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ESTABLISHMENT OF CELL LINES FOR MANUFACTURING RECOMBINANT ANTIBODIES

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- Issues
- Selection of a high producing cell line
- Selection for process compatibility

Cell line development and clinical supply

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- Early phase clinical supply
 - Speed essential
 - Select cell lines that fit manufacturing process
 - Cell line productivity and process economics less important


- Late phase clinical supply
 - Process optimisation required to reduce cost of goods
 - Cell line improvement
 - re-clone cell line, select higher producer
 - re-express product in efficient expression system

- Fifteen licensed rMabs and large number in development
- High dose requirement leads to large volume demand (10's to 100's kg/year)
- Challenge: produce large quantities with cost and time efficiency

The slide features a white background with two large, overlapping, light gray circles. A horizontal gray bar spans the width of the slide, intersecting the circles. On the left side of this bar, there is a small, colorful strip of images showing various scientific or industrial scenes. In the top right corner, the word "Lonza" is written in a bold, black, sans-serif font.

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Selection of a high producing cell line

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- A decorative banner with a collage of scientific and industrial images, including microscopes, test tubes, and laboratory equipment, is positioned below the title bar.
- A high yielding antibody manufacturing process is the result of:
 - Selecting highly productive cell lines
 - Efficient gene expression and stringent selection
 - Cell culture process supporting high viable cell concentration
 - Optimised process
 - Minimising losses in primary recovery and purification
 - Optimised process

High level gene expression

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- Strong promoter to drive expression of product gene(s)
 - Viral, elongation factor
- Increased copy number of product gene(s) that give proportional increase in gene expression
 - Co-amplification of product and selectable marker genes (e.g. DHFR) in presence of cytotoxic drugs (e.g. methotrexate)
 - Lower cell line stability compared to un-amplified cell lines
- Vectors with elements (e.g. SAR/MAR) that create genomic environment for high transcriptional activity
- Targeting of expression vector to genomic hot spot by homologous recombination

Cell line selection: The issues

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- By definition, the transfectants with potentially the highest specific productivities are rare
 - Current approaches are successful in making productive cell lines
 - Specific productivities for antibody-producing GS cell lines: 15 to 65 pg/(cell·h)
 - Production concentrations exceeding 1 g/L are common
- How can the hit rate for finding highly productive cell lines be increased?

Why do I need large numbers of high producers?

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- Selection against process criteria reduces the number of cell lines
 - If only a small pool of high producers, none may be compatible with process

Background: Glutamine synthetase (GS) gene expression system

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- Expression vector encoding product genes plus GS gene, allowing glutamine synthesis
- Selection: combination of glutamine-free medium and GS inhibitor methionine sulphoximine (MSX)
- Only cells with GS gene (and linked product genes) survive

Glutamine synthetase (GS) gene expression system

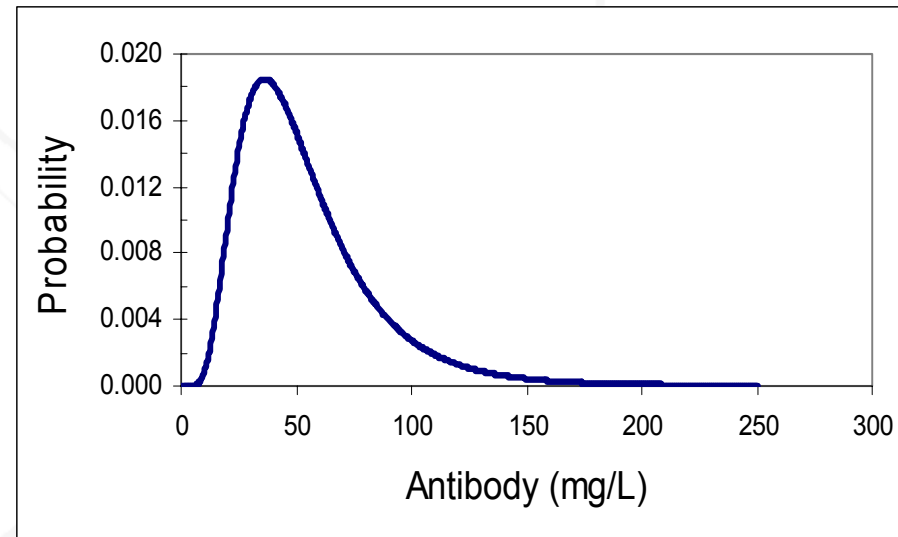
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- Increase selection stringency - use weak promoter on GS gene plus high MSX concentration - selects for rare integration into transcriptionally efficient sites in genome
- Expression of linked product genes, driven by strong promoter, enhanced by favourable integration site

Cell line selection: High producers are infrequent

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- Probability distribution of antibody productivities for primary GS-CHO transfectants (24-well plates)
 - Mean 48 mg/L
- 90% transfectants produce less than 90 mg/L
- 1.5% transfectants produce more than 150 mg/L



