

# MAKING COMPETENT CELLS AND PLASMID TRANSFORMATION

## Introduction

This week, we will treat bacteria to make them **competent** to take up plasmid DNA. The process of DNA uptake is **transformation**, since it alters the genetic complement of the bacteria (they now have a new plasmid).

### LEARNING GOALS:

1. Learn how to treat bacteria to take up plasmid DNA.
2. Understand the process of transformation.
3. Know how we select for bacteria that have taken up a plasmid.

## Topic I: BACTERIAL COMPETENCE

### 1. Natural Competence

Bacteria are able to take up DNA from their environment (**exogenous DNA**) in three ways; conjugation, transformation, and transduction. 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.

For a bacterial cell to take up DNA from its surroundings, it must be in a special physiological state called **competence**. Experiments by Frederick Griffith in 1929 using competent *Streptococcus* (now *Enterococcus*) *pneumoniae* were instrumental in showing that DNA was the transforming principle – the genetic material.

Natural competence is highly regulated in bacteria, and the factors leading to competence vary among genera. For some genera, only a portion of the population is competent at any time; for others, the entire population gains competence. A series of competence proteins is produced, which have some homology but differ in the Gram negative and the Gram positive bacteria.

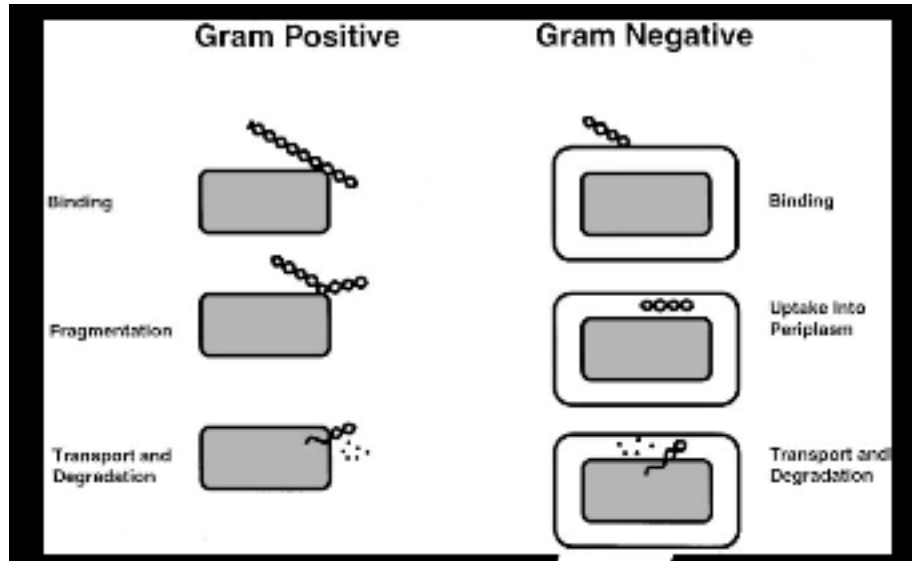


Figure 4.1: Transformation pathways in gram-positive and gram-negative bacteria. From Dubnau, Ann Rev Microbiol, 1999.

Once the DNA has been brought into the cell's cytoplasm, it may be degraded by cellular nucleases, or, if it is very similar to the cell's own DNA, enzymes that normally repair DNA may recombine it with the chromosome. Natural transformation is very efficient for linear molecules such as fragments of chromosomal DNA but not for circular plasmid DNAs.

## 2. Artificial Competence and Transformation

Artificial competence is not encoded in the cell's genes. Instead it is a laboratory procedure in which cells are passively made permeable to DNA, using conditions that do not normally occur in nature. These procedures are comparatively easy and simple, and can be used to genetically engineer bacteria. However, **transformation efficiency** is, in general, low. Only a portion of the cells become competent and a fraction of those are successful in taking up DNA.

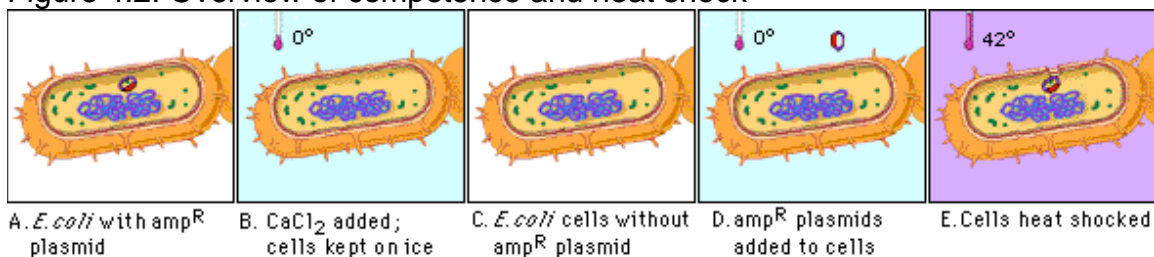
### TRANSFORMATION EFFICIENCY

$$\frac{\text{number of transformants (colonies)}}{\text{microgram of plasmid DNA}} \times \frac{\text{final volume at recovery (mL)}}{\text{volume plated (mL)}} = \text{number of transformants per microgram of plasmid DNA}$$

Chilling cells in the presence of divalent cations such as  $\text{CaCl}_2$  or  $\text{MgCl}_2$  prepares the cell walls to become permeable to plasmid DNA. Cells are incubated with the DNA. Then, during **transformation**, the cells are briefly **heat shocked** ( $42^\circ\text{C}$  for 60-120 seconds), which causes the DNA to enter the cell.

