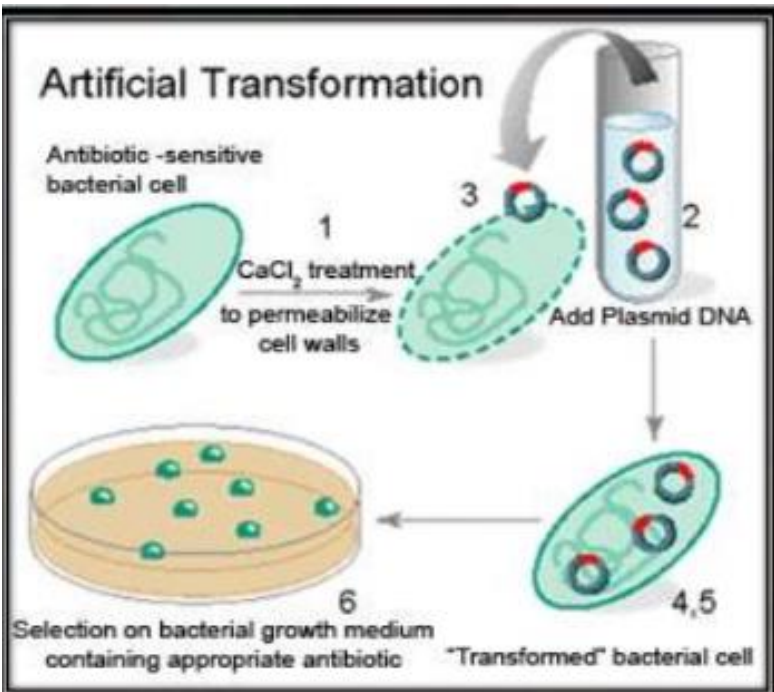


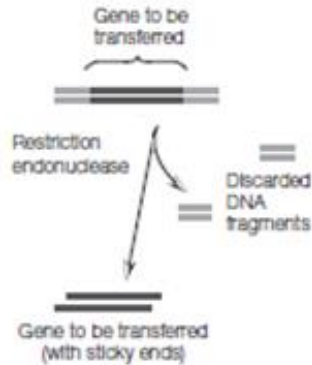
Competent Cells Formation and Transformation

Prepared by Latifa Aljebali

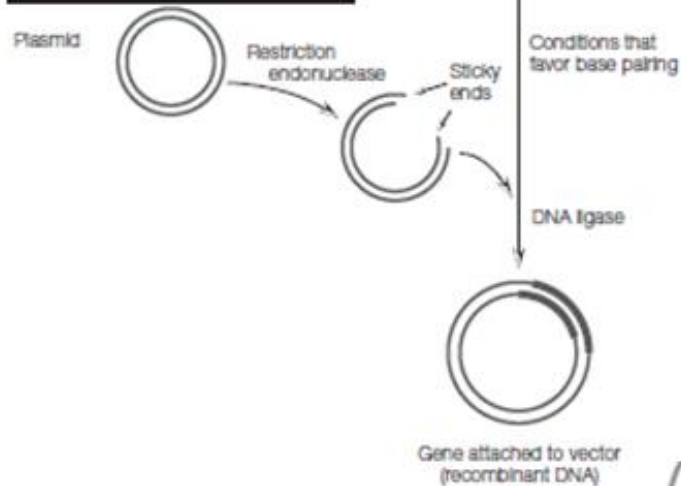


DNA Cloning

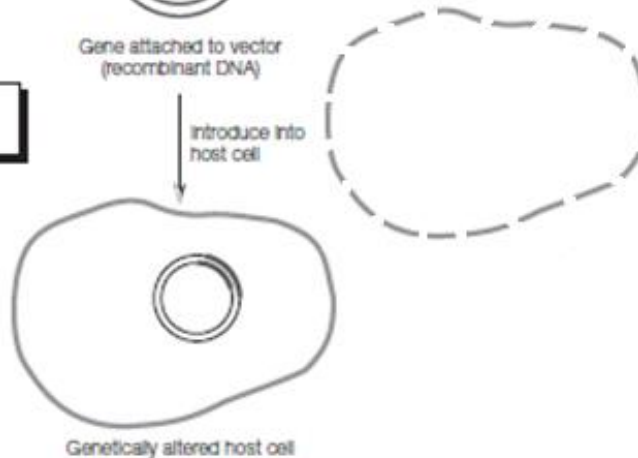
Step 1. Obtain gene to be transferred.



Steps 2 and 3. Attach gene to vector.

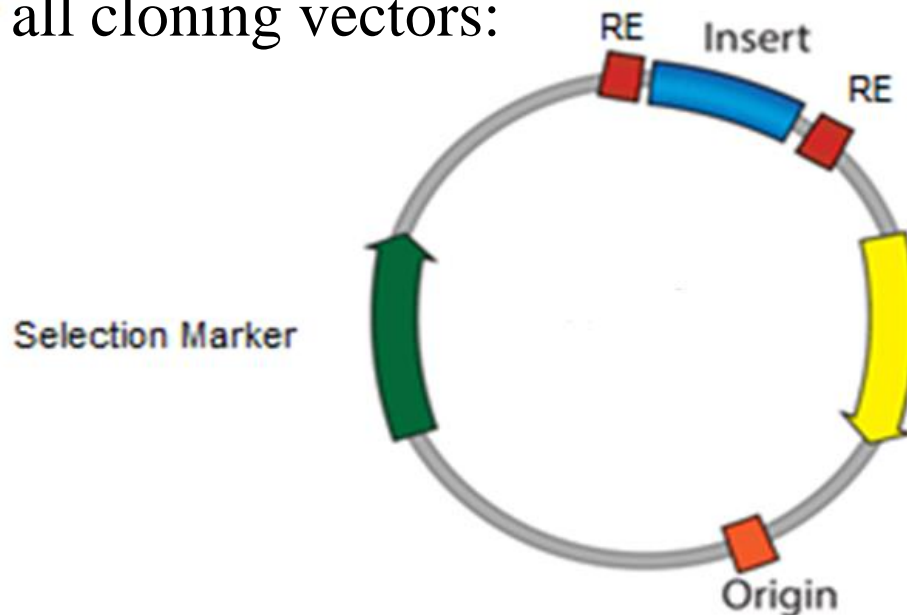


Steps 4 and 5. Introduce recombinant DNA into host cell.



