# GREEN is the new BLACK TEAM MACQUARIE 2013 • In 2009 Australia relied on non-renewable energy from fossil fuels for 95% of its energy needs - 41% coal, 36% oil and 19% gas attributed to this. Successful production of chlorophyll in a bacterial host is the first step towards the synthetic construction of photosystem II, and the eventual creation of a new renewable energy source • Our project aimed to express the thirteen genes (from Chlamydomonas reinhardtii) necessary for the chlorophyll biosynthesis pathway in

## Background

a bacterial host (Escherichia coli)

**Chlorophyll Synthesis** 

**Pathway** 

3. Chlorophyll a

ChIP - BBa\_K1080008 DVR1 - BBa\_K1080012 ChIG - BBa\_K1080009

- Chlorophyll is the green pigment responsible for the absorption and transfer of light energy
- During photosynthesis, light energy is converted into chemical energy: 6CO2 + 6H2O - C6H12O6 + 6O2

Protoporphyrin IX

Mg-Protoporphyrin IX

Mg-Proto ME

Protochlorophyllide

Chlorophyll a

Plastocvanin + YCF54

DVR<sub>1</sub>

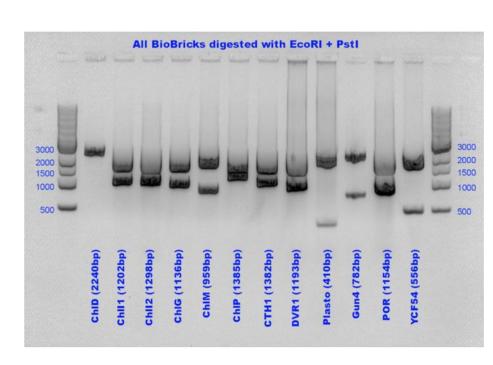
ChIH are subunits

Gun4 is cofactor

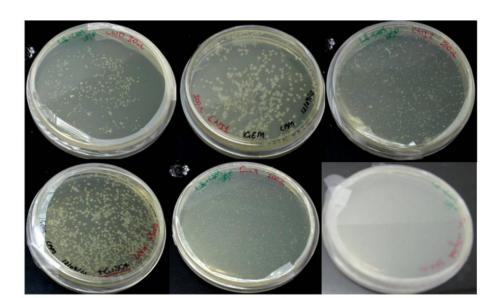




## Results and Characterisation

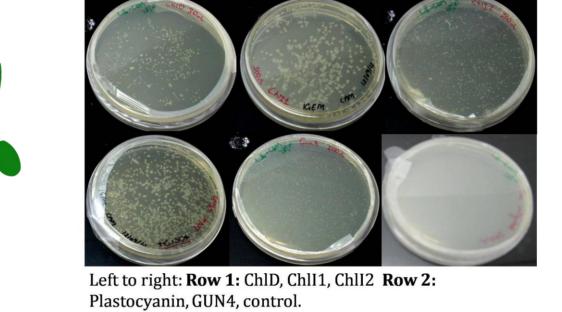


Gene Sequencing Results - All of our genes were assembled correctly from gBlocks, all our sequencing results were submitted, and came back with an identity match of 100%

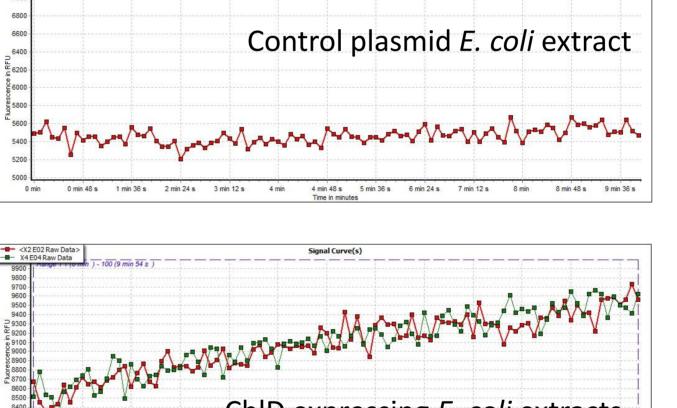


Composite parts: Tac promoter BBa\_K864400 was successfully ligated with the genes: ChID, Chll1, Chll2, Gun4, and Plastocyanin for further characterisation

**ChID** activity assay:



Mg-protoporphyrin IX formation over time (Fluorescence Ex420nm Em590nm)



ChID expressing *E. coli* extracts

fluorescence The increasing shows Mg-protoporphyrin signal indicating a complex formation containing functional ChID has formed

FEBS letters 586 (3), 205-210)

