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RUDBECKS-GYMNASIET

BIOTECH LAB IN SWEDEN

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PRESENTATION

Bacterial Transformation with the pGLO Plasmid

1. INTRODUCTION: What is pGLO transformation?

- Transformation is the process by which a bacterium (in our case, E. coli) takes up foreign DNA from the environment. Using the Bio-Rad pGLO kit, we genetically modify the bacteria to acquire two new traits:

Antibiotic resistance (ampicillin).

Fluorescence (green glow under UV light)

2. Key Player: The pGLO Plasmid

A plasmid is a small, circular DNA molecule that acts as a vector.

It contains three key components:

GFP

The gene for green fluorescent protein (from the jellyfish Aequorea victoria).

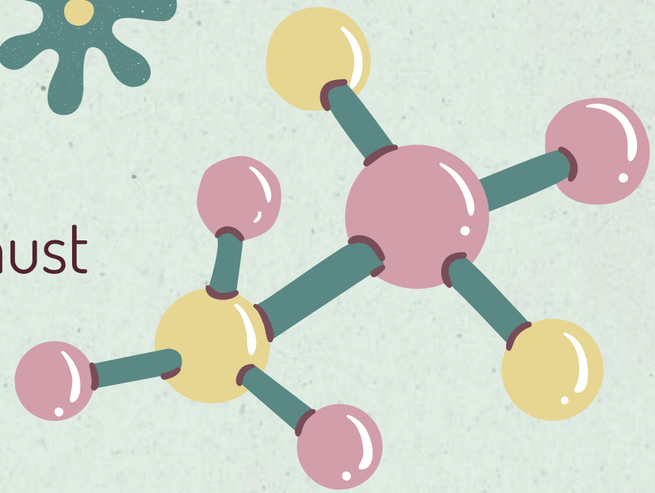
BLA (AMPR)

It confers resistance to ampicillin (allowing survival on a selective medium).

ARA C

A "switch" that activates the GFP gene only when the sugar arabinose is present.

3. Procedure: Heat Shock Method



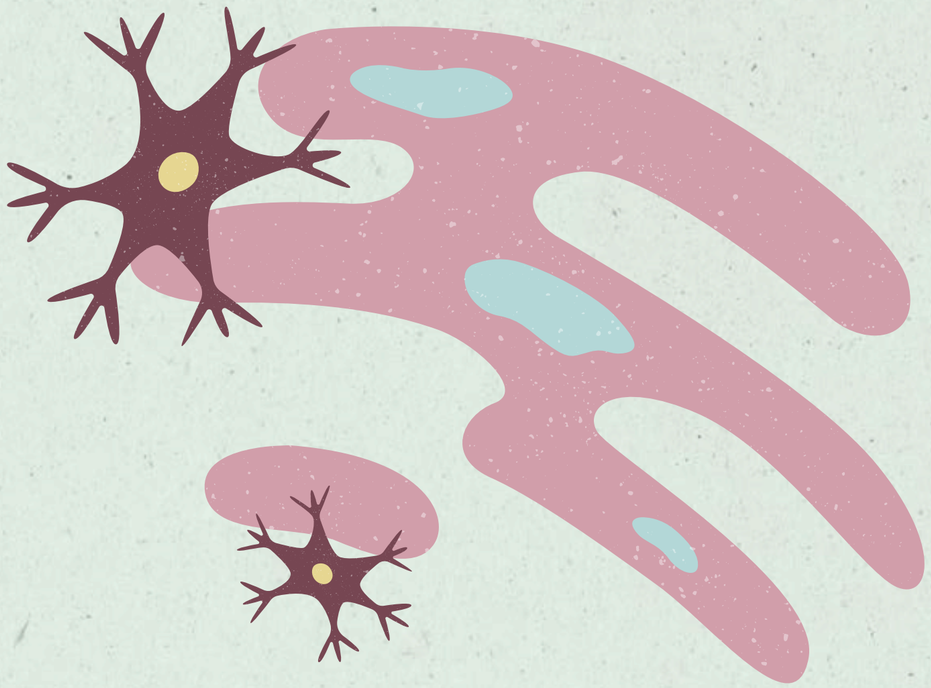
To allow DNA to pass through the bacterial cell wall, we must make the cells "competent":

