Optimization of an electroporation-based transfection system in CHO-DP12 suspension ce

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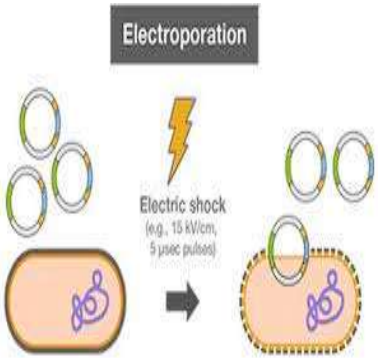
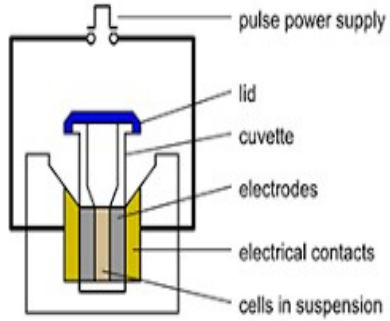
Introduction

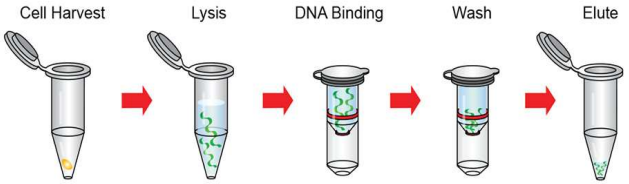
Transfection of DNA plays a major role in production of biopharmaceuticals especially in suspension cells as its easier to upscale the production. An improvement is needed in the process as there is little information about transfection in CHO-DP12 cell line in literature. Here several electroporation experiments were undertaken using the pEZ-M03 plasmid in the CHO-DP12 cel line to optimize transfection efficiency.



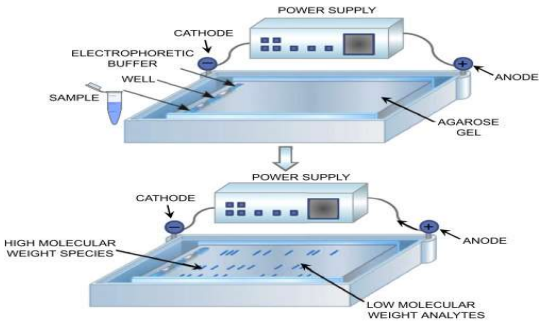


Experimental

➡Isolation of the DNA: Mini-prep and Midi-prep



➡Analysis of the DNA: microvolume spectrophotometer, restriction digestion.



➡Comparison between PBS and Opti-MEM as electroporation buffers.

➡Optimization of electroporation conditions by increasing DNA concentration and voltage.

Results

Comparison between PBS and Opti-MEM as Electroporation Buffers to determine which would be used for the final test.

Table 3.3.1-The concentration of the cells and the % of GFP being expressed in them in each well.

Eppendorf & Well No.	Electroporation Buffer (200µl)	DNA (2µg)	Voltage used (V)	Concentration of cells	% of GFP expressed
1	Opti-MEM	Yes	180	$1.36x10^5$	0
2	Opti-MEM	Yes	300	$2.62x10^4$	0
3	PBS	Yes	180	$3.66x10^4$	7% ($2.62x10^3$)
4	PBS	Yes	300	$2.09x10^4$	0
5	PBS	No	200	$3.71x10^4$	0
6	Opti-MEM	No	200	$5.74x10^4$	0
7	N/A	no	N/A	$3.29x10^5$	0

Optimization of electroporation Conditions

Table 3.4.1- The concentration of the cells and the % of GFP expressed in them in each well

Eppendorf & well No.	Electroporation buffer	DNA (µg)	Voltage	% of GFP expressed (Concentration)
1	PBS	2	200	5% ($5.23x10^3$)
2	PBS	2	230	0
3	PBS	2	250	0
4	PBS	3	190	14% ($1.31x10^4$)
5	PBS	4	190	16% ($7.85x10^3$)
6	PBS	5	190	22% ($5.23x10^3$)
7	PBS	N/A	180	0
8	PBS	N/A	190	0
9	PBS	N/A	230	0
10	PBS	N/A	270	0
11	N/A	N/A	N/A	0

Conclusion

To optimise the electroporation conditions of the pEZ-M03 plasmid in CHO-DP12, the various voltages and concentration of DNA were evaluated.
Voltages more than 200V had a negative impact on the transfection efficiency. As the concentration of DNA increased the transfection efficiency started to increase as they highest transfection % achieved was 22% when 5µg of DNA was used and electroporated under 190V.
Further experiments need to be carried out using lower voltages and higher concentration of DNA to observe the effect on the transfection efficiency and to ensure the acquired results are repeatable. A highly purified DNA could be used alongside a more competent electroporator to improve the transfection efficiency which would help majorly with easing the process of transfection and reducing the time used in research to find methods to improve the transfection efficiency,
It would also be a major contribution to the biopharmaceuticals industry as it would save a lot of money and help with the manufacturing of the recombinant proteins and therapeutic drugs.

References

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