Cloning Definitions

- DNA cloning is a method of rapid isolation and amplification of DNA fragments.
- Cloning vector is a molecule of DNA to which the fragment of DNA to be cloned is joined.
- Three features of all cloning vectors:



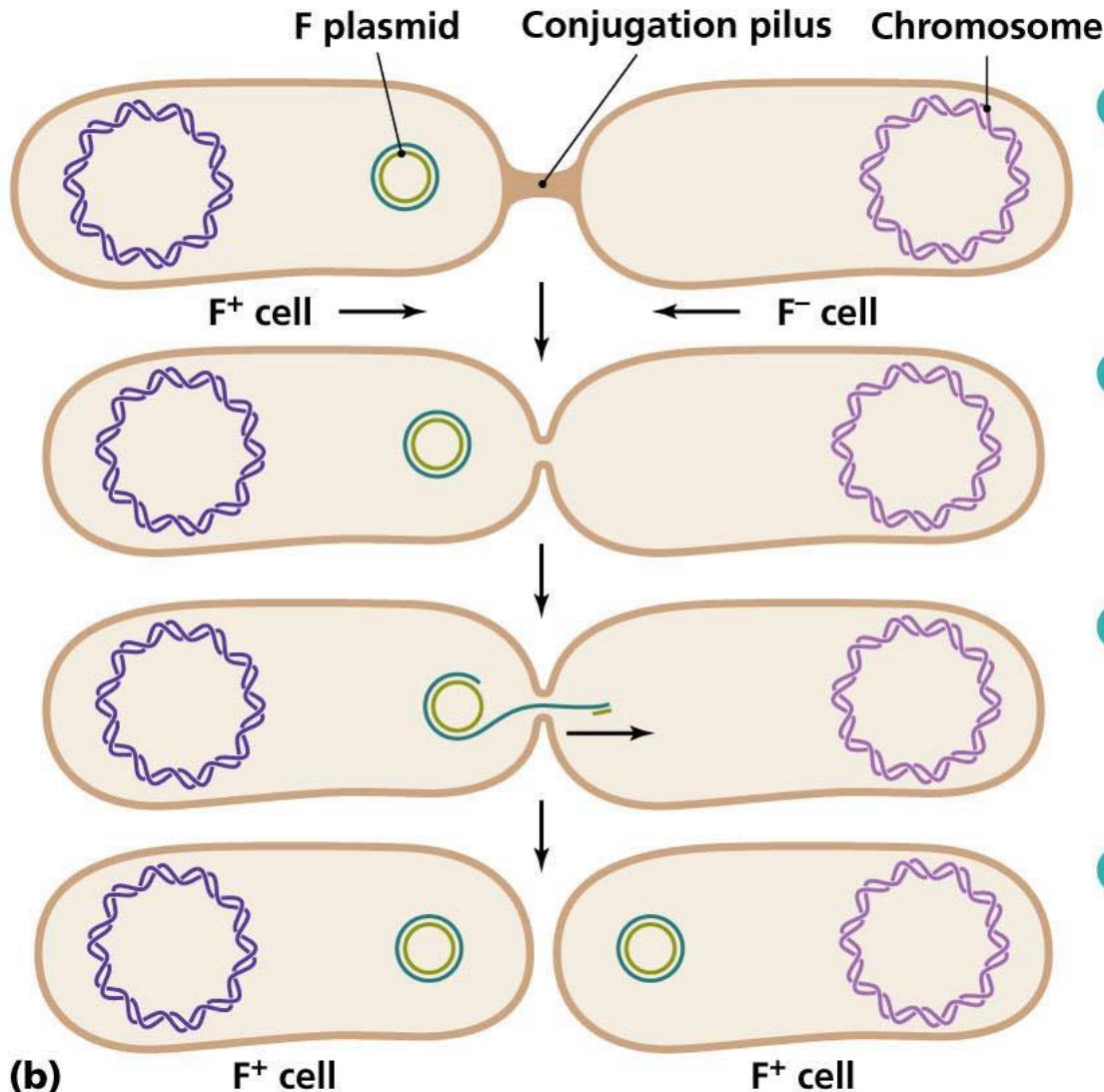
Cloning Vector Types

1. **Plasmid:** an extrachromosomal circular DNA molecule that autonomously replicates inside the bacterial cell; cloning limit: 100 to 10,000 base pairs or 0.1-10 kilobases (kb).
2. **Phage:** derivatives of bacteriophage lambda; linear DNA molecules, whose region can be replaced with foreign DNA without disrupting its life cycle; cloning limit: 8-20 kb.
3. **Cosmids:** an extrachromosomal circular DNA molecule that combines features of plasmids and phage; cloning limit - 35-50 kb.
4. **Bacterial Artificial Chromosomes (BAC):** based on bacterial mini-F plasmids. cloning limit: 75-300 kb
5. **Yeast Artificial Chromosomes (YAC):** an artificial chromosome that contains telomeres, origin of replication, a yeast centromere, and a selectable marker for identification in yeast cells; cloning limit: 100-1000 kb

