High Yield Transient and Stable Protein Production Using CHO Cell Line of Choice: From Transient Transfection to CHO Genome Modification and Cell Line Development.

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Abstract

One aspect of lowering costs and reducing attrition rates during biotherapeutic development is the ability to work in the same host cell from early-stage discovery through biomanufacturing. While the CHO cell background continues to be the gold standard for bioproduction, there are a variety of individual CHO cell lines that are used including CHO-S, CHO-K1, CHO-DG44, CHOZN®, ExpiCHO - - the list expands daily as companies continue to engineer their own unique CHO cell lines through genome modification. Thus, a universal, high performance nucleic acid delivery platform that addresses both transient and stable protein production as well as genome modification is critical to successfully accelerating biotherapeutic development programs from the bench to biomanufacturing. MaxCyte's flow electroporation-based delivery platform is a high efficiency, high viability means of transfecting any cell type, with any molecule of interest, at any scale. In this poster, data are presented demonstrating rapid, gram-scale production of quality antibodies from a variety of CHO cell lines using transient expression. Additionally, we detail the use of MaxCyte's delivery platform for the generation of quality, high-yield stable cell lines (>5.7 g/L) within 8 weeks of transfection. Lastly, we demonstrate the use of Flow Electroporation™ Technology for delivery of CRISPR complexes resulting in highly efficient CHO gene modification.

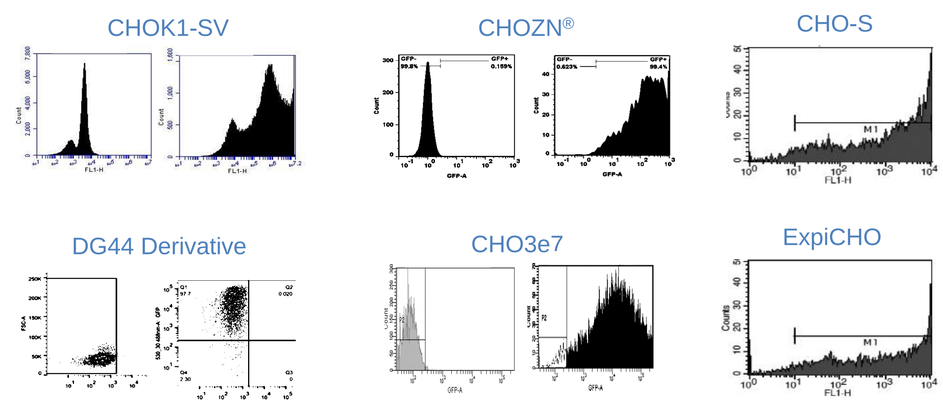
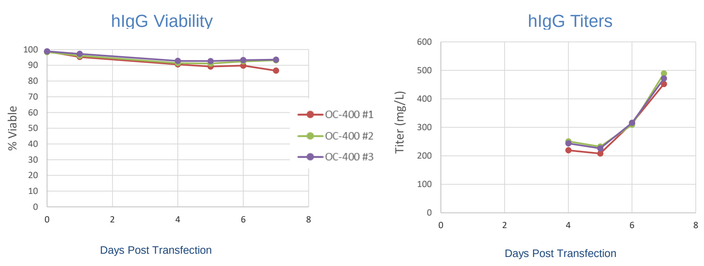


Figure 1: High Efficiency, High Viability Transfection of CHO Cell Line of Choice. Various CHO cell lines were electroporated using the MaxCyte STX with GFP plasmid. All cells, including ExpiCHO cells were cultured in commercially available medium. 24 hours post electroporation, GFP expression was assessed via FACS analysis.

Consistent, High Performance Transfection

Strong Human & Mouse IgG Expression in ExpiCHO Cells

A. Highly Consistent Expression of Human IgG



B. Consistent, High Level Expression of Mouse IgG

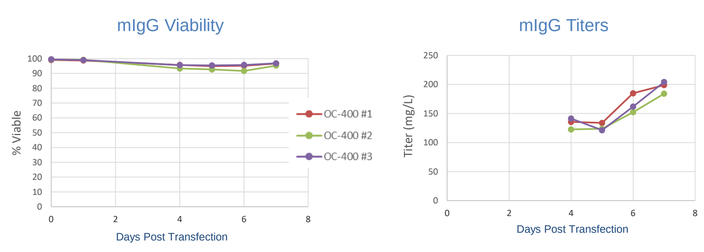


Figure 2: High Titer Production of Human and Mouse IgG Using MaxCye Electroporation of ExpiCHO Cells. ExpiCHO cells were cultured post electroporation in commercially available medium for all experiments. ExpiCHO cells were transfected with a human IgG (**A**) or mouse IgG (**B**) expression plasmid via three independent small-scale electroporations. Cells were seeded post transfection at 4e6 cells/mL and cultured for 7 days.

