Synthetic Transcriptional Hub™ Vectorology for CHO Cell Engineering

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Ryan Taylor, Molly Smith, Cristina Alexandru-Crivac, Kate Fewkes, Renata Caikauskaite, Andy Racher, David James
Email: andy.racher@syngensys.com
SynGenSys Ltd, Sheffield, S1 2JE, UK

Abstract

CHO.SET Transcriptional Hubs™ replace fixed promoter designs with computationally matched assemblies of product-gene and selection-marker promoters that co-vary in transcription factor responsiveness and expression strength. Evaluating vectors with distinct synthetic components enables identification of product-specific promoter stoichiometries that optimise stable transgene expression, Qp, and cell growth. This shifts vector design beyond one-size-fits-all approaches, expanding the genetic design space to de-risk and accelerate CLD, and improve availability of biologics medicines.

SynGenSys's Third Generation Synthetic Promoters

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

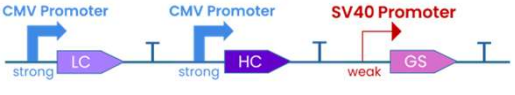
- Transcriptional Hub™ created by combining CHO.SET® promoters with synthetic selectable marker promoters (SMPs) engineered to minimise overlap in TFRE species
- Productivity using Transcriptional Hub™ higher than either CHO.SET® promoters or SMPs in isolation or standard hCMV-MIE/SV40E combination
- Transcriptional Hub™ concept offers approach to increasing selection stringency without the downside of using high concentrations of selective agents

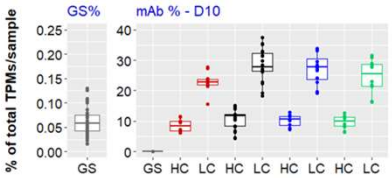
Introduction

Conventional genetic vectors for CHO engineering use fixed promoter combinations that limit control over transgene stoichiometry and selection pressure. Syngensys vectors instead deploy computationally designed synthetic promoter pairs that work synergistically to maximise CHO cell-specific productivity (Qp) and shorten timelines for bulk pool progression. This enables users to identify a product-optimal vector during cell line development without added time or resource burden. CHO.SET Transcriptional Hubs™ comprise more than 100 novel synthetic promoters offering a wide transcriptional activity range in CHO cells. Each hub combines product-gene and selection-marker promoters matched by their transcription factor response element (TFRE) profiles. Evaluating vectors built from these discrete promoter combinations allows selection of constructs that deliver product-specific optimal transgene stoichiometry while maximising host-cell Qp and growth.

1. The problem with standard promoter set-ups

Layout of typical vector for mAb production





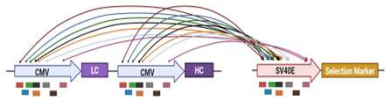
mRNA expression of CMV-driven light chain (LC) and heavy chain (HC) of 4 mAbs, and SV40-driven GS in a GS-KO CHO host cell line.
Fig 3 from Alexandru-Crivac, CN *et al*, 2024
https://doi.org/10.1101/2024.06.27.600995v1

- Low selectable marker (SM) gene expression is potentially growth limiting
- CMV competes with SV40E promoter, a strong promoter in its own right, for transcription factors causing substantial reduction in GS gene transcription
- Fixed transgene and selectable marker expression ratios when using standard CMV-CMV-SV40 set-up
- The need for high levels of selective drugs can cause selection for unwanted phenotypes

2. Transcriptional Hubs™: a route to higher productivities

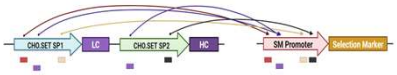
A standard approach to obtaining more productive cell lines is to use higher concentrations of selective drugs to increase selection stringency during CLC. This risks selection for unwanted phenotypes e.g. reduced growth rate or altered cellular redox potential. Increased expression of the selectable 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.

