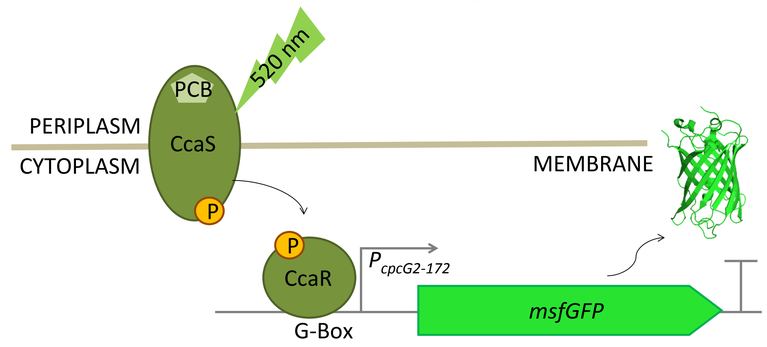
Engineering portability of the CcaSR light switch for the control of biofilm formation in *Pseudomonas putida*

Angeles Hueso-Gil[1], Ákos Nyerges[2], Csaba Pál[2], Belén Calles[1] and Víctor de Lorenzo[1]
1.Microbial Biotechnology Dept., National Centre of Biotechnology (CNB, CSIC). Madrid, Spain
2.Synthetic and Systems Biology Unit, Biological Research Centre. Szeged, Hungary

