



Joint Institute for Nuclear
Research

SCIENCE BRINGING NATIONS
TOGETHER

Dzhelepov Laboratory of Nuclear
Problems (DLNP), JINR



PCR and DNA fingerprinting



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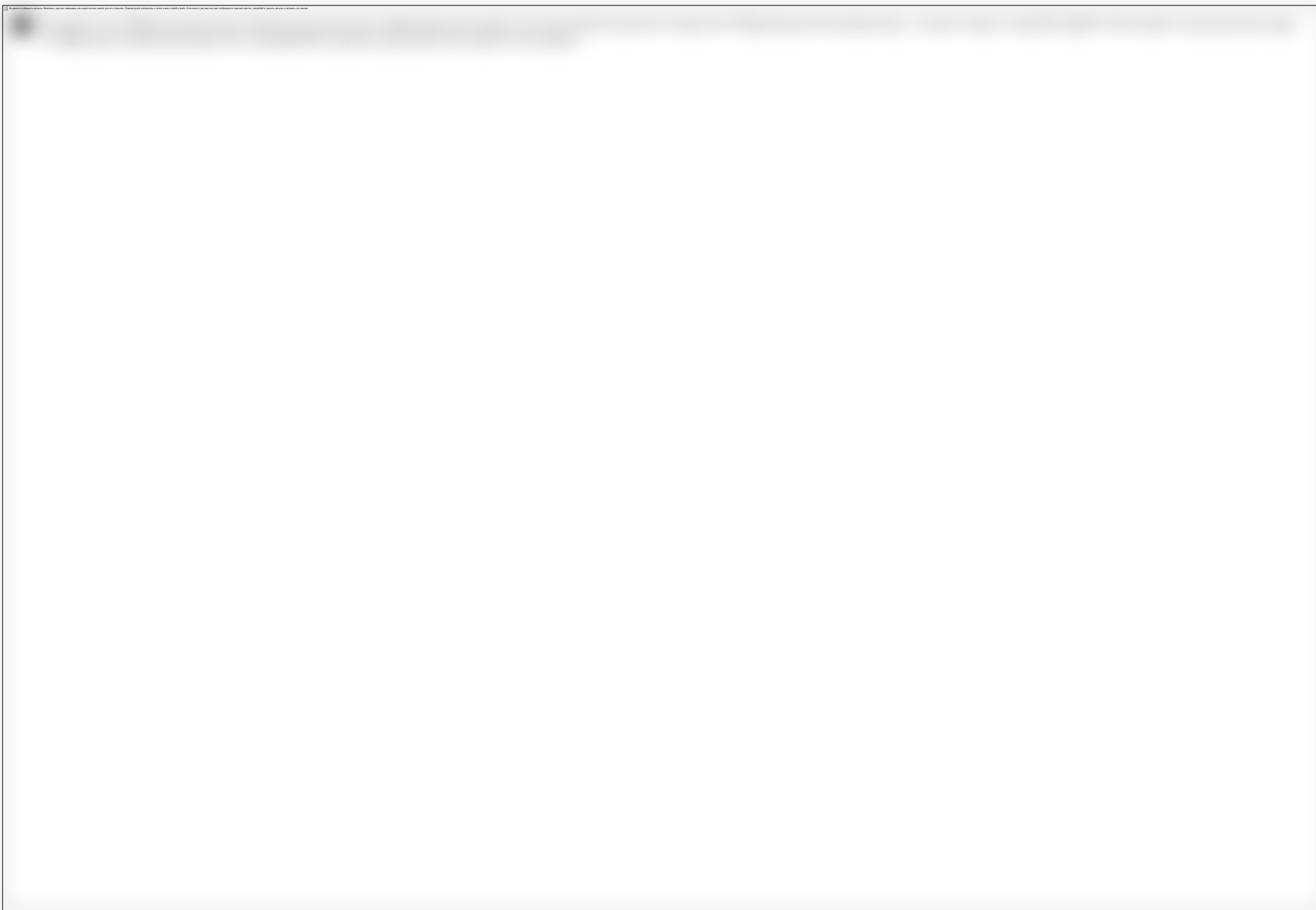
Outline

➤ Introduction and background

- DNA Structure and extraction
- PCR
- Gel electrophoresis (factor affecting fragment separation)

➤ Practical work

- DNA extraction and analysis
- Drosophila using phenol
- Buccal epithelium using chelix
- DNA extraction from agarose gel
- Determination of DNA concentration and purity
- Restriction enzymes
- PCR techniques
- CCR5 gene analysis
- Adducin gene analysis
- Allelic discrimination
- ALU PCR
- Total RNA isolation and electrophoresis
- Real-Time PCR





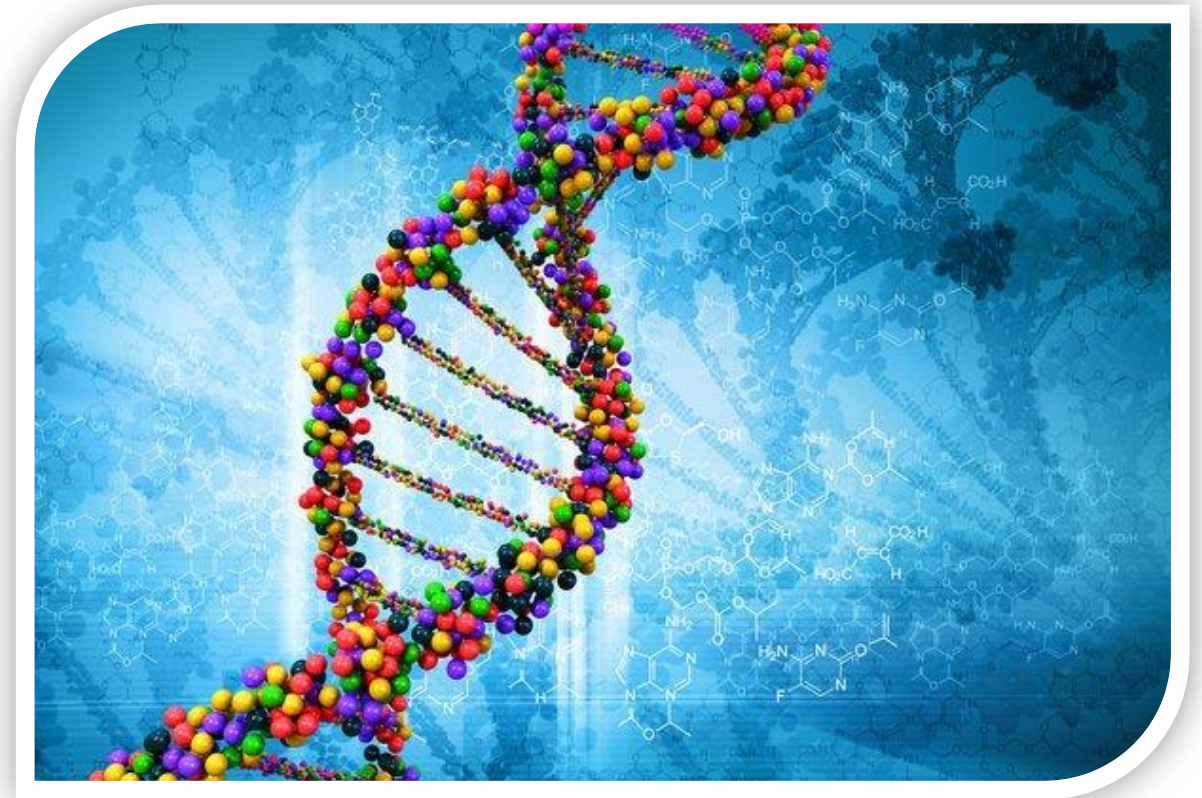
Deoxyribonucleic acid





Role of DNA

DNA is a basics of life and determines parameters of the organism containing it.





Role of DNA

Look at a human and mouse
presented on slide.

Human differs from mouse so much but
in compare to some other species their
DNA are quiet similar.

Information about every living being is
encoded in DNA.





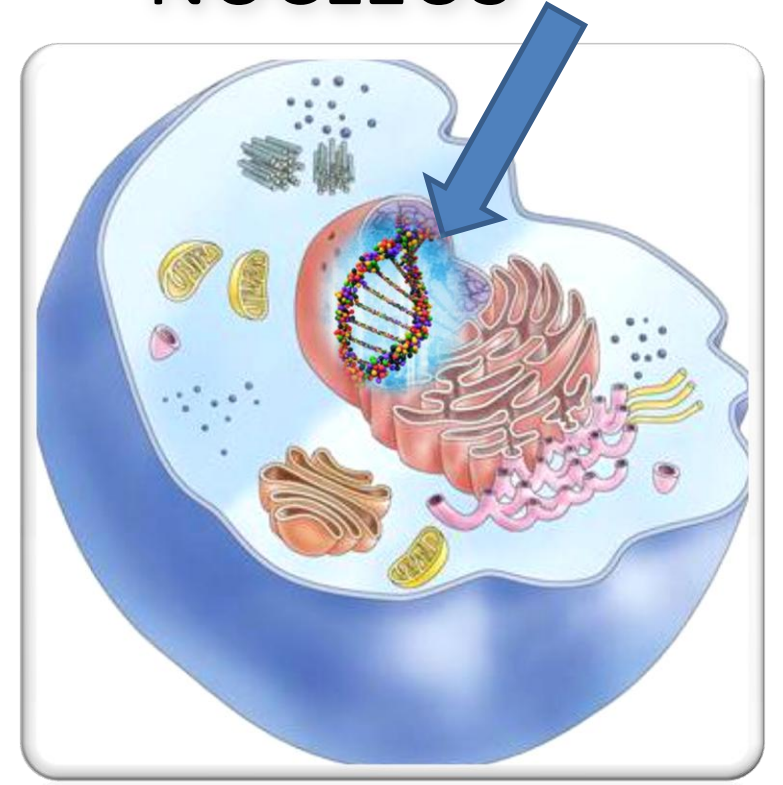
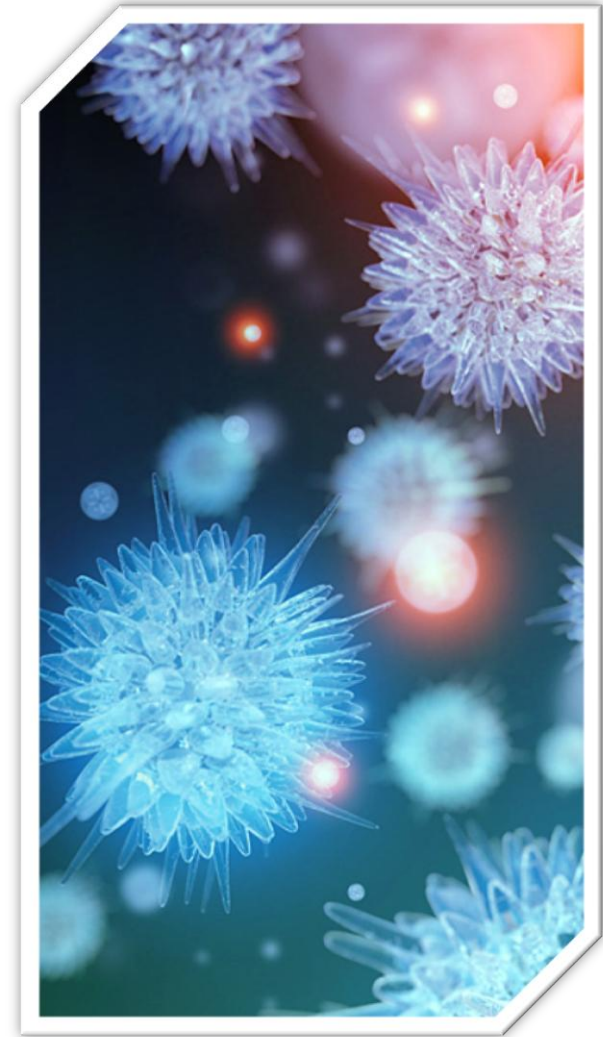
- Animals
- Plants
- Mushrooms

