UNSW IGEM 2020

Synthetic Biology & Our Project

Lecture Outline

- ➢ Introduction: Synthetic biology and the iGEM competition
- ➢ The problem: Coral bleaching and those impacted
- ➢ Our solution: Laboratory workflow and techniques utilised
- ➢ Our results: Our results so far
- ➢ Dry lab: Computational and mathematical analysis utilised
- ➢ Conclusion: Summary and future directions

UNSW iGEM 2020 Team

SYNTHETIC BIOLOGY & OUR PROJECT

Introduction

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Introduction

What is synthetic biology?

- Synthetic biology is a multidisciplinary approach to solving problems through the creation of new biological parts, processes and systems.
- It incorporates a diverse range of fields including:
  - Molecular biology
  - Genetic engineering
  - Computer science
  - Bioinformatics

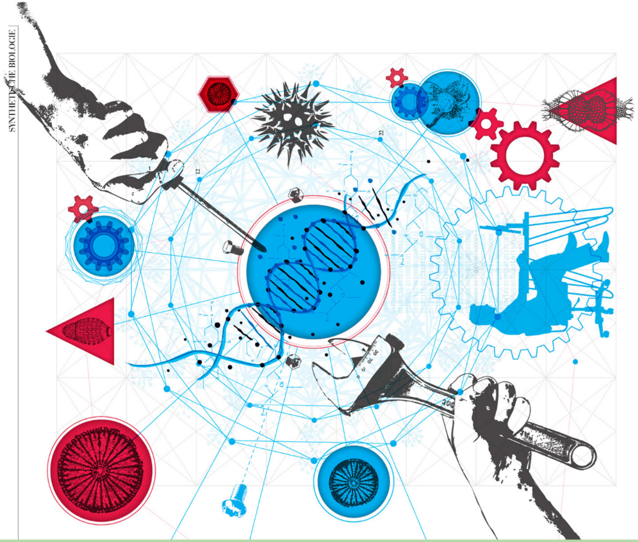

Fig. 1: Synthetic biology[1]

Syllabus points:

What is synthetic biology? (cont.)

Introduction

- Synthetic biology utilises the following engineering principles in a biological setting:
    1. Design
    2. Build
    3. Test

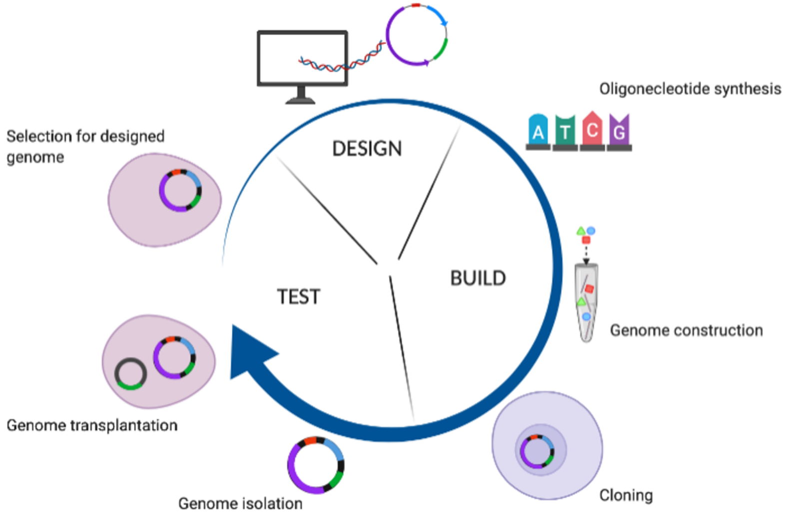

Fig. 2: Flow diagram showing the design, build and test workflow

Syllabus points:

Introduction

What is synthetic biology? (cont.)

- Synthetic biology has many applications such as:
  - Commercial
  - Medical
  - Environmental
- The iGEM competition showcases the diverse applications of synthetic biology.

