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Data Article

Data on the standardization of a cyclohexanone-responsive expression system for Gram-negative bacteria

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ABSTRACT

Engineering of robust microbial cell factories requires the use of dedicated genetic tools somewhat different from those traditionally used for laboratory-adapted microorganisms. We have edited and formatted the ChnR/P_{chnB} regulatory node of *Acinetobacter johnsonii* to ease the targeted engineering of ectopic gene expression in Gram-negative bacteria. The proposed compositional standard was thoroughly verified with a monomeric and superfolder green fluorescent protein (msf●GFP) in *Escherichia coli*. The expression data presented reflect a tightly controlled transcription initiation signal in response to cyclohexanone. Data in this article are related to the research paper "*Genetic programming of catalytic* Pseudomonas putida *biofilms for boosting biodegradation of haloalkanes*" [1].

SPECIFICATIONS TABLE

Subject area	*Biology*
More specific subject area	*Metabolic Engineering*

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Type of data	*Figures and Table*
How data was acquired	*Flow cytometry*
Data format	*Analyzed*
Experimental factors	*Assembly and validation of a standard expression vector for Gram-negative bacteria using regulatory elements from Acinetobacter johnsonii*
Experimental features	*Molecular Biology and Synthetic Biology methodologies (DNA synthesis, PCR, enzyme restriction, DNA ligation), flow cytometry*
Data source location	*Madrid, Spain*
Data accessibility	*Data is with this article*

VALUE OF THE DATA

- Standardized vector designed for tightly regulated gene expression in Gram-negative bacteria.
- Regulatory elements from *Acinetobacter johnsonii* (ChnR transcriptional regulator and P_{chnB} promoter) edited, formatted, and assembled in a minimal DNA segment adopting a Synthetic Biology standard.
- Responsiveness of the expression system to the inducer cyclohexanone demonstrated by using GFP as a reporter.
- The DNA standard described in this dataset could be used as a benchmark for future research on gene expression in Gram-negative bacteria.

1. Data

A cyclohexanone-responsive expression platform was designed based on elements of the cyclohexanol biodegradation pathway of *Acinetobacter johnsonii* (Fig. 1A). The segments bearing the complete *chnR* and P_{chnB} promoter DNA of *A. johnsonii* NCIMB 9871 were edited *in silico* to obtain a standardized SEVA (*S*tandard *E*uropean *V*ector *A*rchitecture [2]) expression cargo (Fig. 1B), and assembled to yield plasmid pSEVA2311 (Fig. 2 and Table 1). Expression data were generated to validate this plasmid. The gene encoding the monomeric and superfolder green fluorescent protein (msf●GFP) was inserted in vector pSEVA2311 (Fig. 3A), and the transcriptional activation of the ChnR/P_{chnB} expression system upon addition of cyclohexanone was evaluated in a wild-type *Escherichia coli* strain (Fig. 3B and C). Key features of this expression vector include [i] a very low expression level in the absence of inducer, [ii] high transcriptional capacity, [iii] an induction kinetics very similar in both minimal and rich culture media, and [iv] linear accumulation of the reporter product along time.

2. Experimental design, materials and methods

2.1. Bacterial strains and culture conditions

Bacterial strains used in this study are listed in Table 1. *E. coli* CC118 was used as the host for plasmid constructs and it was routinely grown at 37 °C in LB medium [3]. For single-cell fluorescence determination by flow cytometry, cells were grown in the semi-synthetic M9CAG medium, which contains the same salts as for M9 minimal medium [3], 0.1% (w/v) acid casein hydrolysate (Becton-Dickinson Diagnostics Co., Sparks, MD, USA), 2 mM $MgSO_4$, 0.1 mM $CaCl_2$, 0.05% (w/v) vitamin B1, and 0.4% (w/v) glucose as the sole carbon and energy source. Kanamycin (Km, 50 μg ml^{-1}) was added to the culture media whenever required. Growth was estimated by measuring the optical density at 600 nm (OD_{600}) after diluting the culture whenever needed [4–6]. Shaken-flask cultures were set in 125-ml Erlenmeyer flasks containing culture medium up to one-fifth of their nominal volume and