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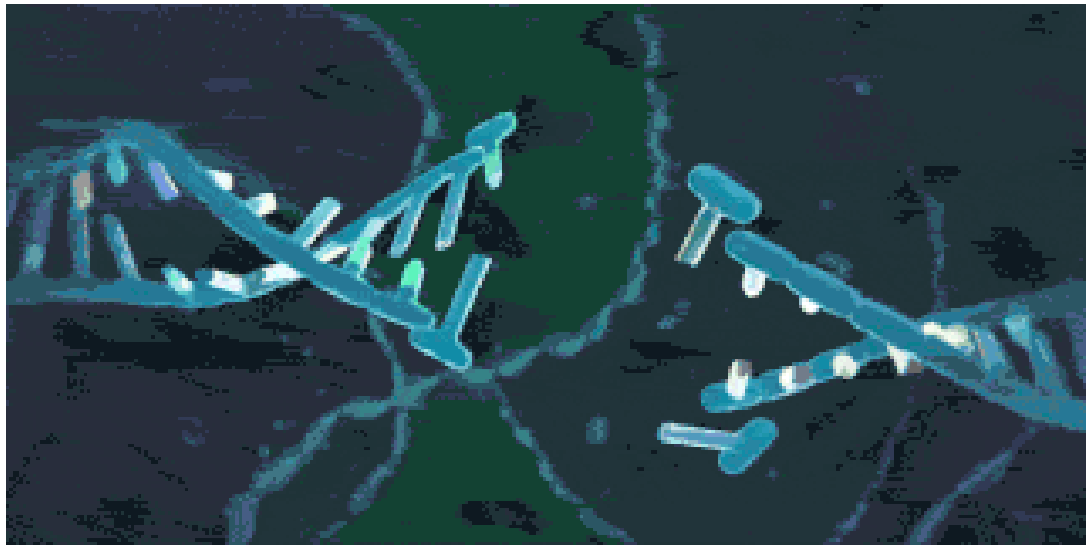


PCR assay of intragenic DNA lesions induced by ionizing radiation at the vestigial gene of *Drosophila melanogaster*

Saif El-Din Al-Mofty[1], Nada Atef Kamal[2], Kristina P. Afanasyeva[3], Igor D. Alexandrov[3]

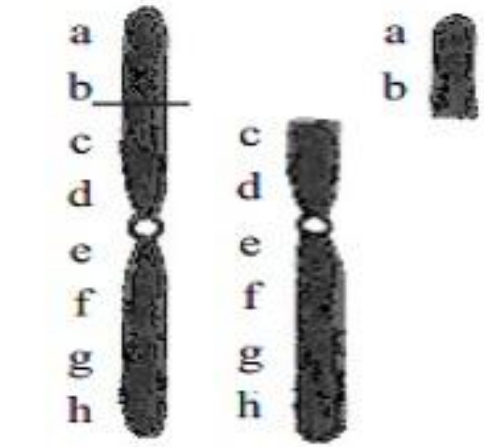
1 Zewail City of Science and Technology, Giza, Egypt.
2 Faculty of postgraduate studies for advanced science and technology, University of Beni Suef, Egypt.
3 Laboratory of Nuclear Problems, JINR, Dubna, Moscow Region, Russia.

Contents

- Introduction
  - *Drosophila melanogaster*
  - Types of mutations in DNA
  - Types of mutants
    - Vg, body color, eye color, body shape
- Aim of project
- PCR method used and purification
- Conclusion

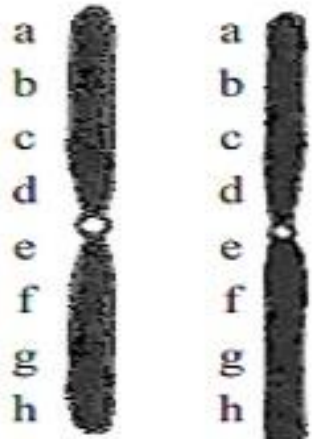
Introduction *Drosophila melanogaster*

- *Drosophila melanogaster* also known as fruit fly is most known species to be experimented on; for whole genome sequencing.
- It has keys for understanding how genes interact with environment and vise versa.
- We could also understand how mutations work by visualizing its phenotypes and sequencing its genotypes.
- *D. melanogaster* has many benefits:
    1. generation time is short.
    2. reproduce many offspring.
    3. Easy cytological analysis for its huge chromosomes

