Switch-a-roo: engineering a photoresponsive ‘E. coli’ light switch’

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Abstract

Phytochromes are ubiquitous proteins that allow an organism to sense light. These proteins have evolved in unique environments to sense light intensity in different colour ranges. This experiment focuses on constructing a biological switch that uses phytochromes from Deinococcus radiodurans and Agrobacterium tumefaciens. The coupling of heme oxygenases supplies our phytochrome proteins with biliverdin, allowing for the self-assembly of the switch within host systems. The switch is the first stage of a two component light sensor and when expressed at high level, there is a noticeable colour change of the cell.

Background

- Phytochromes are light sensors that combine with chromophores to regulate the growth, germination and light mediated responses in plants.
- The phytochrome first binds to the biliverdin to form a complex that undergoes a conformational change. This allows the organism to respond to ambient light.
- Biliverdin is made in the cell from heme by heme oxygenase activity. E. coli does not produce heme oxygenase, so the gene needs to be added.
- These photoreceptors sense red and far red light to undergo photoconversion between green (active) and blue (inactive) forms.

Aims

1. Construct 3 BioBricks which are functionally expressed in E. coli:
   - Heme oxygenase
   - D. radiodurans - phytochrome
   - A. tumefaciens - phytochrome
2. Assemble a light switch mechanism consisting of the HO and bacteriophytochrome parts.

Experimental Design

BioBrick Construction Flowchart

Abstract

BioBrick construction

Digestion of BioBrick shows successful insertion of HO in correct orientation and size – incorrectly orientated inserts yield a single linearised plasmid. This is also confirmed by sequencing results.

Phytochrome PCR

Amplification of the phytochromes from D. radiodurans and A. tumefaciens was successful.

The PCR products can be inserted with the heme oxygenase BioBrick to construct a functional light switch.

Results

Phytochrome PCR

PCR of DR-Bph (left) and AT-Bph (right). Red circle shows desired band size (~2.3kb).

BioBrick construction

EcoRI and SpeI digest of HO-BioBrick.

Demonstration of biliverdin synthesis

Liquid media containing IPTG and ALa (heme precursor), incubated for 72 hours at low temperature, spun down. Pellets are green as heme oxygenase is expressed using T7 promoter and degraded heme into biliverdin.

BioBrick improvement

In 2010, Macquarie iGEM team submitted a HO coding sequence part (Bba_K460000). This year, our team submitted a working version of this BioBrick containing a RBS and HO gene (Bba_K460000) for use by future teams.

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