International Genetically Engineered Machines Competition
Guidebook for High School Students and Instructors

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INTRODUCTION

Emily Hicks and Iain George

Can a team of undergraduate students use today's scientific and engineering tools to build an organism that can solve a problem facing our world? This was the initial question poised by the International Genetically Engineered Machine (iGEM) competition. The goal was simple, to challenge students to become better tinkerers and problem solvers using the power of synthetic biology – a combined field of the engineering to build a solution and science to understand how it works. This is radically different from traditional research where undergraduate students rarely get to drive a design, build and test a research project of their own.

The iGEM competition originated at the Massachusetts Institute of Technology (MIT) in Cambridge, MA in 2003. This was a month long course where students were challenged to make a cell blink. The next steps involved expanding from MIT to other schools in the US in 2004. In 2005, iGEM saw its expansion onto the world stage with the addition of teams from Canada and Europe. The year after saw the introduction of teams from Latin America, Asia and Africa. From iGEM's inception it has been quickly evolving from an class on MIT's campus in 2003 to a competition with 234 teams from around the world in three different divisions in 2013. As iGEM continues to expand, there has been an ongoing push to move beyond just college students.

For the collegiate and entrepreneurial divisions the competition follows the college semester schedule. Project design and recruitment occurs from January to April, main project work occurs from May to August, finalized project work occurs in September and October in preparation of the competitions. There are normally two levels to the collegiate competition, regional jamborees in October and the world jamboree in November.

In 2011, iGEM offered for the first time an entirely separate division for high school based teams to compete in. This provides them a level playing field and the opportunity to interact with other students in their age group. For the high school competition the timeline runs as follows. Team project planning and registration November to January followed by project work from January to May, and then final project work and the world high school jamboree in June.

As in the collegiate competition high school teams need to make three major presentations. First, they need to develop a wiki – the website that full describes their entire project from start to finish. Second, there is a 20-minute presentation plus a 5-minute question session in front of a group of judges and other teams at the world jamboree where the team presents a story showing the world the work they have done. Finally, teams must present a scientific poster to the judges and other iGEM teams where they explain in greater detail the goals of their project and how each of the components fit into the broader story.

1. Types of iGEM projects

Over the years, projects for the competition have been as diverse as the universities entered. At the collegiate level of the competition, teams are sorted into tracks depending on the type of project they chose to undertake. Although these change slightly every year, common examples of tracks include 'foundational advance', 'food and energy', 'environment' and 'health and medicine'. Examples of projects include everything from designing bacterial blood cells 'Bactoblood' to designing bacterial fuel cells and everything in between. A collegiate team from imperial College in 2013 made bacteria capable of degrading plastic while a high school team from China worked on detecting nitrates and

nitrites using bacteria. In short, the types of projects teams tackle can be almost limitless. In fact, picking a project has so few guidelines that it can often be a major stumbling block for many iGEM teams.

2. Tips for Picking a Project

When deciding on a project for your team to undertake, there are several things that need to be considered. The first is the general topic. Generally, there are two main categories of projects that can be undertaken: foundational projects and application projects. While foundational projects aim to add to the synthetic biology toolbox or change the way we clone, manipulate or even think about DNA, an application project aims to use synthetic biology to solve a project. In 2010, the winning Slovenia team created BioBricks that allowed the use of DNA as a scaffold to dock other enzymes in order to speed up reactions involving multiple enzymes. This is a great example of a foundational project. In contrast, in 2013, the 2nd place Imperial College London team used bacteria in a bioreactor to break down different types of plastics, a great example of an application project. Both types of projects are allowed in the competition and can do very well. Foundational projects however can sometimes be more difficult to sell, especially if you are not able to get as much data as you hoped or if your parts didn't work as planned. It can also be more difficult to undertake a human practices component as the implications of the project may be harder to brainstorm and it may be more difficult to engage the general public in what you're doing. While it can be very easy to get people expected about bacteria that clean up oil spills, designing a new way to optimize cloning, while potentially important and innovative, may be more challenging to explain to your community. The students may also have a more difficult time getting excited about a very foundational project. Although these projects can absolutely be done successfully and have been many times, it is something to think about as sometimes teams can make use of a great human practices component to compensate for setbacks experienced in other parts of the project. Whatever you choose, think about how you would tell a story about it and share your work with others, as this is ultimately what you'll need to do to have a good project.

A good place to start for project ideas is in the media. Have there been any hot topics this year that are relevant to your community? Are there certain pollutants that you community cares about or a disease that has been prevalent? Sometimes making use of current events in your area or beyond can help you tell a really great story. Another great place to look is in the literature. Although sometimes access can be challenging, this is something the mentors can help you with. Looking through the literature can help you figure out what's been done on a certain topic in the scientific world. Sometimes, taking a new paper that documents a cool gene or promoter recently discovered and making them into Biobricks to produce in your own cells can be a great project idea that is both feasible and novel to iGEM.

It is also important to think about what kind of experience and human resources you have access to. Picking a project about taking bacteria mars may be very cool, however it may be tough to talk to any 'experts'. Think about who you know and what they could help you with. These don't have to be scientists. For example, if you choose a project trying to detect some kind of chemical that effects crops, it could be very beneficial to talk to a farmer about the problems this chemical causes. If you need some scientific advice, such as someone who studies a particular disease, you can think about contacting a university professor, using resources such as VROC to talk to other scientists or simply talking to your mentors. Often times there are tons of people willing to give you information or opinions that can help your project along.

Once you have a problem or idea in mind, it is a good idea to take a look at previous iGEM projects. This can be done by browsing the wikis (websites) from each year which can be found in a link on the right hand side of the iGEM home page for each year. Looking at what other teams have done can help you ensure that you are not directly repeating what another team has done, and also may give you some inspiration. At all levels of the competition, but especially at the high school level, building off of previous iGEM work is encouraged. For example, many teams may document plans of using a

certain part but may not actually get the time to test it out. Similarly, many teams may use a part in one way, but you may be able to think about another way to use this part. For example, maybe they use an acid-sensitive promoter as a sensor while you decide to use it to only produce your protein of interest when high acid is around. Both of these can situations give opportunities for your own project.

In addition to other projects, it is important to look at the parts registry too (how exactly to navigate this is covered later on). By looking at previously submitted parts, you can also get ideas of natural extensions of what's been done already. Often times a part may be submitted, but it may need another part to function properly. Submitting this second part and showing that the two parts work together could be a useful project. Similarly, you may run into parts that were not submitted or that have problems, and fixing them or re0submitting them could become part of your own project. Carefully looking for parts can also save you form doing needless work. If you want to use a common part such as green fluorescent protein (GFP) for example, it' very likely that other teams have submitted all sorts of parts using GFP. You may want to put GFP with an inducible promoter for part of your system. You'll likely be able to find GFP with different promoters and ribosome binding sites already in the registry. This means that you can just take them and save yourself the hassle of constructing GFP with something else. Although making use of an already existing part doesn't count as submitting something new, making use of what's already there in conjunction with anything new you submit can speed up the process and increase your chance of obtaining results.

3. Necessary Skills for an iGEM team

With varied projects, iGEM teams often involve a variety of students. Although students with a keen interest in biology are clearly needed, iGEM teams often require other skills and interests too. In addition to working in the lab, designing and the wiki- or website, is vital to a successful team. As such, some students on the team may solely focus on building a functional and attractive website as this can be a very time-consuming task. In addition, some teams may implement computer modeling or other types of computer simulation or programming. Some teams have designed simple video games to explain their project while other teams have designed animations to show what is happening in their cells. Although this is not mandatory at high school level, there can definitely be a place for students interested in such topics.

Other important skills needed on an iGEM team include graphic design, public speaking and community engagement. The human practices part of the project for example, which we will go into more detail later on, asks students to think critically about the wider societal implications of their project. Human practices activities may include talking to members of the community, giving presentations to the public (outreach) or producing essays, videos, blogs, etc. that examine some of these implications. As such, students interested in engaging others or examining ethical issues can also contribute to a strong iGEM project. Finally, iGEM teams, being self-funded, often require strong fundraising efforts. This could involve contacting potential corporate sponsors and designing promotional materials. Students interested in these activities are also necessary for a team. In short, iGEM teams require a variety of skillsets and interests that go far beyond what happens in the laboratory. iGEM provides a unique opportunity for students with different backgrounds to work together and as such, should not be advertised solely to 'science-focused' students.

MOLECULAR BIOLOGY

THE BASICS

Magdalena Pop

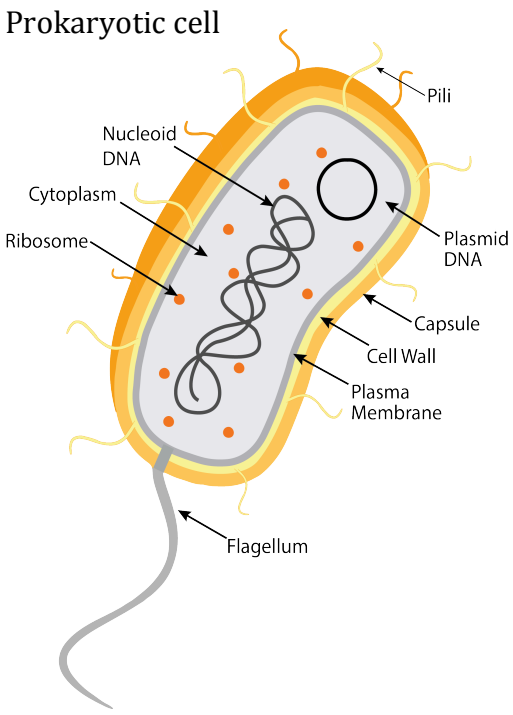
1. Cells

All live biological systems, whether natural or man-made (synthetic), are made of cells. A cell is the smallest unit of life, which means that a single cell is capable of all processes necessary to sustain life: metabolism, growth, reproduction, and adaptation.

Two components essential for any cell are its membrane and its DNA (Deoxyribo Nucleic Acid).

The cell membrane separates and protects the cell from its surroundings, while allowing for selected materials to enter and leave the cell. It is made of a double layer of phospholipids with proteins dispersed in it. This chemical composition results in just enough fluidity for substances to be able to pass through without loss of the integrity of the membrane. The outside of the cell membrane is studded with carbohydrates, which give specificity to cells.

Many small molecules pass through the cell membrane by diffusion from the side of the membrane where concentration is higher to the side where it is lower, i.e. down their concentration gradient. Because it doesn't require energy this process is known as passive transport and may involve the assistance of proteins or not. Passive transport aided by membrane proteins is called facilitated diffusion.



Sometimes small molecules must be moved against their concentration gradient. This is done by specialized proteins embedded in the cell membrane, whose job is similar to that of a pump pumping water uphill. Since it requires energy, this process is called active transport.

Big molecules (macromolecules) get in and out of the cell in transport sacs called vesicles.

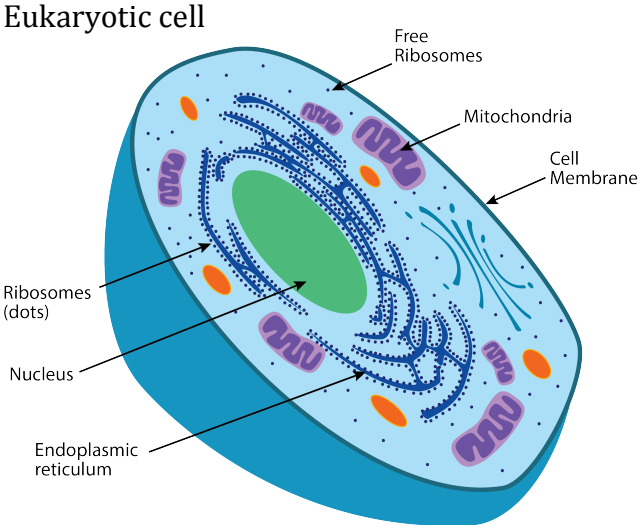
The DNA contains all the information needed by the cell. More about the structure and function of DNA will be discussed in the next sections.

Cells with simple structures, which include little else beside a membrane and DNA, are called prokaryotic cells. They are the oldest type of cells on Earth. The most common prokaryotic cells are the bacteria.

Later in the evolution of life on Earth, a different type of cell emerged, which has a more complicated structure. Most notably, its DNA enjoys extra protection inside an organelle called nucleus. Cells with a nucleus are called eukaryotic cells. Also

unique to eukaryotic cells are additional organelles, such as the mitochondria and the endoplasmic reticulum. All organisms big enough to be seen with a naked eye, as well as some microorganisms, consist of eukaryotic cells.

Eukaryotic cell

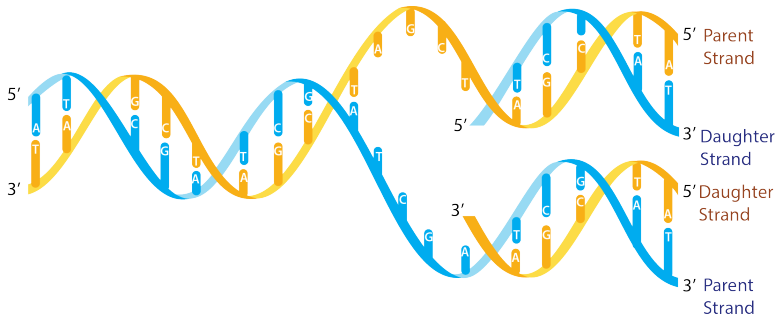


2. DNA

All cells store their information in DNA, which is packaged in one or several chromosomes. The information is coded as a particular sequence of chemical compounds, known as base pairs, along the length of the DNA molecule twisted into a double helix. Four chemicals form the basis of the DNA code: Adenine (A), Cytosine (C), Guanine (G), and Thymine (T). Due to their matching molecular structures, A pairs up with T, and C pairs up with G.

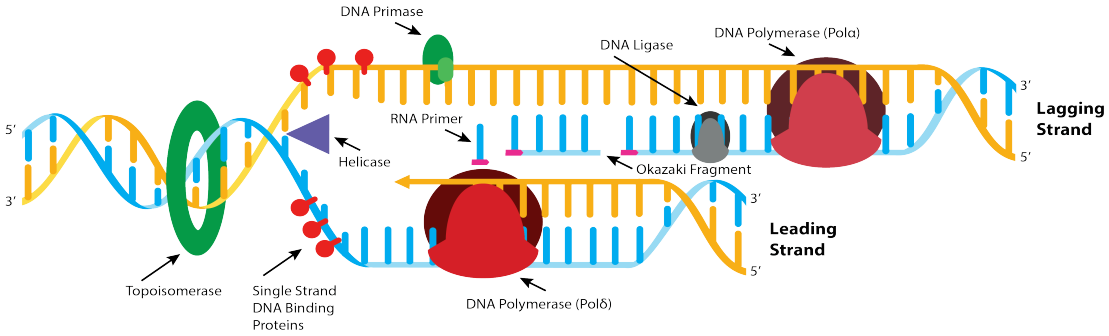
Each time a cell divides the information stored in DNA must be passed along in its entirety to the two new daughter cells. For both daughter cells to carry the exact same DNA as the parent cell, the DNA molecule needs to be copied – or replicated – prior to cell division. Essentially two identical copies of the parental double helix must be produced. This outcome follows elegantly from the complementarity of A and T and that of G and C. Each of the parent strands is used as template and copied separately through insertion of complementary bases across from each base in their sequence. As a result, the two new DNA molecules are identical to the original DNA molecule and consist of both a parent and a daughter strand.

Semiconservative DNA replication



The replication of DNA results from the coordinated activity of several enzymes. It starts with the unzipping of the double helix at a specific sequence called origin of replication, which creates a replication bubble. The bubble then grows in both directions along the DNA molecule, as the main replication enzyme – the DNA polymerase – uses the exposed sequences of the parent strands as template to copy them into the daughter strands. Because of the antiparallel orientation of the two parent strands, the copying of one of them – the leading strand – occurs continuously, while the copying of its complementary strand – the lagging strand – has to be done in short fragments called Okazaki fragments. The Okazaki fragments are then joined into a continuous strand by an enzyme called DNA ligase.

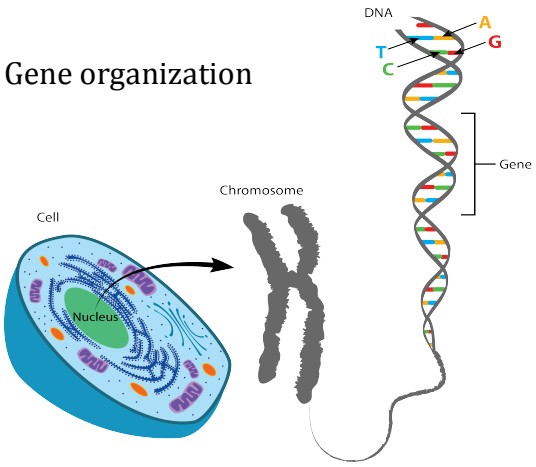
DNA replication machinery



3. Genes

A particular DNA sequence containing a complete unit of information is called a gene.

