LABORATORY 8. DNA GENOTYPING OF *Drosophila* MUTANTS: THE *WHITE-1* LOCUS (W)

LEARNING OUTCOMES

At the end of this lab practice, students should be able to:

- Recognize the relation between genotype and the white-eyes phenotype in *Drosophila*.
- Identify two common molecular genetics methods and its components.
- Perform a DNA extraction and a PCR to identify *Drosophila* mutants and wild-type flies.

INTRODUCTION

In some of the previous labs, you have investigated the inheritance of the white-eye color in *Drosophila*. Early in the twentieth century (1910), Thomas Hunt Morgan discovered that the locus responsible for this trait was inherited as sex-linked. Expressly, he indicated that the locus locates on the X chromosome. Since then, many molecular studies contributed to revealing the mechanisms by which no pigments are present in *Drosophila* eyes.

As you have previously studied, there are two classes of eye pigments in wild type *Drosophila*: **brownish (ommochrome)** and those responsible for **bright red coloration (pteridines**) (Lab # 5). These pigments are synthesized from purine and tryptophan precursor molecules (colorless), whose transportation into the cells are controlled by **transporter proteins**. To be **functional**, these transporters proteins known as the ***brown*** and ***scarlet*** genes **must partner** with a **common protein subunit** coded by the ***white*** **gene (white-1 locus).** The wild-type allele (**w**$^{+}$) is characterized by 6 exons and 5 introns that encode a 687 aminoacids protein located in the membrane of the eye's cells. In the ***white-1* mutation** (**w**), the transcription is disrupted, and the white protein is not produced. Hence, because *white* does not partner with *brown* and *scarlet*, the eye cells are unable to uptake pigment precursors, causing the white-eye phenotype (absence of pigmentation). Further molecular studies on the *white-1* mutation indicated that an insertion of a Doc-retroposon (4,700 bp) in the promoter region resulted in its inactivation, and thus, the disruption of transcription.

Because the gene for **white** eyes is sex(x)-linked, male flies are termed **hemizygous**. That is, they carry **only one** copy of the gene ($X^{w+}Y$ or $X^{w}Y$). Instead, female flies have two X chromosomes and hence, they carry two copies of the white gene. Thus, they can be either homozygous or heterozygous ($X^{w+}X^{w+}$, $X^{w+}X^{w}$ or $X^{w}X^{w}$).

The Polymerase Chain Reaction (PCR)

Back in 1983, Kary Mullis developed a unique molecular biology technique to make multiple copies of a desired piece of double stranded DNA in a test tube. This technique is known as the Polymerase Chain Reaction and is based on the natural process of DNA **replication**. All the nucleotide precursors (DNTPs), a DNA polymerase (*Taq* polymerase), a couple of primers, and the purified target DNA are added to a tube. Using a thermal cycler, the PCR reaction is carried out by alternating different cycles of temperature and

times. There are mainly **three steps** in each cycle of a typical PCR. Each cycle results in the doubling of the amount of target DNA molecules and hence, the exponential reaction results in the production of millions of copies in 30 to 40 PCR cycles.

PCR steps

1. Denaturation

The tube containing the sample DNA is heated to more than 90°C, which breaks the hydrogen bonds between the base pairs and releases two separate single strands.

2. Annealing

During this step, short pieces of commercially prepared single-stranded nucleotide sequences called **primers** are used to amplify the desired region of the target DNA. The primers are complimentary to the desired target DNA sequence and bind (i.e., anneal), when the temperature is maintained within a range of 40°C to 60°C, depending on the nucleotide composition of the primer.

