



ANALYSIS OF BACTERIAL TRANSFORMATION EFFICIENCY USING VIRTUAL SIMULATION

*Investigation of Bacterial Transformation Efficiency
and Selective Gene Expression in E. coli
Using pARA-R Plasmid Simulation*



PREPARED BY

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INSTITUTE

Ideal School and College



CLASS

XI



FIELD OF STUDY

Biotechnology

Title: Transforming Bacteria & Plating Transformed Bacteria

Analysis of Bacterial Transformation Efficiency Using Virtual Simulation

Abstract: In this study the process of bacterial transformation in *Escherichia coli* is explored by using the heat shock method and **assesses** the expression of recombinant DNA through selective plating. To identify successful genetic uptake and expression the recombinant plasmid pARA-R, containing the red fluorescent protein (rfp) gene and ampicillin resistance gene (ampR) was injected. Following transformation, bacteria were cultured on selective media to identify successfully transformed colonies. This investigation demonstrates how recombinant DNA technology and selective growth conditions are used to confirm gene transfer and expression in bacterial systems.

Introduction: Bacterial transformation refers a fundamental technique in biotechnology that enables the introduction of foreign DNA into bacterial cells. In this study, *E. coli* bacteria were transformed using the heat shock method, In the heat shock process, it temporarily increases membrane permeability and allows plasmid DNA to enter the cell.

The recombinant plasmid pARA-R contains:

- **rfp gene** → produces red fluorescent protein (gene expression marker)
- **ampR gene** → provides ampicillin resistance (selection marker)
- **ori (origin of replication)** → ensures plasmid replication in host cells
- **pBAD promoter & araC gene** → regulate gene expression

After transformation, bacteria were plated on selective media containing ampicillin to distinguish transformed cells from non-transformed ones.

Objective: To simulate bacterial transformation using the heat shock method and evaluate successful gene uptake and expression through selective plating of *E. coli*.

Methodology: 1. *Transformation process:*

- *E. coli* cells were exposed to the recombinant pARA-R plasmid.
- Heat shock treatment was applied to increase membrane permeability.
- Cells were allowed to recover to stabilize plasmid uptake.

2. Plating and Culturing:

- Transformed bacteria were plated onto agar containing ampicillin.

- Plates were incubated to allow colony formation.
- Growth was observed under selective conditions

figure-1: Heat shock enables plasmid uptake in *E. coli*. figure-2: pARA-R plasmid structure.

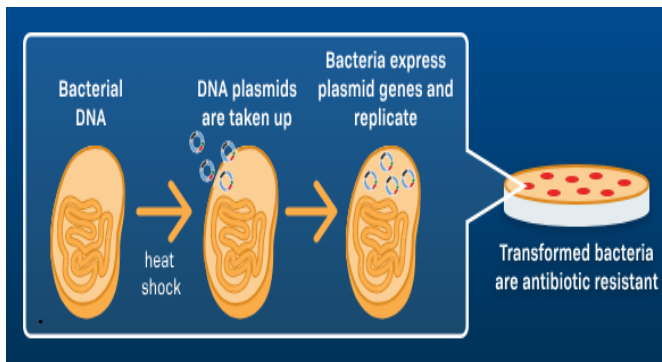


figure-3: Comparison of predicted and actual outcomes of bacterial transformation.

