Integration-helper plasmid mediated rapid gene knock-out in *E. coli*

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In this work, an integration helper plasmid was designed for rapid gene manipulation in *E. coli*. This plasmid (pCW611) expresses two recombinases (Red and Cre) under separate inducible promoters (P_{lacUV5} and P_{BAD}). Thus, iterative plasmid transformation and curing is avoided, resulting in significant time savings compared to the traditional, two-plasmid approach. This enabled target gene deletion in 3 days. The system was verified by deleting *adhE*, *sfcA*, *frdABCD*, and *ackA* individualy and pairwise (*adhE*-*aspA* and *sfcA*-*aspA*). Finally, a fumaric acid producing *E. coil* strain was developed by deleting four genes (*fumB*, *iclR*, *fumA*, and *fumC*) in 10 days as a proof-of-concept.
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