# Strategic vector design to improve asymmetric bispecific antibody assembly and purity with high expression levels in the GOCHO™ platform

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## Introduction

Bispecific antibodies are a fast-growing biotherapeutic modality designed to bind multiple target sites, which increases their significance in clinical applications. An asymmetric bispecific antibody (BsAb) is a heterodimer consisting of three or more chains. Because of the design of an asymmetric BsAb, the correct assembly of the chains can be very challenging. Random assembly of the unwanted homodimers decreases yield and creates purification challenges. High purity of the desired heterodimer can be obtained by effective protein design technologies as well as by strategic vector design. In this study, we designed the vectors to maximize the expression of heterodimers and to select clones expressing high titers of the desired heterodimer. The BsAb in this study has two antigen binding domains with a Fab domain and an ScFv domain targeting two different antigens. We have explored the use of our GOCHO™ platform to stably express this BsAb by modifying our vectors and transfection strategy. Our assumption is that the heavy chain will poorly secrete in the absence of the light chain, therefore two vectors were designed for co-transfection of the GOCHO host. The vectors were selected using two different antibiotic genes. Vector 1 contains one open reading frame (ORF) coding for the heavy chain (HC), and vector 2 contains two ORFs coding for HC-ScFv and light chain (LC). Multiple strategies for transfection and selection were used to generate mini-pools: both vectors were either co-transfected at the same time or sequentially transfected followed by selection with either geneticin (G418), puromycin, or a combination of both antibiotics. The top mini-pools were cloned using a single-cell printer. The top clones were then selected based first on the titer and subsequently by product quality. We were able to successfully select clones producing > 6 g/L of absolute titer in a 14-day, fed-batch culture with a similar overall timeline as a traditional monoclonal antibody workflow (15 wk from transfection to research cell bank). All five top clones had a heterodimer purity of > 80% after 1-step Protein A purification. This work demonstrates the capability of the GOCHO platform to support the isolation of bispecific antibody-producing cell lines with high productivity and quality.

## Co-transfection had more productive mini-pools than sequential transfection

Two different transfection strategies were used. In the co-transfection method (TXN-1 and TXN-2), both vectors were transfected simultaneously, and the mini-pools were selected for either G-418 (TXN-1) or puromycin (TXN-2) in 96-well static plates. In the sequential transfection method (TXN-3 and TXN-4), the two vectors were co-transfected, and the cells were selected as bulk pool using one selection marker followed by a second transfection. The mini-pools were selected 48 h after the second transfection using both antibiotic selection markers in 96-well static plates.

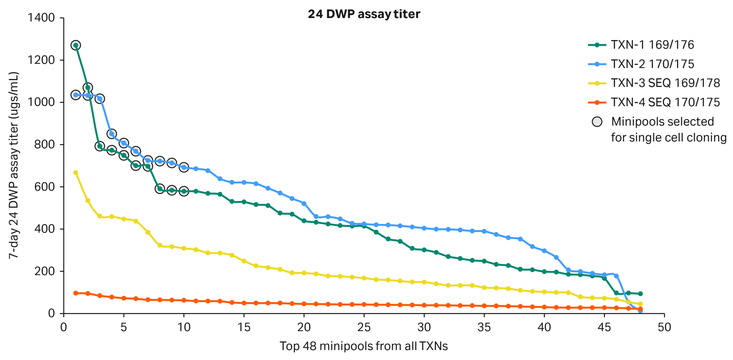


**Fig 2.** The 24 deep-well plate, 7-day assay titers of mini-pools generated by all transfection workflows. The titers for mini-pools generated using the sequential transfection method were significantly lower than the titers for mini-pools from the co-transfection method.

