Work Report

Understanding cellular variability using droplet microfluidics

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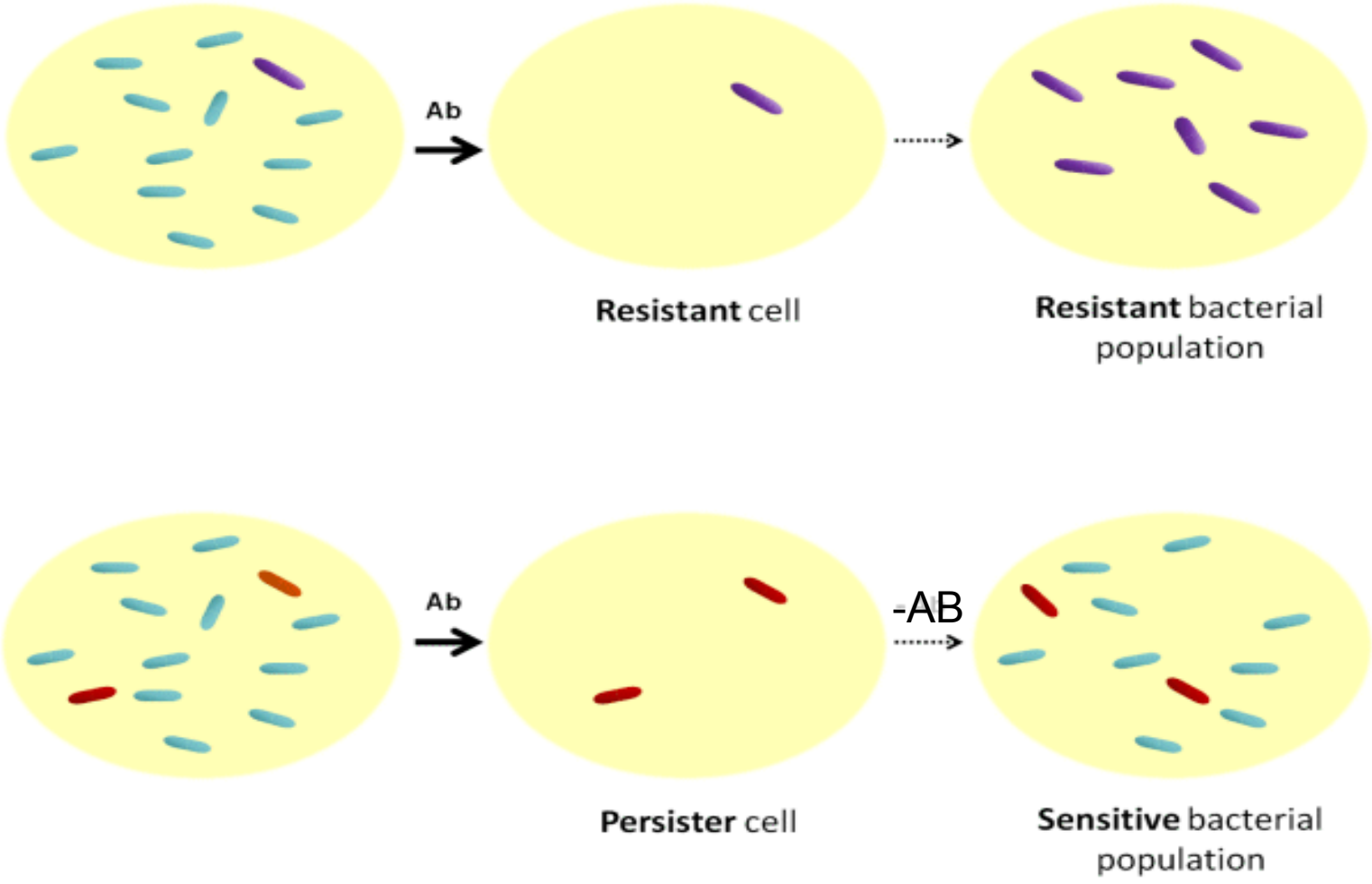
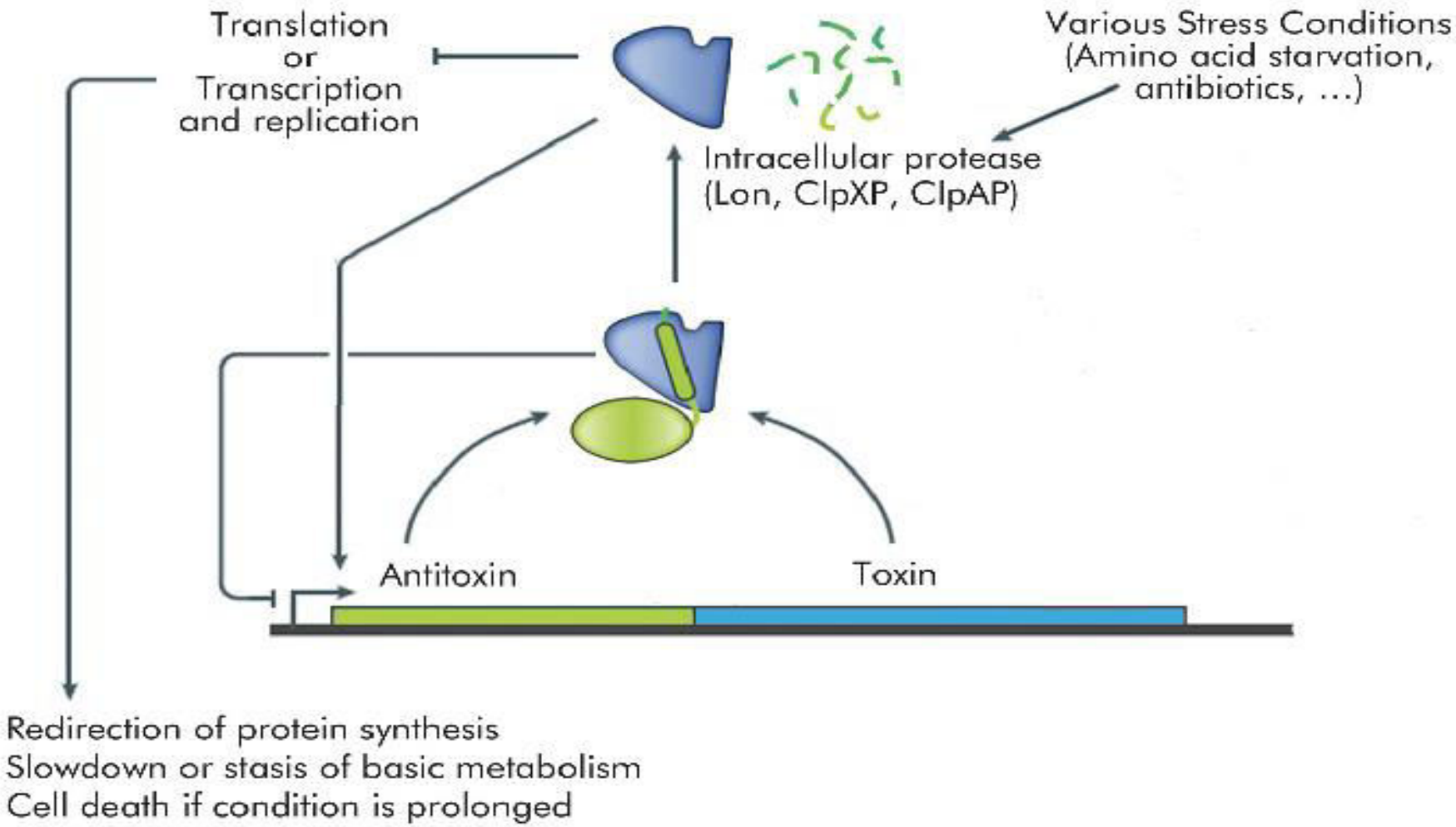
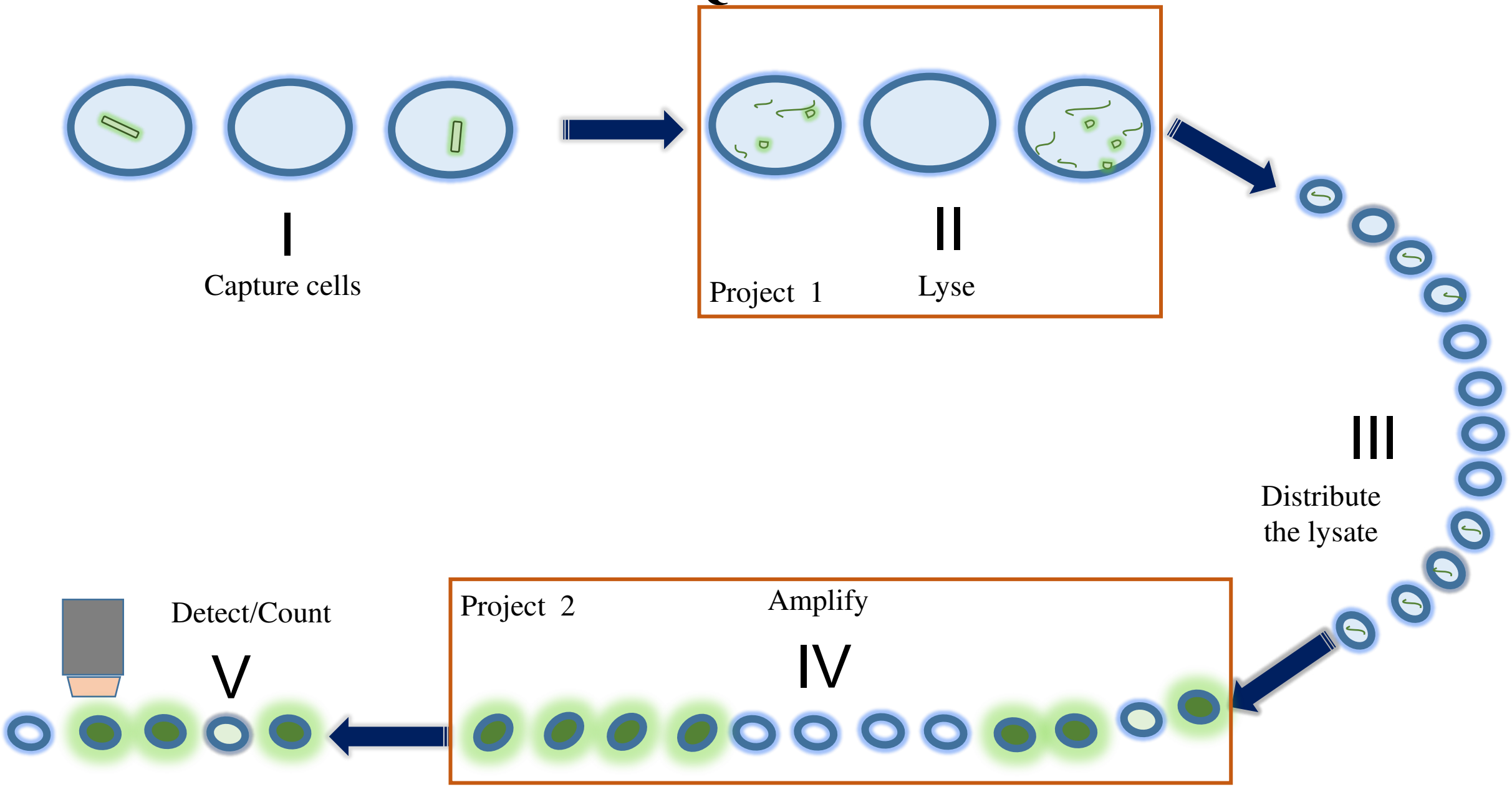
Variation in bacterial cells



