# Genome “dactyloscopy “ (DNA finger - printing) and gene expression: Polymerase Chain Reaction (PCR) and Real Time Polymerase Chain Reaction (RT- PCR) in action

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Erin

## Content:

- Aim of the project.
- Materials.
- Methods, Results and discussion
- Conclusion.

# Aim of the work

- *Provide a genomic identification and determine predisposition to several diseases in the genes of human samples.*

- *Estimate the expression levels for the yellow gene and elongation factor Drosophila melanogaster*

# Materials

Incubator

PCR

RT- PCR

Nano-Volume

RNA work box

Agarose gel electrophoresis

# Methods

First: Human DNA

- ***Isolation of DNA:***
  - Isolation of DNA from buccal epithelium
  - Isolation of DNA from hair
  - PCR reaction
  - Gel electrophoresis
- ***Human finger printing :ALU PCR***
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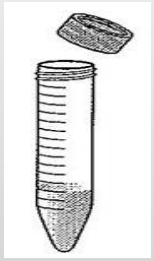
  - Gel electrophoresis
- ***Allelic discrimination :***
  - Isolation of DNA
  - Using TaqMan  PCR

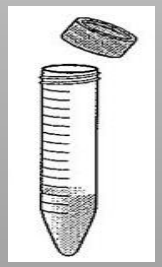
## *Isolation of DNA from buccal epithelium*



Solution with DNA

Supernatant with DNA

## *Isolation of DNA from hair*



Solution with DNA

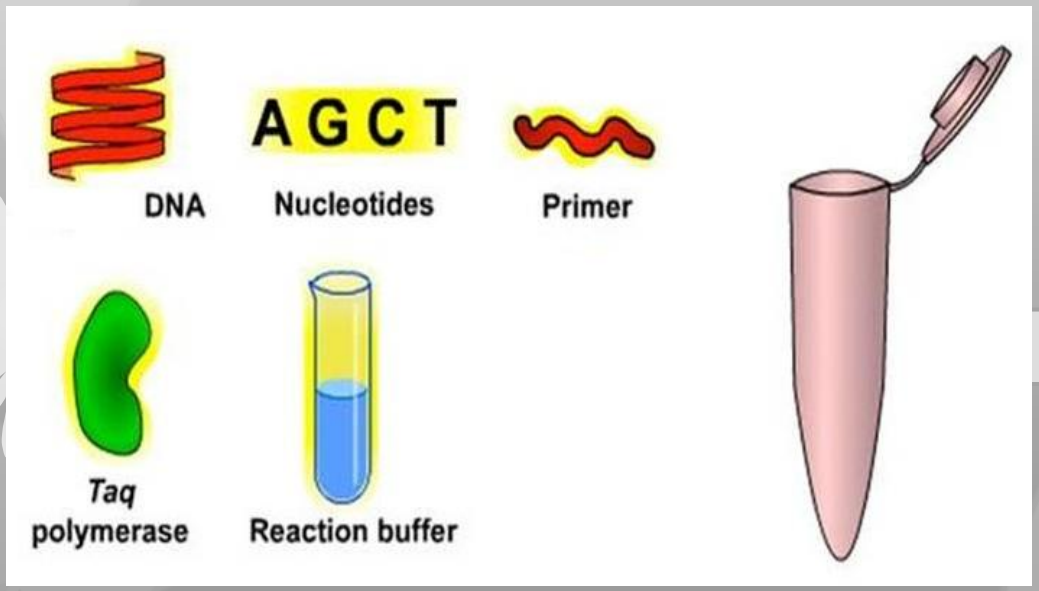
Supernatant with DNA

PCR amplification

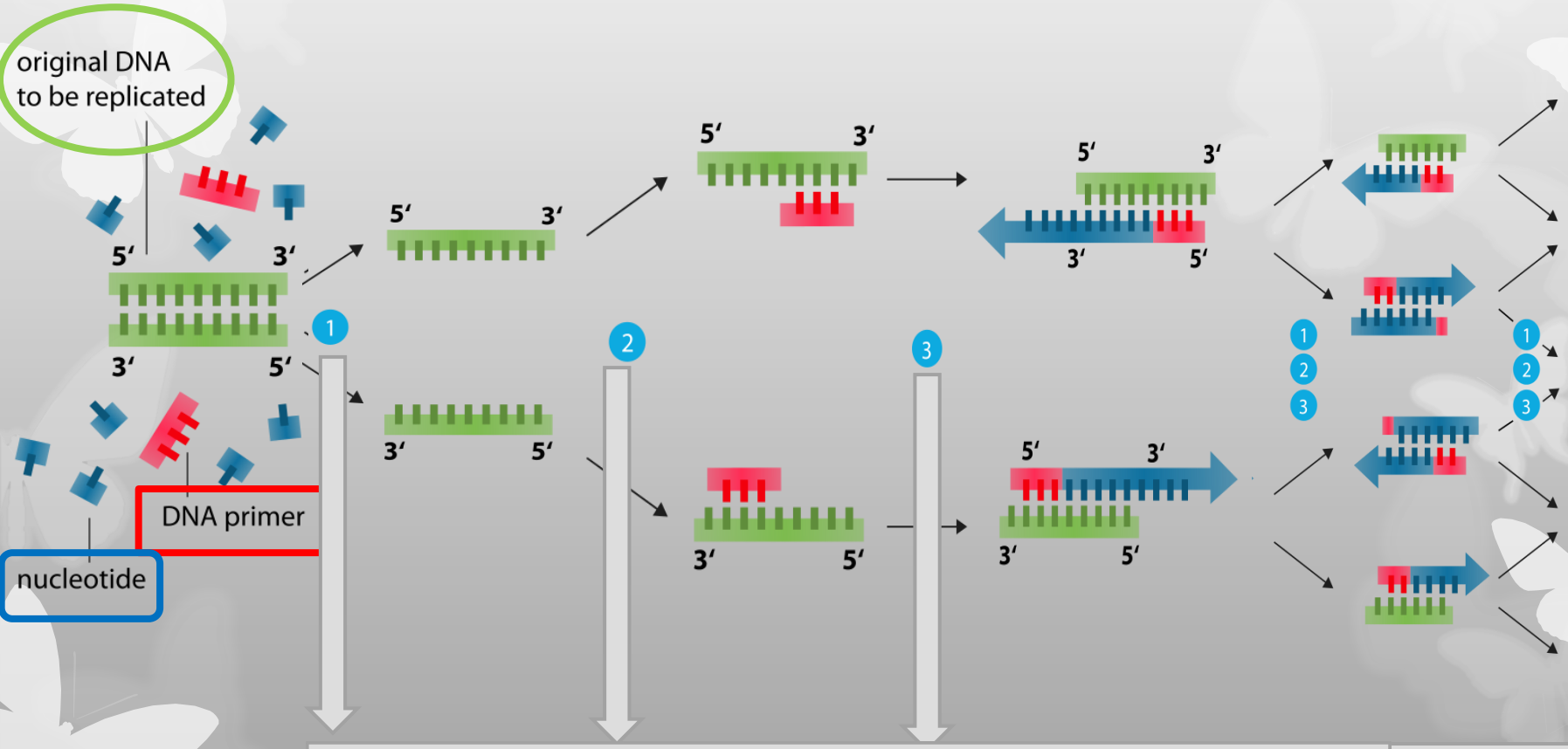
# **P**olymerase **C**hain **R**eaction

- A technique to **AMPLIFY** specific DNA fragments
- Only a single DNA fragment needed to generate a million to billion copies