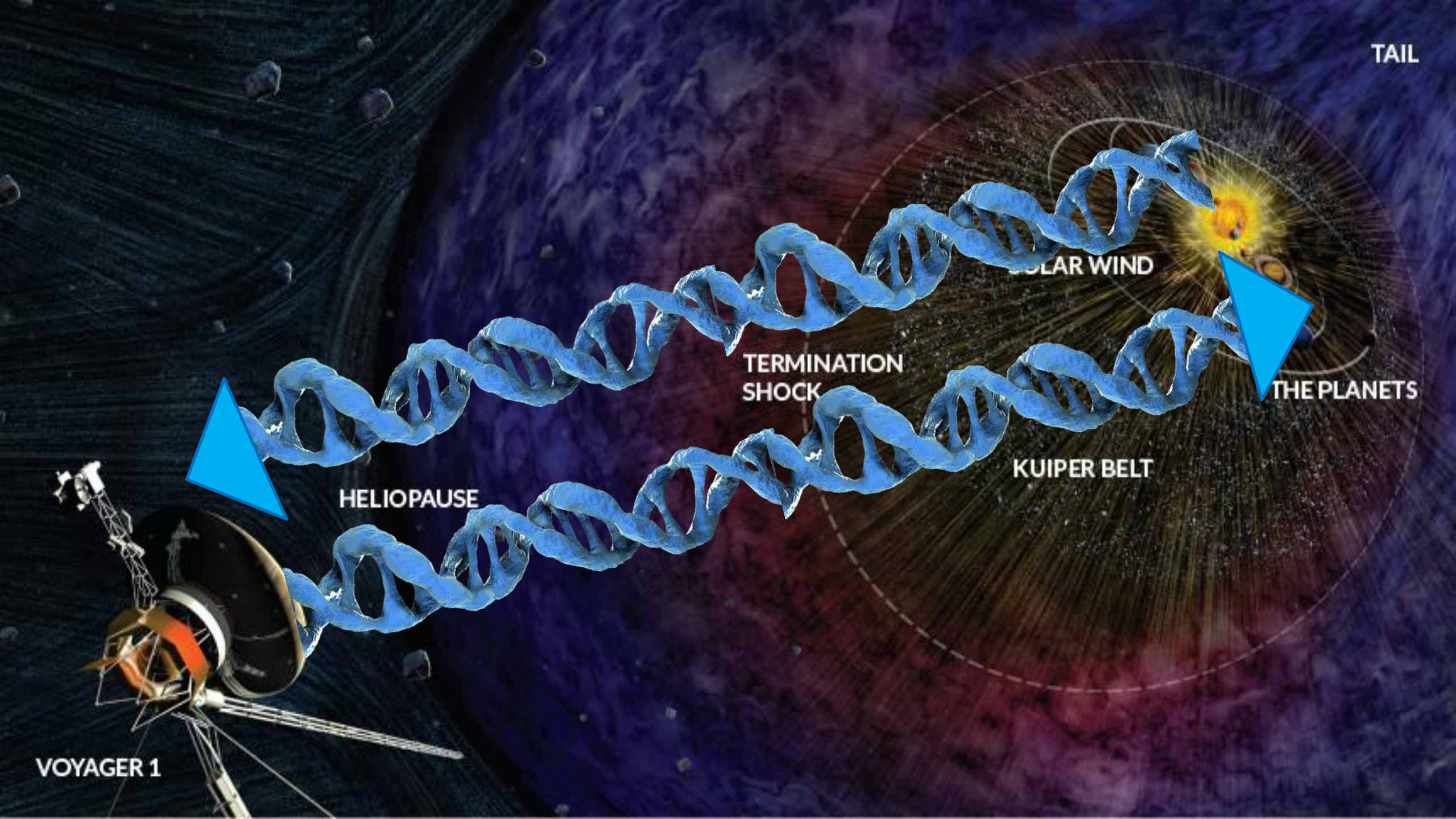
DNA or RNA

- Virus

CELL

NUCLEUS





TAIL

SOLAR WIND

TERMINATION SHOCK

THE PLANETS

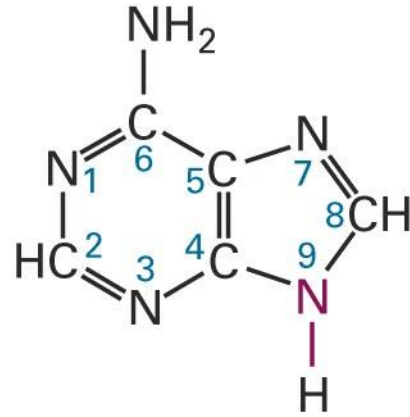
KUIPER BELT

HELIOPAUSE

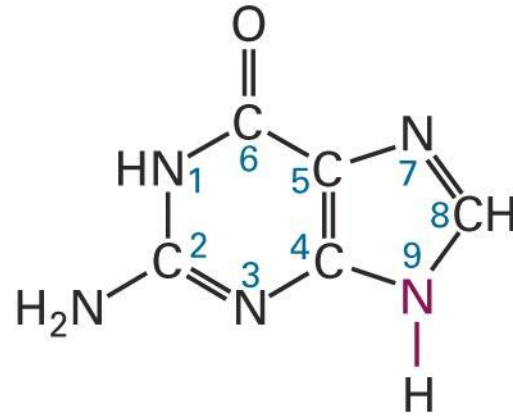
VOYAGER 1



PURINES

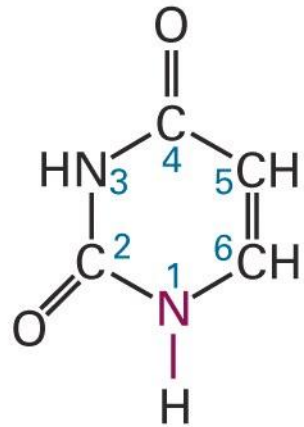


Adenine (A)

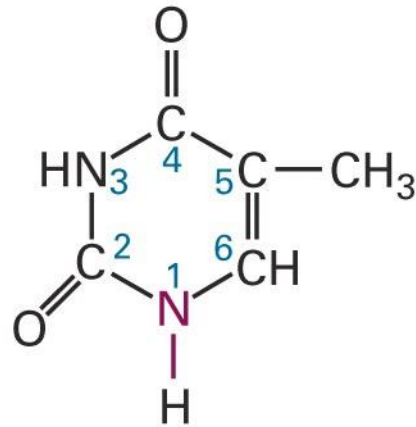


Guanine (G)

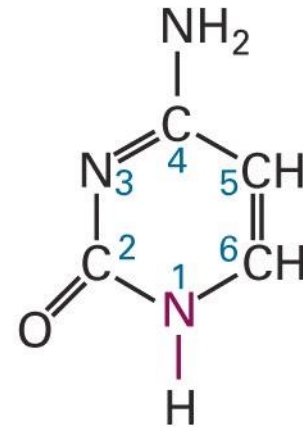
PYRIMIDINES



Uracil (U)



Thymine (T)

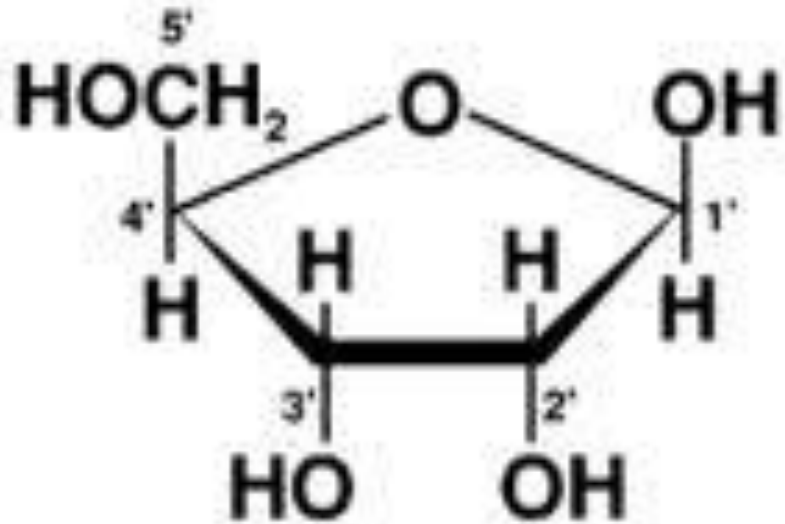


Cytosine (C)

Pentose sugars:

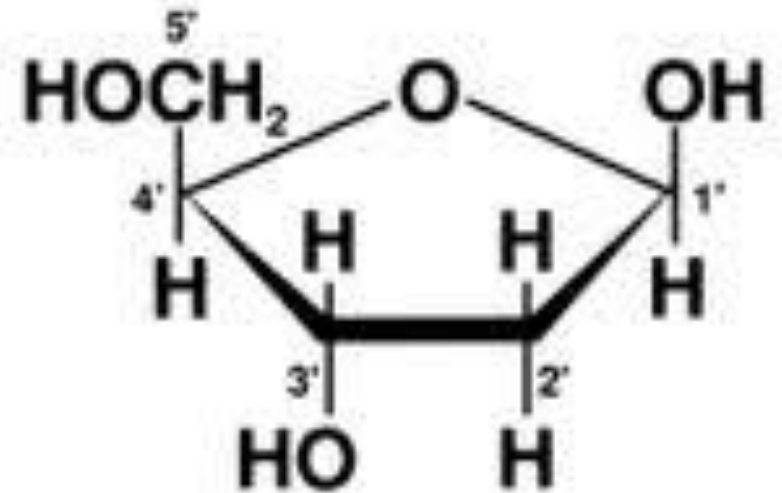


in RNA



ribose

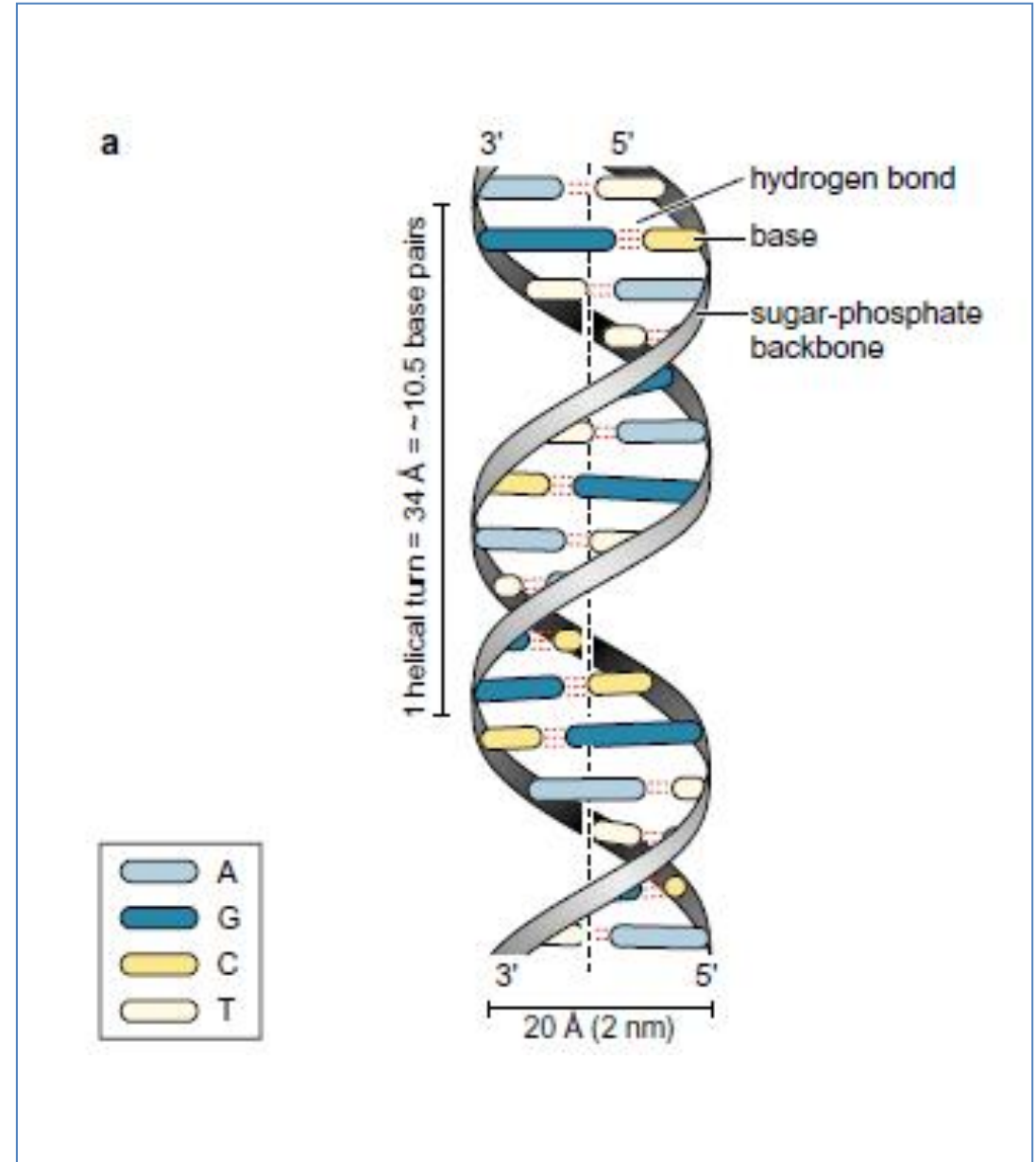
in DNA



2'-deoxyribose

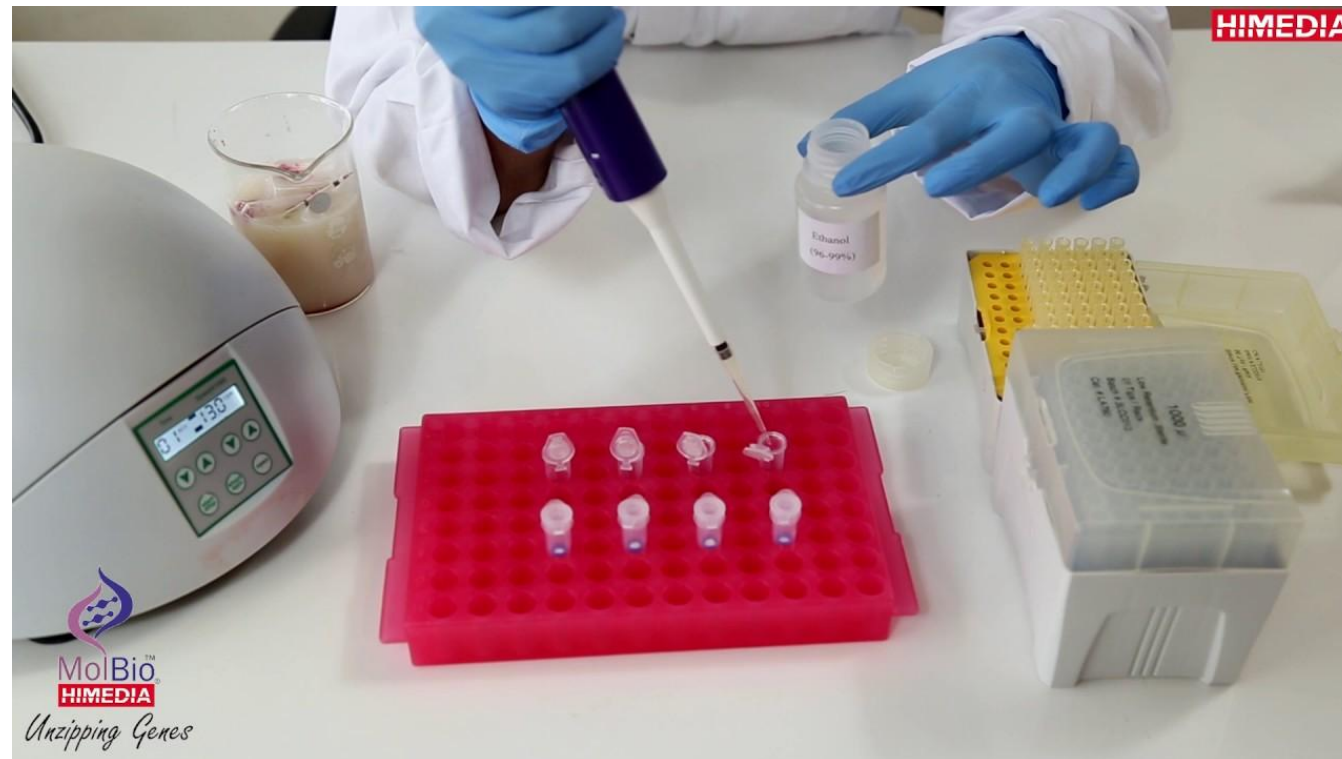


- ❑ 2 polynucleotide chains running in opposite directions = antiparallel
- ❑ Double helix is right handed
- ❑ 2 chains connected by H-bonding between G & C and between A & T = base pairing / bases are complementary
- ❑ Sugar –phosphate backbone on outside & carries –ve charges on PO_4 groups.





Drosophila Genomic DNA Extraction



**Extraction is often the first step involved in
genetic research**



Why Drosophila?



- Well studied example, gene structure known
- Has common principal DNA structure with humans
- Short life cycle
- Permits the study of heritable gene mutation
- Low cost



Drosophila Genomic DNA Extraction



Phenol–chloroform extraction is a liquid-liquid extraction technique in molecular biology used to separate nucleic acids from proteins and lipids.



How Does Gel Electrophoresis Separate DNA Fragments

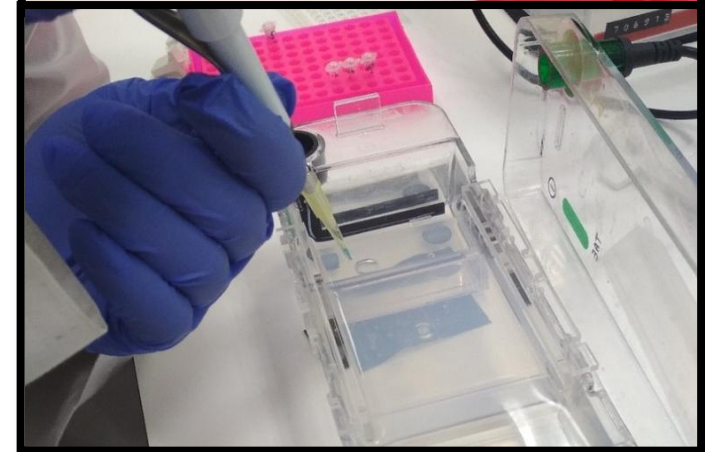
Gel electrophoresis is a technique used to separate fragments of macromolecules such as DNA, RNA, and proteins based on their size and charge.

DNA is a negatively-charged molecule that migrates towards the positive electrode on an electric field.

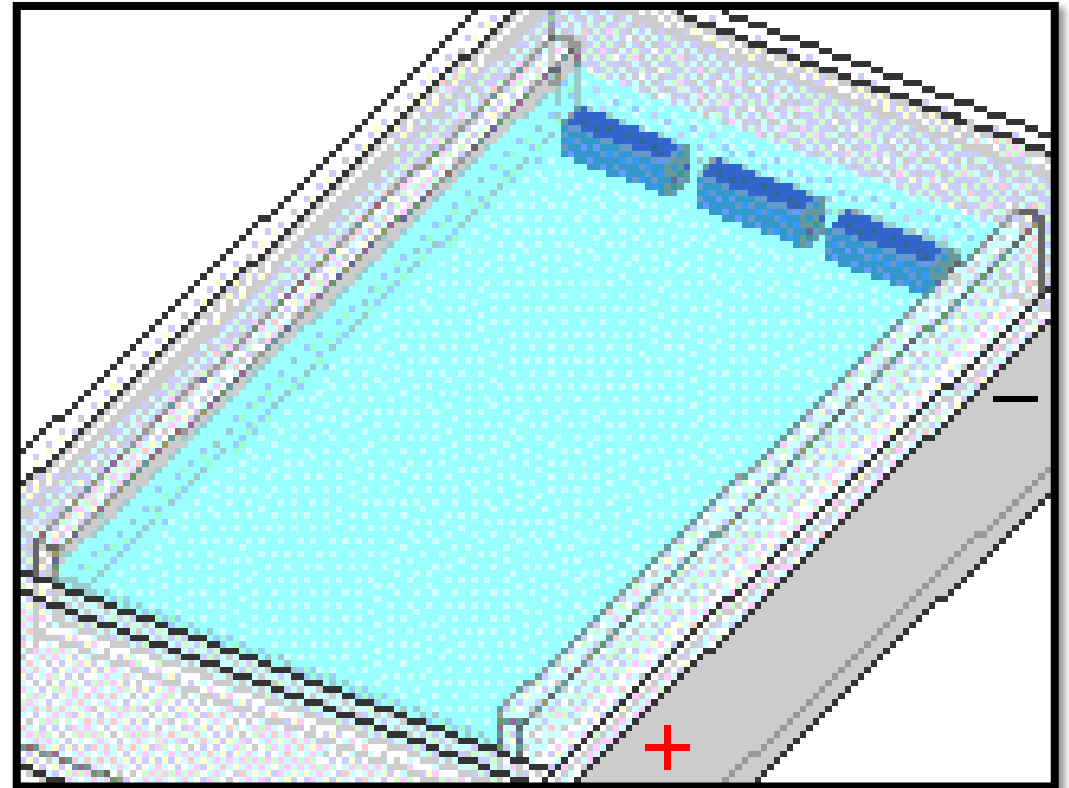
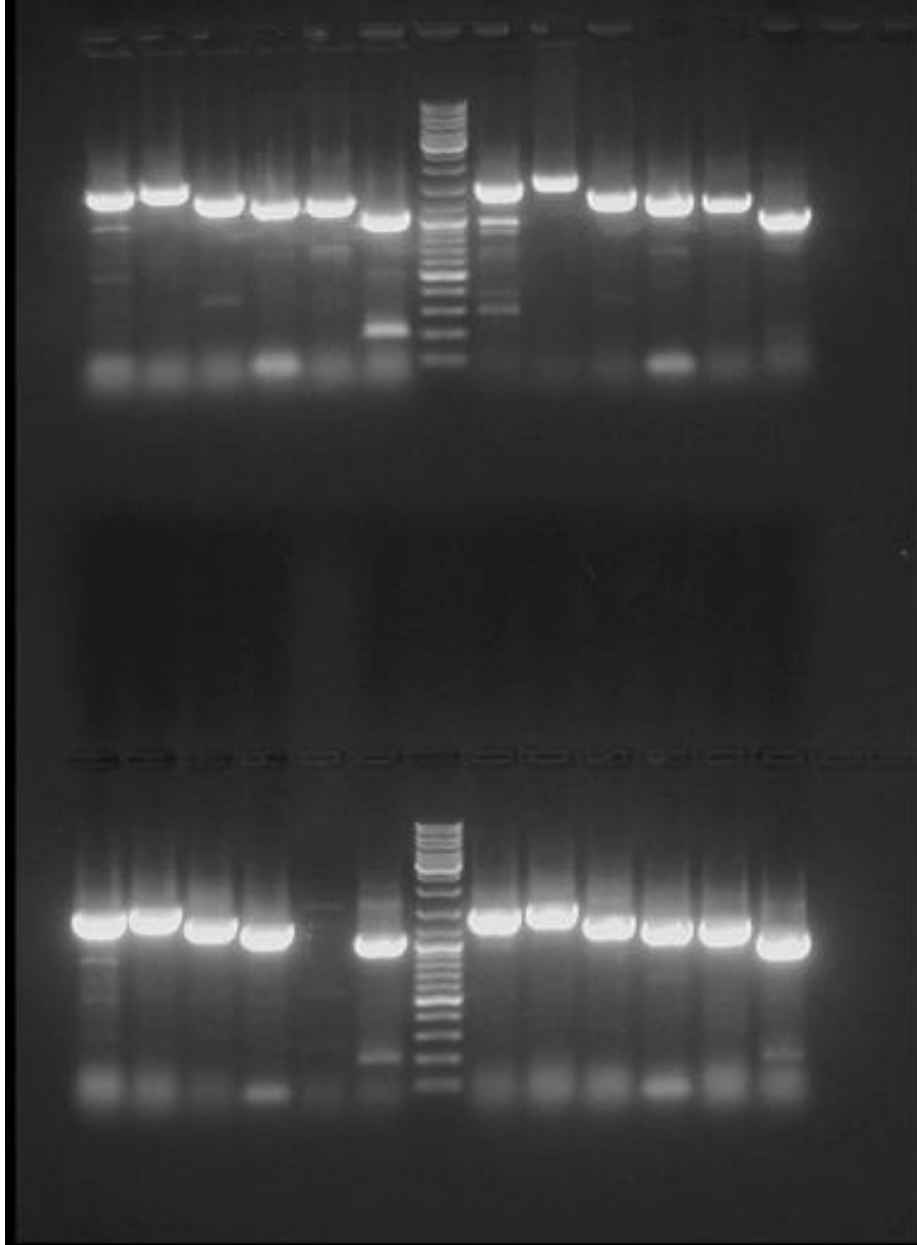
DNA fragments are separated based on the size of the molecules during the migration of DNA molecules through the pores of the gel.



