BACKGROUND

CHEMOTAXIS: The ability to control bacterial motility is important to synthetic biology due to the variety of potential applications such as the “biotweet” project of the WITS-CSIR SA team of 2011.

LIMITATIONS: Some of the shortcomings of the “biotweet” project design is the asynchronous behaviour of the bacteria resulting in reversal of direction before reaching the “recipient” position. Further, the original design does not communicate any information in “reply” from the recipient.

IMPROVED DESIGN: The Pretoria_UP team proposes designs for a synchronized “reply-tweet” that is conditional on a large enough quorum presence and are able to detect and communicate information from the recipient such as whether a chemical of interest is present.

CHARACTERISATION: The genetic switch which would induce chemotactic reversal in the original design has not been characterised. It is our aim to characterise several designs for LuxP-Cre recombinase based genetic switches.

DESIGN

- Quorum sensing is employed to ensure the bacteria behave as a swarm and not individual units.
- A theophylline riboswitch is used to perceive when the bacteria cross a concentration gradient threshold.
- AND gate logic is used to activate a gene switch when input from quorum AND the theophylline riboswitch modules are received.
- Chemotaxis is controlled by a recombinase gene switch under the control of the AND gate. This module induces a change in the direction of chemotaxis.
- A reporter module relays information about a substance of interest.

RECOMBINASE GENETIC SWITCH: AND gate output induces recombination and downstream “reply-tweet”

SPECIAL NOTE: Six synthetic DNA constructs were ordered from Integrated DNA Technologies (IDT). Parts BBa_K1876000 through BBa_K1876006 were documented on the iGEM Registry.

RESULTS

CLOSING: Block parts cloning from a restriction digest (Left) and PCR amplification of the Biobricks (recombinase switch module) corresponding to their expected sizes (right).

SEQUENCING: Analysis of the sequence data was found that the Cre recombinase construct contained two large deletions in the coding region of the construct. The second bp deletion causes frame shift and would render the recombinase protein deactivated. The other gblock fragment sequences were correct with no mutations.

POSITIVE CONTROLS: RFP (left top, BBa_J19522) and GFP (left bottom, BBa_J18511) positive control experiments performed as expected (100% of cells expressed the marker).

CONCLUSIONS AND FUTURE WORK: Our characterisation work was interrupted due to a problem in the Cre recombinase construct. The Cre recombinase construct BBa_K1876004 will have to be re-ordered. Cre-mediated recombinase switches will be tested after IPTG induction of Cre using fluorescent microscopy on RFP positive control and DNA sequencing of RFP expressing colonies. A His tag was fused to the Cre protein to monitor its expression via Western Blot analysis.

MEET THE TEAM

Seven undergraduate students and one BSc. Honours student were selected for team Pretoria_UP, the first iGEM team at the University of Pretoria. Our subject areas range from human genetics to plant science, microbiology to computer science. We are instructed by Dr. Steven Hussey, advised by Prof. Zander Myburg and Dr. Eshchar Mizrahi, and hosted in the Forest Molecular Genetics Programme.

HUMAN PRACTICES

South Africa has a turbulent history of racial segregation and its impact can still be seen today in education. Due to the contrasting socio-economic circumstances which still prevail, we realised that learners from previously disadvantaged areas may not have been exposed to the promise of synthetic biology (SynBio). We issued surveys to Grade 11 learners in two schools situated in different socio-economic areas, Leihlabile Secondary School (LSS) and Pretoria Boys High School (Pret).

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Instructors and advisors:
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  - Prof. Zander Myburg
  - Dr. Eshchar Mizrahi

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  - Forestry Molecular Genetics
  - Sappi Ltd
  - Mondi Ltd

SOUTH AFRICAN INNOVATION ACADEMY: For previous, please see attachment. South Africa: where you can develop skills in technology for primary needs, whereas R&D can develop broader applications.

South Africa: where you can develop skills in technology for primary needs, whereas R&D can develop broader applications.

Conclusion: Previously disadvantaged (LSS) and advantaged (Pret) learners showed significant differences in SynBio awareness, acceptance and expectations. The latter were more sceptical and dismissive of this field, whereas the former regarded it as a means to providing primary needs.

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