Competent Cells Formation and Transformation of Competent Cells with DNA

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Introduction

DNA cloning is a method of rapid isolation and implication of DNA fragments. Cloning involves constriction of hybrid DNA molecules that are able to self-replicate in a host cell(usually bacteria). This is accomplished by:

- Inserting DNA fragments into a cloning vector(Plasmid or bacteriophage).
- Introducing the vector into bacterial cells .
- Amplifying vector DNA using bacterial DNA replication machinery.

What is the Cloning Vector?

It is a molecule of DNA to which the fregmet of DNA to be cloned is joined.

Vector must be capable of independent replication within the bacteria host cell; also they must contain at least one specific nucleotide sequence recognized by a **restriction endonuclease**.

Two major types of cloning vector can be found in bacterial cells, they are plasmid and bacteriophages.

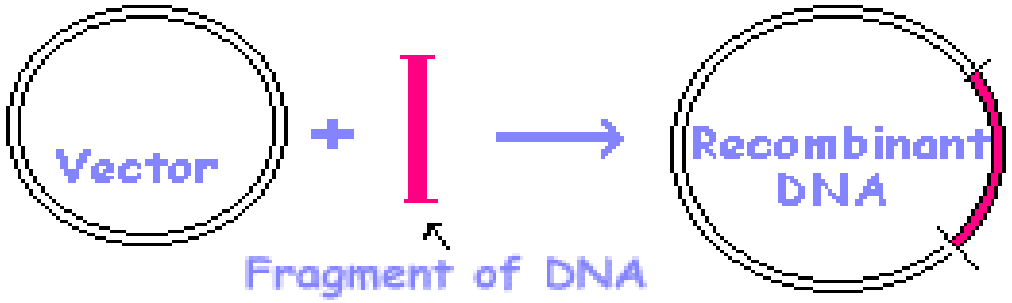


Figure 1: Production of recombinant DNA

How the Host cell can acquired a new genetic information?

Bacteria are able to take up DNA from their environment (exogenous DNA) in **three ways**;

1-conjugation,

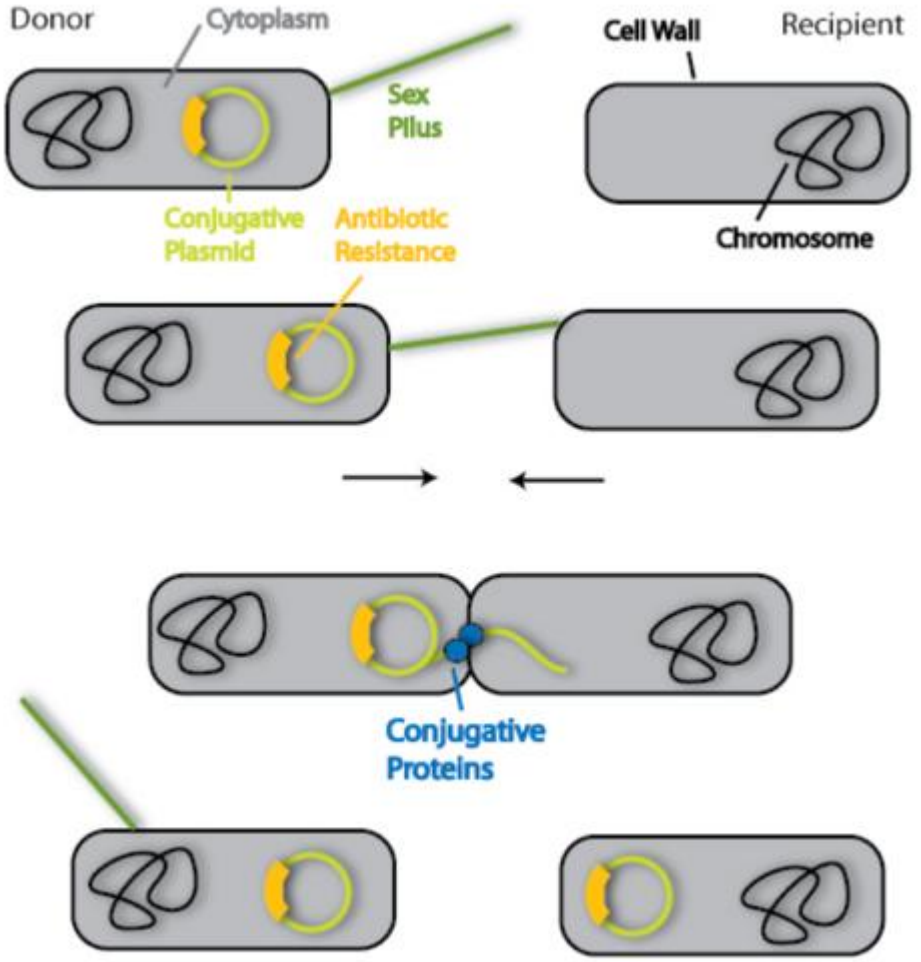
2- transduction

3-transformation.

