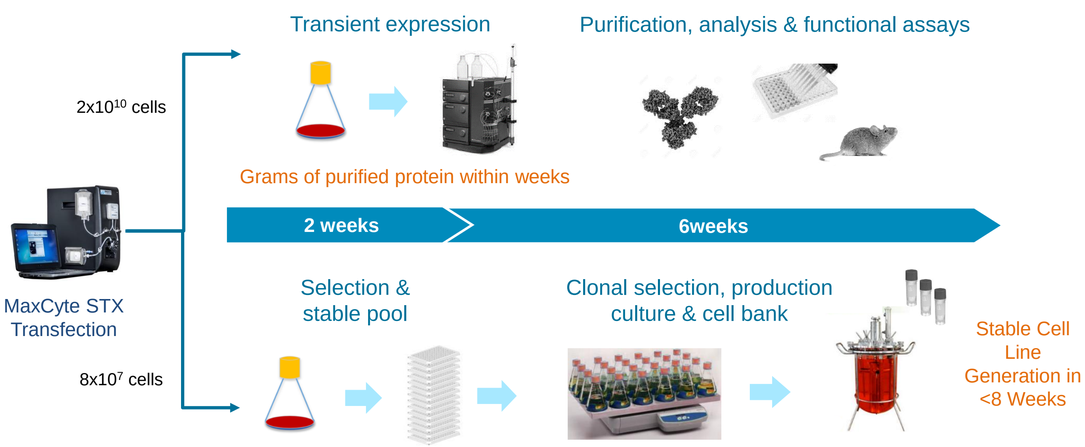
Efficient Cell Line Development and Genome Modification Using Flow Electroporation™ Technology.

Joan Hilly Foster, Weili Wang, James Brady, Rama Shivakumar, Pachai Natarajan, Krista Steger and Madhusudan Peshwa. MaxCyte, Gaithersburg, MD, USA.

Abstract

One aspect of accelerating biotherapeutic development is more efficiently generating high-yield stable cell lines. This can be accomplished through improved expression and delivery platforms, genetic engineering of manufacturing cell lines, and workflow optimization. MaxCyte's flow-electroporation based delivery platform offers significant advantages for rapidly generating stable pools and stable clones using cell backgrounds that are relevant to biomanufacturing. The high levels of transfection efficiency and cell viability post electroporation can significantly reduce the time needed for cell recovery during selection and create stable pools enriched for high producers as well as shorten the timelines and reduce the labor needed for creating clonally-derived cell lines. Flow Electroporation™ Technology also efficiently delivers nucleic acids &/or nucleic acid-protein complexes for CRISPR-mediate genome editing. In this poster, we detail the rapid generation of stable pools as well as a high-yield stable cell line with a titer of 5.7 g/L within 8 weeks of transfection. We share data highlighting the stability of antibody titers, quality, and glycosylation profiles over 60 days in culture to illustrate the robustness of cell lines generated using. Lastly, we demonstrate the use of Flow Electroporation Technology for delivery of CRISPR complexes resulting in highly efficient gene modification.

Streamlining Transient & Stable Protein Production



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

> 2.7 g/L Antibody Titers in <3 Weeks

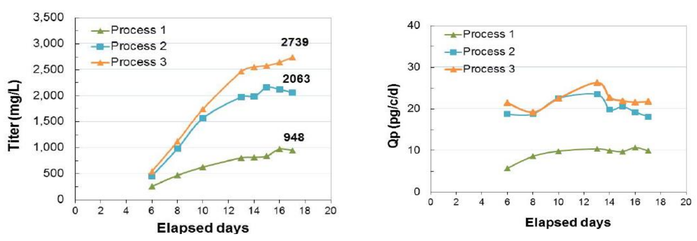


Figure 1: Post Electroporation Culture Process Optimization Produces CHO-S Antibody Titers >2.7 g/L. CHO-S cells were transfected with an antibody expression plasmid (1µg DNA/1E6 cells) via small scale electroporation on the MaxCyte STX®. Cells were plated at approximately 4E6 cells/mL post electroporation.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

