Optimisation of CHO-DP12 transfection using Lipofectamine 2000, TransIT LT1 and electroporation

Department: Department of Life Science

Course : BSc (Hons) in Medical Biotechnology

Introduction

Cell line development is critical for recombinant protein production for use in the medical sector. CHO cells are the work horse of the Biopharm industry with over 70% of currently approved biopharmaceuticals utilising this system (Perkin Elmer 2022)

- Currently the gold standard for transfection is viral, however it is not widely used in industry due to its high expense, procedurally complex nature.
- As a result, there is a need for alternative methods, that offer safer, cheaper and less procedurally complex solutions.
- Transfection is the process of introducing a foreign gene into a host cell using biological, physical or chemical means.
- This investigation focuses on physical and chemical means, in particular electroporation, and the chemical agents Lipofectamine 2000 and TransIT LT1.
- **Electroporation** overcomes the electrostatic repulsion of the cell membrane disruption of the plasma membrane through controlled electronic pulses (Potter, H. and Heller, R., 2018).
- **Lipofectamine 2000** works through the formation of liposomes with a cationic headgroup. This headgroup complexes with the plasma membrane allowing its entry to the cytoplasm through endocytosis.
- **Trans IT LT1** uses a combination approach employing polymers and lipids. The polymers bind to the DNA to form a polyplex to shield the DNA package from nuclease degradation.
- A lipid bi-layer surrounds the polyplex allowing its effective passage through the cytoplasm and into the cell nucleus.

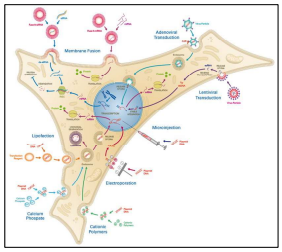

Figure 1 Image depicting the current transfection techniques and the mechanisms in which they achieve stable or transient transfection (IBIDI. 2022)

Aims:

- To optimise the transfection methods Electroporation, Lipofectamine 2000 and Trans IT LT1 for CHO DP12 cells.
- To identify the method with the highest transfection efficiency with minimal toxicity.
- To identify the transfection method that can produce reproducible transfection efficacy, suitable for both industrial and academic applications.

Methodology

- The p-Receiver plasmid was extracted and analyzed using the monarch miniprep kit and the GeneJET midi prep kit
- The resulting extract identity was confirmed using restriction digest, purity and quantity was analyzed using a nanodrop.
- The resulting plasmid DNA was used for transfection optimisation in terms of cell density DNA concentration and reagent DNA ratio
- Transfection efficacy was evaluated by Countess II

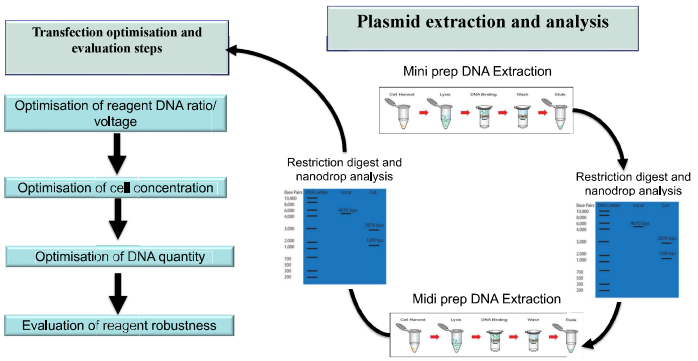

Figure 2 Schematic showing method utilised for plasmid extraction and transfection optimisation. Note cell culture and E-Coli glycerol stock recovery is not shown.

Results

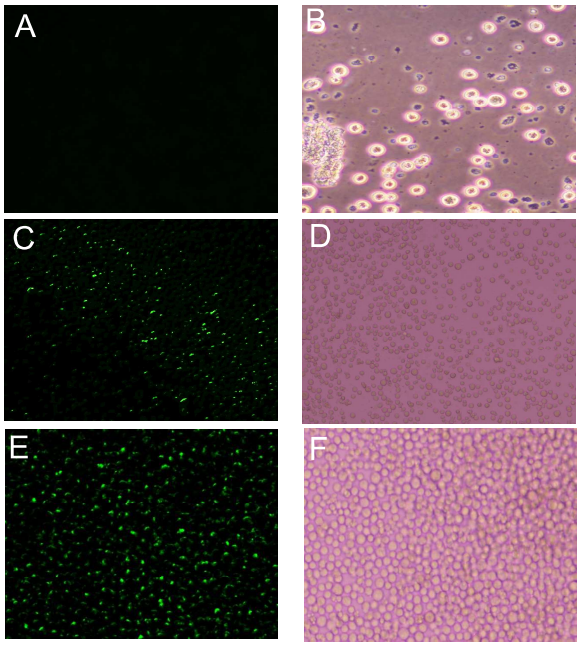

Figure 3 Image of CHO-DP12 cells 48h post transfection. *On the left shows cells under UV light and on the right show's cells under phase contrast. Image taken with Nikon Eclipse Ti-S microscope under the following conditions, fast focus (3x8bit 2880x2048) Quality capture (3x8bit 1440x1024) Continuous AE (300ms) Analog gain (3.4x) and 10x magnification. On the right shows cells under phase contrast. A and B , cells post electroporation* (260V, 2×105 cells/ml, 5µg DNA); C, D cells post Lipofectamine 2000 transfection (Reagent/DNA ratio 2:5, 2x10^5 cells/ml, 5µg DNA) ; E, F TransIT LT1 (Reagent/DNA ratio 3:1, 2x10^5 cells/ml, 5µg DNA)

