THE VIRAL BLUEPRINT

IMMUNEMAP 2025

A Hands-on Workshop on PCR Innovations, Dengue NS1 RT-PCR, RNA Techniques & 3D Epitope Mapping

Organised By

Graduate School o Science

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WORKSHOP DURATIONS

3 Days

ELIGIBILITY

Any UG & PG Students in

Life Science / Allied Disciplines are Eligible

Fees *1800/- Only*

Pay & Send a Screenshot to The Given WhatsApp Number

Scan This QR & Fill Up The Form to Register

Introduction to Different Types of PCR (Polymerase Chain Reaction)

PCR, or Polymerase Chain Reaction, is a revolutionary molecular biology technique used to amplify specific DNA sequences. Since its invention by Kary Mullis in 1983, PCR has evolved into various specialized forms tailored for diverse applications in research, diagnostics, forensics, agriculture, and medicine. Each variant of PCR is designed to overcome specific challenges or meet unique experimental goals.

1. Standard Diagnostic PCR

- **Description:** Basic PCR method to detect the presence or absence of a pathogen or gene.
- **Application:** Used in routine diagnostics (e.g., detecting bacteria or viruses like Dengue, TB, COVID-19).
- **Output:** Amplified DNA visualized using agarose gel electrophoresis.

2. Nested PCR

- **Description:** Involves two rounds of PCR using two sets of primers; the second set is "nested" within the first.
- **Purpose:** Increases specificity and reduces non-specific amplification.
- **Application:** Detecting low-copy targets or degraded DNA.

Introduction to Different Types of PCR (Polymerase Chain Reaction)

3. Hemi-nested PCR

- **Description:** A variation of nested PCR where only one primer in the second round is internal.
- **Purpose:** Offers high specificity with less complexity than full nested PCR.
- **Application:** Diagnostic assays requiring enhanced sensitivity with fewer primers.

4. Touchdown PCR

- **Description:** Starts with a high annealing temperature that decreases gradually in subsequent cycles.
- **Purpose:** Reduces non-specific binding and improves primer-template specificity
- **Application:** Useful in amplifying targets with complex or GC-rich sequences.

5. Touch-up PCR

- **Description:** Opposite of touchdown PCR—annealing temperature increases gradually.
- **Purpose:** Encourages primer binding to low-affinity sites in early cycles.
- **Application:** Used when initial binding is weak or template is highly variable.

Introduction to Different Types of PCR (Polymerase Chain Reaction)

6. Quantitative PCR (qPCR / Real-Time PCR)

- **Description:** Amplifies and simultaneously quantifies DNA using fluorescent dyes or probes.
- **Purpose:** Measures gene expression levels or viral load in real-time.
- **Application:** Diagnostics (e.g., HIV, COVID-19), oncology, pharmacogenomics.

7. Reverse Transcription PCR (RT-PCR)

- **Description:** Converts RNA into complementary DNA (cDNA) using reverse transcriptase, followed by PCR.
- **Purpose:** Detects and quantifies RNA (e.g., viral RNA, gene expression).
- **Application:** Dengue, Influenza, SARS-CoV-2 diagnostics, mRNA studies.

8. Multiplex PCR

- **Description:** Uses multiple primer sets in a single PCR reaction to amplify several targets.
- **Purpose:** Saves time and reagents; allows simultaneous detection.
- **Application:** Respiratory virus panels, genetic screening, forensic profiling.

9. Next-Generation PCR (NGPCR)

- **Description:** High-efficiency PCR methods integrated into NGS workflows.
- **Purpose:** Enables large-scale, high-fidelity amplification with barcoding for sequencing.
- **Application:** Genomic studies, metagenomics, personalized medicine.

