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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AIMS OF THIS PROJECT:

- to study two basic molecular biological techniques and apply them in practice - Polymerase Chain Reaction (PCR) and real-time polymerase chain reaction (Real- Time PCR), also known as quantitative polymerase chain reaction (qPCR) - most powerful tool for quantitative nucleic acids analysis.
- to estimate the expression levels for the *yellow* gene *Drosophila melanogaster* at different stages of fly development.

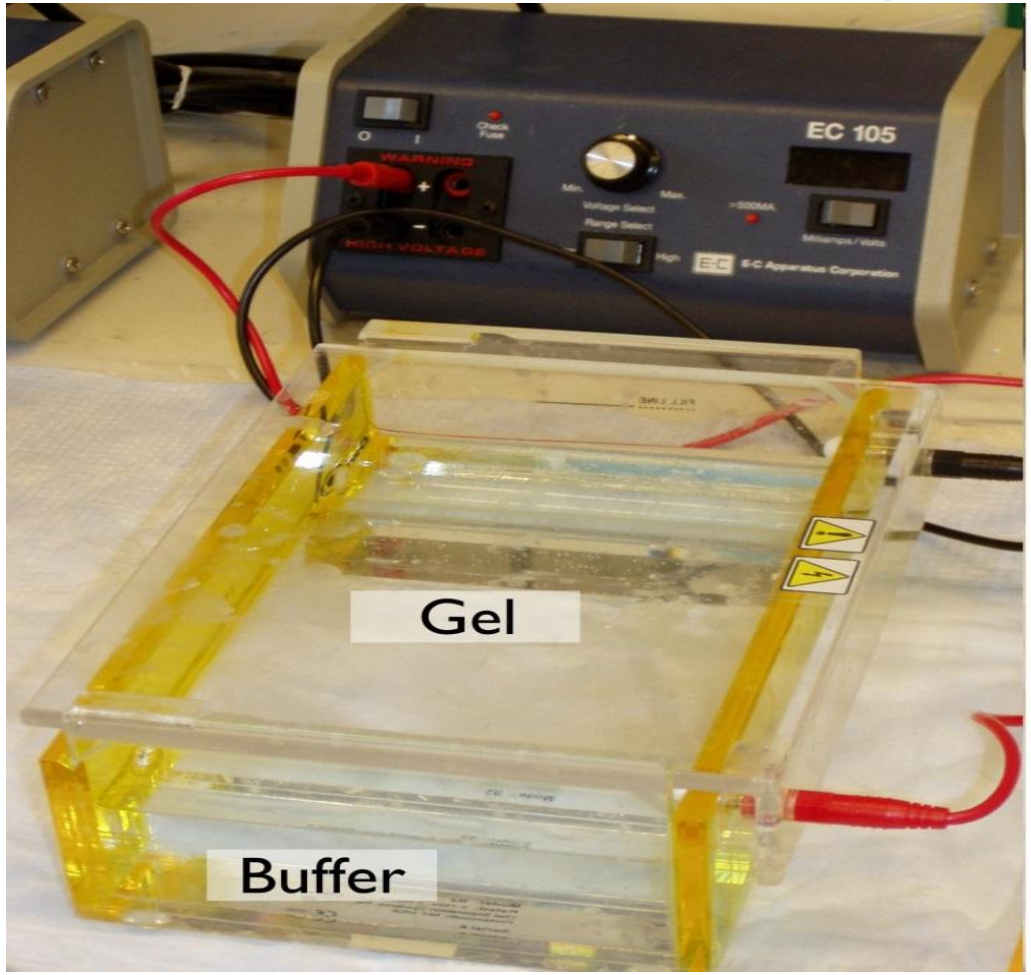
EQUIPMENT:

- *Thermocycler (DNA amplifier)*

- *Laminar box*

EQUIPMENT:

