

# Canadian iGEM NEWSLETTER

# iGEM 2017 Calgary

Hello,

Thank you for taking time out of your day to read the August 2017 issue of the Canadian iGEM Newsletter! iGEM is an international genetic engineering competition that encourages interdisciplinary cooperation within and between institutions.

The University of Calgary team created this newsletter with the hope of bridging communication between Canadian teams to foster more collaboration opportunities.

Special thanks to all of the teams that made this project possible. We could not have done this without you!

Best,  
The iGEM Calgary 2017 Team



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# SyntheINK

## Environmentally Friendly Pigment Production

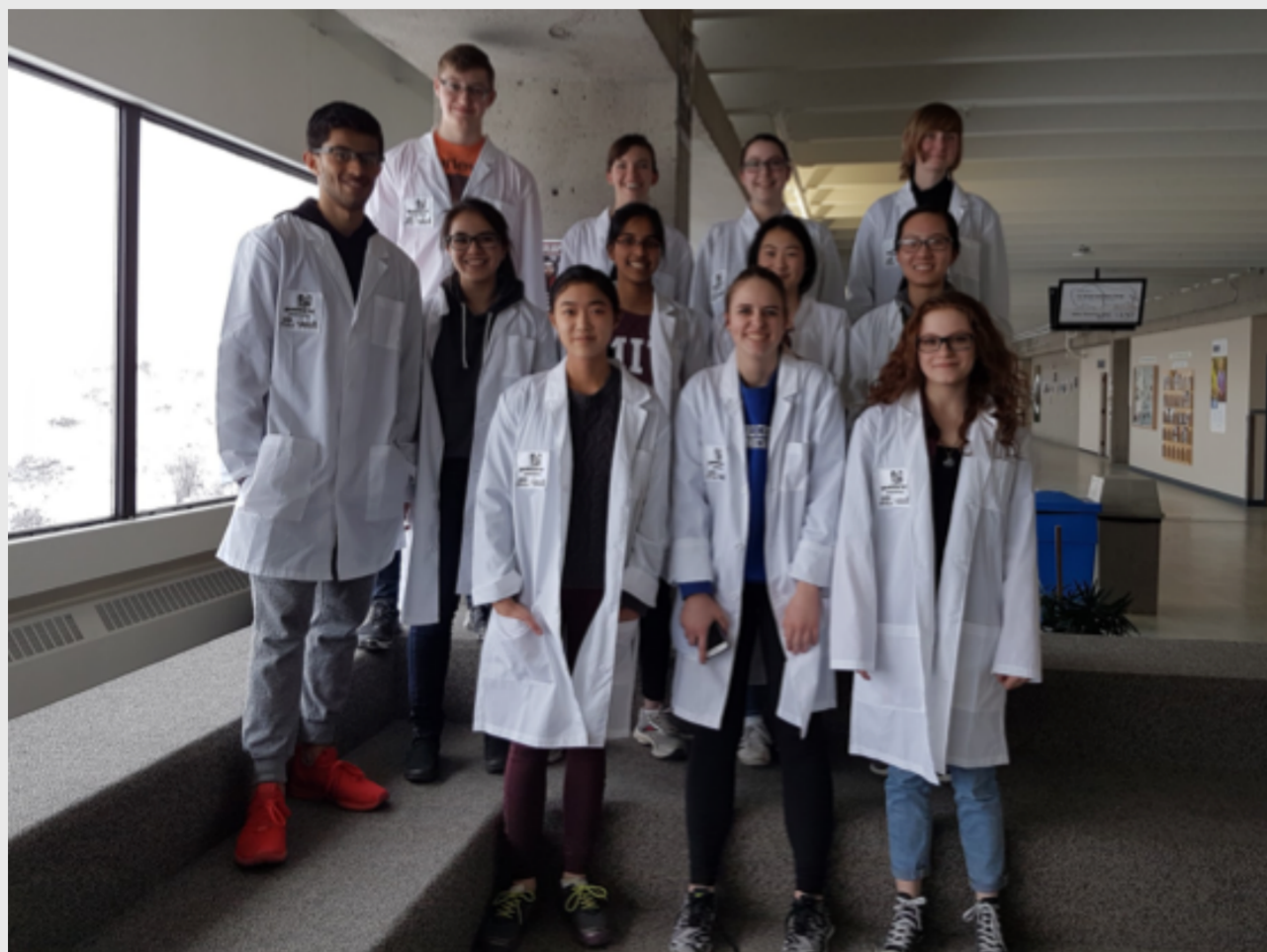


Fig 1. HS iGEM team at the GeekStarter Lab Skills Workshop in March. Missing: Candace Chan, Brian Dempsey

The Lethbridge High School iGEM Team, consists of 3 male and 9 female student members from 3 high schools in our city. This season, we have decided to tackle the issue of ink production. The current pigments used to colour conventional inks and their production may be costly, harmful to the environment, and harmful to one's health. Our goal is to produce biological (environmentally-friendly and non-hazardous) pigments in *Escherichia coli*. We have created four genetic constructs, to produce melanin, indigoidine, anthocyanin, and zeaxanthin, which are black, cyan, magenta, and yellow pigments, respectively. Pigments will be extracted and purified from bacterial culture and incorporated with a solvent and resin into an ink solution.