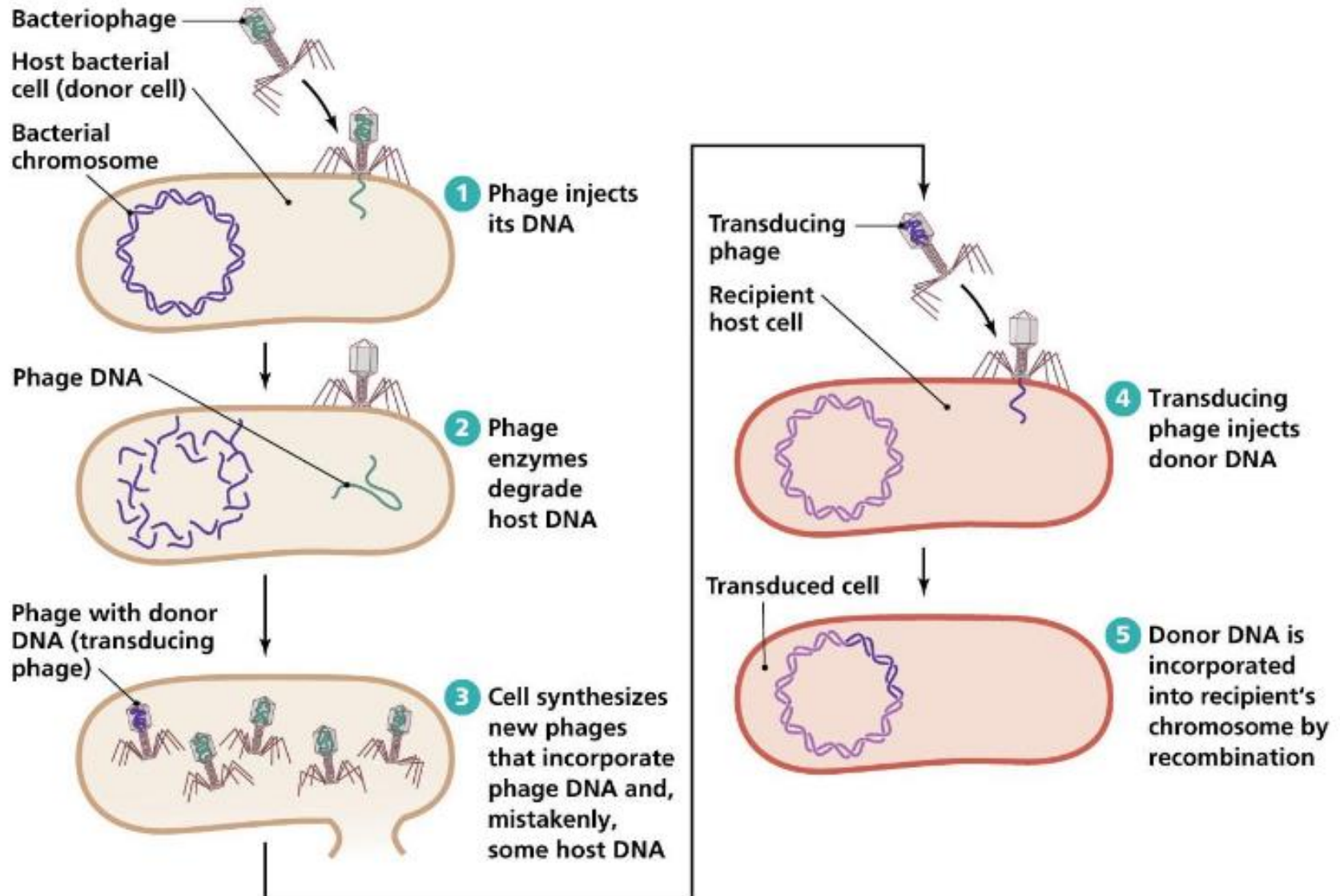
Competence and its Types

- It is the ability of a cell to take the DNA from the environment.
- It has two main types:
 1. Natural competence.
 2. Artificial competence.

