

# Transformation in plasmids



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This experiment aims to demonstrate the phenomenon of transformation. A plasmid was introduced into competent *Escherichia coli* (*E. coli*) cells by electroporation, plated out on a solid medium and left to divide and multiply. Through this experiment, the idea and importance of gene transformation in genetic engineering can be understood. The procedure and observations brought home the concept of genotype and phenotype being directly controlled by the genes which are made of DNA.

Genetic engineering consists of the direct manipulation of an organism's genes. There are five main steps involved. The first involves the isolation of the genes of interest. Then the genes have to be inserted into a vector such as a plasmid. Thirdly, the vector has to be transferred to the organism that needs to be modified and this is followed by transformation of the cells in the organism. Lastly, selection has to be carried out to distinguish successfully modified cells from those that have been unsuccessful.

In this laboratory protocol, the phenomenon of transformation was established. Transformation occurs when a cell uptakes a naked DNA molecule or fragment from the medium and incorporates this molecule into the recipient chromosome in a heritable form through recombination.

However, not all bacteria can undergo transformation and not all extracellular DNA is competent for transformation. For competency, the extracellular DNA must be double stranded and the bacterial cell must have the surface protein, competence factor, which binds to the extracellular DNA in an energy-requiring reaction.

Transformation can also be carried out by introducing a plasmid into a bacterial cell. A plasmid is a small, circular, self-replicating DNA molecule which differs from the bacterial chromosome. It has only a small number of genes and these genes are not required for the survival and reproduction of the bacterium under normal conditions. However, the genes of plasmids can confer advantages on bacteria living in stressful environments.

In this experiment, the plasmid pUC19 was introduced into competent *Escherichia coli* (*E. coli*) cells. *E. coli* is a non pathogenic strain of bacteria which is not harmful. The plasmid contained genes which code for resistance against antibiotic ampicillin. This is essential in the final step of selecting for transformants.

Figure : Genetic map of pUC19 plasmid

The introduction of the plasmid was carried out by electroporation. Electroporation, also known as electroporation, increases the conductivity and permeability of the cell plasma membrane by applying an external electric field. This, thereby, makes it easier for the plasmid to enter the *E. coli* cells.

Electroporation\_Diagram

Figure : Diagram of an electroporator

This experiment consisted of two controls. The negative control is made using *E. coli* without the plasmid while the positive control contains *E. coli* with the plasmid. The positive control will be able to code proteins for ampicillin resistance while the bacterial cells from the negative control will

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develop no such resistance. Therefore, when both controls are placed on the antibiotic containing agar plate, only bacterial cells in the positive control will survive and multiply.

At an ideal dilution, bacterial cells which are plated onto solid media will divide and multiply. A single cell will give rise to daughter cells which aggregate and form clumps which are also termed as colonies. Each colony contains approximately  $10^7$  cells which are derived from a single parent cell. They also contain identical genomes. Single colonies indicate that the population has no contamination with unwanted bacteria and they also confirm the genetic makeup of the bacteria.

## **Equipment and Materials**

37°C shaking incubator

37°C non-shaking incubator

LB agar plates containing 100µg/mL

Competent cells

pUC-19 plasmids

Electroporator

Cuvettes (0.1cm)

Ultrapure H<sub>2</sub>O

S. O. C medium (room temperature). \*S. O. C. medium: Tryptone (pancreatic digest of casein) 2% (w/v), Yeast extract 0. 5% (w/v), NaCl 8. 6 mM, KCl 2. 5 mM, MgSO<sub>4</sub> 20 mM, and Glucose 20 mM,

15 ml snap-cap tubes (one for each transformation)

## **Procedures**

### **Preparation for Electroporation**

In order to attain high efficiency electroporation, it was necessary to keep the cells and cuvettes cold up to the time when the pulse was applied. After which, SOC was added to the cells immediately. The steps below were followed to prepare for electroporation.

0. 1 cm cuvettes were pre-chilled in ice or at -20 oC.

The electroporation device was set up for bacterial electroporation using the following setting. BioRad GenePulser® II

Capacitance: 25 µF

Resistance: 200 Ω

Voltage: 2. 5 kV

Cuvette: 1 mm

An ice bucket was prepared for the cells and the DNA.

The SOC medium to be added to the cells was thawed and kept at room temperature.

Sterile 15 mL tubes were prepared for use after the pulse had been delivered.

1 vial of One Shot® Electrocomp™ cells, plasmid, and H<sub>2</sub>O was thawed on ice for each transformation.

