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Bioproduction

Rapid GS cell line development for production of high-titer bispecific antibodies with the CHOvantage GS CLD Kit

Introduction

Monoclonal antibodies (mAbs) are a therapeutic cornerstone in cancer and other disease treatments. However, too often, the traditional approach of using one mAb to target one antigen cannot adequately address the adaptive multiple signaling pathways that diseases use to evade effective treatment [1]. Multispecific mAb variants, like bispecific antibodies (bsAbs), have emerged to overcome these pathway challenges by using a multiple antigen–binding approach within a single mAb molecule.

Like traditional mAb developers, multispecific mAb developers strive to bring treatments to patients and market as quickly as possible, while minimizing development costs. However, beyond the additional challenges to design an effective multitarget molecule [2], development teams must also rapidly:

- Establish a stable high-producing cell line
- Identify or develop an optimal medium and feed system
- Establish a development strategy and process
- Navigate a rapidly evolving regulatory landscape

The Gibco™ CHOvantage™ GS Cell Line Development (CLD) Kit was created as an integrated solution to these challenges by providing:

- An efficient transposon-based transfection system with DNA vectors and transposase mRNA
- A high-producing Gibco™ CHOvantage™ GS parental cell line (cGMP-banked)
- A catalog medium and feed system pre-optimized for production in insulin-dependent CHO-K1 GS cells: Gibco™ Efficient-Pro™ Medium (+) Insulin, Efficient-Pro™ Feed 3, and Efficient-Pro™ Feed Enhancer
- A comprehensive user guide with protocols for developing a stable cell line, along with documentation to support the regulatory process

A transposon-based transfection system can expedite more efficient stable cell line generation by providing a larger pool of stable high-producing clones that require less screening. Using a cGMP-banked CHO-K1 GS cell line with a chemically defined (CD), animal origin–free (AOF) pre-optimized catalog medium and feed system can support improved production consistency and help ease the regulatory process.

Our results show that the CHOvantage GS CLD Kit enables rapid selection of stable, high-producing bsAb clones within 4 months using a typical integrated workflow that utilizes 14-day fed-batch assays. Stability of multiple clones to 60 generations, and the lead clone to 100 generations, was demonstrated and can be completed within ~2–3 months.

Stable high-producing clones with the CHOvantage GS CLD Kit in ~14 weeks

- CHOvantage GS cells cotransfected with DNA vectors and transposase mRNA
- Stable pools developed with selective pressure without L-glutamine
- Lead pools selected in microbioreactors
- Single-cell cloning by cell sorting
- Lead clone screening in microbioreactors
- Lead clone confirmation scale-up in a 5 L bioreactor

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Development of a bsAb cell line

The transposon-based CHOvantage GS CLD Kit was used in a typical workflow to generate and confirm stable bsAb cell lines within ~14 weeks, as outlined in Figure 1. At each stage, performance metrics were assessed, including viable cell density (VCD), cell viability, and titer. BsAb N-glycan profiles were evaluated at the stages of lead pool screening, lead clone screening, and lead clone confirmation. Additionally, clone production stability to 100 population doubling level (PDL) for the lead clone and 60 PDL for other screened clones was conducted within ~2–3 months.

