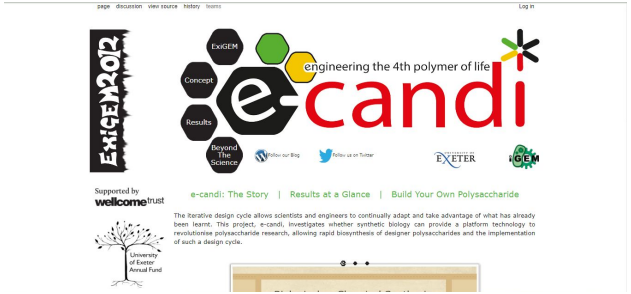
Exeter iGEM 2018

Juliana Sackey and Amy Hewitt

What is iGEM?

IGEM is a synthetic biology competition that gives students all around an opportunity to explore how synthetic biology can make technological advances and solve real world problems.



2012
e-candi
http://2012.igem.org/Team:Exeter

2013
Paint by *E.coli*
http://2013.igem.org/Team:Exeter

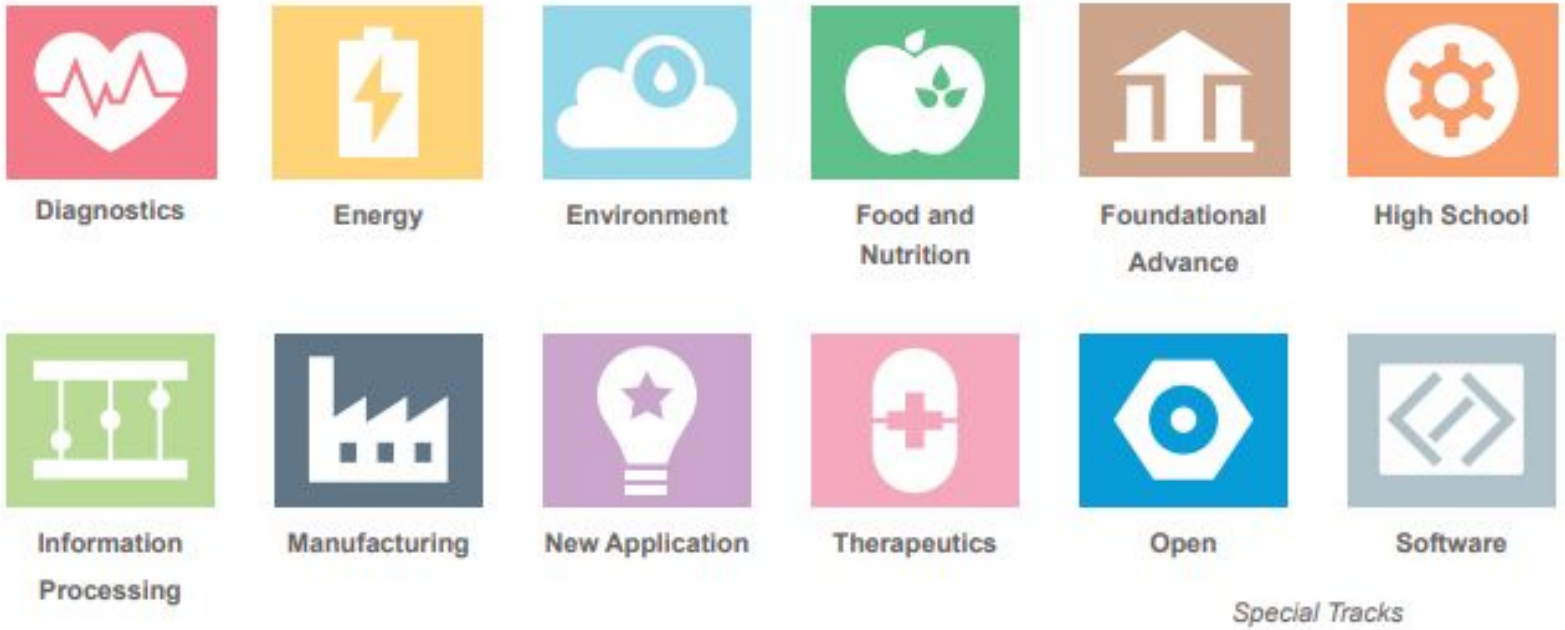
2014
E.R.A.S.E
http://2014.igem.org/Team:Exeter

2015
Ribonostics
http://2015.igem.org/Team:Exeter

2016
Project:EXEpire
http://2016.igem.org/Team:Exeter

2017
Pili⁺
http://2017.igem.org/Team:Exeter

iGEM has 12 tracks



Bronze	All Criteria must be met		
1	**Registration and Giant Jamboree Attendance**	Register for iGEM, have a great iGEM season, and attend the Giant Jamboree.	
2	**Competition Deliverables**	Convince the judges that you have completed the following Competition Deliverables from this page (**link**): #1 Wiki #2 Poster #3 Presentation #4 Judging Form	
3	**Attributions**	Convince the judges that you have completed Competition Deliverable #5 Attributions from this page (**link**). Please note: This requirement is not about citing literature references. Attributions is about describing what work your team did and what other people did for your project.	
4	**Characterization / Contribution**	Do one of these two options: (1) Successfully complete the InterLab Measurement Study (**link**). This means you have met all requirements of the InterLab Measurement Study, including acceptance of data. (2) Convince the judges that you have added new, high quality experimental characterization data to an existing BioBrick Part (Basic or Composite, must be **RFC10** compatible) from the Registry. Clearly document the experimental characterization on that **Part's Main Page on the Registry** (see the **iGEM Registry Contribution page** for instructions). The part that you are characterizing must NOT be from a 2018 part number range. Sample submission is not required.	Document on your team wiki at least one new substantial contribution to the iGEM community that showcases a project related to BioBricks. This contribution should be central to your project and equivalent in difficulty to making and submitting a BioBrick Part.