Gene organization



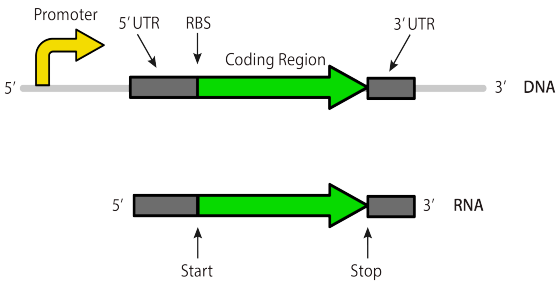
A gene includes the code for a functional product, which is typically a protein, together with sequences that serve regulatory functions, such as instructions for where to start decoding the information and where to end it.

A prokaryotic gene has a relatively simple organization. It starts with a promoter, which is followed by an uninterrupted coding region flanked by short stretches that play regulatory roles (UTRs).

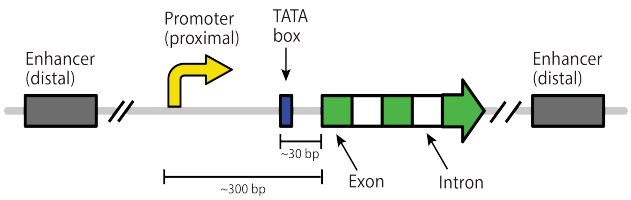
The organization of a eukaryotic gene is more complex. In addition to the promoter, a eukaryotic gene has extra regulatory sequences, such as enhancers, and its coding region is interrupted by non-coding sequences called introns.

A particular gene will serve its purpose when its DNA code is unlocked and used to make a protein. In this process, known as gene expression, the decoding of the information in the gene occurs in two main steps, transcription and translation. In transcription the DNA sequence is copied into a messenger RNA (mRNA). In translation the mRNA is translated into a product – a protein.

Typical bacterial gene

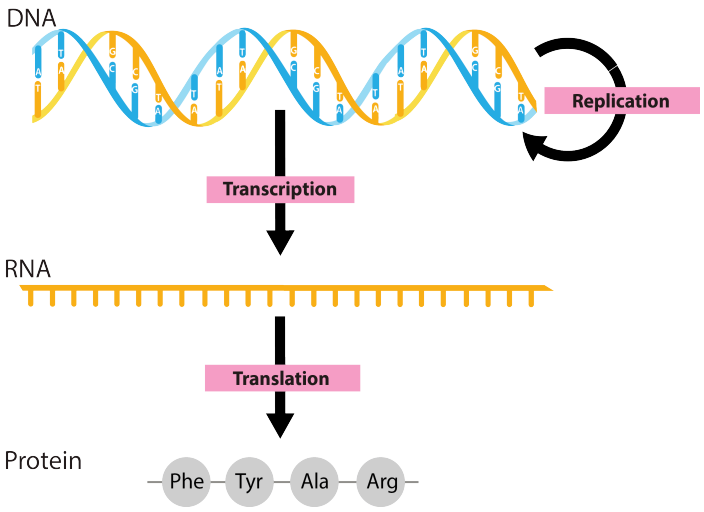


Typical human gene



4. Transcription and Translation

Gene expression



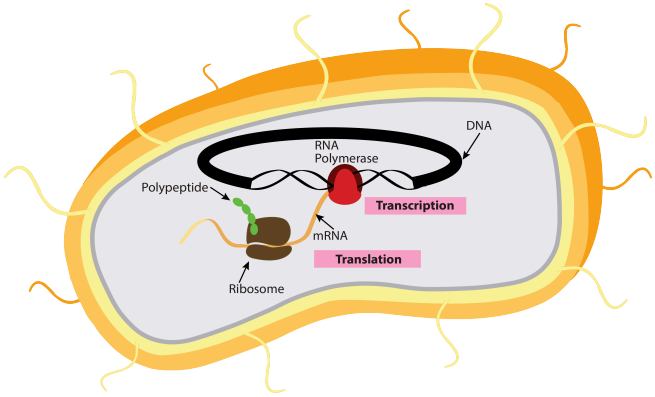
Transcription is carried out by an enzyme called RNA Polymerase. After binding to the promoter the RNA Polymerase opens the double-stranded DNA and then copies its sequence of bases starting at a specified site. The copy is created according to the base pairing rules, e.g. a C is copied into a G, and a T is copied into an A. However, Adenines are not copied into Thymines. Instead, they are transcribed as Uracils (U), which are chemicals unique to RNA molecules.

In a prokaryotic cell the resulting mRNA is immediately available for translation. This is because there is no nuclear enclosure and therefore the ribosomes, which do the translation, have easy and immediate access to the mRNA. Translation results in the production of multiple copies of the polypeptide (or protein molecule) encoded by the gene. To carry out the translation process ribosomes need amino acids – the main building units of proteins.

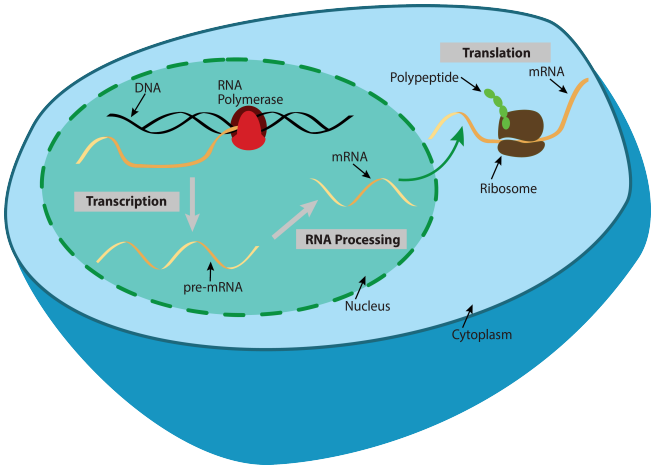
In a eukaryotic cell the process of transcription takes place inside the nucleus and it produces an initial, draft copy of the gene, called precursor mRNA (pre-mRNA). This draft copy is further edited (or processed) until the final mRNA is generated. It is the final mRNA that is sent outside the nucleus to be translated by the ribosomes.

During translation the ribosomes read the mRNA sequence as a series of three-letter words, or codons. For each successive codon the protein chain is extended by one amino acid. The matching of codons to amino acids occurs based on the genetic code. Since there are more codons available (64) than there are amino acids (20), the same amino acid is represented by multiple codons. There is only one exception: AUG is the only codon for the amino acid Methionine, and it is found at the beginning of all mRNA molecules, i.e. it functions as the START codon. And three of the codons, UAA, UAG and UGA function as STOP codons, signaling the ribosomes to stop the translation.

Prokaryotic transcription and translation



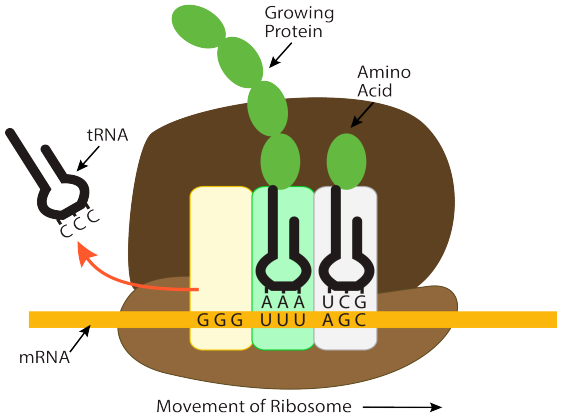
Eukaryotic transcription and translation



The genetic code

First Letter	Second Letter: U	C	A	G	Third Letter
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

Translation

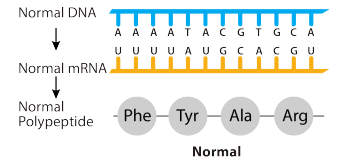


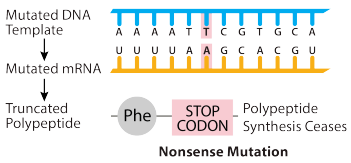
The amino acids cannot find their respective codons by themselves. Instead the matching is done by molecules of transfer RNA (tRNA). There are as many tRNA molecules as there are codons and each of them specializes in transferring one particular amino acid to the ribosomes. The feature of the tRNA molecule that enables the match is its anti-codon sequence, which is the exact complement to the codon for the particular amino acid that tRNA is carrying. For example, there are two tRNA molecules that transfer the amino acid Phenylalanine (Phe) to the ribosomes. The codons for Phe – UUU and UUC – are recognized by the complementary anti-codons – AAA and AAG, respectively – that these tRNA molecules have.

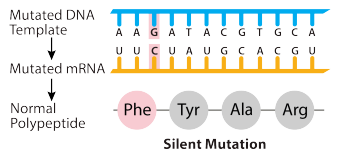
5. Mutations

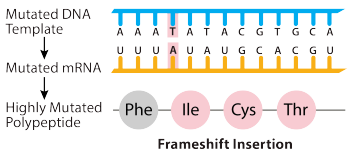
Mutations are changes in the DNA sequence of a gene. Many different changes may occur, affecting one or multiple base pairs in the sequence. Some mutations change one base pair into another without changing the total length of the sequence (for example, changing an adenine to a cytosine). Other mutations eliminate or add one or more base pairs thus altering the length of the sequence. Ultimately what is important is the effect on gene expression, i.e. how the mutation affects the protein encoded by that gene.

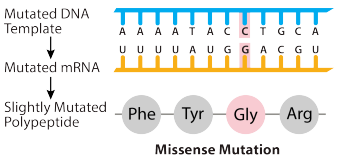
The effect that mutations have on gene expression can vary widely. Silent mutations are changes that have no effect whatsoever on the protein being made. Missense mutations result in a slightly altered protein, while nonsense mutations have more drastic effects ranging from a mis-functioning to a non-functioning protein.

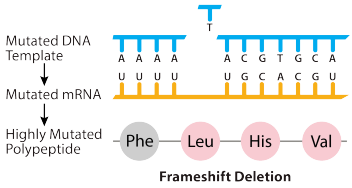












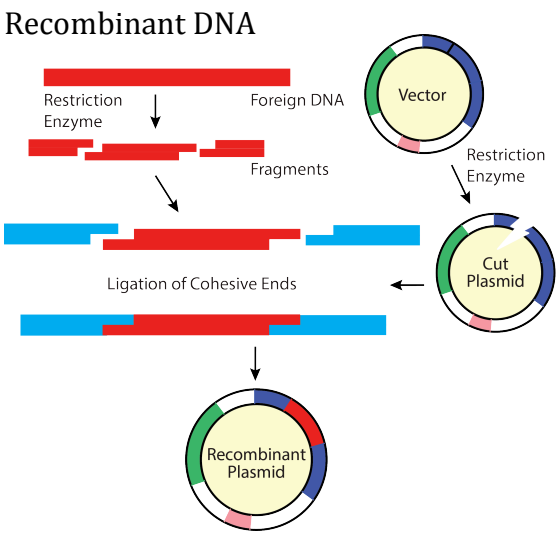
Sometimes mutations occur that shift the correct reading frame of a gene. Such frameshift mutations cause the ribosomes to misalign and to misread the mRNA. The mRNA is now read as a series of three-letter words different than the ones in the original code. Typically, a frameshift mutation has a drastic effect on gene expression, resulting in a defective, nonfunctional protein.

6. Bioengineering

This is a broad and rapidly evolving field of human endeavor, which takes an engineering approach to life sciences and aims to solve problems through redesigning biological systems and processes. Products of bioengineering are used in many different areas and industries, most notably in agriculture, medicine, energy and the environment.

The branch of bioengineering called synthetic biology produces artificial life forms with useful properties by modifying the DNA of existing, natural organisms. Synthetic biology emerged about half a century ago when recombinant DNA technology, or molecular cloning, was first developed.

One of the earliest synthetic organisms created in the lab was an Escherichia coli (E. coli) bacterium, which carried a frog gene. The experiment involved the cutting of the frog gene DNA out of the frog chromosome followed by its insertion into the bacterial DNA.



The procedure used in this early experiment is still central to synthetic biology, and it involves a few essential components: Scissor-like enzymes called restriction endonucleases, which cut DNA at specific sites, and a glue stick-like enzyme called DNA ligase, which glues back together the free ends of the cut DNA. Also essential to the success of the procedure is the use of a special kind of bacterial DNA, called plasmid DNA, which carries genes that render the bacteria resistant to particular antibiotics. This is important because it enables the experimenter to screen out the bacteria that do not carry the recombinant plasmid DNA, simply by adding the antibiotic to the growth medium.

Resources

http://ghr.nlm.nih.gov/handbook/mutationsanddisorders/possiblemutations

SYNTHETIC BIOLOGY PRINCIPLES

Magdalena Pop

1. Introduction

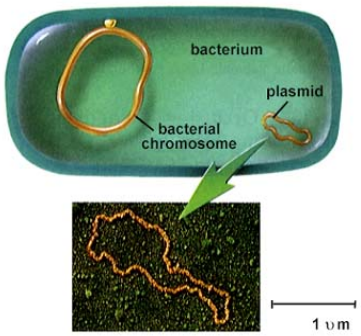
Specialists in synthetic biology regard cells as programmable machinery, whose components work together in an intricate yet predictable manner. In their perspective, components of cells – such as DNA and proteins – interact and function similarly to the components of human-built machines. One can take apart all sorts of cells, study and catalogue their components, and then design new ways to reassemble them to produce biological machines with novel functions. In the process, a growing collection and catalogue of DNA parts with specific functions – a Registry of BioBricks – emerged as an essential resource to reprogram living cells.

2. How to Produce a New Protein in Bacteria

Say you wanted to make bacteria produce protein Z, a protein not normally made by bacteria. How would you go about this project?

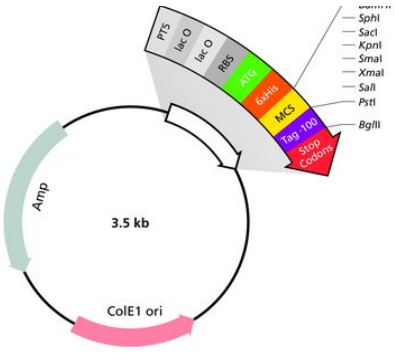
In order for bacteria to produce protein Z, the gene for protein Z – its DNA code – must be put inside the bacteria. It is useful to know that bacteria accept new DNA code relatively easily if carried by a plasmid vector. This is a small circle of DNA with its own origin of replication, which allows it to replicate independently of the bacterial chromosome. Plasmids also contain a gene for some antibiotic resistance (e.g. to Ampicillin), as well as multiple sites for restriction enzymes, both of which are essential features for plasmids to work as effective vectors. The antibiotic resistance gene works as a filter. Because antibiotics normally kill bacteria, only those that have the plasmid and are therefore resistant will survive in the presence of the antibiotic. The sites for restriction enzymes are locations in the plasmid DNA where engineering can be done, i.e. new DNA parts can be inserted. You will learn more about restriction enzymes later in this section.

Bacterial plasmid



In other words, your project will be to assemble the gene for the expression of protein Z in a plasmid, and then introduce that plasmid into bacteria. For this you will first want to find the DNA code for the amino acid sequence of protein Z – its entire coding region from the start to the stop codon. But remember that a gene is made up of more than just the coding region for a particular protein. Additional parts necessary for the gene to function are a promoter and a terminator – to mark the start and the end of transcription, respectively, and a ribosome-binding site (RBS) – needed for the correct initiation of translation.

Plasmid restriction sites



Assembling your gene will be like assembling a circuit using the following DNA parts: (1) a promoter, (2) an RBS, (3) the coding region, and (4) a terminator. For this genetic circuit to function, i.e. produce protein Z, the order in which the four DNA pieces are

assembled is of essence.

Promoter RBS Coding Region Terminator

3. Promoters

How does the RNA Polymerase locate a gene targeted for transcription? How does it know where to start copying the DNA to create the mRNA (the transcript)? This information is provided by the promoter sequence, which signals the RNA Polymerase where to begin.

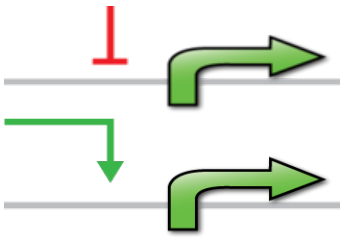
There are many different types of promoters. How are they different and how would you choose the promoter that suits your purpose the best?

