

Free Coli: Improving accessibility to synthetic biology by engineering a naturally transformable *Escherichia coli*

BIOLOGY EDUCATIONAL RESOURCE FOR SECONDARY STUDENTS

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1. Introduction

Synthetic biology and genetic engineering will be key players in the fight against current and future global issues and public health issues, such as genetic diseases, pandemics, food security and climate change. Wealthy developed nations with greater access to research infrastructure, expertise and a skilled workforce are leading the way advancing these technologies.

There is a need to address this inequity to ensure synthetic biology is used as a tool to improve human flourishing, rather than to increase the inequity between those with and without resources.

Increasing accessibility to synthetic biology is partly dependent on ensuring low-resource communities, such as those in developing nations or regional or low socio-economic areas of developed nations, have access to a pipeline of educated and skilled synthetic biology researchers.

Our team has tailored our research to target United Nations Sustainable Development Goal 4 – Quality Education, and its sub-goal 4.3:

“By 2030, substantially increase the number of youth and adults who have relevant skills, including the technical and vocational skills, for employment, decent jobs and entrepreneurship.”

Our design for a naturally transformable lab strain of *E. coli* will provide a cheaper, more efficient and safe host organism for synthetic biology to facilitate greater use in secondary and tertiary education, both in Australia and in the wider global community.

In concert with the accessible synthetic biology technology we have researched and designed, we identified a need to improve and expand the pipeline of highly skilled synthetic biology researchers to fulfill future demand.

We believe that shaping the scientists of tomorrow starts by sowing the seeds of scientific curiosity in children. A key focus for our project was to engage with primary and high school students to teach and spark their curiosity in the synthetic biology.

2. Overview of the Biology Educational Resource for Secondary Students

We consulted the Australian High School and Higher School Certificate Biology Curriculum and Syllabus.

We identified numerous points of alignment between our project and the Preliminary and HSC Syllabus, and were able to develop an educational resource for students that outlines and explains the role of the scientific method in our research and uses features of our project's background literature, research question and design to teach parts of the syllabus using real-world research examples.

We have also linked concepts explored in our research project to the Stage 5 (Years 9 and 10) Syllabus to prepare Stage 5 students with an interest in studying biology at a senior or university level.

This educational resource allows students to gain a deeper understanding of the research process, including identifying limitations and challenges to research such as COVID-19 restrictions.

3. The Role of the Scientific Method in Free Coli

3.1 Think of a question

While the inspiration for our object was our concern with the potential for advances in synthetic biology to increase inequality, we had to think of a more targeted research question. *E. coli* is the preferred host organism of synthetic biology, but is not naturally transformable, unlike other related gram negative bacteria. We asked whether we could improve the preferred host organism of synthetic biology by genetically engineering it to have natural transformation competence. A couple of questions came up:

How does natural transformation occur in naturally transformable bacteria?

Why is *E. coli* not naturally transformable?

Does *E. coli* have the genes for natural transformation? If not, could we genetically edit them into the *E. coli* genome?

Which bacteria should we copy the genes from?

How will we insert the genes?

3.2 Formulate a hypothesis informed by existing literature

Despite the *E. coli* containing all but one of the natural transformation genes possessed by related naturally transformable bacteria, it has never been successfully shown to be naturally transformable. We spoke to an expert in bacterial natural transformation at the University of British Columbia, Canada, and found out that there might be something wrong with the *E. coli* natural transformation genes or their promoter (a promoter is a piece of DNA that can 'switch on' a gene or set of genes). Our literature revealed the best candidate natural transformation genes would be found in the related, non-pathogenic bacteria *Acinetobacter baylyi*. Our hypothesis was that by editing the genome of *E. coli* to contain *A. baylyi*'s natural transformation genes and placing them under the control of an inducible promoter (which means we need to do something to turn the genes on, like place the cells in a special medium), we would be able to make the *E. coli* cells naturally transformable.

3.3 Develop predictions

We had to predict how the genes we inserted would be expressed, whether they would be successful at natural transformation, and whether they would be toxic to the *E. coli* cells. We predicted that we would need twenty-five genes to create naturally transformable *E. coli* cells. We found out we couldn't order a DNA sequence larger than 5kb, so we also needed to divide our 25 genes up into smaller clusters so they were small enough to be inserted

3.4 Design an experiment to test predictions

The first step to test our predictions was to use mathematical modelling to predict how genes would be expressed in the E. coli cells (frequency of transcription/translation/degradation). We also needed to model the optimal order of the genes and how best to cluster them.

Unfortunately, the Sydney COVID-19 outbreak and lockdowns prevented us from conducting our laboratory research to test our predictions and validate our design. We hope to hand down our research, design and predictions to a new generation of researchers so that they can test and validate our design.

3.5 Perform experiment numerous times

Future work.

3.6 Analyse data

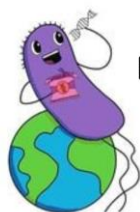
Future work.

3.7 Results

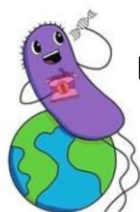
Future work.

