From gene to manufacturing: Application of efficient cell line development strategies to deliver reliable, high quality biomanufacturing processes

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Xtalks webinar: 03 December 2014
Presentation scope

► Introducing FUJIFILM Diosynth Biotechnologies
► Innovation goals
► Apollo™: Mammalian Expression Platform
  • Host cell line development (CLD)
    – Directed evolution approaches
  • Cell line development process
► Media and feed development
  • Platform media for Apollo™
  • Toolbox media for client cell lines
► Single-use technologies
  • Development of single-use platform
  • Successful GMP Manufacturing
► Summary
Dedicated, experienced, global CDMO

- EU and USA operations
- ~950 staff
- Over 1000 cGMP batches manufactured
- 5 Commercial Products
- Mammalian and microbial track record: 230 molecules
- FDA & MHRA Inspection history
- Flexible program management
- Extensive LSS application

Combining >35 years of Biologics CDMO experience
Our mission and innovation goals

Fujifilm is committed to the ‘continuous innovation of technologies, service delivery and quality…’ for our CDMO clients

> 20 FTE years of innovation to further develop systems and approaches which rapidly lead to efficient, robust and high quality biomanufacturing processes

- Apollo™ Mammalian Expression Platform
- Streamlined approach to Analytical and Process Development
- Development of a single-use process platform that is well proven in GMP manufacturing
Presenting the Apollo™ mammalian expression system
Components of the Apollo™ mammalian expression system

- **Host cell line**
  - Adapted to chemically defined medium and suspension culture

- **CHO DG44 derived**
  - Selected for superior growth characteristics and expression capability

- **Developed using a directed evolution approach**
  - Fully characterized cGMP cell bank of host cell line with maximum traceability
Isolate a cell line with more desirable attributes from a heterogeneous cell population.

Approaches taken to obtain a host cell line with improved features/attributes:

- **FACS**: Multiple rounds of sorting for cells with extended viability during batch shake flask screening.
- **Cloning**: Isolate a cell line with more desirable attributes from a heterogeneous cell population.
- **Chemostat**: Allows for evolution of rare mutants with desirable growth properties within a large population.
- **Subculture regime**: Continuous subculture (different regimes) and ability to grow in low [Gln] for lower [NH₃] production.
Screening host cell lines: Doubling times during subculture

- Improved doubling time with host cell line C2 compared to original system (DG44-2B): ~27hrs to ~21hrs
  - Potential faster doubling time of subsequent recombinant cell lines made from new host cell lines
  - Shortens the manufacturing seed train duration
Screening host cell lines: Transient expression capability

- Improved titer obtained from C2 compared to DG44-2B
- Directed evolution had variable effect: up to 3-fold increase compared to DG44-2B
Assessing components of the Apollo™ mammalian expression system

Assess the new host cell line and vector in cell line development
Clone 27 and Sort 5 assessed in CLD run-through

- **New Fujifilm expression vector** used with the new host cell lines

- Original host/vector system included as a comparator up to RCB stage
CLD run-through: Growth profiles and productivity from fed-batch shake-flask screen

- **Clone-27**
- **Sort-5**
- **DG44-2B**

Note: different medium/feed system required for DG44-2B

- A greater number of cell lines from Clone-27 host grew to $>10 \times 10^6$ cells/mL
- A higher proportion of cell lines from Clone-27 had product titers $\geq 2$g/L
No substantial difference in product characteristics between cell lines from different hosts

**Size exclusion chromatography**
- The majority of the product from all recombinant cell lines in monomeric form ($\geq 98\%$)
- No considerable aggregation detected

**Capillary electrophoresis-SDS (reduced)**
- Major peaks identified as LC and HC
- Very low percentage of non-glycosylated (~0.5%) and other variants.

**Cation-exchange chromatography**
- High percentage of main peak for new host cell lines
- Low percentage of acidic and basic variants for new host cell lines

**N-linked glycans**
- Predominant glycan species did not change between host cell lines
Assessing productivity in the ambr15™

The highest titer was achieved by a Clone-27 derived cell line: 3g/L

Cell lines from Sort-5 generally had higher Qp but lower IVC
Polling question

What are your titer targets for CHO expression:

a) <1 g/l  
b) 1-3 g/l  
c) 3-5 g/l  
d) >5 g/l
Components of the Apollo™ mammalian expression system

- **Animal component-free media and reagents throughout cell line development**
- **Robust cloning strategy: high probability of monoclonality**
- **State-of-the-art screening strategies**
- **Up to 3 g/L typically seen pre-optimization**
- **Pooled transfection and/or clonal cell line development**

