

Biobrick Safety Sheet

Risk level: 1

Plasmid: pSB4C5

Chassis: *Escherichia coli* (BW25113 strain Δ *cyaA*)

paraBAD

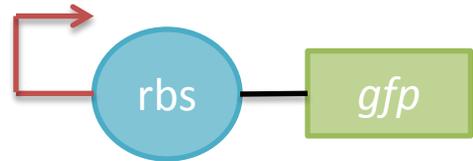


Diagram of the construction

BioBrick code : none for the moment

Construction method

- Technic: Restriction/ligation
- Biobricks:
 - *paraBAD* - *rbs* - *gfp*

Promoter



:

Arabinose Binding Dipeptide

Origin and initial function :

This promoter is present in *E. Coli* BW25113 strain.

It regulates the production of three proteins (AraA, AraB and AraD) which form the arabinose operon. Their production enables the use of arabinose as a carbon source.

It has six regulation sites, five of them are devoted to AraC fixation (three are repressing, one has a dual activity and one is an activator); the last one is a CRP binding site (positive activity).

This promoter is activated when both activated CRP and AraC are bind to it.

E.coli: are bacteria commonly used in laboratories. Some strains are dangerous but most of them are harmless.

Purposes in the system :

It act as an "AND" gate to produce GFP.

Size :

499 bp



Origin and initial function :

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Purposes in the system :

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Size:

bp



Origin and initial function:

The protein comes from *Aequorea victoria*. This jellyfish uses GFP (Green Fluorescent Protein) in order to convert the blue luminescence emitted by the aequorine into a green luminescence. Apparently the resulting fluorescence has a repulsive effect on predators. The gene is also composed of a LVA tail. This tag was used for the degradation of GFP. It is attached on the C-terminal end of the GFP and is also attached at the end of the eCFP.

Aequorea victoria: is a jellyfish that can be found off the coast of north America.

Purposes in the system :

It is used as a reporter for the activation of the paraBAD promotor.

Size :

717 bp

Feedback

Theoretical interactions:

- For the moment we do not know what would happen if the microorganism were scattered outside of the laboratory. Therefore the question to answer is: in which environment can this microorganism live?

The environment in which it has been used and the consequences :

Environment	Consequences
This biobrick is only used in a biology laboratory of level 1 for the moment	The construction has not been built yet. Therefore we do not know if there is any consequences. Theoretically there would be no dangerous effect.

Safety issues:

- For the moment we do not know what would happen if the microorganisms were scattered outside of the laboratory.

Tests to do in order to answer safety issues :

- test the organism survival in sewers.
- check the organism's presence in the researchers' bodies.
- Test interactions with organisms known to be sensitive to cAMP concentrations.

Limitation :

- Because no tests have been done in a different environment than a biology laboratory of level 1, the use of those microorganisms should be forbidden in other environments until a study proves that the risk is low enough.
- when using this microorganism good laboratory practice must be followed

characterization :

put here the information about the functioning of the BioBrick and experimental results.

- Zaslaver A, Bren A, Ronen M, Itzkovitz S, Kikoin I, Shavit S, Liebermeister W, Surette MG, Alon U. Nat Methods. 2006 Aug;3(8):623-8. [A comprehensive library of fluorescent transcriptional reporters for Escherichia coli.](#)
- Stoltzfus L, Wilcox G (1989). "[Effect of mutations in the cyclic AMP receptor protein-binding site on araBAD and araC expression.](#)" J Bacteriol 171(2);1178-84. PMID: 2521619

Author : LINKS Jérôme (iGEM Grenoble 2012)

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