

# Optimization of Cell Culture Processes for the Production of Therapeutic Monoclonal Antibodies

**Lonza**



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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

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- Sales of \$27bn in 2001 expected to grow to \$59bn 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

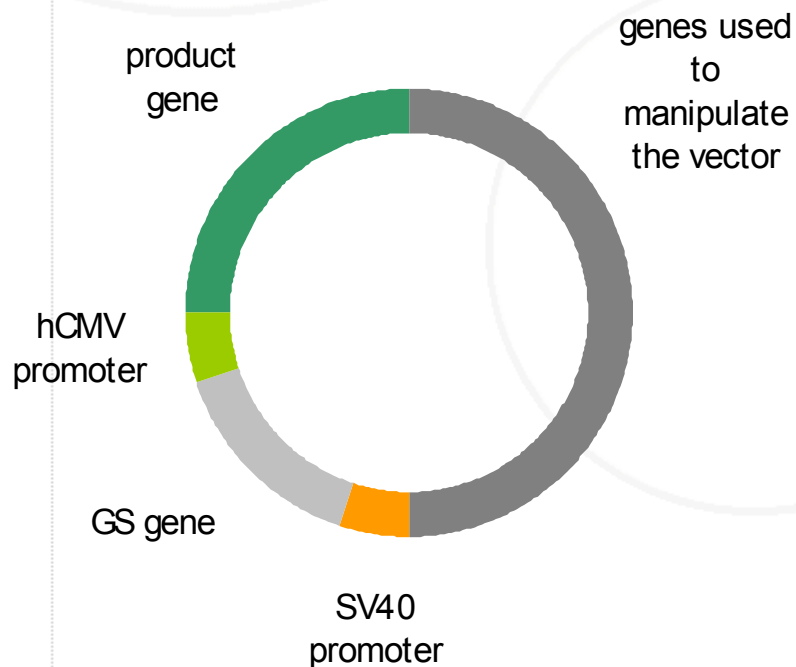
# Improved Upstream Process Efficiency

- 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
  - Optimisation of culture medium, feeds etc


# Design of Gene Vector to Maximize Transcription

- Strong promoter to drive expression of product gene(s)
- Amplification of gene copy number
- Vectors with elements that create genomic environment for high transcriptional activity (positional independence)
- Targeting of expression vector to transcriptionally active site in genome by homologous recombination

## Glutamine Synthetase (GS) Gene Expression System

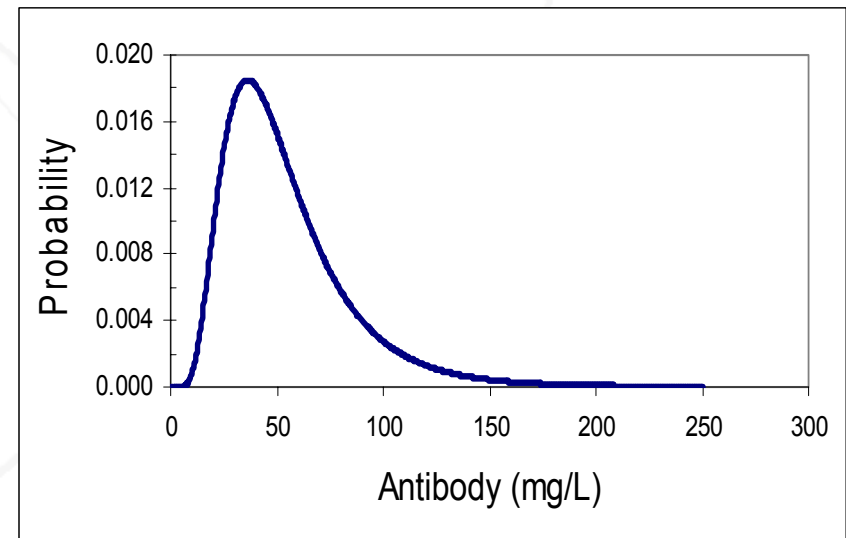


- Many mammalian cells require glutamine
- Use a gene vector which contains the product gene plus the GS gene allowing glutamine synthesis
- Only cells with GS gene (and hence product gene) survive
- Use weak promoter on GS gene and strong promoter on product gene – selects for rare integration into 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