- *Galvanic element for electrophoresis*

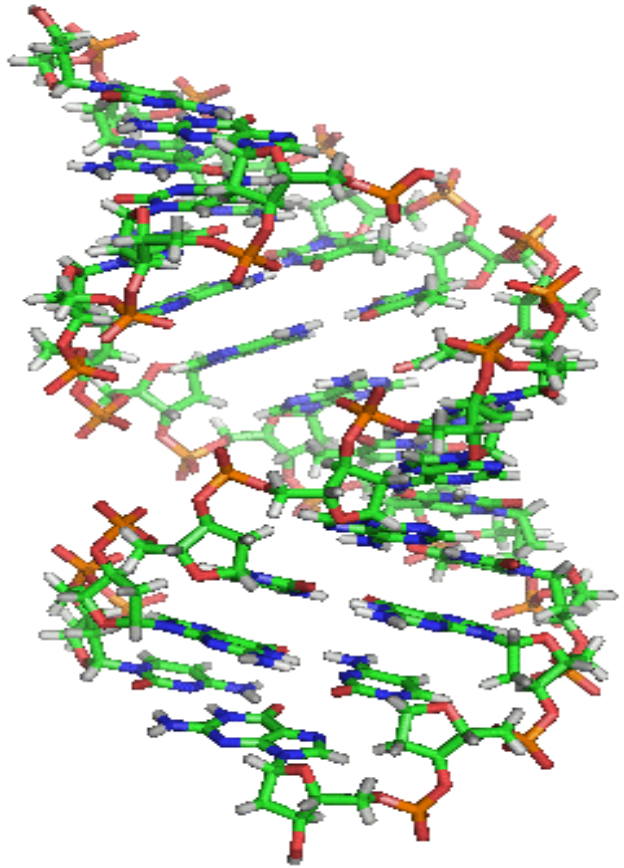


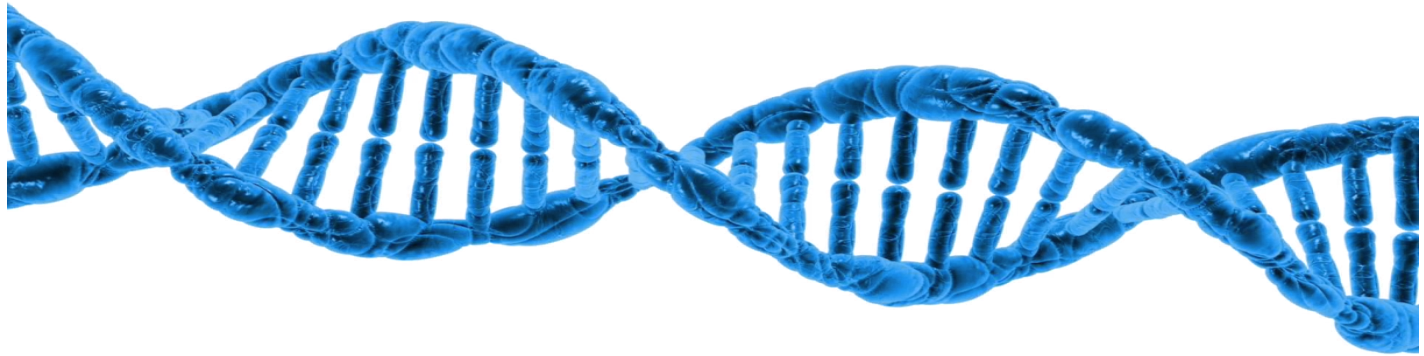
Power supply

Cathode ⊖

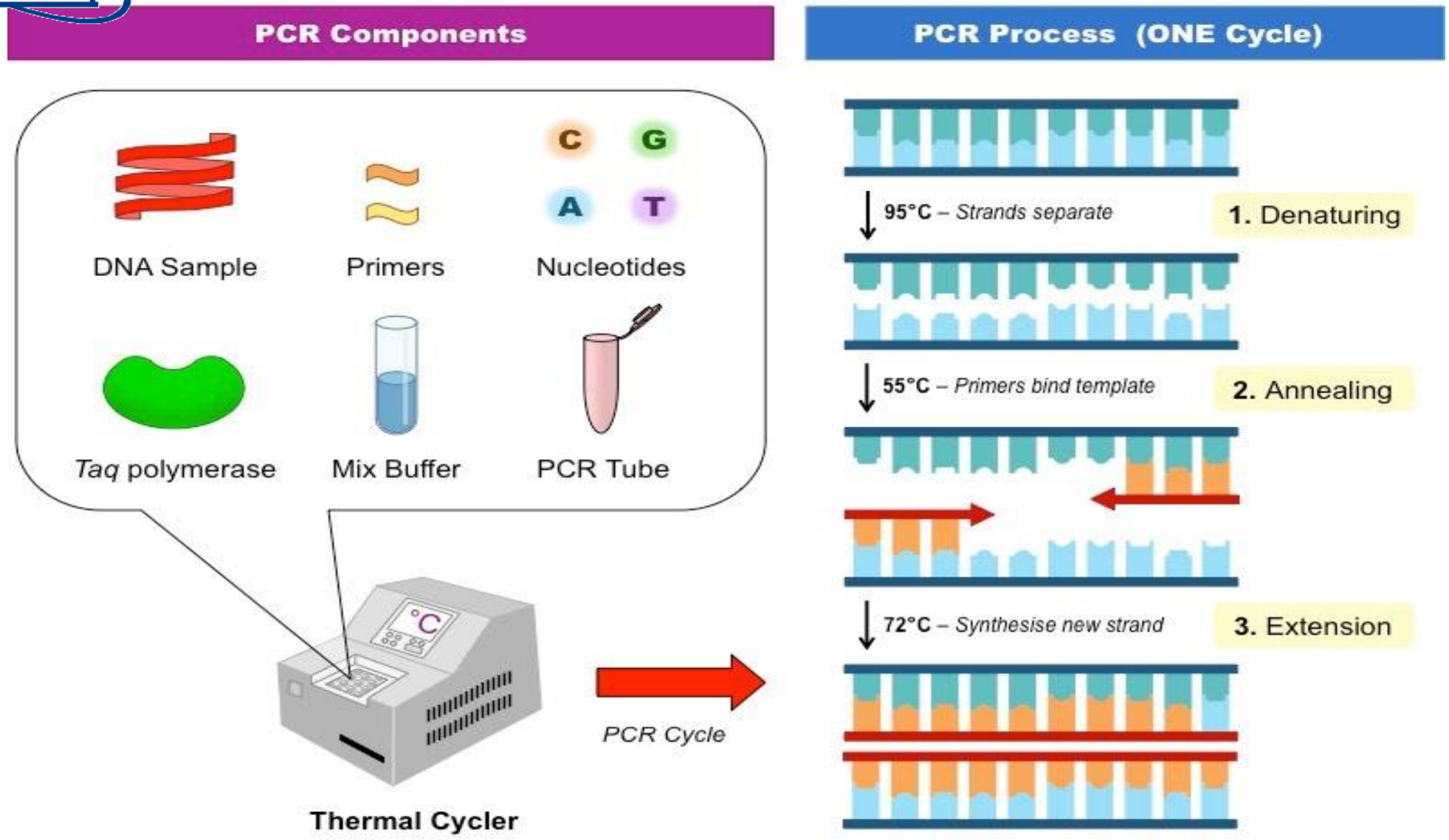
Anode ⊕

WHAT IS DNA:





WHAT IS PCR:


PCR Components
DNA Sample
Primers
C
G
A
T
Nucleotides
Taq polymerase
Mix Buffer
PCR Tube
°C
Thermal Cycler
PCR Cycle
PCR Process (ONE Cycle)
95°C – Strands separate
1. Denaturing
55°C – Primers bind template
2. Annealing
72°C – Synthesise new strand
3. Extension

ISOLATION OF DNA

- PCR can be performed directly from various human samples (buccal swabs, hair, amniotic fluid, skin, saliva, fingernails..) with NO prior DNA purifications.

- We collected DNA from:
  - buccal epithelium
  - hair

- **PCR amplification program ≈ 50 minutes:**

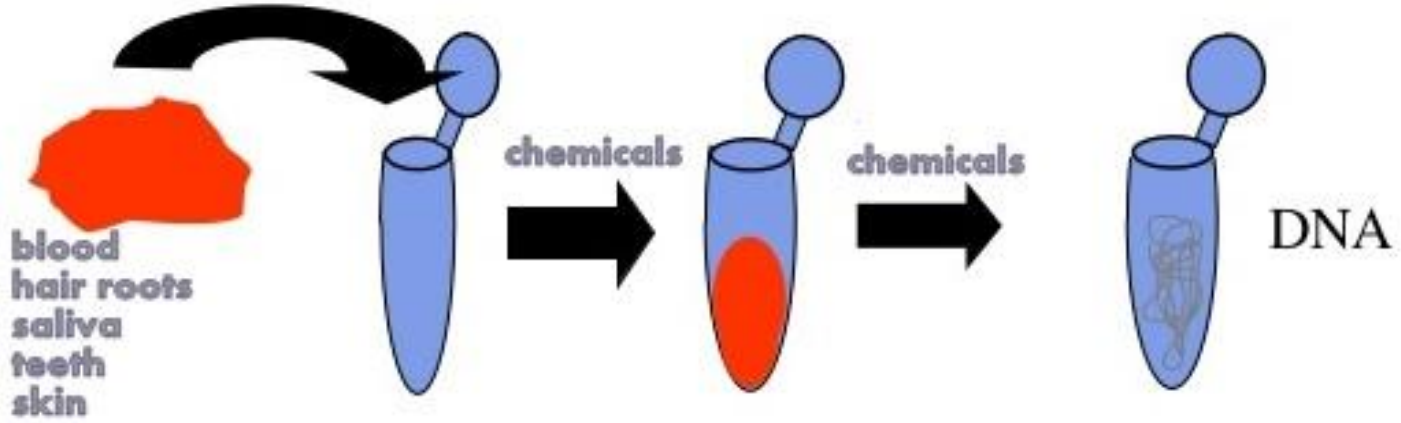
-1 cycle 95˚C for 3 minutes (initial denature)

-30 cycles : 95˚C for 30s (denature)

58˚C for 30s (anneal)

72˚C for 30s (extend)

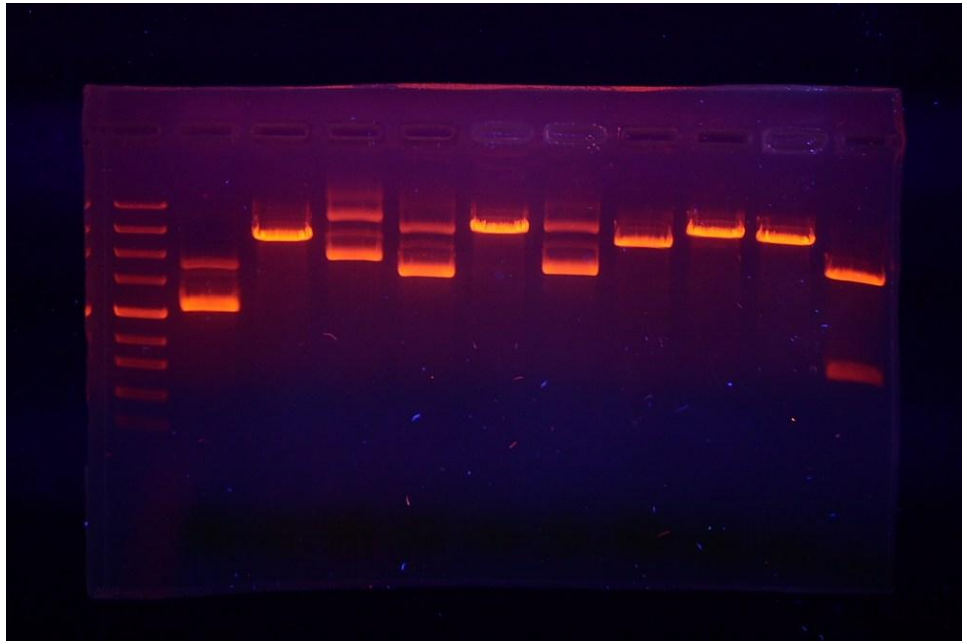
-1 cycle 72˚C for 3 minutes (final extension)

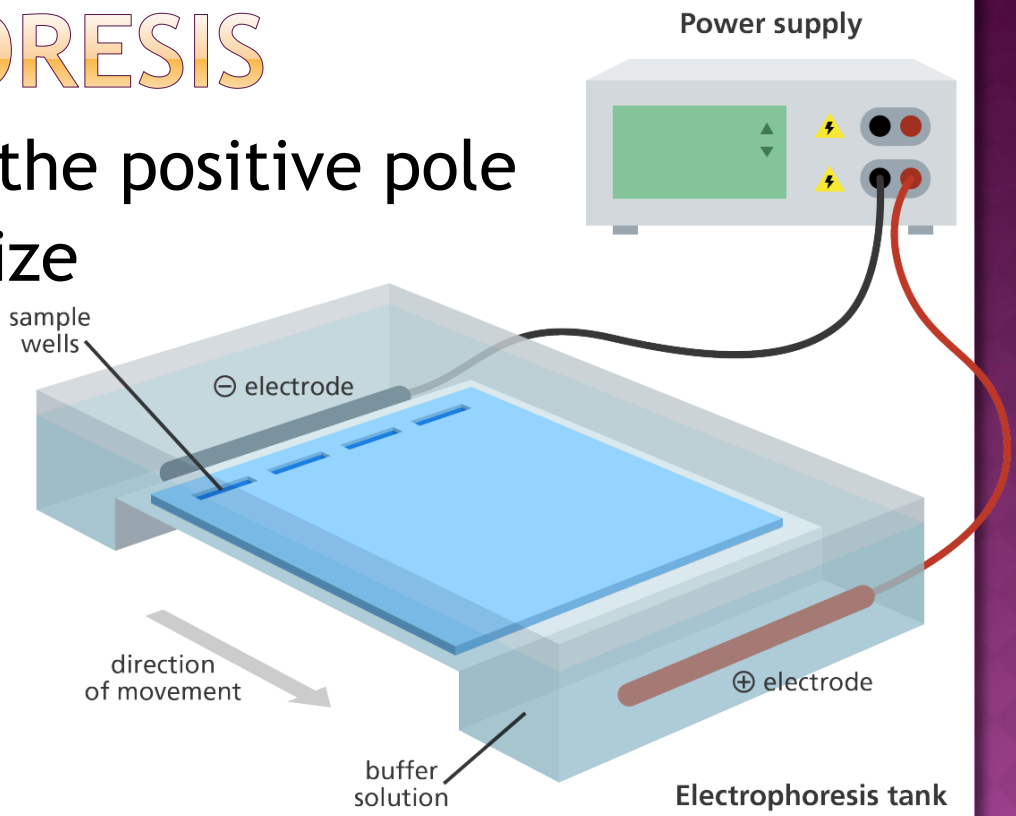


$$2^{32} \approx 4\,000\,000\,000$$

ELECTROPHORESIS

- Negatively charged DNA moves toward the positive pole
- Separation of DNA fragments by their size
- Agarose gel (2%)
- Ethidium bromide-fluorescent dye
- DNA molecular weight marker-ladder