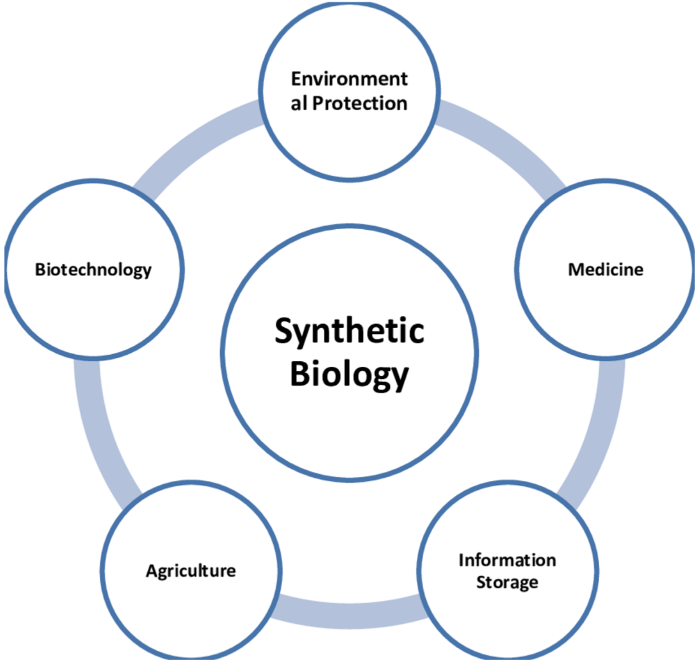

Fig. 3: Synthetic biology application fields[2]

Syllabus points:

Introduction

What is iGEM?

- The iGEM (International Genetic Engineered Machine) is an annual international synthetic biology competition where individual teams design a project to create new biological systems or parts to solve a problem.
- The UNSW 2020 iGEM team is excited to be working on a new project.

Syllabus points:

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The Problem

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The Problem

Coral

- Coral forms the backbone of coral reefs and supports entire ecosystems.
- The Great Barrier Reef (GBR) is one of Australia's most iconic landmarks, and the world's largest coral reef.

Fig. 4: The Great Barrier Reef (GBR)[3]

Syllabus points:

The Problem

Coral structure

- Coral is composed of many organisms living together known as coral polyps[4].
- Coral polyps secrete the white calcium carbonate skeleton which forms the backbone of coral reefs

Fig. 5: Photograph of Coral Polyps[5]

Syllabus points:

The Problem

Coral structure (cont.)

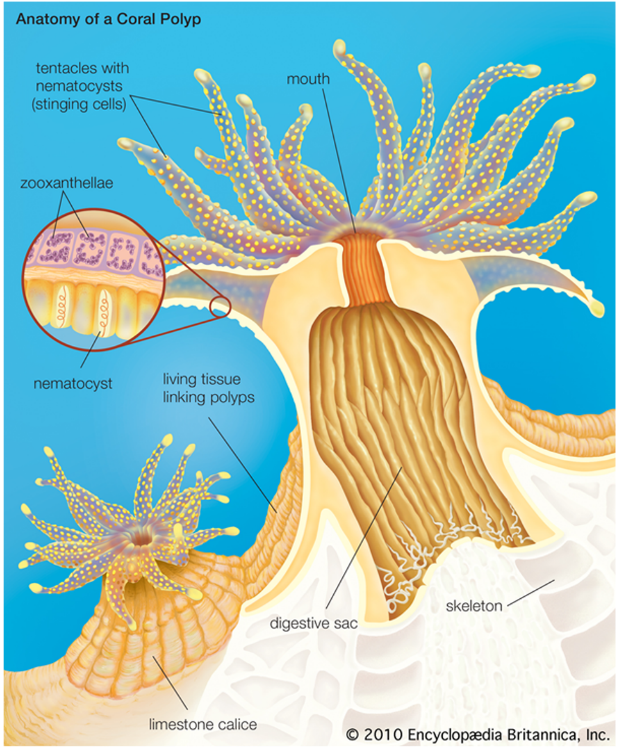

Fig. 6: Diagram of Coral Polyp Anatomy[7]

- Within the coral polyps tissues there are many algae, most belonging to the genus *Symbiodinium* (a type of zooxanthellae) in a symbiotic relationship
  - The algae provide the coral with nutrients through photosynthesis.
  - The algae contained within tissues also give coral its rich and beautiful colours[6].

Syllabus points:

Investigate and determine relationships between biotic and abiotic factors in an ecosystem, including: the impact of biotic factors, including predation, competition and symbiotic relationships, the ecological niches occupied by species

The Problem

Coral bleaching

- Coral bleaching is caused by cellular stress arising from environmental variables such as:
  - Rising sea temperatures
  - Change in pH
- Algae produce toxic reactive oxygen species (ROS) as a result of the stress.
- The coral ejects the algae to prevent the ROS spreading causing a drastic loss of colour, hence the term bleaching.