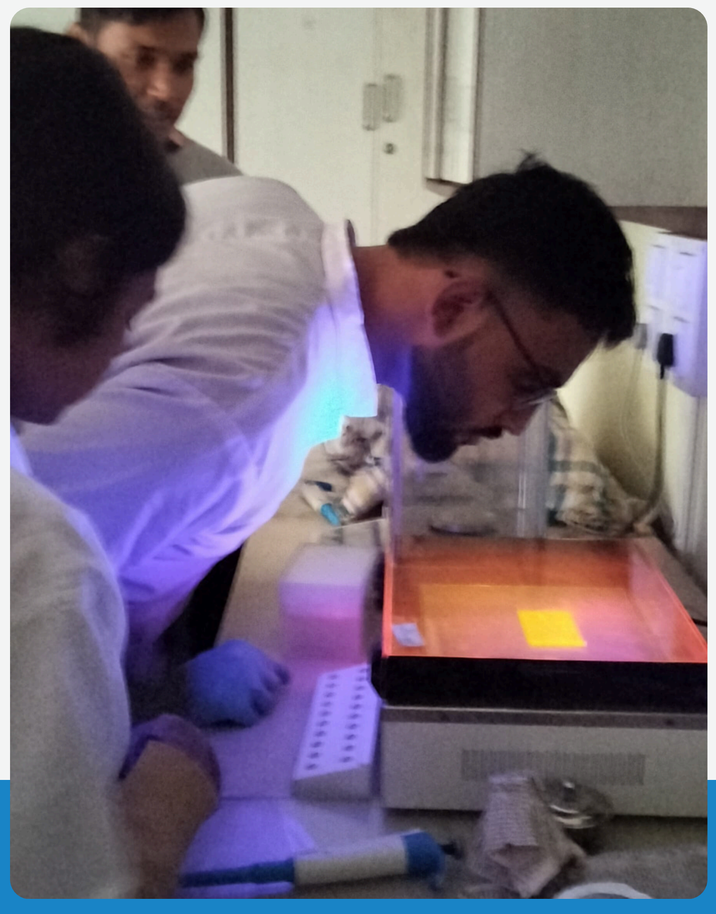
Gene Targeting and Primer Design in Diagnostics

