

Advances in The Development of Biopharmaceuticals



The application of modern technologies and services to the development of Biopharmaceutical processes

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The New Challenges in Biopharmaceutical Development

Increased Numbers



Reduced Time

Decreased Cost's

Potential Solutions to these Challenges



Automation



Platforms

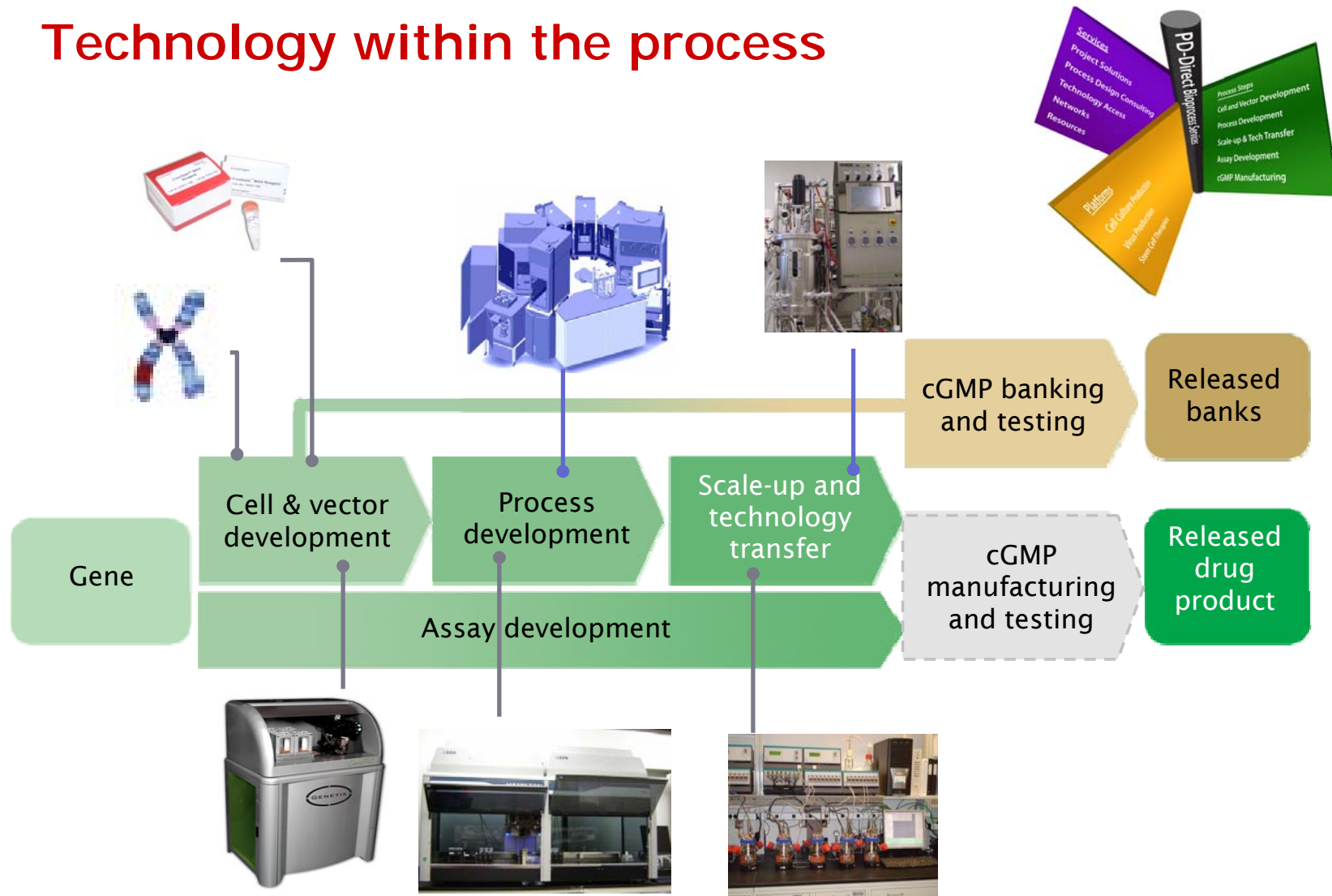


Outsourcing

Invitrogen's Approach to These Challenges

- Develop lock and key products to shorten the development cycle.
 - Kits to Simplify Workflows.
 - Innovative products to enhance development projects
- Offer outsourced services based upon new technologies and products.
 - Cell Line Development
 - Medium and Process Development
- Develop a Platform For CHO Cell Lines
- Invest in Automation
 - ClonePix^{FL}
 - SimcellTM
- Invest in innovative technologies such as Revolution

