Efficient Generation of Highly Productive Cell lines
Utilising the Glutamine Synthetase (GS) Gene
Expression System for High Level Protein Expression in
CHO cells

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Creating highly productive cell lines
- GS gene expression system
- Alterations made to the GS system to improve expression
- Methodology for evaluation of the system

Speed in Development
- Cell line creation
Creation of highly productive cell lines depends on
- Gene vectors
- Translation and secretion
- Improved host cell lines (likely to be more important in future). Also relevant to obtaining desired product characteristics
- Cell line selection

Optimisation of fermentation process
- Design of media and feeding strategies
- Optimisation of physicochemical conditions

For antibodies, concentrations of >1 g/L standard and for the future 10 g/L probably achievable

Caveat – no increase in overall timeline, ideally a reduction.
Glutamine synthetase (GS) gene expression system

- Gene of interest driven by **strong promoter**
  - (hCMV-MIE)

- GS is inhibited by methionine sulfoximine (MSX)

- GS cDNA driven by **weak promoter**
  - SV40E

- Increase **selection stringency**
  - Weak promoter on GS gene - selects for rare integration into transcriptionally efficient sites in genome
Alterations to GS Vectors to Test For Antibody Production

- Is the transcription unit optimal?
  - HC - LC or LC – HC ?

- Do balanced levels of LC and HC lead to increased antibody production?
  - Transcription blocker

- Can stronger promoters be used?
  - Murine cytomegalovirus (mCMV) promoter

- Can sequence elements provide greater promoter accessibility?
  - Matrix attachment regions (MARs)

- Can gene optimisation result in elevated expression?
Evaluating Alterations to GS Vectors with Lonza’s model chimeric IgG₄ antibody

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**STATIC CULTURE**

- Transfect host cells with vector
  - 3 - 4 weeks
  - 100 transfectants
  - Identify single colonies per well

- Transfer to 24 well plate
  - 2 weeks
  - 100 transfectants

- Productivity assessment (quantitative)
  - 100 data points

- Compare against control
Alterations to GS Vectors to Test For Antibody Production

- Is the transcription unit optimal?
  - Heavy chain is thought to be toxic to the host cell, therefore the light chain transcription unit was placed first.
  - However, free light chain can be secreted into the media.
  - What happens if the heavy chain is placed first?

- Do balanced levels of light and heavy chain lead to increased antibody production?
  - The transcription blocker described by Proudfoot (from Clontech vector, pSEAP2) was used.
  - It consists of an efficient poly A signal and strong transcriptional terminator.
Testing Alterations to GS Vectors (1)
Alterations to GS Vectors to Test For Antibody Production

- Is the transcription unit optimal?
  - HC - LC or LC – HC ?

- Do balanced levels of LC and HC lead to increased antibody production?
  - Transcription blocker

- Can stronger promoters be used?
  - Murine cytomegalovirus (mCMV) promoter

- Can sequence elements provide greater promoter accessibility?
  - Matrix attachment regions (MARs)

- Can gene optimisation result in elevated expression?
Testing Alterations to GS Vectors (2)
Alterations to GS Vectors to Test For Antibody Production

- Is the transcription unit optimal?
  - HC - LC or LC – HC?

- Do balanced levels of LC and HC lead to increased antibody production?
  - Transcription blocker

- Can stronger promoters be used?
  - Murine cytomegalovirus (mCMV) promoter

- Can sequence elements provide greater promoter accessibility?
  - Chicken lysozyme Matrix attachment region (MAR)

- Can gene optimisation result in elevated expression?
Testing Alterations to GS Vectors (3)
- Incorporation of MAR elements
Testing Alterations to GS Vectors (3)
- Incorporation of MAR elements

![Box plot diagram showing antibody levels across different conditions.](slide13.png)
Alterations to GS Vectors to Test For Antibody Production

- Is the transcription unit optimal?
  - HC - LC or LC – HC?

- Do balanced levels of LC and HC lead to increased antibody production?
  - Transcription blocker

- Can stronger promoters be used?
  - Murine cytomegalovirus (mCMV) promoter

- Can sequence elements provide greater promoter accessibility?
  - Matrix attachment regions (MARs)

- Can gene optimisation result in elevated expression?
Successful Improvement
- The Effect of Codon Optimisation on Production
Vector Alterations

– In Summary

- The GS vectors are probably optimal
  - The existing vector has been used as part of a process that has led to fermentation yields of 5.5 g/L
  - Modifications to the vector thus far have had no observable positive effect or in the worst cases have been detrimental

- Translation, Assembly and Secretion are the next rate-limiting steps
  - The data from the gene optimisation experiment presented here suggests that further gains can be made at steps down-stream of transcription.
Speed in Development

- **Cell line creation often a rate-limiting step** – need to complete as rapidly as possible, ideally without compromising productivity or clonality criteria.