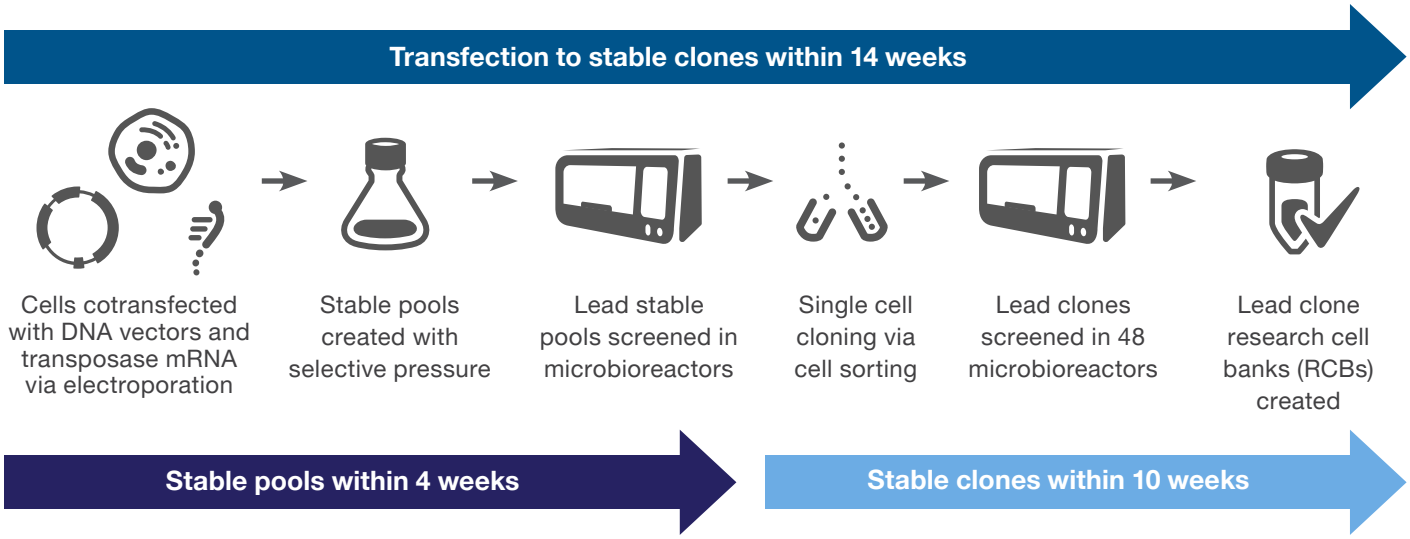

Figure 1. The CHOvantage GS CLD Kit workflow. The integrated kit workflow, as outlined from transfection to stable clone confirmation, can be completed within 14 weeks. Stable pool screening, clone screening, and clone confirmation were conducted with 14-day fed-batch assays.

Stable pool development

A four-chain bispecific antibody was developed with two different CHOvantage transposon-based DNA vector configuration sets (Figure 2). Configuration set A paired each heavy chain (HC) or light chain (LC) together on a vector. Configuration set B paired each HC with its corresponding LC on a vector.

As outlined in Figure 1, stable pools were generated by cotransfecting CHOvantage GS cells with each configuration set and transposase mRNA, followed by applying selective pressure for ~25 days without L-glutamine supplementation. The lead stable pools were identified by screening for titer performance with 14-day fed-batch assays conducted in microbioreactors by three individual operators. Further assay details are in the methods section.

The titer results of the stable pools were consistent and showed a higher average of 3.1 g/L titer with configuration set A, compared to an average of 2.4 g/L with configuration set B (data not shown). Therefore, the stable pool with configuration A was moved forward for single-cell cloning.

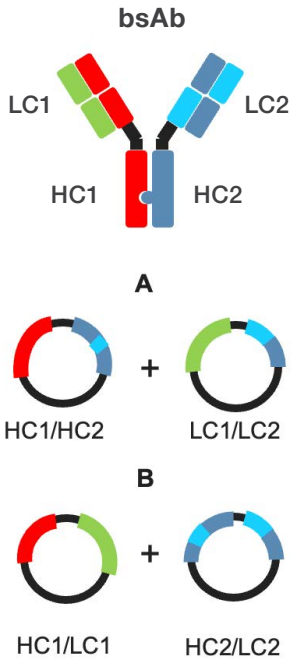

Figure 2. DNA vector configuration sets A and B.

Lead clone screening

After single-cell cloning, the lead-performing clones were identified by screening for growth, productivity, and N-glycans using 14-day fed-batch assays in microbioreactors, as outlined in Figure 1. Further assay details are in the methods section. Overall, the results showed six of seven lead clones yielded a similar or higher average titer than the pool from which they were derived. Three top performers (clones A, B, and C) showed high cell growth, viability, and average titers of ~4–6 g/L, with consistent bsAb N-glycan profiles as shown in Figures 3–5.

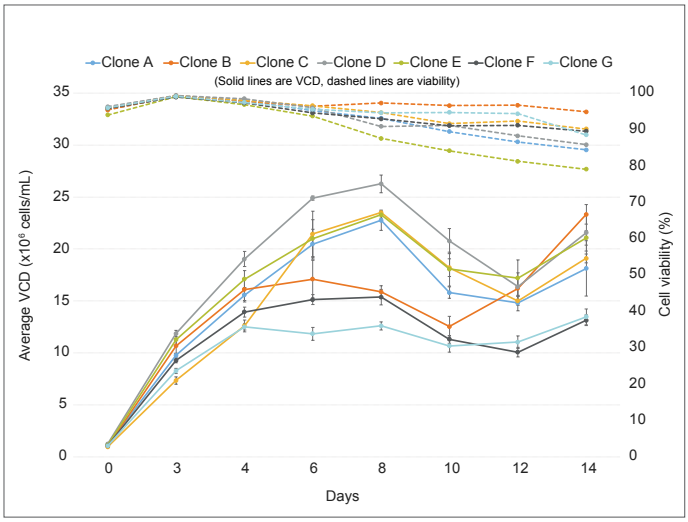

Figure 3. Lead clone screening: VCD and cell viability. Five of seven lead clones (A–E) showed high cell growth with peak VCDs of >20 x 10^6 cells/mL. All seven clones demonstrated high average cell viability near 80% or higher through Day 14 (n = 2–3 microbioreactor vessels per condition).

