

Cell Culture Engineering XI

Lonza

Antibody Manufacture: The Search for 'Desirable' Cell Lines

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Disclaimer

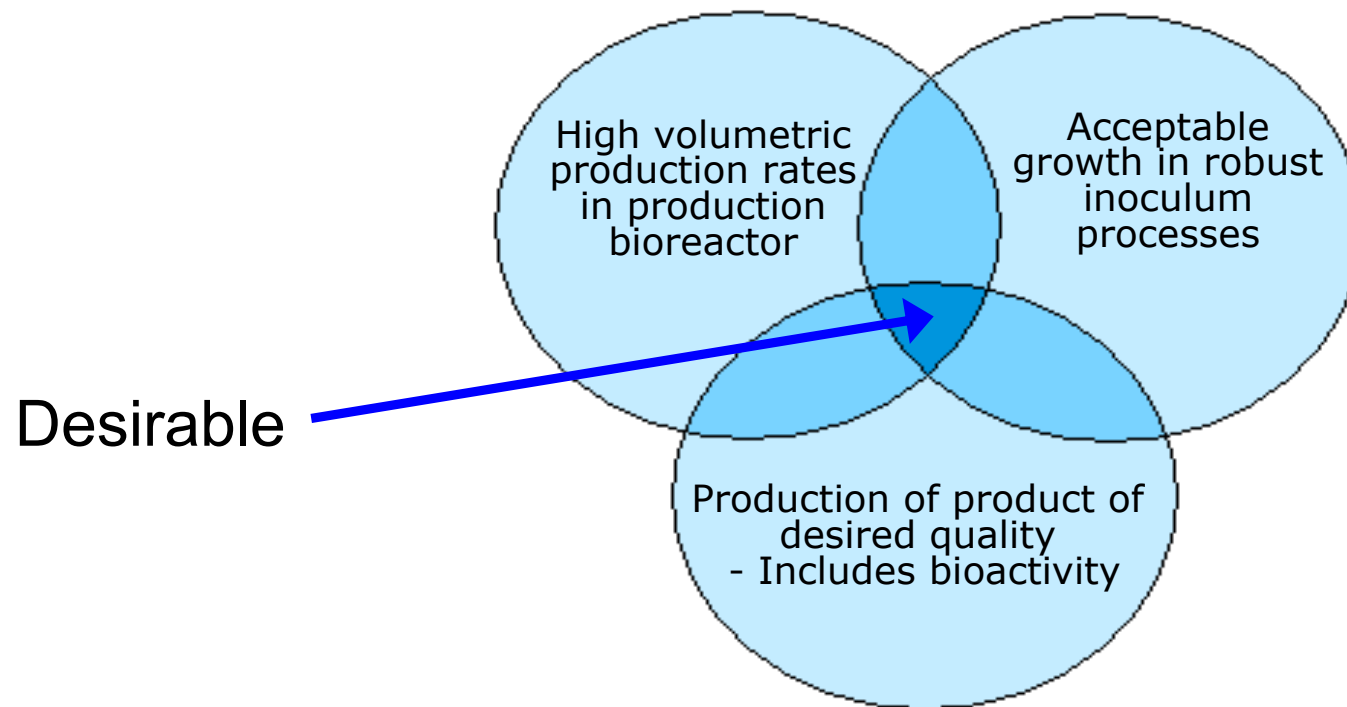
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Scope of Talk

- Cell line construction: issues and strategies to select 'desirable' cell lines
- Understanding the behaviour of cell lines in a selection strategy
- Can the selection strategy be improved?

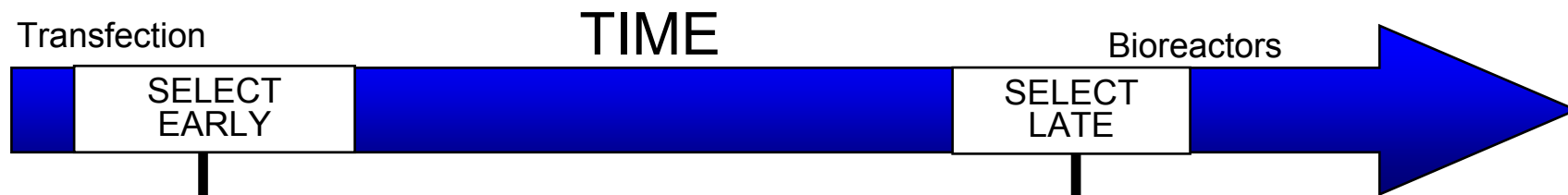
Cell Line Construction: Issues and Strategies to
Select 'Desirable' Cell Lines

Issue



- Measurements made early in cell line selection may not reflect behaviour in final production process
- Strategy required to isolate a 'desirable' cell line

Selection Strategies



- Requires prediction of manufacturing behaviour at very early stage
 - Good predictive markers

