Lab Exercise: Transformation

OBJECTIVES

1. Understand the process of transformation and how it is used in a laboratory setting for expression of genes (i.e. production of proteins).
2. Perform a successful transformation using the pTOM plasmid.

INTRODUCTION

Genetic transformation is used in many areas of biotechnology, and, at its heart, requires two things: Donor DNA and recipient cells. Cells which receive the donor DNA are considered genetically recombined, that is, they have their original DNA (their chromosome) and new DNA (the plasmid) and whatever genes that plasmid carries. Before considering the details of recombination, we will consider each of these players individually.

Plasmids were discovered as extra-chromosomal genetic material in the late 1960s. Like the bacterial chromosome, they are circular but they are much smaller (2,000-10,000 bp), and usually contain genes for one or more traits that may be beneficial to bacterial survival. In nature, bacteria can transfer plasmids back and forth allowing them to share these beneficial genes and adapt to new environments. For instance, the quick dissemination of bacterial resistance to antibiotics is due in part to the transmission of plasmids.

These naturally occurring plasmids have been engineered to contain not only antibiotic resistance (which is used in the laboratory as a selective marker for successful transformation) but other “genes of interest.” If a plasmid is transformed into an *E. coli* cell that is sensitive to the antibiotic ampicillin, it will confer resistance to that antibiotic. Growing the transformants in the presence of ampicillin is an easy way to select for recombined *E. coli* cells. However, *E. coli* that have become antibiotic-resistant are not themselves useful in biotechnological applications. As such, plasmids must be engineered to include some other gene of interest. This gene of interest is spliced into the plasmid at its multiple cloning site.

Many plasmids are commercially available, and many contain the following components:

1. A selectable marker (*i.e.*, a gene that encodes for antibiotic resistance);
2. An origin of replication (ori) for plasmid replication;
3. A multiple cloning site (MCS), containing the restriction enzyme recognition sites;

DNA is a hydrophilic molecule which will not normally pass through the plasma membrane of a bacterial cell. In order to accomplish transformation, the bacterial cells must first be made competent to take up the plasmid DNA. This is done by neutralizing the negative charge of the DNA molecule using CaCl₂. This is followed by a heat shock step that causes the plasma membrane to become permeable. Together the CaCl₂ treatment and heat shock will cause the cells to take up the plasmid DNA. Once the cells have had a chance to recover, they can be plated out on antibiotic-containing media to select for successful transformants.
In this experiment, you will be working with the pTOM (or pARA) plasmid. This plasmid contains three important genetic segments:

1. An **ampicillin resistance gene** to select for transformants by plating on a medium containing ampicillin
2. An **arabinose inducible operon system**, which allows you to express the gene of interest (in this case the red fluorescent protein RFP or "TOM" for tomato), by growing cells on a medium containing arabinose.
3. The gene for the **red fluorescent protein (RFP)**.

Through the course of the experiment you will do the following:

**To move the pTOM plasmid DNA through the cell membrane you will:**
- Use a transformation solution of CaCl$_2$ (calcium chloride).
- Carry out a procedure referred to as "heat shock."

**For transformed cells to grow in the presence of ampicillin you must:**
- Provide them with nutrients and a short incubation period to begin expressing their newly acquired genes

**For transformed cells to exhibit the red phenotype you must:**
- Provide them with the operon inducer arabinose.

### LAB EXERCISES

<table>
<thead>
<tr>
<th>Class supplies</th>
<th>Table supplies</th>
<th>Team supplies</th>
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<tbody>
<tr>
<td>42° C water bath</td>
<td><em>E. coli</em> starter plate</td>
<td>1 tube of 10 µl pTOM plasmid (labeled P+)</td>
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<tr>
<td></td>
<td></td>
<td>1 tube of 10 µl water (labeled P-)</td>
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<td></td>
<td></td>
<td>2 tubes of Transformation Solution (100mM CaCl$_2$ solution)</td>
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<tr>
<td></td>
<td></td>
<td>1 tube of recovery media (LB)</td>
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<td></td>
<td></td>
<td>Ice bucket with ice</td>
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<td></td>
<td></td>
<td>1 LB plate</td>
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<td></td>
<td></td>
<td>2 LB/Ampicillin plates</td>
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<tr>
<td></td>
<td></td>
<td>1 LB/Ampicillin/Arabinose plate</td>
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<td>Pipets</td>
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<tr>
<td></td>
<td></td>
<td>Pipet tips</td>
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<td>Metal spreader, alcohol and turntable</td>
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**Protocol:**
1. Take 2 microcentrifuge tubes: one labeled "P+" and one labeled "P-". These tubes will have 10 µl of either plasmid (P+) or water (P-). The "P-" tube will act as a negative control as it will not be exposed to the plasmid.
2. Take 2 tubes containing 250 µl of ice-cold Transformation Solution (TS) and place on ice.
3. Pick three small colonies of *E.coli* from the culture plate with a sterile loop and mix it into
one of the TS tubes. Repeat with other TS tube and place on ice for 10 mins. (This treatment stresses the bacterium, rendering its cell membrane and cell wall permeable to the plasmid. This process will make the recipient E.coli "competent" to uptake the plasmid.)

