



4<sup>th</sup> stage of international student practice from  
EGYPT, December 2019

**Joint Institute for Nuclear Research (JINR)**



*Genome “dactyloscopy” DNA finger-printing  
&  
gene expression PCR and RT-PCR in action*

*Dzhelepov Laboratory of Nuclear Problems (DLNP), JINR*

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# Project plan

## Part (1)

### *DNA fingerprinting in Homo sapiens*

- *Conventional PCR for detection of dimorphic mutations (Deletion / Insertion) in the following genes :*

1. *TPA-25.*

2. *ZFPM-2.*

3. *XPR-1.*

4. *ACE .*

5. *CCR-5 (HIV-Resistance )*

6. *RHD (Rhesus factor)*

**Cardiovascular  
diseases tests**

- *Real time-PCR (TaqMan probes) for detection of polymorphic mutations (SNP) in:*

1. *OXR gene (Oxytocin test system).*

2. *FCER-1A gene (Allergy test system):*

- *Alrg-1 (For rs2251746).*

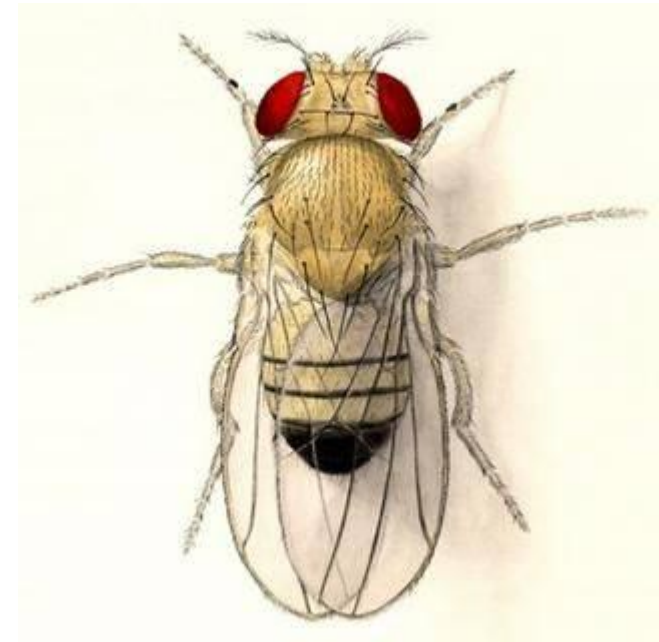
- *Alrg-2 (For rs2427837).*

- *Sanger sequencing of mitochondrial DNA (mtDNA).*

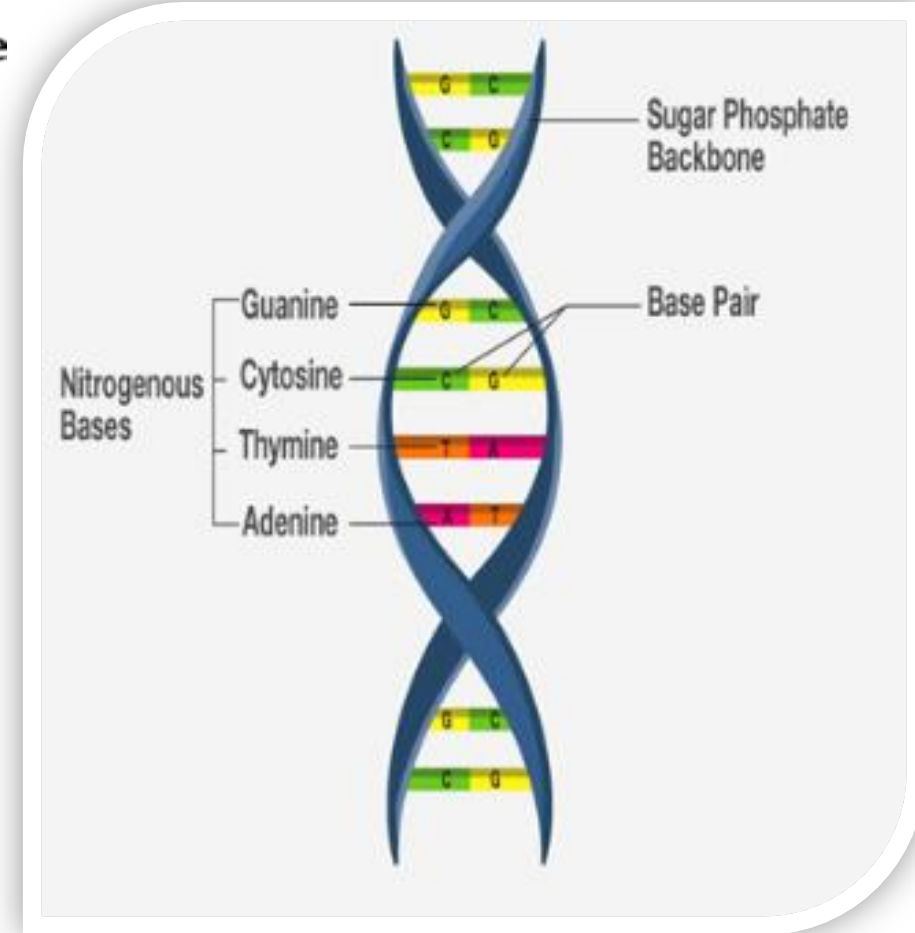
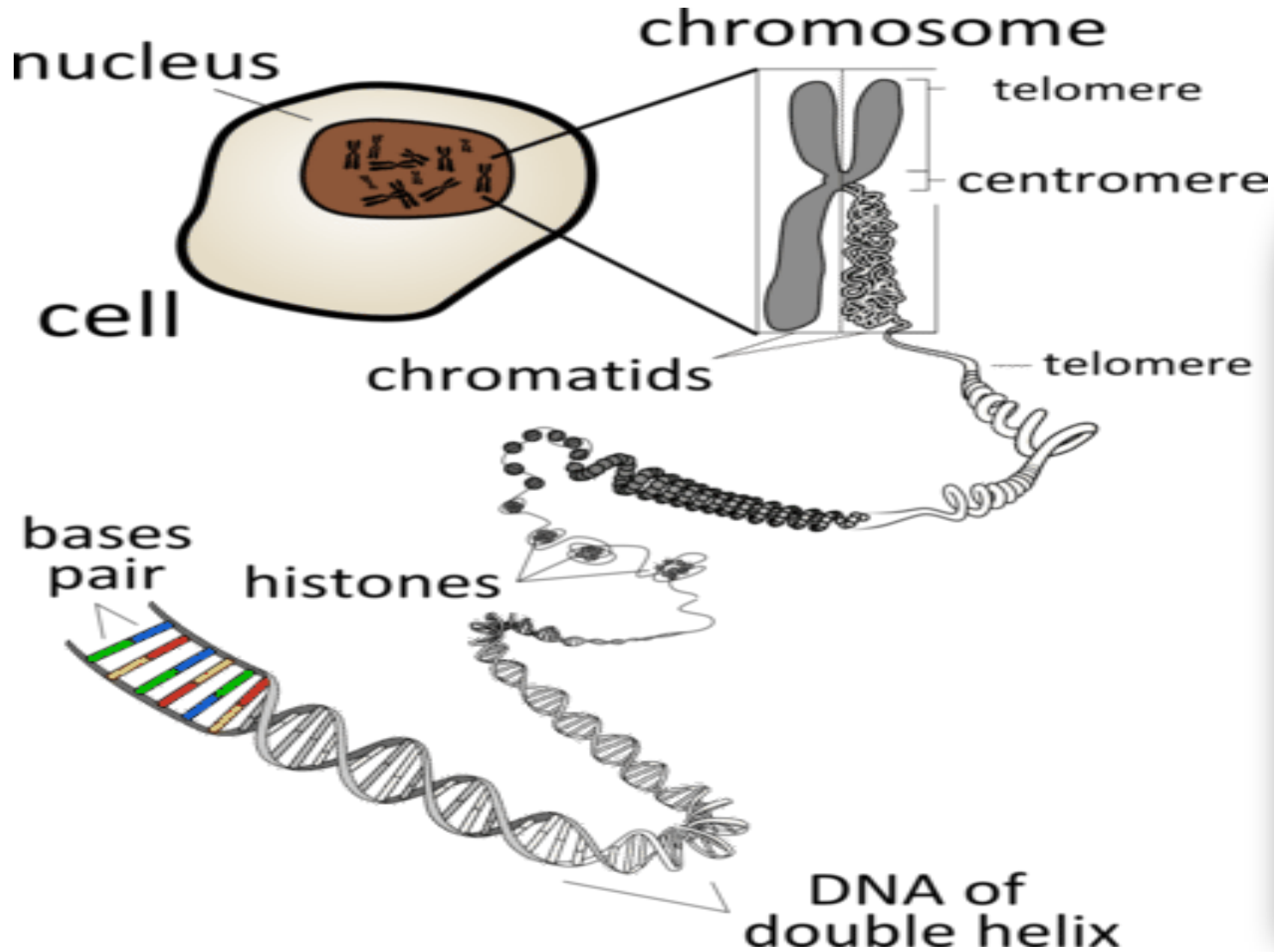
## Part (2)