## PCR components:



# Steps of PCR



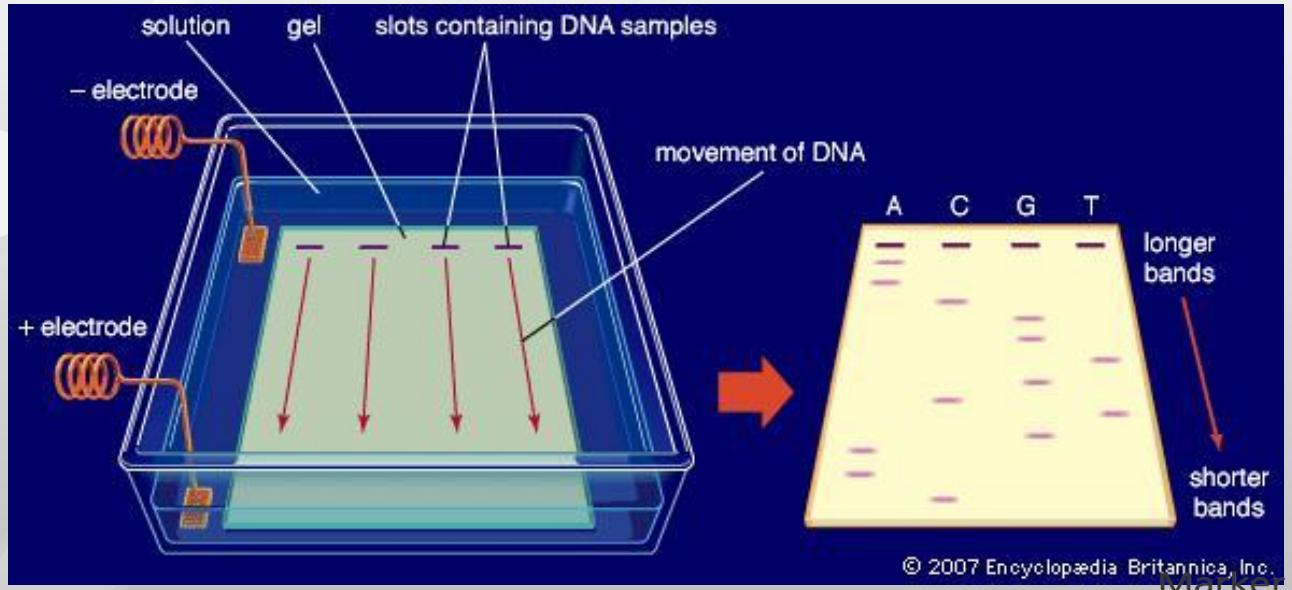

St

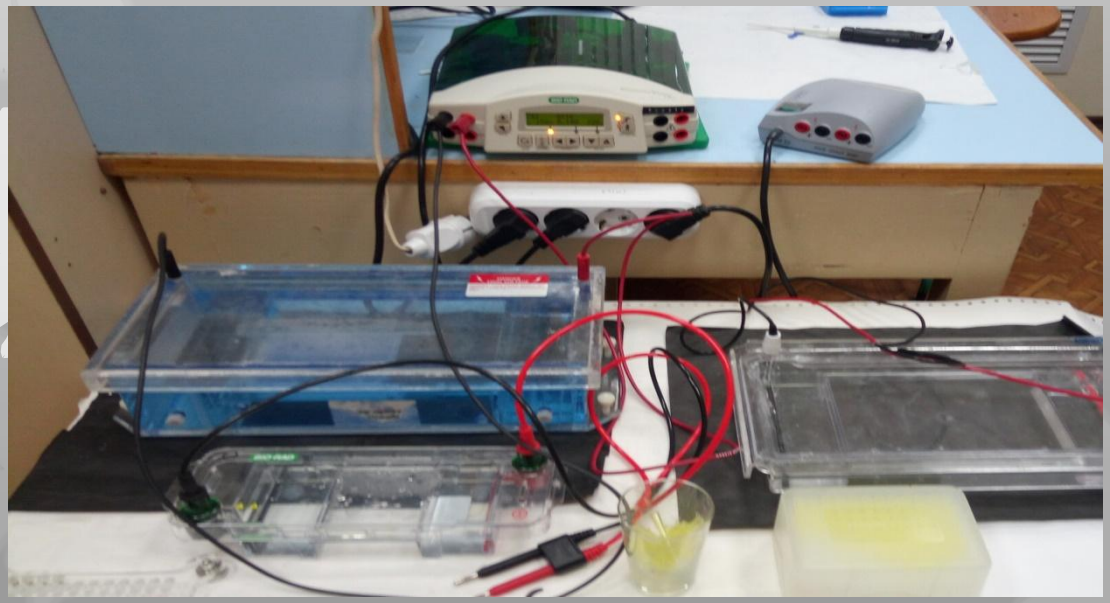
Denatu
9

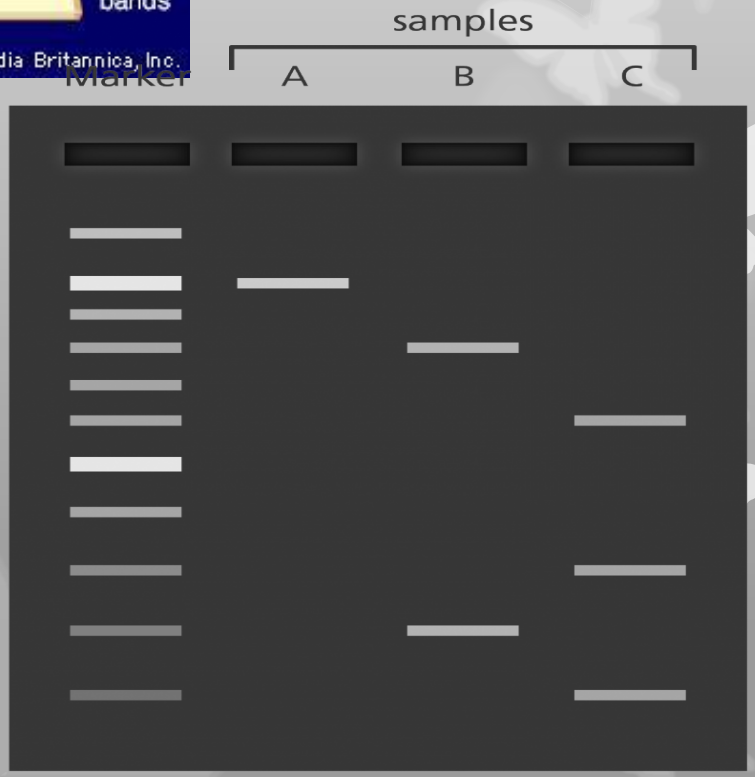
30 cycles produce ≈ 10,000,000 copies

cycles

# Agarose gel electrophoresis





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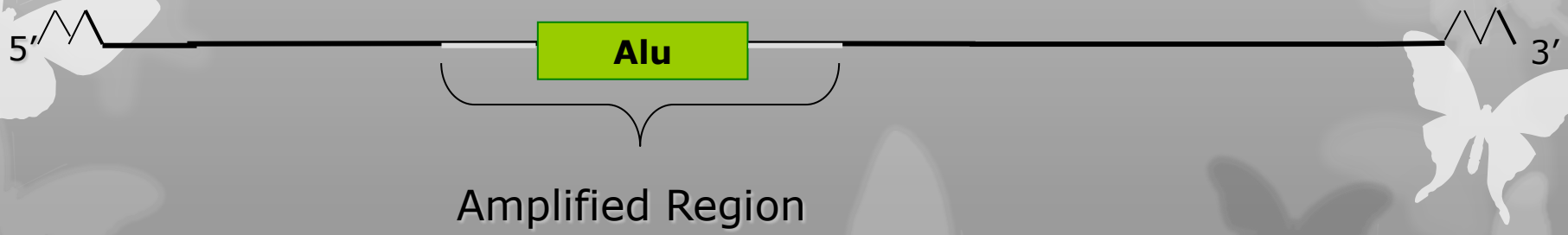
# Human finger printing :ALU PCR

