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Linking Genotype to Phenotype in *Drosophila melanogaster:* PCR Genotyping the *White-one* Eye Mutation

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This lab exercise adds a molecular genetics component to a traditional undergraduate lab that investigates eye color inheritance in *Drosophila melanogaster*. This two-week activity is based on polymerase chain reaction (PCR) and is used to DNA fingerprint (genotype) wild-type (red-eyed) and mutant (white-eyed) flies. The use of this genotyping exercise as a companion activity to a traditional *Drosophila* investigation provides students "hands-on" experience establishing the link between genotype and phenotype. In addition, this lab-based activity introduces students to common techniques used in molecular genetics, including the extraction of DNA, PCR, and agarose gel electrophoresis.

Keywords: *Drosophila*, PCR, *white-one* mutation, genotyping, phenotype, wild-type, eye color, DNA gel electrophoresis

Introduction

This lab exercise was developed to add a molecular genetics component to a traditional undergraduate lab that investigates eye color inheritance in *Drosophila melanogaster*. At Hood College, our sophomore-level cell biology and genetics course includes a multi-week lab exercise that uses *Drosophila* to investigate the classic sex-linked inheritance of "white eyes" discovered by Thomas Hunt Morgan in the early twentieth century. Students culture their own fly stocks, set up a genetic cross examining eye color inheritance and follow the cross through the F_2 generation. Statistical analysis of data is also included. To broaden the learning experience of students, we have developed a genotyping exercise that instructors can use as a companion activity to this classic lab investigation. This two-week activity is based on polymerase chain reaction (PCR) and is used to DNA fingerprint (genotype) wild type (red-eyed) and mutant (white-eyed) flies. The use of this genotyping exercise as a component of a classical *Drosophila* laboratory experience provides students "hands-on" experience establishing the link between genotype and phenotype, reinforcing the connection between classic and molecular genetics. In addition, this lab-based learning activity introduces students to common techniques used in molecular biology, including the extraction of DNA, PCR, and agarose gel electrophoresis.

To help students with the understanding of the biological concepts associated with this lab, we present a Powerpoint presentation during the first week of the two-week activity. This presentation summarizes the fly crosses students have been conducting and provides background information on the cell and molecular biology associated with the red and white-eyed phenotypes. In addition, the presentation introduces students to the principles and applications of PCR.

Student Outline

Introduction

This semester we have investigated the inheritance of eye color in *Drosophila melanogaster* first discovered by Thomas Hunt Morgan in the early twentieth century (Morgan 1910). Wild-type *Drosophila* have red eyes and Morgan's observation of a male with white eyes in his laboratory culture led to the discovery of sex-linked inheritance and the establishment of the chromosomal basis for heredity. Since the pioneering work of Morgan, the genetics and molecular biology of *Drosophila melanogaster* have been extensively studied, including the creation of FlyBase, a database that lists and describes *Drosophila* gene sequences and functions (Tweedie et al. 2009).

This model organism has four pairs of chromosomes designated I, II, III, and IV; females have one pair of X chromosomes, males have one X and one Y chromosome (Fig. 1).

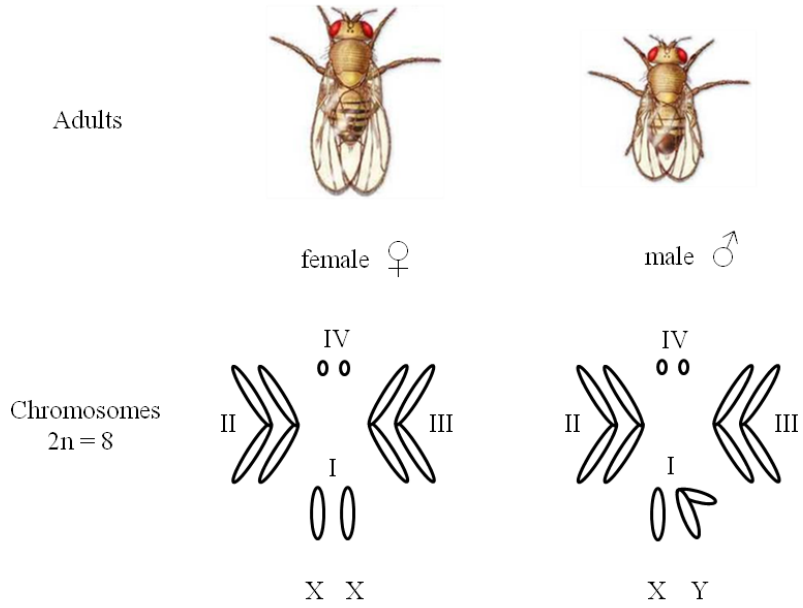


Figure 1. Comparison of female (left) and male (right) *Drosophila melanogaster*.

The sex-linked inheritance of eye color discovered by Morgan (1910) is controlled by the *white* allele found on the X chromosome. This wild-type allele (X^{w+}) is composed of a gene characterized by 6 exons and 5 introns, encoding a protein (designated "white") of 687 amino acids that is located in the membrane of eye cells (and cells of other tissues). White protein partners with the protein "brown" encoded by the *brown* gene to produce a heteromeric transporter protein. In wild-type flies, the transporter moves extracellular precursors for eye pigments into the cytoplasm of eye cells. Once inside the cell, the precursors are biochemically converted to the final pigment products that give wild-type *Drosophila* eyes that are red in color (Tweedie et al. 2009). In the *white-one* mutation (X^{w1}), transcription from the *white* gene is disrupted, the white protein is not synthesized, and the functional transporter protein is not produced. Therefore, this mutation blocks the ability of eye cells to uptake pigment precursors and the eyes appear white (Fig. 2).

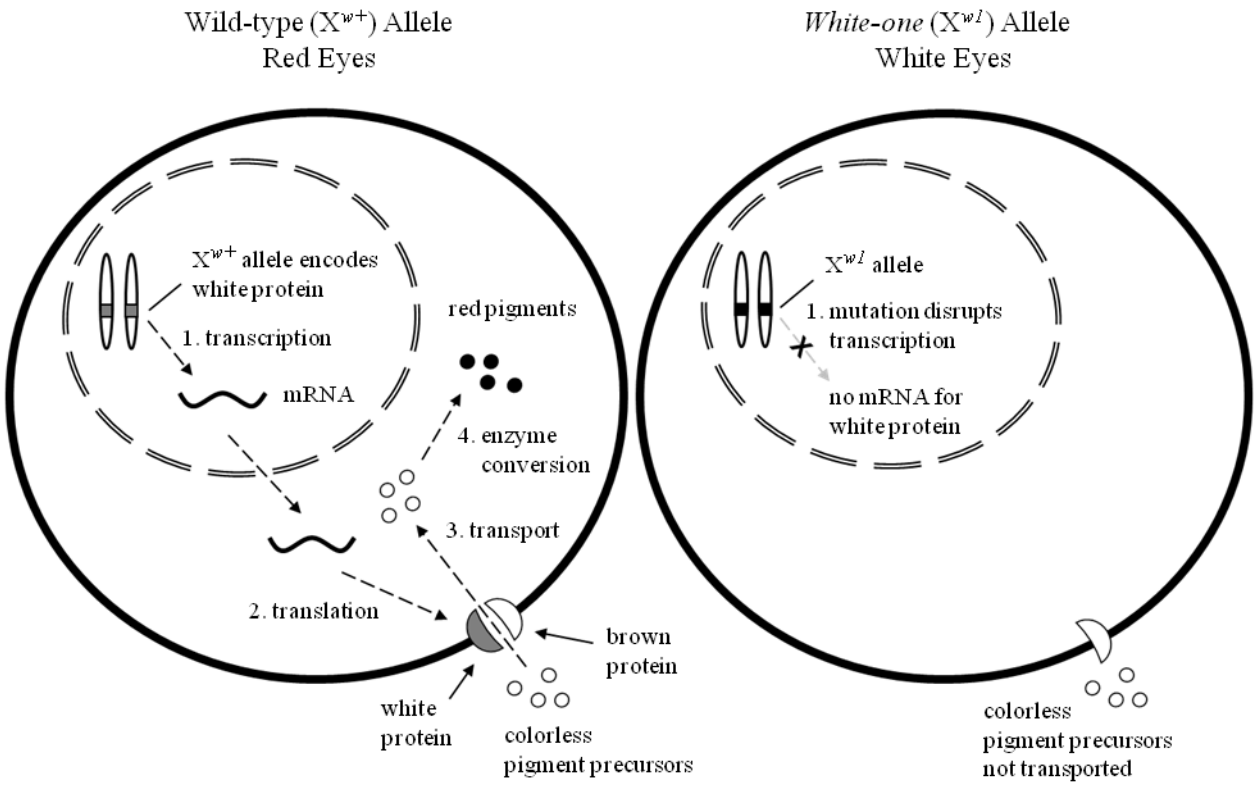


Figure 2. Summary of the wild-type (left) and *white-one* (right) alleles in the eye cells of *Drosophila melanogaster*.

Work on the molecular genetics of the *white-one* (*w1*) mutation has determined that this mutation results from insertion of a 4,700-bp Doc retroposon in the promoter region of the *white* gene resulting in inactivation of the promoter and, thus, disruption of transcription (Driver et al. 1989). In addition, DNA sequence analysis of the 4,700-bp Doc element has been completed and it has been shown to be closely related to other retroposons reported for *Drosophila* (O'Hare et al. 1990).

Objectives

The goal of this two-week lab activity is to analyze individual female and male flies from the F_1 and F_2 generations you have worked with this semester. Each student will choose a fly (male or female; red-eyed or white-eyed), extract fly DNA, and conduct the polymerase chain reaction (PCR) procedure (Bloom et al. 1996; Dieffenbach and Dveksler, 2003) followed by agarose gel electrophoresis to identify the genotype of the fly.

The promoter region for the wild-type (X^{w+}) and mutated (X^{w1}) alleles, including the location of the three primers which will be used to genotype individual flies using PCR are shown in Figure 3. Primers P1 and P3 are specific for the wild-type allele, whereas, primer P2 is specific for the mutated allele. Amplification of the wild-type allele by PCR is expected to produce a product of 467 bp while amplification of the *white-one* allele is expected to produce two products: 5,211 bp and 704 bp. The upper limit of size for PCR products using *Thermus aquaticus* (*Taq*) DNA polymerase approximates 5,000 bp (Barnes, 1994; Dieffenbach and Dveksler, 2003), therefore, the 5,211-bp product generated by primers P1 and P3 is not made in amounts that are detectable by agarose gel electrophoresis.

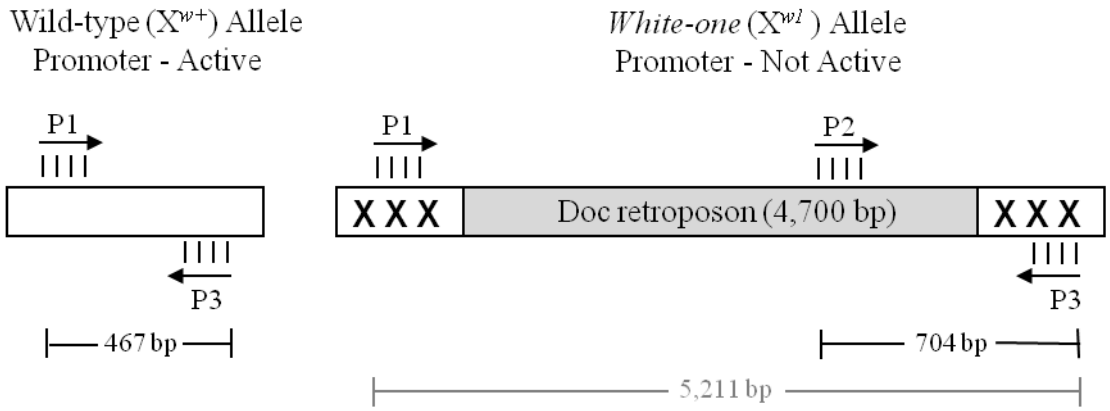


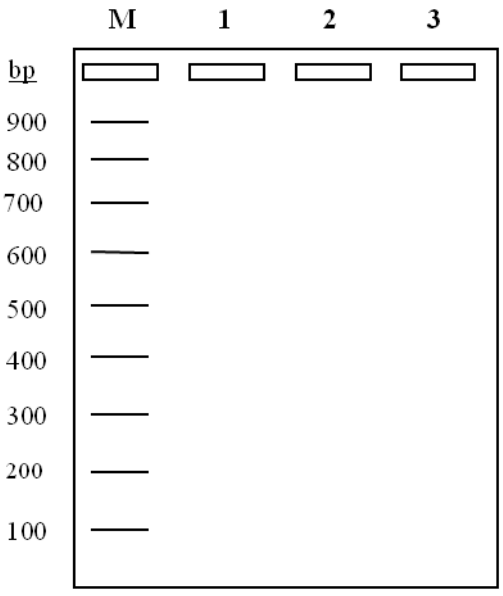
Figure 3. The PCR products produced for the wild-type (left) and *white-one* (right) alleles of *Drosophila melanogaster*. The binding sites for primers P1, P2, and P3 used for PCR are shown with arrows.

The nucleotide sequences for primers P1, P2, and P3 are presented in Table 1. In addition, the sequences for the wild type and *white-one* alleles are available from your instructor, including the location of the binding (annealing) site for each primer.

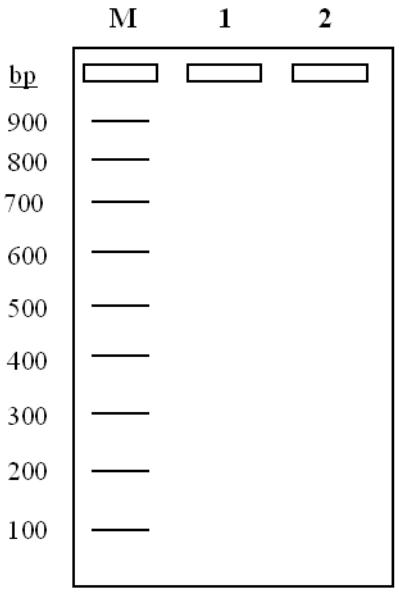
Table 1. Sequence information for primers.

Primer	Sequence (5' to 3')	Length
P1	GTGCAAAGGTGGTCGAATTT	20
P2	TCTGGGAGTTCATCTGGACA	20
P3	GAGAGGAGTTTTGGCACAGC	20

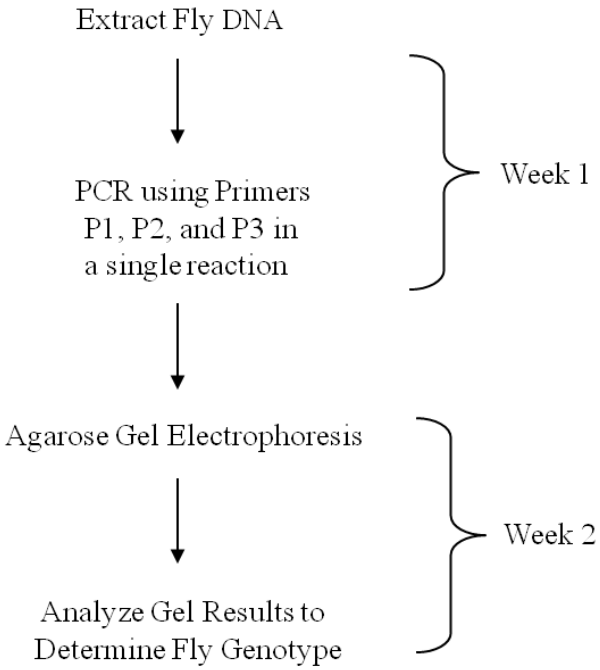
This is a schematic diagram of an agarose gel used to analyze the DNA fragments generated by analyzing individual female flies with primers P1, P2, and P3 in a single PCR reaction. Lane M represents DNA fragments that are size markers (standards). In lanes 1, 2, and 3, sketch in the DNA fragments expected to be produced by PCR for female *Drosophila* homozygous for wild-type, heterozygous, and homozygous for the *white-one* mutation, respectively:



Analysis of male *Drosophila* using the same primers would produce two genotypes. In lanes 1 and 2, sketch in the results expected for wild-type males and those with the *white-one* mutation, respectively:



Flowchart for Two-Week Lab Activity



Extraction of DNA from Individual Flies -Week 1

Place a single fly in a 0.5-mL tube and then report to the instructor's station where the instructor will transfer 50 µL of fly squishing buffer to this tube. After returning to your bench, use a 200-µL pipet tip to squish (mash) the fly for 2 minutes at room temperature. After the fly is squished (yes, you will still see fly parts when you are done!), add 25 µL of mineral oil to the tube and quick-spin (5 seconds) in the microcentrifuge. This ensures the mineral oil forms the top layer, thus forming a barrier against evaporation when the tube is incubated. (Note: the mineral oil is omitted if a thermal cycler with a heated lid is used). Following the instructor's directions, transfer your tube to the thermal cycler which is programmed to incubate the sample at 55ºC for 60 minutes followed by 94ºC for 2 minutes and then cooling to 4ºC. At the completion of this process, you will use 4 µL of the fly DNA lysate as a template for PCR. The residual lysate will be stored at -20ºC.

