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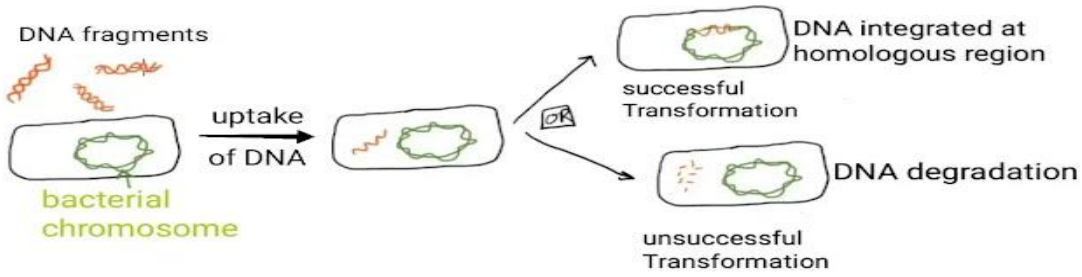
Lab (3) Transformation:

Transformation is the process of a competent bacteria picking up DNA from its environment and integrating it into a chromosome or plasmid. The DNA in the environment could have come from a dead bacterial cell that lysed (exploded), a live bacterial cell which released a copy of its DNA, or a scientist who made the DNA and put it in a test tube. Because this DNA could have come from anywhere, the DNA is said to have come from the environment. While it is common for bacterial cells to pick up genetic material from their environment, not all bacterial cells can do this. Only competent cells can undergo transformation. This means being competent is a requirement of a recipient cell to undergo transformation. Only competent cells have enough membrane permeability to take up DNA from their environment. These cells have large pores in their membrane that the DNA can fit through. In a lab, it is possible to induce competency, make a bacterial cell competent, so it can undergo transformation.



Transformation in bacteria

Procedure:

1) Prepare overnight culture from:

a) *E. coli* sensitive to ampicillin (suspension 1)

b) *E. coli* resistant to ampicillin (suspension 2)

2) Lyse suspension 2 by incubation for 15 min. at 70C and allow ampicillin resistance gene to be free and up taken by suspension I through transformation

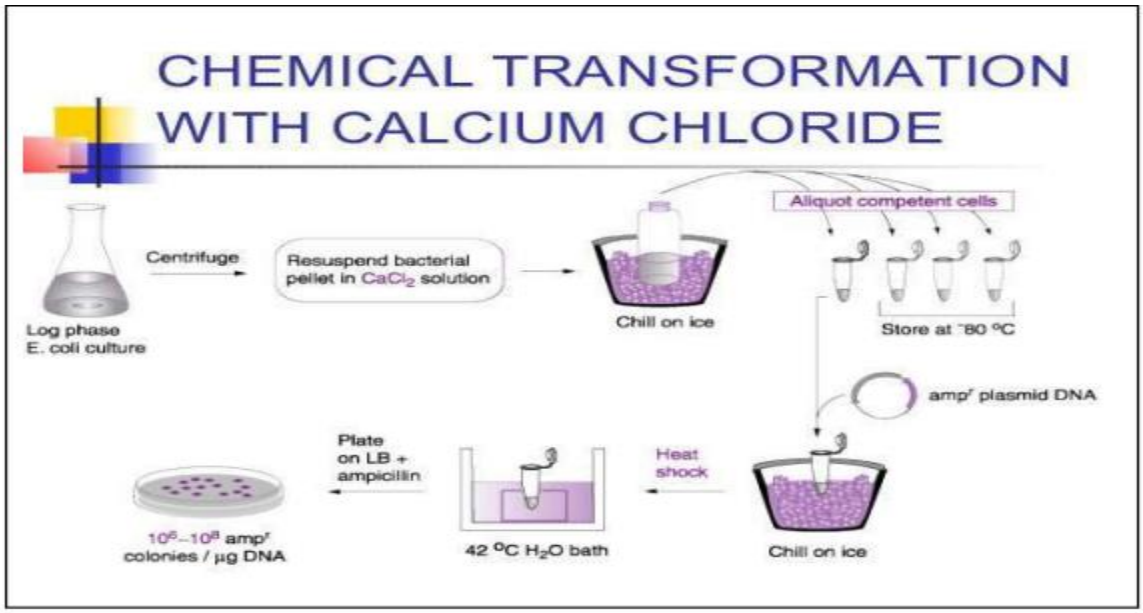
3) Take 3 ml of suspension 1 and centrifuge for 3000 rpm for 10 min.; then add (100 mM CaCl2 with 2.5 ml) to pellet and keep it for 10 min. in ice bath. The suspension is centrifuged again for 300 rpm for 10 min. This step is required to prepare competent cells.

4) Mix (1 ml) from 1 & 2 suspensions, then put the mixture at 0 C for 20 min.

5) Transfer the mixture to water bath at 42 C for 90 sec., then again transfer it to ice bath at 0 C for 2 min. to make a shock in the cell wall of suspension 1 (competent cells) and receive the DNA

6) Incubate the mixture for 15 min. at 37 C, then add it to ampicillin agar plate with 300 µg/ml and incubate for 24 hrs. at 37 C.

** The incubation after heat shock is to enhance and give a period time which could be enough for transformation to occur.*



To remember:

• Calcium chloride partially disturbs the cell membrane, which allows the recombinant DNA to enter the host cell.

• The surface of bacteria is negatively charged due to phospholipids and lipopolysaccharides on its cell surface; and the DNA is negatively charged too. One function of the divalent cations therefore, would be to shield the charges thereby allowing a DNA molecule to adhere to the cell surface.

• The heat pulse is through to create a thermal imbalance across the cell membrane, which forces the DNA to enter the cells through either pores or damaged cell membrane.