

Construction of glutamate biosensor using chimeric two-component regulatory system in
Escherichia coli

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In this study, there was constructed the glutamate biosensor that would be used for a high-throughput system to screen microorganisms that produce glutamate. The biosensors are based on two-component regulatory systems combined with GFP reporter protein. A chimeric DegS/EnvZ (DegSZ) TCRS was constructed by fusing the N-terminal domain of the sensor kinase, DegS from *Planococcus* sp. PAMC21323, with the catalytic domain of the osmosensor, EnvZ from *Escherichia coli*, to control expression of the *gfp* gene in response to glutamate. The *gfp* gene was controlled by the *ompC* promoter through the activated response regulator, OmpR-P. The chimeric TCRS-based biosensors showed a four-fold increase in fluorescent signal after the addition of glutamate. A linear correlation was observed between the fluorescence intensity and exogenously added glutamate concentration. The chimeric TCRS-based biosensor was used successfully to determine glutamate concentration at the single cell level by fluorescence-activated cell sorting.