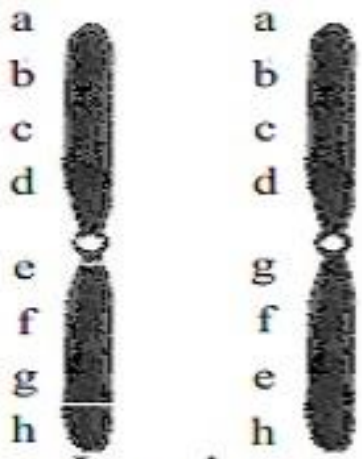


Terminal deletion

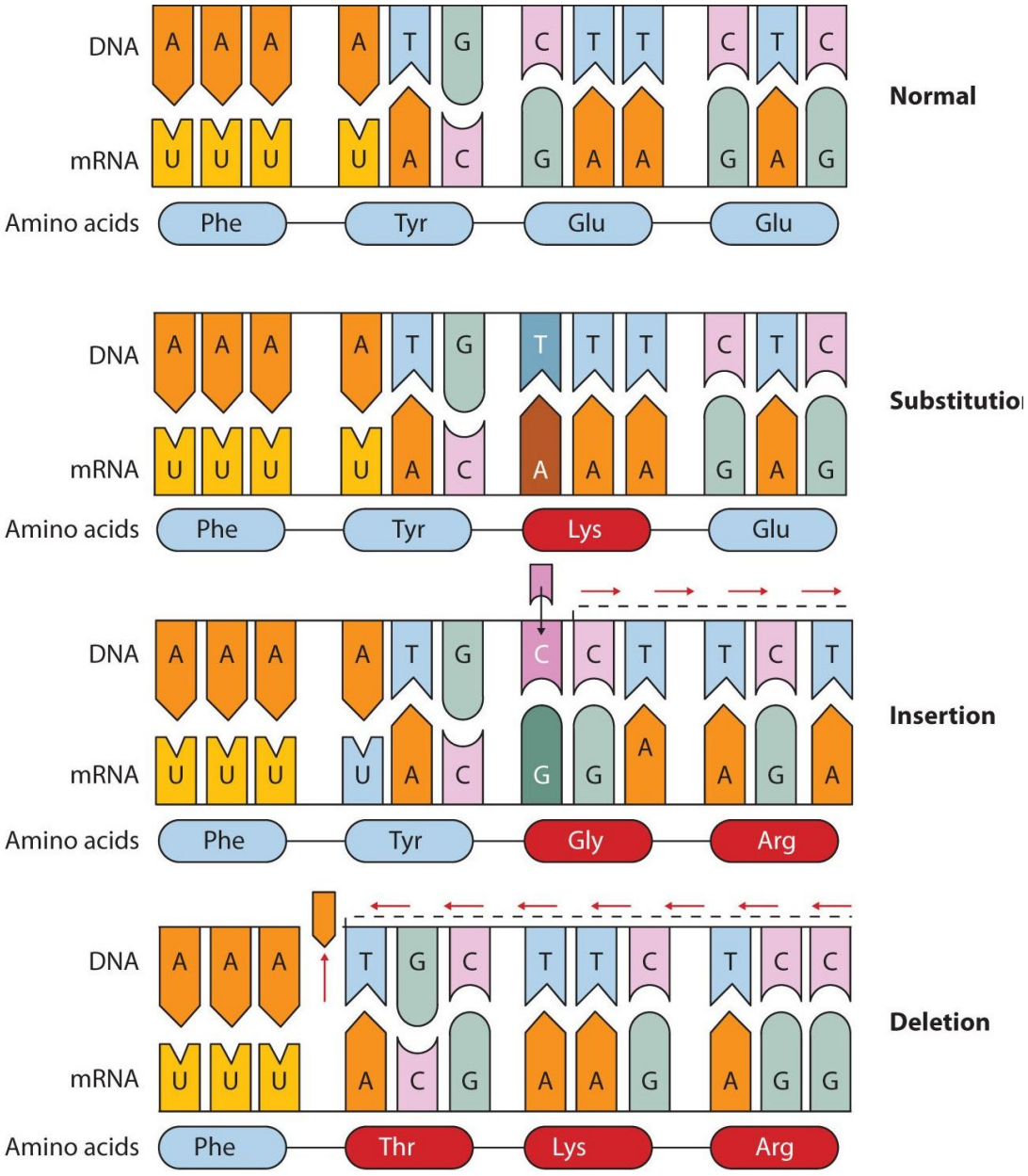
Intercalary deletion



Duplication