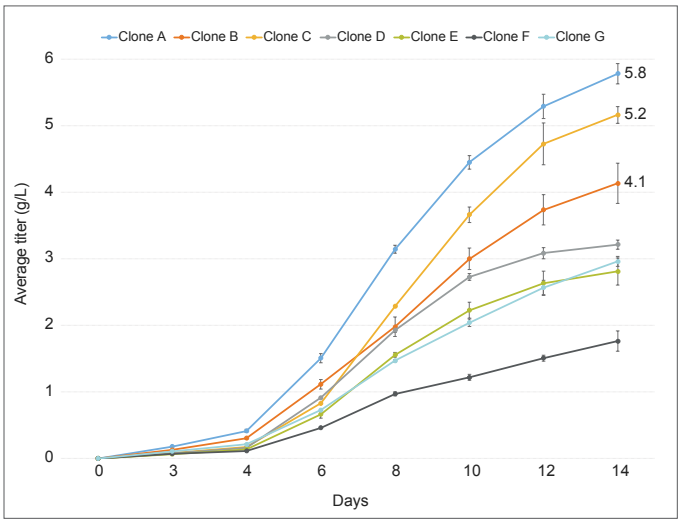

Figure 4. Lead clone screening: titer. The top three lead clones (A, B, and C) produced the highest titers with a maximal titer of 5.8 g/L for clone A, and greater than 5 g/L or 4 g/L for clones B and C, respectively.

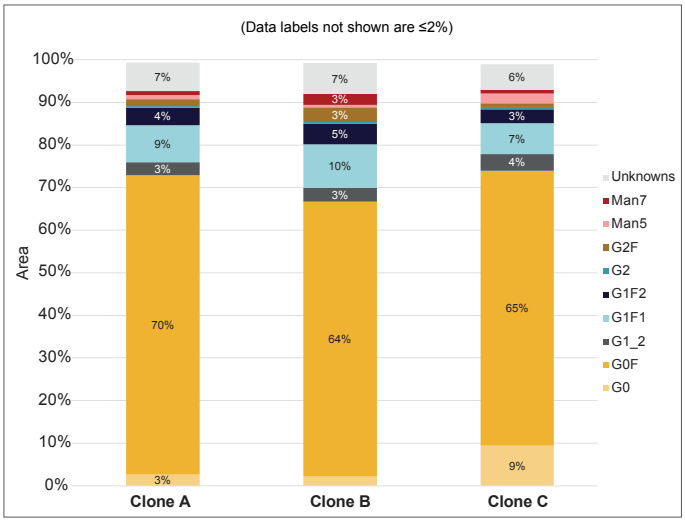

Figure 5. Lead clone screening: N-glycans. Across the three top lead clones (A, B, and C), the bsAb N-glycan profiles were consistently maintained with <10% difference between each structure.

Lead clone scale-up confirmation

As outlined in Figure 1, the performance of the top lead clone (clone A) was evaluated with 14-day fed-batch assays scaled up to 5 L bioreactors. Cell growth and viability, as well as bsAb titer and N-glycans, were assessed, and the results were compared between the two 5 L runs and across the microbioreactor and 5 L scales. Further assay details can be found in the methods section.

Overall, as shown in Figures 6–8, between the two 5 L runs, strong similarity was shown in cell growth and viability, as well as bsAb titer and N-glycan profiles. Across scales, the growth, titer, and N-glycan results were considered acceptable and generally consistent. One minor exception was observed with a desirable slight shift in G0F at the 5 L scale to more galactosylated G1F1, G1F2, and G2F structures [3,4].

