

RAPID SELECTION OF HIGH YIELDING GS-CHO CELL LINES USING THE GS EXPRESSION SYSTEM IN A PROTEIN-FREE, FULLY CHEMICALLY DEFINED, ANIMAL COMPONENT-FREE PROCESS

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Introduction and Aims

The Chinese hamster ovary (CHO) cell line is often used by the bio-pharmaceutical industry for the manufacture of therapeutic proteins. The rapid construction of high yielding recombinant CHO cell lines is economically important. Gene amplification is commonly used to create high yielding CHO cell lines but is a time consuming process that can substantially increase the length of a cell line development programme. Lonza Biologics' glutamine synthetase (GS) gene expression system facilitates the high expression of proteins using the GS gene as a selectable marker. GS is the enzyme responsible for the biosynthesis of glutamine using glutamate and ammonia as substrates. The activity of the GS gene is selectively inhibited by methionine sulphoximine (MSX). This system allows the rapid selection of high-expressing cell lines without amplification.

We report the rapid creation of an IgG₂ expressing GS-CHO cell line using Lonza's proprietary constant region GS vectors. Optimisation of transfection and selection conditions was performed to produce high expressing cell lines. In addition a host cell line pre-adapted to growth in suspension culture (CHOK1SV) was used to speed up the adaptation of transfectants to suspension growth.

As a result a panel of 10 lead candidate cell lines was selected. Antibody concentration of greater than 1.2 g/L were obtained for five of these cell lines, in a chemically defined, animal component-free (CDACF) fed-batch process. In this project the time taken from the transfection to the selection of the production cell line was less than 20 weeks.

Methods

Vector Construction

- Lonza's proprietary GS constant region vectors contain a range of human antibody constant region isotypes and allotypes (Figure 1)
- For vector construction see Figure 2
- Variable regions of heavy and light chains cloned by PCR, adding required restriction enzyme sites
- Single-gene vectors (SGVs) constructed and transiently tested in CHO-K1 cells
- Double-gene vector (DGV) constructed, transiently tested in CHO-K1 cells

Optimisation of Electroporation and Selection

- Aliquots of 5x10⁶ cells were electroporated using three different conditions
- Cell suspensions were diluted with glutamine-free medium and distributed across twenty 96-well plates
- 24 hr post-electroporation glutamine-free medium supplemented with MSX was added to each well
 - for ten plates in each set, MSX was added to a final concentration of 25 µM
 - to the remaining ten plates MSX was added to a final concentration of 50 µM
- Plates incubated at 37°C and 10% CO₂ in air.
- After 4 weeks colonies identified and supernatants diluted 1 in 200 before assembly ELISA assay

Cell Line Construction

- Linearised DGV electroporated into CHOK1SV (Suspension Variant) cells using condition EC3 – see Table 1
- Cell lines selected in serum containing glutamine-free media, containing 50 µM MSX for 3 to 10 weeks
- Transferred to 24 well plates
- Successive productivity assays performed to reduce the number of candidate cell lines
- Candidate cell lines expanded in static culture
- Cell lines transferred to suspension growth in protein-free, CDACF medium
- Cell lines expanded and productivities re-assessed in batch and fed-batch cultures
- Promising cell lines cryopreserved

Figure 1. Heavy Chain Isotypes and Allotypes currently available (* Brusco *et al*, 1998. † Angal *et al*, 1993, and Bloom *et al*, 1997)

