Getting the Most from Your Manufacturing Cell Line: Reducing the Time and Cost of Progressing to the Clinic Using MaxCyte's Delivery Platform.

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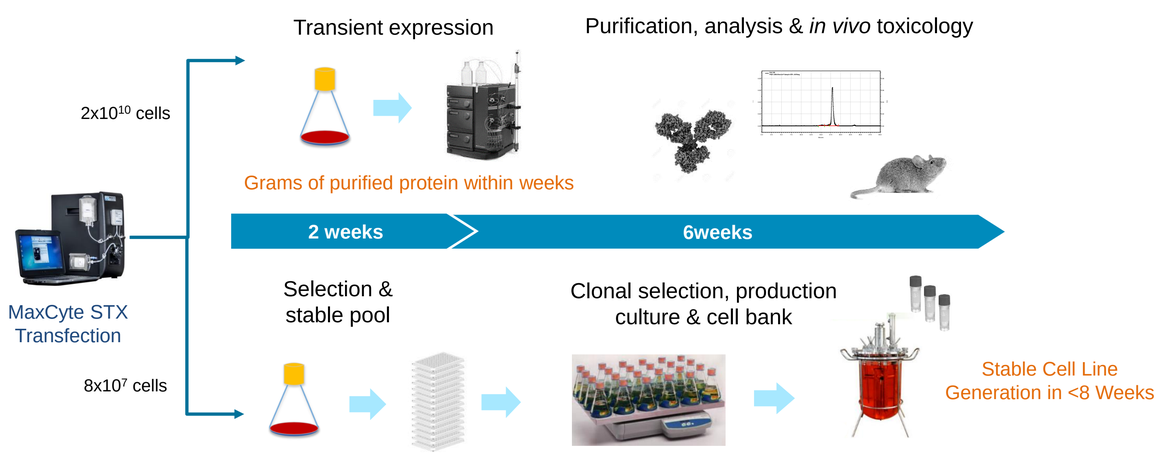
Abstract

The combination of choosing the correct host cell line and the right delivery platform both have significant impacts on the time and cost of progressing to the clinic. Different host cell backgrounds have far reaching implications including differences in culturing requirements, protein attributes such as glycosylation patterns, as well as licensing requirements and intellectual property (IP). Employing an advanced delivery platform augments the benefits of a chosen host cell background including the use of the manufacturing cell line during early-stage development, improving transient and stable pool productivity and streamlining stable cell line generation. In this poster, data are presented demonstrating the high-level efficiency & viability and post-transfection process flexibility of MaxCyte's Flow Electroporation® Technology for a range of CHO cell lines and how the associated increase in transient productivity can delay the need to invest in stable cell generation. Additionally, we share data showing faster cell recovery of stable pools and their enrichment for high producers which shortens the timeline and reduces the labor needed for creating clonally-derived cell lines. Lastly, we highlight the efficient delivery of CRISPR machinery using Flow Electroporation for the creation of customized CHO cell lines.

Unifying Early & Late Stage Development Using Host Cell of Choice



High-level Performance Enables Use of Host Cell-of-Choice Throughout the Development Pipeline

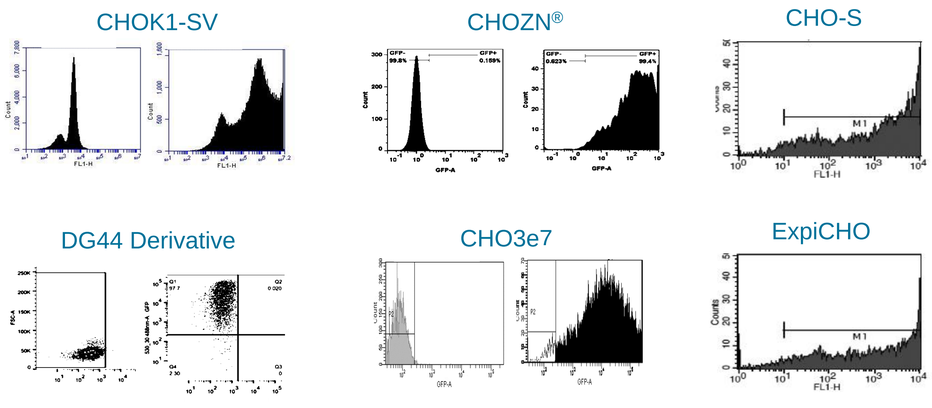


Figure 1: High Efficiency, High Viability Transfection of Any CHO Cell Line. Various CHO cell lines were electroporated using the MaxCyte STX with GFP plasmid. All cells, including ExpiCHO cells were cultured in commercially available medium. ExpiCHO transfected cells were cultured in manufacturer recommended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