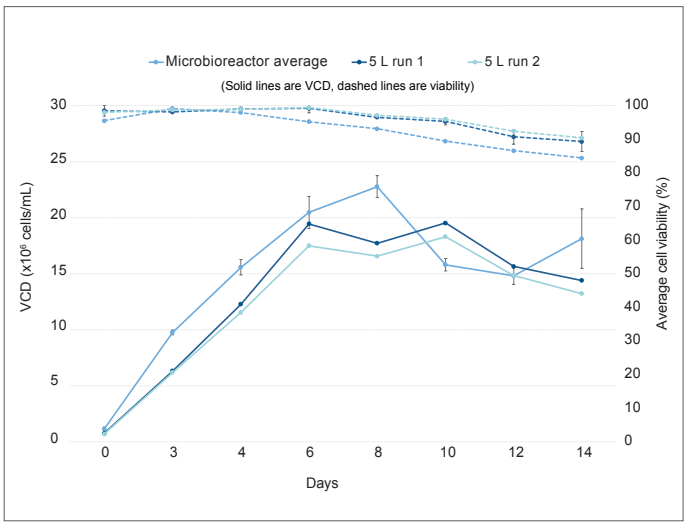

Figure 6. Scale-up confirmation: VCD and viability. Between 5 L runs, clone A showed strong similarity with peak VCDs near 20 x 10^6 cells/mL and ≥90% viability through Day 14. Across the scales, peak average VCDs ranging from 19 x 10^6 to 23 x 10^6 cells/mL and consistent average cell viabilities of >80% were observed through Day 14 (n = 2–3 in microbioreactor vessels, n = 2 in 5 L bioreactors).

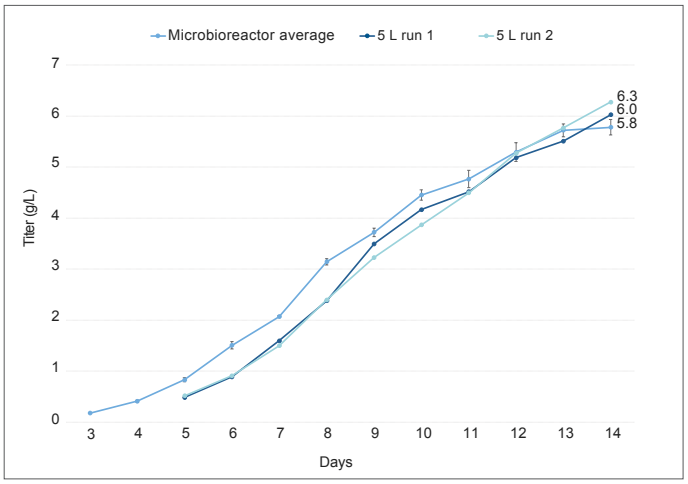

Figure 7. Scale-up confirmation: titer. Consistent bsAb titer performance was confirmed for clone A between 5 L runs and across scales with titers ranging from 5.8 to 6.3 g/L (5 L scale titer evaluation began on Day 5).

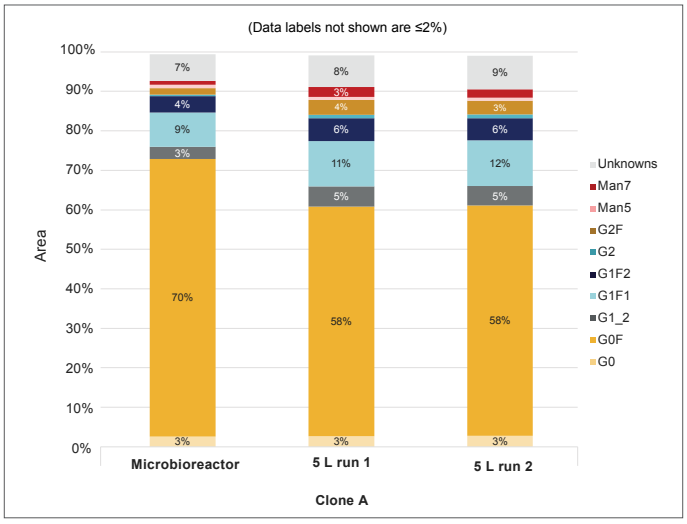

Figure 8. Scale-up confirmation: N-glycans. Clone A yielded generally consistent bsAb N-glycan profiles (<10% difference between each structure) between 5 L runs and across scales, except for the beneficial shift toward less G0F and more galactosylated species (G1F1, G1F2, and G2F) in the 5 L scale relative to the microbioreactor.

Clone production stability

Titer performance for multiple clones was assessed up to 60 generations in passage, with the lead clone A evaluated further to 100 generations, using a 7-day simple (glucose only) fed-batch assay in shake flasks. Clone stability tracking across generations commenced at thaw and continued through cryorecovery and stability assessment, with cell counts recorded at both harvest and cell seeding to monitor generations. Clones were designated as stable if they maintained ≥70% of the relative initial titer performance across the population doubling levels (PDL) tested. Acceptable stable titer production to 60 PDL was demonstrated across all 12 clones tested, while the lead clone A exhibited stability through 100 PDL (Figure 9). Further assay details can be found in the methods section.

