Biobrick Safety Sheet

Risk level:

Plasmid: pSB3C5

Chassis: *Escherichia coli* (BW25113 strain)

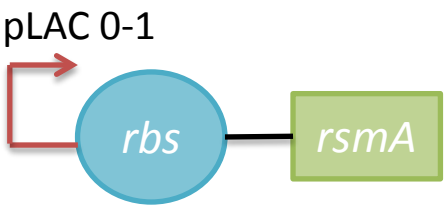

Diagram of the construction

BioBrick code : none for the moment

Construction method

- Technic: Gibson Assembly
- Biobricks:
  - pLAC -rbs coms from BBa I13601
  - *rsmA* originates from iGEM Grenoble 2011 team work

Promoter : plac 0-1(BBa_R0011)

Origin and initial function :

This promoter is a hybrid one made up of two natural promoters. It consists of the phage lambda promoter P(L) which activates the pathogenicity by increasing the transcription. The phage lambda destroys E.coli using a toxin which destroys the membrane. In this regulatory region, instead of the cI binding site, there is lacO1 (from E.coli LacI operon). LacO1 is an operator from lactose operon, it binds LacI (the lac repressor) which is released upon complexion with IPTG, the inducer.
E.coli: are bacteria commonly used in laboratories. Some strains are dangerous but most of them are harmless.
Phage lambda: is an E.coli virus without any pathogenicity towards humans.

Purposes in the system :

It can let the production of RsmA, which binds to the *fha1* RBS. This prevents the production of mCherry.

Size :

55 bp

RBS rbs : Elowitz RBS (B0034)

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 rsma.

Size:

12 bp

Coding sequence *rsmA*

Alternate name: carbon storage regulator

Origin and initial function:

RsmA is a coding sequence from Pseudomonas aeruginosa which increases the pathogenicity by allowing type VI secretion. Taken alone, it does not code for a dangerous protein and it does not increase the risk level.
Pseudomonas aeruginosa: it is a level 2 pathogenic bacterium that leads to nosocomial infection. It is ubiquitous.

Purposes in the system :

It binds to the *fha1* RBS to prevent the production of mCherry.

Size :

Around 200 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	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 microorganism were scattered outside of the laboratory. Therefore the question to answer is: in which environment can this microorganism live?

Tests to do in order to answer safety issues :

- test organism's survival in sewers.
- check organism's presence in the researchers' bodies. What are the consequences?

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 environnements 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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