Synthetic Genetic Elements for Improving Recombinant Protein Expression

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Abstract

High productivity cell lines are essential for improving the availability of mAb drugs. Recombinant gene transcription ultimately controls the recombinant protein production rate by mammalian cells. Synthetic promoters can significantly increase productivity and facilitate an optimal stoichiometry of recombinant polypeptide co-expression. We describe improved mAb expression in GS-KO CHO cells by using novel transcriptional hubs that utilise an optimal combination of synthetic promoters and modified SV40E promoters to enhance expression

SynGenSys's Third Generation Synthetic Promoters

- Rationally designed using rules formulated by SynGenSys
- Not variations of hCMV-IE promoter
- Access more of CHO cell transcriptional landscape than hCMV-IE promoter
- High level of sequence diversity
- Higher productivity, compared to hCMV-IE, seen for a range of products when transcription driven by a CHO.SET® promoter

- Transcriptional hubs created by combining CHO.SET® promoters with SV40E promoter variants engineered to minimise overlap in TFRE species
- Productivity using transcriptional hubs higher than either CHO.SET® promoters or SV40E variants in isolation or standard hCMV-IE/SV40E combination
- Transcriptional hub concept offers approach to increasing selection stringency without the downside of using high concentrations of selective agents

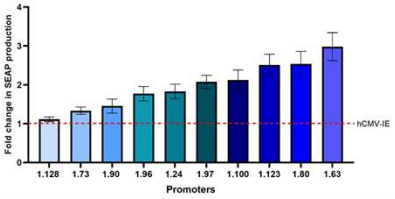
Introduction

Biotherapeutic proteins are made primarily using mammalian cells. Such processes are associated with complex implementation and high cost-of-goods, which limit more widespread patient access. The need to overcome access limitations drives efforts to enhance productivity and reduce costs. In the bioreactor, product concentration is a function of cell specific production rate (Qp), mean viable cell concentration, and process duration. To date, most efforts have focussed upon improving the space-time yield of viable biomass with relatively few efforts focussing upon improving Qp. An assumption is that the widely used hCMV-IE promoter, used to drive transgene transcription, cannot be improved upon. The work undertaken by SynGenSys and its co-founders shows that rules for designing mammalian promoters can be formulated and used to build better promoters. These promoters are not variants of naturally occurring viral or endogenous promoters but true *de novo* designs leading to better Qp values compared to hCMV-IE.

1. Identifying strong promoters

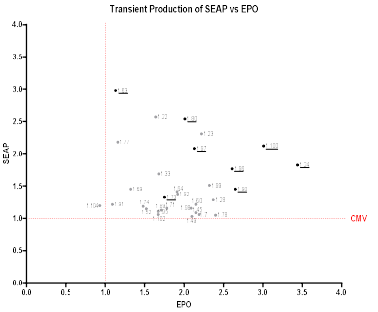
The CHO.SET® master library, containing over 100 promoters, was screened using SEAP expression. These novel promoters are built from 67 unique transcription factor response elements (TFREs) using SynGenSys proprietary design rules.

- Ten members of the CHO.SET Promoter Library®
- Transcriptional strengths up to >3-fold greater than hCMV-IE
- Encompass a range of design criteria and sizes
- High sequence diversity
- Difficult to reverse engineer

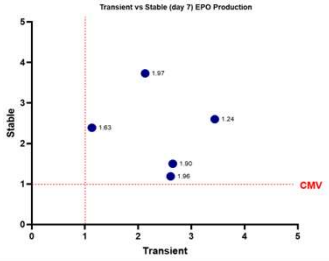


2. EPO production

- Over 30 CHO.SET® promoters, including members of CHO.SET Library®, were tested with EPO and SEAP
- With SEAP, all were stronger than hCMV-IE
- Data showed that synthetic promoters, except from 1.104, resulted in higher EPO titre compared to hCMV-IE
- Breadth of library design allows identification of promoters with yield benefits across broad range of expressed proteins (e.g. 1.97) and/or high specific yield benefits for individual proteins (e.g. 1.63 vs 1.24)