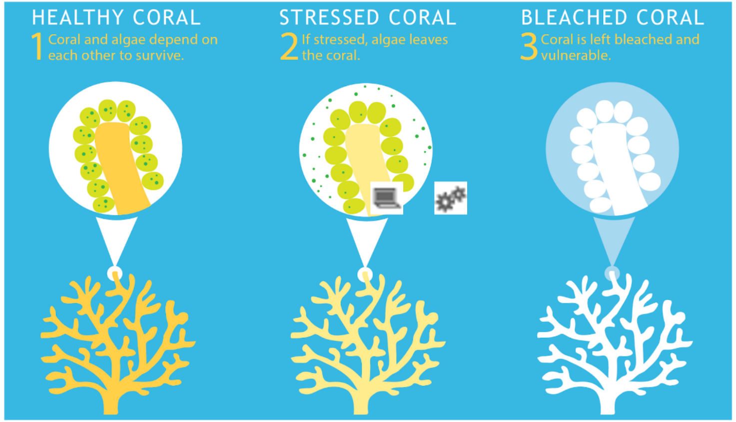

Fig. 7: Diagram showing coral bleaching[8]

Syllabus points:

The Problem

Impacts of coral bleaching

- Coral bleaching has far reaching effects on a range of groups, industries and greater environment.
- Stakeholders impacted by coral bleaching include:
    - Biodiversity
    - Bioprospecting
    - Coastal protection
    - Traditional owners
    - Tourism industry
    - Commercial & recreational fishing

Fig. 9: The Great Barrier Reef[11]

Syllabus points:
Interpret a range of secondary sources to assess the influence of social, economic and cultural contexts on a range of biotechnologies

Coral bleaching (cont.)

- Coral bleaching has devastating effects.
- It is caused by rising water temperatures.
- Coral bleaching is a global problem affecting reefs across the world.
- An effective solution is necessary[8, 9].

Fig. 8: Photograph depicting the effects of coral bleaching between December 2014 and February 2015[10]

Syllabus points:

The Problem

Biodiversity

- The Great Barrier Reef is home to the world's largest coral reef ecosystem.
- There are also an estimated 4,000 species of marine life, and an estimated 1-8 million undiscovered species.
- Of these species, many consider coral their natural habitat, including
  - The world's most important dugong population
  - Six out of seven of the world's marine turtle species


Fig. 10: Reef turtle[12]

Syllabus points:
Evaluate the effect on biodiversity of using biotechnology OR
Inquiry question: How do genetic techniques affect Earth's biodiversity?

The Problem

Biodiversity (cont.)

- The Great Barrier Reef is managed by the Great Barrier Reef Marine Park Authority (GBRMPA).
- THE GBRMPA aims to protect and preserve the Reef, and communities dependent on it, through available science.
- Biodiversity is a central aspect of what makes the Great Barrier Reef such a pillar of Australia's national identity[14].

Australian Government
Great Barrier Reef Marine Park Authority

Syllabus points:
Evaluate the effect on biodiversity of using biotechnology

The Problem

Bioprospecting

- Bioprospecting is the search for valuable compounds in biological sources, such as plant and animals.
- These compounds are utilised in:
  - Medicinal drugs
  - Restoration and bioremediation
  - Cosmetics
  - Nanotechnology

Fig. 11: The Great Barrier Reef surveying activity[13]

Syllabus points:
Evaluate the benefits of using genetic technologies in agricultural, medical and industrial applications

Coastal Protection

The Problem

- Coral reefs provide an important buffer against ocean waves and storms, helping reduce coastal erosion.
- Coral reefs help protect[15,16]:
  - Coastal residences
  - Human infrastructure, such as roads, shops and entertainment areas.
  - People, lifestyles and livelihoods

Fig. 12: Snorkelling as a form of lifestyle activity[17]

Syllabus points:
Interpret a range of secondary sources to assess the influence of social, economic and cultural contexts on a range of biotechnologies

The Problem

Traditional Owners

- Indigenous Australians are the traditional owners of the Great Barrier Reef and surrounding lands.
- The GBR and surrounding lands play a deep seated role in the culture and history of the various traditional owner groups[18, 19].
- Traditional owners play an important role in the management of land and its long-term survivability[18, 20].