Silver	All Bronze criteria must be met, plus all Silver criteria below must be met		
1	**Validated Part / Validated Contribution**	Convince the judges that at least one new BioBrick Part (Basic or Composite, must be **RFC10** compatible) of your own design that is related to your project works as expected. Clearly document the experimental characterization on that **Part's Main Page on the Registry** (see **this page** for details). You must submit a sample of this new part to the Registry (following the **Registry submission requirements**). This part must be different from the new part documented for Gold #2. Teams must follow all of the DNA Submission Requirements and Shipping Guidelines (**link**) to qualify for medals. Failure to follow these guidelines will result in a rejected shipment or sample, which may prevent your team from winning medals and awards.	Convince the judges that something you created (art & design, hardware, software, etc.) performs its intended function. Provide thorough documentation of this validation on your team wiki.
2	**Collaboration**	Convince the judges you have significantly worked with one (or more) currently registered 2018 iGEM team(s) in a meaningful way. For example, mentor a team (or be mentored by a team), characterize a part, troubleshoot a project, host a meetup, model/simulate a system, or validate a software/hardware solution to a synbio problem. Document your collaboration in detail on your wiki. Judges will look at your collaborator's wiki to see what they say about your interaction. Simply filling out a survey for a team is not enough to demonstrate a significant interaction.	
3	**Human Practices**	Convince the judges you have thought carefully and creatively about whether your work is responsible and good for the world. Document on your team wiki how you have investigated these issues and engaged with your relevant communities, why you chose this approach, and what you have learned. Please note that surveys will not fulfill this criteria unless you follow scientifically valid methods. See the Human Practices Hub (**link**) for more information and examples of previous teams' exemplary work.	

Gold	All Bronze and Silver criteria must be met, plus at least two (2) Gold criteria below must be met		
1	**Integrated Human Practices**	Expand on your silver medal activity by demonstrating how you have integrated the investigated issues into the purpose, design, and/or execution of your project. Document on your team wiki how your project has changed based upon your human practices work. See the Human Practices Hub (**link**) for information and examples of previous teams' comprehensive and innovative activities.	
2	**Improve a Previous Part or Project**	Convince the judges that you have created a new BioBrick Part (must be **RFC10** compatible) that has a functional improvement upon an existing BioBrick Part (must be **RFC10** compatible). The sequences of the new and existing parts must be different. You must perform experiments with both parts to demonstrate this improvement. Clearly document the experimental characterization on the **Part's Main Page on the Registry** for both the existing and new parts (see **this page** for details). The Main Pages of each part's Registry entry must link to each other. The existing part must NOT be from your 2018 part number range and must be different from the part you used in Bronze #4. The new part must be different from the new part documented for Silver #1. Submit a sample of the new part to the iGEM Parts Registry (following the **Registry submission requirements**).	Improve the function of an existing iGEM project (that your current team did not originally create) and document your achievement on your team wiki.
3	**Model Your Project**	Convince the judges that your project's design and/or implementation is based on insight you have gained from modeling. This could be either a new model you develop or the implementation of a model from a previous team. You must thoroughly document your model's contribution to your project on your team's wiki, including assumptions, relevant data, model results, and a clear explanation of your model that anyone can understand. The model should impact your project design in a meaningful way. Modeling may include, but is not limited to, deterministic, exploratory, molecular dynamic, and stochastic models. Teams may also explore the physical modeling of a single component within a system or utilize mathematical modeling for predicting function of a more complex device.	
4	**Demonstration of Your Work**	Convince the judges that your engineered system works. Your engineered system has to work under realistic conditions. Your system must comply with all rules and regulations approved by the iGEM Safety Committee (**link**). Your system can derive from or make functional a previous iGEM project by your team or by another team. For multi-component projects, the judges may consider the function of individual components.	

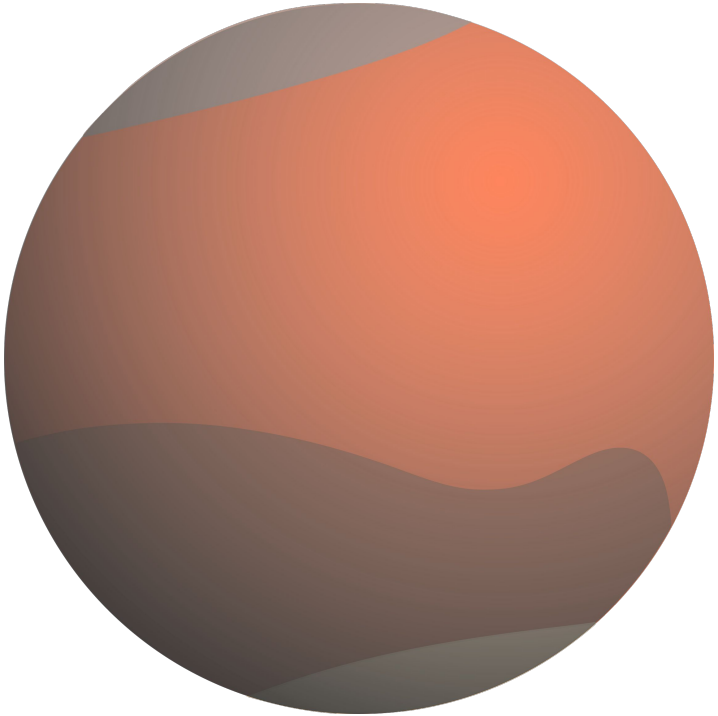

iGEM
Giant
Jamboree


iGEM

Project Perchlorate:

Turning a problem on Earth into a resource on Mars

Why Mars?