Migration of Custom Manufacturing Cell Line into Earlier Development

Fc-Fusion Titers >1 g/L Within Two Weeks in GSN Knockout

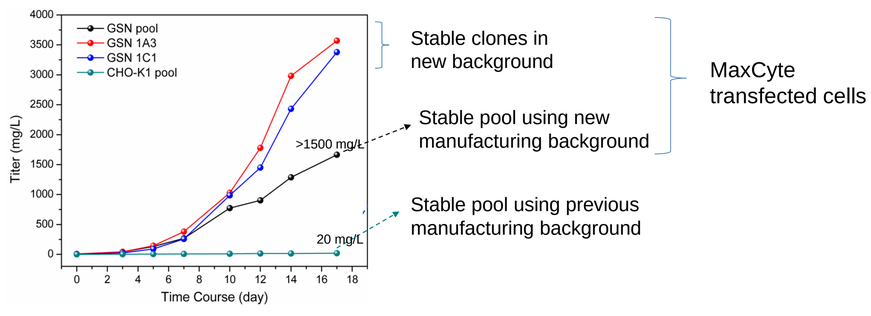


Figure 4: Improved Stable Expression Using Custom CHO Cell Line. A suspension-adapted, serum-free CHO GSN-/- cell line was developed from a parental CHO-K1 cell. The parental cell line and GSN-/- cells were electroporated with a plasmid encoding an Fc-fusion protein. Stable pools and a stable cell line were created and protein production assessed over a 17 day culture. Client met their goal of significantly increasing titers through the construction of a custom cell line & high performance of MaxCyte electroporation. Data courtesy of LakePharma.

Superior Productivity in Manufacturing Host Cells Extends the Role of Transient Expression

Improved CHO-K1SV Antibody Production: Titers >1 g/L Within Two Weeks

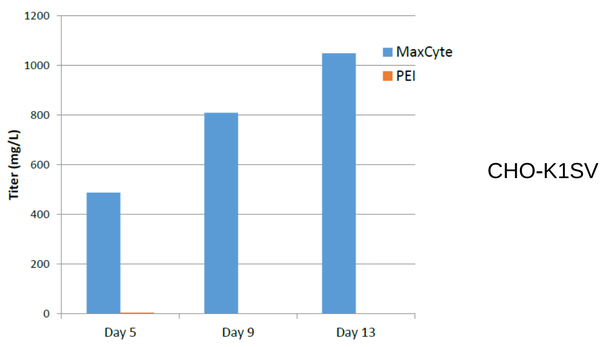


Figure 2: High Titer Antibody Production in CHO-K1SV Cells via MaxCyte Electroporation. CHO-K1SV cells were transfected via static EP or PEI with an IgG expression plasmid and cultured for 13 days or 5 days, respectively. Titer was assayed in the transfected cell on days 5, 9, and 13 post EP. Titers were measured in the PEI transfected cells on day 5. The day 5 titer data indicated clear superiority of MaxCyte Electroporation vs PEI, and the titer data on day 13 revealed productivity exceeding 1 g/L in MaxCyte STX-transfected CHO-K1SV cells.

Accelerated Cell Recovery for Rapid Migration to Stable Expression

50% Faster CHO Cell Recovery

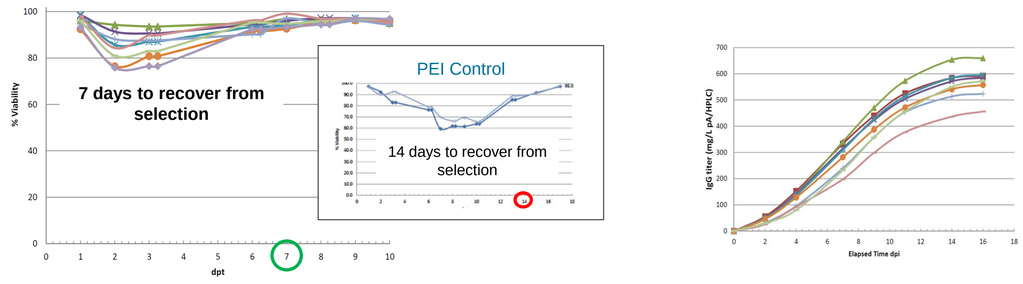


Figure 5: Rapid Recovery of Stable Pools Following MaxCyte Electroporation. CHO cells (proprietary cell line) were transfected via small-scale electroporation with varying concentrations of an antibody expression plasmid. Transfected cells were cultured in shake flasks and were subjected to MSX selection beginning on the day of electroporation. STX transfected cells recovered from selection 7 days quicker than compared to cells transfected with the same plasmid using PEI.

