we can see the phenotype of each fly, why do we bother with extracting the DNA to determine the genotype?

Activity 3: Polymerase Chain Reaction (PCR)

Pre-Lab Questions

1. What is the purpose of a PCR?

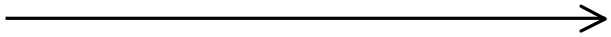
2. What are the chemical components of the PCR and what is the role of each of those ingredients?

3. What is the purpose of each of the three temperatures in each cycle of the PCR?

Analysis Questions

1. What naturally occurring biological process does PCR imitate?

2. Using the single stranded DNA template provided (a) identify the complementary sequence the primers are designed to locate and (b) locate that complementary sequence on the single stranded DNA template.

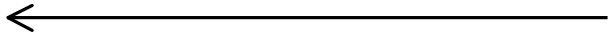


Forward Primer: 5' – GTT TCT CCG TCG TTT TCC CC – 3'

– –

– –

Reverse Primer: 3' – CCG AAA ACA AGT TGG CAT TT – 5'



5' – CGTCGTCCAGACGCCAGTCGACAAAGAAAAAACACAAAGAGGCAG

CAAAAGGGGAGACGAAACTGGCCAAATATTCGAAAATGTTGTCCGTCA

AATCGGATTTGAGTTGAGCAGGTCAGTTAATATTACTAACTGCGATTTT

ATCCTTAAAGTGTTAGTTTATTAATCAATTTTGATATATTCATTCAAAAA

TACAGCCAAAATTAAATTAACTCATATACCTGACAACATGTTTAAGTGA

TTAAAATGTATATTTTCAATTTTCACTTTTACCATTCACACCACCAAAAA

AGCCATCACAGAGCTGCTAACTTACTTGAAATATCCTTCAAATTCCTTT

AAATCCTTTTCCAAAGGCAGTTTAAACTTTTATGTGCCTGTGTTTCCCA

AATTGCAGTTAAGTAATCACAAAAATGCCAACTTGTTTTCGGAACACA

AAAGAAGTGCTCACGGCTTTTGTTCAACCGTAAAGTCTGCA – 3'

3. Thought question: What ingredient(s) of the PCR would have to change if we were targeting a gene other than the *eya* gene?

Activity 4: Hardy-Weinberg Equilibrium

Pre-Lab Questions

1. What is the biological definition of evolution?

2. What are the five conditions a non-evolving population must meet under the Hardy-Weinberg Model?

_____ _____

_____ _____

_____

3. Which Hardy-Weinberg equation describes **genotype** frequency? Which Hardy-Weinberg equation describes **allele** frequency?

genotype frequency: _____

allele frequency: _____

Analysis Questions:

1. In Scenario 1, was the fly population in Hardy-Weinberg Equilibrium?

Was the population in Scenario 2 in Hardy-Weinberg Equilibrium?

2. Which population, the population from Scenario 1 or the population from Scenario 2, is no longer evolving?

3. Thought question. Which genotype - homozygous dominant, heterozygous, or homozygous recessive – do you think plays a large role in promoting evolutionary change?

Worksheet 1: *Drosophila* Eye Phenotypes and Hardy Weinberg Equilibrium.

1. Use Table 1 to record the phenotypes observed by the researchers in **Scenario 1**. Calculate the frequency of each phenotype.

Table 1: Observed Phenotypes:

	Wild Type (Red)	Eyeless	Total
Number of Flies			
Frequency of Phenotype			

2. a. Which phenotype do we believe? Wild Type (Red)? Eyeless?
In other words which is the LEAST subjective phenotype?

b. What is the genotype of this phenotype?

c. From the equation $p^2 + 2pq + q^2$, which term is the Hardy Weinberg notation for this genotype?

3. a. What is the frequency of the recessive allele (q)?

b. Use the equation $p + q = 1$ to calculate the frequency of the dominant allele (p).