### *Gene expression analysis in Drosophila melanogaster*

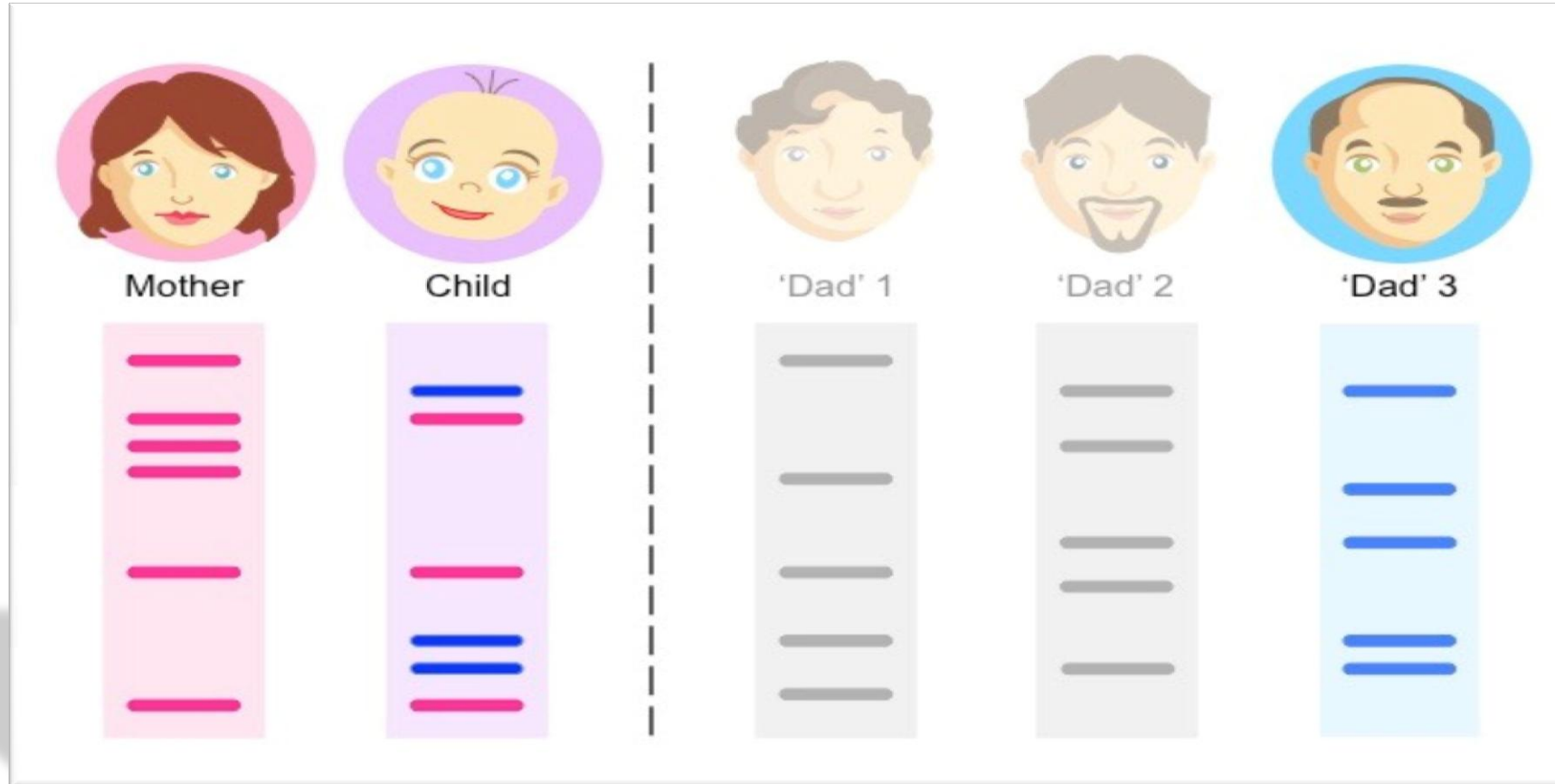
- *Drosophila eye color gene.*



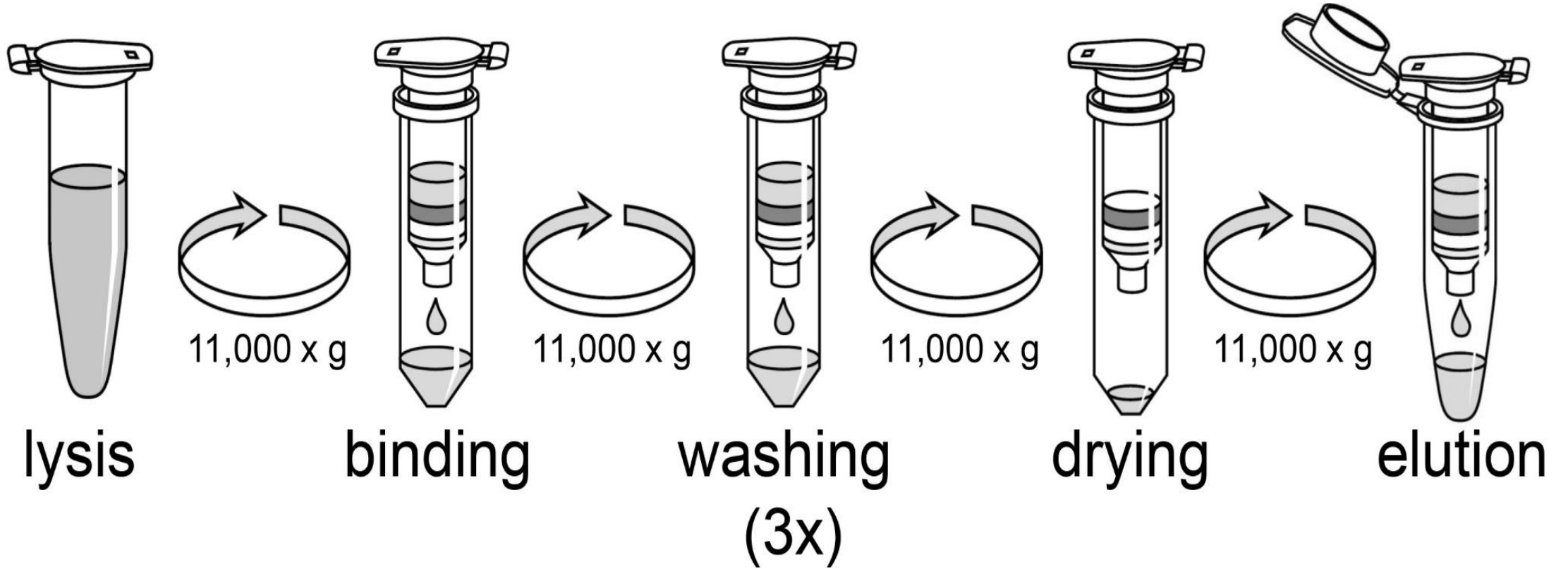
# Chromosome and Gene



# DNA fingerprinting



# DNA Extraction



# ***DNA Concentration and Purity***

Sample No	Extraction method			
	Chelex		DIAGENE Kit	
	Concentration	Purity	Concentration	Purity
<b>1</b>	56.900	1.262	15.150	1.870
<b>2</b>	71.800	1.108	138.25	1.779
<b>3</b>	60.750	1.020	4.350	1.800
<b>4</b>	64.400	1.115	7.500	2.00
<b>Mean</b>	63.46	1.13	41.31	1.86
<b>S.D.</b>	9.56	0.10	64.78	0.099
<b>Mean ± S.D.</b>	63.46 ± 9.56	1.13 ± 0.10	41.31 ± 64.78	1.86 ± 0.099



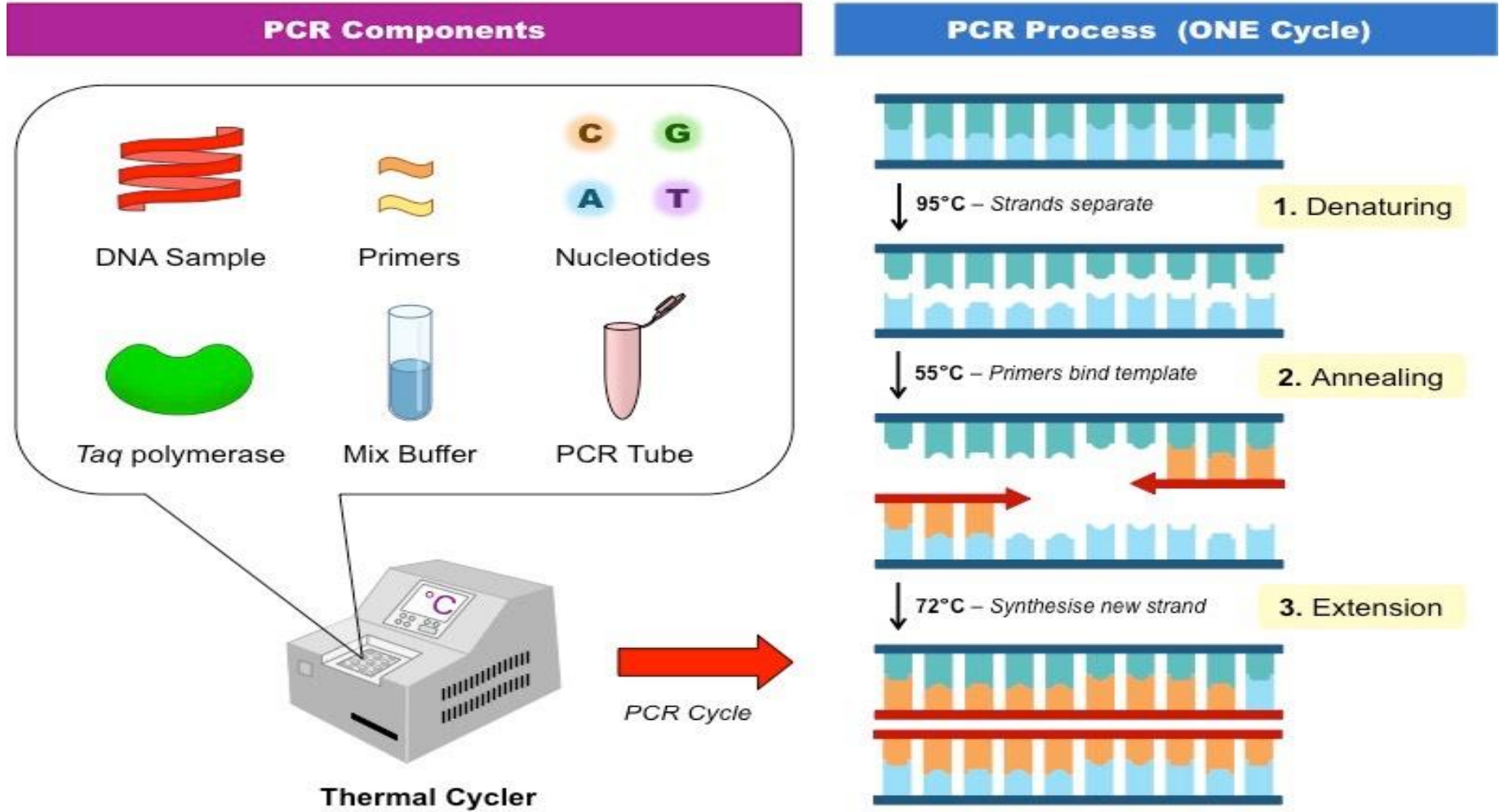
# Conventional PCR

Conventional PCR

Gel Electrophoresis

SNPs  
by qPCR

Sequencing





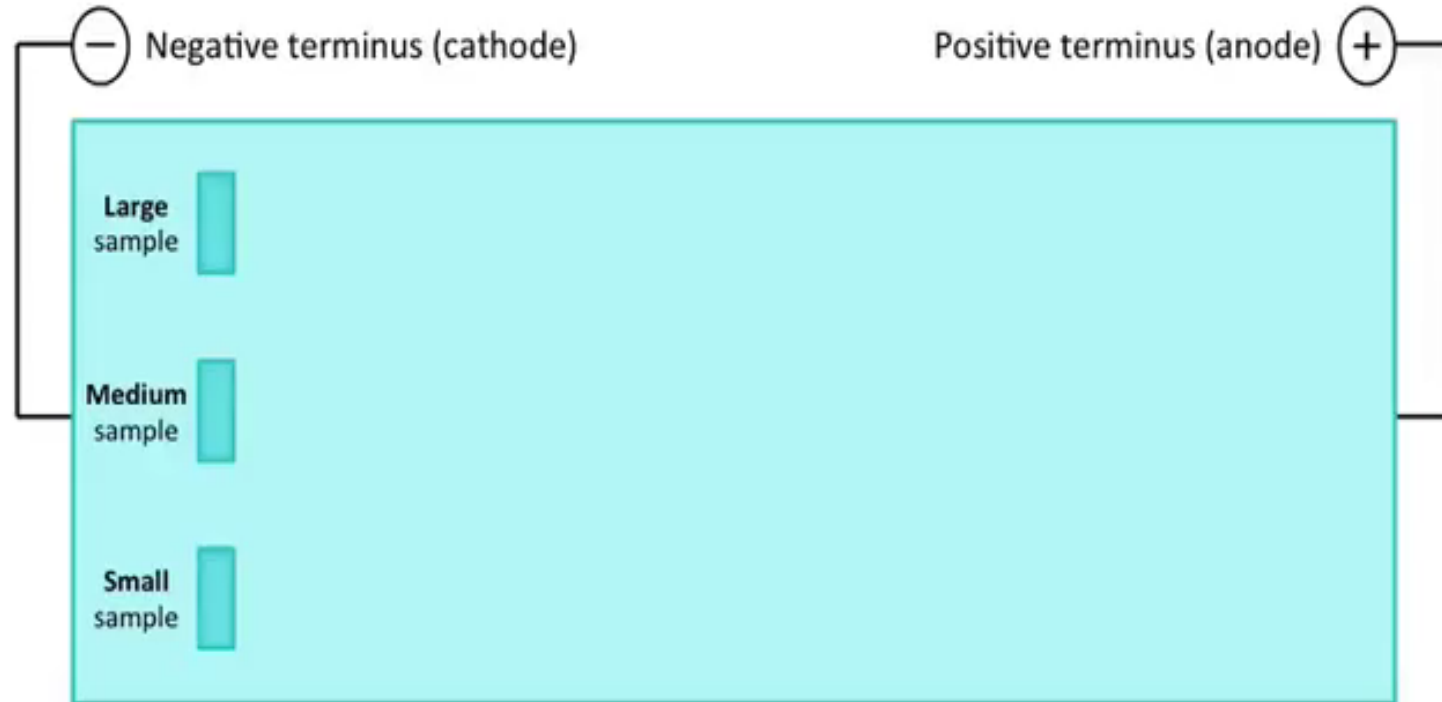
# Agarose Gel Electrophoresis

Conventional PCR

Gel Electrophoresis

SNPs  
by qPCR



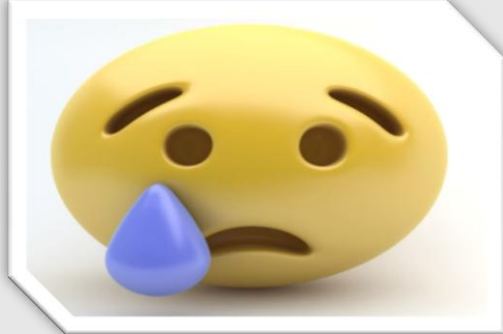
Sequencing



# TPA-25 *Alu* sequence

**Chromosomal location:** 8p11.21

**Function:** A fibrinolytic enzyme involved in breakdown of intravascular blood clots.

<b>Homozygous</b>	<b>Heterozygous</b>	<b>Homozygous</b>
<b>D – D</b>	<b>D – I</b>	<b>I – I</b>
<b>Single band 112 bp</b>	<b>Two bands 112 bp + 412 bp</b>	<b>Single band 412 bp</b>
		

