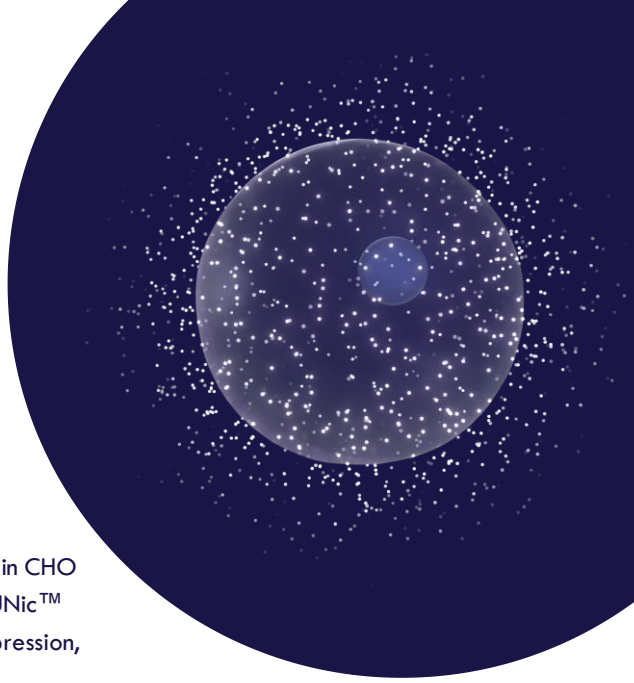
ProteoNic Biosciences

Enhancing Protein Expression in CHO Cell Lines: Unleashing the Potential of 2G UNic™ technology



Introduction

Protein expression in Chinese Hamster Ovary (CHO) cell lines plays a crucial role in the production of biopharmaceuticals. To meet the growing demand, cell line development researchers are actively seeking innovative solutions to enhance protein expression levels. This technical application note delves into the groundbreaking impact of 2G UNic™ technology on improving protein expression in CHO cell lines. By effectively enhancing transcription and translation processes, the 2G UNic™ technology demonstrates its remarkable ability to significantly increase protein expression, even for highly complex and difficult to express (DTE) proteins.

2G UNic™ Genetic Elements

The 2G UNic™ technology utilizes strategic enhancer elements (~1.2 kb) positioned 5' of the gene of interest (GOI) to optimize transcription and translation. These elements work synergistically to enhance protein expression. They are applied to generate stable cell lines for clinical and commercial development.

Transcription Enhancer Elements

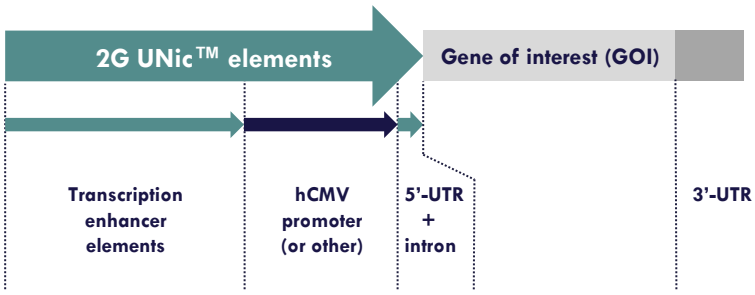
The inclusion of a dual promoter system in the 2G UNic™ technology leads to increased transcription levels. By combining two promoters in a proprietary manner, transcription is significantly boosted. Additionally, the enhancer element is epigenetically optimized to enable high- level expression, irrespective of the genomic locus.

Translation Enhancer Elements

2G UNic™ technology offers a notable advantage in enhancing translation efficiency. The combined improvement of mRNA transport, ribosome affinity, and mRNA stability, comprised in the 5'-UTR element, results in enhanced protein production per mRNA molecule and higher protein yields.

Implications for Protein Expression in CHO Cell Lines

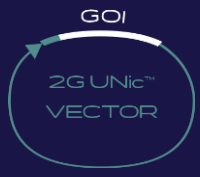
The combined impact of the 2G UNic™ technology transcription and translation enhancer elements on CHO cell lines is significant. By optimizing both processes, the technology enables substantial improvements in protein expression, even for highly complex and DTE proteins.



Adopting 2G UNic™ technology

2G UNic™ technology does not require any change to your current workflow and offers two approaches for adoption. Firstly, researchers can integrate the 2G UNic™ technology genetic elements directly into their existing vector, benefiting from enhanced protein expression and optimized mRNA processing. Alternatively, they can utilize the 2G UNic™ technology optimized vector by inserting their gene of interest (GOI) into it.

This streamlined approach ensures efficient transcription, translation, and expression of the GOI, resulting in improved protein yields. These flexible options empower researchers to leverage the full potential of the 2G UNic™ technology and enhance protein expression in CHO cell lines.



