One-step Gene Knockout Strategy in *E.coli* Using New Integration-helper plasmid

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We developed more rapid and efficient engineering method using integration helper plasmid in E. coli. The integration helper plasmid, pCW611, has two recombinases which are expressed in reverse direction by two independent inducible systems. By using this system, required time and effort can be significantly reduced because the iterative transformation of the helper plasmid and curing steps are not required. We could disrupt one target gene in 3 days by using pCW611. To verify the usefulness of this gene manipulation system, the deletion experiments were performed for knocking out four target genes individually (adhE, sfcA, frdABCD, and ackA) and two genes simultaneously for two cases (adhE-aspA and sfcA-aspA). Also, fumaric acid producing E. coil strain was developed by deleting four target genes (fumB, iclR, fumA, and fumC) in 10 days as a proof-of-concept study. [This work was supported by the Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries from the Ministry of Science, ICT and Future Planning (MSIP) through the National Research Foundation (NRF) of Korea (NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557)]