

Cell Line Development and
Engineering

Lonza

Improving Cell Line Selection Strategies

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Scope of Talk

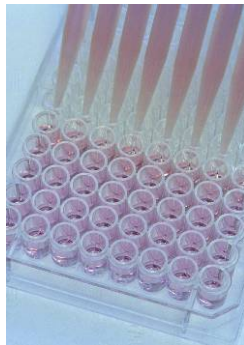
- Cell line construction challenge
- Current cell line construction strategy
- Improving selection strategies

Cell Line Construction Challenge

Cell Line Construction Challenge

■ Issue:

- Transfection results in heterogeneous population
- Measurements made early in construction of a cell line may not reflect how it behaves in the final production process



■ Challenge:

- Selecting a cell line with the desired growth and productivity characteristics

Cell Line Construction Challenge

- A possible strategy
 - Take large numbers (possibly thousands) of cell lines through to the final production process
 - Scientifically sound
 - Lengthy and resource intensive

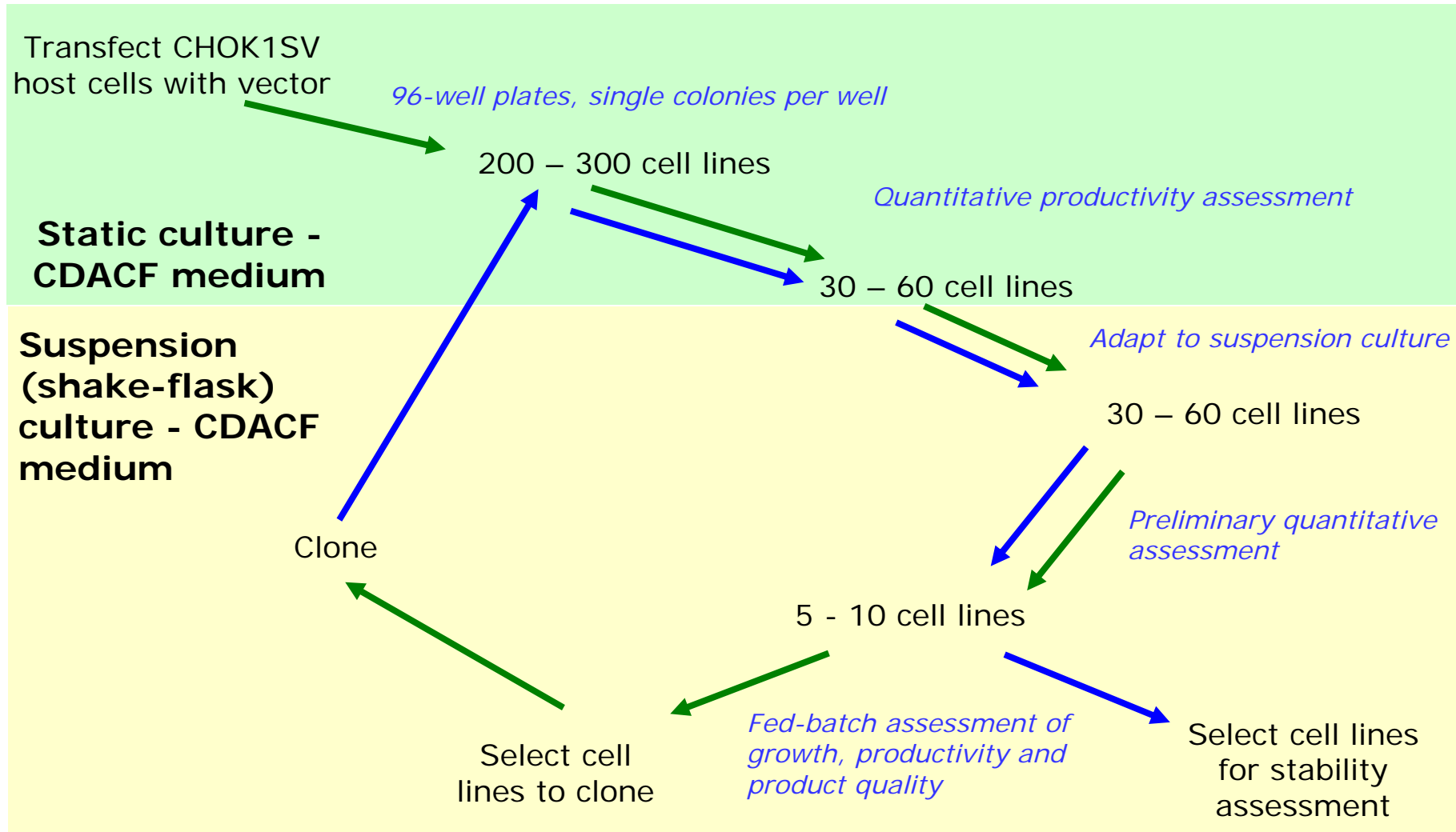
- Developing other strategies
 - Designed with reference to the final production process
 - Requires prediction of manufacturing behaviour of cell lines at very early stage
 - Economic
 - Compatible with resources

