Fast Tracking Early Biotherapeutic Development & Clinical Manufacturing Using Flow Electroporation Technology for Streamlining Migration from Transient Expression to Stable Cell Line Generation.

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Abstract

Companies must efficiently identify, develop, and bring to market new therapeutics with the highest level of efficacy while reducing costs. In this poster, we illustrate a number of means to attaining these goals using MaxCyte's flow electroporation-based delivery platform including: i). expanding the use of transiently produced protein in early stage biotherapeutic development to delay costly generation of stable cell lines; ii). transiently producing proteins in the manufacturing host cell background to ensure relevance and manufacturability of identified candidates; iii). streamlining generation of high-yield stable pools & stable cell lines to reduce development timelines; and iv). harmonizing transient and stable protein expression to reduce risk and fast-track development. Data are presented demonstrating rapid, gram-scale antibody production using CHO cells, including the evaluation of feed strategies to improve antibody yields. We detail the rapid generation of stable pools as well as a high-yield stable cell line within 8 weeks of transfection. We share analytical data to demonstrate that the transiently and stably expressed antibodies are qualitatively equivalent. Additionally, data highlighting the stability of antibody titers, quality, and glycosylation profiles of a stable clone over 60 days in culture is presented to illustrate the robustness of cell lines generated using MaxCyte electroporation. Lastly, we present a workflow solution to parallel track large-scale transient expression and rapid stable clone generation for maximum efficiency that stems from integration of MaxCyte's technology into biotherapeutic development programs.

MaxCyte Transient Transfection Platform

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

MaxCyte VLX®
Up to 2E11 Cells in <30 min

The MaxCyte STX® and MaxCyte VLX® Transient Transfection Systems use fully scalable flow electroporation for rapid, highly efficient transfection.

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

High Efficiency, High Viability CHO Transfection

Multi-Gram, CHO-based Antibody Production

>500 mg/L Achieved in <1 weeks OR > 2.7 g/L Antibody Titers in <3 Weeks

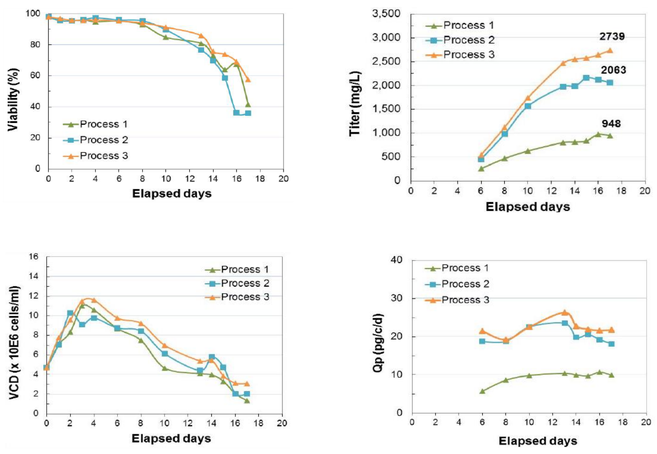


Figure 1: Post Electroporation Culture Process Optimization Produces CHO-S Antibody Titers >2.7 g/L. CHO-S cells were transfected with an antibody expression plasmid (1µg DNA/1E6 cells) via small scale electroporation on the MaxCyte STX. Cells were plated at approximately 4E6 cells/mL post electroporation. 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 2.74 g/L at day 17 post EP as a fed batch.

Rapid Stable Pool & Stable Clone Generation

MaxCyte Transfected CHO Cells Recover Quickly

8 Days Faster than Chemical-based Methods

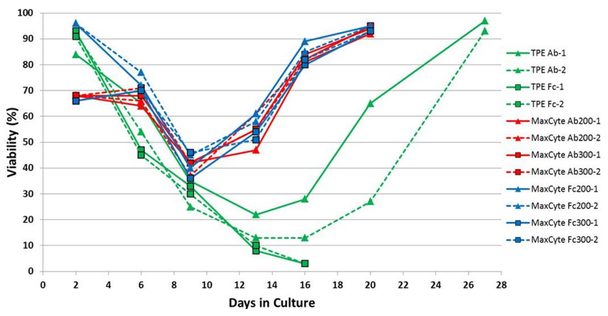
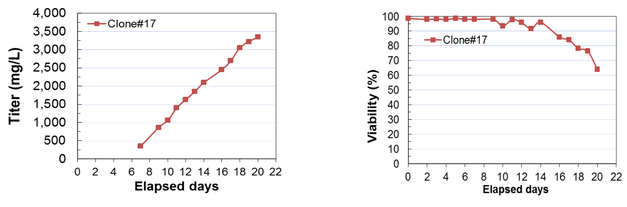


Figure 2: Rapid Recovery of Stable Pools Following MaxCyte Electroporation. Twelve independent CHO cells transfections were performed – four via chemical transfection which are shown in green, and eight via MaxCyte small-scale electroporation, which are shown in red and blue. Half of the transfections for each method were for the expression of a full IgG, while the other half were for the expression of an Fc fragment. Two of the four chemical transfections, specifically those transfected with an Fc expression plasmid, did not fully recover, with almost complete cell loss at day 16 post transfection. Cells from all eight MaxCyte transfection, so those expressing either the IgG or the Fc fragment, all recovered 8 days earlier than the two remaining chemically-transfected cells.

