Laboratory of Systems and Synthetic Biology
Wageningen University and Research Center
Dreijenplein 10, Wageningen
6703 HB, the Netherlands

Thesis project:

Synthetic Biology - Assesement of recombineering systems in Pseudomonas putida.

Stamatios Damalas
E: stamatios.damalas@wur.nl
Building 316
Room 0.023

Background

Pseudomonas putida is a gram-negative soil bacterium that is renowned for its metabolic flexibility. This metabolic flexibility underlies an inherent toxin and solvent tolerance, which make P. putida an organism of interest for laboratory, industrial and environmental applications. It can (i) tolerate toxic intermediates, (ii) survive in biphase production processes, (iii) produce a large range of products, and (iv) handle harsh growth conditions. Besides this flexibility, and in comparison to its relatively close model organism Escherichia coli, the available synthetic biology tools are very little.

Goal

We want to screen the suitability recombineering as an appropriate method for genome editing of P. putida. Recombineering is an efficient method of in vivo genetic engineering applicable to chromosomal as well as episomal replicons. This method circumvents the need for most standard in vitro cloning techniques. Recombineering allows construction of DNA molecules with precise junctions without constraints being imposed by restriction enzyme site location. Bacteriophage homologous recombination proteins catalyze these recombineering reactions using double- and single-stranded linear DNA substrates, so-called targeting constructs, introduced by electroporation. Gene knockouts, deletions and point mutations are readily made, gene tags can be inserted and regions of bacterial artificial chromosomes or the bacterial genome can be subcloned by gene retrieval using this technique (Sharan et al., 2009).

This will involve:

1. Theoretical discussion on the design of the recombineering parts.
2. DNA engineering and parts construction.
3. Screening of recombineering success in P. putida – 2 systems RecTE (Swingle et al., 2010) and lambda Red (Datsenko and Wanner, 2000).
4. Application of the system for the manipulation of P. putida genome.

General

This project will include a concise written report (in English, with critical assessment of the work) and an oral presentation of the work. The project will include synthetic biology and molecular biology techniques along with the usage of biophysical tools.

Contact

Stamatios Damalas (stamatios.damalas@wur.nl) and Rita Volkers (rita.volkers@wur.nl).

DATSENKO, K. A. & WANNER, B. L. 2000. One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. *Proceedings of the National Academy of Sciences,* 97**,** 6640-6645.

SHARAN, S. K., THOMASON, L. C., KUZNETSOV, S. G. & COURT, D. L. 2009. Recombineering: a homologous recombination-based method of genetic engineering. *Nat. Protocols,* 4**,** 206-223.

SWINGLE, B., BAO, Z., MARKEL, E., CHAMBERS, A. & CARTINHOUR, S. 2010. Recombineering Using RecTE from Pseudomonas syringae. *Applied and Environmental Microbiology,* 76**,** 4960-4968.