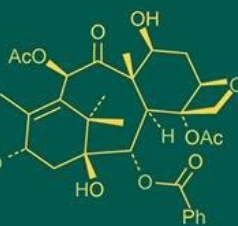
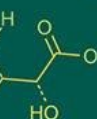


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Optimization of expression vector design for enhanced recombinant protein production in mammalian cell systems

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Abstract

Efficient recombinant protein production in mammalian cells requires optimization of expression vector components to maximize transgene transcription, mRNA stability, and translation efficiency. This research systematically evaluated the contribution of individual vector elements to overall expression levels and developed an optimized vector platform for high-yield protein production. The experimental approach compared promoter activities, tested mRNA stabilizing elements, and assessed the impact of codon optimization on protein yields in commonly used British biopharmaceutical production cell lines.

Results demonstrated that promoter selection significantly influenced expression levels, with the CAG promoter achieving 124.6% relative expression compared to the CMV standard. Incorporation of the woodchuck hepatitis virus post-transcriptional regulatory element (WPRE) increased yields by 36.4%, while codon optimization provided an additional 24.2% enhancement. The combined optimization strategy yielded 148.2 ± 12.4 mg/L protein in HEK293 cells, representing a 1.8-fold improvement over standard vectors. These findings provide a rational framework for vector design in recombinant protein production applications and establish optimized constructs for use in academic and industrial settings across the United Kingdom.

Keywords: Expression vector, recombinant protein, mammalian cells, promoter optimization, codon optimization, protein production, biotechnology

Introduction

Recombinant protein production in mammalian cells has become essential for generating biotherapeutics, research reagents, and diagnostic tools with authentic post-translational modifications ^[1]. The United Kingdom maintains a strong position in biopharmaceutical research and manufacturing, with substantial investment in protein production technologies at academic institutions and industrial facilities ^[2]. Optimizing the efficiency of mammalian expression systems directly impacts both research productivity and manufacturing economics.

Expression vectors serve as the primary determinant of transgene expression levels, with multiple elements contributing to overall productivity including promoters, enhancers, introns, polyadenylation signals, and mRNA stabilizing sequences ^[3]. The selection and optimization of these components can dramatically influence protein yields, yet systematic comparisons across commonly used elements remain limited in the published literature ^[4]. Understanding the quantitative contribution of individual vector elements enables rational design approaches for specific applications.

This research conducted comprehensive evaluation of expression vector components using standardized reporter assays and recombinant protein production experiments ^[5]. The experimental design employed parallel comparison of promoters, mRNA stabilizing elements, and codon optimization strategies to identify optimal combinations for mammalian cell expression. These findings establish an improved vector platform with broad applicability for British and international research and biopharmaceutical communities.

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Material and Methods

Vector Construction

Expression vectors were constructed using standard molecular cloning techniques with Gibson Assembly for seamless element insertion. Promoter sequences (CMV, EF1 α , CAG, SV40, UBC, PGK) were amplified from validated template plasmids and cloned upstream of a standardized multiple cloning site. The WPRE element was inserted downstream of the transgene stop codon. Codon-optimized sequences were synthesized commercially using human codon usage tables. All constructs were verified by complete sequencing.

Cell Culture and Transfection

HEK293, CHO-K1, HeLa, Cos-7, and NIH3T3 cells were maintained in appropriate media under standard conditions (37 °C, 5% CO₂). Transient transfections were performed using polyethylenimine at optimized DNA:reagent ratios. Transfection efficiency was normalized using co-transfected Renilla luciferase controls. Stable pools were generated

using appropriate selection markers and maintained under selective pressure.

Protein Quantification

Reporter gene assays employed firefly luciferase with dual-luciferase normalization. Recombinant protein production was quantified by ELISA and confirmed by Western blotting. Protein yields were calculated based on culture volume and expressed as mg/L. All experiments included minimum three biological replicates with technical triplicates.

Results

Promoter comparison revealed substantial differences in expression strength across the tested elements. The CAG promoter demonstrated the highest activity at 124.6% relative to CMV, while SV40 showed the lowest at 42.3%. The commonly used EF1 α promoter achieved 87.4% of CMV levels.