If your aim is to make bacteria that produce protein Z constantly and regardless of the growth conditions, you will need to use one of the constitutive promoters. These are promoters that depend on little else beside the bacterial RNA Polymerase. In other words, if the DNA is in the bacteria, protein Z will be continuously expressed. An example of constitutively expressed promoters are the Anderson promoter collection, which are available on the registry of standard parts (see The Registry section). These promoters are all constitutively expressed, but each of the promoters in this collection express the protein in different amounts.

Promoter

How about if you wanted to control the expression of protein Z in the bacteria? In that case, you would look for a promoter that can be switched on or off by a specific agent, e.g. a certain substance, or temperature. Controllable promoters exist that are either positively or negatively regulated. The activity of positively regulated promoters increases in the presence of certain inducing agents. The activity of negatively regulated promoters decreases in the presence of specific repressors.

Promoter types



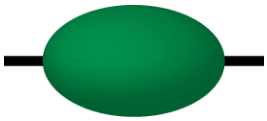
Some common examples of controllable promoters are the LacZ promoter, the araBAD promoter, or the T7 promoter. In the absence of an inducing agent, the activity of the LacZ promoter is blocked (or repressed) through the binding of a repressor protein. Transcription of genes from the promoter will not occur. When an inducing agent is present – e.g. Lactose or IPTG (IsoPropyl – beta D Thio Galactoside) – the repressor becomes inactive and the promoter is switched on. For the araBAD promoter the most common inducer is Arabinose. The T7 promoter needs T7 RNA Polymerase – a protein found in the T7 bacteriophage (a bacterial virus) – to get switched on.

4. Other DNA Parts Essential for Gene Expression

In addition to a suitable promoter and the protein coding region, your circuit must also include an RBS, and a terminator.

As suggested by the name, Ribosome-binding Sites (RBS) are sequences where the ribosomes – the translation machinery – attach in preparation for making the protein. Without an RBS sequence, the

RBS Biobrick symbol



ribosomes would not be able to position themselves properly onto the mRNA transcript and, as a result, the translation would fail. RBS sequences are typically short and they are found only a few nucleotides upstream of the start codon.

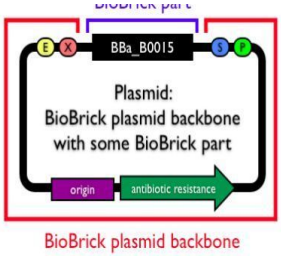
Terminator Biobrick symbol

Whereas an RBS is needed for proper translation, a terminator sequence is a part required in transcription. Once again, the name says it all! The particular sequence of nucleotides in a terminator causes the DNA at the end of a gene to fold, which blocks the progress of the RNA Polymerase and effectively terminates transcription.

5. Designing the Genetic Circuit

Let's assume that all four DNA parts are available in the Registry. This usually means that all four (or more if you are producing more than one protein) are available as BioBricks carried by plasmid vectors; some may even be pre-assembled in the same plasmid. For example, we can assume that a construct made of an RBS – Protein Z coding region – Terminator has already been pre-assembled or BioBricked in a plasmid. We will call this plasmid A. And we will assume that the appropriate promoter is present as a BioBrick in plasmid B.

Biobrick

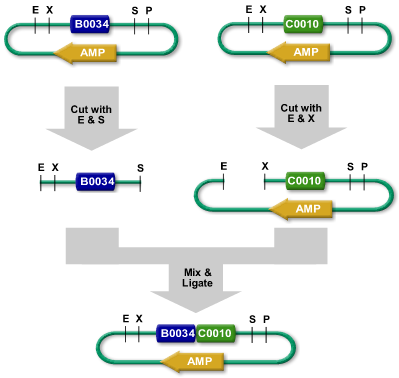


As long as both plasmid A and B are available, you are ready to build the complete genetic circuit for the expression of protein Z. You have two options. One option would be to cut out the promoter from plasmid B and insert it in front (or upstream) of the construct in plasmid A. Alternatively, you could cut out the RBS – coding part – terminator construct from plasmid A and insert it after (or downstream from) the promoter in plasmid B. We'll go with the first option.

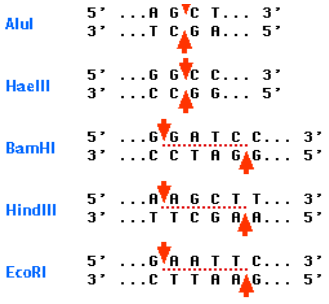
6. Assembling the DNA – Cutting and Ligating

To cut the plasmids you will need restriction enzymes, which are scissor-like protein molecules specialized in cutting DNA. There are many different restriction enzymes, and what makes them useful is that each of them cuts a unique DNA sequence with palindromic properties, which means that the sequence reads the same in both directions. Restriction enzyme cuts are either staggered – producing sticky/cohesive ends – or straight – resulting in blunt ends.

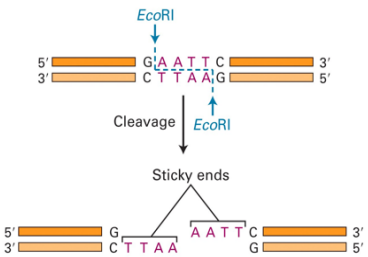
Biobrick assembly



Restriction cut sites

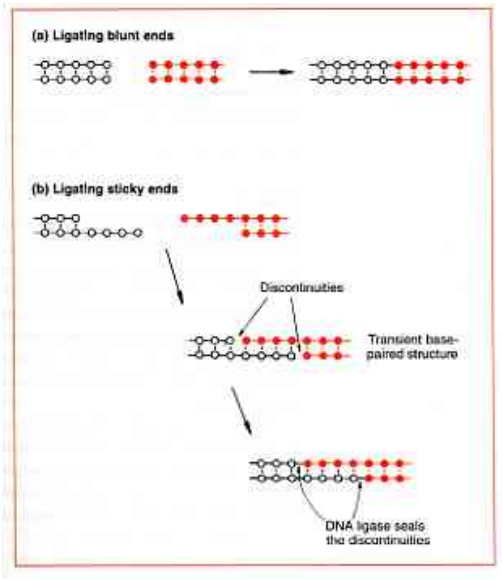


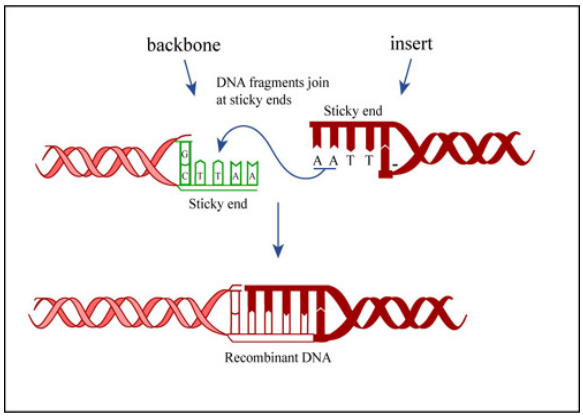
'Sticky' ends



Whether sticky or blunt, free DNA ends resulting from restriction can be joined or glued together later by an enzyme called DNA ligase. Usually sticky ends are easier to join because the base-pair complementarity between the single-stranded overhangs favors the ligation.

Ligation





Ends cut by the same restriction enzyme can always ligate. Ends cut by different restriction enzymes cannot ligate unless they are blunt or their overhangs are complementary. For example, XbaI (X) and SpeI (S) are two different restriction enzymes whose cuts are complementary and will stick together in ligation. That is why these two restriction enzymes are used frequently for the assembling of BioBricks.

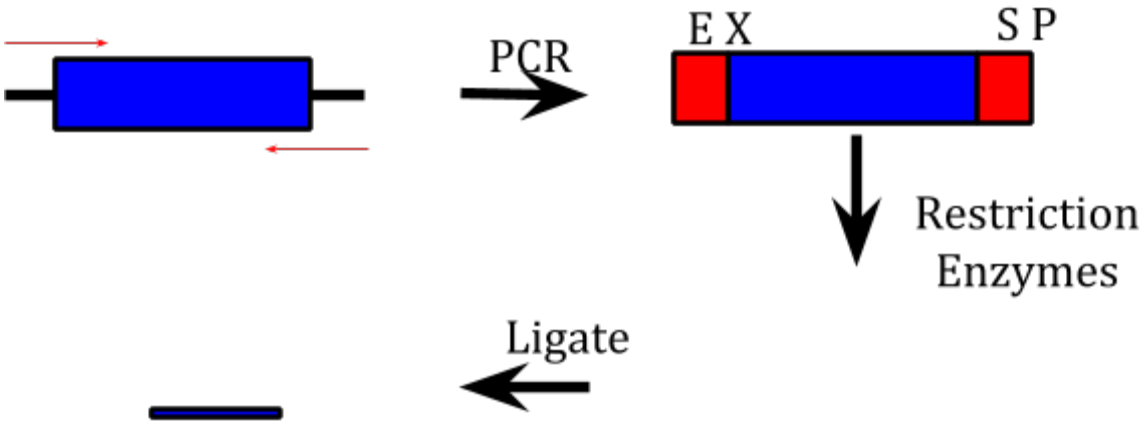
XbaI	5' – T^ CTAGA – 3'
	3' – AGATC^T – 5'
SpeI	5' – A^CTAGT – 3'
	3' – TGATC^A – 5'

Going back to your project now, the promoter will be cut out from plasmid B using restriction enzymes E (EcoRI) and S (SpeI), while plasmid A will be opened by cutting it with E (EcoRI) and X (XbaI). The E sticky ends of the two parts will then join together in ligation, and so will the S and X sticky ends (see above). This will result in the desired genetic circuit, with all four necessary parts for the expression of protein Z assembled in the correct order within the plasmid A backbone. The newly engineered plasmid will then have to be added to the bacterial cells via transformation. You will learn how to do transformation of bacteria in the Methods section. Finally, to ensure that the project has been successfully completed, you will need to verify that the transformed bacteria are indeed capable of expressing a functional form of protein Z.

7. Making a New BioBrick Using PCR

What if the gene for protein Z is not available as a BioBrick in the Registry? In that case you will need to make the BioBrick for protein Z yourself. For this you will use a method called Polymerase Chain Reaction (PCR – see Methods section). This method will enable you to select the gene for protein Z out of a DNA pool, and amplify it. How do you find your target gene? If you know its DNA sequence, it's easy. You place detective-like DNA molecules called primers in the DNA pool. These are short fragments of single-stranded DNA that match the start and the end of your target gene only and nothing else in the DNA pool. The primers will be able to find their matching complementary sequences, bind to them, and prime – or initiate – the copying of the in-between gene. The power of PCR is that – in a very short time – only one copy of the target DNA sequence can turn into millions of copies.

PCR assembly

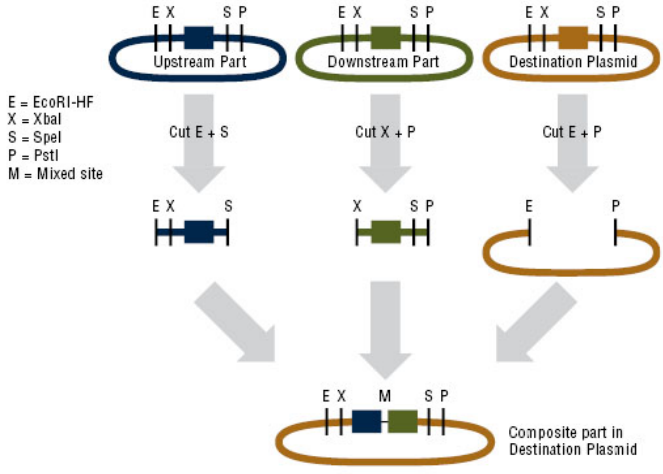


But what do you do with those many copies? Remember that your aim is to biobrick the gene, which means that you want to insert it (or paste it) in a plasmid. This requires that your gene is flanked by suitable restriction enzyme sites, to allow it to stick into an opened plasmid. Meeting this requirement is simple: when you design or choose the primers for PCR, in addition to sequences matching your gene make sure to fit them with appropriate restriction enzyme sites. This will allow you to restrict the PCR product and conveniently ligate it in a destination plasmid.

8. BioBricking Using the 3A Assembly Method

Traditional assembling of DNA parts takes fairly long. Why? Because ligation requires that DNA parts first go through a time-consuming purification procedure called gel extraction (see Methods). This purification gets rid of any DNA pieces that you don't want to have in the final construct. If no purification is done after cutting, and all DNA pieces are left together, chances are high that ligation will regenerate the original plasmid along with the new construct. And because both the original and the newly constructed plasmid have the same antibiotic-resistance, you can't screen out the original simply by throwing a new antibiotic in the growth medium.

3A assembly



To eliminate the need for gel extraction and save time, an alternative method was developed called the 3A Assembly method.

In this method, the final construct is assembled in a destination plasmid whose antibiotic-resistance gene differs from those carried by the original plasmids. Three distinct antibiotic-resistance genes (3A is short for 3 Antibiotics) are thus involved, two found in the original plasmids that provide the parts, and a third in the final, destination plasmid. This means that right after restriction all the cut parts along with the empty plasmids can be thrown together in the ligation

mixture, without prior separation by gel extraction. Although ligation will result in several plasmids, all of them except for the correctly assembled one will be screened out. How? By making sure to add the third antibiotic to the growth medium.

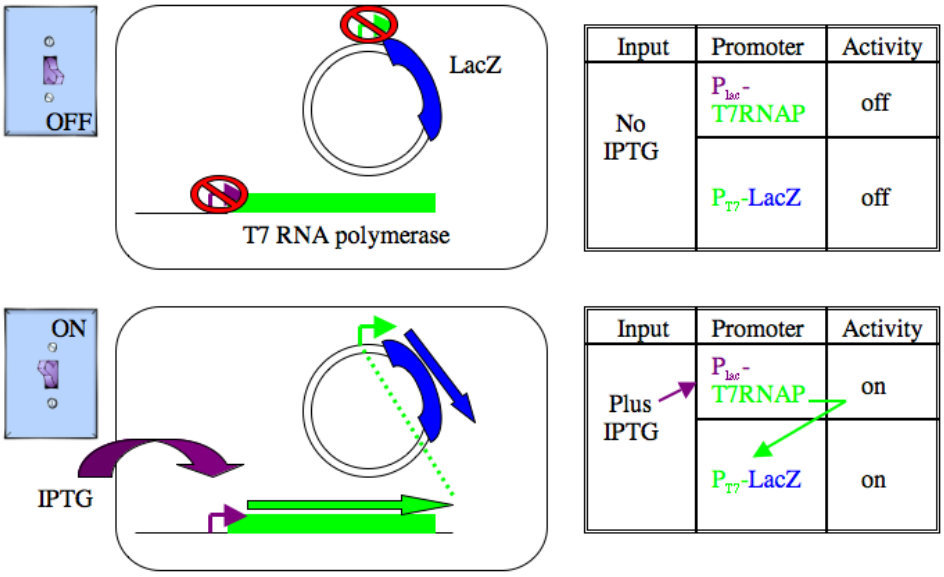
9. Reporter Genes

If you designed a new genetic circuit, where a gene's expression is controlled by a novel regulatory system, a fast way to check that the new system works as expected is by testing it first with a reporter gene. What is a reporter gene? It's a gene that produces an easily detectable output – e.g. a colored or fluorescent product – and can provide you with a quick report on how well your circuit functions.

Say, for example, that you wanted to place your gene of interest (e.g. the gene for protein Z) under the control of the T7 promoter. As described above, the T7 Promoter needs the T7 RNA Polymerase to become active. And where does the T7 RNA Polymerase come from? In your circuit design, it comes from the gene for T7 RNA Polymerase placed under the control of the LacZ promoter. Your plan is to switch on the LacZ promoter with IPTG, which will make T7 RNA Polymerase, which will activate the T7 Promoter, which will finally make the protein you want (Protein Z). In other words, your target output response (production of protein Z) is expected to occur every time the T7 promoter is switched on by input of T7 RNA Polymerase from the IPTG-induced LacZ promoter.

To test that your system works, instead of the gene for protein Z, you would initially place a reporter gene downstream from the T7 promoter. You could for instance use the gene for beta-galactosidase as a reporter. And, in short, you will know if beta-galactosidase was expressed if the bacteria turned blue. With no IPTG there would be no T7 RNA Polymerase to switch on the reporter and the bacteria should be white. Adding IPTG should switch on expression of T7 RNA Polymerase, which in turn will switch on expression of the reporter and turn the bacteria blue.

