# Scalable Transient Gene Expression in CHOZN® GS$^{-/-}$ cells using Thomson Optimum Growth® Flasks

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## Introduction

Transient gene expression (TGE) is widely used in biopharma for early discovery research due to its ease of use for high-throughput screening and shortened timelines compared to stable cell line development. Consistency between transient and stable systems, especially protein quality, is critical for this early discovery work so use of a reliable CHO cell line is highly desirable. However, obtaining high expression levels and consistent results at larger scales can be challenging.

We have combined three off the shelf solutions for consistent and scalable transient expression in CHO with minimal optimization. MilliporeSigma's CHOZN® GS$^{-/-}$ cell line is a high-performing platform for stable biotherapeutic expression that can also be used for transient production. Polyplus FectoCHO® Expression System provides a robust transfection reagent (FectoPRO®) and FectoCHO® CD Medium for high-yield transient expression in CHO. Thomson Instrument Company (Carlsbad CA) Optimum Growth® plates and flasks are designed for high aeration, low shear, and have a uniform design across sizes, enabling scalability up to 5L flasks without loss of viability or growth limitations.

## Methods

CHOZN® GS$^{-/-}$ cells were adapted to the FectoCHO® CD medium with 0.2% poloxamer-188 and 6mmol/L L-glutamine (CHOZN® technical bulletin and the FectoCHO® transient kit protocol recommendations). Adapted CHOZN GS$^{-/-}$ cells were transfected following the FectoPRO® transient protocol with a cell density of 3x106 cells/mL and transfection efficiency was assessed utilizing a fluorophore-encoding vector. We then screened plasmid:FectoPRO® transfection reagent ratios with an unmodified pCGS3.2-IgG1 vector to optimize performance in 24 deep well plates. Cultures with and without Polyplus FectoPRO® Booster and EX-CELL® Advanced CHO Feed 1 were assessed to understand the benefit to growth and performance. Initial titers were enhanced by nutrient feed optimization in Thomson 24 deep-well plates utilizing EX-CELL® Advanced CHO Feed 1 , L-glutamine and glucose additions. Finally, the optimal conditions were scaled across the Optimum Growth® culture vessel sizes available from Thomson up to 5L flasks (24-well plates, 125mL flask, 1.6L flask, 2.8L flask, 5L flask). Problems post-transfection survival prompted optimization of shake speeds and fill volumes. Finally, all plate and flask sizes were transfected following our protocol and the shake speed and fill volumes in table 1. Protein quality was assessed at flask sizes 1.6L, 2.8L and 5L.

| Thomson Catalog Number | Description | Recommended Protocol Growth: Throw (mm)/RPM/Fill Volume | Recommended Protocol Transfection: Throw (mm)/RPM/Fill Volume | Recommended Protocol Assay: Throw(mm)/RPM/Fill Volume |
|---|---|---|---|---|
| 931568 | 10.4mL Square well round bottom, sterile | 12.5mm/ 320rpm/4.5mL | 12.5mm/ 300rpm/3mL | 12.5mm/ 300rpm/3.5mL |
| 931110 | 125mL Optimum Growth® Flask | 50mm/ 110rpm/63mL | 50mm/ 90rpm/47mL | 50mm/ 90rpm/55mL |
| 931113 | 1.6L Optimum Growth® Flask | 50mm/ 110rpm/900mL | 50mm/ 90rpm/675mL | 50mm/ 110rpm/780mL |
| 931114 | 2.8L Optimum Growth® Flask | 50mm/ 110rpm/1.4L | 50mm/ 90rpm/1.05L | 50mm/ 110rpm/1.21L |
| 931116 | 5L Optimum Growth® Flask | 50mm/ 90rpm/2.5L | 50mm/ 90rpm/1.9L | 50mm/ 90rpm/2.19L |

**Table 1. Shaking condition recommendations for each vessel size.**

## Results

Scalability is a challenge that can be overcome utilizing CHOZN® GS$^{-/-}$ cell lines, Polyplus FectoPRO® transfection and Thomson Instrument Company Optimum Growth® plates and flasks. CHOZN® GS$^{-/-}$ cells were adapted to FectoPRO® CD Medium and were found to adapt quickly to the medium with little growth or viability affects (figure 1).

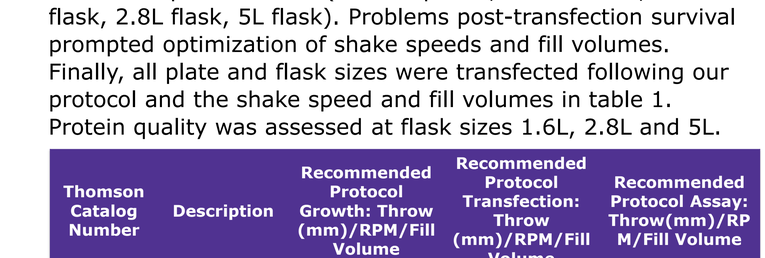


**Figure 1. CHOZN® GS$^{-/-}$ cells fully adapted to FectoCHO® medium with minimal affect on growth.**
Purple lines indicate cultures grown in EX-CELL® CD Fusion; blue lines indicate cultures adapted to Polyplus FectoCHO® CD Expression medium. ● = VCD (viable cell density) and Δ = viability of cultures.