3. Extension

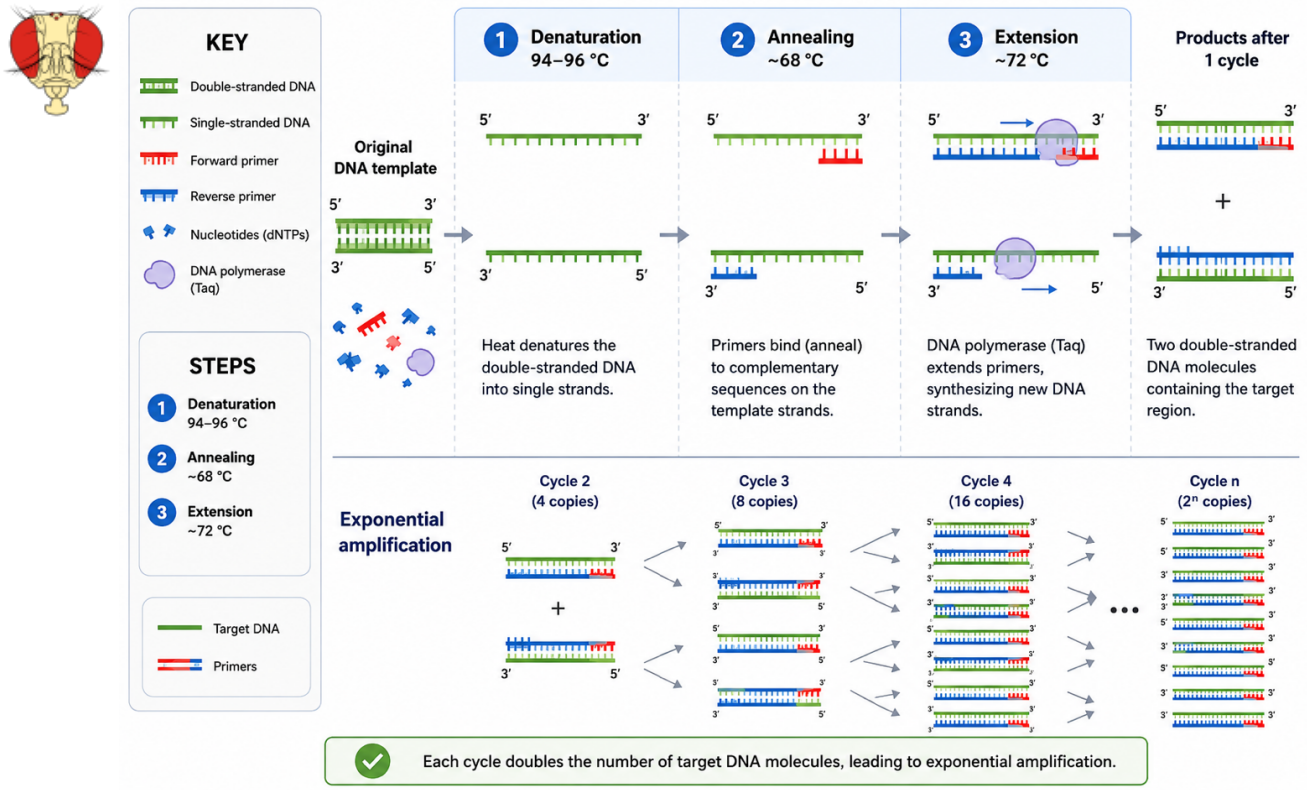
During this step, the copying and extension of single-stranded DNA occurs when the temperature is raised to approximately 72°C (ideal for a particular enzyme: *Taq* DNA polymerase). After the completion of the extension step, a new strand of DNA complimentary to the original strand is made. Thus, one PCR cycle results in two copies of the target DNA.

Since the entire PCR process is automated, the cycle begins again using the duplicated DNA resulting in four copies. After approximately 30 to 40 PCR cycles, more than one billion copies of the original DNA have been made. A common setup for genotyping in a PCR machine includes:

- Initial denaturation: 95-98 °C x 1-5 min.
- Denaturation: 95-98 °C x 40-60 sec.
- Annealing: 50-70 °C x 40-60 sec.
- Extension: 70-72 °C x 40-120 sec.
- Repeat steps 2-4 for 25-32 cycles.
- Final extension: 70-72 °C x 2-5 min.