Fig. 13: Aboriginal art of the Great Barrier Reef[21]

Syllabus points:
Cross-curriculum priorities-Aboriginal and Torres Strait Islander Histories and Cultures

Tourism

The Problem

- Data collected by Tourism Australia shows that 42% of international visitors rank the Great Barrier Reef as the most appealing tourist attraction in Australia.
- The Great Barrier Reef contributes $6.4 billion to Australia's national economy.
- It also provides 64,000 jobs to Australians.
- Tourism is an effective way to educate and communicate issues relating to reef health and marine ecosystems[22].

Fig. 14: The Great Barrier Reef tourism activity[23]

Syllabus points:

The Problem

Commercial & Recreational Fishing

- The Great Barrier Reef Marine Park provides valuable opportunities for:
  - Commercial fishing
  - Recreational fishing
  - Cultural fishing and activities
- Commercial fishing generates approximately $104 million annually for Australia's seafood industry.
- A healthy marine ecosystem is vital for commercial fishing industries, recreation, and a source of local seafood[24].

Fig. 15: Recreational fishing in the Great Barrier Reef[25]

Syllabus points:

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Our Solution

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Our Solution

So how can we prevent coral bleaching?

- Our project, PROTECC coral (Prevent Reactive Oxygen Thermal Extreme Caused Carking) ultimately aims to increase the thermotolerance of *Symbiodinium* sp. in corals
- Through our research we were able to discover two essential elements with the potential to increase thermotolerance of coral:
  - Small Heat Shock Proteins (sHSP)
  - Glutathione production system
- The genes for these elements will be incorporated into *Symbiodinium* sp.

Inquiry question: How is an organism's internal environment maintained in response to a changing external environment?

Our Solution

Glutathione System

- ROS produced by heat stressed algae is toxic at high concentrations
  - ROS causes oxidative damage to DNA and other cellular components
- Glutathione is an antioxidant molecule
  - It is capable of neutralising ROS and preventing oxidative damage
- This results in reduced cellular stress and expulsion of the algae[26, 27].
- Glutathione synthetase/reductase enzymes are used to produce and recycle glutathione

Syllabus points:

Our Solution

Small Heat Shock Proteins (sHSPs)

- sHSP’s are a group of proteins that respond to heat shock by acting on cellular proteins to prevent or correct denaturing.
- This in turn serves to reduce the stress response.
- Certain algae are more thermotolerant, this includes *C. reinhardtii*
    - Proteins such as HSP22E and HSP22F contribute to this increased thermotolerance
- HSP’s have the potential to be integrated into coral algae species where they may increase the thermal tolerance[28].

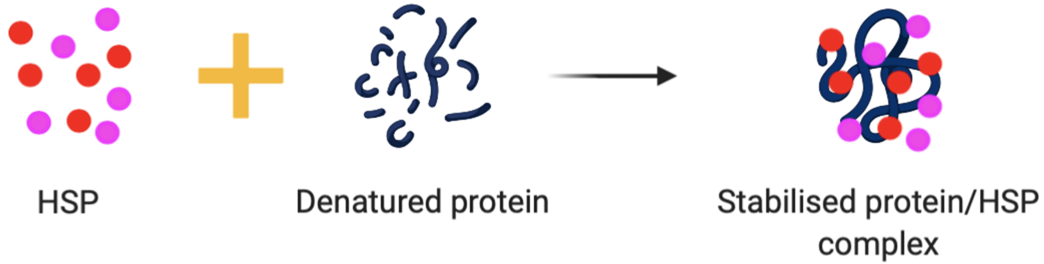

Fig. 16: HSP stabilising protein diagram

Inquiry question: How is an organism’s internal environment maintained in response to a changing external environment?