4. Using the allele frequencies (p and q) calculate the expected frequency of each genotype ($p^2 + 2pq + q^2$) and the expected number of students with each genotype. Record these values in Table 2.

Table 2: Expected Genotypes:

	Homozygous Dominant (p^2)	Heterozygous (2pq)	Homozygous Recessive (q^2)
Frequency of Genotype			
Expected Number of Flies			
Observed Number of Flies (*from the gel*)			

Use these results to answer Activity 4 Analysis Questions.

Worksheet 2: *Drosophila* Eye Phenotypes and Hardy Weinberg Equilibrium.

1. Use Table 1 to record the phenotypes observed by the researchers in **Scenario 2**. Calculate the frequency of each phenotype.

Table 1: Observed Phenotypes:

	Wild Type (Red)	Eyeless	Total
Number of Flies			
Frequency of Phenotype			

2. a. Which phenotype do we believe? Wild Type (Red)? Eyeless?
In other words which is the LEAST subjective phenotype?

b. What is the genotype of this phenotype?

c. From the equation $p^2 + 2pq + q^2$, which term is the Hardy Weinberg notation for this genotype?

3. a. What is the frequency of the recessive allele (q)?

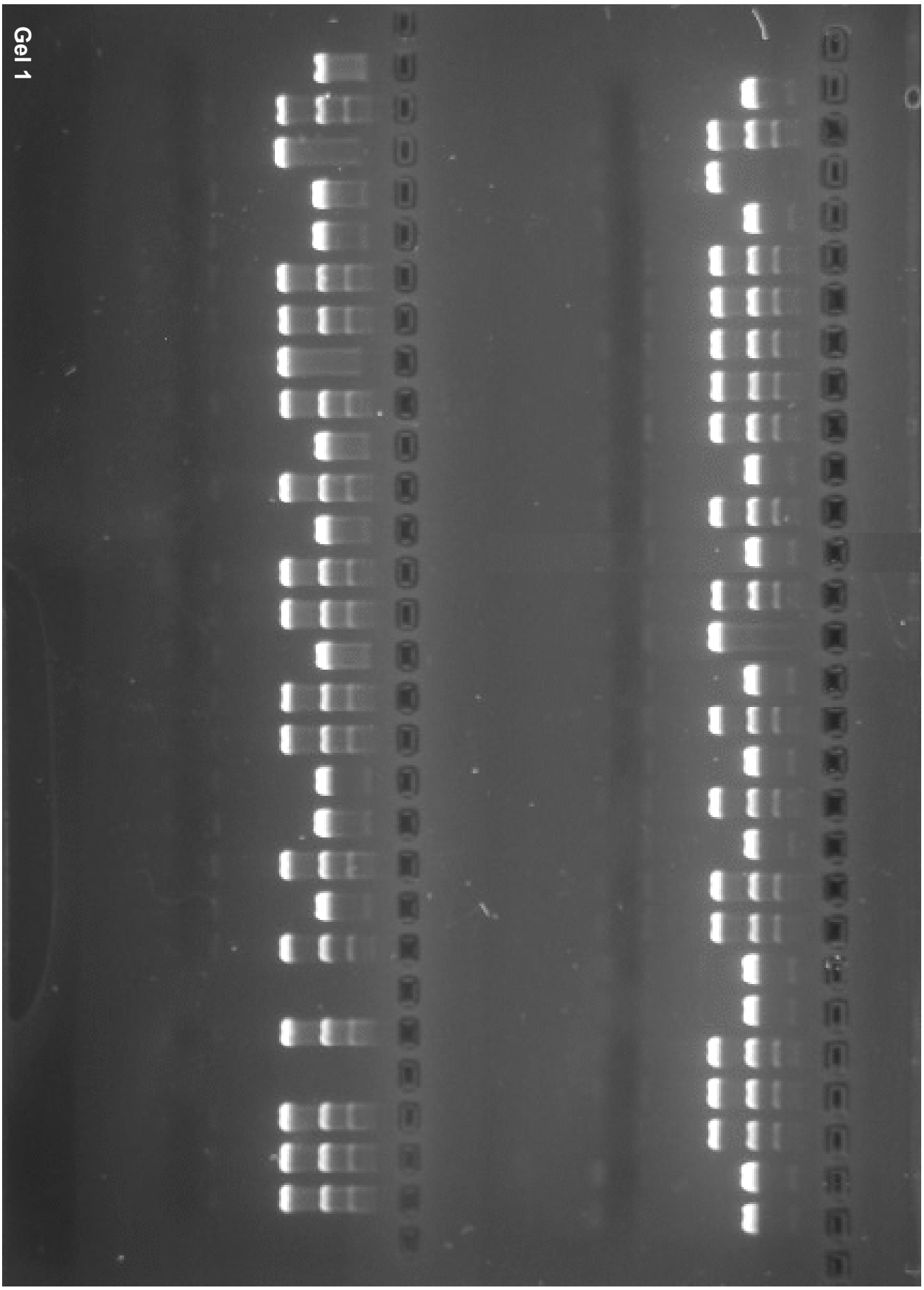
b. Use the equation $p + q = 1$ to calculate the frequency of the dominant allele (p).

