

Optimisation of upstream processes for antibody production from mammalian cell culture

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Biopharmaceutical Proteins

- Sales of \$32bn in 2003 expected to grow to \$53bn by 2010 (Datamonitor)
- MAbs are the fastest growing category
- High dose requirement for antibodies leads to large volume demand (10's to 100's kg/year)
- Estimated 2004 protein demand > 2000kg (UBS)
- This is a driver for –
 - ◆ Increased capacity and reactor size
 - ◆ Improved technology, especially upstream, to increase cost efficiency (taking note of downstream implications)

Facilities



U.K. – R &D
+ small scale manufacture

200 litre airlift reactors
2000 litre airlift reactors
Disposable reactors



U.S.A. – Large Scale Manufacture

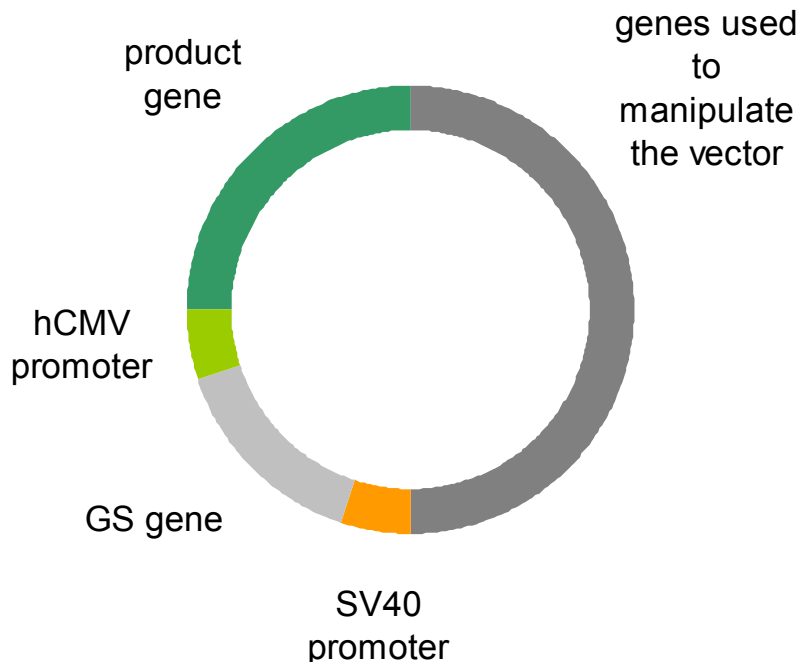
2000 and 5000 litre airlift reactors
2 x 1500 litre perfusion reactors
3 x 20000 litre stirred reactors

Improved Upstream Process Efficiency

- Parameters Targeted
 - ◆ Reactor throughput
 - ◆ Specific production rate
 - ◆ Growth rate
 - ◆ Maximum viable cell concentration
 - ◆ Maintenance of culture at high cell viability

- Routes to optimisation
 - ◆ Design of gene vector
 - ◆ Properties of host cell line
 - ◆ Cell line screening
 - ◆ Optimisation of culture medium, feeds etc

Glutamine Synthetase (GS) Gene Expression System



- Many mammalian cells require glutamine
- Use a gene vector which contains the product gene plus the GS gene allowing glutamine synthesis
- Only cells with GS gene (and hence product gene) survive
- Use weak promoter on GS gene and strong promoter on product gene – selects for rare integration into transcriptionally efficient sites in genome
- Allows rapid creation of highly productive cells without the need for further rounds of selection which can take many months

GS and Choice of Cell Line

- GS widely used for antibody expression in NSO cells (which lack endogenous GS)
- GS can also be used in CHO cells using Methionine Sulphoximine to inhibit endogenous GS
- High productivity (>1 g/l) achieved for non-amplified NSO and CHO in chemically defined medium

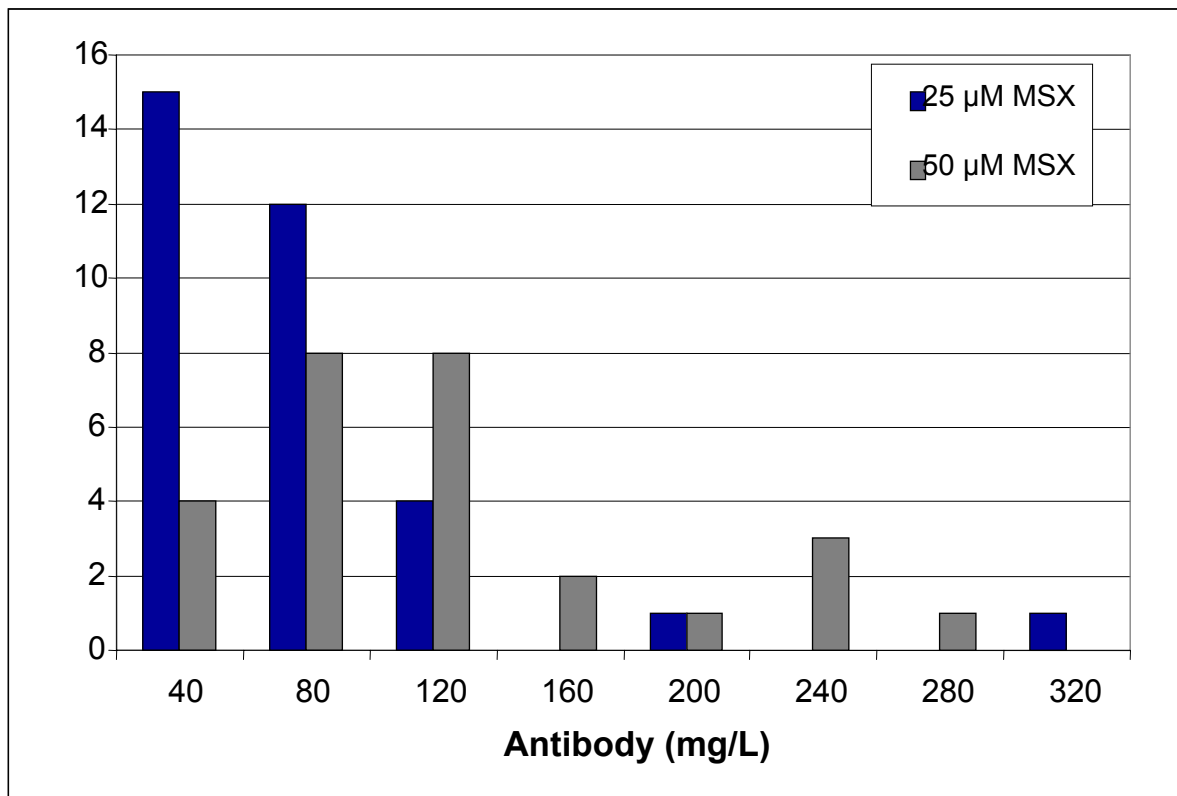
Improved cell lines

- Significant opportunities to create cell lines with improved growth and productivity characteristics
- Example ; suspension variant of CHO with improved growth characteristics

Cell Line Screening

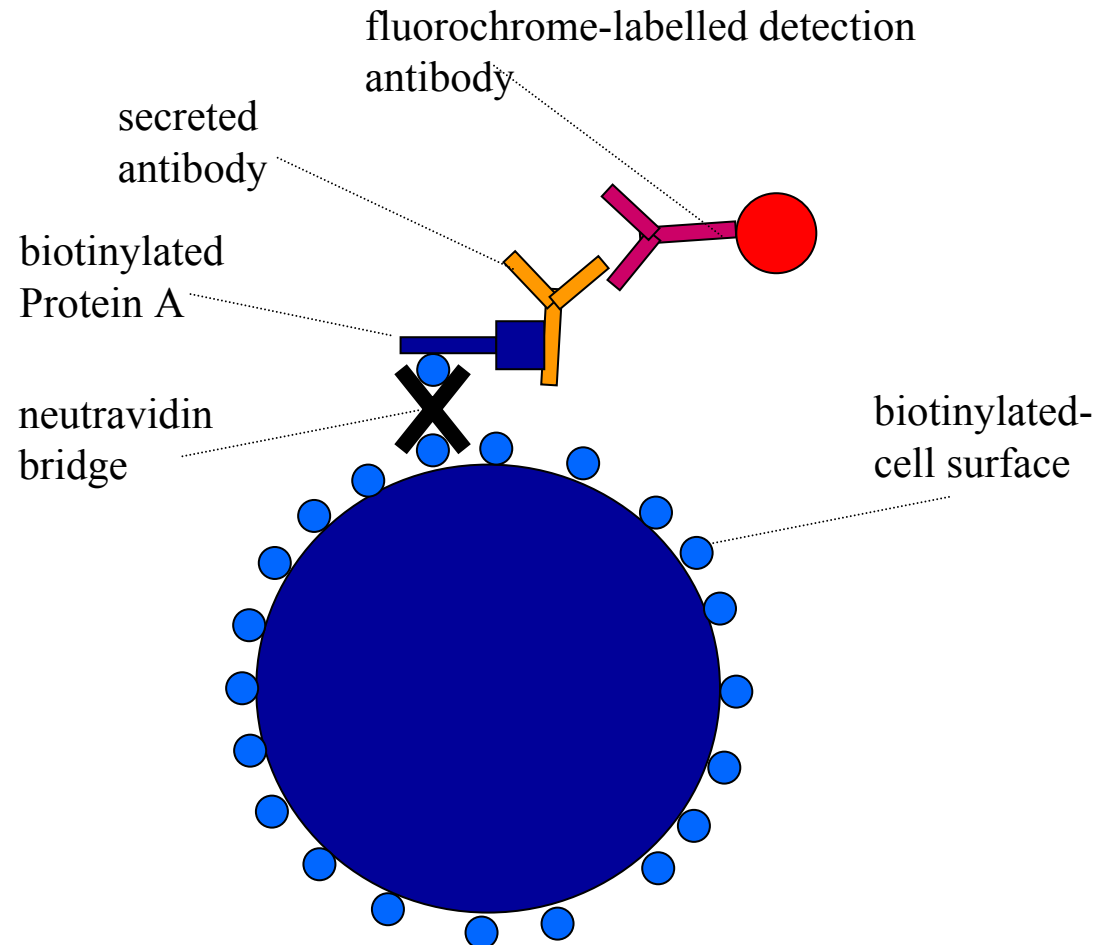
- Highly productive transfectants are rare even with a good selection system
- How can the hit rate for finding highly productive cell lines be increased
- Various approaches to improve screening process
 - Increase transfection efficiency
 - Improve stringency of selectable marker to eliminate low producers
 - High throughput methods (FACS + cell surface product capture)
 - Early screening needs to predict cell specific production capability AND growth characteristics at production scale