Robust: 2G UNic™ technology has successfully and reproducibly been applied to multiple proteins and cell lines

Flexible: Ready to be incorporated into your vector or vectors ready for your GOI

Reduced costs: Increase protein production to significantly decrease COGs

Clinically validated & industry proven: 2G UNic™ technology applied to therapeutic molecules in PII and beyond by Top 10 Pharma

About ProteoNic

ProteoNic is a leading provider of premium vector technology, validated through clinical applications. Our advanced vector platform, 2G UNic™ technology, significantly enhances production levels of therapeutic proteins and biologics, resulting in immediate and substantial cost savings, while expanding production capacity. 2G UNic™ technology is made available through licensing and partnership arrangements.

Business Development Contacts

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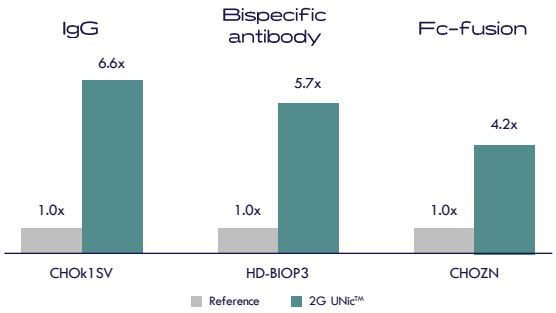
Enhancing Protein Expression: A Comparative Analysis of Clonal Cell Lines Generated with Reference and 2G UNic™ Vectors

In order to evaluate the influence of 2G UNic™ technology on protein expression enhancement, we collaborated with our partners to generate a series of CHO cell lines. These cell lines were constructed with either a conventional reference vector or incorporated 2G UNic™ technology.

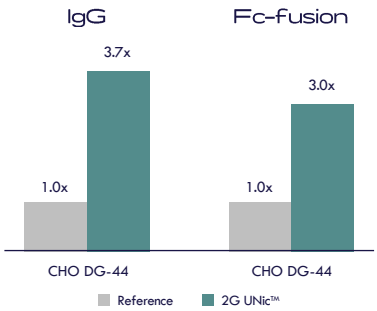
CHO GS-/- Selection

In this study, we compared the fed-batch titers of clonal cell lines generated using a reference vector and the 2G UNic™ vector in CHO cell lines, namely CHOk1SV, HD-BIOP3, and CHOZN. As demonstrated in Figure 1, the results revealed a significant increase in protein expression with the incorporation of 2G UNic™ technology. This increase was observed for complex, more difficult to express proteins including a bi-specific antibody and Fc-fusion protein.



CHO DG-44, DHFR Selection

CHO DG44 cells (DHFR-/-) were used in the study. A conventional CMO vector was compared to a modified vector incorporating 2G UNic™ technology. The results demonstrated a significant 3- to 4-fold increase in protein production using the vector containing 2G UNic™ technology elements. Further improvements in absolute production levels were achieved through additional measures such as MTX amplification, sub-cloning, and scale-up to 300 L (data not shown).



CHO-S and CHO-k1, Antibiotic Selection

Cells were grown with a reference CMO vector or a vector incorporating 2G UNic™ technology. In both CHO-S and CHO-k1 the low expression was elevated by 2- to 3-fold with the addition of the 2G UNic™ technology. Further optimization by our client (data not shown) with media and scale-up achieved production levels exceeding 6 g/L.

Conclusion

In summary, the 2G UNic™ technology offers a transformative approach to enhancing protein expression in CHO cell lines. Through its integrated approach of transcription and translation enhancers, this technology optimizes every stage of the protein synthesis process. Furthermore, 2G UNic™ technology does not impact the product quality, scalability, and stability of the cell lines (data not shown). By leveraging 2G UNic™ technology, cell line development researchers can unlock the potential for significantly improved protein yields, paving the way for advancements in biopharmaceutical production and other protein-related applications.

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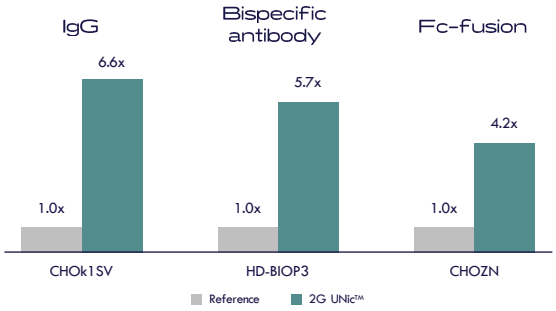

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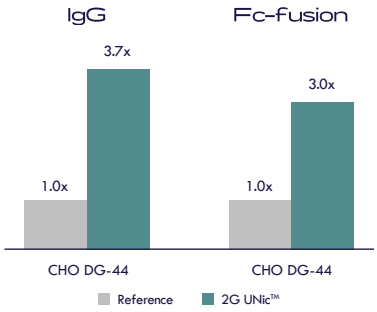

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