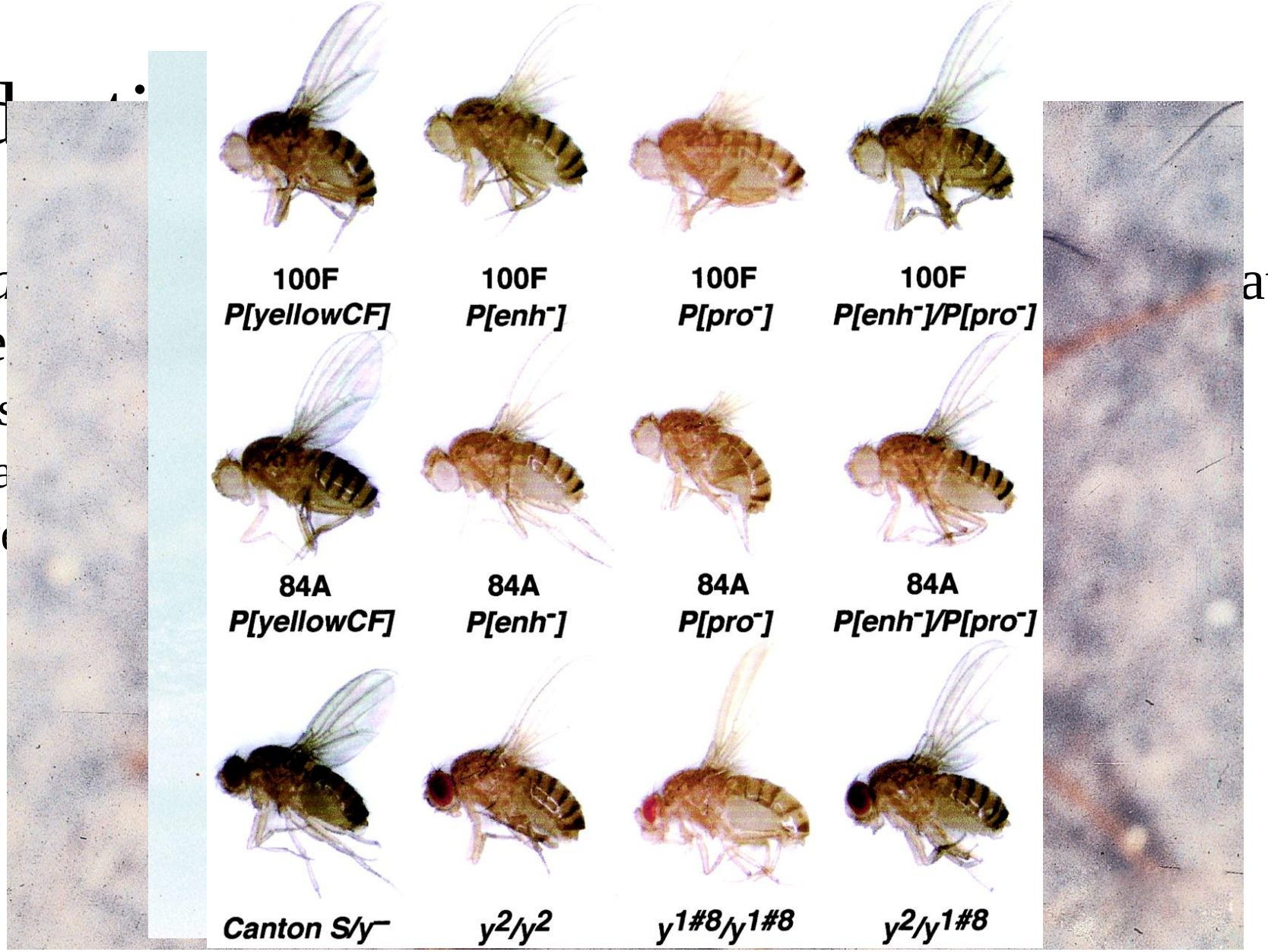


Inversion



Introd

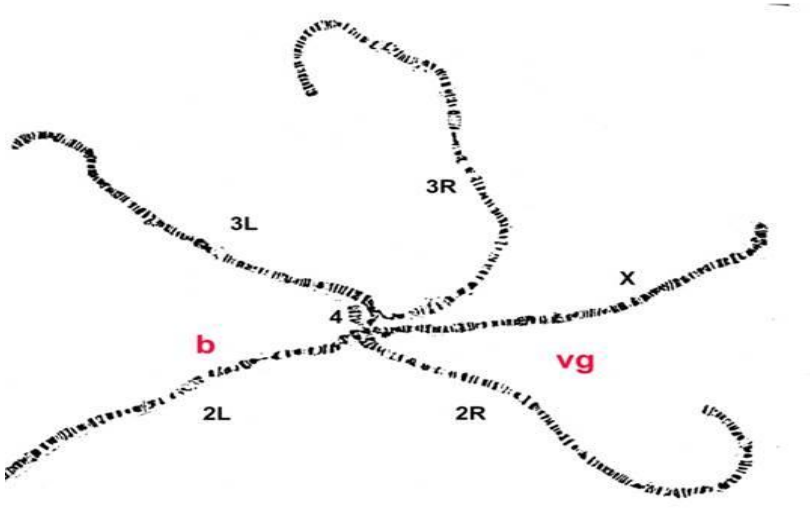
- *D. melo* at occurre
  1. Ves
  2. Bla
  3. Eye
  4. …