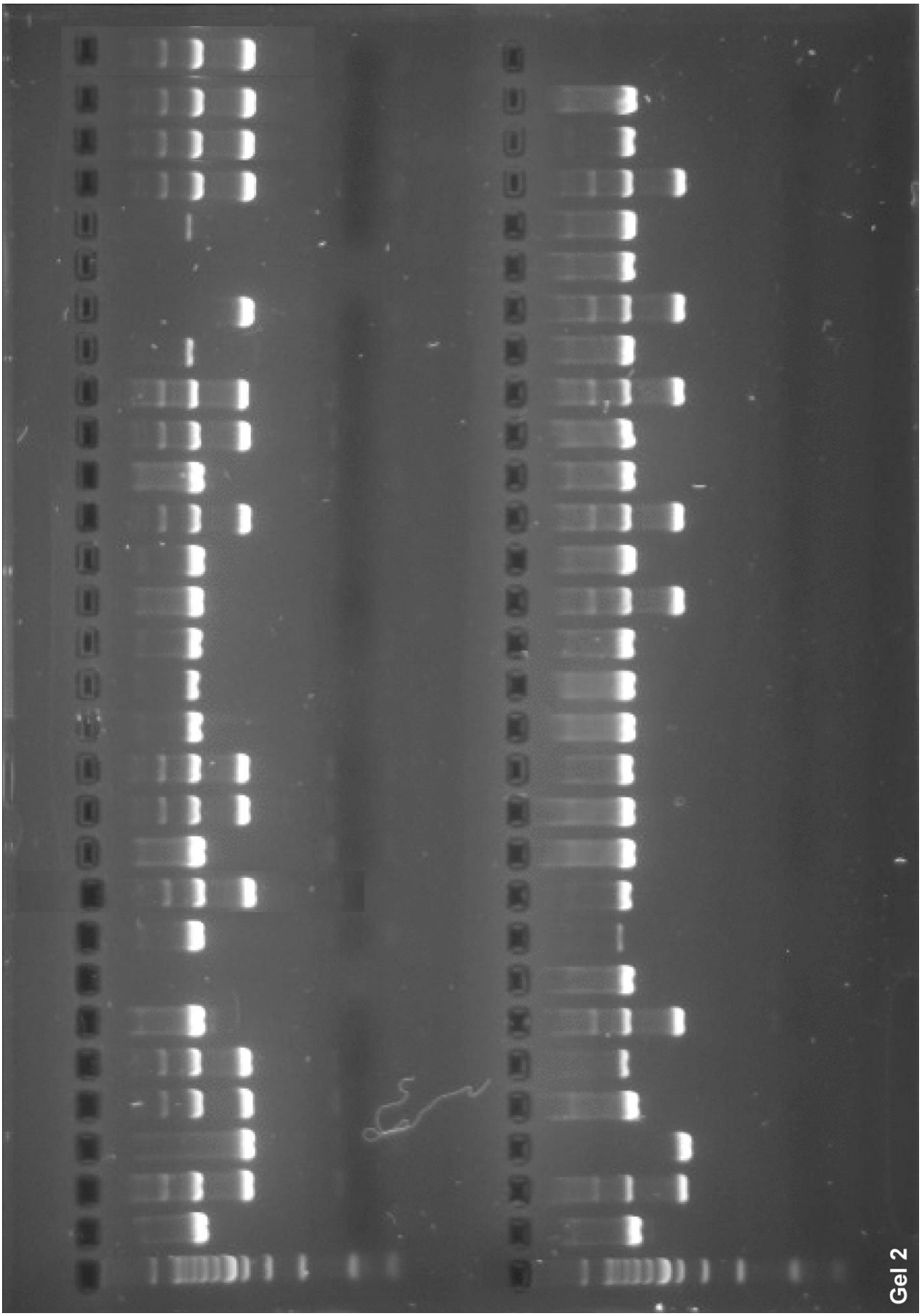
4. Using the allele frequencies (p and q) calculate the expected frequency of each genotype ($p^2 + 2pq + q^2$) and the expected number of students with each genotype. Record these values in Table 2.

