ENGINEERING CHO CELL LINE FOR ENHANCED PRODUCTION OF RECOMBINANT PROTEINS

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Introduction

The development of new mammalian expression platforms represents an ongoing need for advancing biopharmaceuticals towards human clinical trials.

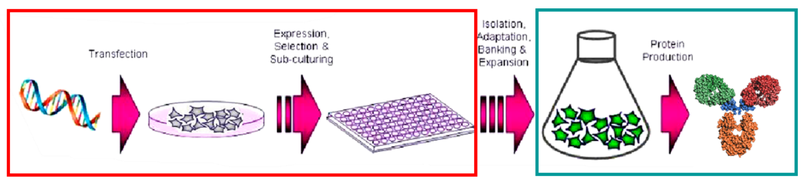
In an effort to address this issue, we have developed new solutions for more efficient identification of individual protein candidates in stably transfected CHO cells. We have shown that the combination of high transfection efficiency with the presence of chromatin structural elements (Selexis Genetic Element) in the expression vectors results in higher efficiency of expression and stability of engineered cells. This allows the protein of interest to be readily secreted and recovered in high yield from the medium. Based on this approach, combinatorial human antibody libraries are generated in CHO cells to allow the identification of the most promising human antibody for binding and expression.

However, additional bottlenecks linked to translation processes such as inefficient processing and secretion remained to be addressed. We have developed metabolically engineered cell lines to co-express chaperones in an attempt to further increase production yields. Indeed, we reached 400% improvement with difficult to express monoclonal antibodies.

The efficiency of the platform allowed significantly increased numbers of high affinity therapeutic antibody candidates to be evaluated for pharmacological and physicochemical properties. Furthermore, high yielding cell lines have been isolated from these pools, thus minimising the risk of product heterogeneity in subsequent toxicology studies.

This novel platform offers considerable time and labour savings as it enables the development of the production cell line by simple cell cloning from the initial cell pool.

1. HOW PLATFORM COMPONENTS IMPACT ON YIELD



- Expression vector ➔ expression level
- Transfection method ➔ copy number
- Host cell line ➔ secretion, p-t modifications
- Selection method ➔ strength of expression

GENETICS

- Media ➔ yield, structure
- Culture conditions ➔ yield, structure

PROCESS

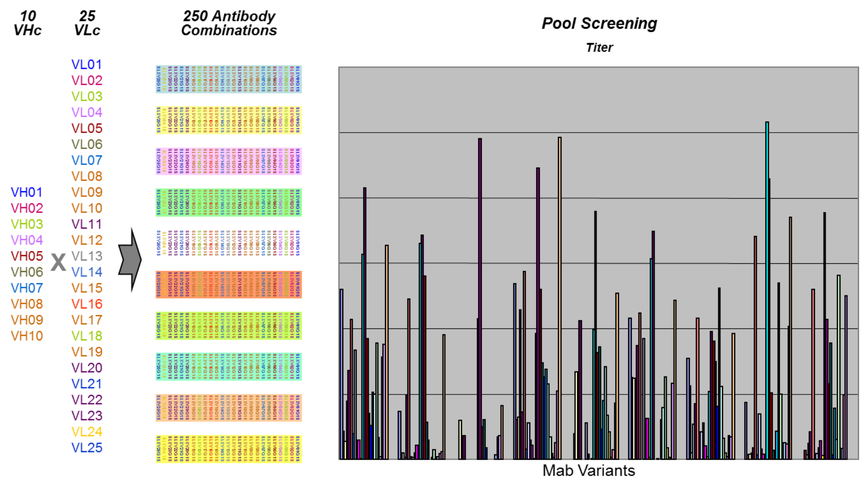
2. BOTTLENECKS IN PROTEIN PRODUCTION

	Gene copy Nb* (Hc + Lc)	mRNA/gene* (Hc + Lc)	IgG amount (batch culture; d3)
mAb1	1.525	166	0.1
mAb2	1.472	5707	22.7
mAb3	7.740	35740	85.4
Other 1	0.493	6344	20
Other 2	4.21	29	7

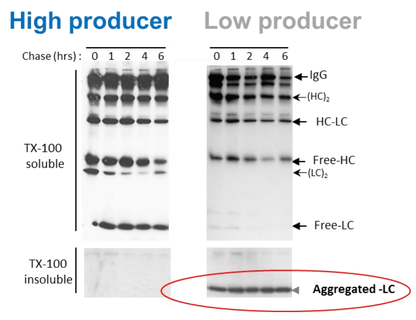
* Data normalized to GAPDH used as endogenous control

- Limited integration ➔ sequence-dependent ?
- Limited transcription ➔ mRNA instability ?
- Limited translation ➔ saturation of secretion pathway?

