In vitro evolution of glutathione s-transferase by plasmid display technology

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The plasmid display system based on GAL4 DNA binding domain was applied to in vitro evolution of glutathione s-transferase. Direct enrichment for high activity was achieved from a mutant library of GST by this plasmid display system, and thermostable mutants were screened on the basis of the residual enzyme activity at 50°C. Computational study was also performed to investigate the residual characteristics of the obtained mutants of high activity, and this has given lessons for in vitro evolution of other target proteins, such as stable secondary structure formation, mutation in similar properties, and mutation to amino acids of similar and smaller size. This study showed that the methodology was one of efficient methods for the mutant selection of high activity and stability. In this aspects, the experimental and computational results will be presented.