Current Cell Line Construction Strategy

Current Cell Line Construction Strategy

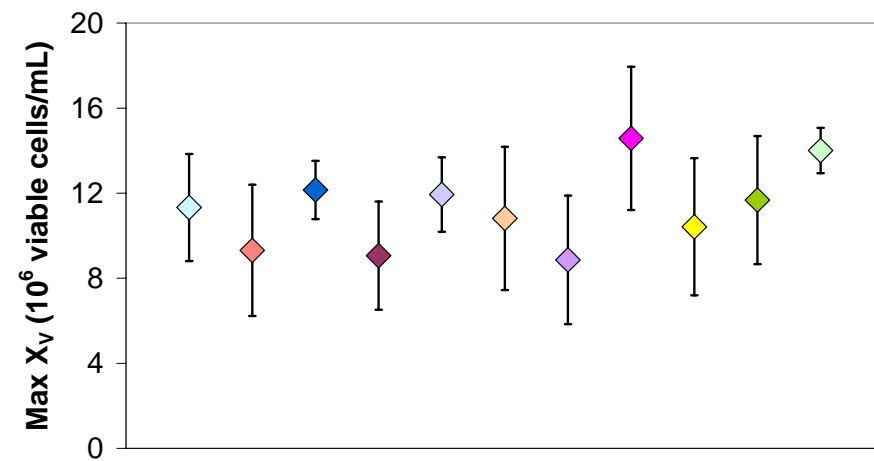
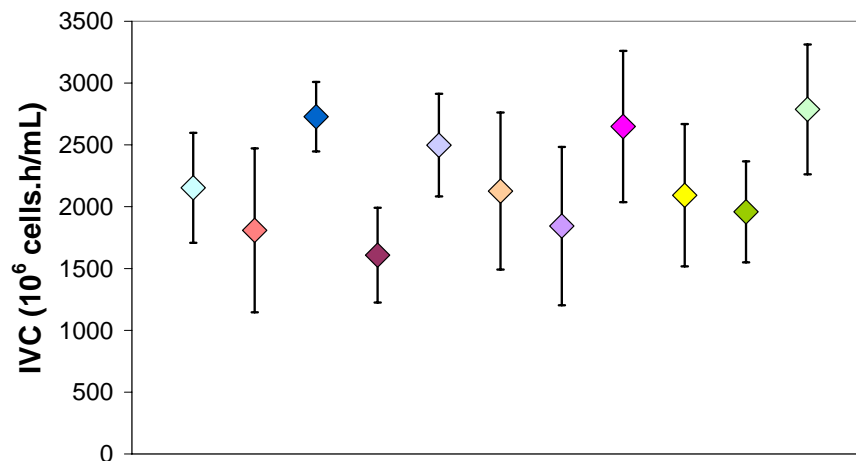
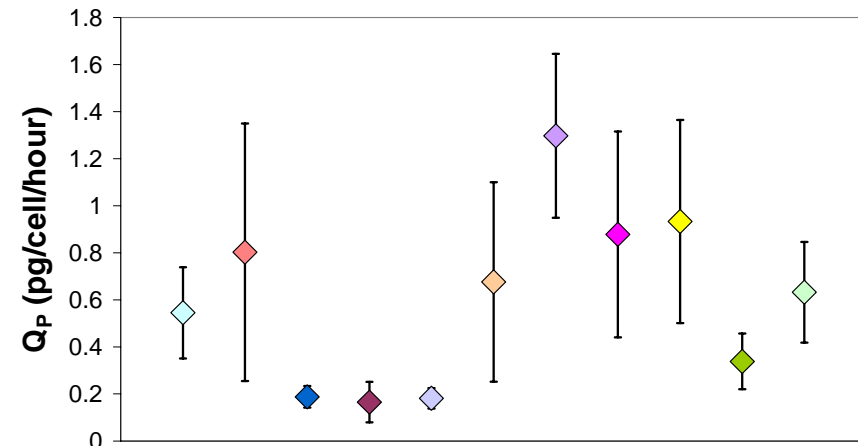
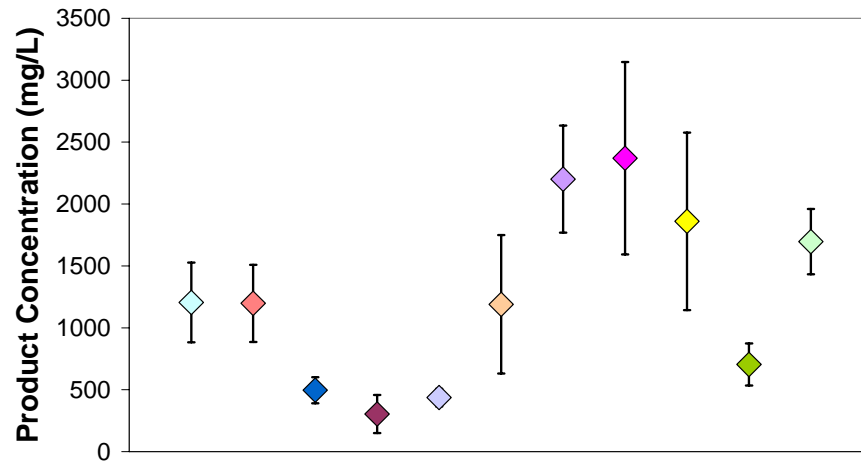
- High stringency selection
 - Weak promoter on GS; strong promoter on gene of interest
 - Selects for integration into transcriptionally efficient sites in genome
 - Inhibition of GS by methionine sulphoximine (MSX) used to further increase stringency of selection

Current GS-CHO Cell Line Construction Method



Variation in Growth and Productivity

Characteristics Seen in Fed-Batch Shake-Flask Screen of GS-CHOs Making Different Antibodies