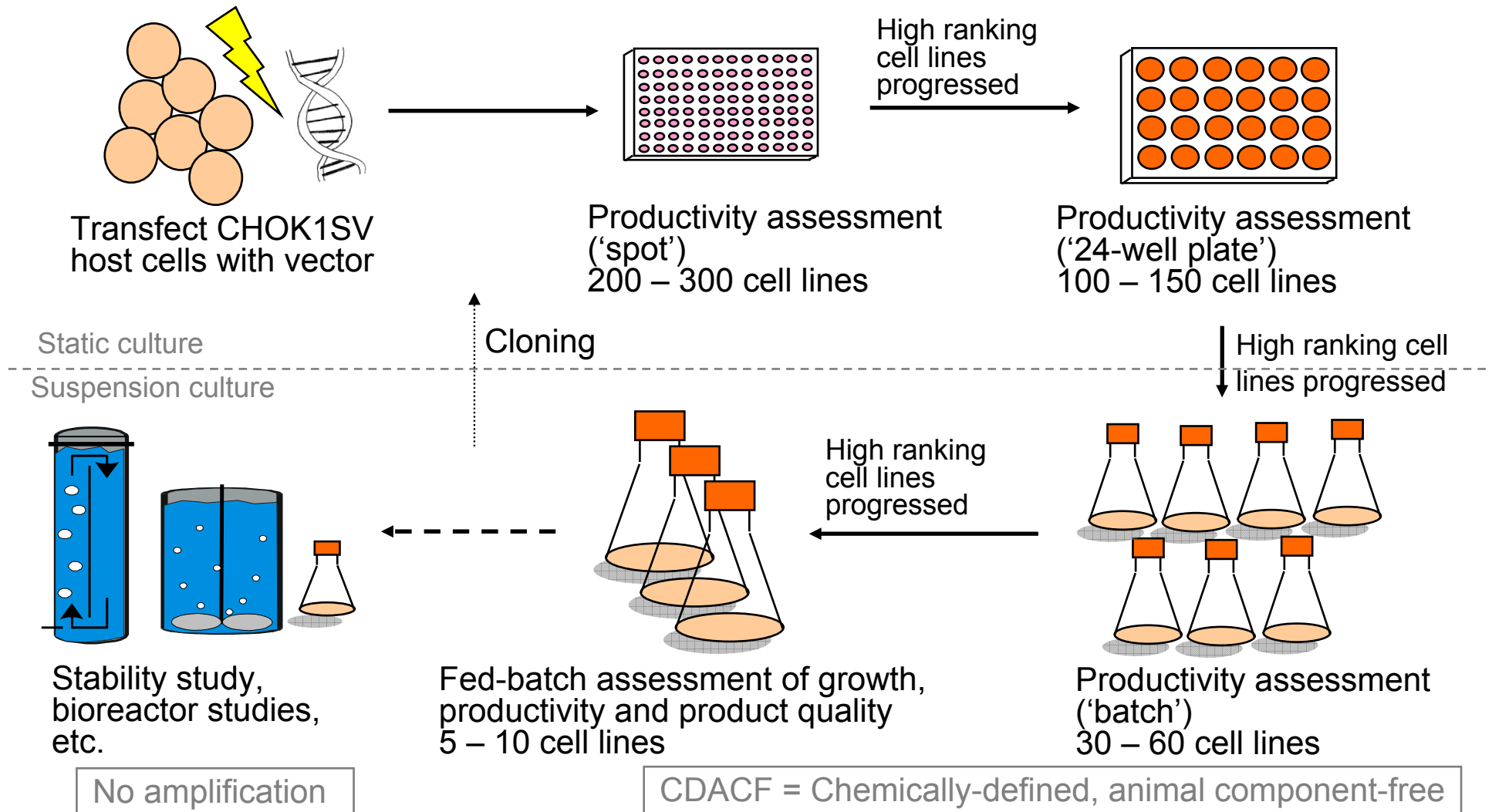
- Selection occurs in manufacturing process
- May require assessment of large numbers of cell lines in the manufacturing process
 - Lengthy and resource intensive
 - Impractical