DNA molecules with a higher number of base pairs migrate slowly through the pores of the gel while small DNA molecules migrate quickly in the gel.



Agarose gel electrophoresis





Polymerase chain reaction or PCR



Polymerase chain reaction or PCR was developed by Cary Mullis in 1980s



PCR is a method of molecular biology that is used to create multiple copies of a segment of DNA



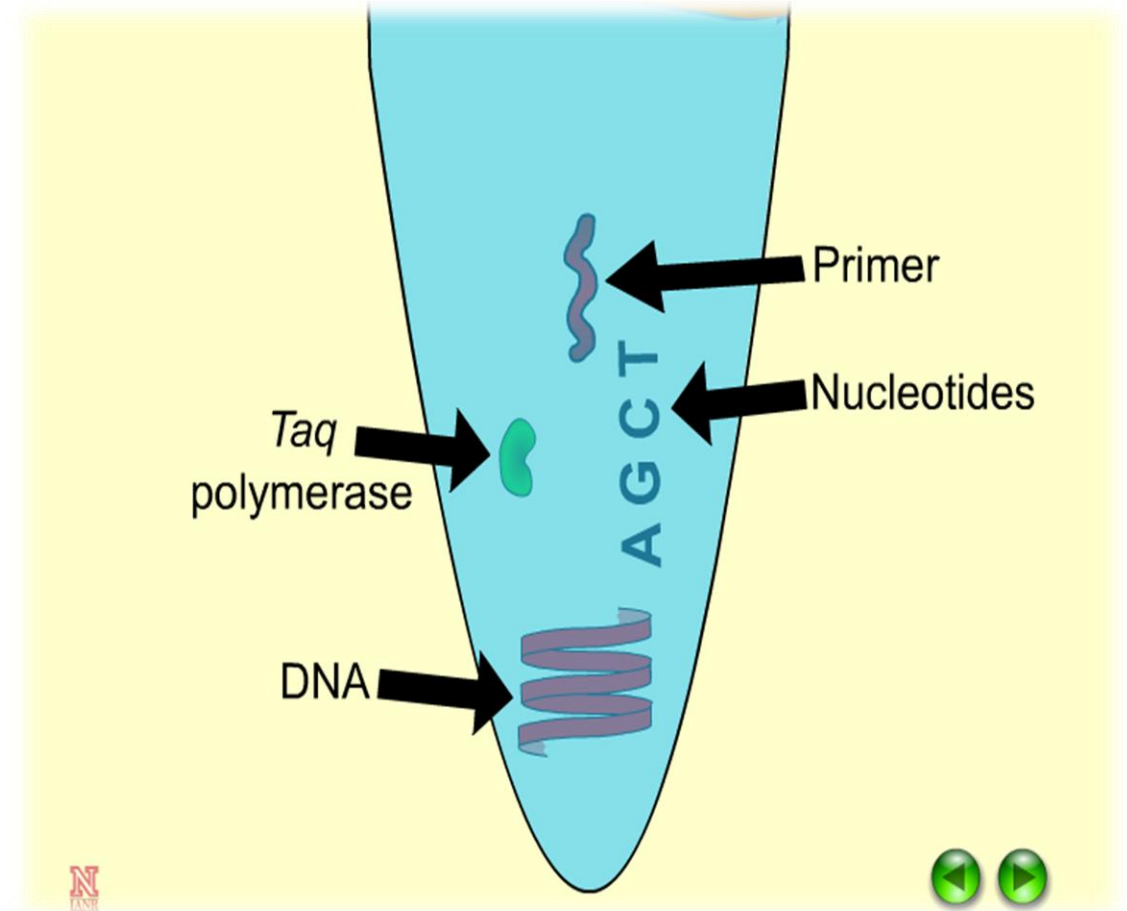


DNA can be extracted from different types of biological material:



- Blood
- Semen
- Teeth
- Skin
- Hairs
- Urine
- Bones
- Muscle

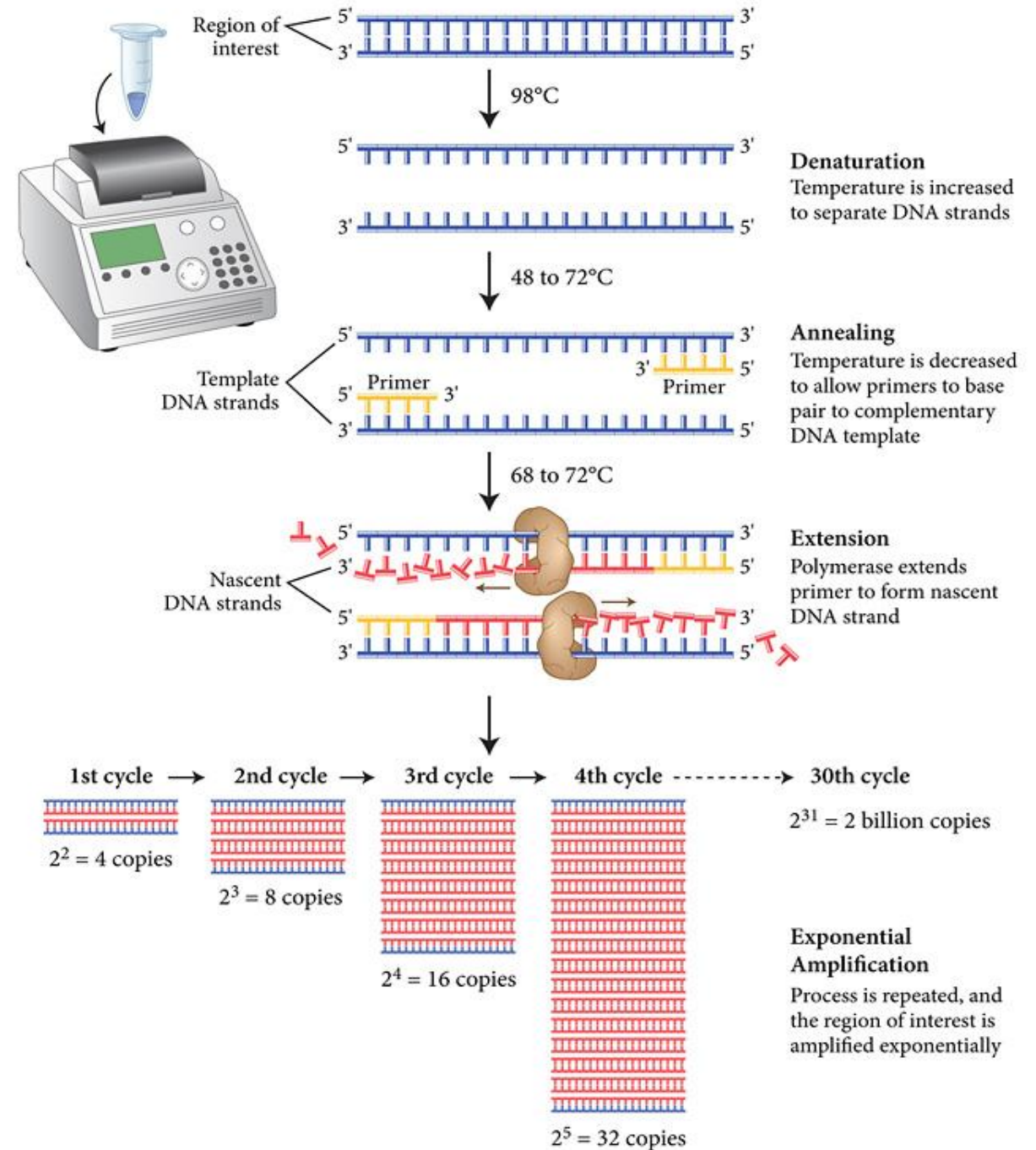
The main components of PCR include:





PCR includes 3 main stages:

1. Denaturation
2. Primer annealing
3. Extension or elongation



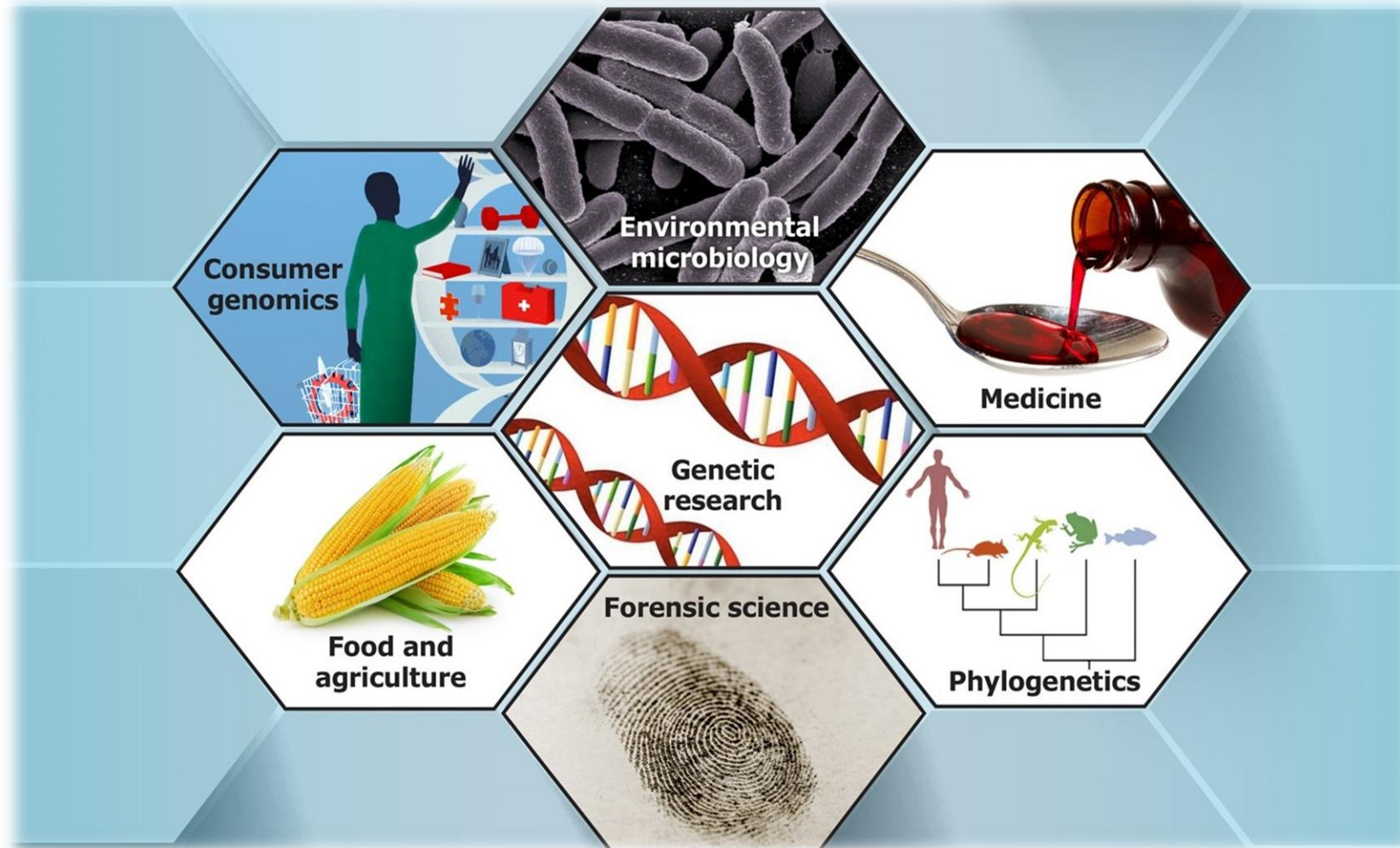
PCR is carried out in a thermocycler





PCR is used for:

- ✓ diagnostics of infectious diseases;
- ✓ diagnostics of cancer diseases;
- ✓ diagnosis of genetic diseases;
- ✓ identification of a person;
- ✓ diagnostics of pathogens in food.

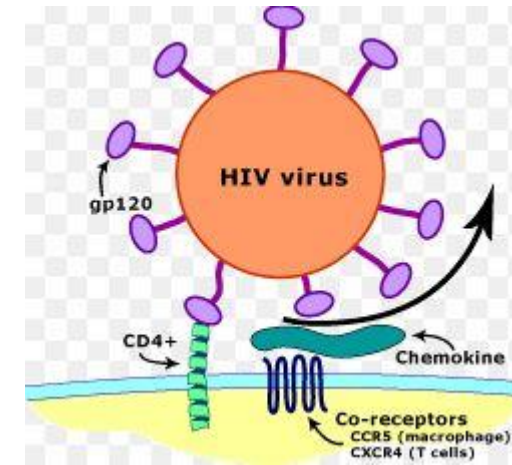




➤ PCR and genetic diseases analyses

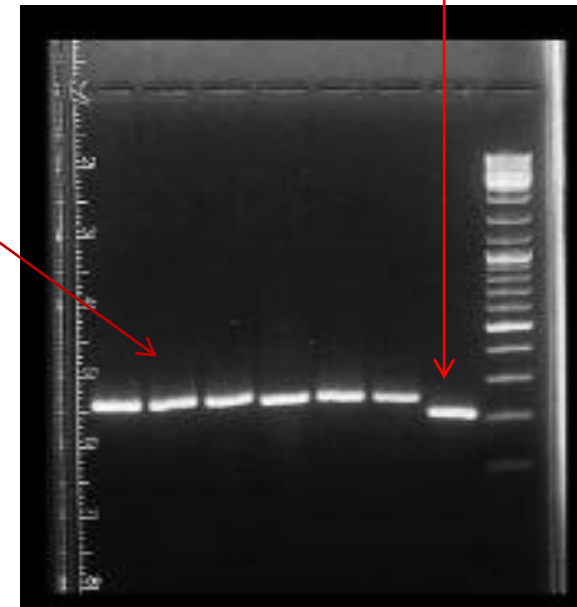
1) CCR5 genes and HIV resistance

- CCR5 predominantly expressed on T cells, macrophages, dendritic cells, eosinophils, and a subpopulation of either breast or prostate cancer cells.
- HIV-1 most commonly uses the chemokine receptors CCR5 as co-receptors to enter target immunological cells



Positive control

Student sample

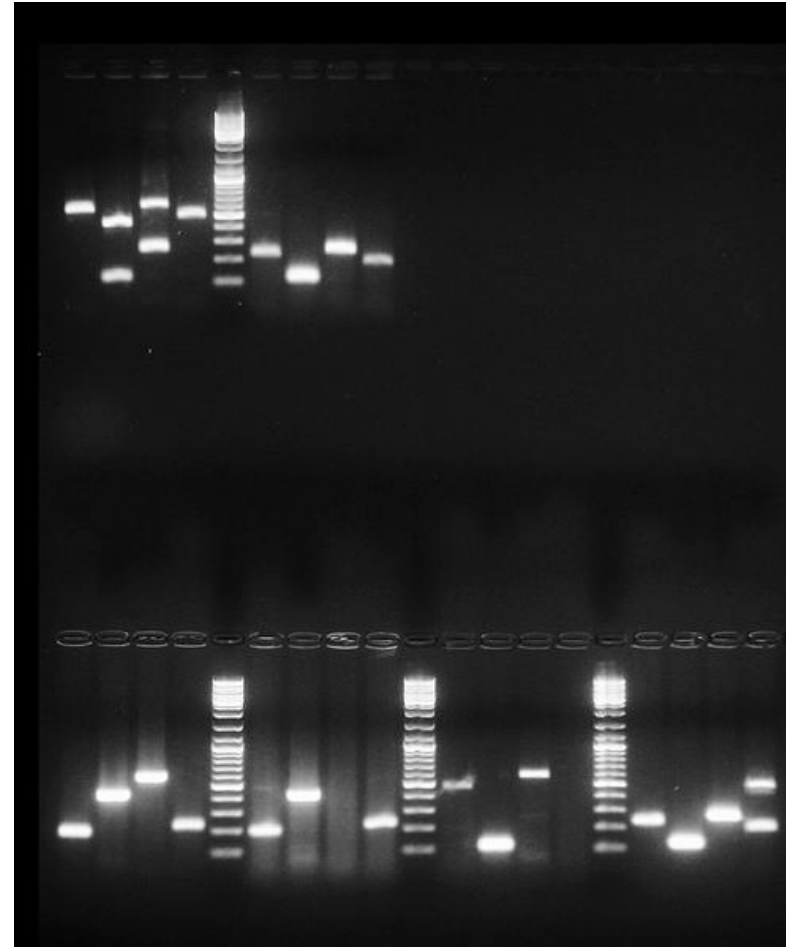


All the samples have no resistance to HIV-1



2) ACE gene and cardiovascular diseases

- Angiotensin I gene is present on chromosome 17 and contain the alu-insertion (ACE-1)
 - The absence of the Alu- sequence allow high risk for heart diseases
- S1 has lower risk for heart disease, while the other five persons show lower risk for heart disease indicated by longer migration in the gel
- All samples are homozygotes except sample 6 is heterozygotes



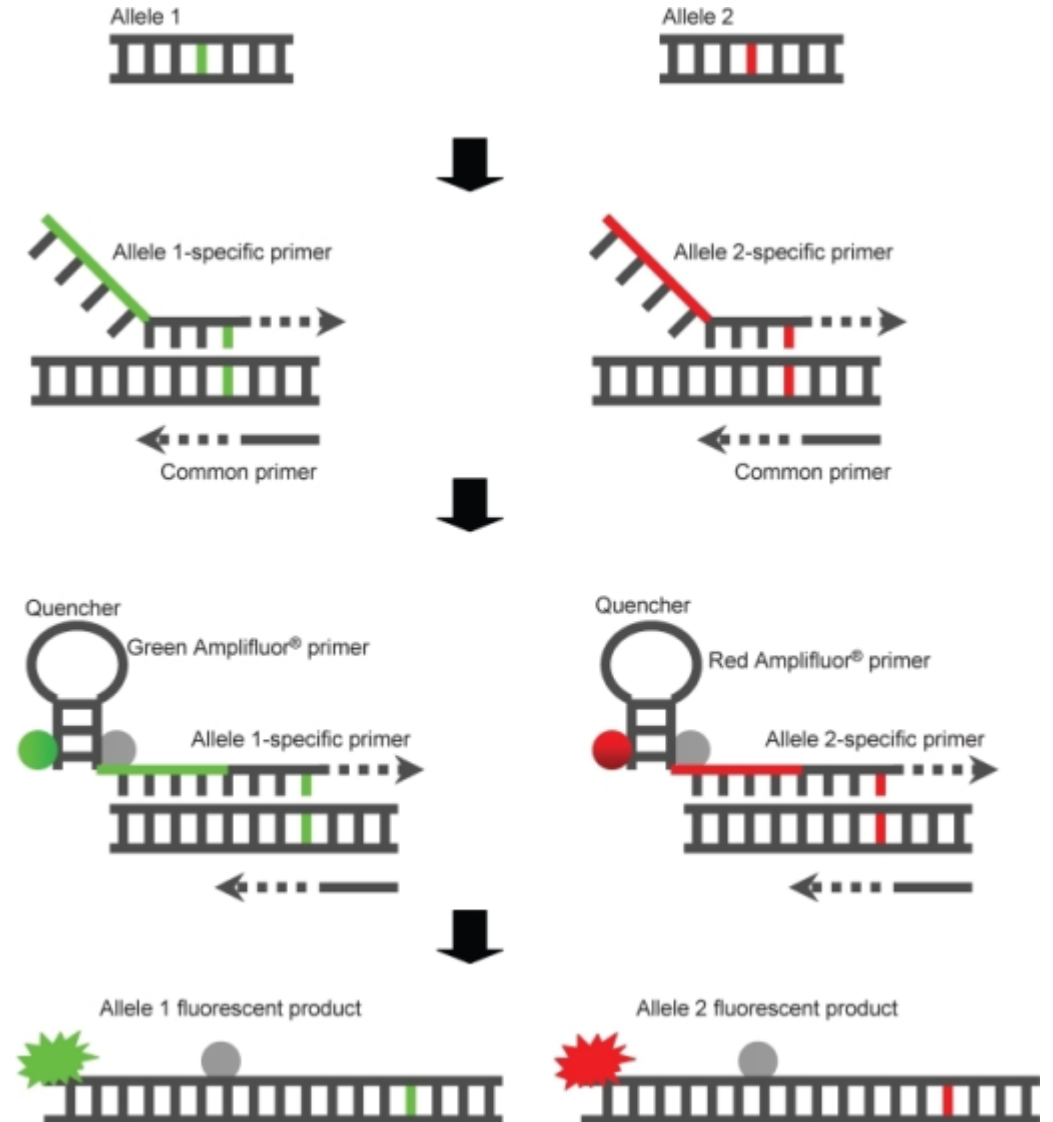
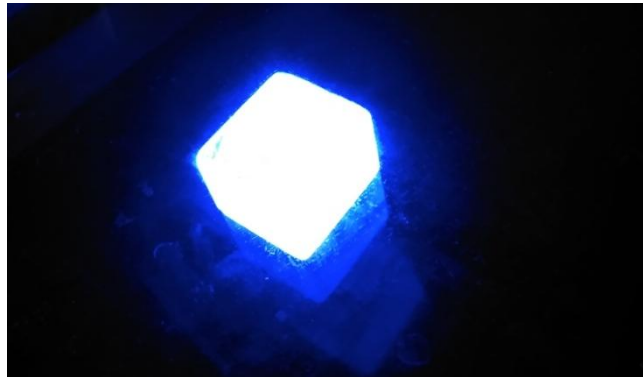
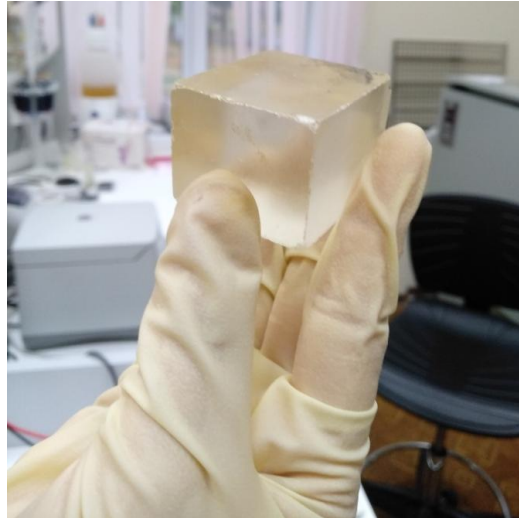
Real-time PCR technique

A real-time polymerase chain reaction monitors the amplification of a targeted DNA molecule, during the PCR, i.e. in real time, and not at its end, as in conventional PCR. Real-time PCR can be used quantitatively, semi-quantitatively, i.e. above/below a certain amount of DNA molecules or quantitatively.