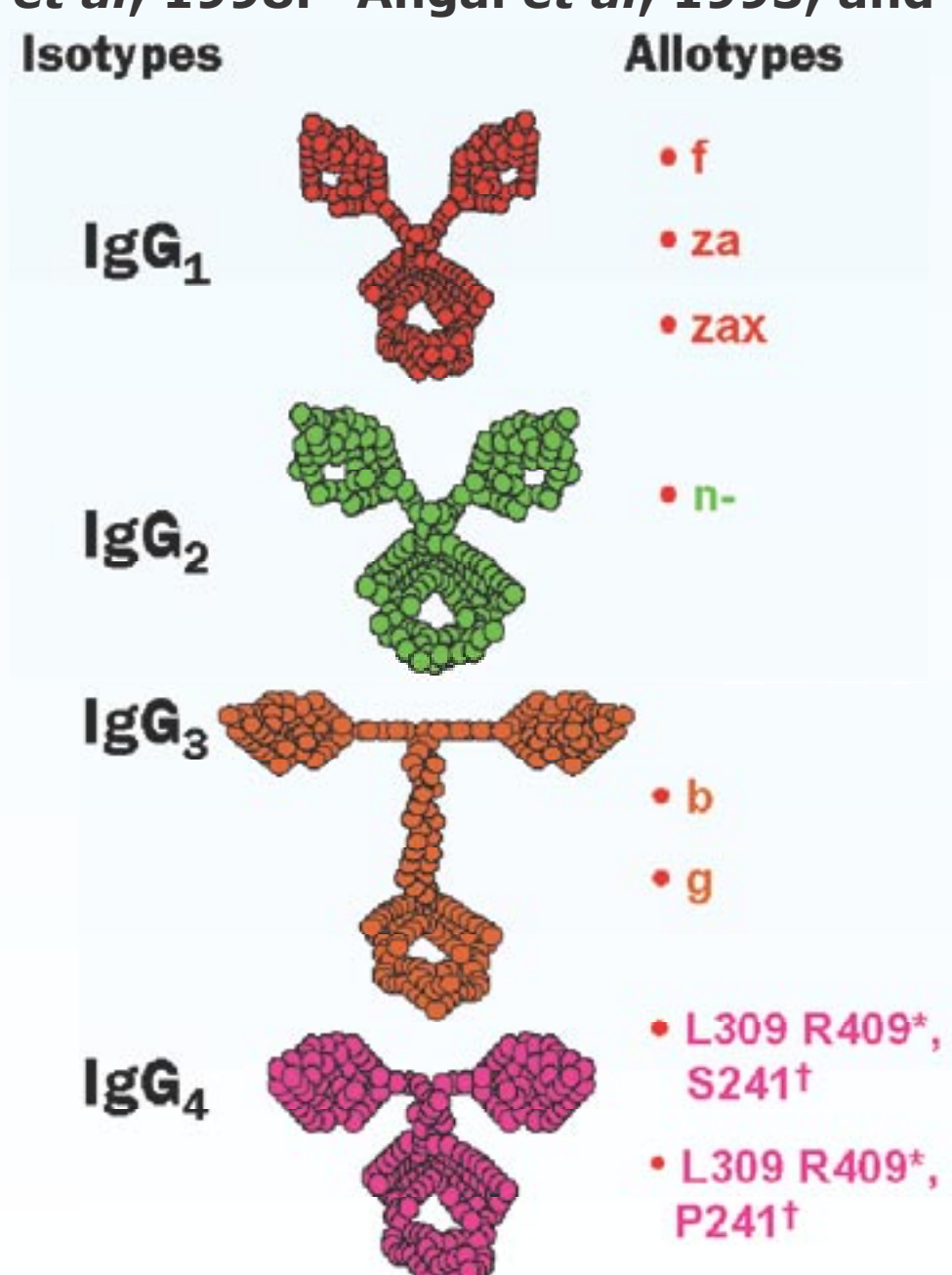


Figure 2. Cloning Method for IgG₂/Kappa Monoclonal Antibody

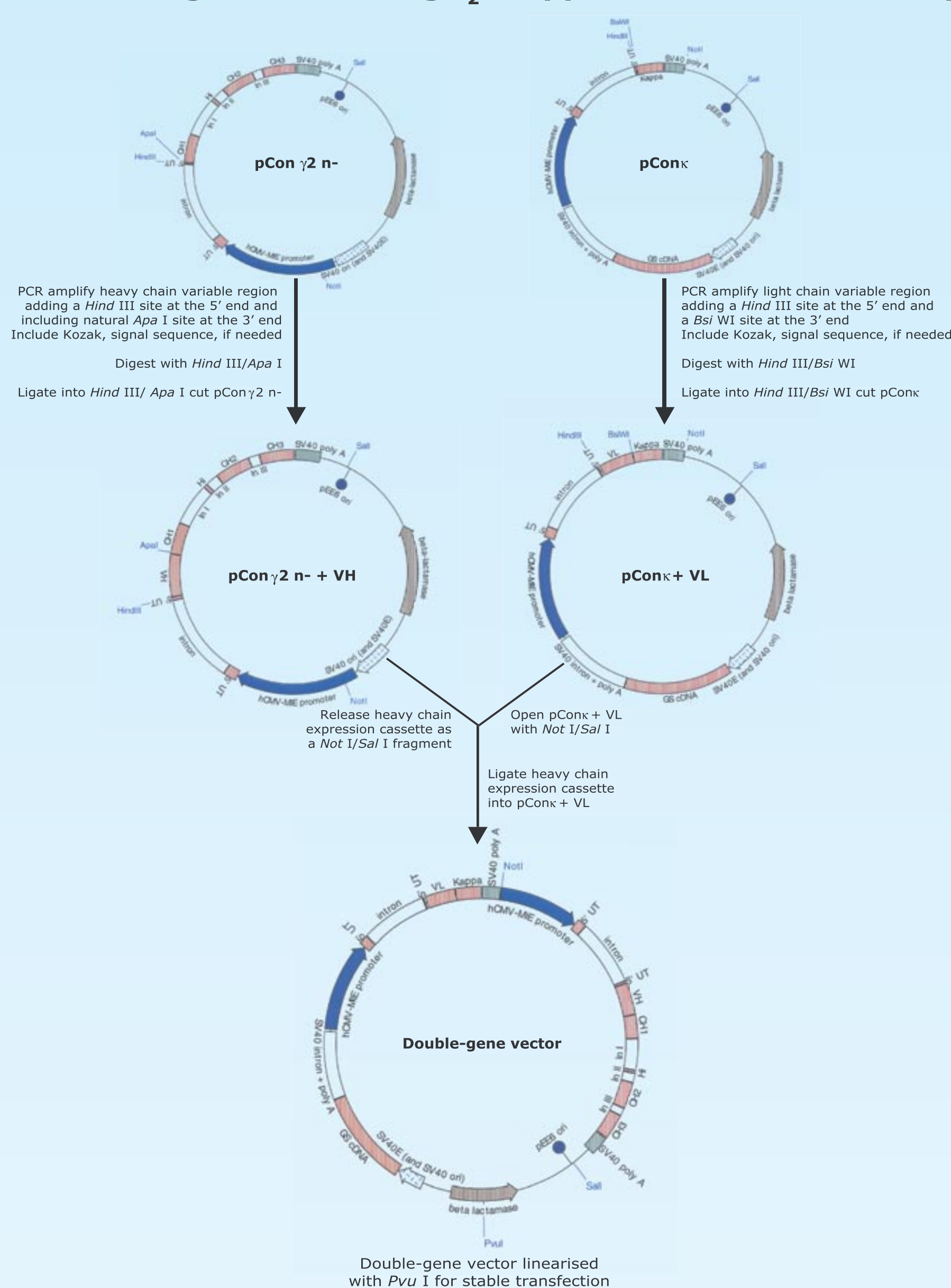


Table 1. Transfection and selection conditions for GS-CHO cell lines expressing model antibody cB72.3

Electroporation conditions	MSX concentration (µM)	Transfectant numbers
EC1	25	68
	50	32
EC2	25	124
	50	57
EC3	25	197
	50	70

Figure 3. Boxplot summary of transfectant productivities: shows 10th, 25th, 50th, 75th and 90th percentiles plus outliers. Samples were diluted 1:200 prior to being assayed for presence of assembled antibody