How does this process extract fly DNA? The squishing buffer contains proteinase K (Gloor et al. 1993). This enzyme is a broad-spectrum protease that is very active at 55ºC, resulting in the digestion (hydrolysis) of cell membrane proteins and the lysis of cells which releases (extracts) fly DNA from the nucleus. The subsequent heating of the tube for 2 minutes at 94ºC inactivates the proteinase K.

Polymerase Chain Reaction (PCR) – Week 1

A. As summarized below, you will conduct a 40-µL PCR reaction that uses 4 µL of the fly DNA lysate you prepared. Components 1-8 will be provided to you as a 36-µL aliquot (termed a "master mix") by your instructor:

Component	Volume	Final Concentration
1. Distilled Water	27.4 µL	
2. 10X PCR Buffer	4.0 µL	10 mM Tris (pH 8.3), 50 mM KCl
3. $MgCl_2$, 50 mM	1.2 µL	1.5 mM
4. dNTPs, 10 mM	0.8 µL	0.2 mM
5. Primer P1, 2.5 µM	0.8 µL	0.050 µM
6. Primer P2, 10 µM	0.8 µL	0.200 µM
7. Primer P3, 10 µM	0.8 µL	0.200 µM
8. *Taq* DNA Polymerase (5 Units/µL)	0.8 µL	25 Units/mL
	36.0 µL	**Components 1-8, from Instructor**
9. Fly DNA Lysate	**4.0 µL**	
	40.0 µL	**Total Volume**

B. Preparation of one PCR reaction by each student:

1. Label a PCR (0.5-mL) tube and aliquot 25 µL of mineral oil into the tube. As described for the fly DNA extraction, the mineral oil is used as a barrier to minimize evaporation during the PCR process. (Note: the mineral oil is omitted if a thermal cycler with a heated lid is used.)
2. Report to the instructor's station where the instructor will transfer 36 µL of the "master mix" to your tube.
3. Return to your bench and transfer 4 µL of your fly DNA lysate to the tube. Your tube now contains mineral oil (25 µL), master mix (36 µL), and your fly sample (4 µL). Close the tube completely and quick-spin (5 seconds) in the microcentrifuge to ensure the mineral forms the top layer. Place the tube on ice.

C. Use of the Thermal Cycler:

1. Following the instructor's directions, place your tube in the DNA Thermal Cycle 480. After all students have transferred tubes to the machine, press "Start" for File #50. The total PCR run time will approximate 3 hours.
2. After the completion of the PCR method, store the reaction tubes at -20ºC for analysis by agarose gel electrophoresis. This analysis will reveal the genotype of your fly (next week).

D. Description of PCR method

File #	Steps	Description
50	1. 94ºC, 1 minute 2. 1 cycle 3. linked to File 49	• Preamplification denaturation of fly DNA
49	1. 94ºC, 1 minute 2. 55ºC, 1 minute 3. 72ºC, 2 minute 4. 35 cycles 5. linked to File 48	• Denaturation of DNA templates • Annealing (binding) of primers • Extension (synthesis) of new DNA • Amplification (steps 1 to 3, repeated 35X)
48	1. 72ºC, 5 minutes 2. linked to File 47	• Final extension (synthesis) of DNA to ensure products (amplicons) are completely synthesized
47	1. 4ºC, hold	• Method completed

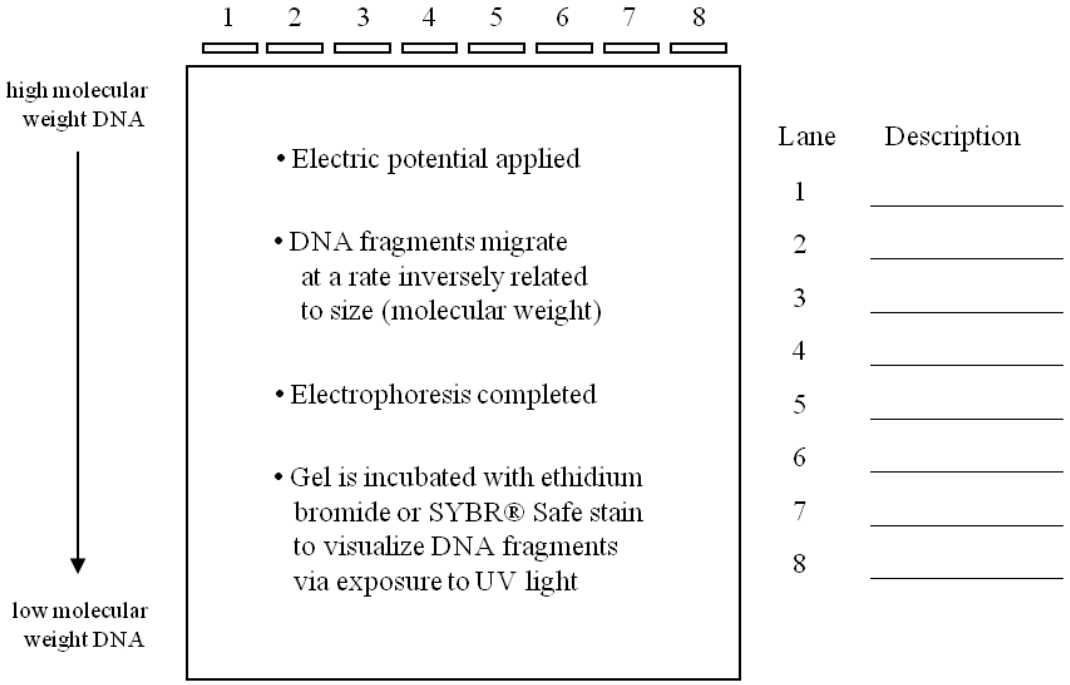
Gel Electrophoresis – Week 2

A. Sample Preparation

Label a 1.5-mL tube and transfer 8 µL of your PCR reaction to this tube followed by 2 µL of 5X DNA loading buffer (25% glycerol, 0.05% bromophenol blue) to produce a total volume of 10 µL. The addition of loading buffer adjusts the sample to 5% glycerol and 0.01% bromophenol blue. The glycerol increases the density of the sample for loading into the well of the agarose gel and the bromophenol blue provides a "dye front" to monitor the length of time for electrophoresis. (Bromophenol blue migrates at a rate equivalent to a 300-bp fragment of DNA.)

B. Gel Electrophoresis

Load your 10-µL sample into the appropriate well of 1.0% agarose gel. Be sure to reserve one well (lane) for DNA size markers. Electrophorese for approximately 60-70 minutes at 80 volts (constant voltage) until the bromophenol marker dye migrates 3/4 the length of the gel.



C. Gel Staining and Documentation

The lab instructor will demonstrate how to stain the gel with ethidium bromide or Sybr® Safe stain and to produce a gel photograph. The final photograph will be posted on Blackboard.

Questions

1. Based on the results of agarose gel electrophoresis, what is the genotype of the fly you processed by PCR. Explain your reasoning.

2. The DNA extraction protocol includes the heat inactivation of proteinase K. Why is this step necessary?

3. Melting temperature (T_m) is the temperature at which a primer dissociates from its complementary target during the PCR process. This parameter is an important consideration for PCR because annealing of primers to target sequences occurs 3 to 5 degrees below the calculated T_m for each primer. In addition, for primers less than 21 bases in length, the following formula can be used to calculate T_m:

$$T_m \text{ (in } ^oC) = 4(G + C) + 2(A + T)$$

Using this formula, complete the following table by calculating the T_m values for the primers employed to genotype the flies by PCR (show all work):

Primer	Sequence (5' to 3')	Length	T_m (°C)
P1	GTGCAAAGGTGGTCGAATTT	20	
P2	TCTGGGAGTTCATCTGGACA	20	
P3	GAGAGGAGTTTTGGCACAGC	20	

4. In the formula provided in Question 3, the (G + C) component makes a greater contribution to the value of T_m as compared to (A + T). Based on your knowledge of DNA structure, explain why this makes sense.

