Overview

**Design**
Self-excising ribozyme

**Modelling**
UW 2010 Staphiscope

**Human Practices**
Marketing plan: UW Staphiscope

**Outreach**
Workshops/ESQ
In Vivo Protein Fusion Assembly Using Self-Excising Ribozymes
Background

- Self-Excising (Group 1) Introns
- Trans-esterification reactions
- Fusion proteins

Image from: Self-Splicing RNAs
Applications

Novel fusion proteins: Antibody generation

DNA shuffling experiments: Cry toxins
Experimental Design

Construction at the DNA level

Functional protein GFP and self-excising elements in Staphylococcus viral phage twort ORF 142.2
Experimental Design

Construction at the DNA level

Functional protein GFP and self-excising elements in
Staphylococcus viral phage twort
ORF 142.2
**Experimental Design**

**Construction at the RNA Level**

- Post-transcriptional modification loops formed
- Loops facilitate splicing
- After splicing, fusion protein code remains
Fused GFP protein expected to fluoresce when translated
BioBricks

Construct pieced together using following submitted BioBricks:
Controls

Positive: RFC 53 flanked by GFP 1 & 2

negative

Negative: Intervening stop codon does not allow functional protein expression
Modelling

Discussion

- Possible presence of other compounds
  - Fluorescing in cells
  - Untransformed cells appear to be expressing
  - Cells in bottom of well and positioning of detector may have allowed readings

Conclusion

- The present method of
  - Methods and materials
  - Conclusions
  - Acknowledgements
  - References

Extended MD 2000

8th World Congress on Clinical and Information Sciences
October 2009

Empirical data for the promotion of parameters

Motivation & Goals

- The goal is to achieve a deeper understanding of the
  - Analysis of data
  - Statistical significance

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Extended UW 2010 Staphiscope

- Use is to detect staph rapidly in clinical settings (native Agr quorum sensing system)
- Wished to characterize the amplifier system independent of promoter
  * Would allow for choosing correct promoter based on desired sensitivity
- Aimed to calculate steady state per cell GFP concentration
  > For both promoters
  > Promoter strength in RPU

*Measuring the Activity of Bio-Brick Promoters using an in-vivo reference standard. (Kelly et al.)

## Motivation & Goals
Extended UW 2010 Staphiscope project

- Amplifier parts characterized by Cambridge 2009
- Different promoter
- Empirical data to evaluate Hill parameters
- Calculate relative promoter units (RPU)

**Expected to observe steady increase of fluorescence, eventually plateauing at steady state**

## Results

- Results were anomalous due to variability in fluorescence
- Observed fit of fluorescence curve did not match predictions by the model
- Observed high initial fluorescence, then a steady-state value
- Untransformed cells used (BB02781) exhibited higher fluorescence than the transformed

## Conclusions

- Other possible sources of error are being examined
- Research is still ongoing
- Computational tools designed
Method

- Assayed promoter activity*
- Cells grown until in exponential phase
- Untransformed cells to determine background absorbance and fluorescence
- Aimed to calculate steady state per cell GFP concentration
  > For both promoters
  > Promoter strength in RPU

*Measuring the Activity of Bio-Brick Promoters using an in-vivo reference standard (Kelly et al.)
**Model**

- araC represses for the pBAD promoter
- Same inducer-repressor ODE model as Cambridge in 2009
  * Promoter activity as hill function
- ODE model used to describe the transcription of GFP in each cell
- Promoter activity assay result shows RPU is given by (steady state assumption):

\[
RPU = \frac{S_{SS_{cell},\phi}^{SS}}{S_{SS_{cell},J23101}^{SS}} = \frac{(dF_{\phi}/dt)/ABS_{\phi}}{(dF_{J23101}/dt)/ABS_{J23101}}
\]
Results

- Results were anomalous: too unreliable to be conclusive
- Fluorescence curve obtained did not match predictions by the model
- Observed high initial fluorescence, then drop to lower steady-state value
- Untransformed cells used (BW27783) exhibited higher fluorescence than the transformed cells
Fluorescence time series for cells at 6.4 μM arabinose; depicts abnormality in untransformed cells.
Fluorescence time series for cells at 6.4 μM arabinose; depicts abnormality in untransformed cells
Discussion

- Possible presence of other compounds fluorescing in cells
- Untransformed cells appear to be expressing GFP
- Cell settling in bottom of well, and positioning of detector may have skewed readings
Conclusions

- Other possible sources of error are being examined
- Research is still ongoing
- Computational tools designed
Human Practices
- Lack of basis
- Rate of technological advancement surpasses one’s ability to adequately understand the technology
- Risks innovation of synthetic biology technologies
Staphiscope
What are the inputs required to devise a marketing strategy:

- Competitive advantage
- Alleviate unfounded stigmas
- Pitch product to both a scientific/non-scientific community
- Utilize a knowledge transfer strategy
Competitive Analysis
BD GeneOhm StaphSR
Genotype MRSA
Brilliance Agar
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Market Pitch: R&D Institution

In order for a synthetic biology technology to be competitively advantageous, it must excel in at least one of the following areas:
Avoid “not invented here” syndrome
Patent protection
Develop a commercialization plan
Achieving Public Acceptance

- Supported as part of the New and Emerging Science and Technology Programme
- Expert committee developed and presented a roadmap
- Attributes required in sustaining the field of synthetic biology
Achieving an interdisciplinary network through the development of a knowledge transfer strategy

The “Target Market”: Who do we want to educate?

1) Engineers
2) Industry Representatives
3) Non-governmental organizations
4) Non-scientific Community (ie. Hospital Staff)
5) Decision Makers
6) Natural & Social Scientists
7) Other Funding Agencies
knowledge transfer

1. Interdisciplinary Network
2. Sustainable Dialogue
3. Involving Stakeholders in the R&D phase
4. Knowledge Brokering
5. Building Partnerships
Outreach

Grades 3-4
How clean are your hands?

- Create informative opinions
- How does symbio affect the world?
What we stand for...

- Better understanding of synbio
- Create informative opinions
- How does synbio affect the world?
Grade 12 Workshop

- More than 85 students
- Effect of synbio in biofuels/pharmaceuticals
- Hands-on activities
  * Enviro-pig
  * Design Your Own Pathway
- Possible career paths
Engineering Science Quest

Grades 3-6
More than 100 students
Grades 3-4

How clean are your hands?
Grades 5-6
DNA extraction from cheek cells
Community

- Alumni events
- Open houses
- Growth of current events
Questions?

WEEF
Waterloo Science Faculty
Waterloo Biology Department
SFF
Bio Basic Inc.