## Strategic vector design and cell line development (CLD) approach to express asymmetric BsAb in GOCHO platform

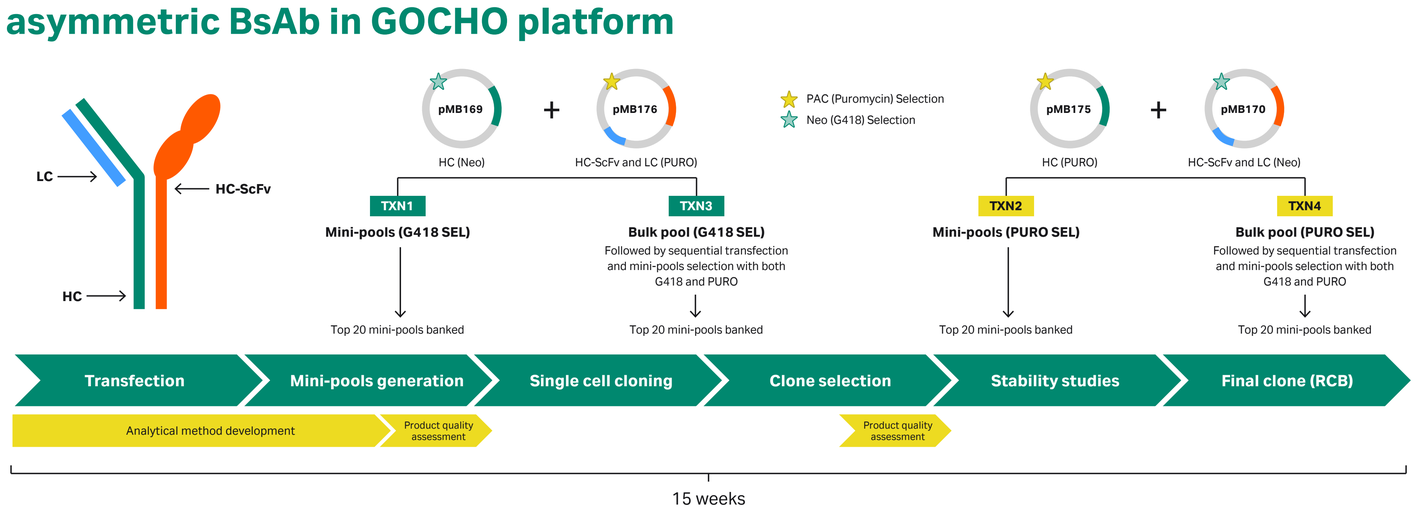


**Fig 1.** Three-chain BsAb vector and workflow design. Two vectors were used to either co-transfect or sequentially transfect the GOCHO host to express the BsAb. Our assumption is that the HC will poorly secrete in the absence of LC. If correct, selecting for the HC may give the best heterodimer ratio. The timelines to develop the asymmetric BsAb were similar to those for a traditional monoclonal antibody (15 wk from transfection to clone banking).

## Clones selected with puromycin had higher titer and higher percent heterodimer than clones selected with G418

**Top clones were assessed in a 14-day, fed-batch assay followed by product quality analysis using HPLC IEX assay**

The resulting clones from puromycin selection were more productive than the clones obtained from the G418 selection. The stringency of puromycin as compared to G418 may have contributed to this outcome. Overall, the top producing clones had titers of > 6 g/L in a 14-day, fed-batch assay. In addition, the percent heterodimer for the top clones was > 80% after 1-step protein A purification.

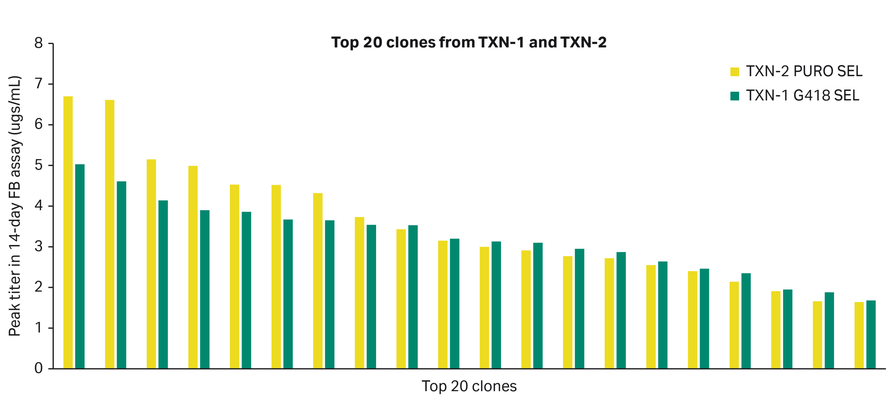


**Fig 3.** Fed-batch assay titers. Top clones from the puromycin selection strategy produced > 6 g/L in a 14-day, fed-batch assay. Clones selected with puromycin had higher titer than clones selected with G418.