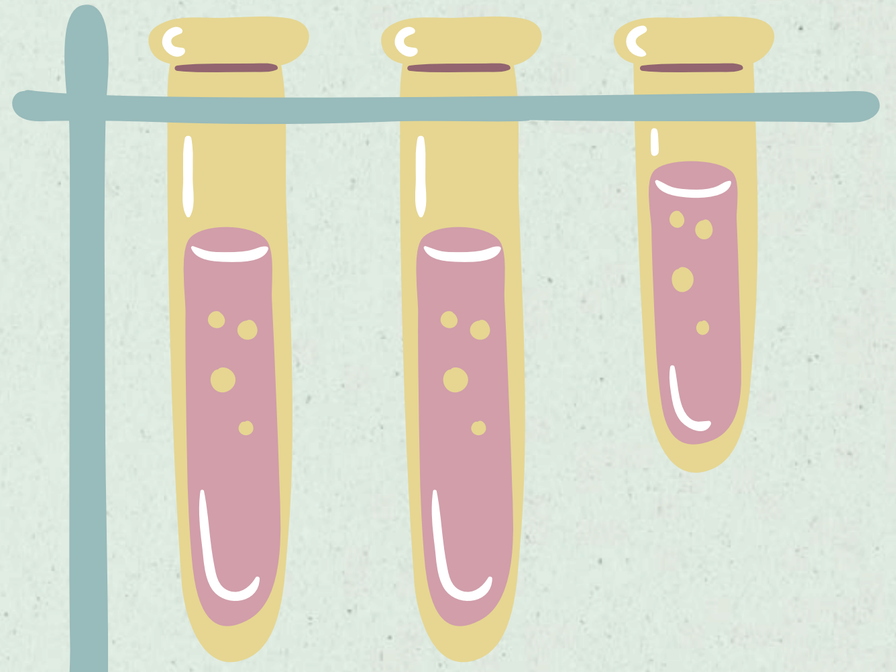
1. **Preparation:** Suspend the bacteria in a $CaCl_2$ solution, which neutralizes the charges on the DNA and the cell membranes.
2. **Heat shock:** Heat the mixture to 42°C for exactly 50 seconds, then immediately return it to ice. This creates temporary pores in the membrane through which the plasmid enters.
3. **Recovery:** Add nutrient broth (LB) so that the cells recover and begin to express the new genes.

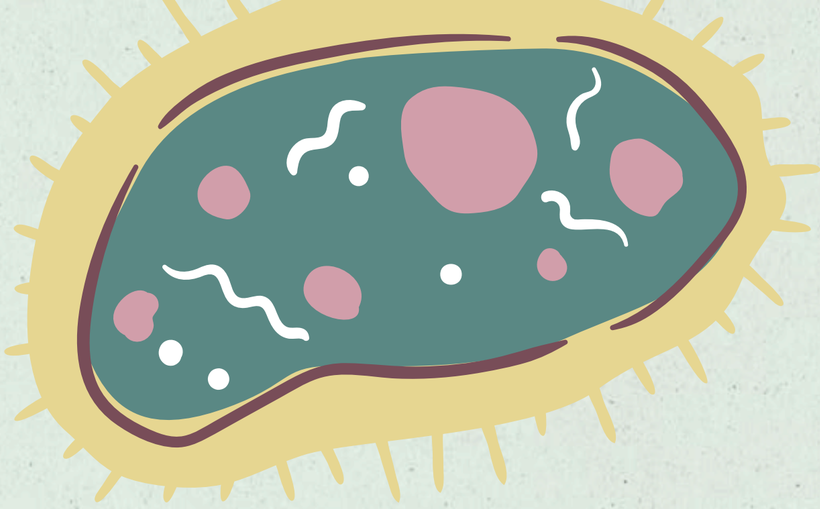
4. Plating and Results: What Will We See?

We plate the bacteria onto different agar media to verify the success of the experiment:

- **LB / ampicillin:** Only transformed bacteria grow (the colonies are white).
- **LB / ampicillin / arabinose:** Transformed bacteria grow, which glow green under UV light.
- **LB (Control):** Dense growth of all bacteria (no selection), which do not glow







5. Conclusion: Why is this important?

This experiment demonstrates the basic principles of genetic engineering. The same principle is used in biotechnology to produce medicines, such as insulin, where bacteria are made to produce human proteins.

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