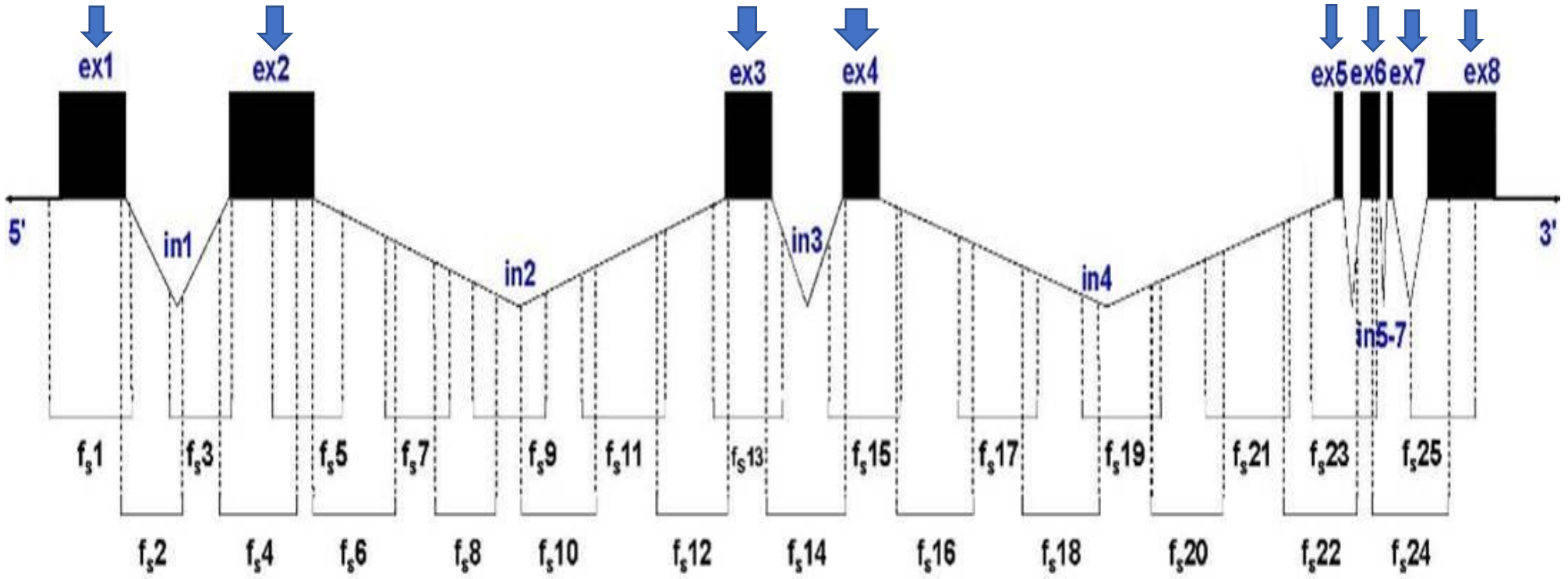
Chen, J. L., Huisinga, K. L., Viering, M. M., Ou, S. A., & Geyer, P. K. (2002). Enhancer action in trans is permitted throughout the Drosophila genome. *Proceedings of the National Academy of Sciences*, *99*(6), 3723-3728.

• Aim OF Project

- To study the nature and location of DNA alterations induced from different irradiations sources that emits γ-rays and neutrons at the fragments of *vestigial* gene of *Drosophila melanogaster*.



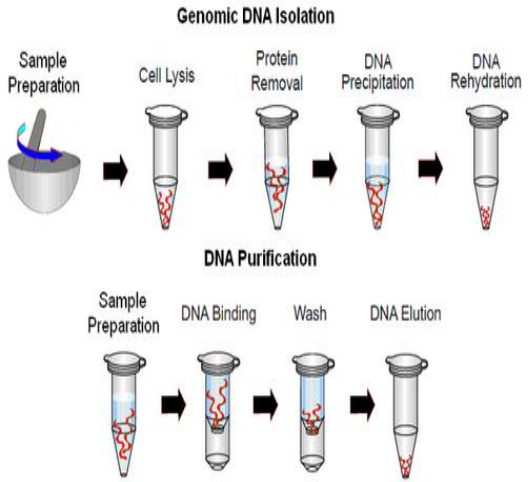
- # Aim of project

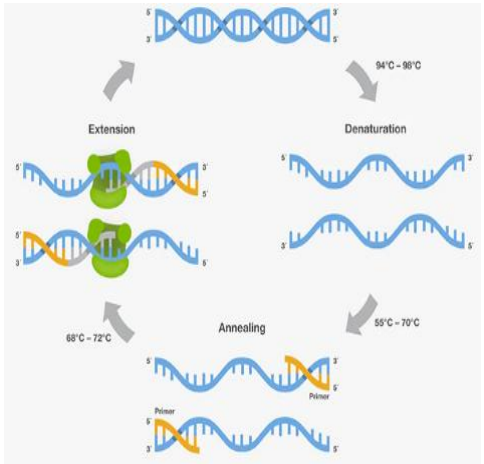


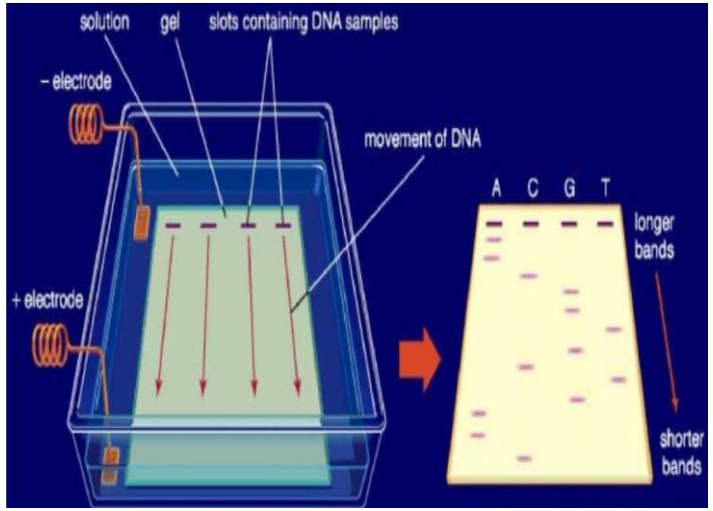
- ***vestigial gene exon-intron structure (15112 bp).***