- **Use of platform technology** – culture media, feeds, growth conditions, purification and assays etc.

- **Predictive scale up systems** – challenge is to predict manufacturing behaviour of cell lines at very early stage and select clones with e.g. appropriate growth characteristics.
Traditional Development Process (Hypothetical)

- Cell Line Development
- Purification development & evaluation of virus reduction
- Cell bank preparation & testing
- Fermentation process development
- Analytical development
- cGMP manufacture

Year 1: Q1 to Q4
Year 2: Q5 to Q10
Approximate Timelines for Constructing and Selecting High-yielding Clonal Cell Lines

**Traditional**
- Transfection & selection
- Amplification & selection (1 round)
- Suspension evaluation (low serum)
- Limiting diln. cloning (2 rounds)
- Suspension evaluation (serum-free)
- Select lead cell lines

**Non-amplified (e.g. GS-CHO)**
- Transfection & selection
- Suspension evaluation (protein-free)
- Capillary-aided cell cloning (1 round)
- Suspension evaluation (protein-free)
- Select lead cell lines
Speed in Development

- Stable Cell line creation – how can timelines for cell line creation be reduced?
  - Avoid amplification
  - Avoid multiple cloning steps
  - Use of host cells for genetic manipulation that have been preselected to grow under manufacturing conditions (e.g. suspension culture in chemically defined medium)

- Alternatively, transient expression or un-clonal cell line can be used for early development studies
Approximate Timelines for Constructing and Selecting High-yielding Clonal Cell Lines

- **Traditional**
  - Transfection & selection
  - Amplification & selection (1 round)
  - Suspension evaluation (low serum)
  - Limiting dil. cloning (2 rounds)
  - Suspension evaluation (serum-free)
  - Select lead cell lines
  - Approximate timeline: ~10 wks for 1 round

- **Non-amplified (e.g. GS-CHO)**
  - Transfection & selection
  - Suspension evaluation (protein-free)
  - Capillary-aided cell cloning (1 round)
  - Suspension evaluation (protein-free)
  - Select lead cell lines

**Graphical representation:**

- X-axis: Weeks
- Y-axis: Processes
- Timeline comparison between Traditional and Non-amplified methods.
Eliminating the Need for Amplification

- High stringency selection for integration events into chromosomal sites of highly transcriptional activity.

- High throughput screening for (rare) highly productive variants
Stringent Selection with Elevated MSX

![Box plot showing antibody concentration (mg/L) under different MSX concentrations (25 μM and 50 μM).](image)
Speed in Development

- Cell line creation – how can timelines for cell line creation be reduced?
  - Avoid amplification
  - Avoid multiple cloning steps
  - Use of host cells for genetic manipulation that have been preselected to grow under manufacturing conditions (e.g. suspension culture in chemically defined medium)
Approximate Timelines for Constructing and Selecting High-yielding Clonal Cell Lines

- Traditional
  - Transfection & selection
  - Amplification & selection (1 round)
  - Suspension evaluation (low serum)
  - Limiting diln. cloning (2 rounds)
  - Suspension evaluation (serum-free)
  - Select lead cell lines
  - ~28 wks for 2 rounds

- Non-amplified (e.g. GS-CHO)
  - Transfection & selection
  - Suspension evaluation (protein-free)
  - Capillary-aided cell cloning (1 round)
  - Suspension evaluation (protein-free)
  - Select lead cell lines
  - ~9 wks
Cloning of Cell Lines

- Limiting dilution cloning commonly used and requires two or more rounds to achieve a high probability of clonality
- Single step method developed – capillary aided cell cloning
- Based on method described by Clarke and Spier (1980) Archives of Virology 63, 1-9
- Small droplets (1 μl) of culture drawn into capillary and deposited in microwell plates
- Visual confirmation of drops containing single cell
- Statistical analysis to establish probability of monoclonality
Speed in Development