Influence of Selection Stringency for GS-CHO Cell Lines Making cB72.3 Antibody

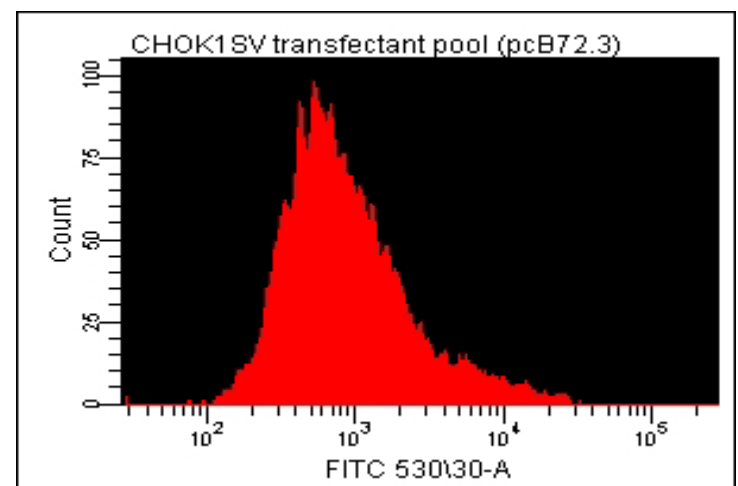
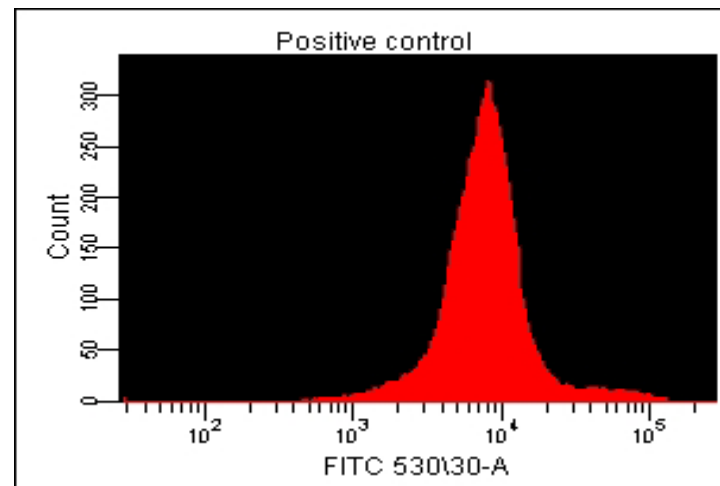
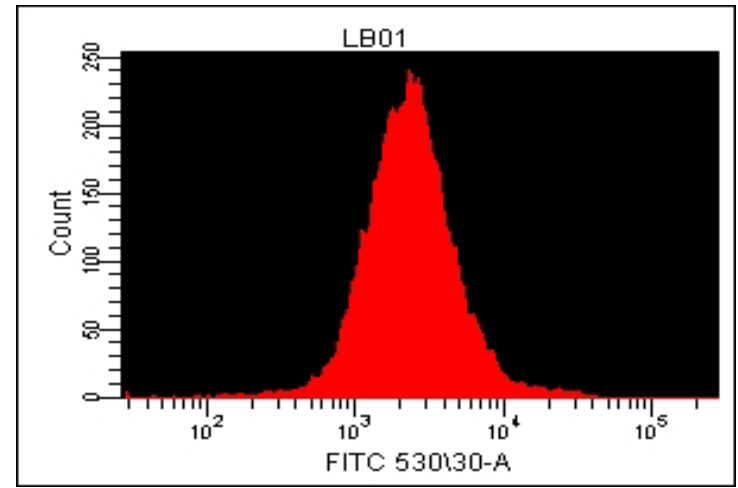
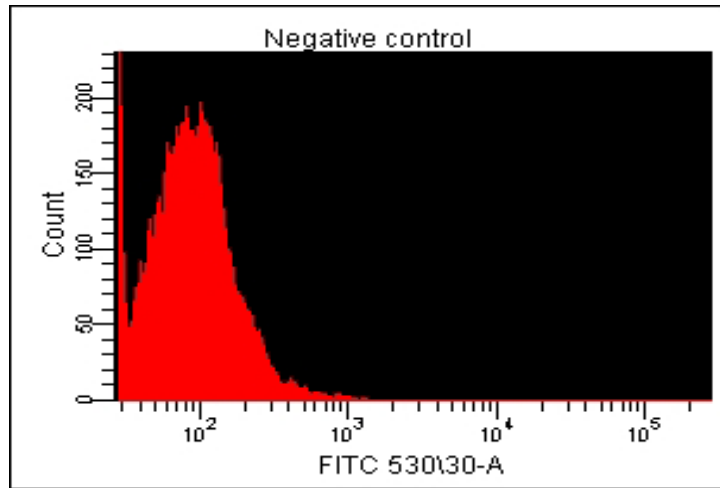


Cell lines have not been amplified.