LacZ reporter



Input	Promoter	Activity
No IPTG	P_{lac}-T7RNAP	off
	P_{T7}-LacZ	off

Input	Promoter	Activity
Plus IPTG	P_{lac}-T7RNAP	on
	P_{T7}-LacZ	on

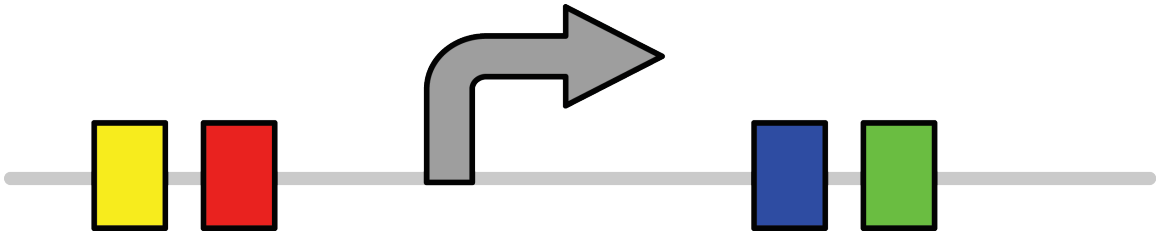
The bacterial gene for beta-galactosidase is one of the earliest reporters used in bioengineering. More recently, other genes useful as reporters were discovered, such as a jellyfish gene that makes a green fluorescent protein (GFP), a firefly gene for a bioluminescent protein, and various genes from corals coding for proteins of different colors.

THE BIOBRICK

Emily Hicks and Lisa Oberding

1. What's a Biobrick?

A very important component of the iGEM competition is the DNA pieces that are generated. These are known as Biobricks. Biobricks are DNA 'parts' that have some sort of function. There are three main components of a Biobrick. There is the 'part' sequence that encodes for the protein or regulatory device that we're interested in. This could be a sequence encoding the gene for green fluorescent protein, a sequence encoding a promoter element or a terminator, etc. This 'part' sequence is flanked by specific sequences of DNA on either end. These specific sequences are called cut sites, as they are recognizable by certain restriction enzymes whose function is to cut DNA. A Biobrick has three of these restriction enzyme sequences on each side of the part sequence. These sites include: *EcoRI*, *NotI*, *XbaI*, *SpeI*, and *PstI*. A schematic of a Biobrick can be seen below.



EcoRI (yellow),
NotI (not shown),
PstI (red)
PREFIX

Promoter Sequence

SpeI (blue)
NotI (not shown)
PstI (green)
SUFFIX

It's important to note that the three cut sites that come before the gene are called the prefix, while the three sites that come after are called the suffix. The 'part' sequence, together with this prefix and suffix sequence together make up what we call a Biobrick.

2. Why are Biobricks important?

Biobricks are important for two main reasons: standardization and modularity. By creating Biobricks, teams then test these Biobricks and find out certain parameters for them. For example, they may test a Biobrick promoter and find that it has high activity levels in the presence of high pH, and low activity levels in the presence of low pH. By having a 'standard' part that they are testing, other people can use this information to use that promoter in a different application. The hope would be that the part would behave exactly the same way as it is a 'standard part' (we know of course that science is rarely this straight-forward however!). The other reason is modularity. By having this standard for Biobricks, there is a simple method to put these parts together. This method is called the Biobrick assembly standard. In brief, if we wanted to put together two parts: part A and part B, there are two approaches we could take using this assembly standard. First we have to figure out which part needs to go upstream (in front) of the other part. If we're putting together a promoter and a gene for example, we would want the promoter to come first to enable transcription of our gene. With that in mind, we have two choices: we can use the promoter as our vector, and we can insert the gene directly downstream of the promoter, or we can insert the promoter directly upstream of the

gene, using the gene as a vector. The details of how this is done are covered in much more detail in the protocols section.

This assembly method allows us to string together single Biobrick parts and build increasingly complicated genetic circuits. Using a promoter Biobrick, a Biobrick encoding green fluorescent protein and a terminator Biobrick for example, it is possible to string these three basic parts together to build a simple genetic circuit, termed a 'composite' part. Teams can use basic parts contributed by other teams, couple with their own novel parts to create new genetic circuits.

3. Limitations to the BioBrick Assembly Method

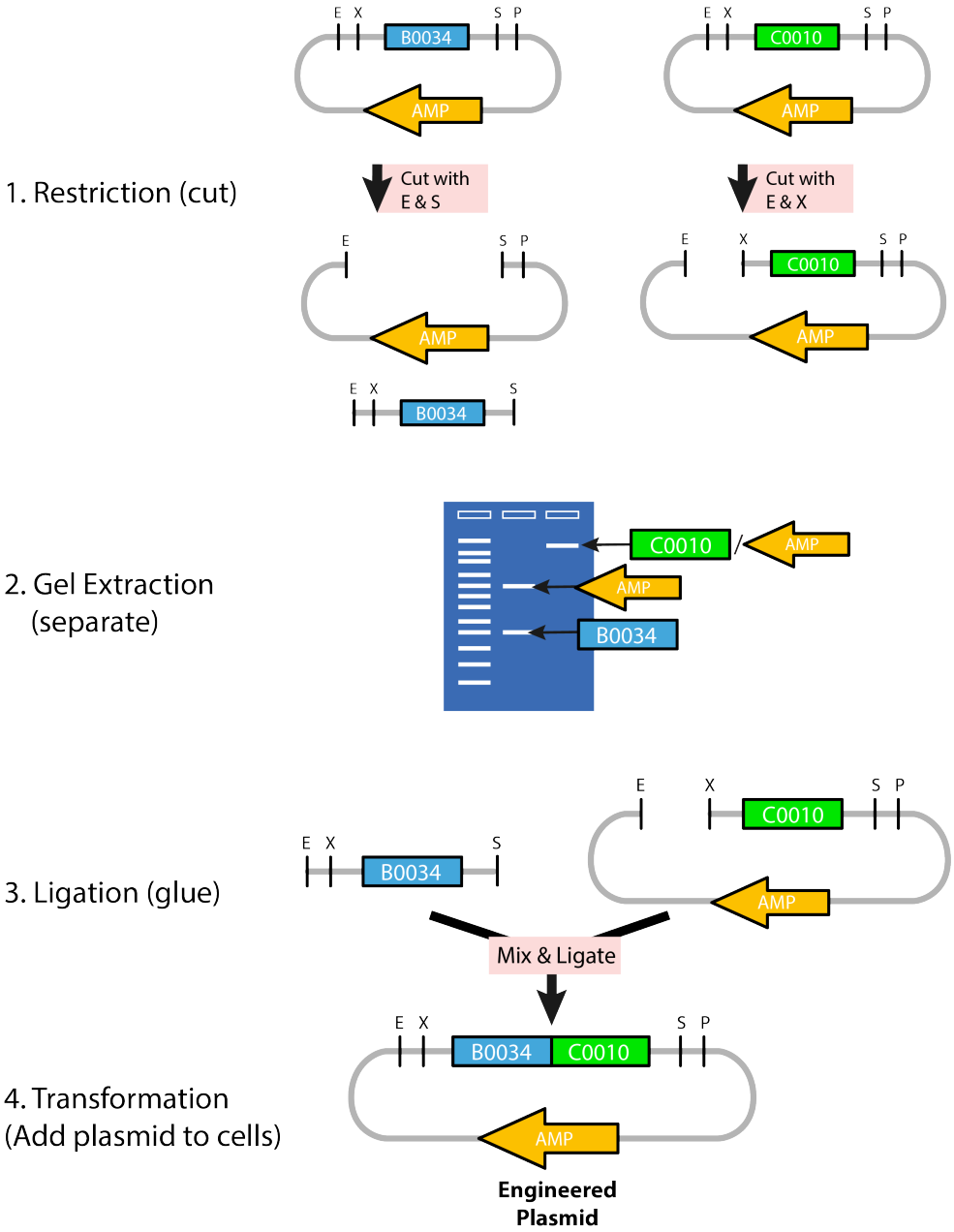
Although the Biobrick assembly method makes it, in theory, easy to put Biobricks together, it does have several shortcomings. With it's very nature, it can be difficult to deal with more 'abnormal' parts. For example, if you needed to create a fusion protein, where two proteins were put together, the Biobrick method needs to be altered. Another example is if you were using a large gene and creating a new biobrick, it would be necessary to remove all the 5 Biobrick cut sites, often called 'illegal' cut sites from this gene sequence. This means that in order to sybmit your new Biobricked gene, you would need to change any naturally occurring *EcoRI*, *NotI*, *XbaI*, *SpeI* or *PstI* sites in the gene. This is generally done by introducing a silent mutation to change the cut site without interfering with the amino acid code. Although this is completely doable and relatively easy if you were to synthesize your parts from a synthesis company such as IDT, it can be time-consuming if you are not using synthesis.

The Biobrick assembly standard is also not always the most efficient way of putting DNA pieces together. Outside of iGEM, there are a variety of different assembly strategies such as golden gate or Gibson assembly. Although we will not go over the details of these other methods, it's important to note that they do exist and should your team consider putting together a large number of parts, this may be something to consider. These methods do however lack the simplicity that the Biobrick method offers and often require additional reagents.

ASSEMBLY METHODS

Zak Stinson and Sutherland Dube

1. DNA assembly outline

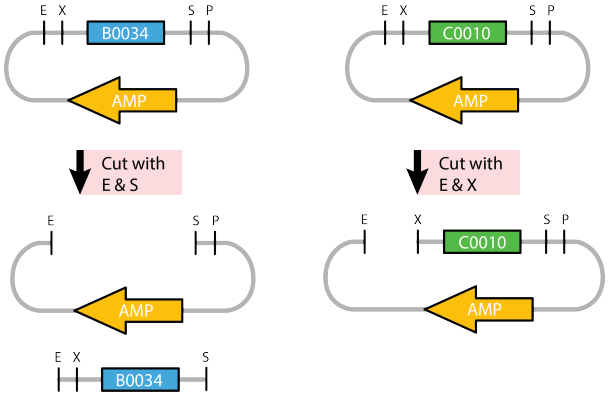


2. Restriction

A restriction is the process of cutting DNA at specific sequences with molecular scissors. These scissors are known as restriction enzymes, and the sequences they cut at are called restriction sites. Each restriction enzyme has its own unique restriction site. Within a Biobrick plasmid, there are specific restriction sites on either side of the gene of interest (GOI). The GOI in this picture is blue (labeled as B0034) in the picture on the right, and green (labeled as C0010) in the picture on the left. By using a specific restriction enzyme on either side of the GOI, we can "cut" it out

The restriction sites located within the Biobrick plasmid are:

-EcoRI (E)
-XbaI (X)
-SpeI (S)
-PstI (P)



Here, we used E and S to cut one plasmid (left) and E and X to cut the other (right).

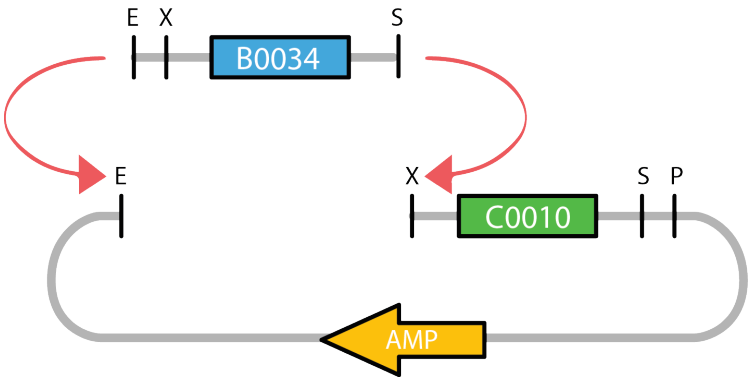
However, there are two restriction sites on either side of the GOI, and **it makes a difference which ones you use.** That is because the in the next step we want to "glue" the GOIs together in a process known as a ligation. However, only specific cut sites can be ligated together.

Restrictions sites that can be glued together are as follows;

-E and E
-X and X
-S and S
-P and P
-S and X

All restriction sites can be "glued back together with themselves

The ends to be "glued" together are E and E, as well as S and X. Both of these are present in the above list, so the parts can be ligated together later on.



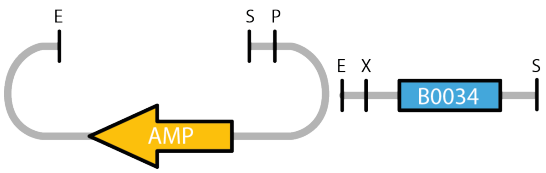
One other issue that can arise is if you wanted the part B0034 (blue) to be placed **after** the part C0010 (green). For example, if C0010 was a promoter, you would want it to be before your gene. In this case, you would have to change which enzymes you restrict with, so that you would "open up" the plasmid behind C0010, rather than in front.

3. Gel Electrophoresis

Before you "glue" your DNA pieces back together (with the extra DNA part), there is one more step. Because you use many reagents as well as enzymes in your restriction reaction, and you want to get rid of this extra stuff before you move on to ligating your DNA together. To isolate just the DNA we want, we use a process called gel extraction. Gel extraction is based on a technique known as agarose gel electrophoresis, which separates DNA based on its size.

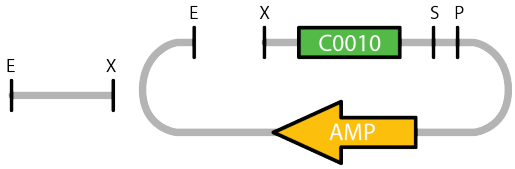
To do this we make an agarose gel (see protocol book)), and add our DNA to "wells". DNA is negatively charged, so by running a current through the gel, the DNA in the wells will migrate towards the positively charged end. Because smaller pieces of DNA can travel faster through the gel, it will separate the different sized strands of DNA, with larger ones closer to the wells, and smaller ones closer to the end of the gel. This method will also get rid of any enzymes and reagents from your restriction.

If you recall, these are the pieces of DNA you would get from your restriction:



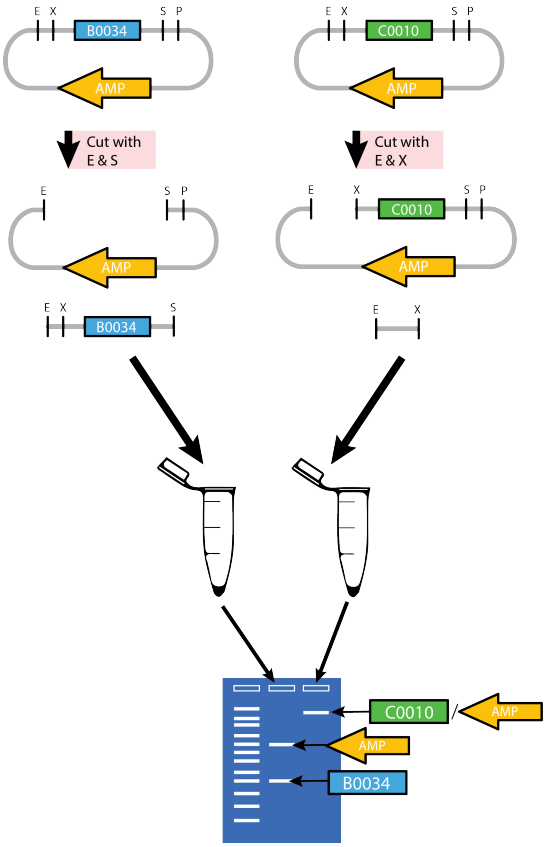
These two parts were originally together but the part was "cut out" of the plasmid.

The empty plasmid is no longer needed but it will still be present on the gel



These two parts were originally together but the green piece of DNA was "cut out" of the plasmid.

Because this green part is so small, it will not be seen on the gel.

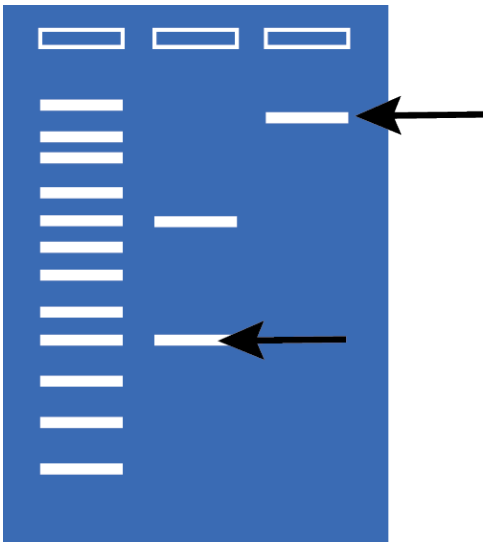


If we separate these four pieces based on their size, we will see three distinct bands of DNA, because the small part cut out of the plasmid (just DNA, no GOI) is too small to be seen. The part B0034 will be relatively small. The plasmid that it is cut out of will be larger. Because the other plasmid contains the part C0010, it will be even larger than the empty plasmid.

