

Cell Engineering 2007

Lonza

Cell Line Creation: Behaviour of Cell Lines in a Selection Strategy

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Scope of Talk

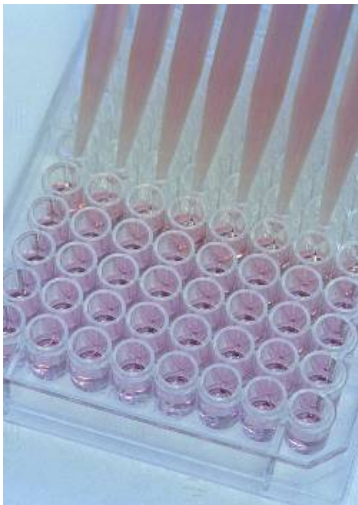
- Cell line construction – issues and strategies to select ‘desirable’ cell lines
- Understanding the behaviour of cell lines in a selection strategy
- Can the cell line selection strategy be improved?

Cell Line Construction

Issues and Strategies to Select 'Desirable' Cell Lines

Issue

- Issue:
 - Transfection results in heterogeneous population
 - Measurements made early in cell line selection may not reflect behaviour in final production process



- Strategy required to isolate a 'desirable' cell line

Cell Line Selection Objectives

- Objective of any cell line selection strategy is to isolate 'desirable' cell line(s)

- 'Desirable' cell lines:
 - Have high volumetric production rates in production bioreactor

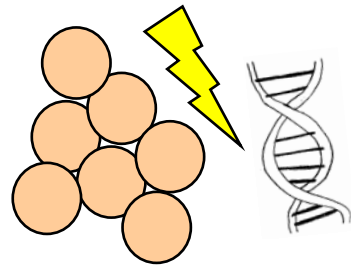
 - Show acceptable growth in robust inoculum processes

 - Produce product of desired quality
 - Including bioactivity

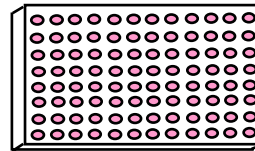
Selection Strategies

- Three classes of strategies to isolate a 'desirable' cell line:
 - Select early
 - Requires prediction of manufacturing behaviour of cell lines at very early stage
 - Good predictive markers
 - Select late
 - Selection occurs in manufacturing process
 - May require assessment of large numbers of cell lines in the manufacturing process
 - Lengthy and resource intensive - impractical
 - Combination
 - May have multiple steps
 - Economic
 - Compatible with resources

Selection of a GS-CHO Cell Line – Using a Chemically-Defined, Animal Component-Free Medium

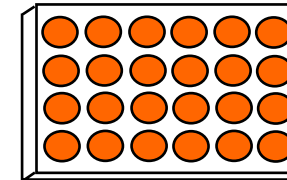


Transfect CHOK1SV host cells with vector



Productivity assessment ('spot')
200 – 300 cell lines

High ranking cell lines progressed

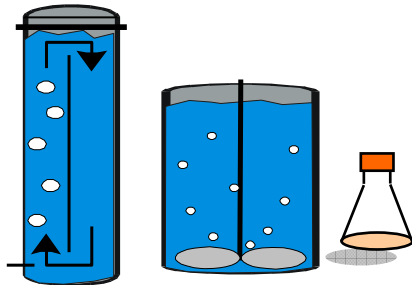


Productivity assessment ('24-well plate')
100 – 150 cell lines

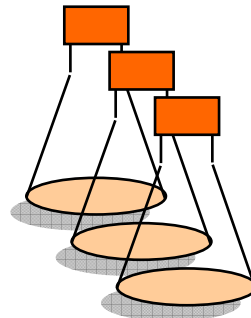
Static culture

Suspension culture

High ranking cell lines progressed

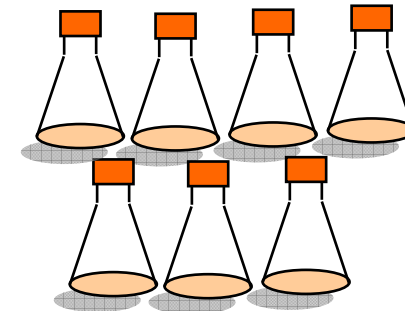


Cloning, stability study, bioreactor studies, etc.



Fed-batch assessment of growth, productivity and product quality
5 – 10 cell lines

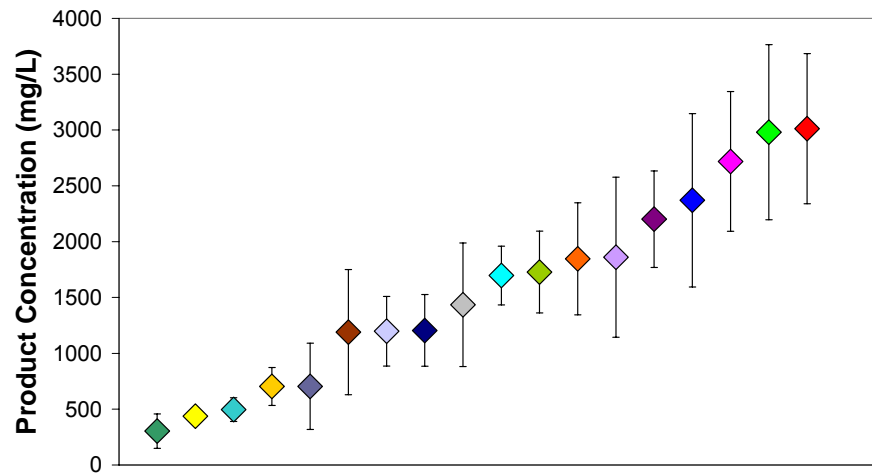
High ranking cell lines progressed



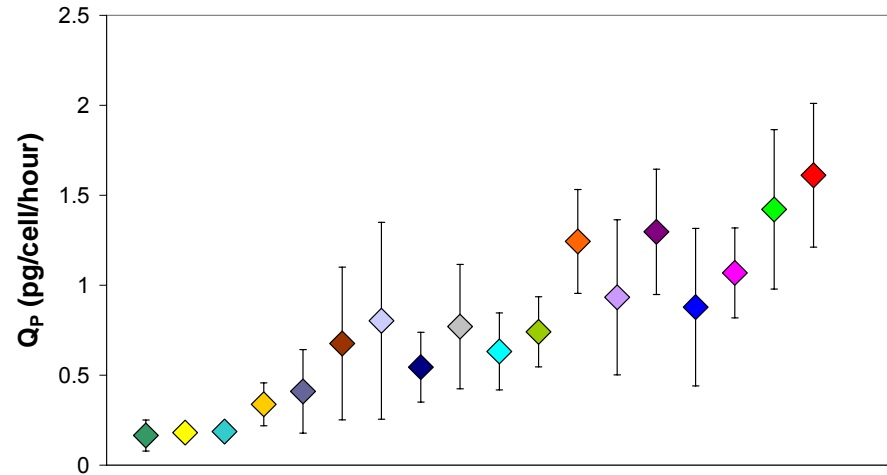
Productivity assessment ('batch')
30 – 60 cell lines