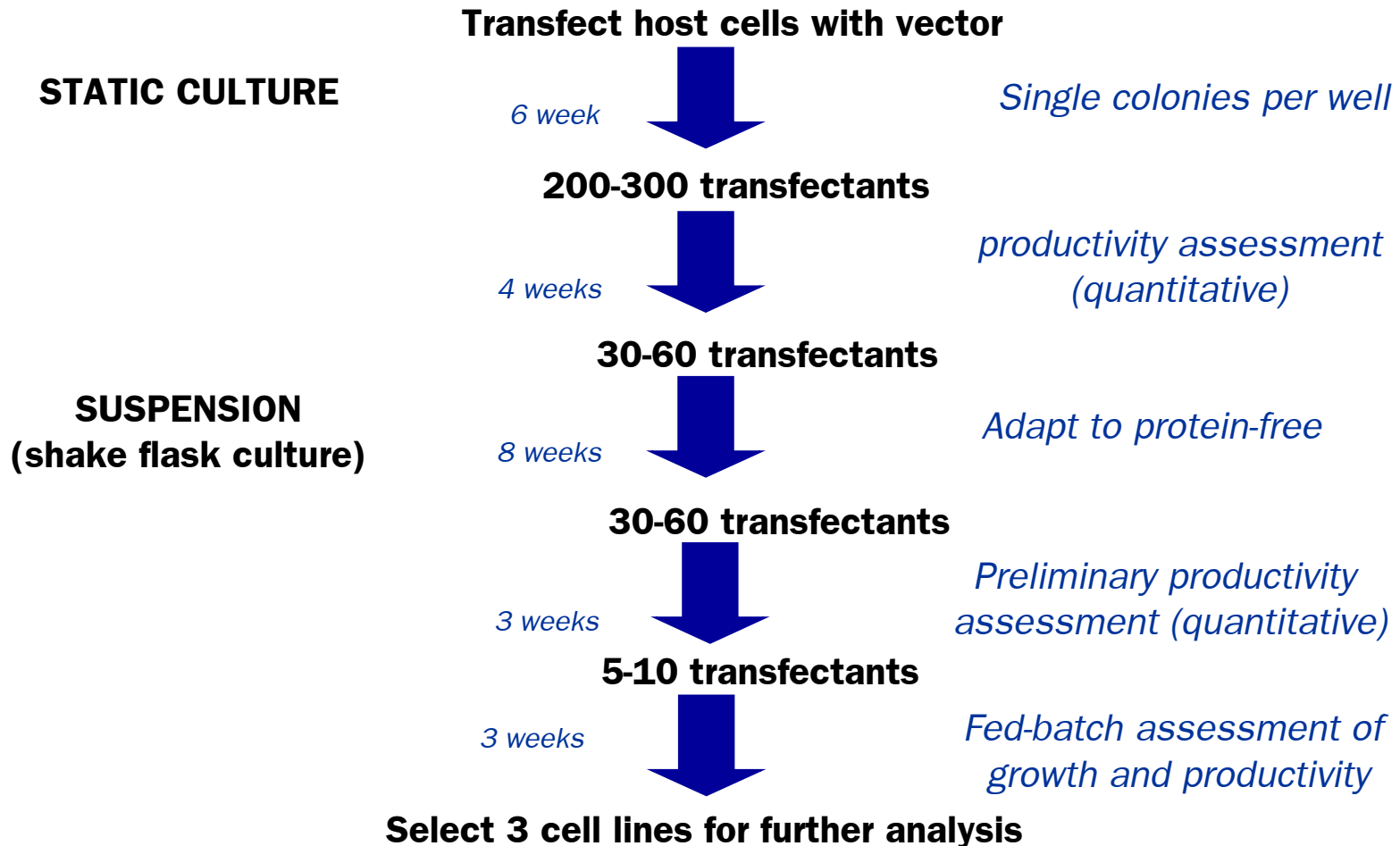
Affinity-matrix surface capture



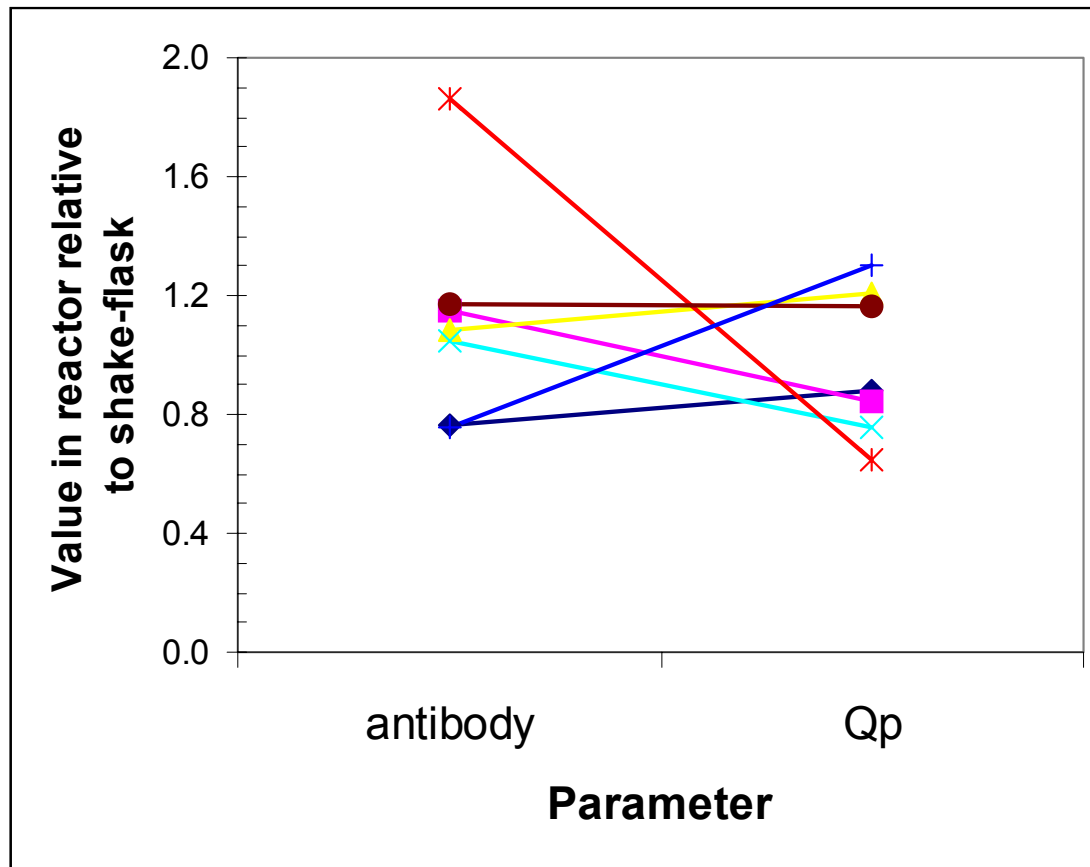
Flow cytometric analysis of AMSC-labelled GS-CHO cells



Selecting high producing cell lines



Prediction of bioreactor behaviour from shake-flask model



Improving the Fermentation Process

