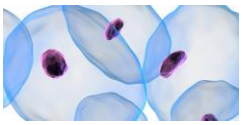

Development of novel CRISPR tools to readjust metabolic pathways

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Type of thesis: Experimental

Required competences: Basic molecular biology techniques: Bacterial cultivation and transformation, PCR, gel electrophoresis, plasmid assembly methods.

Acquired competences: Advanced synthetic biology techniques: novel CRISPR/Cas tools, DNA and gene synthesis, fluorescent essays and HPLC analysis

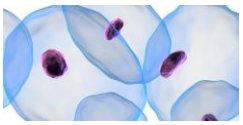
Date: 28-02-2020

Description

Subtle changes in bacterial gene expression can have significant biological consequences. Being able to fine-tune target gene expression is essential for constructing efficient bacterial cell factories. In particular, when introducing heterologous pathways for the production of a target compound, optimal gene expression control and minimal bacterial energy expenditure is desired[1-2].

The development of simple and high-throughput genome engineering tools is indispensable for extensive metabolic engineering, as well as a full exploration of *P. putida*. The CRISPR-Cas9 genome editing technology has provided a precise and high-throughput technique for genome edition, paving the way for a new era in molecular biology[3-4].

We aim at developing novel CRISPR tools to simultaneously regulate the expression of multiple genes, readjusting the metabolism of *P. putida* in a controllable manner. Gene expression will be studied in terms of strength (amount of protein that is produced), genetic stability (the long-term reproducibility), and orthogonality (cross-talk levels with the native genetic pathways).



References

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[2] Vickers, C., Blank, L., & Krömer, J. (2010). Grand Challenge Commentary: Chassis cells for industrial biochemical production. Nature Chemical Biology, 6(12), 875-877. doi: 10.1038/nchembio.484

[3] Amitai, G., & Sorek, R. (2016). CRISPR–Cas adaptation: insights into the mechanism of action. Nature Reviews Microbiology, 14(2), 67-76. doi: 10.1038/nrmicro.2015.14

[4] Marraffini, L. (2015). CRISPR-Cas immunity in prokaryotes. Nature, 526(7571), 55-61. doi: 10.1038/nature15386