Technology within the process

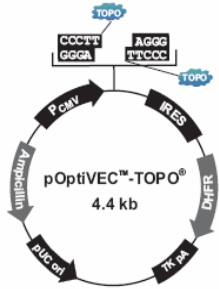


Examples of Our Approach

- Development of the Invitrogen CHO Platform and CLD kits.
- Use of the Simcell for automated Process Development

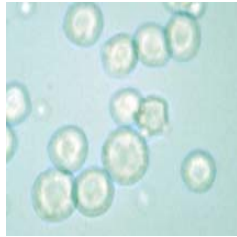
The Invitrogen CHO Platform and cell Line Development Service





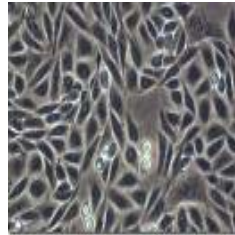
Molecular biology

Vector design and optimisation / Transfection kits
 GIBCO OptiCHO Antibody / Express Kit



Cell lines

Pre-adapted DG44 (Stable Expression),
 HEK293 and CHO-S (Transient Expression)



Dedicated serum-free cloning media

2xOptiCHO™ Cloning medium
 Custom cloning media



Dedicated CD Media
 CDCHO / CD OptiCHO

Feeds for fed-batch

CHO CD EfficientFeeds™
 Foamaway™



Invitrogen CHO Platform



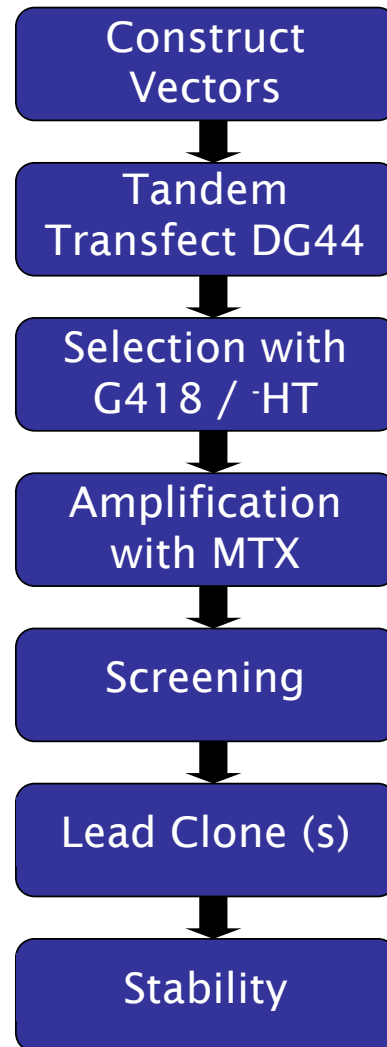
invitrogen™

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OptiCHO Antibody Express Kit

- Contains
 - all of the elements required to construct, clone and produce the vectors
 - Cell line
 - Medium for transfection and selection (with and without HT)
 - Protocols

