Biobrick Safety Sheet

Risk level: 1

Plasmid:

Chassis: *Escherichia coli* (BW25113)

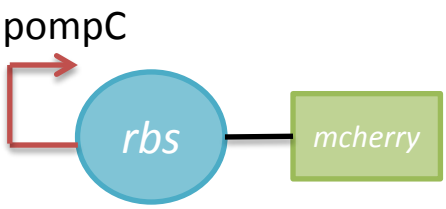

Diagram of the construction

BioBrick code : none for the moment

Construction method

- Technic: restriction-ligation (not constructed yet)
  - pompC
  - Rbs-mCherry (Bba_J06702)

Promoter : pompC

Origin and initial function :

This promoter comes from *E.coli* and is involved in osmotic regulation. In case of high osmolarity in the medium, the protein kinase EnvZ, located in the cytoplasmic membrane, phosphorylates the transcription factor OmpR. Phosphorylated OmpR binds the ompC promoter and activates the transcription.

E.coli: are bacteria commonly used in laboratories. Some strains are dangerous but most of them are Harmless.

Purposes in the system :

It allows to activate the transcription of mCherry when phosphorylated OmpR is present. In our case, it was used to test the hybrid receptor TapZ.

Size :

55 bp

RBS rbs : Standard Elowitz RBS (Bba_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 *mcherry* gene.

Size:

30 bp

Coding sequence mCherry : Monomeric fluorescent protein(**BBa_J06702**)

Origin and initial function:

mCherry is a fluorescent protein, deriving from monomeric RFP (Red Fluorescent Protein) through directed evolution and optimized for expression in bacteria. Pst1 restriction site had been eliminated by point mutation.
RFP 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.
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 :

Around 700 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	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 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.

- 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. [online] (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

- Nathan C Shaner, Paul A Steinbach & Roger Y Tsien (2005). A guide to choosing fluorescent proteins, Nat Method. 2005 Dec;2(12):905-9.

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Update: September 26, 2012