For example, the black pigment used will be melanin and the gene *melA* from *Rhizobium etli* will be used to produce the pigment. The *melA* gene codes for tyrosinase which converts L-tyrosine into dopaquinone, which then polymerizes into melanin.

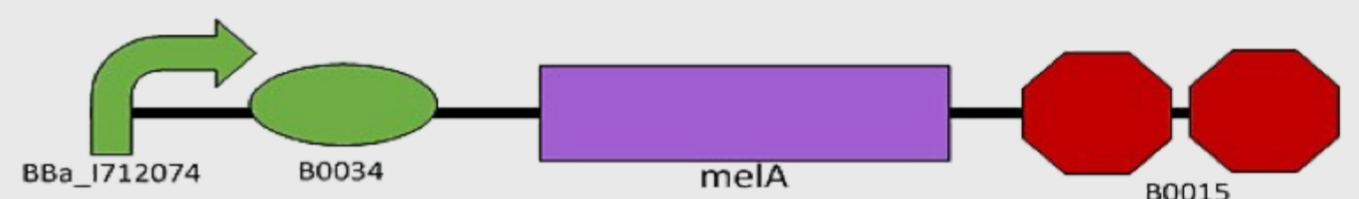


Fig 2. Genetic construct for melanin production. The construct includes an inducible promoter (BBa\_I712074), an *E. coli* rbs (B0034), the *melA* gene for Melanin tyrosinase and a double transcriptional terminator from *E. coli* (B0015). The *MelA* gene has been codon optimized for expression in *E. coli*. This construct will be cloned into pSB1C3.

We hosted a Parent and Teacher night where we invited the parents and teachers of the team members to the University of Lethbridge and performed similar experiments to what we do in iGEM, like extracting DNA and running an agarose gel. This got the community involved and interested in synthetic biology thus promoting integrative, hands-on education.



# SyntheINK

## Environmentally Friendly Pigment Production



interviews, create our wiki, create a prototype, have a good idea of the maximum pigment product of our bacteria through mathematical modelling, and investigate the viability of an ink business.



Fig. 3 (Top) Parents, teachers, and iGEM members in the University of Lethbridge lab-space extracting DNA from a strawberry.

Fig. 4 (Bottom) Parents, teachers, and iGEM members in the University of Lethbridge lab-space running an agarose gel with food colouring.

Our future directions for this project include many goals, leading up to the completion of our project. The goals are to: follow through with the production of pigments with constructs and the purification of the pigments, present to the school board and city council, organize more interviews, follow up with past

# Biofuel Production using Cellulose and Hemicellulose degradation



## The Problem

Dwindling fuel resources and rising environmental concerns have put pressure on companies to look into more sustainable solutions in waste processing and disposal. In Nova Scotia, the softwood lumber industry is an untapped source for low-cost biofuel feedstock. The waste from pulp and paper processing contains valuable cellulose that can be turned into glucose, then ethanol. However, the cellulose in the waste is not easily accessible by traditional means.

## The Project

This year the Dalhousie iGEM team is continuing to tackle the growing problem of fuel scarcity by exploring biofuel production in a synthetic biology context. Last year's team looked to nature to provide insight into the growing field of cellulosic ethanol. The team examined the microbiome of the porcupine (a mammal that feeds on bark, but lacks the enzymes to digest cellulose) and identified bacteria containing enzymes necessary to convert cellulose to glucose. By cloning these enzymes into pET26b and expressing them in *E. coli*, the team aims to co-culture these

recombinant *E. coli* with yeast to produce ethanol for biofuel production.

## Wet Lab

This year's team aims to identify more enzymes key to the cellulose and hemicellulose degradation pathway through a bioinformatics metagenomic pipeline and a metagenomic library done in collaboration with Dr. Trevor Charles at the University of Waterloo. These enzymes will also be cloned into pET26b and expressed in *E. coli* to complete the pathways. Once the pathway is complete, the team aims to co-culture in a bioreactor to generate ethanol. The team has successfully cloned endoglucanase, a key enzyme in the breakdown of cellulose, and is hard at work cloning other enzymes such as beta-glucosidase.

## Dry Lab

The Dry lab team is currently at work with two major goals in mind. The first, to process the DNA sequences collected by the team over the past two years. The second purpose is to model



# Biofuel Production using Cellulose and Hemicellulose degradation



the bioreactor that will ultimately be used to breakdown complex cellulose. So far, the dry lab team has made excellent progress in analyzing the mentioned DNA sequences; a bioinformatic pipeline has been created and used to successfully locate several key enzymes in the cellulose and hemicellulose degradation pathway. The pipeline uses three programs: megahit, prodigal and hmmer to stitch sequences, search for open reading frames and compare those open reading frames to an enzyme database respectively. The goal of the pipeline is to identify known and novel enzymes in the microbiome sequences that are similar to known proteins whose functions have been determined.

better communicate science, and help people identify “fake” science news.