COMPROMISE

- May have multiple steps
- Economical
- Compatible with resources

Example of a Strategy for GS-CHO Cell Line Selection in a CDACF Medium



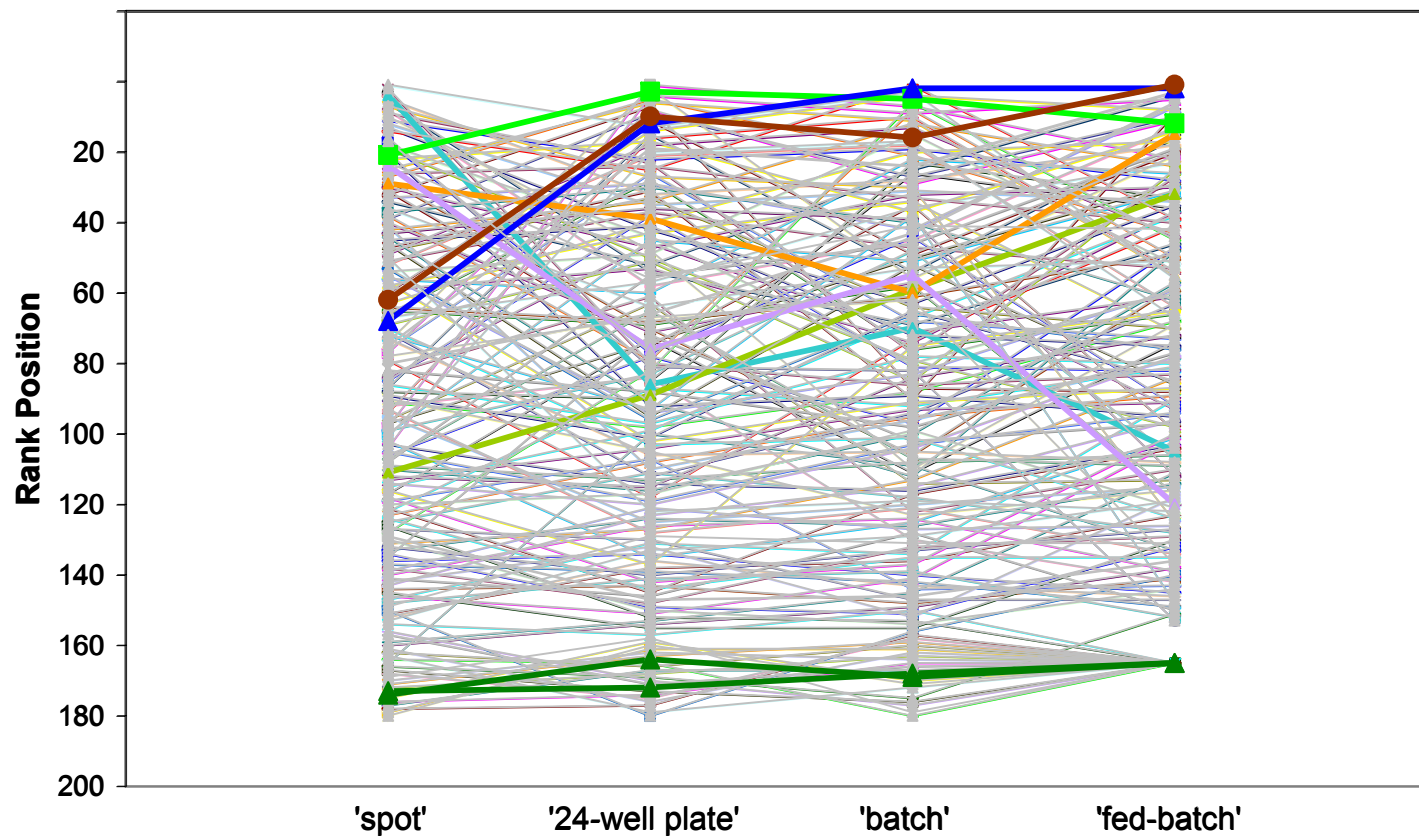
Understanding the Behaviour of Cell Lines in a
Selection Strategy

How Good are the Screens?

- Current selection strategy allows isolation of high producing cell lines
- However:
 - Are these cell lines the highest producers in the population of primary transfectants?
 - Do we discard cell line(s) at each stage that may do well in the production process?
 - Do we retain cell line(s) that may not do well in the production process
 - How will these cells behave in the production process?
- Improved understanding of what happens to different cell lines during the selection strategy may help answer this question
- How?
 - Progressed 175 randomly selected cell lines from transfection through all selection stages prior to bioreactor evaluation
 - Assessment of a subset of these in bioreactors

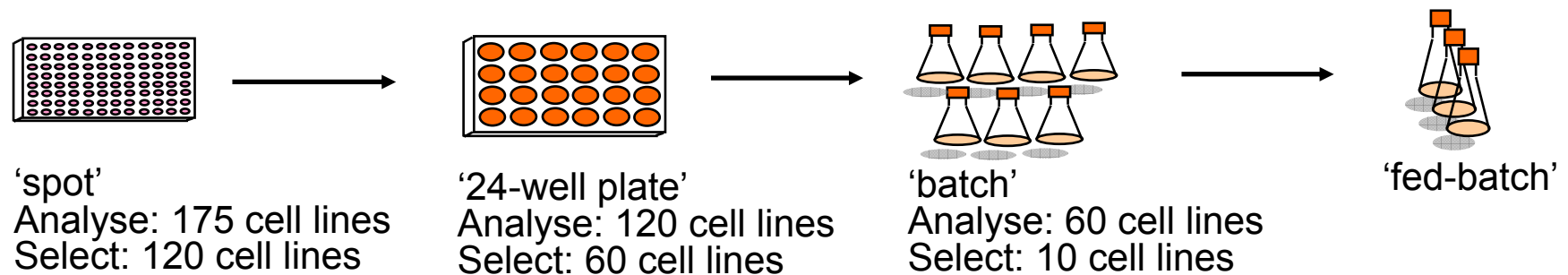
Ranking

- As rank position is used to select cell lines for progression, examined how this changes between each screen