5. Predict the expected outcome in terms of the size of PCR product if a mutation occurs in the promoter by a retroposon that is 300 bp in size. The two primers (P_a and P_b) used in this new PCR method produce a product 250 bp in size for the normal allele. Diagram the promoter with and without the retroposon, similar to Figure 3.

6. Your instructor discovers a new "white-eyed" mutant of *D. melanogaster*. In addition, cell biology students confirm that white and brown proteins are present in the membrane of eye cells of this mutant fly. Based on this information (and Figure 2), suggest one hypothesis for this novel mutation.

7. As for many genes, the link between genotype and phenotype is rarely simple. In fact, the protein encoded by the wild-type allele (*white* gene) is expressed in tissues other than eyes, thus mutations in this gene can alter more than eye color producing a pleiotropic effect. Based on information available at the website (**http://www.sdbonline.org/fly/aimain/1aahome.htm**) for "The Interactive Fly," describe three characteristics (other than eye color) of *Drosophila* that are associated with the *white-one* gene.

Materials

The section describes reagents and supplies for twenty students.

Drosophila and Supplies

For a Pair of Students

- Culture of Wild-type *Drosophila* – Cat. No. 172100 (Carolina Biological Supply)
- Culture of *White-one Drosophila* – Cat. No. 172220 (Carolina Biological Supply)
- FlyNap® Anesthetic Kit – Cat. No. 173010 (Carolina Biological Supply)
- Metal pan, labeled with names of students
- Soft paint brush
- 3 x 5 index cards
- Timer

Shared Material

- Stations for setting up vials of medium: well-sealed bins of Formula 4-24 Instant Medium, Blue – Cat. No. 173210 (Carolina Biological Supply)
- Yeast (if used)
- Vials
- Sterilized stoppers
- Disposal bags for used medium
- Spatulas for scooping medium from vials
- 10-mL pipettes
- Sterile tap water
- Labels and markers for vials
- Alcohol "morgues" for disposing of flies
- Stereoscopes

DNA Extraction

- Fly squishing buffer (10 mM Tris, pH 8.0, 25 mM NaCl, 1 mM EDTA, and 200 µg/mL proteinase K) - 1.5 mL, prepared fresh for each lab, placed on ice.
- DNA Thermal Cycler 480* (Perkin Elmer, Inc.)
- 0.5-mL PCR tubes
- P-20, P-200, & P-1000 micropipettors
- Aerosol-resistant pipet tips
- Ice buckets
- Markers to label tubes
- Mineral oil*

* Mineral oil is required as an overlay for tubes placed in this machine and is omitted for a thermal cycler with a heated lid.

Polymerase Chain Reaction (PCR) - Equipment and Plasticware

- DNA Thermal Cycler 480* (Perkin Elmer, Inc.)
- 1.5-mL microcentrifuge tubes
- 0.5-mL PCR reaction tubes
- P-20, P-200, & P-1000 micropipettors
- Microcentrifuge
- Aerosol-resistant pipet tips
- Ice buckets
- Markers to label tubes
- Mineral oil*

Polymerase Chain Reaction (PCR) - DNA and Reagents (stored at -20ºC)

- Student-prepared Fly DNA lysate samples (50 µL per sample)
- Primer P1 (2.5 µM) - 100 µL
- Primer P2 (10 µM) - 100 µL
- Primer P3 (10 µM) - 100 µL
- 10X PCR buffer (100 mM Tris, pH 8.3, 500 mM KCl) - 100 µL
- 10 mM dNTPs -100 µL
- 50 mM $MgCl_2$ - 100 µL
- PCR-grade distilled water
- Platinum *Taq* DNA Polymerase (5 Units/µL) – Cat. No. 10966-026 (Invitrogen)

Gel Electrophoresis

- Mini-sized horizontal gel unit – Model No. Horizon 58 (Whatman)
- Agarose
- 50X TAE buffer (2.0 M Tris-acetate, pH 8, 0.05 M EDTA)
- 5X DNA loading buffer (50 mM EDTA, pH 8.0, 25% glycerol, 0.05% SDS, and 0.05% bromophenol blue)
- Ethidium bromide (10 mg/mL) or SYBR® Safe stain (10,000X concentrate) – Cat. No. S33102 (Invitrogen)
- 100-bp DNA ladder – Cat. No. 15628-019 (Invitrogen)
- UV-Transilluminator
- Power supply
- Camera system

Notes for the Instructor

Drosophila Crosses

We have found students to be more invested in this semester-long project if they maintain *Drosophila* cultures and are responsible for doing their own crosses. It is very helpful to have teaching assistants maintain fly stocks and to conduct crosses simultaneously with students in the class to provide "spare" cultures or crosses when needed. Individual students conduct one cross through the F_2 generation, either Cross A or Cross B. These are reciprocal crosses and produce the

complete range of fly genotypes. We recommend periodic fly checks where students must fill out forms documenting their progress with the crosses. Students must also show their *Drosophila* cultures when turning in the forms to complete the fly check. Students store flies (F_1 and F_2 generations) at -20ºC in 1.5-mL microcentrifuge tubes which are later processed for DNA extraction and PCR analysis. During the time students are making their crosses through the F_2 generation, students should maintain counts of phenotypic classes. These counts can be used for Chi Square calculations.

The genotypes and phenotypes for the two *Drosophila* crosses through the F_2 generation are summarized in the figure at the bottom of the page.

DNA Extraction – Preparation of Fly Squishing Buffer

Label a 1.5-mL microcentrifuge tube "FSB" (for fly squishing buffer) and place on ice. In the order indicated, add the following components to the tube:

Component	Volume
1. Distilled Water	1,320 µL
2. 10X TNE	150 µL
3. Proteinase K (10 mg/mL)	30 µL
	1,500 µL Total Volume (1.5 ml)

Note: 10X TNE is 100 mM Tris, pH 8.0, 250 mM NaCl, 10 mM EDTA. Proteinase K (Cat. No. 25530-015, Invitrogen) is from a stock solution (10 mg/mL) prepared in distilled water and stored at -20ºC in 35 µL aliquots.

Polymerase Chain Reaction (PCR)

As outlined in the student handout, each student conducts a single PCR reaction using 4 µL of fly DNA lysate, and a 36-µL volume of all the other components that allow amplification of the DNA via PCR. This reaction uses more than two primers, therefore, is termed a "multiplex" PCR protocol (Dieffenbach and Dveksler, 2003).

For a class of 20 students, we provide the 36-µL amounts from a 25-reaction (900-µL) master mix. To prepare the master mix, we label a 1.5-mL microcentrifuge tube "MM" (for master mix), place on ice, and add the components in the order described in Table 2.

This is followed by directing the students to the instructor station where you add the 36 µL aliquot to each student's tube. Students then return to their bench and add 4 µL of their fly DNA lysate to the tube followed by a brief microcentrifugation step (5 seconds). When all students have completed this step, the tubes are placed in the DNA Thermal Cycler 480 and File #50 is started.

