SYNCHRONIZED, CONDITIONAL, GENETIC CHEMOTAXIS PROGRAMMING
Our Story

Forest Molecular Genetics research group community project
Winners of the prestigious “dot furthest from any other dot” award
Time Frame

- Advertise for iGEM team: 02-2015
- First iGEM meeting: 03-03-2015
- Team selection: 04-24-2015
- Project Proposal: 19-05-2015
- Part Designs: 24-06-2015
- DNA synthesis order: 13-08-2015
- DNA synthesis arrival: 28-08-2015
- Experimentation Wiki creation Human Practices: 09-2015
- Jamboree: 24-09-2015
Background

Chemotactic programming – Some application

• Targeted Enzymatic Delivery
• Environmental Detection
• WITS-CSIR_SA Bio-tweet
“Bio-tweet”


Limitations to this bacterial messaging system:

• Asynchronous behaviour - disparate signal
• Premature reversal of motility
• No communication module
Design

- Quorum sensing: “Swarm” behaviour
- AND Gate Logic: Combine Signals
- Recombinase Gene Switch: Chemotaxis Direction
- Reporter module: Relays Information about Receiver state
Design

Quorum sensing module (input 1)
- LuxI
- HSL
- LuxR
- LuxR receptor
- ipgC

"AND" gate
- ipgC chaperone
- mxiE

Riboswitch-activated TF (input 2)
- Theophylline
- Riboswitch B_mxiE

Reporter module
- X
- Reporter-TF
- RFP reporter

Chemotaxis toggle switch
- Cre

WITS-CSIR_SA
Recombinase Gene Switch

Recombination using Lox71 and Lox66 produce a stable genetic change.

**Inverter Switch**
- Promoter expressing GFP
- Recombination - Cre protein inverts DNA
- Inverted Promoter expressing RFP

**Excision Switch**
- Promoter expressing GFP
- Recombination - Cre protein binds DNA
- Spliced DNA - Promoter expressing RFP
Experimental Outline

**IDT synthesis**
- gBlock gene synthesis
  - Resuspend
  - Nanodrop Spec

**Cloning**
- Parts in pUC19
  - Restriction digest: pUC 19, Smal
  - Ligation: pUC, gBlock
  - Transformation
  - Blue white screen
  - Miniprep
  - Screen: PCR, M13 Primers

**First Phase**
- Parts in pSB vectors
  - Restriction digest: pUC construct, pSB1C + pSB1A, EcoRI + PstI
  - Purification: Sticky vector, Sticky insert
  - Transformation: Amp + Chlor
  - Ligation
  - Induce Cre: IPTG, LC Dilution series
  - Transformation
  - Fluorescent microscopy
  - Sequence: VF, VR

**Second Phase**
- Characterisation
  - Basic Parts
  - Restriction digest: GFP, RC: E + S, Inverters: X + P
  - Screen
  - Ligation
  - Transformation: IPTG, LC Dilution series
  - Screen
  - Induce Cre: PCR, VF and VR Primers
  - Fluorescent microscopy
Results

Restriction digests of the constructs were cloned into pSB plasmids and amplified using the standard VF and VR primers
Results

Sequence data analysis revealed deletion in ORF of Cre generator resulting in frame shift.

Time constraints:
- Re-clone
- Re-order

All the other fragment had correct sequences.
Future

Recombinase switches characterisation from *E. coli* cell lines containing the different iterations of the switches and correct inducible Cre construct.

- IPTG Dilution series
- Fluorescent Microscopy controls – GFP and RFP

**pLac Induction**

**BBa_I13522**

**BBa_I13521**
Human Practices

SYNTHETIC BIOLOGY AWARENESS AND ATTITUDES AMONG SOUTH AFRICAN YOUTHS: A COMPARISON OF SOCIO-ECONOMIC INFLUENCES
Rationale

- South African turbulent history of racial segregation
- Free and fair elections - 1994
- Impact of this history can still be seen as income inequality and inferior education in previously disadvantaged areas
Approach

- Educational outreach for Gr. 11 learners from contrasting socio-economic areas
- Survey of awareness and attitudes of synthetic biology (~100 learners per school)
- Statistical analysis: Kolmogorov-Smirnov and Fisher’s Exact Tests
Differences in age distributions and family education

How old are you?

![Age Distribution Chart]

What is the highest qualification of your parents?

A. Neither of my parents have their matric
B. Matric
C. Diploma
D. Degree
E. Postgraduate
F. I don’t know/I prefer not to answer

P < 0.05
Differences in age distributions and family education

From which source did you first find out about synthetic biology?

P < 0.05
Perception of Synthetic Biology

In your opinion, synthetic biology:

A. Will revolutionise medicine
B. I don’t know what to expect in the future
C. Will not affect me directly
D. Is ethical but should be carefully regulated
E. Is unethical or wrong, and should be banned
F. I am afraid that it will be abused

Do you think the public is sufficiently informed and understands advances made in synthetic biology?

P < 0.05
Consumer Choice and Future Projections

Would you make use of synthetic biology products?

Which field do you think is the most important in terms of focusing future synthetic biology research?

P < 0.05
Summary

Improved on the Bio-tweet design by adding quorum, and gate and reporter modules as well as started characterisation of the recombinase switches forming part of the project design (7 parts added to registry)

Results of our human practices survey reveal and confirms the challenges facing synthetic biology in the South African context.

We’ve had a fun first experience of what it means to take part in iGEM – we’ll see you next year with an ambitious new project!
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