

Lonza

Novel approaches for the improvement of transient expression using CHO cells

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Overview

- Introduction

- Transient expression
 - Methods to increase protein yields

- Review of expression options
 - Introduction to pooled transfection techniques

Transient expression

- A procedure capable of generating small to moderate quantities of recombinant protein

Advantages

- Quick¹
- Can be simple²

Disadvantages

- Protocols have been enhanced with serum¹
- Concentrations may be low, i.e. 5-8 mg/L^{3,4}
- Can be complicated
- Significant quantities of DNA may be required⁴

1. Durocher Y, S et al 2002, Nucleic Acids Research 30(2)

2. Jordan M, A. et al. 1996, Nucleic Acids Research 24(4)

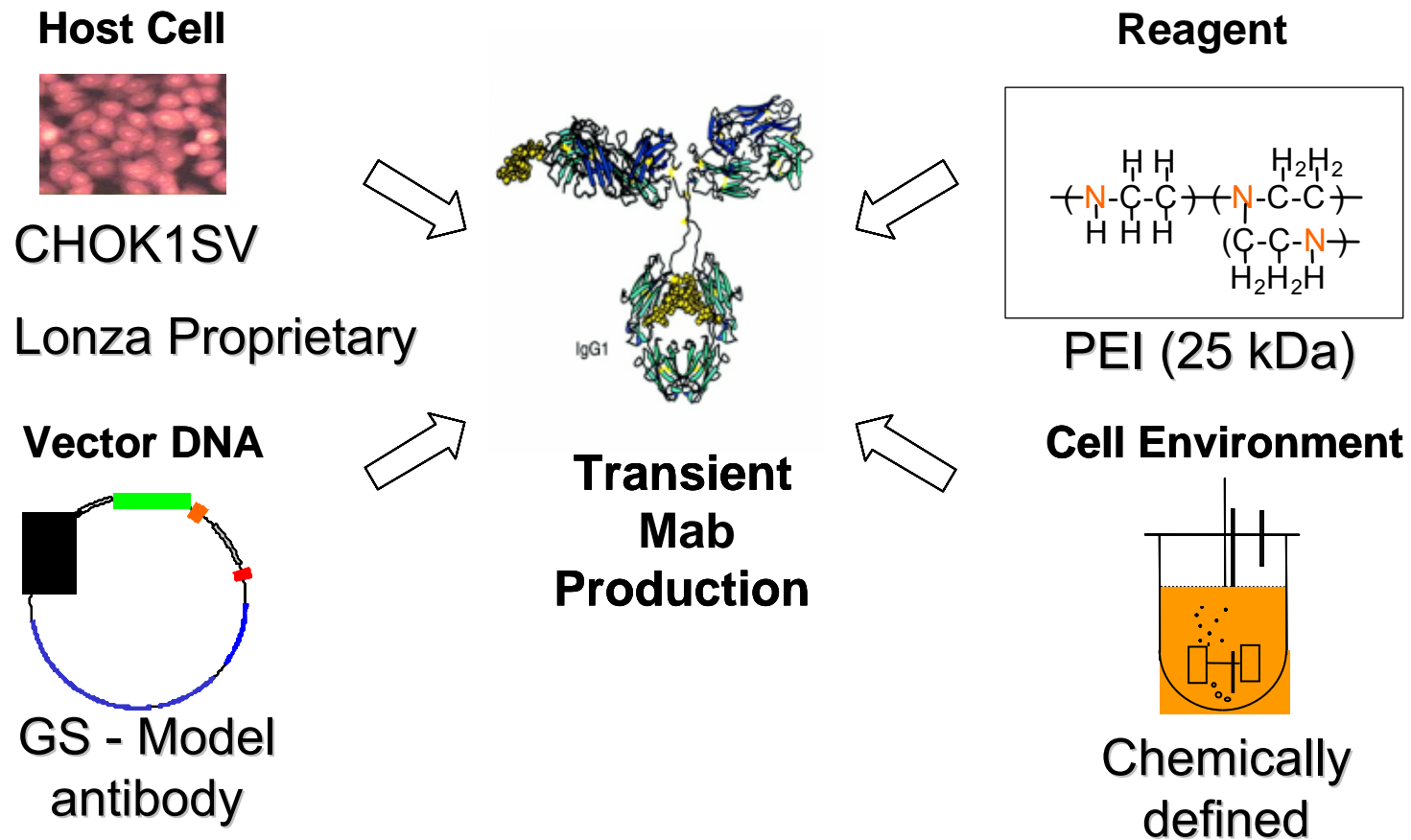
3. Derouazi M, P et al. 2004, Biotechnology and Bioengineering 87(4)