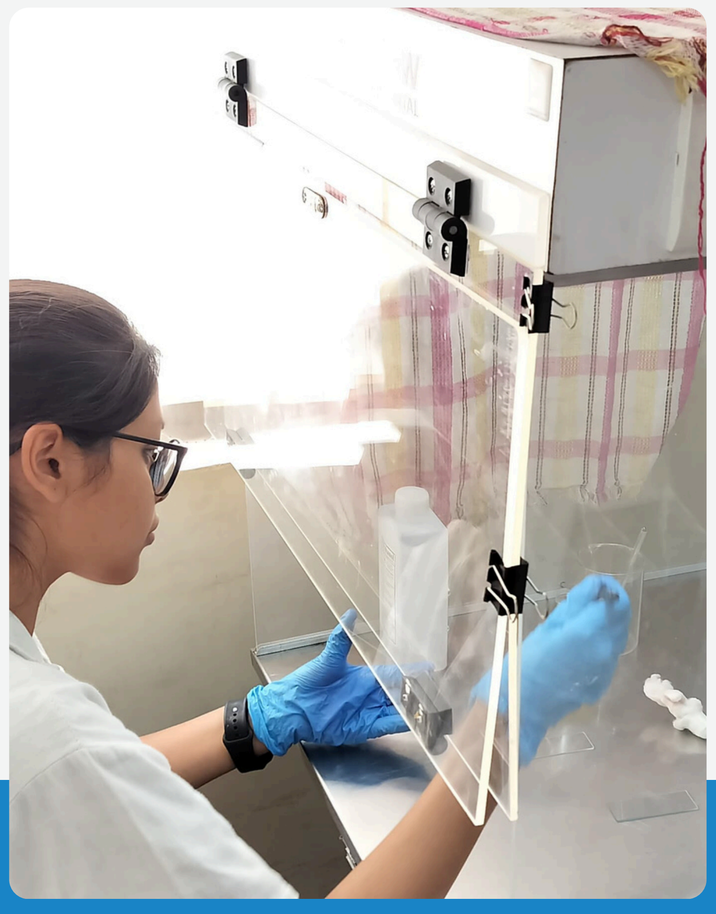
🔬 Gene Targeting Strategy

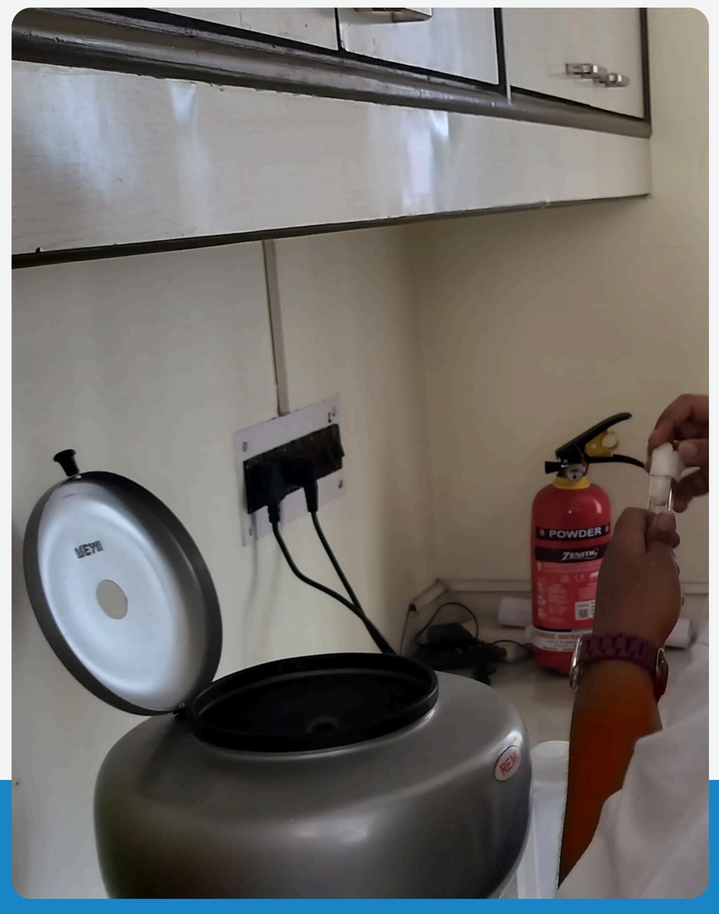
- To select suitable gene regions and design primers with high specificity and sensitivity for accurate diagnosis.
- Gene Targeting Strategy: Use conserved gene regions specific to the pathogen (e.g., NS1 gene in Dengue, RdRp in SARS-CoV-2). Avoid regions prone to mutation or recombination.

🧬 Primer Design Criteria

- Length: 18–24 bp
- Tm: 55–65°C
- GC content: 40–60%
- No significant secondary structures or self-complementarity
- Designed using tools like Primer3, NCBI Primer-BLAST, SnapGene







RT-PCR for NS1 Gene Amplification and Analysis

- This workflow outlines the step-by-step procedure to detect the NS1 gene of the Dengue virus using Reverse Transcription PCR (RT-PCR), a critical molecular diagnostic technique. Each step is designed to ensure accuracy in RNA handling, gene amplification, and visualization.

1. Sample Setup

The first and most crucial step in RT-PCR is the collection and preparation of biological samples, typically serum or plasma from suspected dengue-infected individuals.

RNA Extraction:

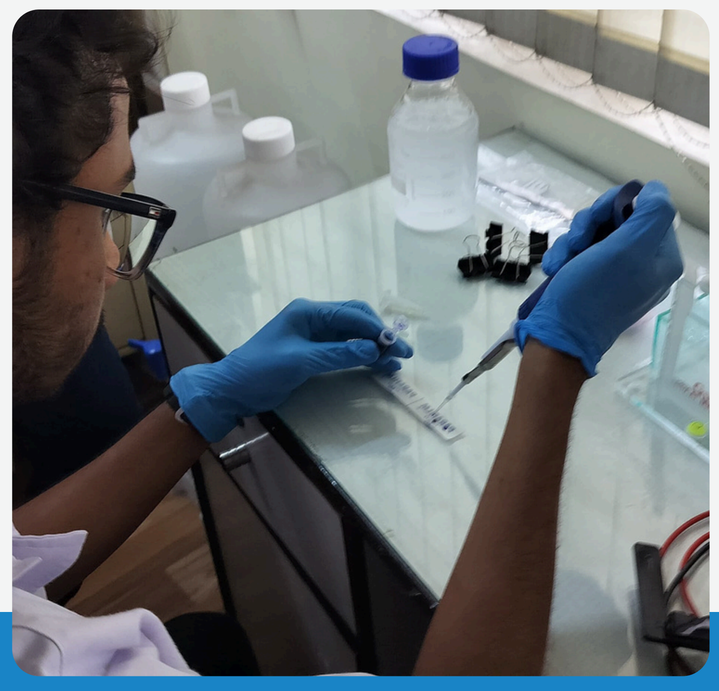
- Use a high-quality viral RNA extraction kit or TRIzol reagent.
- Maintain an RNase-free environment to prevent degradation.
- Work in chilled conditions and use RNase inhibitors.