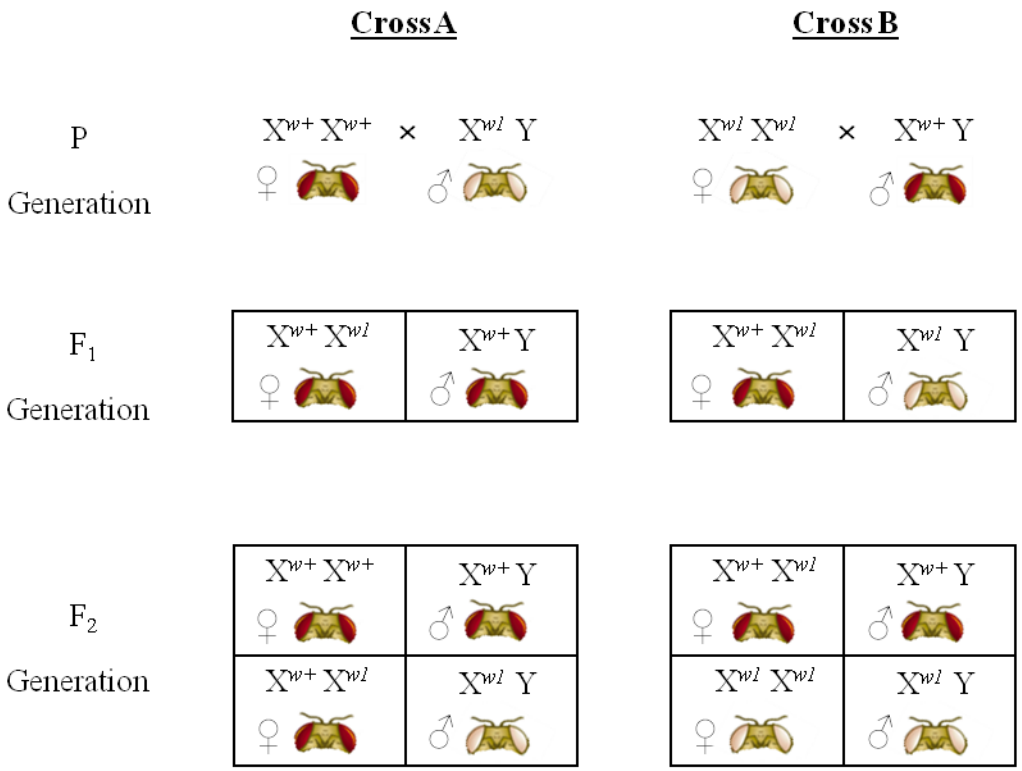


Table 2. Components for PCR master mix.

Component	1 Reaction Vol.		25-Reaction Master Mix Volume
1. Distilled Water	27.4 µL	x 25 =	**685 µL**
2. 10X PCR Buffer	4.0 µL	x 25 =	**100 µL**
3. $MgCl_2$, 50 mM	1.2 µL	x 25 =	**30 µL**
4. dNTPs, 10 mM	0.8 µL	x 25 =	**20 µL**
5. Primer P1, 2.5 µM	0.8 µL	x 25 =	**20 µL**
6. Primer P2, 10 µM	0.8 µL	x 25 =	**20 µL**
7. Primer P3, 10 µM	0.8 µL	x 25 =	**20 µL**
8. *Taq* DNA Polymerase (5 Units/µL)	0.2 µL	x 25 =	**5 µL**
			900 µL Total Volume

Primer and Gene Sequence Information

The program Primer 3 (Rozen and Skaletsky, 2000) was used to assist with the design of primers for this PCR-based lab activity. The DNA sequence for the *white* locus (Genbank accession X02974) of *Drosophila* was employed to design primers P1 and P3 for the wild-type allele (O'Hare et al., 1984). The DNA sequence of the Doc retroposon in the *white-one* mutant of *Drosophila* (Genbank accession X17551) was used to design primer P2 for the *white-one* allele (O'Hare et al., 1991). Information for the primers is summarized in Table 3.

Table 3. Primer sequence information & T_m values.

Primer	Sequence (5' to 3')	Length	GC%	T_m
P1	GTGCAAAGGTGGTC GAATTT	20	45	58
P2	TCTGGGAGTTCAT CTGGACA	20	50	60
P3	GAGAGGAGTTTTG GCACAGC	20	55	62

We have presented the nucleotide sequences for the promoter regions of the wild-type and *white-one* alleles in Appendix A and B, respectively. We recommend using this information and the primer sequences to conduct computer-assisted PCR via the Primer3 (**http://frodo.wi.mit.edu/primer3/**) website (or a similar website) to verify the primer annealing sites and the size of the expected amplicons. Table 4 summarizes the primers and sequences to use to verify PCR product sizes of 467 and 704 bp for the wild-type and *white-one* alleles, respectively. For example, to confirm the PCR product associated with the wild type allele, go to the Primer3 website (do not change the default options) and import the sequence from Appendix A, followed by the sequences for the left (P1) and right (P3) primers from Table 2. To run Primer3 and to produce the output file showing the annealing sites and PCR product size, click on "Pick Primers." Repeat these steps for the *white-one* allele using the information in Table 4.

Table 4. List of primers and sequences to use with Primer3 to confirm primer annealing sites and PCR product sizes.

Allele	Left Primer	Sequence	Right Primer	PCR Product Size
wild type	P1	Appendix A	P3	467 bp
white-one	P2	Appendix B	P3	704 bp

We also encourage you to conduct this activity with students because it introduces them to a useful bioinformatics tool that reinforces PCR concepts. Please note, however, that Primer3 will report T_m values slightly different from those reported in Table 3 because we employed the formula T_m (in ºC) = 4(G + C) + 2(A + T) and Primer3 uses an alternative calculation for T_m.

Preparation of Primer Stock Solutions (100 µM)

Primers were synthesized commercially (Invitrogen) and reconstituted to a final concentration of 100 µM in TE buffer (10 mM Tris, pH 7.2, 1 mM EDTA) followed by storage at -20ºC. For the lab activity, primers P1, P2, and P3, were diluted to concentrations of 2.5, 10, and 10 µM, respectively, using distilled water and stored at -20ºC.

Table 5 summarizes the volume of TE buffer added to each lyophilized primer to produce stock solutions with concentrations of 100 µM.

Table 5. Preparation of primers to yield stock solutions of 100 µM.

Primer	Quantity in tube (from Invitrogen)	Vol. TE buffer added to tube	Concentration
P1	27.62 nmoles	276 µL	100 µM
P2	26.26 nmoles	263 µL	100 µM
P3	28.66 nmoles	287 µL	100 µM

Gel Electrophoresis

1. Electrophoresis Buffer: Prepare 250 mL of 1X TAE buffer (pH 8.0) by mixing 5 mL of 50X TAE buffer with 245 mL of distilled water.
2. Gel Preparation: A 1.0% agarose gel is prepared by adding 0.25 g of agarose to 25 mL of 1X TAE buffer in a 125-mL Erlenmeyer flask. Bring to a boil by microwaving (1 minute). Swirl and pour into the gel unit that has a comb inserted. Allow to solidify (20 minutes).

3. Sample Preparation: Transfer 8 µL of each PCR reaction to a 1.5-mL tube followed by the addition of 2 µL of 5X DNA loading buffer. Mix briefly.
4. Gel Electrophoresis: Pour the remaining amount of 1X TAE buffer (225 mL) into the gel unit and remove the comb. Load each 10-µL sample into the appropriate well. Conduct electrophoresis for approximately 60-70 minutes at 80 volts (constant voltage). The bromophenol blue dye front should migrate about 3/4 the length of the gel.
5. Gel Staining: At the completion of electrophoresis, transfer the gel to a staining tray and add 100 mL of 1X TAE buffer followed by the addition of 5 µL ethidium bromide (10 mg/mL) [caution: ethidium bromide is a mutagen] or 10 µL SYBR® Safe stain (10,000X concentrate). Mix and incubate for 15 minutes. At the completion of staining step, photograph the gel using a UV- transilluminator.
6. **Important Note**: Handle ethidium bromide and SYBR® Safe stain, including waste disposal, in accordance with the safety regulations of your institution.

Expected Results for PCR Genotyping Activity

For a class of 20 students, we suggest dividing the class into three groups and running three DNA gels. It is important, of course, that students in each group have a variety of fly samples that will demonstrate the full range of fly genotypes.