# Clinical relevance of TPA-25 gene dimorphic mutation

Presence of *Alu* element

Associated with 50 % increase in risk of MI




So there is an association between the number of *Alu* repeats and arterial thrombosis

# ZPFM-2 *Alu* sequence

**Chromosomal location:** 8p23.1

**Function:** Important regulators of hematopoiesis and cardiogenesis in mammals.

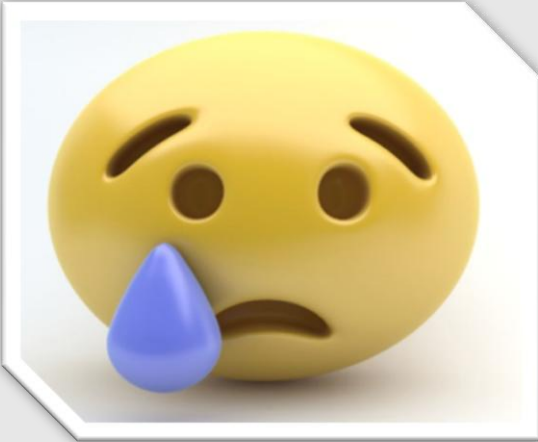
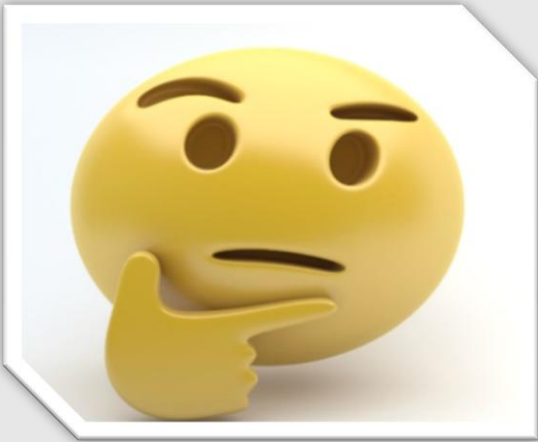

**Clinical relevance:** Mutations in genes encoding this transcription factor have been associated with cardiac septal defects.

Homozygous	Heterozygous	Homozygous
D – D	D – I	I – I
Single band 251 bp	Two bands 251 bp + 551 bp	Single band 551 bp
		

# ACE *Alu* sequence

**Chromosomal location:** 17q23.3

**Function:** Indirectly increases blood pressure by causing blood vessels to constrict.

Homozygous	Heterozygous	Homozygous
D – D	D – I	I – I
Single band 191 bp	Two bands 191 bp + 491 bp	Single band 491 bp
		

# Clinical relevance of ACE gene dimorphic mutation

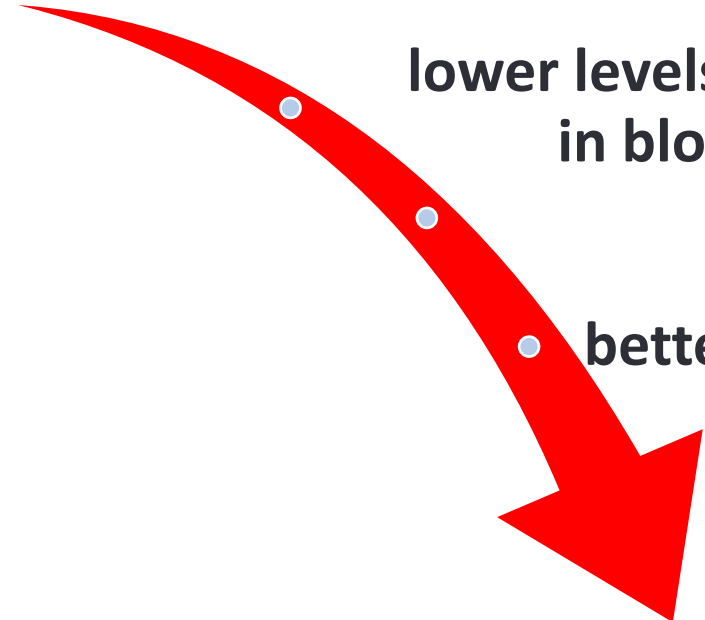
## ACE (Angiotensin I converting enzyme)

Presence of *Alu* sequence  
in ACE gene

lower levels of ACE  
in blood

better vascular tone

less risk of developing  
hypertension





# Clinical relevance of ACE gene dimorphic mutation

Presence of the *Alu* element

Associated with better performance in endurance-oriented events (Triathlons)



Absence of the *Alu* element

Associated with strength- and power-oriented performance (Sprints)






# **XPR-1 *Alu* sequence**

**Chromosomal location:** 1q25.3

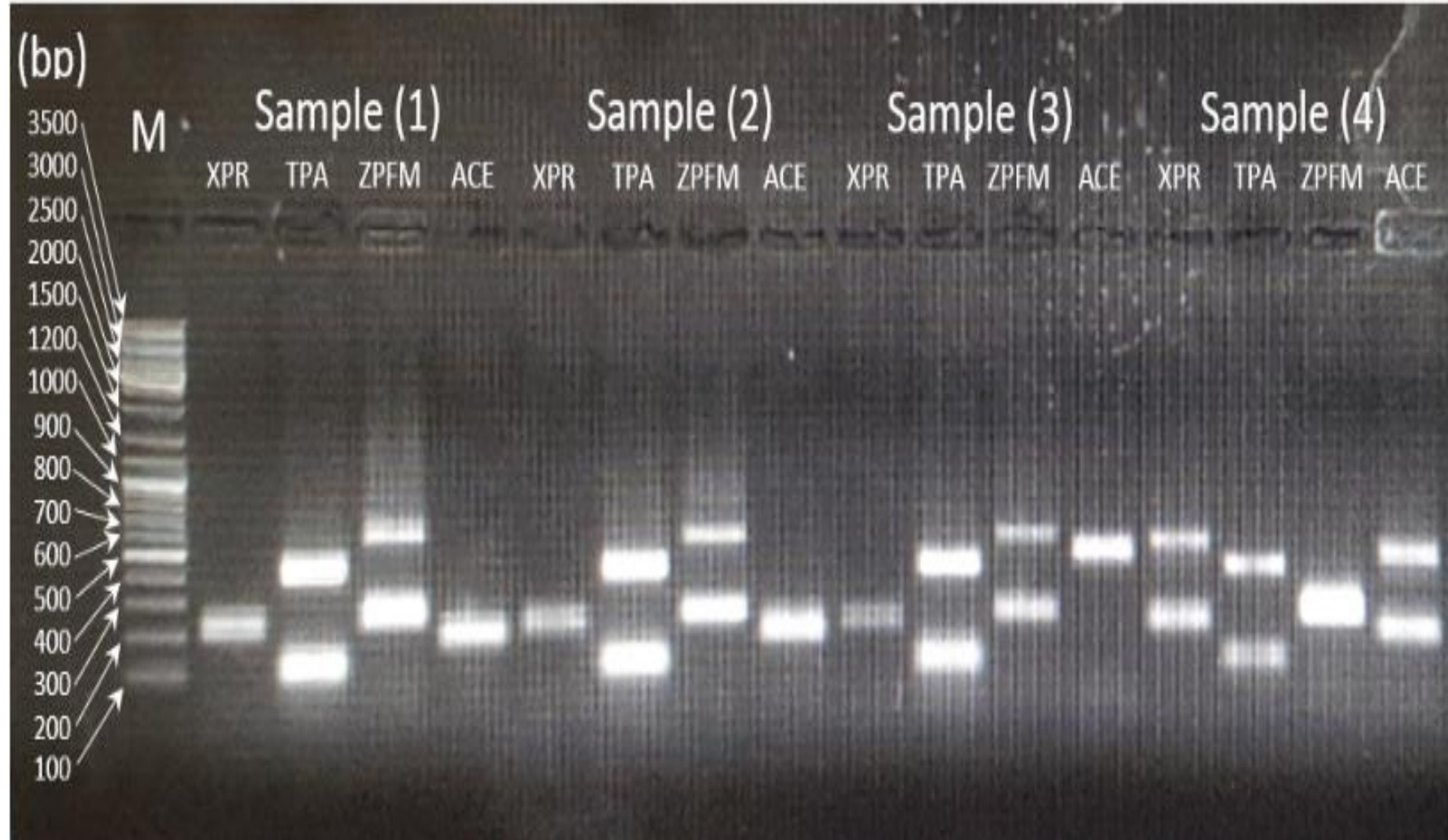
**Function:** Membrane protein plays a role in phosphate homeostasis. It mediates phosphate export from the cell.

**Clinical relevance:** Increased phosphate level (Hyperphosphatemia) results in adverse outcomes due to the direct effect of phosphate on vascular calcification and hence cardiovascular events.

# Clinical relevance of XPR-1 gene dimorphic mutation

-	<b>Homozygous</b>	<b>Heterozygous</b>	<b>Homozygous</b>
	<b>D – D</b>	<b>D – I</b>	<b>I – I</b>
	<b>Single band 223 bp</b>	<b>Two bands 223 bp + 523 bp</b>	<b>Single band 523 bp</b>
			

# *Alu* repeat for cardiovascular diseases



# Finger printing of *Alu* repeats for cardiovascular diseases

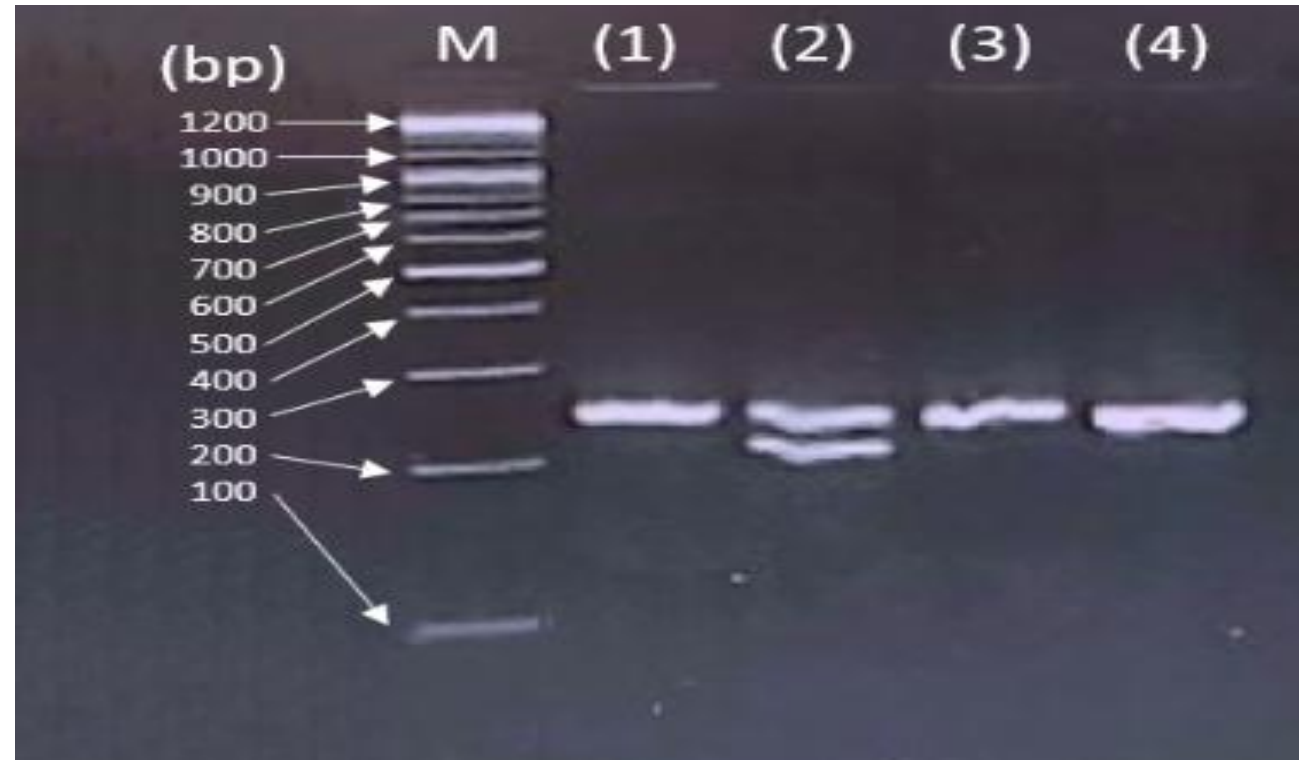
Sample No.	<i>Alu</i> Repeats											
	XPR-1			TPA-25			ZFPM-2			ACE		
PCR product (bp)	223	523	Genotype	112	425	Genotype	251	551	Genotype	191	491	Genotype
1	+	-	Homo	+	+	Hetero	+	+	Hetero		-	Homo
2	+	-	Homo	+	+	Hetero	+	+	Hetero		-	Homo
3	+	-	Homo	+	+	Hetero	+	+	Hetero	-		Homo mutant
4	+	+	Hetero	+	+	Hetero	+	-	Homo			Hetero