Toxin anti-toxin system

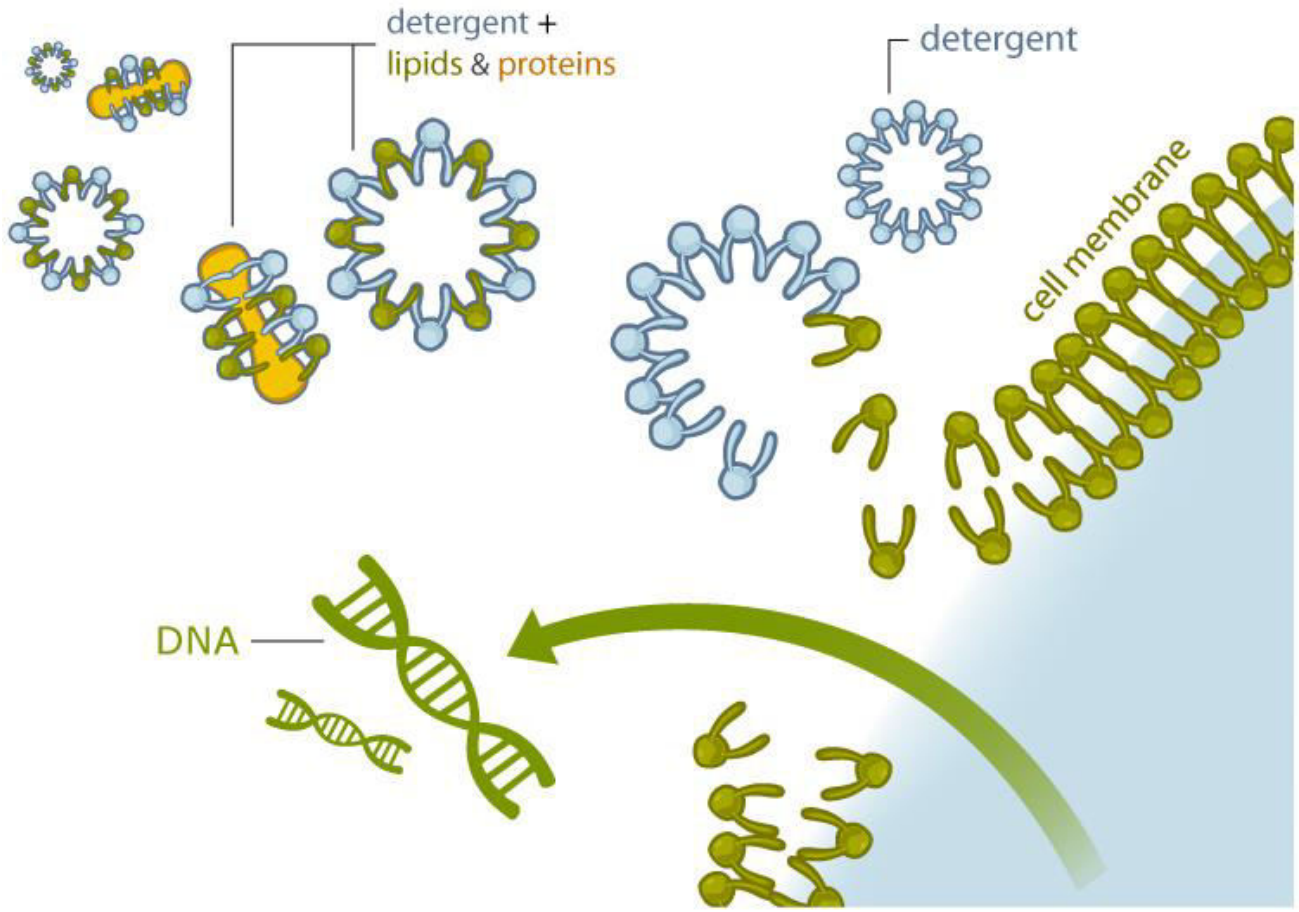


ScNAQ flowchart



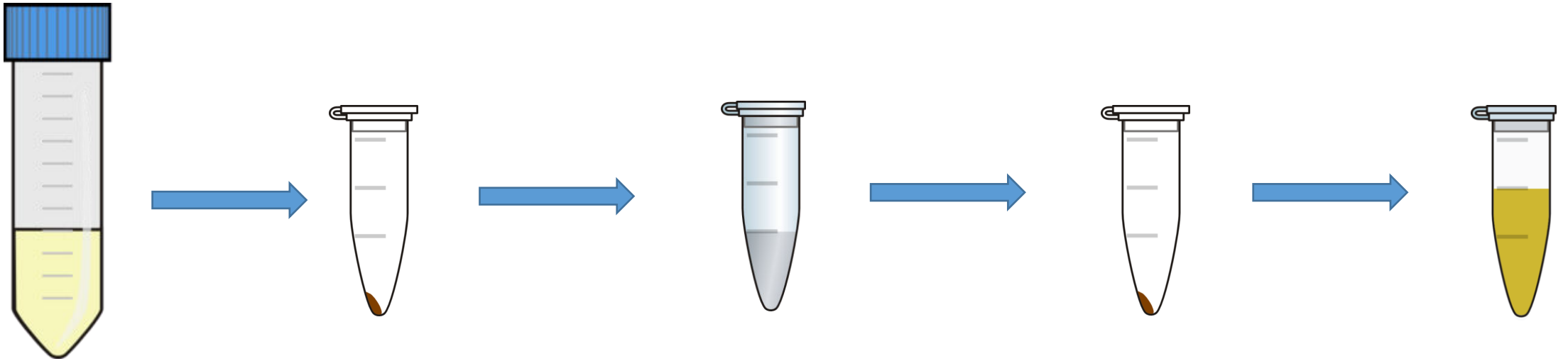
Designed by: Jatin Panwar, PhD scholar, Dr. Rahul Roy's Lab, Chemical Engineering , IISc.

Project 1:Cell lysis



Lysis buffer: Mixture of BugBuster & Lysozyme

Process:



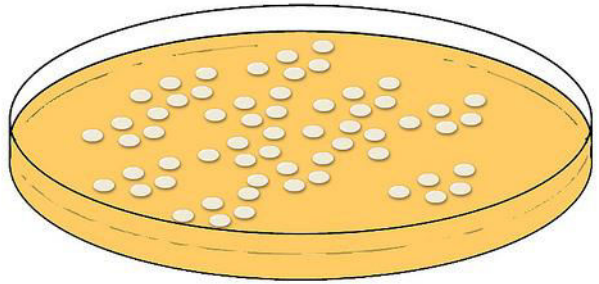
Cells cultured in LB broth
Overnight @ 37˚C

