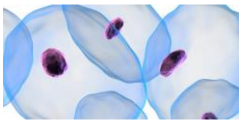

Design and characterize novel synthetic CRISPRi circuits

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Type of thesis: Experimental

Required competences: Basic microbiology (cell culture, transformation) and molecular biology (PCR design, cloning)

Acquired competences: advanced assembly methods, genetic circuit logic, knock-down, growth and fluorescence analysis, integrating modelling and experimental data

Date: 16-09-2021 date the project was proposed

Description

With the advent of synthetic biology, an increasing amount of synthetic genetic circuits are being designed for different applications (biosensors, metabolic control, computing). Many genetic circuits have been built using common transcriptional and translational regulators (e.g. lacI, tetR, riboswitches), and are often compared to computation or electronic devices (switches, logic gates, loops, oscillators). However, the metabolic load and the "limited" number of regulators available hinder the multilayering of such circuits. Recently, the CRISPRi technology has been used in the construction of synthetic genetic circuits, exploiting the ability of dCas9 complexed with sgRNA to repress desired target genes, while keeping a low metabolic burden for the host and allowing the use of plenty of orthogonal sgRNAs (Santos-Moreno et al. 2020). In this way, the authors built and tested some basic circuits such as a toggle switch.

At SSB, we aim at designing and building complex circuits that would allow to solve problems or generate new knowledge. To aid the design of complex circuits, quantitative and predictive mathematical models (developed by the computational researchers at our team) can be used to predict novel experiments or functions. However, to develop good models, data from the basic circuits is needed to help model development, whilst also showing us the experimental limitations of our system. Hence, in this project, we will build simple genetic circuits using CRISPRi and test them under different conditions (i.e. concentration/time of induction). We will work together with the computational team to design the appropriate experiments to obtain all the information needed to characterise the biological system and computationally design more extensive genetic circuits.

References

1. Santos-Moreno *et al.* 2020. *Multistable and dynamic CRISPRi-based synthetic circuits.* Nature communications.
2. Didovyk, A. *et al.* 2016. *Orthogonal modular gene repression in Escherichia coli using engineered CRISPR/Cas9.* ACS Synth. Biol.
3. Brophy J. *et al.* 2014. *Principles of genetic circuit design.* Nature Methods volume.