Engineering a Bacteriophytochrome Switch – Creating a Controllable *E. coli* Chameleon

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Abstract

Photoreceptors are utilized by almost every organism to adapt to their ambient light environment. Our aim is to engineer a novel reversible molecular 'light switch' within *E. coli* by introducing a photoreceptor from non-photosynthetic bacteria (*D. radiodurans and A. tumefaciens*).

By cloning the bacteriophytochrome coupled with heme-oxygenase, an enzyme that produces biliverdin from heme, the created colonies are able to respond to red and far-red light environments. This novel approach results in the colour of the *E. coli* 'switching' from blue to green.

Our *E. coli* chameleon will serve as a fundamental 'bio-brick' for future applications by providing a simple and photo-reversible switch.

Background

- Phytochromes are red/far red light sensors, used in the growth, germination and other factors effected by light.
- The family of phytochrome proteins found in bacteria are known as bacteriophytochrome photoreceptors.
- Phytochromes bind covalently to chromophores such as biliverdin to create a complex.
- The complex undergoes photointerconversion between two stable isoforms allowing proteins to respond to changes in light in the surrounding environment (Figure 1).
- The complex, when exposed to red and far red light, produces change colour to express blue and green, respectively.
- The two stable isoforms create a photoreversible switching mechanism within the organism.

Creating the Switch

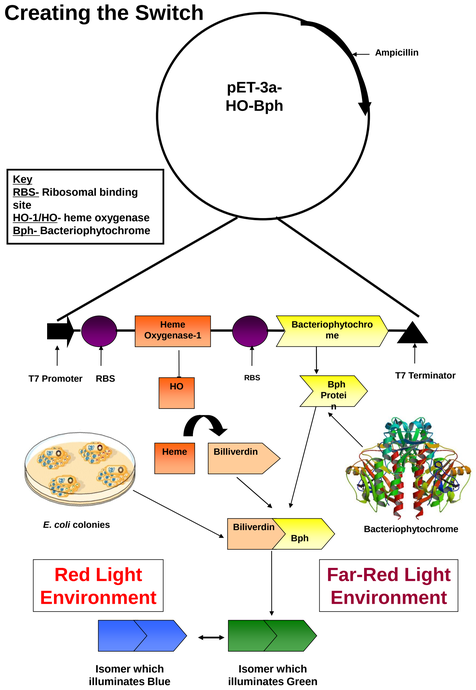


Figure 1. Vector created to transform into *E. coli* to express biliverdin and bacteriophytochrome proteins which form two conformational isomers when exposed to red and far red light to turn *E. coli* blue and green!

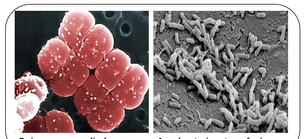
Aims

1. **Isolate a bacteriophytochrome gene** from two bacterial species *Agrobacterium tumefaciens* and *Deinococcus radiodurans*.
2. **Clone bacteriophytochrome into a pET-3A vector** that contains the heme oxygenase gene and transform it into an *E. coli* strain, BL21(DE3).
3. **Create a biological light switching mechanism**: *E. coli* transformants will express both biliverdin produced by the heme oxygenase enzyme and the bacteriophytochrome protein enabling the *E. coli* to turn from blue to green when red and far-red light is absorbed by the colonies.

Experimental Design

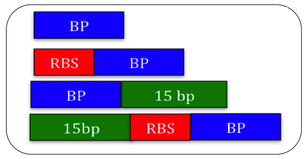
DNA EXTRACTION OF *DEINOCOCCUS* AND *AGROBACTERIUM*

Genomic DNA extracted from *D. radiodurans* and *A. tumefaciens* using salting extraction methods. DNA quality checked by Nanodrop UV-Vis and 1% agarose gel.



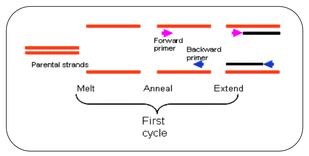
PRIMER DESIGN FOR PCR (*Bph* + RBS)

Designed to amplify bacteriophytochrome genes, introduce ribosome binding sites as well as adding 15bp homology to primers for In-fusion PCR.