Process Flow

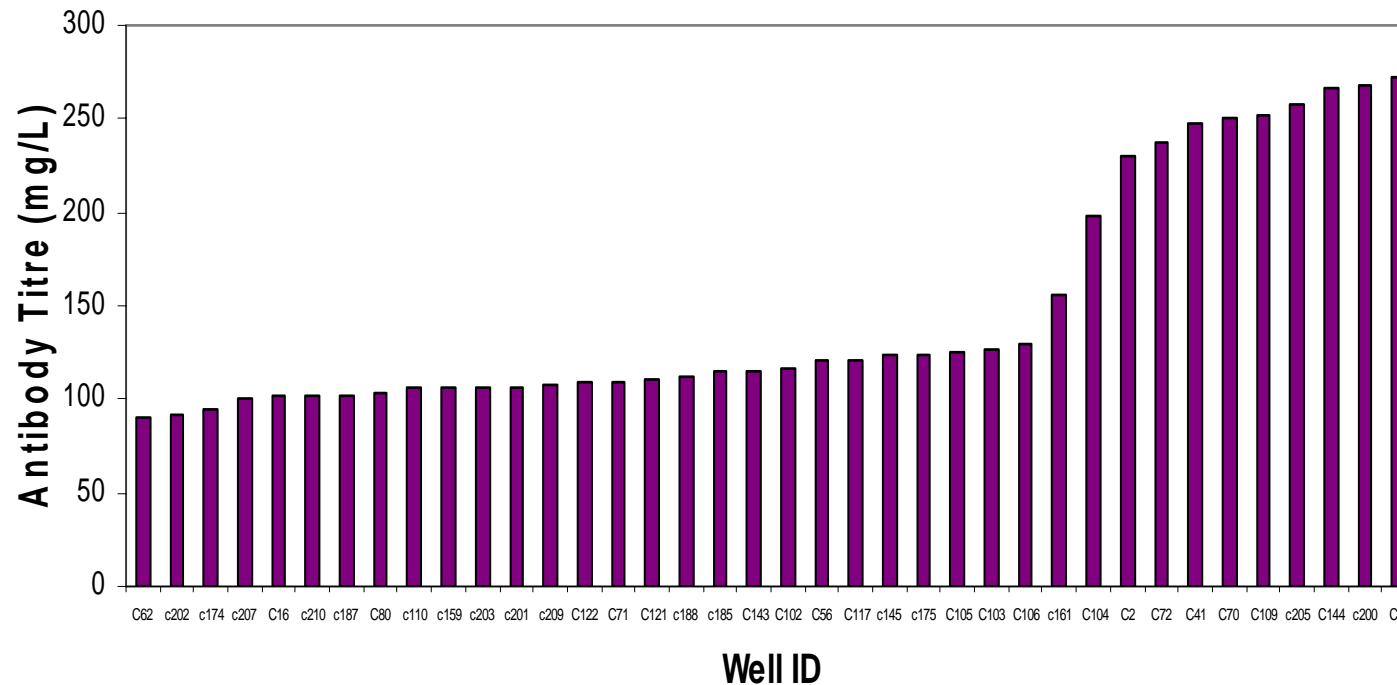


Case Study – Generation of Cell Lines Expressing Recombinant IgG

- Serum and suspension adapted DG44 CHO
- pOptivec vector tandem transfection
- OptiCHO Antibody Express Kit used for transfection and cloning
- Serum free transfection and cloning using lipid based transfection reagents.
- Top forty clones evaluated in a shake flask

Cell Line Development using OptiCHO Antibody Express

Antibody Expression from a DHFR DG44 CHO



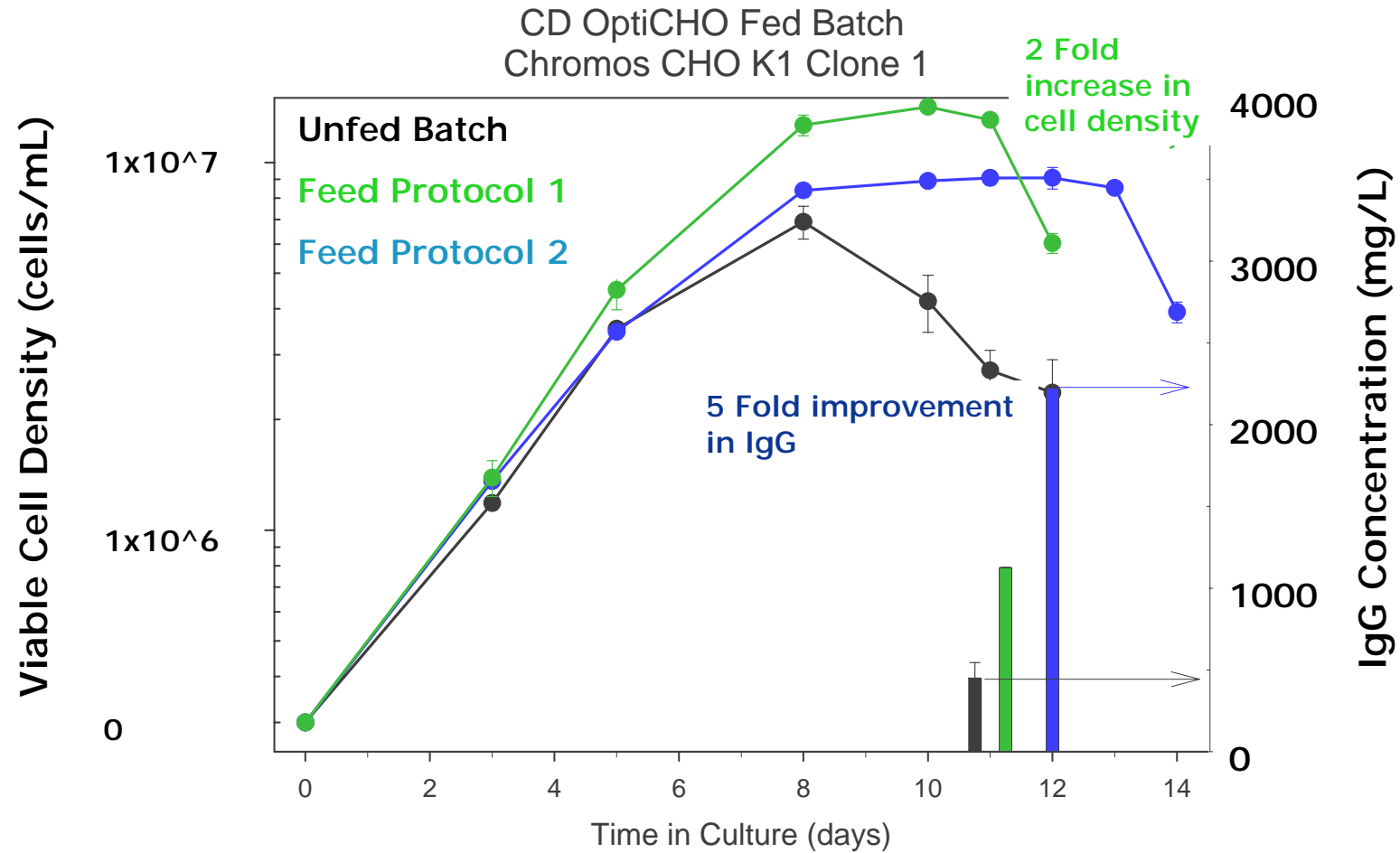
**Cell pool transfected, selected and amplified.
Clones screened by FACS. Top 40 clones isolated.
Clones screened in batch in shake-flask culture
9 clones express over 200 mg/L in an unfed shake-flask**