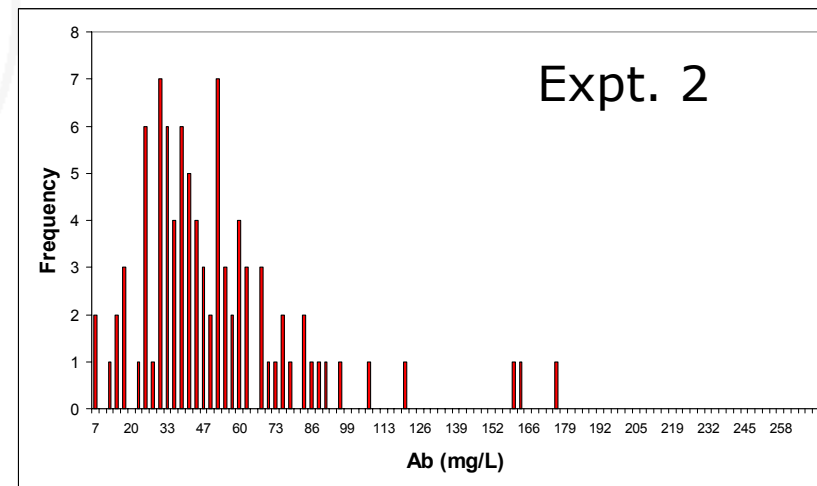
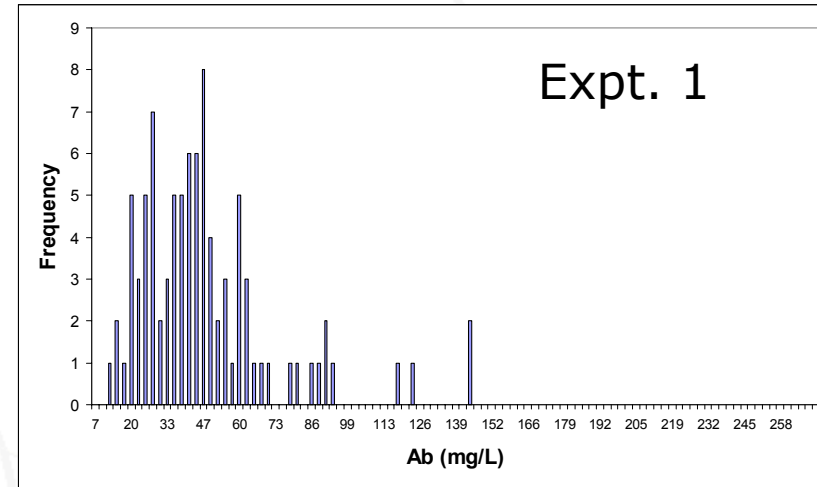
Cell line selection

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- To find these rare events, use a combination of:
 - Transfection method that generates large numbers of stable transfectants
 - maximise the range of productivities
 - Stringent selection to eliminate lower producers
 - High throughput methods, e.g. FACS + cell surface product capture, to screen large numbers efficiently

Cell line selection: The numbers game

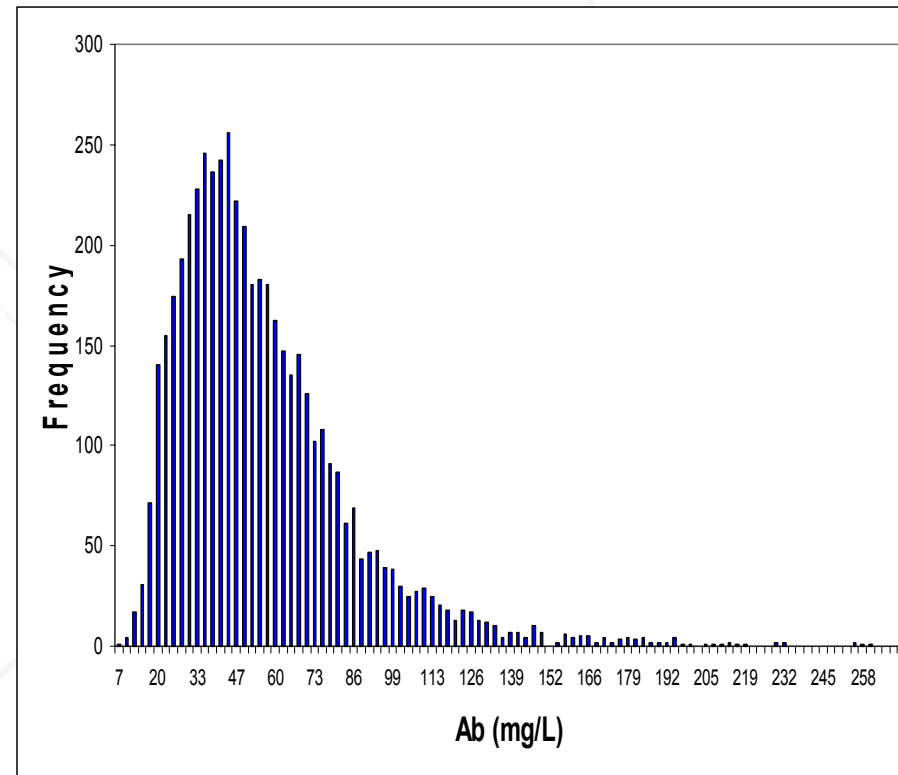
- Expt. 1: 0/92 GS-CHO transfectants produce more than 150 mg/L
 - Mean: 49 mg/L
- Expt. 2: 3/92 GS-CHO transfectants produce more than 150 mg/L
 - Mean: 46 mg/L
- Replicate experiments may (or may not) reveal transfectants making more than 150 mg/L