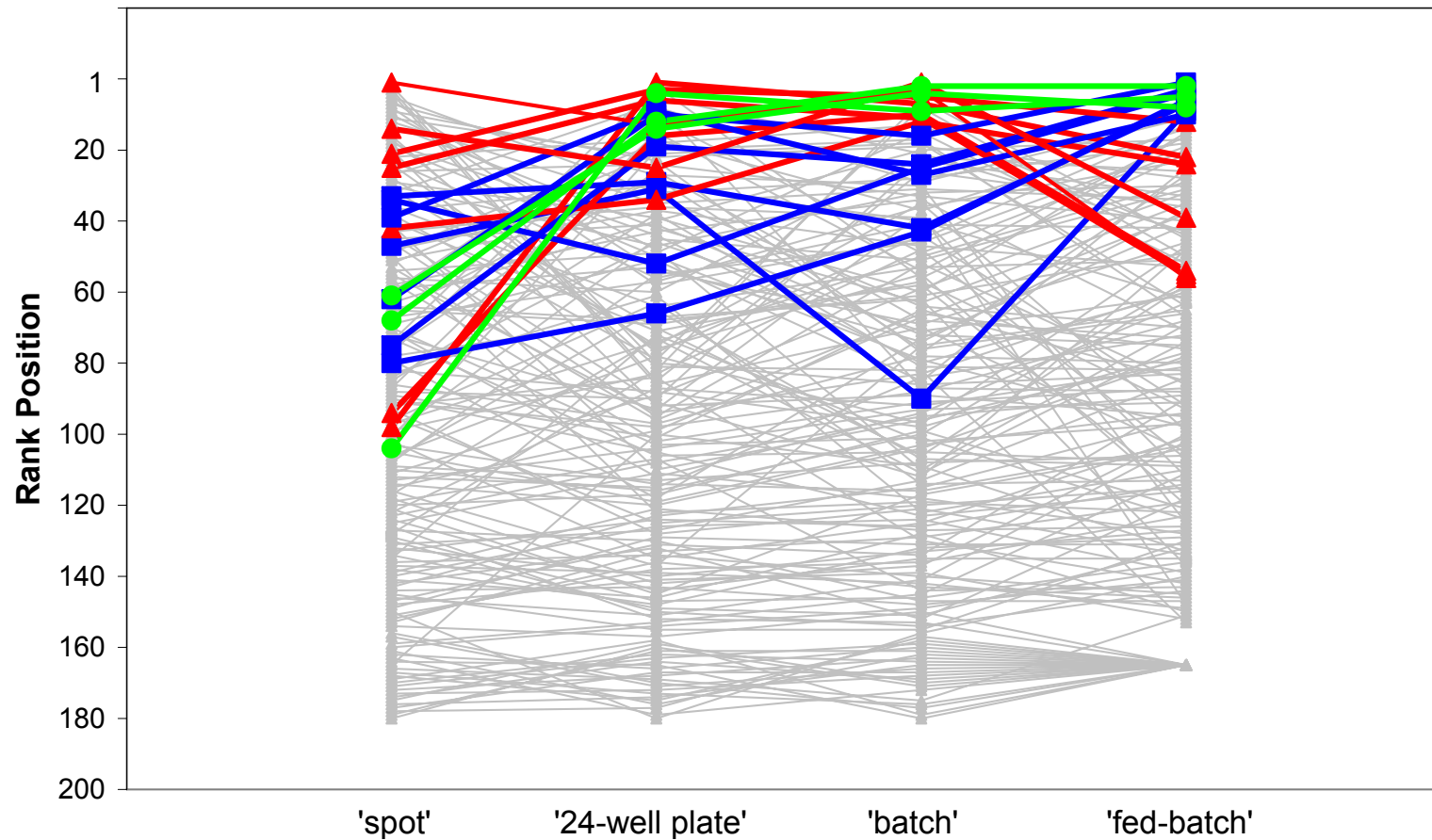
Location of ‘Selected’ and ‘Real’ Top 10 Cell Lines

- Data can be used to model which cell lines would be chosen with different selection strategies
 - Applied to strategy illustrated earlier
 - These cell lines designated the ‘selected’ top 10



- Where are these ten cell lines at each screen stage?
- How does this compare with the ‘real’ top 10?
 - ‘real’ top10 = ten highest producing cell lines in entire population in fed-batch evaluation stage