ALU-PCR

- Short DNA sequence, ≈300 bp
- ``The jumping gene``

❖we examined presence of Alu in:

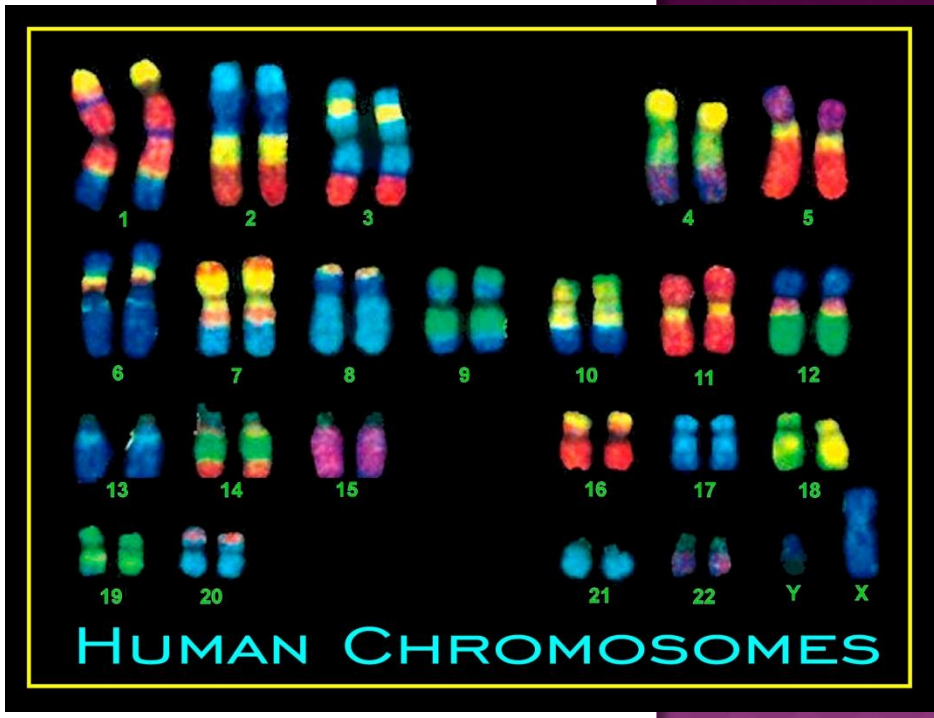
-gene TPA-25 (tissue plasminogen activator), #8

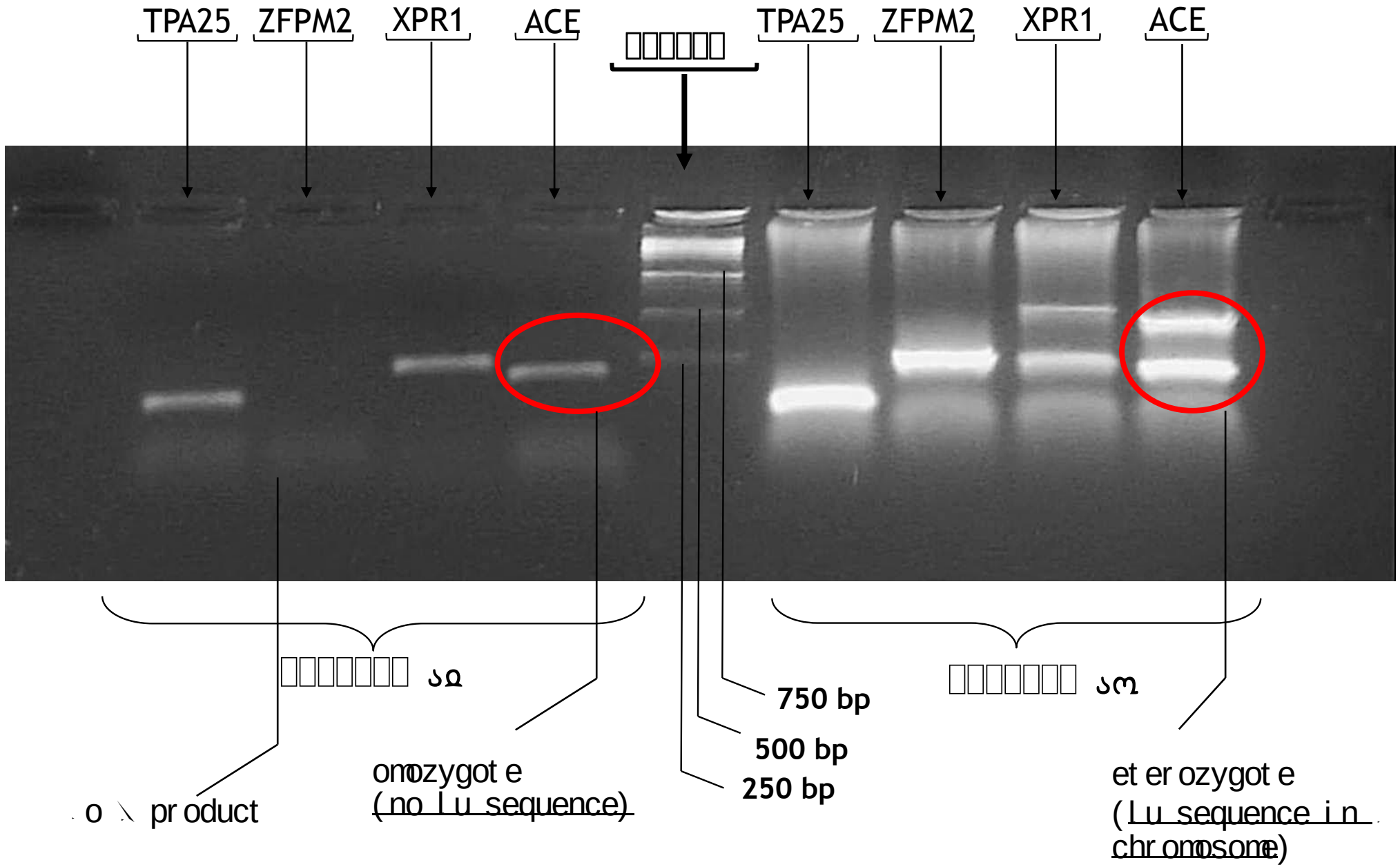
-gene ZFPM2 (zinc finger protein), #8

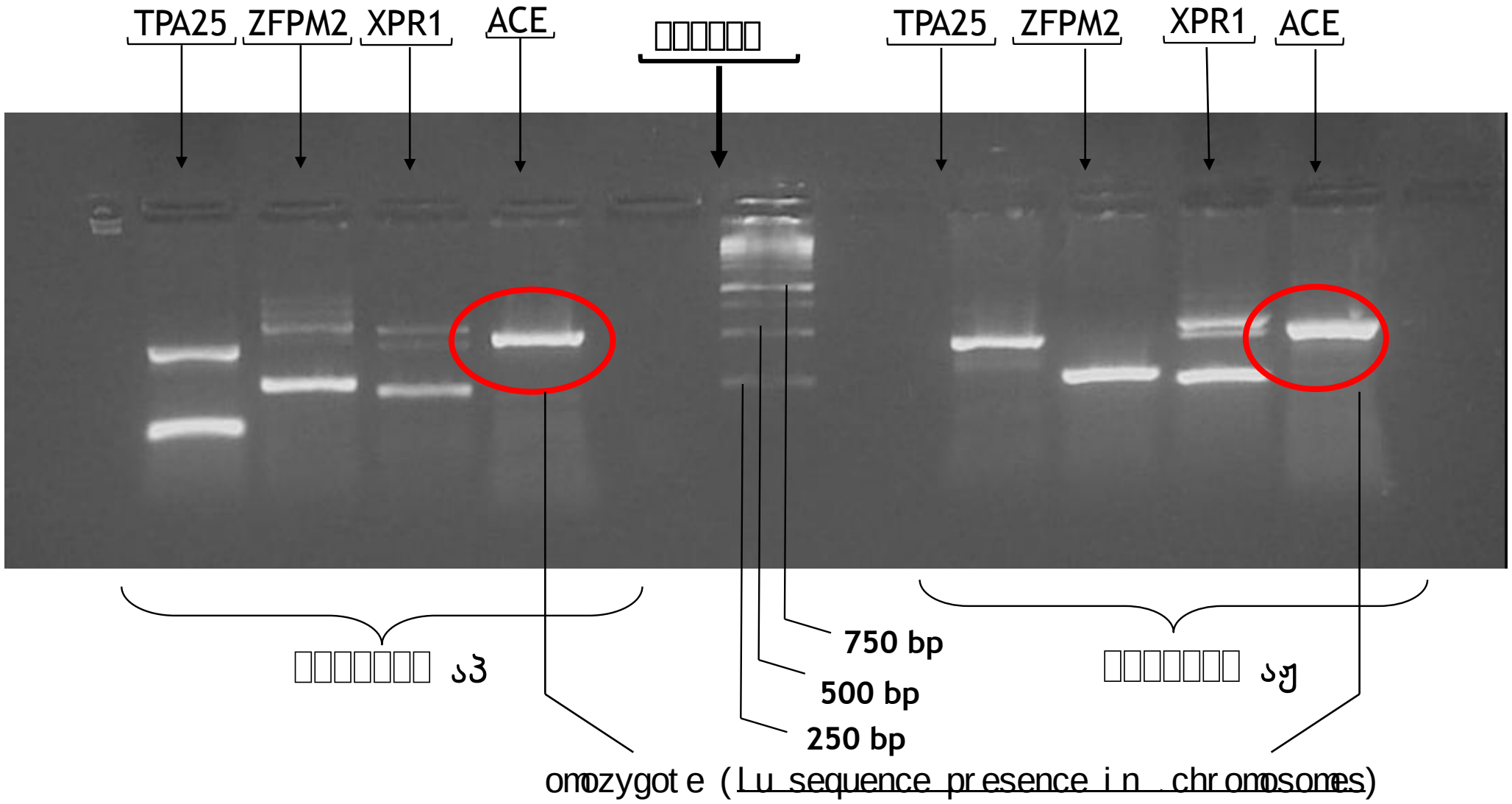
-gene XPR1 (xenotropic and polytropic retrovirus receptor), #1

