CHO CELL LIBRARY FOR THE SELECTION OF IMPROVED RECOMBINANT THERAPEUTIC PROTEIN PRODUCTION CLONES

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1. INTRODUCTION

In an effort to improve product yield of mammalian cell lines, we have previously demonstrated that our proprietary DNA elements, Selexis Genetic Elements (SGEs), increase the transcription of a given transgene[1], thus boosting the overall expression of a therapeutic protein drug in mammalian cells.

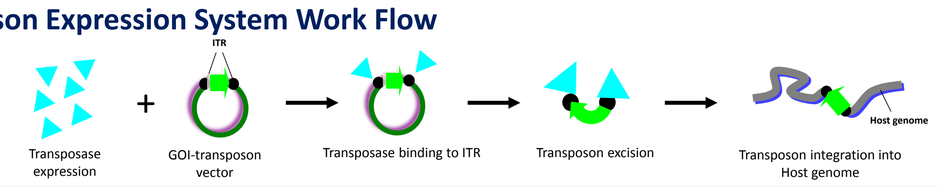
However, there are additional cellular bottlenecks, notably in the molecular machineries of the secretory pathways. Most importantly, mammalian cells have some limitations in their intrinsic capacity to manage high level of protein synthesis as well as folding recombinant proteins. These bottlenecks often lead to increased cellular stress and, therefore, low production rates.

Our specific approach involves CHO cell line engineering. We constructed a CHO-M library based upon the CHO-M genome and transcriptome and using unique proprietary transposon vectors harboring SGE DNA elements to compensate for rate-limiting factors. This CHO-M*plus* library displays a diversity of greater than $1x10^7$ auxiliary proteins involved in secretory pathway machineries and cellular metabolism.

We show that our CHO-M*plus* library enabled the selection of a clonal cell line expressing 10 fold more product by comparison to standard approaches.

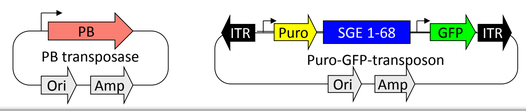
2. TOOLS FOR LIBRARY CONSTRUCTION

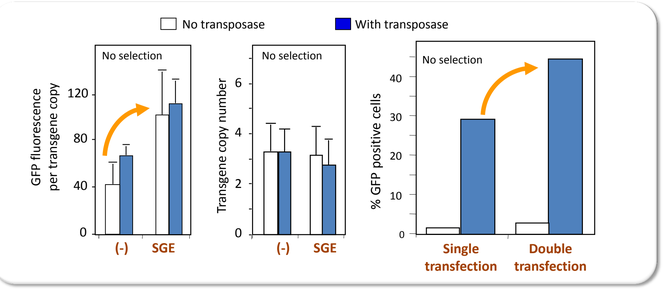
Transposon Expression System Work Flow



Stable Expression by Combining SGEs and Transposon Vectors

Transposon expression vectors:



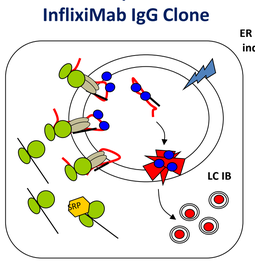


The addition of SGEs to GFP-transposon vectors enhances the expression of GFP per transgene copy number.

The combination of SGEs containing transposon vectors with double transfection results in 50% of the CHO-M cells expressing the transgene without any antibiotic selection.

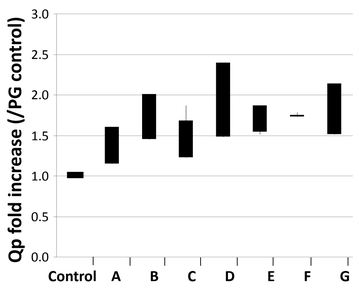
3. CELLULAR PATHWAY ENGINEERING

Low Expressor: InflixiMab IgG Clone



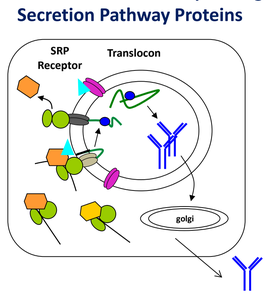
IgG Light Chain misprocessed, IB aggregation induce UPR Stress responses, cell cycle delays, …