Allelic discrimination with TaqMan probes

Fluorophores

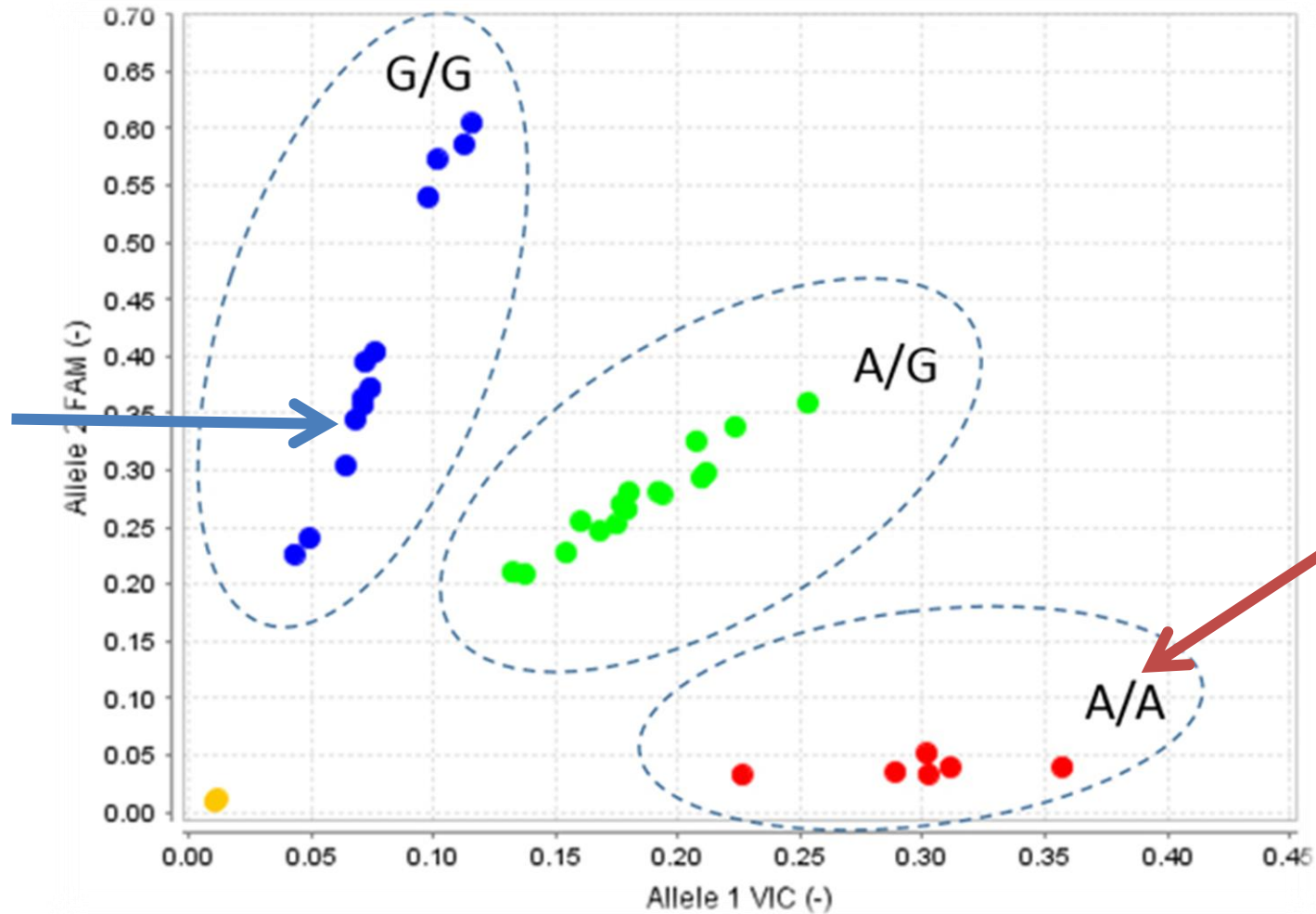




Oxytocin system plays an important role in human socially related personality traits and behaviors, referred to as “sociality.”



Results for Oxytocin hormone

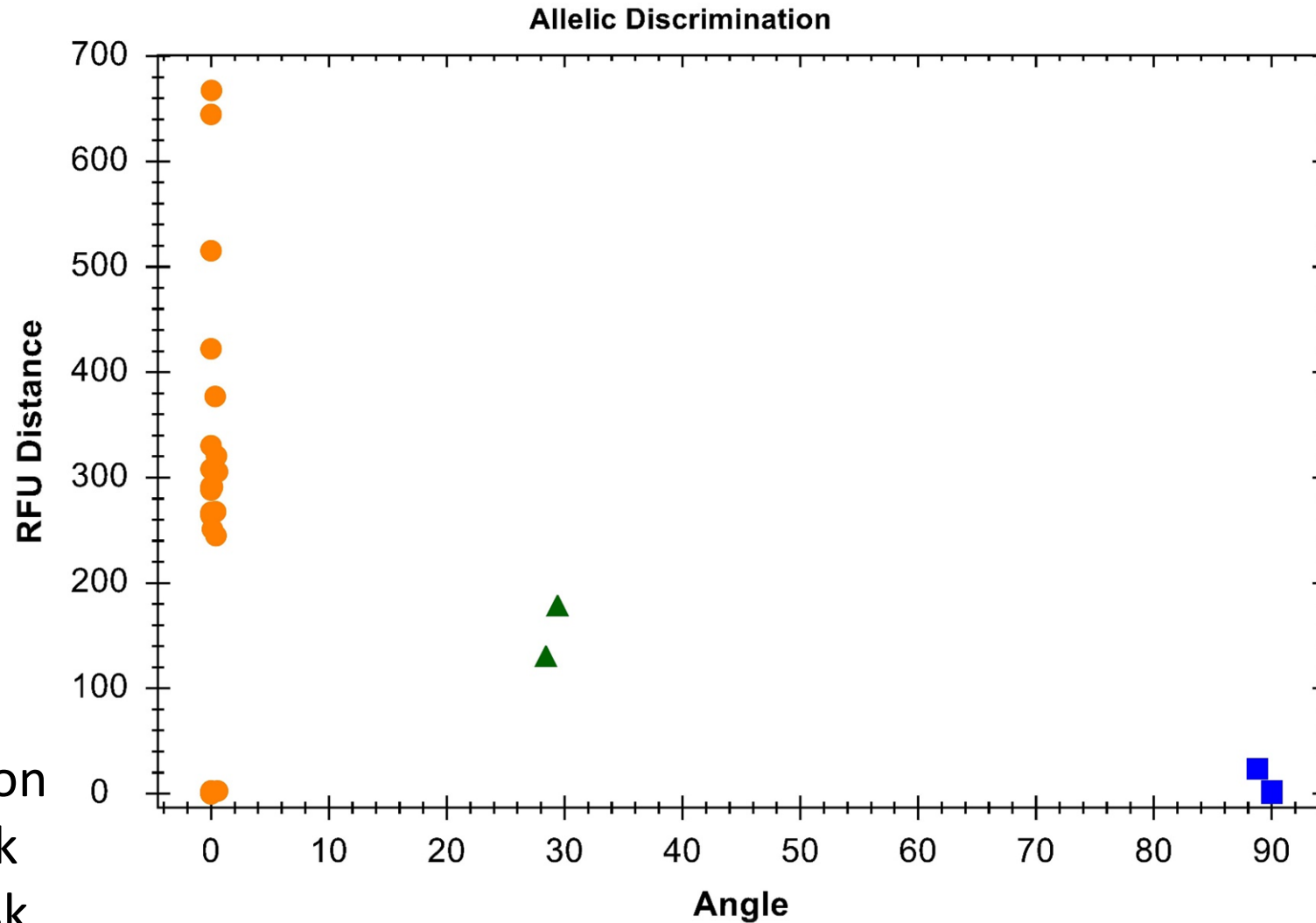


- G/G – social person
- A/A – unsocial person
- A/G - middle social

ROX – specific for allele G
FAM – specific for allele A



Results for Adducin gene



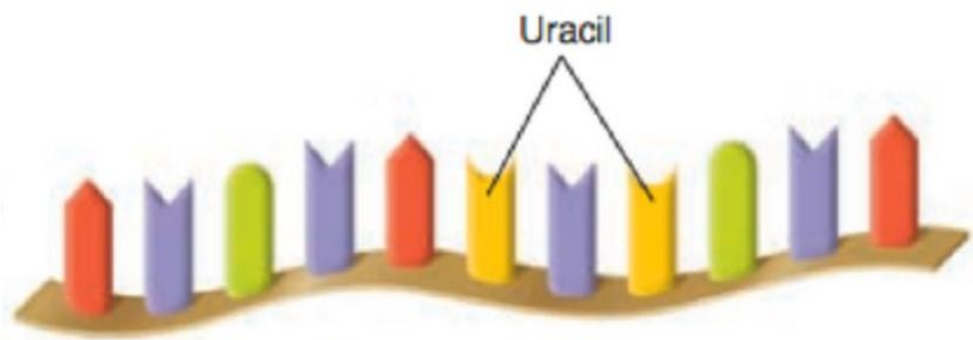
- G/G – normal person
- T/T – 1.8 higher risk
- G/T – 1.8 higher risk