4. Tait A, S. et al, 2004, Biotechnology and Bioengineering 88(6)

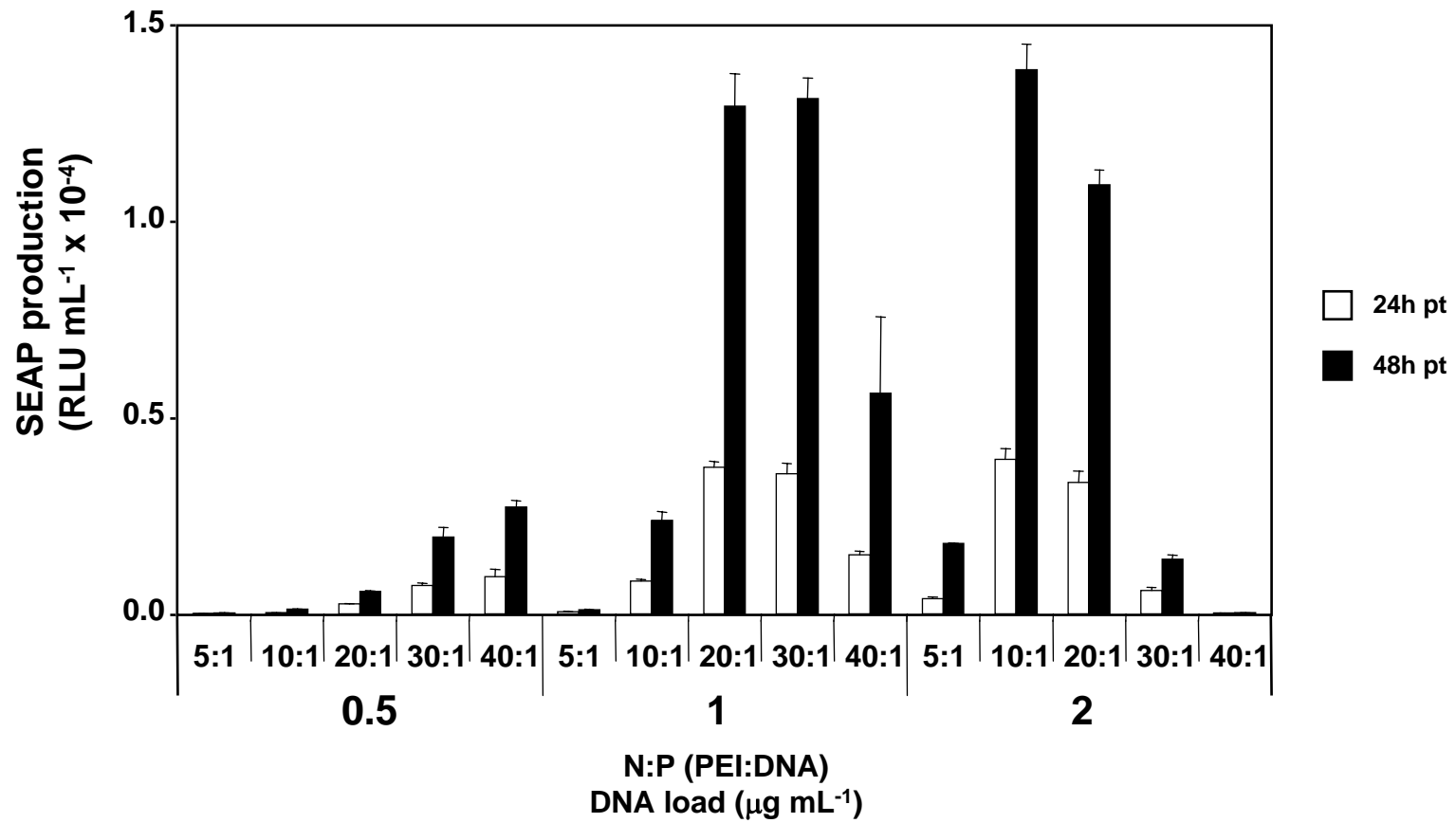
Imposed conditions of the system

- CHO based system
- CDACF
- Scalable
- Relatively inexpensive

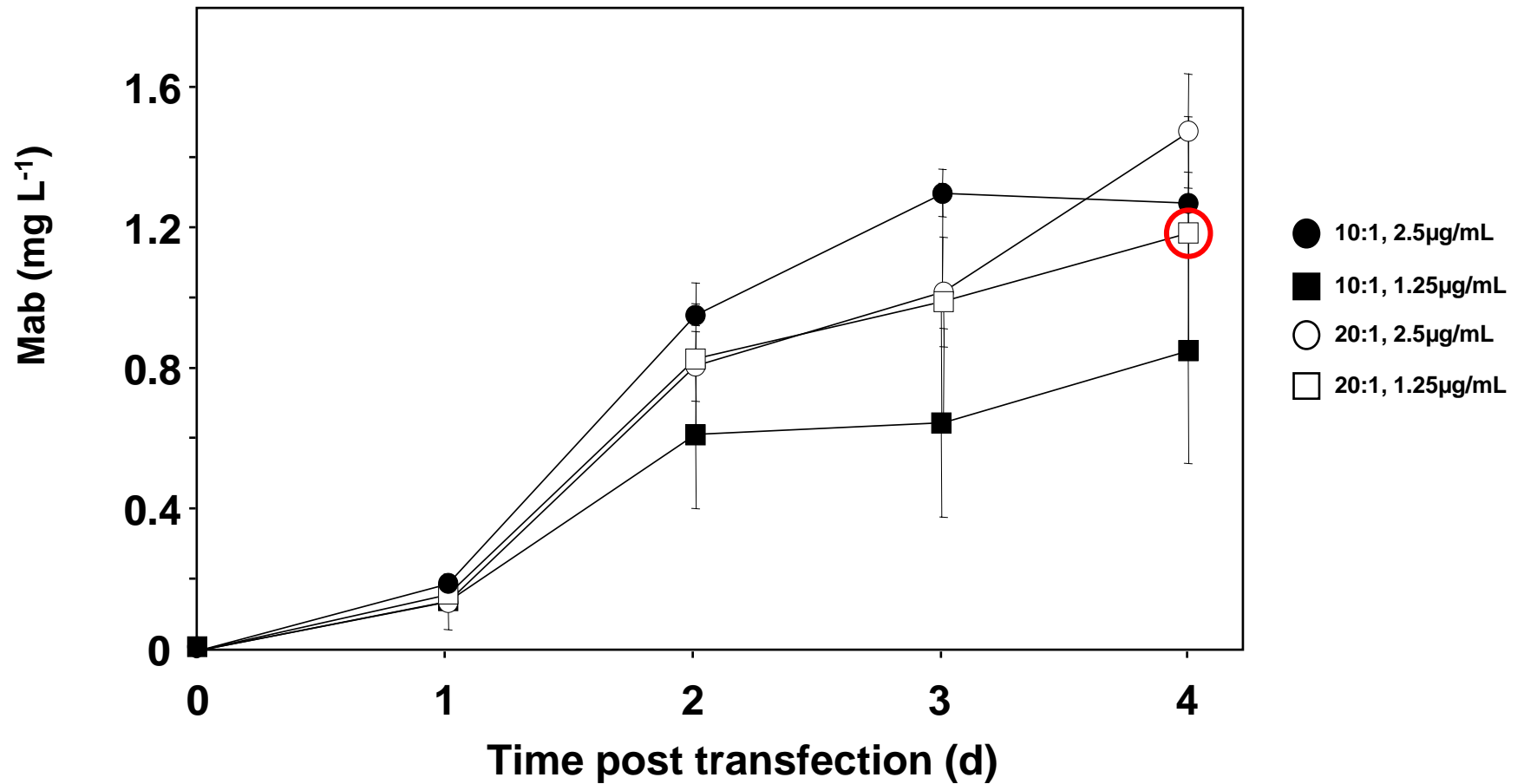
Utilized system



Initial optimization



Initial Mab concentrations - 1.5 mg/L



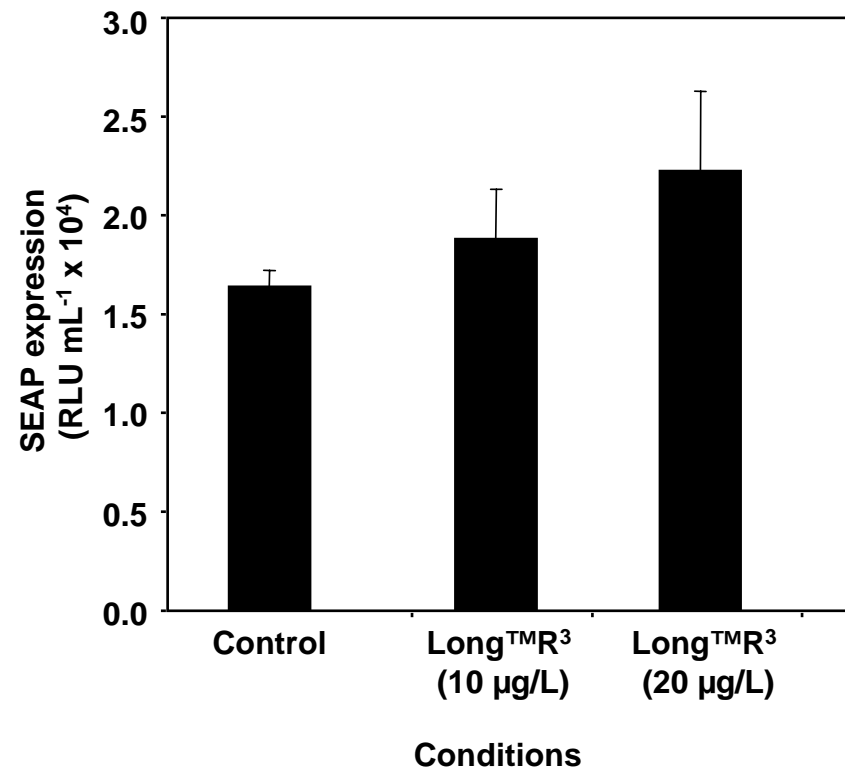
Optimized parameters

- DNA concentration = 1.25 µg/mL culture
- PEI:DNA (N:P) = 20:1
- Mab harvest concentration ~ 1.2 - 1.5 mg/L

Strategies required to increase Mab production

- Previous observation: FBS boosts transient expression in some systems – would BSA help?
