

### Development of plasmid display system and its application for in vitro evolution

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Display technologies which allow proteins or peptides to be physically associated with the encoding DNA have become a tool for enriching molecular diversity and producing novel types of proteins. In this manner, a plasmid display system using GAL4 DNA binding domain was constructed for enriching molecular diversity and in-vitro selection of functional proteins. The possibility and feasibility of the display system was tested by selective enrichment experiments of model proteins and the identification of encoding genes, and thermostable protein screening was also studied based on this plasmid display system. An in vitro selection method for high affinity DNA binding protein was also developed and experimentally demonstrated based on the plasmid display technology, mutants of GAL4 DNA binding domain having high affinity were selected from the mutant protein library of protein-encoding plasmid complex. This study suggests that the display technology can be used to discover a functional protein, such as high affinity and high stability protein, from large libraries by relatively simple steps. In this aspect, the strategy for the selection of functional proteins and the experimental results will be presented.