Ops Gene Knock-out and Dps Gene Expression for Coenzyme Q₁₀ Production in *E.coli* BW25113

Hyeong-Hun Choe¹, Gi-Sub Choi¹, Jin-Ho Seo² and Yeon-Woo Ryu¹,

¹ Department of Molecular Science and Technology, College of Engineering, Ajou
University, Suwon, 443-749, Korea

TEL: +82-31-219-2455, FAX: +82-31-216-8777

²School of Agricultural Biotechnology, Center for Agricultural Biomaterials, Seoul National University, Seoul, 151-742, South Korea

Ubiquinone (Coenzyme Q), composed of a benzoquinone group and a side chain of varying length of isoprenoid group, is an essential component of the electron transfer system in the plasma membrane of prokaryotes and inner mitochondrial membrane of eukaryotes.¹⁾ Additional important physiological function of ubiquinone is an antioxidant activity to prevent DNA damage, lipid peroxidation, protein oxidation, etc.²⁾ To produce CoQ₁₀, useful to human, dps(decaprenyl diphosphate synthase) gene is an essential enzyme, which combines ten isoprenoid diphosphate as CoQ10 tail. Usually, E. coli uses CoQ8 to transfer the electron in the plasma membrane of prokaryotes, nervertheless this pathway is efficient to manipulation, and to acquire electron carrier enzyme. In our study, we've disrupted ops(octaprenyl diphosphate synthase) known to combine eight isoprenoid diphosphate in E. coli and have introduced dps gene to E. coli to construct CoQ_{10} production system. That is to say, we've amplified and cloned the chloramphenicol acetyl transferase(Cm^r) gene of pKD3 into yT&A vector to help useful selection. dps gene containing promoter region amplified by PCR and cloned into upper region of Cm^r gene. In next, the recombinant plasmid is transformed in E. coli BW25113 by electroporation and cultured LB broth, induced of IPTG 1 mM for functional expression . In recombinant E. coli, an expression of the dps gene was confirmed by SDS-PAGE and the produced CoQ₁₀ are observed in HPLC.

References

- 1. Kawamukai M. Biosynthesis, bioproduction and novel roles of ubiquinone. *J. Biosci. Bioeng.* 2002, 94, 511-517.
- 2. Szkopiska A. Ubiquinone. Biosynthesis of quinone ring and its isoprenoid side chain. Intracellular localization. *Acta Biochim Pol* 2000, 47, 469-480.