## What is ALU?

- The genome contains small repetitive DNA elements that have become randomly inserted into the human genome.
- Classified as SINEs (Short INterspersed Repetitive Element) and about 300 base pairs long repeated Approx.
- Useful as a measure of genetic variation, associated with disease or used for DNA typing



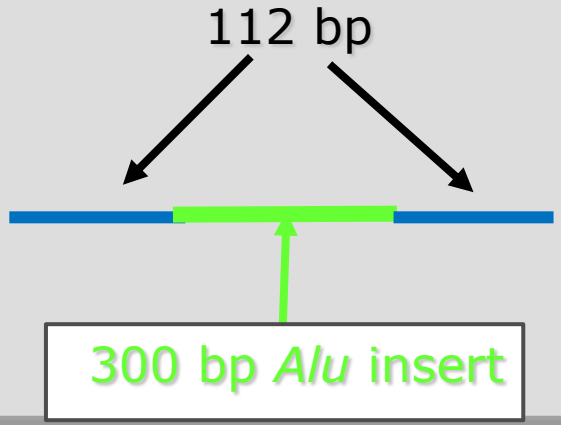
# Alu inserted genes

## TPA25

- Tissue plasminogen activator
- An Enzyme to dissolve blood clot
- In chromosome 8

No insertion: 112 bp

Alu insertion: 412 bp

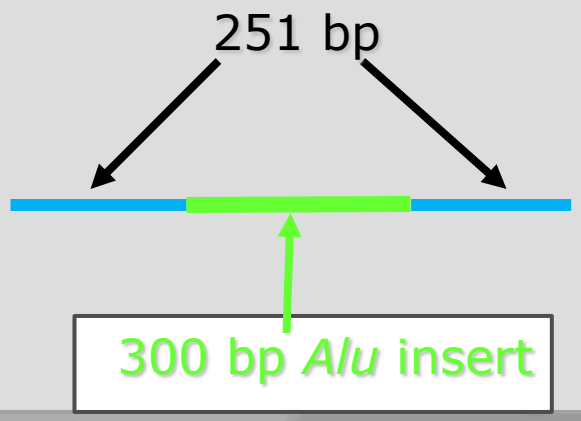



## ZFPM2

- Zinc finger protein
- regulation of apoptosis
- In chromosome 8

No insertion: 251 bp

Alu insertion: 551 bp

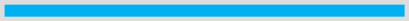


# Alu inserted genes

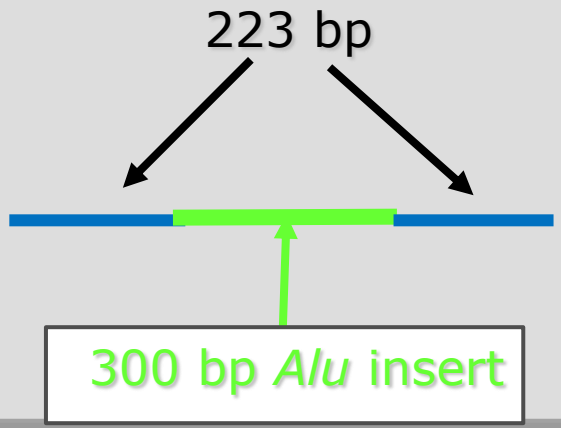
## XPR1

- Xenotropic and polytropic retrovirus receptor
- Plays a role in phosphate homeostasis
- In chromosome 1

No insertion: 223 bp



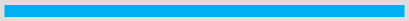
Alu insertion: 523 bp



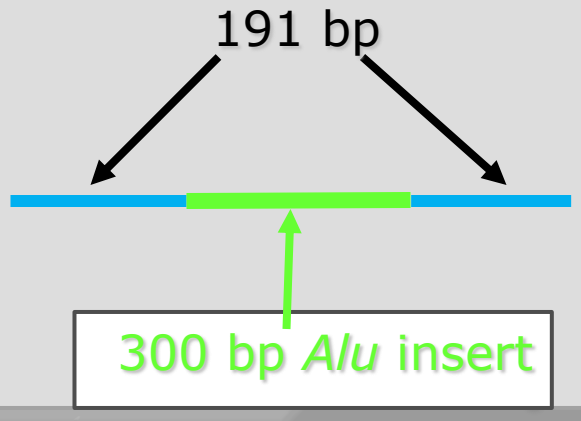

## ACE

- Angiotensin converting enzyme
- inactivate the vasodilator, proinflammatory peptide, bradykinin.
- In chromosome 17