Cells pelleted
6000 rpm , 2 min

Re-suspension in lysis buffer,
2 min

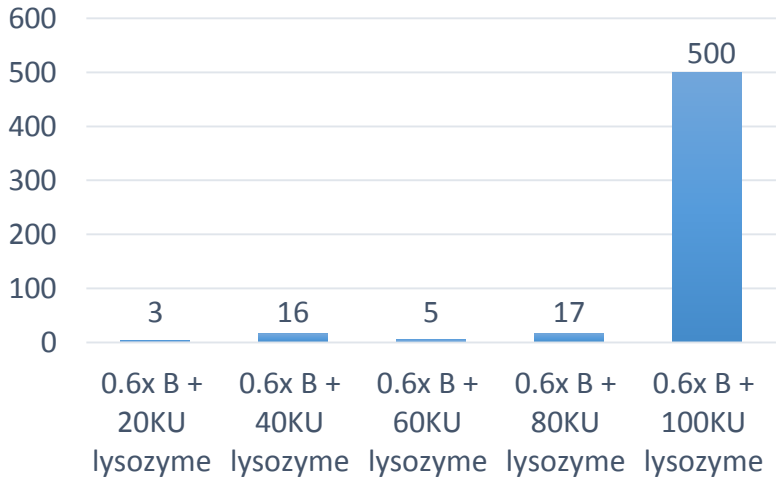
Cell lysate pelleted
6000 rpm , 2 min

Re-suspension in LB broth ,
2 min

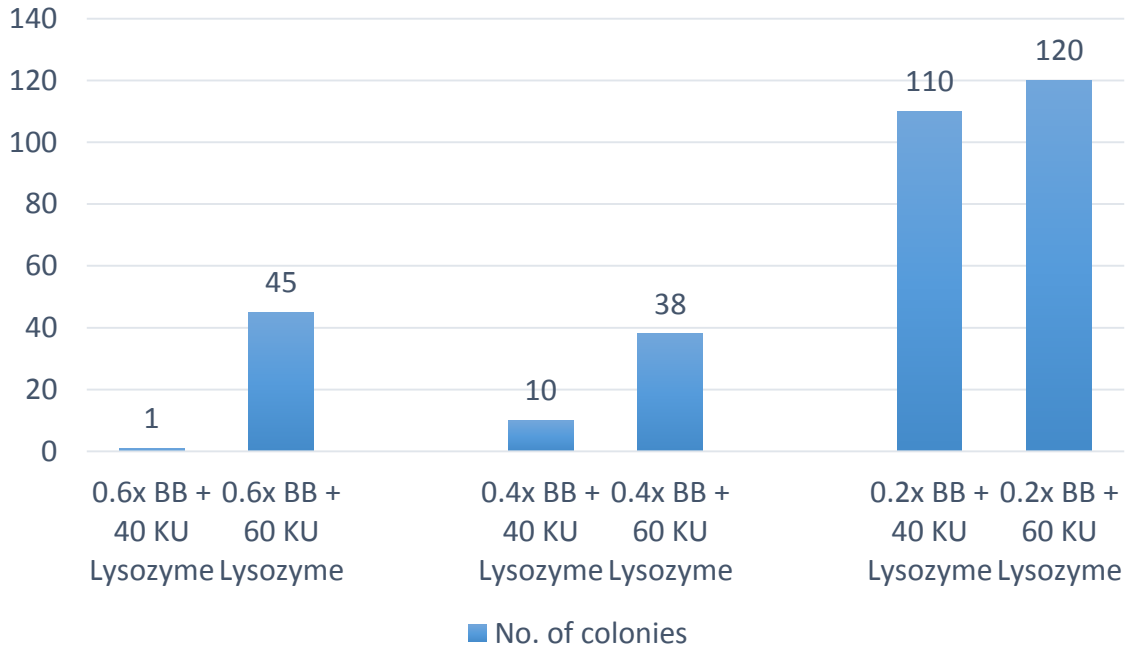


Plating in LB Agar plates (overnight culture @ 37°C) and CFU was measured.

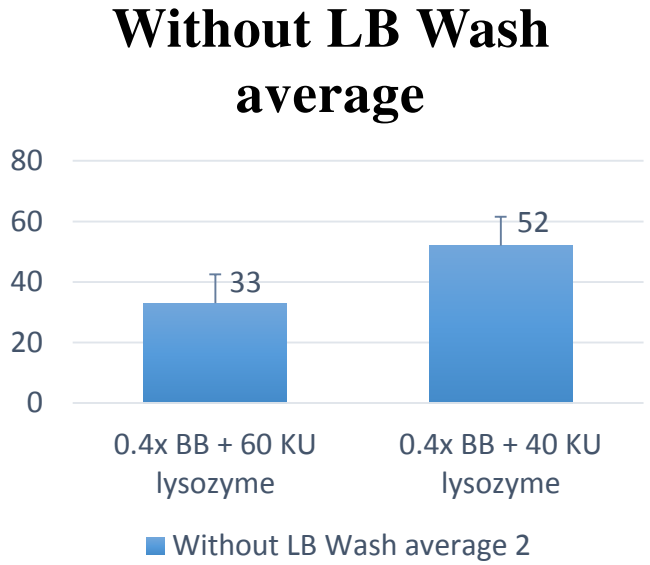
Optimization of Lysis buffer Concentration

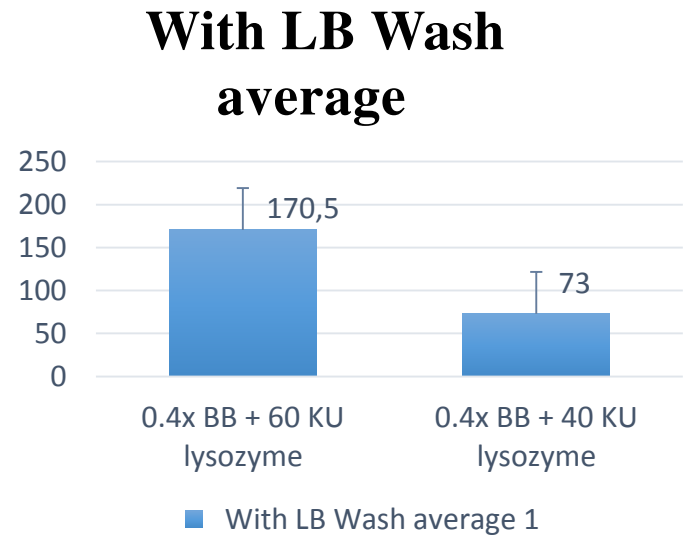


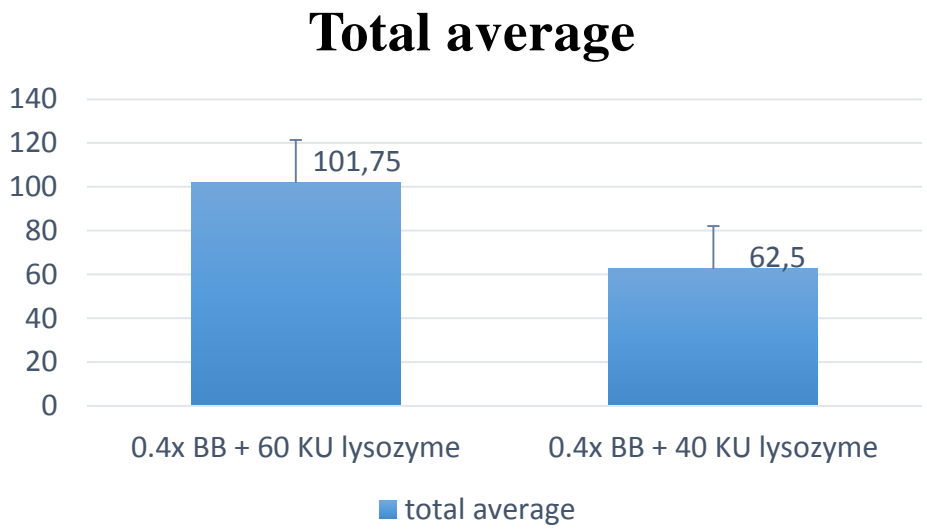
Varying lysozyme concentration, Keeping BugBuster concentration as 0.6x



Varying BugBuster concentration, Keeping lysozyme concentration as 40 KU and 60 KU







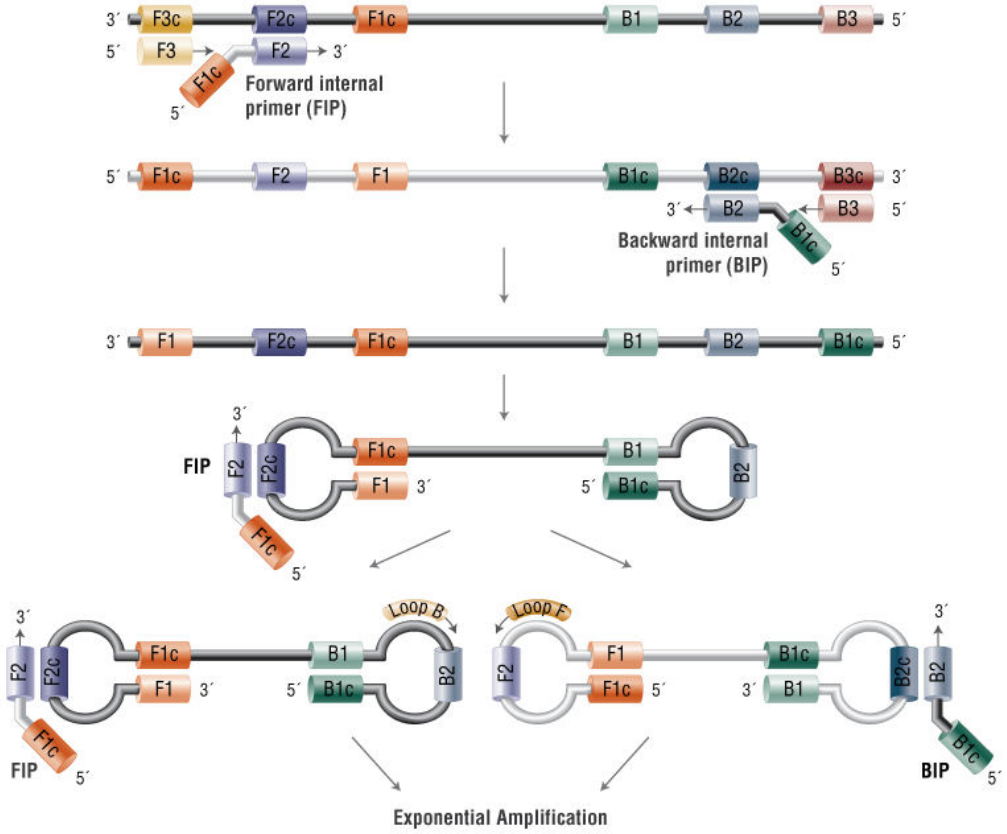
Conclusion:

Cell lysis is best achieved at a mixture of **0.4x BugBuster and 40 KU lysozyme** concentration, as confirmed by repeated experimentation.

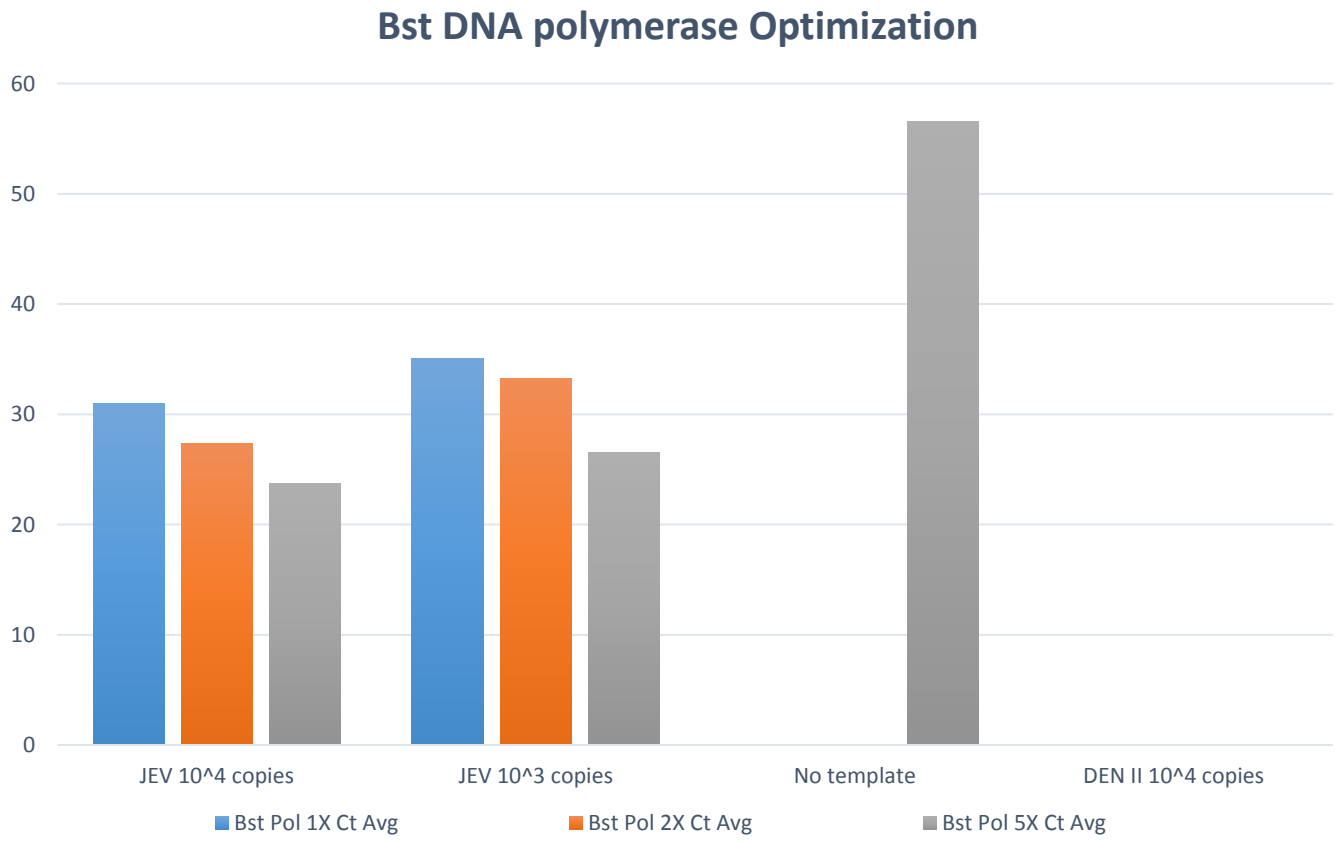
Loop- mediated isothermal Amplification (LAMP)

- Bst DNA polymerase used
- No thermocycler required
- 6 primers used
- High product concentration
- Amplification can be achieved in shorter period of time compared to PCR

Process:

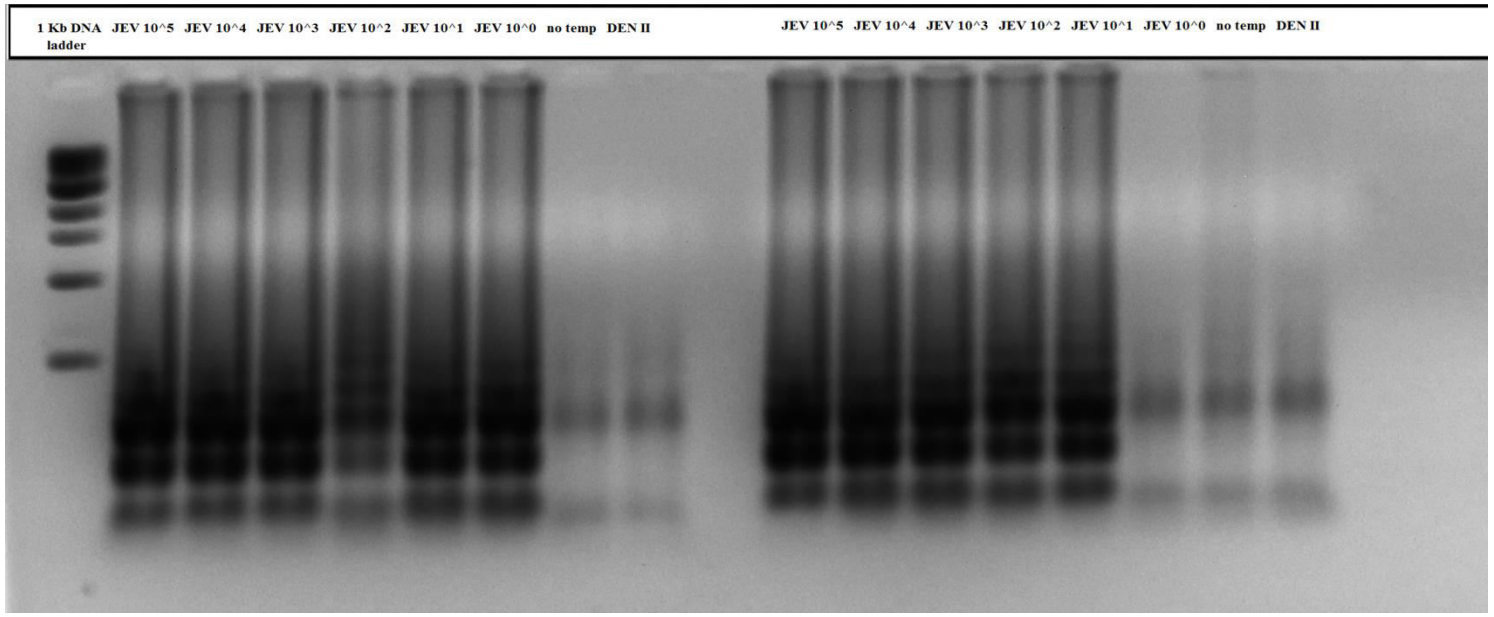


LAMP

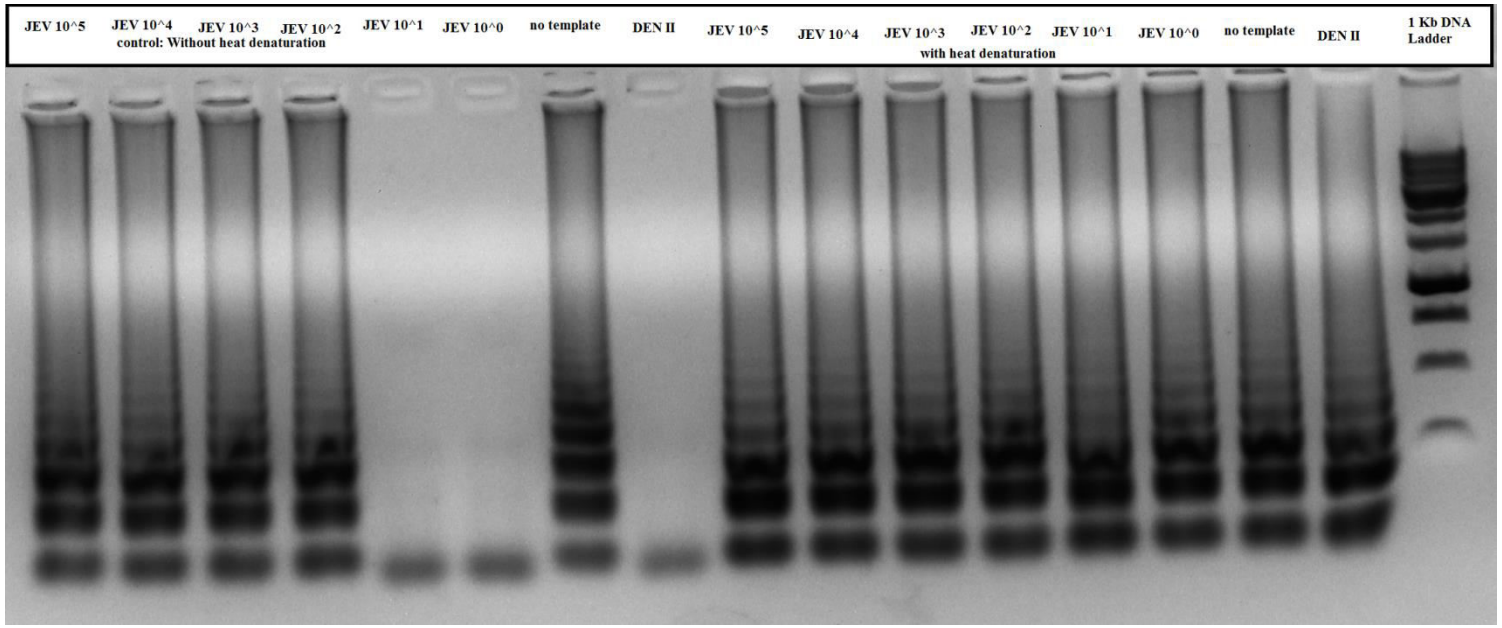


Ct values average obtained after optimization of Bst DNA polymerase concentration by evaluating LAMP reaction at to 1x, 2x and 5x concentration..

Gel Image:

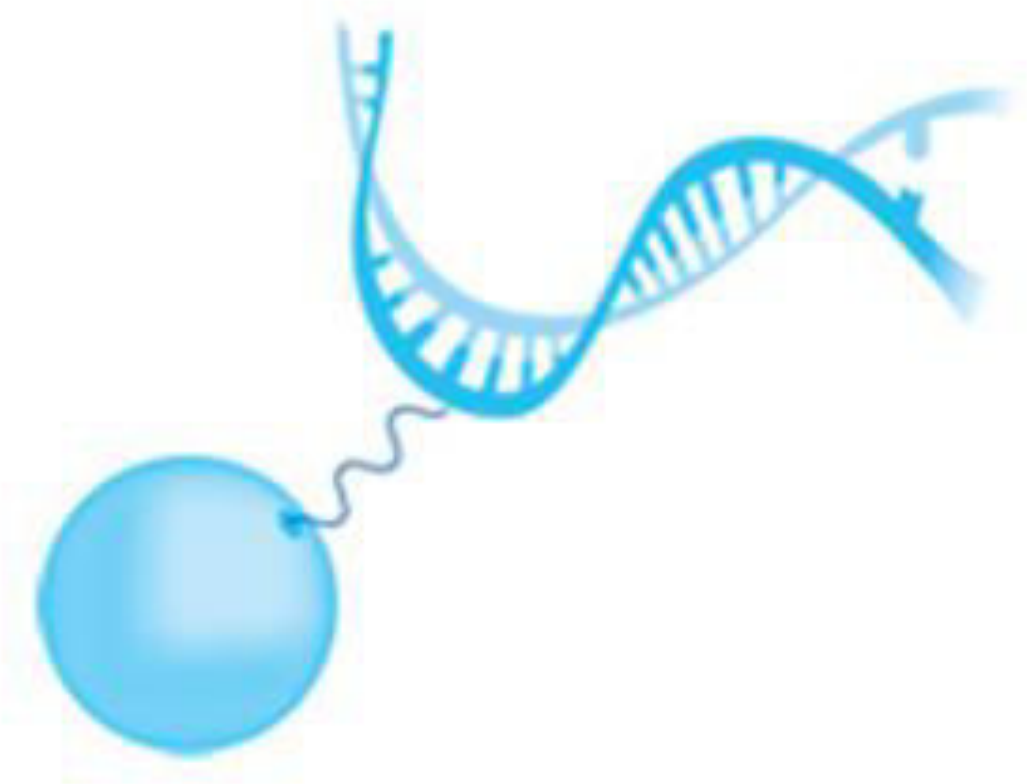


Gel image showing duplicates of a LAMP reaction , decreasing the JEV DNA copy number from 10^5 to 1