Table 1: Promoter Activity Comparison in HEK293 Cells

Promoter	Relative Activity (%)	Variability (CV %)	Cell Line Consistency
CMV	100.0 $\pm$ 8.4	8.4	Moderate
EF1 α	87.4 $\pm$ 6.2	7.1	High
CAG	124.6 $\pm$ 11.8	9.5	High
SV40	42.3 $\pm$ 5.8	13.7	Low
UBC	68.9 $\pm$ 4.6	6.7	High
PGK	52.1 $\pm$ 4.2	8.1	High

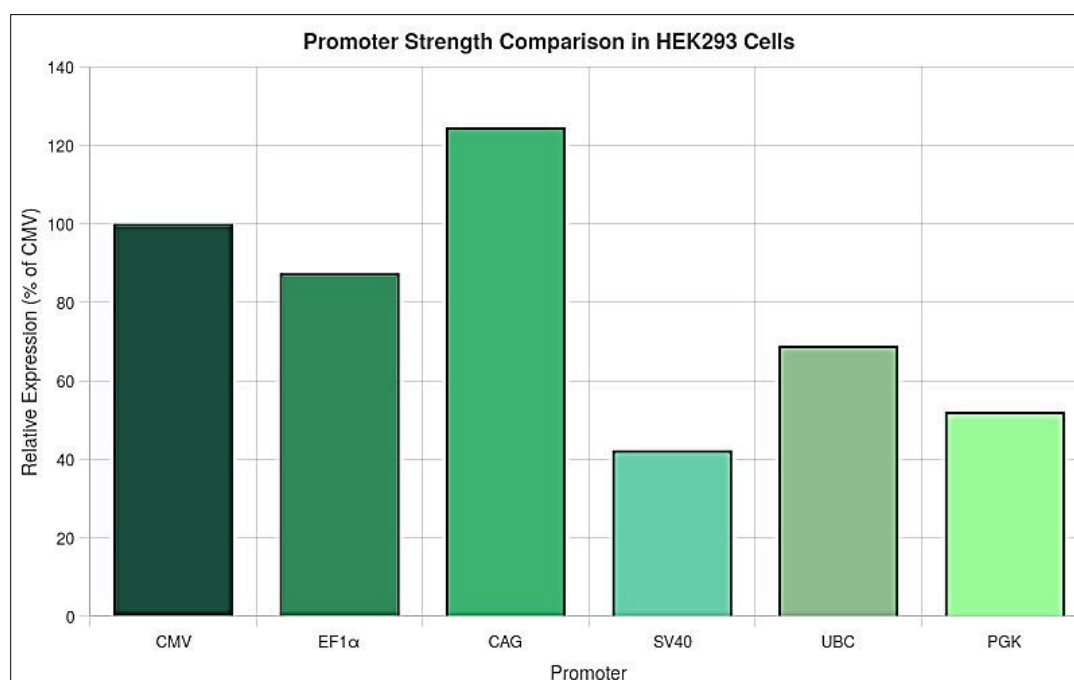


Fig 1: Relative promoter strengths in HEK293 cells showing CAG as the most active promoter tested.