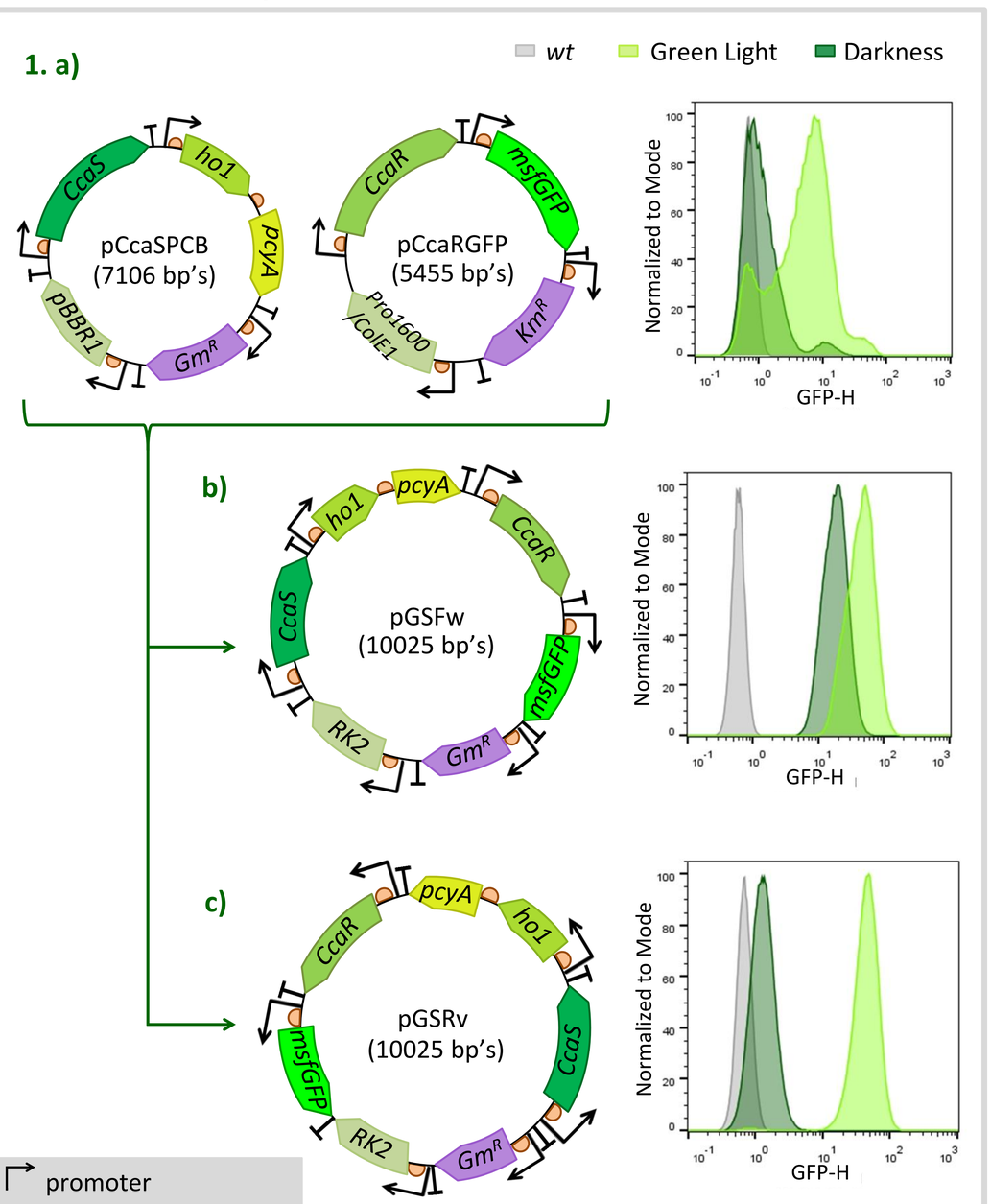
Abstract

Two of the technical challenges faced by contemporary Microbiology involve controlling gene expression using light and regulating the formation of bacterial biofilm, determined by the intracellular levels of the secondary messenger c-di-GMP. CcaSR system is one of the light switches repeatedly used for the induction of transcription in *Escherichia coli*. This two-component system represented a good candidate for its adaptation to *Pseudomonas putida*. Previous attempts have tried to use this microorganism as chassis for the implementation of new pathways, being biofilm formation an important function to control. To this end, we unified CcaSR components in one single construct and randomly mutagenized their regulatory regions to find a clone with a balanced expression of the key parts of the system inside *P. putida*. The combination of this novel mutagenization process with a proper screening, which included a first sorting of the libraries and the later isolation of colonies, lead us to a clone with a much improved induction by green light. The selected variant had a notable capacity in response to green light. Finally, optimized CcaSR was used to control the expression of super-efficient variant of PleD, a diguanylate cyclase of *Caulobacter* which allowed a tight control of c-di-GMP levels, and therefore, of biofilm production.