High promoter interference
Many TF binding elements shared between CMV and industry relevant SM promoters



- hCMV-MIE and SV40E **promoters interfere** through shared TFREs, significantly dampening SV40E-driven transcription
- Co-located **CMV promoters may compete with each other** but retain activity due to TFRE redundancy
- Low SV40E activity leads to less Selectable Marker protein and **slower outgrowth** of desired cell populations
- This mechanism may favour clones with silenced CMV activity, leading to **low Qp levels**

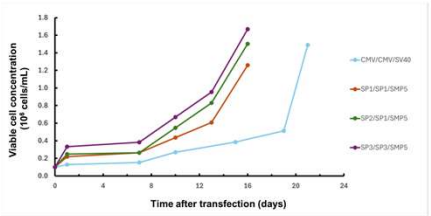
Tuneable promoter interference in Transcriptional Hub™
***Fewer** TFREs shared between CHO.SET™ and SM promoters*

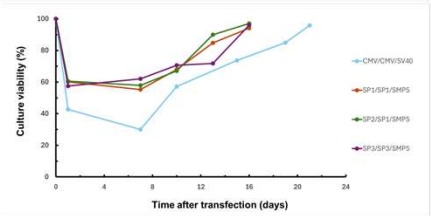


- Designed with varying compositions of active CHO-specific TFREs
- Transgene and SM promoters are co-optimised for minimal interference and hence **rapid outgrowth of high Qp cells**.
- This increases the likelihood of both improved growth and higher productivity than cells containing the standard vector architecture
- No population dynamic pressure to select for unstable clones with silenced transgene synthetic promoter activity

3. Transcriptional Hub™: faster recovery following transfection

- GS-KO CHO host transfected by nucleofection with vector encoding LC and HC genes for trastuzumab plus glutamine synthetase (GS) and allowed to recover according to vendor's guidelines
- Transcription of HC and LC genes driven by hCMV-MIE (CMV) or the CHO.SET promoters SP1, SP2 and SP3
- Transcription of the GS gene driven by either the SV40E promoter (SV40) or a synthetic promoter (SMP5) designed to form a Transcriptional Hub™ when paired with a CHO.SET® promoter
- Data are means of three replicates





- Cells transfected with Transcriptional Hub™ vector recover faster compared to standard CMV/SV40 vector
- Cell concentration at day 10 0.5-0.7 x 10^6 viable cells/mL for cultures using Transcriptional Hub™ compared to 0.3 x 10^6 viable cells/mL for CMV-CMV-SV40 vector, with culture viabilities of about 70% compared to 59% for CMV-CMV-SV40
- For Transcriptional Hub™ vectors, cell concentration achieves 10^6/mL between days 13-16 compared to 19-21 for CMV/SV40 vector
- Similar results seen for CHO GS-KO hosts obtained from two different vendors and different hCMV-MIE promoters

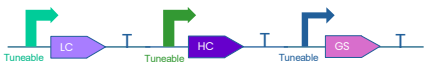
4. Transcriptional Hubs™: higher productivities

CHO.SET Transcriptional Hub™	Promoter strength	Qp (pg/(cell.d))	Harvest titre (mg/L)	IVC (10^6 cells.d/mL)
CMV#2/CMV#2/SV40E	--	5.6	660	117
CMV#3/CMV#3/SV40E	--	5.7	679	118
1.20/1.20/2.5	Low	2.0	226	113
1.97/1.97/2.5	Medium	11.7	1418	121
1.128/1.128/2.5	High	19.9	2202	111

Gene order: LC-HC-GS
Strength assigned on SEAP productivity ranking and relative to hCMV

- Trastuzumab production by GS-KO CHO host tested in fed-batch culture according to vendor's guidelines
- CHO.SET® promoters and Transcriptional Hub™ designed, through use of more powerful promoters to drive transgene transcription and reduced overlap of TFREs, to improve mAb gene expression of each cell. The expected result of an increase in Qp compared to the standard vector set-up was observed.
- Substantially improved harvest titre compared to the standard vector architecture primarily due to large increase in Qp and not IVC

5. Transcriptional Hubs™: answering the productivity question



- Work done at SynGenSys shows that treating the transgenes and SM gene as parts of a single system, a Transcriptional Hub™, can result in substantial improvements in Qp, even over the use of strong synthetic promoters in isolation
  - Lower likelihood of interference between promoters as less competition for TFs
  - Good growth and fewer unwanted phenotypes as high selective agent concentrations are not required