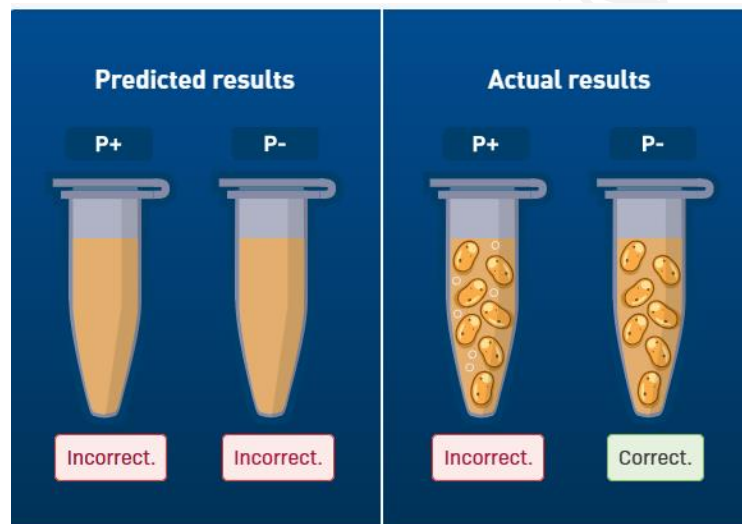
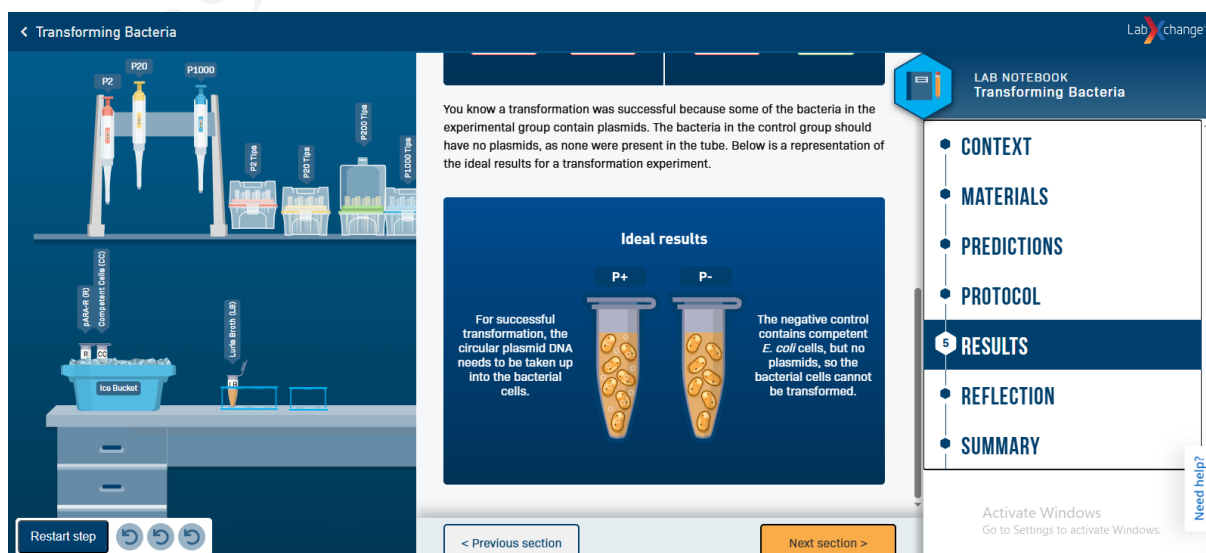


figure-4: Virtual simulation setup illustrating the transformation process and expected results.



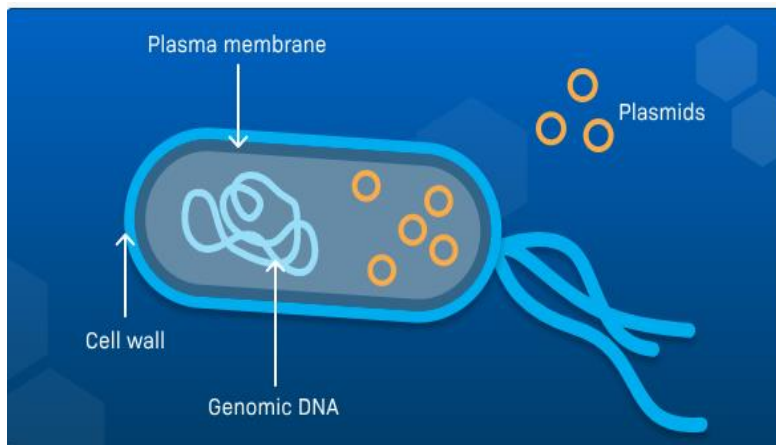


figure-5: Bacterial cell structure with plasmids.

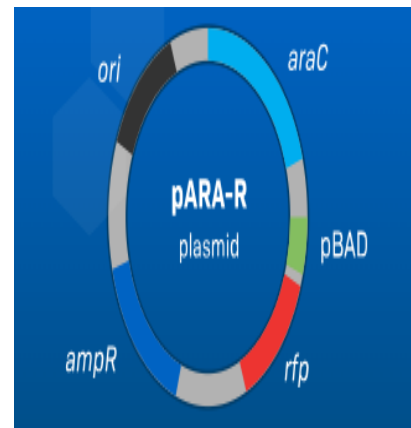


figure-6: pARA-R plasmid.

