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BioBricks for measuring carbon starvation

Table of contents

Carbon starvation & how to measure it

System overview

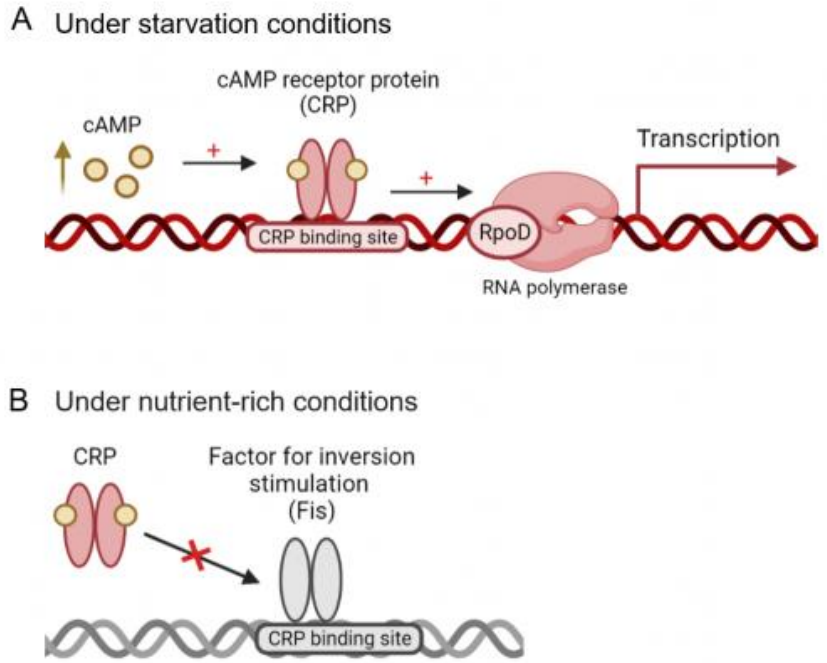
Selected parts & details

Carbon starvation

- The cells undergo carbon starvation once they are running low on the carbon source (glucose)
- Insufficient carbon supply to metabolism causes a transition from the exponential growth phase to the stationary phase, reducing the reaction yield
- Carbon starvation in E. coli cells can lead to shrinkage of the bacterial cytoplasm

Measuring carbon starvation

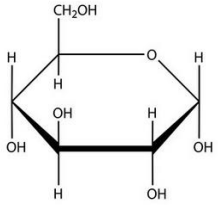
- Low glucose concentrations in bioreactors lead to carbon starvation
- Low glucose concentration leads to the activation of adenylate cyclase, leading to formation of cAMP
- The PcstA promoter is activated by the binding of cAMP to the cAMP receptor protein (CRP) upstream of the transcription site, leading to transcription of the gene
- Thus, when glucose is not present, GFP will be produced, and glucose acts as a sort of repressor on transcription



http://parts.igem.org/wiki/index.php/Part:BBa_K4115003

System overview – Biological NOT gate

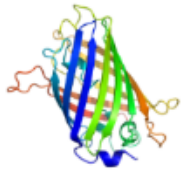
Glucose repressor



Promoter PcstA | GFP gene | Double terminator BB0010 BB0012

Promoter PcstA | GFP gene | Double terminator BB0010 BB0012

GFP



GLUCOSE	GFP
1	0
0	1

SELECTED PARTS

- BBa_K118011: PcstA, a glucose-repressible promoter
- BBa_E0040: coding GFP
- BBa_B0015: double terminator containing B0010-B0012
- Chassis: *E. coli* strain BL21(DE3)
- BBa_K1362091: plasmid pSB1A30, a high copy BioBrick cloning/expression backbone carrying Amp resistance
- Measurements: fluorescent microscopy (visualization of GFP location and expression) and flow cytometry (fluorescent intensity)

DETAILS OF THE SELECTED PARTS

- BBa_E0040: coding GFP, gene of interest
  - Green fluorescent protein gene derived from jellyfish Aequeora Victoria
  - Excitation wavelength 545 nm (light absorbation)
  - Emission wavelength 475 nm (light emission)
  - GFP chosen to easily detect the expression
- BBa_B0015: double terminator containing B0010-B0012
  - B0010: transcriptional terminator derived from E. coli, 64 bp stem-loop
  - B0012: transcriptional terminator from coliphage T7, for E. coli RNA polymerase, promoter in the reverse direction
  - very commonly used terminator

DETAILS OF THE SELECTED PARTS

- BBa_K1362091: plasmid Psb1a30
  - a high copy BioBrick cloning/expression backbone
  - contains an Ampicillin resistance gene

MEASUREMENTS

- fluorescent microscopy
  - visualization of GFP location and expression
- flow cytometry
  - laser used to detect and analyze cells
  - measuring the intensity of fluorescence

Thank you!

Sources:

Shi, H., Westfall, C. S., Kao, J., Odermatt, P. D., Anderson, S. E., Cesar, S., Sievert, M., Moore, J., Gonzalez, C. G., Zhang, L., Elias, J. E., Chang, F., Huang, K. C., & Levin, P. A. (2021). Starvation induces shrinkage of the bacterial cytoplasm. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(24), e2104686118. https://doi.org/10.1073/pnas.2104686118)

Part:BBa K4115003 - parts.igem.org. Igem.org. Retrieved March 20, 2023, from http://parts.igem.org/wiki/index.php/Part:BBa_K4115003

Wei T, Dai H. Quantification of GFP signals by fluorescent microscopy and flow cytometry. Methods Mol Biol. 2014;1163:23-31. doi: 10.1007/978-1-4939-0799-1_3. PMID: 24841297.