- Significant potential to improve processes
 - Physicochemical environment
 - Medium design and feeding strategies
 - Use of chemically defined media (reduces risk of introducing adventitious agents and assists process optimisation)

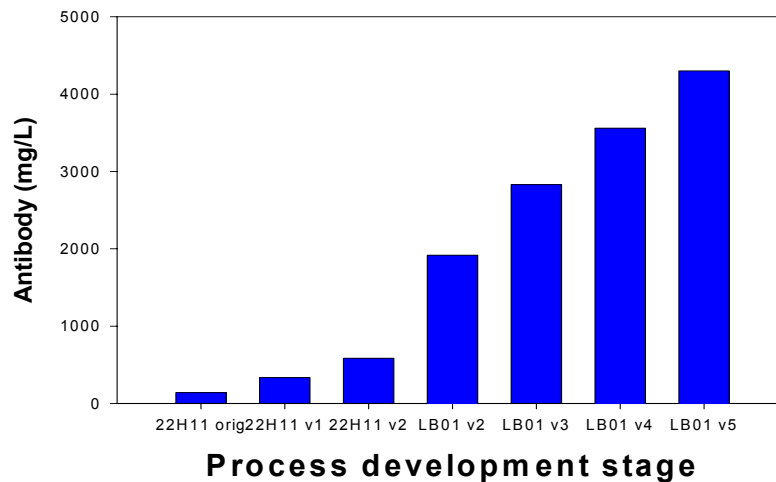
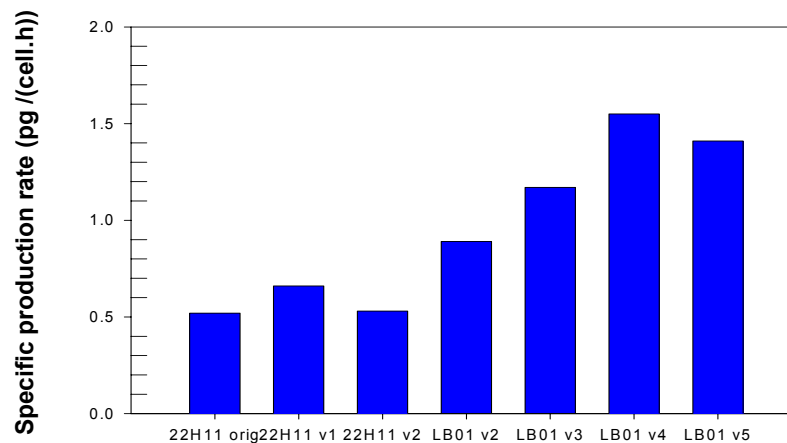
Optimisation of a GS-CHO Process