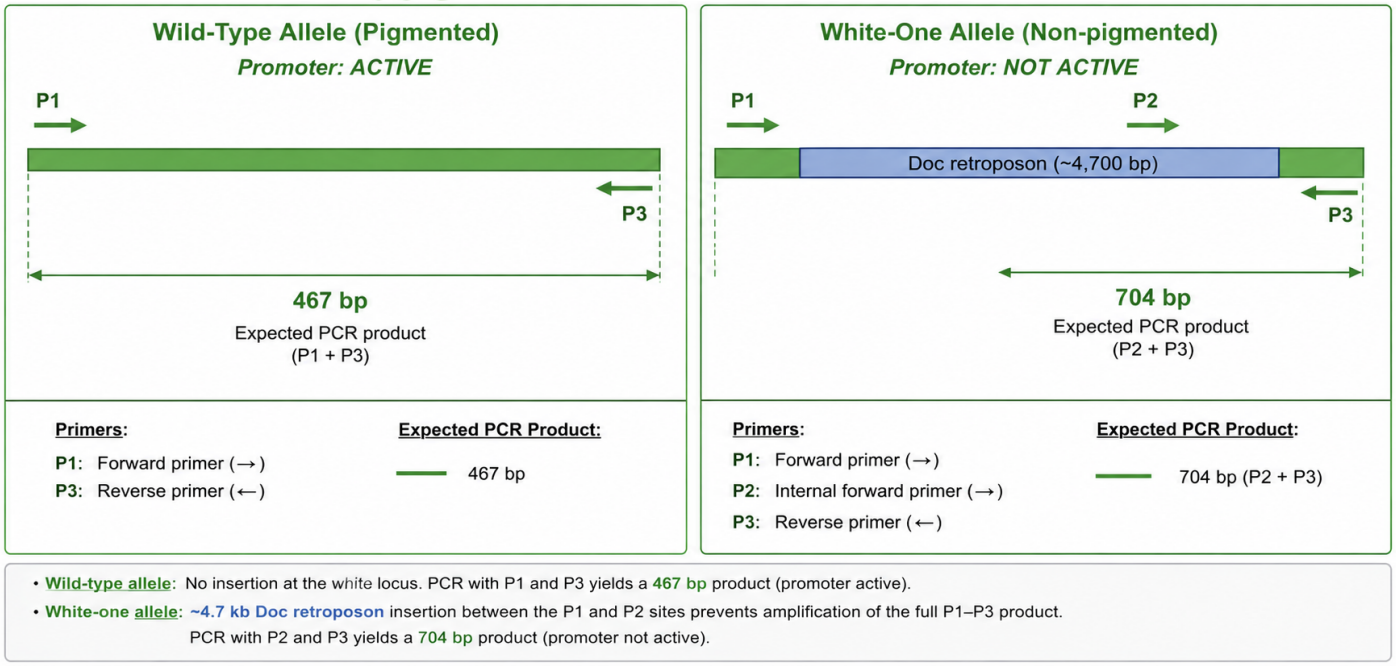
As a complement to the laboratory exercise, in this laboratory you will simulate the amplification of DNA fragments using a virtual environment, known as *in-silico* PCR. This tool is not new, and it is used for many scientists to perform trial-tests of PCR reactions before the real experiment is performed. **Please watch the video available in Canvas on how to use the *In-silico* PCR platform.**

White phenotype **Wild-type (red-dull eyes)**



Genotyping of the White-1 locus

By analyzing extracted DNA of wild-type (red eyed) and mutant flies (white), the genotypes can be easily determined through PCR. This is possible because specific **primers** bind to the **promoter region** on both, wild type and mutant flies (see figures below). For instance, the combination of primer 1 and primer 3 are specific to the **wild-type** and will produce a **467** bp DNA fragment (**active w+ allele)**. The combination of primer 2 and primer 3 will produce a **704** bp fragment specific to the doc-retroposon (**non-active allele**). The following diagrams represents the location of primers on the white-1 locus and the expected DNA fragments after PCR.