- Earth won't last forever
- Mars is similar to Earth
- Area of scientific interest
- Practice for later extrasolar missions

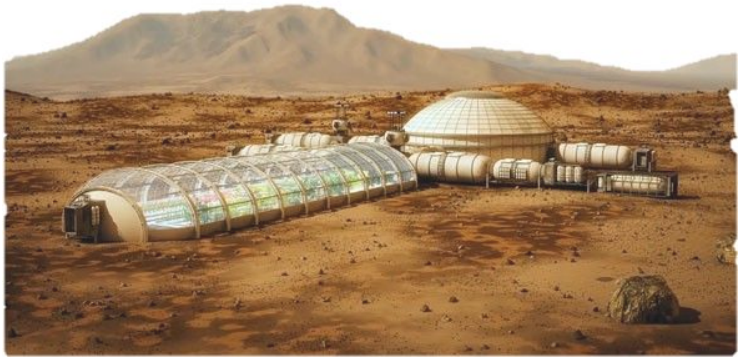
Our vision

NOT terraforming

Biodomes on Mars for colonisers to live in containing:

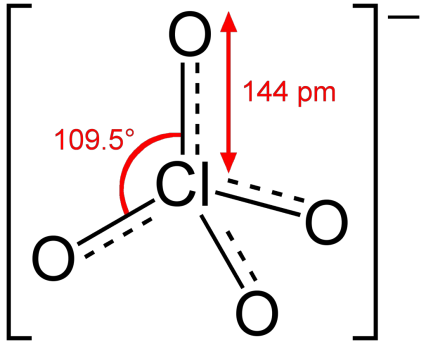
- Breathable air produced before arrival
- Fertile soil for growing plants
- Scientific experiments

http://gaygothamchorus.org/img/700073.jpg



What is the significance of perchlorate (ClO^-_4)?

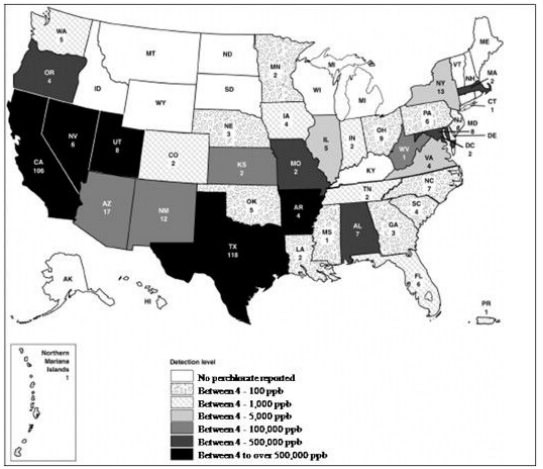
- Ammonium perchlorate (NH_4ClO_4) and sodium perchlorate ($NaClO_4$) have extensive uses in industry
  - Such as in rocket fuel
- Leads to hypothyroidism, especially in children
  - Toxic to humans through ingestion and skin contact



https://images.pexels.com/photos/73871/rocket-launch-rocket-take-off-nasa-73871.jpeg?cs=srgb&dl=rocket-rocket-launch-smoke-73871.jpg&fm=jpg

Perchlorate on Earth

- Perchlorate pollution occurs from multiple sources
- Can leach into soil and groundwater - found in water systems in the USA
- Due to this, DPRBs have evolved



https://www.sciencedirect.com/science/article/pii/S0944501310001114?via%3Dihub

Perchlorate on Mars

- Martian regolith is 1% perchlorate salts
- Processing perchlorate would:
  - Produce oxygen to fill a Martian biodome
  - Remove perchlorate from soil
- Chloride salts are a useful resource

http://spacehabs.com/

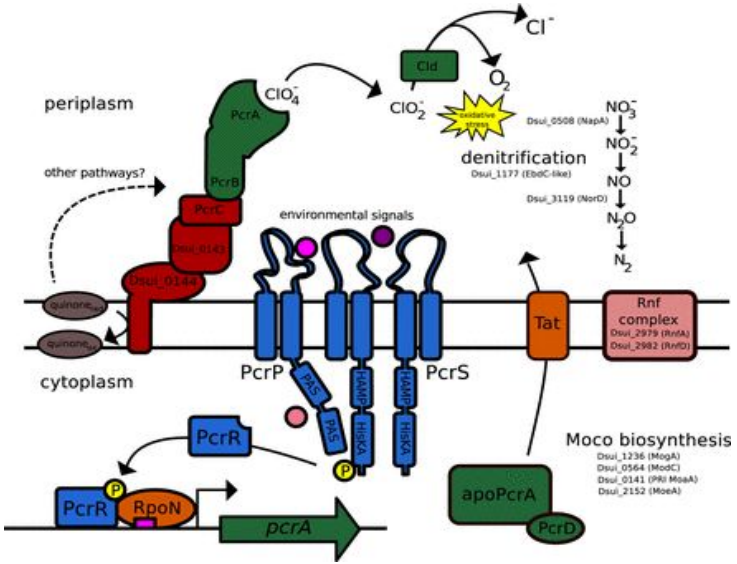
Exeter iGEM 2018: Planning Project Perchlorate

Our Synthetic Biology Solution

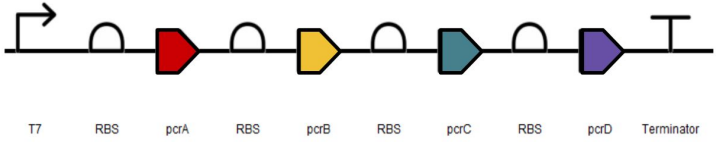
Producing oxygen on Mars...
... filtering water on Earth