Only transformation is the direct uptake of DNA, since conjugation requires cell-cell contact via a sex pilus and transduction requires a bacteriophage intermediary to transfer DNA from one cell to another

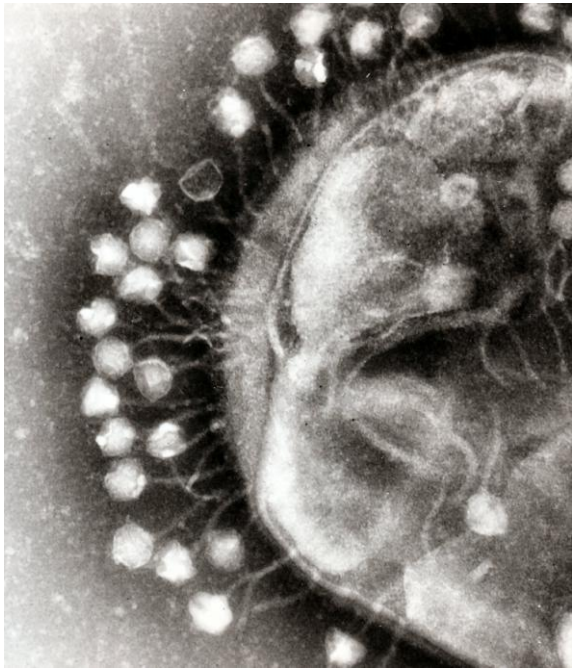
1-conjugation

During conjugation , DNA is transferred directly from one organism to another and it requires direct cell-cell contact



2-transduction

The DNA carried by bacteriophages i.e. transduction **requires a bacteriophage** intermediary to transfer DNA from one cell to another



3- transformation

It involves the acquisition of extracellular DNA from the environment, and genetic competence is the ability to undergo transformation which means the ability of the a cell to take the DNA from the environment.

For a bacterial cell to uptake DNA from its surroundings, it must be in a special physiological state called **competence**.

Competence can be classified as :

- **Natural competence** ; genetically specified ability of bacteria that is occur under natural condition
- **Artificial competence** ; when cells in laboratory cultures are treated to be permeable to DNA.

Methods of transforming E.coli

E.Coli can be used in cloning procedures:

1-Electroporation transformation:

highly efficient but requires expansive instrument.

2-Chemical transformation:

Less efficient than electroporation but does not requires expansive instrument.

3- transformation by microwaves:

Very simple but has low transformation efficient

Principle:

- Transformation of E.coli cells with plasmid DNA is done by using **Chemical transformation.**
- Since DNA is a very hydrophilic molecule, it won't normally pass through a bacterial cell's membrane!!→In order to make bacteria take in the plasmid, this is done by **creating small holes** in the bacterial cells **by suspending** them in a **solution** with a high concentration of **CaCl2** , that renders them competent to take up DNA.

Principle :

DNA uptake is facilitated by brief heat shock and transformed cells are selected by **positive selection** on LB plates with the appropriate antibiotic. Each colony on an antibiotic plates presents a single transformation event.

To use this method it is **necessary to induce competence** for DNA uptake in E.coli, because this bacterium does not possess a natural mechanism for transformation. The efficiency of transformation for this method is between 10000 to 1000000 **transformants /µg of plasmid**

Materials :

Chemicals:

A- Competent Cell Formation

- *E. Coli strain*
- LB medium (Tryptone, yeast extract, NaCl)
- Calcium chloride.
- LB plates (agar)

B-Transformation of Competent cells with DNA:

- Competent cells of *E. Coli strain*
- LB medium (Tryptone, yeast extract, NaCl)
- Appropriate antibiotics
- Plasmid DNA

Experimental protocol

As in lab sheet

Thank You