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- A decorative horizontal bar is located below the title. It features a series of overlapping, semi-transparent circles in shades of blue, green, and yellow, creating a molecular or network-like pattern.
- 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

# Cell line selection: High producers are infrequent

- Probability distribution of antibody productivities for primary GS-CHO transfectants ( 24 well plates )
- 90% transfectants produce less than 90 mg/L
- 1.5% transfectants produce more than 150 mg/L





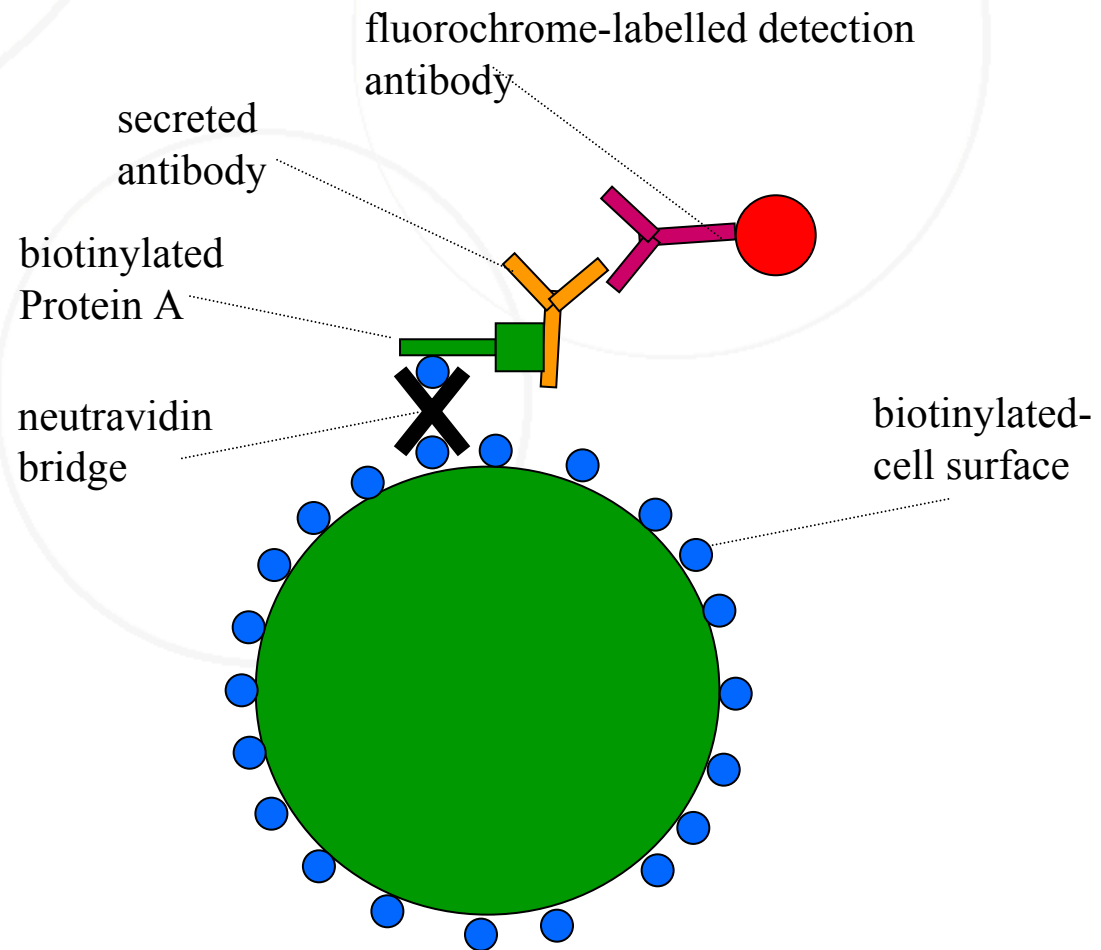
- Highly productive transfectants are rare even with a good selection system
- How can the hit rate for finding highly productive cell lines be increased
- Various approaches to improve screening process
  - Increase transfection efficiency
  - Improve stringency of selectable marker to eliminate low producers
  - High throughput methods (FACS + cell surface product capture)
  - Early screening will not necessarily indicate growth characteristics in manufacturing process

