Risk level: 1

Plasmid: pSB4C5

Chassis: *Escherichia coli* (BW25113 strain ΔcyaA)
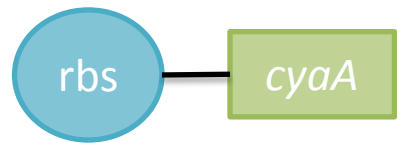
rbs ── cyaA

Diagram of the construction

BioBrick code : none for the moment

Construction method

- Technic: Restriction/ligation
- Biobricks:
    - *rbs - cyaA* comes from a colony PCR on E. Coli (BW25113 strain)

RBS (rbs) : *cyaA* Ribosome Binding Site

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 translational regulation of the Adenylate cyclase production.

Size:

30 bp

Coding sequence `cyaA`   Adenylate cyclase

Origin and initial function:

This protein is present in E. Coli . It catalyzes the production cyclic AMP (cAMP) by an intramolecular transfer of the adenylyl group of ATP to the 3'-hydroxy group. cAMP is an important signaling molecule. Via binding to the CAP/CRP protein, it acts as a partner in the transcription of many genes.

Purposes in the system :

The adenylate cyclase is used to produce cAMP in E. Coli *ΔcyaA* strain.

Size :

2547 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	Overexpression of *cyaA* is lethal in wild type strains. (REF)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. However we do know that cAMP acts as a messenger between cells (Prokaryotes and Eukaryotes except for plants); it overexpression could lead to unexpected consequences on the environment. Therefore the question to answer is: in which environment can this microorganism live?

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.

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Update: August 16 2012