-gene ACE (angiotensin I converting enzyme), #17

❖ Genetic passport




TPA25
ZFPM2
XPR1
ACE
TPA25
ZFPM2
XPR1
ACE
750 bp
500 bp
250 bp
o \ pr oduct
om ozygot e
(no l u sequence)
et er ozygot e
(l u sequence i n
chr om osom e)


TPA25
ZFPM2
XPR1
ACE
TPA25
ZFPM2
XPR1
ACE
750 bp
500 bp
250 bp
omozygot e (Lu sequence pr esence i n chr omosome

ACE GENE

-I/I normal level of ACE in blood (2x 491bp→one line)

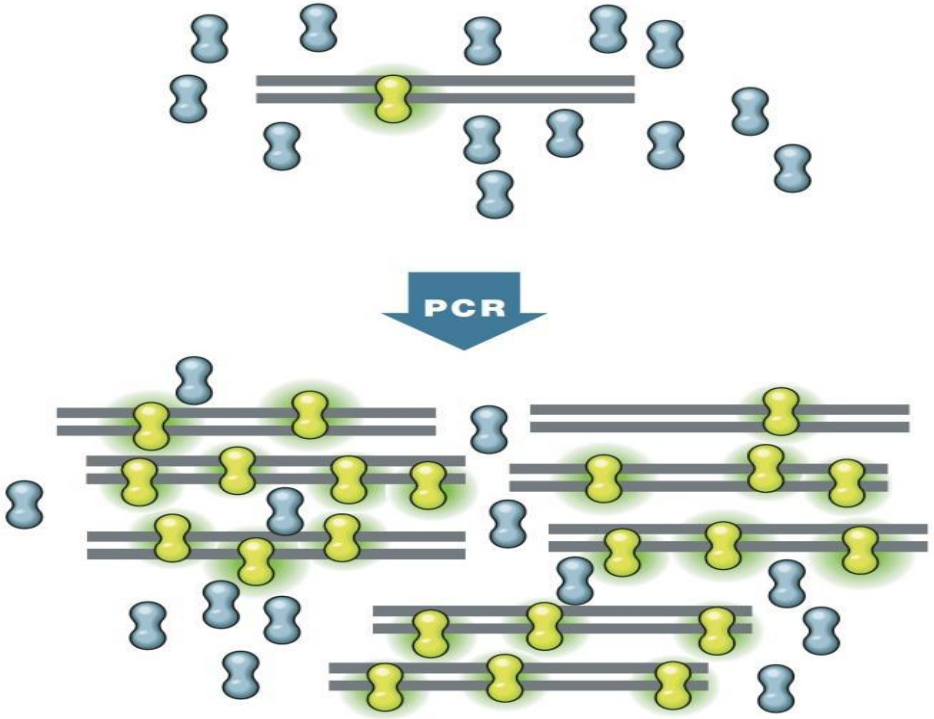
-I/D elevated level of ACE in blood (191 and 491bp→two lines)

-D/D significantly elevated level of ACE in blood (2x 191bp→one line)

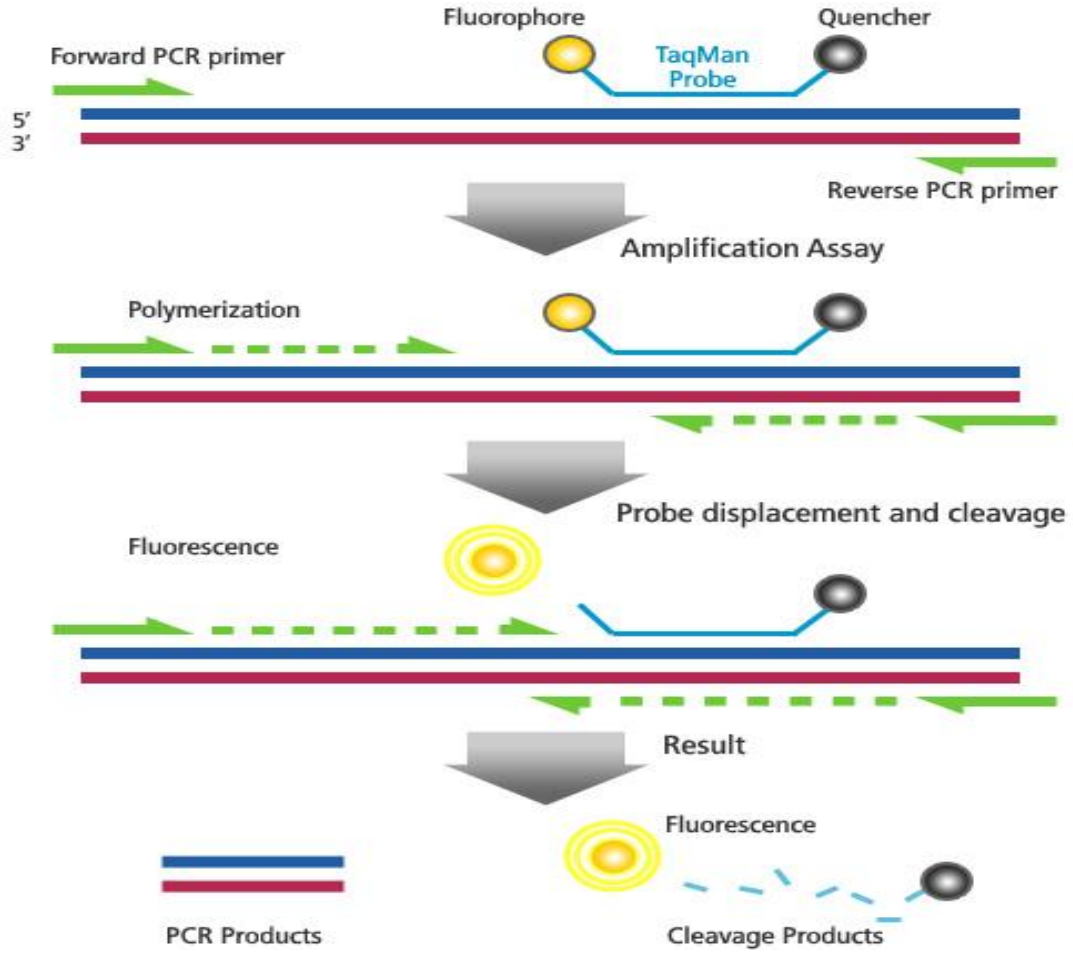
REAL TIME PCR:

- The main principle of real time PCR is the detection of the PCR product as it accumulates.