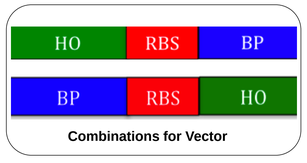
POLYMERASE CHAIN REACTION FOR *Bph* + RBS

In-Fusion PCR used amplified genes with RBS + 15bp homology to pET-3A vector.



CLONING STRATEGY- INTO pET-3a VECTOR

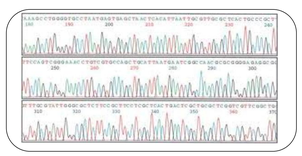
PCR products ligated into vector in various combinations. Bacteriophytochrome gene inserted either before or after heme oxygenase gene.



Combinations for Vector

DNA SEQUENCING OF VECTOR + BP

Cloned pET-3a vector was sequenced at Macquarie University Sequencing Facility to ensure gene sequences were accurate.

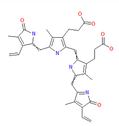


'SWITCH' CLONING - pET-3a into *E. coli*

pET-3a vector transformed into *E. coli* strain BL21(DE3) in order to express the heme oxygenase and bacteriophytochrome proteins.

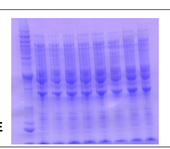
'*CHANGE OF PLANS*' - BILIVERDIN ADDED TO *E. coli*

***E. coli* colonies were not expressing a sufficient amount of heme oxygenase to produce biliverdin therefore biliverdin was added directly into liquid cultures.**



TESTING THE SWITCH ASSEMBLY - SDS-PAGE GEL

Test for band at correct molecular weight using zinc acetate stain under 700nm using an Odyssey Imaging system.



Results

AMPLIFICATION OF THE BACTERIOPHYTOCHOME GENE

PCR was optimized to include a ribosomal binding site (RBS) and 15bp homology either before or after the bacteriophytochrome (*Bph*) gene (Figure 2).

The amplified PCR product can be inserted into the pET-3A vector either before or after heme oxygenase (HO) to create an operon.

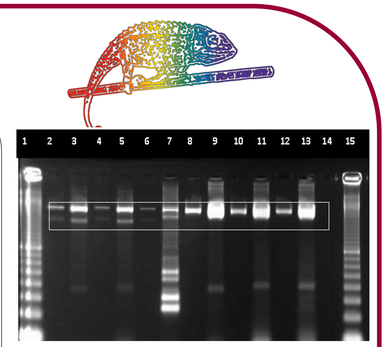


Figure 2. PCR optimization. Amplification of bacteriophytochrome gene from *A. tumefaciens*.

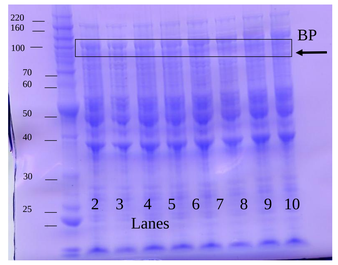


Figure 3. SDS-PAGE of *E. coli* proteins being expressed including bacteriophytochrome gene.

EXPRESSION OF THE BACTERIOPHYTOCHOME GENE

SDS-PAGE gel confirms that bacteriophytochrome gene is expressed by *E. coli* (Figure 3).

Band at ~80kDa represents the bacteriophytochrome protein.

SDS-PAGE – BP + BILIVERDIN COMPLEX

SDS-PAGE gel stained with zinc acetate detects the assembly of the BP + biliverdin complex. At a wavelength of 700nm, the complex fluoresces (Figure 4).

Fluorescence confirms that the experiment was successful in creating a molecular 'switch' in *E.coli!*

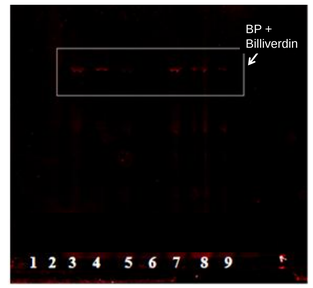


Figure 4. SDS-PAGE gel of *E. coli* stained with zinc acetate and visualized at 700nm to detect BP + biliverdin complex.

Conclusions

Macquarie team successfully created a functional molecular 'switch' within *E. coli* using bacteriophytochrome gene from *A. tumefaciens* & biliverdin.

For further studies and confirmation of the functional molecular 'switch', an analysis by tandem mass spectrometry of the proteins would verify the presence of the bacteriophytochrome protein within *E. coli*.

Acknowledgements

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References

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