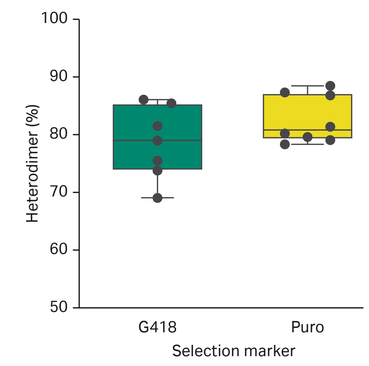


**Fig 4.** BsAb product quality. Puromycin-selected clones had slightly higher % heterodimer (82.7% ± 4.1%) than G418-selected clones (78.6% ± 6.2%).

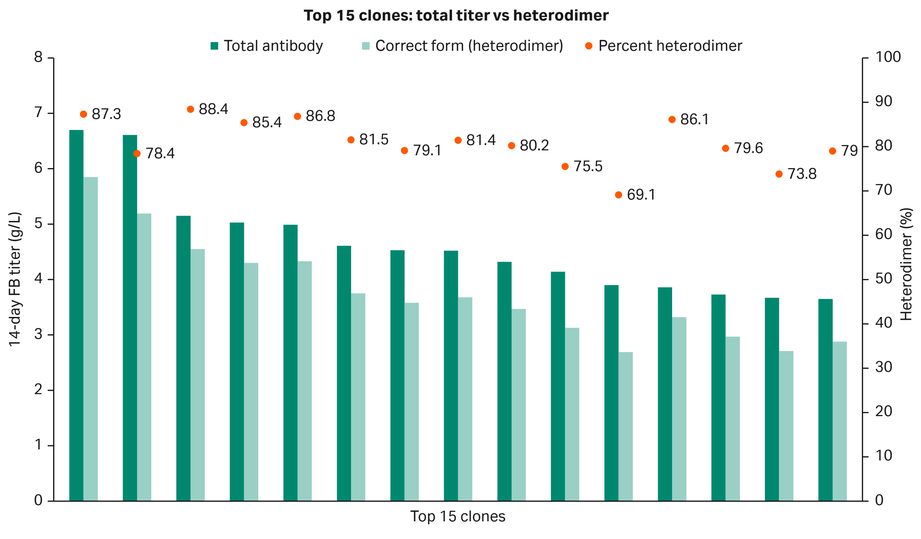


**Fig 5.** Heterodimer-adjusted titers. The top 15 clones from both puromycin and G418 selection yielded clones with > 70% heterodimer after 1-step protein A purification.

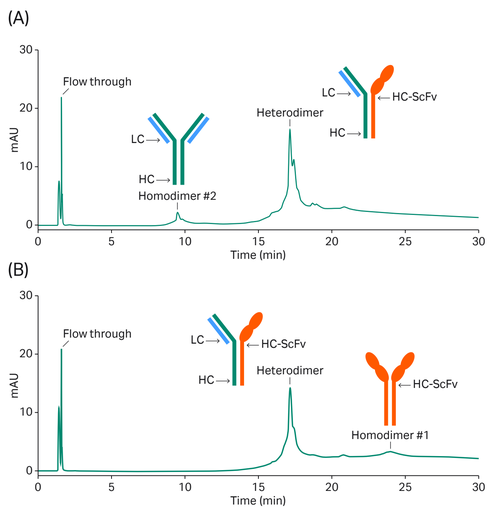


**Fig 6.** HPLC IEX profile for top BsAb clones. Heterodimers predominate in lead clones.

## Conclusions

- We successfully developed vector and workflow strategies to generate clones expressing a 3-chain asymmetric BsAb with a total titer of > 6 g/L in a non-optimized 14-day fed-batch assay. The percent heterodimer for the top clones was > 85% after 1-step protein A purification.
- Vectors were designed to maximize the heterodimer yield for this BsAb. The vector design and transfection strategies may vary depending on the number of chains and/or molecule design for future projects. All transfections and mini-pool and clone scale-ups were carried simultaneously in a staggered timeline. The method development for product quality analysis was done in parallel to CLD activities.
- Timelines for development of this BsAb were similar to those for mAbs (15 wk from transfection to clone banking).

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