Fed-Batch Shake-Flask Screen of GS-CHO Cell Lines

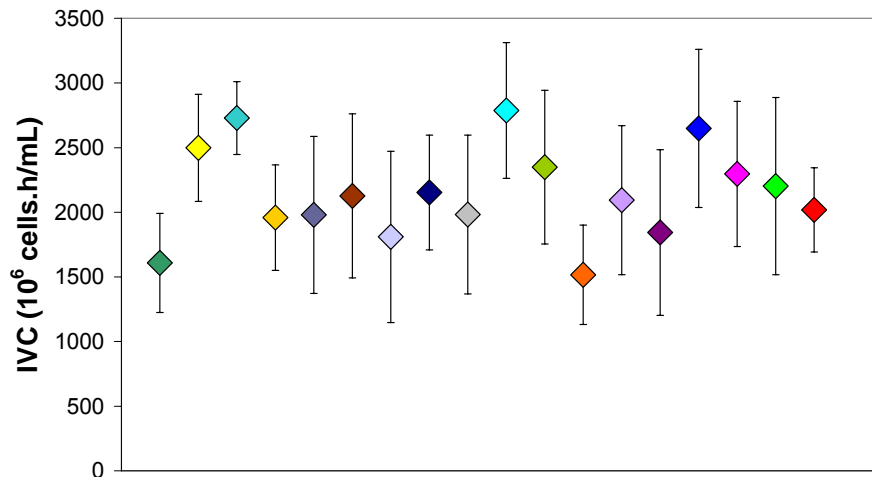
Cell Lines



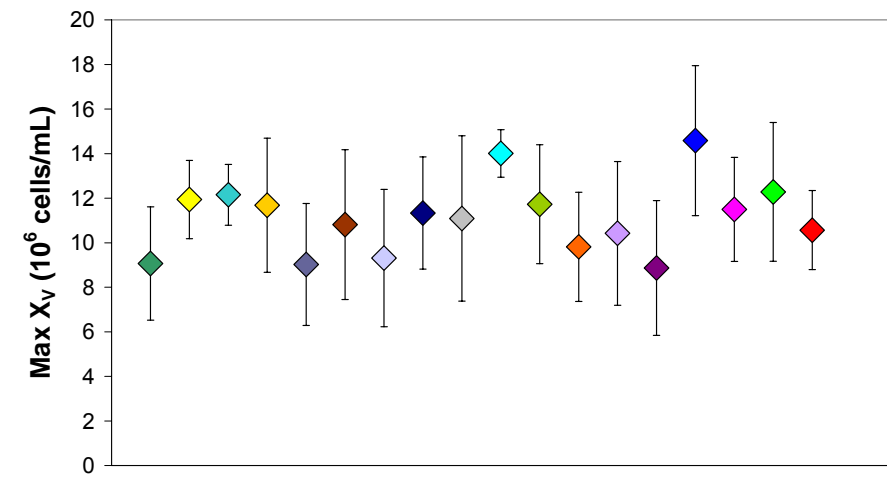
Antibody



Antibody



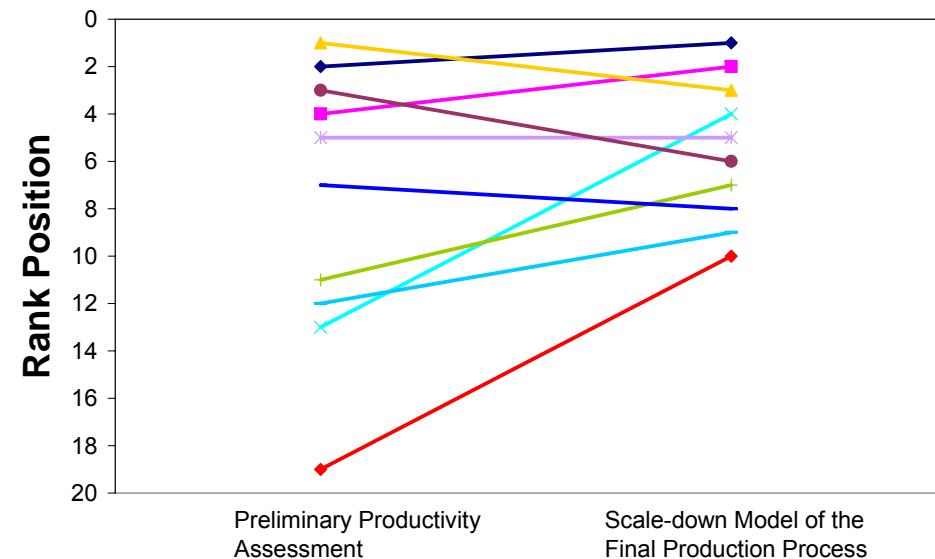
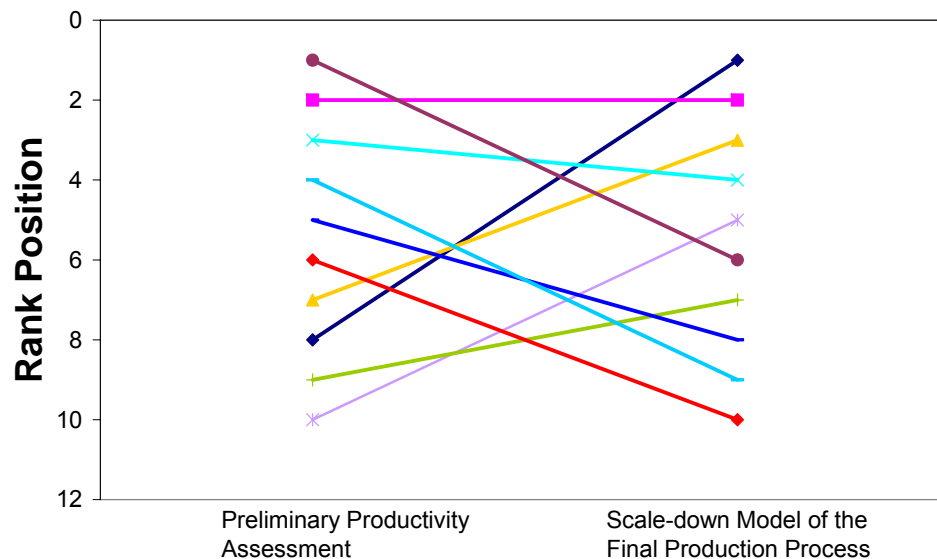
Antibody



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

Understanding the Behaviour of Cell Lines in a
Selection Strategy

Questions

- Although high producing cell lines are isolated using this cell line selection strategy, are these ‘good’ cell lines rather than ‘the best’ cell lines?

- How predictive are the screens?
 - If measurements made early in cell line construction do not reflect how a cell line will behave in the final production process, are we:
 - Discarding cell line(s) at each stage that may do well in the production process
 - Retaining cell line(s) that do not do well in the production process

More Questions

- Can we isolate the 'real top ten' cell lines more frequently, without increasing resource requirement or elapsed time?
 - 'real top ten' = ten highest producing cell lines in entire population in fed-batch evaluation stage

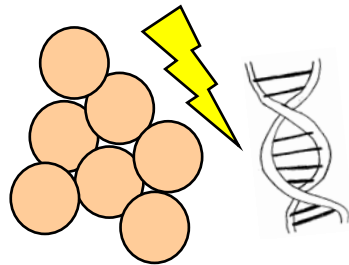
- Answers to these questions were required
 - Limitations with historical data
 - Complete data set only available for limited number of cell lines

Investigation

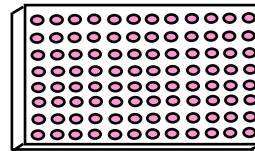
- Improved understanding of what happens to different cell lines during the selection strategy may help answer these questions

- 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

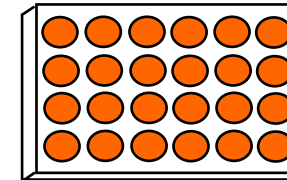
Understanding the Behaviour of Cell Lines in a Selection Strategy - Method



