iGEM USYD

FRES(H)

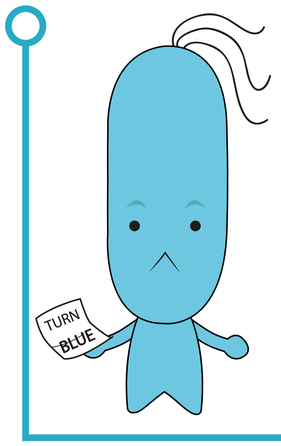
Fruit Ripeness Ethylene Sensor (Hopefully)

Team Sydney_Australia

Almost half of all fruit and and vegetables produced are damaged, lost, or wasted!

What can synthetic biology do about it?

Ethylene is a major hormone involved in the ripening of fruits. High demand for seasonal fruits represents a challenge in terms of long storage and transportation times. Small amounts of ethylene can cause unwanted ripening and spoilage, leading to wastage. We need a way of determining ethylene levels, but current methods are expensive, labour intensive, and frequently not portable. Our solution was to create an ethylene biosensor by engineering bacteria



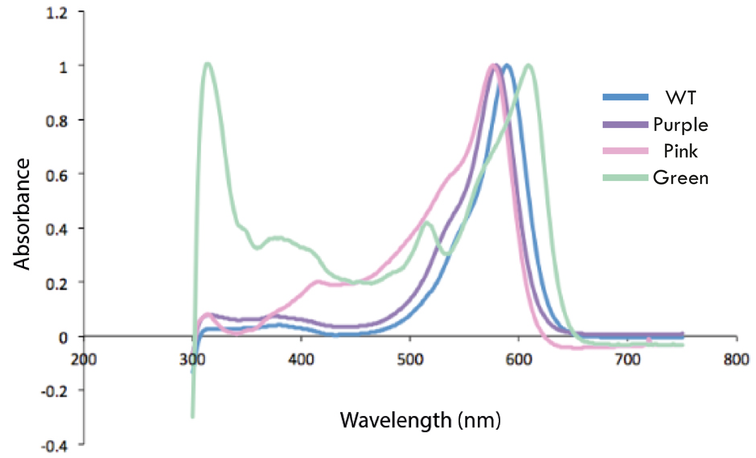
to **SENSE** ethylene

to **EXPRESS** a visible output

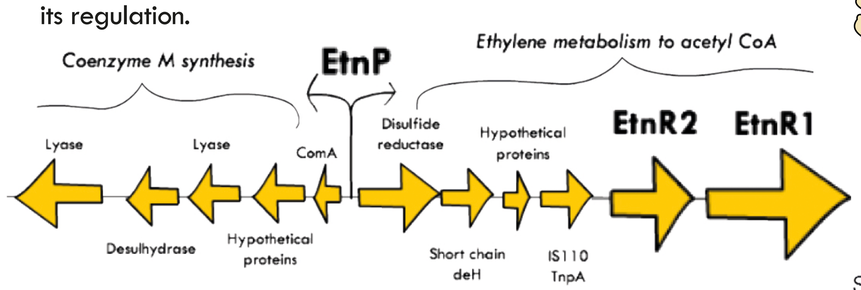
to **KEEP** our produce **FRESH**

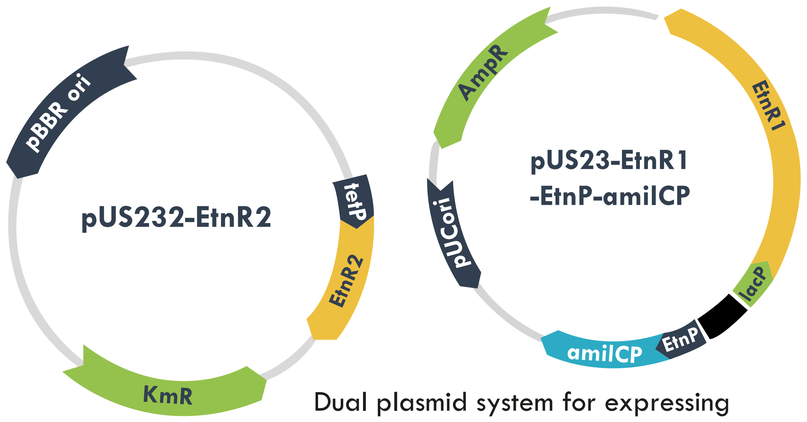
We hijacked the ethylene regulatory system so that *E. coli* could express a chromoprotein (amilCP) upon detection of ethylene.

AmilCP is a blue chromoprotein and an iGEM part that we improved through error prone PCR. We generated 3 colour mutants with shifted absorbance peaks



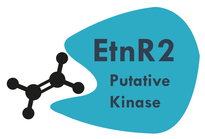
For our biosensor to sense ethylene, we turned to the ethylene-oxidising *Mycobacterium* NBB4. We investigated two genes from its ethylene metabolism operon (below), which we suspected were responsible for its regulation.





Dual plasmid system for expressing EtnR1 and EtnR2, allowing ethylene-inducible expression of amilCP.

Suspected EtnR1 and EtnR2 interactions and the assays to characterise them.