Location of 'Selected' and 'Real' Top 10 Cell Lines

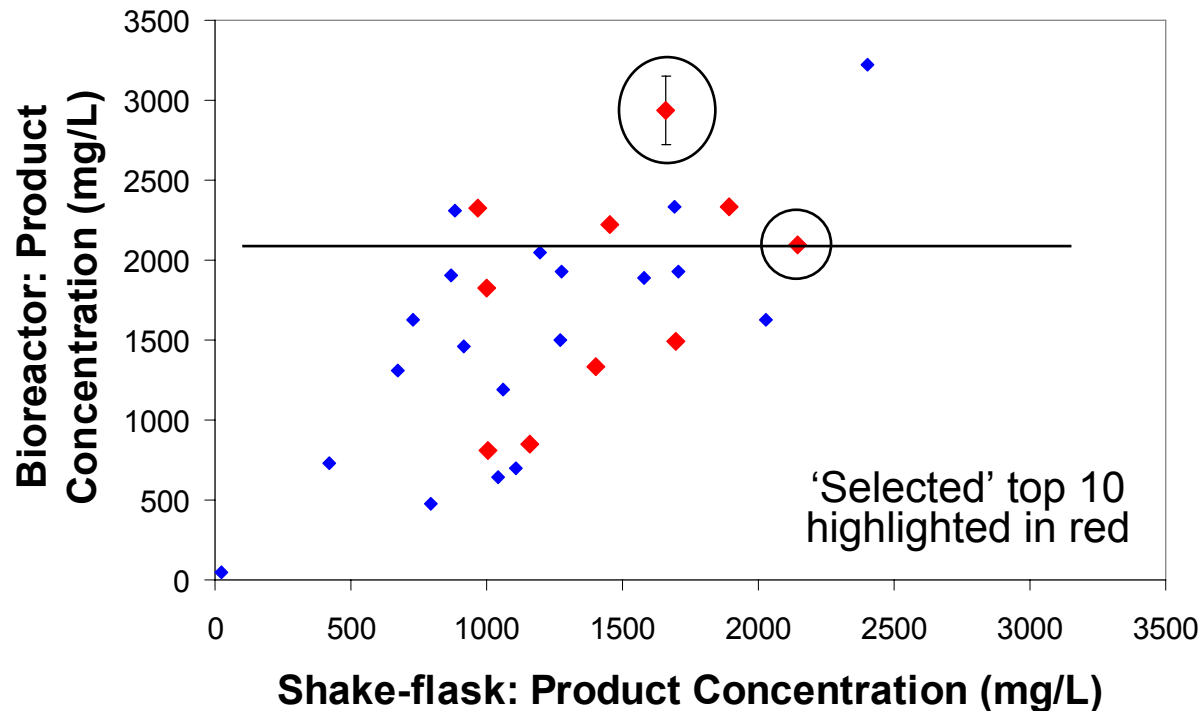


Red = 'selected' top 10; blue = 'real' top 10;
 green = common to both 'selected' and 'real' top 10

The 'Selected' and the 'Real' Top 10

- Current selection strategy identified high producing cell lines
 - Selected cell lines that were in the 'real' top 10
 - 3/10 common between 'real' and 'selected' top 10
 - Did not select all of the 'real' top 10
- As with the 'selected' top 10, the 'real' top 10 were widely distributed in earlier screens
- Initial responses
 - Are these cell lines the highest producers in the population of primary transfectants? **No** – although 3/10 are common with 'real' top 10
 - Are cell line(s) discarded at each stage that may do well in the production process? **Yes**
 - Are cell line(s) retained at each stage that may not do well in the production process? **Yes**

Behaviour of 29 Representative Cell Lines in Fed-Batch Bioreactors



- Strong positive association between behaviour in two culture systems
 - High producer in bioreactor identified from shake-flask data using current selection strategy
 - Not the highest producer

How Could the Selection Strategy be Improved?

- Statistical modelling suggests
 - Only census of all 175 cell lines better than current selection strategy
 - Assessment stages are significant predictors of subsequent screen stages, but are 'not predictive enough'

- Alternative strategies to improve the selection strategy
 - More predictive methodology at assessment stages
 - e.g. make environment in early development similar to final production process
 - Identify alternative predictive markers

