# Developing High Throughput Mammalian Expression Platforms

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The University Of Queensland
AUSTRALIA
AIBN Australian Institute for Bioengineering and Nanotechnology

# AIBN, University of QLD

- AIBN houses > 350 researchers
  - Varied human health research areas
    - Biotherapeutic production
    - Vaccines and delivery systems

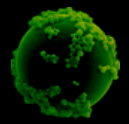
- NCRIS Biologics Facility
  - 200m$^2$ of dedicated clean rooms for mammalian cell culture and downstream processing
  - Bioreactors to 100-L scale
  - Protein analysis and characterisation

- Acyte Biotech Pty. Ltd.
  - Spin-off company (UNSW/UQ)
  - Novel IP for mammalian expression


St. Lucia Campus

# Presentation Outline

- Background and drivers
  - Use of mammalian cells for biotherapeutic manufacture
    - mAbs and CHO cells
- Developing high throughput platforms
  - Transient protein production
    - *Epi*CHO system
  - Stable cell lines
    - Leveraging high throughput instruments
    - Fed-batch optimisation

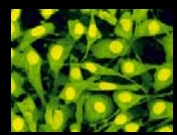


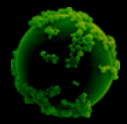
# Mammalian Biotherapeutic Manufacture

- Widely used host system
  - Account for ~ 50% of marketed recombinant proteins
    - > 70% produced in CHO[1]
  - Well characterised → platform processes to 20KL
- Produce bioactive molecules
  - Correct folding and desirable post-translational modifications
  - Proven track record / acceptance by regulatory bodies
- Ideally suited for mAb production
  - > 30% of biopharmaceuticals in current clinical trials[2]
    - Sustained double digit growth through 2007
    - Surpassed growth factors as the highest selling category of biologics

[1] Aggarwal S, 2007, Nat. Biotechnol
[2] Walsh G, 2006, Nat. Biotechnol.





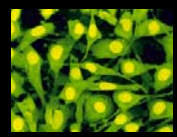


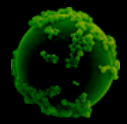
# Challenges with Mammalian Systems

- Time and resource-intensive
  - Technically complex, lower cell density and yields
    - Higher COGs
  - Selection of high-producing, quality cell lines
    - Clone identification / verification ranges from 4-12 months
    - Cell line instability issues

- Restricted access
  - Major advances of the last decade are not ‘freely’ available
    - Heavy licensing costs for access to production cell lines / vectors
    - Proprietary media and fed-batch processes





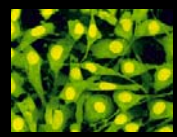


# Rapid Production of mAbs

- Recognising these challenges our group directs research towards:
  - Improved cell lines (focus on CHO and mAbs)
  - Molecular engineering of expression platforms
  - High-throughput and intelligent single cell isolation

- Consortium formed under Federal ICIP grant
  - Low-cost mAb production (transient and stable mammalian cells)
    - Acyte Biotech Pty. Ltd
    - Agen Biomedical Ltd.
    - BioPharmaceuticals Australia
  - Demonstrate that this technology is available to Australian researchers and companies
    - There exist alternative options for mammalian expression
    - Adding value to your process





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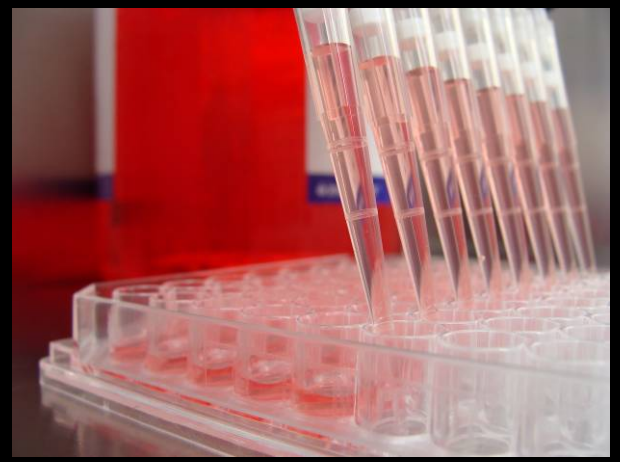
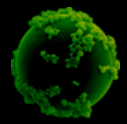




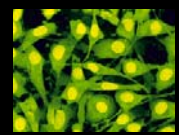
# Transient Expression Systems

- Rapid generation of recombinant protein
  - Allows for timely screening of pre-clinical drug candidates
    - Gram quantities in 1-3 weeks
  - Expression in a wide variety of mammalian systems
    - Transient systems in HEK continue to outperform CHO

- Challenges for transient systems:
  - Rapid dilution of plasmid DNA as cell proliferates
  - Continuity of host cell line
    - Early stage material often manufactured in HEK, while final production host is CHO

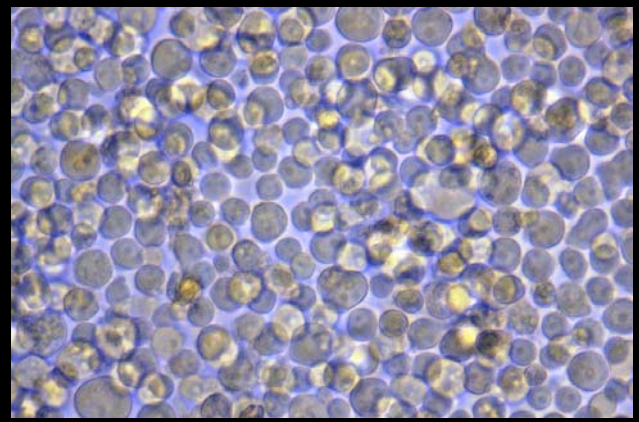
► Ideally require a CHO-based transient system that maintains high expression levels over an extended period



