

Enhancing the efficiency of cell-free protein synthesis through the polymerase-chain-reaction-based addition of a translation enhancer sequence and the in situ removal of the extra amino acid residues

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In this study, we developed a method for the rapid generation of intact proteins in a cell-free protein synthesis system. It is demonstrated that the productivity of recombinant proteins from the PCR-amplified templates can be remarkably enhanced by use of an optimized translation enhancer sequence (first 3, 5, 7, 10 or 13 amino acids of the CAT). The extra amino acid residues that were derived from the translation enhancer sequence were completely removed by use of appropriate detergent (Brij-58) and peptide cleavage enzyme (Factor Xa) in the reaction mixture. The described results demonstrate the versatility of cell-free protein synthesis to provide an optimized and customized reaction conditions for the efficient production of the desired proteins. Through the combined approach to improve the productivity and 'quality' of the expressed protein as described herein, cell-free protein synthesis will find expanded applications as a versatile tool for the production and engineering of important proteins.