

EDVOTEK® Quick Guide: 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.

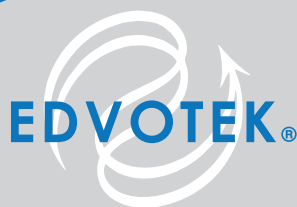
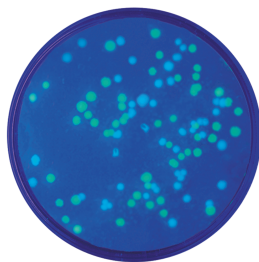
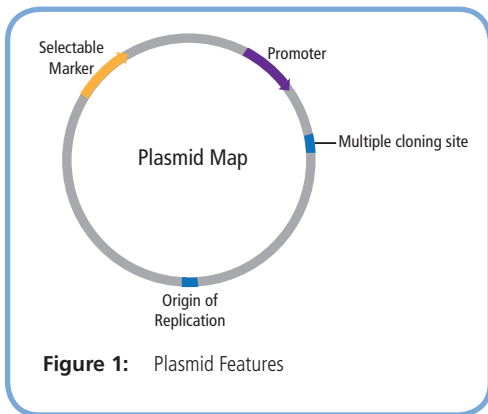
In the laboratory, scientists can force bacteria like *E. coli* to take up DNA and become transformed, even though many bacteria are not naturally competent. It is believed that the combination of calcium chloride and a rapid change in temperature—or “heat shock”—alters the permeability of the cell wall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

What is a plasmid?

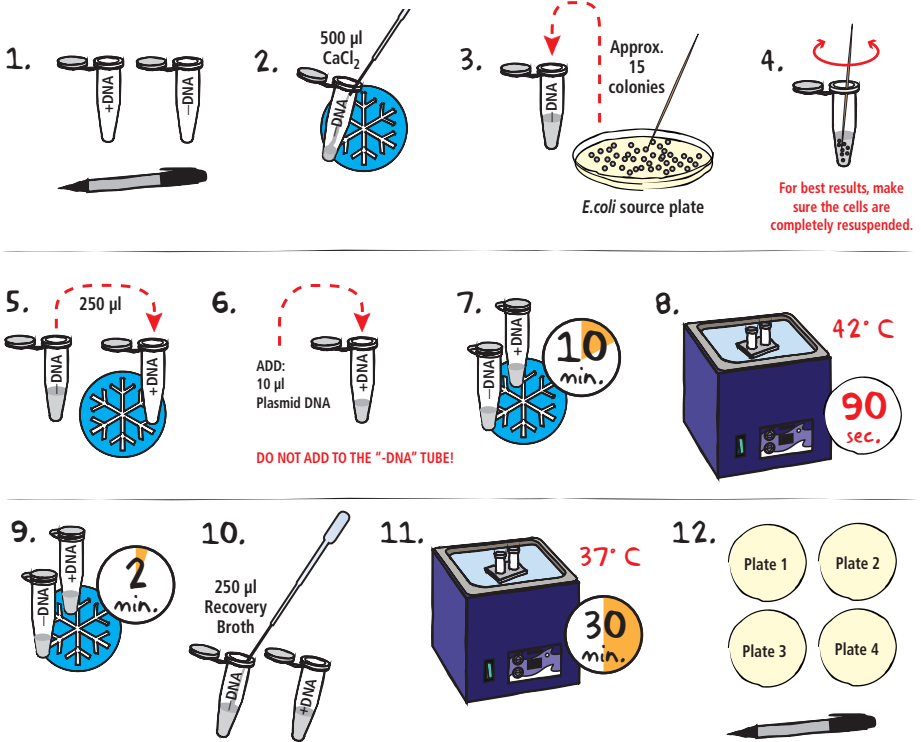
In addition to their chromosomal DNA, many bacteria possess extra, non-essential genes on small, circular pieces of double-stranded DNA. These pieces of DNA, known as plasmids, allow bacteria to exchange beneficial genes. For example, some genes that confer antibiotic resistance can be transferred between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

What is genetic engineering?

Genetic engineering is the use of biotechnology to alter an organism’s DNA. Recombinant DNA technology has allowed scientists to insert genes from different sources into bacterial plasmids. Once transformed, the bacteria can produce large amounts of important proteins from such plasmids, essentially converting cells into living factories. Insulin, which is used to control diabetes, was the first medication for human use to be produced by genetic engineering.