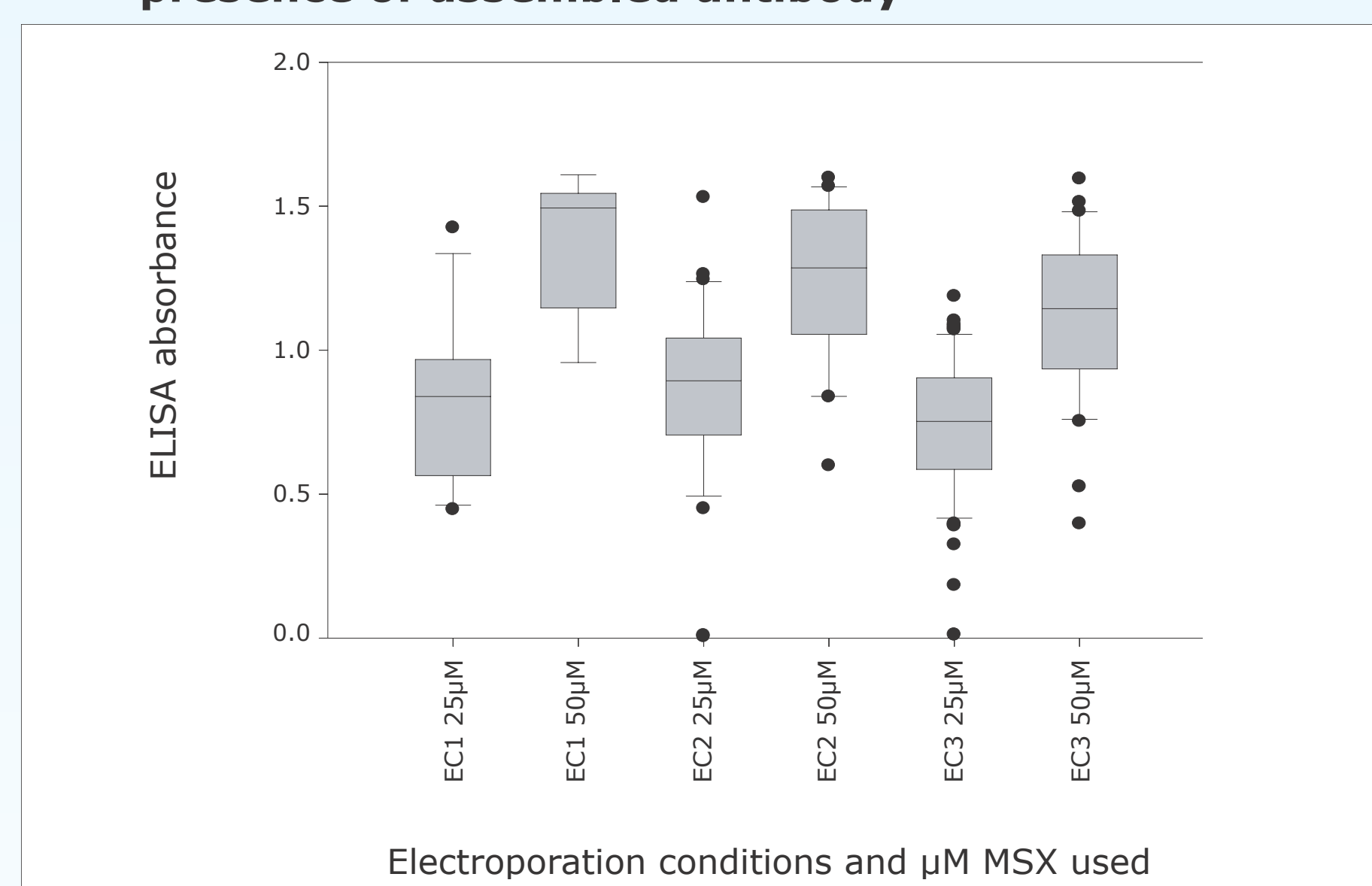


Figure 4. 24-well plate productivity assessment data. Histogram demonstrates numbers of cell lines expressing antibody within given ranges of product concentration

