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Now hand us with questions...

Cambridge iGEM 2012
Parts for a reliable, cost-effective, biosensing standard
The Problem...

- Panama 2011 - Nitrate
- Munich 2011 - Iron
- Munich 2011 - Nickel
The Solution...

- Compatible
- Reproducible
- Simple
- Safe
Human Practices
Our Motivation

- Sharing
- Safety
- Innovation

Figure 4. Predicted probability of fluoride concentration in the groundwater exceeding the WHO guideline for drinking water of 1.5 mg L$^{-1}$. 

**Probability of**

- $F > 1.5$ mg/l
- Poor estimation
- 0 - 0.2
- 0.2 - 0.4
- 0.4 - 0.6
- 0.6 - 0.8
- 0.8 - 1
Ribosense

BioBricks:
- **BBa_K911003 Fluoride Sensitive Riboswitch**
  DNA: Submitted; Experience: Works
- **BBa_K911001 Magnesium Sensitive Riboswitch**
  DNA: Planning, Experience: Issues
Fluoride Riboswitch
All concentrations in mM
Magnesium Riboswitch

**OUR CONSTRUCT:**

- **REPRESSOR**
- **sFGFP**
- **RIBOSWITCH**
- **LAC I**

**Mg^{2+}**

**IPTG**
Ribosense

BioBricks:
- **BBa_K911003 Fluoride Sensitive Riboswitch**
  DNA: Submitted; Experience: Works

- **BBa_K911001 Magnesium Sensitive Riboswitch**
  DNA: Planning, Experience: Issues
Sporino
Cambridge iGEM 2012

Parts for a reliable, cost-effective, biosensing standard

Biobrick:
- BBa_K911008 Fast Germination (B. subtilis)
DNA: Submitted. Experience: None
Ratiometric Outputs
Most reporters

Prone to noise and variation from:

- Culture density
- Cell state
- Substrate availability
- Substrate toxicity (F-)
- Other assay differences
...With an internal control?
Two testing constructs

Fluorescence - based

Luciferase - based
Construct Architecture

BBa_K911009
Relationship between CFP and YFP during steady state region of bacterial growth
CFP and YFP vs OD600

Relationship between OD600 and YFP expression in Ratiometrica

Relationship between OD600 and CFP expression in Ratiometrica

- Pre-exponential phase
- During exponential phase
Two testing constructs

Fluorescence - based

Luciferase - based
mOrange - LuxA fusion

Dachaun Ke & Shiao-Chun Tu
Lux Construct

BBa_K911004
Codon - optimised
Consensus RBSes
Colour Change?
This is not definitive

No access to appropriate luminometer
Ratiometrica

Biobricks:
- BBa_K911004
  Ratiometric Luciferase Construct
  DNA: Submitted; Experience: Working
- BBa_K911009
  Ratiometric Fluorescent Construct
  DNA: Planning; Experience: Issues
Biologger
Instrumentation - Aims

• Develop interfaces for easy interpretation of data provided by our luciferase ratiometrica
• Allowing for the use of multiple different biosensors
• Cheap, open-source, cross-platform
• Automated, easy to use in the field
• Portable
• Wireless
• Bio-containable
• Numerical output
Instrumentation - Theory
Simple electronics and filter gels
Solution - Bilogger

Hardware

Cuvette holders with reflective film

Rotary chassis to support multiple biosensors
Solution - Biologger

GUI for laptop use
Solution - Biologger

Android app for wireless connection

Can exploit mobile platform functions e.g. GPS
Results

Normalised sensor values for different light intensities (setting the dark room measurement at zero)

Normalised percentage of blue frequencies (removing the additional offset of 0.2V for every dilution)

Comparison of sensor responses under different illumination conditions (V: High brightness, V: Low brightness)
Sporage & Distribution

Biobrick:
- BBa_K911008 Fast Germination (B. subtilis)
  DNA: Submitted. Experience: None
Our chassis: Bacillus subtilis

- Sporulation can be induced, cheaply and easily
- No need for freeze drying
- Long term viability
Distribution work through - see wiki for full version...

1.  
2.  
3.  
4.
DIC microscopy of spores (yellow) in germination medium

See our youtube uploads and wiki for time lapse videos!
Fast Germination BioBrick
BBa_K911008

Promoter

Insertion sequence
Data from P. Setlow's lab, Connecticut
Biocontainment

- Include toxic chemical
- Suicidal cells??
Sporage & Distribution

**Biobrick:**
- **BBa_K911008 Fast Germination (B. subtilis)**

  DNA: Submitted. Experience: None
Sporudoino

- Key natural BioBrick: Fluoride
- Two sensitivities

- Predictable BioBrick: Luciferase construct
- No need for complicated controls

- Open-source hardware and software
- Simple to use

Sporage and Distribution
Human Practices

- Market
- Safety
- Direction
Ribosense

- Key natural BioBrick: Fluoride Riboswitch
- two sensitivities
Ratiometrica

- Key engineered BioBrick: Luciferase construct
- Reproducible data
- No need for complicated controls
Biologger

- Open-source hardware and software
- Simple to use
Sporage and Distribution

- Complete work flow
- Fast Germination BioBrick
Ian Practices
- Market
- Safety
- Direction

BioLogger
- Simple to use
- Open-source hardware and software
- Need for completed models
- Ready for completed models
- Collect and analyze data
- Key natural BioBrick: Fluoride
- two sensitivities

Storage and Distribution

Spordino
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