## Policies and Practices

The Human Practices team is working hard on increasing the scientific literacy in the general public. The team is currently at work on a survey which tests the participant’s scientific literacy, as of today the survey garnered over 200 responses. The survey will also be coupled by a set of interviews aimed towards the general public, scientists, science communicators and journalists to try and further understand how we can



# DNAzymes, Biosensing, and Antibiotic-Resistant Bacteria



The McMaster iGEM team has experienced drastic growth since its beginnings. Going into our third year, we are currently a team of almost 40 members making up 6 different subteams: Wet Lab, Dry Lab, Human Practices, Business Development, Community Outreach and Public Relations. Our previous projects have included the use of light to control recombinant protein production (2015) and the use of quorum sensing with genetically engineered lactic acid bacteria as a novel therapy for gastrointestinal tract cancers (2016), the latter of which won us a bronze medal at the Jamboree. This year, the wet lab team is focused on developing a plate-based biosensor for E. coli through the use of fluorescent DNAzymes in an effort to tackle antibiotic-resistant bacteria - a growing public health issue both in Canada and overseas.

DNAzymes (deoxyribozyme) are synthetic, single-stranded DNA that exhibit enzyme-like catalytic abilities. Our engineered DNAzymes specifically detect E.coli by cleaving an attached fluorophore-quencher in the presence of E.coli RNase protein. The separation of the quencher from the fluorophore is accompanied by a dramatic increase in

fluorescence intensity, which can be measured through fluorometry to serve as an indicator for the presence of E.coli. The ability of DNAzymes to carry out species-specific or even strain-specific catalysis can be potentially leveraged as an approach to efficient pathogen detection in the future.

The McMaster dry lab team is focused on using computational and statistical approaches to streamline the future development of DNAzymes for biosensing applications. By analyzing sequencing data to gain insight into the DNAzyme selection process, we hope to construct a machine learning algorithm which can modify and combine existing DNAzymes as well as predict their specificity and function. Additionally, collaborations are in place with the wet lab team to help automate the image analysis process necessary to quantify fluorescence. While we are using E.coli as a proof-of concept target, we are hopeful that our efforts will eventually be applied towards the discovery of highly efficient DNAzymes specific to antibiotic-resistant bacteria strains.





# DNAzymes, Biosensing, and Antibiotic-Resistant Bacteria



The Human Practices team are investigating the ethical, social, and political aspects associated with developing biosensors to address antibiotic resistance, both in Canada and in the developing world. Through consultation with experts across public health, biomedical research, public policy, citizenry and advocacy groups, we hope to understand how biosensors can be developed, brought to market, and implemented in low-resource settings to detect bacteria quickly and inexpensively. We hope to produce a discussion paper that will outline the end-to-end process of biosensor development in Canada and abroad. We also would like to build case studies that focus on patient groups such as seniors, infants, and the immunocompromised. Findings from the human practices team will continue to supplement and inform the wet lab and dry lab teams as they continue their important work on validating our proof of concept.

Always seeking to grow, McMaster iGEM is open to collaboration with other chapters and organizations. In the spirit of the scientific community, we hope to facilitate an exchange of knowledge,

