Enhanced in vitro protein synthesis through the optimal design of PCR primers

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Abstract

Functional stability of mRNA is one of the crucial factors affecting the efficiency of in vitro translation. As the rapid degradation of mRNA in the cell extract (S30 extract) causes the early termination of translational reactions, extension of mRNA half-life will improve the productivity of the in vitro protein synthesis. We have developed a PCR-based, simple method to increase the stability of mRNA in S30 extract. Target genes were PCR-amplified with the primers designed such that both ends of a transcribed mRNA molecule anneal to each other. Compared to a normal mRNA, mRNA with the annealing sequences resulted in approximately 2-fold increase of protein synthesis in an in vitro translation reaction. In addition, by sequentially conducting transcription and translation reactions in a single tube, we were able to directly express proteins from the PCR-amplified genes without a separate purification of mRNA.

References

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