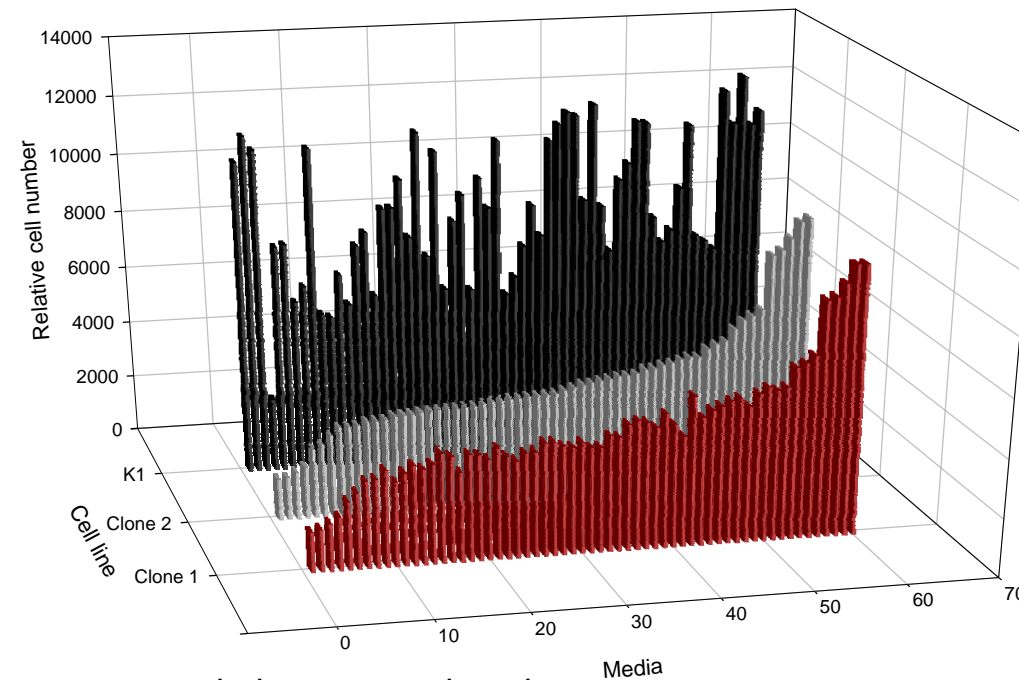
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Enhancing Screening by Process Optimisation

Chromos CHOK1 SV Line Expressing IgG



Response of three cells lines to a Chemically Defined Cloning Medium.



Relative response to 55 panel plus 5 control media.

Response to Clone 1 was used to rate the panel (medium resulting in the lowest to highest proliferation). The other two cell lines were rated based on the Clone 1 response. The two clones respond to the same media in a similar fashion while the third cell line demonstrates a different pattern of response to the same media.