Transfect CHOK1SV host cells with vector



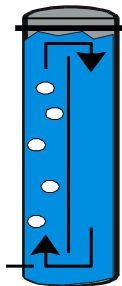
Productivity assessment ('spot')
175 cell lines



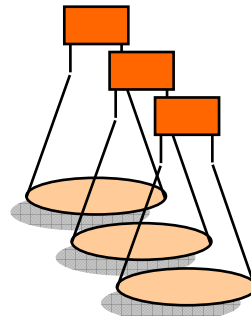
Productivity assessment ('24-well plate')
175 cell lines

Static culture

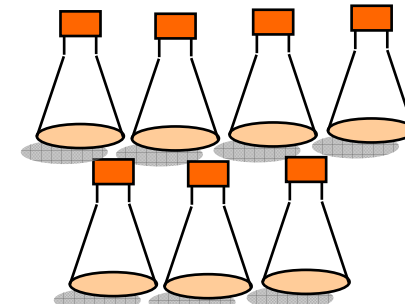
Suspension culture



Bioreactor assessment
29 cell lines

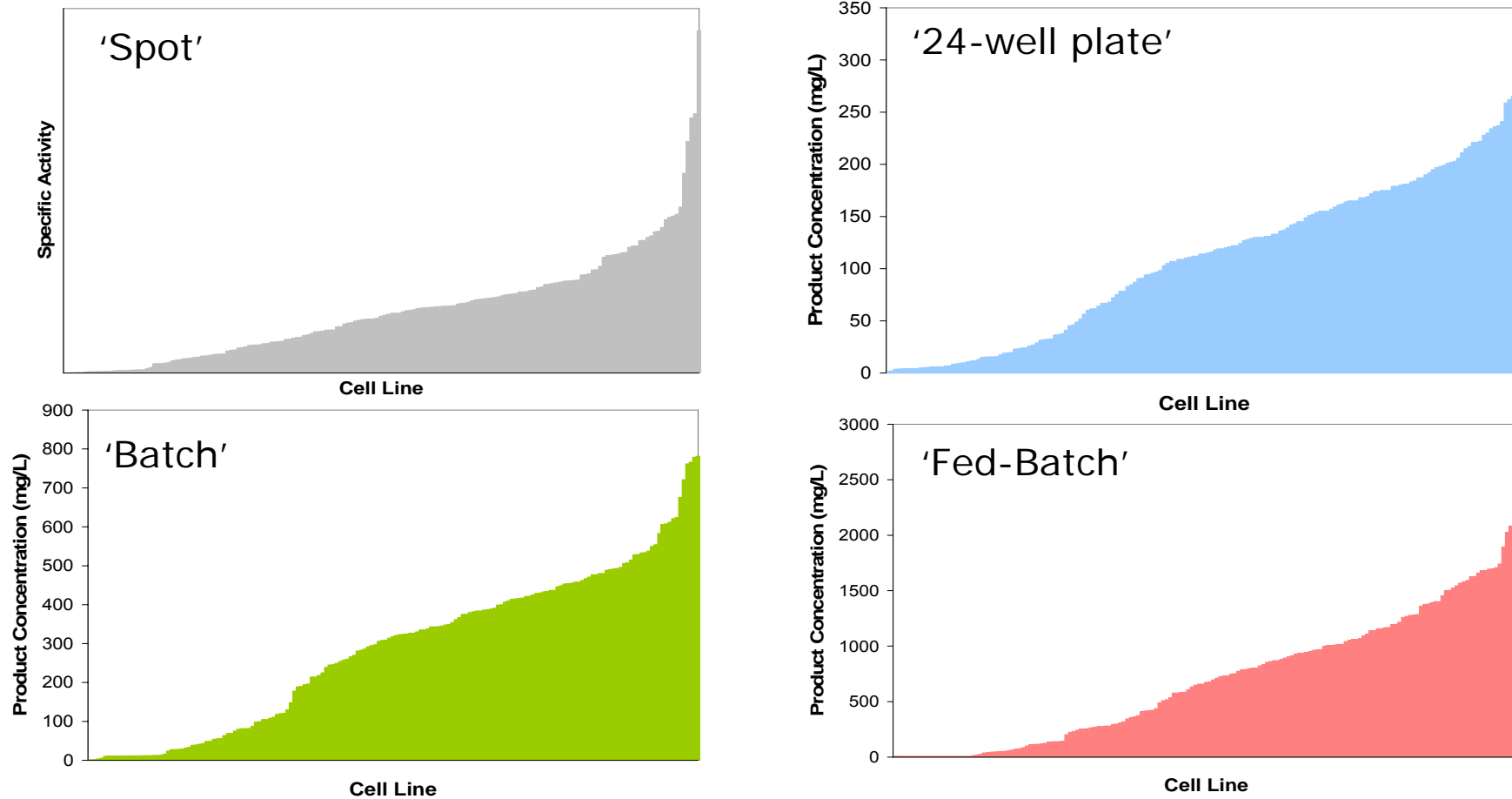


Fed-batch assessment of growth, productivity and product quality
175 cell lines



Productivity assessment ('batch')
175 cell lines

Productivity at Different Screening Stages



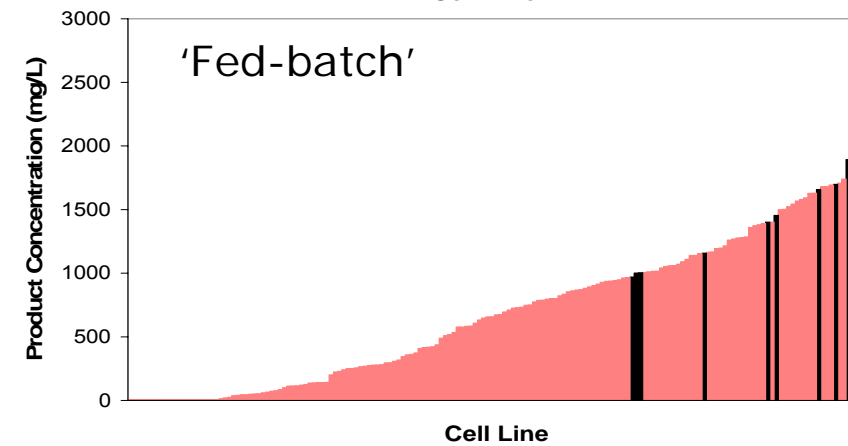
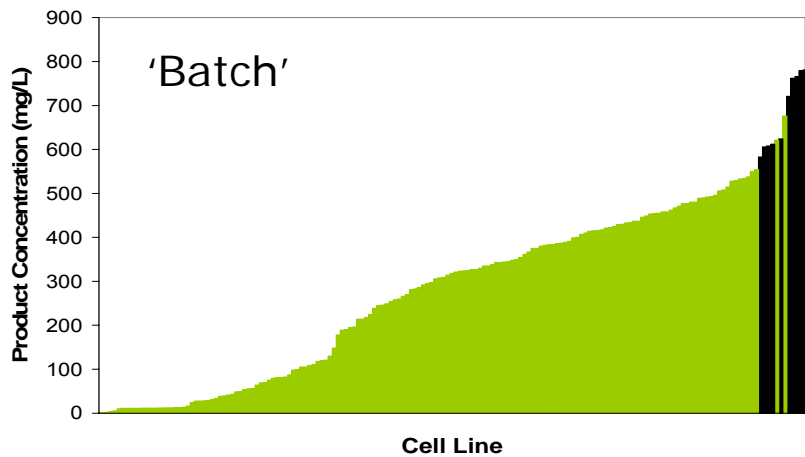
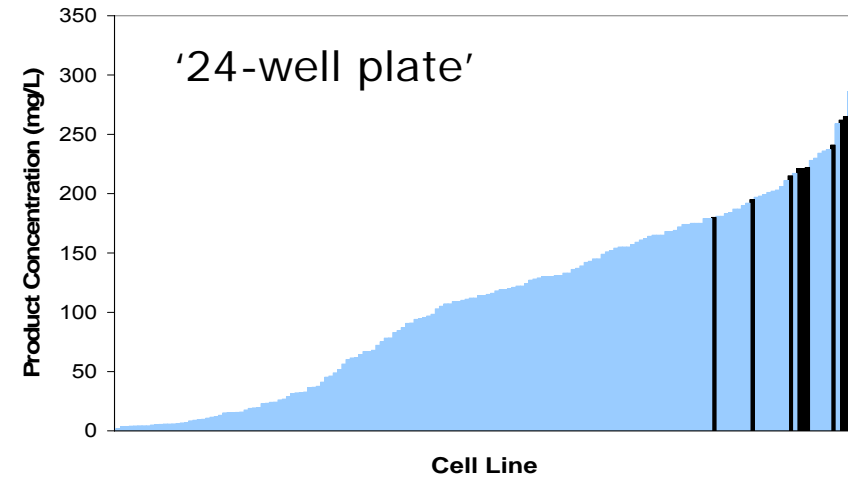
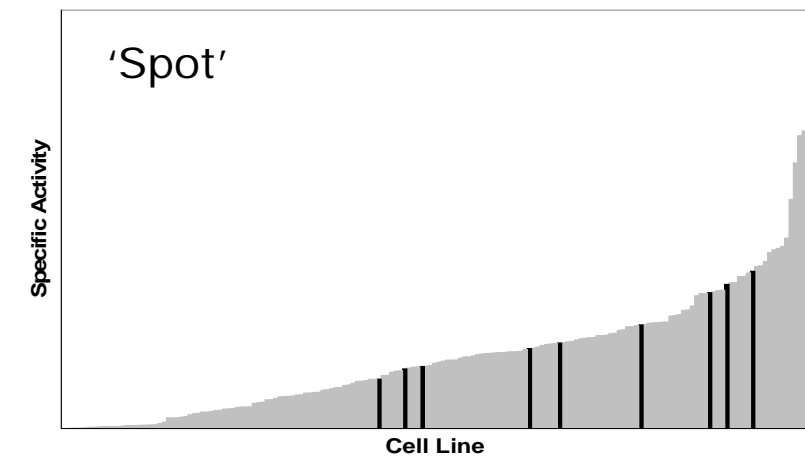
- Wide distribution in productivity observed at each screening stage