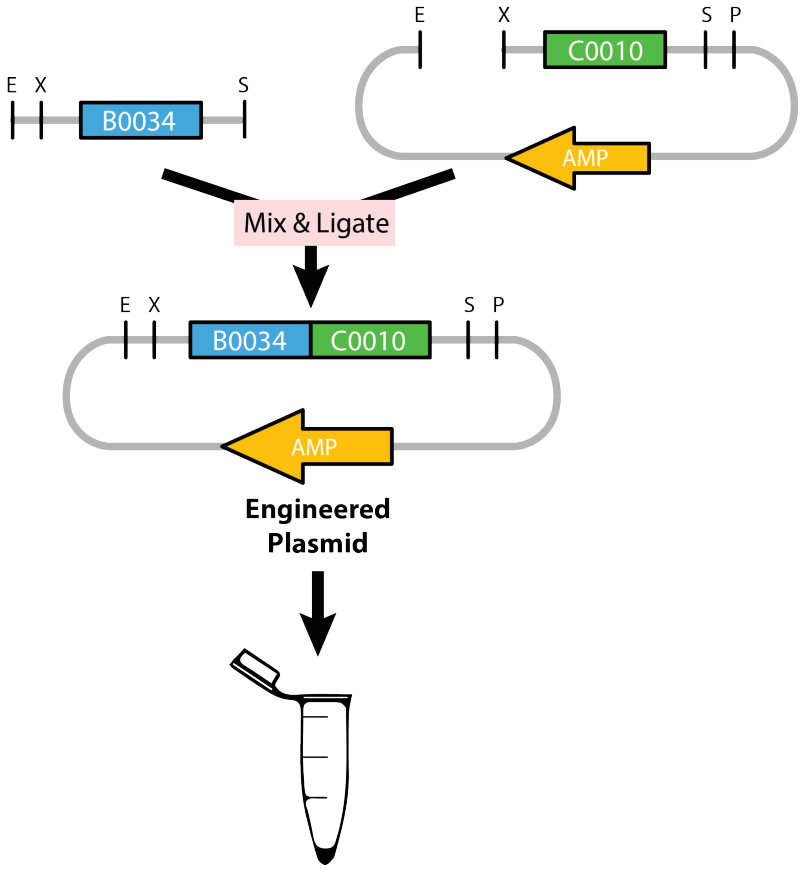
After it is finished, we note where on the gel the two parts we want to "glue" or ligate together are in the next step. In this case, we want to ligate together B0034 and C0010/AMP.

Using a razor blade, we then cut the part out of the gel. This ensures that we have only the DNA that we want. In the picture below, the areas we cut out are outlined in black.

When we cut the DNA out it will still be embedded in the agarose gel. In order to get rid of the gel, we purify the DNA from the gel using a standard protocol (see protocol book).



4. Ligation

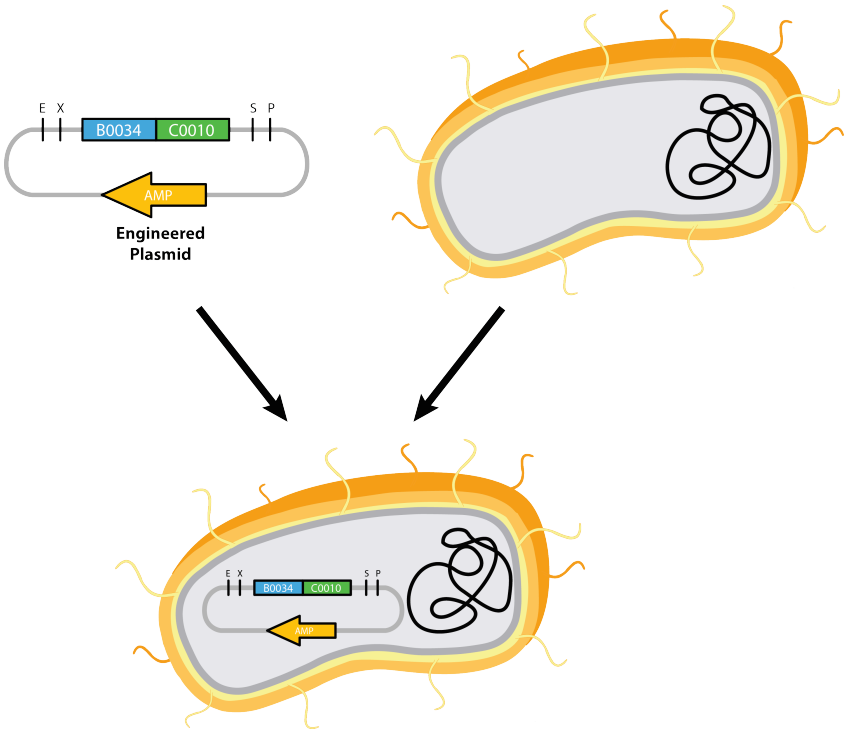


A ligation is the process of "gluing" two DNA parts together using the enzyme DNA ligase. In order to do this we take both DNA parts that have been restricted and gel extracted (and that have been purified out of the gel), and we combine them with DNA ligase and a few other reagents.

5. Transformation

Now that we have a full plasmid with both of our parts together in it, we need to make more of it. In order to do this, we put it into e. coli, so the organism will make lots of the plasmid. We get the plasmid into the bacteria through a process called transformation.

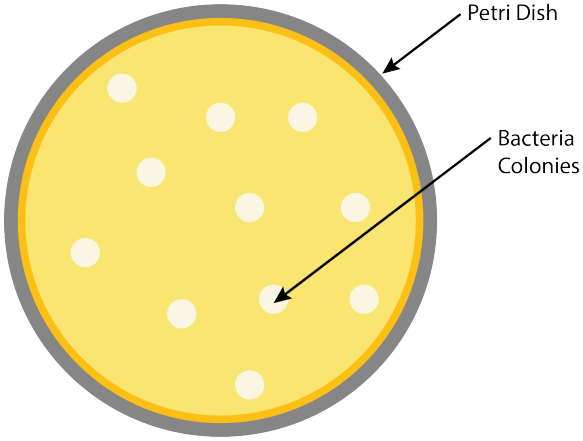
Cells are surrounded by a lipid membrane, so in order to get the DNA through the membrane, we disrupt it through heating it up. This enables the plasmid to enter the cell, and the natural machinery in the cell replicates (makes more of) the DNA.



In this picture of the *E. coli* cell, the yellow inside of the cell is the genomic DNA of the cell, which is different than the plasmid DNA that we insert into the cell.

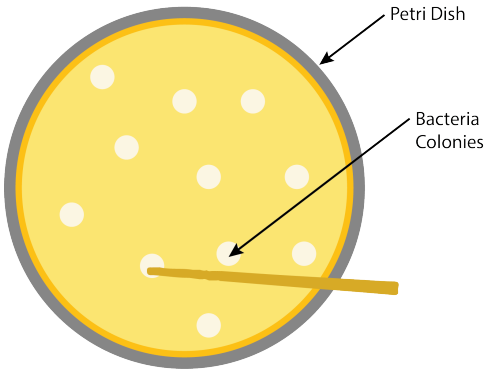
In order to determine which cells have our plasmid inside of them, we utilize the antibiotic resistance found on the plasmid of our cells. To do this, we spread our cells on petri dishes that contain a growth medium (nutrients for the cells to grow) as well as an antibiotic. In this example, the plasmid contains the resistance for AMP, or ampicillin. So, if we grow our cells on a growth medium containing ampicillin, all cells that don't contain our inserted plasmid will die. Therefore, and cell colonies that we see on the plate should contain our plasmid.

The plate in the figure to the right contains the antibiotic ampicillin, so all of the bacterial colonies growing should contain our plasmid. All of the cells from our transformation that did not have our plasmid would die due to the ampicillin.



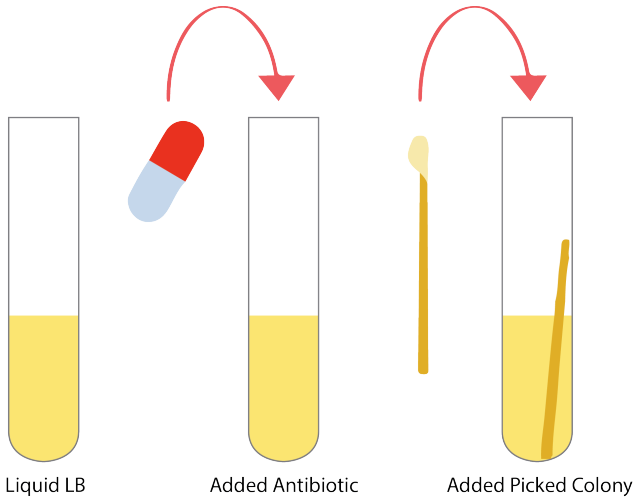
6. Picking colonies from plates

Each colony on the plate was started by a single cell that was successfully transformed with a plasmid from our ligation. In order to see if that plasmid has our successfully assembled part, we need to grow some of these colonies in liquid media so that we can isolate the plasmid DNA minprep and size confirmation. Doing these things will allow us to see which colonies have our successfully assembled part.



To grow up these colonies, we label them on the plates and label tubes in the same way. This way we know which liquid cultures came from which colonies on the plate. To make sure we are only growing cells that contain a plasmid that might be our final assembled part, we put the same antibiotic that was on the plate into a test tube with 5ml of liquid LB media.

Once you have the colony picked, you can put the toothpick or pipette tip into a 5ml tube containing liquid LB media and the same antibiotic that was on the plate. After leaving the tube in the shaking incubator over night, you will have enough cells in the liquid media to do a miniprep and size confirmation to make sure your ligation worked. You should pick a few colonies at a time to make sure that you pick at least one containing copies of the plasmid you wanted to make.

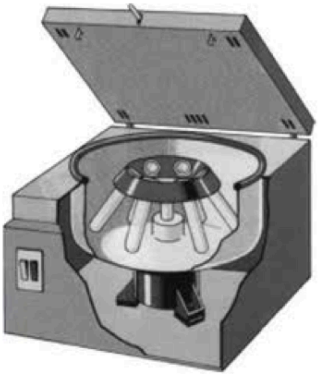


7. Glycerol stocks

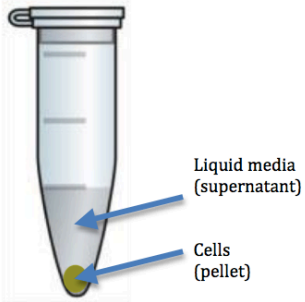
After determining the cells that have our plasmid, we choose one colony and grow those in a liquid growth medium overnight. After that, we mix our cells with glycerol in a tube, and then flash-freeze them. Glycerol protects the cells from damage caused by freezing, so if we need more of the cells (in order to get more DNA), we can just thaw them and they can be used again.

8. Miniprep (plasmid purification)

If we do need more DNA, we can take some cells from our glycerol stocks, and grow them overnight in a growth medium containing antibiotic. When the cells grow they will replicate, so we will have lots of cells, and therefore lots of DNA.



Centrifuge



After we have grown our cells we get rid of the growth medium that we grew them overnight in, and we do this through centrifugation. This step basically spins the tubes very fast, so our cells sink to the bottom of our tubes, and we can then get rid of all of the liquid growth medium.

After this, we then lyse cells, or break the membrane, in order to release our DNA. However, there are many different components inside the cells, so when we lyse

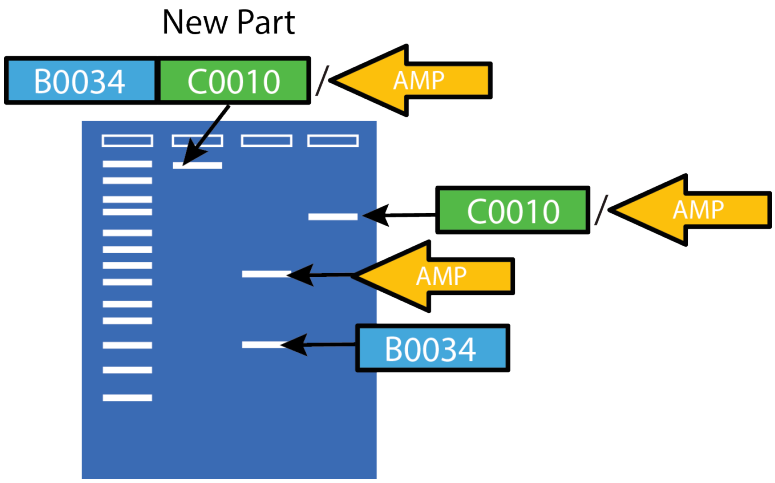
them, it will also release other (genomic) DNA, RNA, proteins and everything else inside the cell. The thing we want to do, then, is separate our plasmid DNA from everything else.

To do this we add reagents that precipitate the genomic DNA as well as the proteins. We then centrifuge this so that all of the precipitated components fall to the bottom. As well, we add a reagent that contains Rnase, which is an enzyme that breaks up RNA.

After we centrifuge this solution, the liquid at the top contains our plasmid DNA (since the precipitated proteins and genomic DNA have been forced to the bottom through centrifugation). We can then put this liquid into a chromatography column, and this "grabs on" to our plasmid DNA and lets everything else flow through. We can then put the column

into a fresh tube, and "release" the plasmid DNA by adding a different solution (called elution buffer). Now, we have a tube that contains only our plasmid DNA in a buffer.

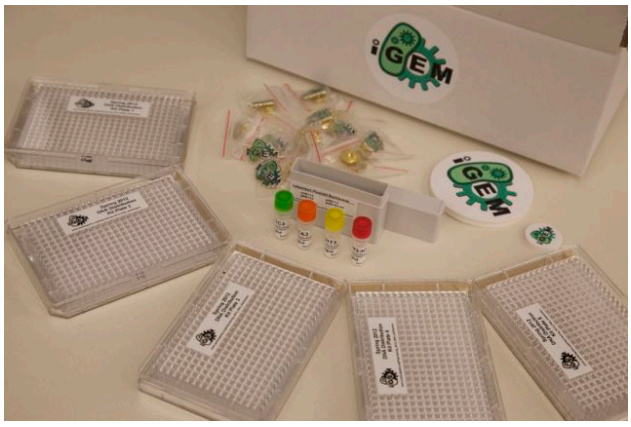
9. Size confirmation



Following the miniprep, an additional gel electrophoresis should be run in order to confirm the size the new DNA part. In order to do this, the DNA should be restricted with a single restriction enzyme in order to linearize it (make it a string of DNA as opposed to a circle of DNA). Following this, the DNA should be run on an agarose gel (see gel electrophoresis above) and the size should be compared to a standard ladder. The size of the new part should be the size of each individual part (in this case, C0010 + B0034), plus the size of the plasmid. Expected sizes can be looked up on the registry of standard parts.

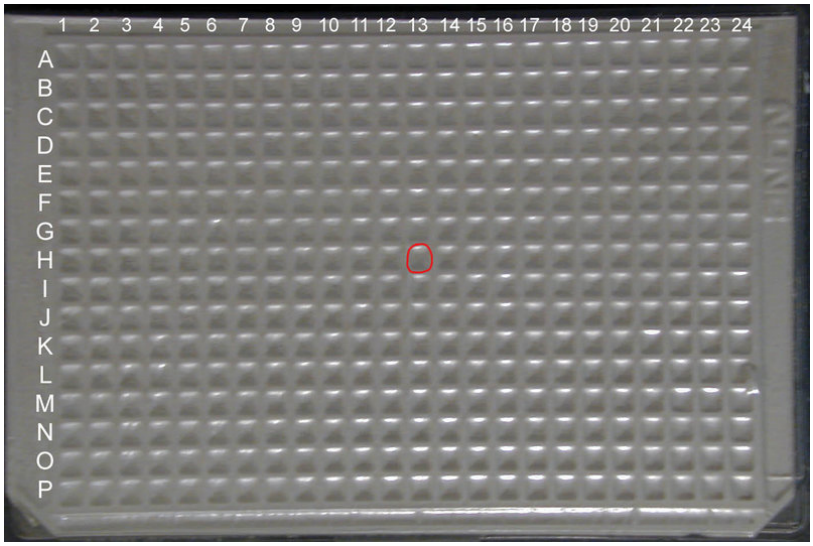
Once the size is confirmed, we can use our DNA for whatever else we need, for example, starting another assembly to add another DNA part on to the one we just made.

10. Kit plates



Before we start doing any assemblies, we first need parts to put together! Every year, iGEM sends out a collection of the best characterized and most useful parts that have been sent to them by teams in the past. These parts come in the form of the Kit Plates.

In order to use the parts that are sent in the Kit Plates, you need to know where to find them. The Kit Plates each contain 384 parts in wells organized in a coordinate system. Each well has a letter and a number associated with it (for example B13).