Cell line selection: The numbers game – how many?

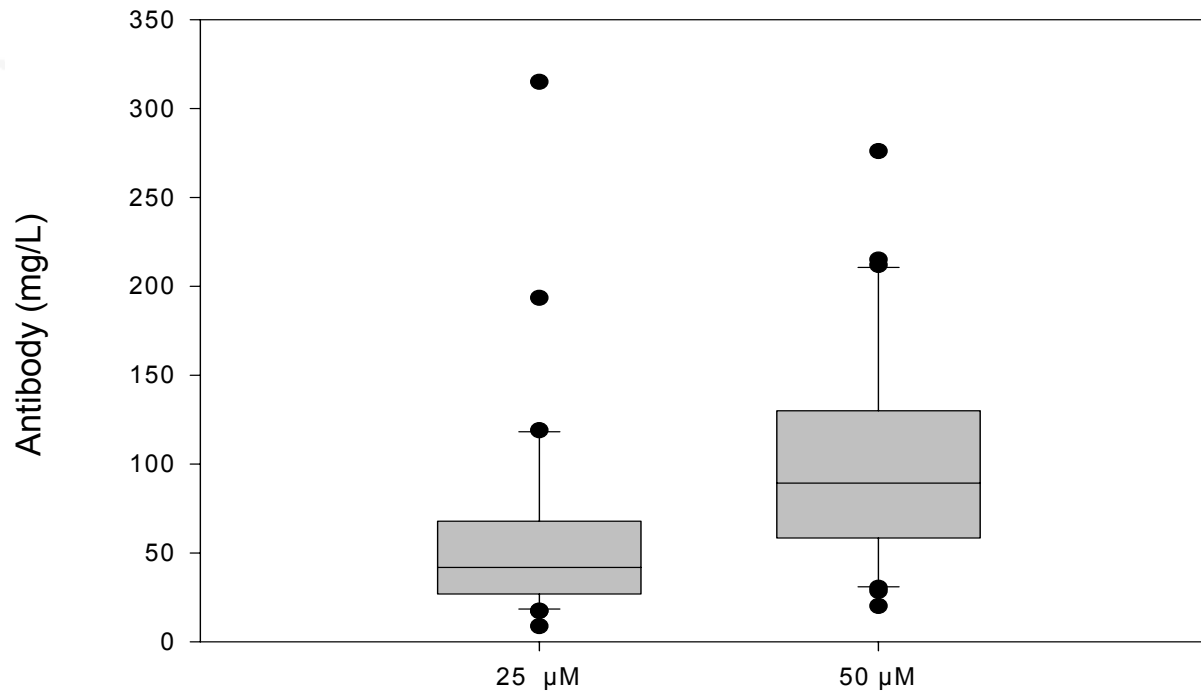
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- Simulated screen of 5000 transfectants
- 75/5000 make more than 150 mg/L
 - Mean: 48 mg/L
- Screen large numbers of transfectants
 - How many?
 - Function: current and target concentrations; shape of distribution
- Alternatively, eliminate lower producers