 - No
- Examined lipid mixtures for their ability increase production
 - Similarly ineffective
- Investigated the use of insulin and insulin like growth factor (IGF)
 - Insulin exhibited no positive effect
 - IGF showed promise
 - In the form of Long™R³ Insulin like growth factor (IGF)-1

Introduction of LongTMR³ IGF

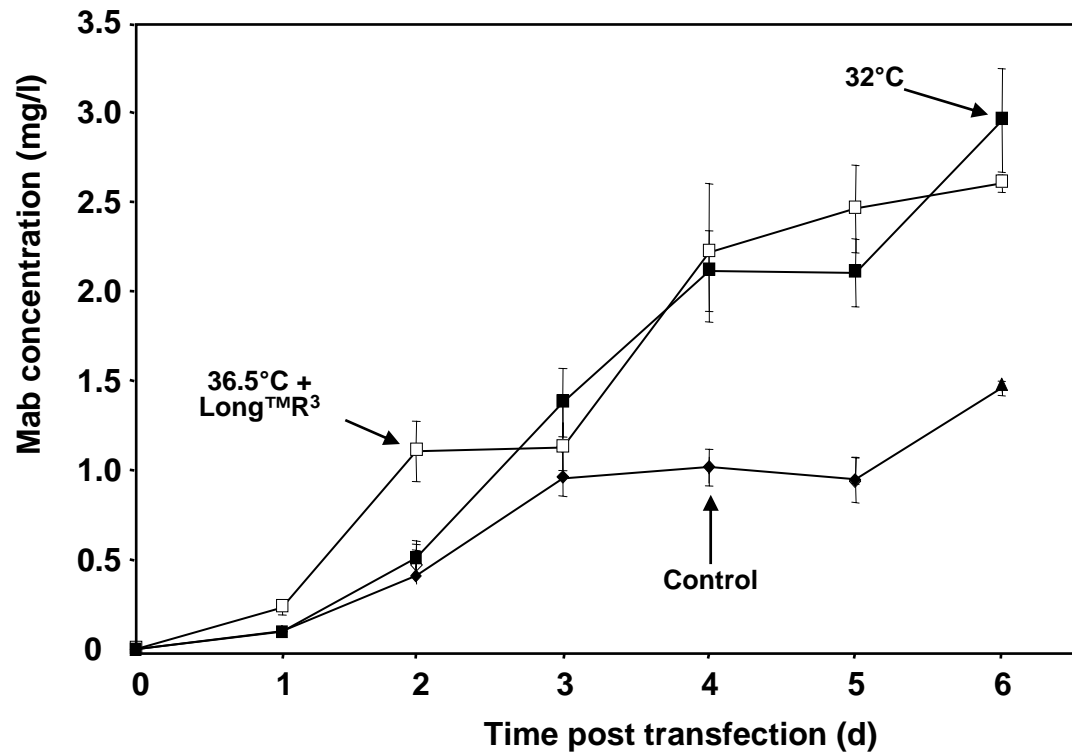


Mild hypothermic strategy

- Previous research has shown a benefit in using mild hypothermic conditions in transient systems¹
- Other research at University of Queensland demonstrated a similar positive effect