Engineered Translation and ER Translocation Pathways

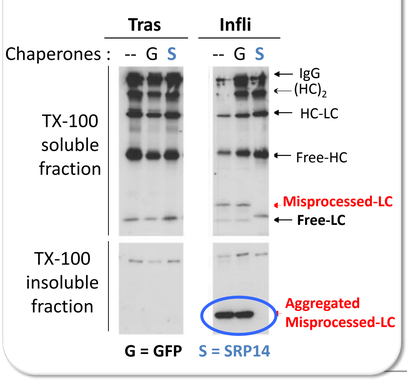


SRP receptor; translocon subunits; SRP9, 14, 54 proteins overexpressed in various combinations

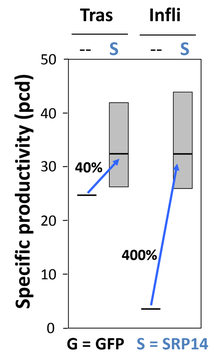
InflixiMab Clone Overexpressing Secretion Pathway Proteins



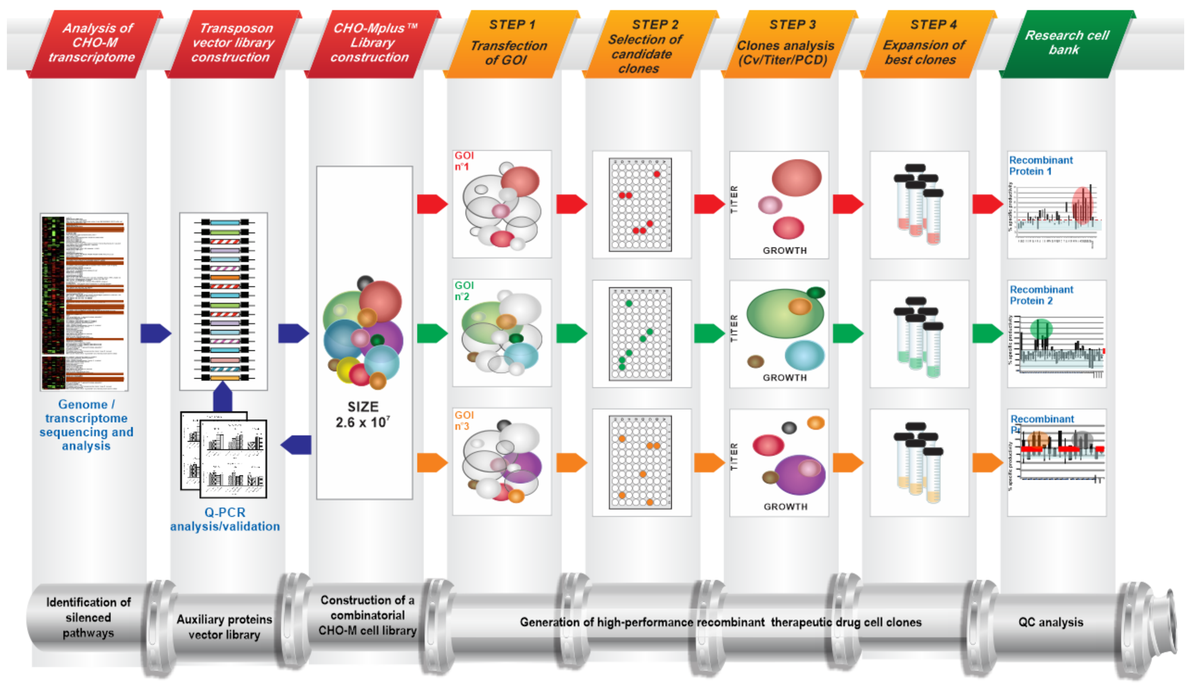
Delayed Light Chain elongation results in correct processing and folding of IgG Light Chain and higher antibody titer in supernatant.