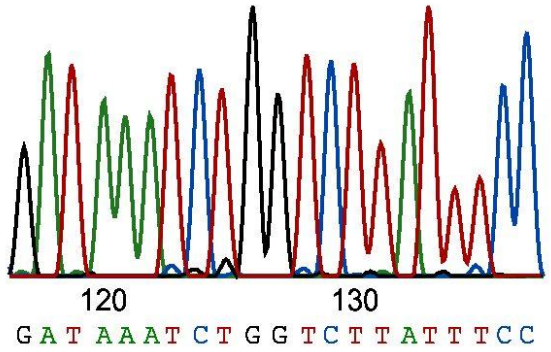
- **Main stages of our work**

DNA Extraction → PCR → Electrophoresis → Sequencing







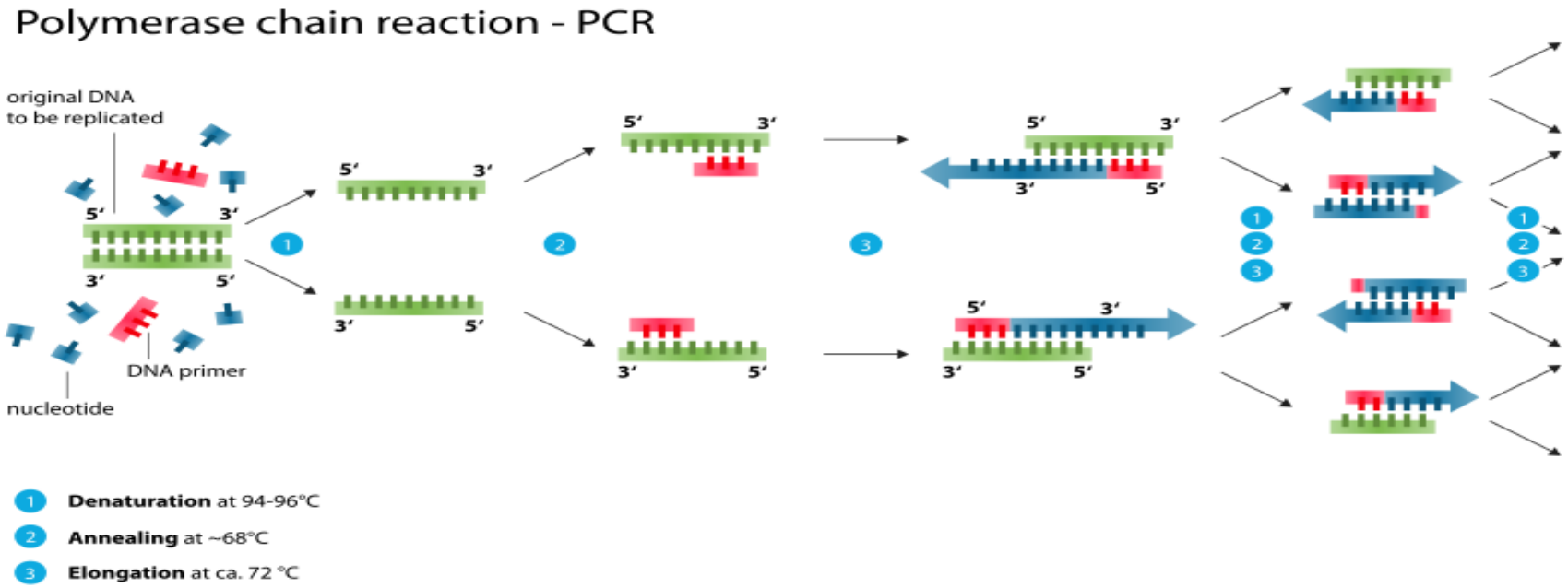


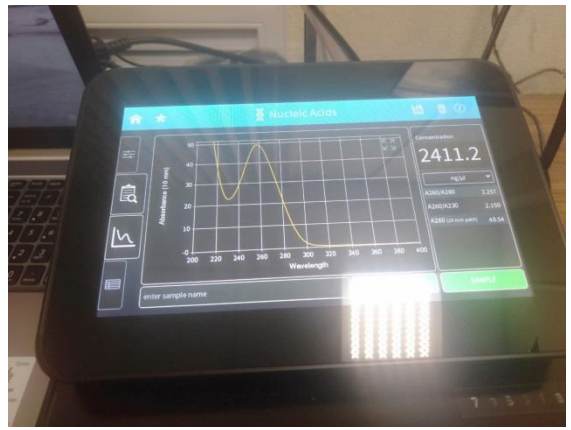
Stages of work: DNA Extraction

- There are different protocols for preparing lysates depending on
  1. DNA yield
  2. Sample size of the Drosophila
  3. Ease of DNA extraction
- We used two methods of DNA extraction
  - **Co-precipitation with silica solution**
  - **Precipitation with Ethanol**

Stages of work: PCR basics

- **Equipment**
  - **Thermal cycler**
  - **Gel electrophoresis unit**
  - **Nanodrop spectroscopy**
- **PCR stages**