Cell line selection: Selection stringency

Influence of selection conditions for GS-CHO cell lines with cB72.3 antibody

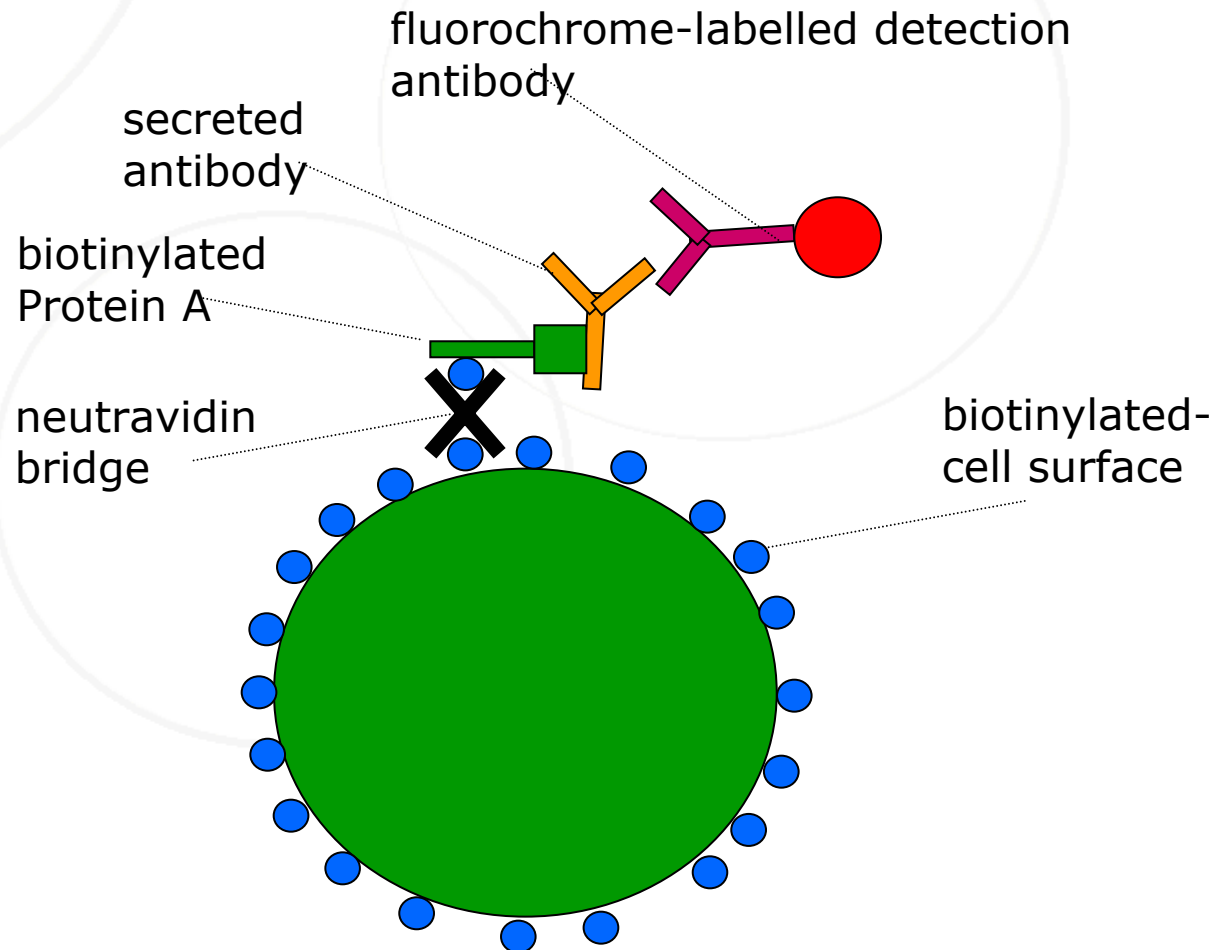


Cell lines have not been amplified.

