

Comparison of three different transfection methods biology essay



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Transfection is a procedure that introduces foreign nucleic acids into cells to produce genetically modified cells. There are three main categories of transfection methods, Biological, Chemical and Physical. In this report the chemical and physical transfection methods will be compared i. e. Physical-Liposome, Calcium Phosphate and Physical- Electroporation. The reason why the biological method was not carried out is that this method is potentially hazardous to laboratory personnel.

The main purpose of transfection is to study the function of genes or gene products, by enhancing or inhibiting specific gene expression in cells, and to produce recombinant proteins in mammalian cells i. e. gene therapy delivering a gene of interest into cells to cure a disease or improve symptoms. (Tae Kyung 2010) . The transfected gene can either be stably transfected or can transiently transfected. Transient transfection is where the gene is expressed for a short period of time and is not introduced in the genome; this is primarily used for fast analysis of genes and for small scale protein production. Stable transfection on the other hand is a constant expression of the gene whereby the gene is integrated into the genome; this is long term, reproducible and has a well defined gene expression. This is used for large scale production, drug discovery and gene therapy. (Adtogen 2007)

Chemical transfection methods: Lipofectamine and Calcium Phosphate:

Chemical transfection methods are the most widely used methods were the first to be used to introduce foreign genes into mammalian cells. Chemical methods commonly use cationic polymer calcium phosphate, cationic lipid

and cationic amino acid. This works on the basis of positively charged chemicals make complexes with negatively charged nucleic acids i. e. the DNA . These positively charged complexes are attracted to the negatively charged cell membrane. It is still unknown as to how the complexes pass the cell membrane, endocytosis and phagocytosis are believed in be involved in the process. The tranfected DNA must be delivery to the nucleus to be expressed and this again is unknown.

Physical Transfection methods: Electroporation:

This method is relatively new this includes direct micro injection, biolistic particle delivery, electroporation, and laser-based transfection. In this report electroporation is the physical method to be used. This involves short electrical pulses that disturbs the cell membranes and make holes in the membrane which the DNA can pass through. This is a very quick and easy method. Electroporation is the most commonly used method for transfection of mammalian cells.

The purpose of GFP (Green fluorescent protein) is that this protein is a marker; this enables one to determine if the gene of interest has been tranfected. The protein glows green under UV light. GFP has many different uses i. e.

Quantify the expression level of a target protein

Determine target proteins solubility

Discover which domains of a protein are soluble

Evaluate how a protein interacts with other proteins (protein - protein interaction)

Reveal the effect a small molecule on the proteins folding (Lol Alomos 2009)

Methods and Materials: Refer to preparation books

Results:

Table 1: Concentration selected for ampicillin G418.

Antibiotic

Concentration Selected

Ampicillin G418

200ug/ml

The above table shows the concentration selected of ampicillin G418 of the selection of tranfected cells. This was selected out of a variety of different concentration ranging from 100-1000 ug/ml(kill curve) of Ampicillin G418 and carried out over 14 days.

Table 2. Concentration of GFP and Insulin GFP

Concentration of GFP

Concentration GFP Insulin

0. 25 $\hat{1}$ /₄g/ul

0.07 $\frac{1}{4}$ g/ul

The above table shows the concentration of GFP (control) and GFP Insulin, this is important to know when carrying out different dilutions of various transfection methods. The concentration values were converted from ug to ul as seen in table 3 and table.

Table 3: Lipofectamine Data results.

Dilution Ratio

DNA: Lipofectamine

Viability of CHO cells (%)

Transfection efficiency (%)

Level of expression (%)

1: 1

95%

15%

10% high level, 5% low level

1: 2

90%

10%

5% high level. 5 % low level

1: 3

85 %

10%

5% high level, 5 %low level

The above table indicates the dilution ratios, Viability of CHO cells, transfection efficiency and the level of expression of this.

Table 4: Calcium Phosphate data results:

Dilution no.

Quantity of GFP

Viability of CHO cells (%)

Transfection efficiency (%)

Level of expression (%)

1

5ul

80%

0%

0%

2

10ul

75%

0%

0%

3

15ul

80%

0%

0%

Table 5: Electroporation data results:

Cell Viability (%)

Transfection efficiency (%)

Level of expression (%)

Sample

5%

0%

0%

Control

100%

0%

0%

The above table indicates the quantity of GFP added to the various dilutions, the viability of CHO cells, the transfection efficiency and the level of expression.

Discussion:

The insulin plasmid and GFP which were already genetically modified was inserted into a vector i. e. E. coli , the GFP and the insulin were already inserted into the plasmid at this stage. This is carried out to allow the plasmid to grow and replicate. The E. coli simply houses the plasmid. The removal of the plasmid from the E. coli bacterial cells is carried out using a spin column, this technique involves centrifuging the E. coli bacterial cell down numerous times and discarding the supernatant until the insulin plasmid/GFP is left. The plasmid is then measured to determine the size, the plasmids concentration is also determined using a spectrometer as seen in table 2 . The plasmid when contained in the vector was grown on ampicillin to acquire resistance to this for the selection of transfected cells from the non

transfected cells further down the line. The concentration of the ampicillin i. e. G418 to be used was determined using a “ kill curve”. This was carried out over 14 days using various concentrations ranging from 100-1000 mg/ml of G418, day 0-7 and day 7-14 the cells were observed for viability/shape and general health using a microscope and the minimum concentration of G418 was determined as stated in table 1.

