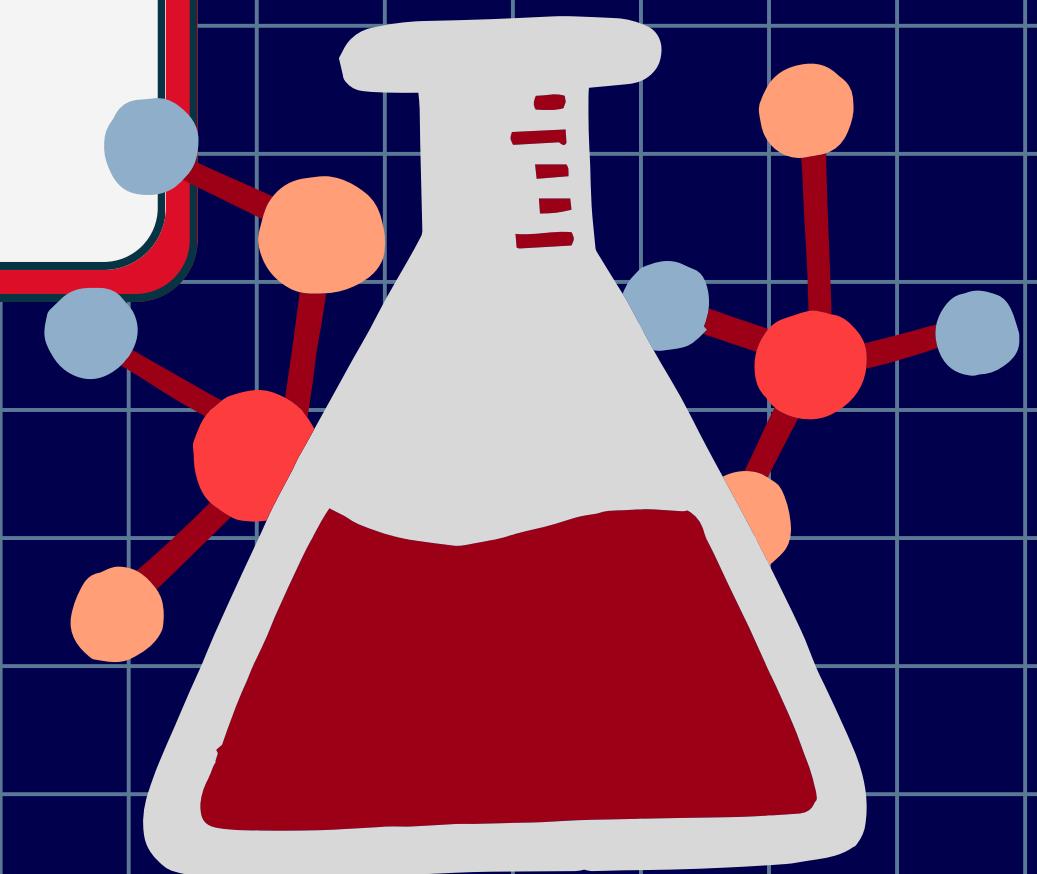


MOLECULAR BIOLOGY TRAINING PROGRAM (ON-JOB TRAINING REPORT)

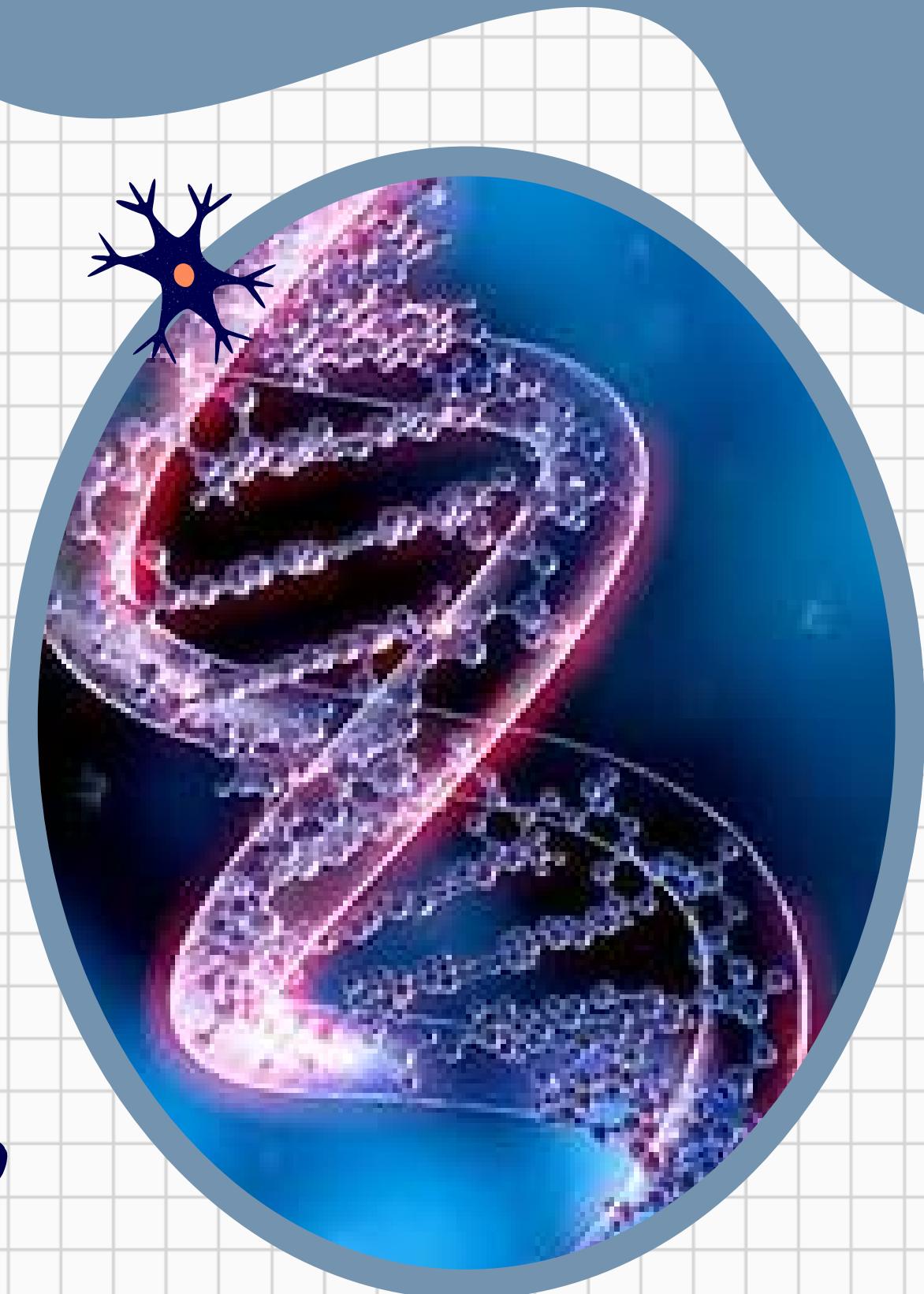
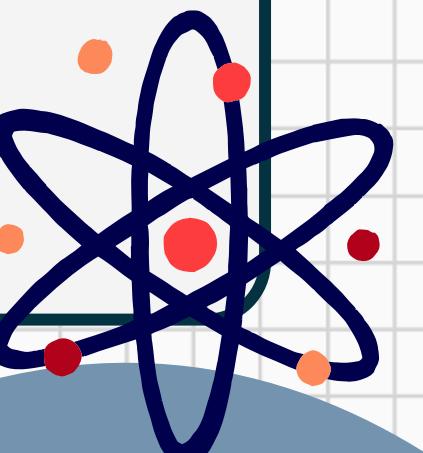
**NATIONAL FACILITY FOR
BIOPHARMACEUTICALS (NFB)**

**- PRARTHI KOTHARI
M. SC. BIOINFORMATICS (PART I)
ROLL NO. - 115**



CONTENTS

- Mentors
- Molecular Biology
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- DNA Ligation
- SDS-PAGE
- Polymerase Chain Reaction (PCR)
- DNA Sequencer
- Flow Cytometry
- RT-PCR
- References



MENTORS



Dr. Dinesh Kumar
Head of the Proteomics
and Protein
Characterization
Laboratory



Mr. Vikas Jha
Head and Laboratory
In-charge - Molecular
Biology Laboratory



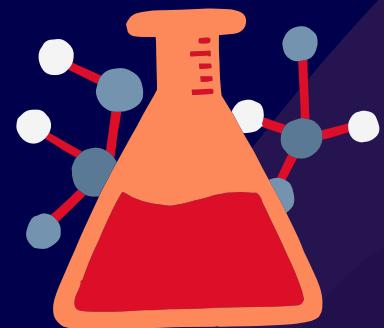
Ms. Valencia D'Souza
In-charge -
Mammalian Cell Culture
Department

