Application of RecET Recombineering System for Markerless Chromosome Editing in *Pseudomonas putida*

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Pseudomonas putida has been widely used as a workhorse for producing several valuable natural products. Here we report a RecET recombinase-based recombineering tool for markerless integration of heterologous genes into the chromosome of *P. putida*. By knocking out various genetic loci on the chromosome in *P. putida*, demonstration of efficiency and capacity of constructed system were shown. Further, the system allowed successful integration of gene clusters synthesizing four proof-of-concept bioproducts into the target locus of *P. putida* chromosome. The markerless genome engineering system developed here for efficient gene knockout and integration will advance metabolic engineering of *P. putida*, a bacterial host strain with increasing interest. [This work was supported by the Novo Nordisk Foundation (CFB core funding and NNF 160C0021746) and further supported by Technology Development Program to Solve Climate Changes on Systems Metabolic Engineering for Biorefineries (Grants NRF-2012M1A2A2026556 and NRF-2012M1A2A2026557) from the Ministry of Science and ICT through the National Research Foundation (NRF) of Korea.]