Three transfection methods were used:

Liposome mediated transfection

Calcium Phosphate mediated transfection

Electroporation mediated transfection

Lipofectamine 2000:

From the above data in table 3, this indicates that the 1: 1 ratio of plasmids to Lipofectamine 2000 proved to be the most efficient with very little cell death as compared to the other two ratios. In fig 1 below there is very high level of low level transfection and there are also high levels of high transfection as indicated by the arrows. This is obvious as the brighter the cell glows the higher the transfection efficiency is there, meaning the CHO cells are expressing the GFP. In the 1: 2 and 1: 3 ratios there showed to be a 5% level of transfection of both high and low, indicating the higher the quantity of Lipofectamine 2000 does not increase the level of expression but only decrease the viability of the CHO cells.

Fig 2 shows the phase picture from the fluorescent microscope this shows vast quantity of live cells indicating that the lipofectamine 2000 in small quantities did not affect the cells as it did in the 1: 2 and 1: 3 ratios as indicated in table 3

Fig 1: 1: 1 GFP (fluorescent microscope)

Low levels of expression.

High levels of expression.

The above diagram indicates the high level of transfection i. e. CHO cells are glowing bright green meaning there are expressing the GFP in much large quantities than the lower level of expression.

Fig 1: 1: 1 Phase

The above diagram illustrates the vast quantity of live cells, the CHO cells here are in good health, based on shape and spreading efficiencies.

Calcium Phosphate:

From the above data in table 4 this shows there was no transfection in any of the three different dilutions.

In the first and second dilution there showed to be high levels of cell viability and precipitate formation, although there was no sign of any transfection.

In the case of the third dilution, there was no transfection; this was due to the calcium chloride which was not added to the plasmid DNA. This was

obvious as there were no phosphate precipitates which indicate the complexes between the DNA plasmid and the calcium chloride. Fig 3 below illustrates the third dilution of a colleague's transfection using calcium chloride; here it is obvious there are high levels of transfection as indicated by the bright green colour of the CHO cells meaning there is very high level of expression of the GFP. In this third dilution there is 80 % transfection efficiency - 50 % of which is high levels of expression and 30% low level expression. Fig 4 illustrates the phase of the third dilution under the microscope; here it is clear this is high level of cells viability as indicated in table 4.

High levels of expression.

Fig 3 : GFP third dilution

Low levels of expression.

Fig 3 illustrates the vast quantity of transfection, arrow 1 indicates the high level of expression as the CHO cells are glowing a much brighter colour than arrow 2 which indicates the low level of expression.

Fig 4: GFP phase

Fig 4 illustrates the vast majority of living cells, the cells here are healthy, and this is based on the size shape and spreading efficiency of the cells.

Fig 5 : DNA Control Fig 6: Calcium Phosphate Control

Fig 5 and Fig 6 were the controls used in this experiment, fig 5 shows the CHO cells are healthy with good spreading exhibited, the CHO cells are not affected by the DNA. Fig 6 shows the CHO cells in calcium phosphate, the cells are not affected by this, they are healthy with good spreading exhibited.

Electroporation

The above data in table 5 shows there was no transfection in the sample and the majority of the cells died due to the force of the electroporation process, electroporation works on the basis of short electrical pulses that disturb the cell membranes and make holes in the membrane which the DNA can pass through and this consequently killed the cells. The control contained the DNA plasmid and media but did not go through the process of electroporation and there was no transfection but the cells were intact and healthy.

Comparison of the three different transfection methods.

From the data provided i. e. tables 4, 5, 6 it is clear that the calcium phosphate mediated transfection showed to yield the highest level of transfection and expression levels, in saying that the % viability was slightly better in the lipofectamine transfection and in this all the three different dilutions showed to yield a certain level of transfection and expression than that of calcium phosphate in which only one of the dilutions showed to be effective. In contrast between lipofectamine and the calcium phosphate method, a lower quantity of lipofectamine showed to yield the greatest transfection efficiency with the GFP staying constant than with the calcium phosphate in which the highest levels of GFP showed to yield the highest level of transfection with the calcium chloride staying constant. In the <https://assignbuster.com/comparison-of-three-different-transfection-methods-biology-essay/>

liposome transfection, the quantity of lipofectamine 2000 could be reduced even further and this may produce a higher level of expression as lipofectamine can be toxic to cells. The same can be said for the calcium phosphate transfection; here the quantity of GFP could be increased to further increase the level of transfection and the expressions levels.

The electroporation showed to have no transfection efficiency; this was due to the electroporation apparatus used in which the voltage was far too high for that of mammalian cells, the apparatus used is typically used for prokaryotes i. e. bacteria which can sustain a much voltage. Ideally the voltage should have been around 0. 1 kilovolts as opposed to 2. 4 kilovolts that were used in this transfection method.

A change that could be made to yield a much higher level of transfection and levels of expression would be to carry out the transfection methods in 96 well plates with the same quantity and dilutions and this ultimately could increase the transfection ability.

Conclusion:

From the three methods of transfection carried out the method with the overall greatest transfection efficiency and level of expression is the calcium phosphate medicated transfection, although it only achieved this in one dilution it expressed a high level of the GFP. The overall purpose of the various dilutions was to determine the most optimum dilution factor which in this case was 15ul GFP to 6ul calcium chloride.

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