Our Solution

Experimental Workflow

- Experimental workflow initial production/characterisation of sHSP

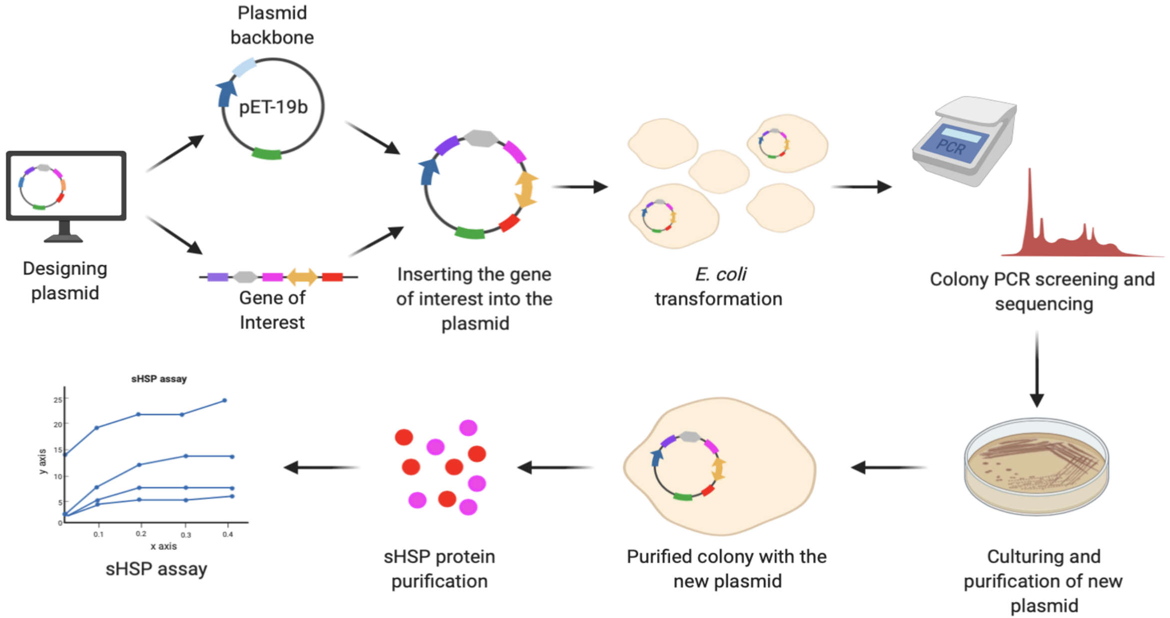

Fig. 17: Laboratory workflow diagram

Syllabus points:

Our Solution

Designed Plasmid

- To have a functional plasmid and successfully produce the appropriate proteins, great care had to be put into the plasmid design. The figure below depicts one of our designed plasmids.

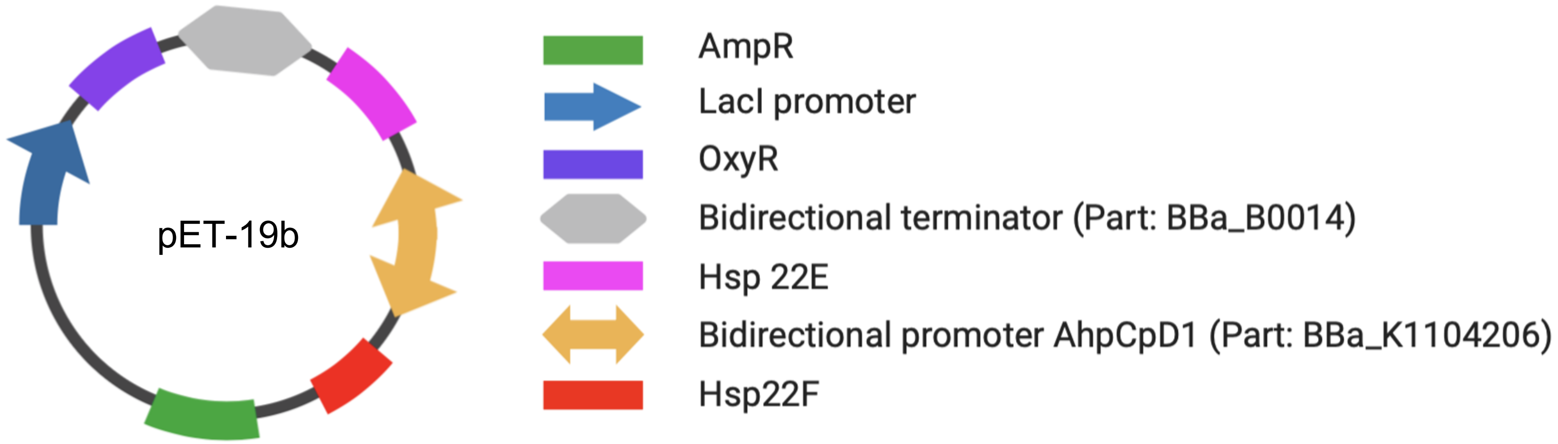

Fig. 18: Diagram of designed plasmid

Syllabus points:
Describe techniques and applications used in recombinant DNA technology

Our Solution

Designed Plasmid (cont.)