Location of 'Selected' Top 10

- Selection strategy used to select 10 cell lines from the 175 cell lines
 - 'Spot': 175 → 120 cell lines
 - '24-well plate': 120 → 60 cell line
 - 'Batch': 60 → 10 cell lines
 - These cell lines designated the 'selected' top 10
 - Would progress to assessment in 'fed-batch' screen

- Where are these ten cell lines at each screen stage?

Location of 'Selected' Top 10



- Current selection strategy identified 3/10 of the highest producers at the fed-batch stage

Responses to Questions

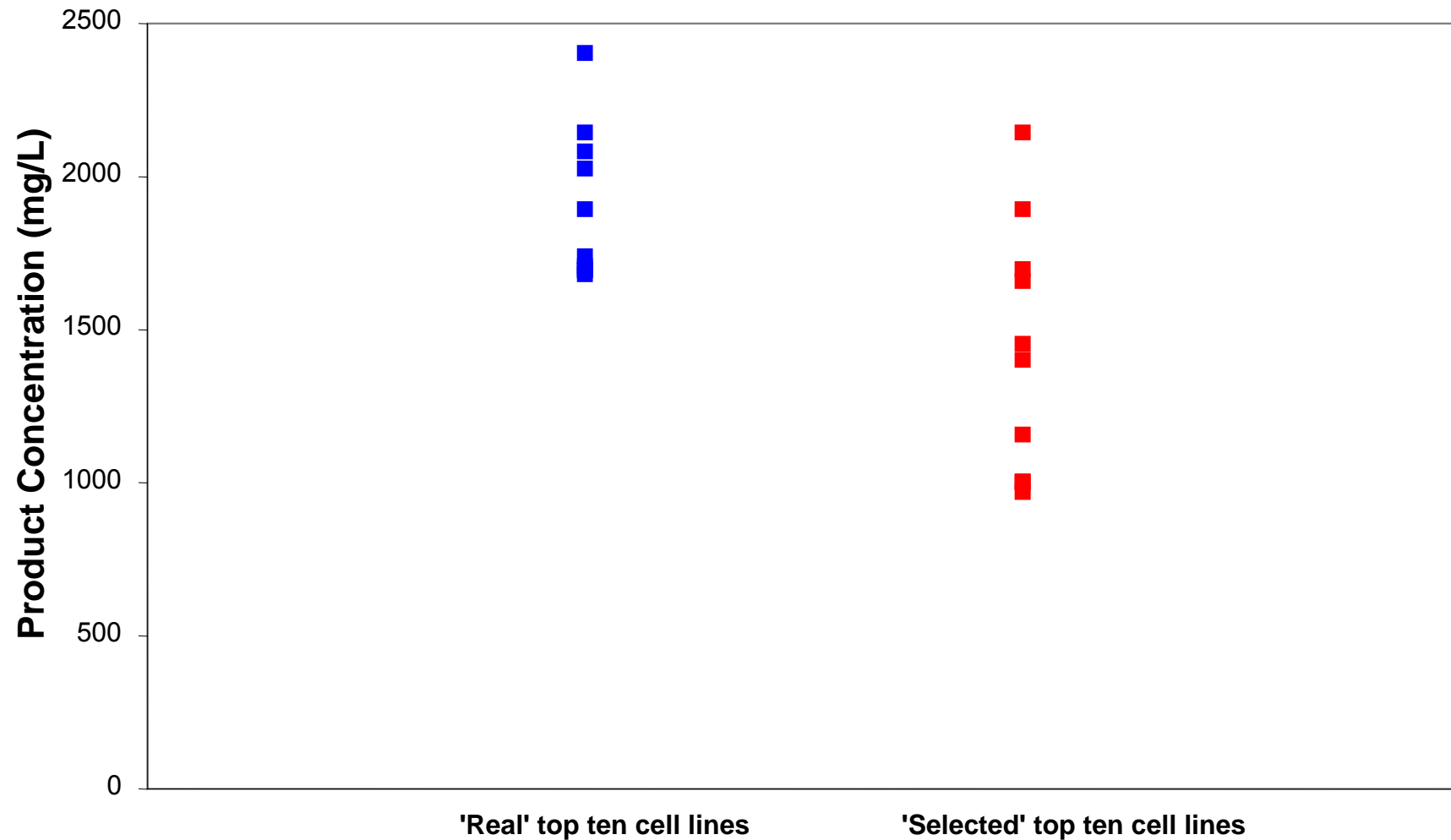
- Does this selection strategy isolate ‘good’ cell lines rather than the ‘best’ cell lines?
 - Selection strategy identifies high producing cell lines
 - Selects cell lines that are in the ‘real’ top ten
 - Does not select all of the ‘real’ top ten