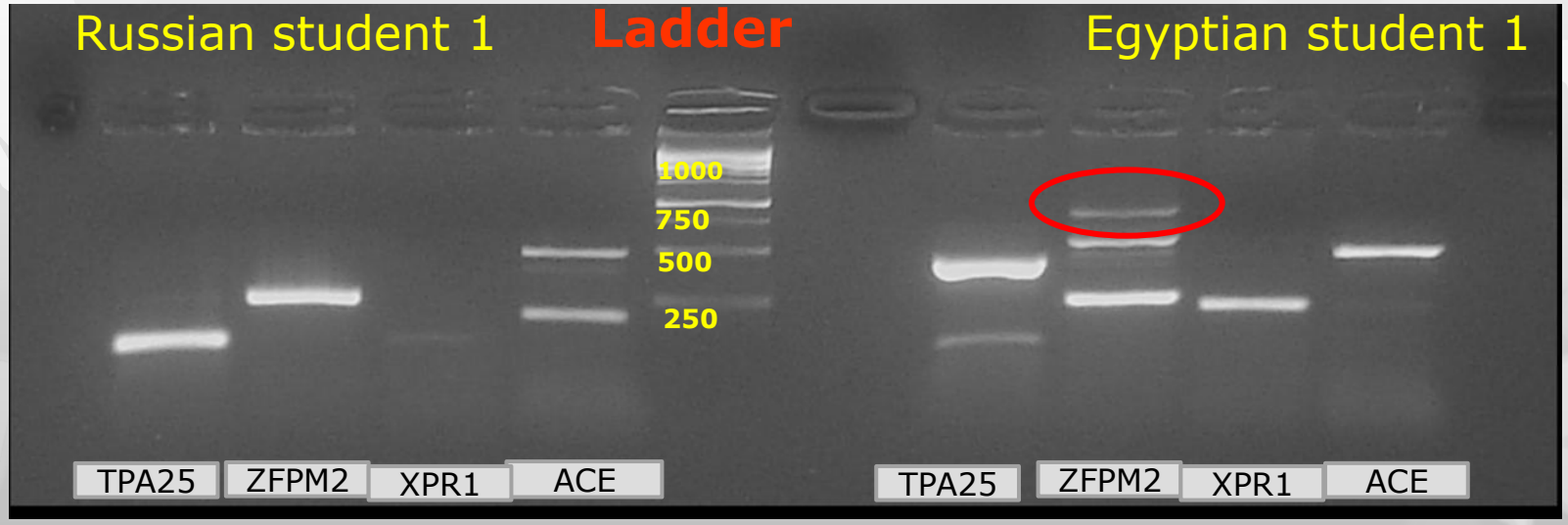
No insertion: 191 bp

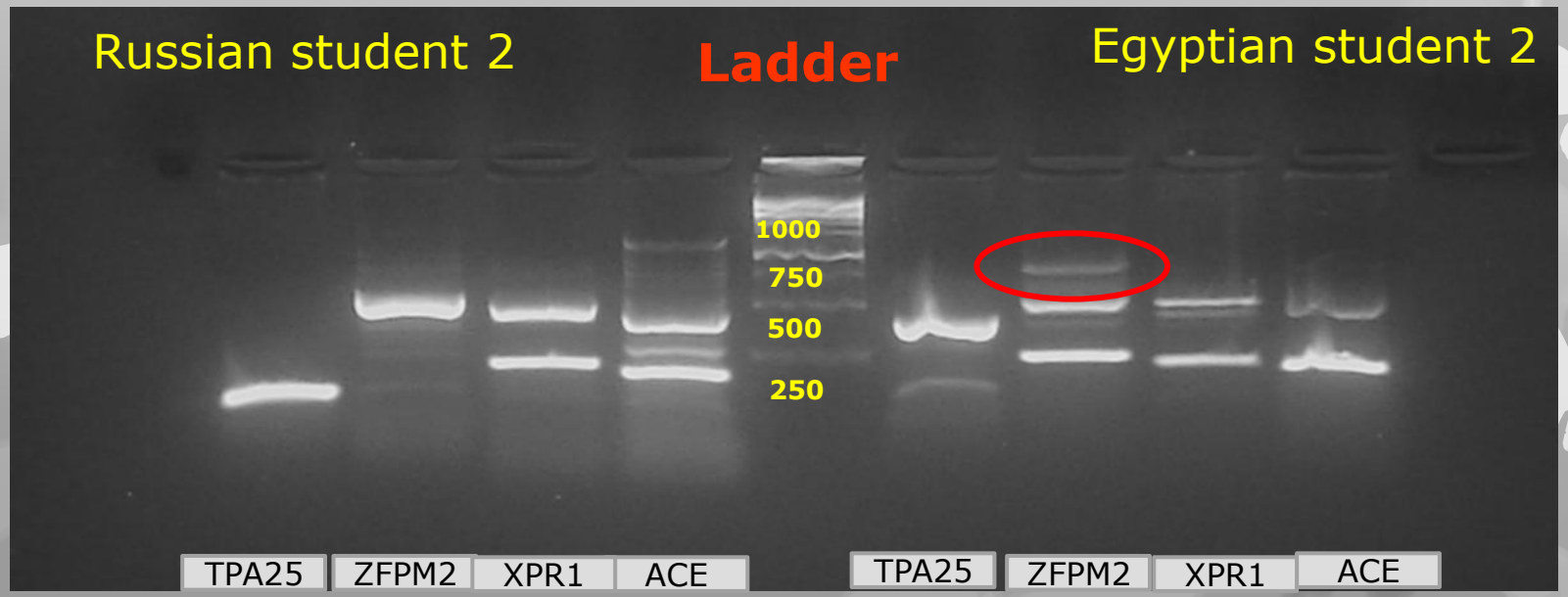


Alu insertion: 491 bp

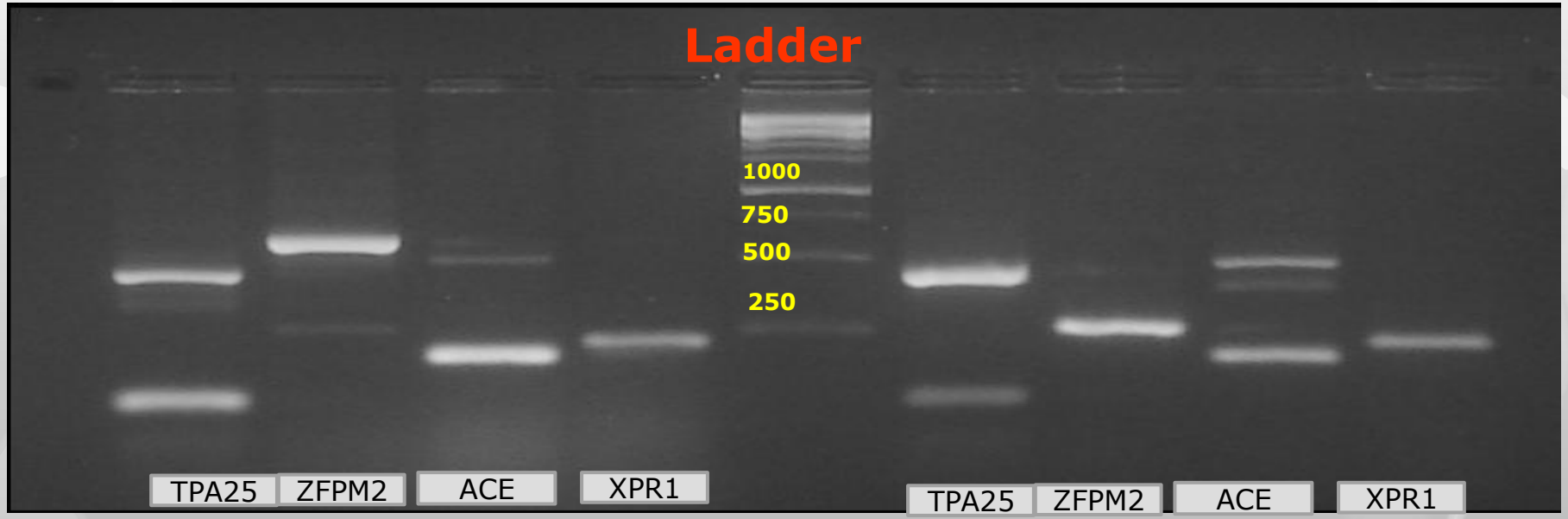


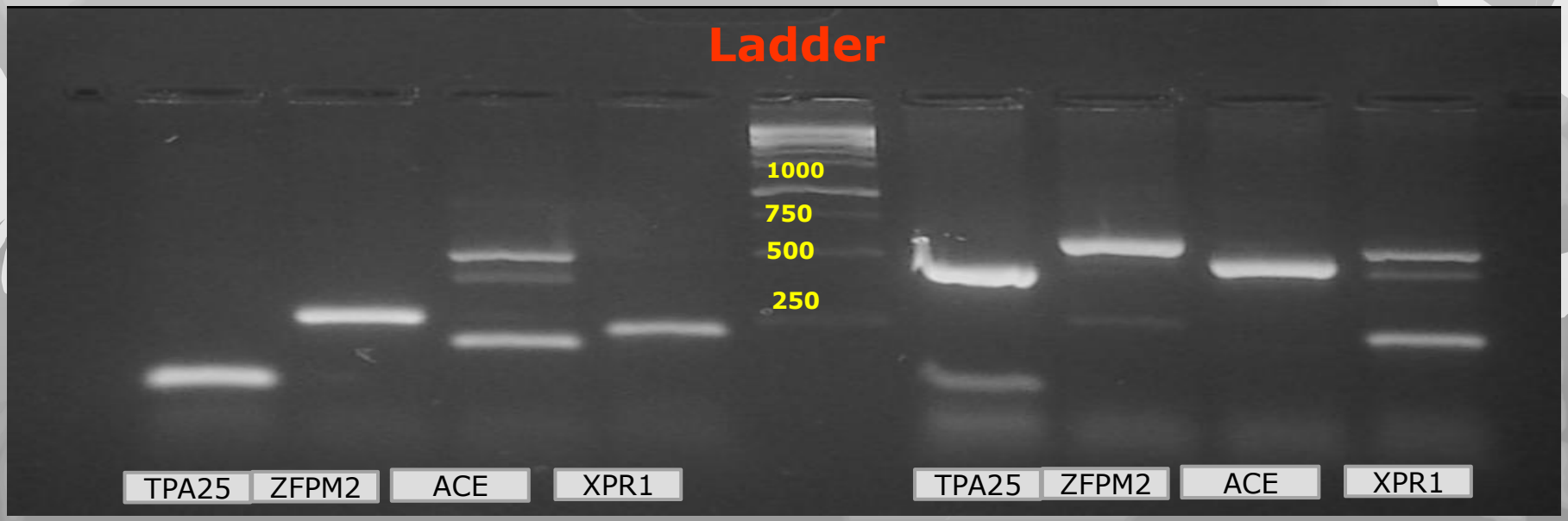
# PCR reaction and gel



# *Other Egyptian