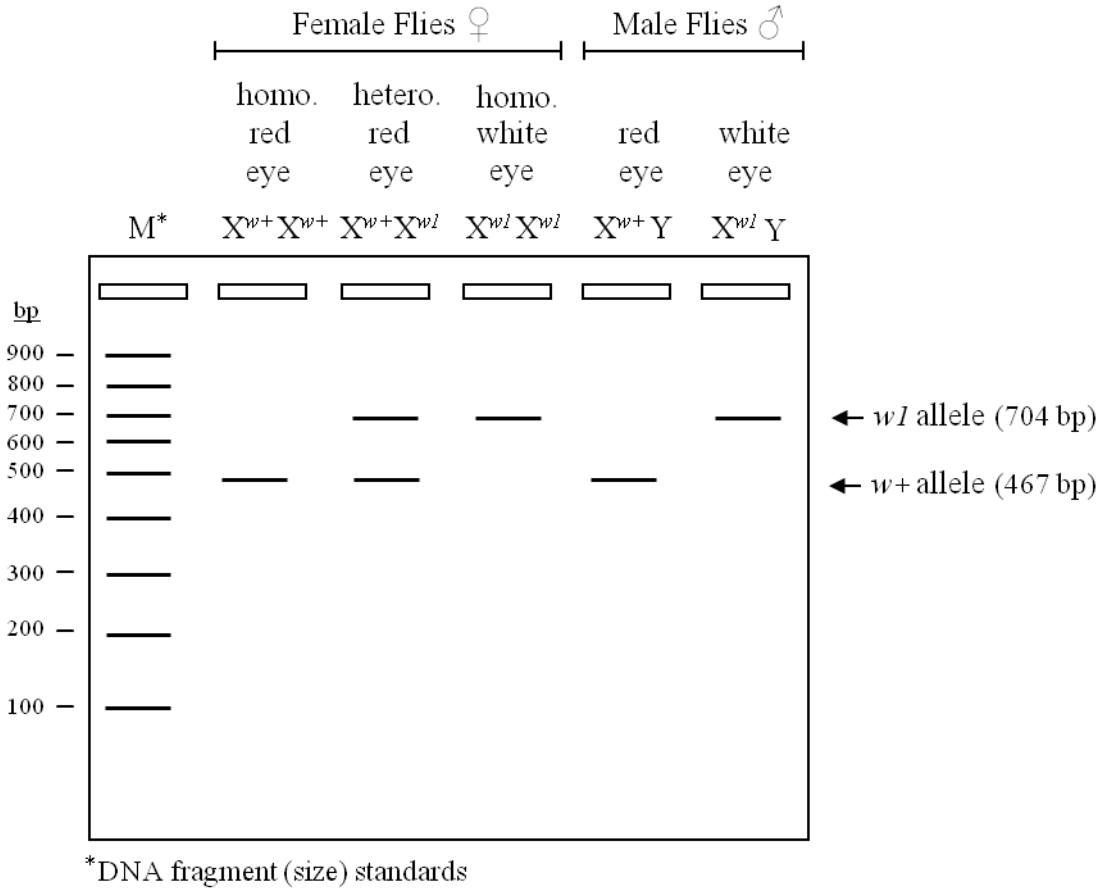
The expected results for gel electrophoresis are summarized in the figure at the bottom of the page.

Sample Student Results

During the spring semester of 2010, Biology 203 students sampled and stored flies at -20ºC followed by genotyping via PCR the week of May 3rd as shown on the next page.

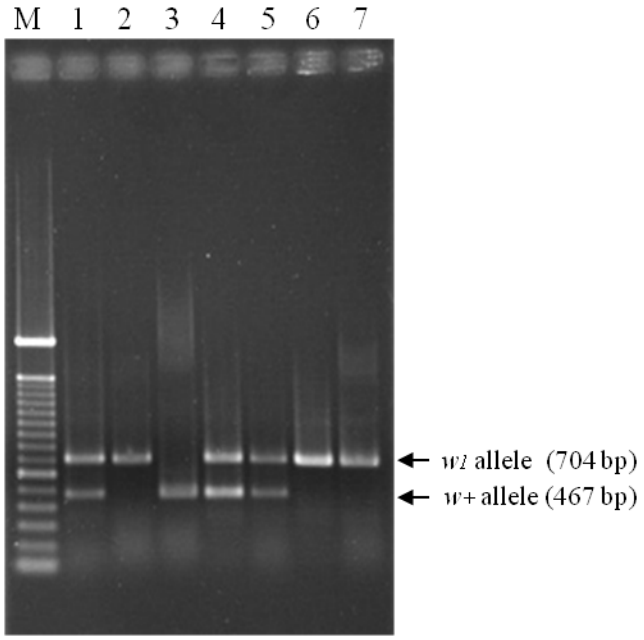
Lessons Learned on the "Fly"

- Fresh flies are preferable to using frozen flies, but often the timing of crosses does not allow students to use fresh flies, particularly from the F_1 generation. Therefore, be very sure that students properly label fly samples that are stored at -20ºC.
- If students are inexperienced using micropipettors, have them practice the proper technique for using micropipettors before they aliquot the small volumes used in this exercise.



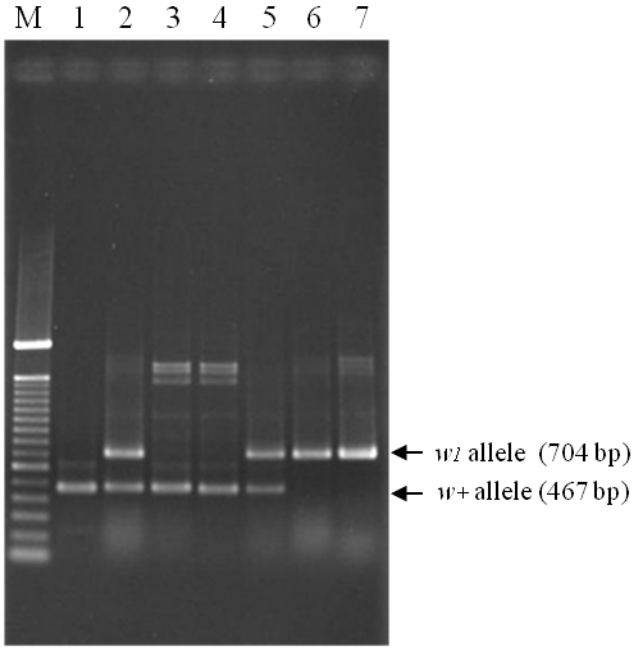
- Squishing the fly for DNA extraction is an important step that students often do not do properly. We have found that toothpicks DO NOT work well during the squishing step. There seems to be a chemical in the wood that interferes with the DNA extraction or the PCR process. When macerating the fly, do this vigorously and hold the 0.5 mL tube on a solid surface so more force can be used.
- To facilitate gel loading by students, we have found it is helpful to have students practice loading a "mock gel" before they load their sample. We use 1% agarose (or agar) in a Petri dish that holds an electrophoresis gel comb the same size as the one used for the gel to make the mock gel. After the mock gel is overlaid with water, students practice pipeting 1X DNA loading buffer into the wells.

Results for Wednesday Lab Section



Lane	Description
M	100-bp ladder (size marker)
1	red-eye ♀ (heterozygous)
2	white-eye ♀
3	red-eye ♂
4	red-eye ♀ (heterozygous)
5	red-eye ♀ (heterozygous)
6	white-eye ♂
7	white-eye ♂

Results for Thursday Lab Section



Lane	Description
M	100-bp ladder (size marker)
1	red-eye ♂
2	red-eye ♀ (heterozygous)
3	red-eye ♂
4	red-eye ♀ (homozygous)
5	red-eye ♀ (heterozygous)
6	white-eye ♂
7	white-eye ♂

Internet Resources

Name	Description	Website
FlyBase	Database of *Drosophila* genes	**http://flybase.org/**
Primer3	Design of primers for PCR	**http://frodo.wi.mit.edu/primer3/**
The Interactive Fly	Guide to *Drosophila* development	**http://www.sdbonline.org/fly/aimain/1aahome.htm**

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Oney P. Smith is an Associate Professor of Biology at Hood College. His education includes a Ph.D. in Entomology from Texas A&M University, a M.S. in Entomology from the University of Maine, and a B.S. in Biology from the University of Vermont. In addition to teaching an introductory laboratory course for the Core Curriculum, "Newsstand Biology," Oney teaches two laboratory courses for biology majors, "Introduction to Cell Biology and Genetics" and "Principles and Methods of Molecular Genetics." His commitment to biology education also involves the active mentoring of undergraduate students on research projects, including the use of molecular genetic approaches to study the biology and gene expression strategies of aphid-transmitted luteoviruses.

Kathy F. Falkenstein is an Associate Professor of Biology and Department Chair at Hood College. She received her B.A. degree in Biology from Gettysburg College, a M.S. in Biology from West Virginia University and her Ph.D. in Botany from The Pennsylvania State University. For two years, Kathy held the position of Lecturer and Post Doctoral Research Associate at Princeton University. At Hood College, Kathy teaches a variety of laboratory biology courses including, "Secret Lives of Plants," "Physiology of Plants and Animals," and "Introduction to Cell Biology and Genetics." She also developed a unique upper-level course for the Core Curriculum entitled, "Reaping the Harvest: Advances in Biotechnology and Global Agriculture."