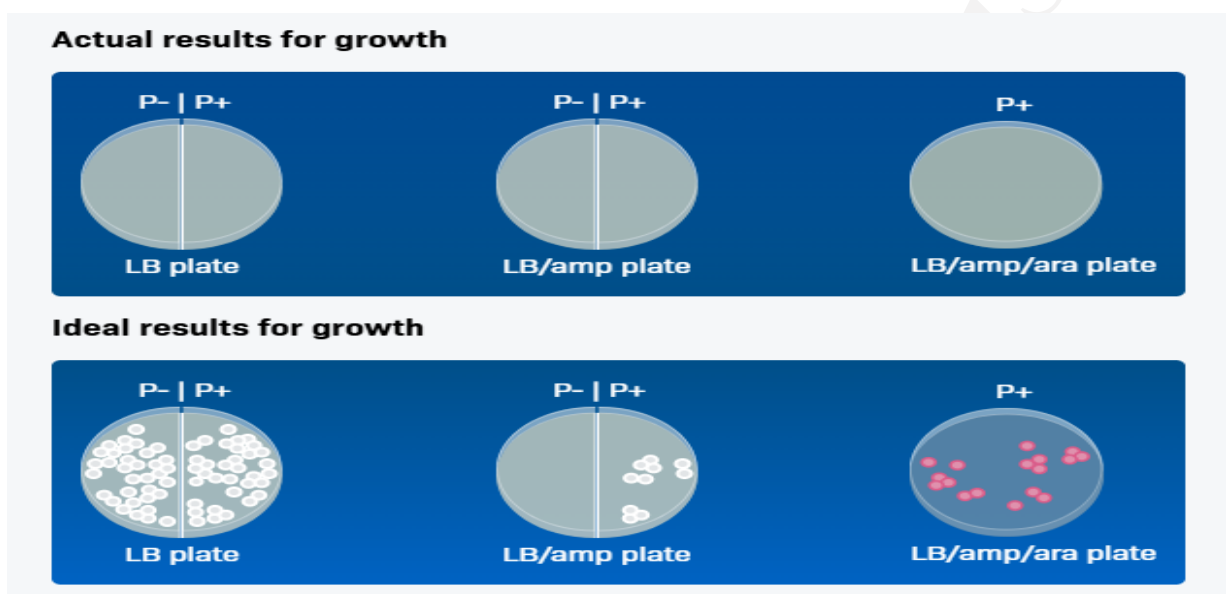


figure-7: Growth of transformed and non-transformed bacteria on selective agar media.

Observations:

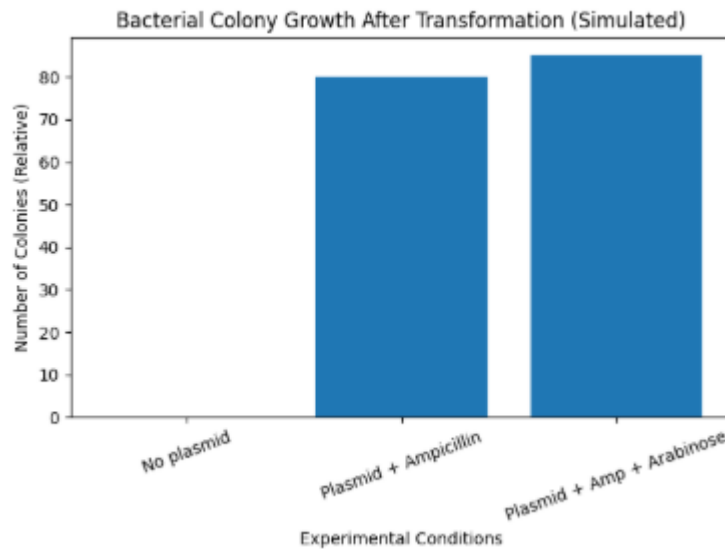
- Only bacteria that successfully absorbed the plasmid survived on ampicillin plates.
- Visible colonies indicated successful transformation.
- Expression of the *rfp* gene resulted in identifiable traits in transformed colonies (depending on simulation settings).

Results:

- Ampicillin-resistant colonies confirmed successful plasmid uptake.
- Selective media effectively eliminated non-transformed bacteria.
- Gene expression was regulated by the pBAD promoter system, showing controlled expression potential.

Showing the Bacterial Growth Under Different Conditions by graph:

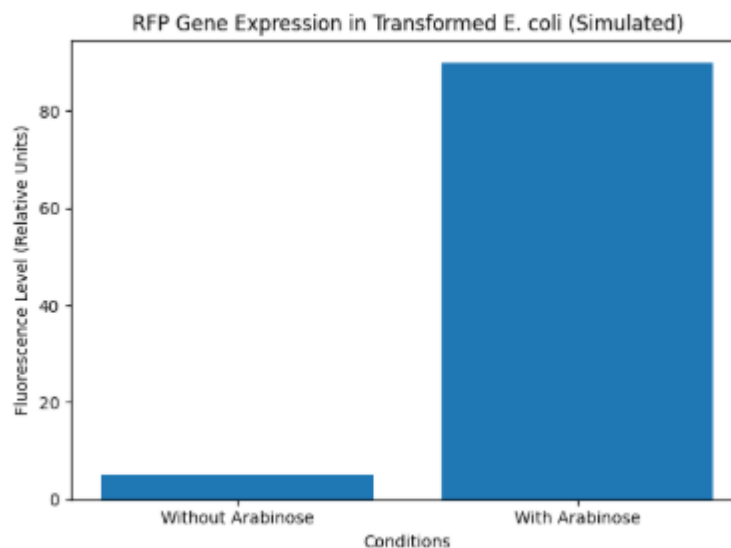
graph-1:



Graph 1. Growth of *E. coli* colonies under selective conditions after transformation with pARA-R plasmid.

