Biobrick Safety Sheet

Risk level: 1

Plasmid: pSB4C5

Chassis: *Escherichia coli* (BW25113 strain *ΔcyaA*)

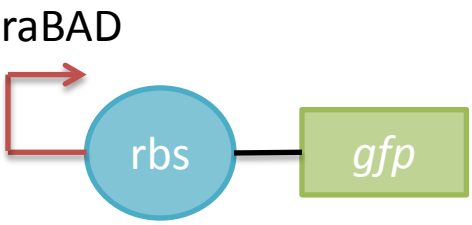

Diagram of the construction

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

RBS rbs : Strong RBS (B0030)

Origin and initial function :

This rbs is hosted in E. Coli . It does not code for a protein and does not increase the risk level.

Purposes in the system :

It allows the transcription of gfp gene.

Size:

15 bp

Coding sequence *gfp* Green Fluorescent Protein

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

Theoritical 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

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Update: August 25th 2012