# CCR5 *Delta* sequence (HIV resistance gene)

- **Chromosomal location : 3p21.31**
- **Function : A chemokine receptors on the surface of WBCs.**
- **Clinical relevance of CCR-5 gene dimorphic mutation :**
  - **Homozygous carriers of this mutation do not express functional CCR-5 receptors on their cell surfaces and are resistant to HIV-1 infection.**
  - **Heterozygous individuals have a greater than 50 % reduction in functional CCR-5 receptors on their cell surfaces and hence exhibit reduced viral load.**



# CCR5 *Delta* sequence (HIV resistance gene)

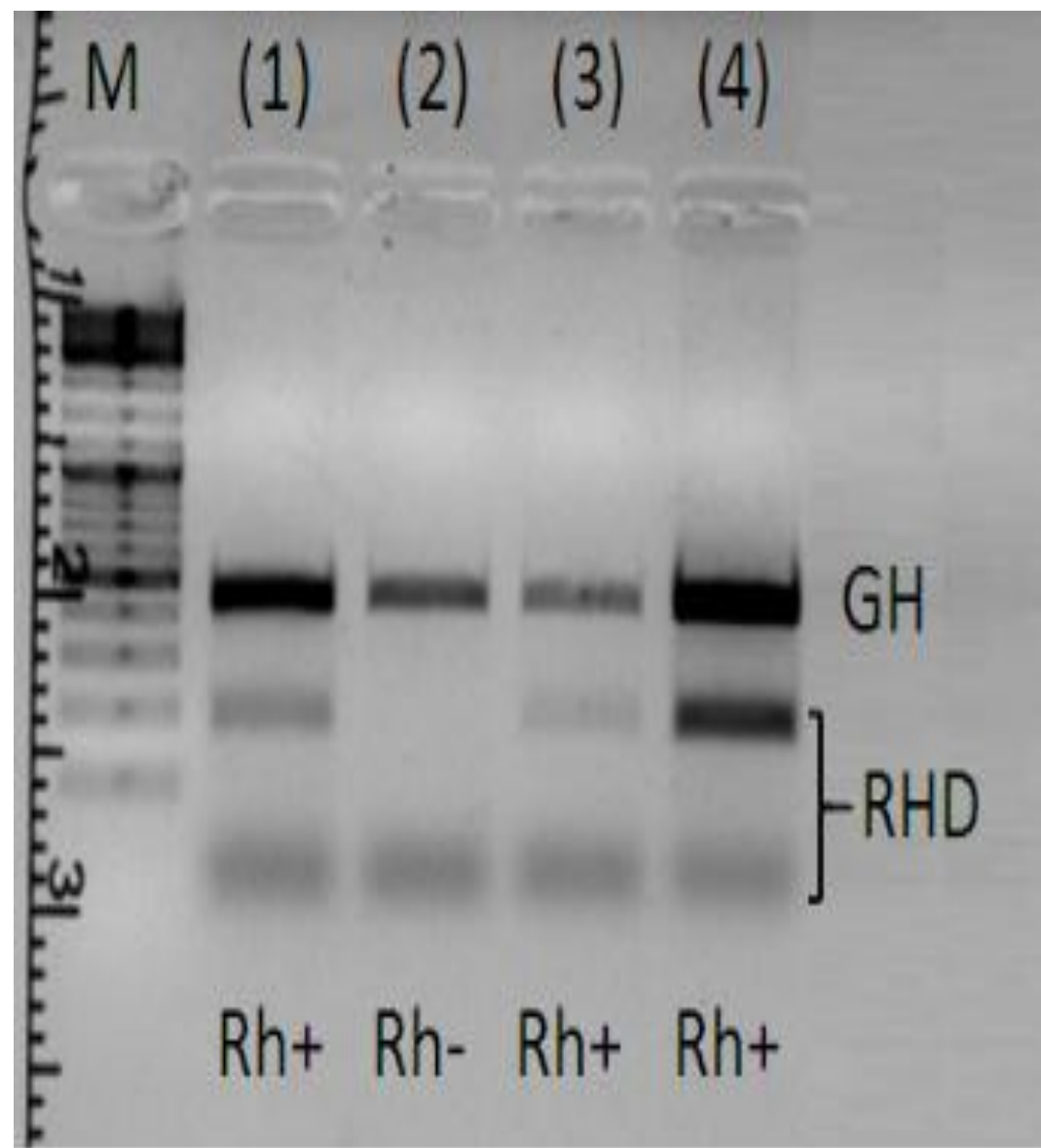
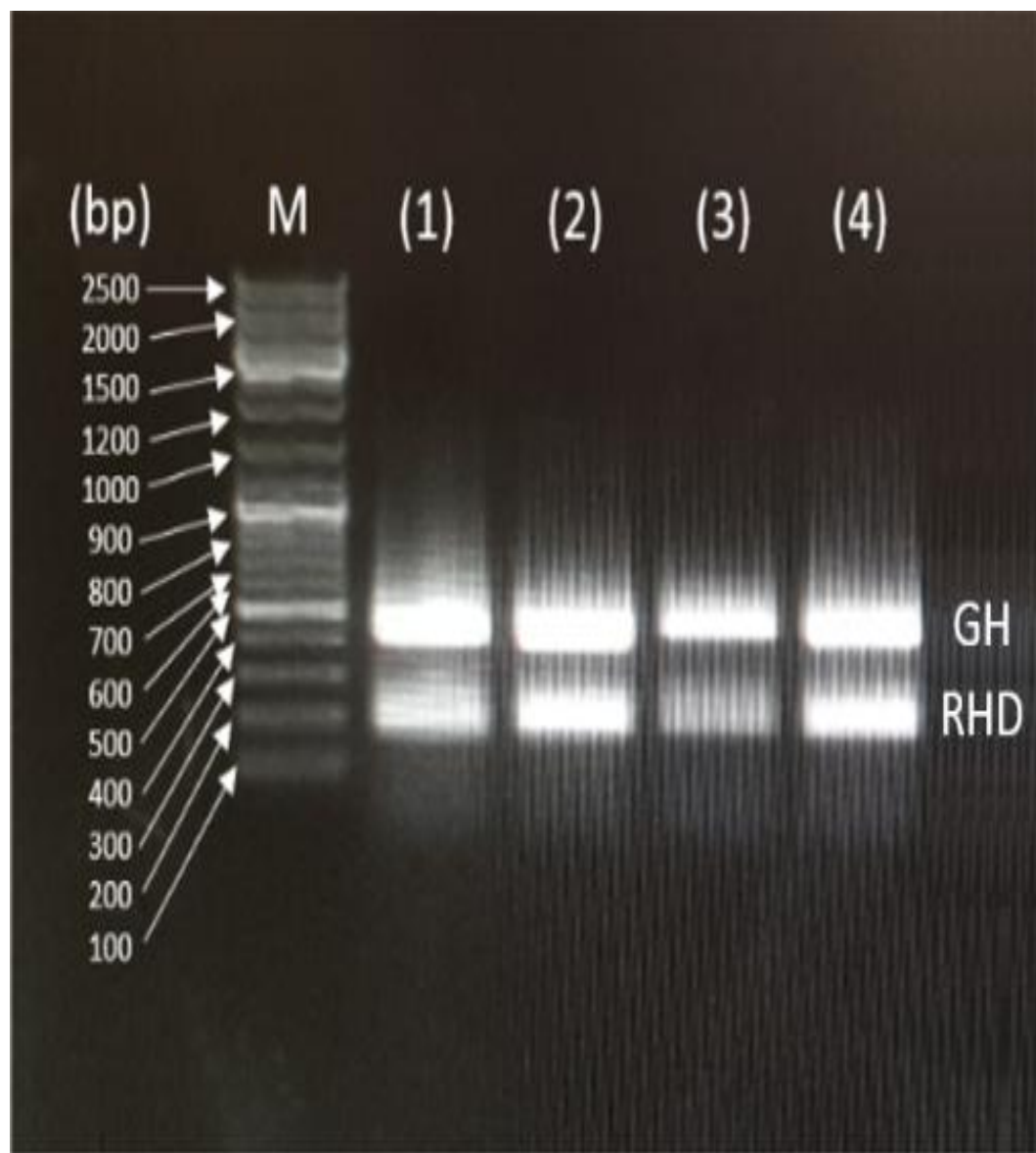


# RHD (Rh blood group-D antigen)

- **Chromosomal location : 1p36.11**
- **Function:** Rh family of proteins is involved in ammonia/ammonium transport.
- **Clinical relevance of RHD-sequence dimorphic mutation :**  
The Rh blood group system is the second in importance to ABO system in blood transfusion medicine for classification of individuals into Rh-positive and Rh-negative.

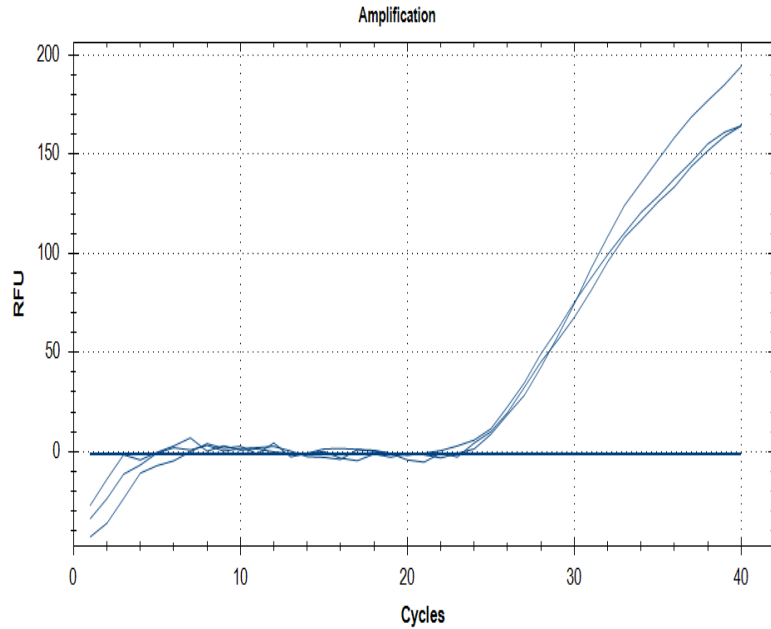
# RHD (Rh blood group-D antigen)

<i>RhD gene sequence (European population)</i>		
<b>Rh- negative individual</b>	<b>Rh-positive individual</b>	
<b>Homozygous</b>	<b>Heterozygous</b>	<b>Homozygous</b>
<b>D – D</b>	<b>D – I</b>	<b>I – I</b>
<b>No bands</b>	<b>Single band 200 bp</b>	<b>Single band 200 bp</b>

