**Cholera**

**What is it?**
Cholera is an acute intestinal infection caused by ingestion of food or water contaminated with the bacterium *Vibrio cholerae*.

**How does it work?**
At low cell density the bacteria attach to the gut wall and express virulence factors. At high cell density the bacteria stop expressing virulence factors and detach from the gut wall. They are then flushed out of the gut.

**Our solution**
Our probiotic bacterium will trick *V. cholerae* into thinking that there is a high cell density of vibrio bacteria. Thus they will detach themselves before virulence factors can take effect.

**Ongoing work**
Created a system of equations to describe the Lux quorum sensing network.


**CqsA – The Cholera Autoinducer Generator**

**Aim**
To design and build a biobrick that would enable E. coli to produce cholera autoinducer. When the autoinducer diffuses out of the cell into the external environment it would be detected by *V. cholerae* bacteria triggering the high cell density response.

**Method**
The CqsA gene was codon-optimised for E. coli. The entire biobrick part was then synthesised and ligated in pSB1C3.

**Conclusion**
The biobrick part successfully built, however, we were unable to complete characterisation. An avirulent cholera strain was acquired so this will proceed in the near future.

**Ribosome Binding Sites**

**Aims**
Analysing the translation rate of an existing RBS and engineering new RBS sequences to optimise the required translation rate, with regard to:
- Upstream sequence of RBS
- Downstream sequence of RBS (including the first 50 nucleotides of protein coding sequence)

**Methods**
Ordering the sequences as primers and ligating them into p13401 to measure the level of protein expression.

**Results**
We were then able to perform parameter tests to investigate which reaction rates affect the level of bistability.

**Conclusions**
- Created a system of equations to describe the cholera quorum sensing network.
- Predicted the RBS strength by the RBS calculator program and measured it in vitro.
- Constructed a biobrick to generate the cholera autoinducer CQS-A.
- New approaches to human practices were introduced with the automated collection of large amounts of readily available web data pertaining to current opinion and trends.
- Supplied Sheffield iGEM with a sample of our CqsA strain for testing purposes.

**Bistability**
- Quorum sensing allows cells to sense their own population.
- Cells synthesise autoinducers, molecules which promote their own synthesis when detected.

**The Lux Quorum Sensing System**
- The Lux system from *Vibrio Fischeri* is the most well studied, it is already present in the registry.
- It gives a switch-like response, turning on rapidly when cell density reaches the threshold (right).
- The Lux promoter, luxI activates transcription of luxS in response to a LuxS-HSL complex (above).

**Re-Engineering the Lux Operon**
- The LuxS protein (shown above in red) acts as part of the “receiver” in this system.
- It forms a complex with the autoinducer (3OC6HSL) which activates transcription from the Lux promoter.
- Increased LuxS concentrations are associated with an increase in system sensitivity to HSL.
- By placing LuxS downstream of the Lux promoter, we can make the system remain “on” until the population has dropped far below the threshold value.
- This is also known as bistability or hysteresis in electronics – these phenomena produce similar graphs (right).
- We set out to construct the rearranged biobrick (above) and model its operation.

**Modelling**

**Aims:**
- To produce a working model of the Lux quorum sensing network
- To investigate the bistability of the system by performing parameter tests
- To develop a working model of the V. cholerae quorum sensing network
- To combine our two models to investigate the effect of our bacterium in the human gut during cholera infection

**Methods**
Using a system of ordinary differential equations, we created a model using the programming language C++ which described the dynamics of the Lux system.

**Results**
Our model produced the bistable behaviour we were expecting:

**Further work**
We created a system of equations to describe the V. cholerae quorum sensing network but due to lack of rate constants were unable to test this. In the future we would propose completing this model and combining it with our existing Lux system.

**Human Practices**
In order to gain an insight into the world view of synthetic biology we developed a series of tools to crawl the web and to parse and analyse web data. Our software crawls through web content including social media, news, blogs, journals etc in order to obtain as wide a range of data as possible. Through the use of natural language analysis tools we were able to examine opinion and how language was used to describe synthetic biology and other topics.

Through analysis of 10 weeks of web data the following trends were observed:
- Opinions towards synthetic biology change frequently and significantly
- There is very little synthetic biology web content when compared to other sciences and even less compared to elements of pop culture
- Compared to traditional science the overall opinion towards synthetic biology is far more prone to change
- When compared against data collected locally using traditional methods, results regarding opinion show almost identical trends.

**Conclusions**
- Created a system of equations to describe the Lux quorum sensing network.
- Introduced two new RBS sequences with measured strength.
- Aims: Downstream sequence of RBS (including the first 50 nucleotides of protein coding sequence)
- BBa_ k356101
- BBa_ k356102
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- To produce a working model of the Lux quorum sensing network
- To investigate the bistability of the system by performing parameter tests
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- Created a system of equations to describe the V. cholerae quorum sensing network but due to lack of rate constants were unable to test this. In the future we would propose completing this model and combining it with our existing Lux system.
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