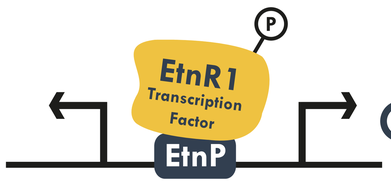


EtnR2 binds to ethylene

Mass spectroscopy for phosphorylation of EtnR2

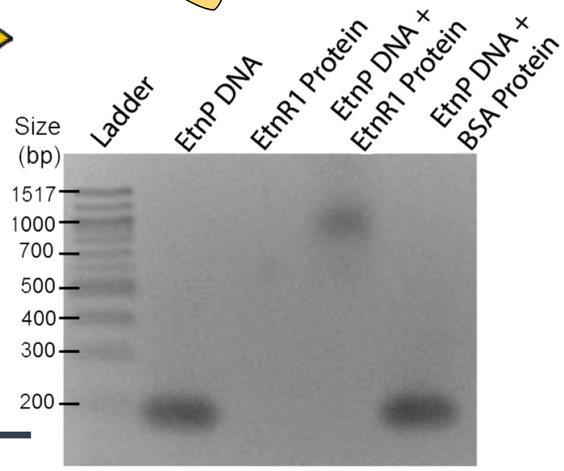
EtnR2 interacts with EtnR1 and leads to EtnR1 phosphorylation

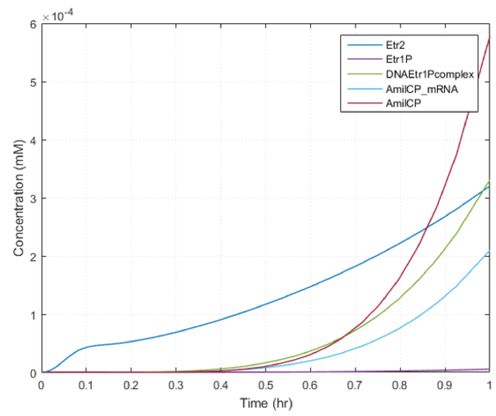
Pulldown assay for binding of EtnR1 and EtnR2



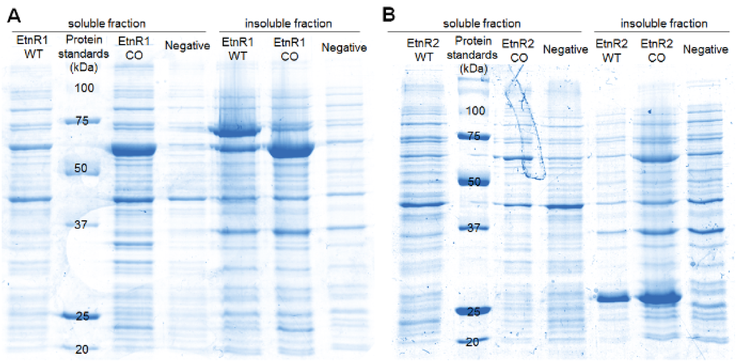
EtnR1 interacts with EtnP and leads to transcription of the operon

Electrophoretic mobility shift assay for binding of EtnR1 to EtnP

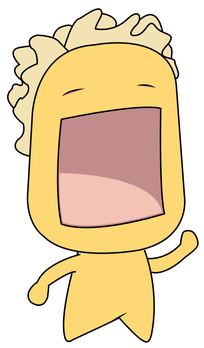




Above: Computational model of protein expression upon stimulation by 20mM of ethylene using MATLAB.

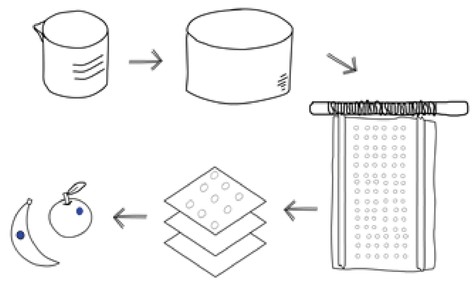


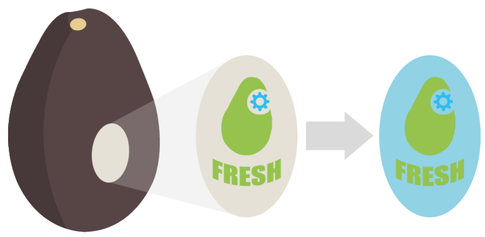
Left: Confirmation of expression of EtnR1 and EtnR2 in *E. coli* using SDS-PAGE.



As an intermediate product whilst cell-free technology is being developed, a latex nanoporous biocoating will be used to immobilised GM *E. coli* on a paper base.

After consulting with potential consumers (Zespri, Avocados Australia, and Fresh Produce Group) we selected three designs for our final biosensor chassis: a fruit sticker, an industrial sticker for use on shipping containers, and a plate and strip system for warehouses.





0.01c vs. 0.04c

Our fruit sticker — Current fruit labelling

0.01c vs. $15,000+

Our fruit sticker — Gas chromotography

Attributions

Flickinger, M., Schottel, J., Bond, D., Aksan, A. and Scriven, L. (2007). Painting and Printing Living Bacteria: Engineering Nanoporous Biocatalytic Coatings to Preserve Microbial Viability and Intensify Reactivity. Biotechnology Progress, 23(1), pp.2-17.

All experiments were performed by the iGEM USyd team.
Thanks should be given to the following for their invaluable help:
Dr Nicholas Coleman (supervisor)
Edward Hancock (cosupervisor)
Brian Jones (cosupervisor)
Various lab companions: Frances, Mark, Deb
Expert advice from:
Antony Allen (Avocados Australia)
Joseph Eckman (The Fresh Produce Group)
Frank Bollen (Zespri Kiwifruits)

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iGEM

NSW Department of Primary Industries

BIOFOUNDRY

BIO-RAD

Fresh Produce Group

NSW Chief Scientist & Engineer

THE UNIVERSITY OF SYDNEY

IDT INTEGRATED DNA TECHNOLOGIES

Zespri Kiwifruit

Australian Avocados