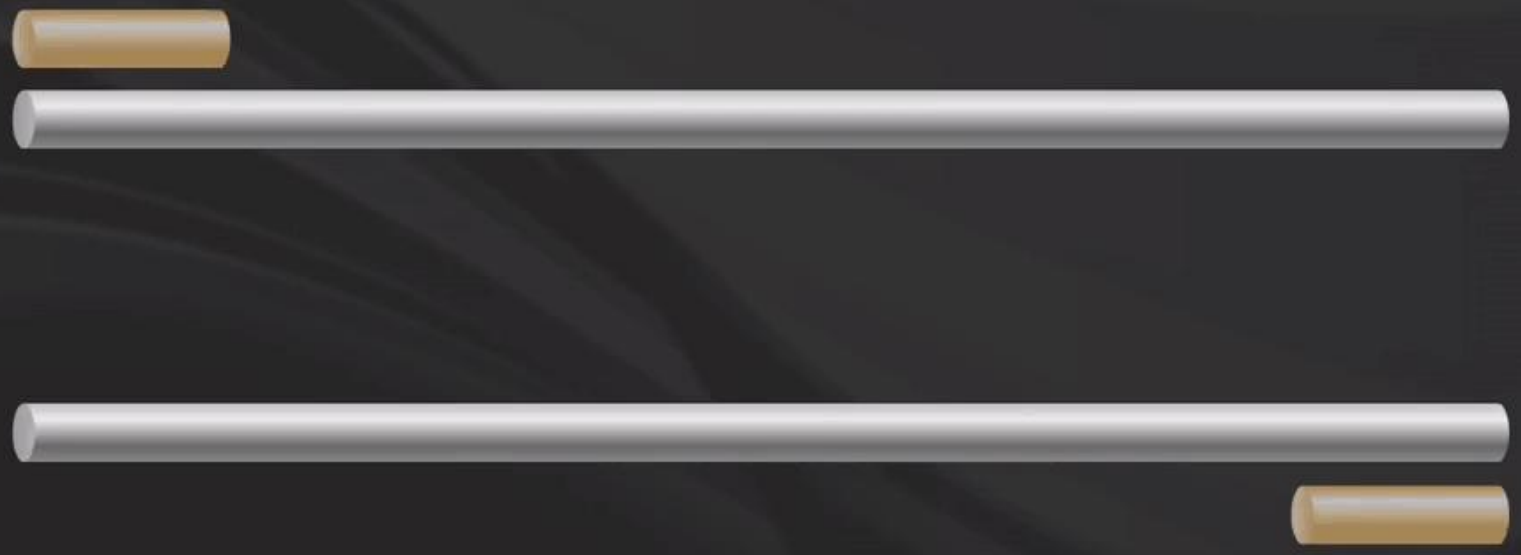
SYBR GREEN



TAQMAN PROBES

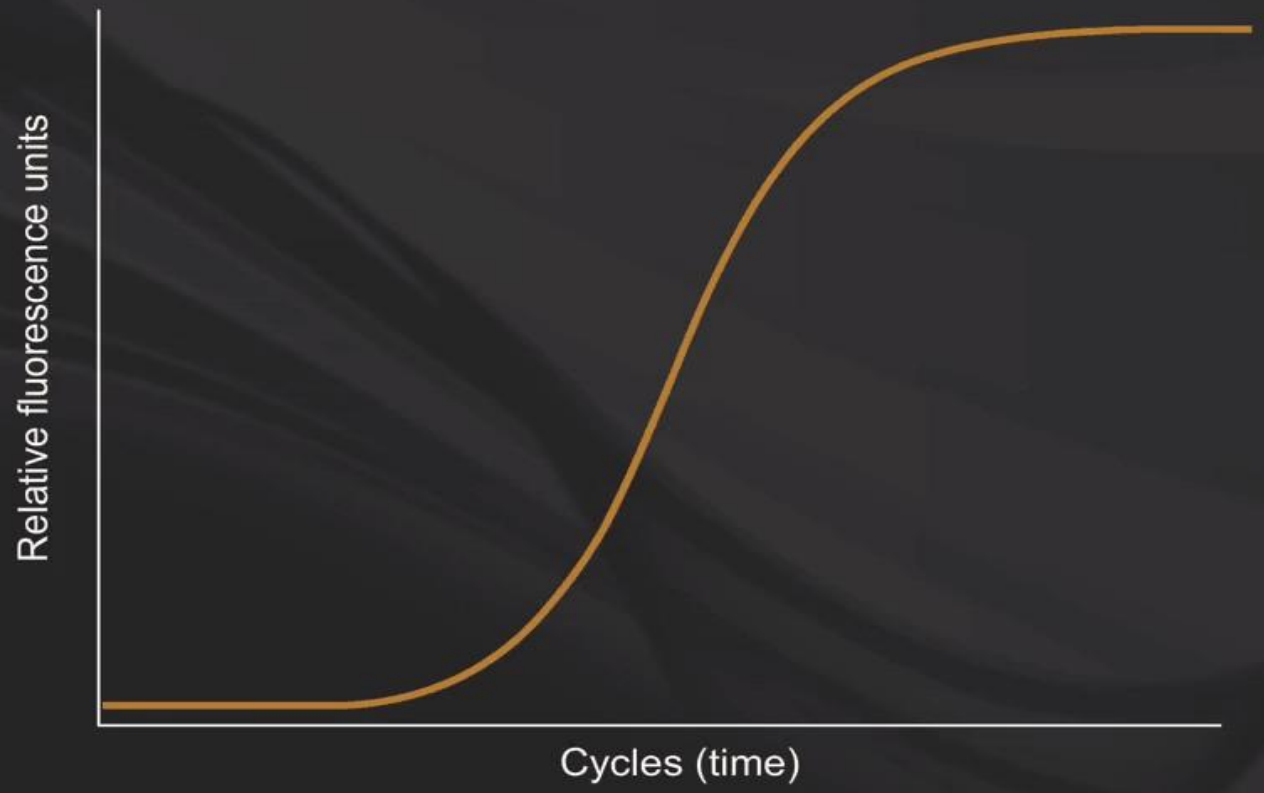


TaqMan assays



qPCR curve

qPCR curve

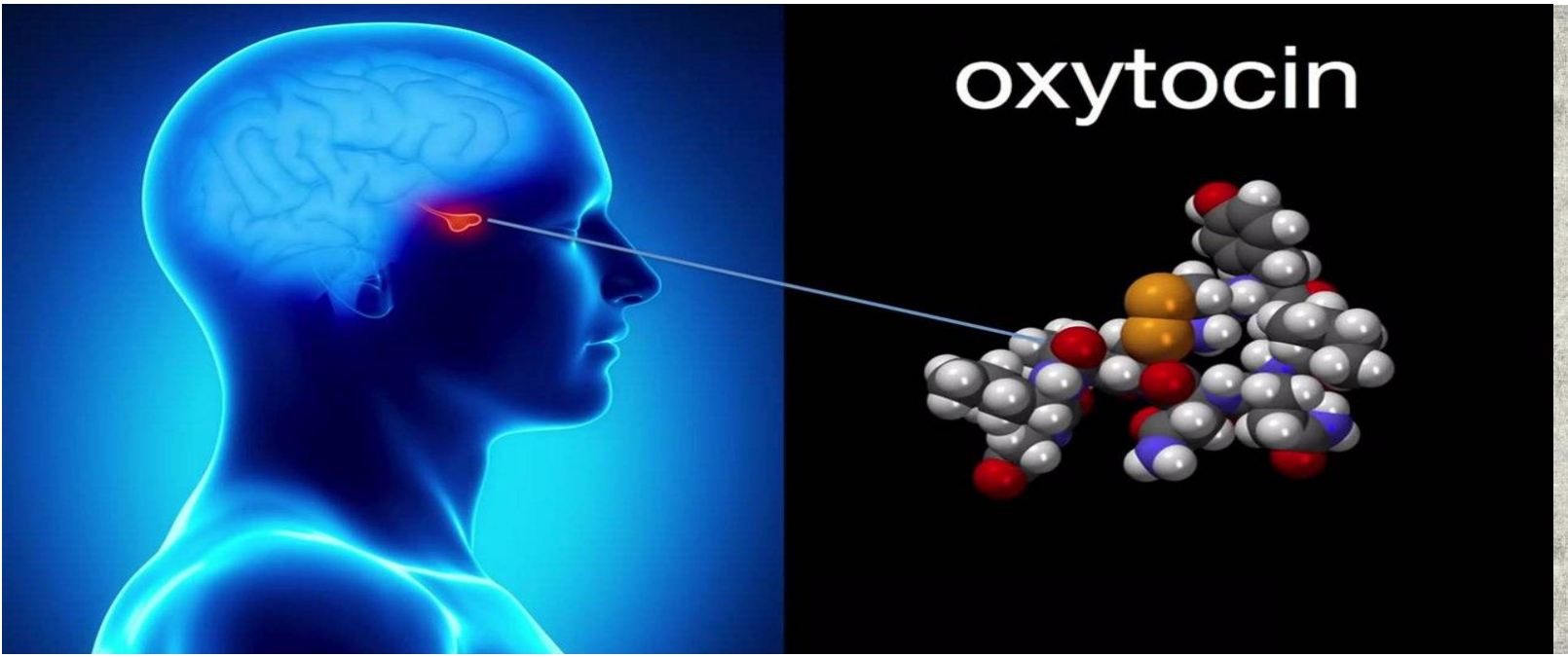


ALLELIC DISCRIMINATION

- It allows to identify SNP in a specific place in a specific gene. The method uses real-time PCR with a TaqMan probe that contains the dyes ROX and FAM.