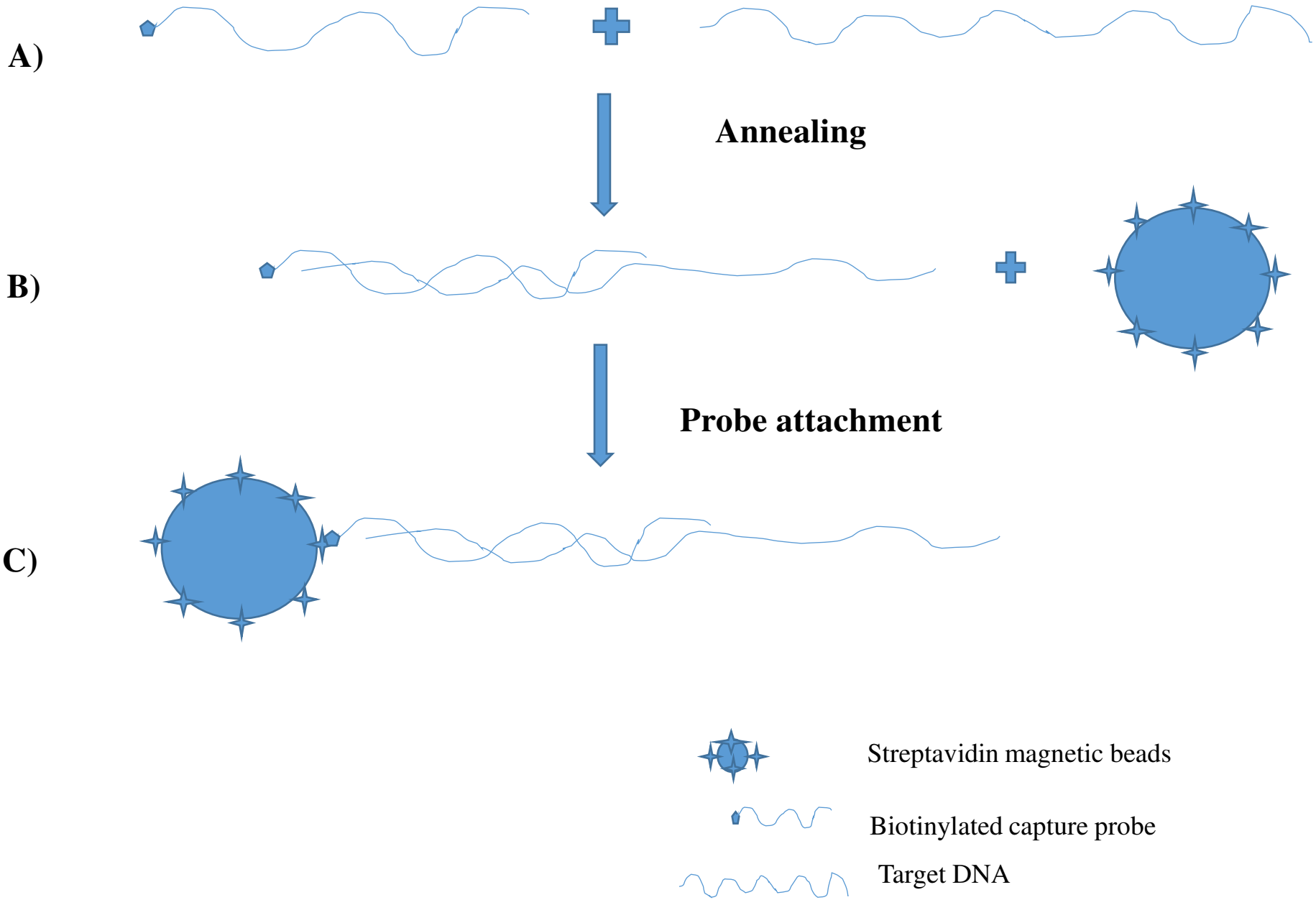


Gel image showing negative control (no template) amplification in case of no heat denaturation (left) and also negative control (no template and Dengue II) amplification in case of additional heat denaturation (right).

Project 3:
Nucleic acid quantification using magnetic beads

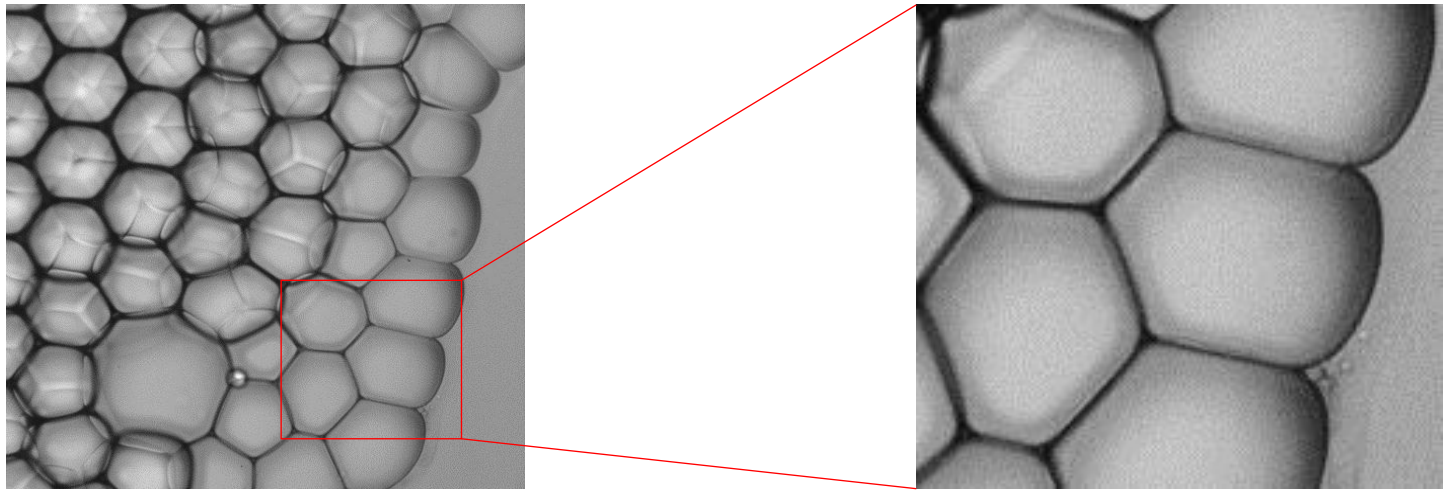


Concept:

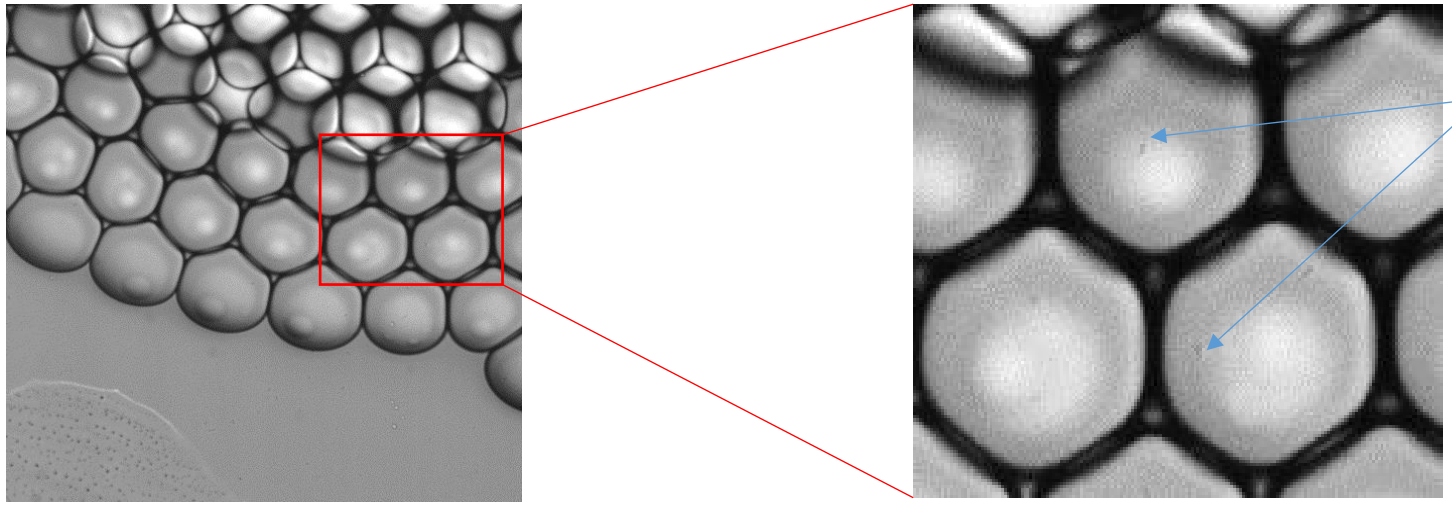

A)
Annealing
B)
Probe attachment
C)
Streptavidin magnetic beads
Biotinylated capture probe
Target DNA

Streptavidin magnetic beads

- Magnetic beads captured inside the droplets (100 µm) (60X magnification)



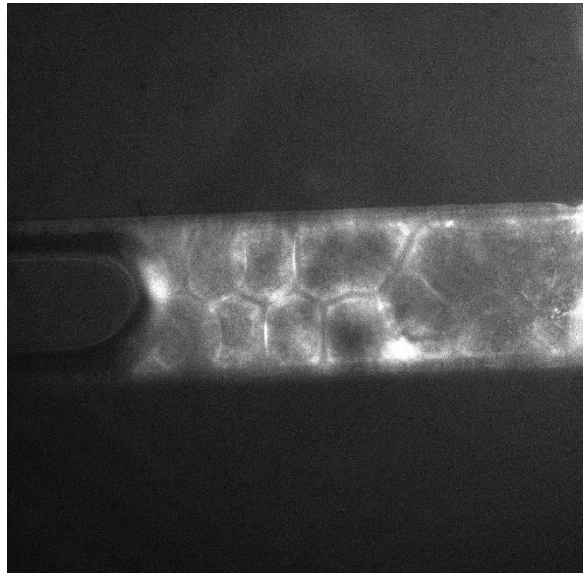
Control: 1X kapa HiFi buffer

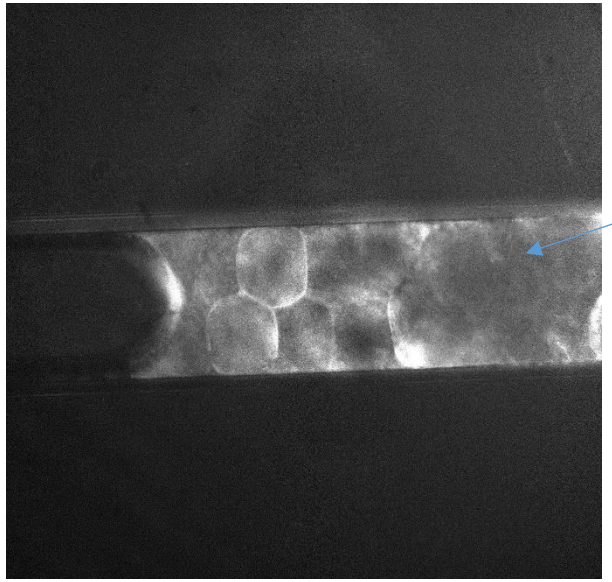


Magnetic beads(1 µm)

7.3 X 10^2 beads/ µL

Microscopic detection using channels

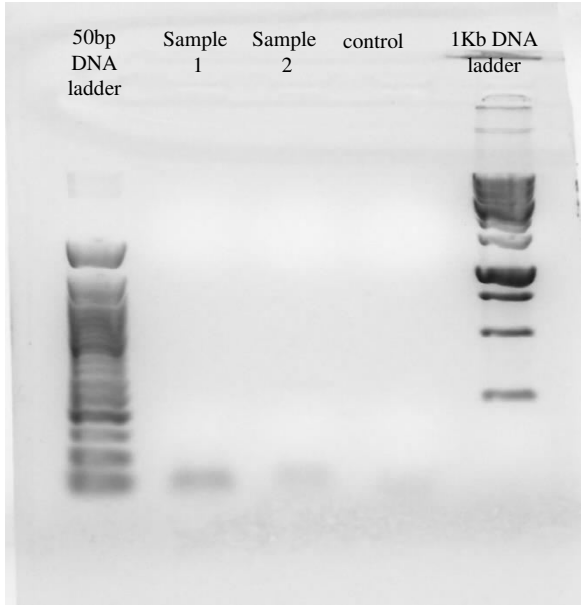




Merged droplets

*The droplets were imaged using 488 nm (blue) illumination

Gel image:



Sample 1: probe attached Template (10^6 copies) + magnetic beads (10^8 beads) – 1000 fold dilution
Sample 2: probe attached Template (10^6 copies) + magnetic beads (10^8 beads) – 10000 fold dilution
Control : Droplets without annealing step with biotinylated probe.

Future work:

- Cell Lysis: Further optimize the concentration of BugBuster and check for efficient release of RNA.
- LAMP: Troubleshoot the contamination problem and optimize the time of amplification. And do in vitro transcription to perform LAMP using RNA as template.
- Droplet PCR: Try making stable droplets with magnetic beads, perform PCR, detect and analyze data.

ACKNOWLEDGMENT

I want to thank centre of BioSystem Science and Engineering for giving this opportunity to pursue my research interest through BioEngineering Summer Training (BEST) program.

I want to express my gratitude to Dr. Rahul Roy for his able guidance and valuable suggestions. I want to thank Ms. Sunanda and Prof. Siddharth and Prof. Ananthasuresh for there able guidance and support throughout the program.

I also want to thank all the research scholars in Dr. Rahul's lab especially Jatin, Monisha, Saranya and Priyanka for their assistance in designing and analyzing the experimental data.

Thank you!