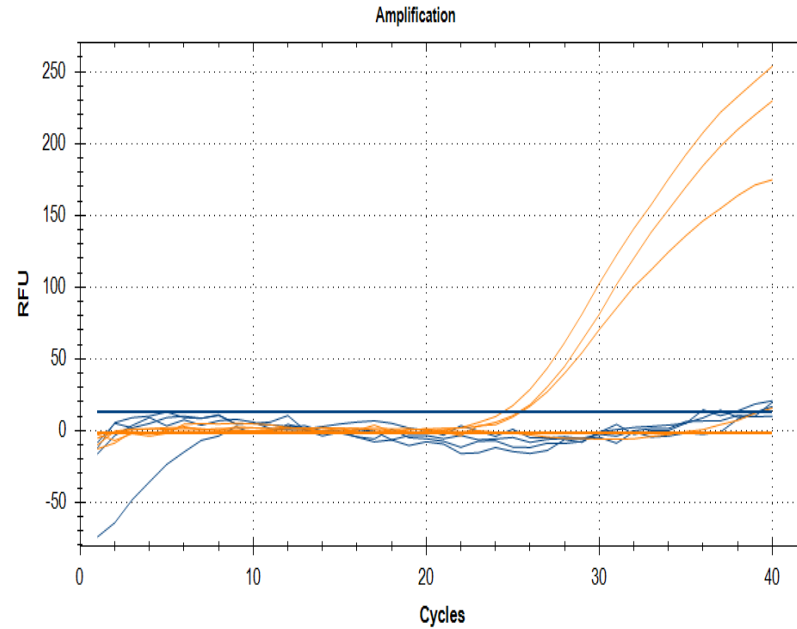


# Allelic discrimination with Taqman probes

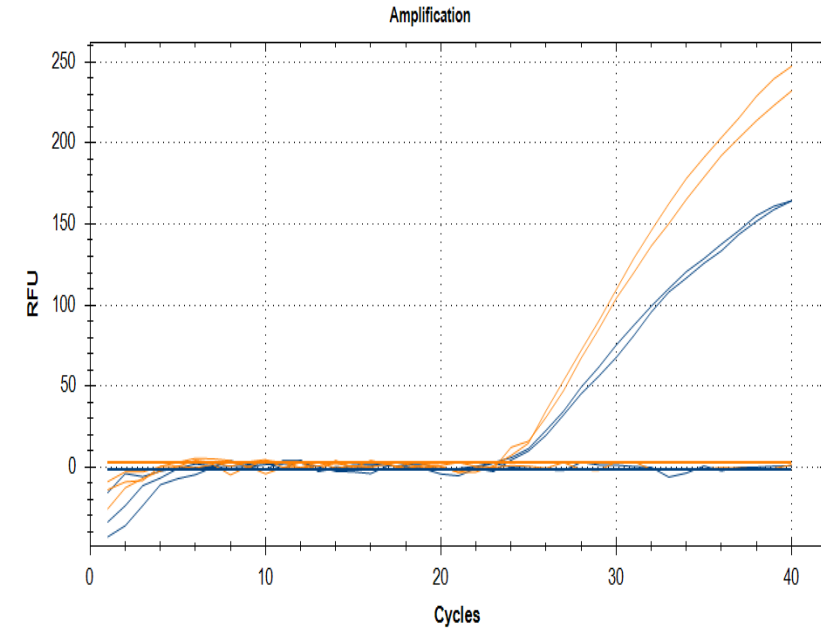
- The possibility of result of each sample may be



Homozygous type  
ROX



Homozygous type  
FAM



Heterozygous type  
FAM & ROX

# Real time-PCR for SNPs detection

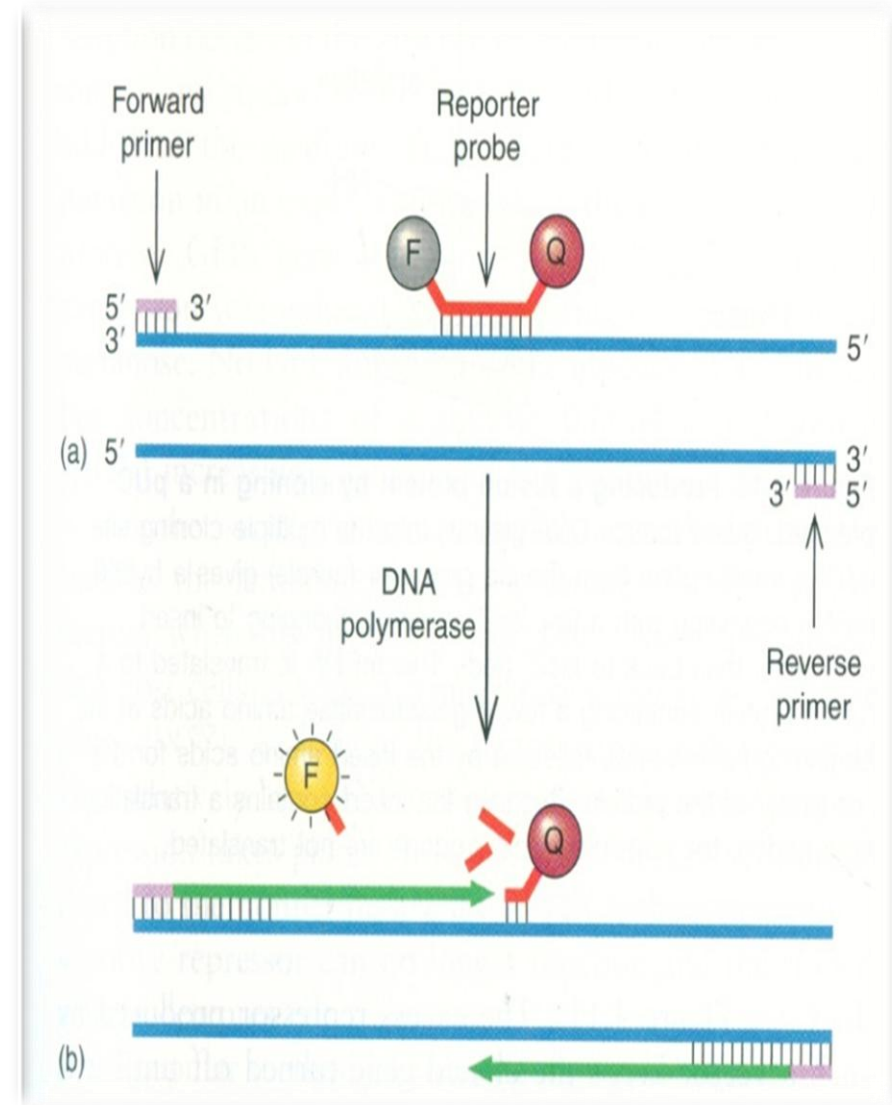
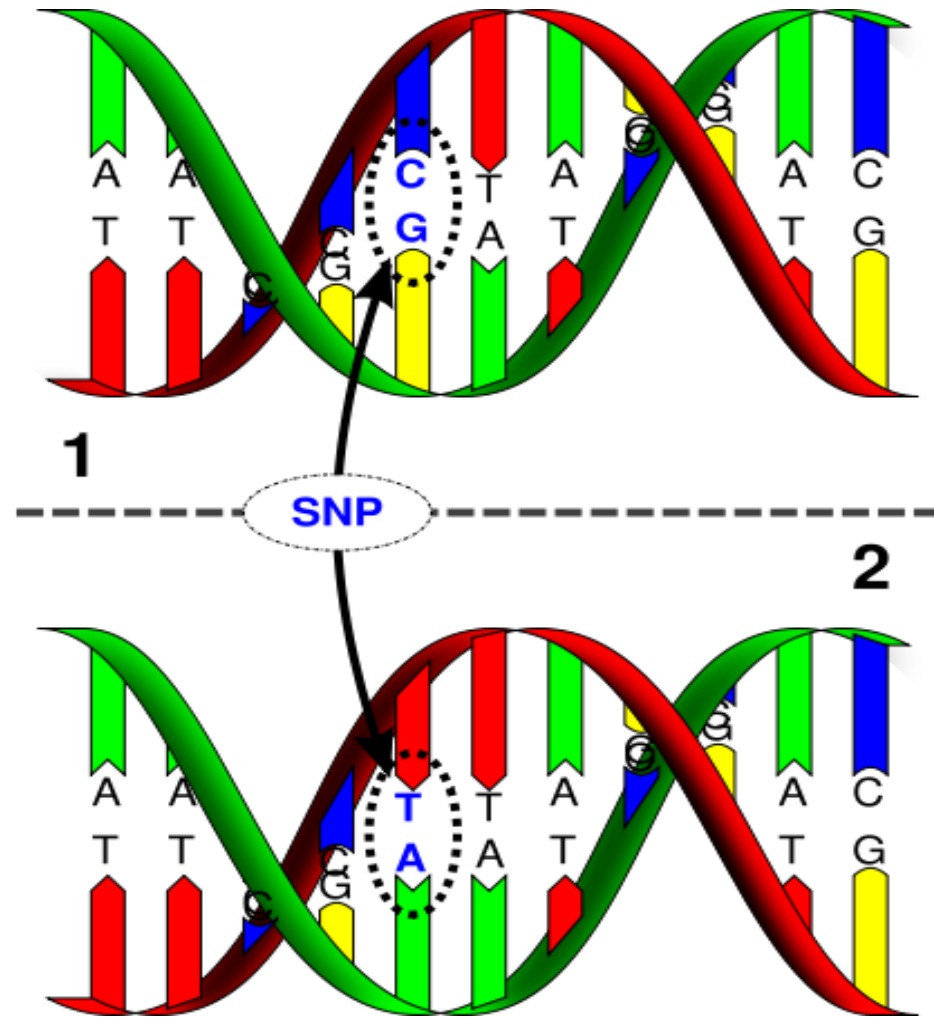
Conventional PCR