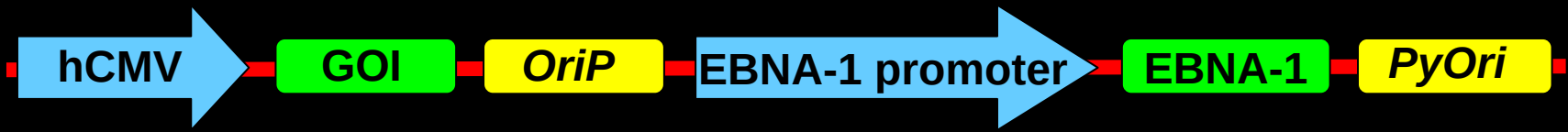


# The *Epi*CHO System

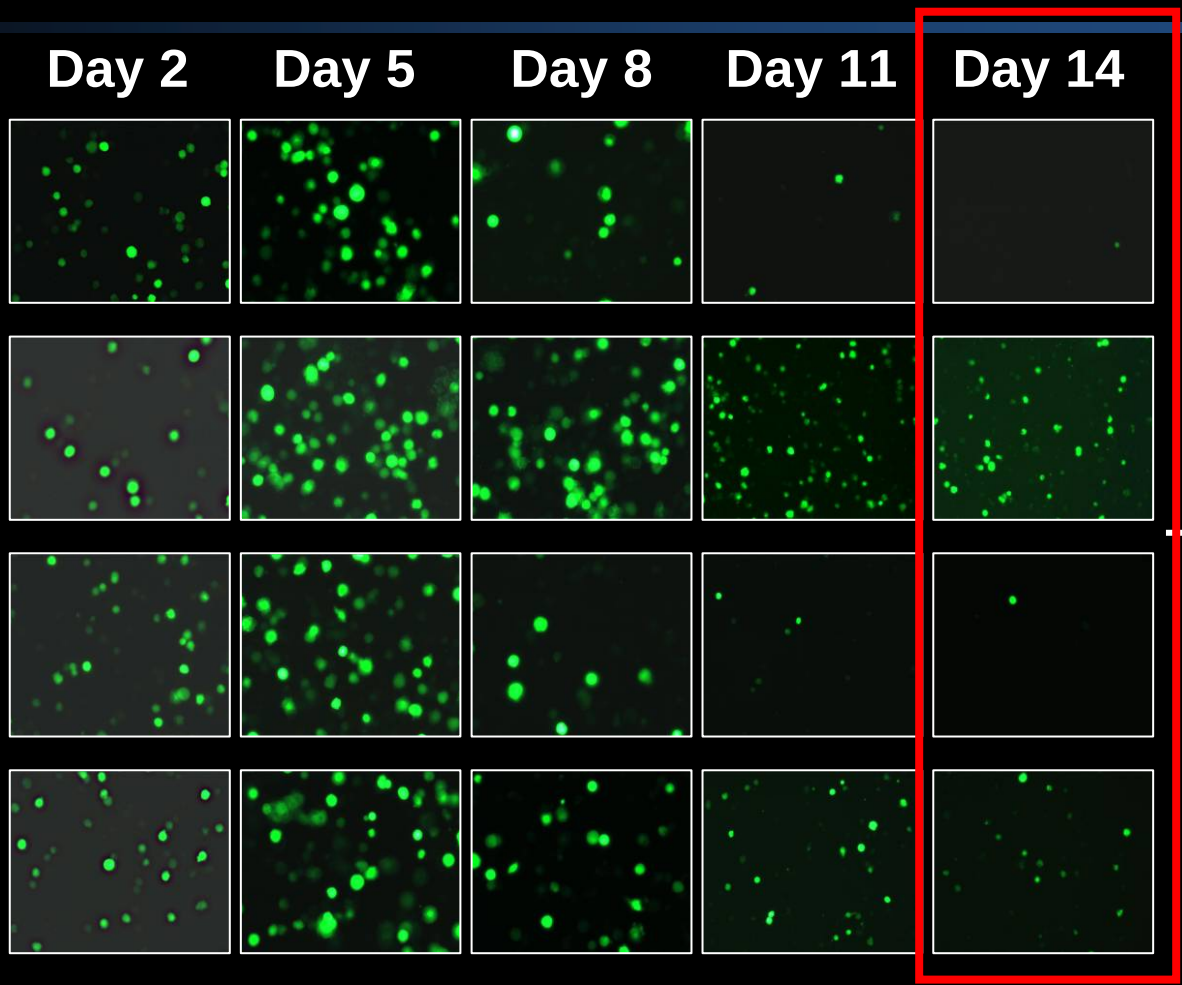
- *Epi*CHO is a transient expression system that provides for amplified and prolonged recombinant protein expression
  - Two key elements:
    1. The host cell line CHO-T
       - Suspension adapted to SFM
       - Constitutively expressing PyLT



    2. The expression vector pPyEBV



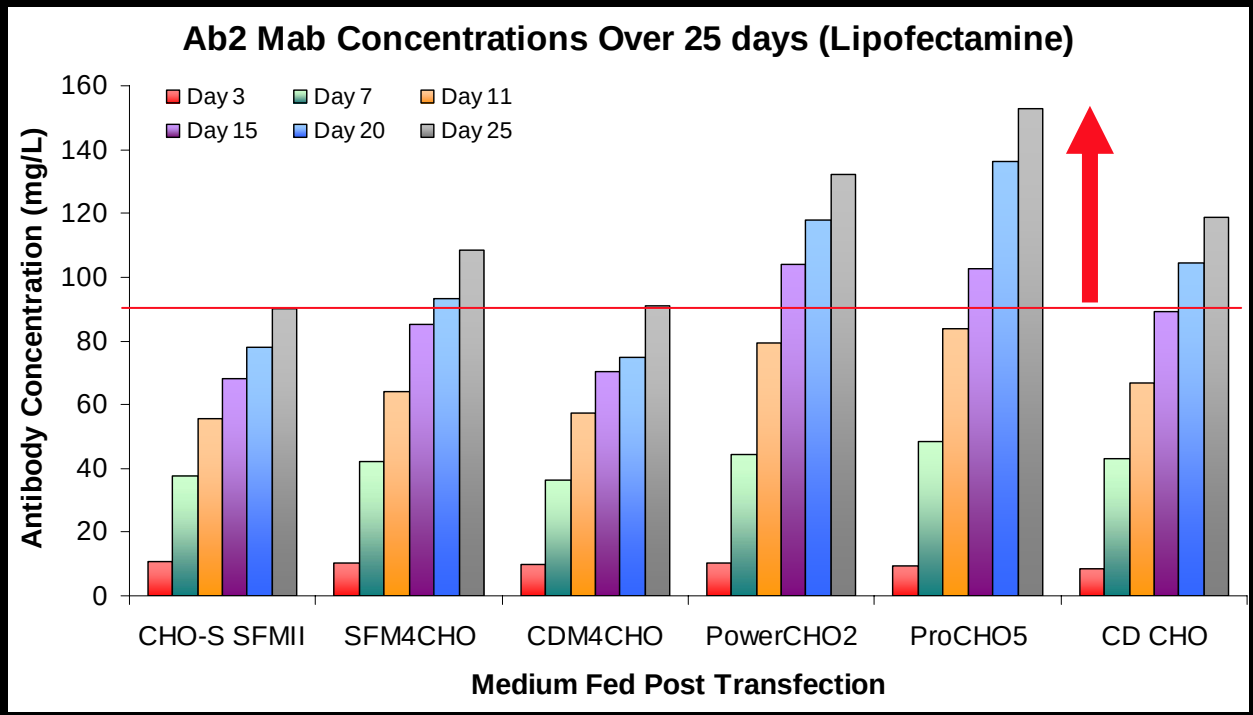
# Prolonged Expression with *Epi*CHO




- Transient transfection with destabilised EGFP reporter

# Prolonged mAb expression with *Epi*CHO

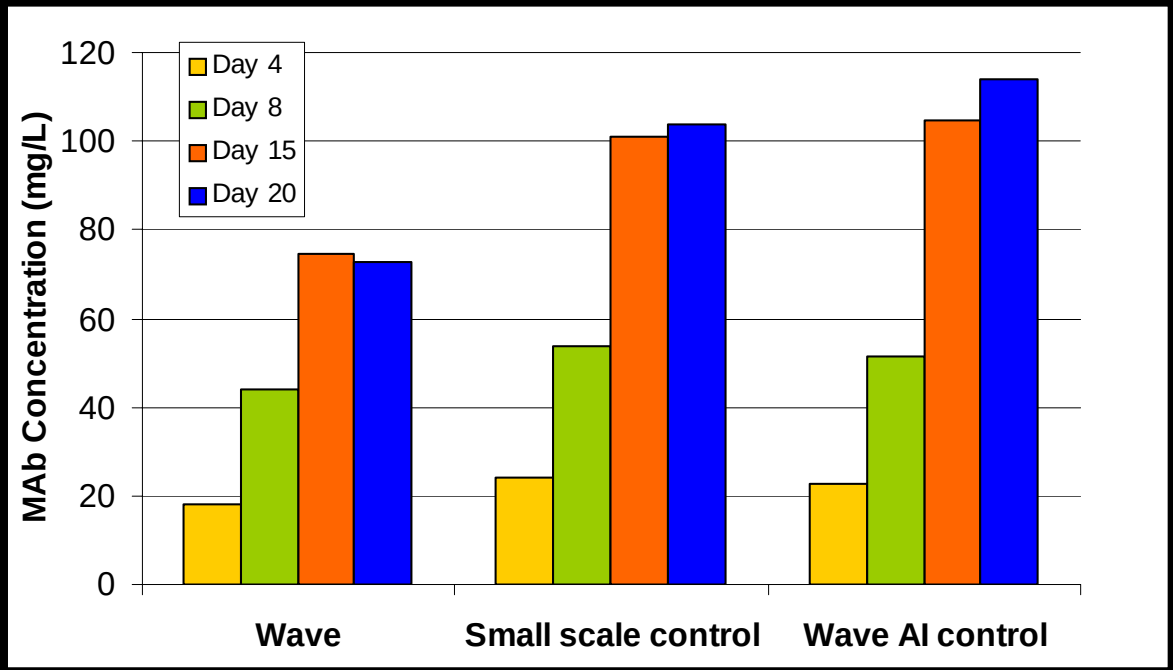
- CHO-T cells transfected with Agen Ab
  - Evaluated productivity in different growth media
    - 50% media dilution 4 hours post transfection
    - Shift to hypothermic conditions (32˚C)