Our Proposed Genetic Modification

- Insert the genetic pcrABCD-cld module from perchlorate reducing bacteria into *E. coli*
- Break down the perchlorate using two separate modules:
  - Perchlorate to chlorate (ClO_3^-)
  - Chlorate to chloride (Cl^-) and oxygen (O_2)
- One module will have perchlorate reductase fixed on the cell membrane
- The other module will secrete chlorate dismutase
  - We will tag signal peptides with GFP



http://mbio.asm.org/content/5/1/e00769-13/F5.expansion.html



pcrABCD and cld gene clusters in Synthetic Biology Open Language

The work so far

Sourcing

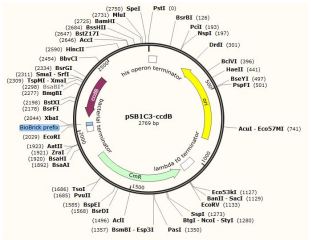
The enzymes necessary for the reaction had to be found, their native organism and their DNA sequence from databases and registries.

Sequencing

The DNA sequences were sent to IDT to be synthesised.

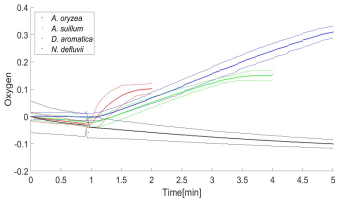
Construction

Sequenced linear DNA fragments were inserted into plasmids to code for the enzymes perchlorate reductase (pcrABCD) and chlorite dismutase (cld) into a cloning strain of E.coli - DH5α,



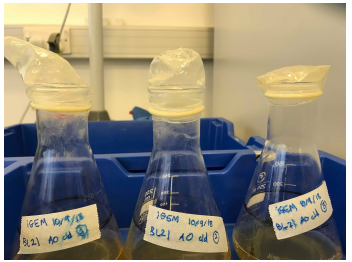
Growth curves

Measured the growth rates of the modified *E.coli* in different concentrations of perchlorate and chlorite



Expression

Expressed the enzyme chlorite dismutase in two reactions producing oxygen; cell free reactions with Clarke Oxygen electrode and whole cell reactions in conical flasks..



UNIVERSITY OF EXETER

COSHH RISK ASSESSMENT

Assessment Reference Number:	*Insert local numbering system if applicable*			
Date of Assessment :	13/7/2018			
Review Date: *Annually as standard or more frequently if (see examples below):* *Change to process or substance* / *Changes in personnel (vulnerability)* *Control measures are failing* / *Following an incident/accident/case of ill health* *Changes in toxicity information/revised MSDS* / *Changes in frequency/quantity used*				
Building / Laboratory / Work Area:	*Henry Welcome Building for Biocatalysis*			
COSHH Assessors Name:	*NAHNSU DAWKINS*			
Identify the persons carrying out the process / using this/these substance(s)	*NAHNSU DAWKINS, VARIOUS AMERICAN IGEM TEAMS*			
Who is likely to be exposed? (circle as appropriate)	Staff and/or Student(s)	Visitors	Maintenance	Other Groups *Give details*
How many people are likely to be exposed? (circle as appropriate)	0-5	6-9	>10	
Any vulnerable or high risks groups likely to be exposed? (circle as appropriate)	Young Person (staff or student under 18)	Pregnant Workers (staff or student)	Other Groups *Give details*	

Process details:

NB: If you are working with micro-organism(s) or biological agents please refer to the Microbiology Risk Assessment for information.
If working with Nano-materials please refer to the Working Safely with Nanomaterials in Research & Development guidance document
For work with chemicals continue completing this form.

1. Collect a beaker of tap water, running the tap until the water is of constant temperature and collecting a representative sample.
2. Make three 20 mL perchlorate solutions, one 10 µg/L, one 20 µg/L and one 50 µg/L, using deionized water as your solvent.
3. Dissolve 1.3 grams of zinc sulphate (equivalent to 8 mmol) and 1.6 grams potassium nitrate (equivalent to 16 mmol) with 20 mL of each of the perchlorate solutions as your positive controls.
4. Dissolve 1.3 grams of zinc sulphate and 1.6 grams potassium nitrate with 20 mL tap water as the test.
5. Dissolve 1.3 grams of zinc sulphate and 1.6 grams potassium nitrate with 20 mL of deionized water as the negative control.
6. Dissolve a solution of 0.005M methylene blue (recommended 0.8 g powder and 500mL water) and using deionized water as your solvent.
7. Place a 20 µL droplet of methylene blue and a 20 µL droplet of the test solution on a microscope slide. Repeat with the positives and negative on their own slides.
8. Mix the two droplets using a rod or pipette tip, then place a slide cover over the liquid. Repeat with the positives and negative.
9. Observe and record the images produced by these slides under the microscope. Try to record representative images and to capture different features that will occur at different locations on the slide. In some cases the slide will push out the liquid underneath it, forming crystals on the sides.