Rapid CHO Cell Recovery

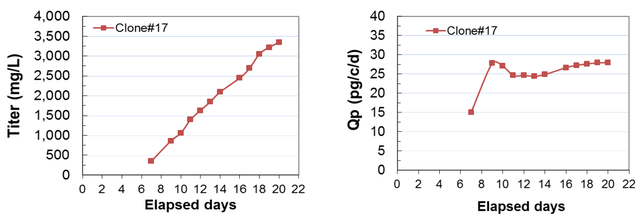
8 Days Faster than Chemical-based Transfection

Figure 2: Rapid Recovery of Stable Pools Following MaxCyte Electroporation. Twelve independent CHO cell 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 -- 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

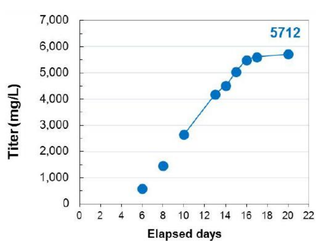


Figure 3: Rapid Identification of High-Yield Stable Clone. A stable pool of CHO cells expressing an hIgG was generated within two weeks of electroporation. **A**). 479 clones were screened following limited dilution cloning. The top clone (clone #17) was selected for production within 6 weeks post transfection.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

Equivalent Antibody Quality & Glycosylation

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

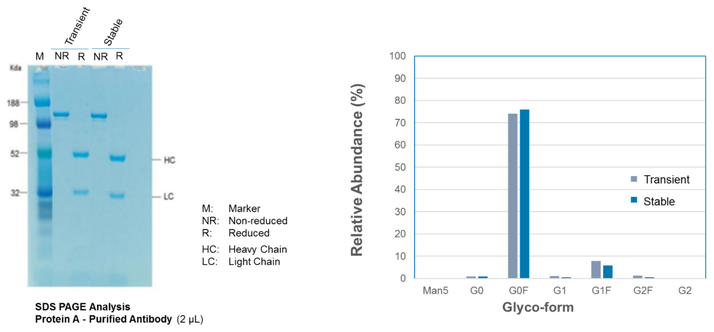
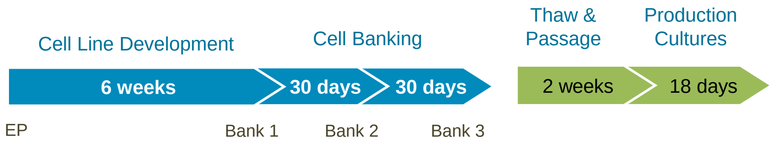


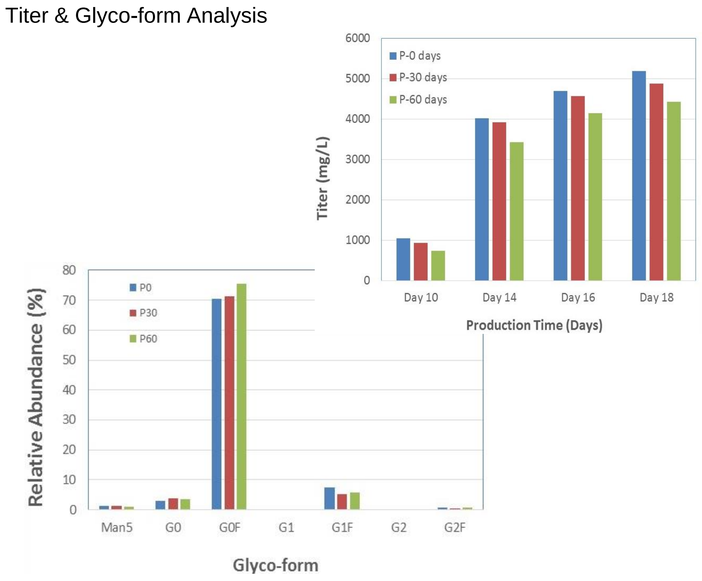
Figure 4: hIgG1 Quality & Glyco-form Comparison - Transient vs Stable Expression. An hIgG molecule was expressed transiently in CHO-S cells using the MaxCyte STX. A stable cell line was generated from an aliquot of the transiently transfected cells by subjecting cells to antibiotic selection, followed by limited dilution cloning. **A**). SDS-PAGE gel analysis indicate equivalent quality (i.e aggregation or degradation) of antibodies produced via transient or stable transfection. **B**.) Glyco-form analysis showed highly consistent patterns of post-translational modification.