60% increase

# Scalable Transient Production

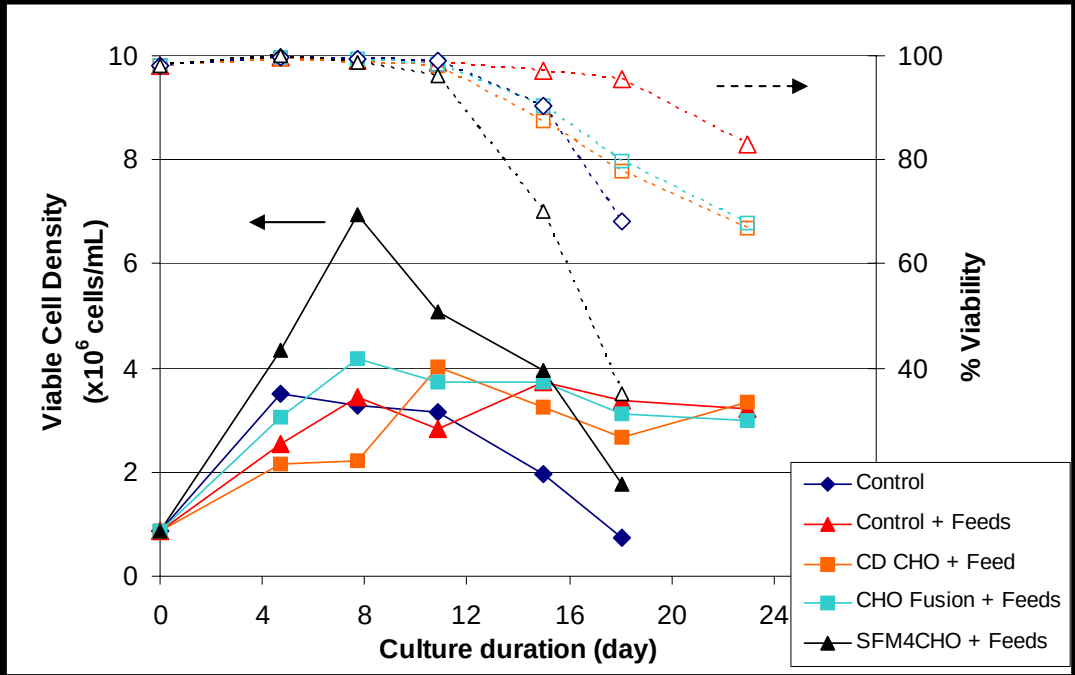
- Traditionally challenging to maintain productivities and efficiency upon scale-up
  - Transfected Agen mAb at 7L volume in a disposable Wave reactor
    - Small scale controls (duplicate 125mL shake flasks)




- Wave culture not linearly scaled
- Gram quantity from a 10L wave should be achievable

# *Epi*CHO Path Forward

- Continue large-scale transfections
  - Optimise wave growth (base addition for pH control)
  - Evaluate alternative culture vessels (3-L disposable flasks)
- Evaluation of new media (data for 32°C growth only)
  - Fed-batch
    - Hydrolysates
  - Supplements
    - IGF-1
    - rTransferrin
  - Extended culture duration and viability
  - 50% increase in IVC



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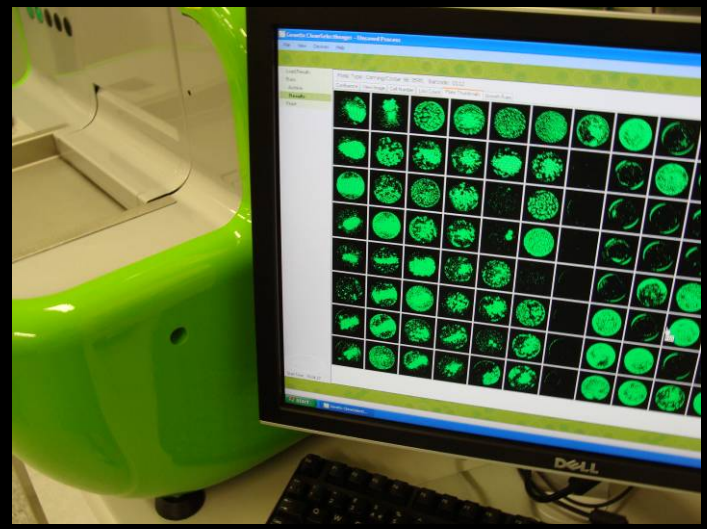
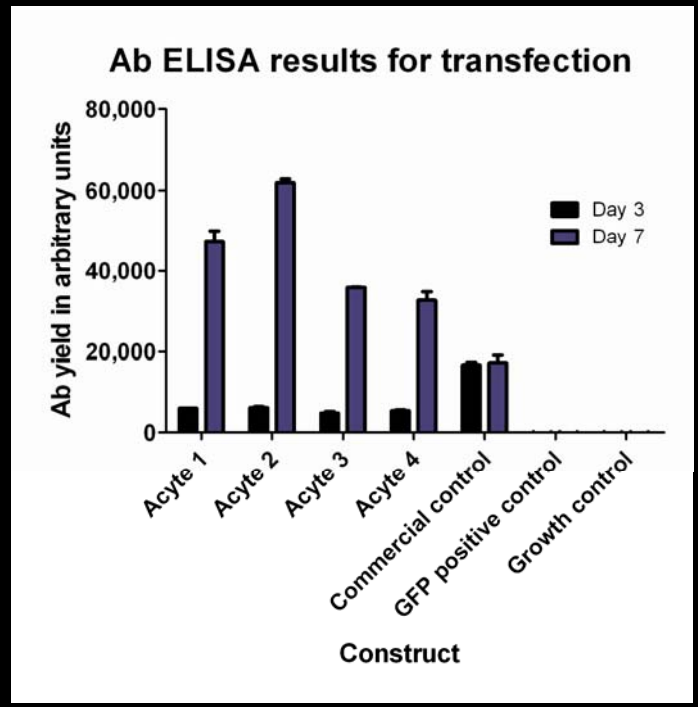


# Stable Expression Technology

- We have assembled a complete set of "in-house" expression vectors
  - Modular design
  - Proprietary high-level expression technology
  - Out-performs commercial mammalian vectors
  - Options for several expression units on a single plasmid

- Stable cell line
  - Based on a low-passage ATCC CHO-K1 variant
    - Suspension adapted
    - Maintained in fully defined conditions
  - Custom designed metal amplification system
  - Modified DHFR+ vectors also in use



