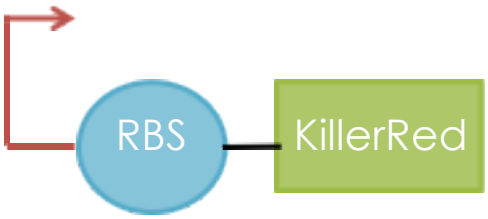
Biobrick Safety Sheet

BioBrick name : BBa_K1141002

Adding on a plasmid : pSB1C3

Chassis used : *E.coli* BW25113



Construction method

The coding gene was obtained by PCR on an eukaryote vector containing the coding sequence of the KillerRed protein (generous gift of Stefan Dimitrov and Yohan Roulland). After putting it in a pQE30 plasmid the part pLac-RBS-KillerRed has been isolated by PCR. An overlapping PCR was performed to remove an *Eco*RI restriction site from the sequence between pLac and RBS. The sequence was then flanked with the iGEM suffix and prefix.

Description:

This BioBrick contains the gene for the expression of the chromophore KillerRed. This protein was engineered via mutagenesis of the gene AM2CP, isolated from an anthomedusa. It is a red fluorescent protein. It's absorption spectrum peaks at 585nm and it emits light with a peak at 610nm. The specificity of this protein is that when excited it produces a large amount of reactive oxygen species (ROS). This release of ROS is potentially dangerous for the cells. We note that after 10min of illumination of KillerRed producing cells, about 96% of them has died.

Size

929 bp

Environment

The BioBrick was only used in a level 1 lab for the moment

Safety Issues

We don't know what would happen if the BioBrick was used in another environment. The light sensitivity of the bacteria carrying it, makes them hardly viable outside. We don't know what would be the consequences of the presence of these bacteria around other living organisms. Note that the ROS produced are highly reactive and therefore only damaging to the cells expressing the protein and not to cells living in their vicinity.

Tests to do

Monitor the survival of bacteria, expressing KillerRed, in a natural light environment.

Observe the consequences of its presence on other living organisms (micro and macro organisms)

References

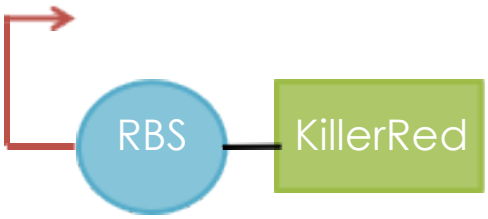
M.E. Bulina et al., A genetically encoded photosensitizer, Nature Biotechnology, January 2006.

Biobrick Safety Sheet

BioBrick name : BBa_K1141001 pLac

Adding on a plasmid : pQE30

Chassis used : *E.coli* BW25113



Construction method

This BioBrick was obtained by PCR on an eukaryote vector containing the coding sequence of the KillerRed protein (generous gift of Stephan Dimitrov and Yohan Roulland) A PCR allowed to flank it with the restriction sites *Kpn*I and *Bam*HI upstream and downstream. It was then ligated into the pQE30 vector.

Description

This BioBrick contains the gene for the expression of the chromophore KillerRed. This protein was engineered via mutagenesis of the gene anm2CP, isolated from an anthomedusa. It is a red fluorescent protein. It's absorption spectrum peaks at 585nm and it emits light with a peak at 610nm. The specificity of this protein is that when excited it produces a large amount of reactive oxygen species (ROS). This release of ROS is potentially dangerous for the cells. We note that after 10min of illumination of KillerRed producing cells, about 96% of them has died.

Size

717 bp (KR)

Environment:

The BioBrick was only used in a level 1 lab for the moment

Safety Issues

We don't know what would happen if the BioBrick was used in another environment. The light sensitivity of the bacteria carrying it, makes them hardly viable outside. We don't know what would be the consequences of the presence of these bacteria around other living organisms. Note that the ROS produced are highly reactive and therefore only damaging to the cells expressing the protein and not to cells living in their vicinity.

Test to do.

Monitor the survival of bacteria, expressing KillerRed, in a natural light environment.

Observe the consequences of its presence on other living organisms (micro and macro organisms)

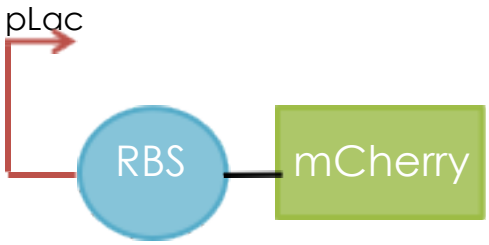
References

M.E. Bulina et al., A genetically encoded photosensitizer, Nature Biotechnology, January 2006.

Biobrick Safety Sheet

BioBrick name : BBa_K1141000
Adding on a plasmid : pSB1C3

Chassis used : *E.coli* BW25113



Construction method

This BioBrick contains the BioBricks BBa_R0010 (http://parts.igem.org/Part:BBa_R0010) and BBa_J06702 (http://parts.igem.org/Part:BBa_J06702).
It was built with standard assembly.

pLac BBa_R0010

Description

This part is a promoter sensible to lactose; it permits the transcription of the gene that follows it as long as there is lactose or IPTG. The concentration of these molecules would define the strength of the promoter.

Purpose in the system

pLac is the promoter that will allow the synthesis of mCherry accordingly to the concentration of IPTG.

Size

200bp

RBS-mCherry BBa_J06702

Description:

This BioBrick contains a Ribosome-binding-site, which is essential for the translation of the RNA into a protein. After the RBS comes the protein mCherry is a Red Fluorescent Protein. Its absorption spectrum peaks at 587nm and it emits light with a peak at 610nm which makes it the closest protein to KillerRed for these settings.

Size

869 bp

Environment

The BioBrick was only used in a level 1 lab for the moment

Safety Issues

The mCherry protein is only slightly toxic to bacteria due to the production of small amounts of reactive oxygen species upon illumination. We don't know what would happen if the BioBrick was used in different environment. We don't know what would be the consequences of the presence of bacteria expressing mCherry around other living organisms.

Test to do

Observe the consequences of its presence around other living organisms (micro and macro organisms)

References

iGEM Registry of biological parts