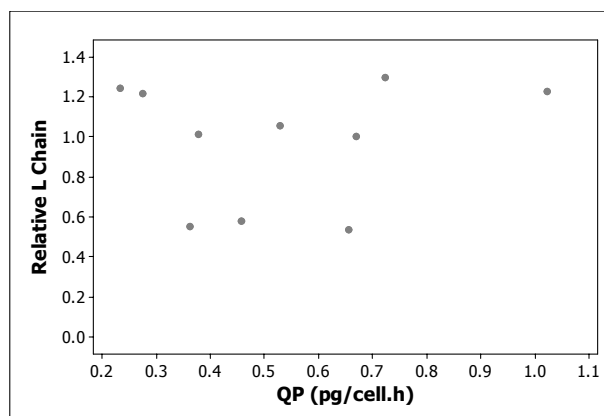
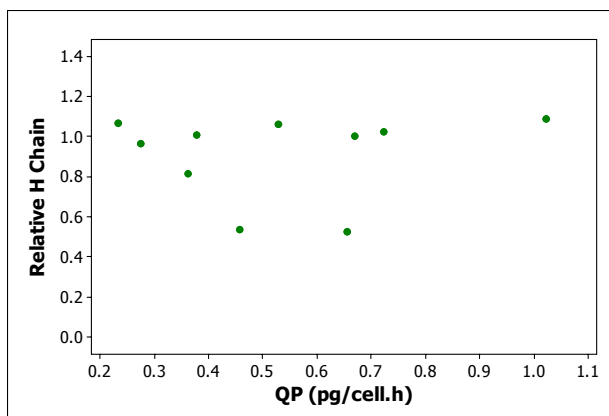
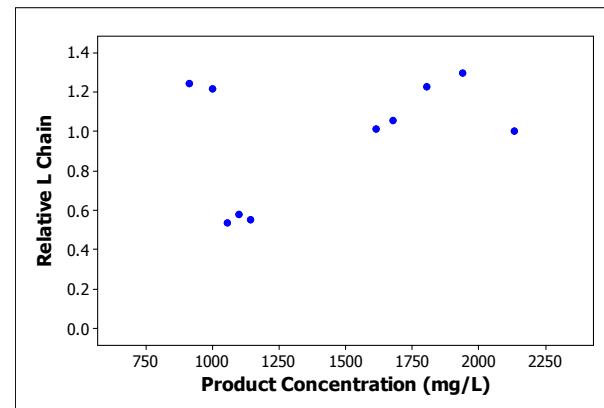
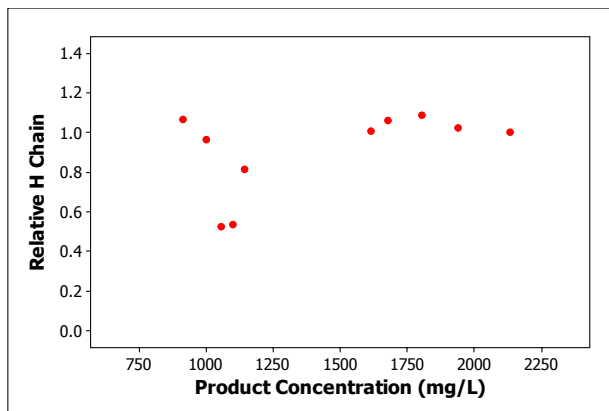
A Potential Alternative Predictive Marker:
mRNA Levels

mRNA Analysis: 'Original Panel' of Cell Lines

- A panel of GS-CHO cell lines, with wide ranging productivities (162 mg/L up to 1.8 g/L), were previously studied
- Positive linear correlation between productivity and mRNA levels observed
 - mRNA levels determined by quantitative RT-PCR
- Questions posed:
 - Would this linear association still be observed with a panel of cell lines 'selected' in a cell line development programme as high producers?
 - Assumes 'selected' cell lines have a narrower range of higher productivities
 - Could mRNA level be used as an alternative predictive marker?
- mRNA levels in 'selected' top 10 cell lines therefore determined

