How to complete a BioBrick Safety Sheet

1 Give the risk level

2 Put a diagram of the biobrick construction using the symbols at the end of this manual

3 Give plasmids on which the BioBrick has been added. Several plasmids are possible

4 Put the name of the biobrick used in the registry

5 Give chassis used. Several chassis are possible

6 Make a brief description of how the construction was made and focus on the biological parts used and created.

7

For each part of the biobrick give the name, the origin, its usage and its size. As far as its origin is concerned, if it is a part composed of different other parts, explain the origin of each part. It is also important to explain their natural function. If there is any BioBrick already registered set up a link with the BioBrick web page.

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 :

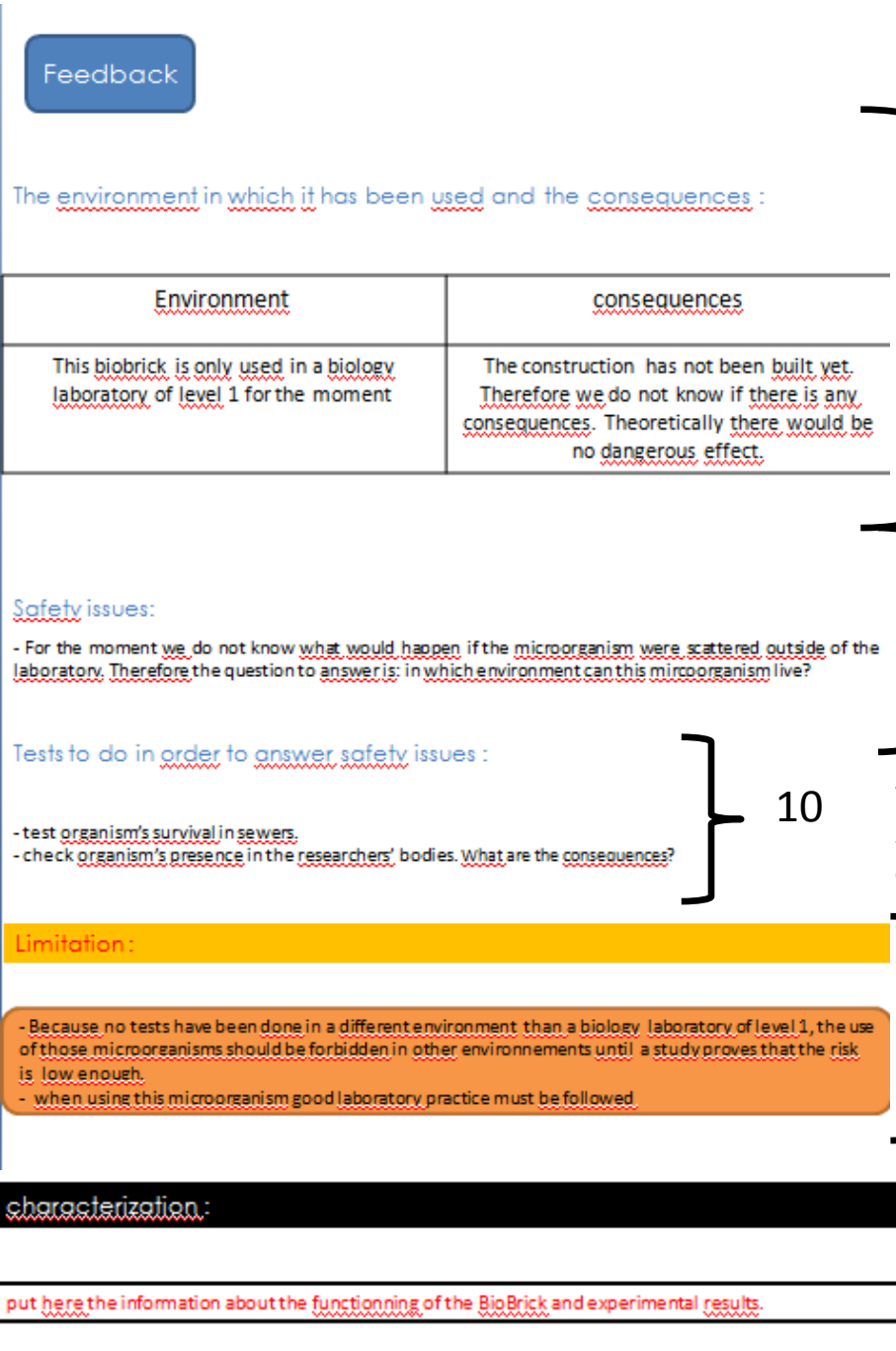
It is used as a reporter. The LVA tail (SsrA tag) coming from E.Coli at the end of the coding sequence reduces the protein stability and strengthens the action of Tsp, a protease.

Size :

762 bp

Example of explanation

Another part deals with the feedback



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.

8 For this part list all environments in which the organism has been used and consequences that happened. If different kind of organism in terms of chassis used or plasmid used, do not forget to define about which construction you are talking about.

9 Sum up the currently state in safety and write the questions that have to be answered to improve the safety use of this BioBrick

10 Write tests and experience that should be done in order to answer on questions above.

11 Thanks to information given previously, give in which ways this Biobrick has to be used or not.

12 in order to give more information on its use, put all the information about the functionning of the BioBrick and experience results

At the end do not forget to write references used and also the name of the person who puts the risk level on the BioBrick Safety Sheet

Author : LINKS Jérôme (iGEM Grenoble 2012)
put the name of the person who gives the risk level

example

How to establish the risk level

1. List every components of the biobrick
2. Trace the origin of each of them
3. Determine the risk level of each components

   (the risk level of each component is the same as their origin organism)
4. Take the higher risk level

   (if study prouves that the construction does not

   correspond to this risk level, identify it in the bibliography, and change the risk level that it corresponds to its real risk level)
5. Write youre name and adress

Symbols to use

RBSs

promoter

Coding sequence

Biobrick Safety Sheet

Risk level: Indicate the risk level

Adding on a plasmid : name of them

Chassis used : Name of theme

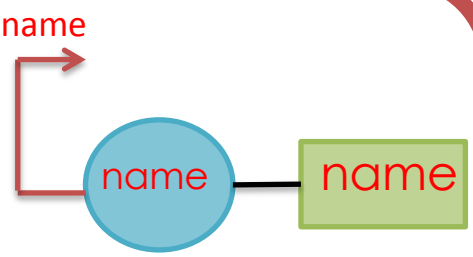


Diagram of the construction

BioBrick name : put the name of the biobrick in the registery

Construction method

Put here the way the construction is built and give the different biological parts used during the construction

Promoter : name(name registry with the link to the web page)

Origin and initial function :

Give the origin of the promoter. More precisely give the origin of the promoter in terms of organisms and what is the goal in the nature.

Purposes in the system :

Give its usage in the system

Size :

55 bp

RBS name : Name and link to the part registry

Origin and initial function :

complete

Purposes in systems :

complete

size:

complete

Coding sequence eCFP

Name and part registry link

Origin and initial function :

complete

Purposes in the system :

complete

Size :

complete

Feedback

environment in which it has been used and consequences :

Environment	consequences
complete	Complete (add lines if necessary)

Safety issues:

- complete

Tests to do in order to answer safety issues :

- complete

Limitation :

-complete

characterization :

put here the information about the functionning of the BioBrick and experience results.

- Put references

Author : put the name of the person who gives the risk level example LINKS Jérôme (iGEM Grenoble 2012)