ALLELIC DISCRIMINATION

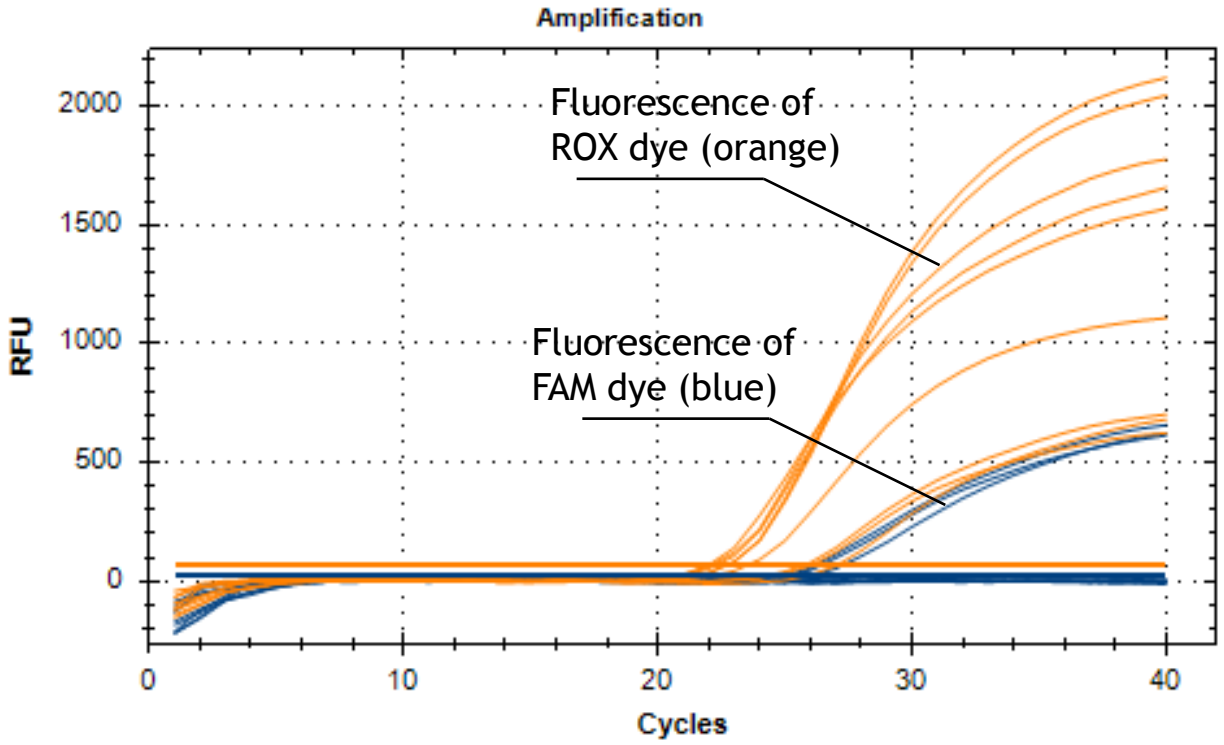
- The different variants of the OXTR gene corresponds to the psychological profile of a person: how resistant it is to stress, whether it is easy to be depressed, and ect .

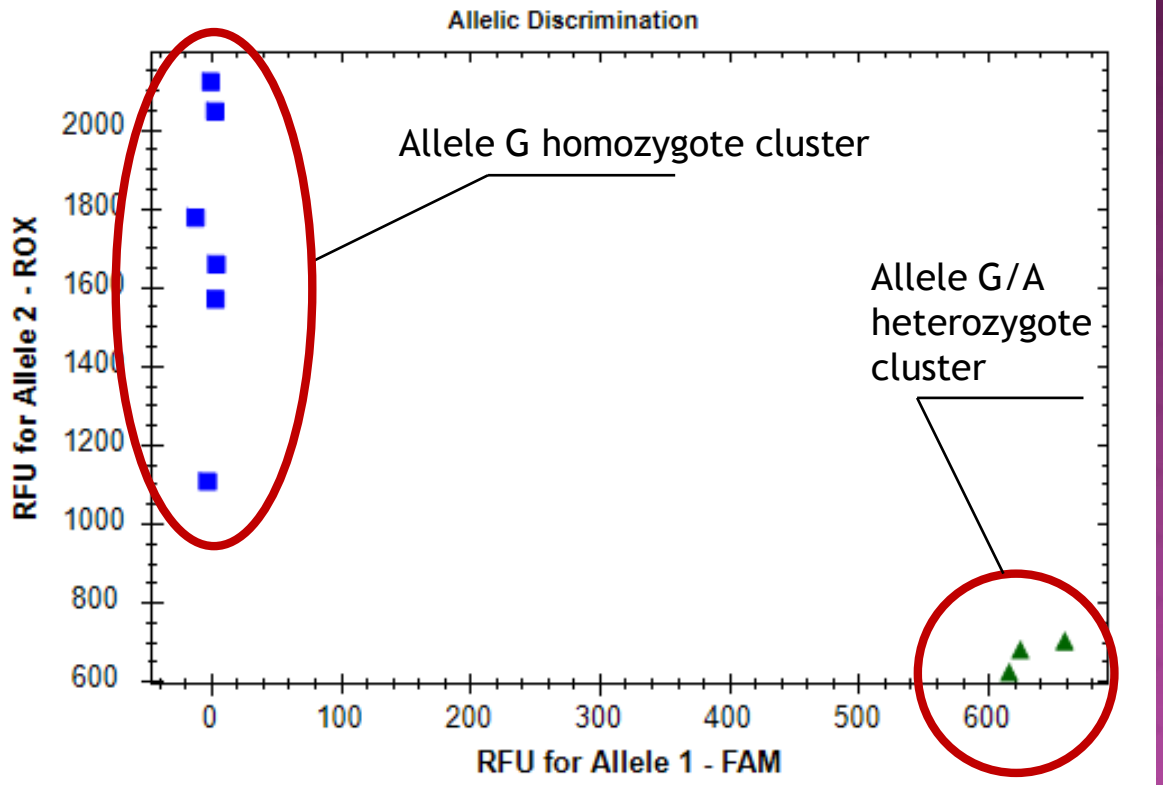


ALLELIC DISCRIMINATION OF G/A ALLELE IN OXTR GENE (3P25)

- Goal: to analyze the rs5376 single nucleotide polymorphism in OXTR gene using quantitative real-time PCR.
- Fluorescent reporter - TaqMan probe, which contains 2 dyes:
  - A-allele – FAM dye,
  - G-allele - ROX dye.

RESULTS OF ALLELIC DISCRIMINATION OF G/A ALLELE IN OXTR GENE (3P25)





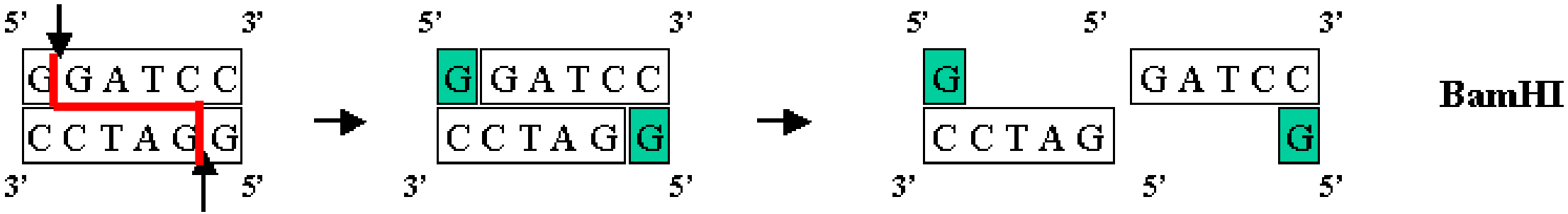
Frequency of G allele = 0.87

Frequency of A allele = 0.13

- People with G/G genotype were better able to discern the emotional state of others
- People with G/A allele are less empathetic, than individuals with G/G genotype

VERIFICATION OF ALLELIC DISCRIMINATION RESULTS WITH RESTRICTION ENDONUCLEASES DIGESTION

- Restriction enzymes “scans” a DNA molecule, looking for a particular sequence. Once it finds the recognition sequence, it stops and cuts the strands.
- BamHI restriction enzyme recognize the palindromic GGATCC sequence.

