

Development of new protein display system suitable for protein engineering in cytoplasm of  
*Escherichia coli*

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Proteins can be engineered toward better properties by directed evolution technologies with high throughput screening and, protein display system in which genotype and phenotypes are physically linked, is the most powerful tool for this purpose. So far, several display systems including phage display cell surface display, etc have been developed and widely used in bacterial and yeast host cells. However, even though many proteins have been engineered successfully with those plasmid systems, each system has several drawbacks to be improved - particularly, no display systems are suitable for cytoplasmic proteins yet. In this study, we developed new protein display system, plasmid display system in which target protein is linked to plasmid DNA in cytoplasm of *E. coli*. DNA binding domain (DBD) of human Oct-1 was employed which can bind to specific DNA sequence (ATGCAAAT) with high affinity ( $KD = 9 \times 10^{-11}$  M). With the His-tag fused model, the conditions for gene expression and isolation of protein-linked plasmid complex were optimized. The results demonstrate that Oct-1 based plasmid display system is suitable for the isolation of engineered proteins from huge libraries.