Higher Titer Protein Production in CHO-K1

3-24 Fold Higher Protein Titers than Variety of Other Methods

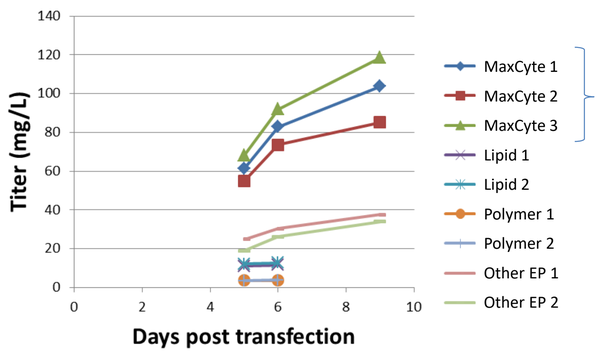
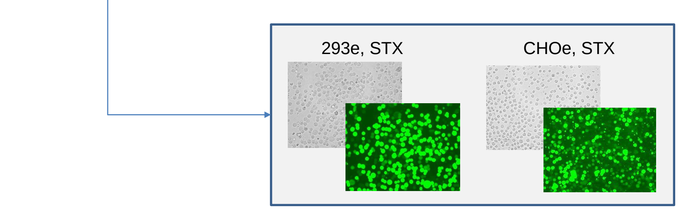


Figure 3: Superior Therapeutic Protein Production in CHO-K1 Cells with the MaxCyte STX Compared to Other Transfection Methods. CHO-K1 cells were transfected via MaxCyte electroporation, an alternative research-scale electroporation instrument, polymers, or lipid reagents with a plasmid encoding a recombinant protein and cultured in 125 mL shake flasks for up to 10 days post transfection. Titers in three sets of cells transfected with the STX were significantly higher than titers generated by cells transfected via all other methods.

Enabling Use of CHO EBNA for Mouse IgG

MaxCyte Outperforms PEI-based Method

	Transfection	% GFP+
Previous Method	293ebna, Polymer	86.0
	CHOebna, Polymer	0.1
	293ebna, MaxCyte	97.7
	CHOebna, MaxCyte	86.0



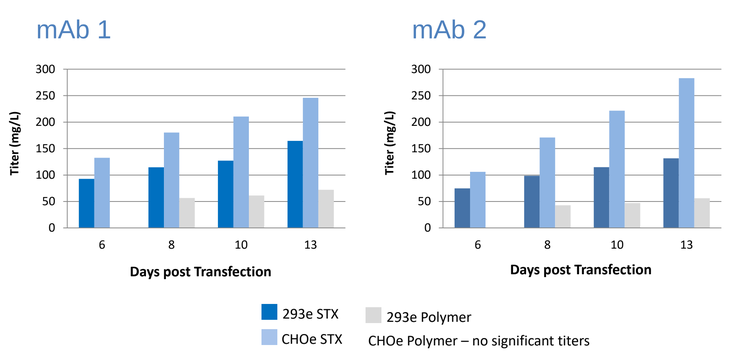


Figure 4: High Titer mAb Expression in CHO EBNA and 293 EBNA Cells. CHO EBNA and 293 EBNA cells were transfected with an IgG expression plasmid via small-scale electroporation (6E7-8E7 cell per condition) and cultured in 125 mL shake flasks for 13 days. Secreted antibody titers in both STX-transfected cell lines greatly exceeded titers generated by an optimized PEI transfection method of 293 EBNA cells.

Multi-Gram, CHO-based Antibody Production Using Transient Expression

> 2.7 g/L Antibody Titers in <3 Weeks

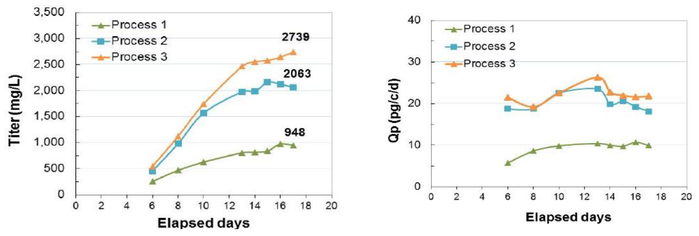


Figure 5: Post Electroporation Culture Process Optimization Produces CHO-S Antibody Titers >2.7 g/L. CHO-S cells were transfected with an antibody expression plasmid (1µg DNA/1E6 cells) via small scale electroporation on the MaxCyte STX®. Cells were plated at approximately 4E6 cells/mL post electroporation. Transfected cells were cultured in media with different additives and culture conditions. Titer was verified by both ELISA and Protein A capture assays. The optimized process produced antibody titers of 2.7 g/L at day 17 post electroporation as a fed batch.