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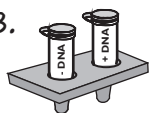


Transformation of *E. coli* with Plasmid DNA

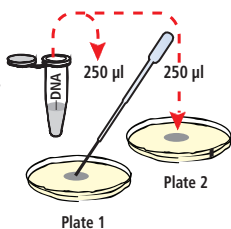
1. LABEL one microcentrifuge tube with "+DNA" and a second microcentrifuge tube with "-DNA".
2. TRANSFER 500 μL ice-cold CaCl_2 solution into the "-DNA" tube using a sterile 1 mL pipet.
3. Using a toothpick, TRANSFER approx. 15 well-isolated colonies (each colony should be approx. 1-1.5 mm in size) from the *E. coli* source plate to the "-DNA" tube.
4. TWIST the toothpick between your fingers to free the cells. RESUSPEND the bacterial cells in the CaCl_2 solution by vortexing vigorously until no clumps of cells are visible and the cell suspension looks cloudy.
5. TRANSFER 250 μL of the cell suspension to the tube labeled "+DNA". PLACE tubes on ice.
6. ADD 10 μL plasmid DNA to the tube labeled "+DNA". DO NOT add the plasmid to the "-DNA" tube.
7. INCUBATE the tubes on ice for 10 minutes.
8. PLACE the transformation tubes in a 42° C water bath for 90 seconds.
9. Immediately RETURN the tubes to the ice bucket and INCUBATE for two minutes.
10. TRANSFER 250 μL of Recovery Broth to each tube using a sterile 1 mL pipet. Gently MIX by flicking the tube.
11. INCUBATE the cells for 30 minutes in a 37° C water bath.
12. While the cells are recovering, LABEL the bottom of four agar plates as specified by the protocol.

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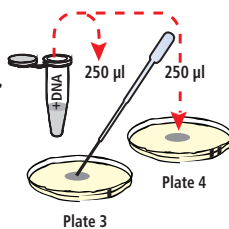
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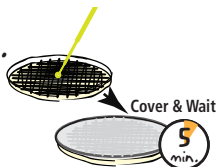
14.



15.



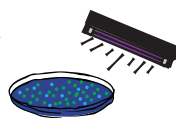
16.



17.



18.



- After the recovery period, **REMOVE** the tubes from the water bath and place them on the lab bench.
- Using a sterile 1 ml pipet, **TRANSFER** 250 µL recovered cells from the tube labeled " -DNA " to the middle of the specific plates as outlined your protocol.
- Using a new sterile 1 ml pipet, **TRANSFER** 250 µL recovered cells from the tube labeled " +DNA " to the middle of the specific plates as outlined your protocol.
- SPREAD** the cells over the entire plate using an inoculating loop. Use one sterile loop to spread both -DNA samples. Change to a fresh loop before spreading the +DNA samples. Make sure the cells have been spread over the entire surface of the plates. **COVER** the plates and **WAIT** five minutes for the cell suspension to be absorbed by the agar.
- STACK** the plates on top of one another and **TAPE** them together. **LABEL** the plates with your initials or group number. **PLACE** the plates in the inverted position (agar side on top) in a 37° C bacterial incubation oven for overnight incubation (16-18 hours). If you do not have an incubator, colonies will form at room temperature in approximately 24 - 48 hours.
- VISUALIZE** the transformation and control plates. Some experiments require the use of a U.V. light source.

Experiment Summary:

E. coli from the source plate are resuspended in an ice-cold CaCl₂ solution. Plasmid DNA is added to half of the cells before they are "heat shocked" in a 42°C water bath. The heat shock step facilitates the entry of DNA into the bacterial cells. Recovery Broth is added to the cell suspension, and the bacteria are allowed to recover for 30 minutes at 37°C. This recovery period allows the bacteria to repair their cell walls and to express the antibiotic resistance gene. Lastly, the transformed *E. coli* are plated on LB plates and allowed to grow at 37°C overnight.

NOTE for Step 17:

It may take longer for the cells to absorb into the medium. Do not invert plates if cells have not completely been absorbed.

Related Equipment

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