Application of Simcell to Process Development

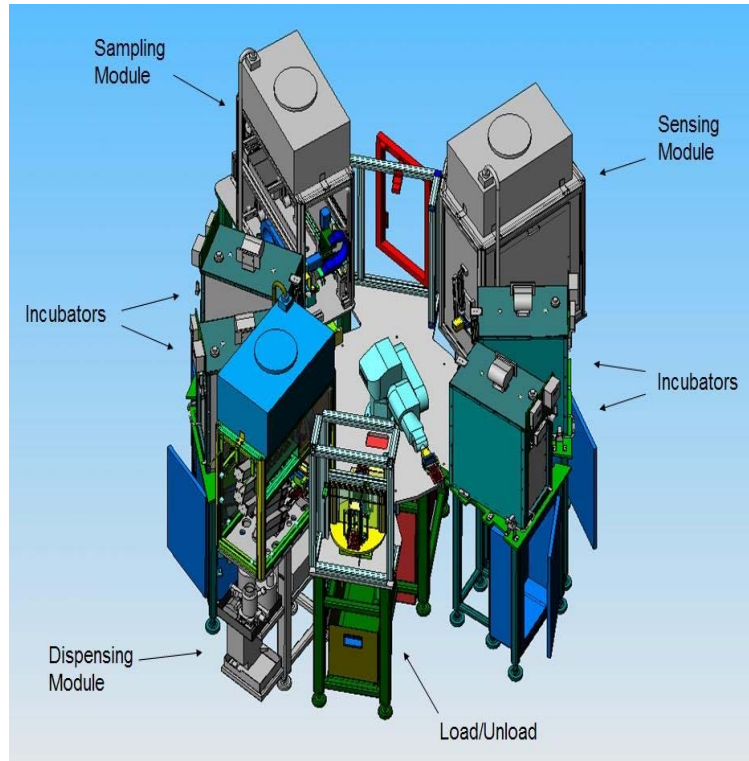


The Simcell™ MicroBioreactor Array (MBA)



- 6 MicroBioreactors per array
 - Working volume: 550 - 700 μL
 - Proprietary gas permeable material
 - Has supported numerous mammalian cell culture types
 - Densities achieved have been up to 2×10^7 cells/mL
 - Practical densities to $\sim 1.2 \times 10^7$
-
- Air bubble traverses perimeter of MicroBioreactors
 - Creates mixing effect / mass transfer

The SimCell™ System at Invitrogen



- Incubators
 - Each holds up to 42 MicroBioreactor Arrays
 - T, CO₂, O₂ and agitation control
- Sensing module
 - Biomass
 - pH
 - DO / Glucose (in mid-2007)
- Sampling module
 - Sample removal to well plates
 - “Sim-fed batch”, “Sim-pH” corrections
- Dispensing module
 - Eight pumps for experimental factors
 - Media / Feed delivery
 - Cell Source delivery
 - pH adjustment

