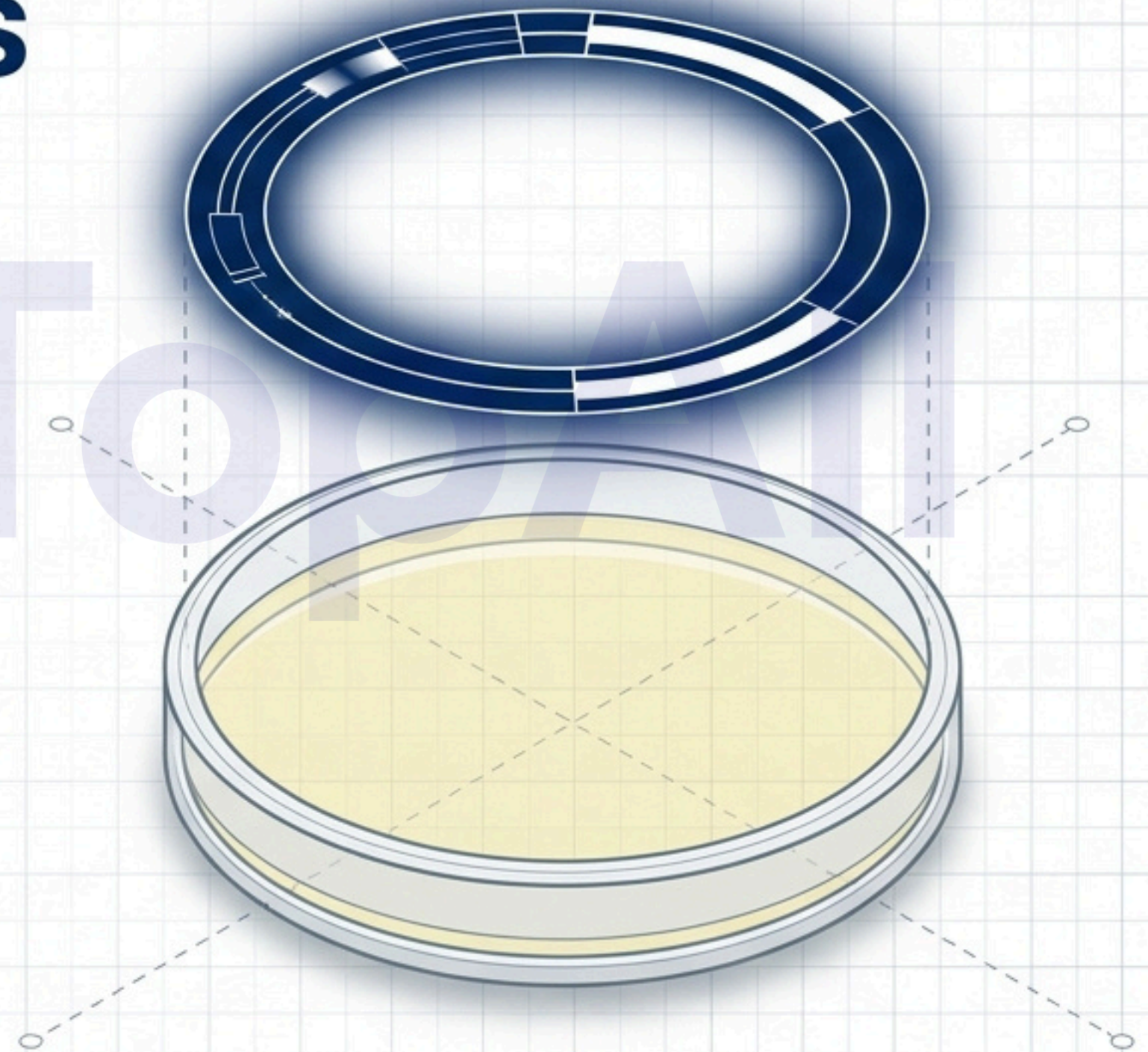


Selecting Transformed Cells

The isolation of recombinant DNA in host organisms



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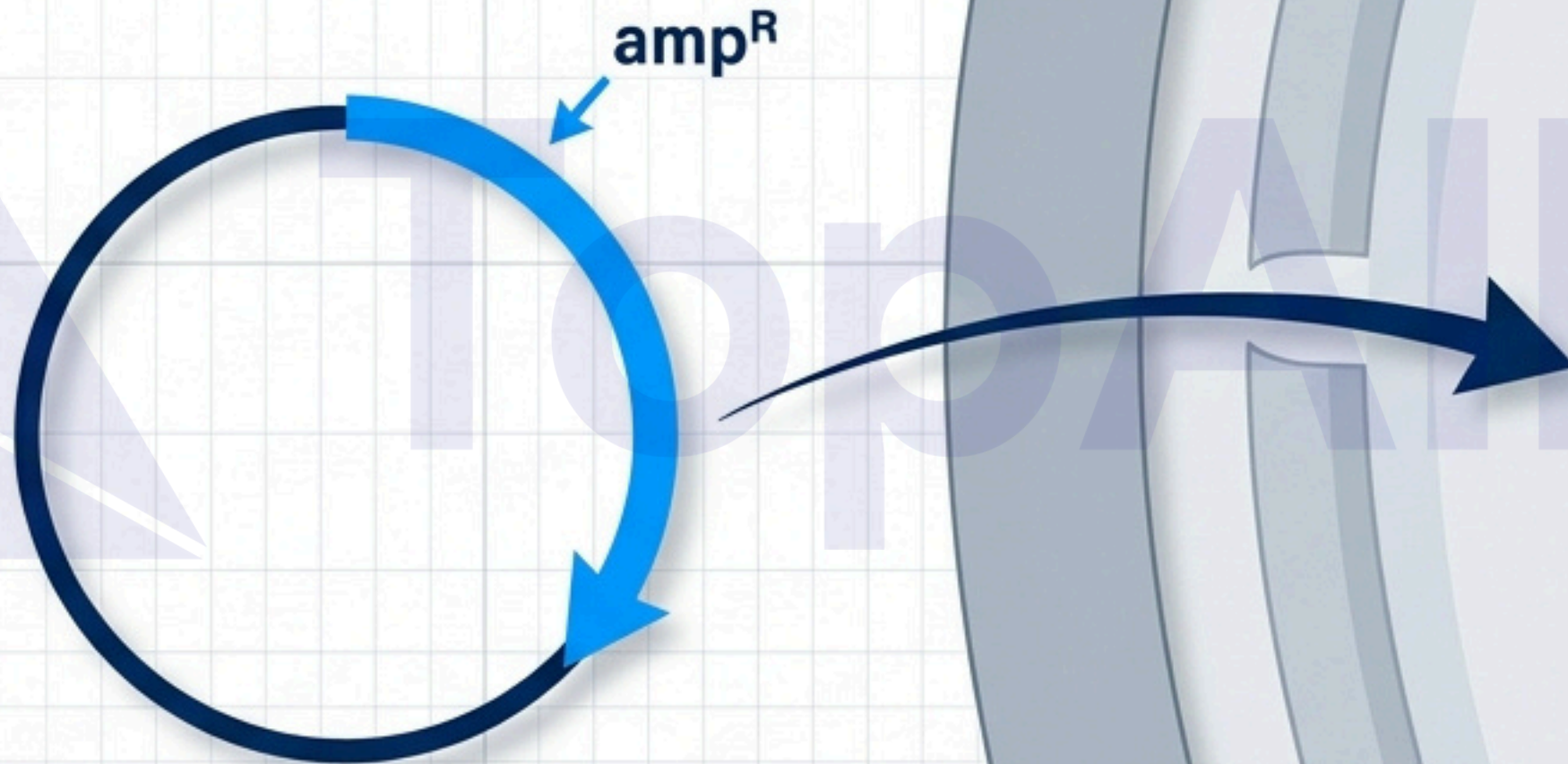
Recombinant DNA with ampicillin resistance enters *E. coli*

Step 1: Chemical Treatment

Bacterial cells are treated with a specific concentration of a divalent cation (e.g., Calcium) to increase the efficiency with which DNA enters through pores in the cell wall.

Step 2: Temperature Cycling

Cells and recombinant DNA are incubated on ice, followed by a brief heat shock at 42°C, and then returned to ice. This thermal shift forces the cell to take up the plasmid.





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$$E=mc^2$$



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Host cells become transformed



Transformation:

The procedure through which a piece of DNA is introduced in a host bacterium.

The Challenge:

Not all recipient cells successfully take up the DNA from their surroundings. The heat shock process leaves a mixed population of Transformants and Non-transformants in the culture tube.