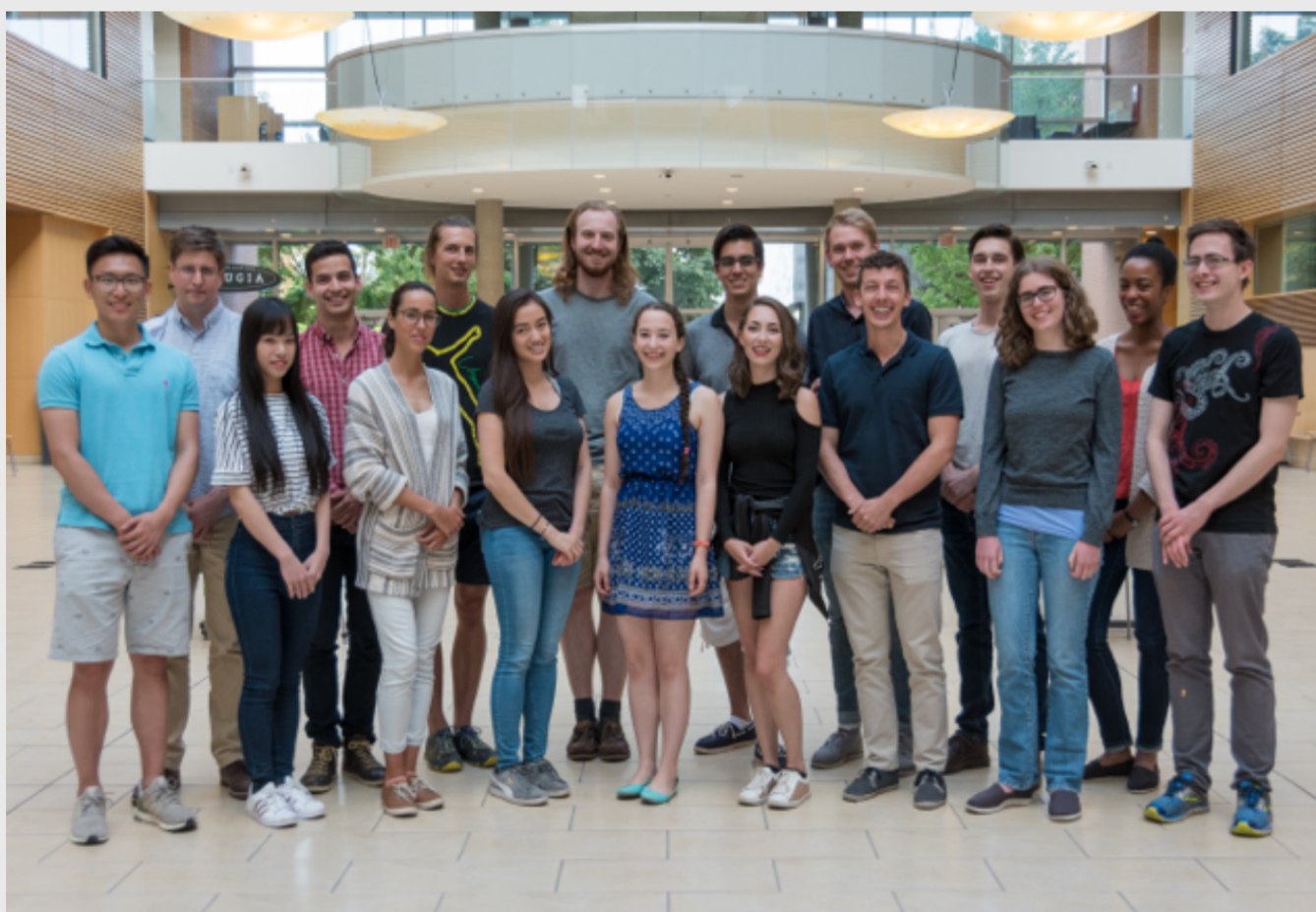
ideas and innovation, as well as shared sub-projects and cross-verification of experimental results. For more information about our progress, visit our website [mcmastergem.com](http://mcmastergem.com), or contact us through Facebook (@igemmcmaster) or e-mail ([igemmcmaster@gmail.com](mailto:igemmcmaster@gmail.com))!

# Biocontrol Mechanism for Crown Gall disease and Hairy Root disease



## Who are we?

This year's UBC iGEM team consists of 16 dedicated undergrad students and 5 advisors. The team is interdisciplinary and comes from many different backgrounds such as Microbiology, Psychology, Computer Science and Chemical & Biological Engineering to name a few. Together, we all share a common passion for synthetic biology and are excited to contribute to the iGEM community.



## Our Project

*Agrobacterium tumefaciens* is a gram-negative soil bacterium responsible for two common plant diseases: Crown Gall and Hairy Root. When infected, plants display bizarre growths and tumors that cause plant cells to reallocate nutrients ineffectively and can lead to plant death.

To prevent infectious spreading, current solutions involve removing the infected plants or chemically treating the soil in an attempt to eliminate the bacterium. However, despite these efforts, the pathogenic *Agrobacterium* can remain in the soil for up to 16 years resulting in large economic losses for farmers.

What if you could protect plants from this pathogenic bacterial infection without the use of harsh chemicals or labour intensive measures? For our project, we have decided to take a synthetic biology approach to combat *Agrobacterium tumefaciens* infections.

## What's happening in our lab?

Our goal is to develop a strain of *Agrobacterium* that can combat pathogenic *Agrobacterium tumefaciens* populations to prevent infections and remediate impacted soil ecosystems. To do this, we want to engineer a plasmid, equipped with a CRISPR-Cas9 system that can target and disarm the virulent region on the native plasmid found in *Agrobacterium tumefaciens* that causes the disease in plants. With the virulent



# Biocontrol Mechanism for Crown Gall disease and Hairy Root disease



genes removed, the pathogenic *Agrobacterium tumefaciens* would be unable to transfer the DNA that causes the plant infection and disease.

## What are we modelling?

For the modeling component of our project, we will predict the effectiveness of our engineered plasmid carrying CRISPR/Cas9 to attack the native, virulent plasmids in *Agrobacterium* populations. To accomplish this, first, we are developing a tool for predicting potential gRNA sequences for Cas9 to target a specific and conservative virulent region with minimal off-target effects. Next, we are modelling the spread of our plasmid with CRISPR/Cas9 relative to the spread of the disease-causing plasmid in *Agrobacterium* population. Finally, we plan to model the overall efficiency of our CRISPR/Cas9 system to fight Crown Gall and Hairy Root diseases.