Antibody

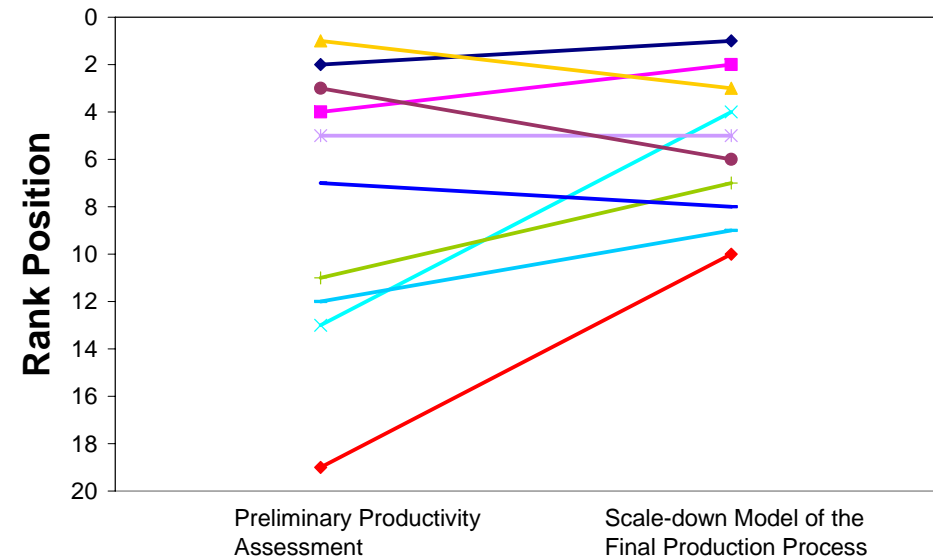
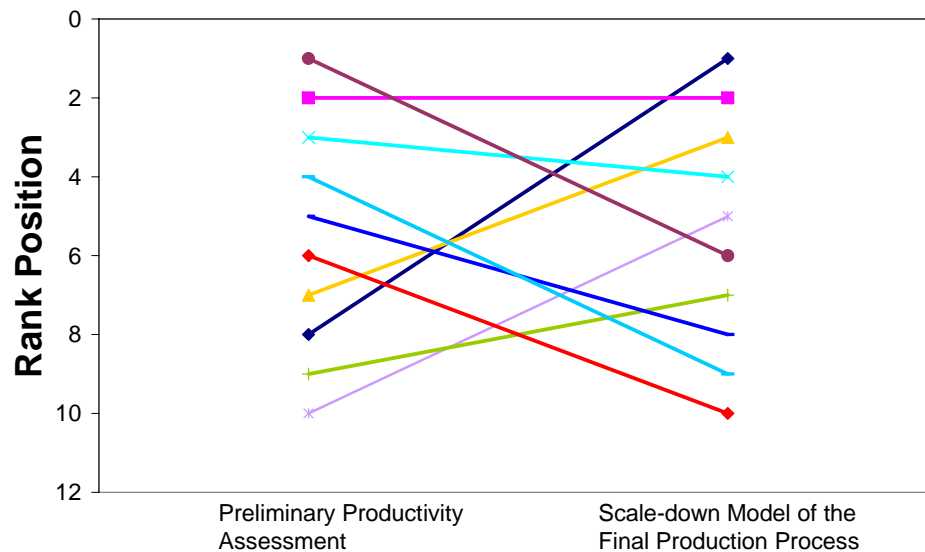
Antibody

Antibody

Antibody

Batch and Fed-Batch Culture: Comparison of Ranking Positions

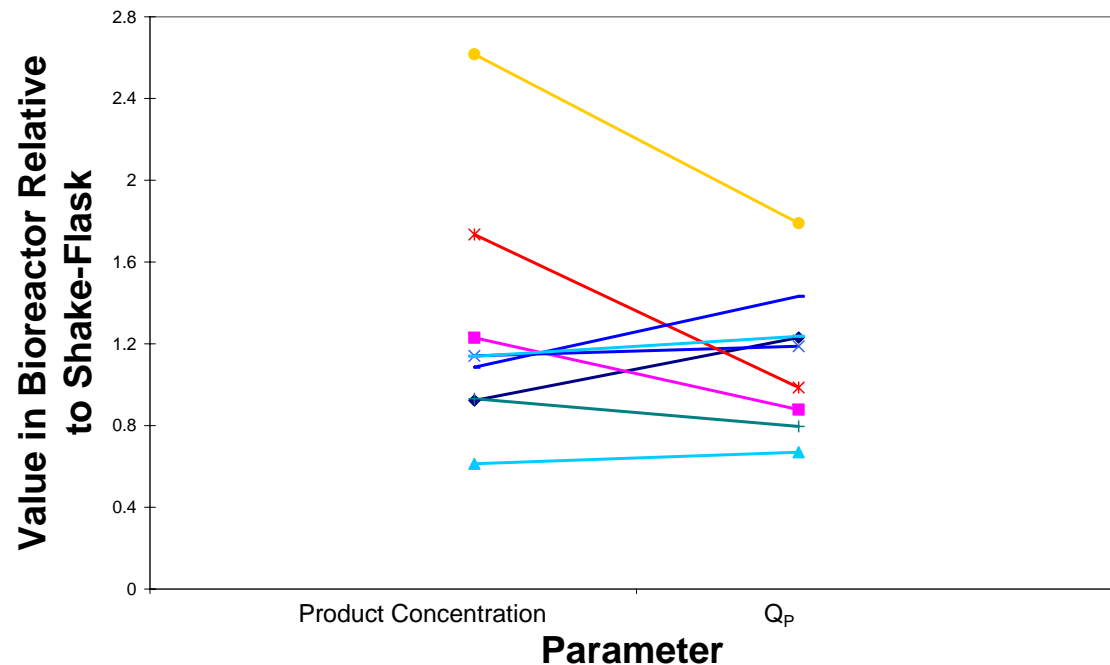
- Examples from two GS-CHO construction programmes



- Individual cell lines respond differently to feed
 - Change in rank position

Fed-Batch Shake-Flask vs. Fed-Batch Bioreactor

- Relationship between productivity characteristics of the lead GS-CHO cell lines making nine different antibodies



- Individual cell lines respond differently to culture in bioreactor

Current Cell Line Construction Strategy - Summary

- Variation seen for cell lines
 - expressing same antibody
 - expressing different antibodies

- No obvious relationship between growth and productivity

Current Cell Line Construction Strategy - Summary

- Individual cell lines respond differently to environment
 - Feed
 - Bioreactor
- Supports requirement to select cell lines in a good model of the production process
 - But no model is perfect

Improving Selection Strategies

Aim: Improve Selection Strategies

■ How?