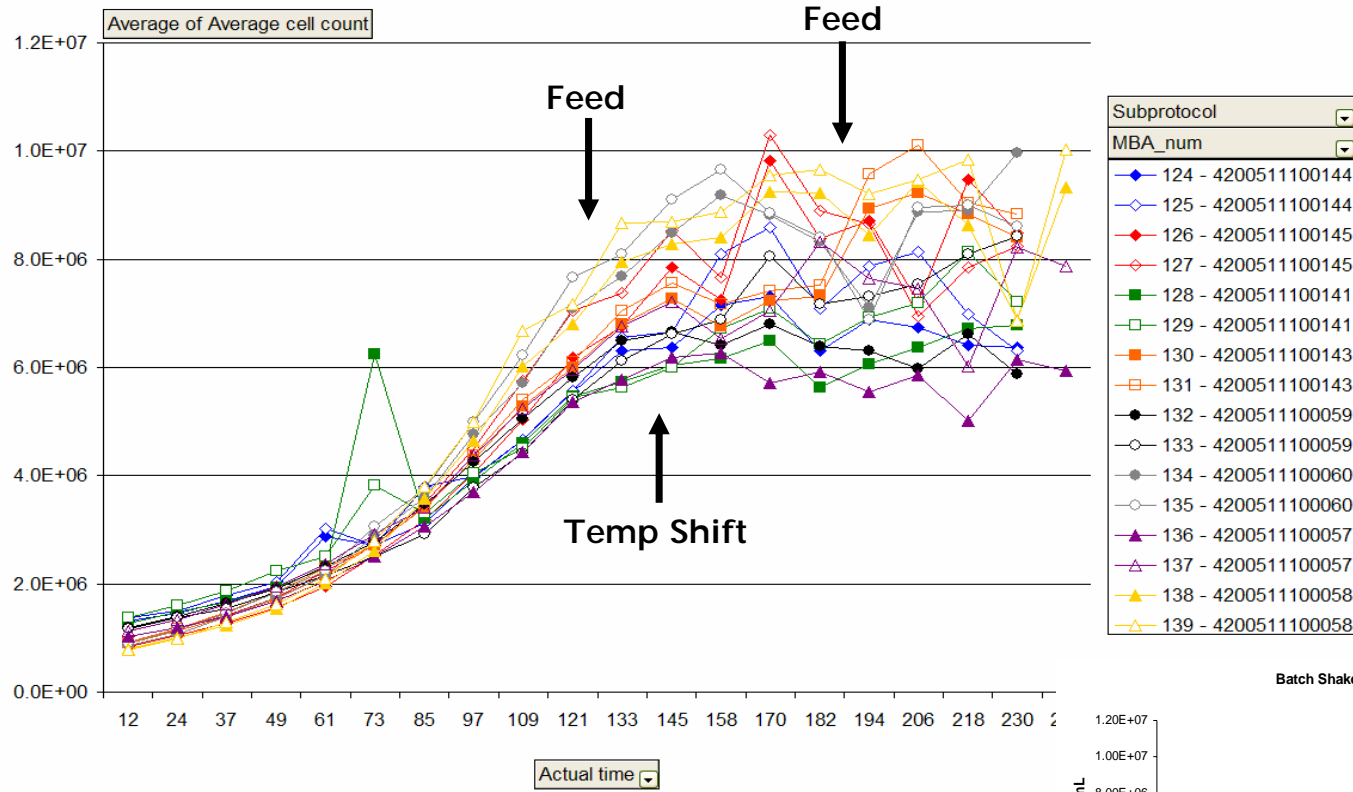
- Miniaturization enables larger, more thorough DoE
- Finding robust media and process solutions faster

CHO – IgG Fed Batch Study

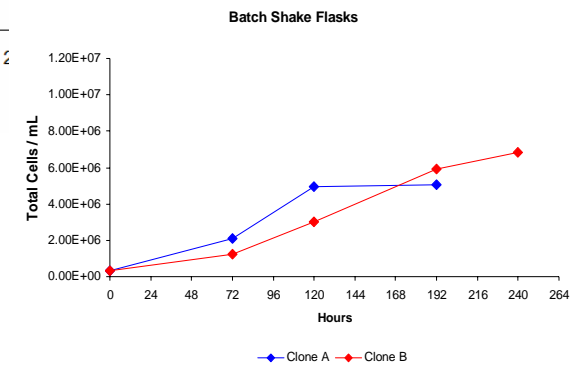
- 4-Factor 2-Level full factorial
 - Cell line
 - pH
 - Feed type
 - Temperature shift
 - CD CHO as production medium

Subprotocol	Clone A	Clone B	Gluc-Gln-Glut Feed	5X CD CHO AGT	Final Temp	pH Setpoint
124	600	0	60	0	37	6.95
125	600	0	0	60	37	6.95
128	600	0	60	0	33	6.95
129	600	0	0	60	33	6.95
132	600	0	60	0	37	7.15
133	600	0	0	60	37	7.15
136	600	0	60	0	33	7.15
137	600	0	0	60	33	7.15
126	0	600	60	0	37	6.95
127	0	600	0	60	37	6.95
130	0	600	60	0	33	6.95
131	0	600	0	60	33	6.95
134	0	600	60	0	37	7.15
135	0	600	0	60	37	7.15
138	0	600	60	0	33	7.15
139	0	600	0	60	33	7.15