Figure shows that no colonies appear in the absence of plasmid, while transformed bacteria survive on ampicillin media. Slight variation in colony count is observed in arabinose-supplemented conditions.

Graph-2:



Graph 2. Relative expression of RFP gene in transformed *E. coli* under presence and absence of arabinose.

The figure demonstrates minimal fluorescence without arabinose, while strong red fluorescence is observed when arabinose is present, indicating activation of the pBAD promoter system.

Discussion:

This experiment narrates the effectiveness of heat shock transformation in introducing recombinant plasmids into bacterial cells. The presence of the ampR gene enables antibiotic-based selection, ensuring that only transformed bacteria survive under selective conditions.

Furthermore, the rfp gene functions as a reporter gene and allows visual confirmation of gene expression. This reflects real-world applications in genetic engineering, where selectable markers and reporter genes are essential tools for identifying and analyzing genetically modified organisms.

However, this study was conducted using a simulation model, which does not fully replicate real laboratory conditions. Factors such as environmental variation, experimental error, and biological complexity are not represented in the simulation.

Conclusion:

The simulation successfully demonstrated bacterial transformation and selection using recombinant plasmid DNA. The combination of heat shock transformation and antibiotic selection gives a reliable method for recognizing genetically modified bacteria. These techniques are widely applied in biotechnology for gene cloning, protein expression, and genetic research.

Author Statement:

I confirm that this independent research project was performed and prepared by me based on virtual laboratory simulations and my own analysis. All sources used have been properly acknowledged. This work reflects my personal understanding and interpretation of bacterial transformation and gene expression.

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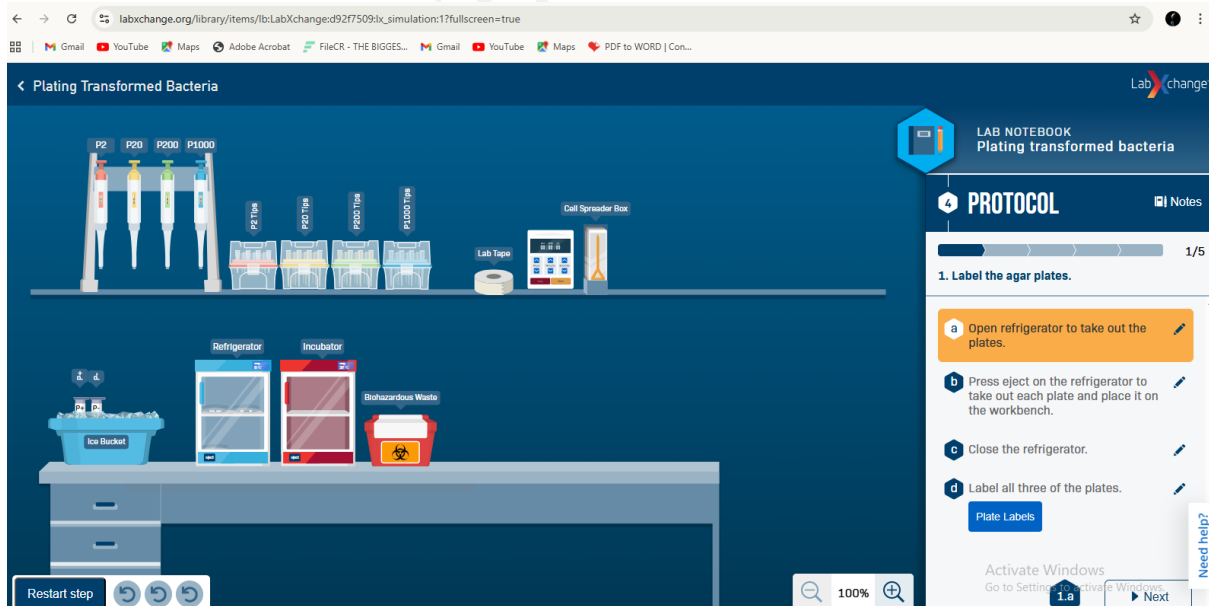
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