4. Using separate sterile pipette tips, add the 250 µl of transformation solution/cells from one of your TS tubes to the P+ tube. Add 250 µl from the second TS tube to the P- tube. Mix tubes well and keep on ice for 10 mins.

5. After 10 minutes, walk with your ice bath and tubes to the water bath. Transfer both the tubes from the ice container into the water bath at 42ºC for 50 sec. The transfer from the ice to the water bath should be immediate. When incubating the tubes in the water bath, be sure that the tube bottoms are all the way into the water below the rack.

This step is called the "Heat-Shock" step, and it will maximize the uptake of the plasmid through the wall and membrane of the cells.

6. After 50 seconds, immediately place these tubes on ice for 2 mins.

7. At room temperature, add 250 µl of Recovery Media to both tubes, mix gently, and leave at room temperature for 15 mins.

8. Using a lab marker label your 4 plates as shown below

   #1 LB with P-
   #2 LB/Amp with P-
   #3 LB/Amp with P+
   #4 LB/Amp/Ara with P+

9. Transfer 100 µl from the P+ tube to the LB/Amp and LB/Amp/Ara plates. Top spread evenly.

10. Transfer 100 µl from the P- tube to the LB and LB/Amp plates. Top spread evenly.

11. Allow liquid to absorb (about 15 minutes), then invert the plates and incubate at the appropriate temperature.
DATA AND OBSERVATIONS

1. Observe and draw the results of your transformation for each of your 3 plates. Make sure to count the CFUs on each plate, and record any observations on color of the colonies.

   - LB plate
     - CFUs __________
     - Color __________

   - LB/Amp plate
     - CFUs __________
     - Color __________

   - LB/Amp/Ara plate
     - CFUs __________
     - Color __________
DISCUSSION

1. Which plate(s) served as control(s)? What is meant by a control plate and what purpose does it serve?

2. What do you expect to see growing on each plate (+ and/or - cells)? Answer this question in terms of growth and phenotype (i.e., color). Compare these to the actual results you obtained. Are they the same? Why or why not?

3. Describe the evidence that indicates whether your attempt at performing a genetic transformation was successful.

4. Look again at your plates; do you observe some *E. coli* growing on the LB plate that does not contain ampicillin or arabinose? From your results, can you tell if these bacteria are ampicillin resistant by looking at them on the LB plate? Explain your answer.
5. Very often an organism’s traits are caused by a combination of its genes and its environment (multifactorial traits). Think about the red color you saw in the genetically transformed bacteria:
   a. What two factors must be present in the bacteria’s environment for you to see the red color?

   b. What is each of the two factors you listed above doing to cause the bacteria to turn red?

   c. Why is it advantageous for an organism to be able to turn particular genes on or off?

6. Calculate transformation efficiency
Your next task in this experiment will be to learn how to determine the success of your transformation of the E. coli cells with the pTOM plasmid DNA. This quantitative measurement is referred to as transformation efficiency. Transformation efficiency is a number which represents the total number of bacterial cells that express the red protein, divided by the amount of DNA used in the experiment (i.e. it tells us the total number of bacterial cells transformed by one microgram of DNA). The transformation efficiency is calculated using the following formula:

\[
\text{transformation efficiency} = \frac{\text{total CFUs}}{\text{DNA (micrograms)}}
\]

Before you can calculate the efficiency of your transformation, you will need two pieces of information:

   a. The total number of red colonies growing on your LB/Amp/Ara plate (data previously collected). This is the total CFUs in the transformation efficiency equation above.

   b. The original concentration of pTOM, and more importantly, the resulting amount
plated on the LB/Amp/Ara plate. You can determine this by completing the calculations below.

In this experiment the pTOM had a stock concentration of _10_ ng/µl (1 µg = 1000 ng). This means that each microliter of pTOM solution has _0.01_ µg of pTOM DNA. If you put _10_ µl of this pTOM solution into your P+ tube, your P+ tube should have _0.1_ µg of pTOM DNA total.

Now calculate the amount of DNA transferred from that P+ tube to your LB/Amp/Ara plate. You plated _100_ µl of the P+ solution onto your LB/Amp/Ara plate. To determine the percentage transferred of the original amount, divide the number above (amount plated) by the total reaction volume (transformation solution + pTOM DNA + LB nutrient medium). This will give you the percentage transferred. In this case 100/510 or we can round to 1/5 or 20% or 0.2. Multiply this number by the total micrograms of pTOM DNA in your P+ tube, this will give you the DNA (micrograms) for your transformation efficiency equation. [0.2 X 0.1 = 0.02 µg pTOM DNA]. For example if you get 50 CFU, you would have 50/0.02 or 2.5 X 10^3 for your transformation efficiency.

Biotechnologists are in general agreement that the transformation protocol that you have just completed generally has a transformation efficiency of between 8.0 X 10^2 and 7.0 X 10^3 transformants/µg DNA.

• How does your transformation efficiency compare with the above?

• How does your transformation efficiency compare with the other groups in class?

References


5. **Madigan, Michael, John M. Martinko, Paul V. Dunlap and David P. Clark.** *Brock’s Biology of Microorganisms, 11th Edition.* Benjamin Cummings. 2005