Gel Electrophoresis

SNPs

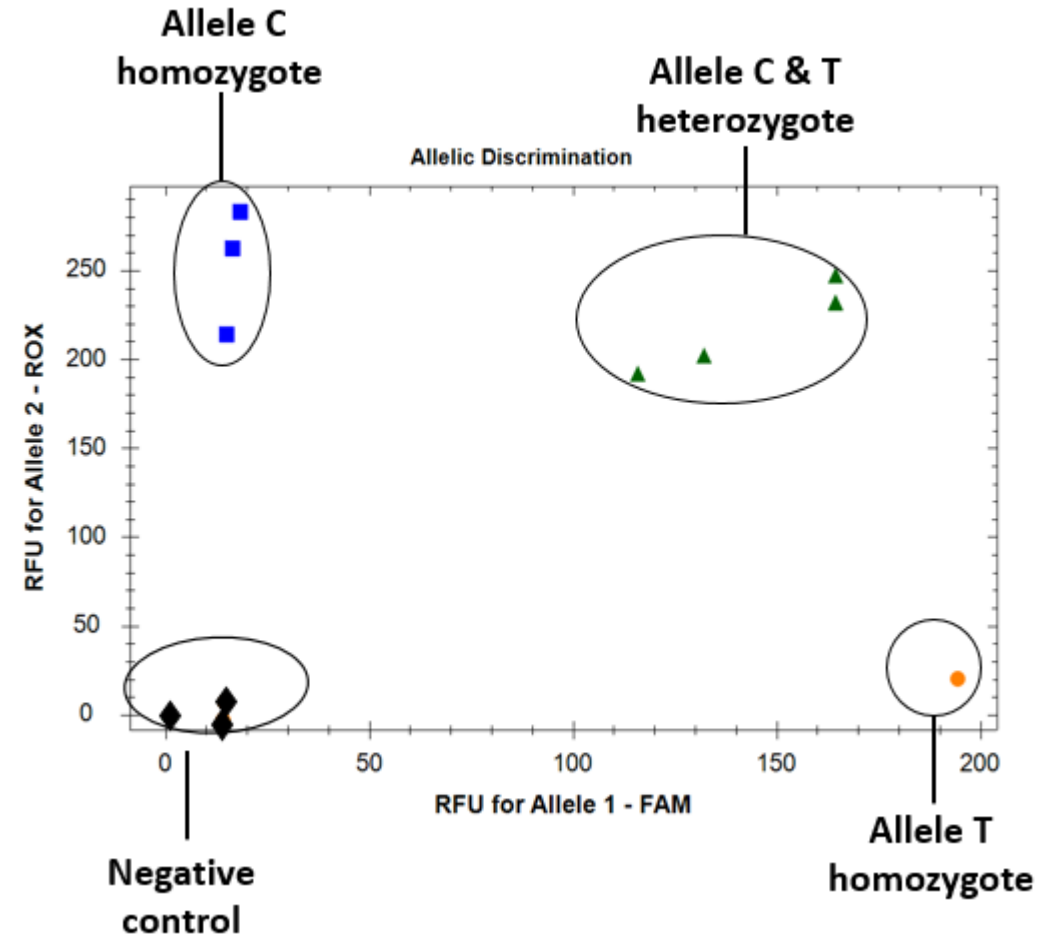
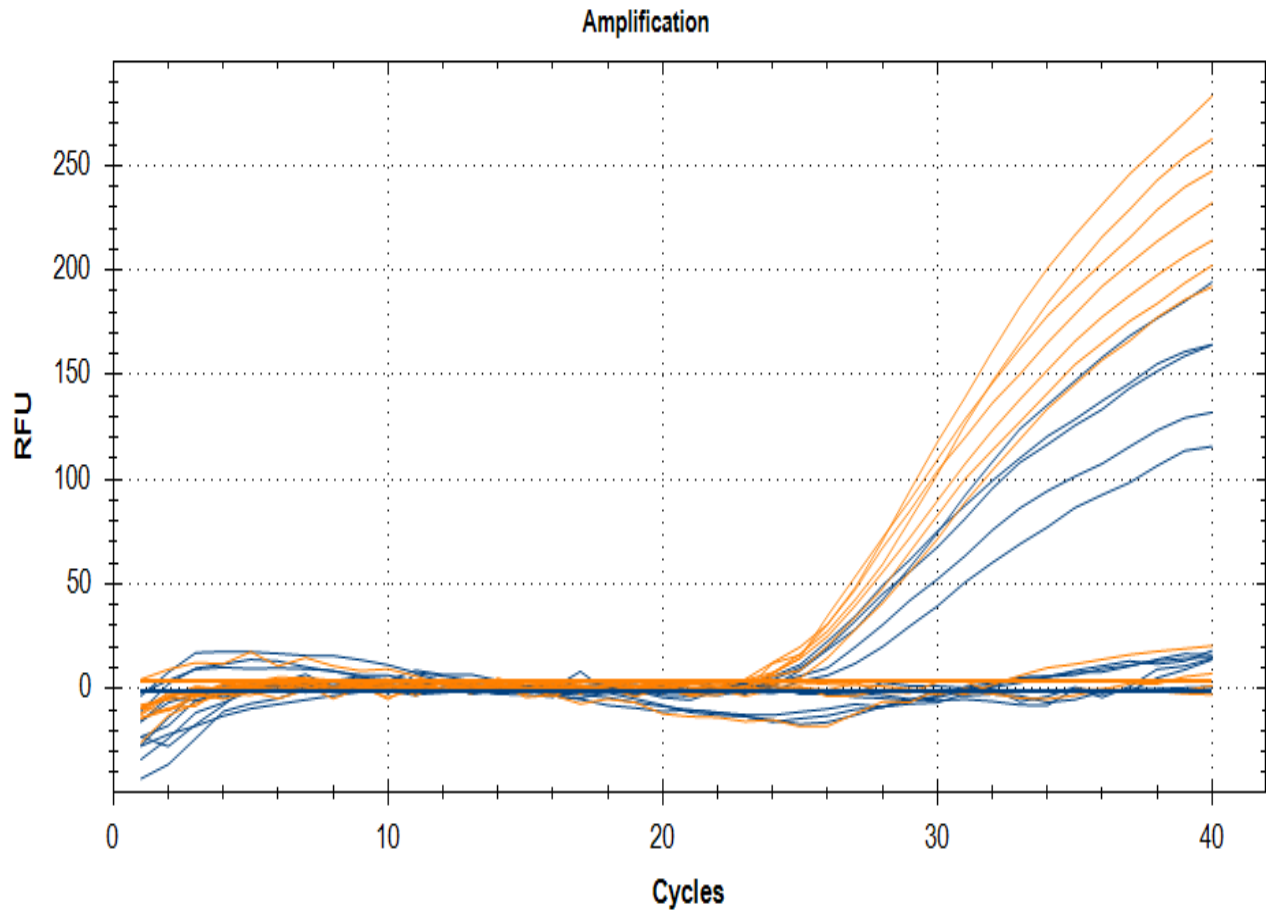
by qPCR

Sequencing

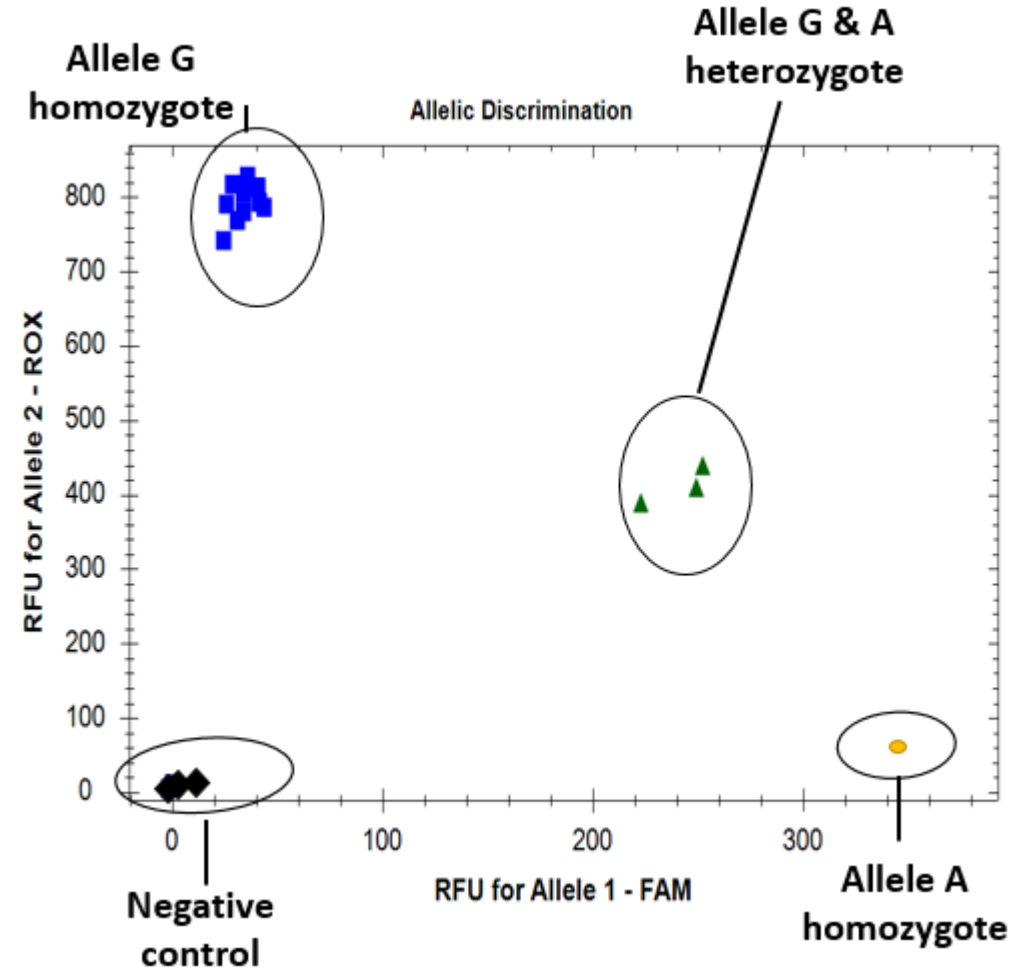
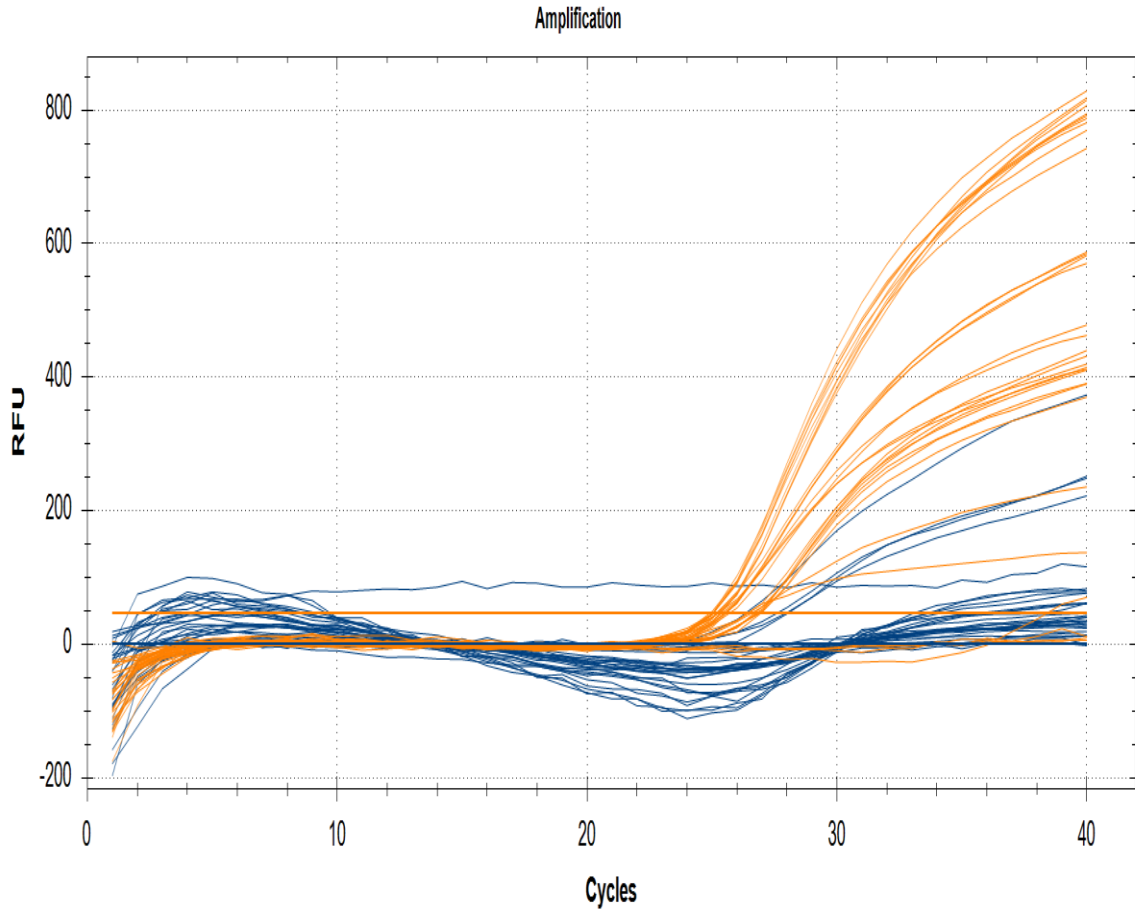