CHOZN® GS$^{-/-}$ cultures were transfected using a fluorophore-encoding vector to assess transfection efficiency in FectoCHO® medium utilizing the FectoCHO® Expression System and found to have >60% efficiency 24 hours post-transfection and >90% efficiency 48 hours-post transfection (figure 2).


**Figure 2. CHOZN® GS$^{-/-}$ showed high transfection efficiency.**

We performed a DOE and obtained optimized transfection conditions, which showed that a 3x106 cells/mL cell density at the time of transfection, as well as a lower DNA quantity of 0.5 µg/mL and 1:2 plasmid-to-FectoPRO® ratio were the best performing conditions. A subset of the ratios tested are shown in figure 3 #1 (2µg/mL:1.5µL/mL), #2 (0.75µg/mL: 4µL/mL), #3 (0.5µg/mL:1.0µL/mL). However, we also noticed that viability was impacted post-transfection, as shown by condition 2 which did not maintain a viability above 70% by day 3 of the assay and therefore protein production is not shown (figure 3A-B). We decided to pursue the optimization by further exploring feed strategies.

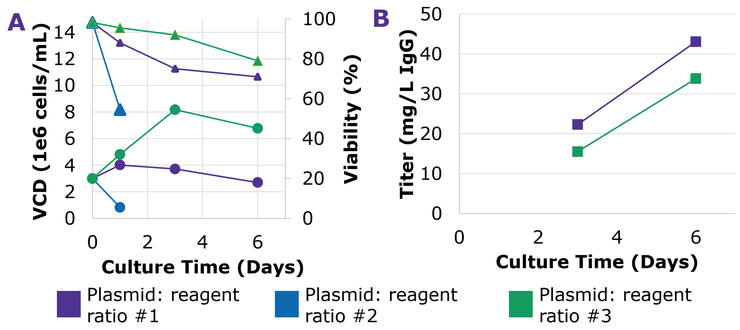


**Figure 3A-B. Transfection condition enabling protein production while maintaining culture health was found by testing several plasmid:FectoPRO® transfection reagent ratios.**
Colors correspond to the condition number, ● = VCD (viable cell density), Δ = viability and □ = titer of all conditions.

We wanted to improve the protein output and well as increase the longevity of the cultures in the batch assay and therefore, we assessed the benefits of adding EX-CELL® Advanced CHO Feed 1 as well as FectoPRO® Booster on the performance of CHOZN® GS$^{-/-}$. An addition of EX-CELL® Advanced CHO Feed 1 at 15% of the initial volume was necessary to maintain viable cultures past day 6 of the fed batch (figure 4A). Adding EX-CELL® Advanced CHO Feed 1 with FectoPRO® Booster resulted in a more than 2-fold increase in protein production (figure 4B) without compromising longevity of the culture (figure 4A). Careful monitoring of metabolites led to a feeding schedule of 8mmol/L glutamine and 8g/L glucose additions on a bidaily scale (data not shown).

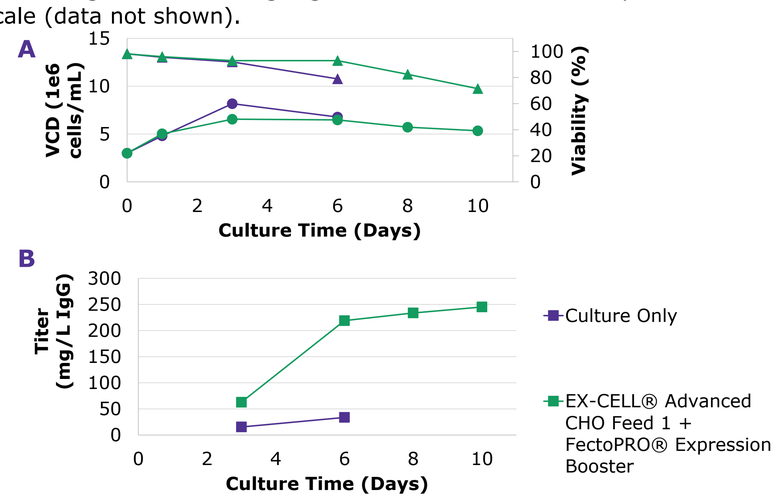


**Figure 4A-B. EX-CELL® Advanced CHO Feed 1 with the addition of FectoPRO® Expression Booster increased protein production while maintaining high viability through the fed batch.**
Colors correspond to the condition number, ● = VCD (viable cell density), Δ = viability and □ = titer of all conditions.

