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.

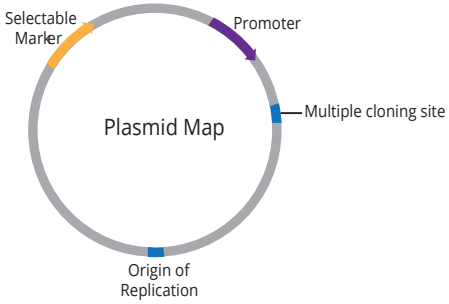

Figure 1: Plasmid Features

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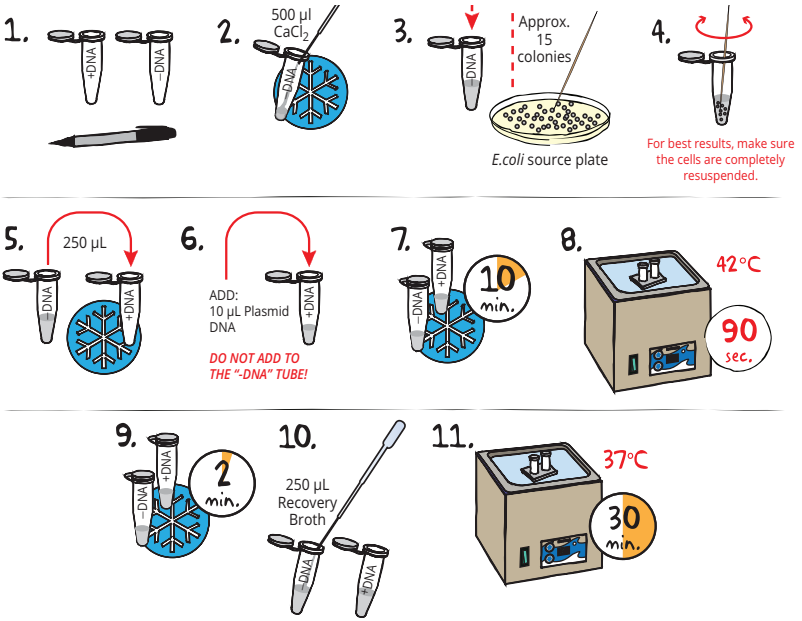
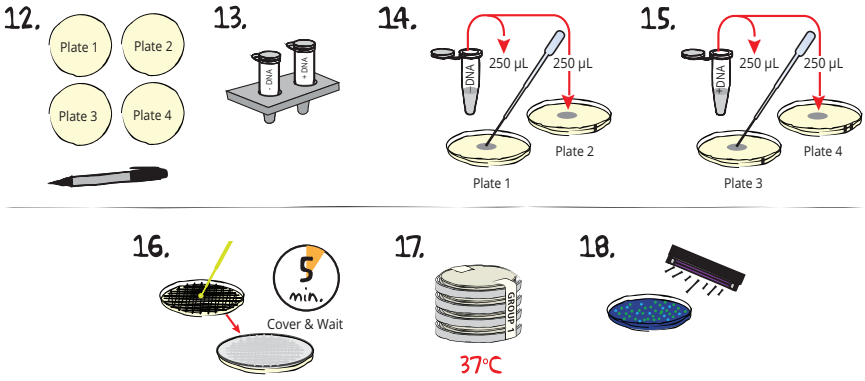
Colony Transformation of *E.coli* with Plasmid DNA



In colony transformation, the host *E. coli* bacteria will be grown for 18-22 hours on LB-agar "source plates", collected using a sterile loop, and made competent in $CaCl_2$. Next, the plasmid will be added to half of the cells before they are briefly heat shocked. Finally, the bacteria will be allowed to briefly recover before they are plated on LB-Agar plates and incubated at 37°C overnight. This protocol does not require a centrifuge.

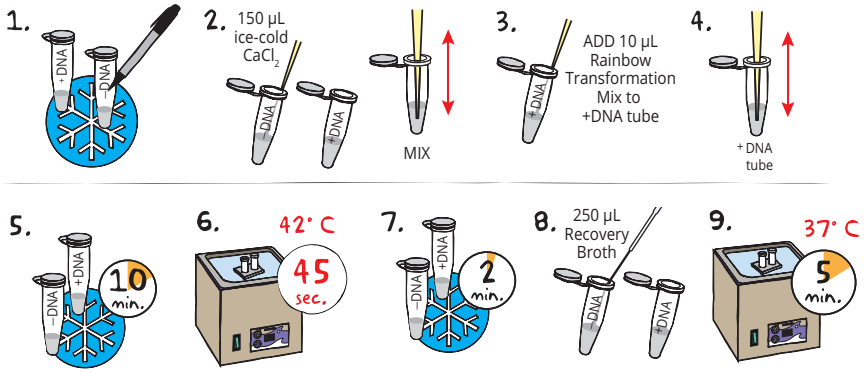
1. **LABEL** one microcentrifuge tube with "+DNA" and a second tube with "-DNA".
2. **TRANSFER** 500 µL ice-cold $CaCl_2$ solution into the "– DNA" tube using a sterile 1 mL pipet.
3. Using a toothpick, **TRANSFER** approx. 15 well-isolated colonies (each colony should be approx. 1-1.5 mm in size) from 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.