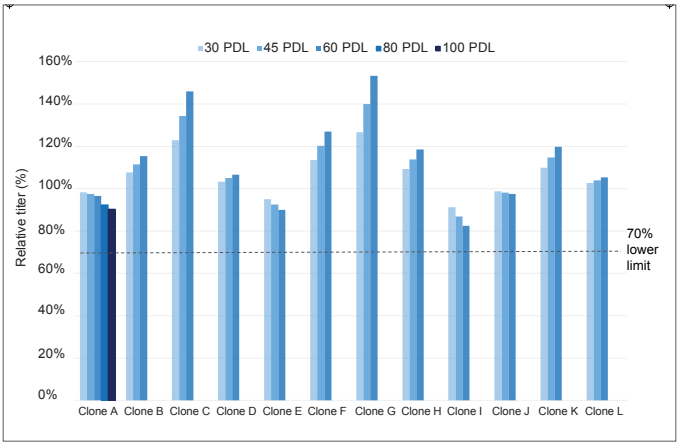

Figure 9. Clone production stability. Across the 12 clones tested to a 60 PDL, all clones demonstrated acceptably stable titer production above the 70% lower relative titer limit. The lead clone A was assessed and shown stable to 100 PDL (n = 1 in shake flasks).

Conclusions and discussion

Our bsAb CLD results show that the CHOvantage GS CLD Kit supports:

- Accelerated generation and confirmation of a stable bsAb cell line within 4 months
- Consistently high cell growth, viability, titer, and N-glycan product quality
- Confirmed benchtop-scalable performance
- Stable cell productivity up to 60 PDL (100 PDL for the lead clone)

Multispecific antibodies are poised to help usher in a new era of more effective treatments for patients. Rapid and efficient delivery of improved treatments can begin with using an integrated CLD kit approach.

The CHOvantage GS CLD Kit can help achieve your goals with the following benefits:

- Accelerates development with transposon-based technology that efficiently generates a larger pool of high-performing stable clones requiring less screening
- Supports process consistency and eases regulatory considerations with a cGMP-banked cell line, and a pre-optimized catalog CD and AOF medium and feed platform with regulatory support documentation
- Efficient bioproduction scale-up with liquid and Gibco™ Advanced Granulation Technology™ (AGT™) medium and feed formats in a range of packaging options
- Consultation and guidance available with a detailed user guide and access to highly experienced and knowledgeable field applications and R&D scientists

For over 60 years, Gibco™ products have helped scientists and developers move past the hurdles of cell culture and advance biologics production. We offer the products, support, and guidance you need to navigate the requirements of your multispecific antibody development.

For additional information, visit **thermofisher.com/chovantage**

References

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Methods

Parameter	Stable pools and clone screening	Lead clone confirmation scale-up	Clone stability
Cell line	CHOvantage GS cell line		
Vessels and replicates	Ambr™ 15 microbioreactors (Sartorius) in triplicate vessels	5 L glass stirred-tank bioreactors in duplicate vessels	Shake flasks in singlet
Medium and supplementation	Efficient-Pro Medium (+) Insulin with 4 mM L-glutamine and 1% Gibco™ Anti-Clumping Agent		
Feeding strategies	Bolus feeding of 2% Efficient-Pro Feed 3 on Day 2, and 4.8% feed and 0.5% Efficient-Pro Feed Enhancer daily on Days 3–13	Continuous gravimetric feeding of 3.5% Efficient-Pro Feed 3 on Days 1–13, and daily bolus of 0.4% Efficient-Pro Feed Enhancer on Days 2–13	–
Glucose	Stable pool medium supplemented with 2.5 g/L on Day 0; clones maintained at 3 g/L	Bolus feeding when <4 g/L to 6 g/L	Bolus feeding to 5 g/L on Day 4
Seeding density	~0.75 x 10^6 viable cells/mL		
VCD and viability	ViCELL™ BLU Cell Analyzer (Beckman Coulter)		
Titer	Cedex™ Bio HT Analyzer (Roche)	Cedex Bio HT Analyzer with Day 14 titer confirmed by HPLC	Cedex Bio HT Analyzer (Roche)
N-glycan profiling	–	Size exclusion HPLC	–

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