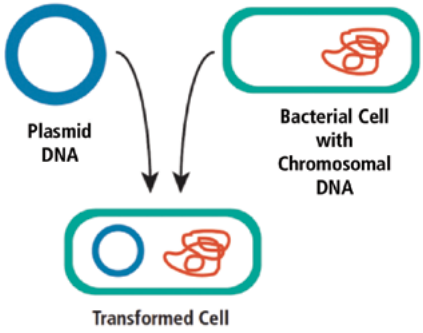
QUICK GUIDE:

# Enhanced Transformation

## WHAT IS TRANSFORMATION?

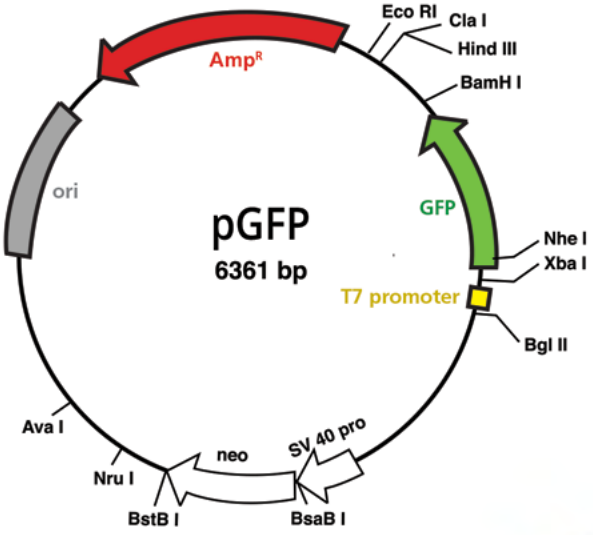
In nature, some species of bacteria can acquire exogenous DNA from the surrounding environment through a process called transformation. The newly acquired genetic information is both stable and heritable.

In the laboratory, scientists can force bacteria like *E. coli* to take up DNA and become transformed, even though many bacteria are not naturally competent. It is believed that the combination of calcium chloride and a rapid change in temperature- or "heat shock" - alters the permeability of the cell wall and membrane, allowing DNA molecules to enter the cell.




## WHAT IS A PLASMID?

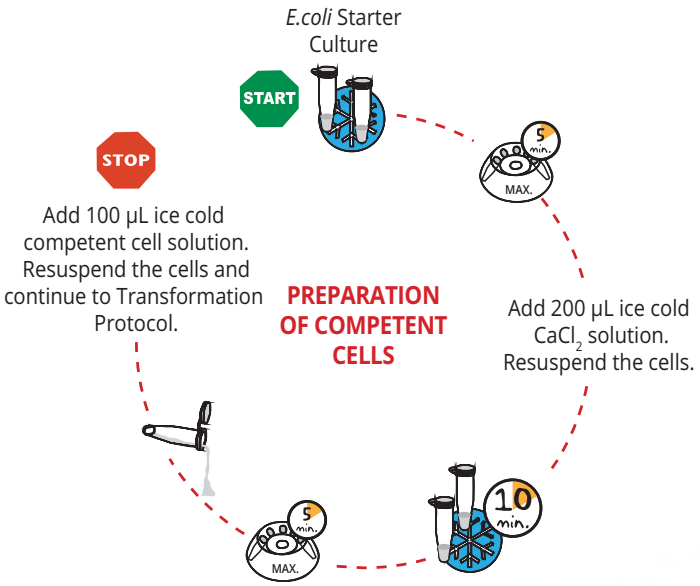
In addition to their chromosomal DNA, many bacteria possess extra, non-essential genes on small, circular pieces of double-stranded DNA. These pieces of DNA, known as plasmids, allow bacteria to exchange beneficial genes. For example, some genes that confer antibiotic resistance can be transferred between bacteria on plasmids




## WHAT ARE COMPETENT CELLS AND WHY ARE THEY IMPORTANT FOR A SUCCESSFUL TRANSFORMATION?

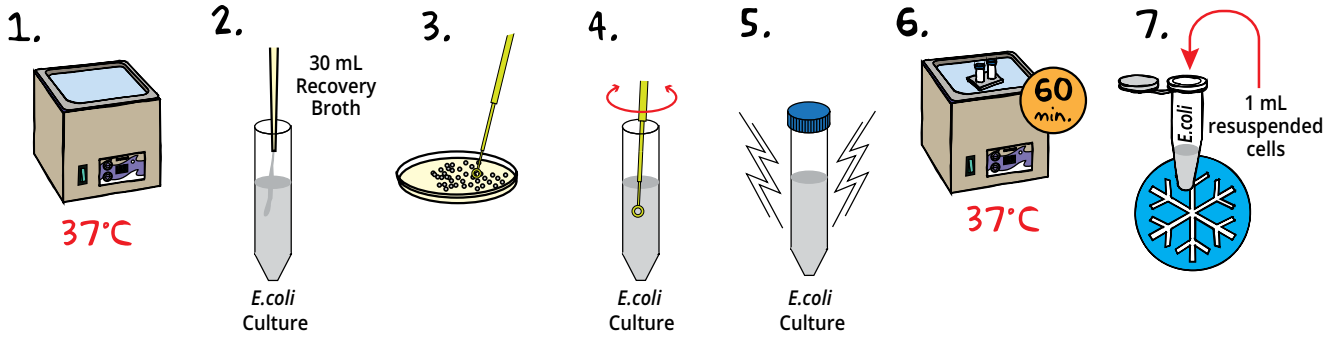
Competent cells are bacterial cells that have been altered to be able to allow exogenous DNA (like the GFP plasmid) into the cell through pores in the cell wall and membrane. This is most commonly done using the heat shock method, where cells are exposed to ice cold $CaCl_2$, the plasmid is added, then the cells are shocked in a 37ºC water bath to open up the pores. This is done quickly, then they are immediately put back onto ice to seal in the plasmid.

Having competent cells determines the success of your transformation. If the cells are not able to take up the plasmid, then they will not be transformed. This is easier to do with fresh colonies, which is why we now recommend the enhanced transformation protocol! The colonies are grown for an hour in liquid media and used that same day for the transformation. If you happen to use a source plate that is several days old, the colonies tend to dry up and it is much harder to make the cells competent.



**WHAT IS DIFFERENT ABOUT THE ENHANCED TRANSFORMATION PROTOCOL?**

The enhanced transformation is different from colony transformation because of the source of the bacterial culture. In colony transformation, students are picking 5-10 well isolated colonies off of a source plate, resuspending them in $CaCl_2$, introducing the plasmid and heat shocking them. In the enhanced transformation, the cells are harvested from the main liquid culture, and resuspended in $CaCl_2$. There are far more cells in this method since a large amount of colonies (a matchstick amount) is resuspended in the liquid media. *E. coli* has a rapid doubling rate, so when the cells are allowed to incubate, there are far more cells present than in colony transformation!

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