

Lab 3 - DNA Transformation

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INTRODUCTION

One of the newest and most interesting areas of current research in molecular biology is **genetic engineering**, where specific genes are isolated from one organism and inserted into another organism of the same or a different species. Through this process, organisms can be given new characteristics. For example, through gene transfer, plant breeders have developed canola plants that are tolerant to specific herbicides or groups of herbicides. Today 80% of the acres sown in Canada are these genetically modified canola plant varieties. Golden rice is another example of genetic engineering. It was created by transforming rice with two beta-carotene biosynthesis genes which allows the rice to produce beta-carotene, a precursor of Vitamin A, in the part of rice that people eat, the endosperm. Golden rice was developed as a fortified food to be used in areas where there is a shortage of dietary vitamin A.

The beginnings of genetic engineering can be traced to 1928 when Fred Griffith, a British bacteriologist, carried out experiments on *Pneumococcus*, a bacterium which causes pneumonia. In these experiments, he studied the effects of virulent (S) and non-virulent (R) strains of *Pneumococcus* on mice. As anticipated, mice that were injected with living S-strain bacteria died and those mice injected with the living R-strain or heat-killed S-strain survived. Unexpectedly, those mice injected with a mixture of live R-strain and heat-killed S-strain bacteria died. Since Griffith did not understand how a mixture of nonvirulent and dead virulent bacteria could produce such a result, he examined the dead mice and found they were full of living S-strain (virulent) bacteria. After several experiments, Griffith concluded that the living non-virulent bacteria had been **transformed** into virulent cells by material from the dead virulent bacteria. It appeared that hereditary material from the dead bacteria had entered the live non-virulent bacteria and changed them into virulent bacteria.

In 1931, experiments by other scientists found that bacterial transformations could occur in test tubes and did not require a mouse host; and in 1944, the agent of transformation was shown to be DNA. In this later experiment by Avery, MacLeod, and McCarty, DNA was isolated from virulent bacteria and inserted into non-virulent bacteria, transforming them into the virulent strain. Aside from being one of the first experiments to demonstrate that DNA was the hereditary material, it showed that genes can be artificially transferred.

Since these early experiments, a vast amount of knowledge on gene structure, function, and control has been acquired. Presently, it is known that **genetic engineering depends on the presence of three factors**:

- (a) **a suitable host organism** into which a foreign gene can be inserted
- (b) **a vector**, a specially manipulated DNA molecule, like a plasmid, into which a foreign DNA segment is inserted so it can carry the foreign DNA segment into the host organism
- (c) **a method of isolating host cells** which have taken up the foreign gene (vectors are not 100% efficient, some cells do not take up the foreign DNA).

In this lab, the bacteria *Escherichia coli*, will be put in an environment containing foreign DNA (in the form of a plasmid), providing the opportunity for some of the bacteria to take up this foreign DNA. This plasmid contains a gene that makes the host bacterium resistant to the antibiotic, ampicillin.

specific.

References:

Include at least three sources other than your lab manual and textbook. No Wikipedia!

PROCEDURE

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1. Slowly thaw, on ice, 6 tubes of competent *E. coli* cells. Label these tubes A, B, C, D, E, and F. They will be treated as follows:

A	<i>E. coli</i> control - heat
B	<i>E. coli</i> control - no heat
C	<i>E. coli</i> control - heat
D	<i>E. coli</i> control - no heat
E	<i>E. coli</i> transformed - heat
F	<i>E. coli</i> transformed - no heat

Then, label the six agar plates with their corresponding tube letter (see above), your group number and the date. Plates for tubes A and B should contain **plain LB agar**, while plates for tubes C, D, E, and F should contain **LB agar with ampicillin**.