**CLD Process**
Screening strategy

Classical screen
- Plate screen:
  - Static
  - Batch culture

Classical screen
- Batch shake flask
- Fed-batch shake

New screen
- Plate screen:
  - Suspension
  - Fed-batch process

New screen
- ambr15™

More predictive of cell line behaviour in final production process
Fed-batch, shaken multiwell plate screening stage

36% of cell lines produced >1g/L in the fed-batch, shaken multiwell plate screen
A robust cloning strategy

Increased interest from regulatory bodies regarding the method of cloning used and the P(monoclonality) achieved

- Fujifilm has therefore looked carefully at the cloning strategy used to ensure the highest quality of the cell lines developed for clients

- Cloning method(s)?
- P(monoclonality) achieved?
Cloning: Why a two-step approach?

**Two rounds LDC**
- Historically accepted cloning method (≥ 2 rounds)
- Achieve P(monoclonality) 99.76% based on a seeding density of 0.1 cell/well

**One round ClonePix 2™**
- Very low seeding densities required to achieve P(monoclonality) >99.5%
- Results in adverse time and cost implications

**ClonePix 2™ + LDC**
- Achieve P(monoclonality) ≥ 99.78%
- Security from having two rounds of cloning
- Includes an early screen

► Supporting experimental evidence for the statistical prediction of P(monoclonality) collected
  - Separating two cell lines
► FDB can provide supporting experimental and statistical data package
Polling question

What method of cloning are you using?

a) FACS
b) FACS + imager
c) Clonepix™
d) Limited dilution cloning
e) Other
Polling question

What do you see as the most important attributes of a recombinant cell line?

a) Licensing fees / freedom of use
b) Regulatory track record
c) High Product Titer
d) Commercial manufacturing ready cell line
e) Product quality attributes
Apollo™ timeline

Time (weeks)
1 . . . 5 . . . . 10 . . . . 15 . . . . 20 . . . . 25

Vector construction

Representative material (transfectant pools)

Pre-clonal cell line for DSP & early supply

Cell line

RCB of clonal cell line
Media development for the Apollo™ mammalian expression system

Apollo™ CLD

Integrate CHO cell line and media/feed/process to define a Cell Culture Platform

Cell culture process development
Media development for the Apollo™ mammalian expression system

- **CHO cell culture platform**: Chemically defined and/or protein free
- **Apollo™ CHO cell line**: Selected for superior characteristics including growth, titer, and product quality
- **Platform media, feed, and process**: Concept of continuous improvement with formulation knowledge

**Concept of continuous improvement with formulation knowledge**

**Platform media, feed, and process**

**Selected for superior characteristics including growth, titer, and product quality**

**Chemically defined and/or protein free**

**CHO cell culture platform**
Approach for media and process development

Apollo™ or Client CHO Cell Lines

Apollo™ High-throughput (HT) platform media development
- Develop high standard platform media and feeds with HT methodologies (collaboration with vendor)

Optimize media nutrient components by understanding cell culture metabolism through spent media analysis

Optimize cell culture bioreactor process parameters to maximize CC performance

Media Toolbox for Client Cell Lines
- Develop a collection of media and feeds for diverse CHO cell lines (client CHOs)
Apollo™ platform media development

- Media Library
  - Apollo™ CHO Lines
    - HT Screening
  - Fed-Batch Process
    - Spent Media Analysis
      - CC Process
- Top 6 Media
  - HT Media
  - DOE Model
  - Blending DOE
    - HT Feed Screening
      - MVA
- Optimized Feeds
  - CC Process
  - MVA
- Platform Media
  - Additives Screening
  - Continuous Improvement
- Output: platform media and feeds

Formulation
A snapshot of HT media development

HT media blending
DOE in 96 DWP
(vendor data)

Confirmation in shake flask
(vendor data)

Peak Productivity: Fed-Batch
Mix A

Average Productivity
Mix A
Mix A’
Confirmation experiments from HT medium development

Media optimization in shake flask (Fujifilm data)

Confirmation in 2L bioreactor (Fujifilm data)

► Product quality attributes within acceptable mAb ranges

M1 = Medium 1 from HT screen
T1 = Toolbox medium 1

M1 (n=2)

T1 (n=2)

Control medium
Media/Feed Toolbox advantage

► What is the “Media/Feed Toolbox”?
  • A collection of cell culture basal media and feed media (n= 4 ~ 5) from varied vendors to rapidly deliver high performance for diverse CHO lines

► Established media/feed “Toolbox”