1

Health and Safety

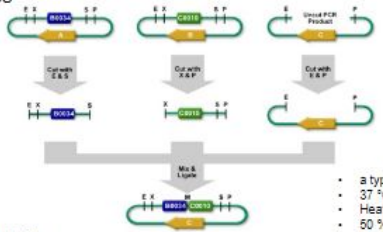
Perchlorate is toxic, necessary safety measures must be taken to use it in a lab, before we sent out our collaborations we had to assess the risks associated with working with perchlorate solutions;

- Requires respiratory wear and gloves to be handled
- Cannot come into contact with metal or any organic material

Biobricks and Gateway

Biobrick one pot reaction

- BioBrick enzymes



EcoRI
G^AATT C
C TTAA^G

- a type II restriction enzyme
- 37 °C
- Heat deactivated
- 50 % activity CutSmart
- May exhibit star activity in CutSmart buffer
- High Fidelity version available

PstI
C TGCA^G
G^ACGT C

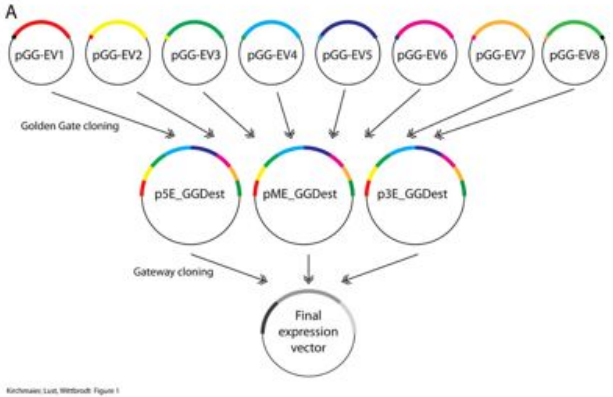
- a type II restriction enzyme
- 37 °C
- Heat deactivated
- 50 % activity CutSmart,
- May exhibit star activity in CutSmart buffer
- High Fidelity version available

XbaI
T^CTAG A
A GATC^T

- a type II restriction enzyme
- 37 °C
- Heat deactivated
- 100 % activity CutSmart
- **Cleaves to leave a 5'CTAG overhang which can be ligated to SpeI**
- Dam sensitive
  - GA^TC

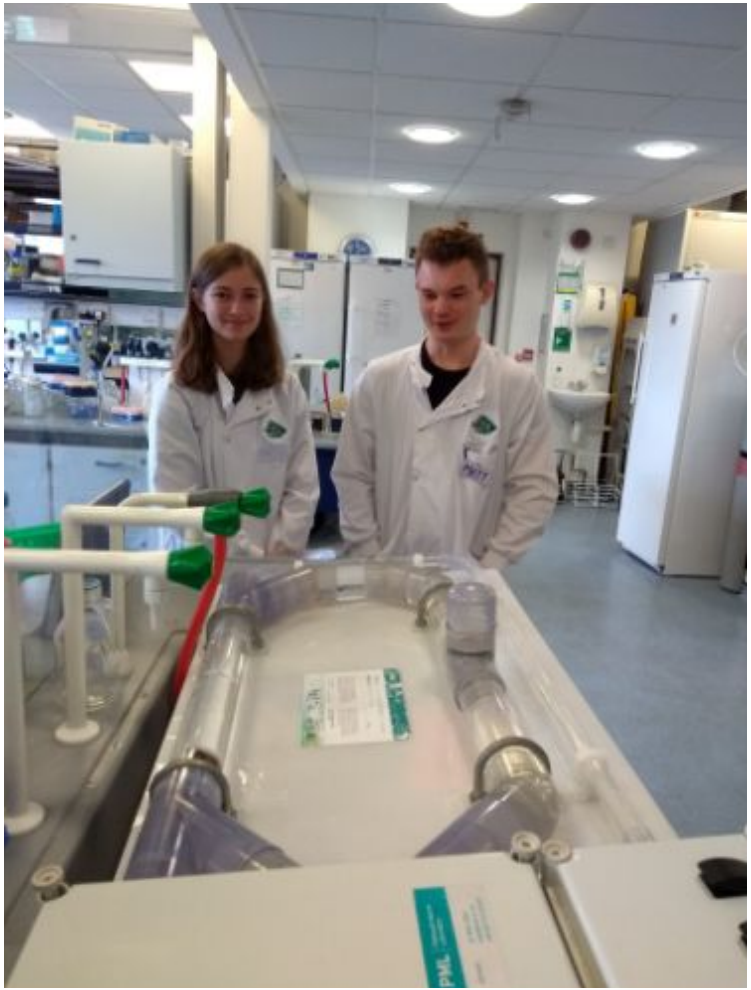
SpeI
A^CTAG T
T GATC^A

- a type II restriction enzyme
- 37 °C
- Heat deactivated
- 100 % activity CutSmart
- **Cleaves to leave a 5'CTAG overhang which can be ligated to XbaI**
- High Fidelity version available