1. Schlaeger E. J et al 1998, Cytotechnology 28(1-3)

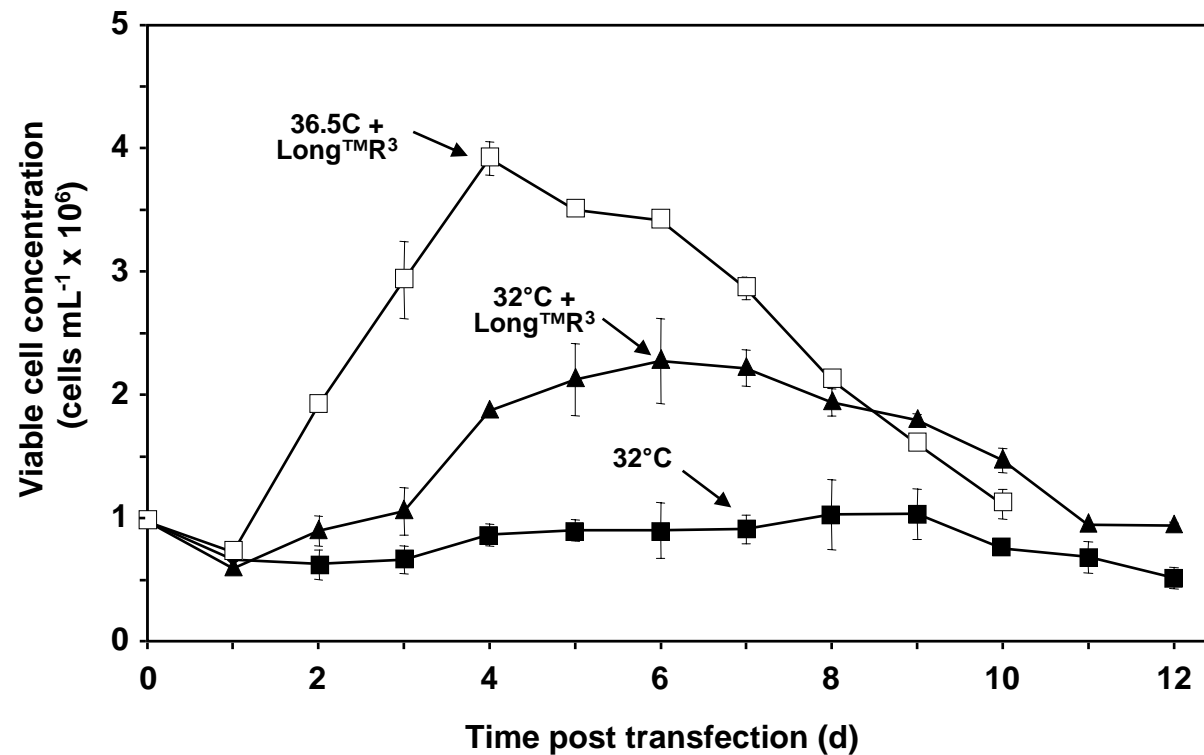
Comparison of LongTMR³ with low temperature conditions



Monoclonal antibody harvest concentration

- Initial harvest antibody concentrations were ~ 1.5 mg/L
- ~ 2.5 mg/L with either the introduction of Long™R3 or mild hypothermic conditions (32°C)
- Hypothesized that superimposing these conditions would result in still further increases in final antibody concentrations

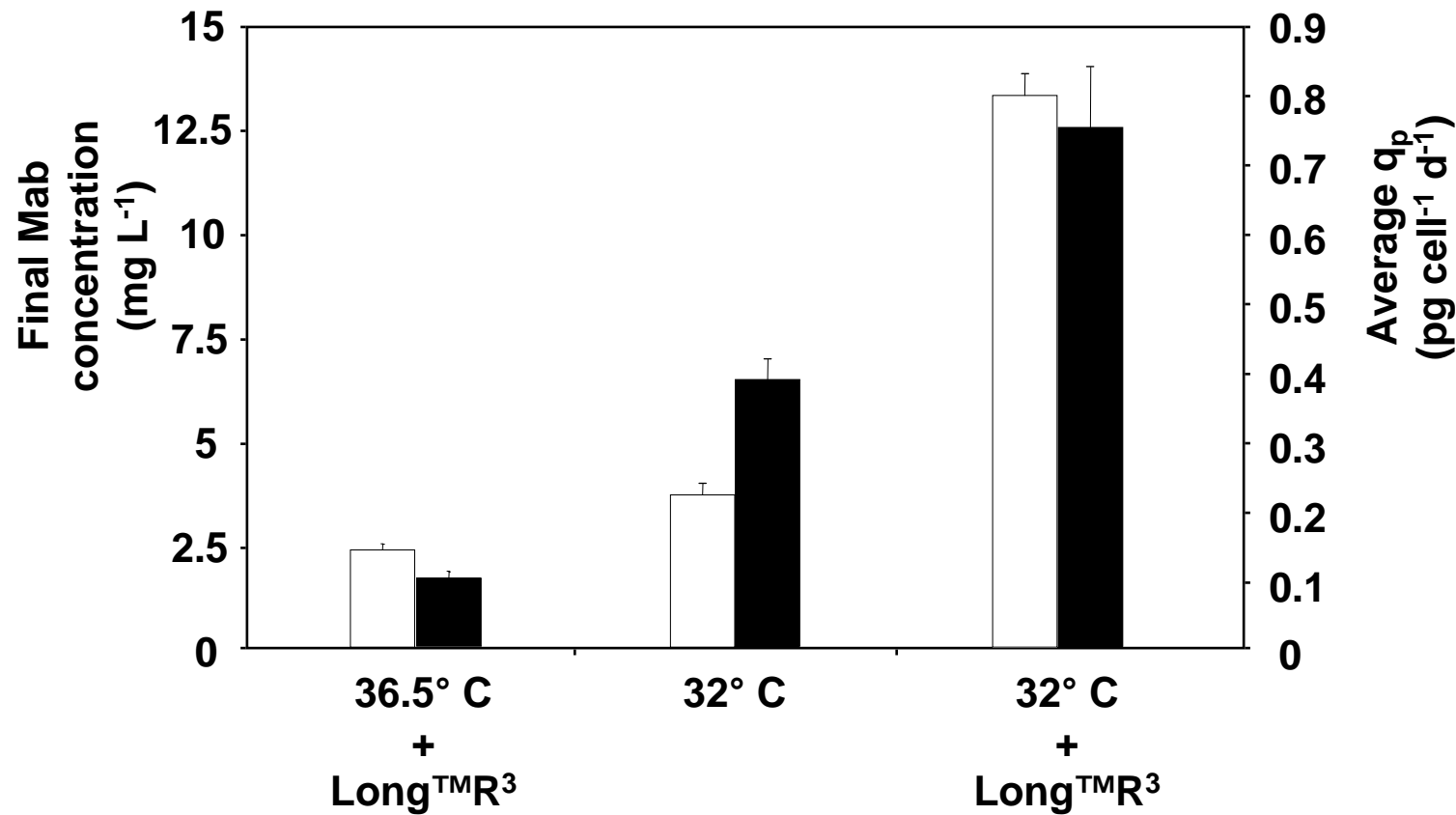
Combination of LongTMR³ with mild hypothermic conditions