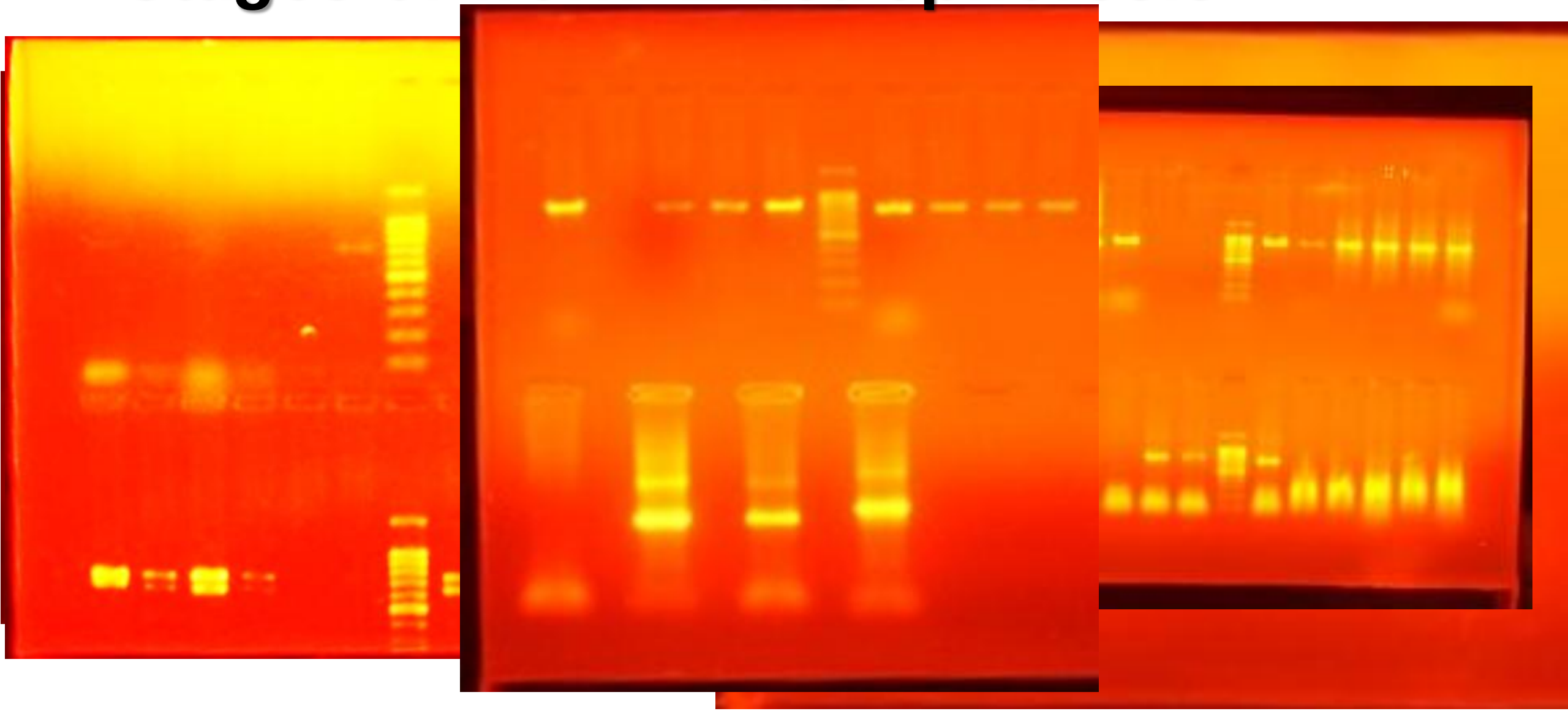


Stages of work: DNA purification

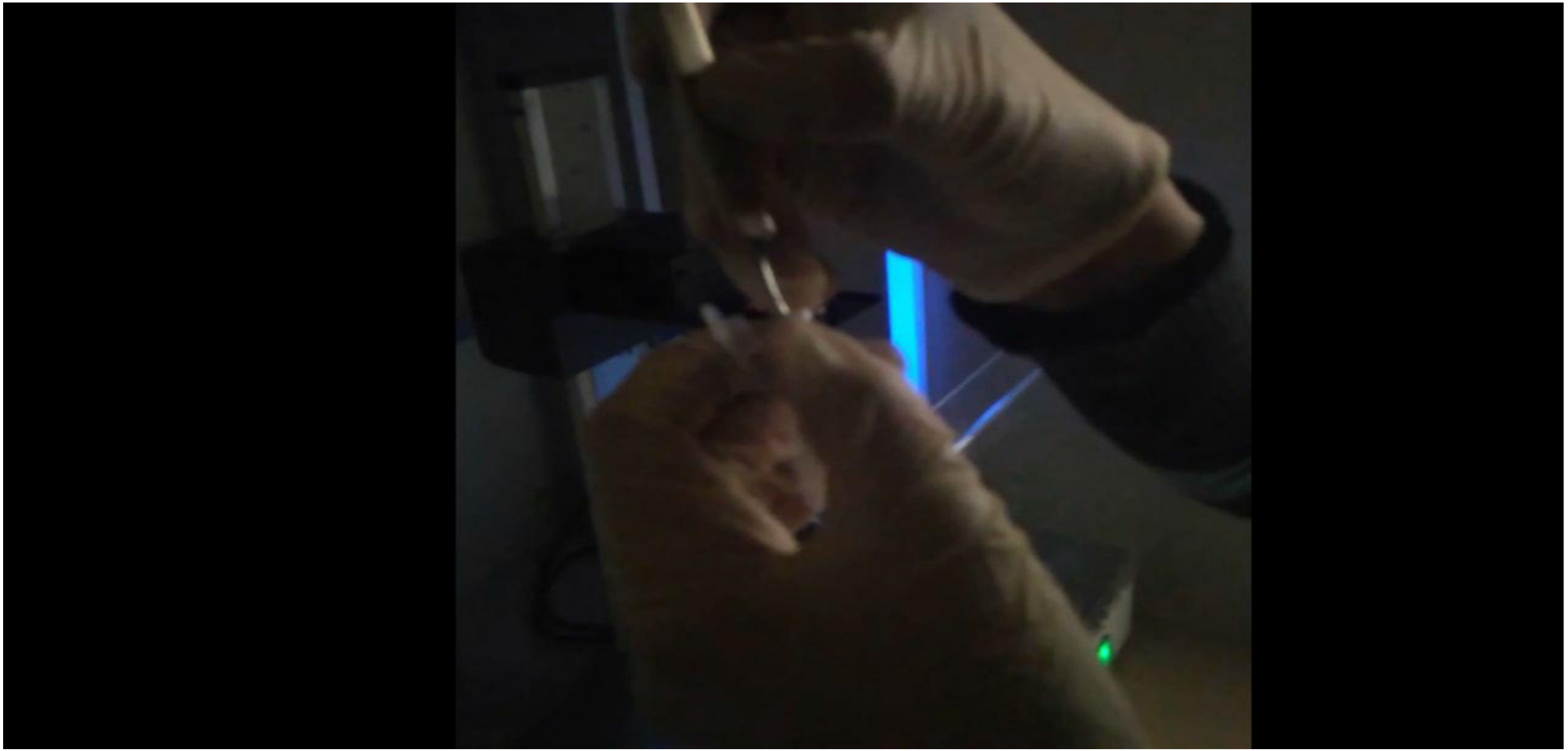
- Two kind starting materials for DNA Purification.