MOLECULAR BIOLOGY

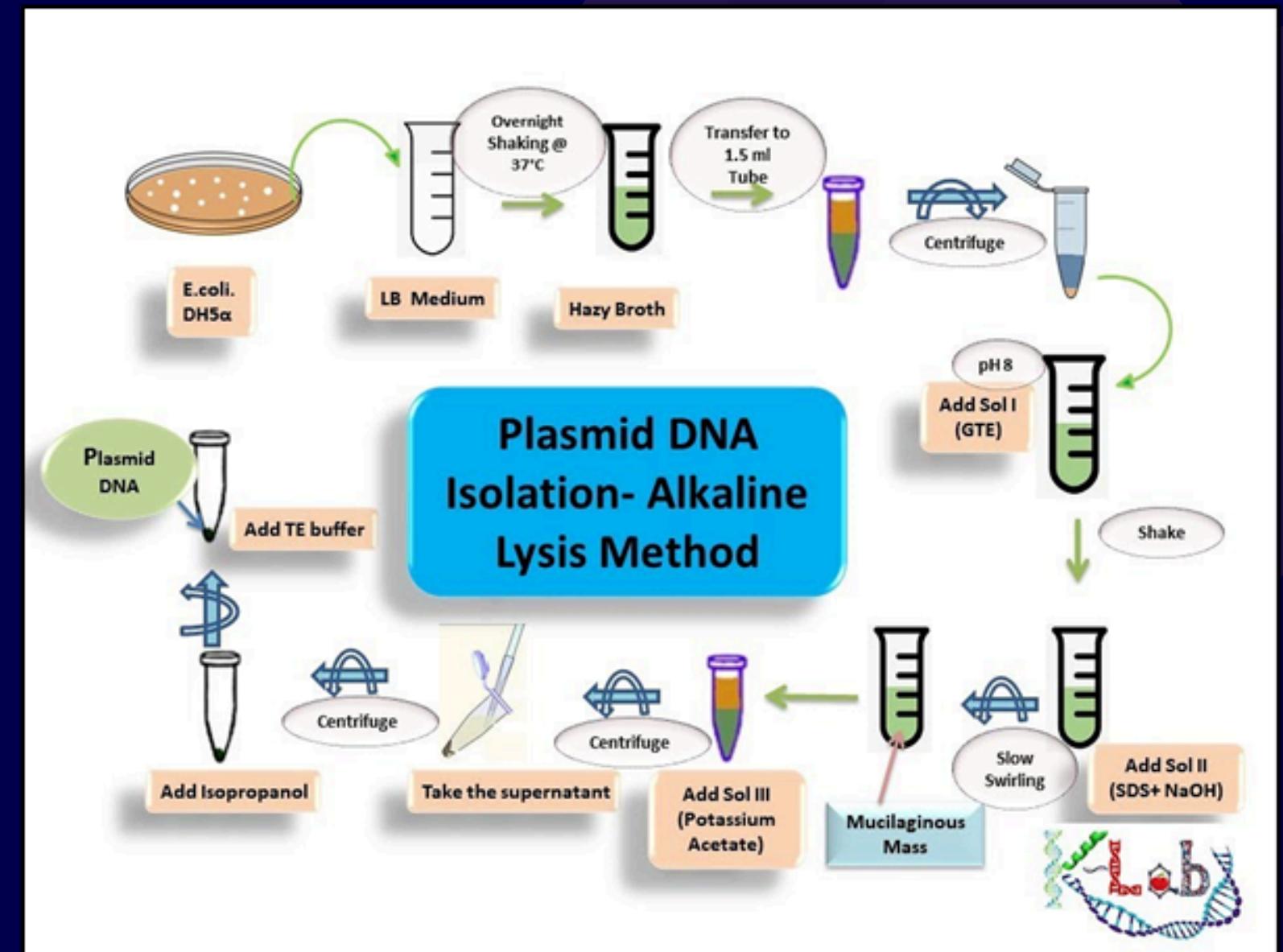
- understanding how molecules interact within living organisms to carry out life functions, with a particular emphasis on DNA, RNA, and proteins.
- Term 'molecular biology' - first used in 1945 by physicist William Astbury
- Advantage - powerful tool for genetic engineering, drug development, and personalized medicine
- Disadvantage - Ethical concerns and potential negative impact on biodiversity
- Applications - Genetic engineering, Biotechnology, forensics, diagnostics, personalized medicine, synthetic biology



ISOLATION OF PLASMID DNA



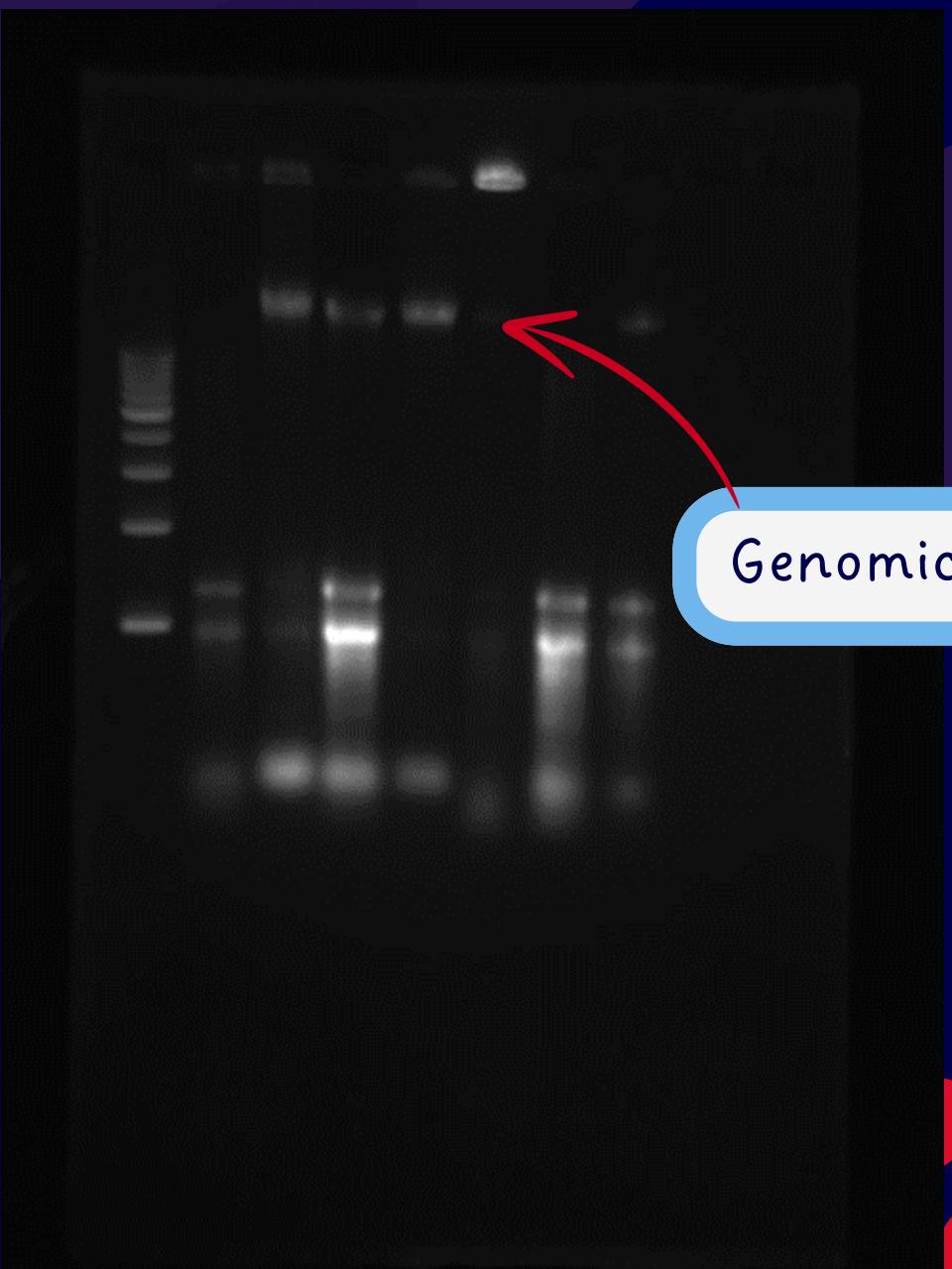
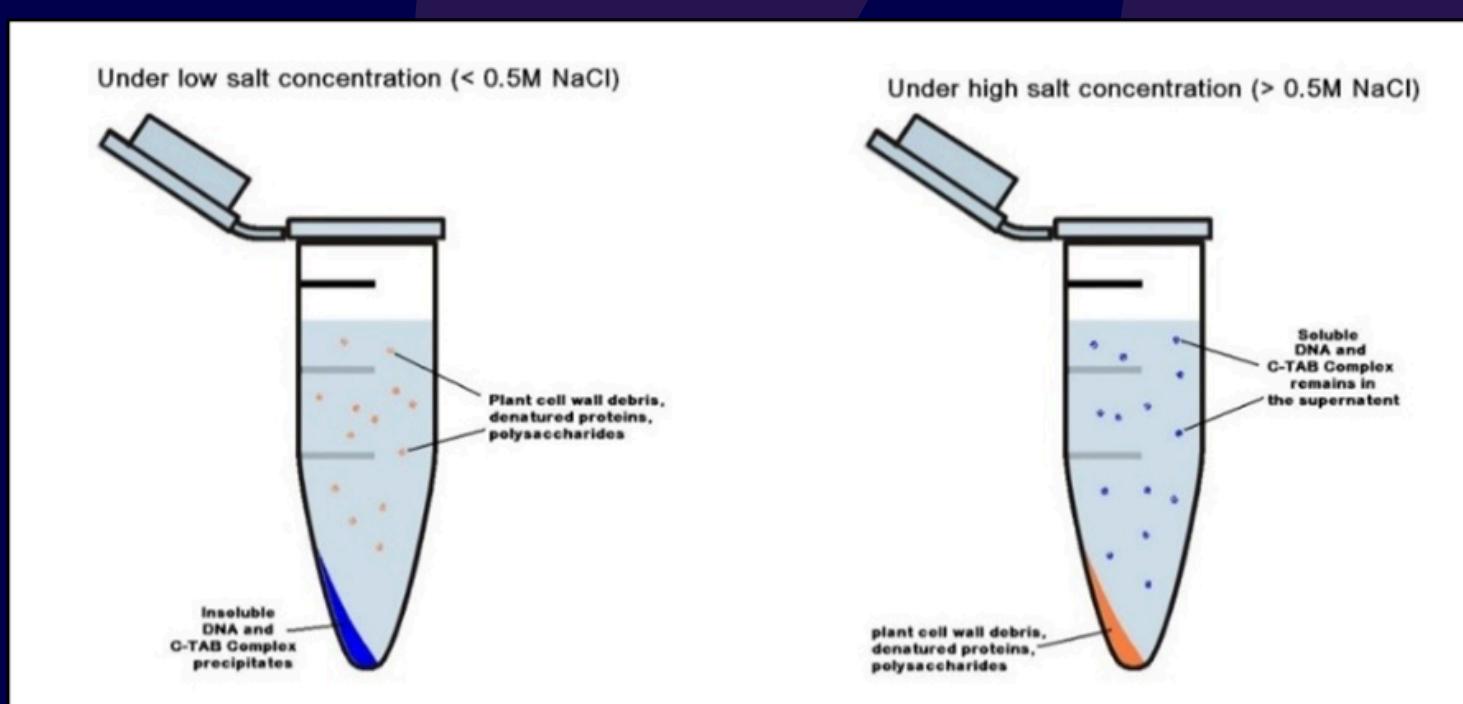
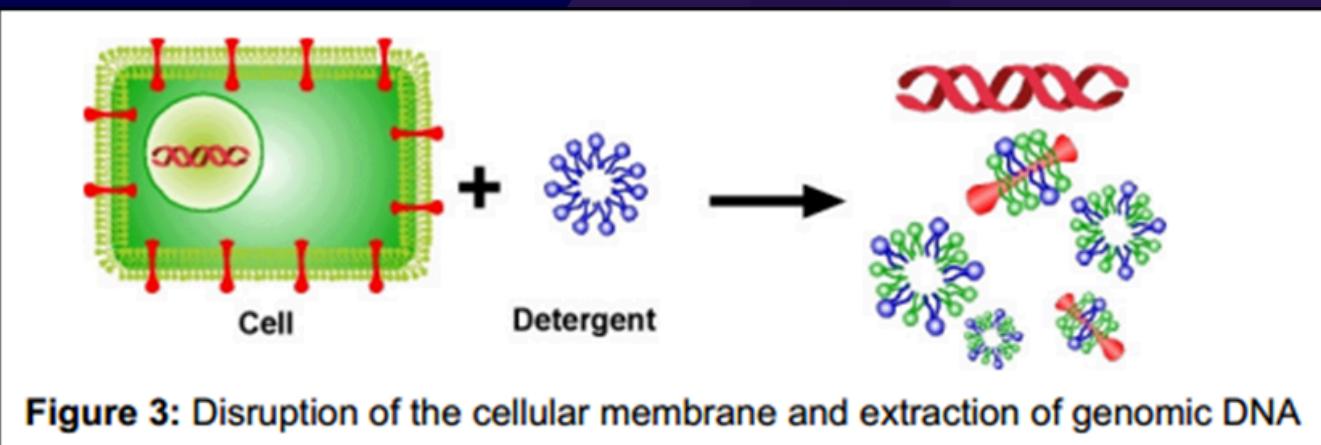
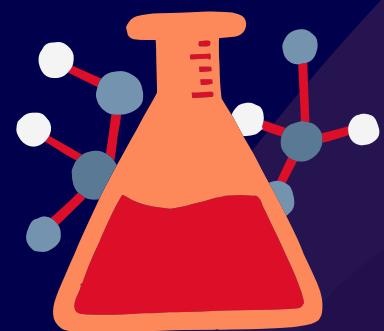
To isolate plasmid pET21b from E. coli culture using the alkaline lysis method.



