

## Biobrick Safety Sheet

Risk level: I

Plasmid: pSB4C5

Chassis: *Escherichia coli* (BW25113 strain)

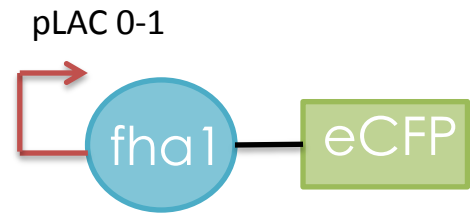


Diagram of the construction

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* *Lacl* operon). *LacO1* is an operator from lactose operon, it binds *Lacl* (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 *Lacl*.

## 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

E 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 aequorin 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.

- Lederberg E. (January 1950). Lysogenicity in Escherichia coli strain K-12 , *Microbial Genetics Bulletin*, v.1, pp. 5-8
- Lederberg J . (1953). Genetic Studies of Lysogenicity in Escherichia Coli . *Genetics* 38. 51–64. [on line]. (august 2012). available on [PMID 17247421](https://pubmed.ncbi.nlm.nih.gov/17247421/)
- St-Pierre F, Endy D (2008). Determination of cell fate selection during phage lambda infection. *Proc. Natl. Acad. Sci. : U.S.A.* 105. [on line] (August 2012). Available on <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2605630/?tool=pmcentrez>
- (2011) Bacteria An introduction to Earth's largest family, PDF generated using the open source mwlib toolkit. [on line] (August 2012). Available on <http://www.scribd.com/doc/74972710/Bacteria>
- iGEM KULeuven 2008. (2008). iGEM KULeuven 2008 team site, [on line] (July 2012). Available on <http://2008.igem.org/Team:KULeuven/Data/GFP>
- Silber K. R., Keiler K C, and Sauer R T. (1992 January 1). Tsp: a tail-specific protease that selectively degrades proteins with nonpolar C termini, *Proc Natl Acad Sci : U S A.* 295–299. [on line] (August 2012). Available on <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC48223/>
- Flynn JM, Levchenko I, Seidel M, Wickner SH, Sauer RT, Baker TA. (2001). Overlapping recognition determinants within the ssrA degradation tag allow modulation of proteolysis, *Proc Natl Acad Sci : U S A.* [on line] (August 2012). Available on <http://www.ncbi.nlm.nih.gov/pubmed/11535833>
- Pseudomonas Genome Database . [on line] (August 2012). Available on <http://v2.pseudomonas.com/>

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