mRNA Analysis: 'Selected' Top 10 Cell Lines

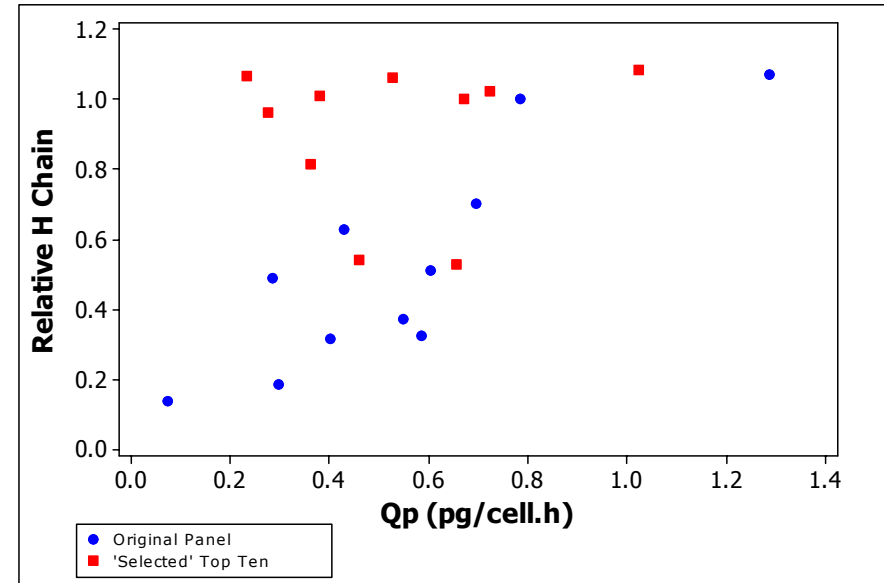
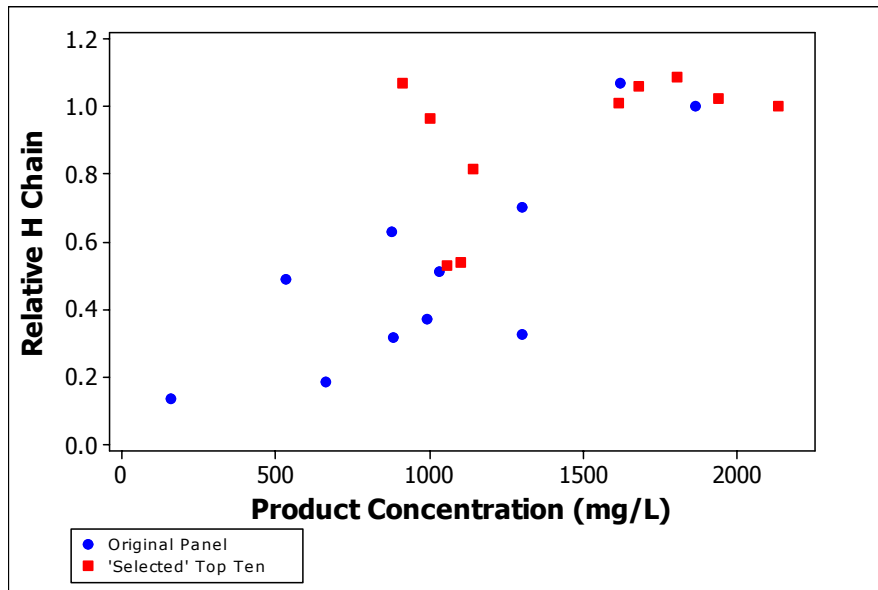
- Relative mRNA expression levels of the heavy chain and light chain were determined by quantitative RT-PCR



Data from fed-batch shake-flasks

- No evidence for linear correlation between mRNA levels and productivity

Heavy Chain mRNA Analysis: Combining Data from 'Original Panel' and 'Selected' Top Ten Cell Lines



- Initial interpretation of the data suggests that a biphasic relationship exists between mRNA level and product concentration