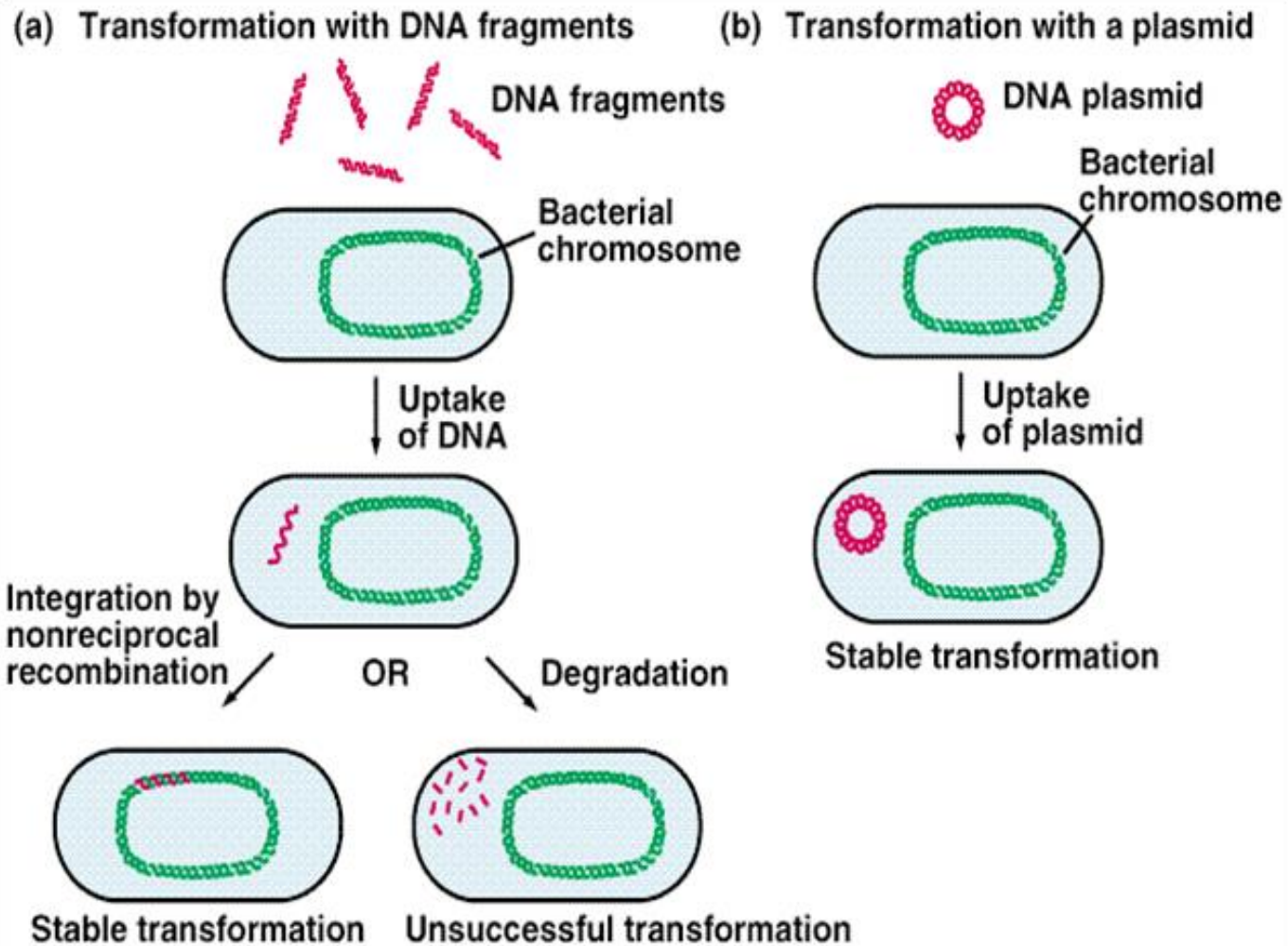
Conjugation



Transduction

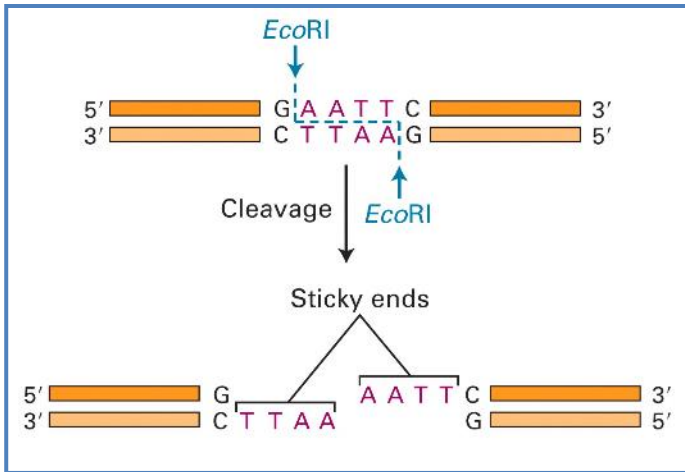


Transformation

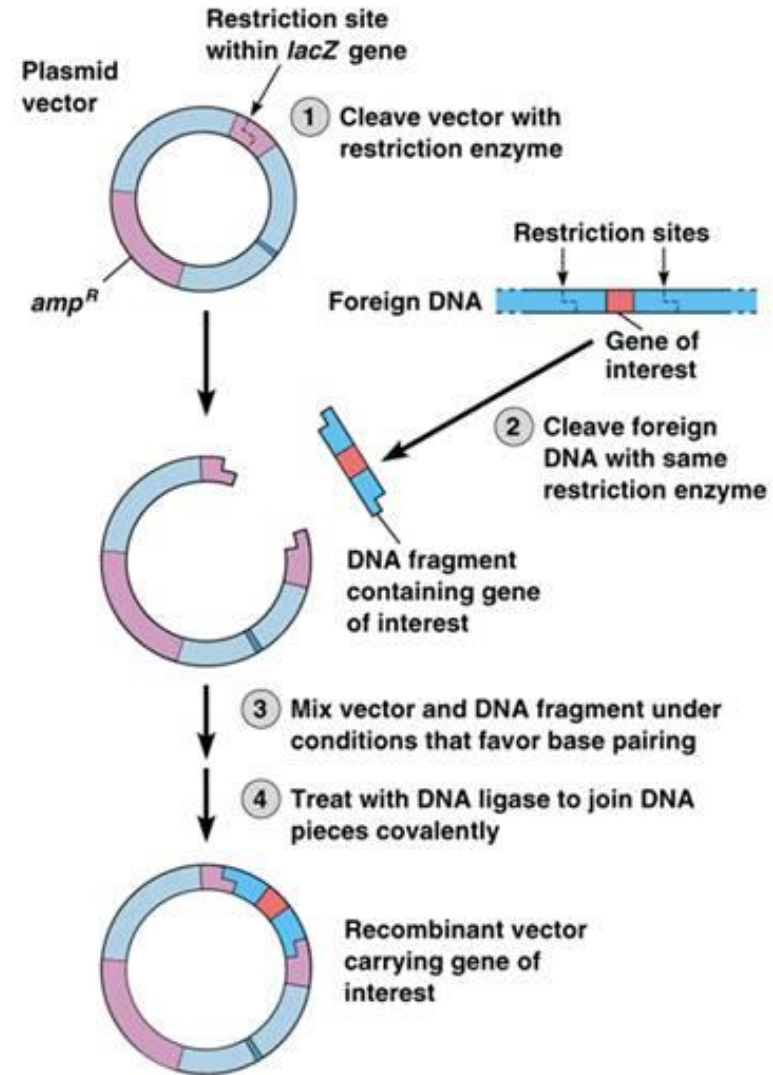


Transformation Cont.

