Biobrick Safety Sheet

Risk level: I

Plasmid: pSB4C5

Chassis: *Escherichia coli* (BW25113 strain)

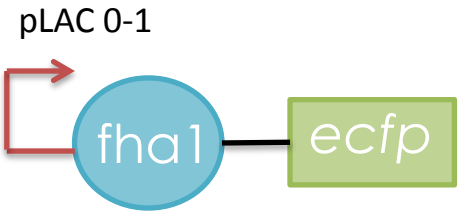

Diagram of the construction

BioBrick code : none for the moment

Construction method

- Technic: Gibson Assembly
- Biobricks:
  - plac originates from BBa_I13601
  - fha1 originates from iGEM Grenoble 2011 team work
  - *ecfp* is extracted from BBa_E0422

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 allows a strong transcription of *ecfp* and which can be induced by IPTG and repressed by LacI.

Size :

55 bp

RBS fha1 : Forkhead-associated domains 1 (not in the registry for the moment

Natural origin and function :

fha1 is a RBS from *Pseudomonas aeruginosa* which increases the pathogenicity by allowing type VI secretion. Taken alone, it does not code for a protein and it does not increase the risk level.
Pseudomonas aeruginosa: is a level 2 pathogenic bacterium which leads to nosocomial infections.

Purposes in the system :

It allows a translational regulation of the eCFP production. fha1 contains a binding site for the RsmA protein. By biding to the RBS, RsmA inhibits mRNA translation. However, this inhibition can be recovered thanks to rsmY (siRNA) which sequestters RsmA.

size:

47bp

Coding sequence ***ecfp***

enhanced Cyan Fluorescent protein (E0022)

origin and initial function:

The fluorescent protein eCFP (enhanced Cyan Fluorescent Protein) derived from *Aequorea victoria* GFP (Green Fluorescent Protein). This jellyfish uses GFP 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. The LVA tail (SsrA tag) coming from E.Coli at the end of the coding sequence reduces the protein stability and strengthens the action of Tsp, a protease.

Size :

762 bp

Feedback

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 microorganism were scattered outside of the laboratory. Therefore the question to answer is: in which environment can this mircoorganism 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 functionning of the BioBrick and experimental results.

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