DNA

RNA

mRNA



Messenger RNA

Ribosome
rRNA



Ribosomal RNA

tRNA

Amino acid

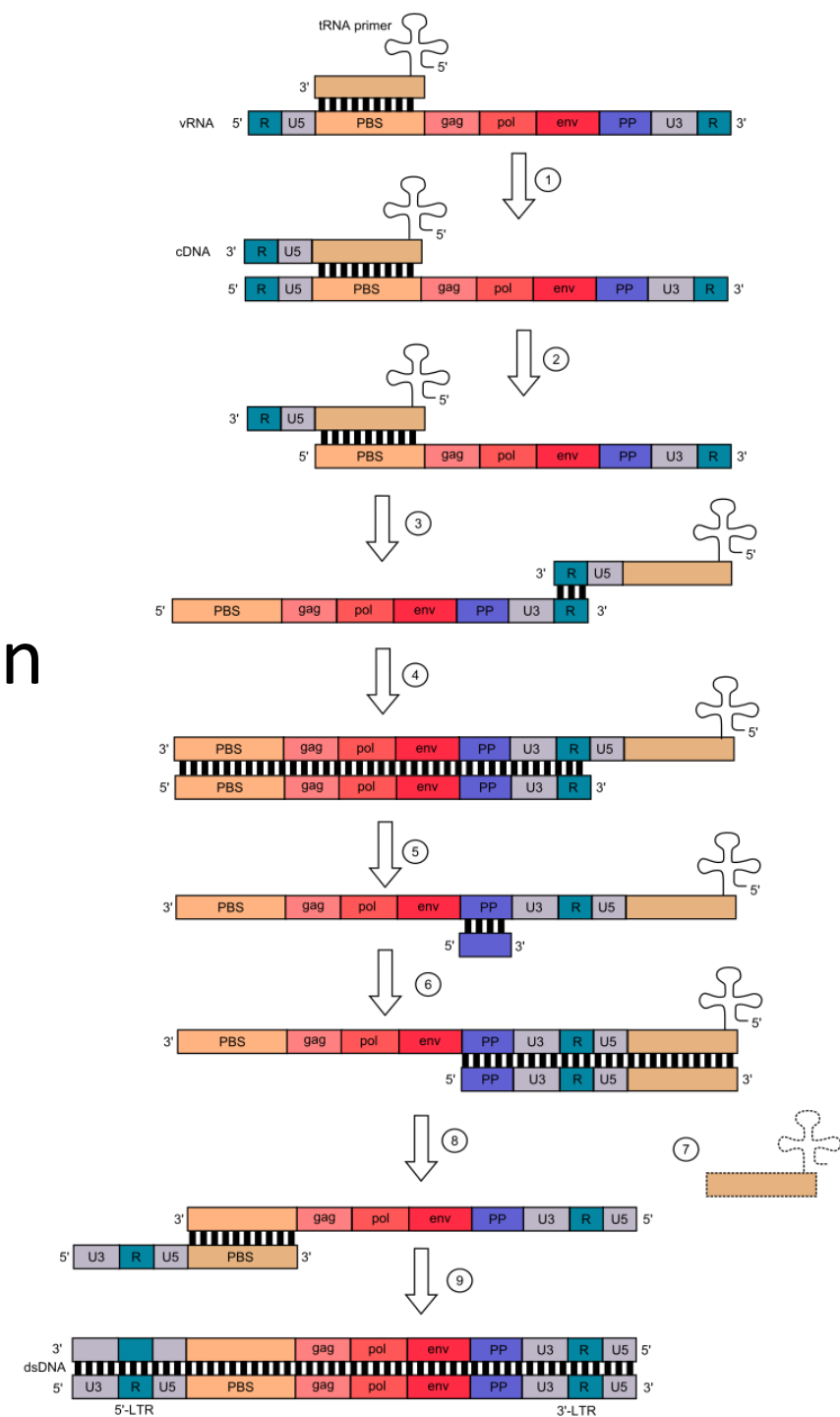
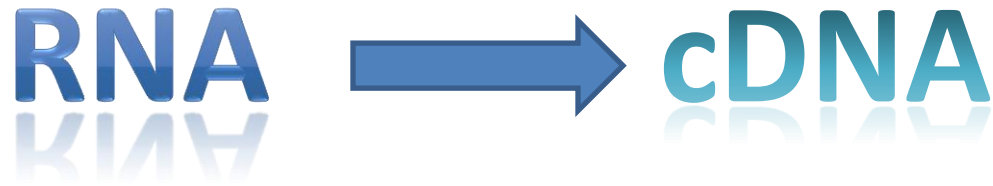


Transfer RNA



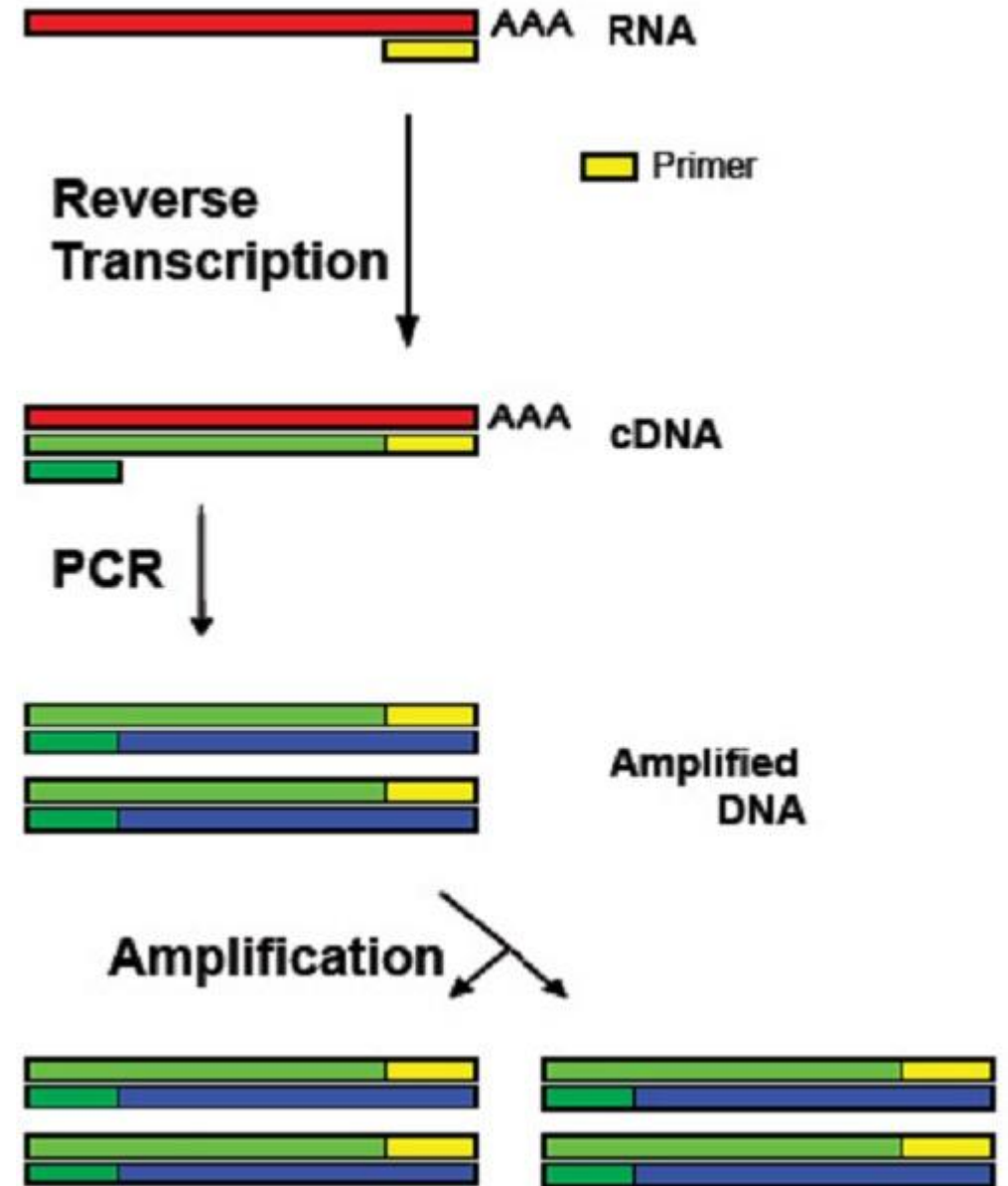
Reverse transcription

This is the process of formation of double-stranded DNA (cDNA) based on information in single-stranded RNA





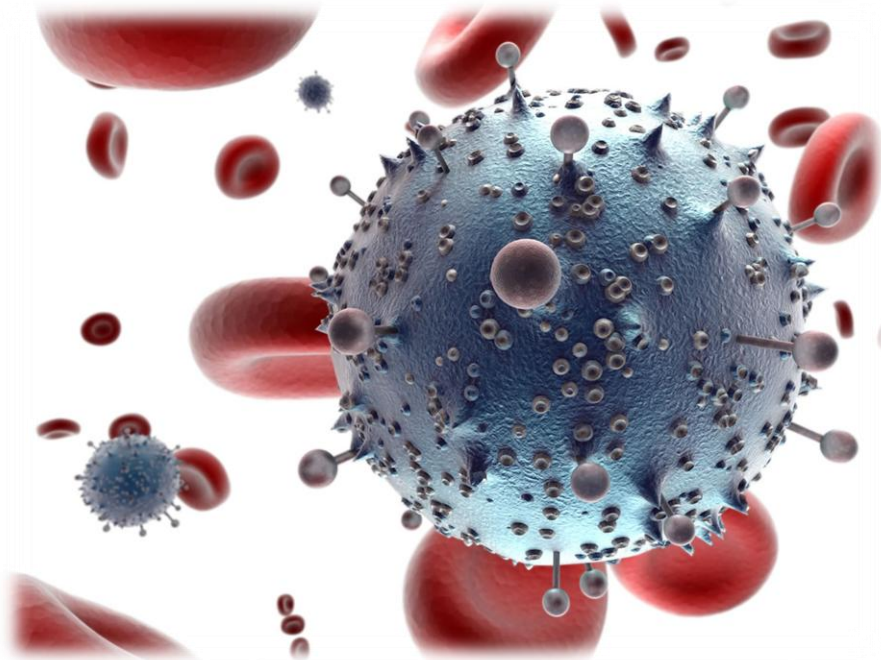
Received cDNA was used in RT-PCR (Reverse Transcription Polymerase Chain action). This method PCR is very sensitive





How can we use RT-PCR?

1. Examine the presence of retroviruses
2. Analysis of mRNA levels over time
3. Diagnosis of genetic diseases



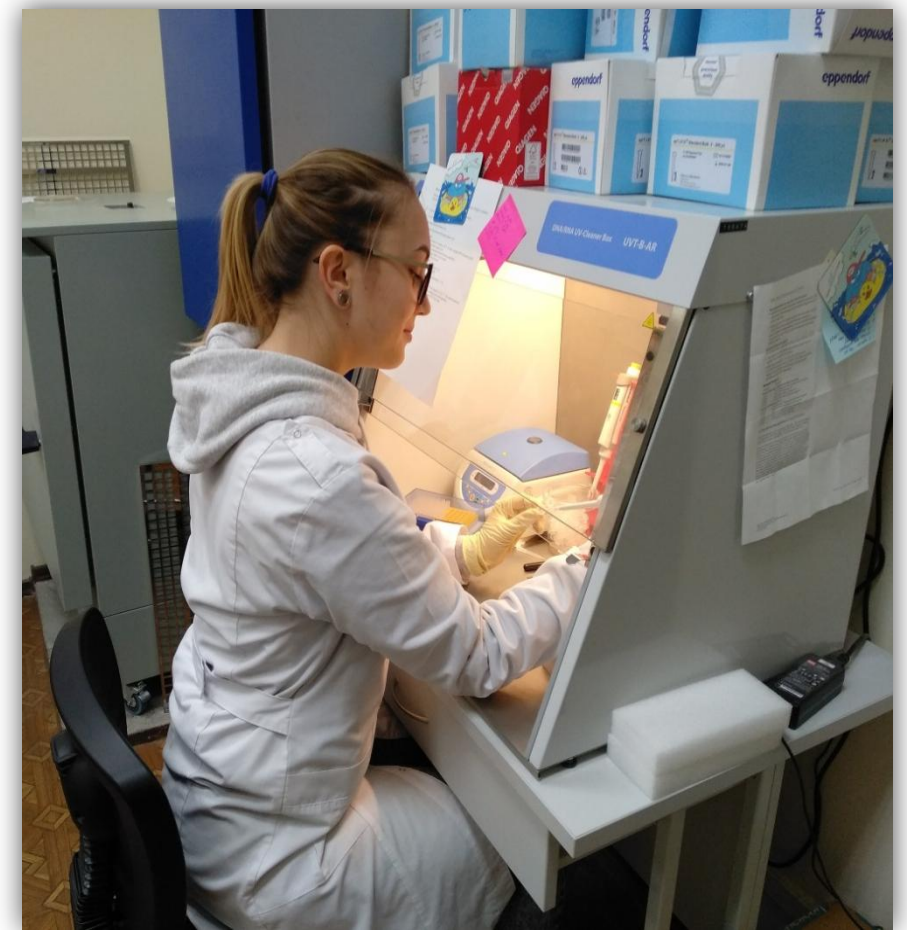


In our research, we estimated gene expression (yellow) of *Drosophila melanogaster* (pupae and flies)





