Development of rapid gene manipulation tool in *Escherichia coli*

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The integrated helper plasmid pCW611 contains two recombinases (Red and Cre) which are expressed in reverse direction by two independent inducible systems. The main advantage of this system is that the time and effort required can be significantly reduced because the iterative transformation of the helper plasmid and curing steps are not required. We could delete 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 (*adhE-aspA* and *sfcA-aspA*). Also, sequential deletion of four target genes (*fumB*, *iclR*, *fumA*, and *fumC*) was successfully performed for the construction of fumaric acid producing strain. The efficiencies of target gene replacement and removal of the resistance marker were nearly 50% and 100%, respectively. (Development of systems metabolic engineering platform technologies for biorefineries; NRF-2012-C1AAA001-2012M1A2A2026556) funded by the Ministry of Education, Science and Technology)