Bacterial Transformation and DNA Electrophoresis

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BIO 1120 – Section 03

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Reagents:

* Competent E. coli cells
* Plasmid DNA (with ampicillin resistance gene)
* Ice-cold CaCl₂ solution
* Sterile water
* LB broth
* LB agar plates
* LB+ampicillin agar plates
* Microcentrifuge tubes (“+” and “–”)
* Pipettes and sterile tips
* Water bath (42°C)
* Incubator (37°C)

Experiment Summary

In this lab, we will perform a bacterial transformation experiment using E. coli and a plasmid containing a gene that provides resistance to ampicillin. The goal is to introduce this plasmid into competent bacterial cells and observe whether they successfully acquire the resistance trait.

To begin, we will prepare two microcentrifuge tubes one labeled “+” (with plasmid DNA) and one labeled “–” (without plasmid DNA)each containing competent E. coli cells treated with ice-cold CaCl₂. These cells will be placed on ice, then briefly heat shocked in a 42°C water bath to facilitate the uptake of DNA. After heat shock, the cells will recover in LB broth.

Next, we will plate both the transformed (“+”) and untransformed (“–”) cells onto two types of agar plates: LB (no antibiotic) and LB+amp (contains ampicillin). Growth on LB plates is expected for both “+” and “–” samples. However, only transformed cells with the plasmid should grow on LB+amp plates, confirming successful uptake of the resistance gene.

This lab demonstrates key molecular biology concepts including transformation, selective antibiotic resistance, and plasmid-based gene transfer. It also reinforces the need for sterile technique and experimental controls in biotechnology.