- Identify correlations between productivity kinetics and various other parameters

■ Why?

- Use correlations to aid prediction of behaviour of cell lines in final production process
 - Ideal if could be used early in the selection process

Improving Selection Strategies

■ Methods

- Assess panel of GS-CHO cell lines encompassing a range of productivities, in triplicate, in a scale-down model of Lonza Biologics' production bioreactor process, using chemically-defined, animal component-free (CDACF) media and feeds.

■ Parameters assessed

- Growth and productivity kinetics
- Nutrient utilisation
- mRNA expression levels
- Gene copy number

Improving Selection Strategies – Growth and Productivity Kinetics

Cell Line	Maximum Viable Cell Concentration 10 ⁶ /mL	Harvest Viable Cell Concentration 10 ⁶ /mL	Viability at Harvest %	Integral of Viable Cell Concentration 10 ⁶ cell.h/mL	Doubling Time hours
LB01	13.65 ± 1.27	9.72 ± 0.97	75 ± 7	2421 ± 218	33.2 ± 2.2
E6	6.53	5.44	90	1423	52.0
Z6	10.50 ± 0.45	5.71 ± 0.13	71 ± 4	1964 ± 59	35.9 ± 2.0
Z2	12.19 ± 1.57	6.97 ± 1.47	67 ± 19	2439 ± 100	32.7 ± 2.0
E9	9.3 ± 0.24	6.73 ± 0.70	69 ± 4	1929 ± 52	39.5 ± 3.7
Z3	10.00 ± 1.29	2.08 ± 1.25	19 ± 12	1903 ± 56	38.3 ± 2.4
Z14	11.11 ± 0.65	7.85 ± 2.17	66 ± 4	2295 ± 37	37.1 ± 7.5
Z10	10.14 ± 0.50	3.79 ± 1.21	34 ± 11	2077 ± 60	35.1 ± 0.5
Z18	10.79 ± 0.66	4.92 ± 1.25	45 ± 16	2275 ± 91	41.5 ± 0.9
Z22	12.70 ± 0.98	1.13 ± 0.08	8 ± 1	2104 ± 98	36.0 ± 2.4
Z1	11.95 ± 0.27	1.02 ± 0.76	8 ± 6	2254 ± 141	36.3 ± 1.0
CHOK1SV	9.43 ± 0.43	1.36 ± 0.22	10 ± 1	1670 ± 25	32.5 ± 2.8
NULL 8	8.97 ± 0.53	4.42 ± 0.55	50 ± 4	1612 ± 41	38.8 ± 1.3

Data were obtained from triplicate cultures, excepting E6 (single culture) and E9 (duplicate culture)

Improving Selection Strategies – Growth and Productivity Kinetics

Cell Line	Product Concentration at Harvest (mg/L)	Specific Production Rate	
		Using Viable Cell Concentration (pg/cell/h)	Using Viable Biomass Concentration (pg/10 ³ fL/h)
LB01	1861 ± 66	0.78 ± 0.07	0.354 ± 0.052
E6	1619	1.29	0.522 ± 0.054
Z6	1303 ± 57	0.69 ± 0.06	0.282 ± 0.026
Z2	1299 ± 128	0.59 ± 0.09	0.243 ± 0.038
E9	1033 ± 72	0.60 ± 0.01	0.269 ± 0.009
Z3	991 ± 25	0.55 ± 0.03	0.243 ± 0.017
Z14	880 ± 7	0.40 ± 0.00	0.192 ± 0.007
Z10	878 ± 41	0.43 ± 0.05	0.190 ± 0.022
Z18	663 ± 16	0.30 ± 0.01	0.109 ± 0.006
Z22	534 ± 40	0.28 ± 0.02	0.137 ± 0.003
Z1	162 ± 4	0.07 ± 0.00	0.032 ± 0.002

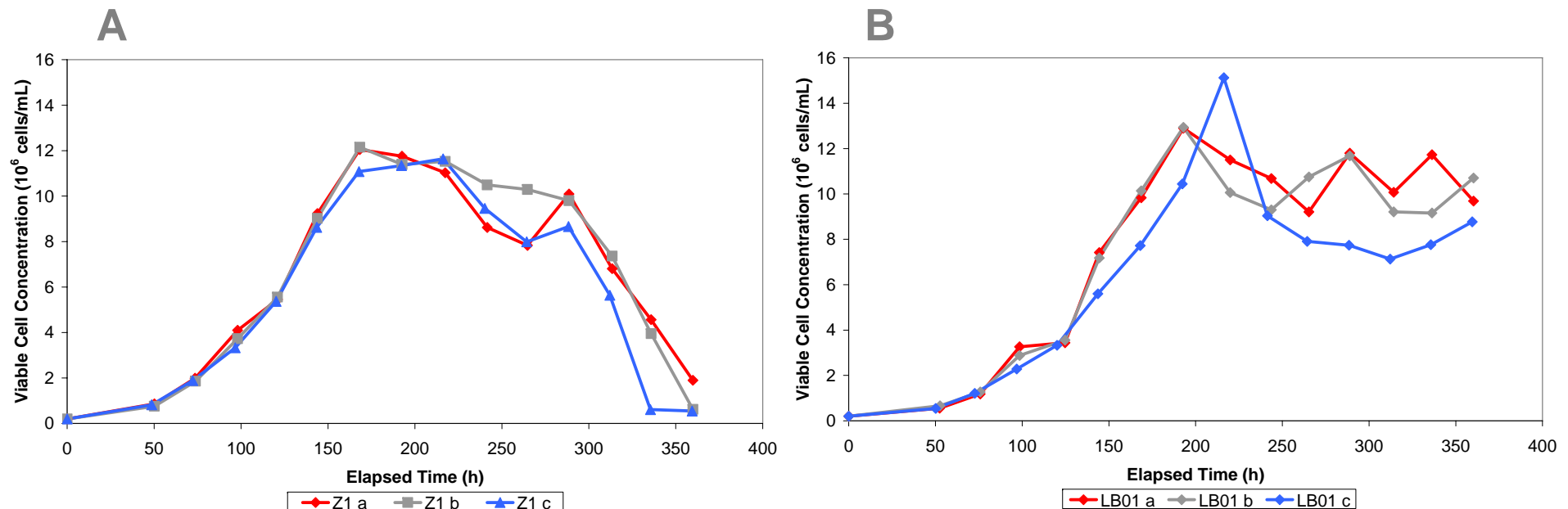
Data were obtained from triplicate cultures, excepting E6 (single culture) and E9 (duplicate culture)