# High Throughput Technologies for Cell Selection

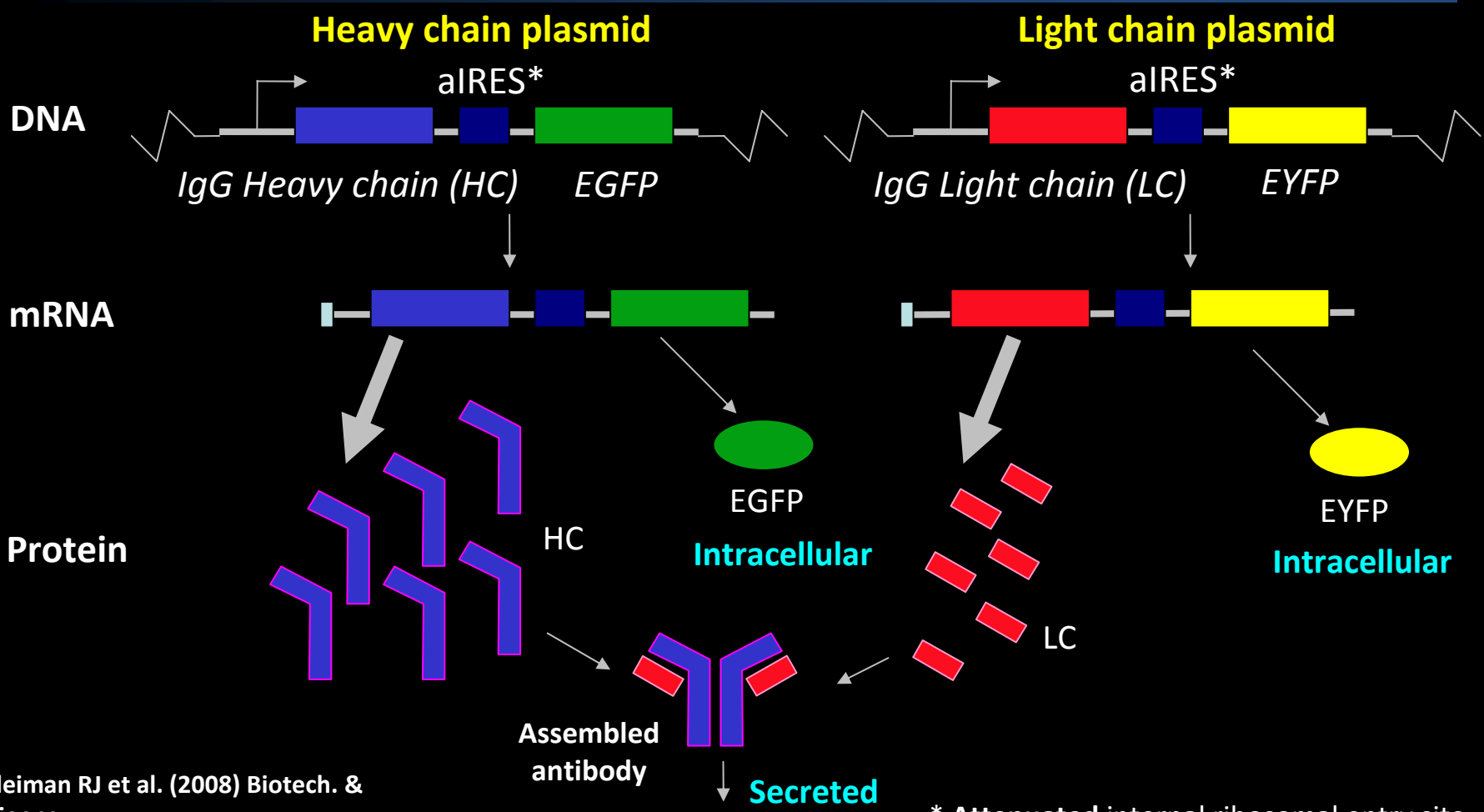
- Automated high-throughput instruments:

Genetix ClonePix FL

BD FACS AriaII

- Facilitates rapid selection of high-expressing clones
  - Utilises Acyte technology
    - Expression vectors with two-colour transcriptional reporters

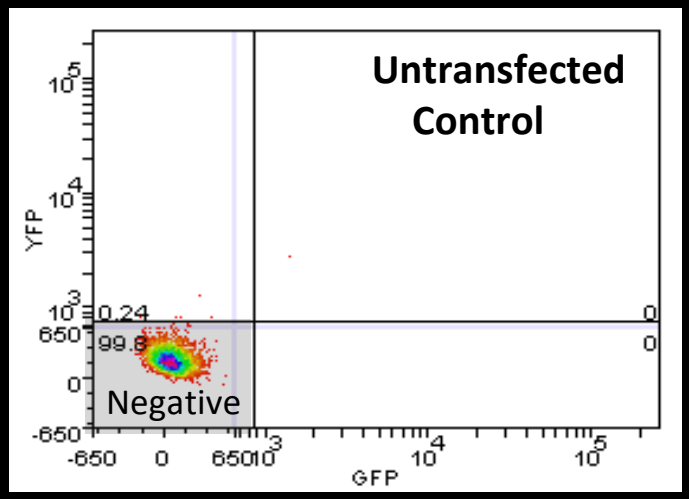
# Acyte Dual Bicistronic Reporter / Expression Vectors

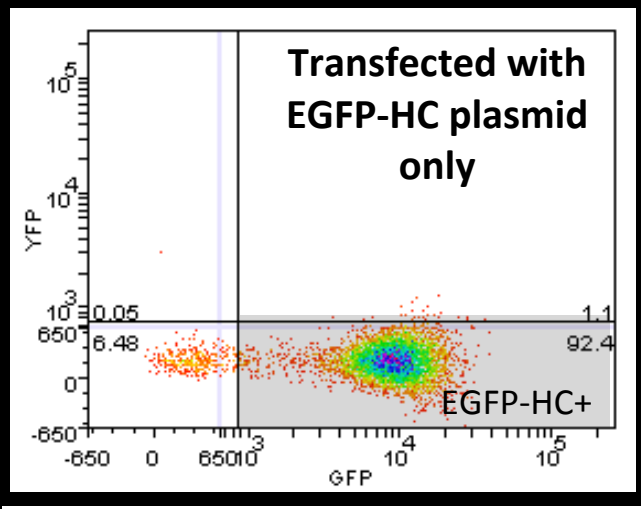


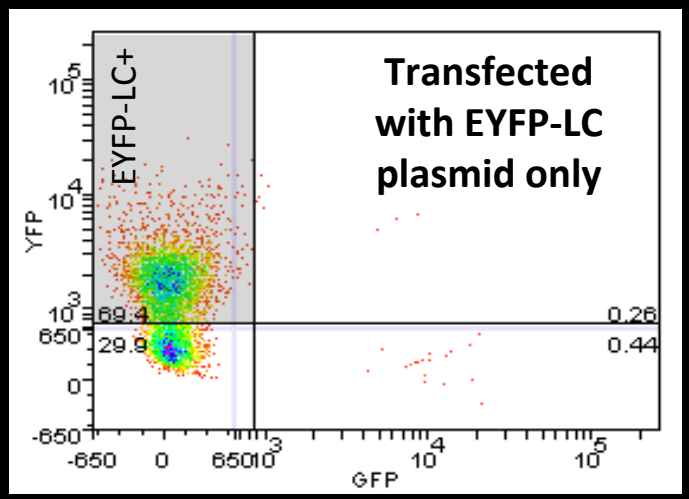

Sleiman RJ et al. (2008) Biotech. & Bioeng.

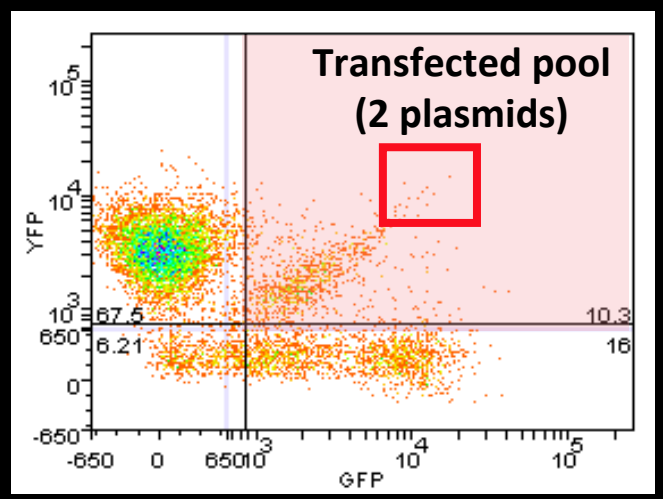
* **Attenuated** internal ribosomal entry site

# FACS Single cell analysis of IgG LC/HC transcription in transfected pools




EYFP Fluorescence (IgG LC Transcription)