DNA gel electrophoresis

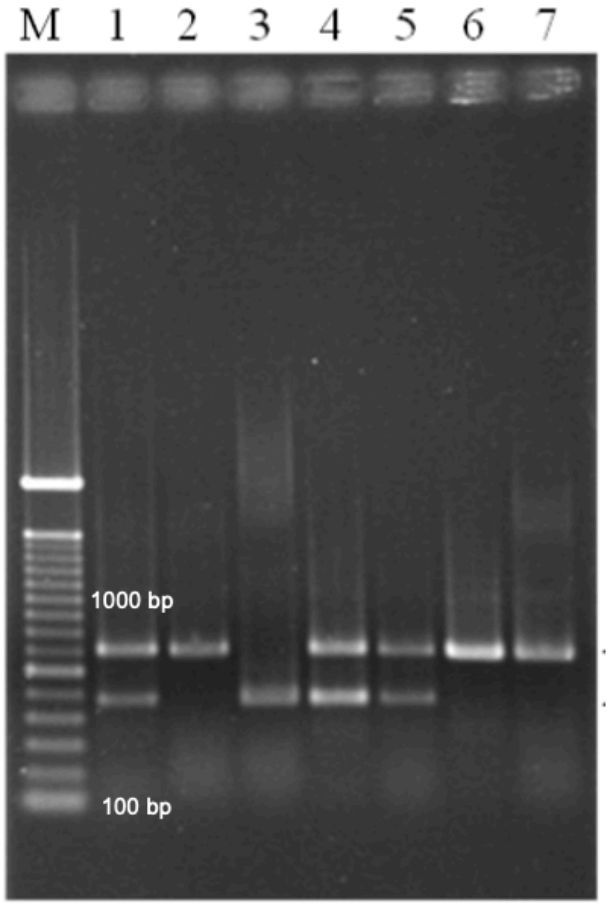
Electrophoresis is a molecular method based on the principle of separating molecules **based on their size** (Ex., base pairs) and **attraction to an electric charge** (+ or -).

After the PCR reaction is performed, the mixture of DNA fragments can be separated on an agarose gel. An electric current (voltage) is applied to the electrophoresis apparatus and then, the charged molecules in the sample enter the gel through the wall of the wells. **Since DNA is negatively (-) charged** (due to the phosphate groups), it moves through the pores of the agarose gel towards the positive electrode.

Usually, the first well on the gel is loaded with a **DNA ladder**, a solution with standard molecular weight markers used to estimate the size of DNA fragments. Larger molecules will migrate less than smaller molecules because the latter ones fit easily on the tiny agarose pores and travel farther in the gel. The current is run for about 15 to 20 minutes, and then, the gel is stained with a fluorescent dye (Ex. ethidium bromide). The end results of the electrophoresis are visualized under UV light and recorded using a digital camera.

MAIN MATERIALS (*PCR*)

- *Drosophila* flies:
  - P_1 white-eyed females
  - P_2 Red-eyed males
  - F1 red-eyed females
  - F1 white –eyed males
- 1.5 ml tubes
- 0.2 ml PCR tubes
- **Solution A**: DNA extraction solution (40 µL dilution Buffer + 1 µL DNA Release Additive).
- **Solution B** (12 µL): PCR reaction solution containing: dNTPs, $MgCl_2$, hot start II DNA polymerase, primer mix (primers I, II, III) and nuclease-free water.
- Micropipettes and tips, 5 µL and 20 µL
- Fine-tipped forceps
- DNA thermal cycler
- sharp-point markers for labeling tubes
- DNA Ladder (1kb DNA ladder)



Gel electrophoresis of DNA fragments and genotyping (*white-one* locus). Line "M" corresponds to a known DNA ladder (Smith and Falkenstein, 2011).