Selection conditions - MSX concentration

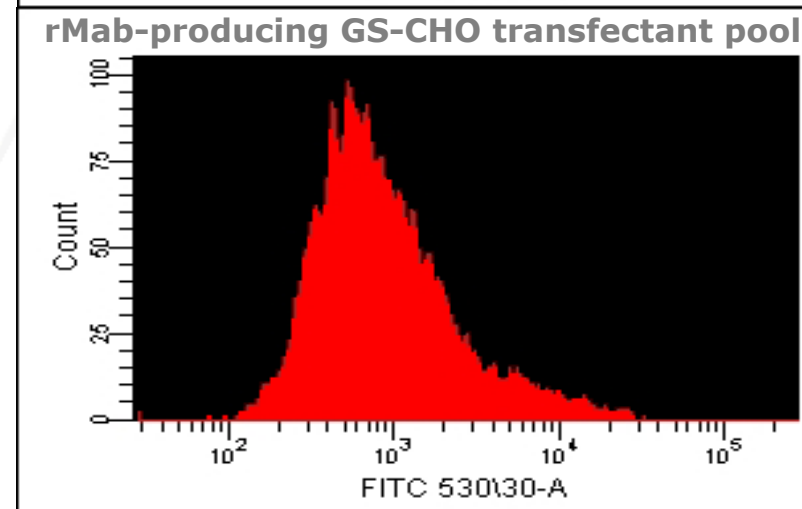
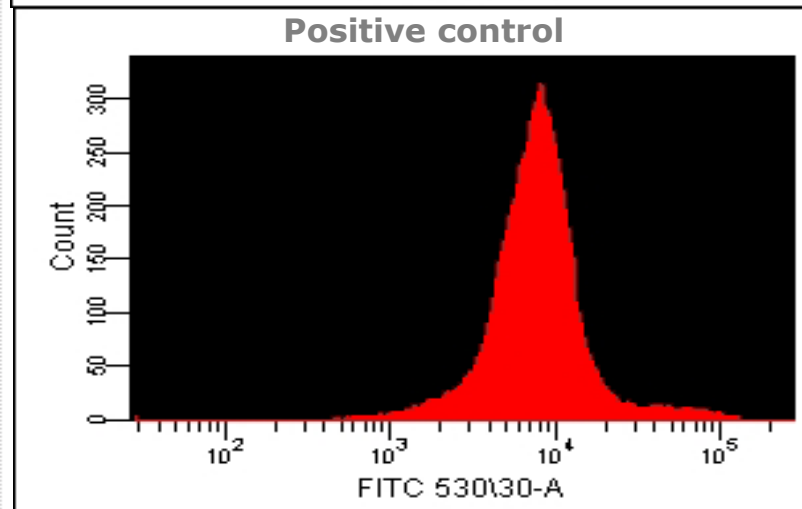
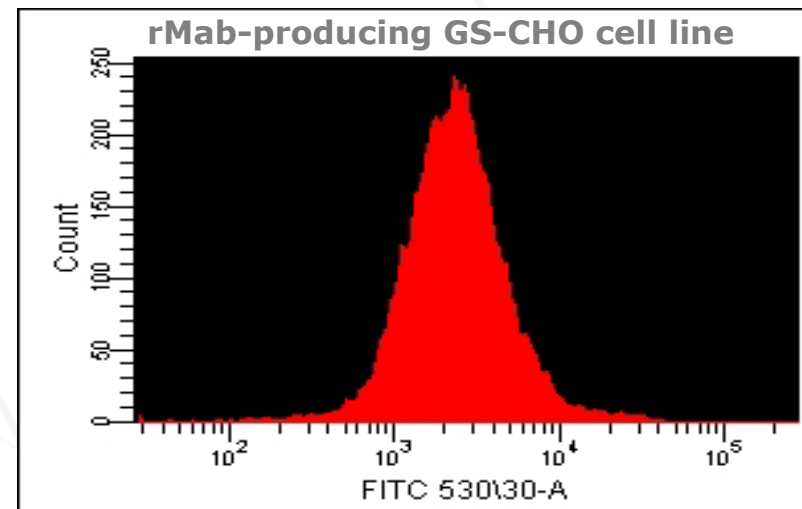
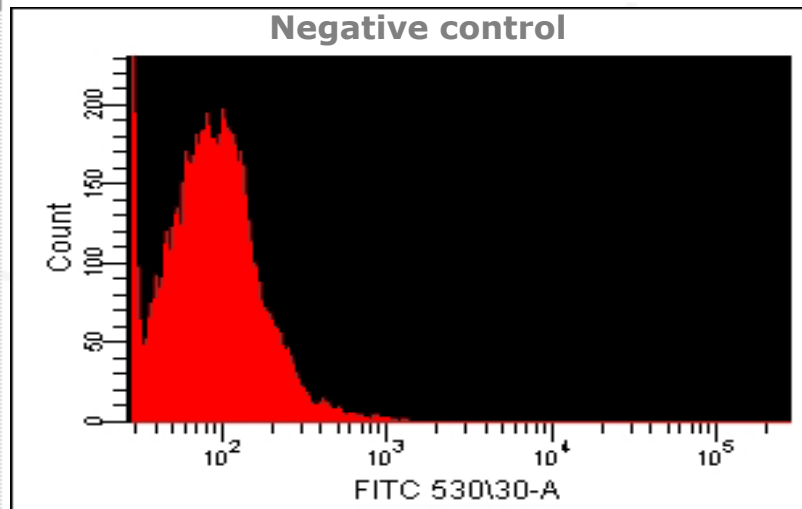
FACS-based methods: Affinity-matrix surface capture (AMSC)