Rapid, High-Yield Stable Cell Generation

Stable Cell Lines Generated in 6 Weeks

A. High-Producing Stable Clone Identified



B. Process Development Boosts Stable Clone Production

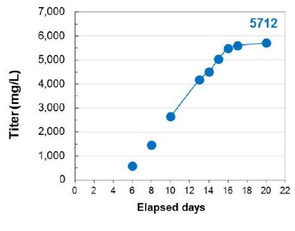
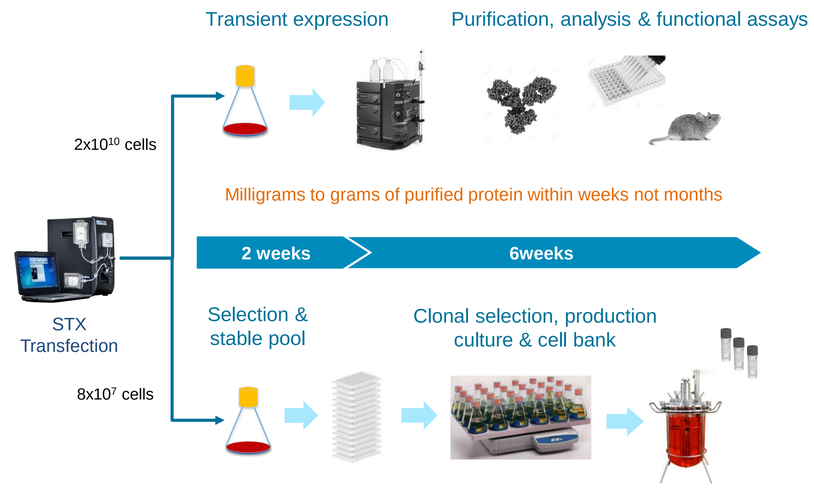


Figure 3: Rapid Identification of High-Yield Stable Clone. A stable pool of CHO cells expressing a hIgG was generated within two weeks of electroporation. A). 479 clones were screened following limited dilution cloning. The top clone (clone #17) was selected for production within 6 weeks post transfection. 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.

Streamlining Transient Expression & Stable Generation



Transient is Consistent with Stably Produced Protein

No Change in Protein Characteristics

Transient & Stable Antibodies Demonstrate Similar Characteristics

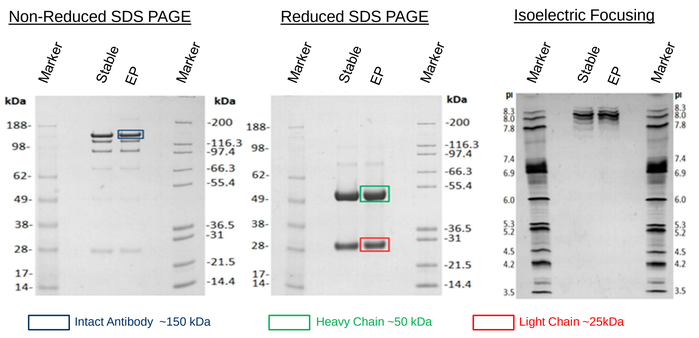


Figure 4. Comparison to Stably Expressed Reference Antibody. CHO suspension cells were transfected by static electroporation with a plasmid encoding IgG heavy and light chain antibody sequences. No differences in size or electrophoretic properties were detectable between the antibody produced via transient gene expression using MaxCyte Flow Electroporation (EP) Technology and antibody produced by stably transfected CHO cells (Stable). *Data courtesy of NovImmune*

Equivalent Antibody Quality & Glycosylation

Similar IgGs Produced via MaxCyte Transient Transfection & Stable Cell Lines Generated Using MaxCyte Transfection

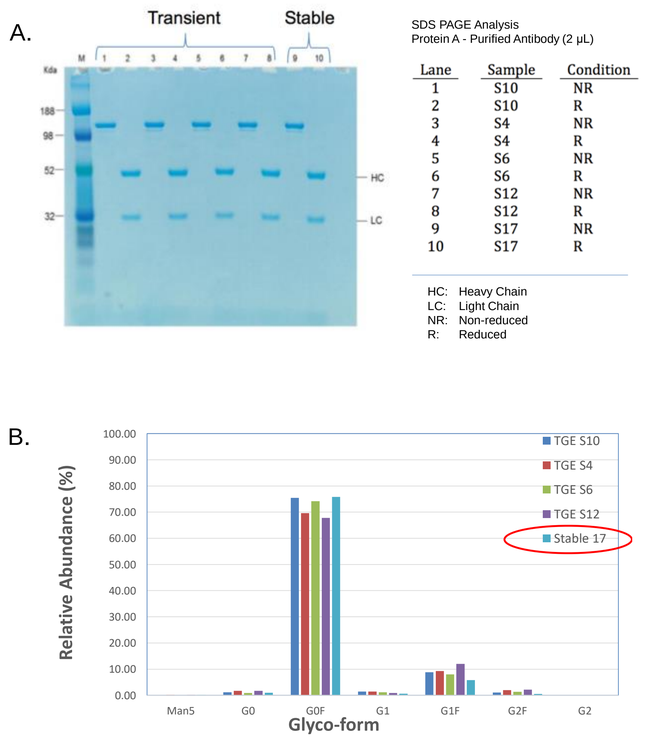
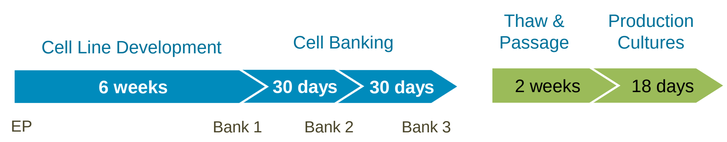