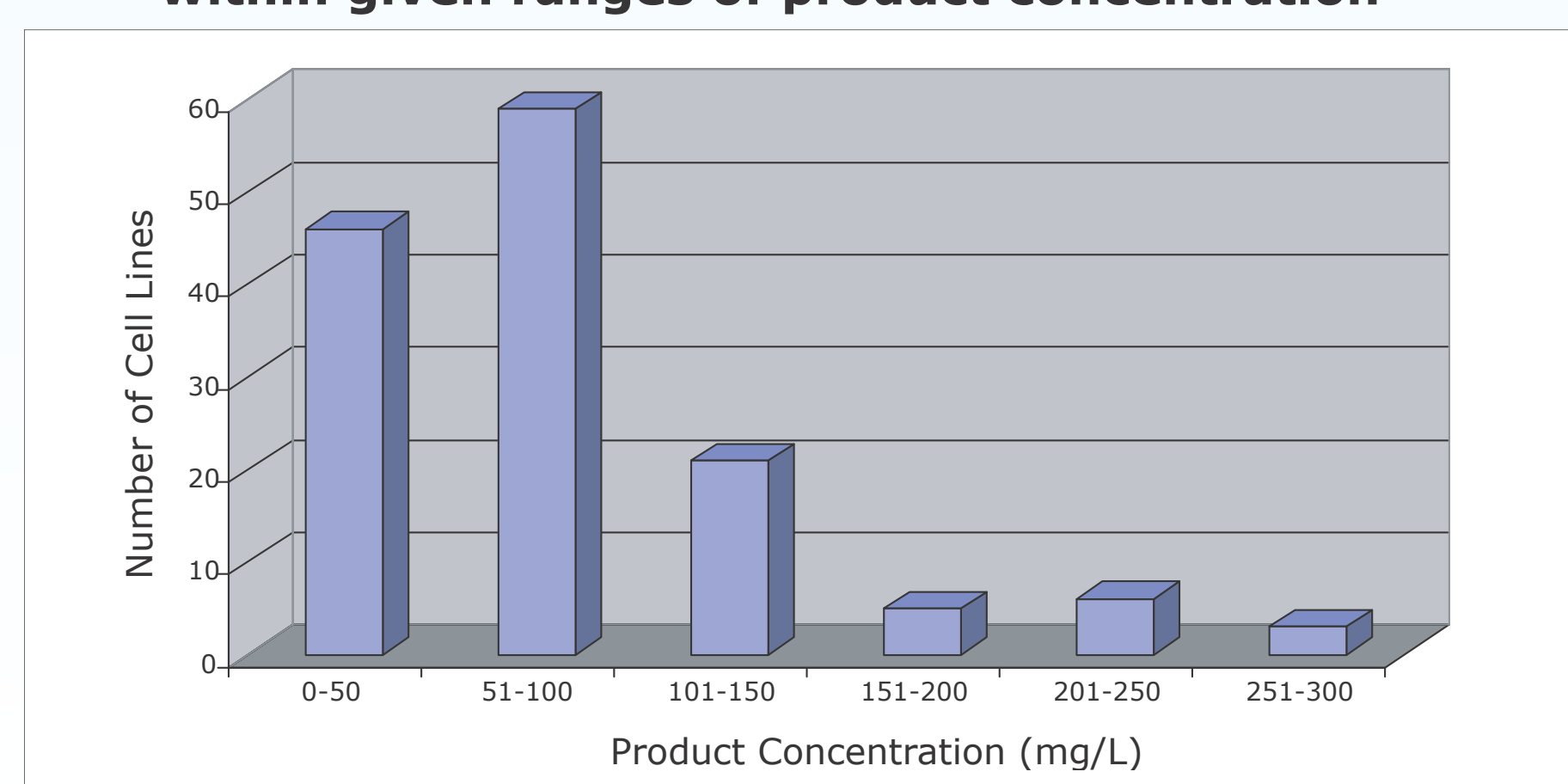


Figure 5. Antibody production data from candidate lead cell lines, in shake-flask culture operated in fed-batch mode.