students*



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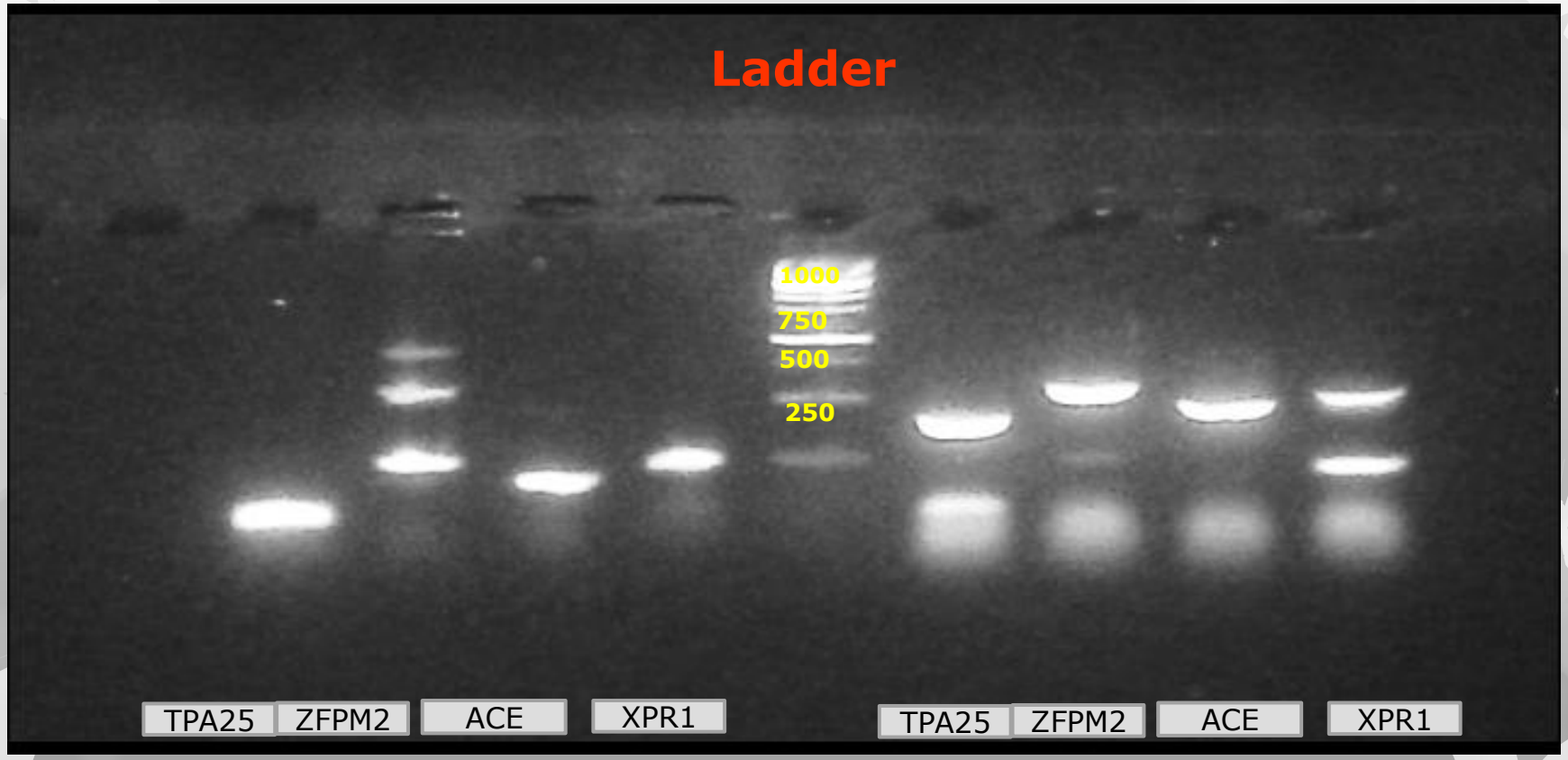

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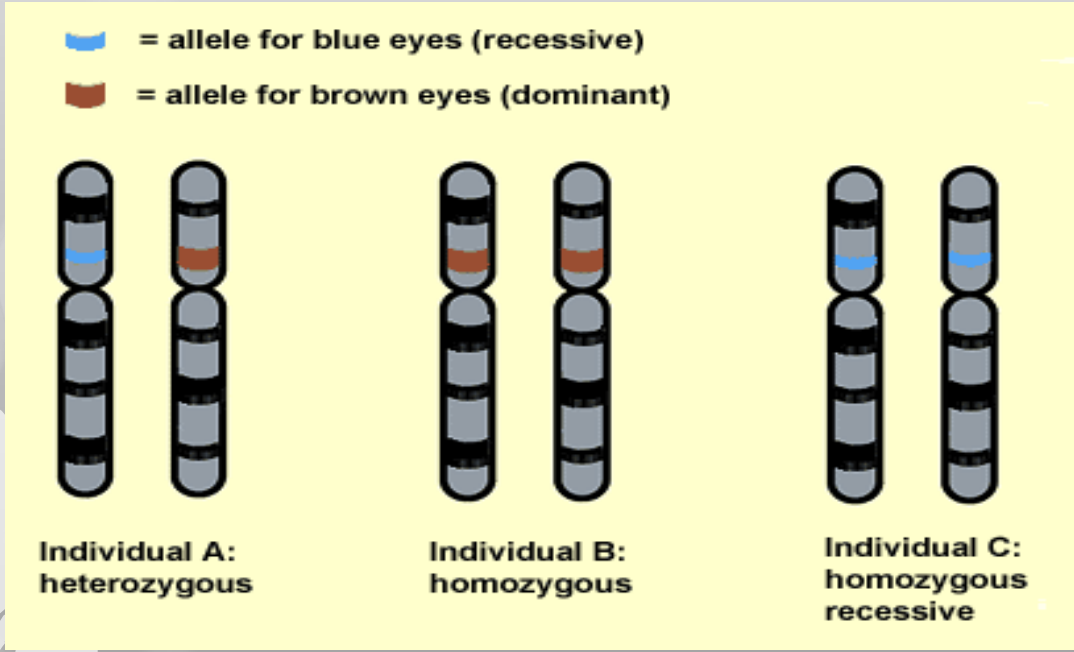
# Allelic discrimination with TaqMan probes RT-PCR