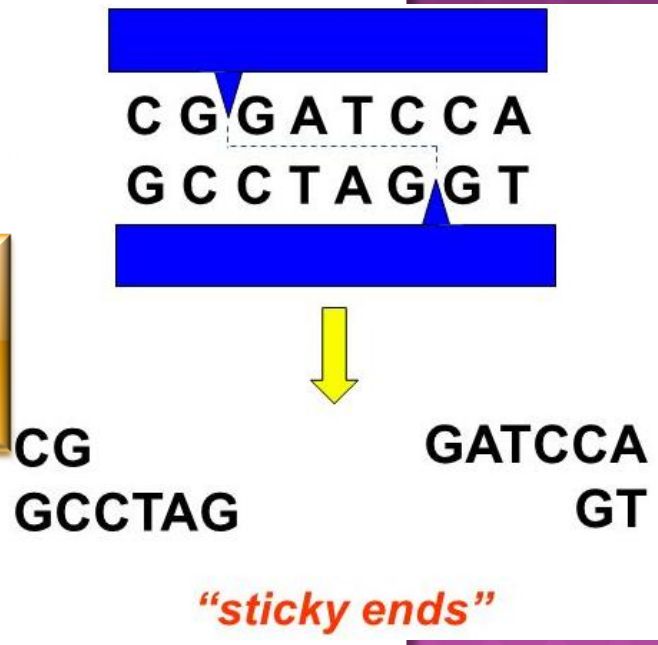


VERIFICATION OF ALLELIC DISCRIMINATION RESULTS WITH RESTRICTION ENDONUCLEASES DIGESTION

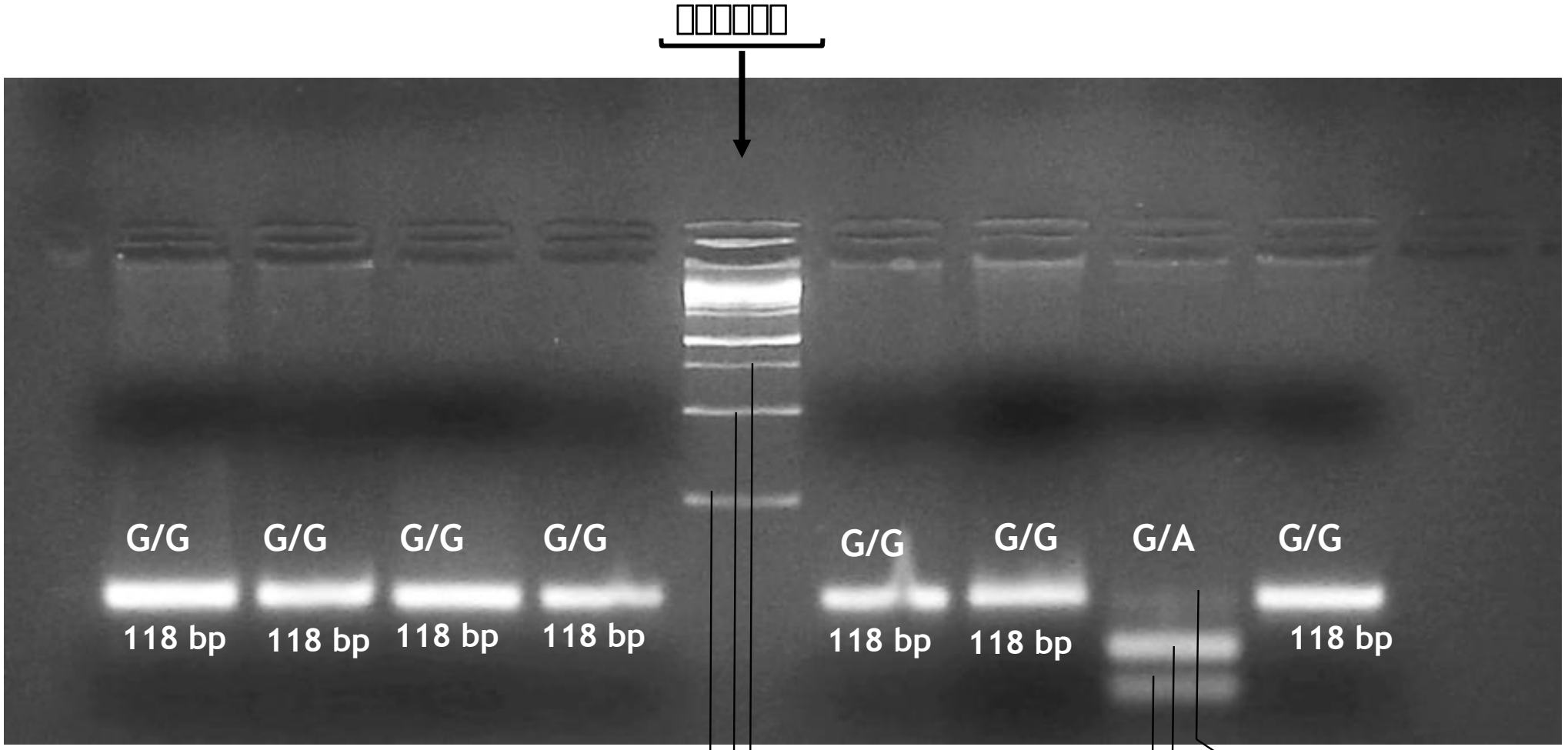
- Incubation the samples of amplified DNA with BamHI restriction enzyme:

- For A-OXTR allele – GGATCC – BamHI recognition site exists
- For G-OXTR allele – GGGTCC – BamHI recognition site is missing.

A/A genotype – 75 bp and 43 bp fragments
G/A genotype – 118 bp, 75 bp and 43 bp fragments
G/G genotype – 118 bp fragment



VERIFICATION OF ALLELIC DISCRIMINATION RESULTS WITH RESTRICTION ENDONUCLEASES DIGESTION



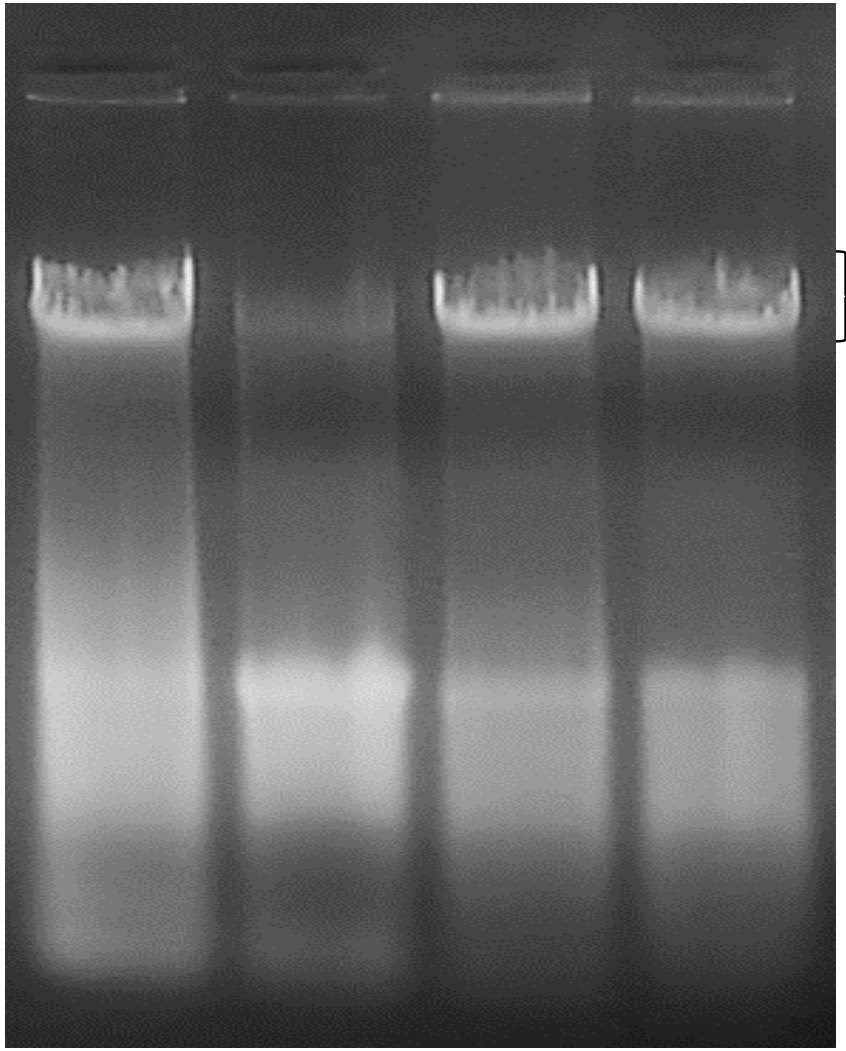
Frequency of G allele = 0.87

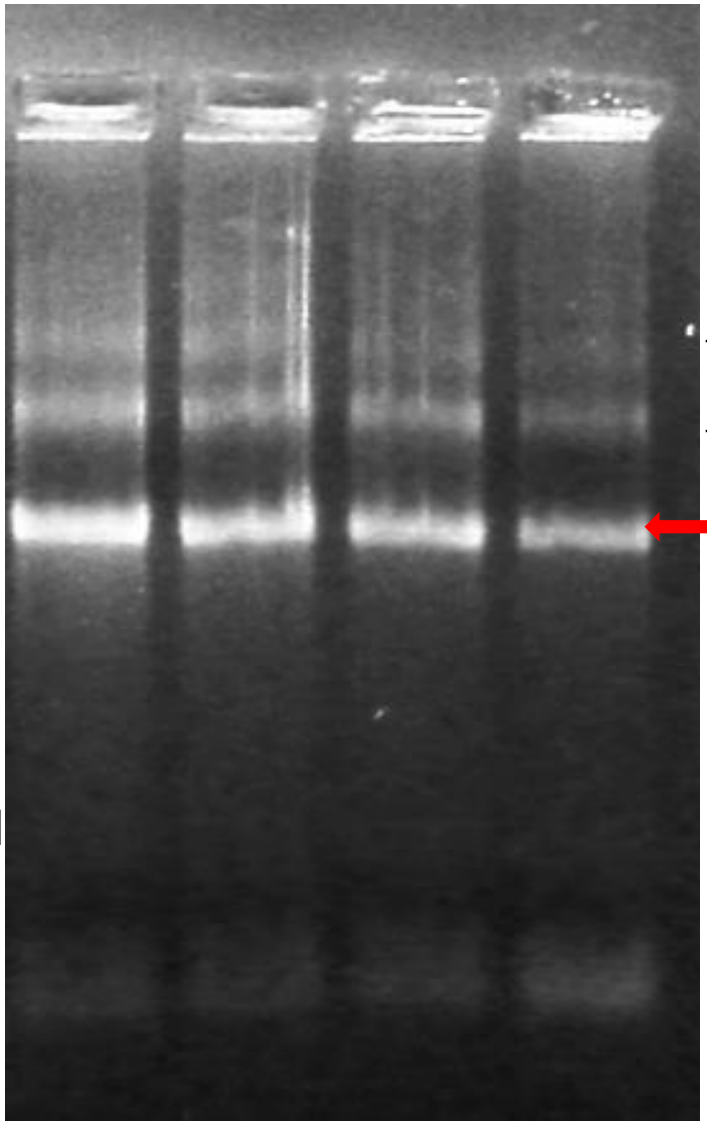
Frequency of A allele = 0.13

EXPERIMENTS WITH *DROSOPHILA MELANOGASTER*

- We obtained the DNA and RNA extraction from larva and flies (adult stage of development) of *Drosophila melanogaster* .
- Then we see information about presence of this material on electrophoresis.