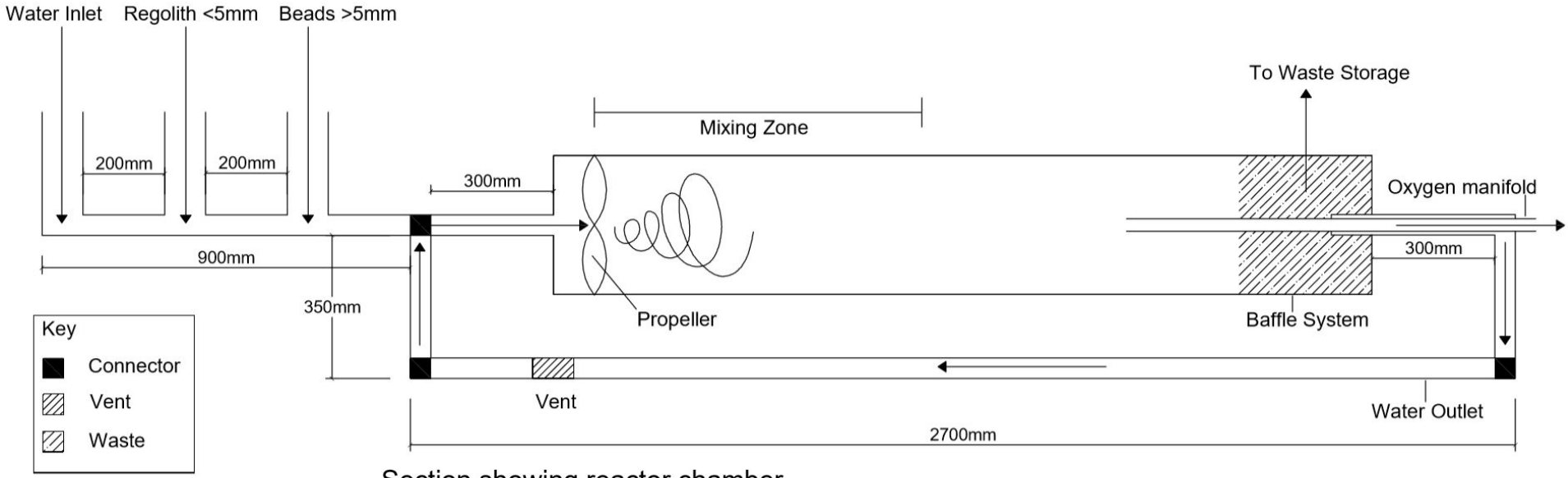


Our bioreactor

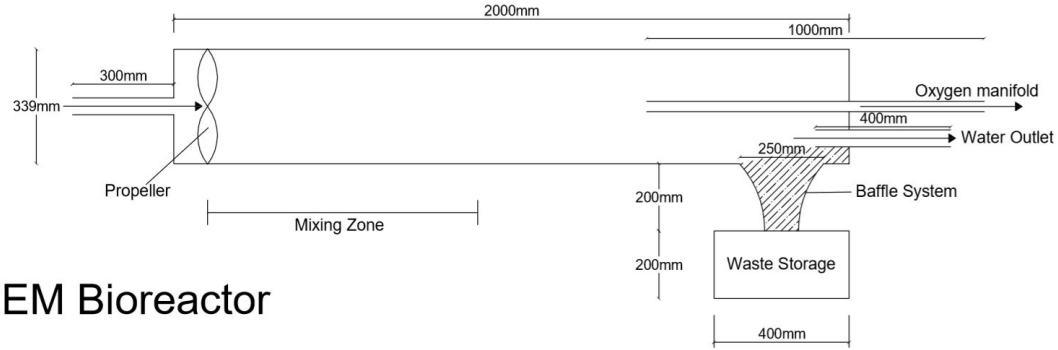
- Worked closely with stakeholders
- Dr Mike Allen, from PML
- 3D printable and light
- Low power consumption
- Durable
- Protected
- Kill switch



Plan View of Bio Reactor



Section showing reactor chamber



Exeter 2018 iGEM Bioreactor

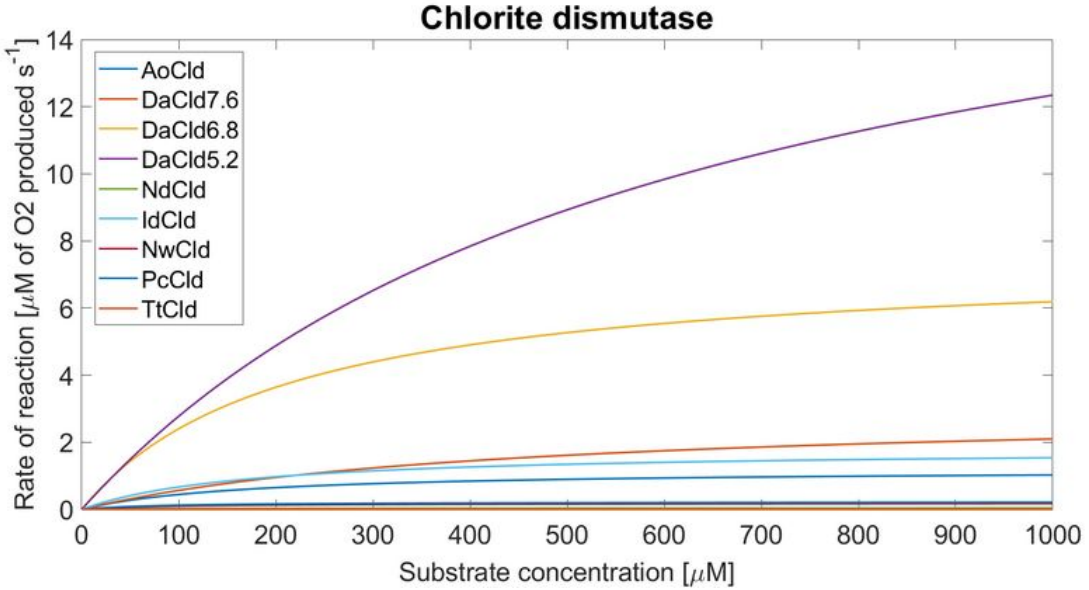
Scale 1:10 @ A3

Modelling our work

Perchlorate reductase - *Azospira oryzae*

Chlorite dismutase;

- *Azospira oryzae*
- *Azospira suillum*
- *Dechloromonas aromatica*
- *Nitrospira defluvii*