We saw that some larger scale flasks had lower post-transfection viabilities (<70%; data not shown) and identified shake speeds and fill volumes that allowed for high survival rates (table 1); increasing the poloxamer level to closer to the CHOZN® Technical Bulletin recommendation was also found to help survival.

## Scalability

### Growth and Productivity

Following our optimized protocol in each vessel size (table 1), we were able to reach peak titers between 200-350mg/L IgG by day 13 (figure 5A) utilizing a CHOZN® GS-/- cell line without any modification. These peak titers were confirmed via HPLC (data not shown).

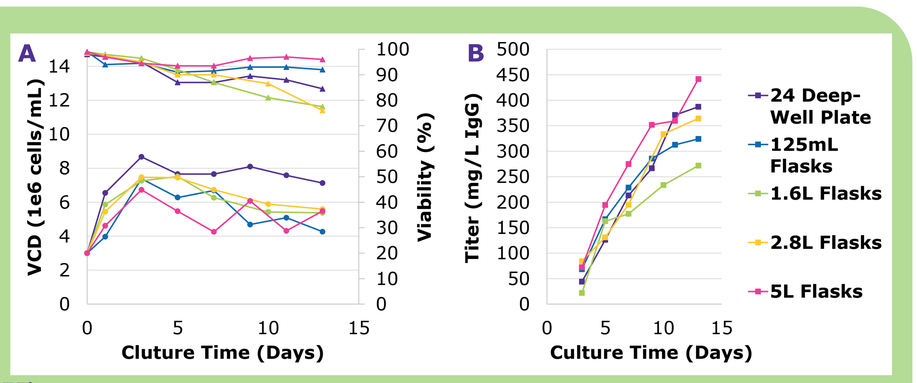


**Figure 5A-B. Growth, viability and protein production were scalable across all vessel sizes. Colors correspond to the condition number, ● = VCD (viable cell density), Δ = viability and □ = titer of all conditions.**

### Protein Quality

Charge variant analysis by imaged capillary isoelectric focusing (iCIEF) showed no significant differences between stably expressed IgG and transiently produced IgG across different vessel sizes (Figure 6).

Glycosylation analysis via intact mass spectrophotometry also showed consistency across vessel sizes (data not shown). However, significant differences were seen in mAb transiently expressed in Polyplus FectoCHO® CD medium versus stably expressed in EX-CELL® Advanced medium (data not shown). Additional studies would be required to confirm whether the glycosylation differences observed were due to differences in basal medium. In prior experiments (data not shown) consistent glycosylation profiles have been observed between transient and stable production when the same medium is used. When consistent glycosylation profiles are needed, a medium exchange post-transfection into a stable production medium or supplementation should be considered.

No significant protein quality changes in transiently produced mAb were seen in protein quality across vessel sizes. Growth, viability and productivity levels were also consistent across vessel sizes, with modest increases in titer seen at large vessel sizes. This indicates that this transient process for therapeutic protein production in CHOZN® GS$^{-/-}$ can be predictably scaled using Thomson Optimum Growth® Flasks for early discovery work.

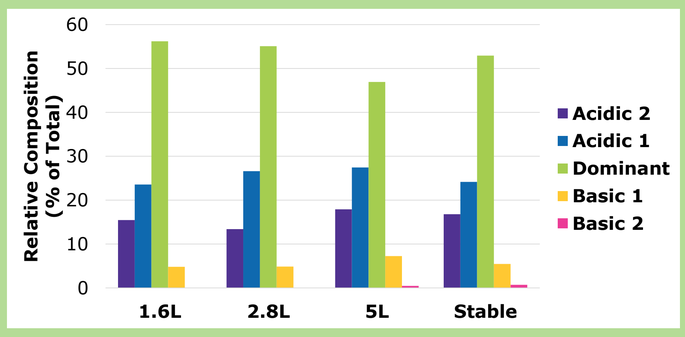


**Figure 6. Protein quality reports of large vessel sizes compared to a IgG1 producing stable cell line showed charged variants.**

## Summary

Scalability is a common challenge within transient transfection to obtain reliable high titer and growth from plates to large scale flask sizes. We have shown that high levels of transient product can be obtained using a combination of off the shelf products: CHOZN® GS$^{-/-}$ cell line and EX-CELL® Advanced CHO Feed 1 and Polyplus FectoPRO® transfection system, with minimal optimization requirements. Importantly, Thomson Instrument Company (Carlsbad CA) Optimum Growth® flasks support seamless scale up from 24 deep-well plates through 5L flasks with consistency in growth, productivity, and protein quality, enabling transient work for biotherapeutic applications.