***What is allele?***



# Quantitative - PCR

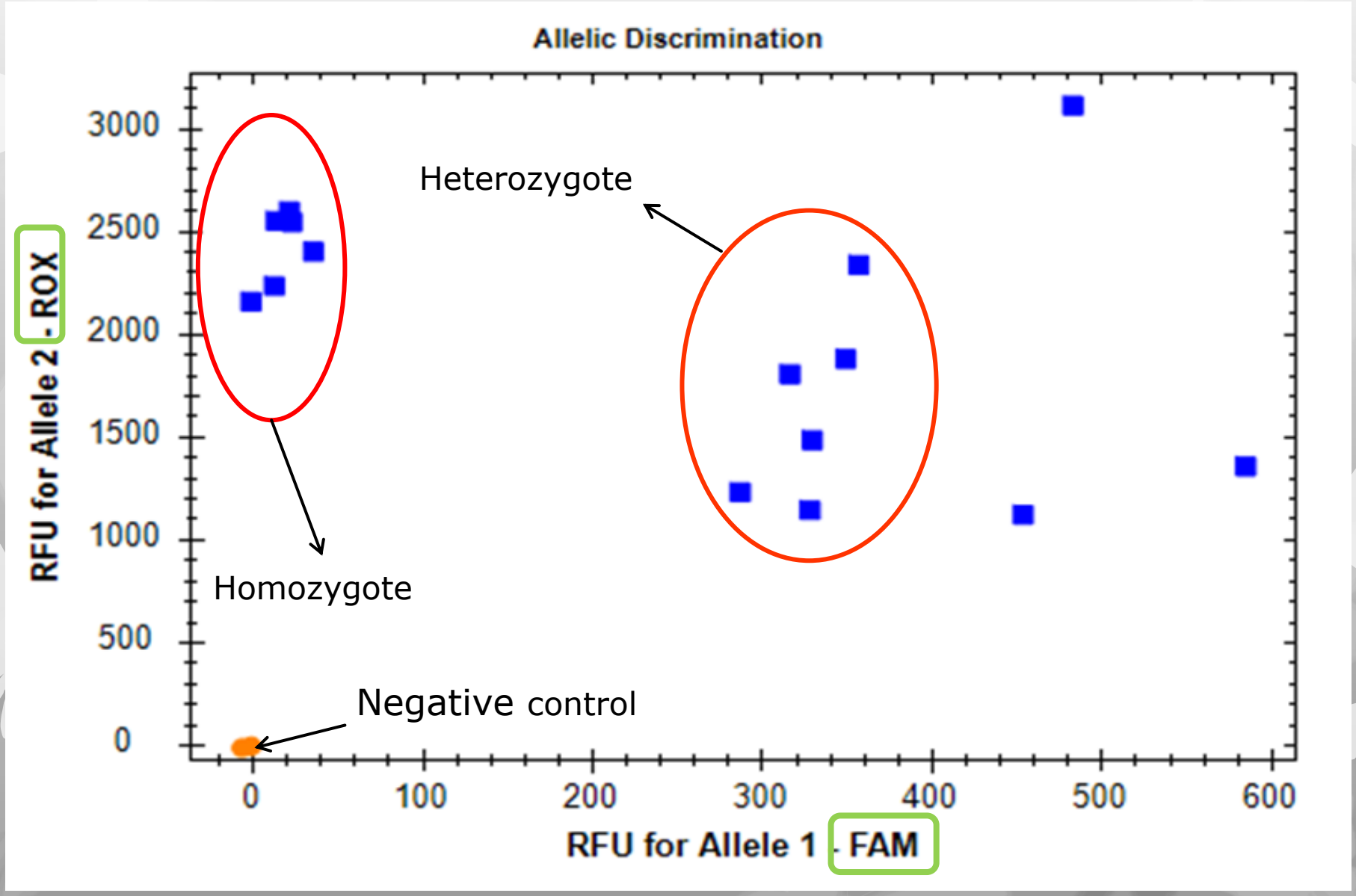
Q-PCR amplifies a specific target sequence in a sample then monitors the amplification progress using **fluorescent technology**

In real-time PCR, the amount of DNA is measured after each cycle via fluorescent dyes that yield increasing fluorescent signal in direct proportion to the number of PCR product molecules (amplicons) generated.