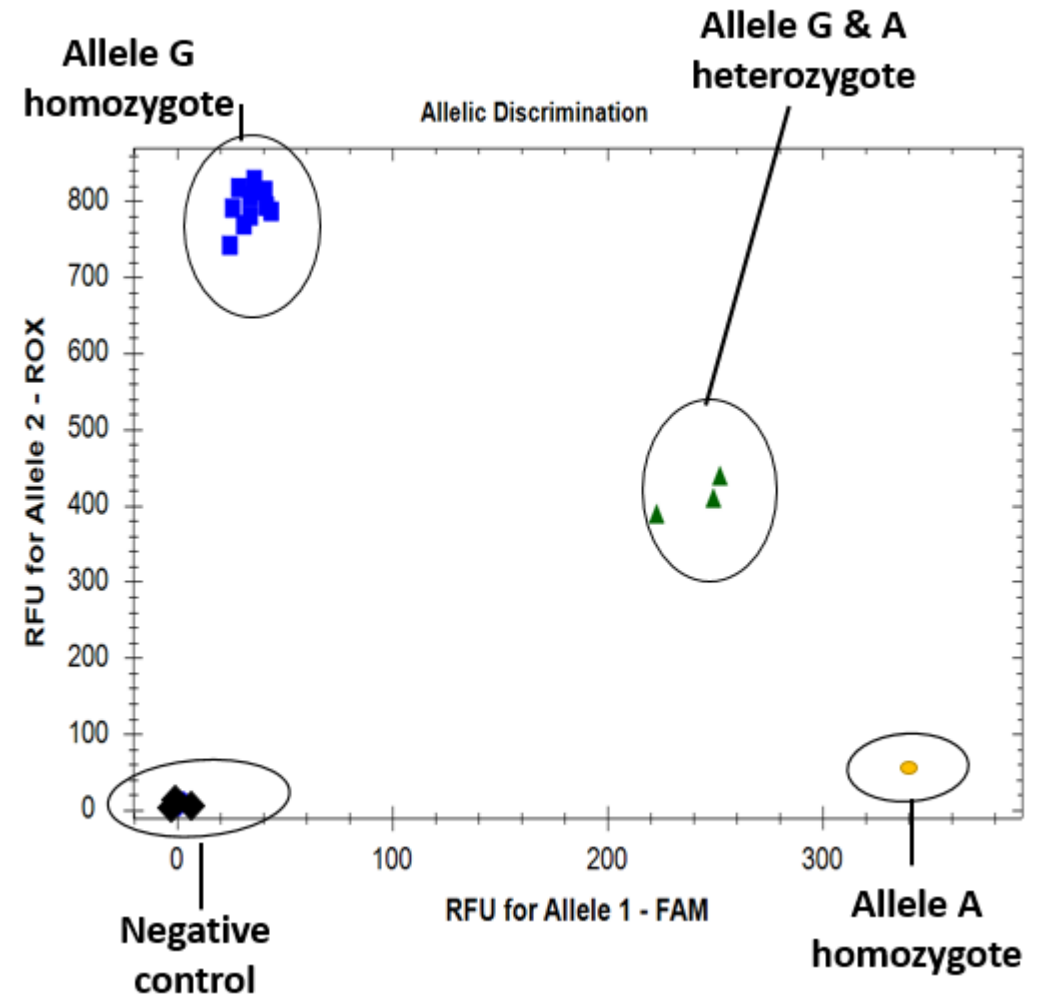
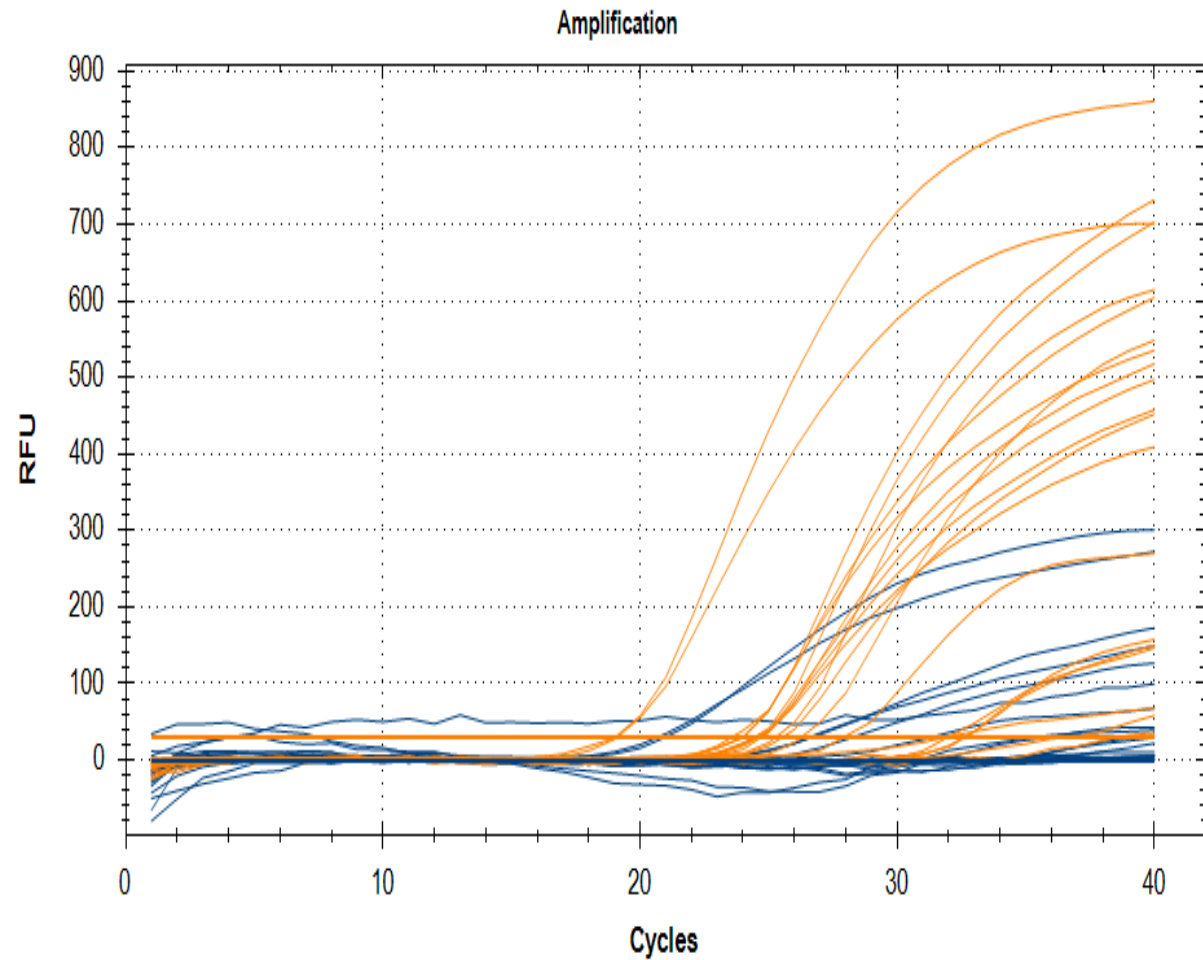
# Allergy 1 test system for 4 samples



