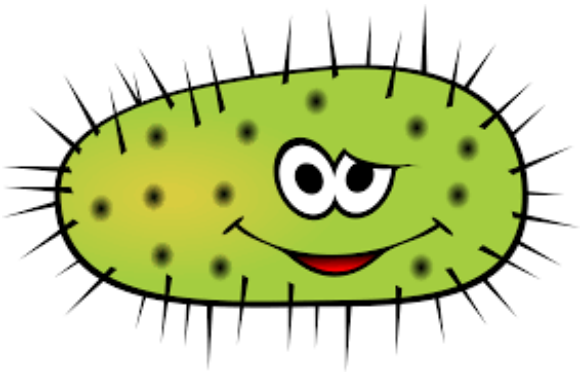
iGEM Minnesota: Biocontainment Strategies

The Minnesota iGEM team created a pamphlet that will contain a variety of safety systems for biocontainment that iGEM teams have made. Examples of safety systems include, but is not limited to, antibiotic resistance plasmids, auxotrophy systems, and kill switches. This is intended to be resource for the future if teams are or will be considering a hypothetical release of their project into the environment and want to study how the system will behave in an ecosystem. It is of the utmost importance to consider safety precautions and implement them into the project beforehand.

Safety Mechanisms



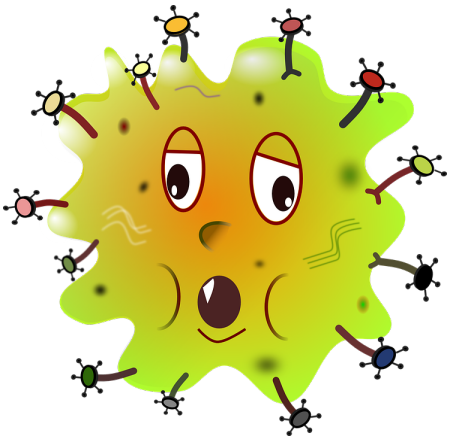
iGEM Minnesota:

Project Description:
Zebra mussels have a large impact locally in Minnesota as well as nationally. Zequanox is a new effective treatment composed of heat-killed Pseudomonas fluorescens, and it is very expensive. The Minnesota iGEM team has proposed a novel synthetic system that is based upon a toxin released by this Pseudomonas fluorescens strain, cytolysin FitD, that could be released into lakes. This designed system entails the creation of a modified Escherichia coli strain that expresses FitD and a biomolecular control system to allow for the release of live bacteria into the lake. With the continuous production of the FitD toxin, an increased number of zebra mussels will be killed, limiting the number of treatments needed. Minnesota iGEM experimented with several biocontainment strategies, including a thymidine auxotrophy system, purine auxotrophy system, SacB control system, and toxin activation system. These control mechanisms help to create robust and safe engineered biological systems for environmental applications.

Potential Hazards:
As this project is intended to one day be used by the general public and thus released into the environment, there are many ecological safety concerns that need to be addressed. Firstly, the concern of horizontal gene transfer from our *P. fluorescens* model into a different bacterium is present. Another possible problem would be the introduction of antibiotic resistance into the wild by virtue of the antibiotics found on our plasmids. Finally, the concern of off target effects as in our toxin targeting native mollusks or other native organisms found in Minnesota lakes and rivers.

Safety Precaution In Use:
A delayed purine auxotrophy system will serve as a control system so the replication of the bacteria can be eventually inhibited upon release into the lakes. In effect, this would result in a delayed auxotrophy. The number of cell divisions would depend on the initial copy number of the plasmid. After reaching the maximum number of divisions, the cells would still survive (to some extent) but they would be unable to replicate because they could not make DNA.

Note that the auxotrophy would also allow us to select transformants without including an antibiotic resistance gene on the plasmid. This would be important if we don't want to introduce antibiotic resistance into the wild. This mechanism wouldn't provide a precise limit on the number of cell divisions, but it is probably simpler and more robust than any system that does. We might be able to tune the allowed number of cell divisions by controlling the initial copy number of the plasmid, which we might be able to do by controlling the concentration of the compound necessary for the origin of replication.

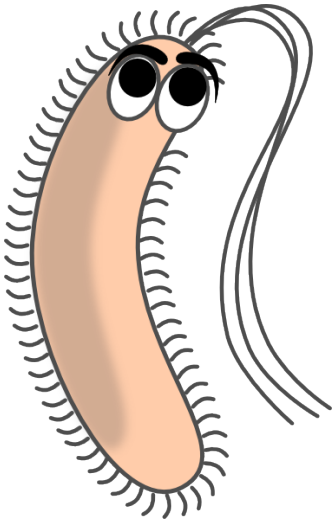


iGEM Linkoping Sweden:

Project Description:
We will express peptides related to Alzheimer's disease fused with eGFP and try to optimize the expression of these fusion proteins. This in the hope that the researchers at our university could use these proteins to discover more about the early stages of fibril formation in Alzheimer's disease. The optimization will be done by changing expression time, induction strength, temperature and chaperones.

Potential Hazards:
We work with a fusion protein of Tau and eGFP. Also as far as we can tell not many iGEM teams work with chaperones.

Safety Precaution In Use:
Sterile working, always autoclaving all the waste, keeping all the GMOs in the lab etc.



iGEM Bulgaria:

Project Description:
Our project is all about evolution and the benefits of it when you can selectively enhance E.coli characteristics - protein production, durability in different environments etc. This scientific breakthrough can carry out a great deal of prosperity for the drug and pharmaceutical industry.

Potential Hazards:
Antibiotic resistance

Safety Precaution In Use:
Working safely with strains that have antibiotic plasmids so to decrease chance of spread.

iGEM Groningen:

Project Description:
We are trying to build a bacteriophage detection system in Lactococcus Lactis based on a dual Cas9 system. We are trying to help the dairy industry with phage detection. A hCas9 incorporates spacers from incoming phages and uses the transcribed tracerRNA to defend against further attacks. A dCas9 will use the same tracer to bind to a predesigned spacer array, which will inhibit GFP output and therefore allow for detection of phages based on the predesigned spacer array. It works off of two Cas9 proteins in L. Lactis, and novel bacteriophage detection system.

Potential Hazards:
Contamination of fermentation system with GMOs, spread of immune system to other Lactis cells

Safety Precaution In Use:
We will be creating a knockout mutant which is nutrient dependent, physical separation with redundant safety systems, and containment in cartridge.