- How predictive are the screens?
 - If measurements made early in cell line construction do not reflect cell line behaviour in the final production process, are we:
 - Discarding cell line(s) at each stage that may do well in the production process **Yes**
 - Retaining cell line(s) that do not do well in the production process **Yes**

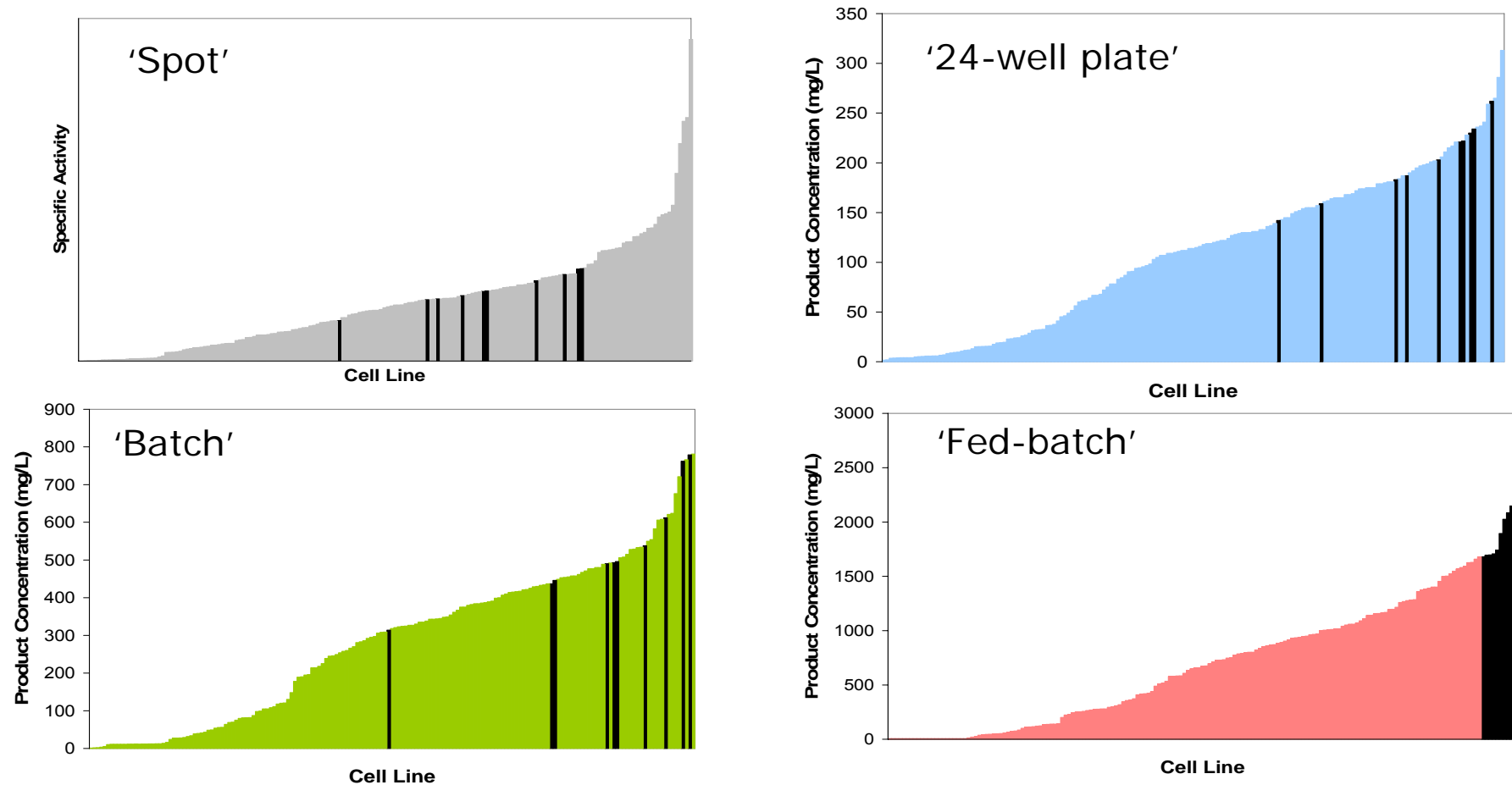
The 'Real' Top 10

- How do the 'real' top ten compare to the 'selected' top ten?
- Where are the 'real' top ten cell lines at each stage?
 - Location of the ten highest producers identified in the 'fed-batch' screen

'Real' vs. 'Selected' Top 10 - Productivity at 'Fed-Batch'



Location of the 'Real' Top 10



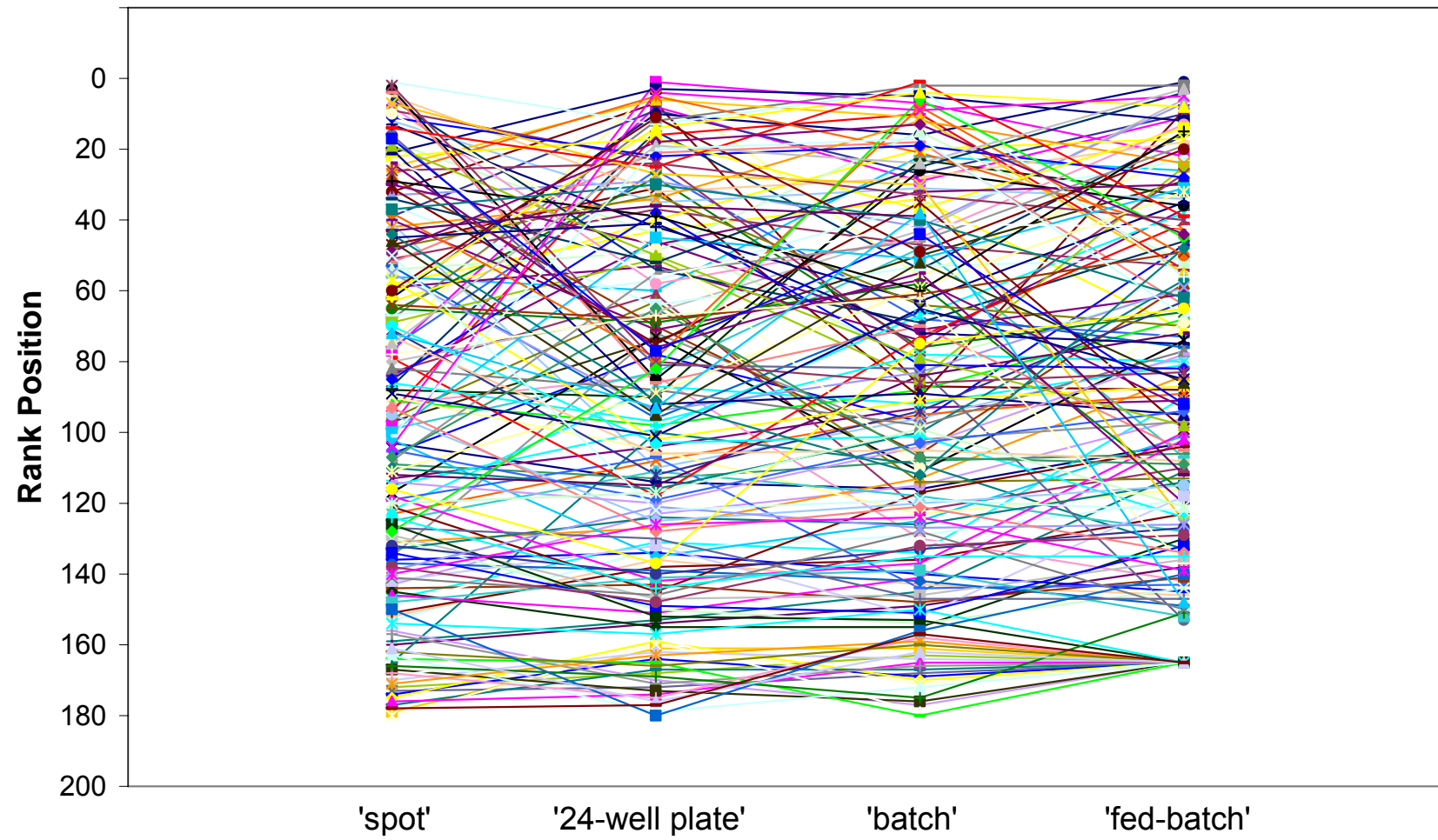
■ As with the 'selected' top ten, the 'real' top ten are widely distributed

How Predictive are the Screens?