ISOLATION OF GENOMIC DNA



To isolate
genomic DNA
from
Salmonella
typhimurium
bacteria



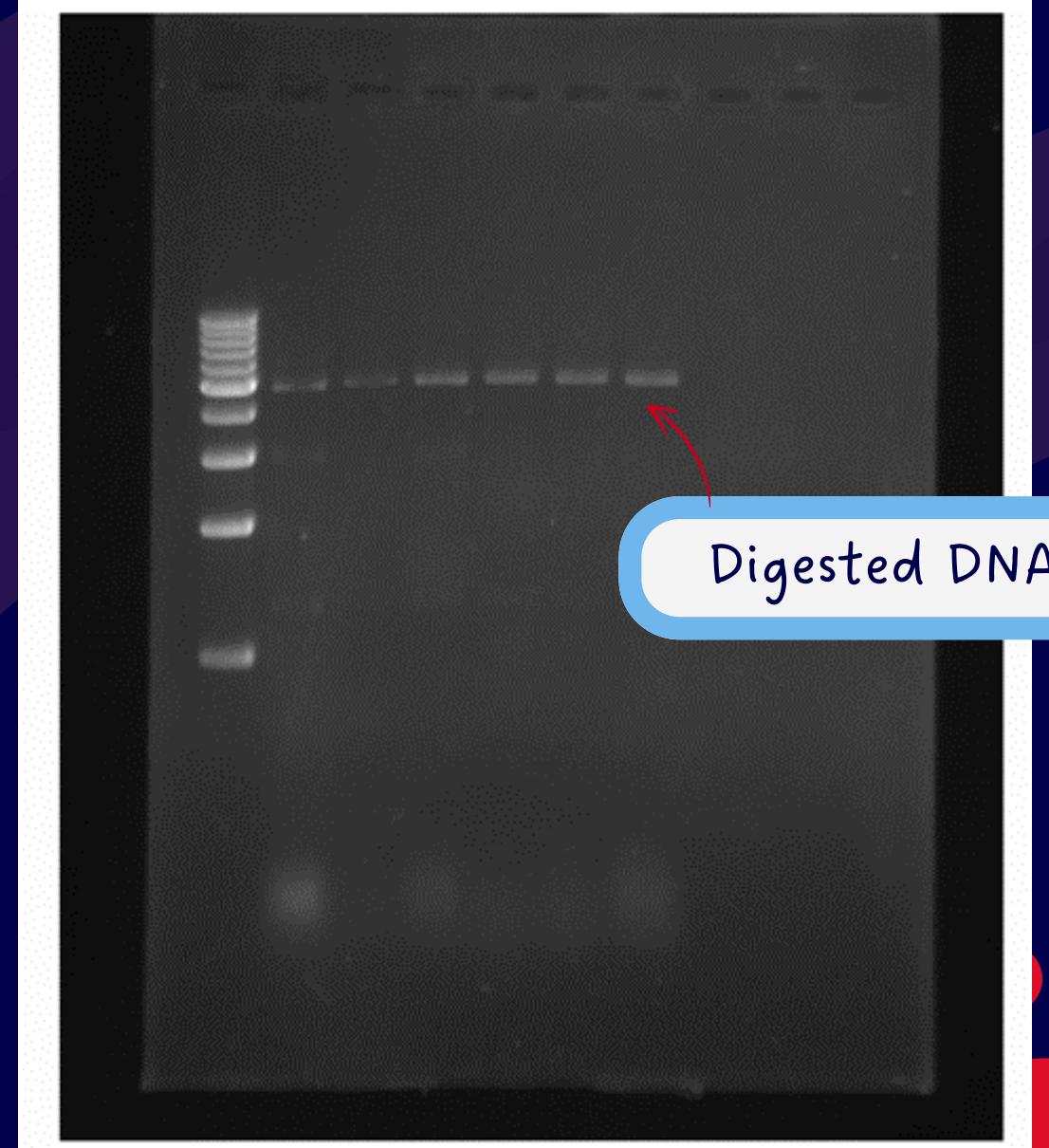
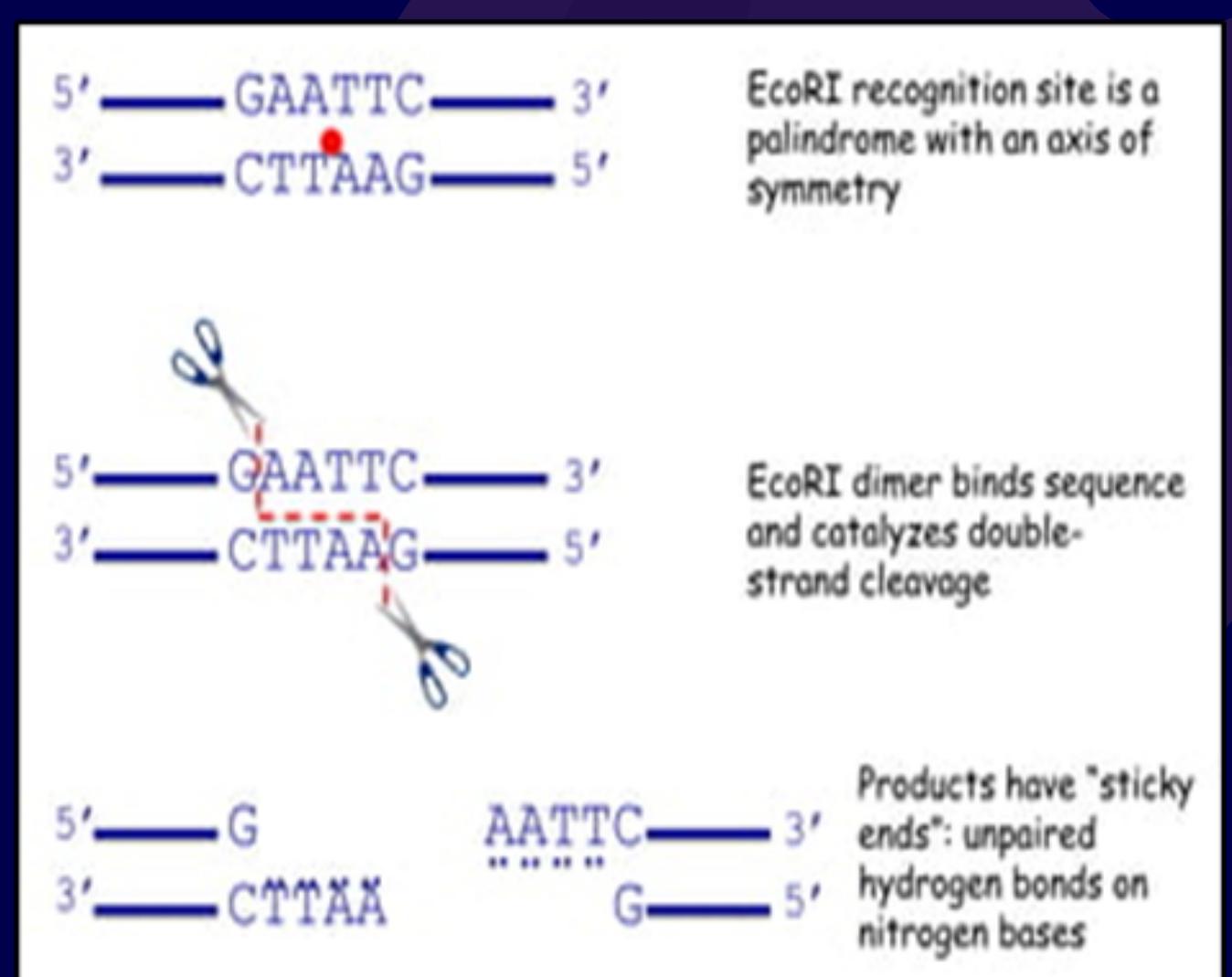
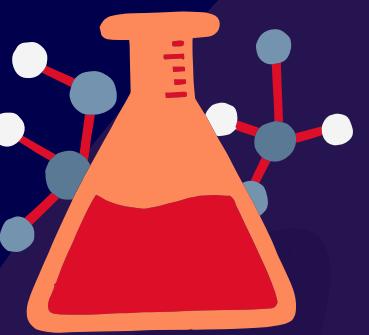
Genomic DNA