ChID from the extract was used to

form the magnesium chelatase

complex with purified Chl11, Chl12,

ChlH and GUN4 (Zhou et al. 2010

• 1μL of cell extract had 2.1ng of active ChID protein





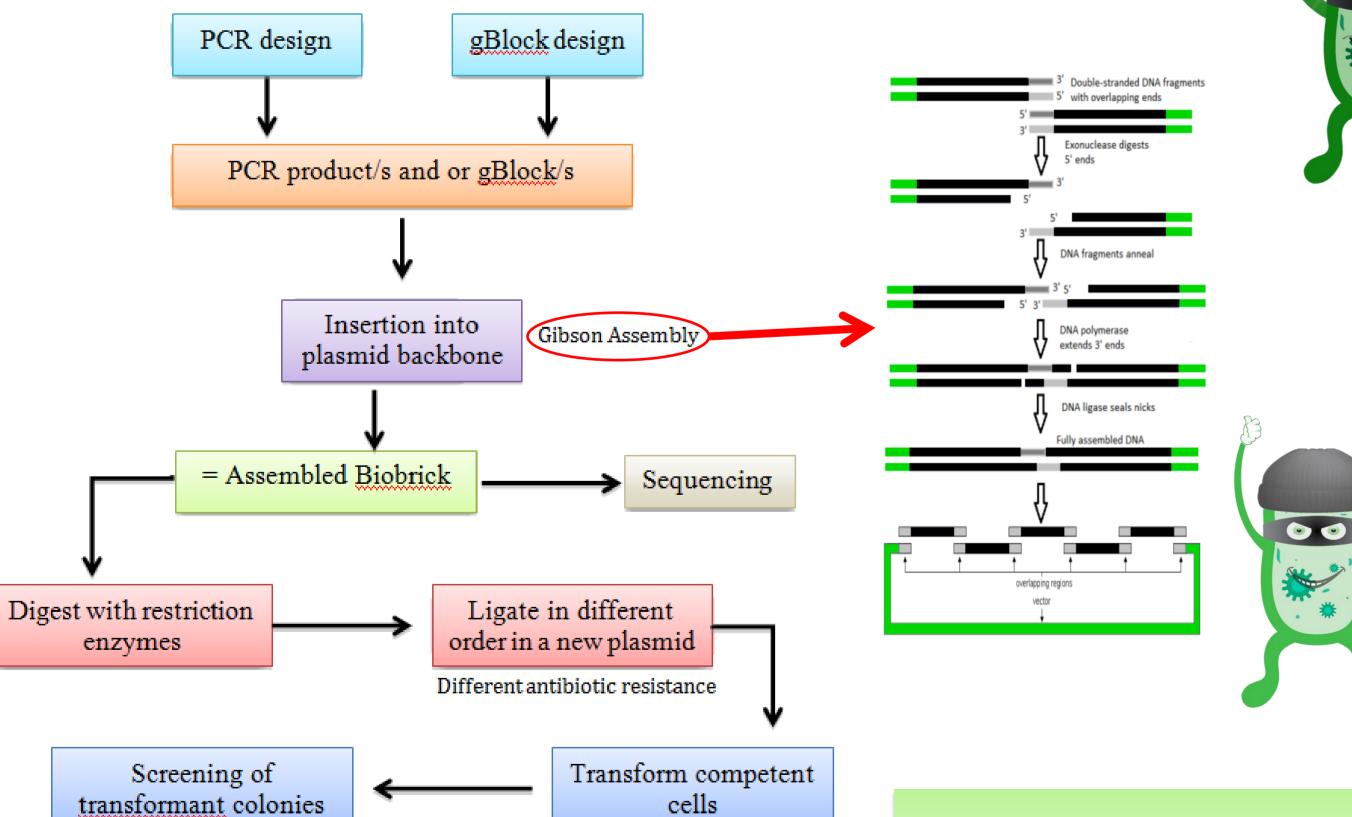
• C. reinhardtii is an algae that synthesises Chlorophyll  $\alpha$  from

A branch in the heme synthesis pathway will allow the use of

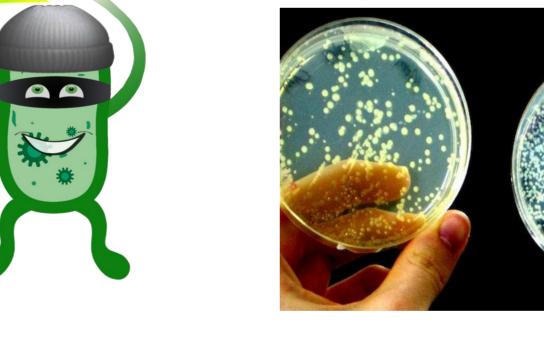
protoporphyrin IX through a multistep pathway

E. coli as an expression host to create chlorophyll

• E.coli uses protoporphyrin IX in the production of heme









- Plastocyanin produces a copper chelated protein
- When exposed to an inducer and copper E.coli expressing this gene will turn blue (right plate)

### Conclusion

- Successfully constructed 12 BioBricks
- Designed 3 operons necessary for chlorophyll biosynthesis
- Improved understanding on how to manipulate plant genes
- Initiated reproduction of photosystem II to act as a cheap and efficient renewable green energy source
- New sources of electrons and hydrogen gas to combat the energy crisis

### **Human Practices**



#### **Australasian Conference of Undergraduate Research**

 Winner- Best Presentation in Molecular Biology or Plant Science research

#### **Education**

- Presented 2<sup>nd</sup> year uni lecture on synthetic biology
- High school synthetic biology workshops

#### **Synthetic Biology Conference**

Organised first conference in Southern hemisphere

#### **Synthetic Biology Society**

Initiators of SynBioNet Society

#### Quarantine

 Recommendations for easy access to information on international standards for shipping regulations

#### **University Open Day**

 Organised a variety of laboratory activities for members

#### of the public

**Collaboration With Sydney Uni iGEM** Helped promote the Strange Nature writing

Mentor program

competition