- Restriction enzymes are endonucleases.
- Each RE recognizes a very specific nucleotide sequences called recognition sequence.



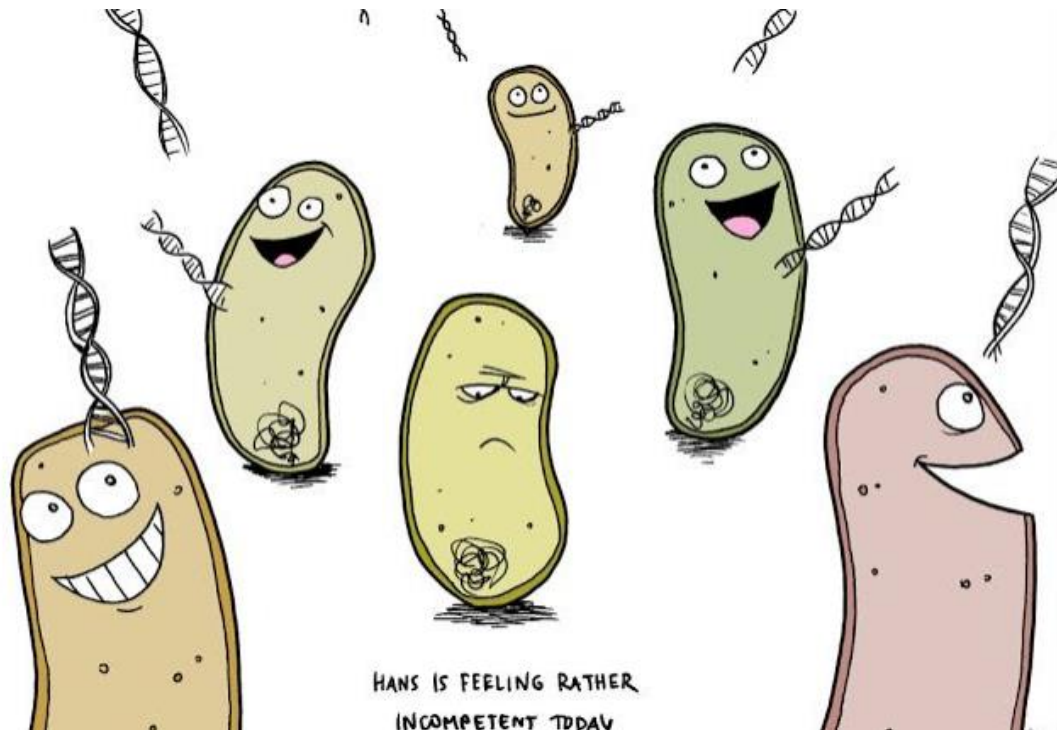
- DNA ligase joins the sticky ends of DNA fragments.