RESTRICTION ENZYME DIGESTION



To perform restriction enzyme digestion of the isolated plasmid.

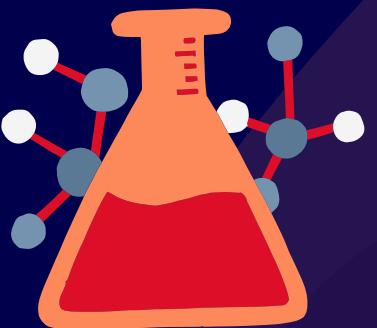
Reagents	Volume(µL)
Sterile distilled water	2
10X assay buffer	1
DNA sample	5
Restriction enzyme (<i>BamH1</i>)	1
Restriction enzyme (<i>Pst1</i>)	1
Total volume	10



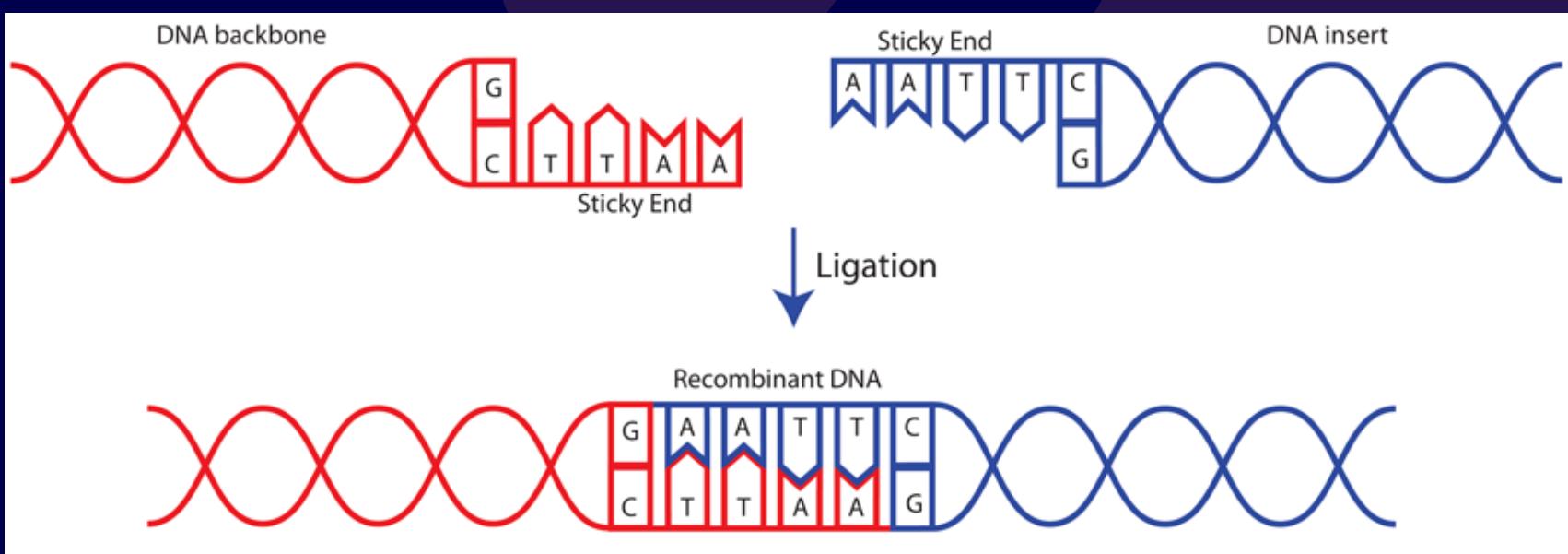
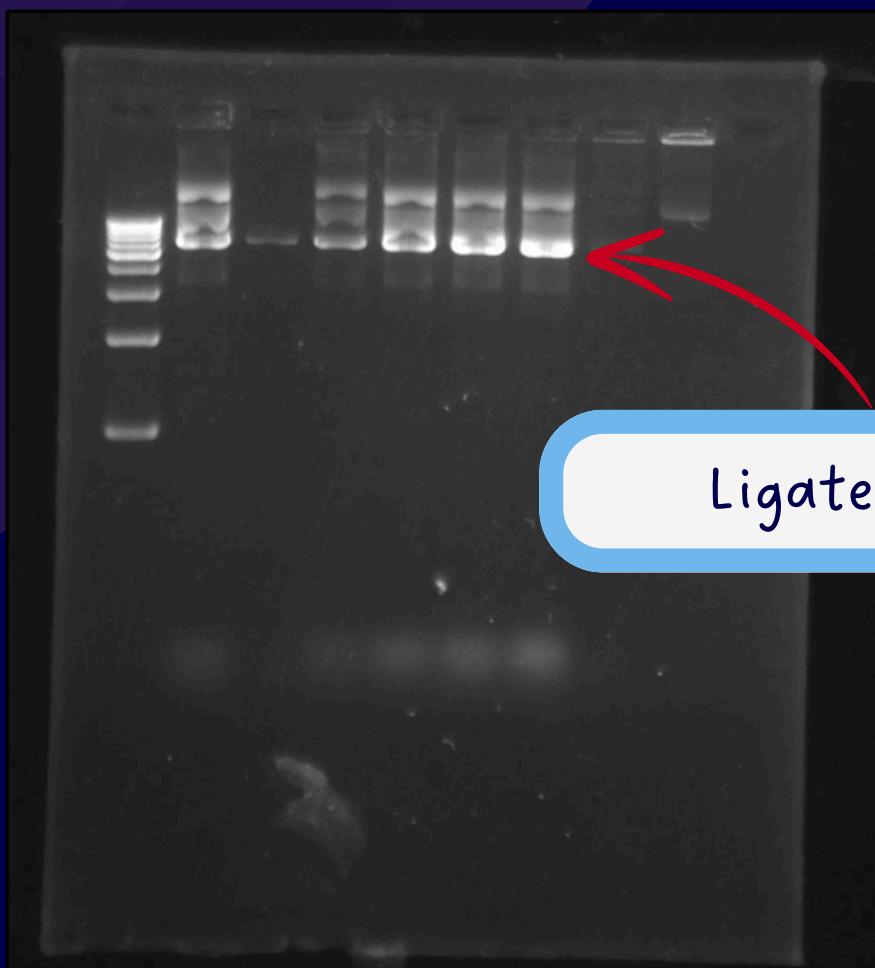
DNA LIGATION



To perform DNA ligation using T4 DNA ligase enzyme.