Initial conclusions

- The presence of Long™R³ results in conditions that allow for growth post transfection both under normal 36.5°C conditions and during mild hypothermic conditions (32°C)

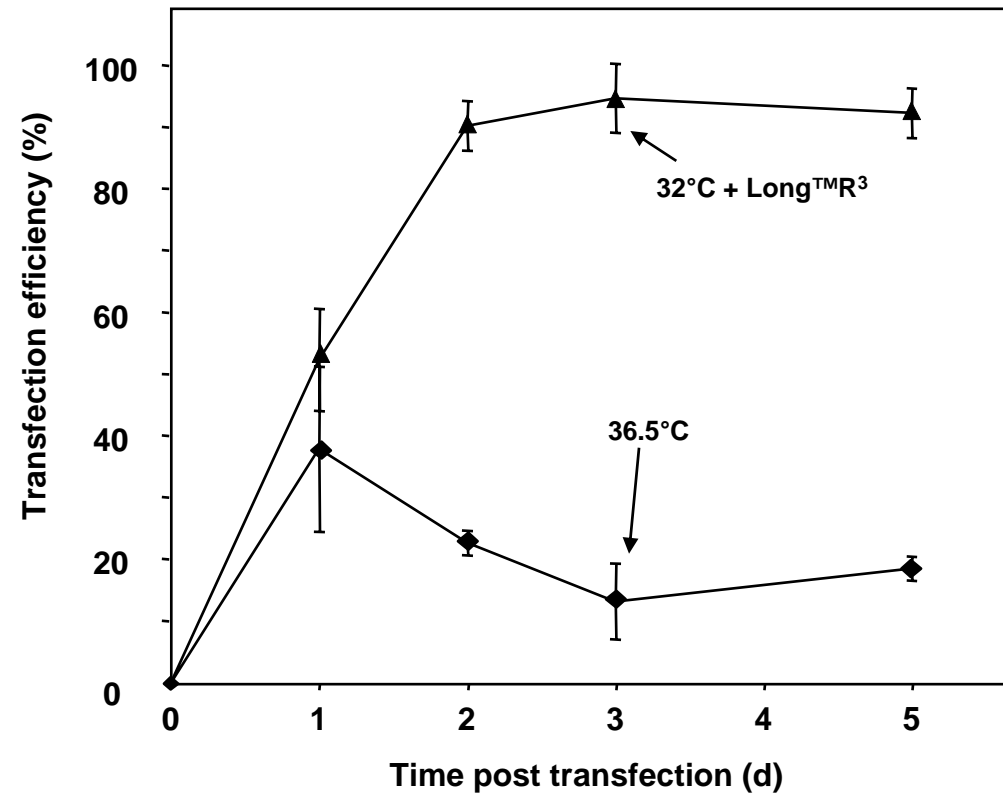
Combination of LongTMR³ with mild hypothermic conditions



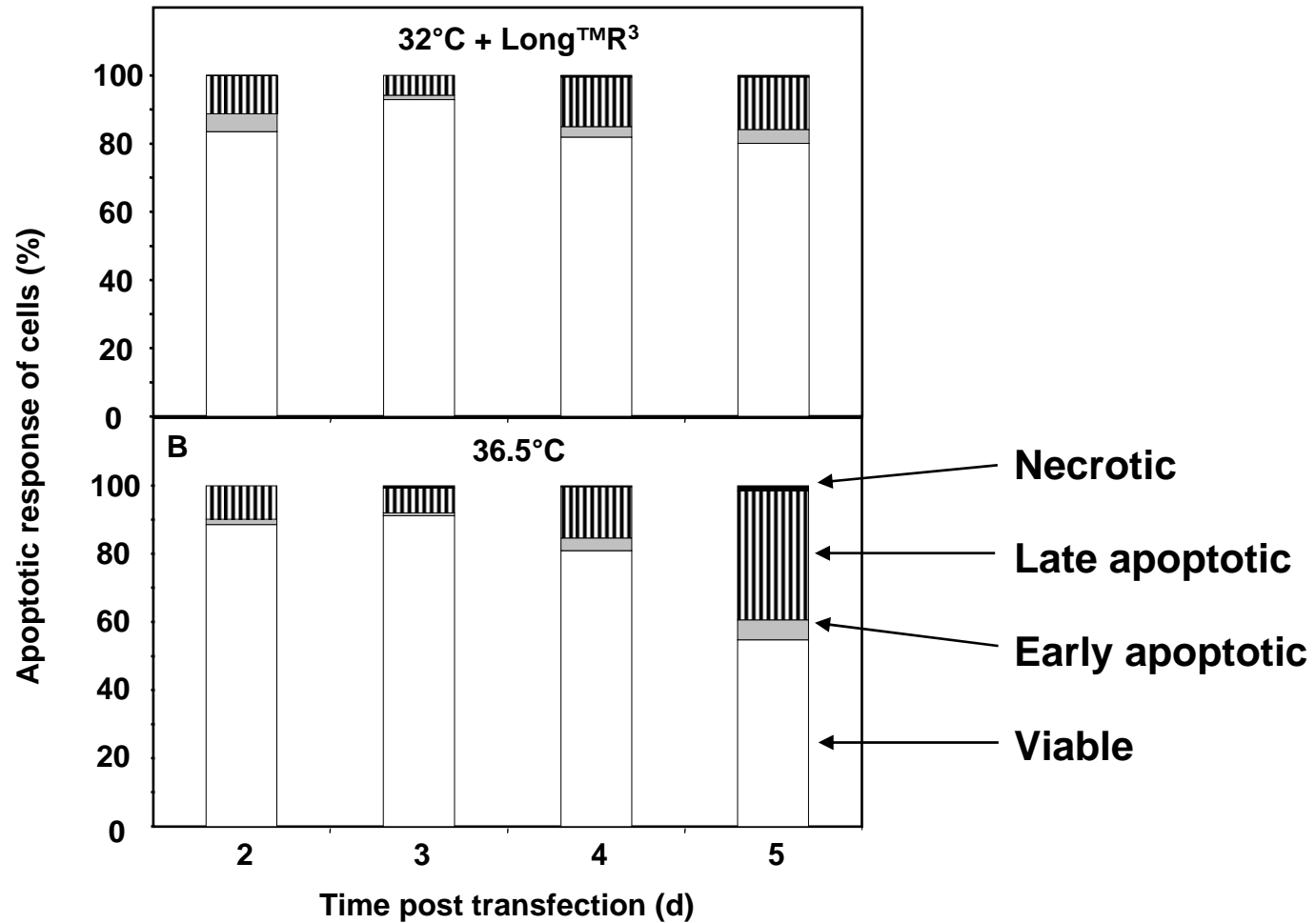
Effects of simultaneous mild hypothermic conditions with the presence of Long™R³