## Transfection and Selection Conditions for GS-CHO Cell Lines Expressing cB72.3 Antibody

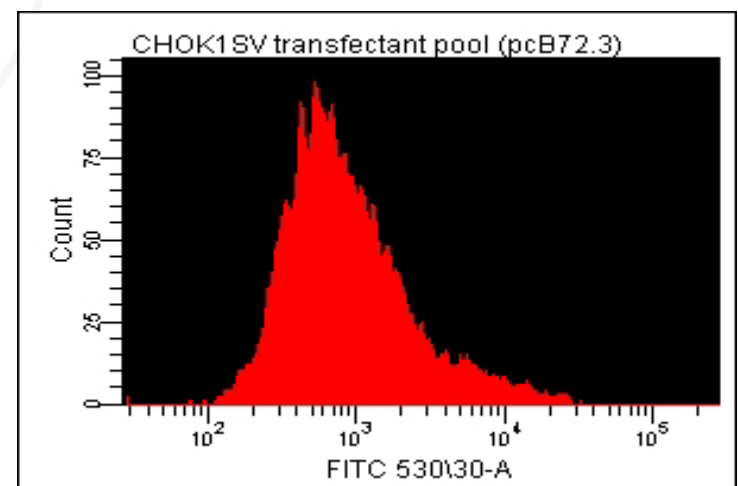
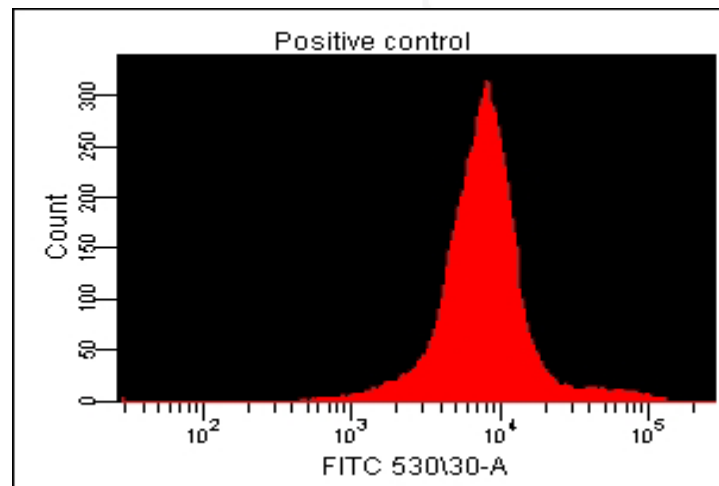
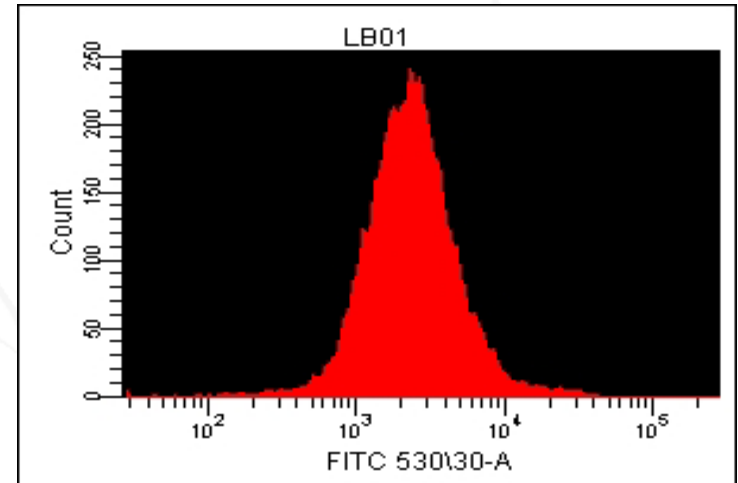
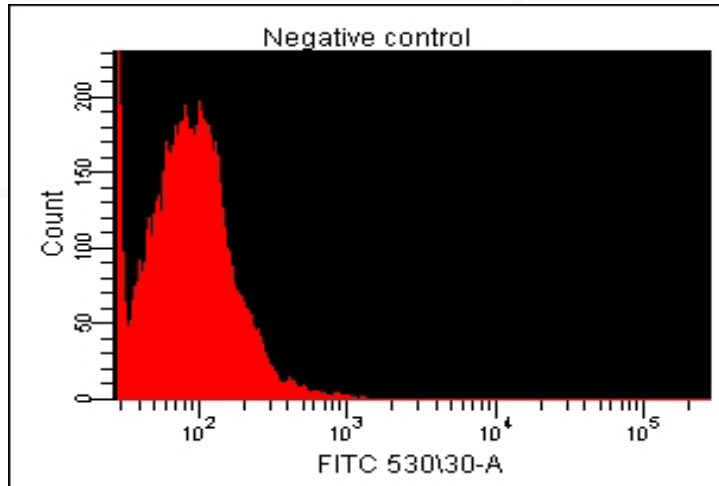
Electroporation condition	MSX ( $\mu\text{M}$ )	Stable transfectant numbers
1	25	68
	50	32
2	25	124
	50	57
3	25	197
	50	70