1. From DNA Product.
2. From Gel product.

Stages of work: Electrophoresis

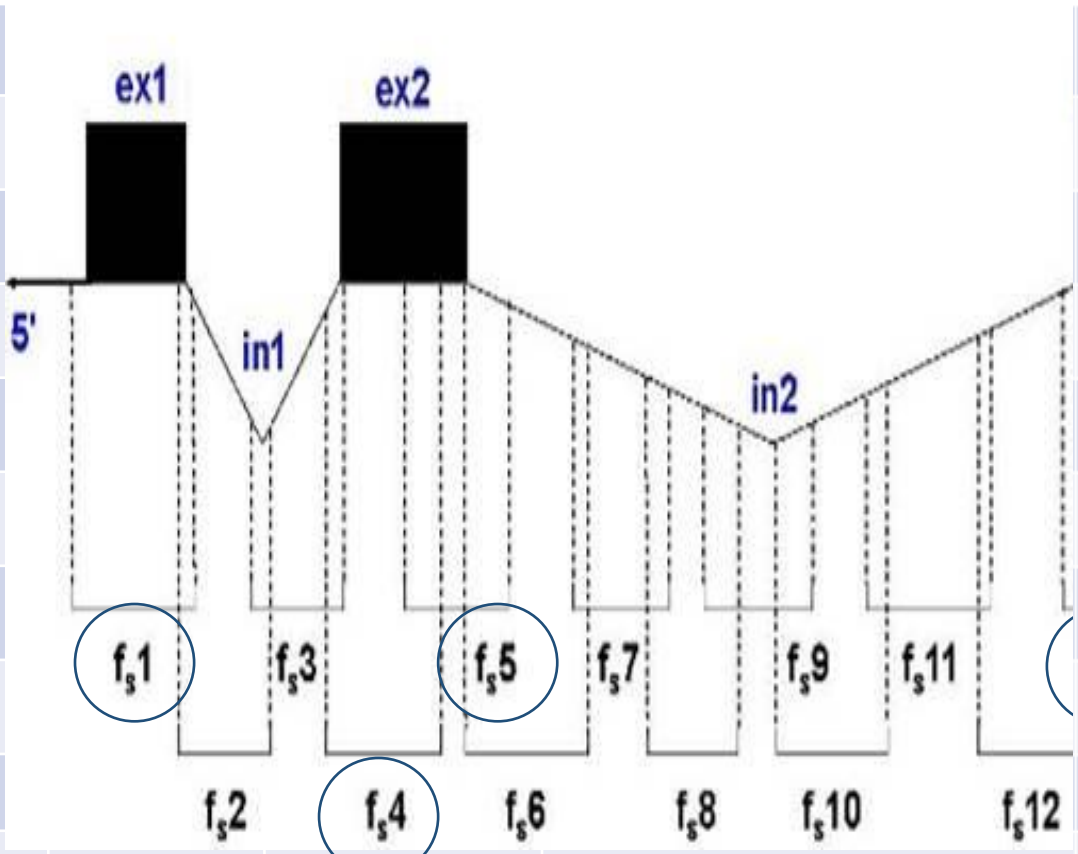


Stages of Work: Electrophoresis (Gel cutter V1.0)



