University of Westminster

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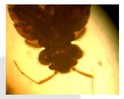
The Team

We are first and second year undergraduates and from various Life Sciences courses at University of Westminster, located in central London.

The Bed Bug Problem

Since 1995 there has been a noticeable increase in the prevalence of bedbugs due to widespread pesticide resistance and has resulted in a surge of interest and study of bed bugs.

Bed bugs (*Cimex lectularius)* are parasitic, blood feeding insects. They are attracted to their host by warmth, CO_2 and other gasses and are well adapted to living with humans.



Eradication of bed bugs frequently requires a combination of pesticide and non-pesticide approaches. This is both expensive and at times ineffective and thus new methods of combating the bed bug problem is required.

Serratia marcescens

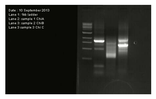
Serratia belong to the family Enterobacteriaceae. They are Gram negative rod shaped bacteria and are found in many environments from soil, bathroom showers and even as a biofilm on teeth. Isolates are also known to be pathogenic to humans.

Chitinases from *Serratia marcescens*

We were kindly gifted the three chitinase genes (*chiA*, *chiB*, *chiC*) isolated from *S. marcenscens* by Prof. Frank Sargent of the Dundee iGEM 2013 team. *S. marcescens* has been identified as the most efficient chitin degrader among 100's of other bacteria. We therefore decided to design a chitinase expressing *E.coli* which would attack to bed bugs.

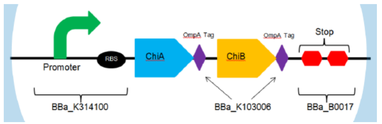
Parts

Name	Type	Description	Length
BBa_K1201000	Coding	Derived from Serratia marcescens	1692
BBa_K1201001	Coding	Derived from *Serratia marcescens*	1500



We have submitted two of the three chitinase genes (chitinase A and chitinase B) to the parts registry.

Construct design

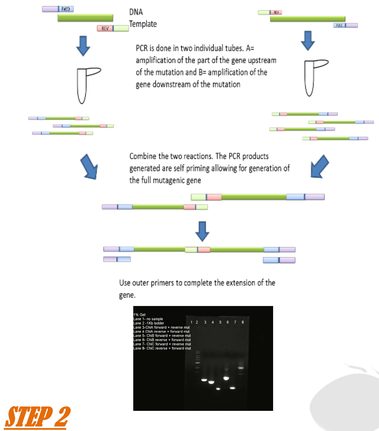


The proposed construct design includes OmpA secretion tags to ensure the chitinases are expressed on the cell surface of *E. coli*.

Mutagenesis PCR

Mutagenesis PCR to remove illegal restriction sites

Site-directed mutagenesis PCR using overlapping primers was our chosen method to perform the removal of restriction sites in the chitinase genes. This PCR involves a two-step reaction and uses four primers. Forward and reverse primers containing the bio brick prefix and suffix were designed. The mutagenic primers were designed to retain the amino acid identity but remove the restriction recognition site.



STEP 1

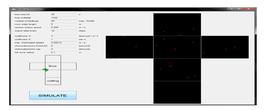
PCR (A) forward-prefix primer and the reverse-mutagenic primer were used in amplification.
PCR (B) forward-mutagenic primer and reverse-suffix primer were used in the amplification.
Thus, PCR (A) amplified the part up stream of the mutation site and PCR (B) downstream of the mutation site for the gene.
The PCR reaction was then run on a gel and the DNA was gel extracted.

STEP 2

For the second PCR, 2µl of each PCR above was added to a PCR mastermix without primers PCR amplified for 10 cycles. The DNA templates contain overlapping regions which self-prime allowing for extension of the full length of the gene, creating template DNA. After this, primers for the outer regions are added. This ensures amplification of the full gene.

Modelling

The model simulated bed bugs moving randomly in a cubic room. One of our proposed blood traps was integrated into the simulation, which visually demonstrated bed bugs being attracted and then subsequently killed by the device. Various aspects such as room size, number of bed bugs and strength of attractant can be inputted to the model to simulate bed bug behaviour. The modelling was made in co-operation with UCL



Chitinase Azure assay

We used the chitin azure assay to determine chitinase activity. Chitin azure is a dispersible colloidal chitin which has been covalently linked to Remazol Brilliant Violet dye. This assay quantatively determines the activity of chitinase In our testing we determined that all three chitinase genes from *S. marcenscens* is required for degradation of chitin .

Human Practice

Dr James Logan

The Westminster iGEM team met up with Dr. James Logan at The London school of Hygiene and Tropical Medicine to discuss our project. We gained new insight into bed bug behavior and discussed how to make our project more effective at targeting beg bug. He suggested using pheromones which cause the bugs to coagulate which would allow our genetically modified *E. coli* to spread easily through contact.

MANTIS

We have also been interacting with a pest control company, MANTIS. This has helped us think about the ethical and practical aspects of our project. They have also supplied us with bed bug samples.

Outreach

Synthetic Biology Speed Debate

The team held a speed debate event at the University of Westminster. During the event, participants were allocated to five groups where they had the opportunity to discuss and debate various topics around expectations, fears and excitement around synthetic biology. Before the event started, the participants were asked to write down what comes to mind when they hear the word synthetic biology?