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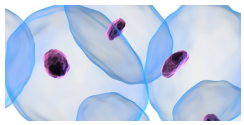

Development of CRISPR Multiplex Recombineering Technologies (CRMAGE) in *P. putida* to enable genome recoding

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Type of thesis: Experimental

Required competences: Basic molecular biology cloning techniques (bacterial cell growth culturing, transformation, PCR, gel electrophoresis, restriction-ligation, assembly…), basic oligonucleotide design.

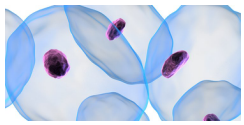
Acquired competences: CRISPR/Cas, recombineering, MAGE, CRMAGE, MASC-PCR, SEVA-Brick cloning and assembly, fluorescence analysis.

Date: 26-09-2019

Description

CRISPR/Cas technologies have been shown to efficiently improve the levels of allelic replacement in recombineering protocols when used as a counter selection tool in several organisms, including *Pseudomonas putida*. This versatile cell factory is being redesigned to substantially enhance its commercial value by increasing its abilities for tailored biocatalysis[1]. While several recombineering protocols for *P. putida* have been published during the last five years[2–4], high levels of efficiency have been only shown when the method has been combined with CRISPR/Cas counter selection[3,5].

In this context, our objective is the development of an alternative method which would allow us to perform genome editing in a much more flexible manner, almost without restrictions, in order to mutate any specific locus and multiple loci at the same time (MAGE)[6]. Moreover, such tool would facilitate complex whole-genome editing projects like recoding or rewriting of genomes[7–10].



References

1. Martínez-García, E., Nikel, P. I., Aparicio, T. & de Lorenzo, V. Pseudomonas 2.0: genetic upgrading of P. putida KT2440 as an enhanced host for heterologous gene expression. *Microbial Cell Factories* **13**, (2014).
2. Aparicio, T., Jensen, S. I., Nielsen, A. T., de Lorenzo, V. & Martínez-García, E. The Ssr protein (T1E_1405) from *Pseudomonas putida* DOT-T1E enables oligonucleotide-based recombineering in platform strain *P. putida* EM42. *Biotechnology Journal* **11**, 1309–1319 (2016).
3. Choi, K. R., Cho, J. S., Cho, I. J., Park, D. & Lee, S. Y. Markerless gene knockout and integration to express heterologous biosynthetic gene clusters in Pseudomonas putida. *Metabolic Engineering* **47**, 463–474 (2018).
4. Ricaurte, D. E. *et al.* A standardized workflow for surveying recombinases expands bacterial genome-editing capabilities. *Microbial Biotechnology* **11**, 176–188 (2018).
5. Aparicio, T., de Lorenzo, V. & Martínez-García, E. CRISPR/Cas9-Based Counterselection Boosts Recombineering Efficiency in *Pseudomonas putida*. *Biotechnology Journal* 1700161 (2017). doi:10.1002/biot.201700161
6. Ronda, C., Pedersen, L. E., Sommer, M. O. A. & Nielsen, A. T. CRMAGE: CRISPR Optimized MAGE Recombineering. *Scientific Reports* **6**, (2016).
7. Isaacs, F. J. *et al.* Precise Manipulation of Chromosomes in Vivo Enables Genome-Wide Codon Replacement. *Science* **333**, 348–353 (2011).
8. Amiram, M. *et al.* Evolution of translation machinery in recoded bacteria enables multi-site incorporation of nonstandard amino acids. *Nature Biotechnology* **33**, 1272–1279 (2015).
9. Boeke, J. D. *et al.* The Genome Project-Write. *Science* **353**, 126–127 (2016).
10. Gallagher, R. R., Li, Z., Lewis, A. O. & Isaacs, F. J. Rapid editing and evolution of bacterial genomes using libraries of synthetic DNA. *Nature Protocols* **9**, 2301–2316 (2014).