The Ssr protein (T1E_1405) from Pseudomonas putida DOT-T1E enables oligonucleotide-based recombineering in platform strain P. putida EM42

Some strains of the soil bacterium Pseudomonas putida have become in recent years platforms of choice for hosting biotransformations of industrial interest. Despite availability of many genetic tools for this microorganism, genomic editing of the cell factory P. putida EM42 (a derivative of reference strain KT2440) is still a time-consuming endeavor. In this work we have investigated the in vivo activity of the Ssr protein encoded by the open reading frame T1E_1405 from Pseudomonas putida DOT-T1E, a plausible functional homologue of the β protein of the Red recombination system of λ phage of Escherichia coli. A test based on the phenotypes of pyrF mutants of P. putida (the yeast’s URA3 ortholog) was developed for quantifying the ability of Ssr to promote invasion of the genomic DNA replication fork by synthetic oligonucleotides. The efficiency of the process was measured by monitoring the inheritance of the changes entered into pyrF by oligonucleotides bearing mutated sequences. Ssr fostered short and long genomic deletions/insertions at considerable frequencies as well as single-base swaps not affected by mismatch repair. These results not only demonstrate the feasibility of recombineering in P. putida, but they also enable a suite of multiplexed genomic manipulations in this biotechnologically important bacterium.