Practical 2: Transformation of Bacterial Cells

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Aim:

To successfully transform a culture of *E. coli* cells to resist ampicillin by creating competent bacterial cells able to take up the plasmid coding for its resistance.

Introduction:

Within this practical the bacterial transformation of competent *E. coli* was performed. The transformation of bacteria is extremely important in the molecular cloning industry as it allows one to synthesize huge quantities of a targeted recombinant DNA molecule. This transformation is mainly performed using plasmids, which contain the targeted recombinant DNA. But the plasmids must first enter these bacteria cells which is performed via artificial means within this practical. The plasmid will then incorporate within the bacteria's DNA which will thereby be copied and replicated within successive generations. This thereby creates a near limitless production of the targeted recombinant DNA molecule. Furthermore, the need for preparing competent *E. coli* cells is a must. A competent bacterial cell is defined as a bacterial cell that is able to take up foreign DNA from the surrounding environment (Tang et al., 1994). Within this practical the transformation of *E. coli* cells into ampicillin (an antibiotic) resistance cells shall take place using plasmid conferring antibiotic resistance. Hence hypothesis, if there exist bacterial colonies within the agar plate containing ampicillin, then the transformation of *E. coli* to resist antibiotics was successfully performed. Furthermore, one will then be able to use the agar plates of different dilution factors of the bacteria to determine the efficiency of this transformation.

Procedure:

Please find protocol by, Cassar. (2009), to this practical via to following link: https://www.um.edu.mt/vle/pluginfile.php/1107910/mod_resource/content/1/BIO3300%20Practical%202%20-%20Transformation%20of%20Bacterial%20Cells.pdf

Modifications to the protocol:

At part 2 "Transformation Reaction", the following modifications where performed:

Step 3. Only one tube was used.

Step 6. 45 ℃ was used instead of 42 ℃.

Step 8. Only one tube was used.

Step 10. 100 μl was used instead of 500 μl.

Step 11. 900 μl was used instead of 500 μl.

Step 12. A, B, C and D should have the following dilution factors, 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}, respectively.

Sources of Errors:

- Proper resuspension of the *E. coli* pellet may not fully be performed, hence not all the bacteria cells were converted to competent cells.
- The plasmids might be old or stored improperly thereby being expired.

Precautions:

- When resuspending the pellet, do not mix vigorously but instead gently.
- When sterilizing the plastic hood, first coat the inside and outside with 70% ethanol, then wipe the ethanol off. Next spray a light coating inside the plastic hood and allow the ethanol to evaporate. This will sterilize the plastic hood.
- Coat every item with 70% ethanol before entering it in the plastic hood.
- Wear disposable gloves since one is working with *E.coli* cells.
- Coat hands with 70% ethanol and do not touch any non-sterile surface/item before entering them into the sterile plastic hood.

Results:

Table 1:

Plates	Dilution factor	Bacterial colonies
W / ampicillin	1	0
A	0.1	TMTC
B	0.01	TMTC
C	0.001	TMTC
D	0.0001	183

Note: This table shows the bacterial colony after plasmid transformation of the *E. coli* for antibiotic resistance. Note, TMTC means To Many To Count. View Appendix for images of the plates.

Using the following formula and *Table 1*, one will be able to find the population concentration and transformation efficiency of the *E. coli* batch solution:

$$Transformation\ Efficiency = \frac{Transformation\ Cells}{\frac{CFU}{ml}}$$

Note: CFU means Colony Forming Unit.

Hence:

$1830\,\frac{CFU}{ml}$ at a dilution factor of 0.0001.

As such:

$18{,}300{,}000\,\frac{CFU}{ml}$ are found at the full batch strength.

Furthermore:

$$Transformation\ Efficiency = \frac{0}{18{,}300{,}000} = 0$$

As such, no *E. coli* cells were transformed to resist ampicillin.

Discussion:

Due to the hydrophilic property of DNA, transformation requires multiple steps in order to open a pore in the cells membrane so as to allow the plasmid to enter the cell and later integrate with the DNA, hence this requirement is known as creating a component *E. coli* cell. This process was first performed using the pellet of bacteria cells and resuspending it in $10\ ml$ of $100mM\ MgCl_2$. Once the $MgCl_2$ was added to the cells it was gently induced to incorporate the Mg^{2+} cation into and onto the cell membrane via low temperatures. Next the incorporation of Ca^{2+} was performed using a $10ml$ of $100mM\ \ CaCl_2$. The Ca^{2+} cations were gently incorporated onto the cell membrane of the *E. coli* by using low temperatures. The purpose of the Ca^{2+} and Mg^{2+} cations are to neutralize the negatively charged components on the cell membrane, thus, preventing the

negatively charged plasmid from electrostatically repelling from the *E. coli*, (Chehal et al., 2007). After this, the *E. coli* cells were then considered as competent cells. Next, one will extract a small volume of these competent *E. coli* cells and mix it with a small volume of plasmids. At first, the competent cells will not be able to take up the plasmids, but once the $MgCl_2$ and $CaCl_2$ treated cells are heat shocked using 45 ℃ for $45\ s$, the cell membrane will become strongly depolarized. This results in a decrease in the membrane potential which in turn reduces the negativity within the cell. As such, thanks to the Ca^{2+} and Mg^{2+} neutralizing the negatively charged membrane, the negatively charged plasmid will be bind to the lipopolysaccharide of the cell membrane which will then create a pore, thus allowing it to enter the cell after the heat shock period, (Nakata et al., 1997; Chang et al., 2017). To increase the efficiency of the transformation one will incorporate magnesium within the cell, (Asif et al., 2017). This is due to the fact that once the plasmid is within the *E. coli* cell, the cell will need to break a DNA linkage from the plasmid and at the gene of interest. Thus, incorporated Mg^{2+} will act as a co-factor to increase DNase activity, thereby increasing the probability of successfully incorporating the plasmid into the DNA (Magnesium Chloride Solution 1 M., 2022).

Within this practical the assumption that the formation of one colony is due to only one *E. coli* cell was taken. This was done so that one can estimate the concentration of the bacteria in a specific amount of solution. Furthermore, this practical found that out of a batch concentration of $18{,}300{,}000\frac{CFU}{ml}$, zero successful *E. coli* transformations were performed, hence a $0\,\%$ transformation efficiency. As such, it can be stated that the experiment was a failure hence no successful *E. coli* transformations. The failure within this practical could possibly be due to a number of factors. Such as, sub-optimal plasmid quality (i.e. the plasmid is expired) or the concentration of the plasmids is too low for transformation. Furthermore, the time and/or at which

the bacteria cells are being converted to competent cells and are incorporating the plasmids might be wrong for the specific *E. coli* strain used. The resultant zero transformations could also possibly be due to incorrect $MgCl_2$ and $CaCl_2$ concentrations. Hence possible modifications to this protocol can be changed as stated by Tang et al., (1994), which used $50mM$ and $80mM$ of $MgCl_2$ and $CaCl_2$, respectively. Or by Tee et al., (2017), which used $20mM$ and $50mM$ of $MgCl_2$ and $CaCl_2$, respectively. Another factor could be that the plasmid was coding for resistance to a different antibiotic than ampicillin or that the ampicillin was of such a high concentration that even the ampicillin resistant *E. coli* cells could not survive.

To conclude, the act of transforming bacterial cells to resist an antibiotic is a simple example of the extremely useful biological tool. This method (if done successfully) results in a cheap and simple way in storing and replicating specific targeted genes and it is used within biological industries. Within this practical, it was shown that this method, although simple, does result in many errors, hence, amendments must be carried out for successful transformations.

References:

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Appendix: