Steps Towards the Design of Complex Quorum Sensing Networks Through Standard Biological Parts: Characterization of a Synthetic LuxR-HSL Repressible Promoter

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Motivation

Synthetic Biology is an emergent discipline which main goal is to define standardized procedures to construct and characterize genetic circuits, introducing into biology the engineering key concepts of standardization and reusability. Different strategies to physically assemble DNA sequences, such as promoters, ribosome binding sites, coding sequences and transcriptional terminators have been proposed; one of them, known as BioBrick Standard Assembly [1], has become very popular among laboratories as it gives the opportunity to create a new standard part by simply merging together two BioBrick parts through simple laboratory protocols. Standardized strategies are also needed in order to characterize the working of genetic circuits: many efforts have been done to propose quantitative methodologies able to measure the performances of the systems. At present Relative Promoter Units (RPUs) [2] are the standard measurement for promoters transcriptional activity. This procedure is reported to be robust to experimental conditions variation. Sharing data also plays an important role; for this reason people interested in Synthetic Biology can find important information about several standard parts, systems and devices in an on line, continuously growing catalog called Registry of Standard Biological Parts (http://partsregistry.org). Some of these parts are involved in cell-to-cell communication, a feature which can coordinate the behavior of a cell population. In nature bacteria, such as V. fischeri, which regulates the expression of luminescence in a cell-density dependent fashion, are able to communicate through the quorum sensing. The characterization of new synthetic parts that can be used for cell to cell communication and construction of novel logic genetic circuits exploiting the quorum sensing are fascinating goals. BBa_R0061 is a synthetic promoter repressible by LuxR-HSL complex, composed by consensus -35 and -10 hexamers and a partially overlapping lux box from the natural luxI inducible promoter of V. fischeri between them. This part has been characterized to demonstrate its possible usages in designing activation-repression logic circuits in E. coli and a mathematical model has been proposed to describe its behavior.
Methods
BBa_R0061 was studied in E.coli TOP10. For repression evaluation, cells were transformed with high copy number plasmid pSB1A2, bearing a system that constitutively expresses LuxR protein and harbors LuxR-HSL repressible promoter regulating the transcription of reporter genes (Green or Red Fluorescent Protein-GFP and RFP). A non-fluorescent strain was used in all the experiments to estimate and subtract the fluorescence background during GFP or RFP measurements. As reference constitutive promoter BBa_J23101 with GFP or RFP gene was used. Long-term bacterial glycerol stocks were streaked on selective LB-agar plates and grown over-night; for each strain, three colonies were inoculated in 1 ml of M9 supplemented medium and grown over-night. After diluting 1:100 in a final volume of 2 ml, cultures were grown for about six hours and diluted to the same Optical Density at 600 nm (OD600) in 2 ml of M9. Cells were grown again for 1 hour then 200 ul for each culture were transferred into a 96-well microplate and incubated in TECAN Infinite F200 microplate reader; OD600 and fluorescence were measured every 5 minutes for 24 hours. After 3 hours, cultures were induced with different concentrations of N-(3-oxo-hexanoyl)-L-homoserine lactone (3OC6HSL). BBa_R0061 transcriptional strength was finally measured in RPUs computing the ratio between the synthesis rate per cell (as the time derivative of the fluorescence divided by OD600) of the promoter of interest and BBa_J23101 reference in the same experimental conditions at the steady state.

Results
BBa_R0061 synthetic promoter shows the expected behavior of a repressible promoter as, in the presence of proper concentrations of 3OC6HSL, it is almost completely repressed. In particular, the transcriptional activity starts decreasing for concentrations of 3OC6HSL equal or greater than 50 nM. This behavior can be exploited in activation-repression logic circuits such as switches or biological NAND gates, together with the HSL-inducible natural luxI promoter. We also propose a mathematical model relating the transcriptional output of BBa_R0061 to the concentration of HSL, in order to quantitatively detail the characterization of this part.

References

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