2.5 million cells electroporated for each condition

# Affinity-matrix surface capture




# Flow cytometric analysis of AMSC-labelled GS-CHO cells





- 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 )

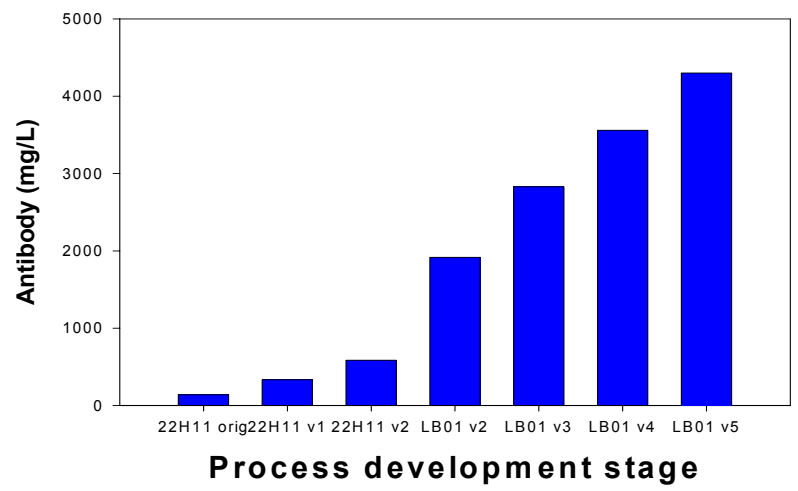
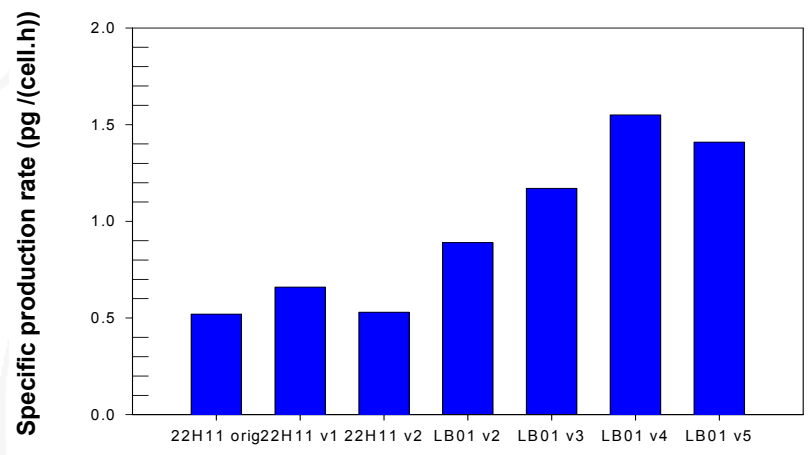
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- Culture conditions for a GS-CHO making cB72.3 antibody were optimised
  - Suspension variant of CHO-K1 isolated:
    - grows in chemically defined medium without need for adaptation (can take several months)
  - Efficiency and stringency of transfection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

