

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

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For two plasmid-mediated co-expression in E.coli, mutual compatibility of the replication origins should be carefully considered. In addition, it is also required that each plasmid carries the gene encoding different antibiotic resistance.

Since cell-free protein synthesis does not rely on the growth of cells, we expected that different plasmid could be expressed in a same reaction mixture without such constraints. However, on the contrary to our expectation, the E.coli-based cell-free protein synthesis system showed "biased" expression of proteins. For example, when two plasmids encoding CAT and GFP were incubated in the same reaction mixture, GFP showed dominant expression while the expression of CAT was substantially repressed. The biased expression was overcome by sequentially expressing the target proteins in a dialysis reactor. We are currently investigating the molecular mechanism by which the different templates compete during cell-free protein synthesis.