Light surrounds us in our everyday lives and is used in numerous engineering applications. However, the Registry has so far lacked a self-sufficient BioBrick for the generation of light. We placed bioluminescent (light producing) systems from fireflies and bacteria into E. coli. Codon optimisation for gene expression in E. coli and single amino acid mutagenesis allowed us to generate bright light output in range of different colours. In addition, we developed a set of tools to aid construction of new BioBrick parts and devices.

Applications
Biosensors exist to measure the amount of a specific factor. There are a number of existing reporters but there are none which are both affordable and quantifiable. Separately tuned wells require lots more work to tune, and add to cost. Using our light outputs, we have proven that the light from bacterial culture can be assayed affordably with our E. glowl (see right). These could be mass produced lowering production costs, and distributed with the bacterial biosensors for a complete system.

Co-reporters
Multiple colours and filters allow use of the co-reporter assay with applications such as:
- Using one output as a control to check for biosensor viability, avoiding false negatives
- Measuring two or more quantities concurrently, e.g. a number of different toxins

Lighting
From an early stage our project was motivated by using our bacteria as a potential light source. By the end of the summer our bacteria was bright enough to read a page of text. Lighting consumes 8% of global electricity. In a number of applications it is used to read a page of text.

Project Firefly
Background
Project Firefly focused on the construction of light-generating BioBricks from genes involved in firefly bioluminescence.

Fireflies are insects from the family of click beetles and are one of the few land animals able to emit light. Larvae use light as a warning signal to predators, adults use it in courtship displays.

Chemistry
The reactions for light production occur in a specialised organ called the lantern. Here the enzyme Luciferase catalyses the oxidation of D-luciferin into excited oxyluciferin, which then rapidly drops down to the ground state emitting a photon of visible light.

Another enzyme involved in the pathway is LRE (luciferin-regenerating enzyme), which converts oxyluciferin back into luciferin via CHBT and D-cysteine. Without this oxyluciferin cannot be regenerated and inhibits luciferase activity.

Coloured Outputs
We generated six different luciferase parts with different colours. As well as being aesthetically pleasing this variety of colour allows light to be tailored to a detector.

Additionally the different colours can easily be placed under various regulatory inputs facilitating useful co-reporter assays.

Kajiya and Nakano (1991) had reported that the colour from the luciferase of Japanese firefly Luciola cruciata can be significantly altered by a variety of single amino acid changes.

We ordered the codon optimised gene from DNA 2.0 and used site directed mutagenesis to create the mutant codon changes.

Luciferin Recovery
We combined both L. cruciata and P. pyralis luciferases individually with their own LRE.

Our plate reader measurements confirmed that upon the addition of D-cysteine light emission was brighter and longer lasting in the presence of LRE compared to its absence.

Firefly Modelling
Current models focus on in vitro reactions, due to luciferase’s common use in reporter assays.

We developed a model of the luciferin cycle in vivo using data published by Marques (2009) and Inouye (2010). We added equations for luciferin regeneration by LRE and after experiments refined our model with diffusion of luciferin from medium to cell.

Project Vibrio
Background
Natural bioluminescence is found in several marine bacteria, allowing them to form symbiotic relationships with deep-sea fish and squid.

The enzymes for light-generation are encoded by the lux operon.

In Vibrio fisheri, this system consists of Lux R CDABEG.

LuxR and I exert quorum sensing control on expression of the lux operon.

LuxA and B encode the bacterial luciferase.

LUXC, D and E products synthesize the substrate tetradecanal.

LuxG’s function is unknown, but its presence increases light output.

In nature, lux genes are repressed by the nucleoid protein, H-NS. This binds sequence-independently to curved DNA around promoter sites and intergenic and coding regions of the lux operon. Activated LuxR relieves this repression. However, when the lux operon is placed under a different promoter in a H-NS wild type strain, repression persists and inhibits maximal levels of gene expression.

The LuxBrick
We extracted luxCD, luxAB and luxEG from a clone of the V. fisheri lux operon and assembled a new operon under the arabinose-inducible promoter pBAD.

This construct gives bright and reproducible light output in E. coli Top10 cells and forms an arabinose > light device. The duration of light output can be increased by expression in a H-NS knockout strain.

The LuxBrick completes our spectrum of emission wavelengths with blue light, removes the need for external substrates and can be combined with various inputs to act as a biosensor output.

Codon Optimisation
Despite their common genetic code, different organisms have preferences for the triplets they use for a given amino acid. Using NCBI sequences of the V. fisheri lux operon, we designed a codon optimised version for E. coli, conserving the amino acid sequence whilst increasing translational speed.

The natural V. fisheri lux operon contains stretches rich in A+T content, resulting in curvature, which the H-NS repressor protein binds to. We raised the G+C content in these regions (occasionally with suboptimal codon usage) using computational prediction as a guide to alleviate H-NS repression.

In V. fisheri, LuxA and B are expressed at five times the levels of LuxC, D, E and G. In order to reproduce physiological expression ratios we put LuxA and B and Lux C, D, E and G under two different promoters.

Gibson Assembly
Gibson Assembly is a cutting-edge DNA ligation technique developed by Dan Gibson at the JCVI in 2009. Combined with PCR, it allows the simultaneous joining of multiple blunt ended DNA sequences without scarring.

We made extensive use of this technique to speed up the creation of our BioBricks and promoted it through a music video and BioBrick Foundation RFC, as well as creating software to aid with the design of the required PCR primers.

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