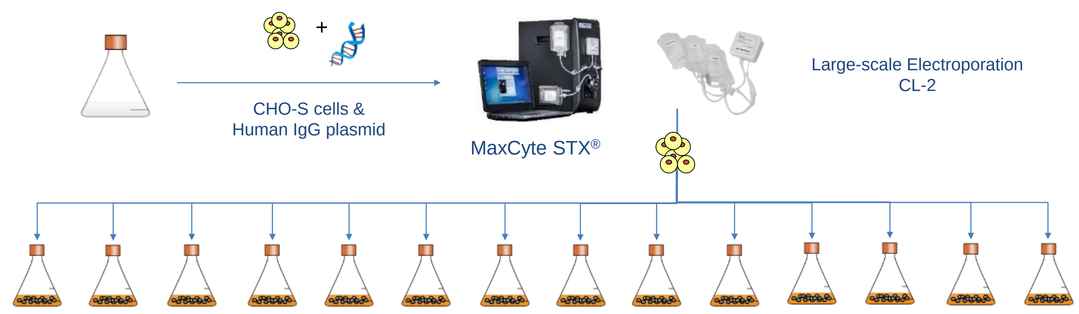
Highly Efficient Construction of Custom Cell Line via Gene Editing

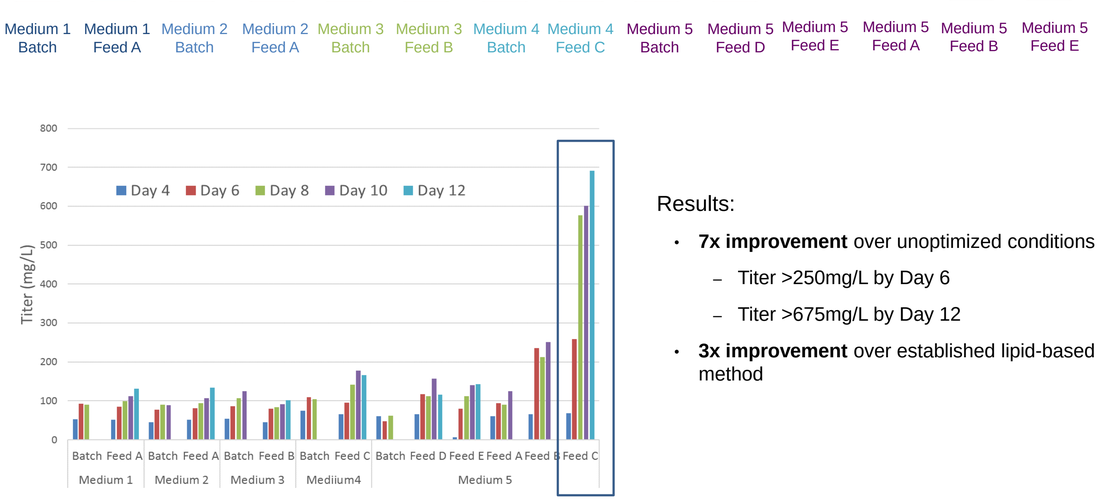
CRISPR/Cas9-mediated CHO Gene Integration

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.

Maximizing CHO-S Productivity Enabled Through Unmatched Flexibility

Media & Feed Optimization Produces 7x Improvement in Antibody Titers





Results:

- **7x improvement** over unoptimized conditions
  - Titer >250mg/L by Day 6
  - Titer >675mg/L by Day 12
- **3x improvement** over established lipid-based method

Figure 3: 7x Improvement in Titer Upon Optimization of Media and Feed. 2e9 CHO-S cells were transfected with a human IgG plasmid via a single large-scale electroporation. 1.2e8 cells were seeded in each of 14,125mL flask. 30mL of media (Medium 1, 2, 3, 4 or 5) was added per flask bringing the initial seeding density to 4e6 cells/mL. Cells were either grown as batch or fed-batch cultures (10% feed on days 1, 3 & 5, 5% feed on day 7).

Summary

- MaxCyte's Flow Electroporation® Technology enables high efficiency, high viability transfection of any cell type, including a variety of CHO cell lines, for high titer transient expression of a range of antibodies & antibody-like molecules.
- The high performance transfection of custom CHO cells enables their use in earlier development activities improving *in vivo* translation and delaying the investment in stable cell line generation.
- MaxCyte's delivery platform significantly outperforms other transfection methods including chemical and lipid-based methods.
- Media and feed strategy flexibility enables large improvements in productivity and reduction in consumable costs.
- MaxCyte's delivery platform generates high quality stable pools that quickly recover from selection greatly reducing development timelines.
- MaxCyte electroporation efficiently delivers CRISPR components to CHO cells for engineering custom CHO production cell lines.
- MaxCyte STX scalability enables a single large-scale electroporation for use in DOE studies and/or simultaneous transient expression and stable cell generation for improved transient productivity & accelerated migration to stable expression.

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