- Culture conditions for a GS-CHO making cB72.3 antibody were optimised
- Suspension variant of CHO-K1 isolated:
 - grows in chemically defined medium without need for adaptation (can take several months)
- Efficiency and stringency of transfection conditions increased to improve selection of highly productive clones
- Growth conditions further optimised

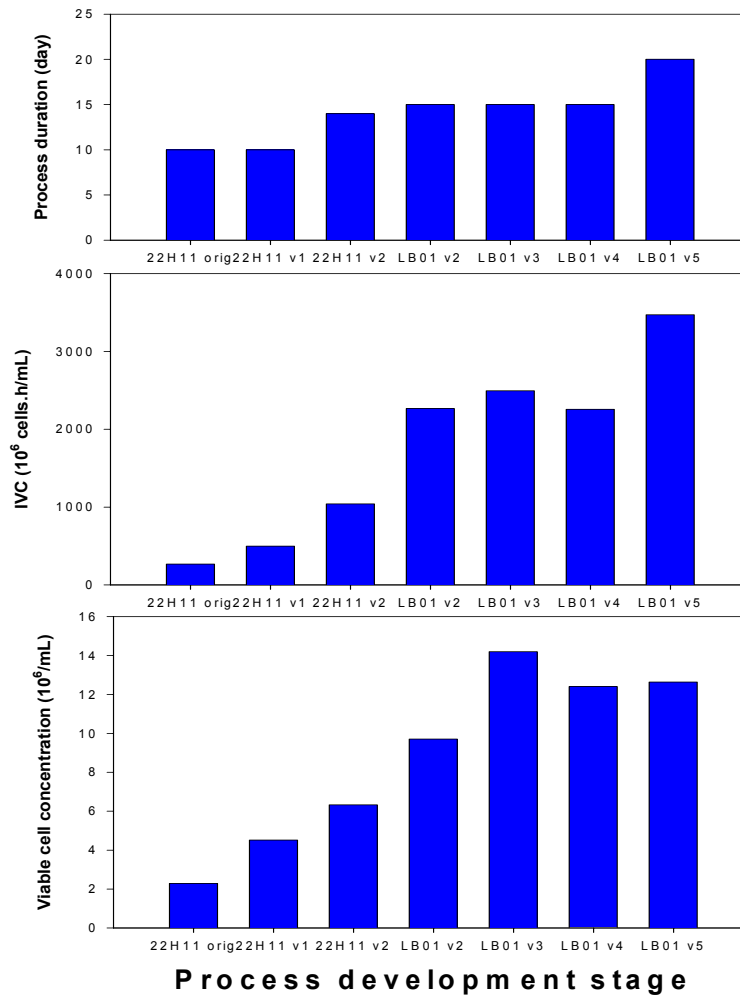
Process optimisation for a GS-CHO cell line

Process	Antibody (mg/L)	Fold increase
Original cell line	139	
Iteration 1	334	2
Iteration 2	585	4
New cell line	1917	14
Iteration 3	2829	20
Iteration 4	3560	26
Iteration 5	4301	31