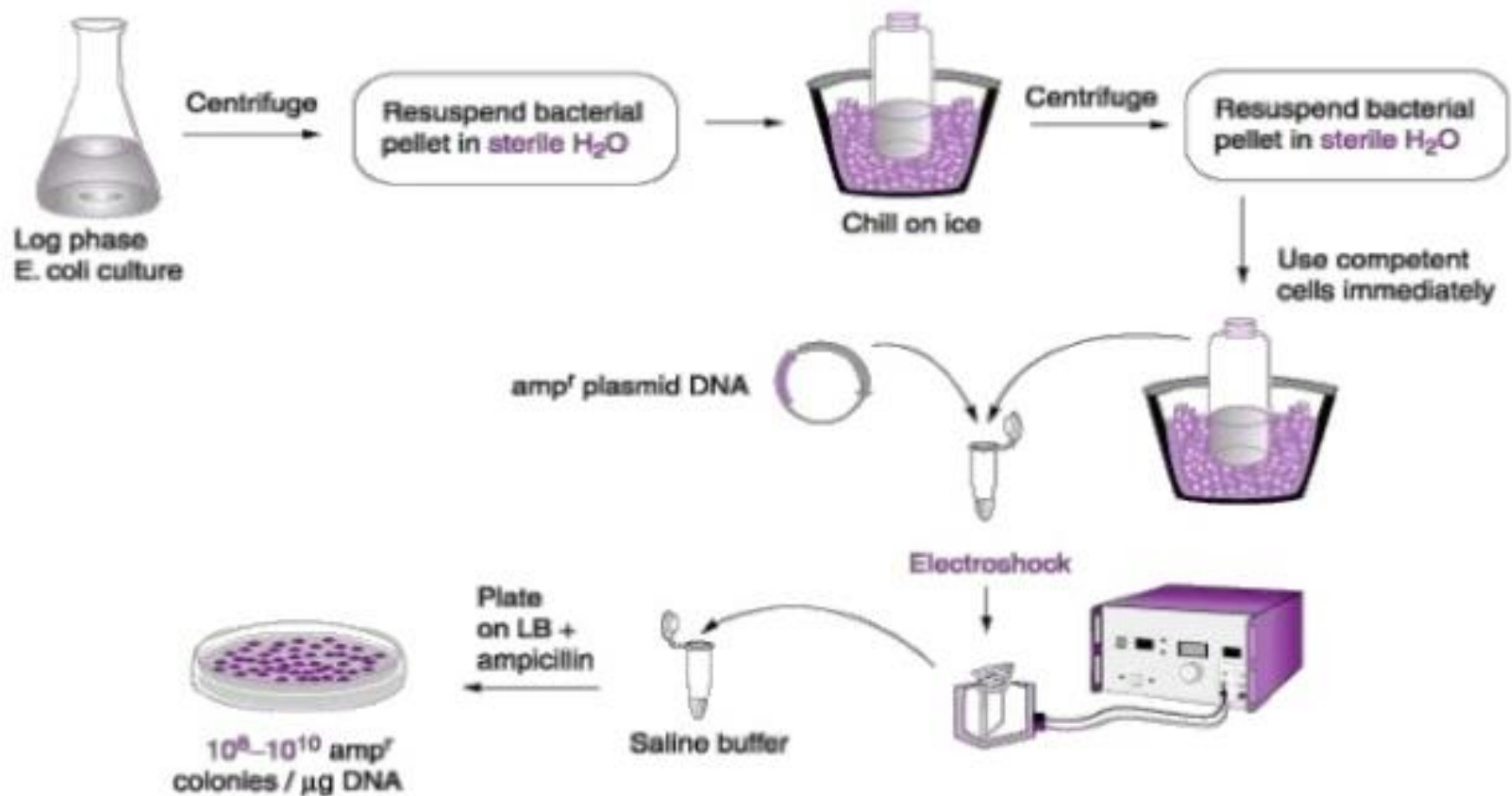
(b) Preparation of recombinant plasmid vector

Competent Cells Formation

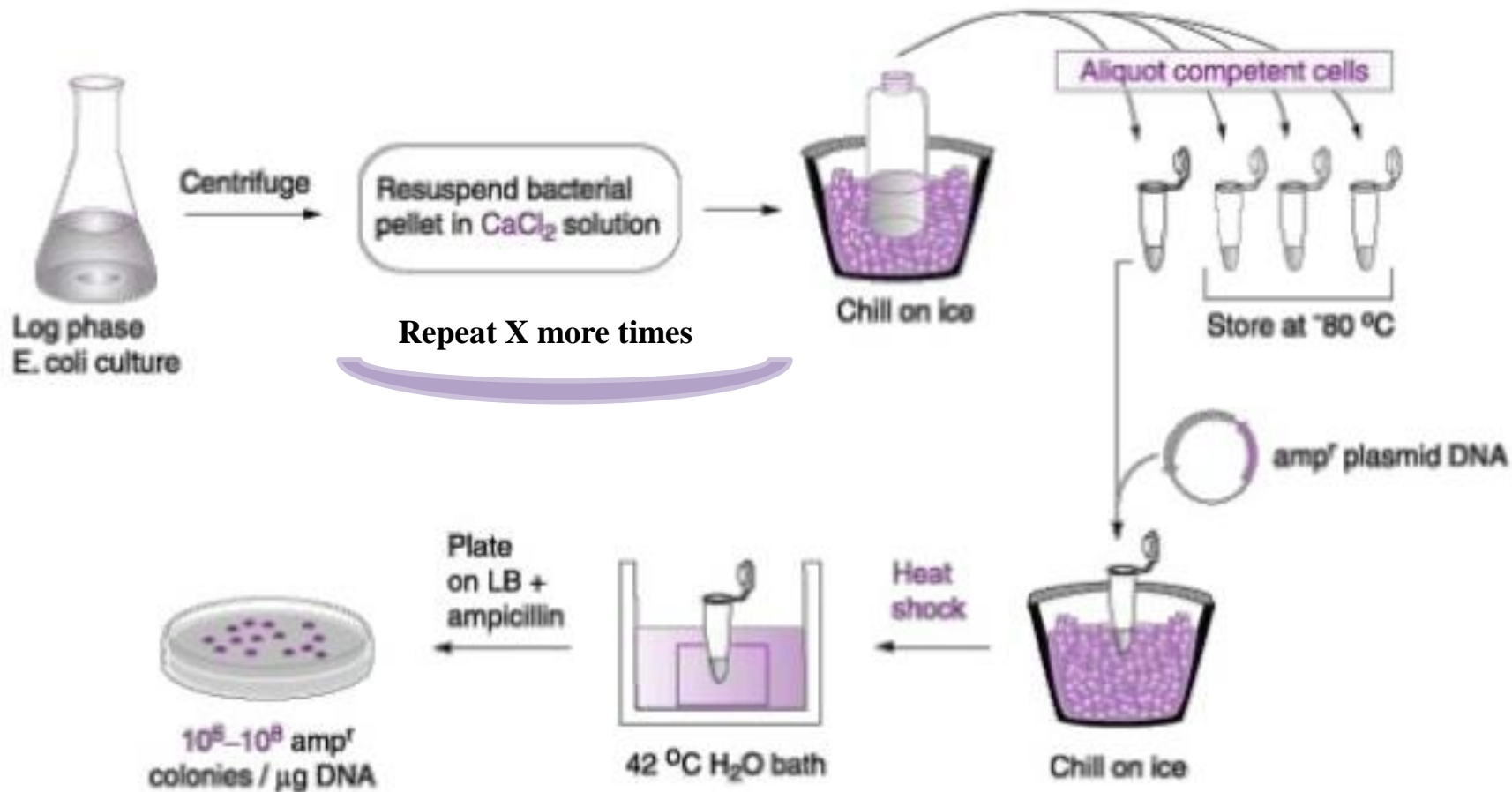
- Either by:
 1. Electroporation.
 2. Chemical transformation.
 3. Microwave radiation.