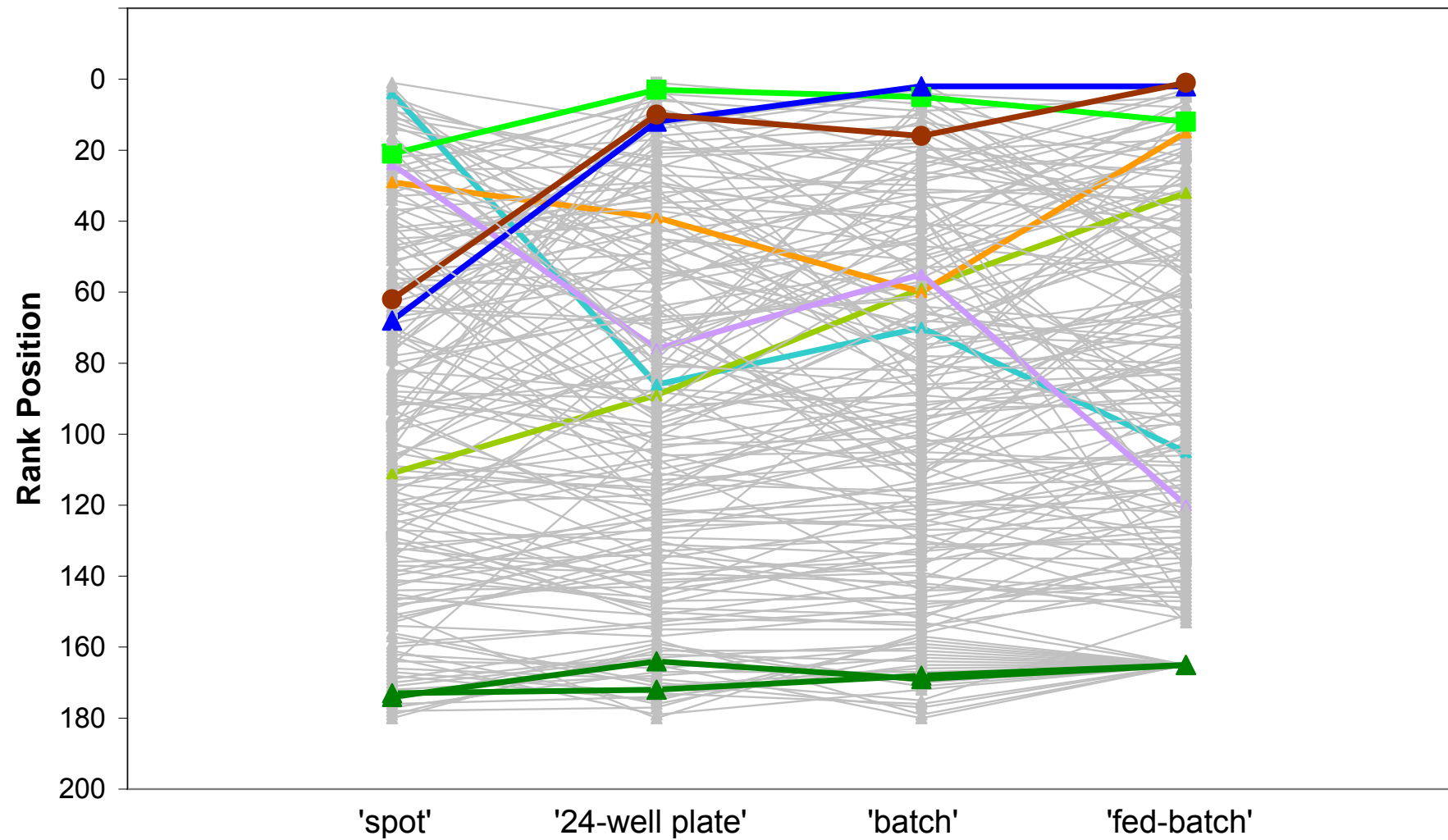
- As rank position is used to select cell lines for progression, examined how this changes between each screen

- Hypothesis:
 - The number of instances where a cell line moves more than x rank places between two successive screens is zero
 - $x = \frac{1}{4}$ of the total number of cell lines assessed in the first of two successive screens if selection strategy is applied
 - 43.75 rank positions between 'spot' and '24 well-plate'
 - 30 rank positions between '24 well-plate' and 'batch'
 - 15 rank positions between 'batch' and 'fed-batch'
 - Historically thought that movement between quartiles is minimal

Ranking



Ranking



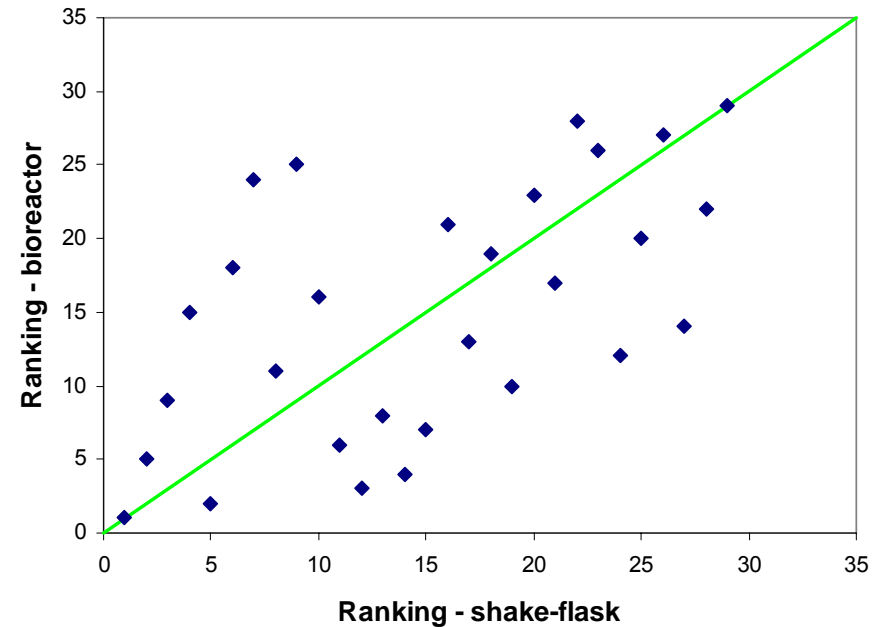
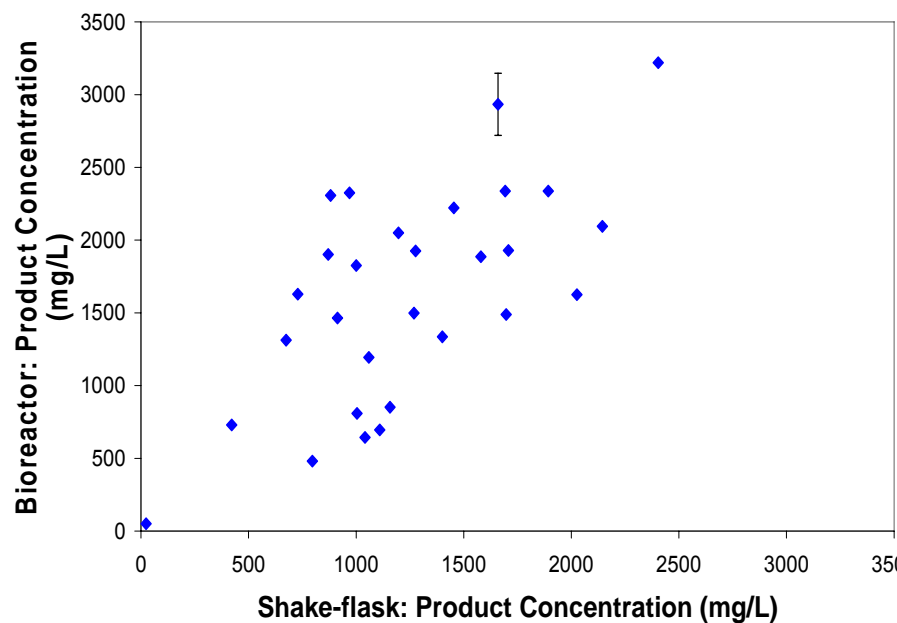
Ranking

