Ethene Oxide Synthesis via Heterologous Expression of Ethene Monooxygenase in *Pseudomonas Putida*

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**Problem**

Ethene oxide is an important industrial compound, used for production of antifreeze (ethylene glycol), and many other chemicals. Current problems with its synthesis include:

- Petrochemical feedstocks running out
- Very energy intensive
- Generates toxic waste

Biocatalysis is more environmentally-friendly, using non-toxic, low-energy catalysts.

**Aim**

Heterologous expression of *Mycobacterium chubuense* ethene monooxygenase in *Pseudomonas putida* to enable ethene oxide biosynthesis.

**Experimental Design**

1. Codon harmonised EtnABCD
2. LacI co-expression
3. Induction
4. Check for ethene oxide

**Modelling**

Our codon harmonisation algorithm, *TransOpt*, aims to preserve ribosome translation rate and mRNA folding profile from the native to the new host to enhance heterologous protein expression and folding.

**Experimental Validation**

*Bacillus subtilis* flaving-binding fluoroprotein (BsFP) was used to test different codon optimisation methods (fast-folding vs. *TransOpt* vs. standard harmonisation).

**Solution**

The ethene monooxygenase enzyme (EtnABCD) from *Mycobacterium chubuense* can synthesise ethene oxide from ethene, but unfortunately, to date, this enzyme cannot be expressed in standard cloning hosts.

- EtnABCD genes cloned by Golden Gate method into pSB1C3, sequenced, deposited as Part, but not expressed in *P.putida*, using pBRR1MCS-2 vector
- New LacI Part with P*LacI* promoter shown to be functional, but this added regulator protein did not help heterologous expression of etnABCD
- New codon harmonisation algorithm (*TransOpt*) used to make *P.putida* version of *etnABCD*, and *E.coli* version of *B. subtilis* fluoroprotein (BsFP)
- *TransOpt* not successful for improving expression, but brightly-fluorescing standard codon-harmonised BsFP deposited as a new fluoroprotein Part

**Results**

- EtnABCD genes cloned by Golden Gate method into pSB1C3, sequenced, deposited as Part, but not expressed in *P.putida*, using pBRR1MCS-2 vector
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- New codon harmonisation algorithm (*TransOpt*) used to make *P.putida* version of *etnABCD*, and *E.coli* version of *B. subtilis* fluoroprotein (BsFP)
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**Human Practices**

We engaged with the public and students via:

- School workshops
- Science festivals
- Public talks
- Media interviews
- “Strange Nature” writing competition
- Collaboration with other iGEM teams

We aimed to raise awareness of the benefits and issues of synthetic biology, and to enhance public understanding of molecular biology and microbiology.