## Human Practices

Science education and communication are the focuses of our human practices! We are developing methods to communicate our project to different audiences and educate synthetic biology through interactive workshops and activities. So far, we have led STEM

based summer camps for kids, talked at a local conference for high school students and are preparing to interview different faculty members here at UBC. Through this work, we hope to provide our community with a better understanding of synthetic biology.



Checking out protists under an iPhone microscope in our kids camp (Left) and our genetic engineering workshop with the Research in Science and Engineering (RISE) conference (Right).

## Collaborate with us!

Do you have any feedback? Know of any resources we might be able to use? We would love to know! If your team is developing your own STEM workshops for students, we would also love to share some of our game and activity ideas with you! Send us an email at [ubcigem@gmail.com](mailto:ubcigem@gmail.com). Make sure to follow us on Facebook and Instagram to stay updated with our project and team!

# Production of Bioplastics from Solid Human Waste on Mars

## Our Team

The iGEM Calgary 2017 team is consisted of 14 ambitious undergraduate students hailing from the Faculties of Science, Engineering, and Medicine. Acting under the supervision of Dr. Mayi Arcellana-Panlilio, we are a research group based within the O'Brien Teaching Labs in the Cumming School of Medicine. We are fortunate to have several past iGEM Calgary team members act as mentors for our project this year, as well as two TA's to advise us in the lab.



The iGEM Calgary 2017 team with Dr. Mayi and our two TA's, Rachele and David.

## Our Project

The goal of this year's project is to engineering a recombinant strain of *Escherichia coli* that will be able to convert the glucose and volatile fatty

acids that are found in human feces into Polyhydroxybutyrate (PHB), which would be secreted out of the cell. These particles can then be used for 3D printing on Mars. Our team this year is split into 4 main subgroups: Synthesis, Secretion. Process Development, and Human Practices. This year, we will be using kinetic modelling and flux balance analysis to optimize our metabolic pathways.

### Synthesis

This summer, our synthesis group is working on optimizing the genes for the glycolysis pathway of PHB production, which uses acetyl CoA as a starting material. We are comparing our optimized part to an existing part that was created by the Tokyo 2012 team. In addition, we are developing a new part which will utilize products of fatty acid beta-oxidation to produce PHB. By utilizing both pathways, we can maximize PHB production in *E. coli*, which is essential given the limited amount of our feedstock, human waste, on long-term space missions with small crews.



## Secretion

The secretion team has been working on implementing a PHB secretion system into *Escherichia coli*. This system functions by coupling the hemolysin secretion system naturally found in *E. coli* with phasin proteins, which bind to PHB granules inside the cell. Once PHB granules are synthesized intracellularly, phasin proteins fused with a hemolysin secretion tag will coat the granule and tag them for secretion through membrane transport proteins. *E. coli* have been successfully transformed with the phasin-tag fusion protein and assays to quantify the secretion of PHB from the cell are currently underway.

## Process Development

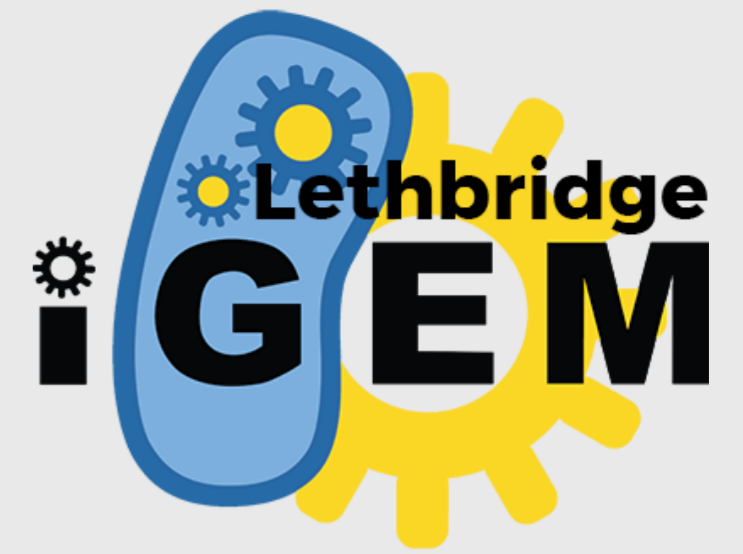
Dubbed as the "Engineers Group", our team is working on creating a step-by-step process for the conversion of biosolid waste into PHB. From our literature search, 6 steps have been identified. We start by fermentation of the biosolid waste to create volatile fatty acids (VFAs). Then, the solids and liquids are separated to extract the VFAs. This separation can be done using a screw press or centrifugation. The VFAs and the liquid

medium are then sterilized and sent to a bioreactor. Here the microbes will use the VFAs to create and secrete bioplastic nanoparticles. The microbes and the bioplastic nanoparticles are then separated and the bioplastic laden output stream is taken for further processing where the PHB is separated from the liquid stream.

## Human Practices

We have spoken to multiple experts in a variety of fields to optimize our project for various applications. Ultimately, we have decided to pursue a space application for our project, as doing so would reduce the costs of restructuring a system to accommodate our project (as would have happened had we pursued terrestrial wastewater treatment). In addition, we have planned and executed a wide array of community outreach events, including speaking with high school students about synthetic biology, conducting workshops for students in grades 7 to 9, and conducting a bioplastic demo and synthetic biology conversation at our local science centre.