Improving Selection Strategies – Growth and Productivity Kinetics

- A correlation was found between product concentration at harvest and specific production rate (Q_p)
- No correlation was found between product concentration at harvest or Q_p and:
 - maximum viable cell concentration ($\max X_v$)
 - the time integral of viable cell concentration (IVC)
 - doubling time (t_d)
- Conclusions that could be drawn based upon viable cell concentration and viable biomass concentration were similar

Improving Selection Strategies – Growth and Productivity Kinetics

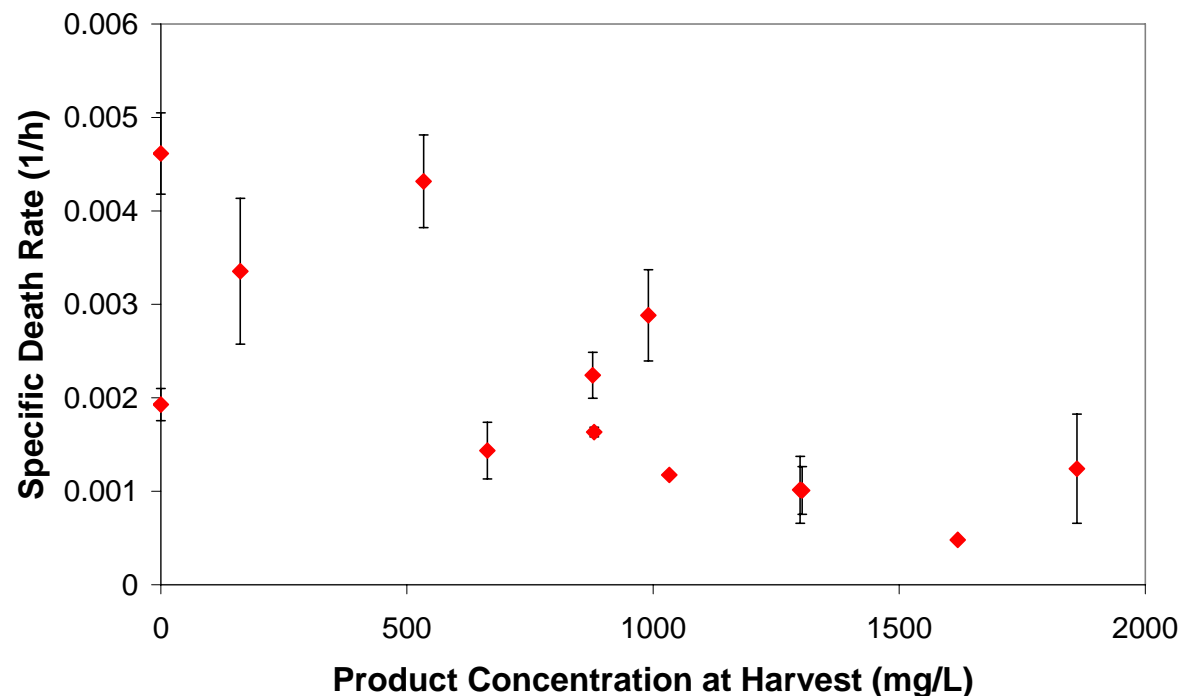
- Example of growth profiles for triplicate cultures of
 - (A) a low producing cell line and
 - (B) a high producing cell line



- The decline in viable cell concentration after the max X_V was more pronounced for the lower producing cell lines

Improving Selection Strategies – Growth and Productivity Kinetics

- Analysis of the specific death rate



- A correlation was found between the specific death rate and product concentration at harvest