To get a specific part from the Kit Plate, look up that part's coordinates on the iGEM DNA Part Libraries (http://parts.igem.org/cgi/assembly/libraries.cgi) and mix the dried DNA in the wells with 10ul of lab grade water. Once the DNA is mixed with the water, let it sit for 5 minutes, then you can move it to a newly labeled tube or use it for a transformation.

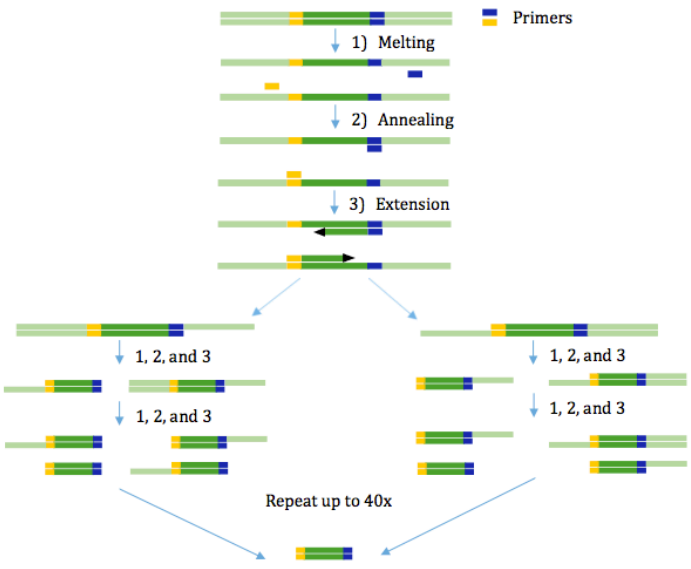
11.PCR

PCR stands for **Polymerase Chain Reaction**. Polymerase, because it uses DNA polymerase to make single stranded DNA into double stranded DNA; Chain Reaction, because each cycle of a PCR makes the starting components for the next cycle. PCR is very useful for making many copies of a piece of DNA that is between regions that have a known sequence. These known sequences are primer binding sites, which are complimentary to small single stranded DNA molecules called primers. These primers, when bound to the primer binding sites, give the DNA polymerase a place to start replicating the DNA you are interested in.

PCR has 3 unique steps in each cycle of the chain reaction:

1) **Melting** – Here the double stranded DNA in the reaction mixture is separated into single stranded DNA molecules by heating up the tube that the reaction mixture is in.

2) **Annealing** – Annealing is the process of two pieces of single stranded DNA that are complementary binding together. In PCR, annealing happens between the primers and the primer binding sites. This gives DNA polymerase a place to start making more double stranded DNA. The temperature must be lowered for annealing to happen.



Exponential increase of copies of sequence between primer binding sites

3) **Extension** – This is the step where the temperature of the reaction tube is just right for DNA polymerase to extend the short primer and turn the single stranded DNA template into a double stranded molecule.

THE REGISTRY

Lisa Oberding and Emily Hicks

1. The Registry of Standard Biological Parts

With iGEM teams all over the world making, putting together and testing Biobricks, there needs to be some kind of repository for both the DNA and the data about these parts and systems that they create. One of the key contributions that iGEM has made to the synthetic biology community has been the Registry of Standard Biological Parts, often abbreviated as 'the registry'. With both a physical and internet entity, the registry serves two major purposes: to store physical DNA for Biobricks and to communicate about these Biobricks in an open-source setting. Located in Cambridge, Massacheusetts, the physical repository allows a common storage location for all the DNA parts created. iGEM teams and other labs send in their BioBricks as plasmid DNA that is stored on site. iGEM Headquarters spends time trying to ensure that the parts have the right sequence and are of good quality. Each year, iGEM teams are then distributed a portion of the total parts in the registry for use in their own projects. Teams use these parts to build their own new systems, contributing novel parts in the process. They share their data on these parts using the parts registry website which we will cover in more detail in the next section. This idea of sharing and building upon previous teams work is a cornerstone of the iGEM competition.

2. How is the regsistry organized?

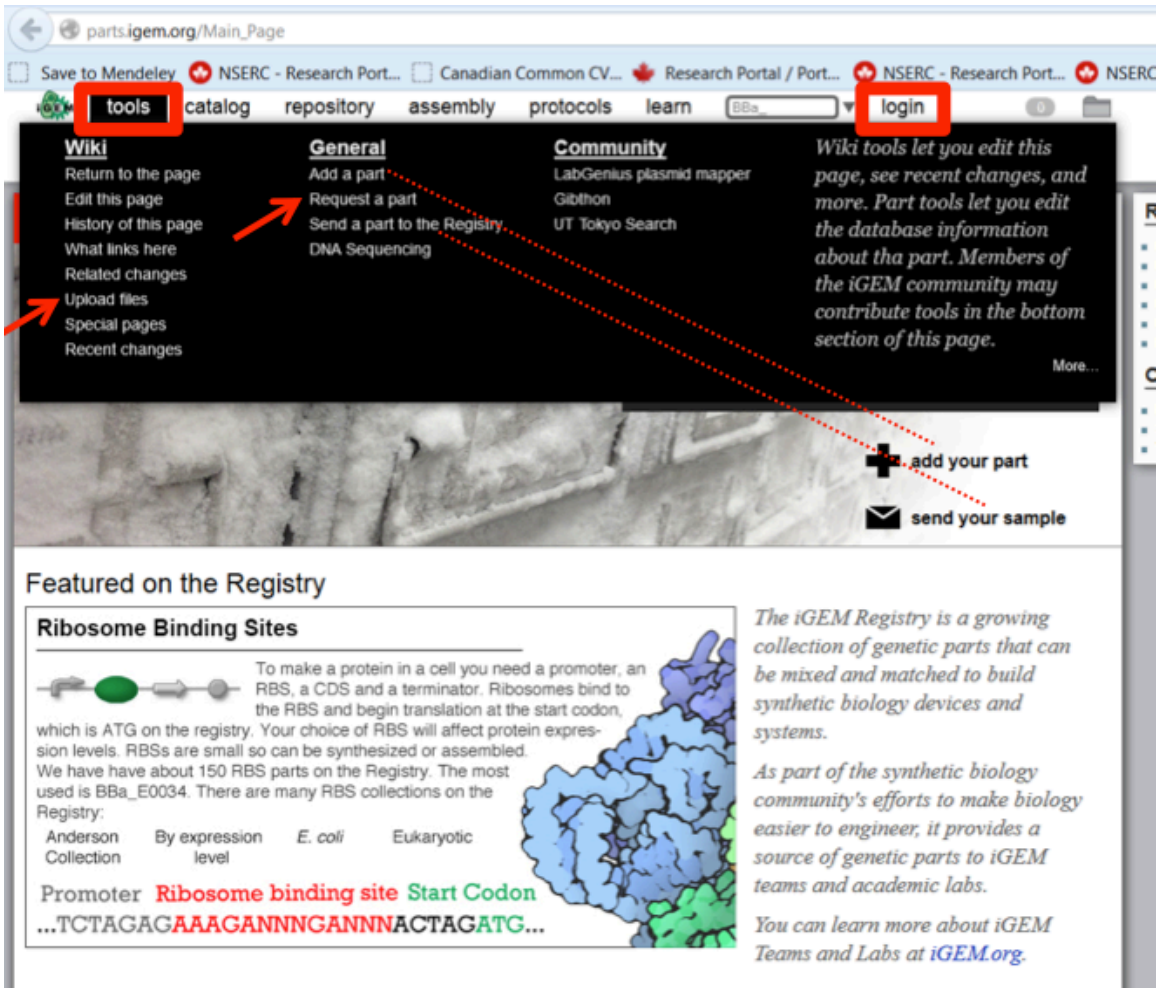
In the registry, each individual Biobrick 'part', whether it be simply a promoter, or whether it be a composite biobrick containing a variety of subunits, has its own part page. As we will explain shortly, each part page contains different types of information about that parts such as it's subunits if it is a composite part, or where the DNA came form if it's a basic part. To make things easier, the registry has assigned a variety of symbols to each 'type' of part, for example, promoter, terminator, ribosome binding site, etc. These symbols match the Synthetic Biology Open Language (SBOL) Visual which is an open-source graphical way of representing parts (http://www.sbolstandard.org/visual). These symbols can be found in the table below.

(Table of Biobricks including: promoter, terminator, RBS, reporter, etc.)

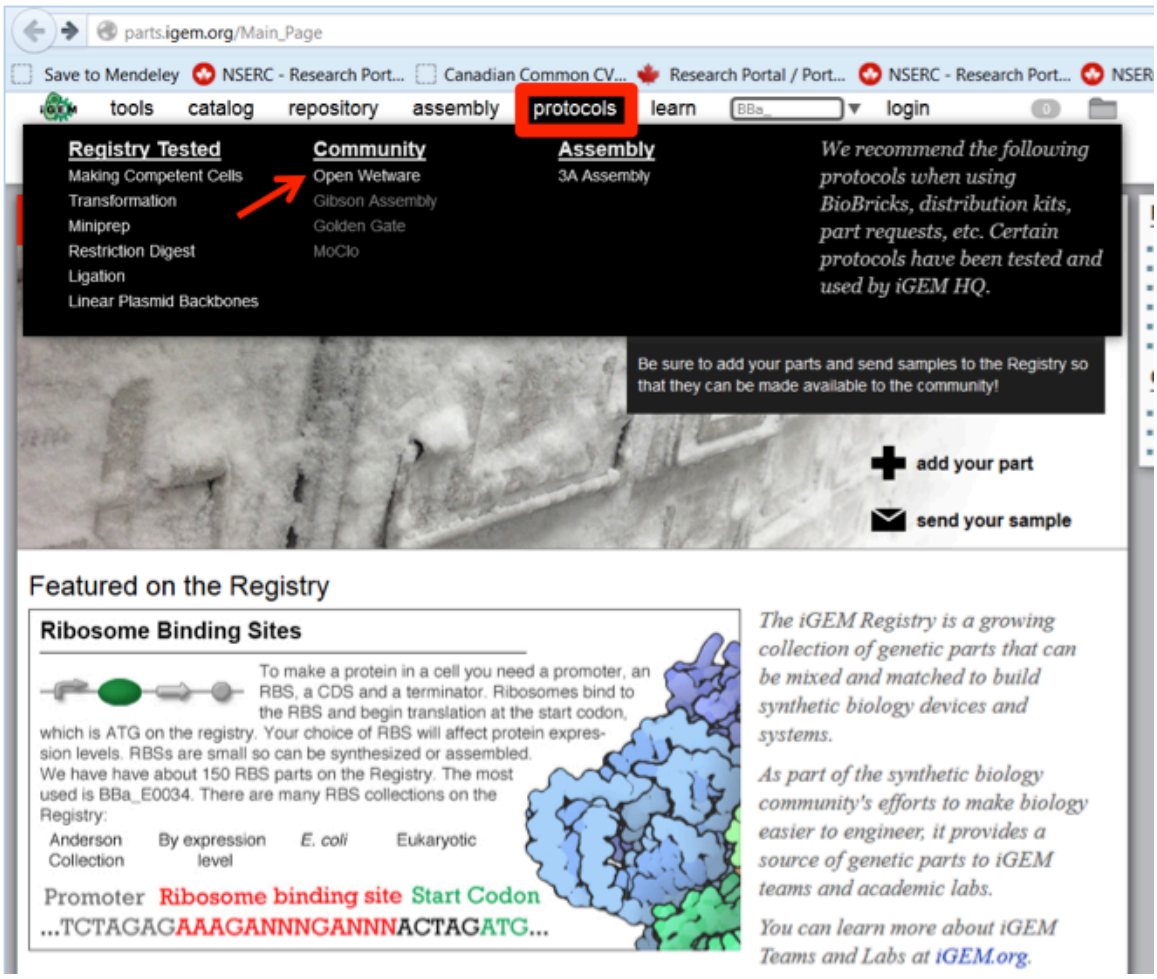
3. How do you search the registry?

Although the registry is a fantastic resource, it can sometimes be difficult finding what you want. Although it can be frustrating, in order to make sure you are not 're-inventing the wheel', it is important to do a thorough search when considering building a new part from scratch. Often teams spend large amounts of time building a part only to discover that it was submitted by a previous team and has been in the registry all along. Below is a guide to to walk you through what's available in the registry, the basic tools availale as well as some tips to make your searches easier.

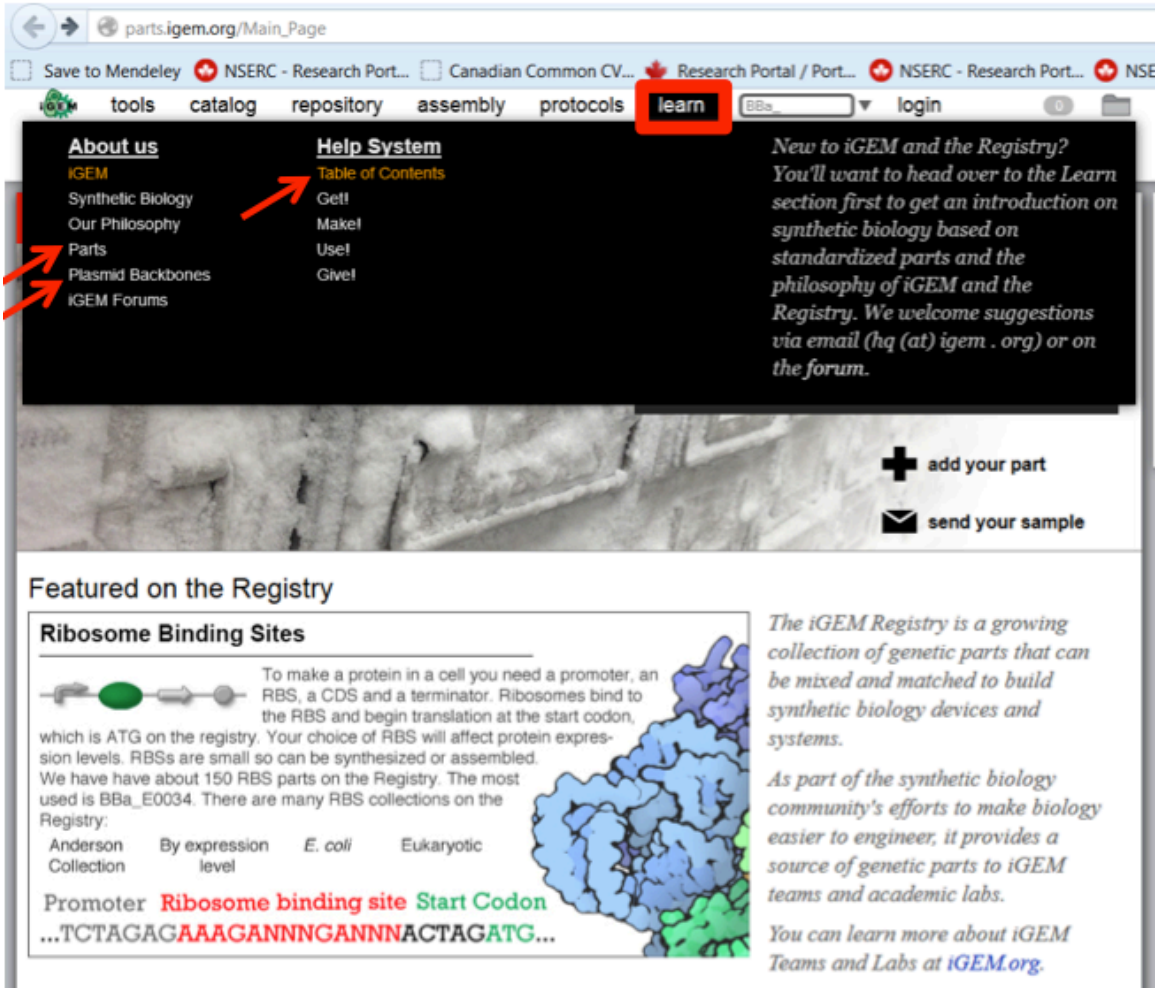
The tools menu will give you options to upload files (such as images), add a page for a part you have created to the registry & send parts to the registry (these links are also available directly on the homepage, and will be explained further later), as well as request a part from the registry for use in your project if it was not sent in the distribution package you received. In order to do any of these things, you must first log in to your iGEM account, using the link in the top right corner.