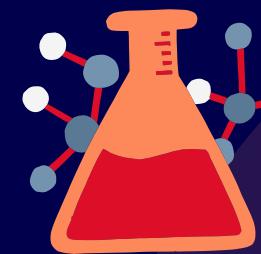
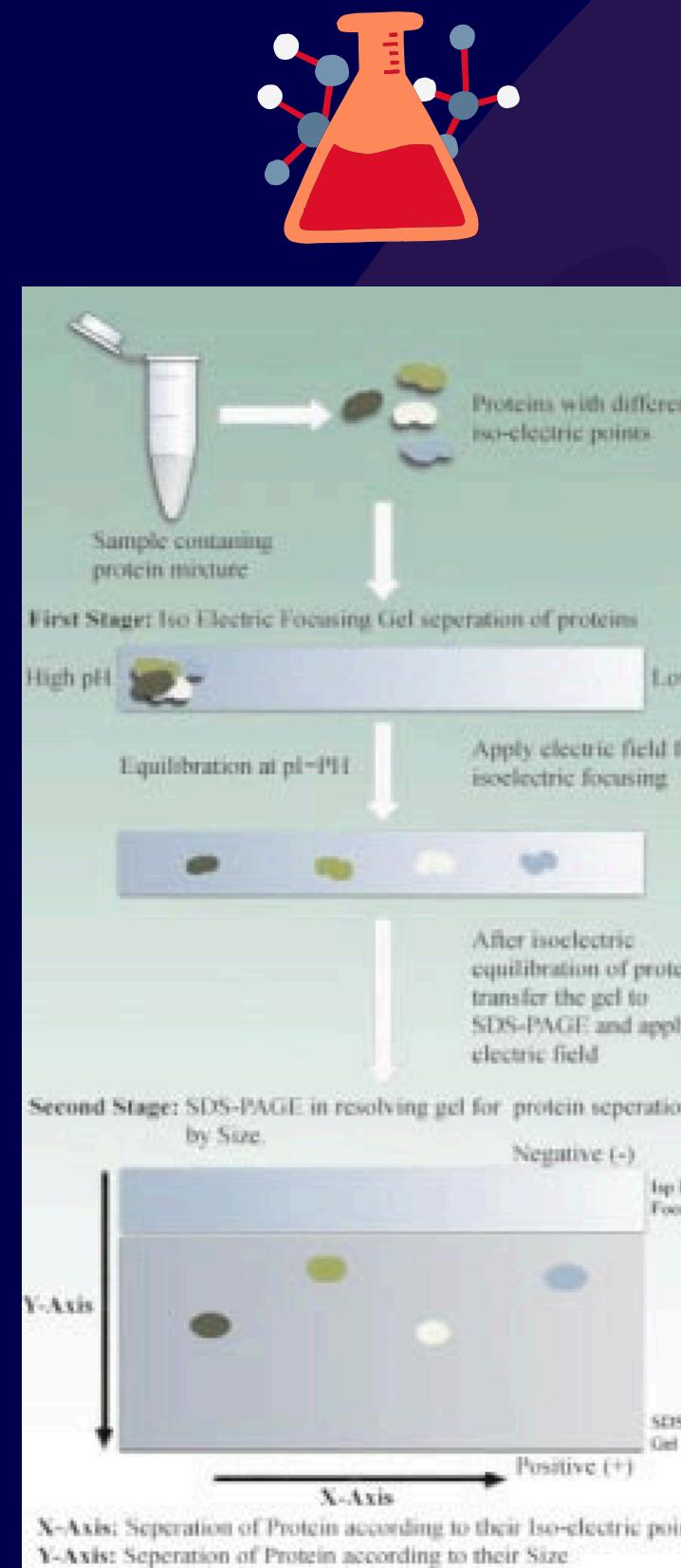
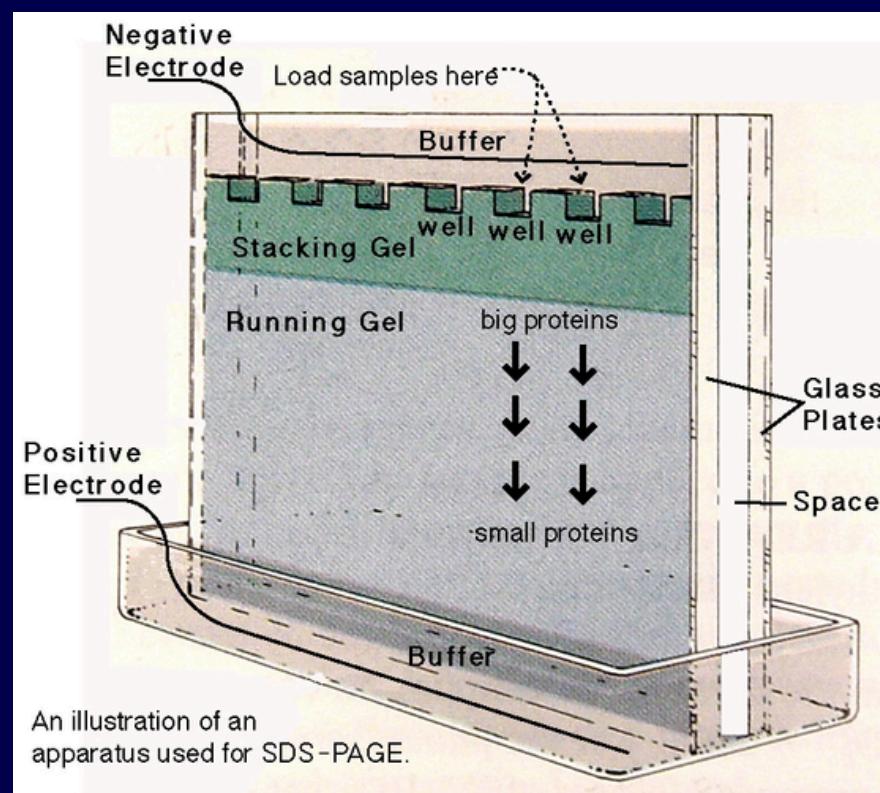
Chemicals	Volume (in μ l)
Vector volume (μ l)	5
Insert volume (μ l)	5
D/W (μ l)	8
10 X ligase buffer (μ l)	1
T4 DNA ligase (5 Weiss U/mL) (μ l)	1
Total volume (μ l)	20



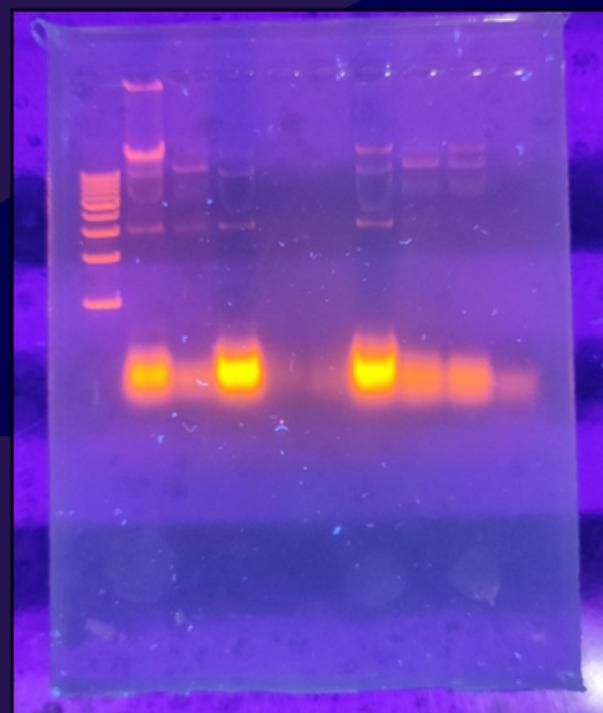
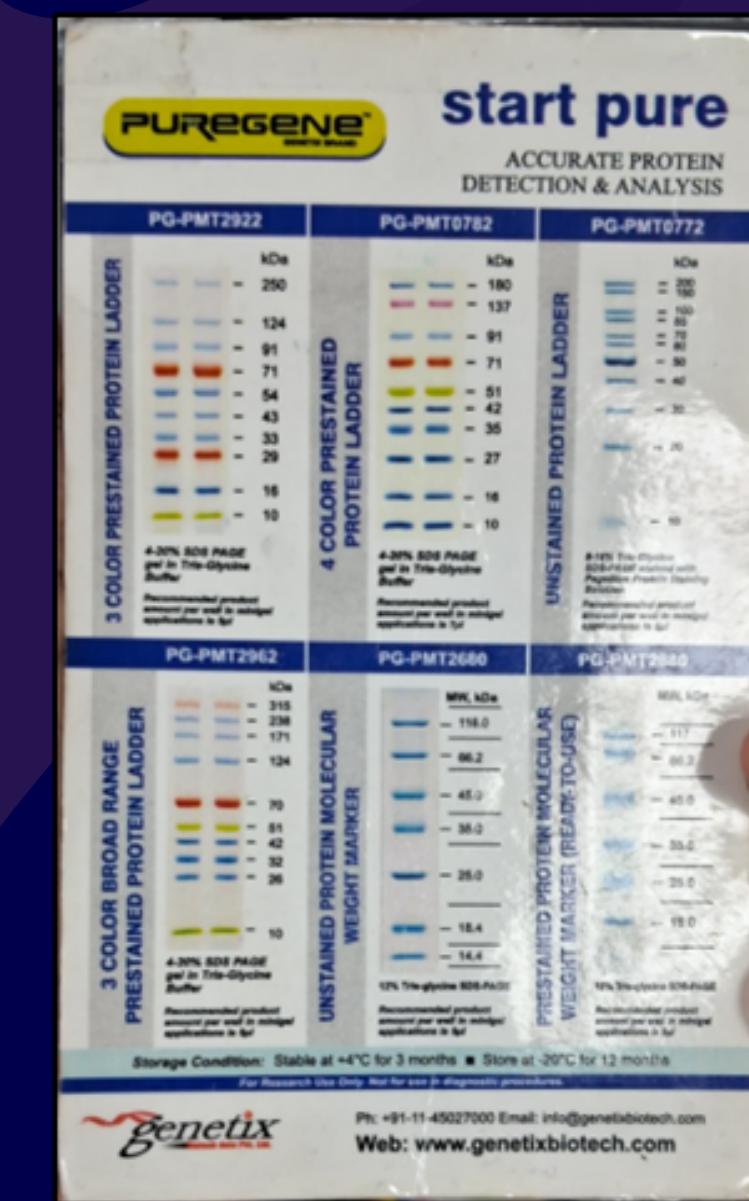
SODIUM DODECYL SULPHATE - POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)