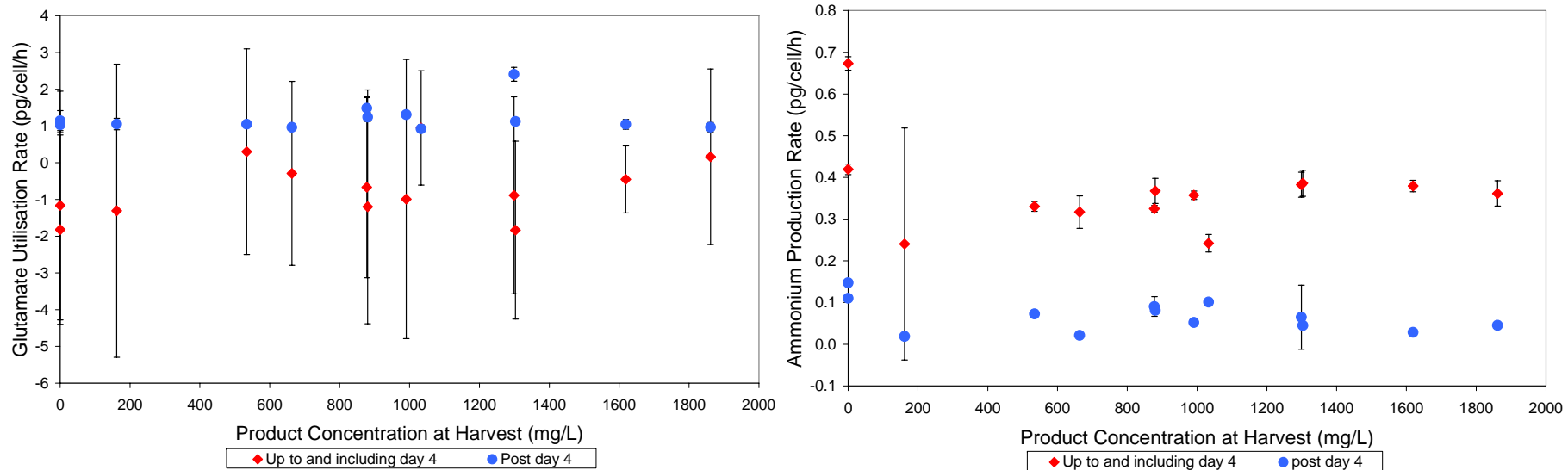
Improving Selection Strategies – Nutrient Analysis

- Daily analysis using Nova BioProfile 400

- Utilisation or production rates calculated for:
 - Glutamate
 - Ammonium
 - Glucose
 - Lactate

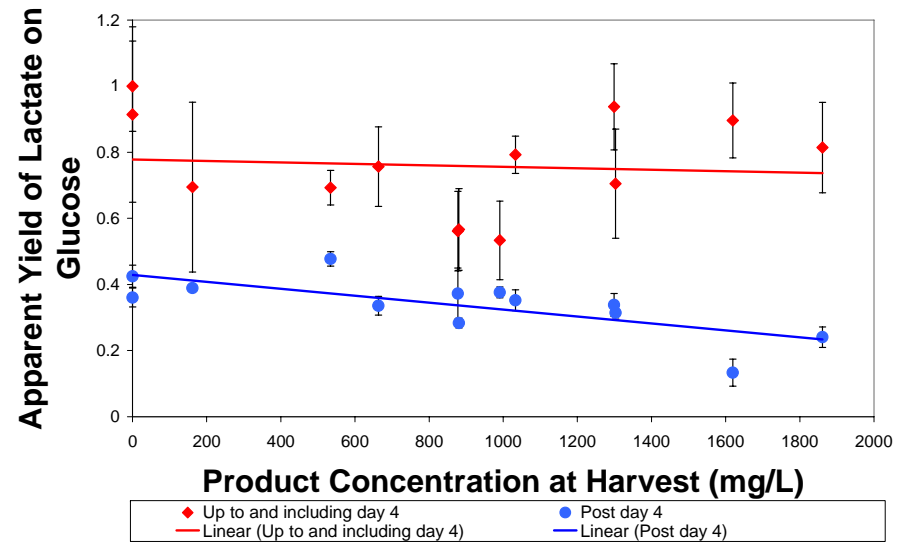
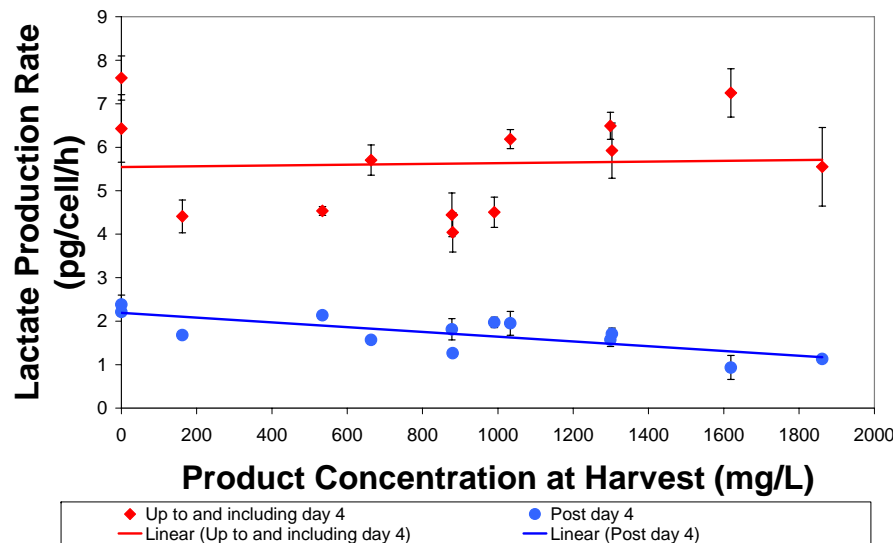
- Change in metabolism at day 4
 - Cause of change unknown
 - Data therefore assessed (i) as a whole, (ii) up to and including day 4 and (iii) post day 4

Improving Selection Strategies – Nutrient Analysis



- No correlation observed between product concentration and
 - glutamate utilisation rate
 - ammonium production rate
 - glucose utilisation rate