I: Media adaptation
  • Adapt multiple CHO cells from original medium to testing media

II: Media Screening
  • Select top media by screening multiple CHO cell lines

III: Feed Screening
  • Select top feed media by screening by screening multiple CHO cell lines

Top basal media and feed media

Continuous Improvement:

V1 vs. V2

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<tr>
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<th>IgG Titer (g/L)</th>
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<tbody>
<tr>
<td>Control</td>
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</tr>
<tr>
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<tr>
<td>V2#3</td>
<td>3.5</td>
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</table>
Apollo™ mammalian expression system

- CHO DG44
- DHFR Vector
- Cell Line Development Process
- Media & Feed Optimisation

Platform media and feed
- Chemically-defined (CD), 4 – 5 g/L
- Formulation “know how”, enable “life-cycle” improvement

Media “Toolbox”
- Good cell line diversity

CHO Media and Feeds

CHO Cell Culture Process
- Optimized process conditions, i.e. seed density, temperature, etc.
Upstream PD standard flow chart

Apollo™ rCHO cell line

Apollo™ CLD

ambr15™ clone screen

Platform basal media for manufacturing ready cell line

ambr15™ /2L DOE 10L supply

2L/10L DOE (optional)

200L Demo

RCB

MCB Manufacturing

MCB vial

Client rCHO cell line

Toolbox media/Feed Screening

Adapt cells into FDB Toolbox media

Identify top media/feed for bioreactor PD

Define cell culture process parameters with DOE design and 10L reactors for material supply

Process demonstration & tox supply

Seed Train, Harvest, Purification PD
Polling question

What are your realistic timeline expectations for the duration of CLD, PD, analytical development and GMP drug substance supply?

a) ≤ 12 months
b) 12 - 14 months
c) 14 - 16 months
d) 16 - 18 months
Process development approach

Fujifilm experience and innovation in mAb development

= Focused, Standardized approach to process development

► See BPI Yearbook* for CHO Development Case Study
► Case study shows a 3-fold titer increase while maintaining product quality

*BioProcess International: July/August 2014 Yearbook, Pg. 78, Vol. 12, No. 7 http://www.bioprocessintl.com
Development of Fujifilm single-use platform

- Fujifilm experience with stainless steel (SS) reactors used as SUB design basis for mixing and mass transfer
- 200L SUB high density CHO runs were used to optimize parameters
- **200L and 1000L SUB growth and productivity were ≥110L SS**
Development of Fujifilm single-use platform

Product Quality: Glycosylation patterns

Successful SUB Performance

<table>
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<th>BEVS</th>
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- Cell growth
- Product titer
- Product quality
- SUB control

1000L SUB process transferred from RTP to UK Manufacturing facility with matching results
Fujifilm experience and value from innovation

► Fujifilm’s CC experience includes 40 mAb and non-mAb programs
► Manufacturing-ready cell line and process platform provides robust clinical and commercial product supply

Process and Analytical platform

- Proprietary DG44 CHO host
- Proprietary vector design
- cGMP cell banking
- Media/Feed platform
- Media/Feed “Toolbox” for client cell lines
- CC development + single-use platform
- mAb DSP platform
- High throughput mAb analytics

CGMP Manufacturing platform

- 145L - 2,000L SS cGMP plant (US)
- 50L - 2,000L SU cGMP plant (US)
- 200L - 2,000L SU cGMP plant (UK)
Cell culture cGMP manufacturing strategy

Matching Capabilities at Fujifilm Diosynth Biotechnologies US and UK Sites

30-60 kgs capacity
- 1000L SUB
- 1000L + 2000L SUB
- 2013

120-240 kgs capacity
- 4 lines of 1000L or 2000L SUB
- 2015-17

Scale-OUT rather than scale-up:
- Expansion in line with clinical needs

2000L Photo Courtesy of GE Healthcare
Acknowledgements

► CLD and upstream teams
  • Adeline Bayard
  • Naz Dadehbeigi
  • Tim Hill
  • Ian Hodgson
  • Clare Lovelady
  • Meghan McCann
  • Stewart McNaul
  • Alison Porter
  • Leon Pybus
  • Fay Saunders
  • Carrie Weymer
  • Alison Young
  • Leisha Youngblood
  • Min Zhang

► Analytical teams
  • Greg Adams
  • James Goromonzi
  • Rebecca Hill
  • Jeff Keen
  • Shaun Lawson
  • Clara Rangel

► FDB Management
  • Mark Carver
  • Gerry Farrell
  • Bo Kara
And you thought we were just the world’s largest film and imaging company...

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