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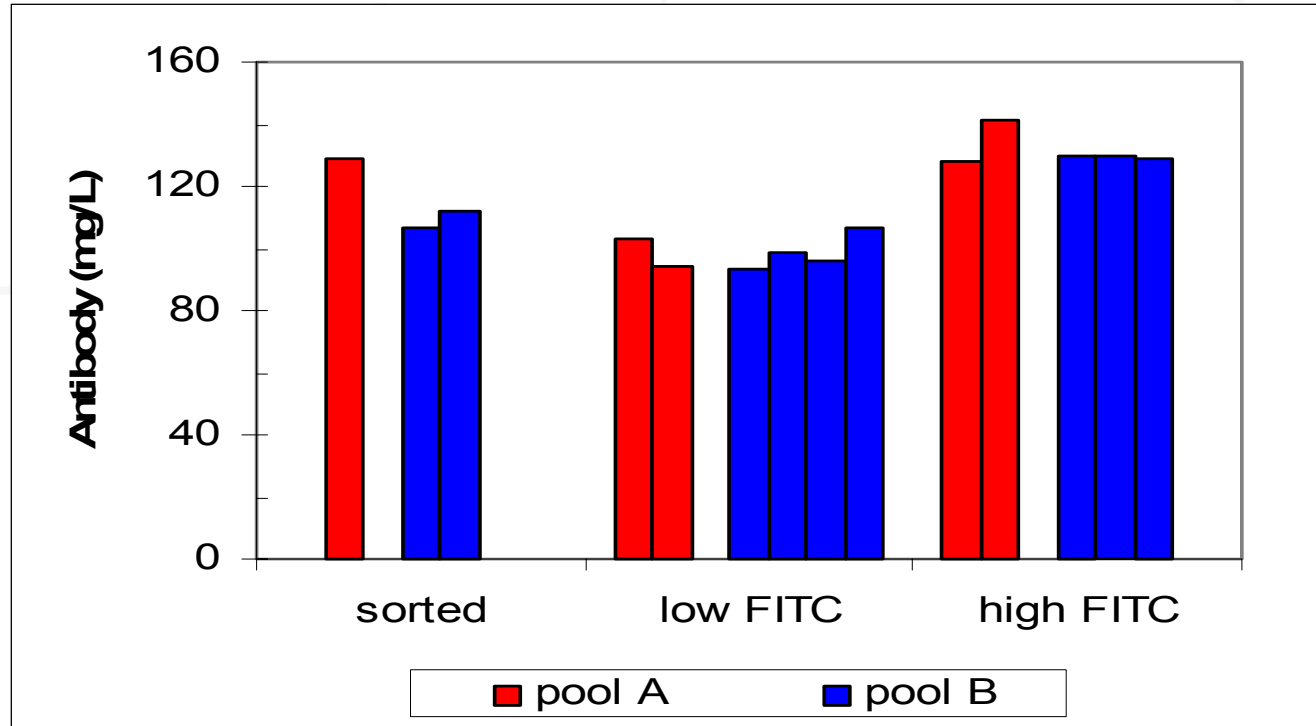
Flow cytometric analysis of AMSC-labelled GS-CHO cells

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Productivity of AMSC-labelled and bulk sorted GS-CHO transfectants

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- Two transfectant pools labelled by AMSC method
- Sorted on FITC signal (high, low) or viability (sorted)
- Significantly more antibody from high FITC population

A horizontal strip of microscopic images showing various cell cultures and laboratory equipment, located below the title bar.


Summary

- Large number of transfectants required
- Variety of approaches to finding high producers in transfectant pool
- Increasing the stringency of the selection conditions substantially increases the median antibody productivity
- Further enrichment possible by FACS-based methods