Organisation is key

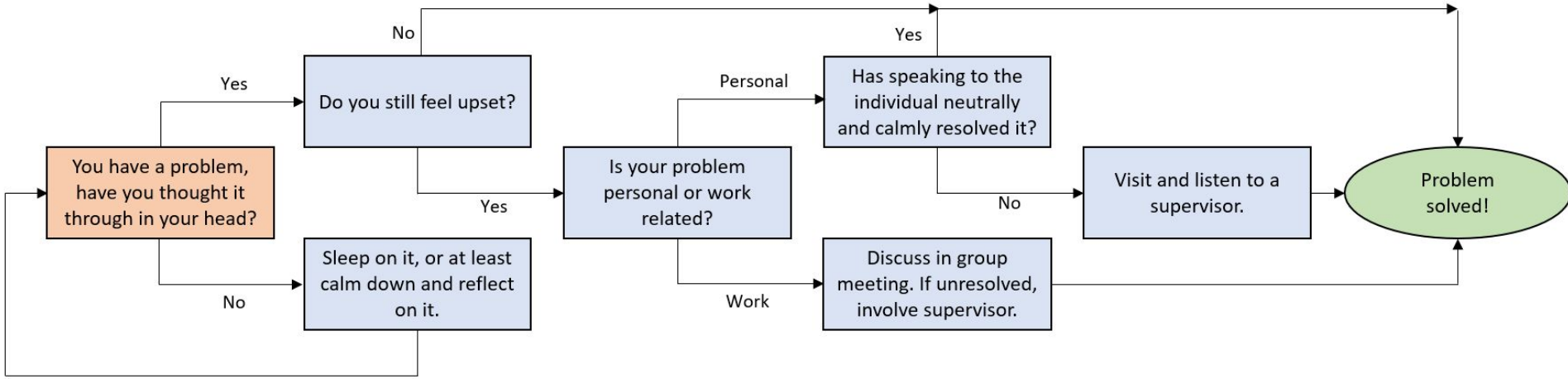
Meetings

Agendas

Goals

Sub teams

"Consult the flowchart"



Exeter iGEM 2018: Entrepreneurship

Unibiome

Bluepha
蓝晶微生物

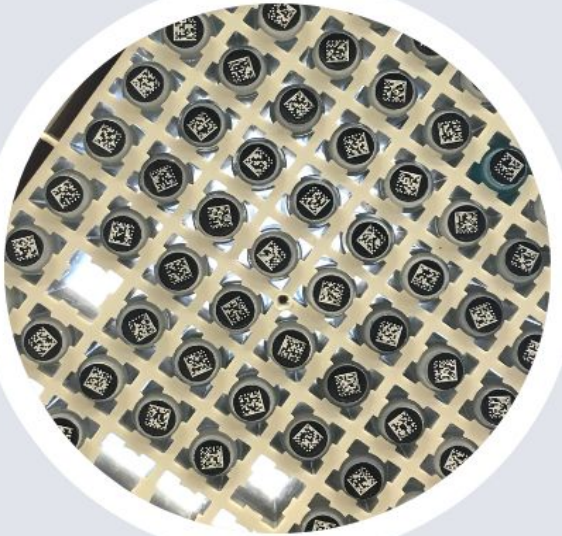
iGEM Projects have become businesses...

So it's important to think about the project long term

BentoLab:
A genetics lab the size of a Bento Box

CULTURED INGREDIENTS

Producing valuable ingredients via fermentation with engineered yeasts for perfumes, foods, cosmetics, and more.



STRAIN IMPROVEMENT

Where fermentation is already used in bioindustrial applications, organism engineering can improve efficiency and sustainability.

ENZYMES

Enzymes are used in applications from cheesemaking to pharmaceuticals to stonewashed jeans. We're discovering better enzymes for more applications.

Things to consider

Scalability

Marketing strategies

Patents and IP

Type of business

Investment opportunities

Exit strategies

Exeter iGEM 2018: Bioethics

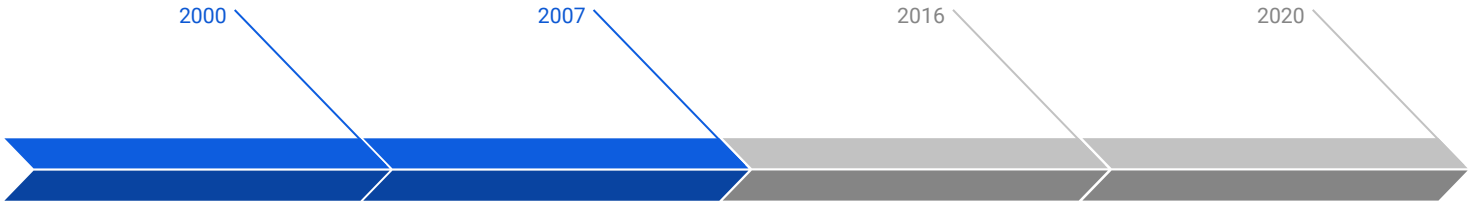
Human Practices

“How your work affects the world and the world affects your work”

science⇄society

Frameworks for Human Practices

Identifying your stream of stakeholders



Market research

What do consumers/end users actually need/want?

What is already available?

What are the current concerns?

Design

How are you meeting the needs and wants of your consumer/end user?

How well does your product/service meet the criteria?

Test

Does the idea work in a real world scenario?

How does the work affect your suppliers, manufacturers etc?