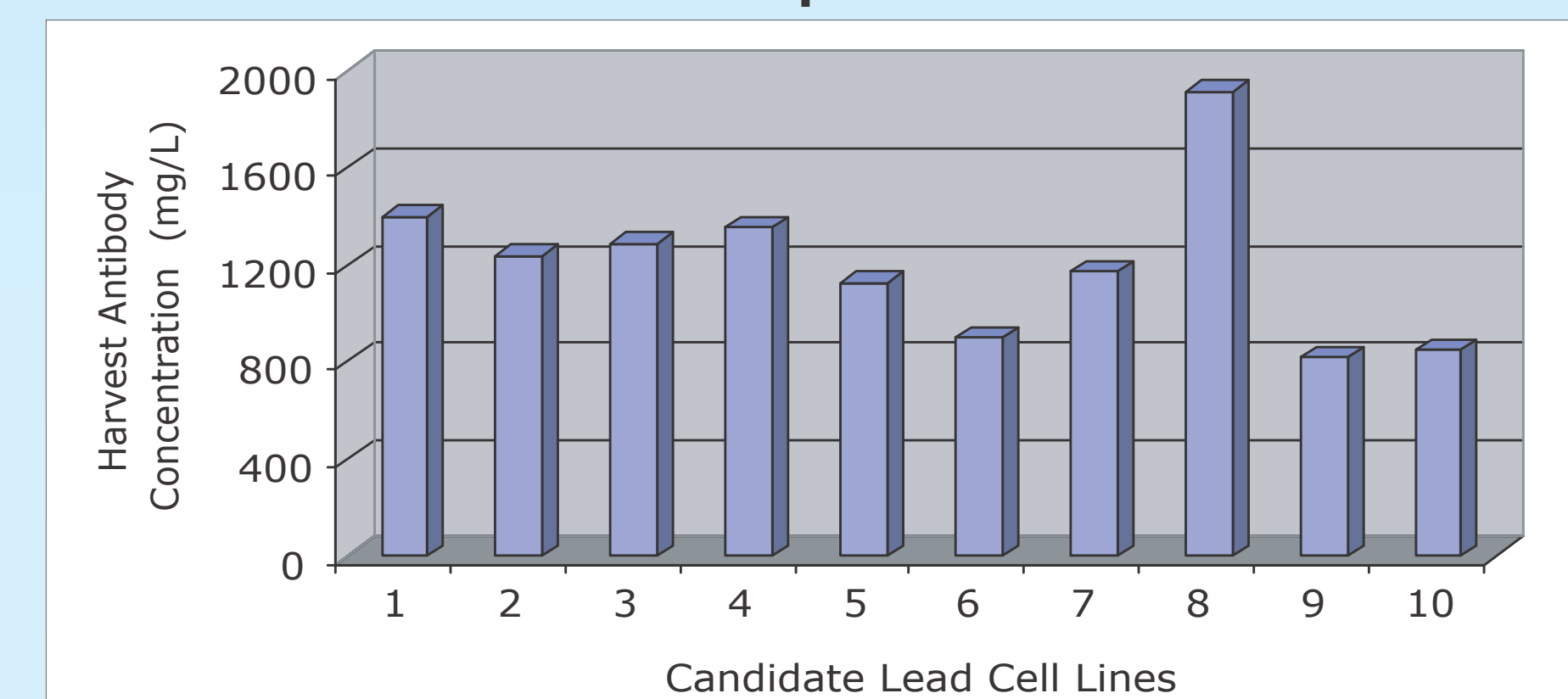
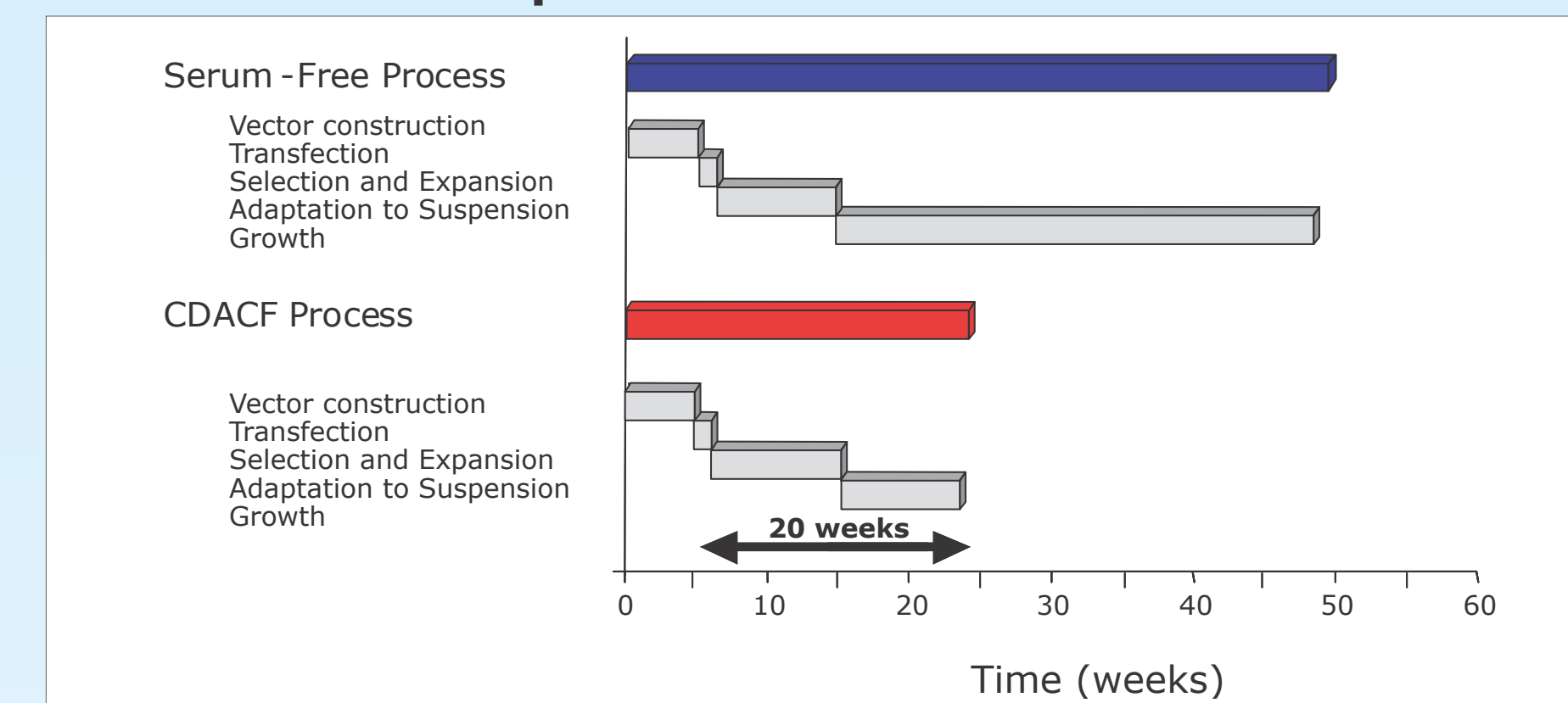