Table 2: Expected Genotypes:

	Homozygous Dominant (p^2)	Heterozygous (2pq)	Homozygous Recessive (q^2)
Frequency of Genotype			
Expected Number of Flies			
Observed Number of Flies (*from the gel*)			

Use these results to answer Activity 4 Analysis Questions.


Gel 1


Gel 2

Classroom Analysis: *Drosophila* Eye Phenotypes and Hardy Weinberg Equilibrium.

1. Use Table 1 to record the phenotypes observed in the population of *Drosophila* flies used by your class. Calculate the frequency of each phenotype.

Table 1: Observed Phenotypes:

	Wild Type (Red)	Eyeless	Total
Number of Flies			
Frequency of Phenotype			

2. a. Which phenotype do we believe? Wild Type (Red)? Eyeless?
In other words which is the LEAST subjective phenotype?

b. What is the genotype of this phenotype?

c. From the equation $p^2 + 2pq + q^2$, which term is the Hardy Weinberg notation for this genotype?

3. a. What is the frequency of the recessive allele (q)?

b. Use the equation $p + q = 1$ to calculate the frequency of the dominant allele (p).

4. Using the allele frequencies (p and q) calculate the expected frequency of each genotype ($p^2 + 2pq + q^2$) and the expected number of students with each genotype. Record these values in Table 2.

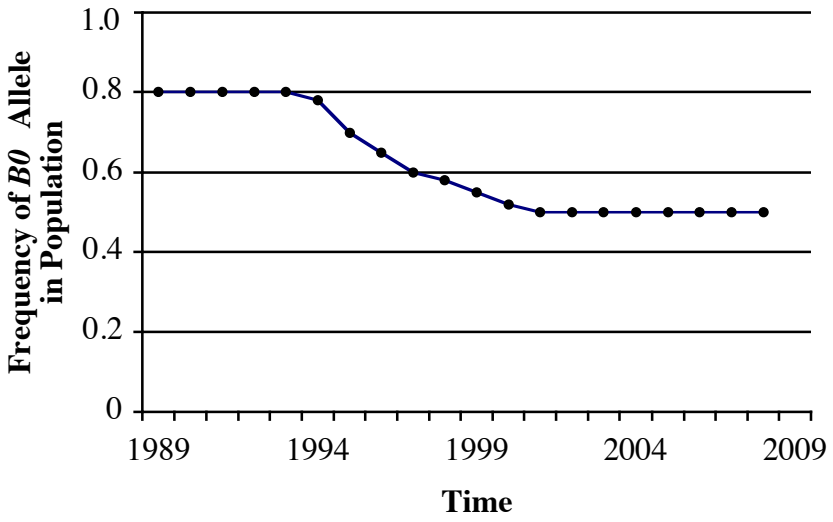
Table 2: Expected Genotypes:

	Homozygous Dominant (p^2)	Heterozygous (2pq)	Homozygous Recessive (q^2)
Frequency of Genotype			
Expected Number of Flies			
Observed Number of Flies (*from the gel*)			

5. Compare the expected genotypes and the observed. Is the classroom population in HW Equilibrium? *Why or why not?*

AP Biology Questions AP1-AP5 refer to the following*

In the land snail *Cepaea nemoralis* (Grove snail) presence of bands on the shell is controlled by two alleles, B^0 and B^B at a single locus. B^0 (unbanded shell) is dominant over B^B (banded shell). A large population of snails was studied over time and the frequency of the unbanded allele B^0 was documented. The results are shown below. In 2009 a random sample of 1000 snail eggs was collected and the snails were allowed to hatch.



AP1. During which of the following time periods could the population have been in Hardy-Weinberg equilibrium for the B^0 allele?

I. 1990-1994
II. 1995-2001
III. 2002-2009

(A) I only
(B) II only
(C) III only
(D) I and III only
(E) I, II, and III

AP2. Assuming the snail population was in Hardy-Weinberg equilibrium for the ***B*** locus, what percentage of the snails in the natural population had banded shells in 1992?

(A) 2%
(B) 4%
(C) 10%
(D) 20%
(E) 64%

AP3. Assuming the population was in Hardy-Weinberg equilibrium for the ***B*** locus, what was the frequency of the B^0 allele in the snails that were hatched in 2009?

(A) 0.33
(B) 0.50
(C) 0.67
(D) 0.75
(E) 1.00

AP4. Assuming that the population was in Hardy-Weinberg equilibrium for the ***B*** locus, what percentage of unbanded snails that hatched in 2009 was heterozygous?

(A) 0%
(B) 25%
(C) 33%
(D) 67%
(E) 100%

AP5. Which of the following is the most likely reason for the observed differences in the frequency of the B^0 allele between 1995-2001?

(A) Emigration of banded snails from the population.
(B) Chance
(C) Selection against unbanded phenotypes.
(D) Speciation
(E) Mutation