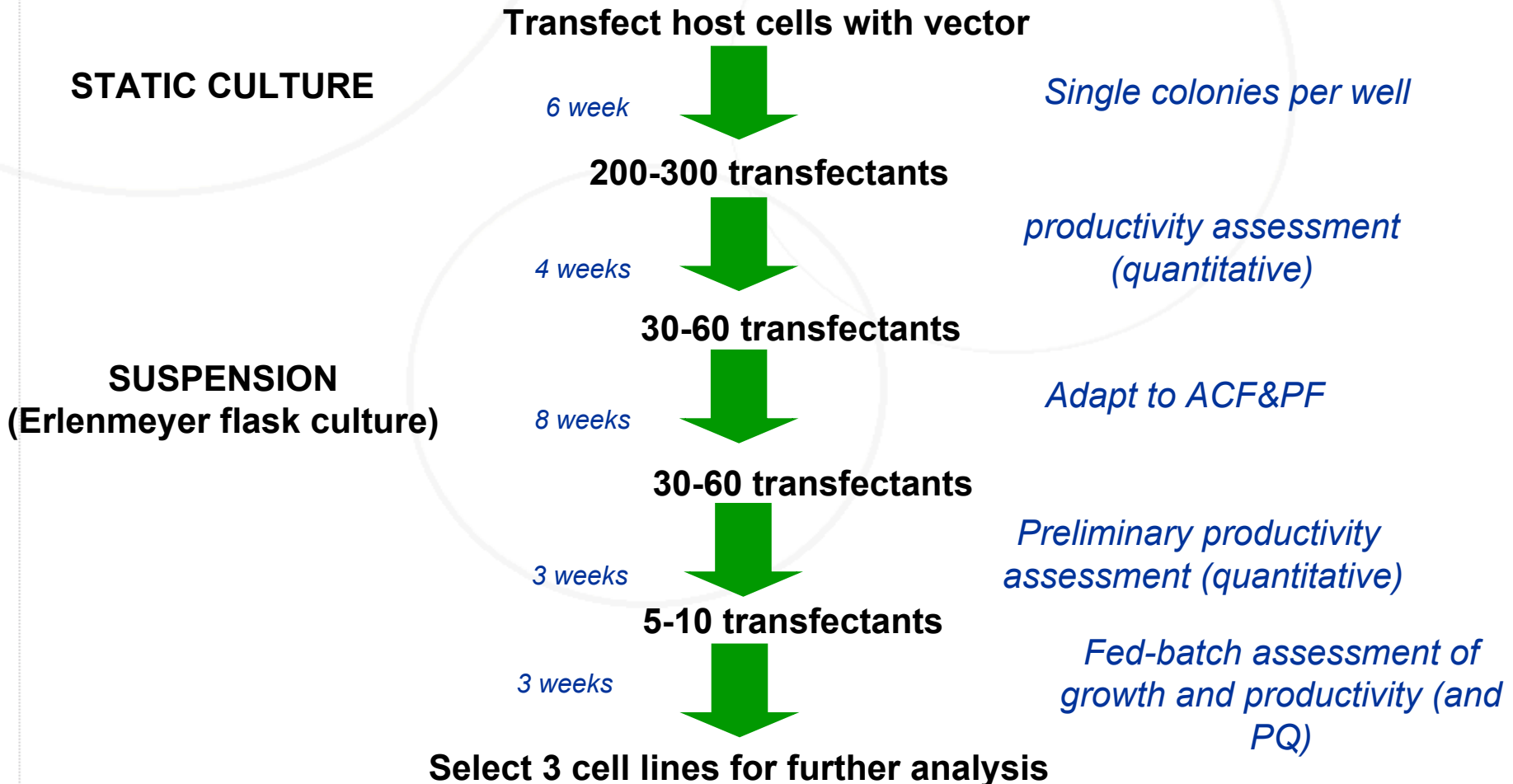


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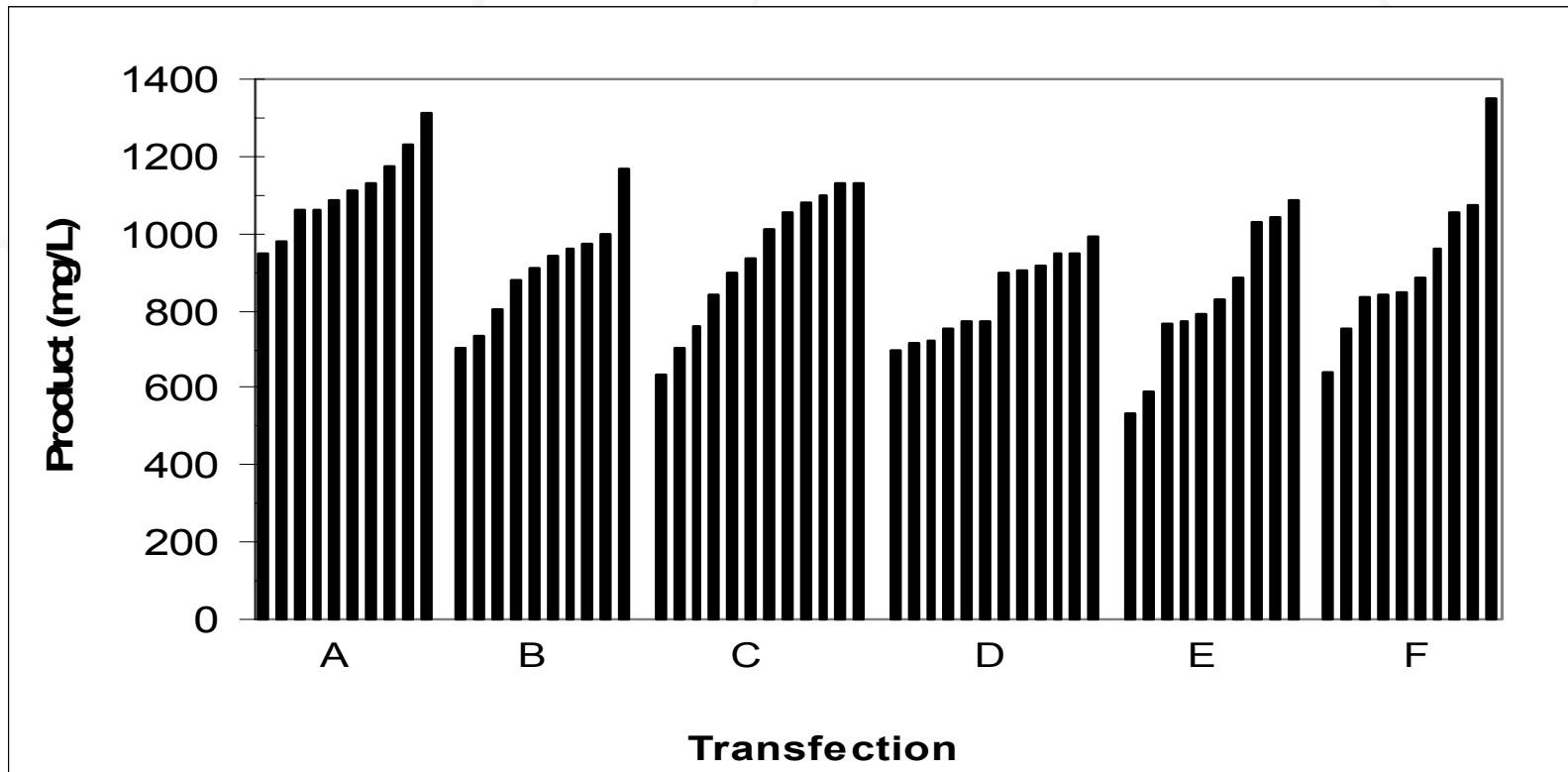
Selection for process compatibility