- Cell line rank position changes as it moves through the different screens
 - Up and down movement can be observed

- Cell lines which are initially the lowest producers tend to remain as low producers throughout each screen

- Reject hypothesis of zero instances where a cell line moves more than x rank places between two successive screens
 - Percentage of cell lines moving more than the defined number of rank positions:
 - Between spot and 24-well plate = 20%
 - Between 24-well plate and batch = 23%
 - Between batch and fed-batch = 49%

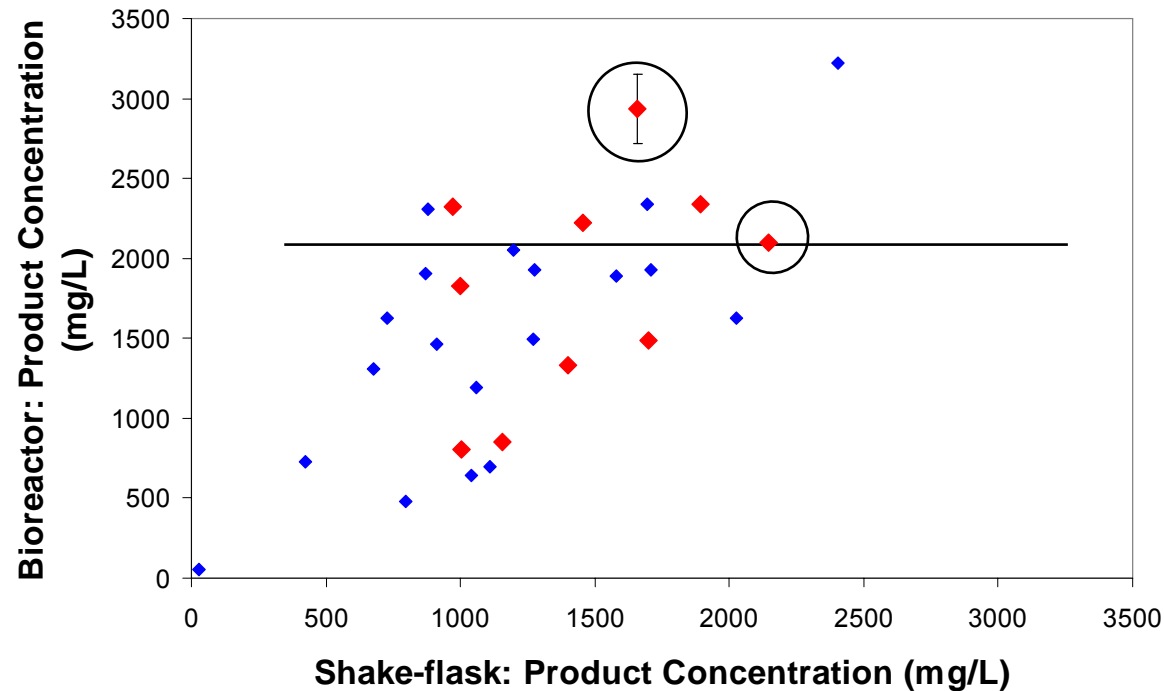
How Did the Cell Lines Behave in Bioreactor Culture?



- 29 cell lines evaluated in bioreactors
- Strong positive association between behaviour in two culture systems
- Generally, high ranking in bioreactor culture reflects high ranking in shake-flasks

Bioreactor Productivities and the Selection Strategy

- ‘Selected’ top ten highlighted:



- A cell line which is highly productive in the final production process can be selected
- Highest producer in bioreactor not identified from shake-flask data using current selection strategy

Can the Cell Line Selection Strategy be Improved?

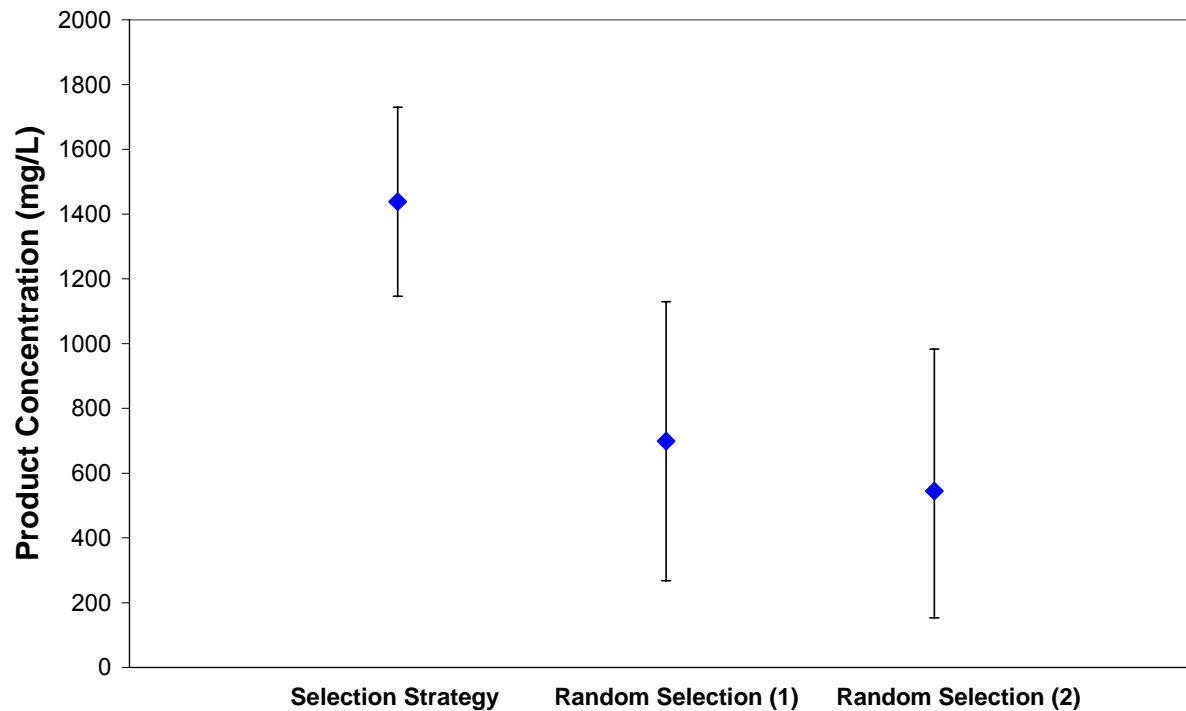
Statistical Modelling

- Is the selection strategy better than random selection for isolating high producing cell lines?