4. Connections to the Stage 5 Curriculum

Module	Topic	Connection to Free Coli
LW3 Advances in scientific understanding often rely on developments in technology, and technological advances are often linked to scientific discoveries	<p>Identify that during reproduction the transmission of heritable characteristics from one generation to the next involves DNA and genes</p> <p>Identify that genetic information is transferred as genes in the DNA of chromosomes</p> <p>Outline how the Watson-Crick model of DNA explains the exact replication of DNA and changes in genes (mutation)</p>	<ul style="list-style-type: none"> • Reproduction in single cellular organisms involves copying of one chromosome • Understanding the E. coli circular chromosome as different to the human diploid chromosomes • The concept of genetic engineering as a human-induced mutation • Demonstration of the link between genotype and phenotype in example of using selection markers e.g. antibiotic resistance to prove whether a bacterial cell has successfully had its DNA and genes changed



	<p>Describe, using examples, how developments in technology have advanced biological understanding</p> <p>Discuss some advantages and disadvantages of the use and applications of biotechnology, including social and ethical considerations</p>	<ul style="list-style-type: none"> • Discussion of the ethical and social considerations of our project: 1) Making sure biotechnology doesn't contribute to public health or environmental problems such as antibiotic resistance, 2) The ethics of genetically engineering plants, animals and humans, 3) GMO foods and vaccines as examples of biotechnology products.
LW4 The theory of evolution by natural selection explains the diversity of living things and is supported by a range of scientific evidence	<p>Explain, using examples, how natural selection relates to changes in a population e.g. in the development of resistance of bacteria to antibiotics</p> <p>Outline the roles of genes and environmental factors in the survival of organisms in a population</p>	<ul style="list-style-type: none"> • Demonstrate how natural selection can be exploited to screen successfully and unsuccessfully genetically changed bacteria through the use of selection marker genes • Discuss the value of natural transformation ability in allowing bacteria to survive
Additional content	<p>Discuss the strengths and limitations of using models to make predictions about change in biological systems</p>	<ul style="list-style-type: none"> • Explain the role of mathematical modelling in our design process, to ensure our natural transformation genes are effective and non-toxic in E. coli cells
	<p>Describe examples of advances in science and/or emerging science and technologies</p>	<ul style="list-style-type: none"> • Discuss the applications of E. coli cloning
	<p>Assess the role of the development of fast computers in the analysis of DNA sequences</p>	<ul style="list-style-type: none"> • Show students what a DNA sequence looks like on SnapGene software • Explain how it was crucial to have this technology to sequence the genomes of naturally transformable bacteria so we knew the DNA sequences for the genes we wanted to insert into E. coli



5. Connections to Stage 6 Curriculum

Module	Topic	Connection to Free Coli
Cells as the Basis of Life	Types of Cells Cell Structure Cell Function	<ul style="list-style-type: none">• Our project works with E. coli, a prokaryote• Prokaryotic cell structure and membranes were an important consideration in our research and design because DNA needs to get past multiple cell layers to the chromosome for integration
Adaptations and Survival Evolution and Natural Selection	Adaptations (Physiological/Structural) Modern Examples of Evolutionary Change	<ul style="list-style-type: none">• Natural transformation as an adaptation for bacteria• Problem of antibiotic resistance• Why is E. coli not naturally transformable but phylogenetically related bacteria are?• Use selectable markers in genetic engineering
Ecosystem Dynamics	Human-Induced Change in Ecosystems	<ul style="list-style-type: none">• What is the impact of human-induced genetic engineering on current and future ecosystems?
Heredity	Asexual Reproduction	<ul style="list-style-type: none">• Binary fission in bacteria - a crucial feature that makes E. coli an ideal host organism (quick growth into large colonies)
DNA and Polypeptide Synthesis	DNA in Prokaryotes Polypeptide Synthesis Functions of Proteins in Living Things	<ul style="list-style-type: none">• E. coli chromosome• Central Dogma of Biology and the need to model transcription and translation rates in target genes• Role of promoters in transcription• Role of natural transformation gene proteins
Genetic Change	Mutations and How they Affect Organisms	<ul style="list-style-type: none">• Exploring the concept of artificial mutations = genetic engineering• Importance of ensuring no unintentional mutations in our DNA sequence when we are assembling in software (Snapgene)
Biotechnology	Past, Present and Future of Biotechnology	<ul style="list-style-type: none">• Applications of genetic engineering/synthetic biology (medical, agricultural, environmental)• Tools to manipulate DNA - recombination, CRISPR• Ethics and social implications of biotechnology - accessibility of the field and the need to address research resource inequity



Genetic Technologies	Cloning	<ul style="list-style-type: none">• E. coli as a host organism for cloning - transformation• Transformation vs natural transformation
Infectious Disease	Pathogenic Bacteria	<ul style="list-style-type: none">• Choosing non-pathogenic bacteria was an important safety and ethical consideration in our design• Pathogen transmission can be aided by pili (the role of the pili in natural transformation and as the focus of our design)