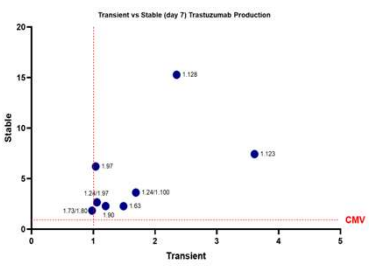


- Five members of CHO.SET Promoter Library® were tested for ability to produce EPO in transient and stable pool systems
- All tested promoters were more active than hCMV-IE
- 3-fold improvement achieved compared to hCMV-IE in both settings
- Promoter selection for transient or stable transfection ensures greatest productivity benefit



3. Trastuzumab

- Stable and transient expression of trastuzumab tested for nine CHO.SET® Promoters
- Five cases: same promoter selected to drive LC and HC expression
- Three cases: different promoters used to drive LC and HC expression. The two promoters (shown as LC then HC in the figure) were chosen to have different design features and promoter strengths
- Majority of promoters / promoter combinations exhibit greater activity than hCMV-IE
- CHO.SET Promoter Library® provides options for increasing productivity in both transient and stable transfections

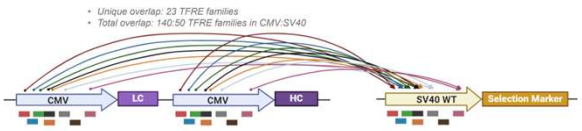


4. Transcriptional hubs: a route to higher productivities

A standard approach to obtaining more productive cell lines is to use higher concentrations of selective drugs to increase selection stringency during CLC. This risks selection for unwanted phenotypes e.g. reduced growth rate or altered cellular redox potential. Increased expression of the selectable marker gene risks damping transgene transcription as both promoters compete for the same transcription factors (TFs). Transcriptional hubs, artificial assemblies of genes whose transcription is driven by distinct promoters, create the possibility of stringent selection using synthetic promoters designed with minimal overlap of TFREs and no increased levels of selective drugs.

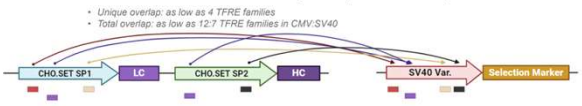
- hCMV-IE competes with SV40E TFREs for TFs
- Co-located CMVs may compete with each other but retain activity due to TFRE redundancy
- Low SV40 activity leads to slow outgrowth of transfected cell populations as insufficient GS leads to a lack of glutamine
- This mechanism may favour clones with silenced CMV activity, affecting Qp levels

High promoter interference risk:
many TFREs shared between CMV & SV40 promoters



- Synthetic promoters were designed with non-redundant, tested CHO-active TFREs for efficient TF recruitment
- Minimal overlap in TFREs between promoters
- Selection for good growth from high GS gene expression does not risk damping transgene expression due to competition for limited number of specific TFs
- Some competition remains as promoters recruit shared transcriptional machinery, but good growth and high productivity possible

Low promoter interference risk:
•fewer TFREs shared between CHO.SET synthetic promoters & SV40 promoters



- Both CHO.SET® (transcriptional power) and SV40E variant (selection stringency) promoters in isolation improve trastuzumab production in fed-batch culture using bulk pools
- Transcriptional hub further improves productivity
- Additive improvement in titre observed for some combinations (blue text)
- Possible synergistic improvement in titre observed for some combinations (red text)

SV40E variant	Dual hCMV-IE	Dual 1.118	1.24 LC / 1.97 HC	Dual 1.128	Dual 1.97
WT	16	212	183	299	107
v2.2	231	833			
v2.3	513		501	841	708
v2.4	97	658			
v2.5	415		395	734	641

Values are trastuzumab titre (mg/L) at harvest (day 9-11) and mean of n = 2 or 3 cultures

- Beneficial impact upon trastuzumab Qp seen by changing either selection stringency (SV40E) or transcriptional power of promoters driving mAb gene expression
- Further benefit seen from changing selection stringency and transcriptional power together
- Possible synergistic improvement in Qp observed for some combinations (highlighted cells)

SV40E variant	Dual hCMV-IE	Dual 1.118	1.24 LC / 1.97 HC	Dual 1.128	Dual 1.97
WT	0.5	4.4	2.7	5.1	1.7
v2.2	1.7	14.0			
v2.3	7.1		12.5	14.4	11.4
v2.4	1.3	12.3			
v2.5	3.7		7.8	14.6	10.3

Values are Qp in pg/(cell.day) estimated by linear regression of titre vs IVCC