- The following table describes the role and origin of the various components of the designed plasmids

Table 1: Designed main plasmid component and its functions

Component	Function
AmpR	Ampicillin resistance (antibiotic) - allows for selection
LacI promoter	LacI promoter to allow downstream genes to be expressed

Syllabus points:
Describe techniques and applications used in recombinant DNA technology

Our Solution

Designed Plasmid (cont.)

Table 2: Continued table of the main plasmid components and its functions

Component	Function
HSP22E/F	Genes encoding for sHSP that prevent protein denaturation
OxyR	Transcription factor that is activated in presence of high H_2O_2 concentrations
Bidirectional terminator	Terminates the transcription of OxyR and HSP22E
Bidirectional promoter (AhpCpD1)	Initiates transcription of genes when activated by OxyR

Syllabus points:

Describe techniques and applications used in recombinant DNA technology

Designed Plasmid Function

Our Solution

- So how does our plasmid function once assembled together?
    - ➢ In high temperatures, ROS is released, this activates the OxyR transcription factor.
    - ➢ OxyR activates the bidirectional promoter (AhpCpD1)
    - ➢ The promoter allows assembly of transcription complex to transcribe both the sHSP
    - ➢ sHSPs limit protein denaturing and provide increased thermal tolerance

Syllabus points:

Our Solution

Recombinant plasmid construction

- Gibson assembly was used to insert our target gene (HSP22E/HSP22F) into plasmid backbone
  - Restriction enzymes chew back DNA at cutting sites
  - Sticky ends of target gene anneal with plasmid
  - Gaps are fixed with ligase to form complete recombinant plasmid

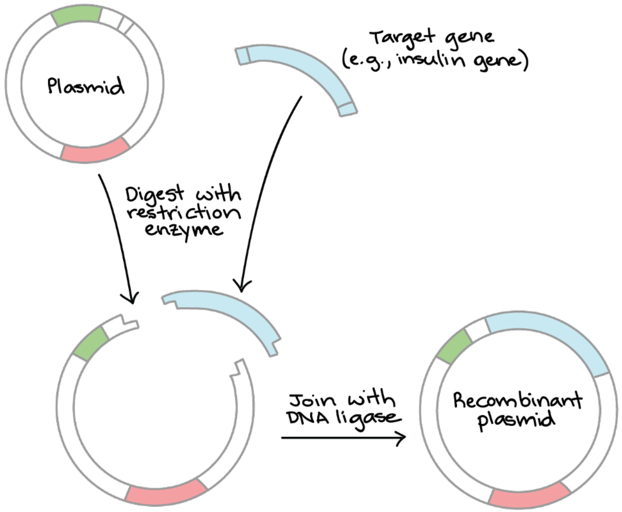

Fig. 19: Construction of recombinant plasmid[29].

Syllabus points:

Describe techniques and applications used in recombinant DNA technology

Our Solution

E.coli transformation

- This stage of the experimental procedure involved transforming the recombinant plasmid into *E.coli* cells via heat shock transformation
- Cells were incubated on agar plates containing ampicillin overnight at 37°C

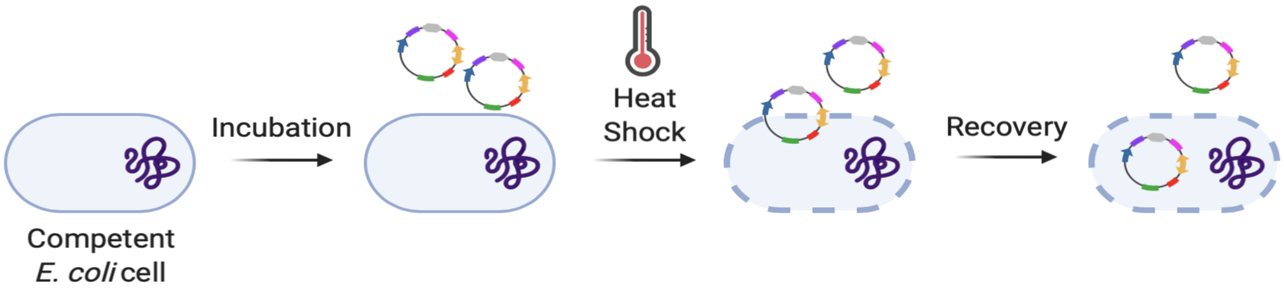

Fig. 20: Diagram depicting the heat shock transformation process

Syllabus points:
Investigate and assess the effectiveness of cloning, including but not limited to:
- gene cloning