Appendix A

DNA Sequence for the Promoter Region of the Wild-type Allele

DNA sequence of the promoter region (abridged) for the wild-type allele (X^{w+}). This is a partial sequence (1,040 bp) obtained from Genbank accession X02974 and was used to select primers P1 and P3 for PCR by Primer3 analysis:

P1 primer binding site - bases 171 to 189
P3 primer binding site - bases 618 to 637

```
   1  aaatcatgca tgcaaagtac agtgcaaacc cccgaaacgg gacgacaaca
  51  ggcggattaa caagaactct cttattcacg ataagaagac gcttcccact
 101  caacctaatc agtattcaaa gacagccact cagcttatga gtactgccca
 151  ggtggggtac tatcatattg gtgcaaaggt ggtcgaattt ttaattattt
 201  tgaacggaac acattagcta aacataaaca tgttgtcact agtatgtatg
 251  taagttaata aaaccctttt ttggagaatg tagatttaaa aaaacatatt
 301  tttttttttat tttttactgc actggacatc attgaactta tctgatcagt
 351  tttaaattta cttcgatcca agggtatttg aagtaccagg ttctttcgat
 401  tacctctcac tcaaaatgac attccactca aagtcagcgc tgtttgcctc
 451  cttctctgtc cacagaaata tcgccgtctc tttcgccgct gcgtccgcta
 501  tctctttcgc caccgtttgt agcgttacct agcgtcaatg tccgccttca
 551  gttgcacttt gtcagcggtt tcgtgacgaa gctccaagcg gtttacgcca
 601  tcaattaaac acaaagtgct gtgccaaaac tcctctcgct tcttattttt
 651  gtttgttttt tgagtgattg gggtggtgat tggttttggg tgggtaagca
 701  ggggaaagtg tgaaaaatcc cggcaatggg ccaagaggat caggagctat
 751  taattcgcgg aggcagcaaa cacccatctg ccgagcatct gaacaatgtg
 801  agtagtacat gtgcatacat cttaagttca cttgatctat aggaactgcg
 851  attgcaacat caaattgtct gcggcgtgag aactgcgacc cacaaaaatc
 901  ccaaaccgca atcgcacaaa caaatagtga cacgaaacag attattctgg
 951  tagctgtgct cgctatataa gacaattttt aagatcatat catgatcaag
1001  acatctaaag gcattcattt tcgactacat tctttttttac
```

Appendix B

DNA Sequence for the Promoter Region of the *White-one* Allele

DNA sequence for the promoter region (abridged) of the *white-one* allele (X^{w1}). This is a partial sequence (5,784 bp) derived from Genbank accession X02974 and X17551 and was used to select primers P2 and P3 for PCR by Primer3 analysis:

P1 primer binding site - bases 171 to 189
P2 primer binding site - bases 4678 to 4697
P3 primer binding site - bases 5362 to 5381
Doc retroposon - bases 556 to 5280 (upper-case letters)

```
   1  aaatcatgca tgcaaagtac agtgcaaacc cccgaaacgg gacgacaaca
  51  ggcggattaa caagaactct cttattcacg ataagaagac gcttcccact
 101  caacctaatc agtattcaaa gacagccact cagcttatga gtactgccca
 151  ggtggggtac tatcatattg gtgcaaaggt ggtcgaattt ttaattattt
 201  tgaacggaac acattagcta aacataaaca tgttgtcact agtatgtatg
 251  taagttaata aaacccttttt ttggagaatg tagatttaaa aaaacatatt
 301  tttttttttat tttttactgc actggacatc attgaactta tctgatcagt
 351  tttaaattta cttcgatcca agggtatttg aagtaccagg ttctttcgat
 401  tacctctcac tcaaaatgac attccactca aagtcagcgc tgtttgcctc
 451  cttctctgtc cacagaaata tcgccgtctc tttcgccgct gcgtccgcta
 501  tctctttcgc caccgtttgt agcgttacct agcgtcagcg tcaatgtccg
 551  ccttcGACAT TCGGCATTCC ACAGTCTTCG GGTGGAGACG TGTTTCTTTC
 601  AAGCTACGAA TAGCAAGTTC TAAAAACTAC AACAGTATAG TGAAAGTTAA
 651  ACACAAAGTG TAAAGTGCAG TTTGCACAAC TAACAATTAT TGACTATAGT
 701  AATTATTTAC TAAAATAAAT AATTATTCCA TATTGTTCTG GTAATTGTTA
 751  TATGTGGACT TAGAACAATG AATCAAAACG ACATACGTTC TCAGCGACAA
 801  TGTGAACAAG ACGAGCGCCG GCTCTCTTTA CAACGCAACA ATGCATACTT
 851  TTCTTTCGTC TCACCGCAAA TCGGTGATCG AGCACCCTCA CCTTCAACTA
 901  ACTCGAAACT TTTGCCCTCA GCGAACGACA GACCGCGTTC TTGCTCTCCC
 951  TCTCTGCCTG CTTCGGCTCA CAAGTCGTGG AGCGAAGAGA CCGCCTCTCC
1001  TACCCCGCTC CTCTCGCAGC GCCAAACGAC CGTCCCGGGT AACTGTAACA
1051  CTGCAATAAC GAGTGCAGTG ACCTCACTGG CAACTGCCAC AACATCAACT
1101  TCGTCAGCGG CCCAACTAAT TATCGCTGTG CCAGCTGTAA ATAATTCAGC
1151  AGCACTGACC GTTTGCAACA ACAATAATGC ACGTAAAGAA GAATCAAAAC
1201  AAAAGCAGAA GTCGATTTCG ACTGTGCAGA CTGGCATGGA TCGCTACATC
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1251  CAAATCAAGA GAAAGCTCAG CCCTCAAAAC AATAAGGCAG GTAATCAACC
1301  CAAAATCAAT CGAACCAACA ACGGCAATGA AAACTCTGCA GTAAATAATT
1351  CAAACCGATA TGCTATCTTG GCTGATTCTG CGACCGAACA ACCCAACGAA
1401  AAAACGGTAG GGGAACCAAA AAAGACCAGG CCTCCACCAA TTTTCATACG
1451  AGAACAAAGT ACAAATGCAC TTGTAAATAA ACTCGTTGCT TTGATTGGTG
1501  ACAGCAAGTT CCACATTATC CCACTTAAAA AAGGAAATAT TCATGAAATA
1551  AAACTACAGA TCCAAACAGA AGCAGACCAC CGTATAGTGA CTAAATACCT
1601  AAATGATGCT GGTAAAAACT ACTACACATA CCAATTAAAA AGTTGCAAAG
1651  GGCTACAGGT AGTACTTAAG GGCATTGAAG CAACAGTGAC ACCAGCTGAG
1701  ATAATTGAGG CTCTGAAGGC CAAAAACTTT TCTGCAAAGA CAGCTATTAA
1751  TATTTTAAAC AAAGACAAAG TTCCGCAGCC ACTATTCAAA ATAGAACTCG
1801  AACCAGAGCT CCAGGCACTA AAGAAAAACG AAGTGCACCC AATATACAAT
1851  TTACAGTACT TGCTACATCG GAGGATCACC GTGGAGGAGC CGCACAAACG
1901  TATCAATCCA GTTCAATGTA CTAATTGCCA AGAATACGGC CACACCAAGG
1951  CATACTGCAC CCTTAAGTCC GTATGTGTTG TCTGTAGCGA ACCTCATACT
2001  ACCGCAAACT GCCCCAAAAA CAAGGACGAT AAGTCTGTGA AGAAATGCAG
2051  TAACTGCGGG GAAAAACATA CTGCAAACTA CAGAGGCTGT GTGGTGTACA
2101  AAGAATTGAA GAGCCGCCTA AACAAACGTA TTGCCACAGC ACATACATAC
2151  AACAAAGTCA ATTTCTACTC TCCGCAACCG ATTTTTCAAC CACCCCTAAC
2201  TGTCCCAAGC ACTACTCCAA CAATTTCTTT CGCTAGCGCC CTAAAATCCG
2251  GACTAGAAGT GCCCGCCCCA CCGACAAGAA CTGCTCATTC CGAACATACA
2301  CCGACAAACA TCCAACAAAC ACAACAAAGT GGCATCGAAG CTATGATGCT
2351  ATCCCTACAG CAAAGCATGA AAGACTTCAT GACGTTCATG CAAAATACTT
2401  TGCAAGAGCT CATGAAAAAC CAAAATATCC TGATTCAACT TCTTGTATCT
2451  TCAAAATCCC CATAATGGCT TCCCTACGGA TATCTCTGTG GAACGCAAAT
2501  GGCGTTTCAC GGCATACACA AGAGCTCACA CAGTTCATTT ACGAAAAAAA
2551  CATCGACGTA ATGCTACTAT CAGAAACGCA CCTCACAAAT AAAAACAATT
2601  TTCATATACC AGGATACTTG TTCTATGGTA CAAATCATCC AGATGGTAAA
2651  GCTCATGGAG GCACTGGAAT ACTCATCAGA AATCGCATAA AACACCACCA
2701  CTTAAACAAT TTTGACAAAA ACTACTTACA ATCTACGTCC ATAGCCTTAC
2751  AACTCAACAA TGGTTCAACG ACTCTAGCCG CAGTCTACTG CCCACCGCGC
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2801  TTTCCAATCT CTGAGGATCA ATTCATGGAA TTCTTTAACA CACTAGGTGA

2851  CAGGTTCATC GCAGCGGGTG ACTATAACGC CAAGCACACC CATTGGGGAT

2901  CTCGACTTGT GTCGCCAAAG GGTAAGCAAT TGTACAATGC GCTTACGAAG

2951  CCAGAAAACA AGCTAGACTA TGTATCCCCG GGTAAGCCTA CATACTGGCC

3001  AGCAGACCCA AGAAAAATCC CAGACCTGAT CGATTTTGCA ATTACTAAAC

3051  ATGTCCCCCG CAACATGGTC ACCGCCGAAG CACTAGCAGA TTTATCATCA

3101  GATCACTCAC CTGTTTTTCT AAATATGCTA ACTCGCCCCC ACATCGTCGA

3151  CCCACCGTAT AGACTCACAA ATTTTAGAAC AAACTGGCCA AGGTATCAAA

3201  AGTATGTCTG TTCACACATA GAACTAACGA CGGCATTATC TACAAAGGAG

3251  GATATAGACA AGTCAACGGA AACTCTTGAA AACATTTTAG TTTCGGCTGC

3301  AAAGGCTTCA ACCCCGCCAG TGACGTATGC AAAACCAAAC TACATCAAAA

3351  CTAATCGCGA AATCGAGCGG CTGGTATTAG ATAAACGACG CCTACGAAGG

3401  GATTGGCAGT CTAATAGATC ACCAATTACT AAGCACATGC TTAAGATAGC

3451  CACACGCAGG CTTACCAATG CTCTCAAACA AGAGGAAAAA AACAGCCAAC

3501  GTTCATATAT CGAGCAACTC TCTCCCACCA GCACTAAGTA CCCTCTTTGG

3551  AGAGCTCACA GAAACCTAAA GACTCCAATA GCGCCAATTA TGCCACTCCG

3601  AAGTCCCTCT GGCACCTGGT TTCGAAGTGA TGAAGAAAGA GCCAGTGCTT

3651  TCGCTGACCA TTTACAAAAT GTATTCCGAC CAAATCCCTC TACCAACACA

3701  TTTATTCTCC CTCCTTTAAT AGCAGCCAAT CTAGATCCTC AAGAACCCTT

3751  TGAATTCCGA CCATGTGAAC TAGCAAAGGT TATCAAAGAG CAACTGAACC

3801  CAAGAAAATC GCCTGGCTAC GACCTAATAA CTCCAAGAAT GCTCATTGAA

3851  CTCCCAAAGT GTGCTATTCT TCACATCTGC CTGTTGTTCA ACGCAATCGC

3901  CAAGCTTGGA TACTTCCCTC AAAAATGGAA AAAGTCGACC ATAGTAATGA

3951  TTCCAAAGCC AGGAAAAGAT AAAACGCAGC CATCATCATA TAGACCGATA

4001  AGCTTACTAA CATGTCTTTC AAAGCTGTTT GAAAAAATGC TACTCCTTCG

4051  GATTAGCCCT CATCTTAGAA TAAACAACAC ACTTCCAACA CATCAATTTG

4101  GCTTTAGAGA AAAACATGGA ACCATCGAAC AGGTCAACCG AATCACGTCA

4151  GAAATTCGTA CTGCTTTTGA ACATCGAGAA TACTGCACAG CCATTTTTCT

4201  AGACGTCGCG CAGGCATTTG ACAGAGTGTG GCTCGATGGA CTTTTGTTTA

4251  AAATAATCAA GCTGTTGCCC CAAAACACAC ATAAGCTACT GAAGTCATAC

4301  CTATATAACA GAGTGTTTGC AATAAGATGC GATACAAGCA CTTCACGCGA
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4351  TTGCGCAATC GAAGCTGGAG TGCCGCAAGG CAGTGTACTG GGTCCAATCT
4401  TATACACCCT GTATACGGCG GATTTCCCCA TAGACTACAA TCTAACAACC
4451  TCCACGTTCG CTGATGATAC CGCGATACTC AGTCGCTCGA AATGCCCAAT
4501  AAAAGCCACG GCACTCCTAT CCCGACACTT AACATCTGTA GAACGATGGC
4551  TTGCCGACTG GAGAATTTCA ATAAATGTTC AAAAATGCAA GCAGGTTACC
4601  TTTACCTTAA ACAAACAAAC ATGCCCACCA CTGGTCTTGA ATAACATATG
4651  CATTCCACAA GCCGACGAGG TAACATATCT GGGAGTTCAT CTGGACAGGC
4701  GGCTCACTTG GCGCAAACAT ATAGAAGCCA AATCGAAACA TCTTAAACTT
4751  AAAGCAAGGA ACCTCCACTG GCTCATAAAT GCTCGCTCTC CACTTAGTCT
4801  GGAGTTCAAA GCTCTTCTAT ACAACTCCGT CTTAAAACCT ATCTGGACTT
4851  ATGGCTCCGA GCTGTGGGGC AACGCATCCA GAAGTAACAT AGACATTATT
4901  CAGCGAGCAC AGTCAAGAAT TCTGAGAATT ATCACTGGAG CGCCGTGGTA
4951  CCTTCGAAAC GAAAACATAC ACAGAGACCT AAAAATCAAA TTAGTAATCG
5001  AAGTAATAGC TGAGAAAAAA ACGAAGTATA ACGAAAAGCT GACCACCCAT
5051  ACAAATCCCC TCGCAAGAAA ACTAATCCGA GTATGCAGTC AAAGCCGGCT
5101  GCACCGCAAC GACCTCCCAG CCCAGCAATA AACTTATTAG GGCATTAATG
5151  AAAAAAAAAA ACTATCACTA AGTGAAAGTT AATTAAGTTA GATTAAGATT
5201  TGAACACTTA TTGTTAGTCT CTTAACACAA AGGGAAGATT CAATAAATAA
5251  TAAAAATTAA AAAAAAAAAA AAAAAAAAAA aatgtccgcc ttcagttgca
5301  ctttgtcagc ggtttcgtga cgaagctcca agcggtttac gccatcaatt
5351  aaacacaaag tgctgtgcca aaactcctct cgcttcttat ttttgtttgt
5401  tttttgagtg attggggtgg tgattggttt tgggtgggta agcaggggaa
5451  agtgtgaaaa atcccggcaa tgggccaaga ggatcaggag ctattaattc
5501  gcggaggcag caaacaccca tctgccgagc atctgaacaa tgtgagtagt
5551  acatgtgcat acatcttaag ttcacttgat ctataggaac tgcgattgca
5601  acatcaaatt gtctgcggcg tgagaactgc gacccacaaa aatcccaaac
5651  cgcaatcgca caaacaaata gtgacacgaa acagattatt ctggtagctg
5701  tgctcgctat ataagacaat ttttaagatc atatcatgat caagacatct
5751  aaaggcattc attttcgact acattctttt ttac
```

Appendix C

Supplier Addresses

Carolina Biological Supply Co.
2700 York Road
Burlington, North Carolina 27215
Phone: 800-334-5551
Website: **http://www.carolina.com**

Invitrogen Corp.
5791 Van Allen Way
P.O. Box 6482
Carlsbad, California 92008
Phone: 800-955-8288
Website: **http://www.invitrogen.com**

Whatman, Inc.
Building 1
800 Centennial Avenue
Piscataway, New Jersey 08854
Phone: 800-942-8626
Website: **http://www.whatman.com**

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