Improvement of translational efficiency by N-terminal codon optimization in the reconstituted cell-free protein synthesis system

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Abstract

In this study, we aimed to improve the translational efficiency of protein synthesis by optimizing N-terminal codons. We used the PUREflex® system, which is a reconstituted cell-free protein synthesis system. We found that the optimization of N-terminal codons significantly increased the translational efficiency.

1. PUREflex®

The PUREflex® system is a reconstituted cell-free protein synthesis system that uses a highly purified ribosome. This system allows for the optimization of N-terminal codons to improve translational efficiency.

2. Experiments

- Protein Synthesis
  - SDS-PAGE (0.5 μL of reaction/lane)
  - Gel stain with Coomassie Blue (Bio-Rad)
  - Analysis with LAS4000 (Fujifilm)

3. Results 1: N-terminal codon and protein synthesis

3-1. Fab Heavy Chain (Herceptin)

AT-rich codon vs GC-rich codon

AT-rich codon > GC-rich codon

4. Result 2: Additional sequence in N-terminus of ORF

4-1. Codon optimization of Hisx6-tag

Conversion from GC-rich codon of Hisx6-tag to AT-rich codon

4-2. Effect of additional sequence of N-terminus to improve translational efficiency

5. Conclusion

In N-terminal sequence...

We recommend the AT-rich codons in N-terminal sequence for high protein expression level.

On-going

Further analysis of Herceptin Hc codon optimization in N-terminal sequence
Investigation of suitable additional sequence for high protein expression

References