PROCEDURES

DNA EXTRACTION FROM INDIVIDUAL FLIES

1) Work in **groups of four** people.

2) Each student is required to extract DNA from only one type of fly:

P_1 white-eyed females
P_2 Red-eyed males
F1 red-eyed females
F1 white –eyed males

3) Take a tube containing the DNA extraction solution (**clear solution).**

4)Add one fly head to the tube using forceps or a brush. Label your tube on the side or the top using the fine markers.

5)Crush the tissue using a plastic pestle or a toothpick until a homogeneous mixture is present. You can mix by vortex or tapping using your fingers.

6) Incubate the solution **during 45 min at 37 ℃ in the thermal cycler.** Please ask your TAs to perform this step.

7) **Spin the tube down** using a centrifuge.

8) Remove 1 µL of the solution and add it to **the PCR reaction tube (blue solution)**. Be careful not to suck up any fly parts.

9) Mix the PCR mixture tube by vortex or tapping, then, perform a quick spin in the centrifuge to collect all the solution at the bottom of the tube.

10) Ask your TAs to place the tubes in the thermocycler and run the PCR program that you selected as appropriate. It will take up to 3 hours to complete.

GEL ELECTROPHORESIS

- After the PCR is completed, the Lab staff will keep your PCR products in the freezer.
- **A gel electrophoresis and further identification will be performed next week.**

MATERIALS FOR VIRTUAL PCRS

- Desk or laptop computer. Small devices as cell phones or tablets **are not recommended**.
- Web browser (Chrome, Mozilla, Safari, etc.)
- Access to the ***Genetics Virtual Lab*** platform (www.ampossot.com/virtual_lab)
- Primers sequences
  - PF1: GTGCAAAGGTGGTCGAATTT
  - PF2: TCTGGGAGTTCATCTGGACA
  - PR3: GAGAGGAGTTTTGGCACAGC
- Five *Drosophila* DNA sequences. These files are available at the LMS (lab website) with the following names:
  - Male_1.txt
  - Male_2.txt
  - Female_1.txt
  - Female_2.txt
  - Female_3.txt
- Notebook and pen (or pencil)

PROCEDURE FOR *VIRTUAL* PCR

1) Open the **lab website** at the LMS (Canvas or Blackboard).

2) Download the DNA sequence files (n=5) available in the Module # 8.

3) **Store** the files in your computer. It may be easier to find them if you use the folder "desktop".

3) Launch the **Virtual PCR** tool at www.ampossot.com/pcr

4) **Upload** the five DNA sequences to the server.

Primers sequences
PF1: GTGCAAAGGTGGTCGAATTT
PF2: TCTGGGAGTTCATCTGGACA
PR3: GAGAGGAGTTTTGGCACAGC

5) Perform **the virtual PCRs** required to complete your assignment. Remember to carefully select the **right DNA sequence and primers** for each PCR

6) Analyze the obtained PCR products (i.e., sizes) and fill the electrophoresis templates.

7) **Complete and submit your assignment by the deadline**.

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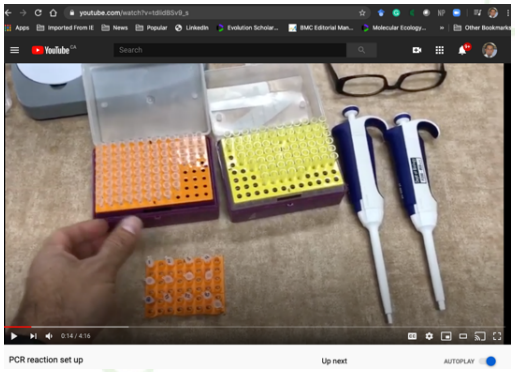
Setting up a PCR reaction and a gel electrophoresis in the lab is a straightforward process. Please check these videos illustrating the process in our lab at the University of Saskatchewan.



PCR reaction:
https://youtu.be/tdIidBSv9_s

Gel electrophoresis:
https://youtu.be/FZ19A0RT_tY