Plate cells on ampicillin agar

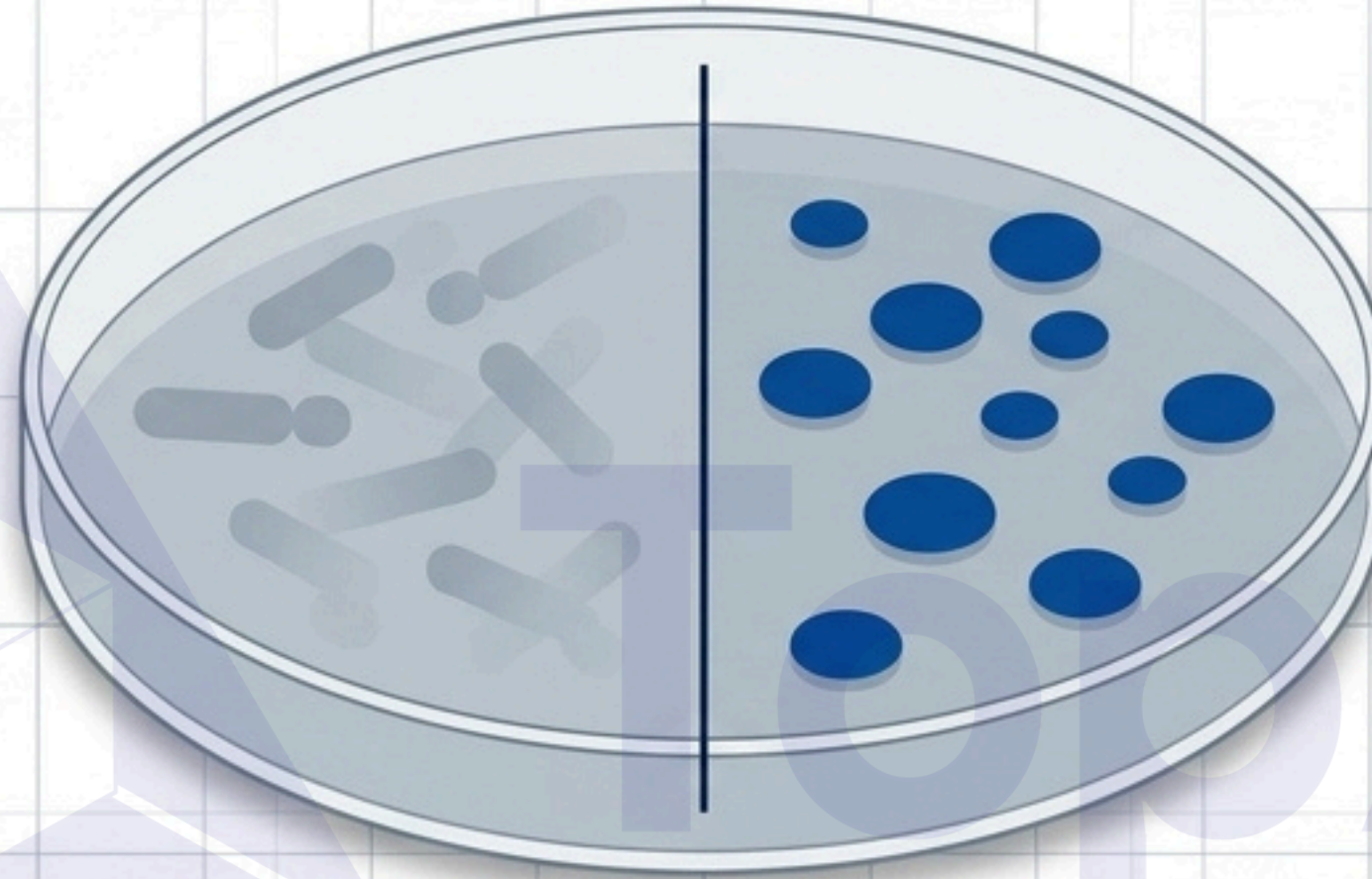
The agar medium contains the antibiotic **Ampicillin**.



Normal *E. coli* cells do not carry **resistance to ampicillin**. Without the recombinant plasmid, **this environment is lethal**.



Transformants survive; untransformed cells die.



The Untransformed

- **Genotype:** No plasmid taken up.
- **Phenotype:** Vulnerable to ampicillin.
- **Fate:** Cells die.

The Transformants

- **Genotype:** Harboring recombinant DNA.
- **Phenotype:** Expressing the amp^R gene.
- **Fate:** Cells survive and multiply into visible colonies.

The amp^R gene acts as a selectable marker, allowing us to easily identify and isolate only the specific cells that successfully integrated the recombinant DNA.



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