# Accessible Modular Reaction Vesicles with Standardized (BioBrick) Components



This year's University of Lethbridge Collegiate iGEM has recruited many new and engaged team members working toward one of our more ambitious projects. This year marks the tenth year our school has competed in the iGEM competition. As such we are planning several celebratory events and outreach projects for past and current iGEM members to gauge how involvement in iGEM/synthetic biology has impacted their career and lives. These events and outreach are above and beyond the scope of our iGEM project this year, in which we hope to make a huge foundational advance for the synthetic biology community as a whole.

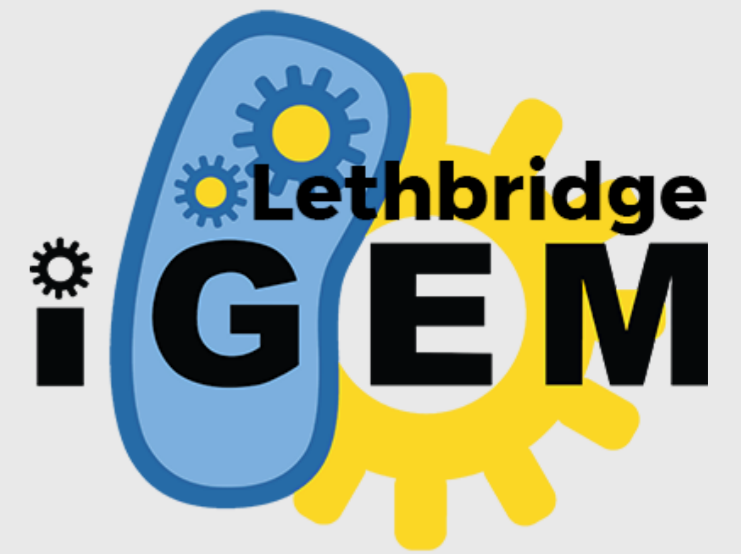


University of Lethbridge Collegiate 2017 iGEM team at the geekStarter workshop in June.

Our goal this season is to create modular, easily attainable, and cost-effective cell-free transcription and translation systems to make synthetic biology accessible to not only scientists and other iGEM teams, but also to the masses including educators. Ex vivo systems are ideal for a variety of applications for they are easily contained, non-proliferating, non-toxic, and allow for the precise control of molecular interactions. Such systems can be lyophilized for use in paper-based applications or encapsulated in lipid vesicles (liposomes), providing advantages over bulk solution by minimizing issues with diffusion. With increased modularity, predictability and freedom of design, these modular cell-free expression units have been explored for use in bioremediation, prototyping, directed evolution of proteins, design of a synthetic minimal cell, and biosensor development. These studies have shown great potential in cell-free expression systems but continued advancements and innovations are required for the widespread use of these devices.



# Accessible Modular Reaction Vesicles with Standardized (BioBrick) Components



accessible to the masses. We are also researching the impact our system will have on industries that use Synthetic Biology as well as scholars who may come across our cell-free system in the future.

Over the past month our team has made progress on all aspects of our project. We have received and begun working with 26 of our 38 constructs required to produce all the components required for a cell-free transcription/translation (TX/TL) system. In the wet lab we have begun cloning, sequence confirming, and transferring our constructs into the iGEM BioBrick standard plasmid of pSB1C3. While the wet lab team is working on getting each individual TX/TL component expressing, our modelling team is working on determining how to best regulate expression of each component to achieve the proper ratio from a one-step purification of all components. We aim to have all the TX/TL components purifiable by a one step, minimal cost protocol thus making it more



# Use of RNAi as Treatment and Detection of methicillin-resistant *Staphylococcus aureus* (MRSA)



## How Our Project Started, How it Changed, and Where We are Today

iGEM at UNBC is still in its infancy; this year marks the second year our University is taking part in the iGEM experience. Our iGEM team is the product of the UNBC Synthetic Biology Club, or USBC, which functions as an entity promoting the field of synthetic biology, extra-curricular research, and student-driven innovation at UNBC. Throughout the school year, members of the USBC met to brainstorm any and all project ideas for the iGEM team to pursue. Come summer time, the iGEM team is chosen from the club via an application process. This year, as many of the students involved with the USBC are interested in the healthcare field as a future career path, we decided to focus on a healthcare related topic. In looking at some of the greatest issues facing modern medicine, it did not take long to come across the issue of antibiotic resistant bacteria, and specifically, MRSA (Methicillin-Resistant *Staphylococcus aureus*).

As the wheels started turning, we started to come up with ways we could try and combat and detect MRSA using synthetic biology. Initially, our idea was to introduce a plasmid into *E. coli* containing genes that would be transmitted through conjugation with MRSA. Our broad idea was that these genes would code for ribozymes that would target specific RNA inside MRSA, thus knocking out things like resistance and virulence. However, after reviewing the literature and coming across a similar project done by Waterloo iGEM in 2014, we decided to shift away from conjugation and focus on sRNA-mediated gene silencing, as conjugation has proven non-viable in the past.