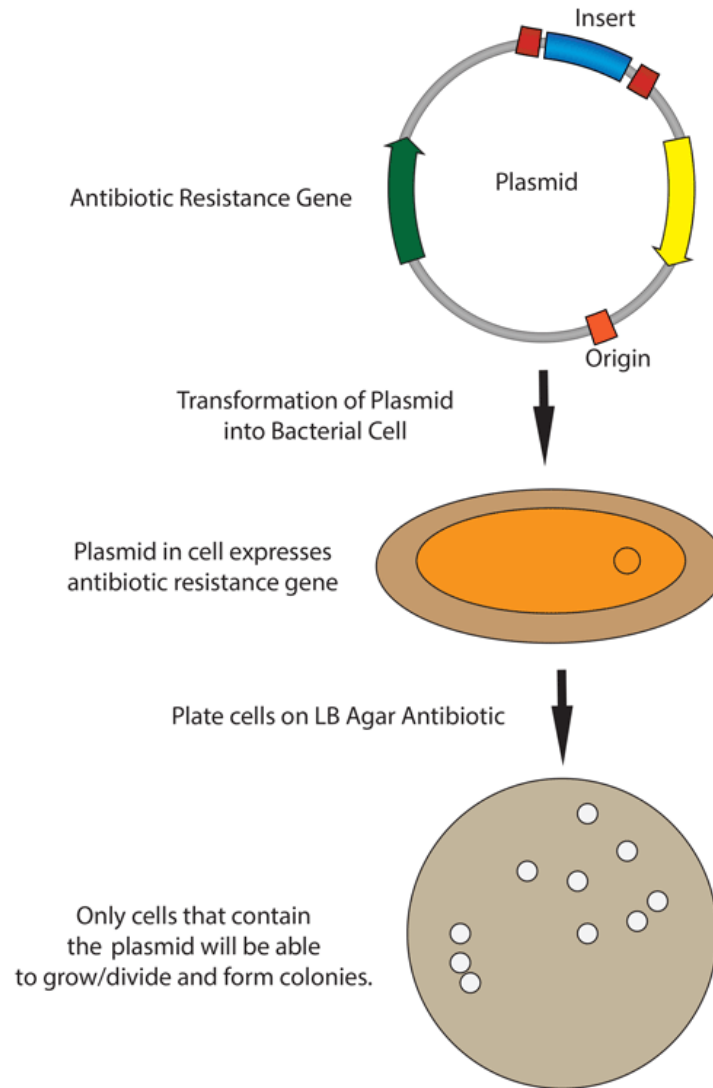
TRANSFORMATION BY ELECTROPORATION



CHEMICAL TRANSFORMATION WITH CALCIUM CHLORIDE

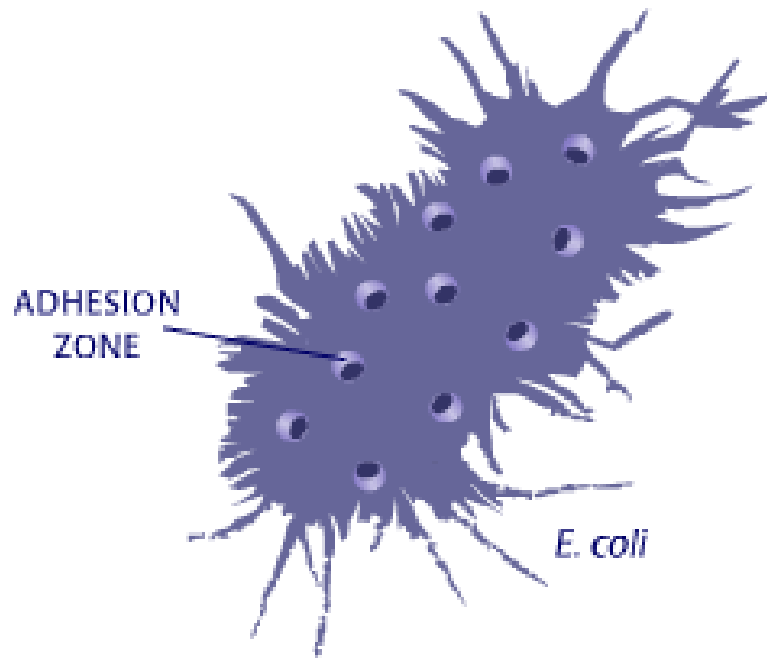


Plasmid Transformation

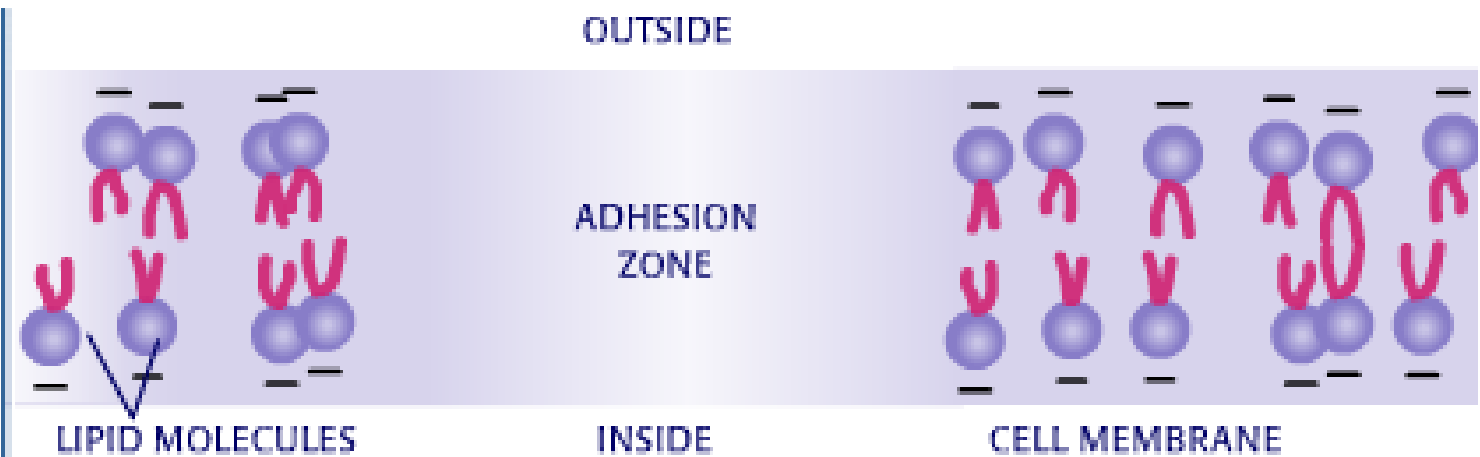
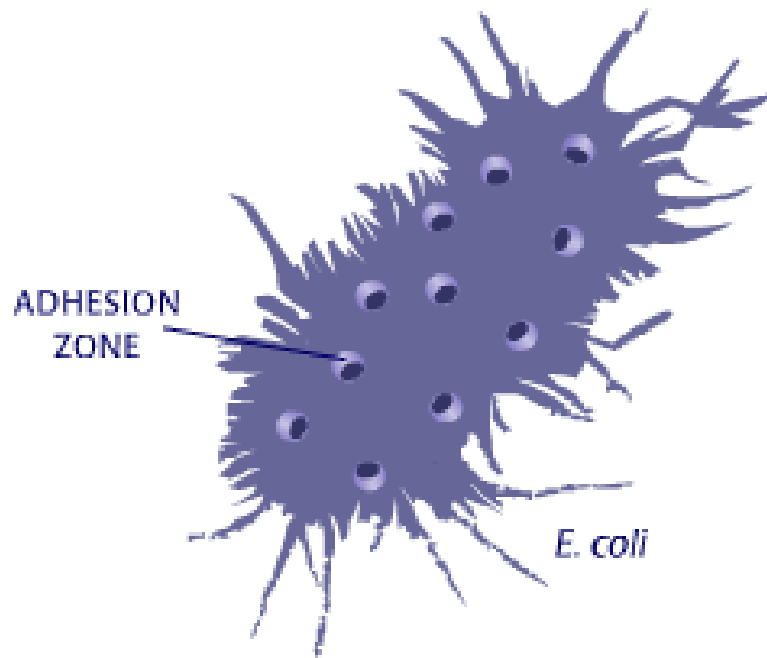


$$\text{Transformation Efficiency} = \frac{\text{Total number of colonies on LB/Amp plate}}{\text{Amount of DNA plated } (\mu\text{g/ml})}$$