- Cell line creation – how can timelines be reduced?
  - Avoid amplification
  - Avoid multiple cloning steps
  - Use of host cells for genetic manipulation that have been preselected to grow under manufacturing conditions (e.g. suspension culture in chemically defined medium)
Approximate Timelines for Constructing and Selecting High-yielding Clonal Cell Lines

Traditional

- Transfection & selection
- Amplification & selection (1 round)
- Suspension evaluation (low serum)
- Limiting diln. cloning (2 rounds)
- Suspension evaluation (serum-free)
- Select lead cell lines

- ~35 wks

Non-amplified (e.g. GS-CHO)

- Transfection & selection
- Suspension evaluation (protein-free)
- Capillary-aided cell cloning (1 round)
- Suspension evaluation (protein-free)
- Select lead cell lines

- ~9 wks
CHO Suspension Variant

- Isolated variant of CHOK1 (CHOK1SV) capable of growth in suspension in chemically defined medium without need for adaptation period

- Used as host with GS expression technology

- High producing cell lines created (grams / litre)
Growth comparison: “old” vs. “new” GS-CHO cell lines (based on host variant adapted to suspension growth in chemically defined medium)

![Growth Comparison Graph]

- **Time (h)**: 0, 40, 80, 120
- **Viable cell concentration (10^5/mL)**: 0, 100, 200, 300, 400

Legend:
- **22H11**
- **LB01**
Speed in Development

- Cell line creation – how can timelines be reduced?
  - Avoid amplification
  - Avoid multiple cloning steps
  - Use of host cells for genetic manipulation that have been preselected to grow under manufacturing conditions (e.g. suspension culture in chemically defined medium)
  - How can timelines be further reduced
Possible Solutions

- **How can programme length be shortened?**
  - Duration can be reduced by moving straight from the transfection stage to the cloning stage, removing intermediate screening stage.
The pool of cell lines can be rapidly enriched for those with desired characteristics prior to cloning.

- Glutamine Synthetase (GS) Expression System indirectly enriches for high producers at transfection stage by use of the GS inhibitor MSX (as discussed earlier).

- Use second, rapid, method (FACS) to enrich directly for high producers between transfection and cloning stages.
Affinity-Matrix Surface Capture (AMSC)

- Neutravidin bridge
- Biotinylated Protein A
- Secreted antibody
- Fluorochrome-labelled detection antibody
- Biotinylated-cell surface

EP1415158 A, Lonza
AMSC

- Does high fluorescence correlate with high productivity?

- Do cell lines generated by both the AMSC and conventional methods produce similar antibody concentrations in a model of the production process?
FACS Enrichment

- Cell line construction and selection methods were run in parallel using a conventional protocol and a flow cytometric protocol.

- Cell line CHOK1SV transfected with GS expression vector encoding HC and LC genes for a human IgG.
Fluorescent signals for antibody-producing GS-CHO cells were substantially higher than for non-producing cells.
Conventional vs. AMSC-Cloning: Fed-Batch, Suspension Culture

- **Individual Value Plot Antibody Concentrations for Fed-batch Cultures**
  - Y-axis: Antibody (mg/L)
  - X-axis: Cultures (Conventional, AMSC)

- **Interval Plot of Antibody Concentration Data for Fed-batch Cultures**
  - 95% CI for the Mean

- **Individual Value Plot of Specific Production Rate in Fed-batch Culture**
  - Y-axis: Up (gpr (g/cell/hr))
  - X-axis: Cultures (Conventional, AMSC)

- **Interval Plot of Specific Production Rate for Fed-batch Culture**
  - 95% CI for the Mean
FACS Enrichment

- Cytometric method enriched cell populations for high producers

- For key selection stage, evaluation in fed-batch suspension culture, mean antibody concentration and mean Qp were the same
Impact of Using AMSC-Cloning Approach

- Both methods generated a panel of cell lines having similar productivities
- Enrichment of transfectant pool before cloning has shortened screening step by 9 weeks and potential for further improvement if FACS is qualified for single cell sorting
Summary

- Significant progress has been made in two key aspects of upstream mammalian cell process development
  - Further increases in yields are obtainable in the initial stages of the process through optimising translation and perhaps assembly and secretion also.
  - Reduced timelines from approximately 100 to 30 wks in cell line development – to lead cell line (including AMSC-cloning)

- There are important opportunities for further improvement in many areas including productivity (cells and process), speed, design of molecule for manufacture and improved design of screens for cell lines
Acknowledgements

- Members of Lonza Cell Culture Process Development and Assay Development Groups