Build

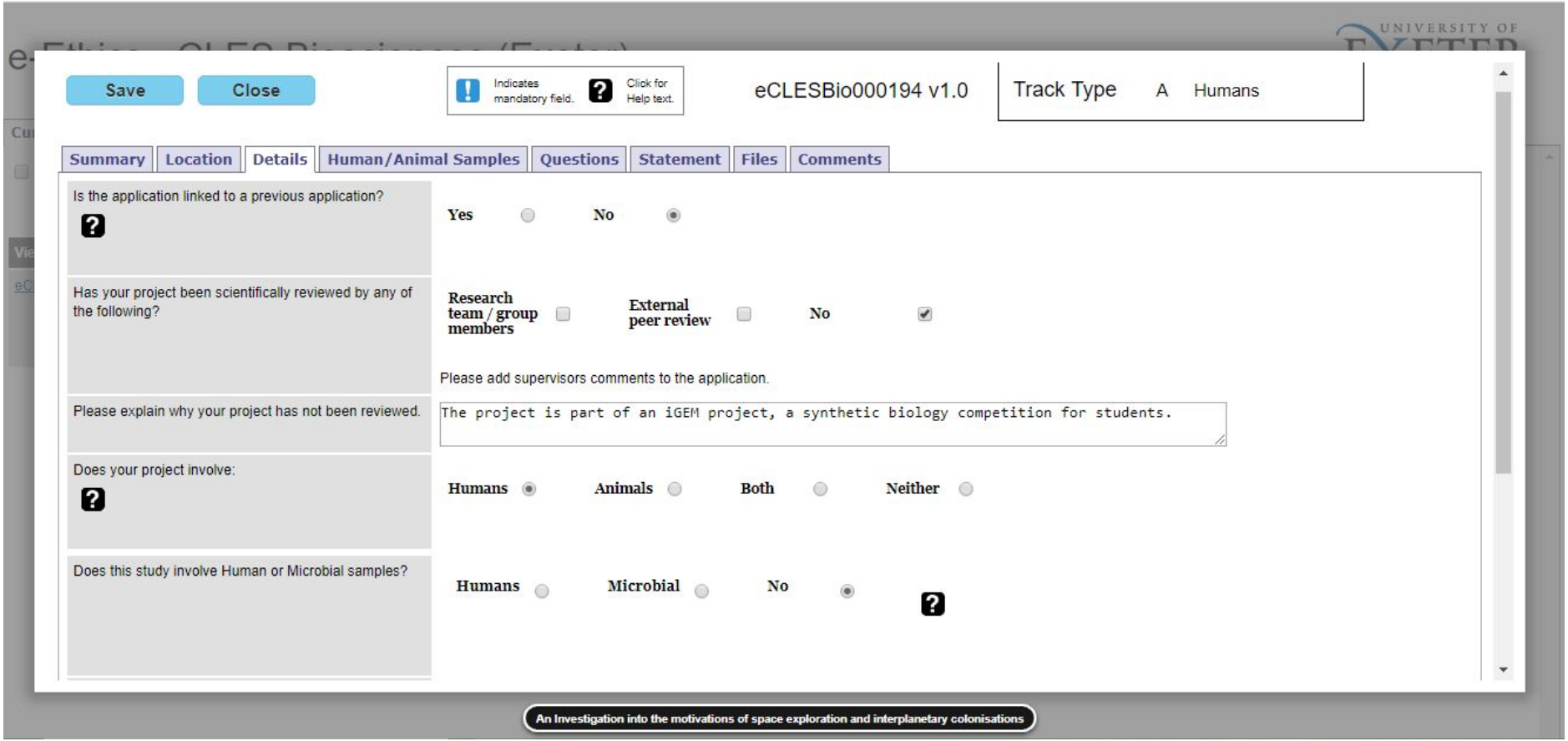
Have you addressed as many concerns as possible?

What are you doing about the unanswered questions?

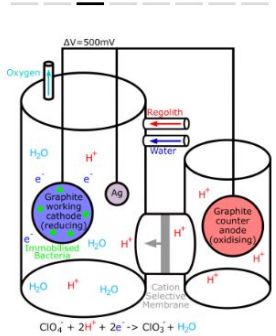
A survey is basic

- You can't phrase a question to get the answer you want
- You have to consider every opinion
- You can't assume responses
- You can't falsify data
- ...but a good survey, following proper scientific method can be very insightful

Getting ethics approval



Some of our work in Human Practices



3: Our Initial Designs

After we had decided that our bioreactor would be designed for Martian use, we contacted Nick Musgrove, commercial director of Infors HT to help us understand the basics of bioreactor design. Using the information gathered from this talk we drafted the features that we envisaged our bioreactor having to optimise it for perchlorate reduction.

In order to develop our idea we needed to ascertain how regolith would be entered into our bioreactor. Ben Reeve, CTO of CustoMem,

Exeter iGEM 2018:
The iGEM Experience

Exeter iGEM 2018: Internships

Making the most of internships

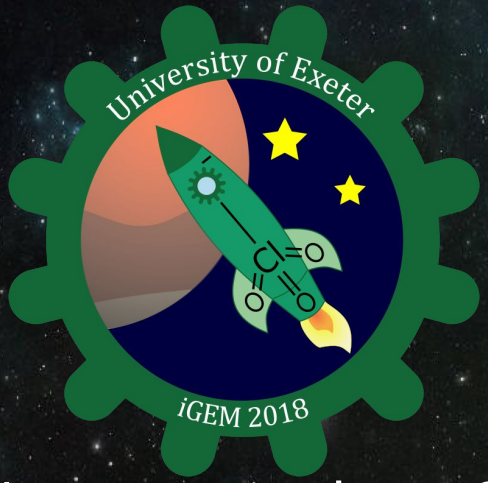
- Speak to your advisors, learn as much and gain as many skills as you can
- Ask questions
- Be present
- Be bold
- Be rememberable
- Breathe
- Learn from every mistake
- Skills to consider

Acknowledgements

Exeter Microbial Biofuels Group

SnapGene®
Software for molecular biology

Thank you for listening



Any questions?

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