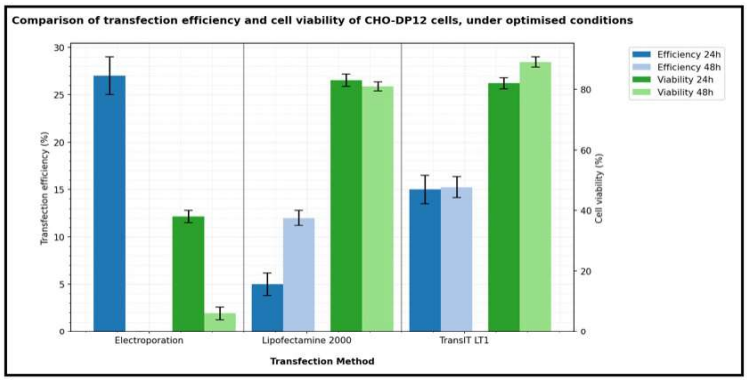

Figure 4. Comparison of transfection efficiency and cell viability in CHO-DP12 cells using optimized conditions. Conditions: Electroporation (260V, 2×10^5 cells/ml, 5µg DNA), Lipofectamine 2000 (2:5 reagent/DNA ratio, 2×10^5 cells/ml, 5µg DNA), and TransIT LT1 (3:1 reagent/DNA ratio, 2×10^5 cells/ml, 5µg DNA)

Table 1 CHO-DP12 cell transfection efficiency post optimisation using Electroporation Lipofectamine 2000 and TransIT LT1 (n=3)

Transfection method	Optimised condition	Time post transfection	Transfection efficacy	Cell viability
Electroporation	260V, 2×10^5 cells/ml, 5µg DNA	24h	25%	39%
		48h	0%	6%
Lipofectamne 2000	Reagent/DNA ratio 2:5, 2x10^5 cells/ml, 5µg DNA	24h	5%	85%
		48h	12%	83%
TransIT LT1	Reagent/DNA ratio 3:1, 2x10^5 cells/ml, 5µg DNA	24h	15%	84%
		48h	15.5%	91%

Discussion and Conclusion

The use of GMOs is a staple of modern biopharmaceuticals. Despite this, there is a niche, in terms of robust chemical and mechanical mechanisms; that offer solutions to the issues seen in viral transfection, in terms of safety, procedural complexity and expense . This investigation demonstrated the capacity of lipofectamine 2000 and Trans IT LT1 to meet that need.

Transfection using Lipofectamine 2000 and TransIT LT1 was successful, with a transfection efficiency of 12% and 15.5%, respectively. The optimal condition determined by this study for Lipofectamine 2000 were reagent DNA ratio 2:5, cell concentration 2x10^{5}cells/ml, and a DNA quantity of 5µg; and for TransIT, reagent DNA ratio 3:1, cell concentration of 2x10^{5}cell/ml and DNA quantity of 5µg. TransIT LT1 displayed no cytotoxicity in conditions tested; However, Lipofectamine 2000 showed significant cytotoxicity, under reagent DNA ratios 4:5 and 1:1 post-transfection.

Although electroporation achieved a transfection efficiency of 27.33%, this was lost after 24h, and the issues in operation of the electroporator prevented further evaluation. The fact that transfection was achieved and there is still potential for further optimisation in terms of media formulation and complex incubation time; and the potential electroporation offers, a non-viral, inexpensive transfection method is offered. This is significant for the medical sector, as an inexpensive and procedurally simple transfection method, could accelerate the development of recombined biopharmaceuticals and ultimately reducing the cost to the patient in a hyperinflated medical sector.

Acknowledgments

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References

IBIDI. 2022. Chemical Transfection Methods | ibidi. [online] Available at: https://ibidi.com/content/263-chemical-transfection. [Accessed 11/Jan/22]

Karthik P.Jayapal, KatieF. Wlaschin, We Shou Hu 2020. Recombinant protein therapeutics from CHO cell – 20 years and counting [online] Available at: https://www.aiche.org/sites/default/files/docs/pages/CHO.pdf.

Potter, H. and Heller, R., 2018. Transfection by electroporation. *Current protocols in molecular biology*, *121*(1), pp.9-3