Figure 6. Timeline comparison



Results and Discussion

Vector Construction

- Rapid cloning scheme allows production of a single vector containing heavy chain, light chain and GS-coding sequences (Figure 2)

Transfection and Selection

- A range of electroporation conditions were assessed to optimise the creation of high expressing transfectants (Table 1). Condition EC3 generated the highest number of transfectants
- Use of MSX at 50 µM immediately following transfection resulted in lower total numbers of transfectants but a greater number of high expressors compared to 25 µM (Figure 3)

Cell Line Construction

- Initially 350 cell lines were assayed by assembly ELISA for antibody expression
- Highest 150 cell lines expanded and moved to 24 well plates
- 24 well plate productivity assessment data shows a range of high and low expression (Figure 4)
- 60 highest cell lines adapted to suspension growth
- All 60 cell lines assessed in batch culture (data not shown) and top 10 selected for fed-batch culture
- Fed-batch culture assessment reveals expression levels between 0.8 and 1.9 g/L (Figure 5)
- Overall timeline was reduced from almost 50 weeks to less than 20 weeks (Figure 6) by the adaptation of host cells to suspension culture and to CDACF media prior to transfection

Conclusions

- We have demonstrated the use of GS constant region vectors to rapidly generate IgG₂ expressing GS-CHO cell lines. Five of the ten candidate lead cell lines yielded greater than 1.2 g/L. Optimisation of transfection and selection conditions and the use of a pre-adapted host cell line drastically reduced the time required to less than 20 weeks.
- We present the GS-CHO expression system as an amplification-free commercially viable option for antibody expression.

References

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