# Allelic discrimination with

## TaqMan

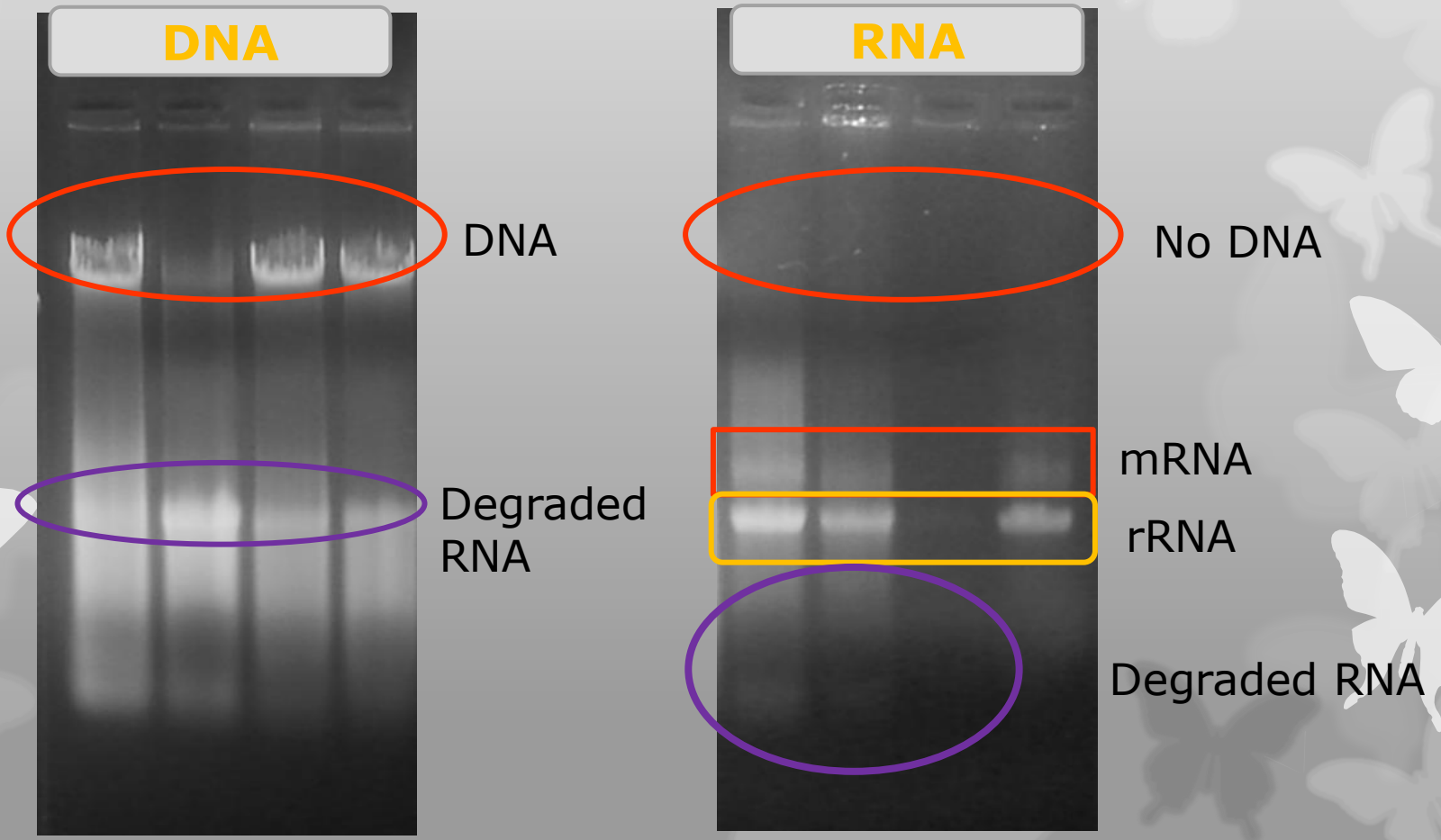
# Results


Allelic Discrimination
RFU for Allele 2 - ROX
3000
2500
2000
1500
1000
500
0
0
100
200
300
400
500
600
RFU for Allele 1 - FAM
Heterozygote
Homozygote
Negative control

**second**

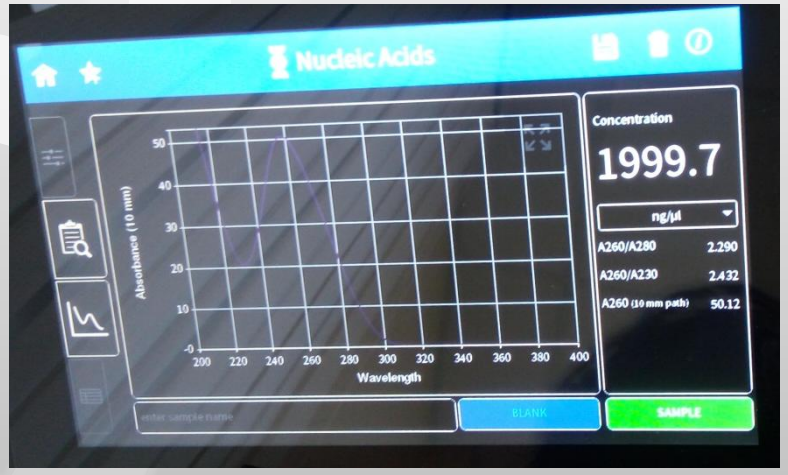
# Experiments with *Drosophila Melanogaster*

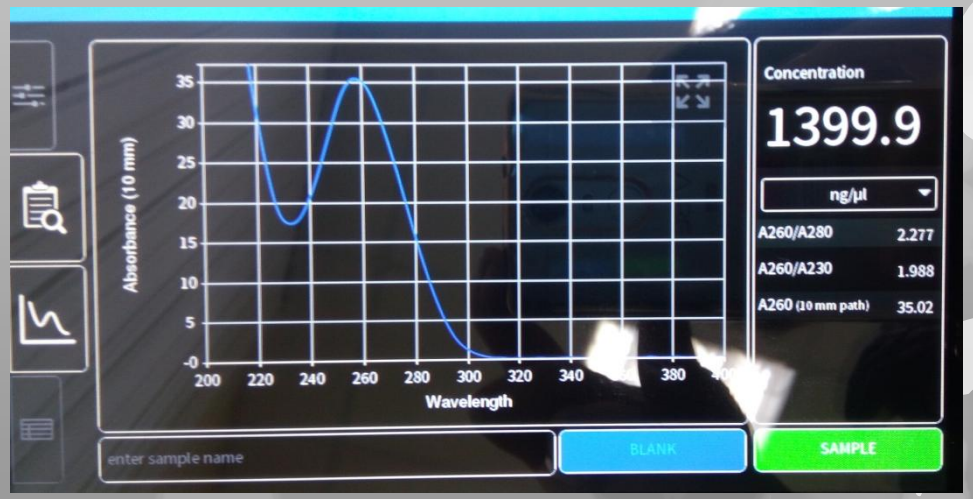
- Isolation of DNA & RNA each one alone
- Agarose gel electrophoresis for both



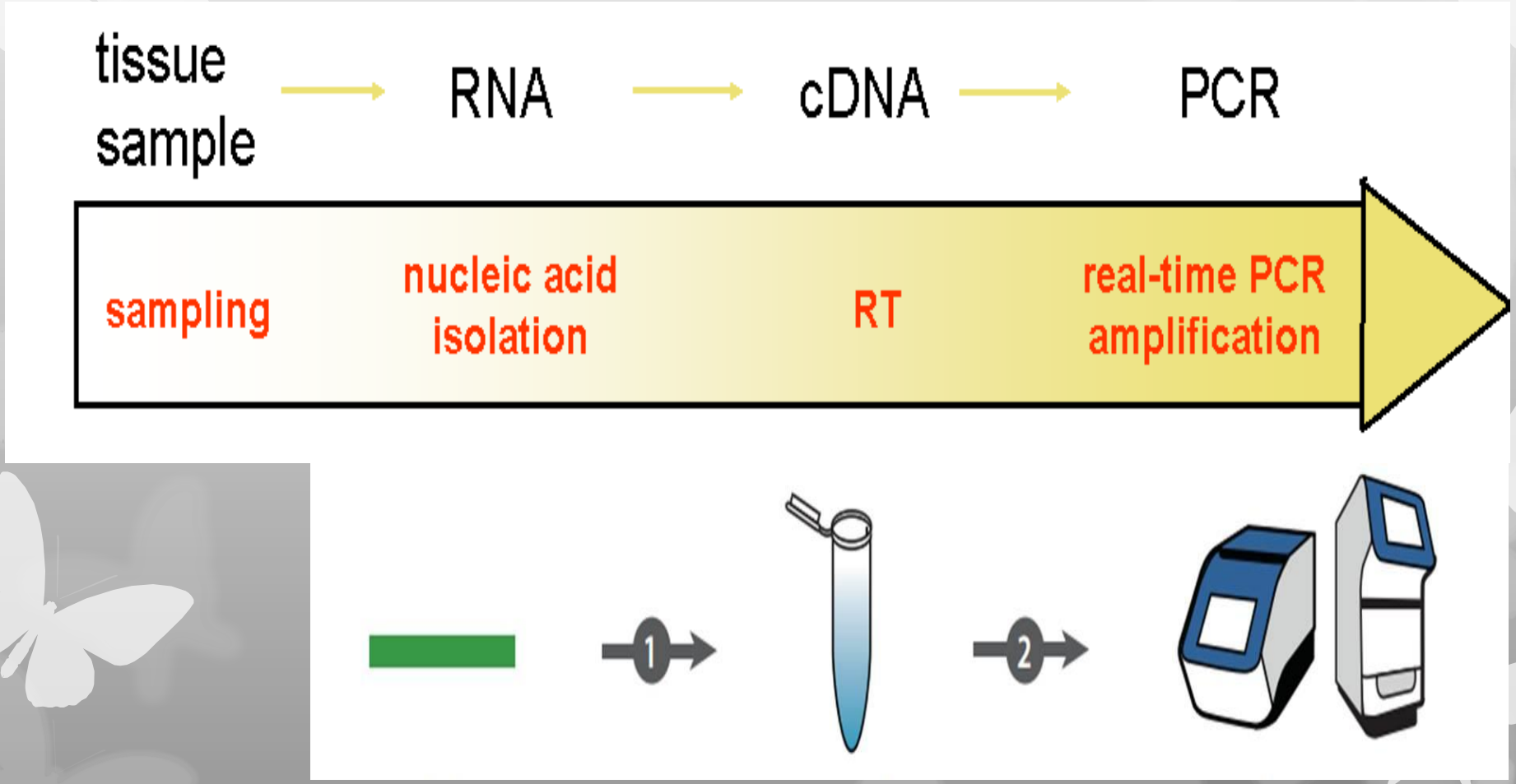
# Determine the concentration and purity of an DNA and RNA samples by using Nano-volume