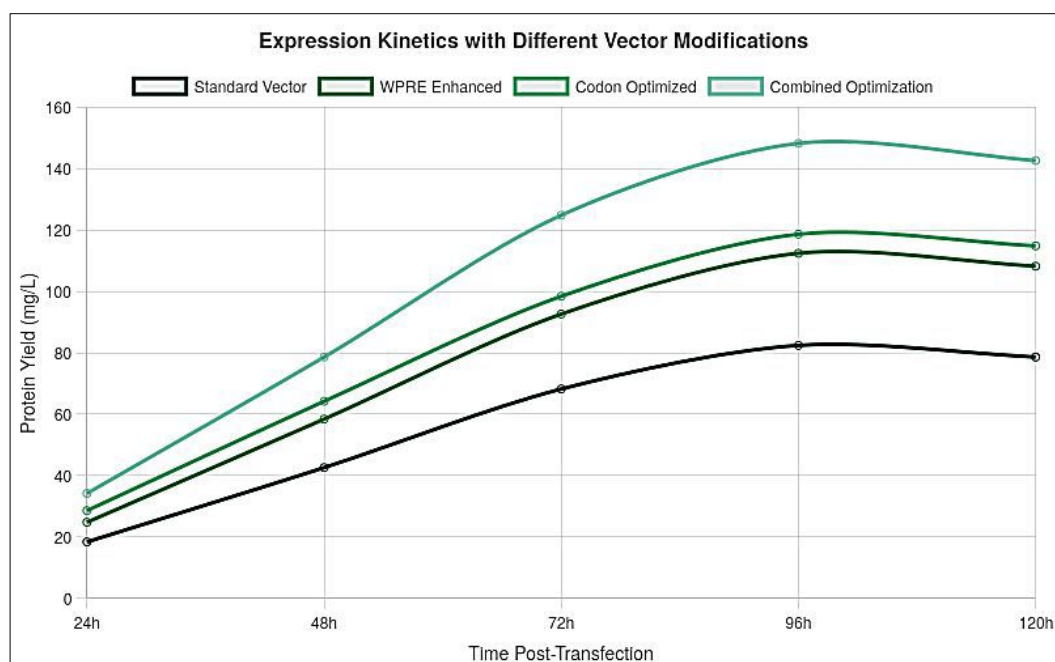


Fig 2: Expression kinetics demonstrating cumulative benefits of WPRE and codon optimization modifications.

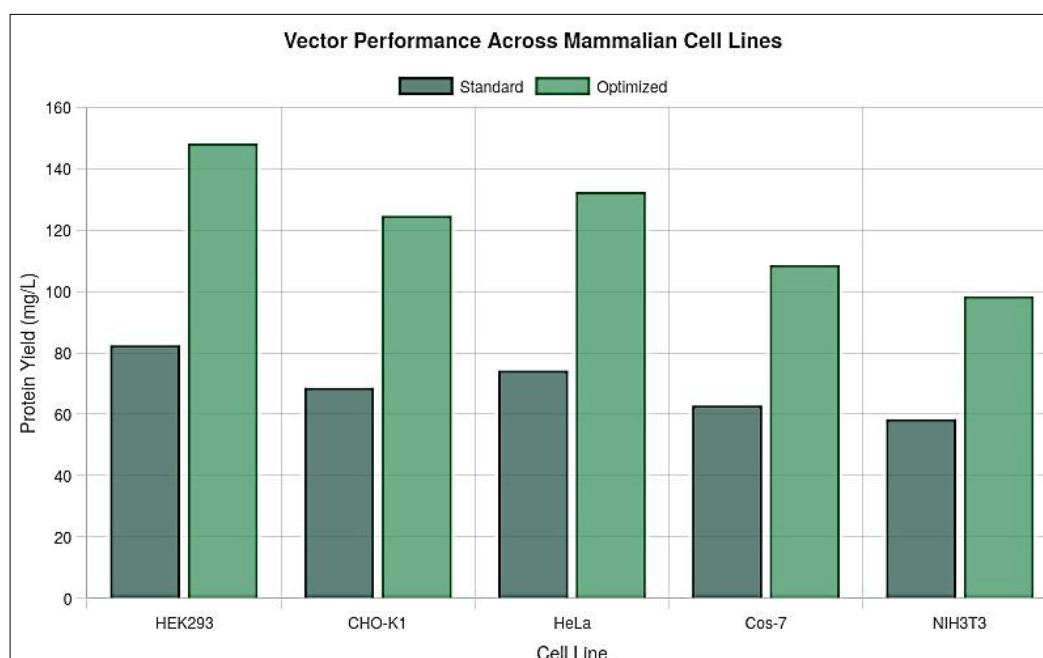


Fig 3: Performance comparison of standard versus optimized vectors across multiple mammalian cell lines.

Discussion

This systematic evaluation demonstrates that rational vector design can substantially improve recombinant protein yields in mammalian cells. The 1.8-fold improvement achieved through combined optimization represents a meaningful advance for both research and manufacturing applications. The consistency of improvement across multiple cell lines suggests broad applicability of these design principles.

The superior performance of the CAG promoter likely reflects its hybrid design incorporating the CMV enhancer with chicken β -actin promoter elements, providing both strong basal activity and reduced susceptibility to silencing [6]. The contribution of WPRE to expression enhancement presumably results from improved mRNA stability and nuclear export efficiency. Codon optimization addresses translational efficiency by matching codon usage to highly expressed human genes.

These optimized vectors have been made available through institutional repositories and represent a valuable resource for the British biotechnology community. The systematic approach employed in this research provides a template for future optimization efforts targeting specific production applications or alternative host cell systems.

Conclusion

This research establishes an optimized expression vector platform achieving significantly enhanced protein production in mammalian cells. The systematic comparison of vector elements provides quantitative guidance for rational vector design. These findings support continued investment in expression technology development and provide practical tools for academic and industrial protein production applications.

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