Currently, our project starts with expressing and purifying a dsRNA chaperone protein, Hfq, which is essential for sRNA-mediated gene silencing. Hfq is a hexameric protein that works by binding of its proximal face to AU rich regions on an sRNA. It then binds the target mRNA on its distal face and sustains the dsRNA





# Use of RNAi as Treatment and Detection of methicillin-resistant *Staphylococcus aureus* (MRSA)



interaction in order for RNase III to recognize the complex and target it for degradation. We hope to characterize the binding affinity to our custom designed sRNAs, containing a 5' AU rich region for Hfq recognition, using a fluorescence polarization assay. We have designed four separate sRNAs to target four distinct pathways within *Staphylococcus aureus*: *mecA*, which codes for PBP2A; *secA*, which is a critical ATPase in the bacterial secretion system involved with virulence; *glmM*, a phosphoglucosamine mutase involved with peptidoglycan synthesis; and D-alanine—D-alanine ligase, also involved with peptidoglycan synthesis through D-alanine metabolism.

Continuing to pioneer iGEM at UNBC, a small school that typically doesn't partake in synthetic biology research, has proven to be difficult and not without time setbacks. Alas, our lab supplies and DNA/RNA are en-route, and we are very excited to become fully immersed in the iGEM experience and build a project representative of our capabilities as a team and of UNBC.

After obtaining and analyzing our FP data, we will transform plasmids

containing genes coding for the sRNAs of interest into *S. epidermidis*, as it is a safer level 1 organism, as well as the level 2 pathogen *S. aureus*, which we obtained approval to work with independently through our university. These plasmids will also contain copies of the Hfq gene to ensure adequate levels of Hfq protein expression within the cell. Gene silencing efficiency will be measured using qPCR and a probe for the target mRNA. Viable targets will then be phage-delivered into *S. epidermidis* and *S. aureus*, time permitting.

# Exploring gRNA-Modulated Genetic Networks



## Introduction

The regulation of a gene of interest using transcription factors is achieved by upstream binding of a transcription factor to a promoter region. Despite the fundamental role transcription factors play, they place many constraints on experimental designs due to the limited types available. As a result, DNA manipulation is often required to place a promoter for which there is a corresponding transcription factor upstream of the target gene. Synthetic guide RNA, sgRNA, can be modulated to match the existing promoter upstream of a gene of interest allowing for targeted regulation without the need for DNA manipulation. The creation of a library of sgRNA corresponding to different promoters would reshape and dramatically improve the efficiency of all work in the field of molecular biology.

## Goal

To explore the potential of sgRNAs in genetic network engineering, our project aims to implement a sgRNA-based logic gate, the NOR gate. Logic gates are basic representations of digital circuits in terms of input and output flow

under binary conditions. The behavior of a 2-input NOR gate (Figure 1) can be demonstrated with a truth table (Table 2). The output Z is true only when both input A and B are not true, which could be represented by the boolean expression:  $(Z=(\text{Not } A \text{ or } B))$ .

Input		Output
A	B	Z
0	0	1
0	1	0
1	0	0
1	1	0

Table 1. NOR logic gate truth table showing possible combinations of input A, B and resultant output Z.



Figure 1. Schematic of a NOR composition showing input A, B and output Z.



# Exploring gRNA-Modulated Genetic Networks



## Wet Lab

Inspired by Gander's study, "Digital logic circuits in yeast with CRISPR-dCas9 NOR gates", we have decided to build a NOR gate whose output is controlled via the inducible production of gRNA-complexed dCas9. First, we will create four different strains of yeast, *Saccharomyces cerevisiae*, with each strain representing a stationary state in the truth Table 1. For instance, one strain contains a constitutive GFP reporter. This strain represents the first state in Table, in which neither gRNA A nor gRNA B are present, allowing for the production of GFP, which leads to an output of 1. Two strains contain only one type of gRNA along with dCas9. Since gRNA is complementary to a region in the promoter of GFP, it will complex with dCas9 to inhibit the production of GFP via steric hindrance, leading to an output of 0. The fourth strain contains both gRNAs as well as dCas9, with an expected output of 0. Finally, we will create another strain containing a NOR gate whose different output states can be modified. This can be achieved by controlling the production of both gRNAs using different inducible promoters. NOR gate parts of this project,

notably dCas9, the GFP reporter, and the different gRNAs, have been adapted from the aforementioned study.

## Dry Lab

Because the GFP promoter is constitutive, we will model the effects of the repression of GFP production via gRNA.

## Human Practices

We will compare the opinion of synthetic biologists on the safe usage of dCas9. We will do a literature study to determine when exactly the use of dCas9 constitutes a gene drive, and when it does not. Finally, we propose a Canada-wide protocol of gene drive manipulation.