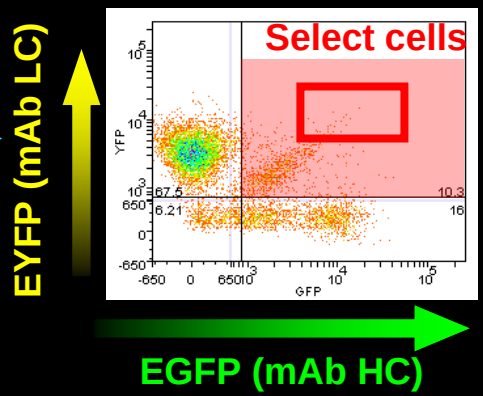
EGFP Fluorescence (IgG HC Transcription)

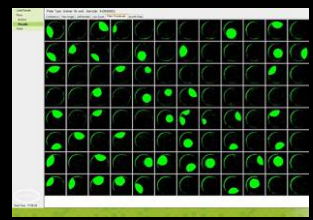
Antibody expressing cells (both chains) High mAb expressers

# Typical FACS Workflow

- BD FACSAriaII™: high speed data acquisition and cell sorting at rates up to 70,000 cells/sec in up to 15 parameters, along with single cell deposition for clonal isolation

BD FACS AriaII



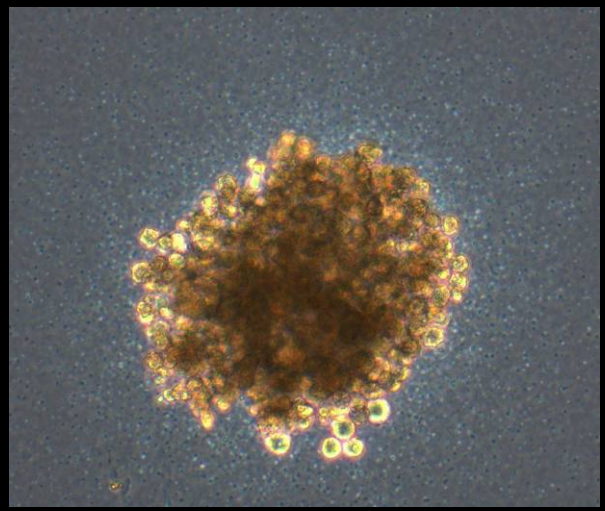
96-well plate culture. Verify clonality, screen for growth and productivity

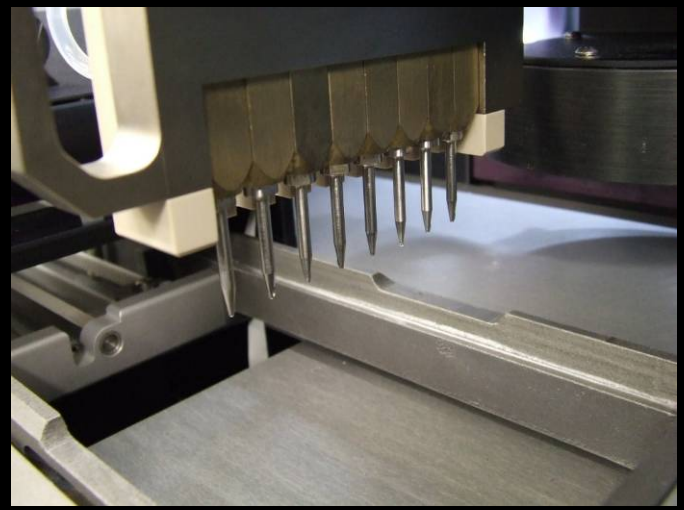
CloneSelect Imager

Expansion and Production

# Genetix ClonePix FL

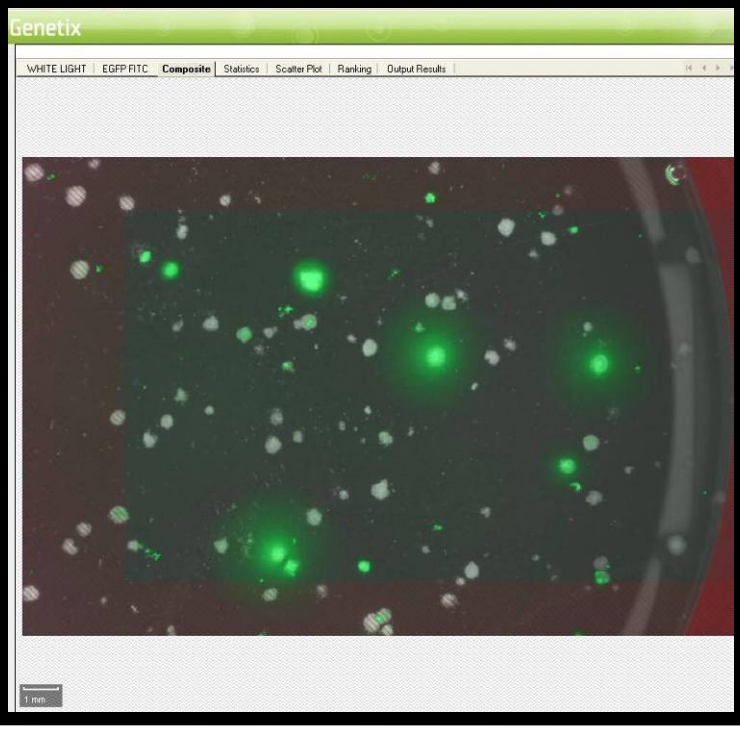
- Automated high resolution imager with sterile robotic picking head
  - Selection of cells secreting recombinant proteins (e.g. mAbs).
  - Plate cells at low concentration in semi-solid media
    - Single cells grow into discrete colonies (7-14 days)
  - Secreted recombinant product detected by fluorescently tagged antibodies specific for the target protein.

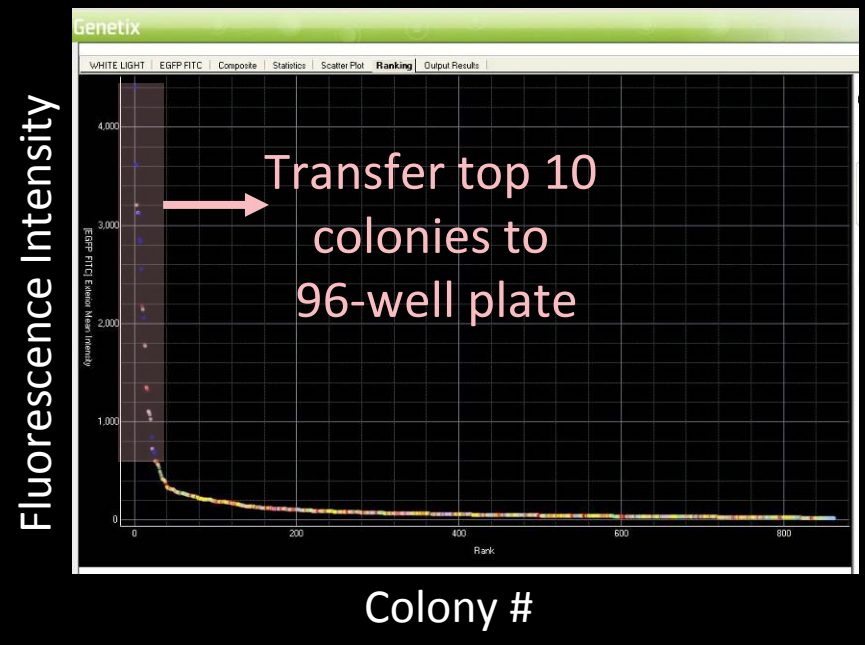




# Selecting mAb Producing Clones

- Example composite image (white-light and florescent images)
  - Clearly identifies secreting colonies of interest
  - Selection criteria can be customised to select high-producing, robustly growing colonies

