



Team iGEM UFSCar-Brasil About our Project

Do you plan to experiment with any other organisms, besides your chassis?

Our project involves the use of *Escherichia coli* DH5 α strain as the main chassis in our experiments of limonene synthase expression. Our genetic circuit comprises 3 modules: the expression of limonene synthase, chaperon expression and the assembly of a Kill Switch. The main experiments consist in purification and quantification of limonene molecules. Finishing the assembly of our genetic circuit, we plan (further, not for Giant Jamboree presentation) to use *Lactobacillus* sp. as chassis. In this way, will avoid some issues that are found using *E. coli*, like inconvenient smell and its pathogenicity.

How will your project work?

Our project aims to develop bacteria that is capable of producing suitable limonene, a volatile terpen that helps in the prevention of mosquitoes transmittable diseases like dengue and malaria. In this way, a repellent with a long life span will be produced using *E. coli* in a solution of Polyethylene glycol (PEG) as a final product. The bacteria will be in a smoldering state, with contraction of its volume, also known as plasmolysis. When applied to skin, the sweat will dilute PEG, leading to an osmotic stress that will promote the activation of a promoter (*UspA*) that regulates limonene synthase. Once expressed, the enzyme will use geranyl-pyrophosphate as substrate to produce limonene. Besides, the stressing situation promotes the co-expression of different classes of chaperones, which are expected to remodel the enzyme correctly. The bacteria will then be able to express chaperones of classes *ClpB*, *DnaK* and *IbpA/IbpB*. The developed engineered bacteria also feature a kill switch system based on *E. coli* operon *znuABC*, preventing the dissemination of bacteria to the environment. Other kill switch systems are being considered, like *BaRNase* that will degrade all the bacterial RNA, and *Killer Red* that promotes the production of reactive oxygen species.

Any further comments about your project:

Our project presents a big appeal in Brazil, where the dengue is a disease that each year grows in great part of the states. In 2015, our city was on an epidemic state and even members of our team were affected. The production of limonene by our bacteria represents a strategic method to fight the mosquito with a simple appliance of our repellent cream, we

avoid its sting. Besides, our molecule presents much lower toxicity compared to other commercial repellents.

Comments about this form: Is it easy or difficult to use? Are the questions confusing?

Some questions were a bit complicated and did not fit in some situations our team. In general the questionnaire was to be answered easily.