Streamlining Transient & Stable Protein Production

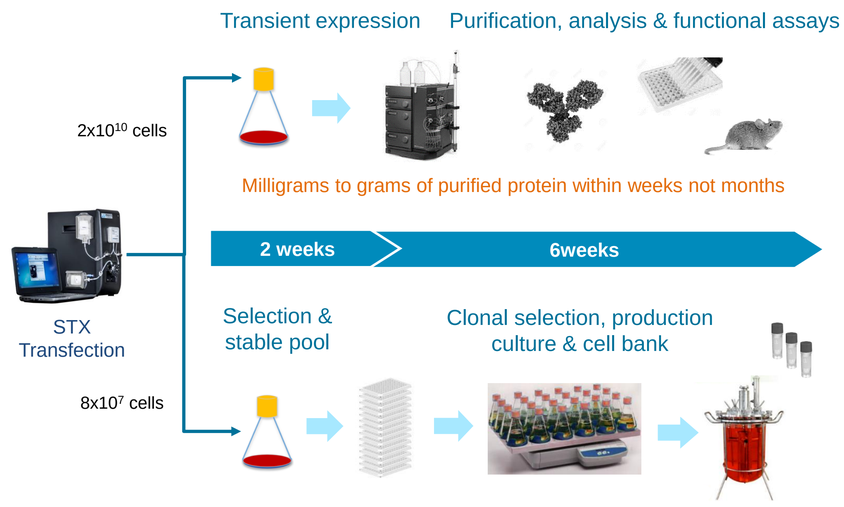


Figure 6: Simultaneous Transient Expression & Stable Cell Line Generation. Cells can be split following a single large-scale transfection, with one portion being used for high titer, gram-scale transient protein production and a small portion used to begin stable cell line generation.

Rapid, High-Yield Stable Cell Generation

Stable Cell Lines Generated in 6 Weeks

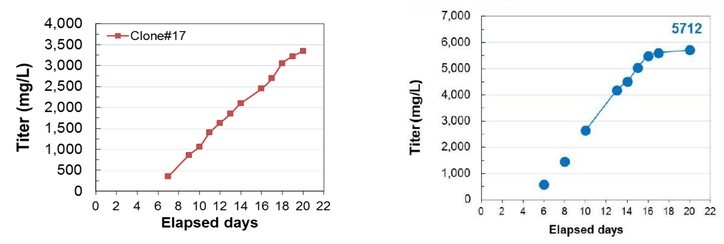


Figure 7: Rapid Identification of High-Yield Stable Clone. A stable pool of CHO cells expressing an hIgG was generated within two weeks of electroporation. **A**). 479 clones were screened following limited dilution cloning. The top clone (clone #17) was selected for production within 6 weeks post transfection. **B**). The production culture was carried out in shake flasks as a fed batch. At day 17, productivity reached >5.5 g/L. Results were verified by both ELISA and Protein A capture assays.

CHO Genome Modification

CRISPR/Cas9-mediated Gene Integration

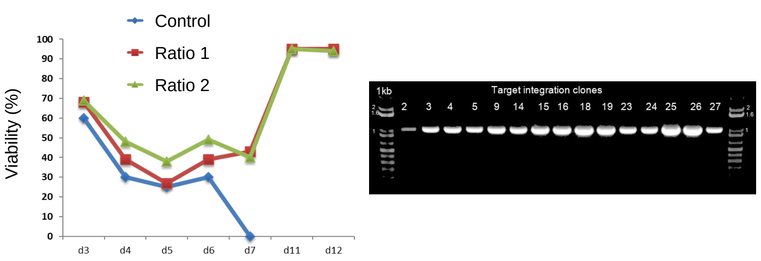


Figure 8: CRISPR-mediated Integration of Protein Expression Construct Within CHO Genome. CHO-S cells were transfected with 2 ratios of donor plasmid to Cas9 & gRNA. Selection was applied 72 hours post electroporation. Cells electroporated with either ratio of CRISPR components recovery within 11 days. 15 of 30 clones isolated from stable pools showed locus-specific integration by PCR.

Summary

- MaxCyte's Flow Electroporation™ Technology enables high efficiency, high viability transfection of any cell type, including a variety of CHO cell lines.
- MaxCyte's delivery platform outperforms other transfection methods including chemical, lipid-based and other electroporation instruments.
- The MaxCyte platform allows the use of any media or culture supplements enabling lower consumable costs and maximum process optimization flexibility.
- High viability and transfection efficiency results in strong expression of more difficult-to-express mouse IgGs.
- With process optimization, gram-scale quantities of antibodies can be produced within 2 weeks following a single transfection.
- High CHO cell viability post electroporation enables generation of stable clones in 6 weeks with yields >5.7 g/L. High cell viability enabled stronger antibiotic selection which enriched for high yield clones. Fewer than 500 clones needed to be screened to identify a high-yield clone.
- MaxCyte's platform efficiently delivers CRISPR components to CHO cells for engineering custom CHO production cell lines.

MaxCyte Delivery Platform

MaxCyte STX®

5E5 Cells in Seconds
Up to 2E10 Cells in <30 min

MaxCyte VLX®

Up to 2E11 Cells in <30 min

- High efficiency & high cell viability
- Broad cell compatibility
- True scalability requiring no re-optimization
- Closed, computer-controlled instruments
- Master file with US FDA & Health Canada

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