Poster

Genexput: A rapid and standarised method for large genome expansion of Pseudomonas putida

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ABSTRACT

The soil bacteria Pseudomonas putida has gained considerable interest in recent years due to its metabolic complexity, which gives the bacteria huge potential in fields as diverse as bioremediation and bio-based industrial production of chemicals [1]. However, the full exploitation of P. putida potential requires of the development of standarised and hightrouput large genome edditing methods in this bacterium. Actual synbiotools for this purpose in P. putida are still in its infancy and need to be optimised. For instance, well-known state-of-the-art methods such as recombineering and CRISPR-Cas approachs have yet low efficiency in this bacteria [2]. In an attemp to address this important shorcoming in the portfolio of P. putida synbiotools, we have constructed a pSEVA-based [3] library of vectors which allows the genome integration via homologous recombination of large fragments of exogenous DNA. Thereof, loci distributed all along the genome were selected and designed as landing paths for the integration of alien DNA. This way it is possible to add an additional level of gene expression control at genome localization level. We further validated our approach by i) monitoring the expression level of a single GFP reported gene along the genome localization and ii) by expanding the metabolic versatility of P.putida KT2440 towards toluene and m-xylene by integrating in its chromosome the genes encoded in the pWWO plasmid. In summary we provide an efficient toolkit that allows a rapid genome expansion of P. putida while allowing the expression level of the desired transgene.

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