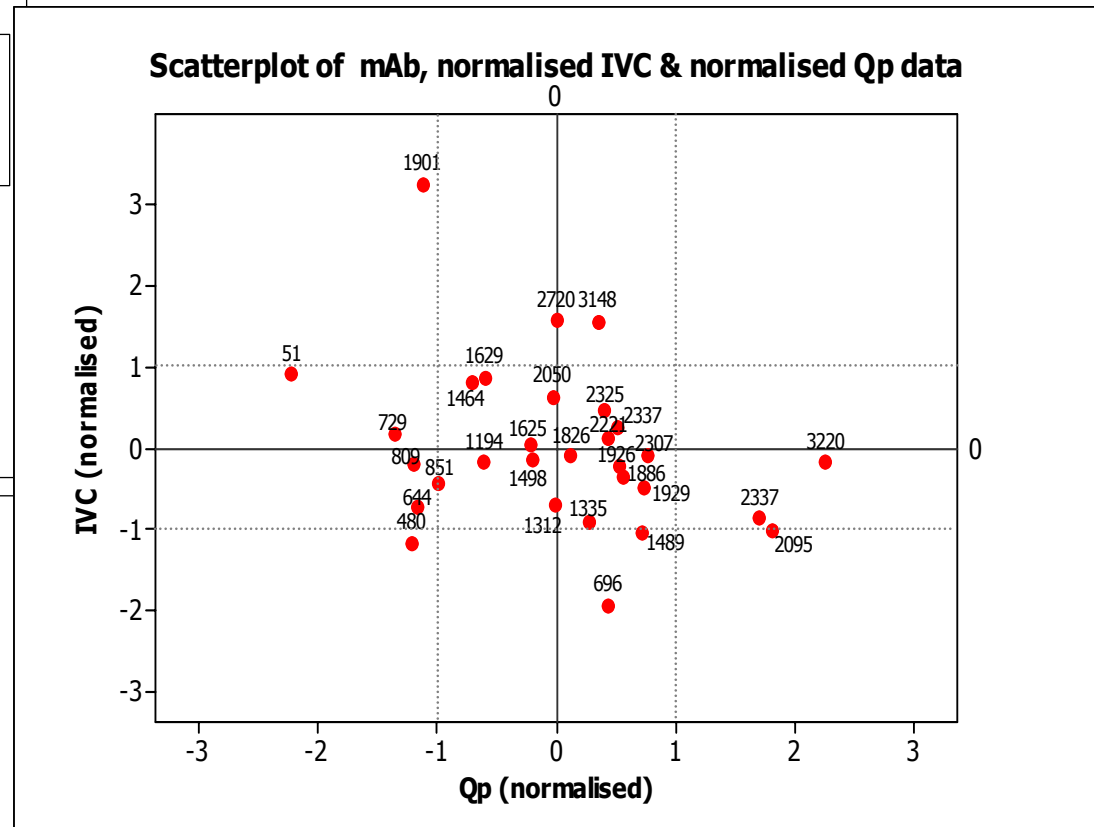
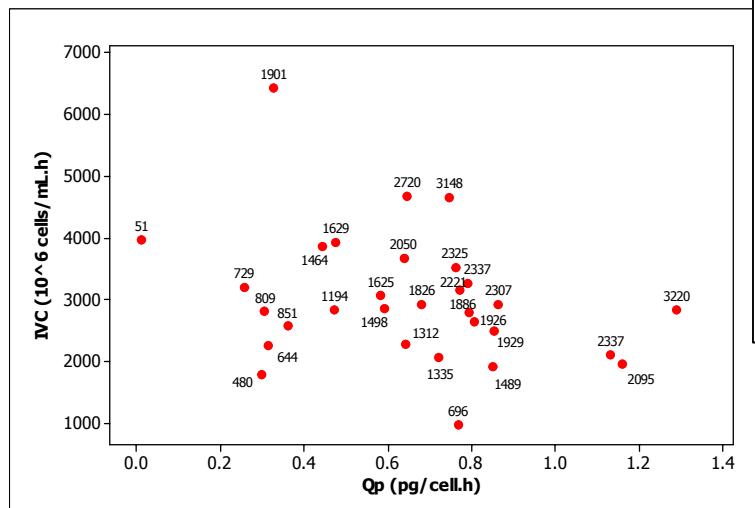
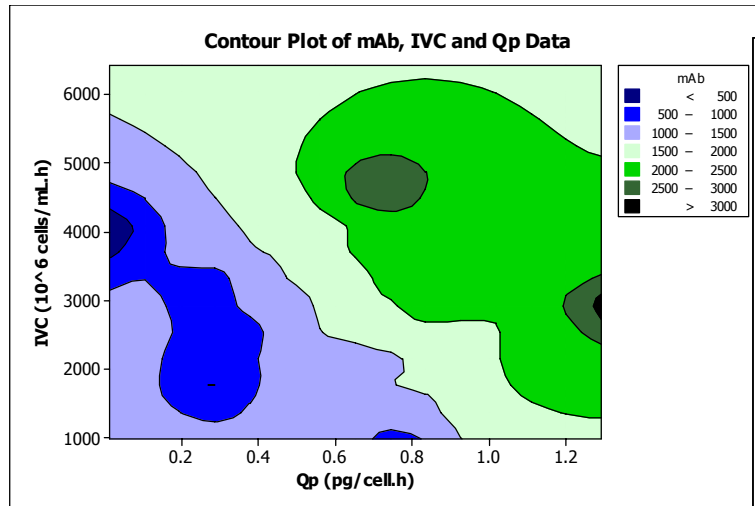
mRNA Levels as a Predictive Marker: Responses to the Questions Posed

- Would the linear association observed with the 'original panel' still be observed with a panel of cell lines 'selected' in a cell line development programme?
 - No linear correlation between productivity and mRNA level for 'selected' top ten cell lines
 - For combined data sets observed:
 - Positive linear association up to a plateau region for mRNA level
 - Suggests that once plateau reached, cause of differences in product concentration may occur at some stage after transcription

- Could mRNA level be used as an alternative predictive marker?
 - Unsuitable due to range of productivities observed with highest mRNA levels

The Future: How 'High' Can We Go?

IVC vs. Qp: Which is the More Important in Achieving High Product Concentrations?



Data from 29 representative cell lines in fed-batch bioreactors

Searching for 'High-Producing Cell Lines'

- For a given production bioreactor process, data suggest greater contribution of Q_p to overall product concentration than IVC
 - Influence of Q_p
 - Lower producers tend to cluster in LH side of graph
 - Higher producers generally found on RH side
 - Influence of IVC
 - Higher producers generally found with mid- to high-range IVC values
 - Lowest producers appear to have combination of low IVC and low Q_p
 - Highest producers have mix of Q_p and IVC values

- Cell lines with combination of high Q_p and high IVC were not observed
 - Raises the question: Are high Q_p and high IVC compatible?

Summary

Summary

- Current selection strategy identifies highly productive cell lines
- Locations of highest producing cell lines, in earlier screens, are widespread
- Ranking positions change as cell lines progress through the different assessment stages
- Alternative strategies to improve the selection strategy
 - Identify alternative predictive markers
 - More predictive methodology at assessment stages

Summary - Continued

- Analysis of mRNA suggested
 - A biphasic relationship exists between mRNA level and productivity
 - mRNA levels are not suitable as an alternative predictive marker

- Observations from behaviour of different cell lines in bioreactors
 - Suggested Q_p greater contributor to product concentration than IVC
 - Raised the question 'are high Q_p and high IVC compatible?'

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