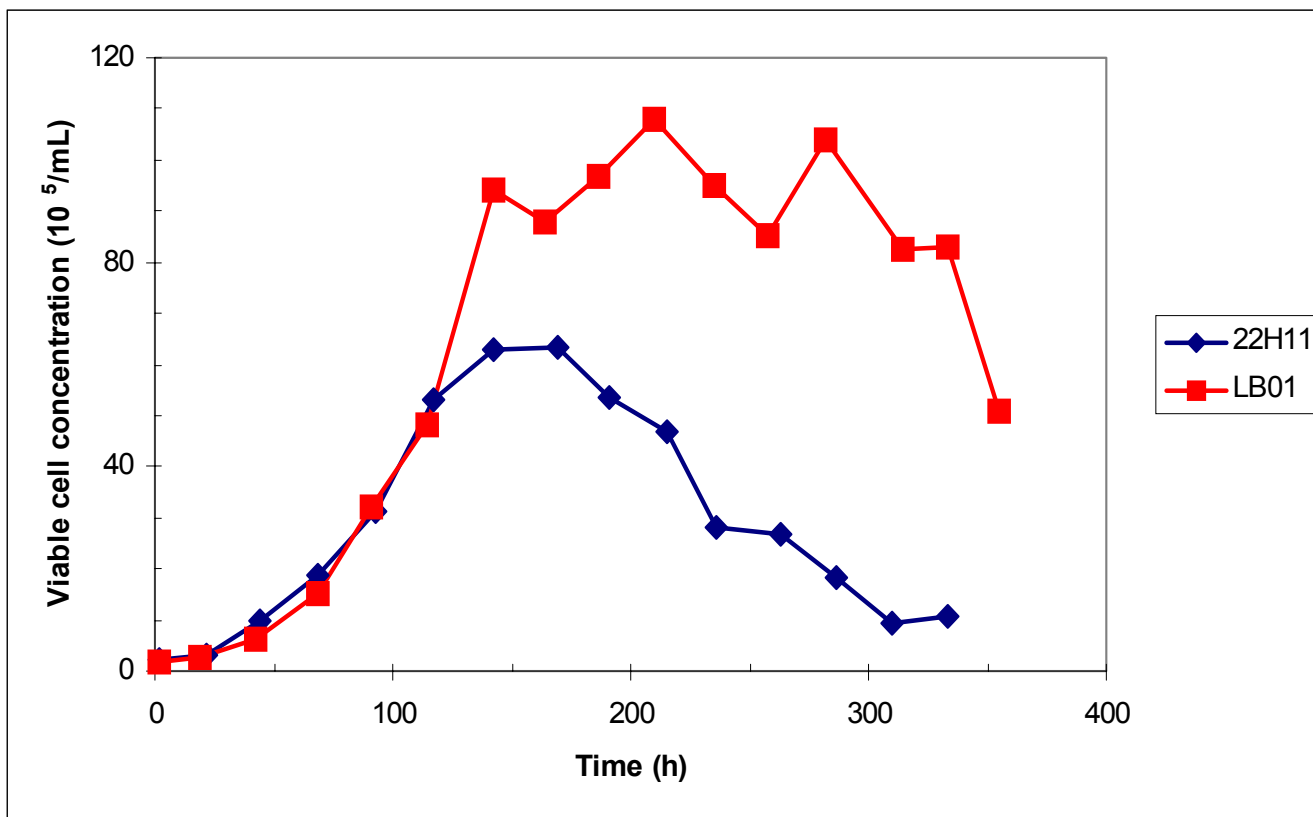
GS-CHO: antibody production



GS-CHO growth



Growth comparison: "old" vs. "new" GS-CHO cell lines



Summary

- Combination of efficient expression system (GS), effective screening and process optimisation gives high productivity for non-amplified NSO and CHO cell lines

Number of approaches to increasing hit rate for productive cell lines

- Screen large numbers
- Improved stringency of selection
- Select cell lines to fit manufacturing cell culture process
 - Scale-down models
 - Multiple selection criteria
- Significant potential for further yield enhancement based on process optimisation and improvements to host cell lines