- Can the selection strategy be improved?
 - Initial objective: Can the frequency of isolating the 'real top ten' cell lines be increased, without increasing resource requirement or elapsed time?
 - 'real top ten' = the cell lines which, at fed-batch, are the ten highest producing cell lines in entire population

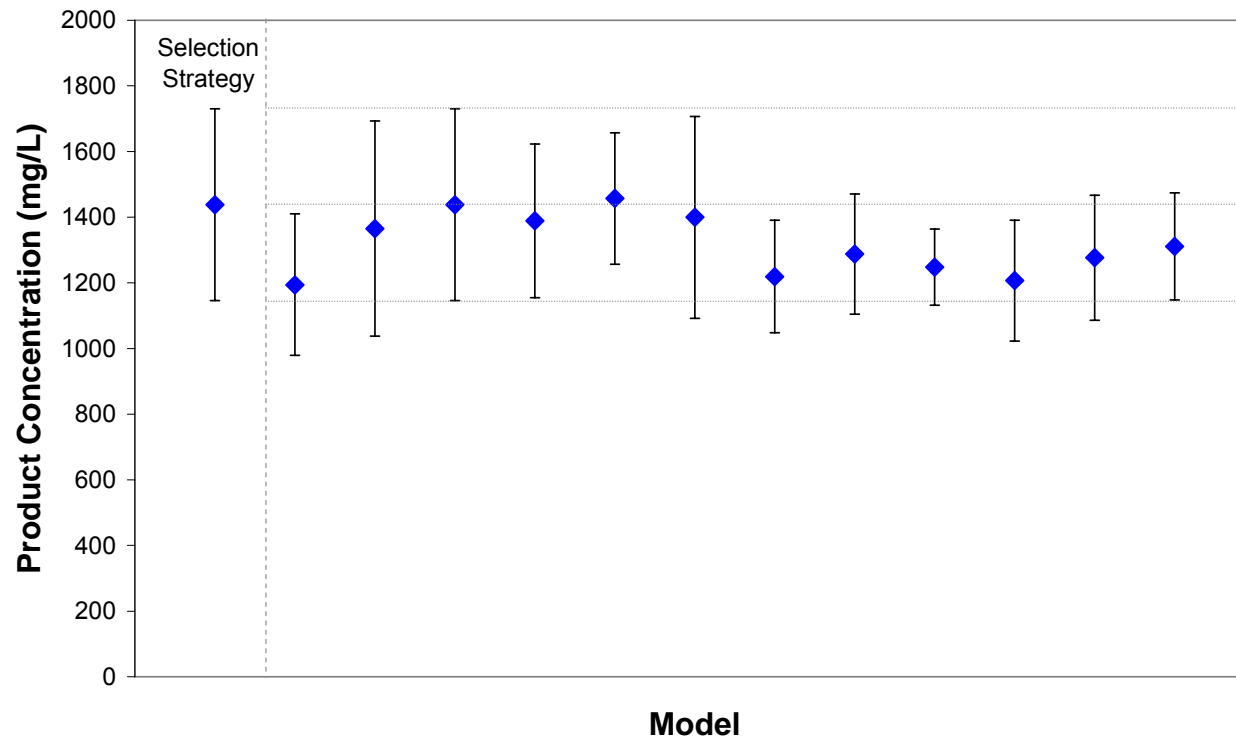
- Using scenarios modelled using discriminant analysis

Selection Strategy vs. Random Selection



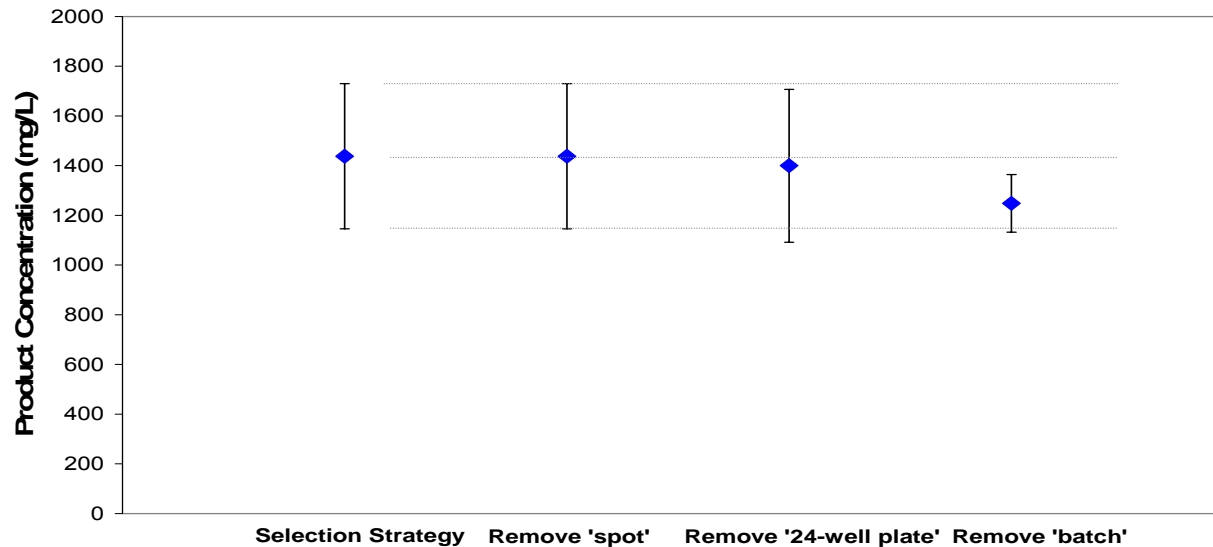
- Current selection strategy better than random selection
 - Higher mean antibody concentration and smaller 95% confidence intervals

Can Alternative Models Improve The Selection Strategy?



- No model tested, except progression of all 175 cell lines, showed substantial improvement cf. the current selection strategy
 - Validates current strategy

Elimination of Assessment Stages



- Lower product concentration range when batch eliminated
- Eliminating 'spot' or '24-well plate' similar to original selection strategy results
 - Risks associated with these scenarios
 - Potential problems with expression not identified early

Questions Raised

- Why isn't frequency of isolating the 'best' cell lines increased?
 - Assessment stages are 'not predictive enough' of the subsequent stages
 - Assessment stages significant predictors of subsequent stages, but not all variation explained (e.g. batch explained approx 75% variation in fed-batch)

- How could the frequency of isolating the 'best' cell lines be increased?
 - More predictive methodology at assessment stages
 - e.g. make environment in early development similar to final production process
 - Identify alternative predictive markers

Statistical Modelling Summary

- Selection strategy better than random selection for selecting high-producing cell lines

- 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'

Summary and the Future

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
- Selection strategy better than random selection
- Only census of all 175 cell lines better than current selection strategy

The Future

- Investigate other strategies to increase the frequency of isolating the 'best' cell lines
 - More predictive methodology at assessment stages
 - e.g. make environment in early development similar to final production process
 - Identify other predictive markers

Acknowledgements

- Lonza Biologics plc, Slough, GB
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 - Juanita Porter
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