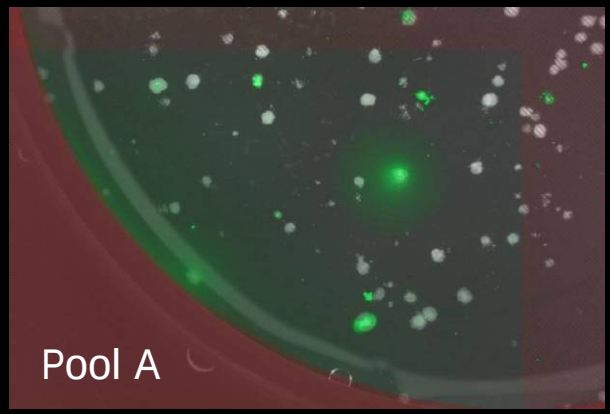


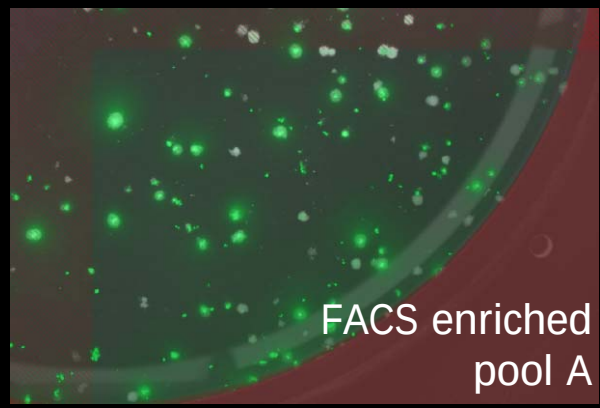



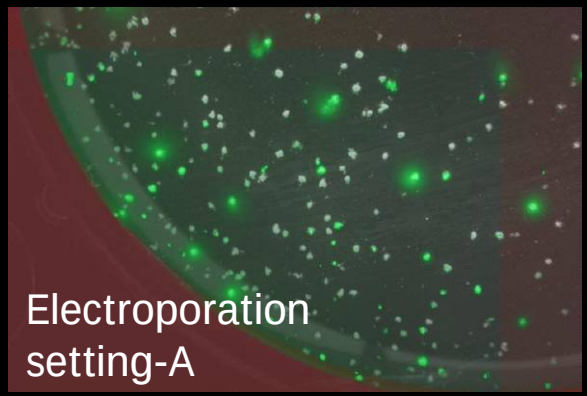

Evaluate 1000's of cells / plate

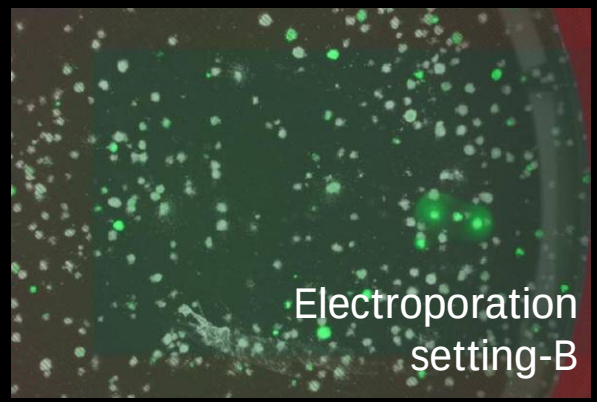
# ClonePix Advantages

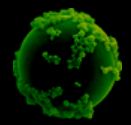
- Increased success rate for growth post picking
- Provides a visually intuitive feedback on cell line and transfection conditions



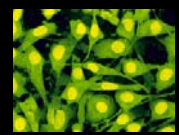





# Stable Expression of the Agen mAb

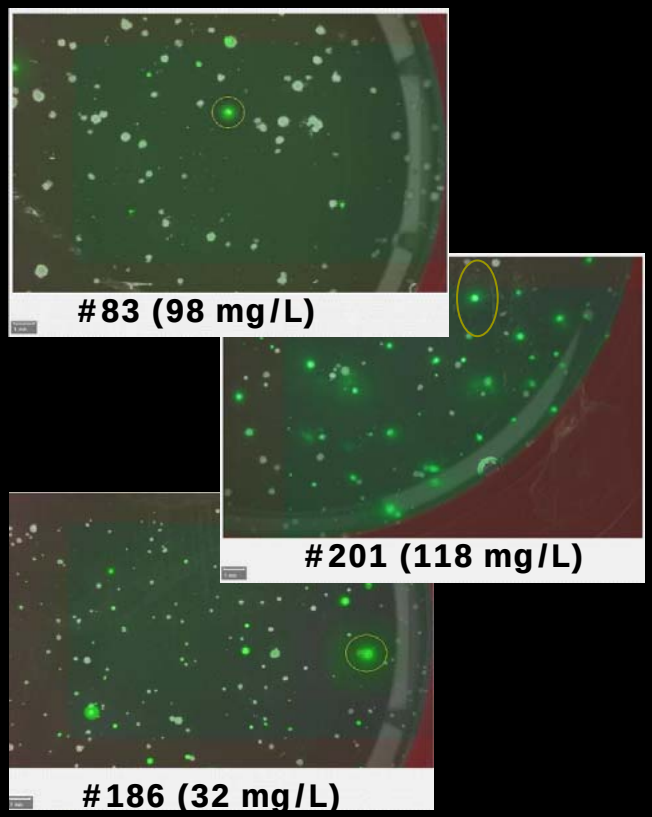
- Stable pools generated with Agen antibody cDNA
  - clones selected for scale-up in ~5 months
  - Project timeline:
    - cDNA resynthesis — 3 weeks
    - Vector construction / verification — 4 weeks
    - Electroporation and G418 selection — 3 weeks
    - Clonal isolation:
      - FACS and ClonePix — 2-4 weeks
    - Clonal screening / adaptation — 2-4 weeks
    - Clone verification:
      - Fed-batch analysis / monitoring stability — 6-8 weeks
  - Note: Pools were not amplified





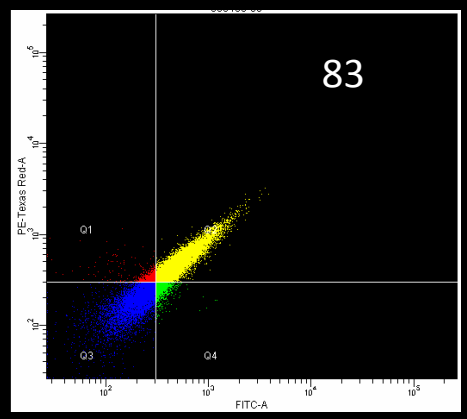
# Analysis of Selected Clones

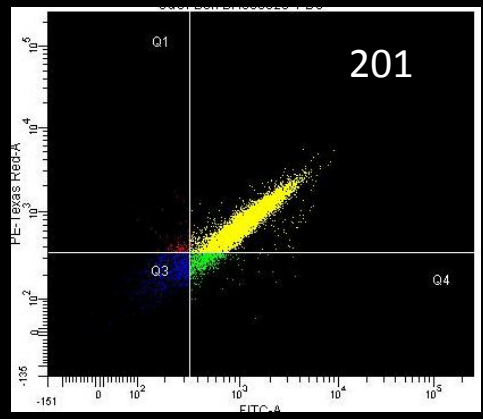
- ClonePix

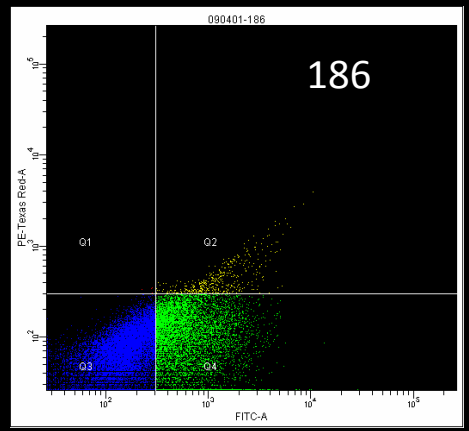



(7-day batch accumulations)

FACS




mCherry Fluorescence (IgG LC Transcription)

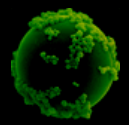
EGFP Fluorescence (IgG HC Transcription)

- Good correlation with ELISA productivity

# Fed Batch Evaluation

- Investigated commercially available media / supplements
  - Spent media analysis
    - Glucose / glutamine
    - Amino acids
    - Trace minerals
  - Hydrolysates
  - Temperature shift
  - CHO Efficient Feed