Quality Check:

- Measure RNA concentration and purity using a spectrophotometer (e.g., NanoDrop).
- An ideal A260/A280 ratio for pure RNA is between 1.8 and 2.1.

Aliquoting and Storage:

- Store extracted RNA at -80°C for long-term use.
- Avoid repeated freeze-thaw cycles.



RT-PCR for NS1 Gene Amplification and Analysis

🧪 2. Master Mix Preparation

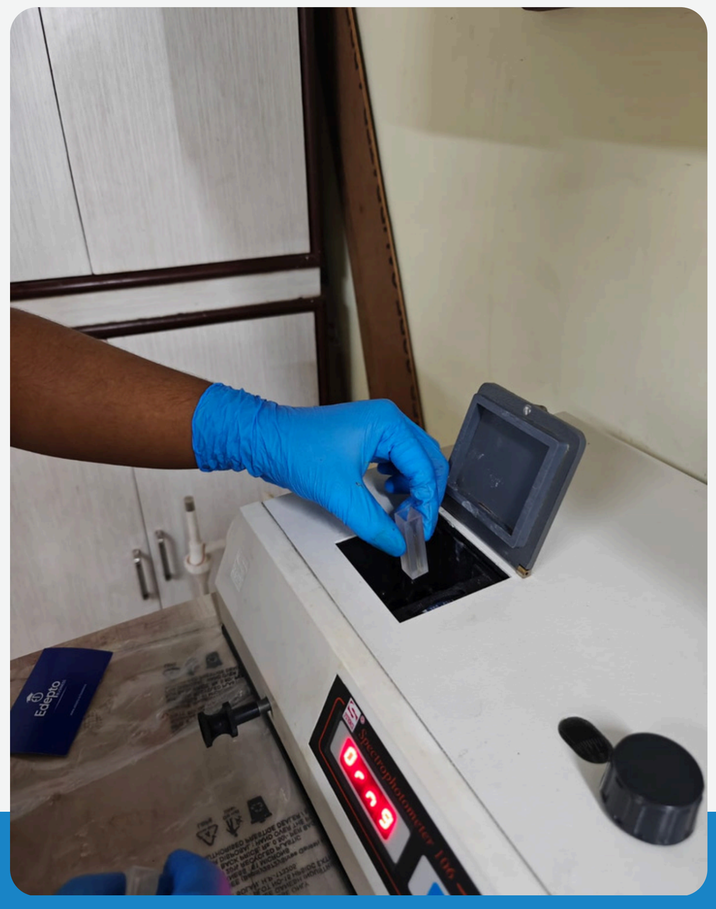
The RT-PCR Master Mix contains all the reagents required for reverse transcription and amplification of the NS1 gene.

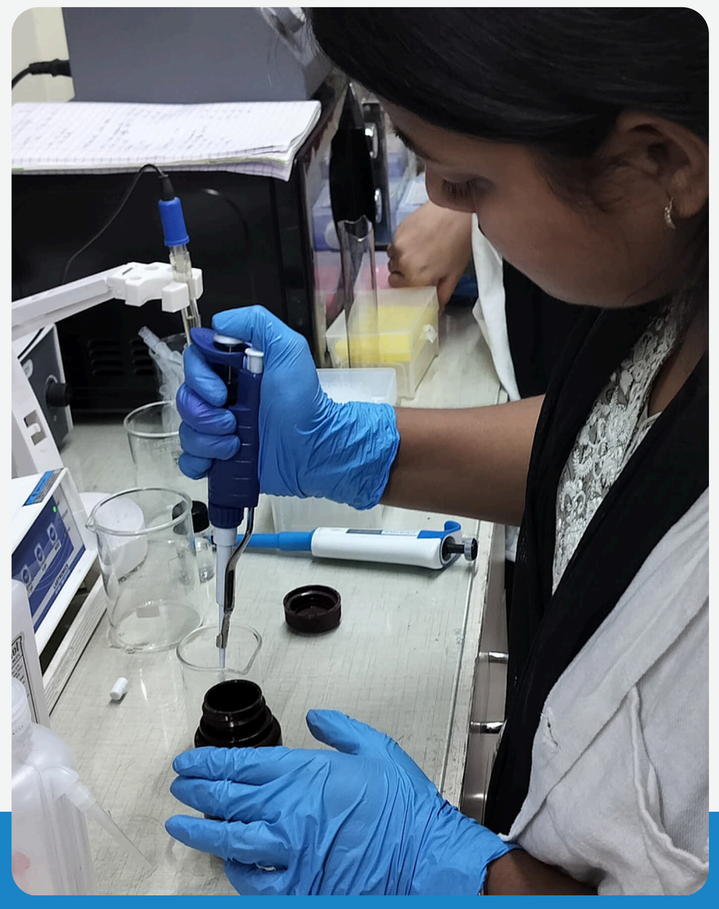
Components:

- Reverse Transcriptase enzyme
- DNA Polymerase (Taq or Hot-start)
- dNTPs
- RNase Inhibitor
- Specific primers for NS1 gene (Forward and Reverse)
- Reaction Buffer and $MgCl_2$
- Nuclease-free water

Steps:

- Thaw all reagents on ice and vortex gently.
- Prepare a reaction master mix to reduce pipetting error.
- Add RNA template last to prevent degradation.





RT-PCR for NS1 Gene Amplification and Analysis

3. PCR Run (Thermal Cycling)

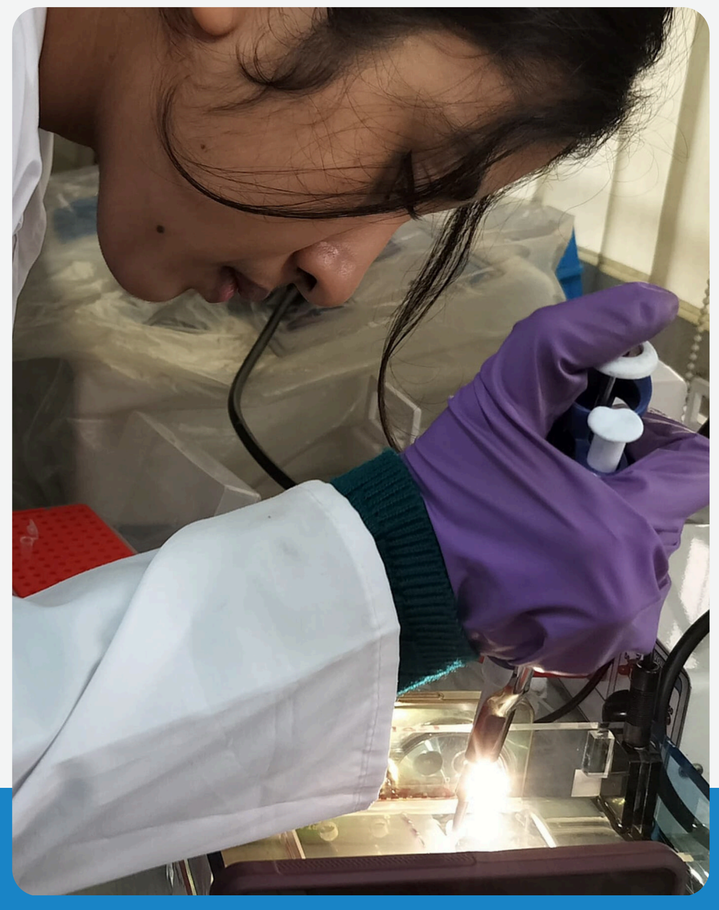
Using a thermocycler, the RT-PCR program proceeds through defined temperature cycles to synthesize cDNA from RNA and amplify the NS1 gene.