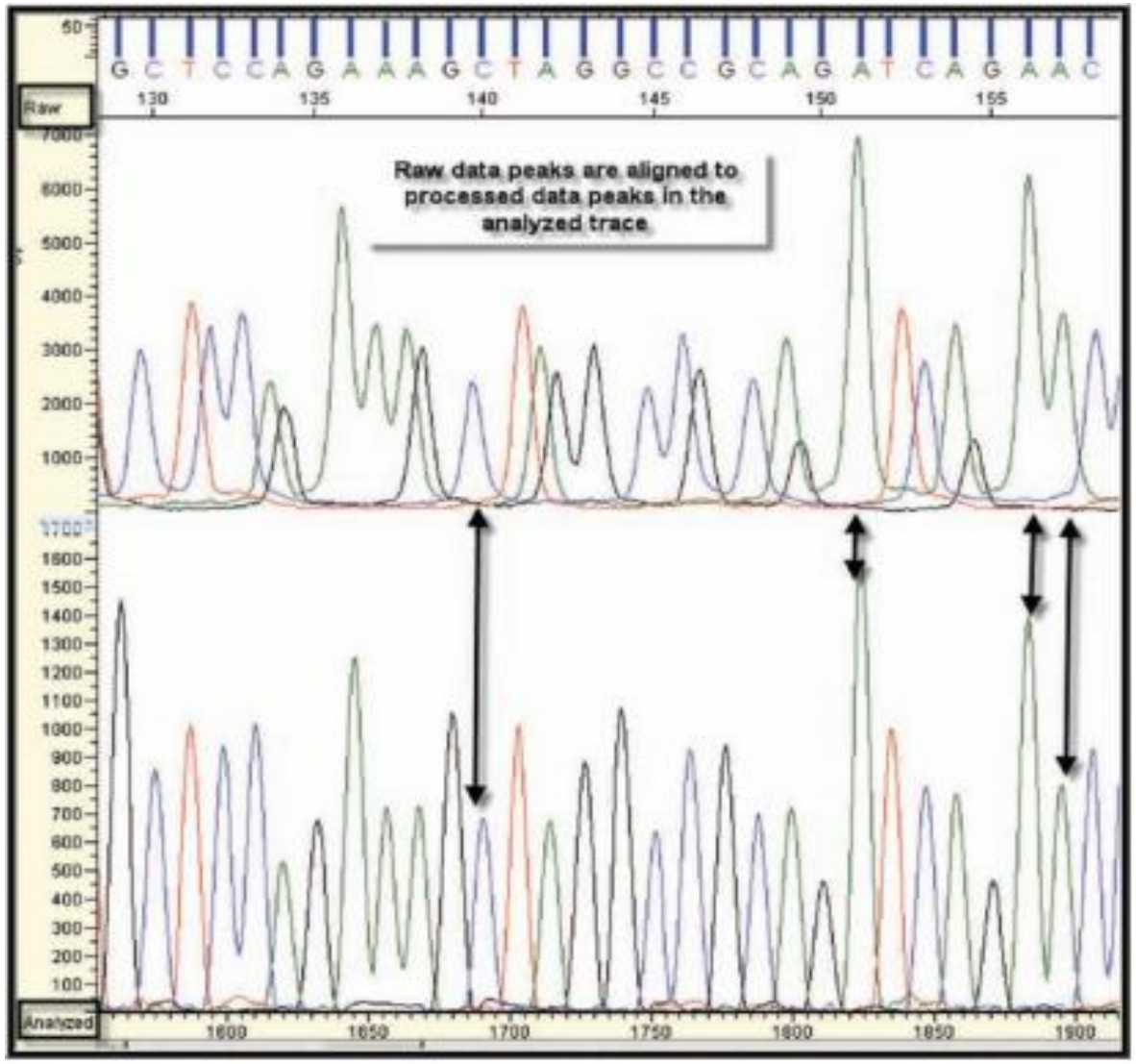
Stages of work: PCR products

NO	Mutant	Source dose (type, Gray units)	Chromosomal Aberration	ex 1 845b (1)	ex 2.1 794b (4)	ex2.2 717b (5)	ex3 710b (13)	ex4 738b (15)	ex5 670b (23)	Ex6-7 782b (24)	ex8 620b (25)
1				+ve	+ve		+ve	+ve			
2				+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
3				+ve	+ve	+ve		+ve	+ve		
4				+ve	+ve				+ve		
5				+ve	+ve			+ve			
6				+ve	+ve	+ve	+ve	+ve	+ve	+ve	
7				+ve	+ve	+ve		+ve	+ve	+ve	+ve
8				+ve	+ve	+ve	+ve				
9				+ve	+ve				+ve		
10	Vg 157	n + γ ,20		+ve	+ve	+ve	+ve	+ve	+ve		



Stages of work: Sequencing

- Sequence Scanner Software.
  1. Forward sequence
  2. Reverse sequence
  3. Analyze the mutations that occurs



Conclusion

- Understand the effect of different radiations on DNA sequence .
- DNA Extraction with two different methods.
- PCR techniques and Purification.
- Do the best to understand what happen in vestigial gene and how this change effect in the function of the gene .
- The role radiation and how it influence DNA are not known exactly due to the behaviour of biological machinery that erases some of the effects of radiation.

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- Chen, J. L., Huisinga, K. L., Viering, M. M., Ou, S. A., & Geyer, P. K. (2002). Enhancer action in trans is permitted throughout the Drosophila genome. *Proceedings of the National Academy of Sciences*, *99*(6), 3723-3728.
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Thank you very much

большое Вам спасибо