Characterization of Promoter Strength at Different DNA Copy Numbers Using RPU Approach

ZUCCA S (1, 2), PASOTTI L (1, 2),
CUSELLA DE ANGE LIS M G (2), MAGNI P (1, 2)
(1) Dip. di Informatica e Sistemistica - Università degli Studi di Pavia, Italy
(2) Centro di Ingegneria Tissutale - Università degli Studi di Pavia, Italy

Motivation
Synthetic Biology is a new emerging discipline that aims at the rational design and implementation of standard biological components to program living cells for the realization of a desired function. The key concepts of Synthetic Biology are modularity and standardization. The fundamental DNA components can be considered as separate modules, easy to study and characterize separately, that can be combined to build up complex genetic circuits showing predictable behaviours. The process of standardization allows the interoperability of these modules and is based on the concept of BioBrick parts (DNA sequences that, despite the biological function, share a common physical interface that facilitates the modules assembly) [1]. All the BioBrick parts are collected in the Registry of Standard Biological Parts, stored at MIT, and available online (http://partsregistry.org). Several efforts have been made to provide an accurate documentation for their functioning and to define standardized units of measurement. Transcriptional promoters are small DNA sequences that regulate the transcription process of the downstream coding sequence. A standard measurement methodology, called Relative Promoter Units (RPUs), was proposed in the literature to evaluate the promoter activity in vivo. The RPUs computation is based on the in vivo estimation of the synthesis rate of a reporter protein using a mathematical model of the reporter protein expression [2]. Since they are computed as the ratio of the absolute activity of a promoter relative to a standard reference (BBa_J23101), RPUs are supposed to be independent of the measurement system and of many difficult-to-measure biological parameters, such as DNA copy number. In order to investigate the robustness of RPUs against the variation of DNA copy number, six constitutive promoters (BBa_J23100, BBa_J23101, BBa_J23118, BBa_R0011, BBa_I14032, BBa_R0051) and one inducible device (BBa_F2620, 3OC6-HSL-inducible) from the Registry were studied using Red Fluorescent Protein (RFP) in E. coli.

Methods
E.coli MG1655 strain was used for cloning and testing. Standard methods for plasmid transformation and preparation were used. The measurement systems, composed by the RFP generator device assembled under the control of the studied promoters, were
obtained by the 2010 DNA Parts Kit Distribution or built using standard assembly techniques. All the measurement systems were assembled in the high copy number vector pSB1A2 (~200 copies per cell), in the low copy number vector pSB4C5 (~5 copies per cell) and in single copy, by integration in E. coli genome using the BioBrick integrative base vector BBa_K300000, designed and realized by the University of Pavia research group (http://partsregistry.org/Part:BBa_K300000). Fluorescence and optical density (OD) measurements were made on the cultures bearing the measurement systems and on proper negative controls using the multi-well microplate reader TECAN Infinite F200. For each part, 5 ul of culture were inoculated from glycerol stock in 1 ml M9 supplemented medium and incubated overnight at 37°C, 220 rpm. In the morning, cultures were 1:500 diluted and incubated for further 6 hours. Cultures were then transferred in a 96-well microplate (200 ul per well) in 3 wells (constitutive promoters) or in 21 wells (inducible promoter, 3 wells for every inducer concentration), induced when required with 3OC6-HSL, incubated in microplate reader (TECAN) and assayed every 5 minutes for ~5 hours with the following protocol: linear shaking (3 mm, 15 s), OD (600 nm) and fluorescence (RFP, lex= 535 nm, lem= 620 nm, gain= 80) measurements. For each promoter x, the three OD600_x and RFP_x time series were averaged and the blank measurement (i.e. the OD600 of M9 and the RFP of the negative control, respectively) was subtracted thus obtaining OD600n_x and RFPn_x signals. Synthesis rate of RFP per cell (Scell_x) was computed as the time derivative of RFPn_x divided by OD600n_x. Scell_x signal was averaged over time considering only the bacterial exponential phase (S_x), identified by visual inspection of the logarithmic growth curve. RPUs were computed for every promoter as: RPU_x= S_x/S_BBa_J23101.

Results
The results obtained for the different DNA copy numbers were compared in terms of RPUs. The ranking of the studied promoters activity was consistent for all the copy number conditions. However, in some cases the relative activities were highly different among these conditions. In conclusion, the results highlighted the need of carrying out quantitative characterization of biological parts as a function of the number of copies. To this aim, standard solutions such as BioBrick-compatible replicative or integrative vectors are important tools to facilitate the construction of biological measurement systems for the desired copy number conditions. On the other hand results confirm the robustness of RPU approach to rank promoter strength.

References

Contact email
susanna.zucca@unipv.it