# Process optimisation for a GS-CHO cell line



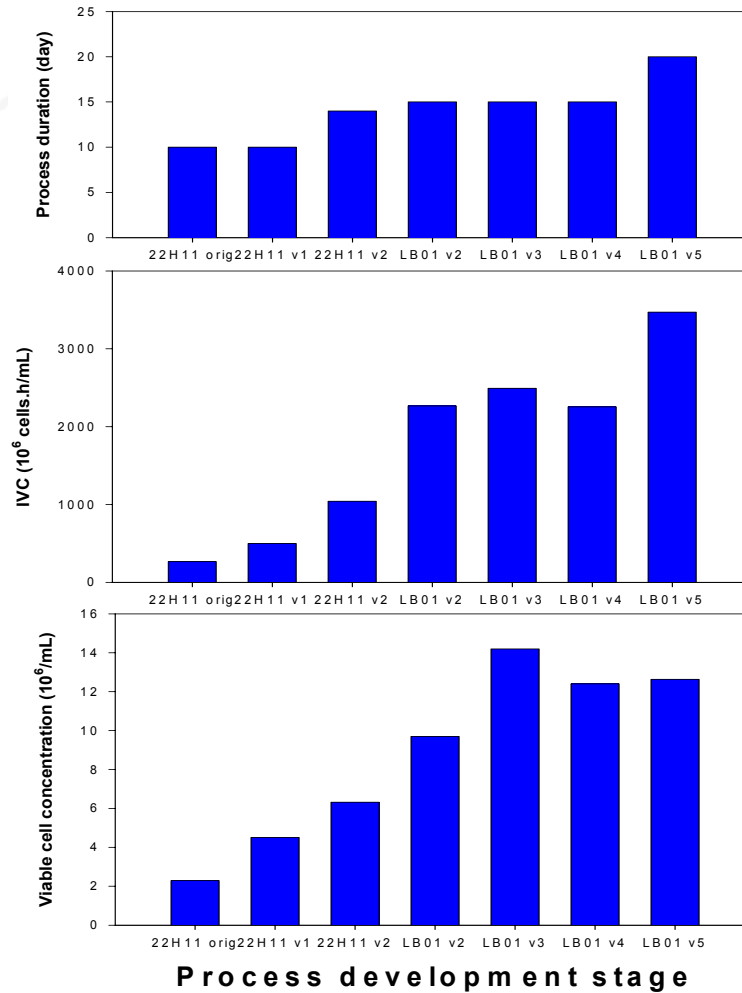
Process	Antibody (mg/L)	Fold increase
Original cell line	139	
Iteration 1	334	2
Iteration 2	585	4
New cell line	1917	14
Iteration 3	2829	20
Iteration 4	3560	26
Iteration 5	4301	31

# GS-CHO: antibody production



**Process development stage**

# GS-CHO growth





- Combination of efficient expression system (GS) and process optimisation gives high productivity for non-amplified NSO and CHO cell lines
- Significant potential for further yield enhancement based on process optimisation and improvements to host cell lines
- Improvements upstream have large impact on downstream operations