

## Selection of positive clones using photodynamic reaction

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Ferrochelatase is the enzyme coded by hemH in *E. coli* and proceeds the reaction from protoporphyrin IX to protohaem by incorporating ferrous ion. In plant, ferrochelatase governs the reaction on magnesium and protoporphyrin IX. This enzyme is important in biological systems for the ability of capturing various metal ions into biological molecules. When microorganism is deficient of this enzyme, reactive oxygen molecules accumulated inside the cell, thus cell can not survive under light emission. Here we suggest the photochemical selection method of positive clones using photosensitive mutant of *E. coli* and vector system. When constructing library, there appeared at least 5% self-ligated clones even if the vector is well treated with CIP. We constructed the light sensitive mutant of *E. coli* which is deficient of hemH and vector which consists of SD sequence and original hemH gene. Without proper promoter or gene with promoter, this mutant simply can not survive under light exposure. From the system constructed, only light radiation was required to select positive clones. Through this simple and comfort method, we could successfully obtain positive clones from metagenome. (This work was supported by a grant from the Korean Ministry of Science and Technology (Korean Systems Biology Research Grant, M10309020000-03B5002-00000) and by the Brain Korea 21 Project. Further support through the LG Chemicals Chair Professorship is appreciated.)

### References

1. Allan Wallace Scruggs, Neal W. Woodbury. 2003. Optical processing of bacterial libraries for directed evolution. *Biotechnol Bioeng* 84:445-451.
2. Daniel, R. 2004. The soil metagenome-a rich resource for the discovery of novel natural products. *Curr Opin Biotechnol* 15:199-204.