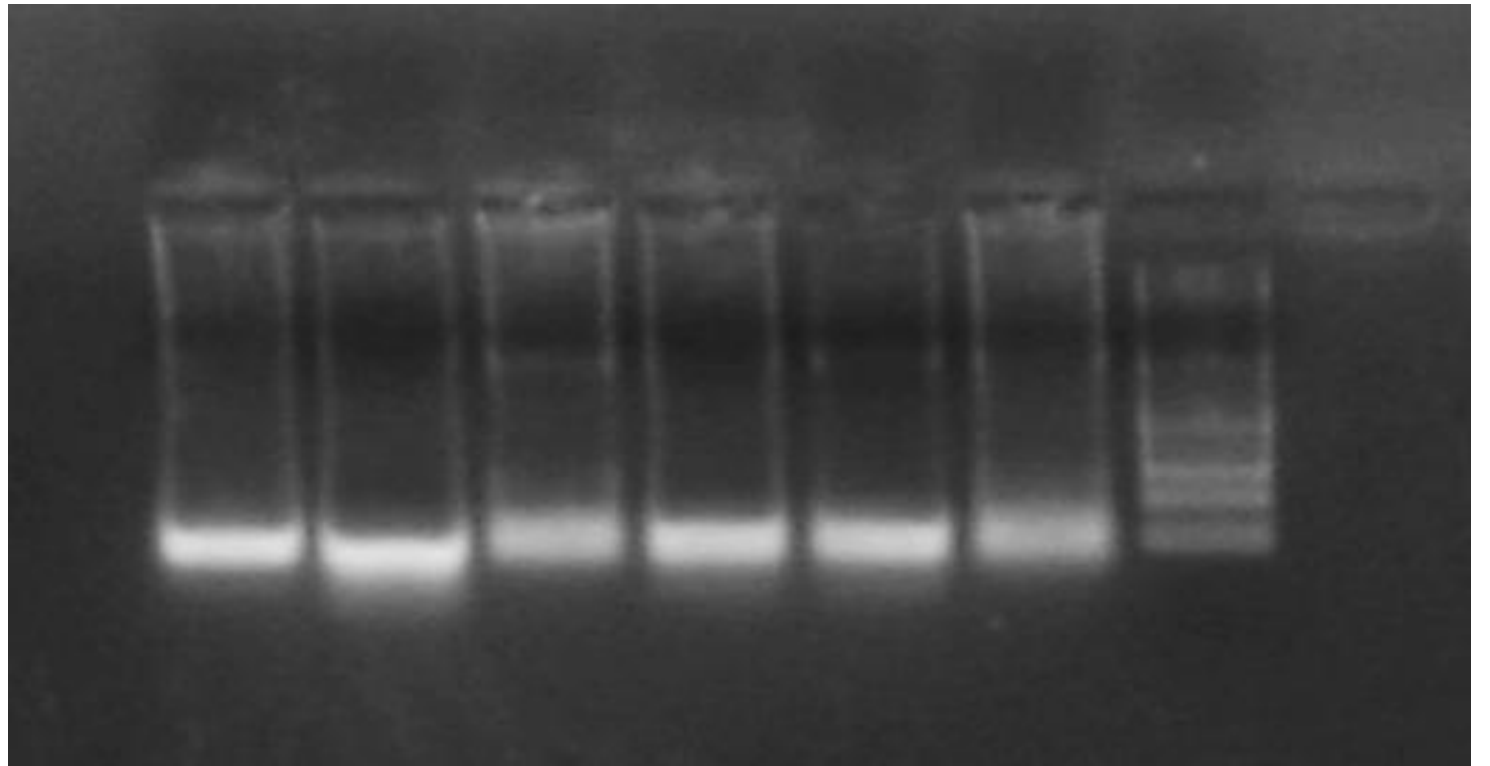
Extraction of drosophila's RNA was carried out in box and work on ice because this molecule is unstable



RNA extraction from Drosophila

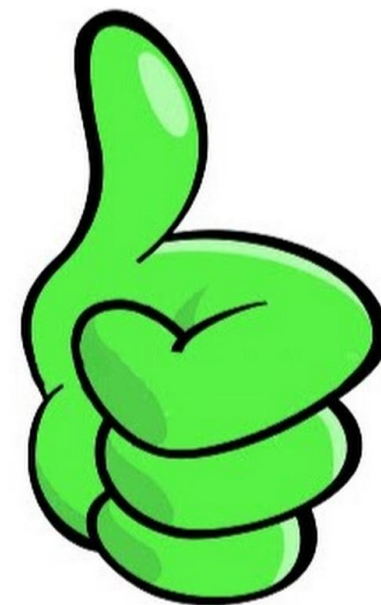
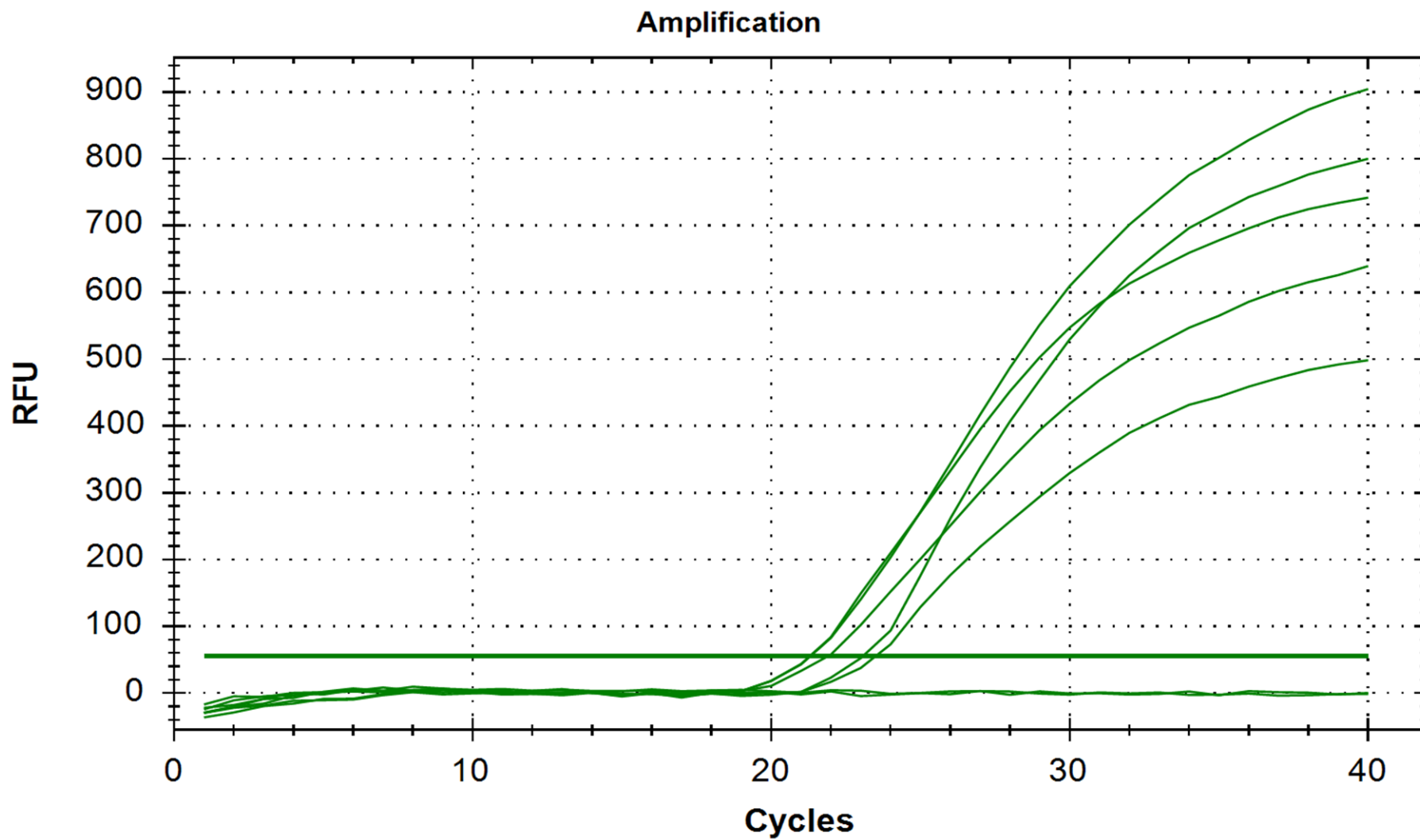
- We extracted RNA from drosophila flies and pupas

- The gel electrophoresis of the 6 samples show a successful extraction process with clear bands



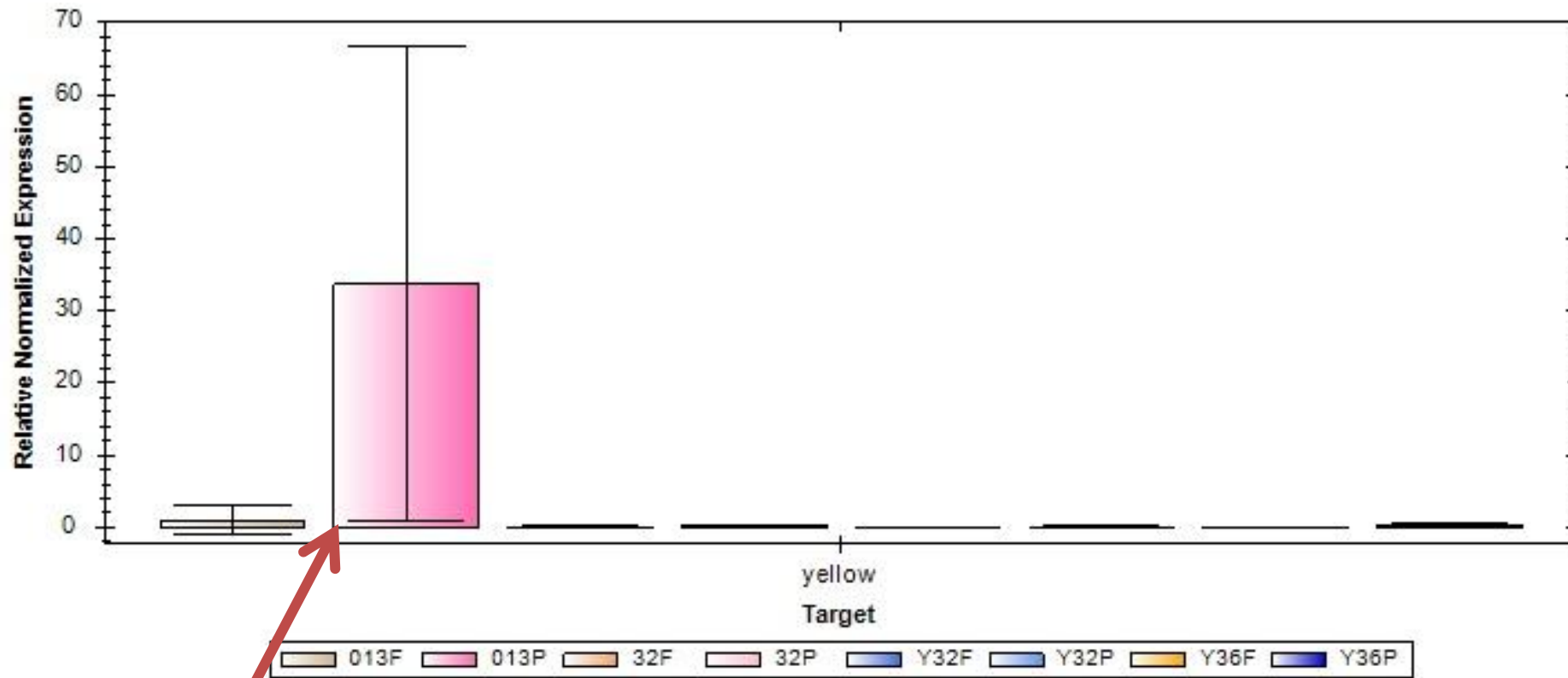


Results





Results



High level expression of pupas

*Thank You for your
attention!*

