Protocols for RecET-based markerless gene knockout and integration to express heterologous biosynthetic gene clusters in Pseudomonas putida

Pseudomonas putida has emerged as a promising host for the production of chemicals and materials thanks to its metabolic versatility and cellular robustness. In particular, P. putida KT2440 has been officially classified as a generally recognized as safe (GRAS) strain, which makes it suitable for the production of compounds that humans directly consume, including secondary metabolites of high importance. Although various tools and strategies have been developed to facilitate metabolic engineering of P. putida, modification of large genes/clusters essential for heterologous expression of natural products with large biosynthetic gene clusters (BGCs) has not been straightforward. Recently, we reported a RecET-based markerless recombineering system for engineering P. putida and demonstrated deletion of multiple regions as large as 101.7 kb throughout the chromosome by single rounds of recombineering. In addition, development of a donor plasmid system allowed successful markerless integration of heterologous BGCs to P. putida chromosome using the recombineering system with examples of – but not limited to – integrating multiple heterologous BGCs as large as 7.4 kb to the chromosome of P. putida KT2440. In response to the increasing interest in our markerless recombineering system, here we provide detailed protocols for markerless gene knockout and integration for the genome engineering of P. putida and related species of high industrial importance.

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