Generation of Quality Stable Cell Line

Antibody Attributes Maintained for > 60 Days



A. Titer & Glyco-form Analysis



B. Protein Quality

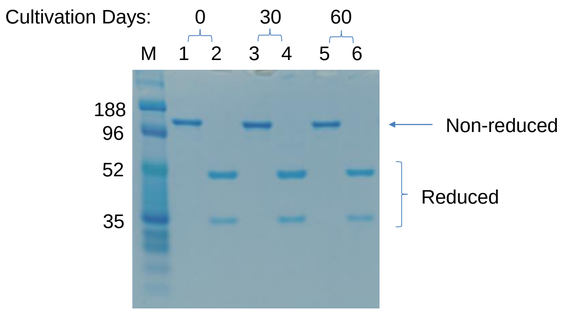


Figure 5: Analysis of IgG Titers, Post-Translational Modifications and Protein Quality of a MaxCyte-produced Stable Cell Line Over 60 Days. CHO-S cells were subjected to antibiotic selection and limited dilution cloning following static electroporation with the MaxCyte STX. The top clone (S17) was selected 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.

CHO Genome Modification

CRISPR/Cas9-mediated Gene Integration

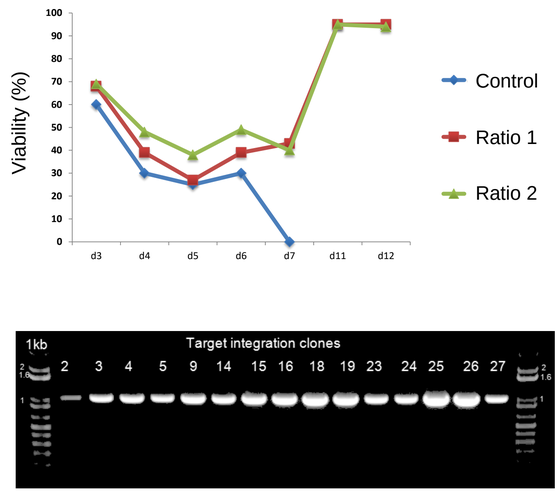


Figure 6: CRISPR-mediated Integration of Protein Expression Construct Within CHO Genome. CHO-S cells were transfected with 2 ratios of donor plasmid to Cas9 & gRNA. Selection was applied 72 hours post electroporation. Cells electroporated with either ratio of CRISPR components recovery within 11 days. 15 of 30 clones isolated from stable pools showed locus-specific integration by PCR.

Summary

- Transient transfection of CHO cells using Flow Electroporation™ Technology produces the gram scale quantities of antibodies needed for early and mid-stage development.
- MaxCyte's delivery platform generates high quality stable pools that recovered over a week faster than chemical 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.
- Antibodies produced using Flow Electroporation™ Technology demonstrate similar protein characteristics and glycosylation patterns to stably produced antibodies, supporting the use of transiently produced protein in early stage biotherapeutic development.
- Flow Electroporation™ Technology enabled generation of stable cell lines that maintained protein titers, quality and glycosylation patterns over 60 days.
- MaxCyte's platform efficiently delivers CRISPR components to CHO cells for targeted genome modification.

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
- cGMP-compliant & CE-marked
- Master file with US FDA & Health Canada

Corresponding Author: James Brady; jamesb@maxcyte.com MaxCyte, Inc., Tel: (301) 944-1700
info@maxcyte.com, *www.maxcyte.com*