## Switch Control for CRISPR-Cas9 Edits



This year's iGEM Toronto project is a light-activated switch to reduce off-target CRISPR-Cas9 edits by controlling CRISPR-Cas9 duration and activity levels. By putting sgRNAs and anti-CRISPR proteins under the control of a novel fusion protein LacILOV (developed by the Mahadevan lab here at the University of Toronto) and the *cl* repressor, respectively, we aim to create a switch that allows CRISPR-Cas9 activity only under a specific wavelength of blue light. In the dark, LacILOV represses downstream transcription of both sgRNA and the *cl* repressor. The lack of *cl* protein expression allows anti-CRISPR protein transcription, which in turn inhibits Cas9 activity – so CRISPR-Cas9 activity is doubly prevented by a lack of sgRNA and anti-CRISPR Cas9 inhibition. In the presence of blue light, however, LacILOV repression is abolished, allowing sgRNA transcription and removing anti-CRISPR inhibition (through *cl* expression) to facilitate CRISPR activity.

The switch will be validated in *E. coli*, where spectrophotometric assays of the reporter proteins YFP and mCherry will characterise the kinetics of light-induced activation and repression.

Measurable interference of metabolic and reporter genes by dCas9 will assay functional control of CRISPR activity by our toggle switch. We will then be using these experimental results to simulate a stochastic model of the gene circuit. Furthermore, we will use the MATLAB Synbio Library to analyse dynamics of the system, optimising a model which can be implemented in the lab.

While this light activated CRISPR-Cas9 system should provide scientists with greater control and accuracy in gene editing, the regulatory framework required to implement this technology as a normalised component of health care is still lacking. To investigate the barriers that human gene editing may face, we will be conducting a systematic analysis of the socioeconomic, legal, ethical, and political considerations through interviews with local politicians, healthcare professionals, members of religious communities, advocacy groups, and end users who will be affected by the inclusion of this technology into mainstream healthcare. Our project aims to contribute



## Switch Control for CRISPR- Cas9 Edits



to the body of research geared towards making CRISPR an accurate, reliable and ultimately safe clinical option as well as to provide a diverse understanding of the manner in which this technology will shape, and is shaped by, the political and social landscape of Canada.

We are also committed to outreach and education through three exciting projects this year: an iGEM podcast series, our high school bioinformatics and synthetic biology camp, and the Iconathon, a day-long event which will pair scientists and artists to enrich the currently meagre synthetic biology icon repository. If any teams are interested in participating in the podcast or other collaborations, please let us know at [igem@skule.ca](mailto:igem@skule.ca).



# Bacterial Biofilm for Arctic Oil Spill Bioremediation

## Project Abstract

The Queen's Genetically Engineered Machine (QGEM) Team is turning to nature as inspiration for building a safer, cheaper method of oil spill cleanup in the Arctic. QGEM is designing and engineering a bacterial biofilm to bind ice and recruit oil-degrading native marine bacteria. The end product will be a dynamic, bifunctional biomaterial that may be deployed in the Arctic marine environment during oil spills, as a bioremediation factory, with limited disturbance to the surrounding ecosystem.



## Wet Lab

Our project involves functionalizing the CsgA amyloid subunits that form the proteinaceous component of E. coli biofilms. *Marinobacter hydrocarbonoclasticus* is a hydrocarbon degrading bacteria that attaches to the biofilm via dextran binding.

The polysaccharide-binding domain (MhLap) of *M. hydrocarbonoclasticus* binds dextran that is linked to the biofilm by the C Lectin-SpyCatcher domain. The SpyCatcher end would bond to the SpyTag proteins displayed on our biofilm, permanently attaching the Lectin domain. Additionally, antifreeze proteins (AFPs) that are fused directly onto CsgA endow the biofilm with the ability to bind to, and colonize, surface ice. QGEM successfully expressed GFP-SpyCatcher, which would allow for proof of concept of the SpyTag-SpyCatcher system. QGEM successfully cloned both CsgA-AFP8 and CsgA-SpyTag into pETDuet-1, and the Csg Operon into pET28. These will be transformed into the same PQN4 E. coli cell line and biofilm expression to test the bifunctionality will begin.





# Bacterial Biofilm for Arctic Oil Spill Bioremediation

## Dry Lab

This summer, Dry Lab is working to create a ratiometric program that builds upon the concept of biofunctional biofilms. By reading user input of desired protein ratios found in the biofilm (ie. 5 Protein A: 2 Protein B), the program produces promoters, RBS sequences, etc., required to achieve such a ratio. This program is centred on determining and manipulating relationships between promoter strength, RBS strength, and resultant protein output. To ensure reliable results, QGEM built upon the ideas of RPUs (Relative Promoter Units) created by the Anderson Lab at UC Berkeley, and the RBS Calculator created by the Salis Lab at Penn State University.

## Policy & Practice

On May 13th, the team participated in Science Rendezvous Kingston. We are also filming an interview series that feature bioremediation researchers and potential stakeholders. QGEM will also be developing a new online synthetic biology course for second year undergraduates at Queen's University.

