**Introduction**

We rely heavily on plastics in our day to day lives - and yet they are currently produced from a non-renewable resource, fossil fuels, in a process requiring large amount of energy. We have found that the majority of plastic products are produced from or derived from the compound Ethylene.

The problem: Ethylene is currently produced in an energy intensive process called steam cracking where large hydrocarbons from oil or natural gas are heated to roughly 900 °C and saturated with steam and subsequently cooled to -157 °C to be compressed and distilled multiple times. This process has a large carbon footprint. To solve this, we have found that plants can produce ethylene from the amino acid methionine at room temperature with renewable and readily available compounds at a much lower carbon cost.

**Aim:** Construct an ethylene generation device in an *Escherichia coli* chassis to produce ethylene using the enzymes involved in plant ethylene biosynthesis.

**Ethylene biosynthesis**

Ethylene biosynthesis occurs in plants through the Yang cycle, also known as the methionine cycle.

**Design & Assembly**

![Alt Text](image)

- **Design uses Lac inducible promoter (R0011) and strong ribosome binding site (RBS) (B0034)**
- **Initially tried 3A Assembly procedure**
- **Experienced problems with attaching RBS to coding sequences**
- **RBS sequence too small?**
- **RBS lost?**
- **Developed alternative assembly procedure (See left) to try and compensate for the possibility of the RBS being lost**
- **Successfully cloned SAM Synthetase from E. coli genomic DNA**
- **Shipped as part K417000**

**Kinetic Modelling**

Used the program Tinkercell to create a simple model of the device.

**Future Work**

- **Finish construction of our design**
- **Measure ethylene output via Gas Chromatography**
- **HCN removal via the enzyme Cyanide Dihydratase**
- **Reconstruct entire Yang’s Cycle to recycle methionine**
- **Optimise ethylene output via alternative RBSs, promoters**
- **Measure enzyme concentration, steady state**

**MONASH University**