Improving Selection Strategies – Nutrient Analysis

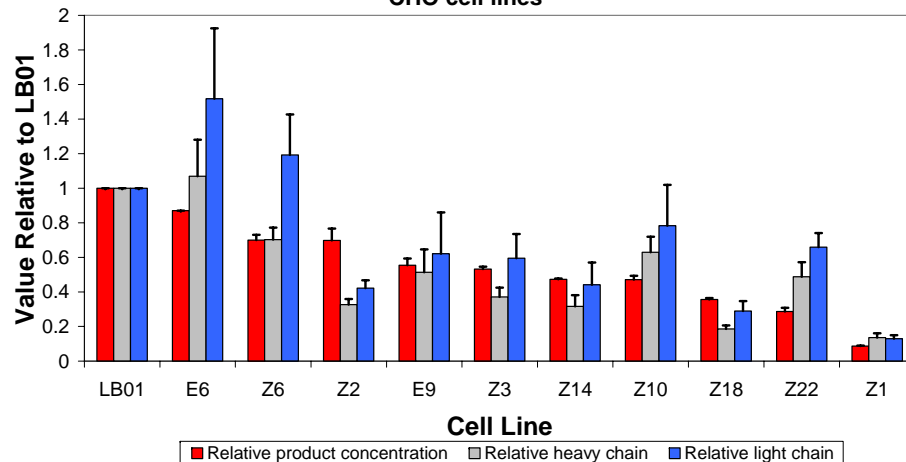


- Correlation observed between product concentration and
 - lactate production rate
 - apparent yield of lactate on glucose
- Higher producers had the lower lactate production rate and apparent yield of lactate on glucose

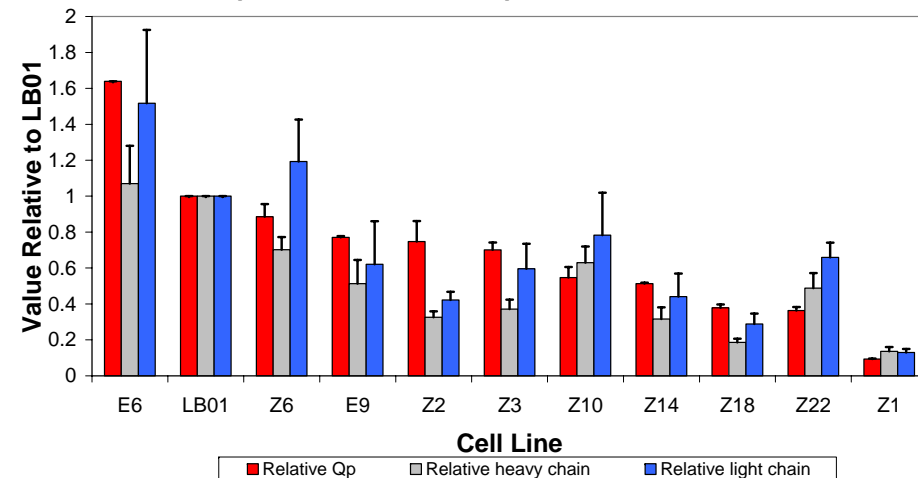
Improving Selection Strategies – mRNA

- Relative mRNA expression levels of the heavy chain and light chain were determined by quantitative RT-PCR

Comparing IgG₄ heavy chain mRNA expression, light chain mRNA expression and product concentration at harvest for a panel of GS-CHO cell lines



Comparing IgG₄ heavy chain mRNA expression, light chain mRNA expression and Q_p for a panel of GS-CHO cell lines



Improving Selection Strategies – mRNA

- Significant correlations were observed between:
 - Heavy chain mRNA expression and
 - product concentration ($r^2 = 0.66$)
 - Q_p ($r^2 = 0.72$)
 - Light chain mRNA expression and
 - product concentration ($r^2 = 0.56$)
 - Q_p ($r^2 = 0.77$)

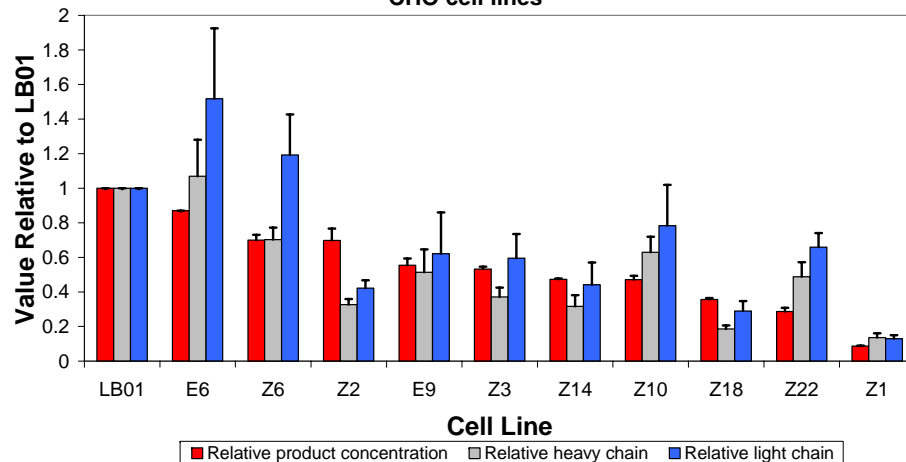
Improving Selection Strategies – mRNA

- Higher levels of mRNA were associated with higher product concentration
- Transcription is probably rate limiting for antibody production in most of these GS-CHO cell lines
 - Difference in effect if performed on a panel of GS-CHO cell lines already narrowed down to those with higher productivities?
 - A couple of cell lines did not follow the trend