Colony Transformation of *E.coli* with Plasmid DNA, continued



12. While the cells are recovering, **LABEL** the bottom of four agar plates as specified by the protocol.
13. After the recovery period, **REMOVE** the tubes from the water bath and place them on the lab bench.
14. 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.
15. 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.
16. **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.
17. **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.
18. **VISUALIZE** the transformation and control plates. Some experiments require the use of a U.V. light source.

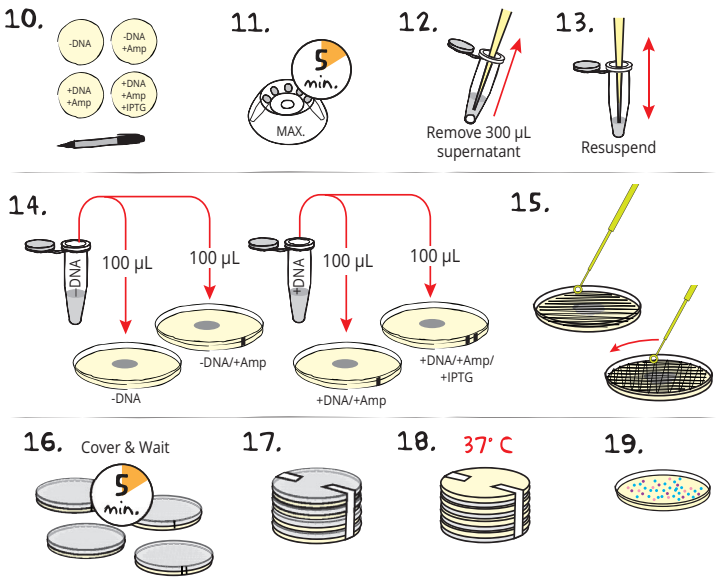
Enhanced Transformation Protocol



This procedure creates a liquid culture of competent cells that can produce superior transformation results. It also offers additional flexibility for the teacher and students. The protocol requires additional recovery broth and a specially formulated competent cell solution (both provided in the kit components). It also requires the use of a centrifuge. In this protocol, you will prepare the starter cultures while your students will prepare the competent cells and perform the transformation. However, if class time is limited, you may decide to prepare the competent cells as part of the teacher's prelab.

1. **RETRIEVE** the competent cell tubes and place immediately on ice. **LABEL** one tube "+DNA" and the other tube "-DNA".
2. **ADD** 150 µL ice-cold $CaCl_2$ solution to both tubes. **MIX** by gently pipetting up and down several times.
3. **ADD** 10 µL of the plasmid DNA to the tube labeled +DNA. Do not add anything to the -DNA tube.
4. **MIX** the cells by gently pipetting up and down several times.
5. **INCUBATE** both tubes on ice for 10 minutes.
6. Quickly **PLACE** both tubes in a 42°C water bath for 45 seconds.
7. Immediately **RETURN** the tubes to the ice bucket and incubate for 2 minutes.
8. **ADD** 250 µL of Recovery Broth (RB) to both tubes using a sterile 1 mL pipet. Gently **MIX** by flicking each tube.
9. **INCUBATE** both tubes for 5 minutes in a 37 °C water bath.

Enhanced Transformation Protocol, continued



10. While the cells are recovering, **LABEL** the bottom of four agar plates as indicated below. Keep labels small and along the edge so you can easily see your colonies at the end.
    - -DNA (plate with no stripe)
    - -DNA/+Amp (plate with one stripe)
    - +DNA/+Amp (plate with one stripe)
    - +DNA/+Amp/+IPTG (plate with two stripes)
11. After the incubation, **REMOVE** the tubes from the water bath and **CENTRIFUGE** for 5 minutes to pellet the cells.
12. **REMOVE** and discard 300 µL of supernatant. DO NOT DISTURB THE PELLET.
13. **RESUSPEND** the pellet in remaining liquid by slowly pipetting up and down.
14. With a new pipet, **TRANSFER** 100 µL of the transformed cells to the center of the appropriate LB agar plates.
15. Using a sterile loop, **SPREAD** the cells evenly and thoroughly over the entire surface. Turn the plate 90° and thoroughly spread again. Use the same loop for both "-DNA" plates, then use a second sterile loop for both "+DNA" plates.
16. **COVER** the plates and allow the liquid to be absorbed for 5 minutes.
17. **STACK** the plates on top of one another and TAPE them together. **LABEL** the plates with your initials or group number.
18. **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.
19. **VISUALIZE** the transformation and control plates. Some experiments require the use of a U.V. light source.

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Cat. #557

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