Selective plates were warmed at 37°C for 30 minutes.

## **Procedure for Gene Transformation**

Each of 1 µl (10 pg) of the pUC19 control DNA and 1 µl of water (negative control) was placed in a sterile microcentrifuge tube and chilled on ice. It was ensured that the DNA was resuspended in water (rather than TE buffer) to keep the ionic strength to a minimum. This prevented arcing from occurring. Arcing can damage the machine and result in transformation failure and cell death. DNA in ligation or restriction buffer was precipitated or desalted before electroporation.

Cells were gently thawed on ice. The cells were used immediately and not left on ice for an extended period of time.

25 µl of cells were transferred to each of the pre-chilled microcentrifuge tubes obtained from Step 1. The volume of DNA added did not exceed 5% of the total cell/DNA mixture. The cells were mixed gently and then left on ice for 1 minute.

After 1 minute, the cells were transferred to a chilled cuvette and gently shaken to the bottom of the cuvette. It was ensured that the cells made contact all the way across the bottom of the chamber without any air bubbles. This was done promptly so as to not warm up the cuvette and cells.

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The condensation from the outside surfaces of the cuvette was removed with a tissue.

The sample was electroporated using the settings on the previous page.

250  $\mu$ l of S. O. C. medium was immediately added to the cells.

The suspension was transferred to a 15 mL tube. Then, it was incubated at 37°C in a rotary shaking incubator at 225 rpm for 1 hour to allow the expression of the antibiotic resistance.

After 1 hour, 10  $\mu$ l of the transformation sample was diluted with 90  $\mu$ l of SOC medium (1: 10). Then, 10  $\mu$ l of the first dilution was diluted with another 90  $\mu$ l of SOC medium (1: 100).

20  $\mu$ l of each of the second dilutions (1: 100) were plated on a prewarmed plate with 100  $\mu$ l/mL ampicillin and incubated overnight at 37°C.

The number of colonies that grew on each plate was recorded on the next day morning.

## **Results**

## **Discussion**

**Define the vocabulary used in this experiment: transformation, electroporation, host, plasmid, and competent.**

Transformation occurs when bacterial cells uptake a plasmid DNA, incorporate it into their genome and express the target gene.

Electroporation uses an externally applied electric field to increase the conductivity and permeability of a cell plasma membrane.

Plasmids are circular, double stranded, extra chromosomal DNA capable of autonomous replication.

Competence refers the ability of a cell to ingest extracellular DNA such as plasmids from its environment.

### **State why E. coli is used in many genetic engineering experiments.**

E. coli is non-pathogenic, can be cultured easily and can also be manipulated with ease.

### **Explain why competent cells, ampicillin and SOC medium were used for the transformation.**

Competent cells were used as they can uptake the plasmids easily. The antibiotic, ampicillin, was used to select for transformants. Cells which take in the plasmid will be able to express the gene for ampicillin resistance. Only these cells will survive on the agar plate which contains ampicillin. Thereby, the ampicillin aids in selecting the cells which have taken up the plasmid DNA. SOC medium provides the environment for cell growth.

### **Explain the purpose of the controls in this experiment.**

The negative control contains cells which do not contain the plasmid.

Therefore, this control provides the outcome of these cells when put on the agar plate containing ampicillin. Easy comparison can then be done with the positive control which contains cells with the plasmid DNA.



**Explain how the colony growth relates to gene transformation.**

Only cells which contain the plasmid DNA are able to survive in the agar plate containing ampicillin as they are able to express the gene for ampicillin resistance. Rightly so, only the positive controls show clumps of colony growth. With an altered genetic makeup, the competent cells were able to grow in the medium.

**Describe how ionic strength of DNA solution affects electroporation.**

Arching might occur in the electroporator if the ionic strength is not kept to a minimum. This is harmful for the sample as it causes cell death.

**If your transformation efficiency is lower than  $1 \times 10^9$  cfu/ $\mu$ g, conjecture and explain potential reasons for the low efficiency.**

Firstly, the cells may have been mixed using the pipette. This could have killed some of the fragile cells. Secondly, cell effectiveness would have been lost if the experiment had not been carried out quickly. This would have reduced the cell count and thus affecting the colony growth. Thirdly, sufficient time may not have been provided for the cells to mix after dilution. This would have caused uneven mixing and cell numbers could have been affected. Lastly, arching may have occurred. This may have killed majority of the cells. Only a small percentage of surviving competent cells will be able to form colonies.

**Discuss current and potential applications of gene transformation techniques in biotechnology.**