Accelerated genome engineering of Pseudomonas putida by I-SceI-mediated recombination and CRISPR-Cas9 counterselection

Pseudomonas species have become reliable platforms for bioproduction due to their capability to tolerate harsh conditions imposed by large-scale bioprocesses and their remarkable resistance to diverse physicochemical stresses. The last few years have brought forth a variety of synthetic biology tools for the genetic manipulation of pseudomonads, but most of them are either applicable only to obtain certain types of mutations, lack efficiency, or are not easily accessible to be used in different Pseudomonas species (e.g. natural isolates). In this work, we describe a versatile, robust and user-friendly procedure that facilitates virtually any kind of genomic manipulation in Pseudomonas species in 3-5 days. The protocol presented here is based on DNA recombination forced by double-stranded DNA cuts (through the activity of the I-SceI homing meganuclease from yeast) followed by highly efficient counterselection of mutants (aided by a synthetic CRISPR-Cas9 device). The individual parts of the genome engineering toolbox, tailored for knocking genes in and out, have been standardized to enable portability and easy exchange of functional gene modules as needed. The applicability of the procedure is illustrated both by eliminating selected genomic regions in the platform strain P. putida KT2440 (including difficult-to-delete genes) and by integrating different reporter genes (comprising novel variants of fluorescent proteins) into a defined landing site in the target chromosome.

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