Practical Biotechnology

Lab 5

Competent cell preparation

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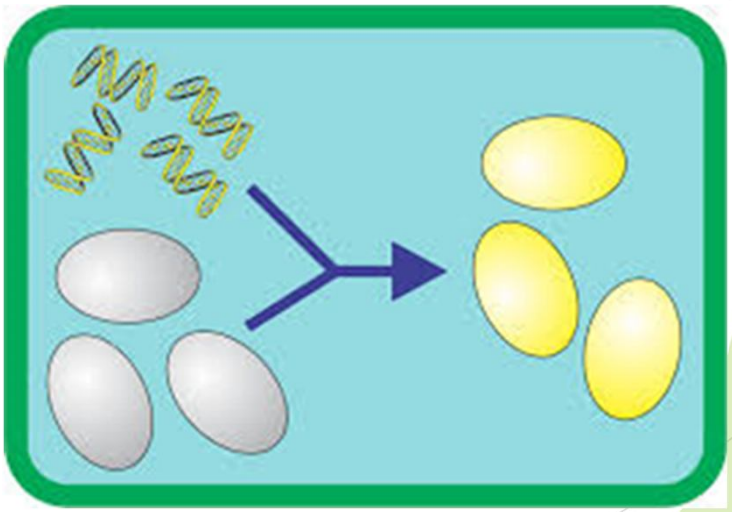
What are competent cells?

Cell competence refers to a cell's ability to take up foreign (extracellular) DNA from its surrounding environment.

The process of genetic uptake is referred to as *transformation*.



Principle of Competent Cells

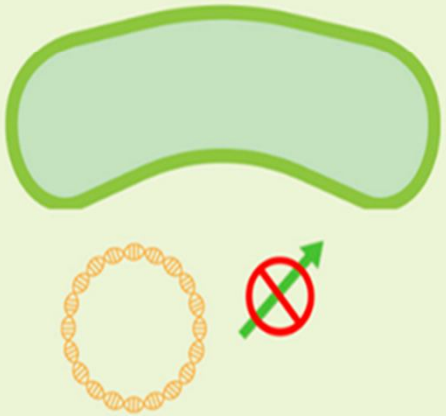
Some cells got to be exposed to some chemical or electrical treatments to transform them into competent cells by altered cell walls that allow the DNA to simply undergo it.

Cell competence has become an essential research tool for cloning because it provides scientist a mechanism to introduce new genetic material into a cell.

Other factors include existing DNA damage within the cell and recombination ability of incoming DNA.

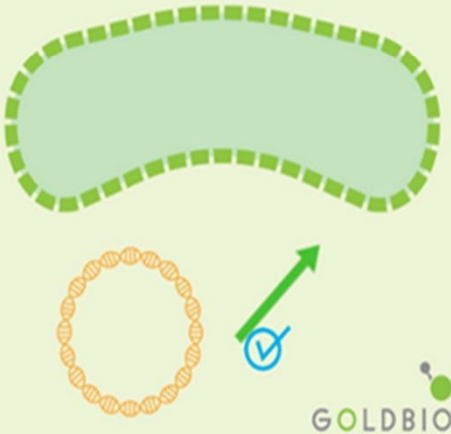
Noncompetent *E. coli*

E. coli that has not been made competent will not take up plasmids introduced into their enviroment.



Competent *E. coli*

E. coli made competent either through $CaCl_2$ and heat-shock or through electroporation will have better membrane permeability (pores), enabling plasmid uptake.



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Methods of Preparation of Competent Cells

Competence is achieved in two ways:

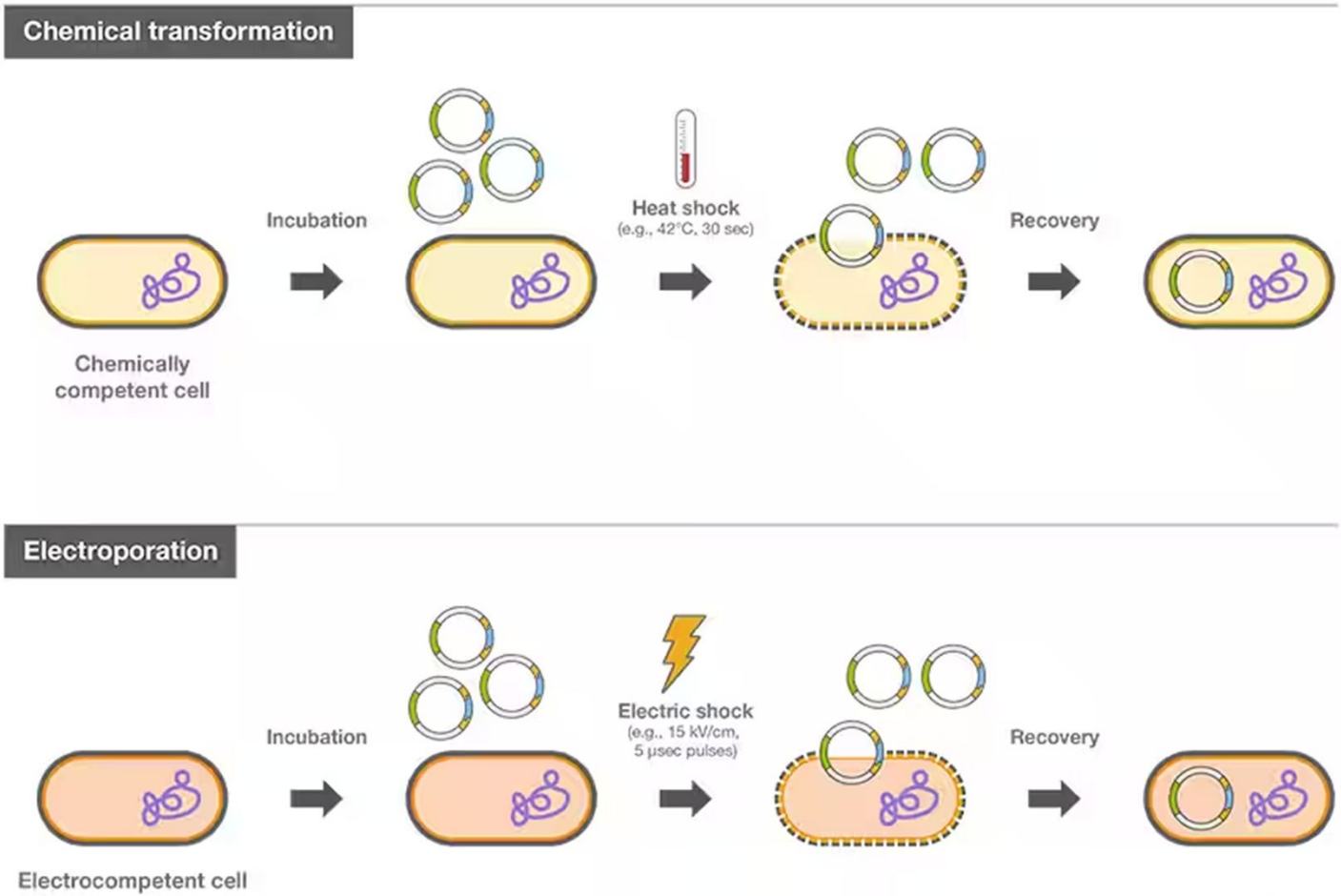
- Natural Competence
- Artificial Competence

Natural cell competence is genetically determined, that is to say, a bacterium is genetically predisposed to take up free genetic material that exists within their environment.

Artificial or induced competent cells are cells researchers have made competent through electrical (electroporation) or chemical manipulation. Electroporation is the process in which cells take up DNA.

Calcium Chloride: The bacterial cells were treated with salt then suddenly exposed to high temperatures. This is known as the heat shock treatment method and it is the standard method for the preparation of those cells.

Electroporation: During this technique, an electrical field is applied to the cells to extend their permeability. It is also known as electropermeabilization.


Chemical transformation
Incubation
Heat shock
(e.g., 42°C, 30 sec)
Recovery
Chemically
competent cell
Electroporation
Incubation
Electric shock
(e.g., 15 kV/cm,
5 µsec pulses)
Recovery
Electroccompetent cell

Procedure:

1. Inoculate 2ml of LB or (nutrient broth) with a single DH5α colony. Incubate culture overnight at 37oC while shaking at 250 RPM.

2. The following morning, inoculate 500ml of LB or (nutrient broth) with 1ml of saturated overnight culture. Incubate culture at 37oC while shaking at 250 RPM until OD600 = 0.5 (3-5 hours).

3. Transfer culture to 2 pre-chilled sterile 250ml centrifuge tubes.

Pellet bacteria cells with a 5000 RPM spin for 10 minutes at 4oC. Discard supernatant. Place pellets on ice.

4. Resuspend cells in 10ml cold CaCl2 solution. Pool cells together into one pre-chilled 50ml Oakridge tube.

5. Pellet cells with a 2500 RPM spin for 5 minutes at 4oC. Discard supernatant and resuspend cells in 10ml cold CaCl2 solution. Set on ice 30 minutes.

6. Pellet cells with a 2500 RPM spin for 5 minutes at 4oC. Discard supernatant and resuspend cells in 2ml cold CaCl2 solution.

*At this point you can leave cells on ice overnight at 4oC – this increases competency in some cases.

7. Dispense cells into 50ul aliquot in pre-chilled sterile polypropylene tubes.
Store cells at –80oC. Test for Competency Transformation.


Competent Cell Preparation
Abnova
Resuspend each tube with 40ml chilled CaCl2 by gentle pipetting

Thank you for listening

8. Remove competent DH5a cells from the –80oC and immediately place on ice. Once thawed, add >10ng of plasmid DNA to a 50ul aliquot of competent cells. Place cells/DNA on ice for 3 minutes.

9. Heat shock cells at 42oC for 3 minutes.

10. Place cells back on ice for 3 minutes.

11. Add 1ml LB to cells/DNA. Tape tube onto shaking incubator platform and incubate cells/DNA for 1 hour at 37oC while shaking at 250 RPM.

12. Pellet cells with a quick spin. Remove 800ul of supernatant. Resuspend cells in the remaining supernatant.

13. Plate 100ul and 200ul of transformation onto 2 LB+Amp plate. Place plates inverted at 37oC overnight.

Solutions:

1. 100 mM MgCl2:
   - 1:10 dilution of lab stock; use sterile ingredients or filter
   - sterilize
2. 100 mM CaCl2:
   - 1:10 dilution of lab stock; use sterile ingredients or filter
   - sterilize
3. 85% 100 mM CaCl2, 15% glycerol:
   - 42.5 ml 100 mM CaCl2
   - 7.5 ml 100% glycerol
   - 50.0 ml total volume; mix well and use sterile ingredients or filter sterilize