Quorum sensing: A tool for autoinducible heterologous protein expression

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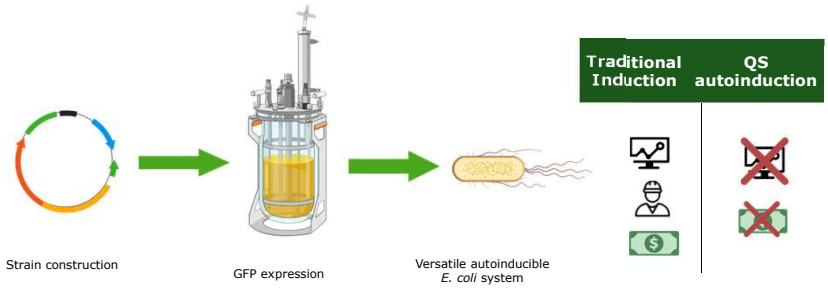
Background

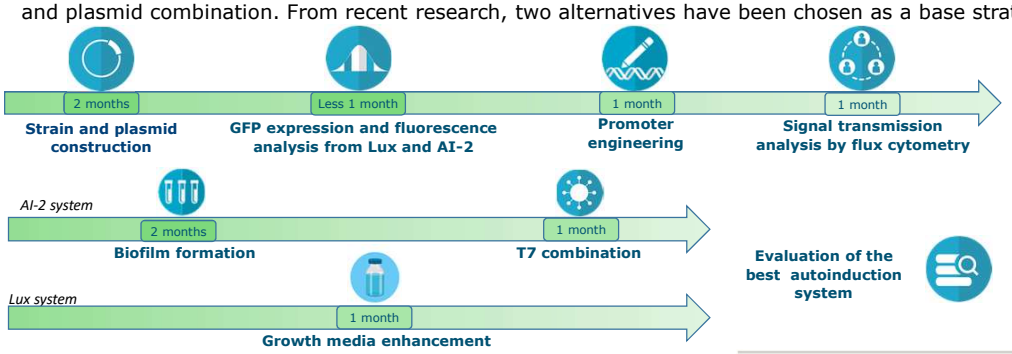
Inducers being used nowadays in industrial protein production by *Eschericia coli* come at a high cost and toxicity. Moreover, the need to monitor cell cultures makes induction-dependent processes labor dependent and costly.

Using quorum sensing (QS) as a tool to induce protein expression at high cell densities without the need to monitor or add inducer is a cheap and sustainable alternative to chemical or thermal induction.



Objective

Generate a versatile autoinducible protein expression system in *E. coli* by using a multi-platform approach to determine the best producing strain and plasmid combination. From recent research, two alternatives have been chosen as a base strategy for autoinduction: Lux_1 and AI-2_2 system

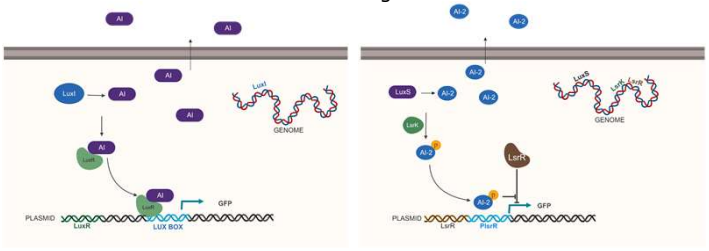


- AI-2 system
  - *E. Coli* native QS system engineering. Using AI-2, autoinducer naturally produced by LuxS gene from *E. coli* to induce protein expression
- Lux system
  - *Vibrio fischerae* QS system applied to *E. coli* by inserting LuxI to *E. Coli*'s genome and LuxR and the promoter LUX BOX in a plasmid for protein expression

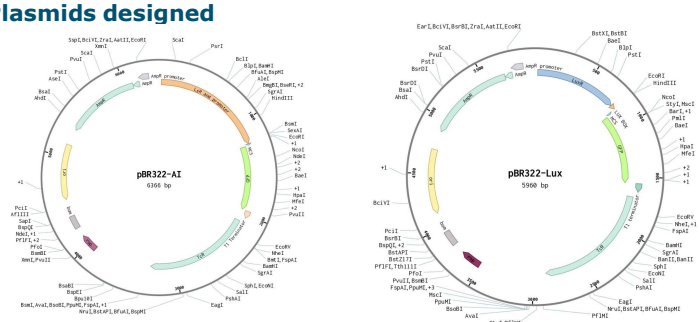
Introduction

LuxI generates AI from S-adenosylmethionine and AI froms a complex with LuxR that will favour the expression of genes regulated by LUX BOX.

LuxS creates AI-2 from DPD. LsrR respresses the lsr genes and once AI-2 is phosphorylated by LsrK expression occurs. The plasmids in this system contain an extra copy of LsrR for tight regulation.



Plasmids designed



Plasmids for AI-2 System (pBR322-AI) and Lux System (pBR322-LUX) are represented.

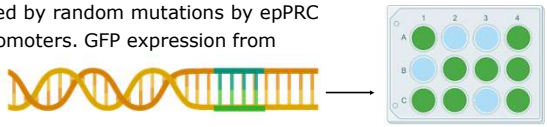
Budget calculation

Expense	Price (€)
Fungible material	6000
External services (Flow cytometry)	150
Equipment (Fluorometer)	2500
Congress/travelling	600
TOTAL	9250

Budget estimation for the total duration of the project (7 months). Project carried out and financially supported by a protein production company.

Promoter engineering

Promoter regions of LuxS (AI-2 system) and LuxI (Lux system) will be modified by random mutations by epPRC to achieve stronger promoters. GFP expression from mutant libraries will be screened for better producing strains.



Signal transmission

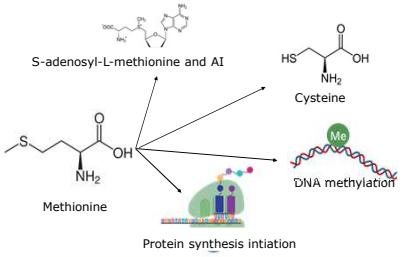
Growing mixed cell populations (QS producers and QS inhibited) to analyse fluorescent population will highlight ability of cells to transmit QS signal. GFP expressing cells will be counted by FACS.

Biofilm formation

Due to a reported higher specific protein production and reduced plasmid loss in biofilm a colonies$_3$ a KO *wza* and *flu* mutants are grown and compared to biofilm producing cells to asses its effect

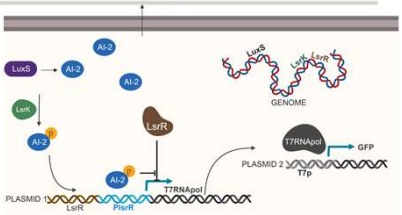
Growth media enhancement

The increased demand of methionine for AI synthesis in Lux system is expected to have an effect on the cell's metabolism. An addition of methionine in the growth media will be analysed for a potential better growth and production.



Signal amplification T7RNApol

QS induction is used to express T7RNApol that will transcribe GFP with a high rate.



References

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3.Gomes, L. et al. (2017). *Process Biochemistry*, *57*, 1–8.

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