Our Solution

Culturing and purification of new plasmids

- Recombinant plasmids from the *E.coli* need to be selected and extracted.
- Bacteria are grown on ampicillin. Only bacteria that have taken up the plasmid with the HSP22E/F genes will grow as they will also have the ampicillin resistance gene (AmpR)

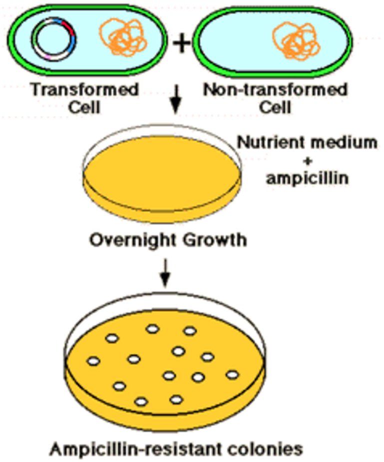

Fig. 21: Ampicillin resistance selection [30]

Syllabus points:

Additional information

Our Solution

- If you are curious about learning more regarding the experimental procedures utilised. You can receive more detailed information in the UNSW iGEM 2020 educational package lab handout. This will include details on our own laboratory work and explanations for some techniques you may be unfamiliar with.
- While our iGEM team is currently making its way through this lab workflow you have just learnt about, let’s have a look at some of the results we have obtained so far.

Syllabus points:

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Our Results

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Lab experiment result

Our Results

- We transformed our plasmid into the *E. coli* following workflow shown earlier
- To check if the transformation worked, we conducted the following experiment
- We picked bacterial colonies and conducted PCR to amplify HSP22E/F gene
- The presence of the HSP22E/F gene was confirmed using gel electrophoresis
  - This is a technique that separates DNA based on size
  - Smaller pieces of DNA move further down the gel

Syllabus points:

Our Results

Lab experiment result (cont.)

- First ten lanes show DNA taken from *E. coli*
- Last lane is a 'ladder'
  - We can compare our DNA to this to find out what size it is
- *E. coli* transformation only worked for colonies in red boxes
  - These show DNA being around 800bp - the expected size of the HSP22E/F gene

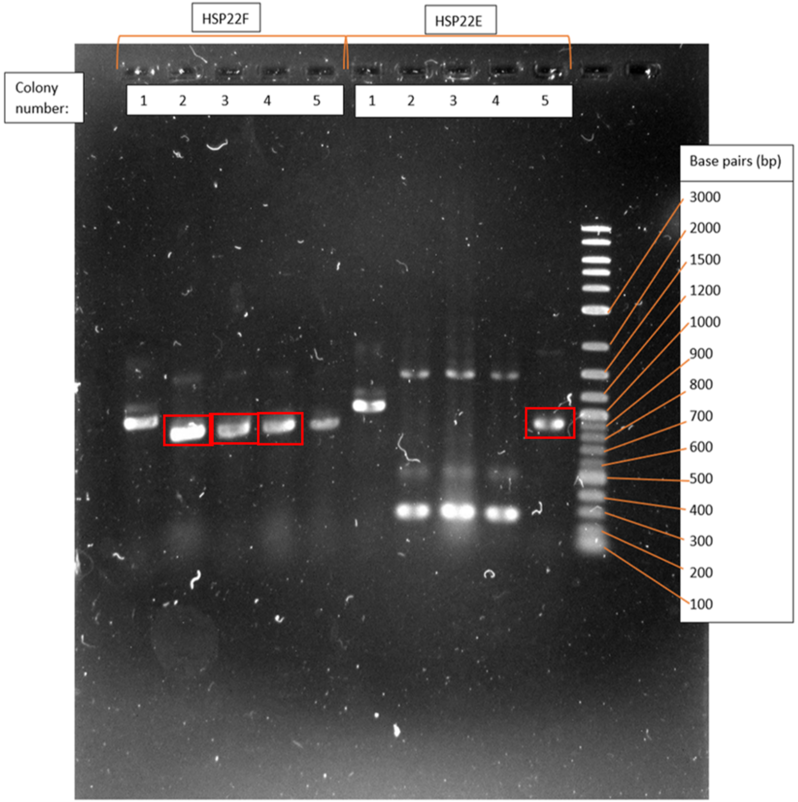

Fig. 23: Photograph of the gel electrophoresis

Syllabus points:

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Dry Lab

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Dry Lab

Dry lab

- UNSW iGEM 2020 team also conducts dry lab work with computational and mathematical analysis
- This aims to verify the effectiveness of the proposed solution
- We adopted a mathematical model to simulate the cellular environment of *Symbiodinium* sp.
    - Utilised the Python libraries to run simulation and mathematical modelling.
- Given the solution involves introducing a new protein, we wanted to evaluate how it would impact the cells survivability

Syllabus points:

Dry Lab

Dry lab

- The diagram provides a visual representation of how the modelling can depict the cellular processes and survivability of our projects cells.
- This allows for us to assess the effectiveness of our project

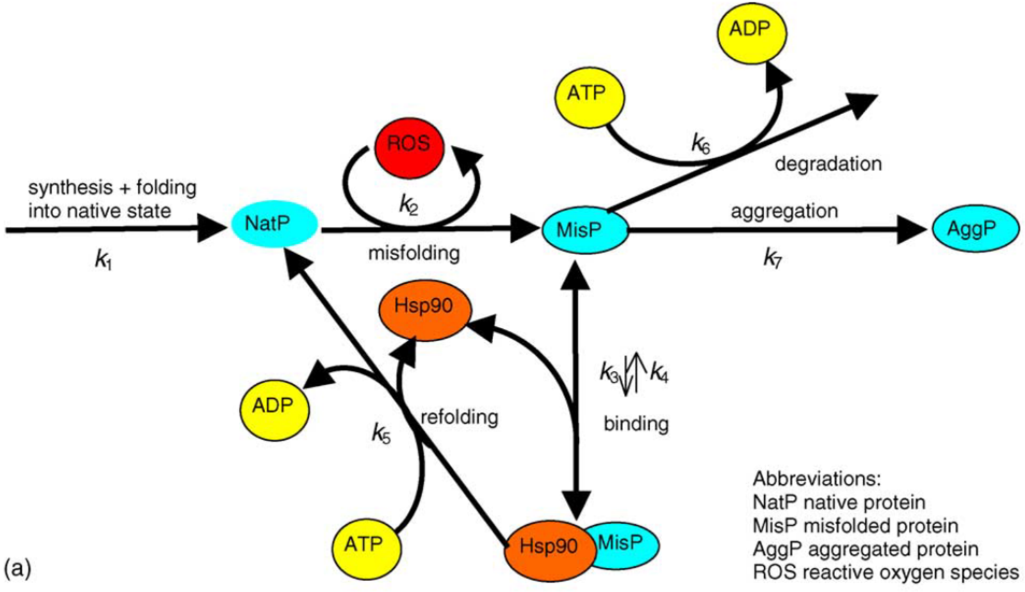

Fig. 24: Diagram of cell survivability model[32]

Syllabus points:

SYNTHETIC BIOLOGY & OUR PROJECT

Conclusion

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Conclusion

Future directions

- Our lab work was cut short due to quarantine restrictions. Due to this the work carried out in the lab was limited.
- Future research could replicate our work done in *E.coli* in a model species and then potentially in a *Symbiodinium* sp.
- Future research could also work at improving the efficiency of our designed plasmid to further improve thermal resistance.

Syllabus points:

Investigate the uses and applications of biotechnology (past, present and future), including: researching future directions of the use of biotechnology

Conclusion

Future directions

- It should also be noted that due to the sensitive nature of many coral reef ecosystems, including the GBR, release of GMOs are prohibited.
    - Due to this it is unlikely any modified Symbiodinium species would be released unless circumstances for the reef were deemed incredibly severe.
- While it may not be directly utilised in protecting the GBR, it may play a role by being a proof of concept.
    - This means it may provide proof and inspiration that coral can develop increased thermotolerance, which can lead to increased funding and continuation of projects such as the joint directed evolution project between CSIRO, AIMS and Melbourne University[33].

Syllabus points:

Investigate the uses and applications of biotechnology (past, present and future), including: researching future directions of the use of biotechnology, analysing the social implications and ethical uses of biotechnology, evaluating the potential benefits for society of research using genetic technologies

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Acknowledgments & References

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- Professor Heath Ecroyd

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