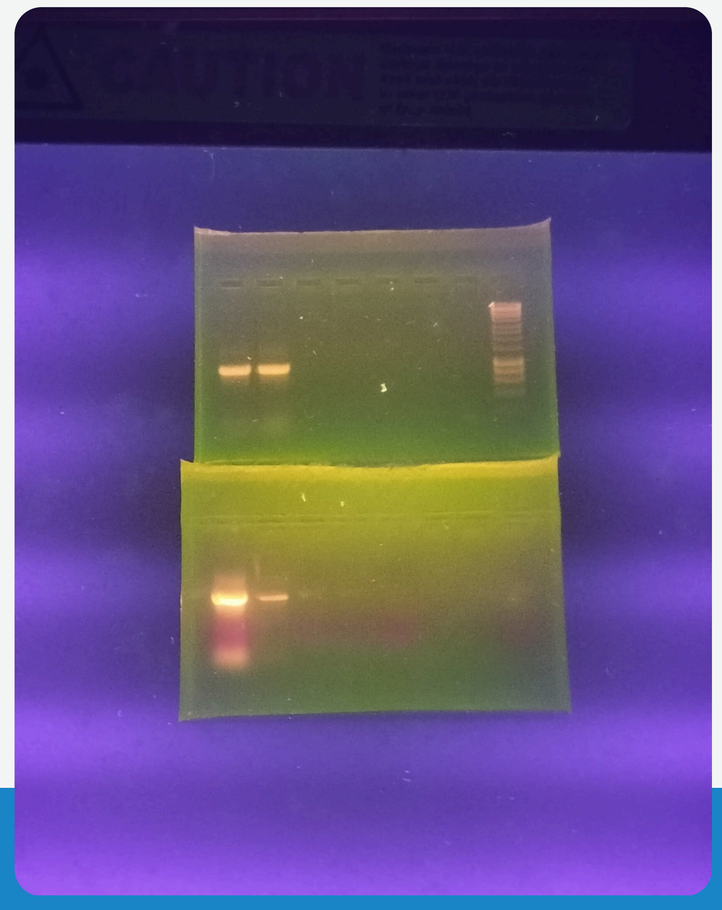
Typical RT-PCR Program:

1. Reverse Transcription: 42°C for 30–45 mins
2. Initial Denaturation: 95°C for 2–3 mins
3. PCR Cycling (30–40 cycles):
    - Denaturation: 95°C for 15–30 sec
    - Annealing: 50–60°C for 30 sec (based on primer Tm)
    - Extension: 72°C for 30–60 sec
4. Final Extension: 72°C for 5 mins
5. Hold: 4°C indefinitely

Controls:

- Include a positive control (known NS1 RNA template)
- Include a negative control (no template control – NTC) to detect contamination





RT-PCR for NS1 Gene Amplification and Analysis

🧬 4. Gel Casting and Sample Loading

After the PCR run, the amplified DNA is visualized using agarose gel electrophoresis, a standard technique for DNA separation.

Gel Preparation:

- Prepare 1.5–2% agarose gel with ethidium bromide or GelRed (for DNA staining).
- Pour into a gel casting tray and allow it to solidify.
- Submerge gel in 1X TAE or TBE buffer.

Sample Loading:

- Mix PCR product with loading dye.
- Load the ladder (DNA marker) in one well and samples in others.
- Run gel at 80–120V for ~30–45 mins.

5. Band Visualization and Result Interpretation

Finally, the gel is examined under a UV transilluminator or blue light imager to visualize the amplified NS1 gene.

Positive Sample:

- A visible band at the expected amplicon size (e.g., ~300 bp for NS1) indicates a positive result for Dengue virus.

Negative Sample:

- Absence of a band means no detectable NS1 gene, implying a negative result (provided controls worked).

Control Check:

- Ensure bands are present in the positive control and absent in the NTC.
- If not, repeat the experiment due to possible contamination or failure.

Serological Epitope Mapping Using PyMOL and Chimera

Objective:

- To visualize, identify, and analyze serological epitopes on antigen structures using PyMOL and UCSF Chimera.

Prerequisites:

- Basic understanding of antigen-antibody interactions, 3D protein structures, and familiarity with structural bioinformatics databases (PDB, IEDB, etc.).

Session 1: Introduction to Serological Epitope Mapping

Duration: 1 hour

Topics:

- Linear vs. conformational epitopes
- Role in vaccine design and diagnostics
- Tools and databases: IEDB, PDB

Hands-On:

- Access epitope data from IEDB
- Retrieve antigen structures from RCSB PDB

Session 2: Getting Started with PyMOL

Duration: 1.5 hours

Topics:

- Interface, commands, plugins
- Loading and coloring structures

Hands-On:

- Load and visualize 6VSB

Session 3: Mapping Epitopes in PyMOL

Duration: 2 hours

Tasks:

- Visualize and label residues
- Display surface and sticks

Serological Epitope Mapping Using PyMOL and Chimera

Session 4: Epitope Mapping with UCSF Chimera

Duration: 2 hours

Topics:

- Visual analysis: distances, hydrogen bonds, surface

Hands-On:

- Select epitope residues
- Highlight, analyze clashes, use Multiscale Models

Session 5: Advanced Techniques and Automation

Duration: 1.5 hours

Topics:

- PyMOL scripting
- Integration of prediction tools (IEDB, ElliPro, DiscoTope)

Tasks:

- Import predictions, generate PyMOL scripts, export images

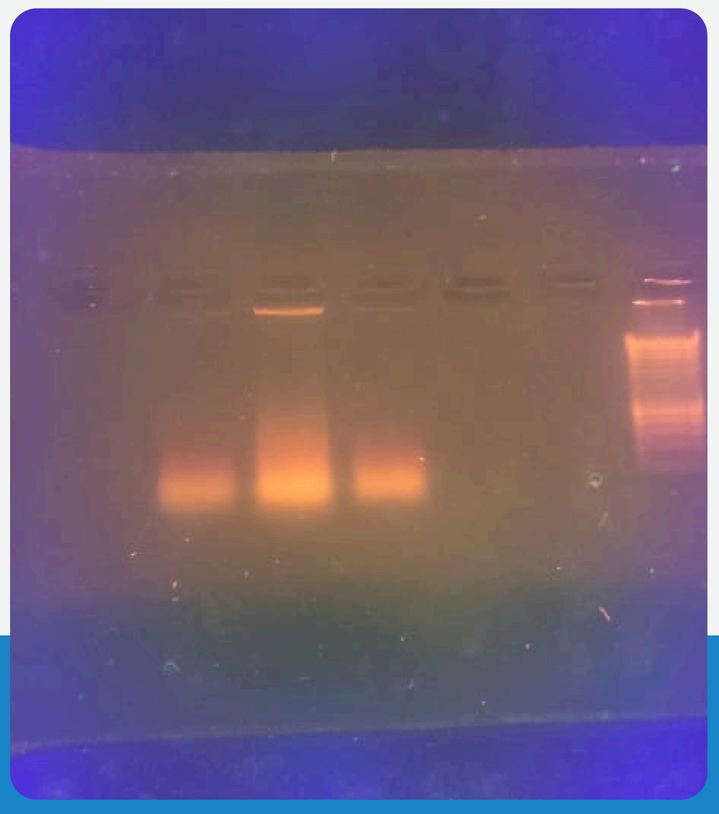
Session 6: Case Study & Assignment

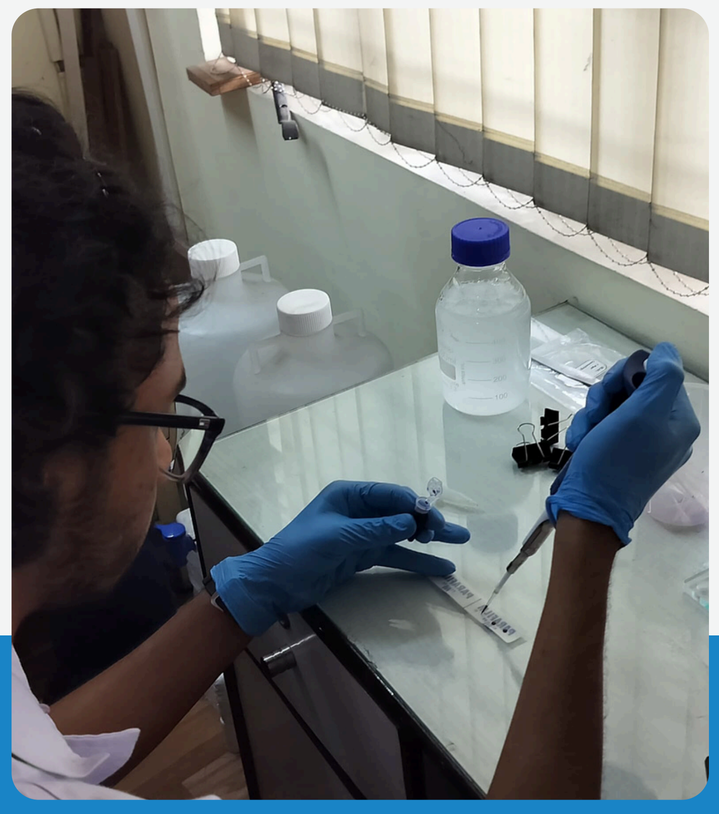
Duration: 2 hours

Example: Dengue E protein with antibody (PDB: 3IXY)

Assignment:

- Choose antigen-antibody complex
- Map epitope with PyMOL & Chimera
- Submit images and report





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