# Concentration of RNA samples



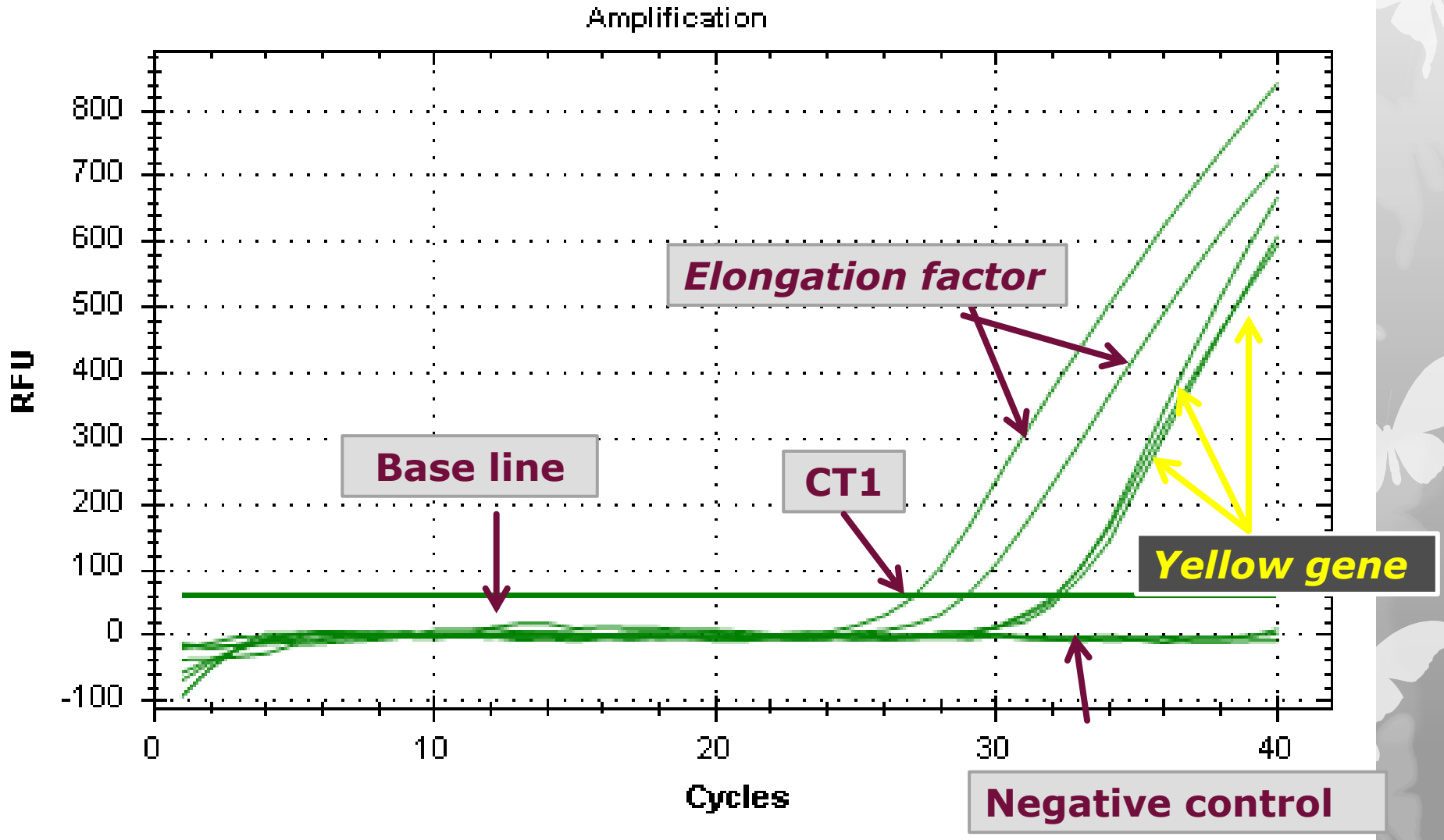


# Reverse Transcription with RT kit



# SYBR green

# Quantification of RT-PCR for yellow gene and elongation factor



# Calculations

***1. The average of yellow gene :***

$$\frac{32.32+32.15+32.06}{3} = 32.17$$

***1. The average of elongation gene :***

$$\frac{29.89+27.08}{2} = 27.98$$

***2. Subtract both results***

$$(Y - EF) =$$

$$32.17 - 27.98 = 4.19$$

***Number of Cycles :***

$$4.19^2 = 17.55 \text{ cycle}$$

| Fluor | Target | Content | Cq | Well | Sample |
|---|---|---|---|---|---|
| SYBR | | Unkn | N/A | A03 | |
| SYBR | | Unkn | N/A | B03 | |
| SYBR | | Unkn | 32,32 | [illegible] | |
| SYBR | | Unkn | 32,15 | [illegible] | |
| SYBR | | Unkn | 32,06 | [illegible] | |
| SYBR | | Unkn | N/A | C03 | |
| SYBR | | Unkn | 28,89 | [illegible] | |
| SYBR | | Unkn | 27,08 | [illegible] | |

*copies of elongation factor > copies of yellow gene*

# ***Conclusion :***

- According to the result we measure fluoresce from SYBR DNA complex that reflects the starting amount of RNA in our samples.

- The relative expression level of *elongation factor* gene is approx. 18 times more than *yellow* gene.

- That is because all living cells need *elongation factor* but only cuticula cells need product of *yellow* gene

# THANK YOU

FOR YOUR ATTENTION

ANY
QUESTIONS
?