3. TYPICAL MPACT OF HEAVY AND LIGHT CHAINS ON PERFORMANCE

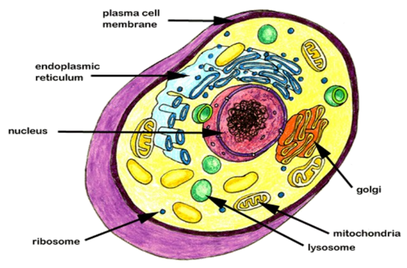


4. INEFFICIENT PROCESSING AND SECRETION



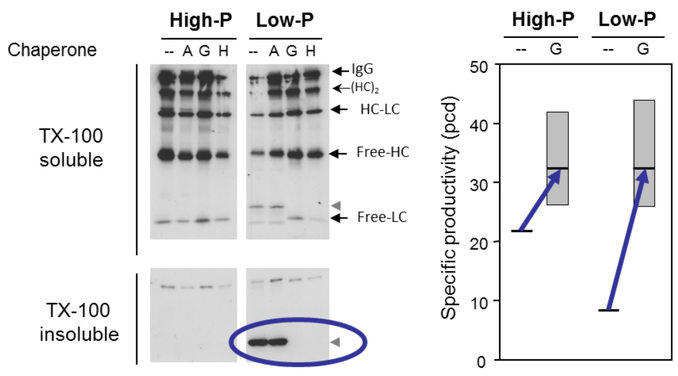
- Codon optimization ➔ no increase in productivity
- Signal peptide ➔ +10% using IL-SP with Low-Producer

5. APPROACHES TO DEBLOTTLENECKING PRODUCTION

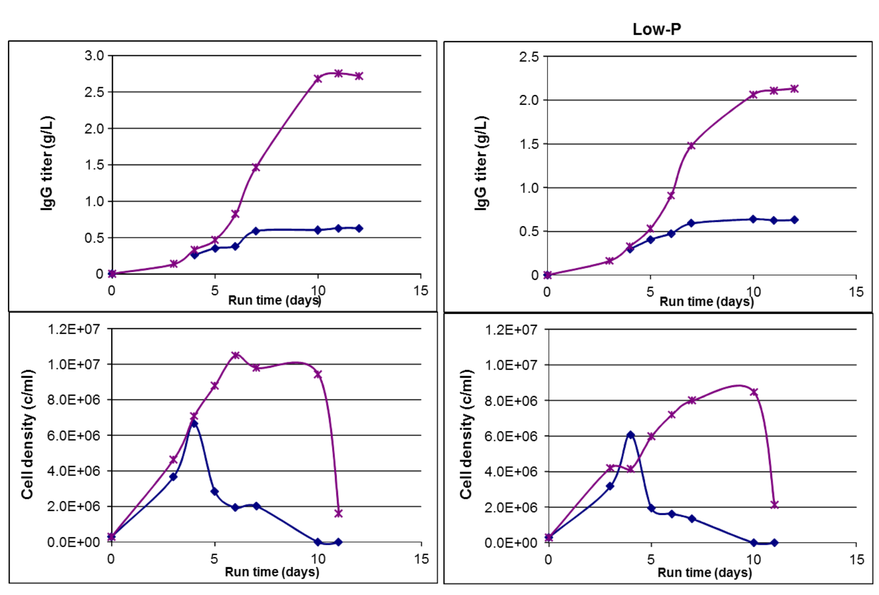


- Limited integration ➔ expression vector design
- Limited transcription ➔ codon optimization, toxic motifs
- Limited translation ➔ culture conditions, media optimization, cell engineering

6. METABOLICALLY ENGINEERED CELL LINE



> More efficient secretion with co-expressed chaperone:
> + 400% on « Difficult-to-express mAb »
> + 40% on « Easy-to-express mAb »



Conclusions

- Bottlenecks are specific to each protein
- Appropriate chaperones can augment the secretion of recombinant protein
- Development of a SuperCHO host cell line by co-expression of chaperones has limitation
- CHO genome sequence will enable genetic engineering deactivation/activation of chaperone systems