

Biobrick Safety Sheet

Risk level: I

Plasmid: pSB4C5

Chassis: *Escherichia coli* (BW25113 strain)

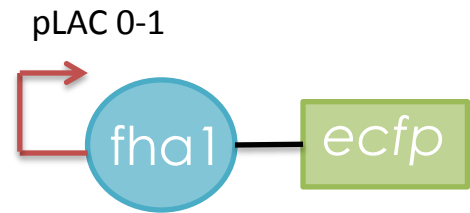


Diagram of the construction

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 *cl* 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 complexation 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 binding to the RBS, RsmA inhibits mRNA translation. However, this inhibition can be recovered thanks to *rsmY* (siRNA) which sequesters 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 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 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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