*modeled after a 2002 College Board Released Exam Excerpt, Section I

Activity 5: Gel Electrophoresis

Pre-Lab Questions

1. What material will be used to make the gel matrix?

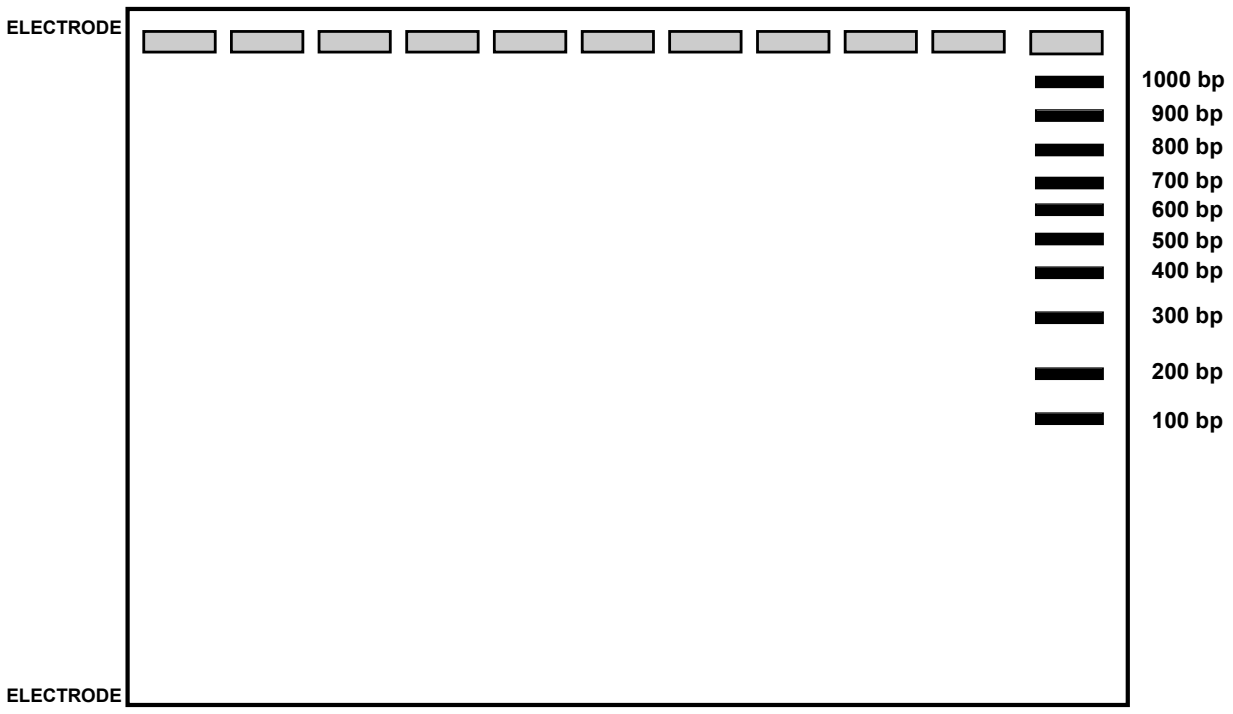
2*. Which fragment will move the fastest, a 687 base pair fragment or a 367 base pair fragment?

3. Why does a heterozygote genotype have two fragments?

Analysis Questions

1*. Why can DNA be seen in the agarose gel under UV light?

2*. Using the gel illustration, make a prediction about the fragment pattern for each of your flies. Draw the DNA fragments, and label the DNA starting point, positive and negative electrodes, and direction of DNA migration. (**use more than one lane for a red eye fly*).



AP Biology Questions

AP1*. Discuss how each of the following factors would effect the ability to visualize DNA on an agarose gel using the gel electrophoresis technique.

a. Voltage used. *For example, if high voltage is used and there is a small difference between DNA fragment sizes then the resolution is POOR? GOOD?*

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b. Running time. *For example if the gel is run for a long time versus a short time; how does that impact the ability to see differences in the DNA fragments created?*

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c. Amount of DNA used. *What if you load a small volume of DNA into the gel, or a large volume of DNA?*

_____

_____

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d. Reversal of polarity. *What if you connected the electrodes so the current was applied with the DNA next to the (+) electrode?*

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AP2. Two very small DNA fragments of nearly the same base pair size look like a single band, even when the DNA is run to the end of the agarose gel. What could be done to resolve (see the difference between) the two fragments? Why would it work? *Hint: think about the relationship between the agarose gel concentration (thickness) and factors (a) and (b) from above.*

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AP3. How does the loading dye function in gel electrohoresis?

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AP4. How can a mutation, like an insertion or deletion, be detected using gel electrophoresis?

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* Pre-Lab question 2, Analysis questions 1 and 2, and AP1-4 modeled after The College Board AP Program (2001), Lab Six Molecular Biology, Exercise 6B analysis questions 1,2, 4-8.

Final Lab Activity: Data interpretation, troubleshooting and review of primary concepts

Be prepared to discuss all of the activities and all of the questions, including the ones below. This time will be used to analyze genotypic data and compare the results to your phenotype and your predictions from the first day.

Discussion Questions for Final Day

Were you able to support the hypothesis you formed on Day 1?

Were you able to correctly predict your flies genotype based on their phenotype? What are some possible explanations for an incorrect prediction?

Was the population of flies (class) in Hardy-Weinberg Equilibrium? If not, what are the possible explanations for being out of Hardy-Weinberg Equilibrium?

Based on what you now know, do you think the information about genetics on television and in movies is accurate? What is an example of accurate and inaccurate information?

How do genetics play a role in your daily life?