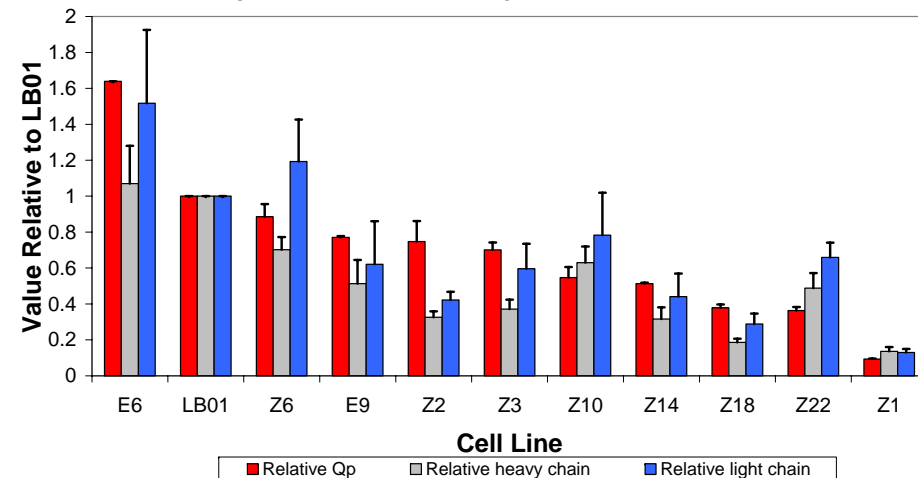
Improving Selection Strategies – mRNA

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Comparing IgG₄ heavy chain mRNA expression, light chain mRNA expression and product concentration at harvest for a panel of GS-CHO cell lines

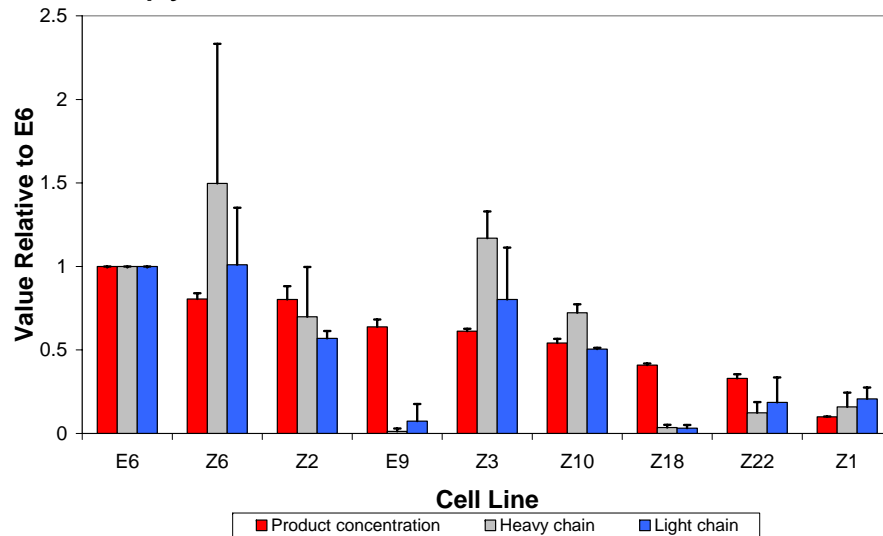


Comparing IgG₄ heavy chain mRNA expression, light chain mRNA expression and Q_p for a panel of GS-CHO cell lines

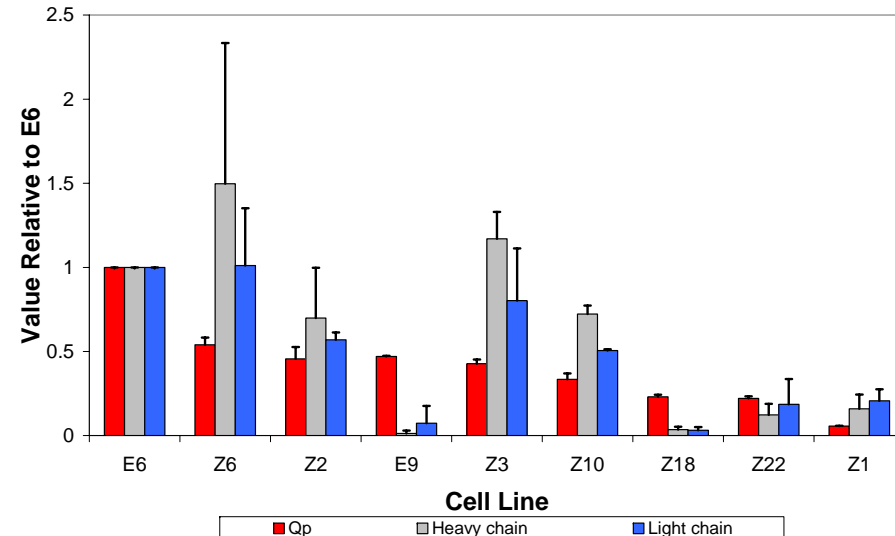


Improving Selection Strategies – Gene Copy Number by Southern Blot Analysis

Comparing product concentration and copy number



Comparing Q_p and copy number



- No obvious trend exists between copy number and product concentration or Q_p

Improving Selection Strategies - Summary (1)

- A correlation between productivity kinetics and mRNA expression
 - Cell lines with higher product concentrations/higher Q_p had higher levels of heavy and light chain mRNA expression
 - Suggests that transcription is probably a rate limiting step for the majority of the panel of GS-CHO cell lines
 - Difference in effect if performed on a panel of GS-CHO cell lines already narrowed down to those with higher productivities?

Improving Selection Strategies - Summary (2)

- A correlation between the specific death rate and product concentration
- A correlation between product concentration and lactate production rate / apparent yield of lactate on glucose
- No correlation between product concentration and glutamate utilisation, ammonium production or glucose utilisation rates
- No obvious trend between copy number and product concentration or Q_p

Discussion

- Heterogeneous population is obtained after transfection
- Cell lines respond differently to their environment
 - Careful interpretation of early data with current selection strategy

- Correlation observed between
 - productivity kinetics and mRNA expression levels
 - specific death rate and product concentration
 - Lactate production and product concentration

- Application
 - Can correlations be used for predicting behaviour of cell lines in the final production process?

Acknowledgements

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