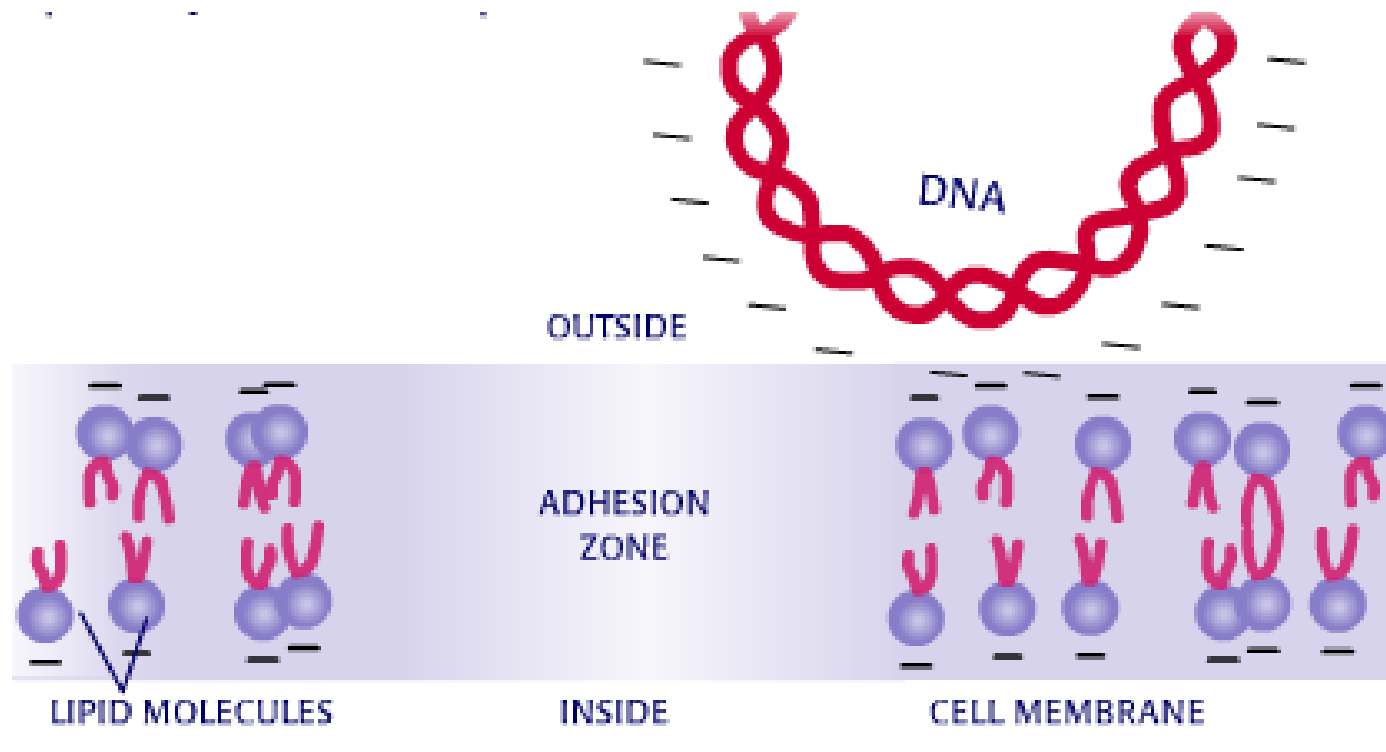
Chemically Formed Competent Cells



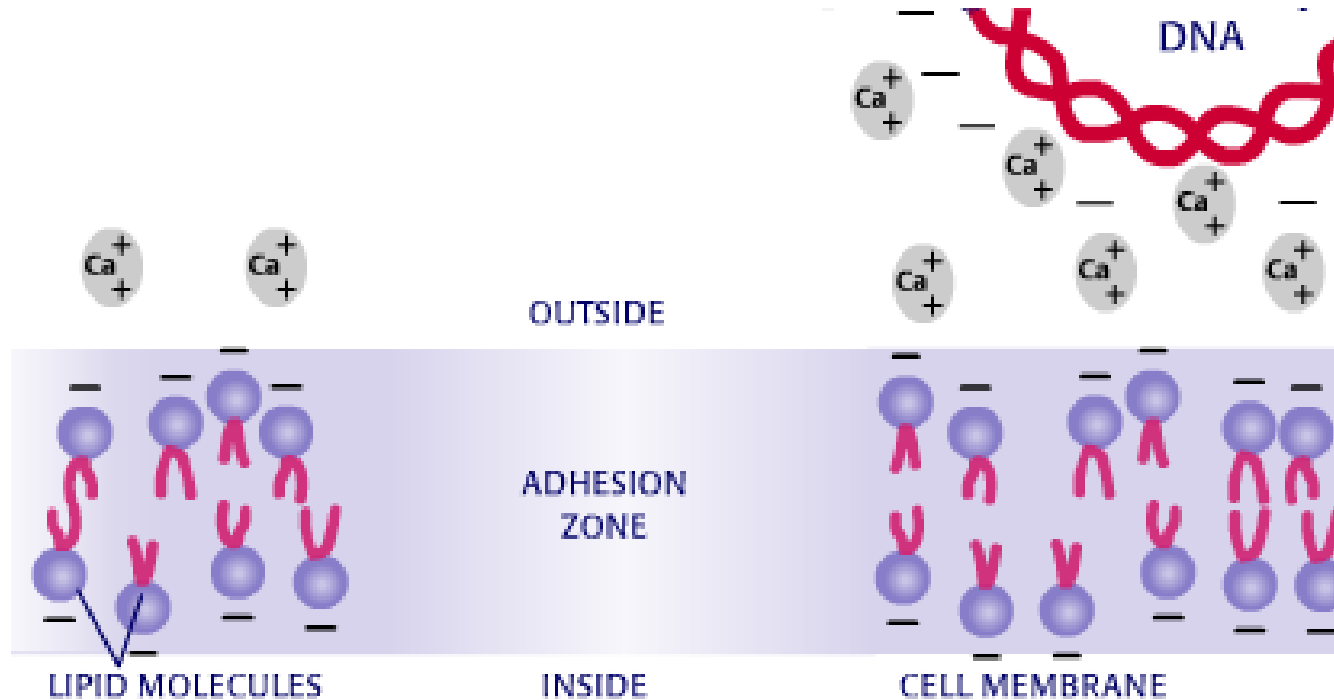
Chemically Formed Competent Cells Cont.



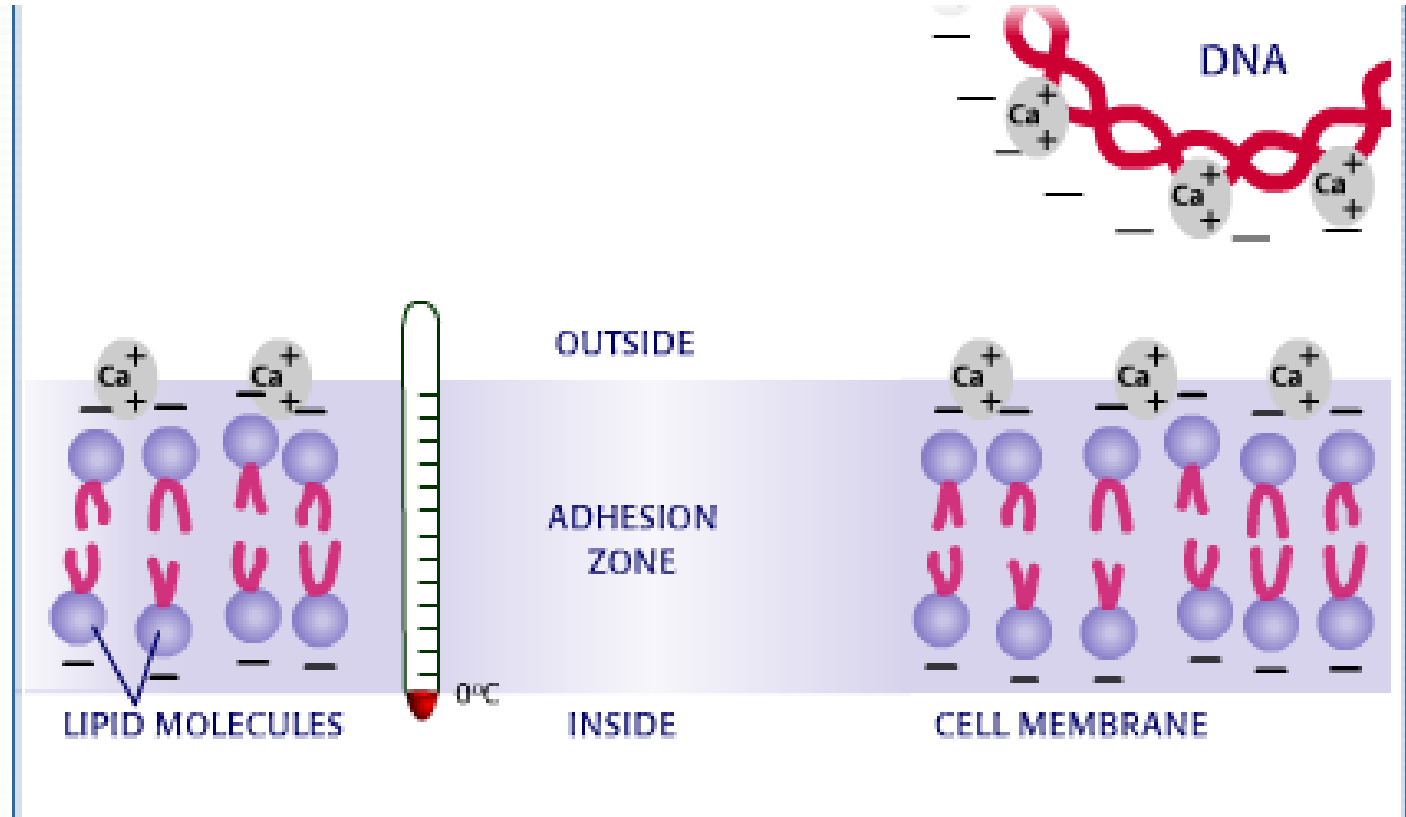
Chemically Formed Competent Cells Cont.



Chemically Formed Competent Cells Cont.



Chemically Formed Competent Cells Cont.



Chemically Formed Competent Cells Cont.