2. To tubes A, B, C, and D add 20µl of **sterile distilled water**. To tubes E and F add 20µl of the **plasmid** preparation. Gently flick each tube for 1 minute to reconstitute the DNA. Incubate all tubes on ice for 30 minutes. *Do you know why?*
3. Heat shock tubes A, C, and E by placing them in a 42°C water bath for 90 seconds. Return them to the ice immediately and incubate for 2-3 minutes. *Do you know why?*
4. To each of the 6 tubes, add 1ml of **LB media** (without antibiotic) and gently invert to mix the tubes.
5. Place the tubes in the water incubator at 37°C for 45 minutes. *Do you know why?*
6. Remove the tubes from the water incubator and spin for 1 minute in balanced positions in the microcentrifuge. A pellet will form at the bottom of the tube. Remove 1ml of the supernatant (**USE SEPARATE PASTEUR PIPETTES TO AVOID CROSS-CONTAMINATION!**).
7. Resuspend the cells in the remaining supernatant by gently pipetting the suspension up and down with a Pasteur pipette (**AGAIN, AVOID CROSS-CONTAMINATING YOUR TUBES!**).
8. Plate each of the suspensions on their appropriate culture plate and spread the suspensions over the agar using the "**hockey-stick**" technique demonstrated by your lab instructor. Make sure you plate tubes A and B on plain LB agar and plate tubes C, D, E, and F on LB agar that contains ampicillin. Let the plates dry right side up for about 5 minutes and then place all plates in the 37°C incubator upside down. *Do you know why?*
9. The plates will remain in the incubator overnight. The next day your lab instructor will remove the plates from the incubator, seal them with a strip of Parafilm and store them in the refrigerator. Your lab instructor will post the results of your experiment on the lab Moodle page when they become available. The results will be recorded as descriptive phrases to indicate how much bacterial growth was present on the plates such as: no colonies, few colonies, many colonies or a lawn of growth.

Complete this table by filling in the expected results for each tube in the first blank row and then add your actual results to the last row.

TUBE A	TUBE B	TUBE C	TUBE D	TUBE E	TUBE F
<i>E. coli</i> ↓	<i>E. coli</i> ↓	<i>E. coli</i> ↓	<i>E. coli</i> ↓	<i>E. coli</i> ↓	<i>E. coli</i> ↓
NO Plasmids	NO Plasmids	NO Plasmids	NO Plasmids	Plasmids	Plasmids
Ice ↓	Ice ↓	Ice ↓	Ice ↓	Ice ↓	Ice ↓
Heat (42°C) ↓	Ice ↓	Heat (42°C) ↓	Ice ↓	Heat (42°C) ↓	Ice ↓
Ice ↓	Ice ↓	Ice ↓	Ice ↓	Ice ↓	Ice ↓
LB ↓	LB ↓	LB ↓	LB ↓	LB ↓	LB ↓
37°C incubator centrifuge ↓	37°C incubator centrifuge ↓	37°C incubator centrifuge ↓	37°C incubator centrifuge ↓	37°C incubator centrifuge ↓	37°C incubator centrifuge ↓
Plain agar ↓	Plain agar ↓	Amp agar ↓	Amp agar ↓	Amp agar ↓	Amp agar ↓
37°C incubator	37°C incubator	37°C incubator	37°C incubator	37°C incubator	37°C incubator

Expected results

Actual results

Lab 4 DNA Transformation Lab Report

Abstract:

Create a "snapshot" of your experiment that summarizes your report.
150-250 words

Introduction:

It includes the background details and the definitions of terms relating to the experiment you have conducted. You will need to provide references here to show where you obtained your information. Make sure within your Introduction you answer the following questions:

What is genetic engineering?

What is transformation?

How does heat shock work to influence the rate of transformation?

What bacteria and extra cellular DNA will you be using?

What is competence?

How does the CaCl_2 make the *E. coli* cell competent for transformation?

Why were the cells also treated with glycerol?

What is the purpose of this lab?

What is the result of this transformation?

What factors affecting transformation will be examined?

How is transformation detected?

What does Ampicillin do? Provide a short, general explanation of *how* it works and *what is the final effect on the cells*

Materials and Methods:

Describe what you did in enough detail so that the experiment can be repeated.

Do not list the materials used or number the methods like in the lab manual. Write the materials and methods section in complete paragraphs.

Do not forget to reference your lab manual as the source for the procedure you followed.

Results:

State the results for each tube in proper paragraph form. Create one table to show your results with both expected and actual results. Make sure to reference your table in your written results.

Discussion:

What should be happening on each plate ideally? Compare each of your plates and draw your conclusion. Did the heat shock increase or decrease the rate of transformation?

Explain possible reasons why your results may have differed from what was expected, be specific.

References:

Include at least three sources other than your lab manual and textbook. No Wikipedia!