- Combination of mild hypothermic conditions and Long™R³ led a significant increase in antibody concentration from 2.5 to 3 to a final antibody concentration of 12.5 mg/L
- Also boosted cell specific production from between 0.1 to 0.4 pg/cell/day to a final production rate of 0.7 pg/cell/day
- Cells were then transfected with eGFP to examine effects on transfection efficiency and apoptosis by using flow cytometry

Synergistic effect on transfection efficiency

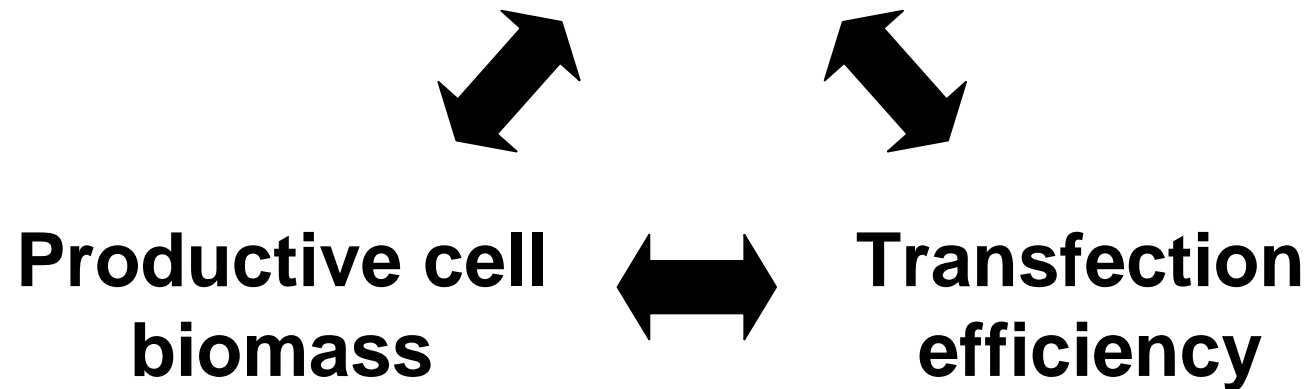


Lowering of apoptosis



Improving Product Yield From Transient Expression

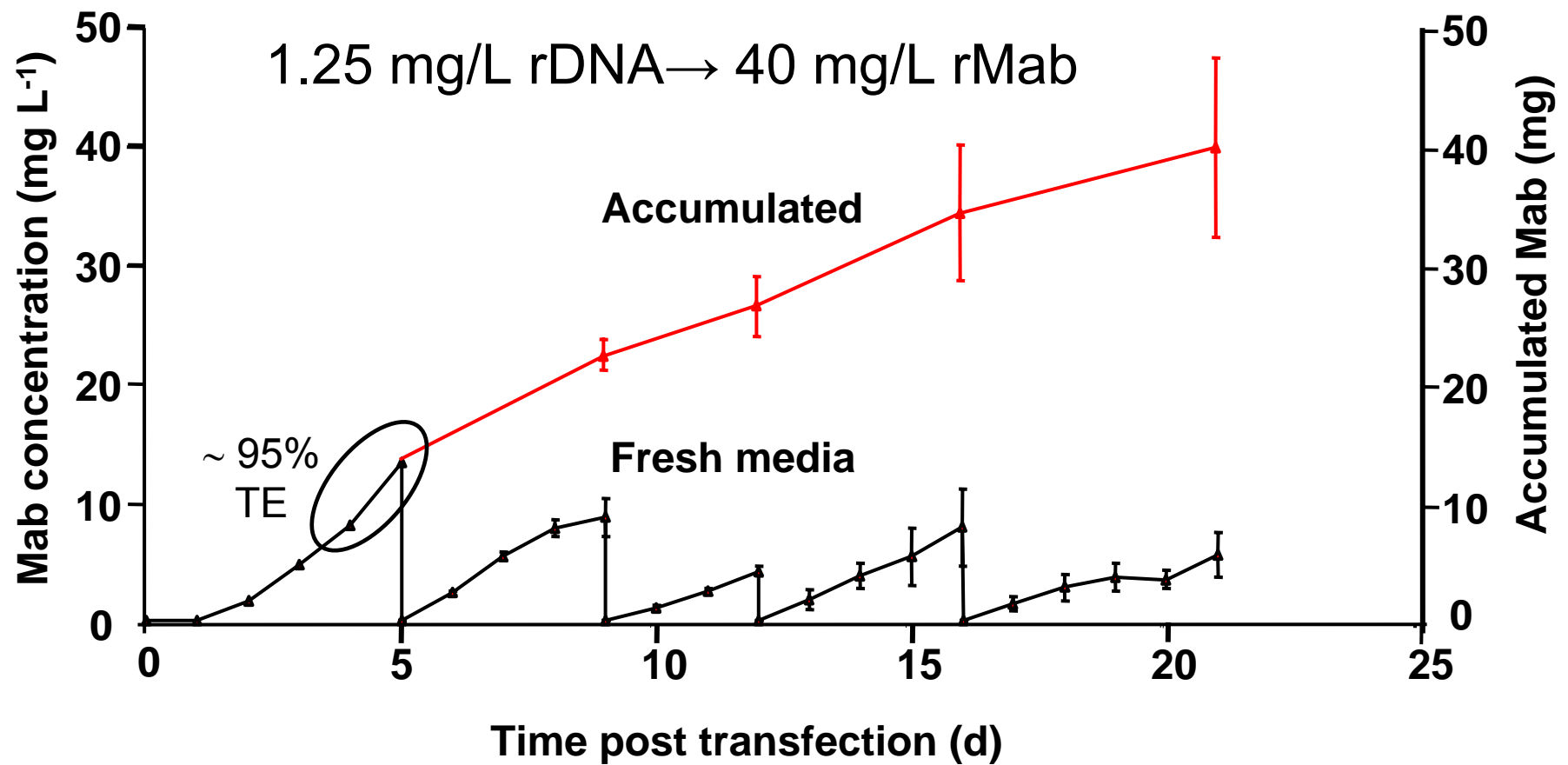
Bioprocess design



New strategy to increase production