CHO - IgG Fed Batch Study



- Enabling diversity in growth patterns



Increasing Titer via DoE

- 2-fold increase over batch titer
- Relatively simple, small design

Design-Expert® Software

Final Titer

- D- -1.000
- ▲ D+ 1.000

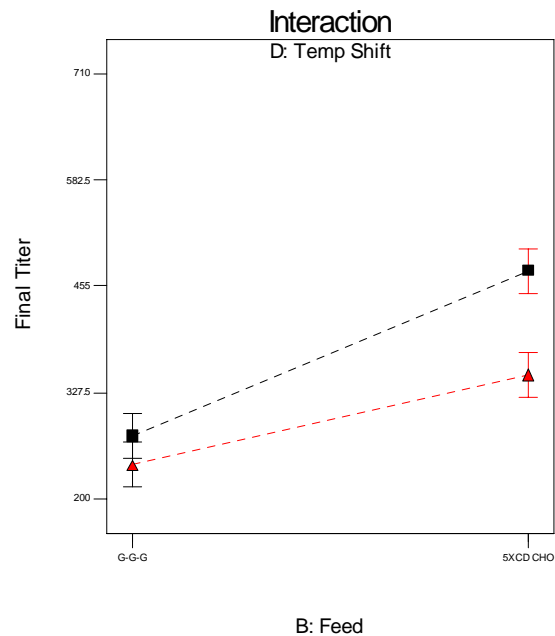
X1 = B: Feed

X2 = D: Temp Shift

Actual Factors

A: Cell Line = Line A

C: pH = 0.00



Design-Expert® Software

Final Titer

- D- -1.000
- ▲ D+ 1.000

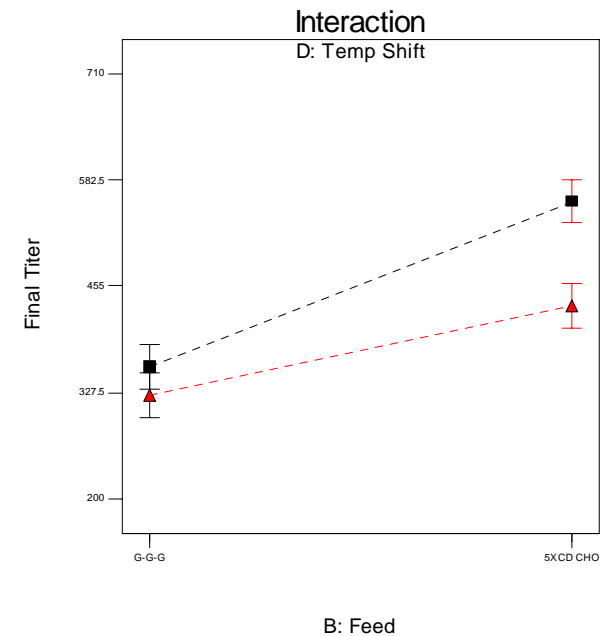
X1 = B: Feed

X2 = D: Temp Shift

Actual Factors

A: Cell Line = Line B

C: pH = 0.00



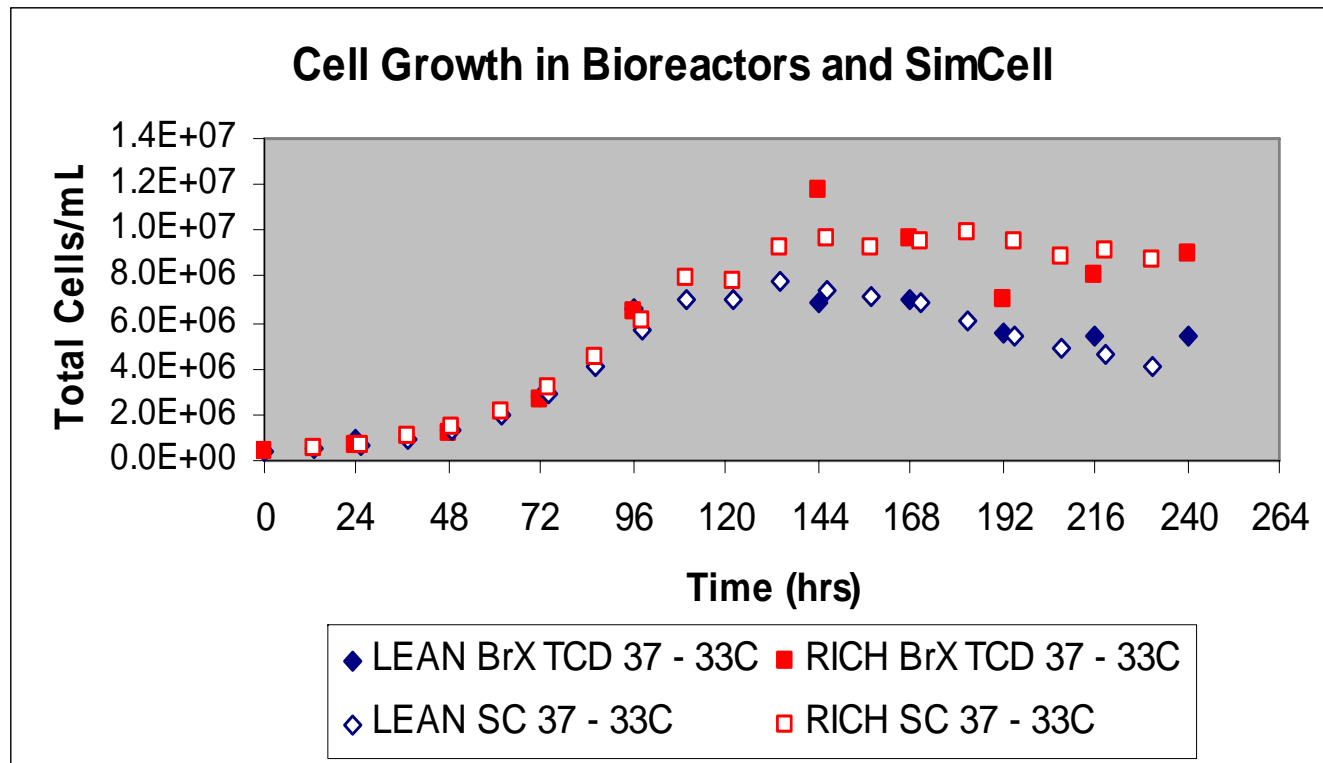
- 6 – 10 factor+ DoE likely to show higher fold-improvement