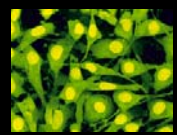
- Standard conditions:
  - ICD = 0.2 x $10^6$ cells/mL
    - 100% media exchange
  - 125mL baffled shake flasks
    - 250mL square bottles
  - 37°C, 7.5% $CO_2$, 170-200 RPM



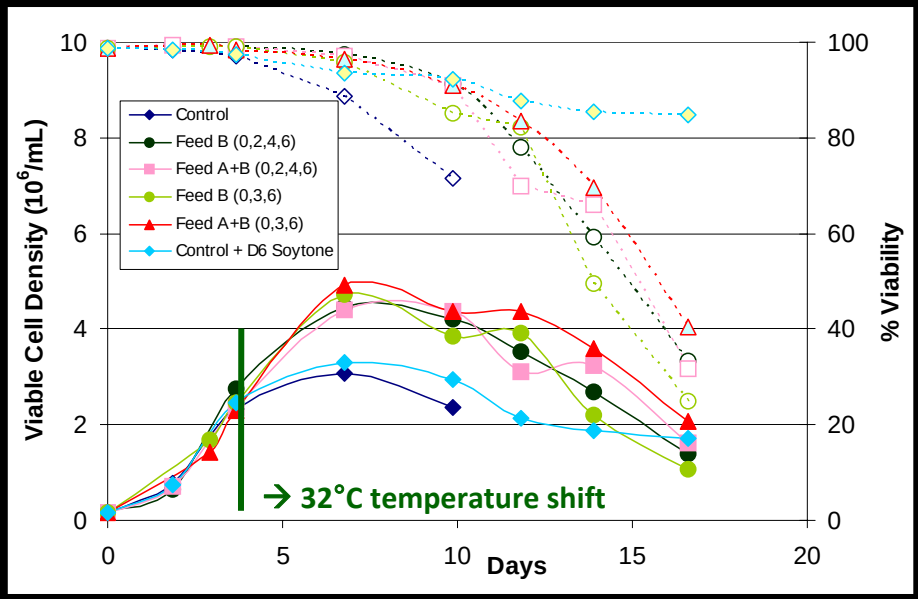
# Efficient Feed Supplements “A” and “B”

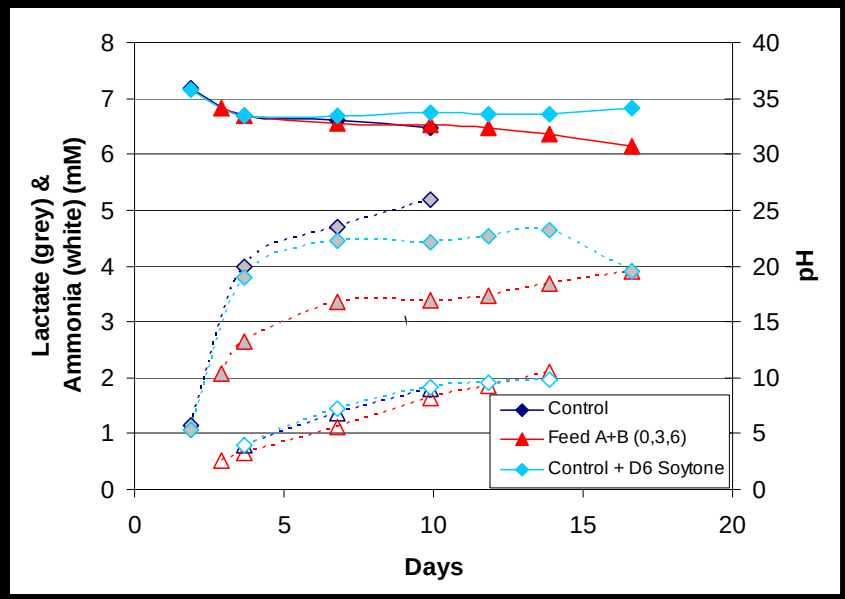
- Chemically defined, protein free
  - *Contains*: Carbon source, concentrated amino acids, vitamins, salts, trace minerals
  - *Does not contain*: Lipids, hydrolysates, or growth factors
- Mini-DOE for multi-day feeding:
  - 15% initial feed was beneficial
  - Adding B or A+B yielded highest titer
    - Noted altered cellular metabolism with reduced lactate /ammonia accumulation
    - Increased cell diameter, higher osomolality (~350 mOsm/L)
- Follow-up experiment with most promising conditions:
  - Additionally investigated addition of hydrolysates
  - Hypothermic temperature shift





# Efficient Feed Optimisation




- Feed A+B (day 0,3,6) yields best cell growth and mAb titer
  - Positive effect of Day 6 Soytone addition (cell growth and pH)

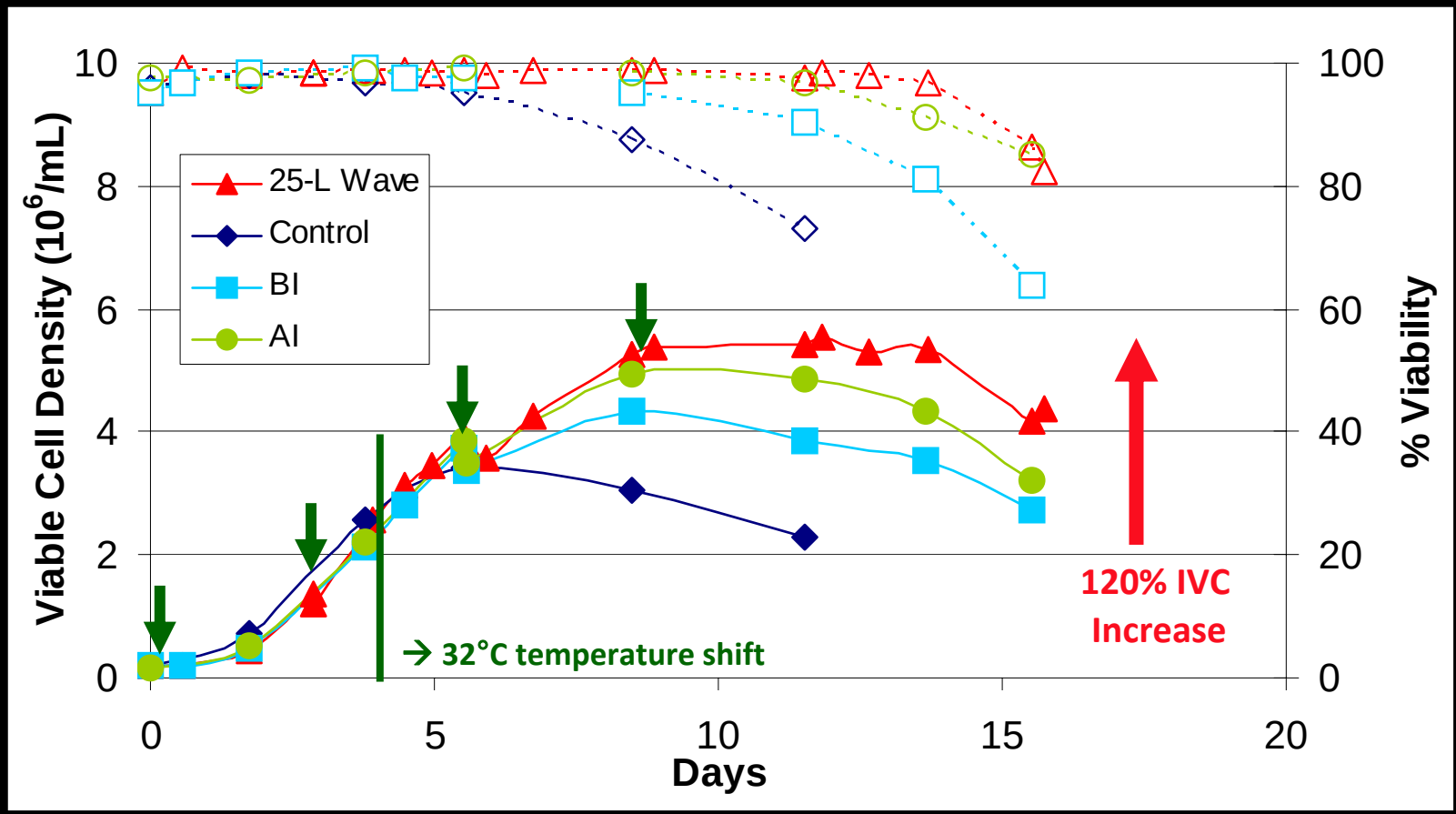
→ Identified optimal feeding conditions

# Scale-up of Fed-batch process

- Performed 25-L fed-batch process (50-L Cultibag)
  - pH manually adjusted (%$CO_2$ and 1M $Na_2CO_3$ addition)
  - Culture Feeds:
    - Efficient Feed A+B
      - 15% Day 0, 3, 10% Day 6
    - L-glutamine
      - 8mM Day 0, 5mM Day 6
    - Soytone Hydrolysate
      - 5g/L Day 9