- Cells were transfected in the presence of Long™R³ under mild hypothermic conditions (32°C) and then after peak transfection efficiencies were reached, they were centrifuged and resuspended in fresh medium

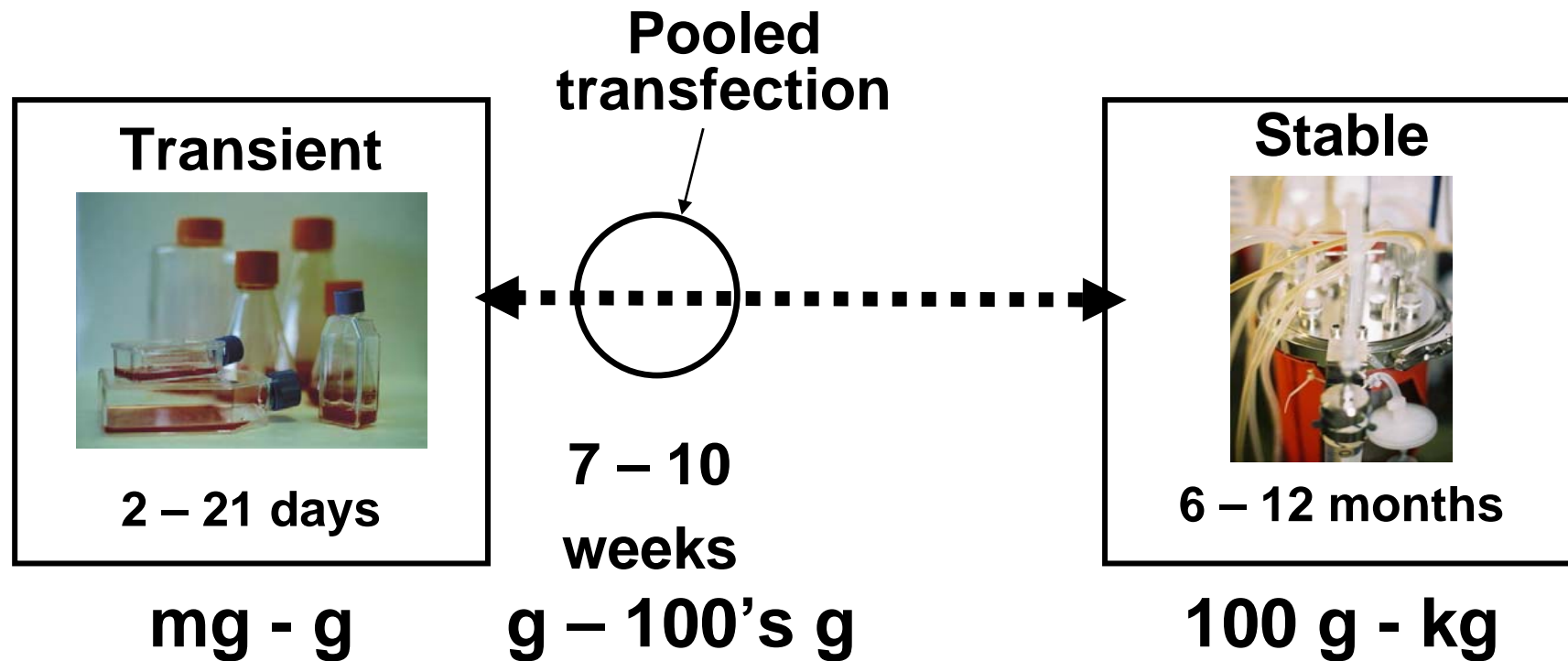
Improved bioprocess



Transient expression conclusions

- Simple bioprocess strategies can have significant synergistic effects on transient expression
- Long™R³ and mild hypothermic conditions work synergistically to boost expression
- Strategies that retain cells in transfected and transcriptionally active state can substantially lengthen the duration of expression and hence boost overall transient Mab production