Efficient folding and secretion of IgG after a multistep pathway engineering yields a secretion increase of 40% to 400% of Trastuzumab (Tras) and Infliximab (Infli).



4. CHO-M*plus* LIBRARY GENERATION

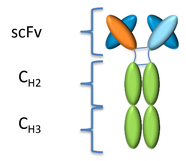


The CHO-M*plus* library expressing combinations of auxiliary proteins to rescue and support secretory pathway machineries and cellular metabolism.

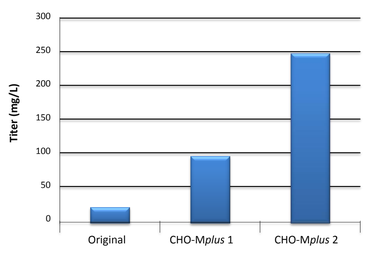
5. APPLICATION: MINIBODY

Novel Scaffold

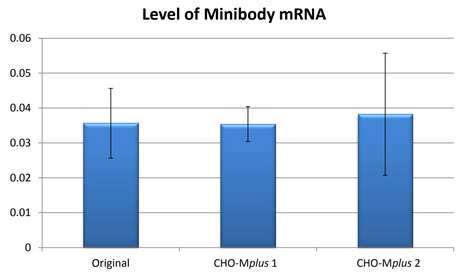
Minibody, single chain variable region (VH-VL) fused to Fc region (H-CH_2-CH_3)



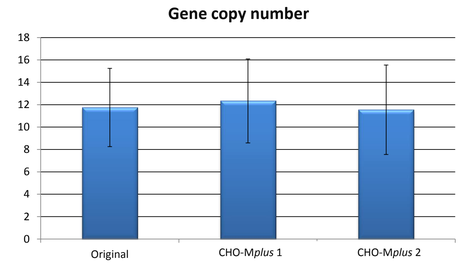
Productivity



Level of Minibody mRNA



Gene copy number



The iterative application of the CHO-M*plus* library enabled the selection of a clonal cell line expressing 10 fold more product by comparison to standard approaches.

6. CONCLUSION

The elucidation of protein expression bottlenecks is time-consuming, labor intensive and is dependent on the gene to be expressed. By mapping both the genome and the transcriptome of its CHO-M cell line, we have developed the combinatorial CHO-M*plus* library which addresses a broad range of expression issues simultaneously.

The combinatorial library metrics are:

1. Specifically tailored to improve production and secretion properties of recombinant proteins in the CHO-M production cell line
2. Combines SGE DNA elements and transposon technology to ensure maximum expression of both secretory components as well as recombinant proteins of interest
3. Addresses > 100 different secretory components of the CHO cell line
4. The combinatorial library complexity is greater than $1x10^7$
5. Easily integrated with selection criteria for most recombinant proteins

We have applied this strategy to select for clonal cell lines expressing secreted proteins such as monoclonal antibodies, enzymes, hormones, blood factors as well as membrane-bound proteins such as GPCRs and ion-channels.

REFERENCES

[1] Ley D, Harraghy N, Le Fourn V, Bire S, Girod PA, Regamey A, Rouleux-Bonnin F, Bigot Y, Mermod N. MAR Elements and Transposons for Improved Transgene Integration and Expression. **PLoS One 2013** Apr 30;8(4).
[2] Le Fourn V, Girod PA, Buceta M, Regamey A, Mermod N. CHO cell engineering to prevent polypeptide aggregation and improve therapeutic protein secretion. **Metab. Eng. (2013)** Feb 1.
[3] Girod PA, Nguyen DQ, Calabrese D, Puttini S, Grandjean M, Martinet D, Regamey A, Saugy D, Beckmann JS, Bucher P, Mermod N. Genome-wide prediction of matrix attachment regions that increase gene expression in mammalian cells. **Nat Methods. 2007** Sep;4(9):747-53. Epub 2007 Aug 5.

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