To separate proteins on the basis of their molecular weights by the technique SDS-PAGE.



Separated proteins

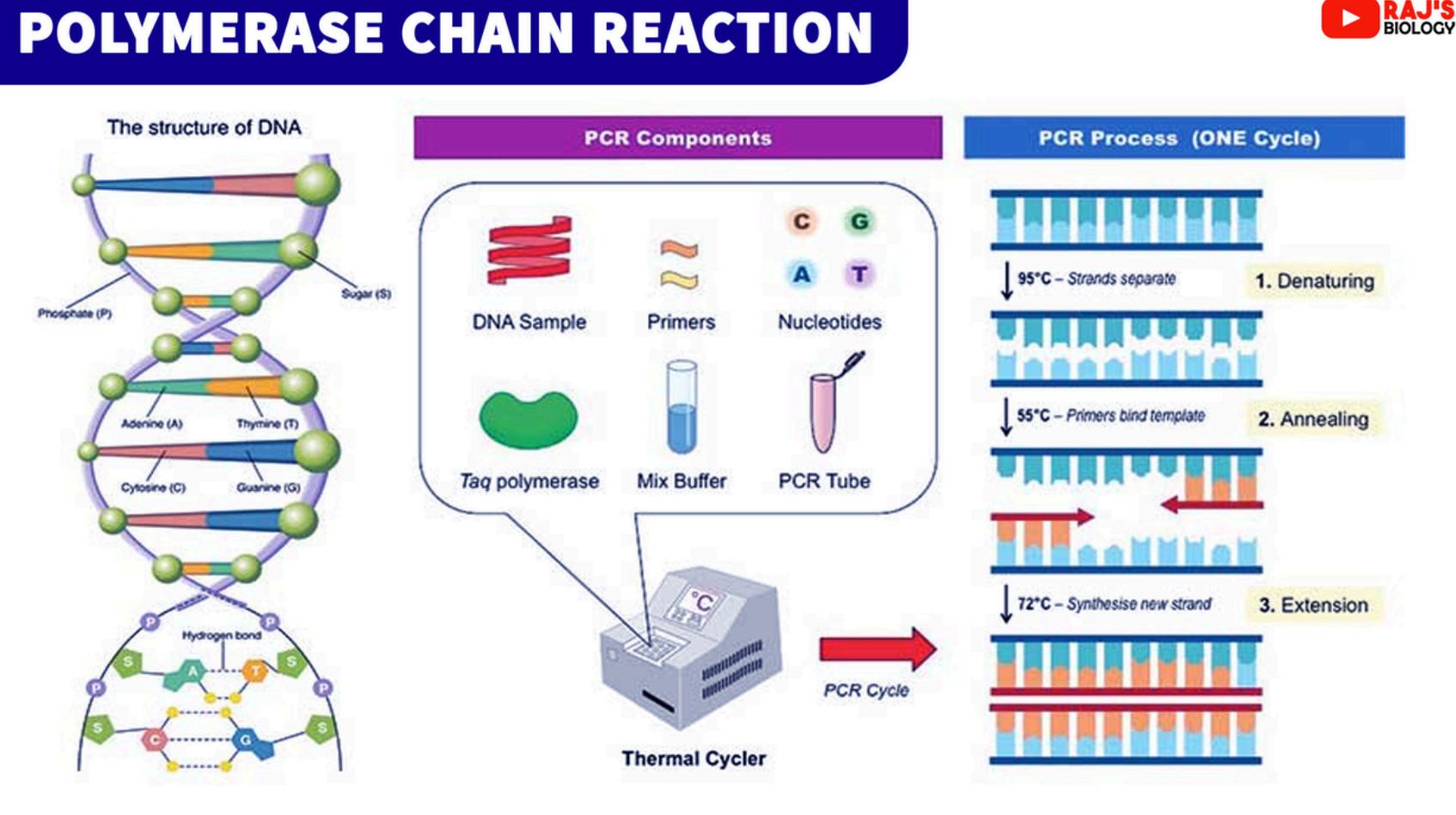
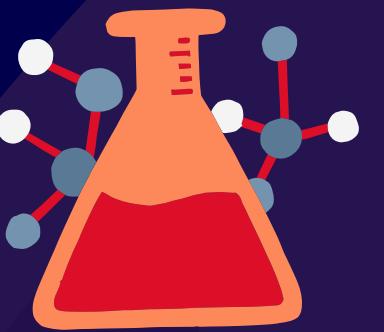


POLYMERASE CHAIN REACTION (PCR)

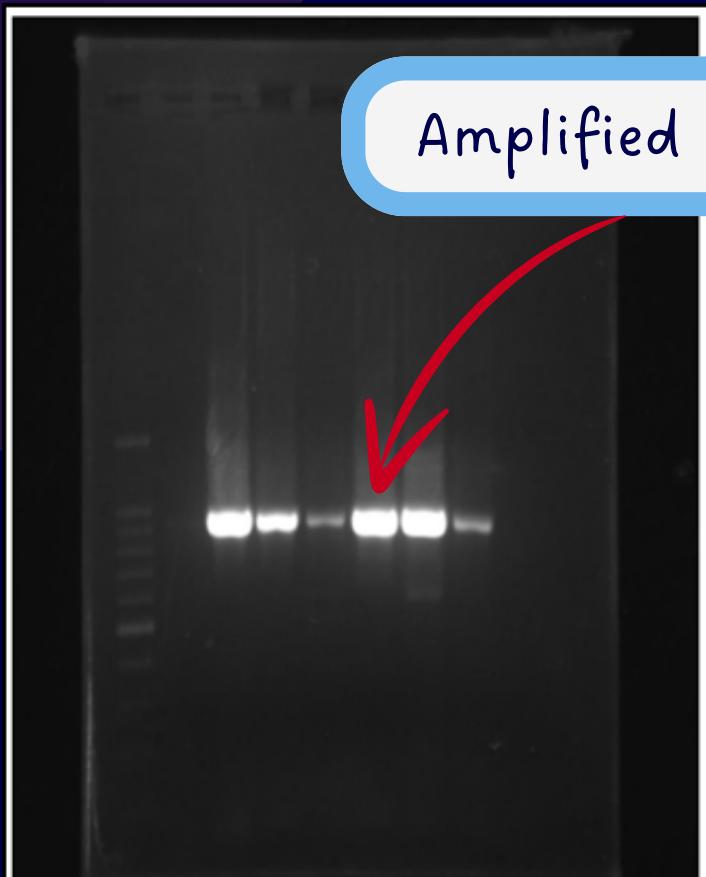


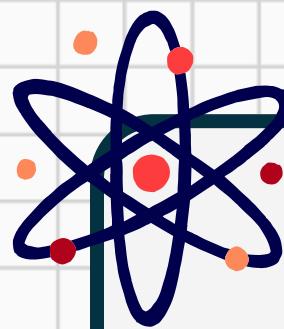
To amplify the given DNA sample by using specific primers in thermal cycler using PCR machine.