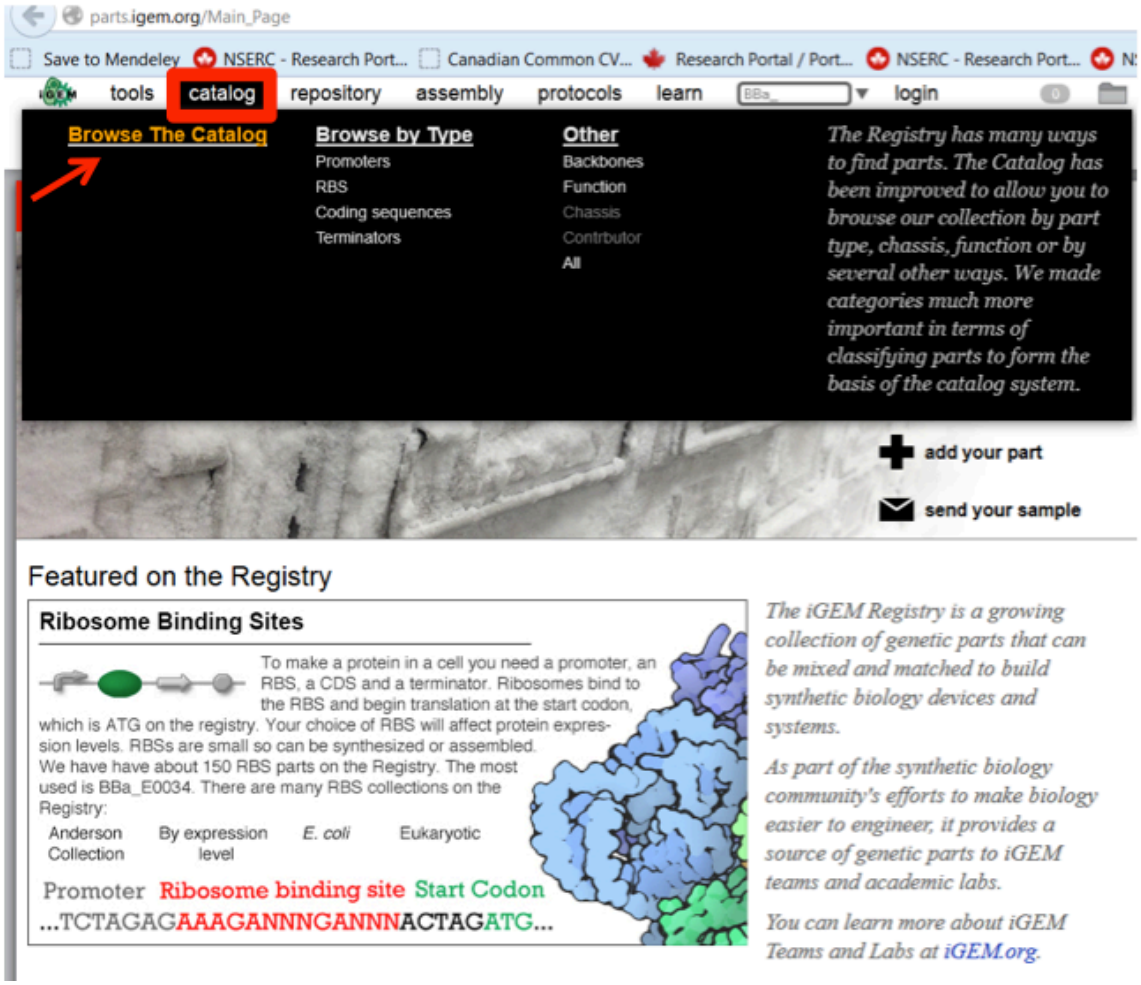
The assembly menu will give you links to information about the standard assembly methods that are used to create BioBricks. The three RFC standards given are the most commonly used. When submitting a part to the registry, your part MUST be compatible with RFC10- that means that it must have the standard BioBrick prefix and suffix sequences, and that the standard cut sites in the prefix/suffix must not be present anywhere else inside of the part sequence. RFC23 has a modified prefix and suffix to create a different scar site that can be used to create protein fusions, and is not mandatory. RFC25 is also not mandatory. It includes additional cut sites that can be used to create protein fusions. To learn more about each of the assembly standards, you can click on the links. Here you can also find information on 3A assembly, which is another way of putting two BioBricks together into a different backbone.



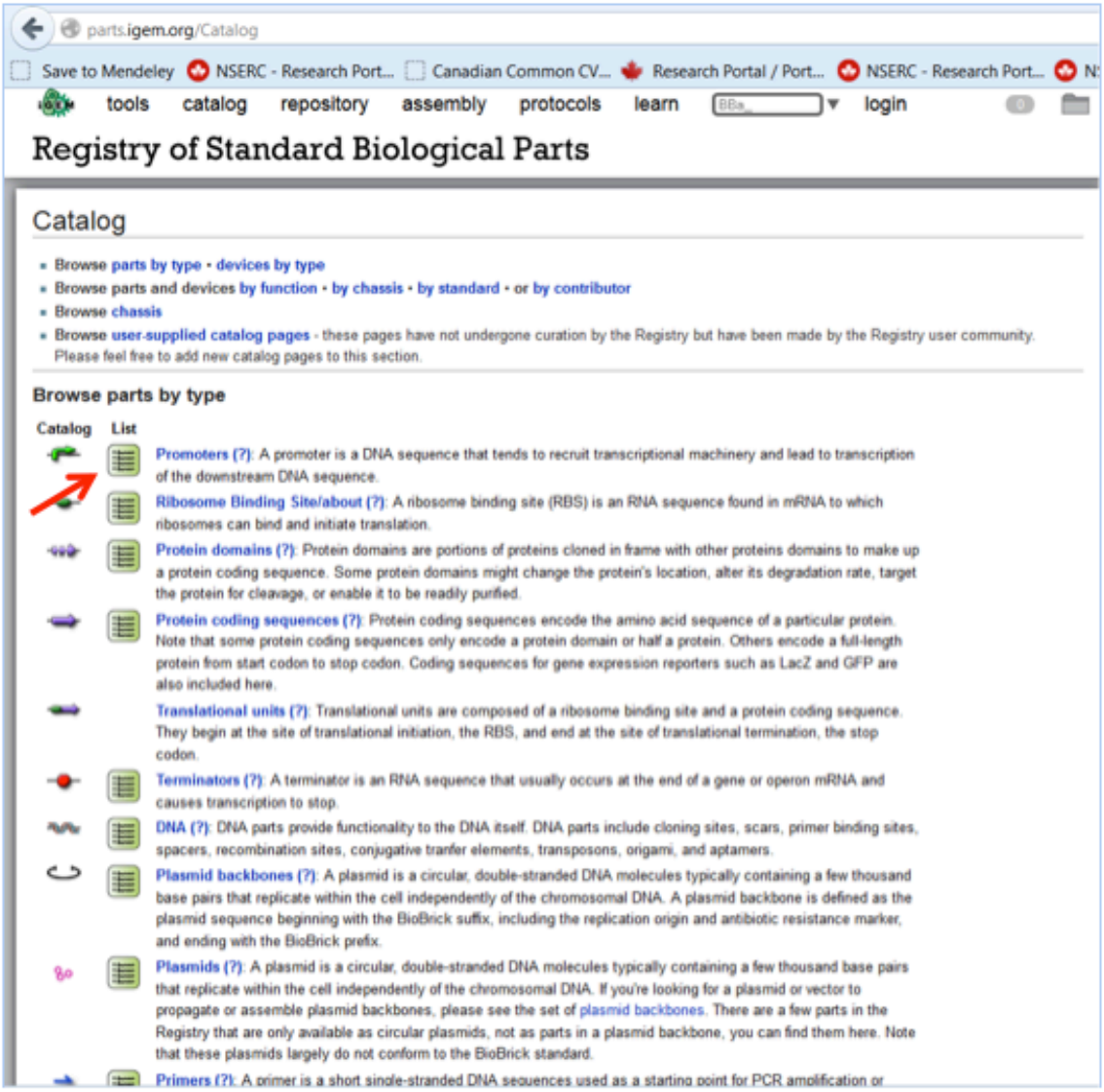
In the protocols section, you can find common lab protocols that you will need during the course of your project. In addition to these, you will find a link to OpenWetware, which is a site that provides many lab protocols not listed in this menu. This site is a great resource for experimental methods that you will need during the course of your project.



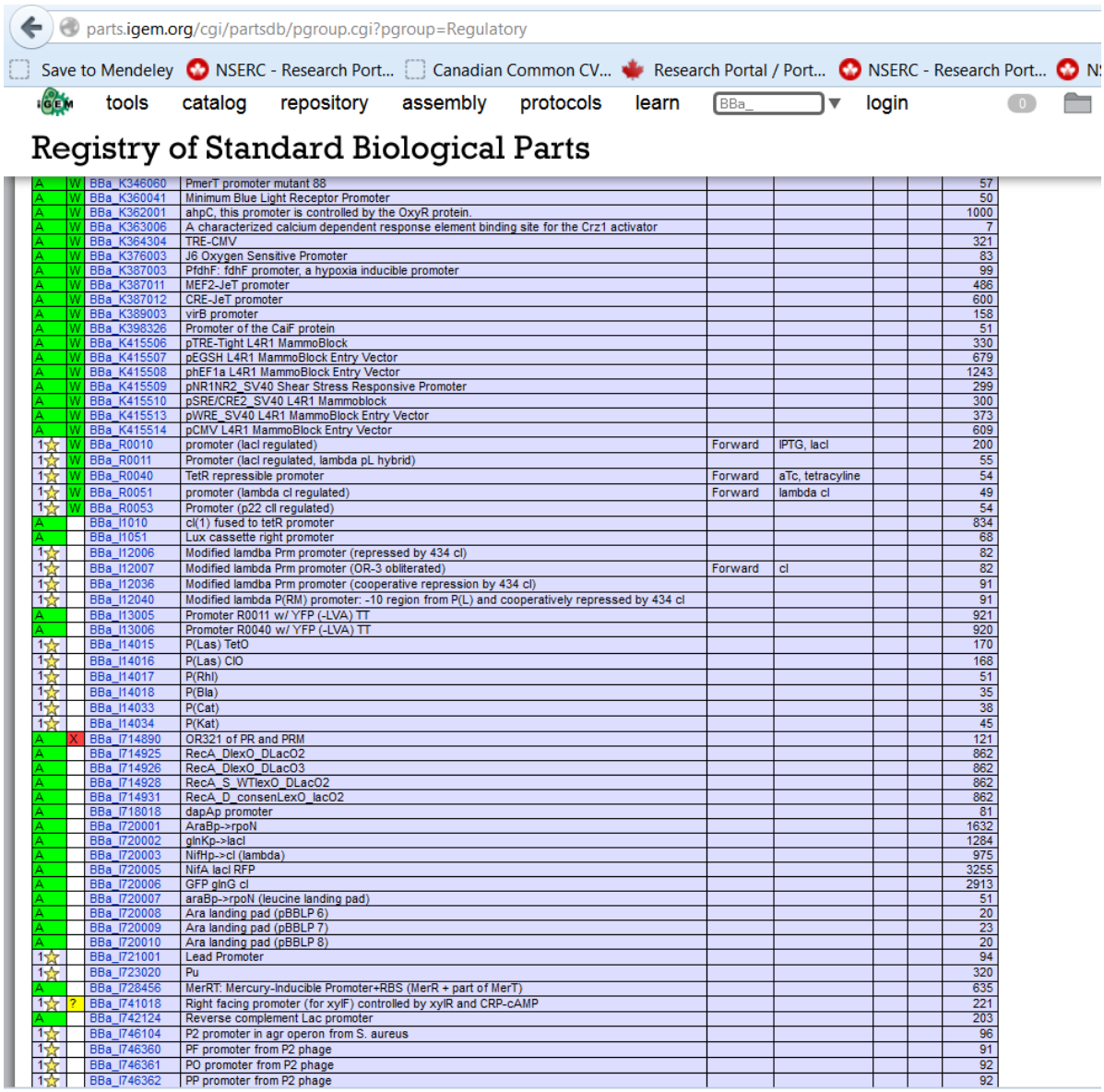
The learn section of the registry menu will provide links to a lot of information that can be useful for new iGEM teams, as well as information on iGEM itself. "Parts" and "Plasmid Backbones" will give you background information on the BioBrick system you will be using in your project, and the "help" system will provide you further information about how to utilize the parts registry which will be helpful in your project beyond what information is given here.



The catalog menu will allow you to browse the parts previously submitted to the registry by type. Note that not all parts of a certain category will be listed under the respective section in the catalog, so often using a search term instead will return more results that have not been categorized in the catalog yet. “Backbones” will give you information on the common plasmids you will be working with for your BioBricks. By clicking “Browse the Catalog”, you will be given more information on the categories of parts.



The catalog organizes parts by their function, and gives a description of what that type of part is for. It also shows the standard symbol that is used to display that type of part on a parts page based on its function. By clicking any of the links, you will be taken to a page with further information on that type of part and its design, as well as a catalog list which will give you further subcategories of that part to help you narrow your search to the exact type of part you need. By clicking on "list" on this main page you will be given a full list of parts of that particular type.



parts.igem.org/cgi/partsdb/pgroup.cgi?pgroup=Regulatory

Save to Mendeley | NSERC - Research Port... | Canadian Common CV... | Research Portal / Port... | NSERC - Research Port...