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Bench scale comparison

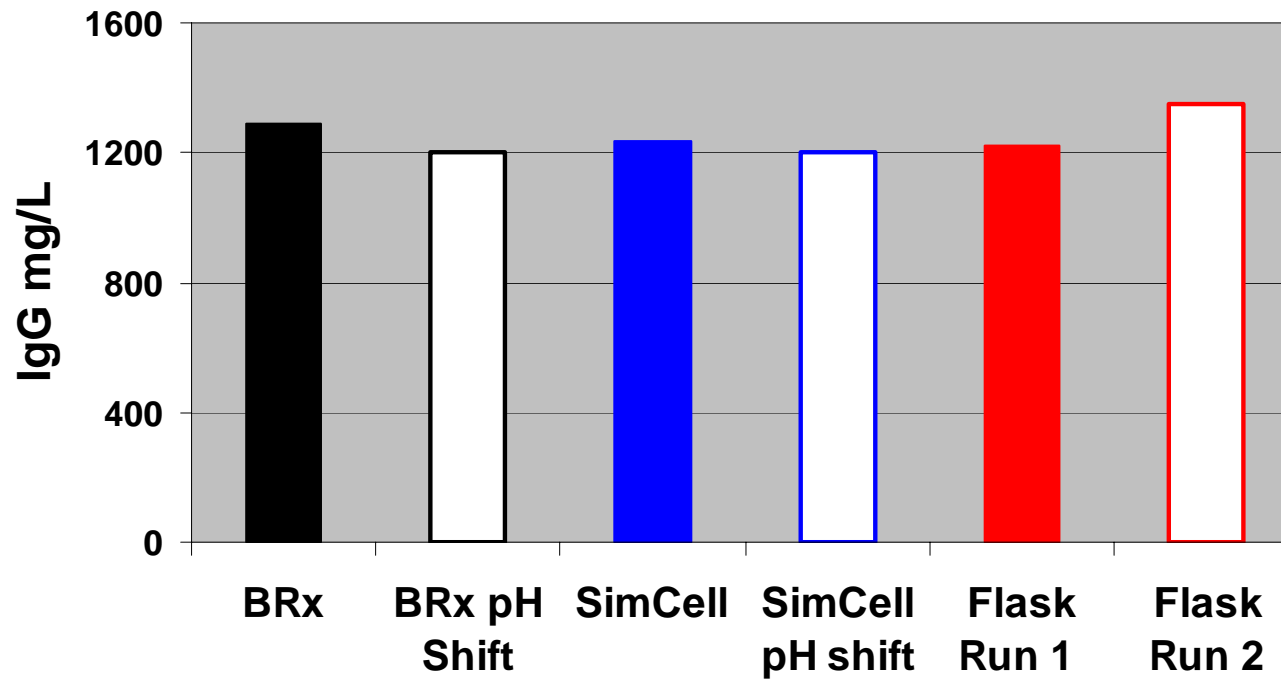
- Two processes expected to yield differences were simultaneously tested in bioreactors and the Simcell
- Saw very good agreement in total cell density,



Optimising IgG Productivity in SimCell™

IgG Production Comparison: Bioreactor, SimCell, Flask

IgG Production in Three Systems



Summary and Observations

- The rapidly evolving field of bioprocess development present new challenges and opportunities.
- Applying the best ideas and technologies can engender improvements and challenge the status quo
- A mix of services and products can leverage the best experiences within companies and suppliers.
- Automation can rapidly enhance biopharmaceutical development.

Acknowledgements

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