D. MELANOGASTER DNA AND RNA ELECTROPHORESIS





MEASUREMENT OF RNA CONCENTRATION

- RNA obtained from *Drosophila* larvae and flies
- nanophotometer Implen NP80
- 1µl of sample

$$\frac{\mathbf{A260}}{\mathbf{A280}} > 2 \rightarrow pure\ RNA$$

Sample Name	Concentration (ng /µl)	A260/A280
Blank 1	0.0000	0.000
Sample 1	748.84	2.287
Sample 2	594.24	2.135
Sample 3	931.68	2.154
Sample 4	1128.8	2.429

- Samples 1,3 and 4 were diluted to the same concentration as the Sample 2.
- RT-PCR – the same concentration of RNA in each sample
- Aim: to see expression of *yellow* gene and housekeeping gene

REVERSE TRANSCRIPTION

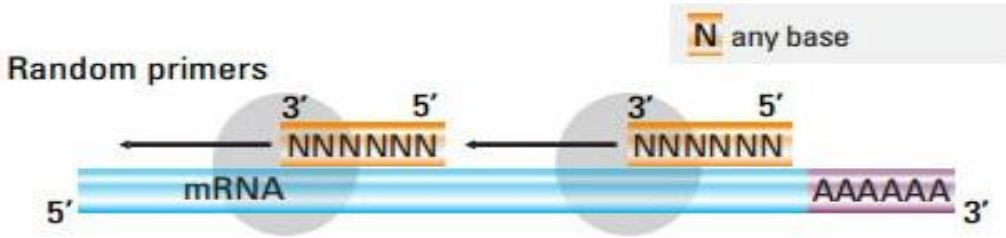
- PCR: RNA → DNA

- Enzyme :reverse transcriptase - convert RNA sequence to complementary DNA (cDNA)

❖RT-PCR Quantitative analysis of mRNA

-RNA → cDNA →PCR amplification

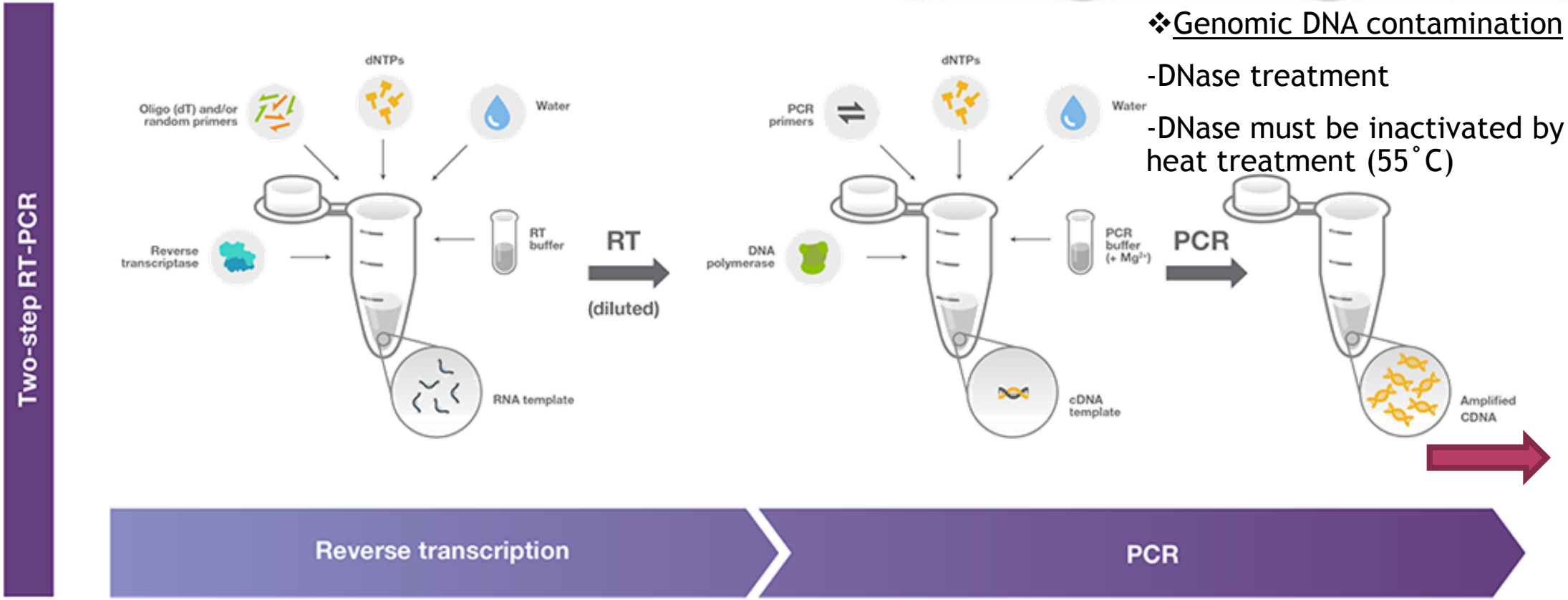
-Levels of amplified cDNA are measured by fluorescence (SYBR Green)



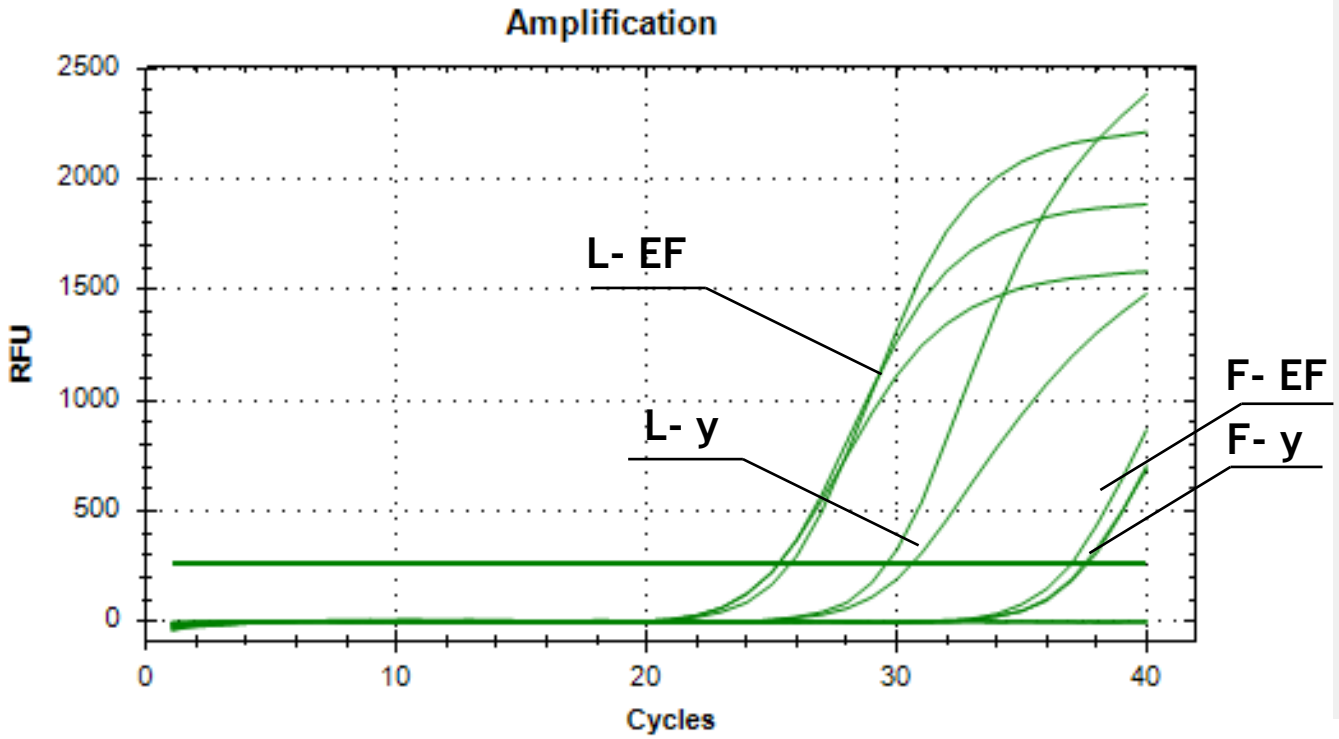
❖Genomic DNA contamination

-DNase treatment

-DNase must be inactivated by heat treatment (55˚C)



QUANTITATIVE RT-PCR OF *YELLOW* GENE OF *DROSOPHILA MELANOGASTER*



Well	Fluor	Target	Content	Sample	Cq
A10	SYBR		Neg Ctrl		N/A
B10	SYBR		Unkn		N/A
C04	SYBR		Unkn		30,64
D04	SYBR		Unkn		29,62
D10	SYBR		Unkn		37,66
E04	SYBR		Neg Ctrl		N/A
F04	SYBR		Unkn		25,31
F10	SYBR		Unkn		37,55
G04	SYBR		Unkn		25,77
G10	SYBR		Unkn		37,03
H04	SYBR		Unkn		25,31

L- EF - Larva *elongation factor* gene
F- EF - Fly (imago*) elongation factor* gene
L-y - Larva *yellow* gene
F-y - Fly(imago) *yellow* gene

- Expression of larvae *yellow* gene is lower in 10 times compared to the expression of larvae *EF* gene.
- Expression of larvae *yellow* and *EF* genes is higher in 1000 times compared to imago (adult flies)

CONCLUSION

- PCR and qRT-PCR is widely used in medicine, molecular biology and genetics.

We have demonstrated this using the example of a study:

1. Genomic identification and determination of predisposition to several diseases.
2. Allelic discrimination of SNPs.
3. Estimation of the expression levels of wild-type and radiation-induced mutant genes.


Thank you for your attention!