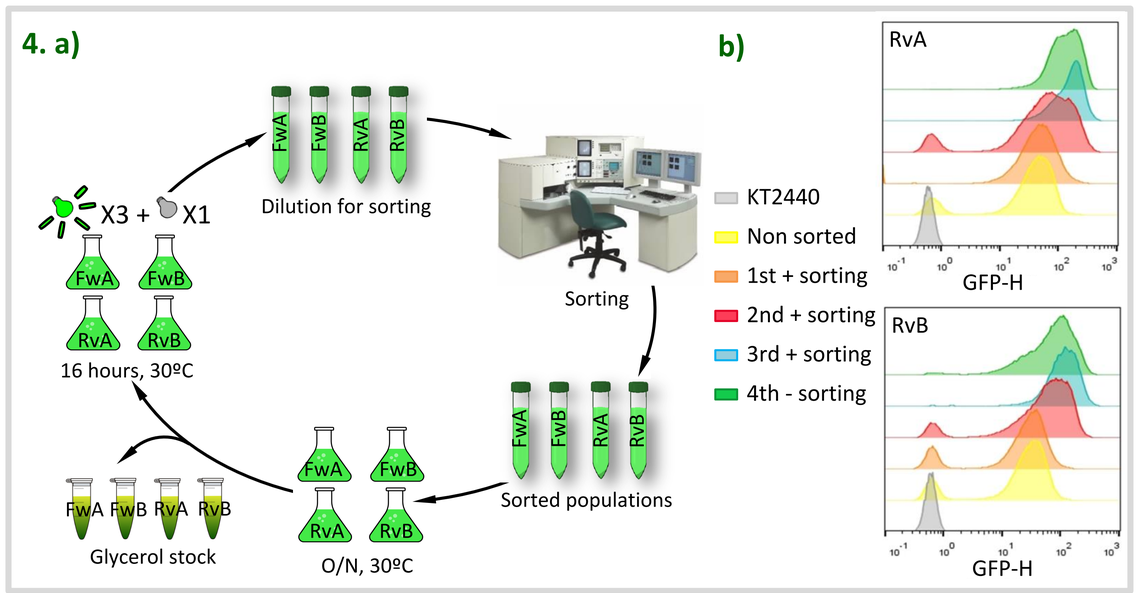
CcaS/CcaR system

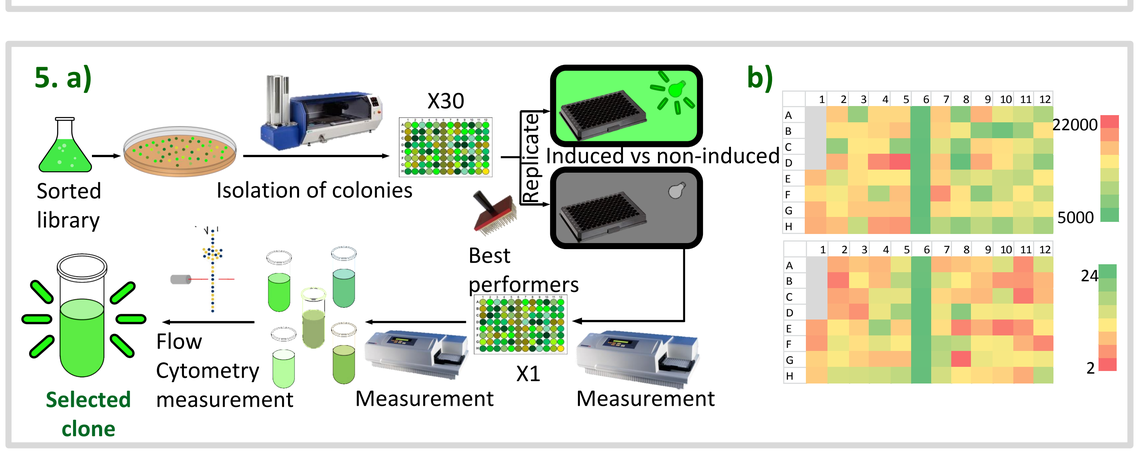


Modules, context and behaviour

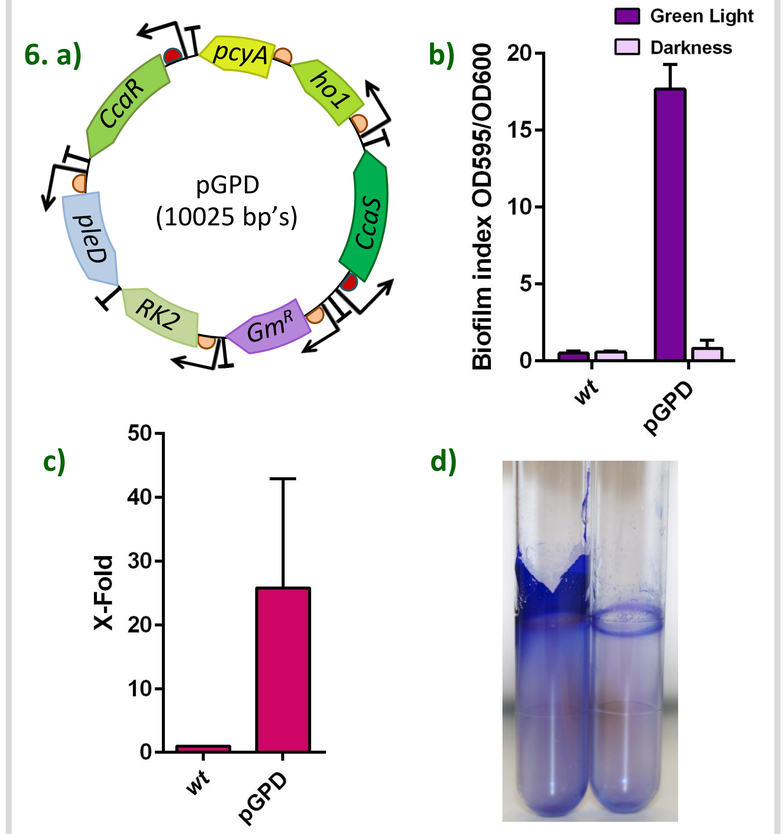


Two-step screening





Light switch for biofilm control



REFERENCES:

Refactoring and Optimization of Light-Switchable Escherichia coli Two-Component Systems, Schmidl SR, Sheth RU, Wu A, Tabor JF. ACS Synth Biol 2014 3 (11) 820-831

Directed evolution of multiple genomic loci allows the prediction of antibiotic resistance. Nyerges Á, Csörgő B, Draskovits G, Kintses B, Szili P, Ferenc G, Révész T, Ari E, Nagy I, Bálint B, Vásárhelyi BM, Bihari P, Számel M, Balogh D, Papp H, Kalapis D, Papp B, Pál C. PNAS 2018, 115 (25)

Conclusions: In this work, we have optimized the balance of CcaSR two-component system for its utilization in *P. putida* as a tool for biofilm control using light. The procedure let us keep biofilm levels to the minimum in the absence of the inducer and increase it 30-fold when cultures were illuminated. Also, the application of DIvERGE soft mutagenesis approach over regulatory regions gave us a libraries with different balances of expression. This randomization was combined with a proper screening that included a first sorting of the sub-populations with higher capacity and a second isolation of colonies for further characterization. This approach lead us not only to the balanced clone for *P. putida*, but also to a method that can be applicable to a wide spectrum of portability challenges. This way, implementation of external devices in new hosts can be accomplished succesfully for endowing them with new useful functionalities.