Reagents	Volume (μL)
Distilled water	15.5
10X Taq polymerase assay Buffer (MgCl_2)	2.5
dNTPs	0.5
Forward primers	2.5
Reverse primers	2.5
Taq DNA Polymerase	0.5
DNA Template	1
Total Reaction Mixture Volume	25



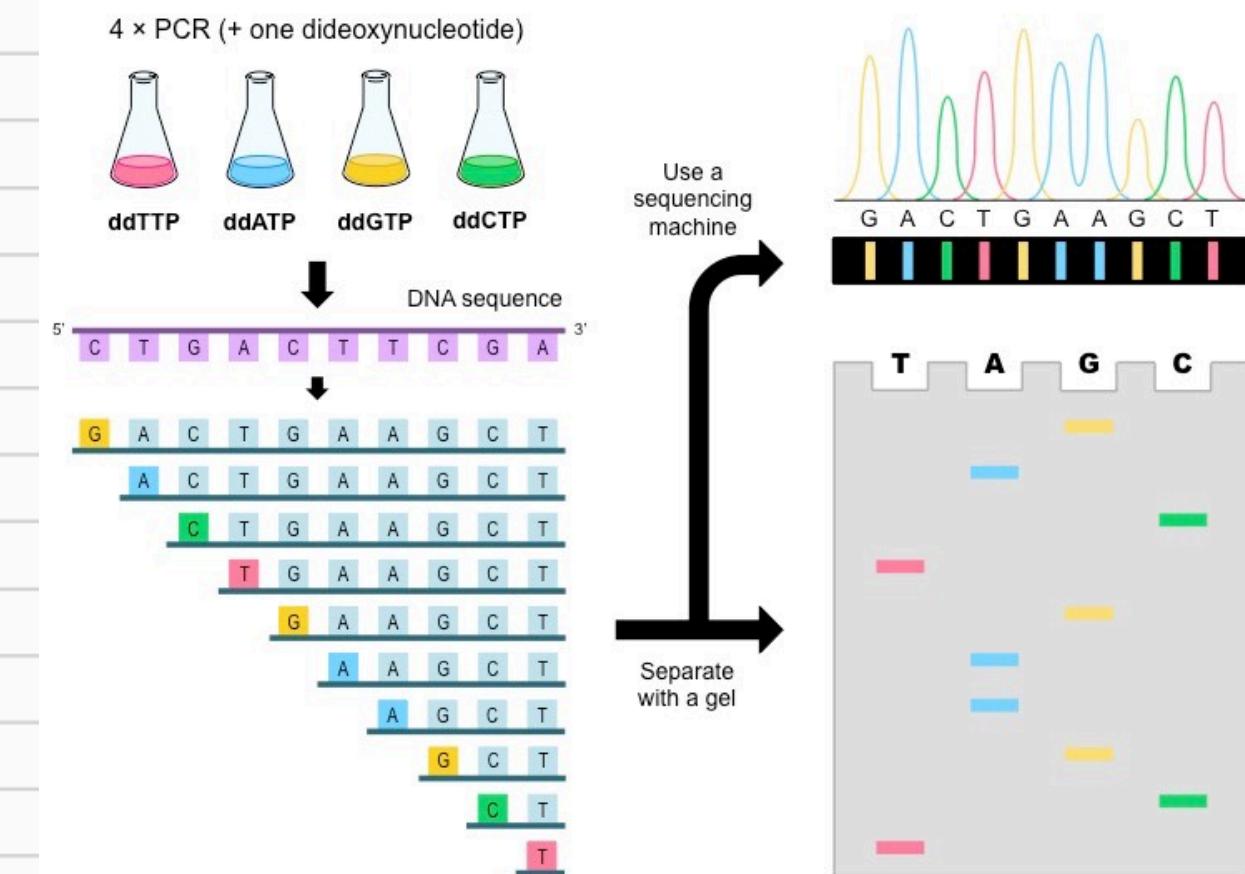
Amplified DNA

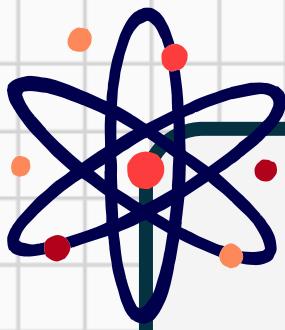




DNA SEQUENCER

- Sequencing = process of determining the nucleic acid sequence - the order of nucleotides in DNA
- DNA sequencers automate the process of identifying the order (sequence) of the four bases (adenine, cytosine, guanine, thymine) in a DNA sample.
- Common Sequencing Methods -
 1. Sanger Sequencing (Chain Termination Method) - Electrophoresis-based technique using dideoxynucleotides to terminate DNA replication during in vitro copying.
 2. Maxam and Gilbert Sequencing (Chemical Sequencing Method) - Chemical modification and subsequent cleavage of DNA backbone
- Advantages - High-throughput, High Accuracy and speed
- Disadvantages - Time-consuming, prone to errors, read length limitations
- Applications - Genomic Analysis, Personalized medicines, Disease diagnosis





STEPS OF DNA SEQUENCING

Sample preparation - DNA is extracted from the sample and purified to remove contaminants.



Library preparation - The DNA is fragmented into small pieces and adapters are added to each end to enable the DNA to be amplified and sequenced.



Amplification - The DNA fragments are amplified using PCR (polymerase chain reaction) to create multiple copies of each fragment.



Sequencing - The amplified DNA is loaded onto the sequencer, which reads the DNA sequence. There are different sequencing technologies available, including Illumina, PacBio, and Oxford Nanopore, among others.



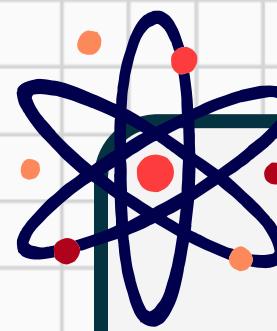
Base calling - After the sequencing is complete, the raw data is processed by a computer to identify the base calls (A, C, G, or T) for each read.



Assembly - The reads are then assembled into longer contiguous sequences (contigs) using specialized software.

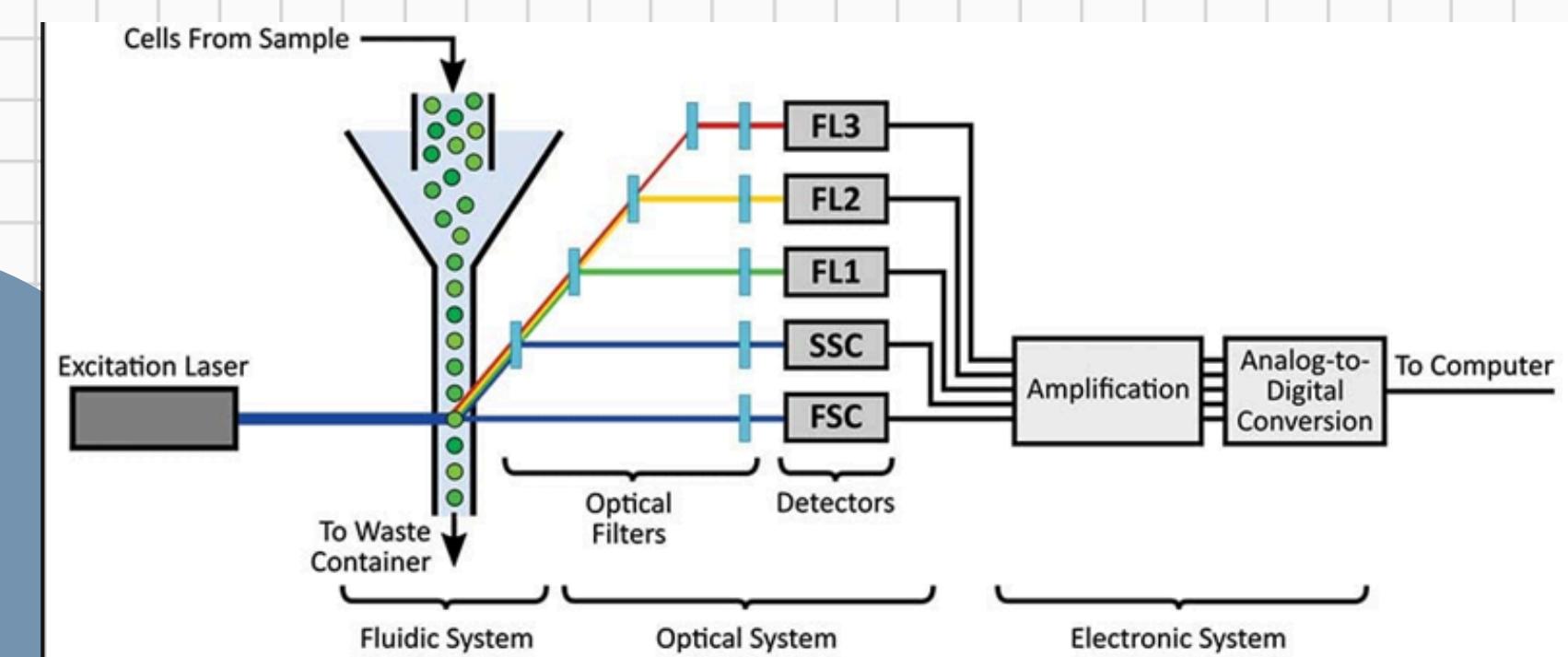


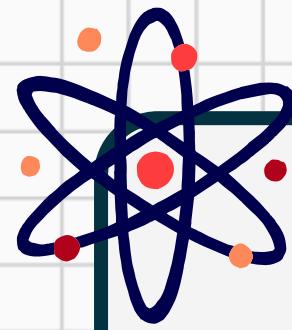
Analysis - The assembled sequences are compared to reference genomes or other databases to identify genes, mutations, or other features of interest.