This method works well for circular plasmid DNAs but not for linear molecules such as fragments of chromosomal DNA.

Figure 4.2: Overview of competence and heat shock



From: [http://www.phschool.com/science/biology\\_place/labbench/lab6/test1.html](http://www.phschool.com/science/biology_place/labbench/lab6/test1.html)

Cells that are undergoing very rapid growth are made competent more easily than cells in other stages of growth. Thus cells are brought into **log phase** before the procedure is begun. The log phase cells are all living, healthy, and actively metabolizing. Because this procedure can be very harsh on cells, the log-phase cells are more able to withstand this treatment.

The basic protocol for artificial competence has been known since the early 1970's, and transformation efficiency has improved since those early days. Competent cells are now readily available commercially. However, despite the importance of this process to molecular biology and biotechnology, the exact mechanisms involved in artificial competence are not known.

An article by Panja *et al.* (J Biotech. 2006) investigated the events producing competence and transformation. In a previous work these researchers showed that "naked" DNA is bound to the lipopolysaccharide (LPS) receptor molecules on the competent cell surface. They suggested that the divalent cations formed coordination complexes with the negatively charged DNA and LPS. However, DNA is a large molecule and it was not known how the DNA crossed the cell membrane to enter the cytosol. Their recent work shows that the heat-shock step strongly depolarizes the cell membrane of CaCl<sub>2</sub>-treated cells. The decrease of membrane potential may lower the negativity of the cell's inside potential and allow the movement of negatively charged DNA into the cell's interior. A subsequent cold-shock raises the membrane potential to its original value. The authors were able to mimic the transformation process by using the protonophore CCCP that reduces membrane potential by dissipating the proton-motive force across the *E. coli* plasma membrane.

## Topic II: IDENTIFICATION OF POSITIVE CLONES

### 1. Spreading a Plate

The technique used to obtain transformation colonies is to spread a plate rather than streaking for single colonies. Both procedures will give isolated colonies that

are *clonal* (all bacteria in a colony are genetically identical). Spreading on a drug<sup>R</sup> plate lets us use the entire plate surface to *select* for antibiotic-resistant bacteria. In this experiment we also *screen* for the presence of a plasmid with an insert. Transformation efficiency varies so we do not know how many bacteria actually took up a plasmid, thus we usually spread several plates with different volumes of cells. This is why it is important to *thoroughly spread the cells over the entire plate* until all of the solution is absorbed into the agar. Please examine the photos on “how to spread a plate” available on the Virtual Lab Book.

## 2. Selection and Screening

Only bacteria that contain a viable plasmid with a drug resistance gene will be able to grow on the drug<sup>R</sup> plate. If the bacteria do not have a plasmid, or if the plasmid is faulty (for example, no origin of replication), then we will never see it. However, while we can **select** for bacteria containing a plasmid this does not guarantee that the plasmid has our insert.

Even though we cannot select for plasmids containing a DNA insert, the plasmid has the genetic tools to **screen** for potential inserts. The gene **lacZ** codes for the **β-galactosidase** enzyme, and this enzyme can metabolize the artificial substrate **X-gal** (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) to produce a **blue** pigment. This is a straightforward concept, but the actual detection system has an interesting twist. The *lacZ* DNA is in two separate locations; a small piece, the **α subunit**, is located in the cloning vector. Within the α subunit is the Multiple Cloning Site. The remaining *lacZ* DNA, the **Ω subunit**, is embedded in the bacterial chromosome. The α and Ω subunits come together to make functional β-galactosidase that metabolizes the X-gal and produces blue colonies. However, if a DNA insert disrupts the α subunit then NO functional β-galactosidase is produced and the colony is the normal “white” color. However, there are reasons *other* than an insert that will cause a colony to be white, including mutations in the α subunit or improper subunit assembly.

This discussion illustrates the important difference between a selection and a screen. A well-designed selection will eliminate all unwanted possibilities. A screen will help you eliminate many of the possibilities and guide you to the desired outcome (a plasmid with an insert) but cannot guarantee that outcome.

## REFERENCES

- Dubnau D., DNA uptake in bacteria, *Annu Rev Microbiol.* 1999;53:217-44.  
[http://www.phschool.com/science/biology\\_place/labbench/lab6/test1.html](http://www.phschool.com/science/biology_place/labbench/lab6/test1.html)  
 Panja S, Saha S, Jana B, Basu T., Role of membrane potential on artificial transformation of E. coli with plasmid DNA, *J Biotechnol.* 2006 Dec 15;127(1):14-20.