Co-expression of recombinant proteins in cell-free protein synthesis reactions

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In our experiments to produce different combinations of recombinant proteins in a cell-free protein synthesis system derived from E.coli, we found that certain pairs of the ORFs were not expressed equally. Instead, only a single DNA species was expressed dominantly while the expression of the other one was almost completely repressed. Such a bias during the co-expression of DNA pairs was eliminated when an identical downstream box sequence was added to the 5'-ends of the template DNA pairs. Using the identical nucleotide sequences of his-tag or the downstream box of chloramphenicol acetyltransferase (CAT-DB) in front of the target genes that had not been otherwise expressed compatibly, both of the encoded proteins were produced at similar productivities. Moreover, in the presence of a common downstream box, multiple numbers of genes were simultaneously expressed in a same reaction mixture. We expect that the proposed approach will offer a powerful tool for the preparation of unbiased protein libraries as well as for studying the structure and functions of interacting proteins.