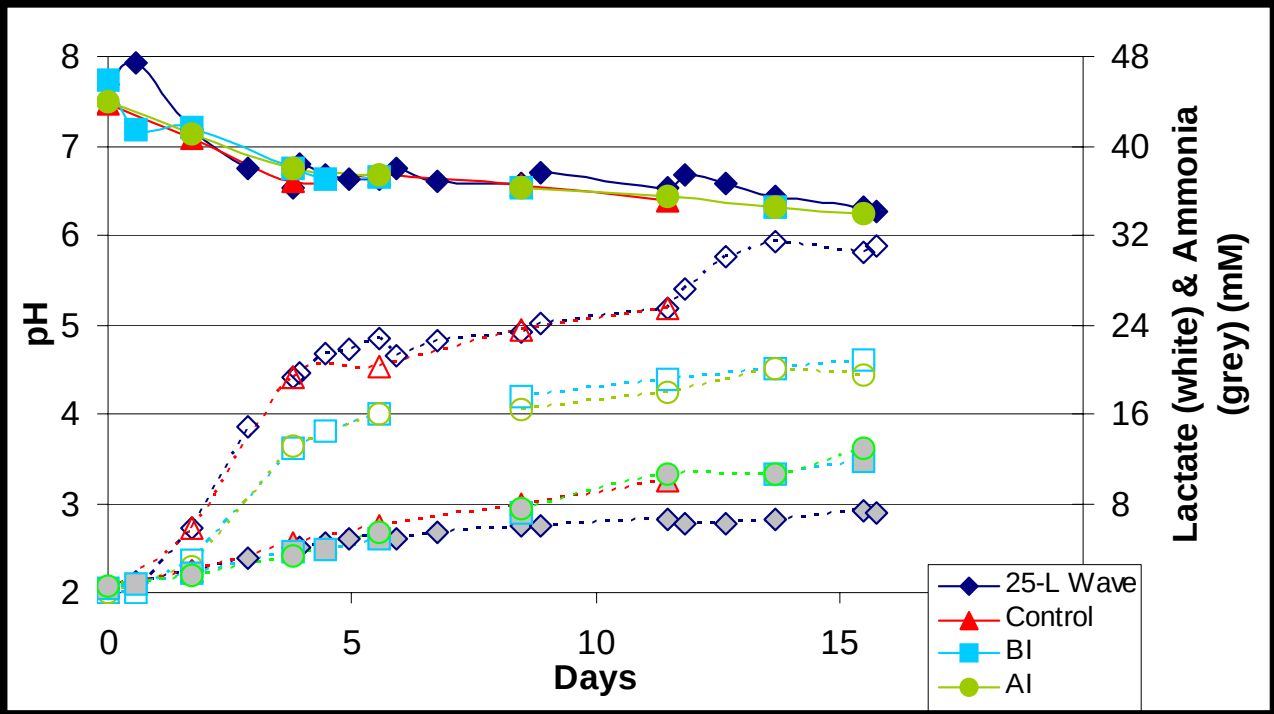
# 25-L mAb Production Run

- Cell growth and viability:

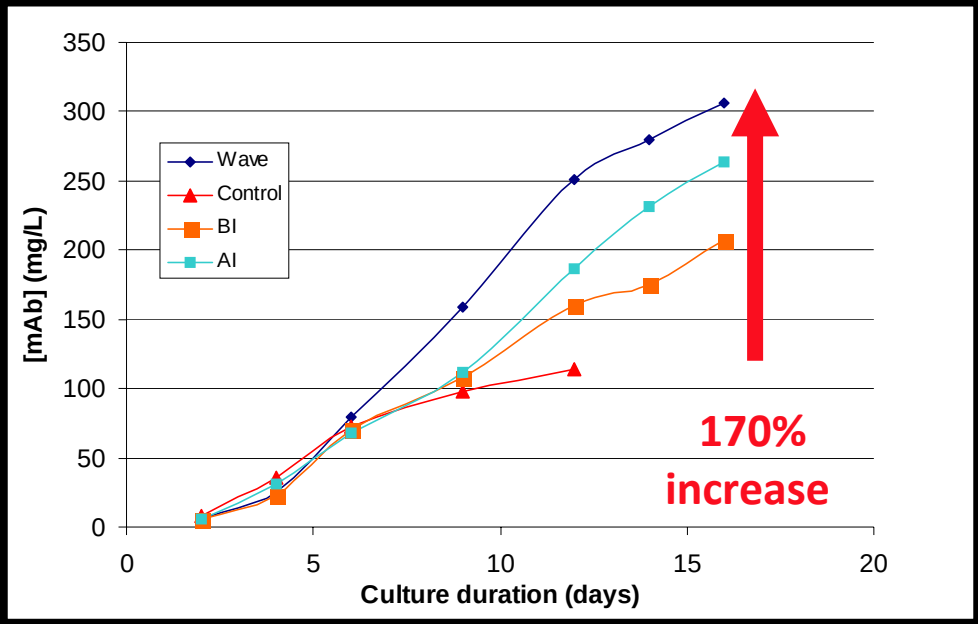


# Metabolites and pH profiles:

- Wave pH maintained at ~ 6.7
  - No base addition to small scale cultures
- Reduced lactate in Efficient Feed cultures
  - Wave suffered elevated pH (7.9) for initial 12 hours



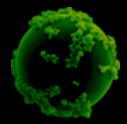
# 25-L mAb Productivity Results




- Significant mAb increase over batch control
  - Product quality retained as compared to Agen reference mAb
- Acceptable productivity for non-amplified clone ($q_p \approx 7$ pg/cell.day)

- Continued fed-batch evaluations
  - Re-adaptation of above clone to alternative media and feeds:

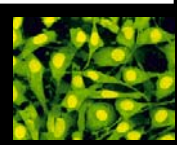
| Media | Feed | IVC increase | Titer increase |
|---|---|---|---|
| CD CHO (control) | 15% A+B | - | - |
| SFM4CHO | 15% A+B | 2% | -54% |
| CHO Fusion | None | 19% | 76% |
| CHO Fusion | Fusion Hydrolysate | 24% | 18% |



# Summary

- Successfully demonstrated mAb production both transient and stable expressions systems
  - This technology is readily available to the Australian biotech industry
- *Epi*CHO is a transient system that promotes elevated and prolonged expression
  - Platform for rapid scalable production
- High-throughput equipment are powerful tools for efficient and intelligent clonal isolation
  - Provide continuous feedback for streamlining clonal selection
- Significant process improvements can be realised with fed-batch optimisation
  - "Off the shelf" solutions (+ a liberal amount of sweat)





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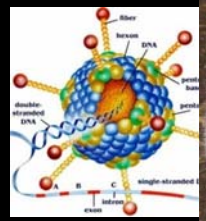

Images courtesy of http:\\en.ec.cn, www.theanimalfiles.com