# Allergy 2 test system for 4 samples



# Oxytocin test system for 4 samples



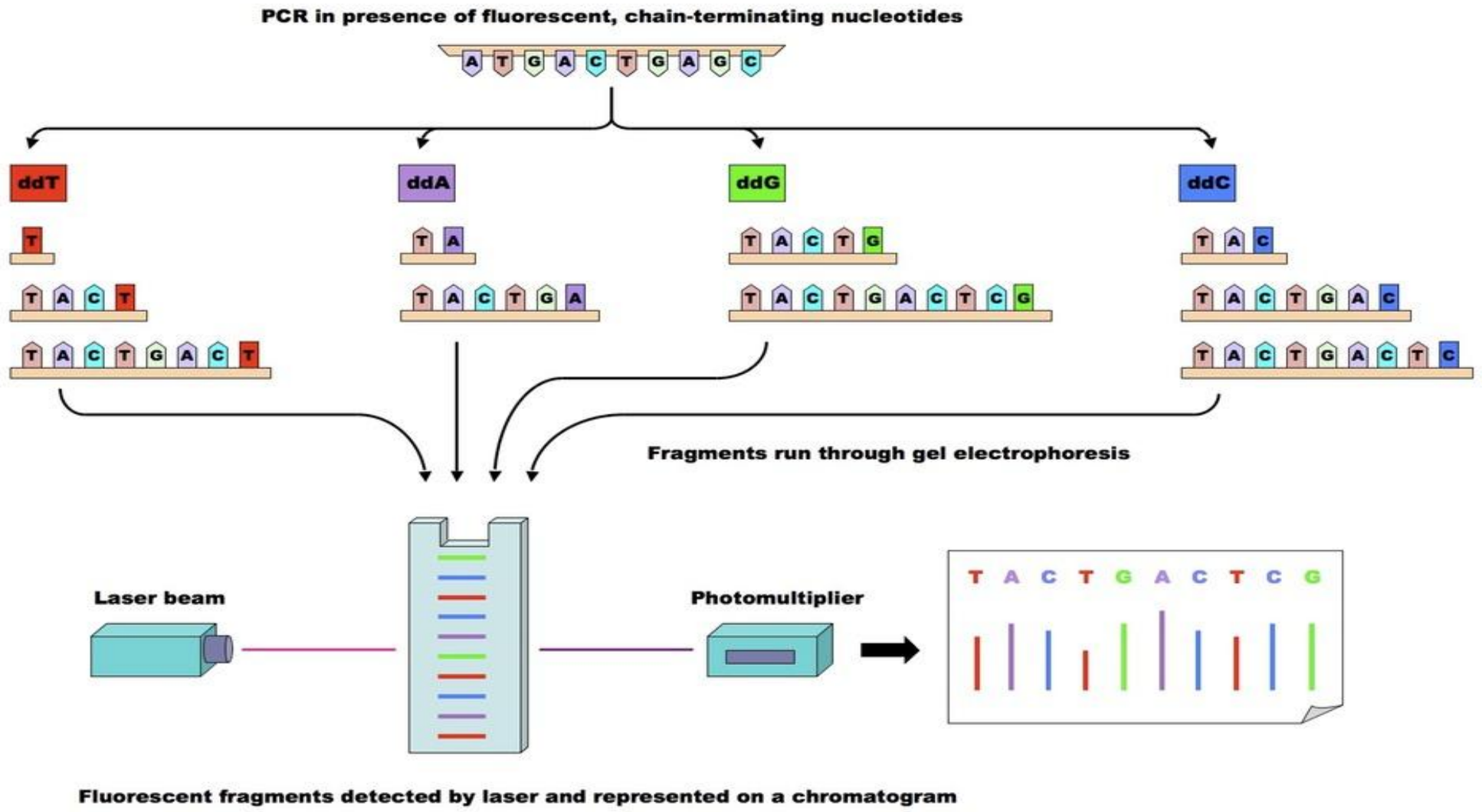
# Sanger Sequencing

Conventional PCR

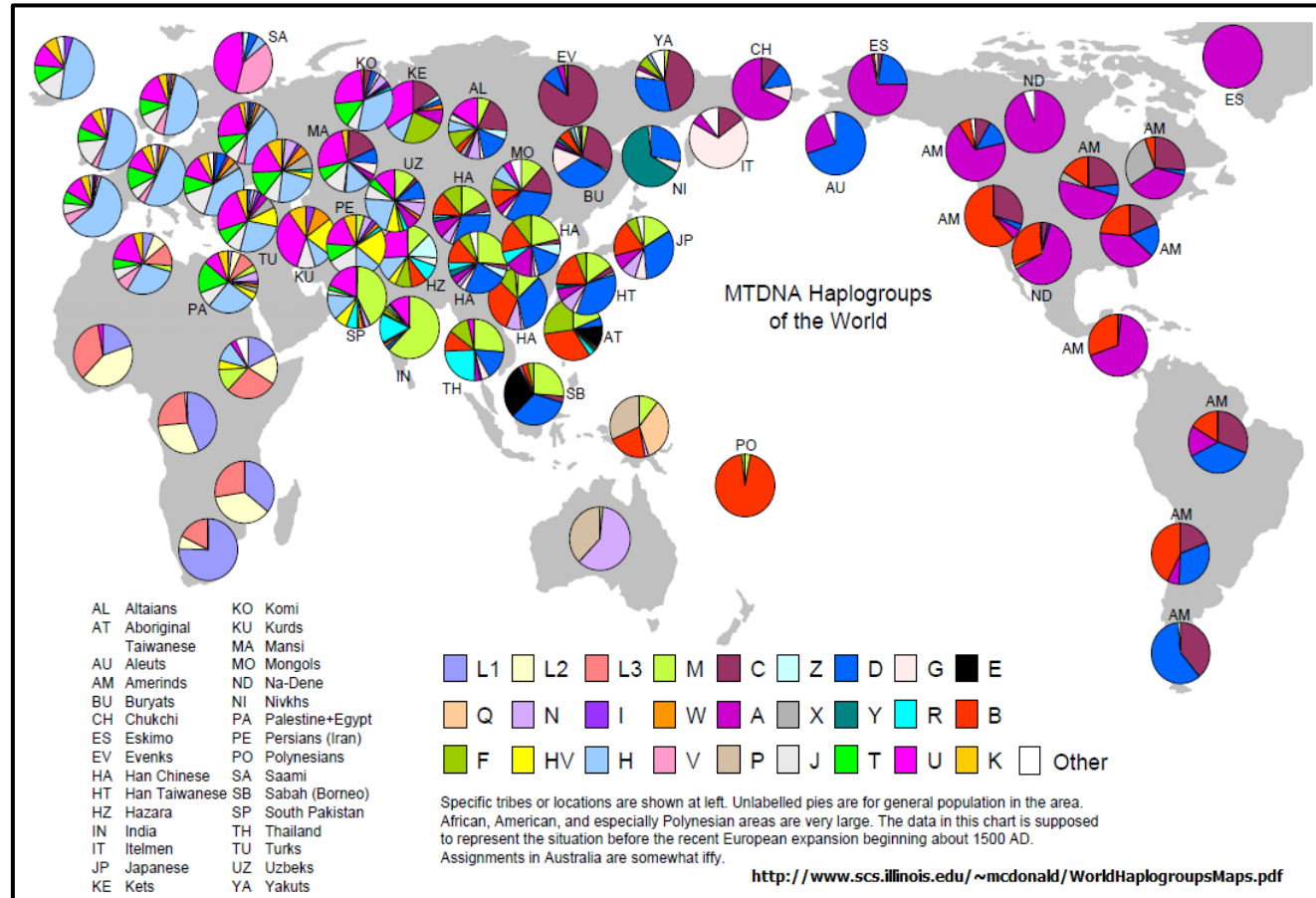
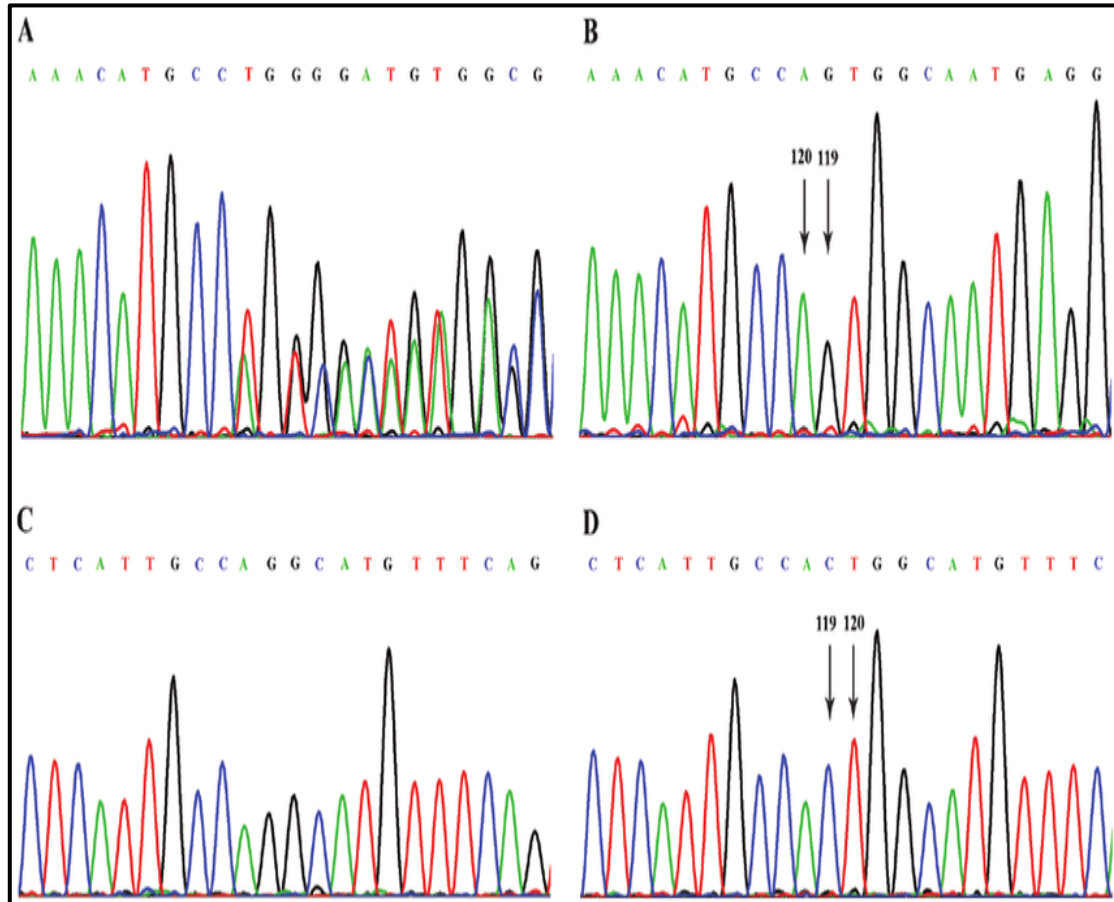
Gel Electrophoresis

SNPs  
by qPCR

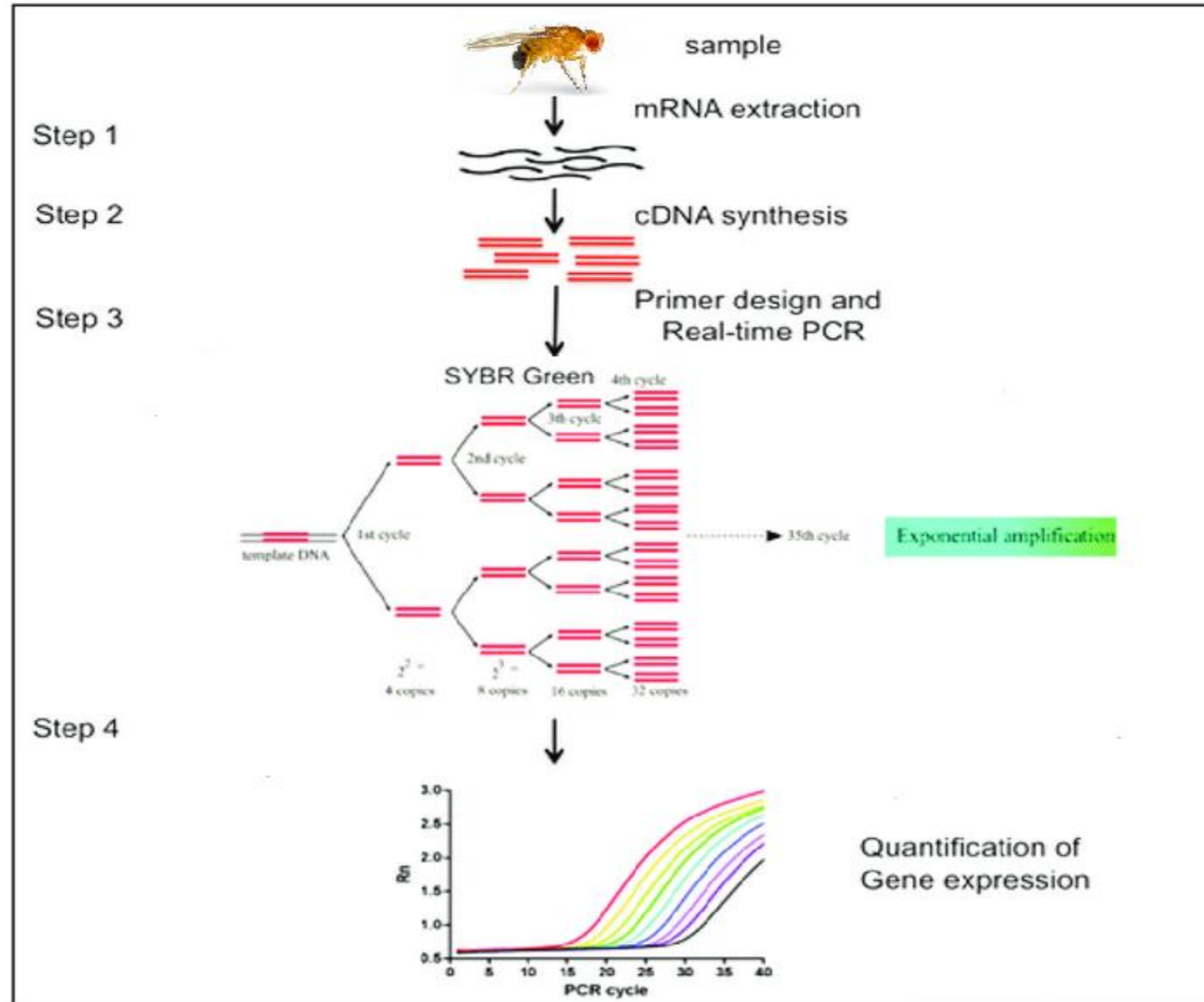
Sequencing



# Sequence Analysis for Mitochondria Hypervariation Region



# RT-QPCR

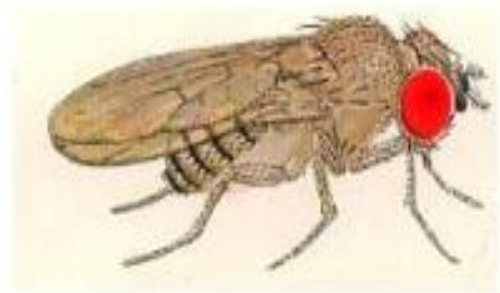




# Expression of White Eye Color Gene in *Drosophila melanogaster*



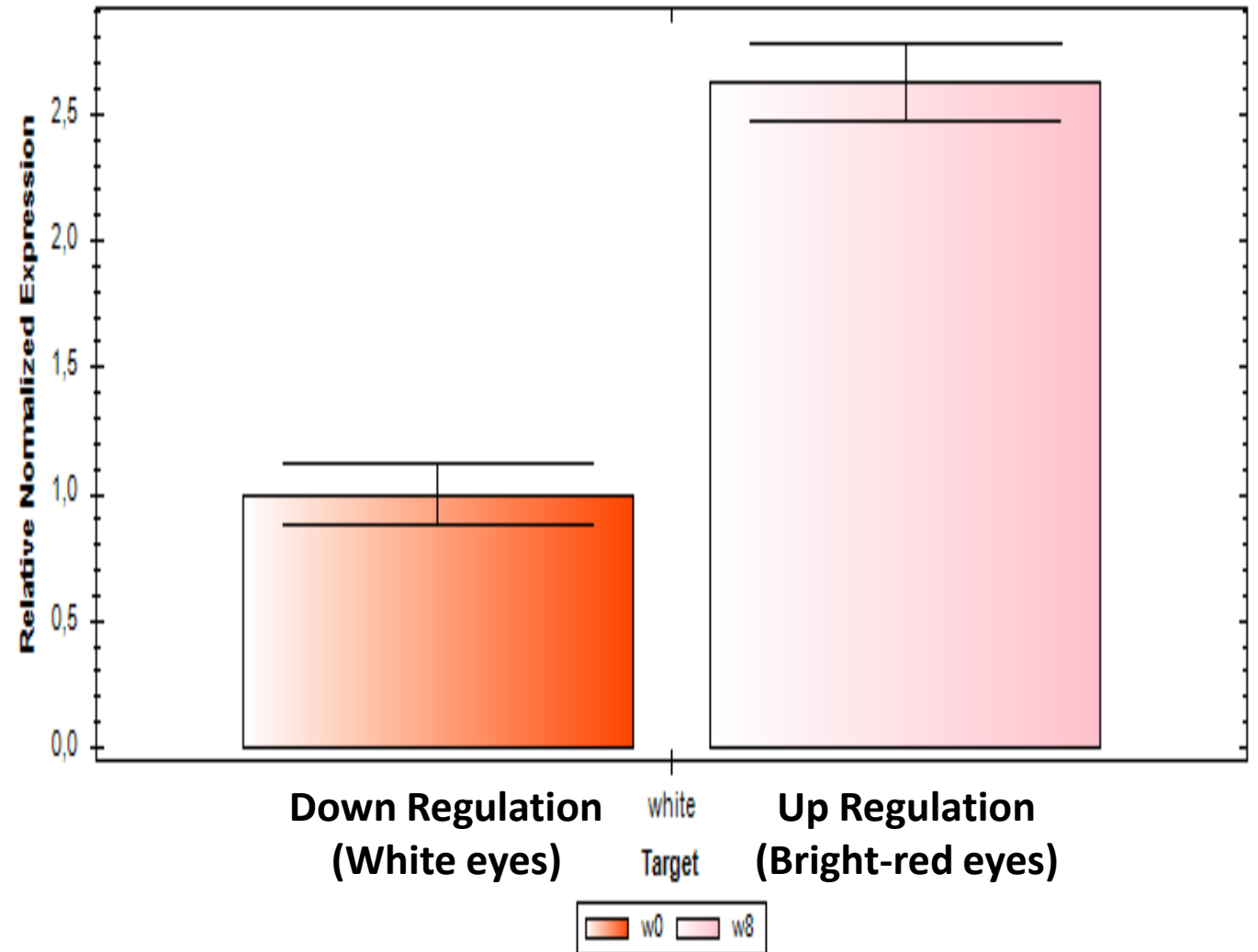
red eyes



bright-red eyes



white eyes



# *Acknowledgment*



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Thank  
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Any  
Question?





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