Review of expression options



Overview of Pooled Transfection



Transfect CHOK1SV host cell line



Static cultures

~ 4 weeks



Transfer from static to suspension culture

Minimum 1 week



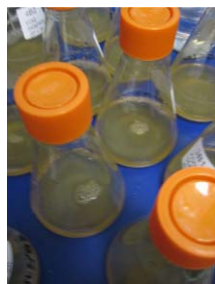
Fed-batch Erlenmeyer flask cultures

15 days



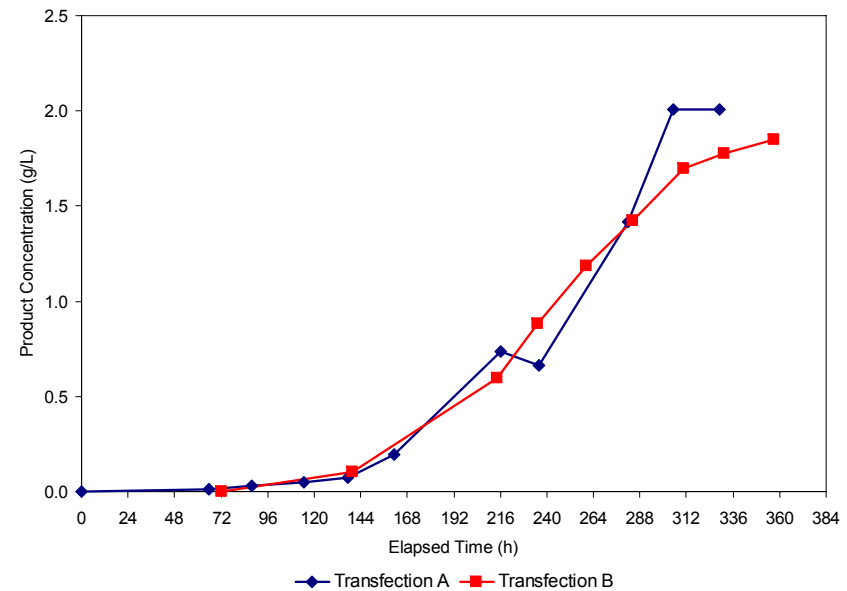
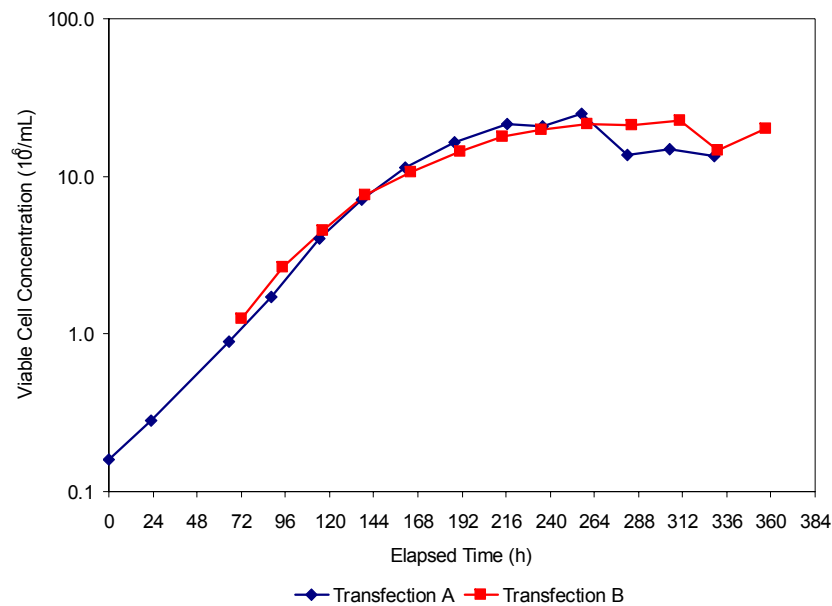
Fed-batch bioreactor cultures

15 days



Transfection to harvest in 7-9 weeks

Growth and Productivity of GS-CHO Pools in Disposable Bioreactor



Inoculated 7 weeks after transfection (7 L culture)

Disposable Bioreactor Cultures of GS-CHO Pools

Run	Maximum viable cell concentration (10 ⁶ /mL)	Time integral of viable cell concentration (10 ⁶ cell.h/mL)	Specific growth rate (1/h)	Antibody concentration at harvest (mg/L)	Q _p (pg/(cell d))	Culture length (days)	Day post transfection at start of culture
A	24.8	3443	0.027	2010	14.0	14	49
B	22.4	4103	0.023	1856	10.8	15	45

***~ 14 g of MAb harvested from 7 L cultures
9 weeks after transfection***

Product Quality Assessment

- Samples analysed from
 - disposable bioreactor cultures
 - flasks derived from same transfection
 - flasks derived from different transfections
 - early and late fed-batch Erlenmeyer flask cultures
 - and compared to product expressed by a stable cell line, in a bioreactor
- Banding patterns were comparable by
 - reduced and non-reduced SDS PAGE
 - isoelectric focusing

Product Quality conclusions

- There is consistency in product between flasks derived from different transfections
- Although product is average of material produced by many cell lines, it is comparable with material made by single cell line

Different products

- To date this procedure has been used on our own internal protein and 3 other proteins
- Mean harvest Mab concentration = 1028 mg/L
- Mean harvest Mab concentration (excluding own model antibody) = 770 mg/L
- This was achieved in a variety of expression formats from disposable bioreactor systems (wave) to more traditional bioreactor formats
- Total quantities achieved have reached hundreds of g's of product within the 9 weeks from transfection to harvest

Conclusions

- The pooled transfection method is capable of quickly generating a significant amount of recombinant product in a method that is very similar to the generic fed-batch and fermentation operations

Comparison with Transient expression

- The pooled transfection technique is slower but produces more protein than most transient expression techniques
- Pooled transfection technique is similar to stable cell culture techniques
- Pooled transfection uses less recombinant DNA than transient expression techniques
 - < mg quantities DNA used regardless of scale compared with mg/L
- Pooled transfection has fewer scale limitations than more traditional transient expression options

Final conclusions

- Expression format chosen depends on the output desired from the fermentation
- Simple bioprocess manipulations can have a synergistic effect on transient expression
- The pooled transfection method is capable of generating up to hundreds of grams of material in a timeframe between transient expression and stable cell line generation

Acknowledgements

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