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- A decorative horizontal bar is located below the title, featuring a collage of images related to biotechnology, including microscopes, test tubes, and laboratory equipment.
- Example of minimum process characteristics
 - Suspension culture
 - ACF&PF medium
 - Sub-culture by dilution, at 1:7 to 1:10 ratio
 - Specific bioreactor configuration and operating parameters
 - To minimise project timelines, cell line development programme should include evaluation against process characteristics

Selecting high producing cell lines: Cell line ranking changes as go through process

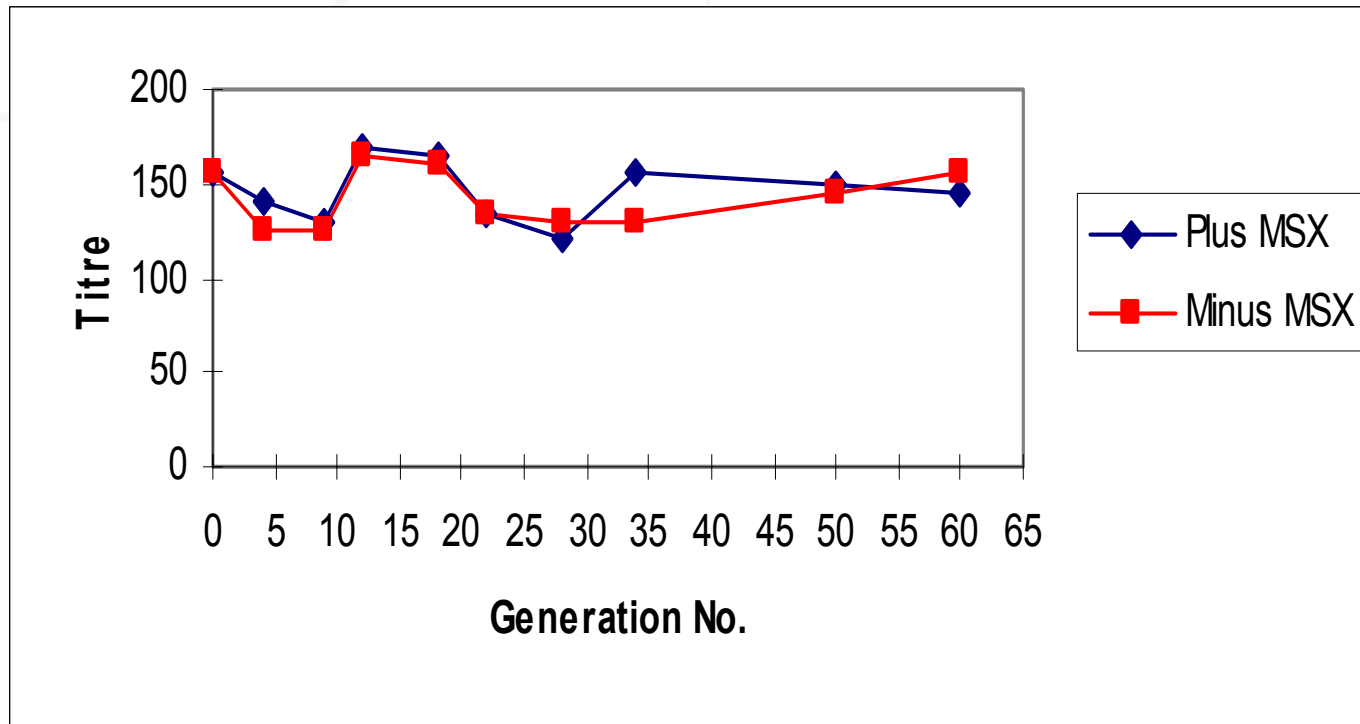


GS-NS0: productivity distributions for different transfections in fed-batch, Erlenmeyer flask culture



Highest ranked cell line will not necessarily be same as highest ranked cell line at 96-well plate stage

Selection of production cell line – consistent product synthesis



What further analysis?

- Key steps include:
- Evidence of clonality required by regulatory authorities
 - Limiting dilution cloning commonly used
- Evaluation in scale-down model of bioreactor process proposed for manufacturing
 - Are product concentration and product quality criteria met?

Selection of production cell line

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- Consistent product integrity and acceptable bioactivity
- Consistent cell growth in production process
- Consistent high volumetric productivity

- Highest producers are rare
- Number of approaches to increasing discovery hit rate
 - Screen large numbers
 - Enrichment
- Select cell lines to fit manufacturing cell culture process
 - Scale-down models
 - Multiple selection criteria