FLOW CYTOMETRY

- technique designed for counting, examining, and sorting microscopic particles suspended in a fluid stream
- In flow cytometry, a focused stream of fluid is illuminated by a single-wavelength beam of light, typically generated by a laser. Several detectors are strategically positioned at the point where the fluid stream intersects with the light beam. Among these detectors are one aligned with the light beam, known as Forward Scatter (FSC), and several positioned perpendicular to it, including Side Scatter (SSC) and one or more fluorescent detectors.
- As suspended particles ranging from 0.2 to 150 μm pass through the light beam, they scatter the light in various ways. Additionally, fluorescent chemicals within the particles or attached to them may be excited, emitting light at a higher wavelength than the light source. The detectors capture this combination of scattered and fluorescent light. By analyzing fluctuations in brightness at each detector (with one for each fluorescent emission peak), diverse information about the physical and chemical structure of each individual particle can be derived.

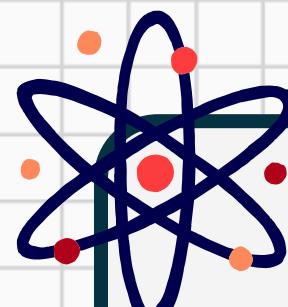




COMPONENTS OF FLOW CYTOMETER

1. Flow cell: liquid stream (sheath fluid) carries and aligns the cells so that they pass single file through the light beam for sensing.
2. Optical system: commonly used are lamps (mercury, xenon); high power water-cooled lasers (argon, krypton, dye laser); low power air-cooled lasers (argon (488nm), red-HeNe (633nm), green-HeNe, HeCd (UV)); diode lasers (blue, green, red, violet) resulting in light signals.
3. Detector and Analogue to Digital Conversion (ADC) system: generating FSC and SSC as well as fluorescence signals from light into electrical signals that can be processed by a computer.
4. Amplification system
5. Computational analysis of the signals.

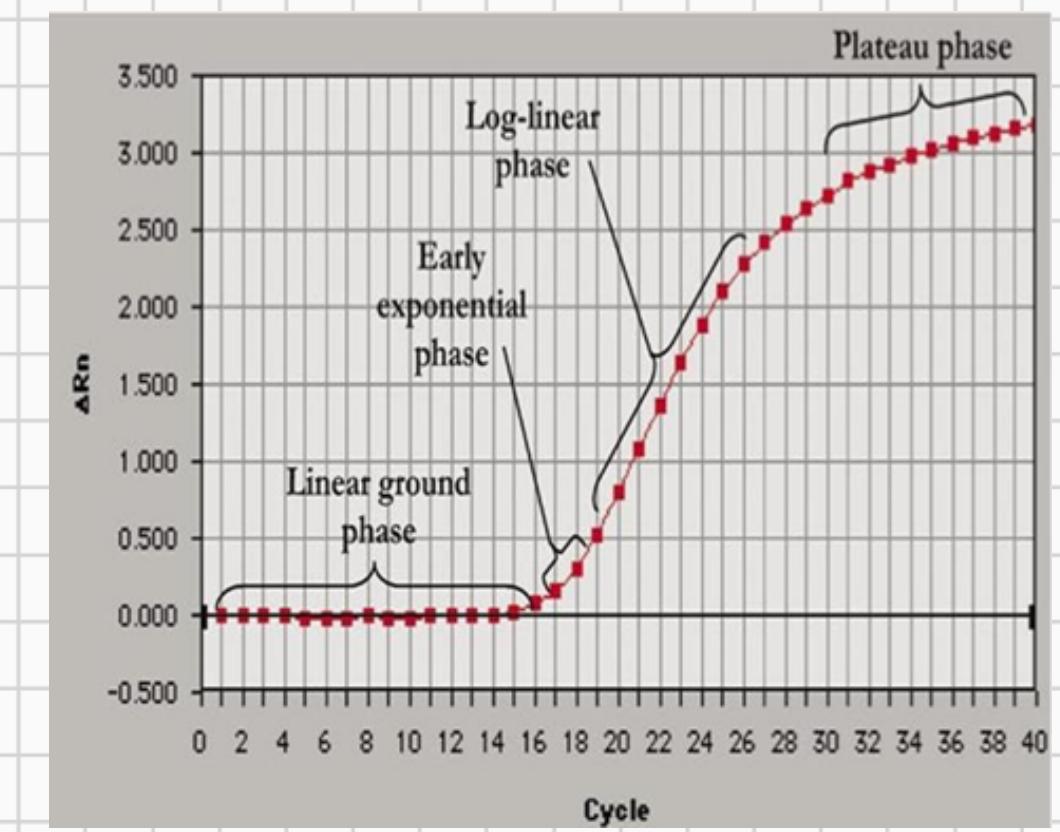
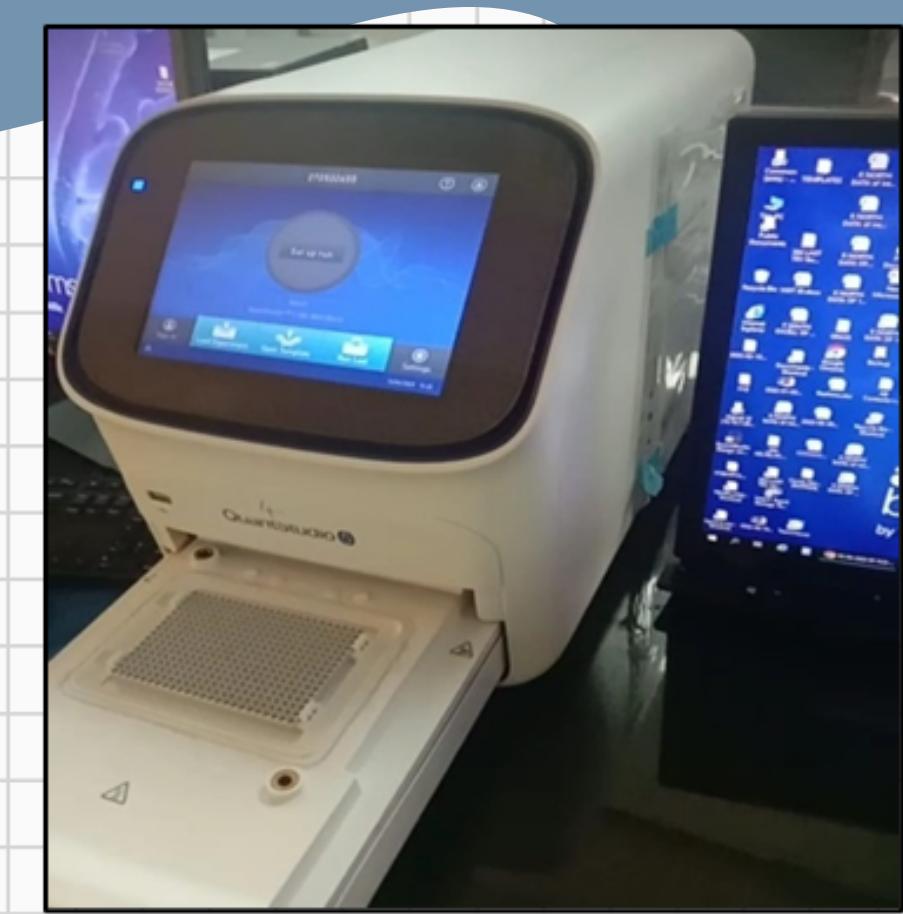


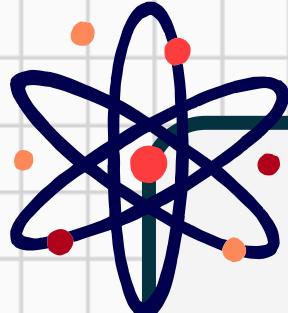


REAL-TIME POLYMERASE CHAIN REACTION (RT-PCR)

- Detects and quantifies specific nucleic acid sequences in real-time by amplifying a target DNA sequence using primers and DNA polymerase.
- Process -
Reverse transcription (RT) - RNA converted to complementary DNA (cDNA)

↓
PCR Amplification - Generates multiple copies of the target cDNA sequence through cycles of denaturation, annealing, and extension.
- PCR Phases -
 1. Linear ground phase (baseline fluorescence established).
 2. Early exponential phase (fluorescence increases, Ct value defined).
 3. Log-linear phase (optimal amplification, product doubles per cycle).
 4. Plateau phase (reaction components limited, fluorescence not useful for data).
- Applications - Gene expression analysis, cancer research





STEPS OF RT-PCR

DNA Extraction

Isolate DNA from the sample.

Primer Design

Create specific primers for the target DNA sequence.

Amplification

1. Denaturation - Separate double-stranded DNA into single strands at high temperature (95°C).
2. Annealing - Hybridize primers to complementary sequences on the target DNA at a specific temperature (based on primer Tm).
3. Extension - Extend primers using DNA polymerase at optimal temperature (70-72°C).

Real-time Monitoring

- o Use fluorescent marker and thermal cycling to detect amplified DNA.
- o Fluorescence intensity increases with more DNA copies.
- o Detect signal exceeding a threshold level.

Data Analysis

Analyze data using software to quantify the original DNA sample.

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THANK YOU!

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