Figure 5: hIgG1 Quality & Glyco-form Comparison - Transient vs Stable Expression. A human IgG molecule was expressed transiently in CHO-S cells via four independent static electroporations with the MaxCyte STX. A stable cell line (S17) was also generated by subjecting transfected cells to antibiotic selection, followed by limited dilution cloning. A). SDS-PAGE gel analysis (reducing and non-reducing) data indicate equivalent quality (i.e aggregation or degradation) of antibodies produced via transient or stable transfection. B.) Glycoform analysis showed highly consistent patterns of post-translational modification among the different transient transfection runs as well as the protein produced from media harvested from the stable cell line.

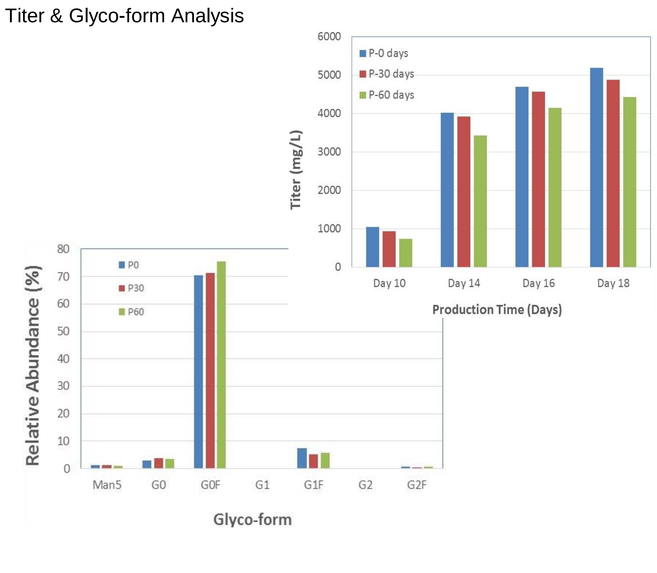
Stability of MaxCyte-produced Cell Lines

High-Yield, Quality Stable Cell Line Generation

Antibody Attributes Maintained for > 60 Days



A. Titer & Glyco-form Analysis



B. Protein Quality

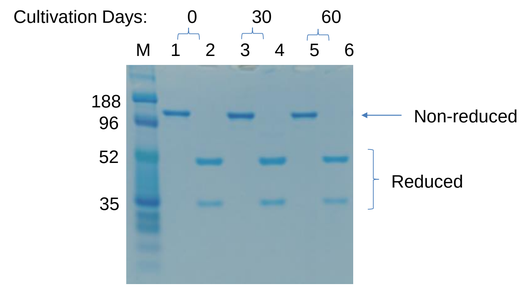


Figure 6: Analysis of IgG Titers, Post-Translational Modifications and Protein Quality of a MaxCyte-produced Stable Cell Line Over Passage. CHO-S cells were subjected to antibiotic selection and limited dilution cloning following static electroporation with the MaxCyte STX. The top clone (S17) was selected for production within 6 weeks post transfection (see Figure 3 for productivity data. A). Cell banks were generated from S17 at three different time points post selection (day 0, day 30 and day 60). Cells from the three cell banks were thawed and proteins were produced in fed batch cultures. Less than 15% loss in titer was observed after 60 days in culture, and glycoform patterns remained consistent. B). Proteins produced by the three cell banks were purified using Protein A and analyzed via SDS-PAGE analysis. No loss in protein quality was observed.

Summary

- Transient transfection of CHO cells produces the gram scale quantities of antibodies needed for early and mid-stage development reducing early reliance on stable cell generation.
- MaxCyte electroporation generates high quality stable pools faster than chemical-based transfected methods. MaxCyte stable pools recovered over a week faster than chemical transfection.
- High CHO cell viability post electroporation enables rapid generation of stable pools and development of stable clones in 6 weeks with yields >5.7 g/L. High cell viability enabled stronger antibiotic selection which enriches for high yield clones. Fewer than 500 clones needed to be screened to identify a high-yield stable cell line.
- Antibodies produced using MaxCyte electroporation demonstrate similar protein characteristics and glycosylation patterns to antibodies produced by stable cell lines, supporting the use of transiently produced protein in early stage biotherapeutic development.
- MaxCyte transfection led to generation of stable cell lines that maintain protein titers, quality and glycosylation patterns over 60 days.

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