tools catalog repository assembly protocols learn BBa_ login

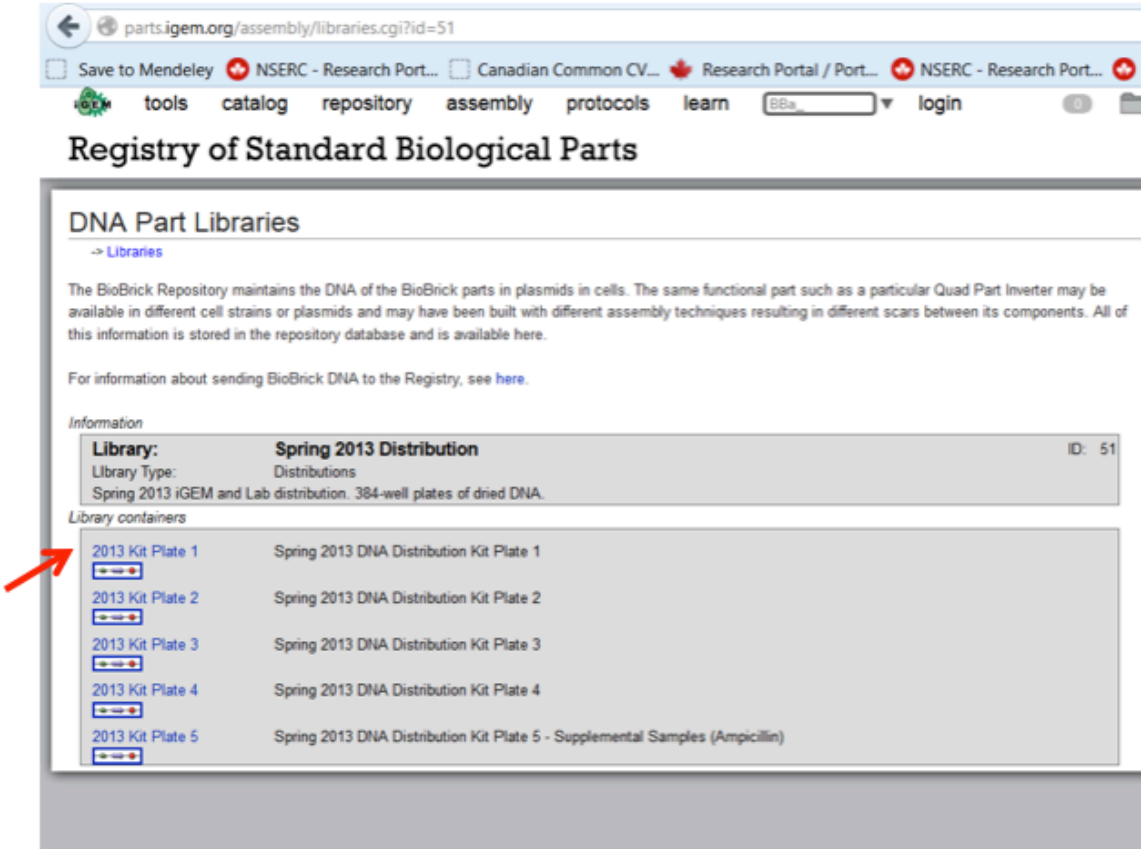
Registry of Standard Biological Parts

A	W	BBa_K346060	PmerT promoter mutant 88					57
A	W	BBa_K360041	Minimum Blue Light Receptor Promoter					50
A	W	BBa_K362001	ahpC, this promoter is controlled by the OxyR protein.					1000
A	W	BBa_K363006	A characterized calcium dependent response element binding site for the Crz1 activator					7
A	W	BBa_K364304	TRE-CMV					321
A	W	BBa_K376003	J6 Oxygen Sensitive Promoter					83
A	W	BBa_K387003	PfdhF: fdhF promoter, a hypoxia inducible promoter					99
A	W	BBa_K387011	MEF2-JeT promoter					486
A	W	BBa_K387012	CRE-JeT promoter					600
A	W	BBa_K389003	virB promoter					158
A	W	BBa_K398326	Promoter of the CaiF protein					51
A	W	BBa_K415506	pTRE-Tight L4R1 MammoBlock					330
A	W	BBa_K415507	pEGSH L4R1 MammoBlock Entry Vector					679
A	W	BBa_K415508	phEF1a L4R1 MammoBlock Entry Vector					1243
A	W	BBa_K415509	pNR1NR2_SV40 Shear Stress Responsive Promoter					299
A	W	BBa_K415510	pSRE/CRE2_SV40 L4R1 Mammoblock					300
A	W	BBa_K415513	pWRE_SV40 L4R1 MammoBlock Entry Vector					373
A	W	BBa_K415514	pCMV L4R1 MammoBlock Entry Vector					609
1☆	W	BBa_R0010	promoter (lacI regulated)	Forward	IPTG, lacI			200
1☆	W	BBa_R0011	Promoter (lacI regulated, lambda pL hybrid)					55
1☆	W	BBa_R0040	TetR repressible promoter	Forward	aTc, tetracyline			54
1☆	W	BBa_R0051	promoter (lambda cI regulated)	Forward	lambda cI			49
1☆	W	BBa_R0053	Promoter (p22 cII regulated)					54
A		BBa_I1010	cI(1) fused to tetR promoter					834
A		BBa_I1051	Lux cassette right promoter					68
1☆		BBa_I12006	Modified lamdba Prm promoter (repressed by 434 cI)					82
1☆		BBa_I12007	Modified lambda Prm promoter (OR-3 obliterated)	Forward	cI			82
1☆		BBa_I12036	Modified lamdba Prm promoter (cooperative repression by 434 cI)					91
1☆		BBa_I12040	Modified lambda P(RM) promoter: -10 region from P(L) and cooperatively repressed by 434 cI					91
A		BBa_I13005	Promoter R0011 w/ YFP (-LVA) TT					921
A		BBa_I13006	Promoter R0040 w/ YFP (-LVA) TT					920
1☆		BBa_I14015	P(Las) TetO					170
1☆		BBa_I14016	P(Las) CIO					168
1☆		BBa_I14017	P(Rhl)					51
1☆		BBa_I14018	P(Bla)					35
1☆		BBa_I14033	P(Cat)					38
1☆		BBa_I14034	P(Kat)					45
A	X	BBa_I714890	OR321 of PR and PRM					121
A		BBa_I714925	RecA_DlexO_DLacO2					862
A		BBa_I714926	RecA_DlexO_DLacO3					862
A		BBa_I714928	RecA_S_WTlexO_DLacO2					862
A		BBa_I714931	RecA_D_consenLexO_lacO2					862
A		BBa_I718018	dapAp promoter					81
A		BBa_I720001	AraBp->rpoN					1632
A		BBa_I720002	glnKp->lacI					1284
A		BBa_I720003	NifHp->cI (lambda)					975
A		BBa_I720005	NifA lacI RFP					3255
A		BBa_I720006	GFP glnG cI					2913
A		BBa_I720007	araBp->rpoN (leucine landing pad)					51
A		BBa_I720008	Ara landing pad (pBBLP 6)					20
A		BBa_I720009	Ara landing pad (pBBLP 7)					23
A		BBa_I720010	Ara landing pad (pBBLP 8)					20
1☆		BBa_I721001	Lead Promoter					94
1☆		BBa_I723020	Pu					320
A		BBa_I728456	MerRT: Mercury-Inducible Promoter+RBS (MerR + part of MerT)					635
1☆	?	BBa_I741018	Right facing promoter (for xylF) controlled by xylR and CRP-cAMP					221
A		BBa_I742124	Reverse complement Lac promoter					203
1☆		BBa_I746104	P2 promoter in agr operon from S. aureus					96
1☆		BBa_I746360	PF promoter from P2 phage					91
1☆		BBa_I746361	PO promoter from P2 phage					92
1☆		BBa_I746362	PP promoter from P2 phage					92

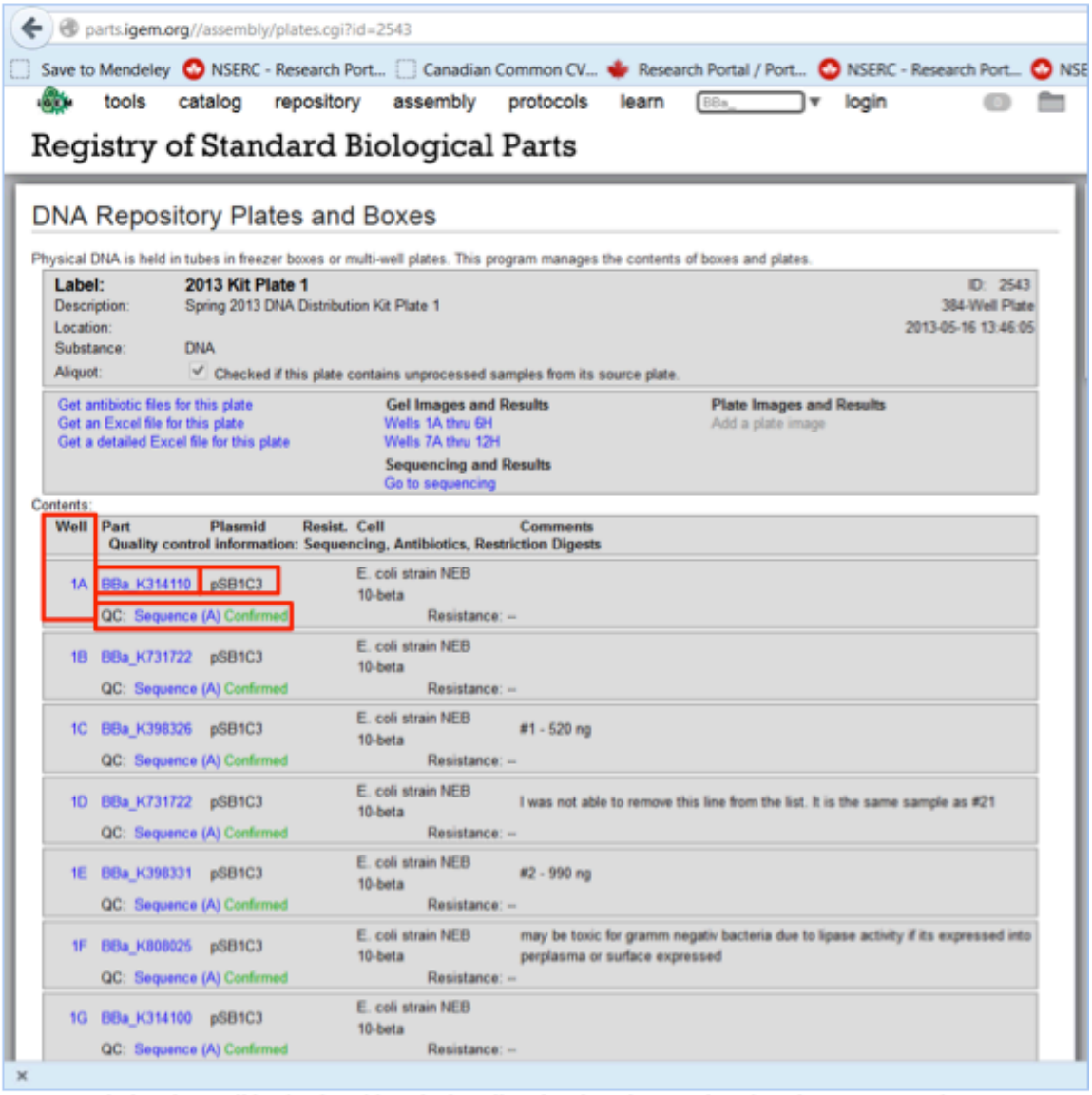
The list that you will be given will look similar to this. The length of the part in base pairs, short description of the part, and the BioBrick number of the part will be shown, as well as some additional information. A green box with an A means that that part is available from the registry, either in a kit plate or by request. A star means that the part has been rated by other teams to be functional and of high quality. If this box is blank, you may still try to contact the registry for the part, however it may not be available.

A green box containing a W means that the part has been shown to work, a red X means that the part does not work, and a yellow ? means that its functionality is questionable. If the box is blank, the part may be untested, or the information may not have been updated. Here, you will want to check the parts page for more information, but these parts can still be requested if you want them for your project. By clicking on the Bba_ links, you will be shown an informational box about that part, with further information about its use. By clicking on the part number in this box, you will be taken to the page for that particular part.

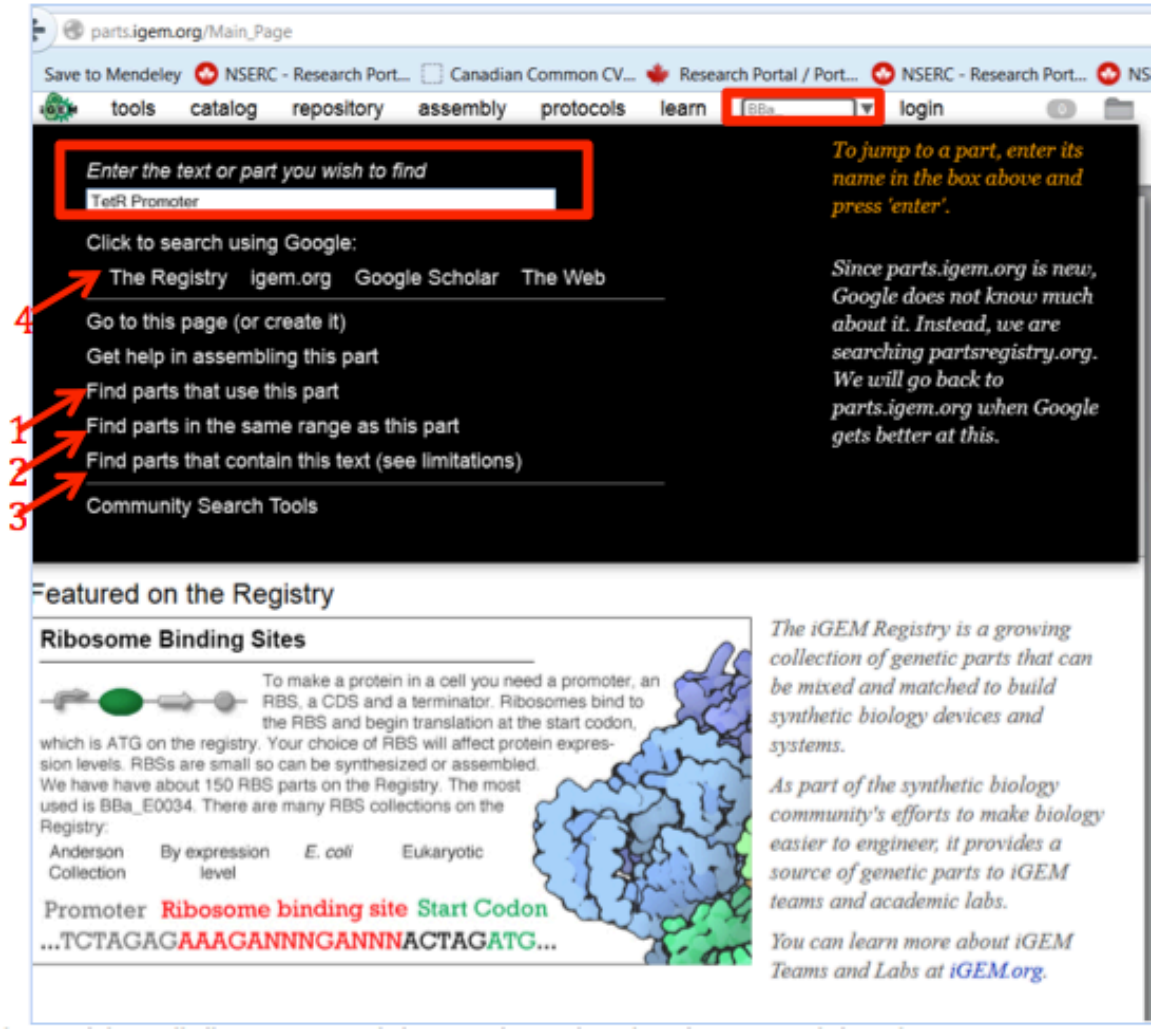
The repository menu on the home page will allow you to search the parts that are specifically found in the DNA distribution kit plates, or display all the parts in a particular distribution. By clicking "Search Distribution", you can search for a particular part of interest to see if it was sent in a kit plate. By clicking a particular distribution, you will be brought to a page with further information.



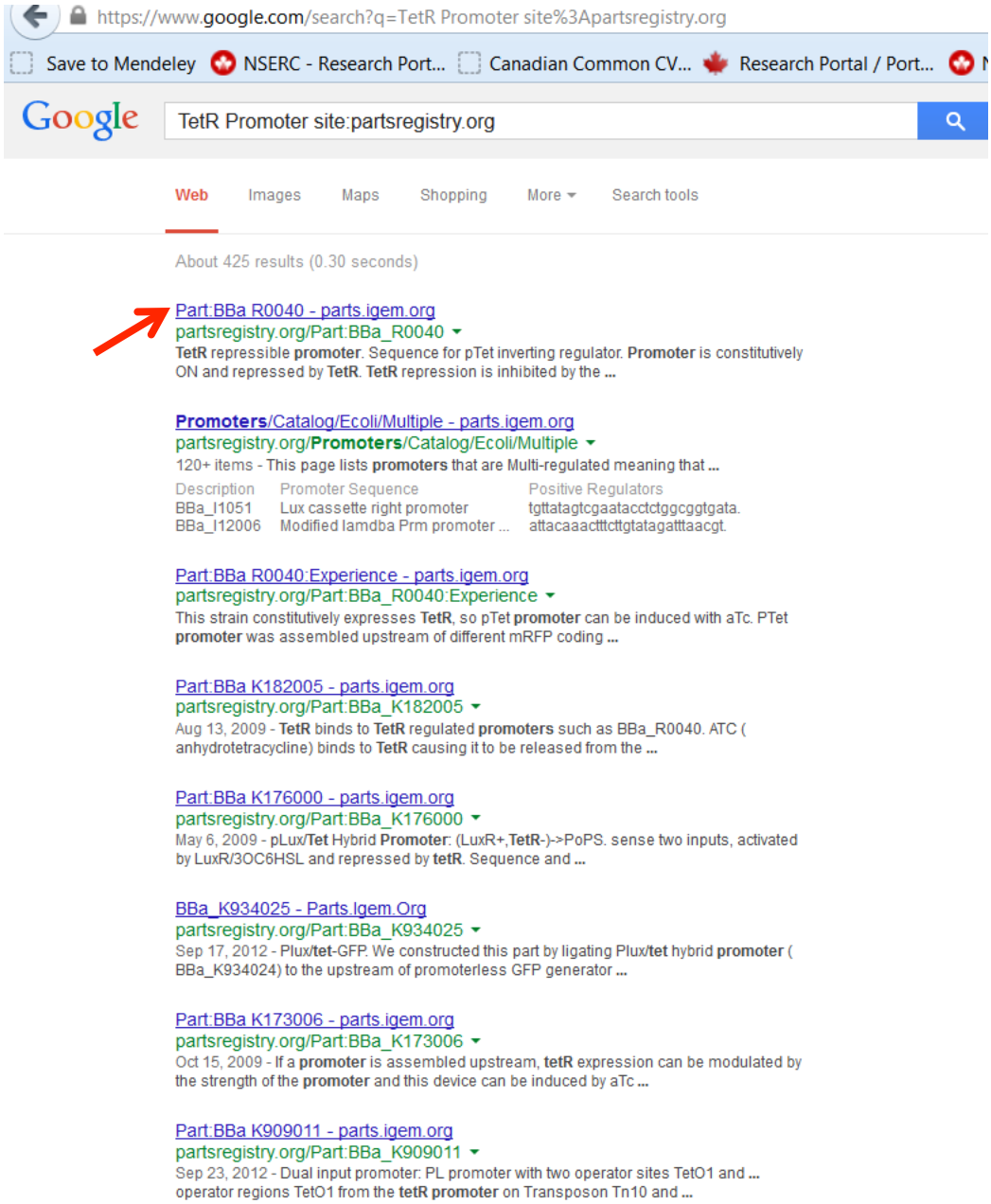
Each distribution is composed of plates, where each well has a single type of plasmid containing a particular part. By clicking the kit plate of interest, you will be taken to a menu that displays what is contained in each well of a particular plate.



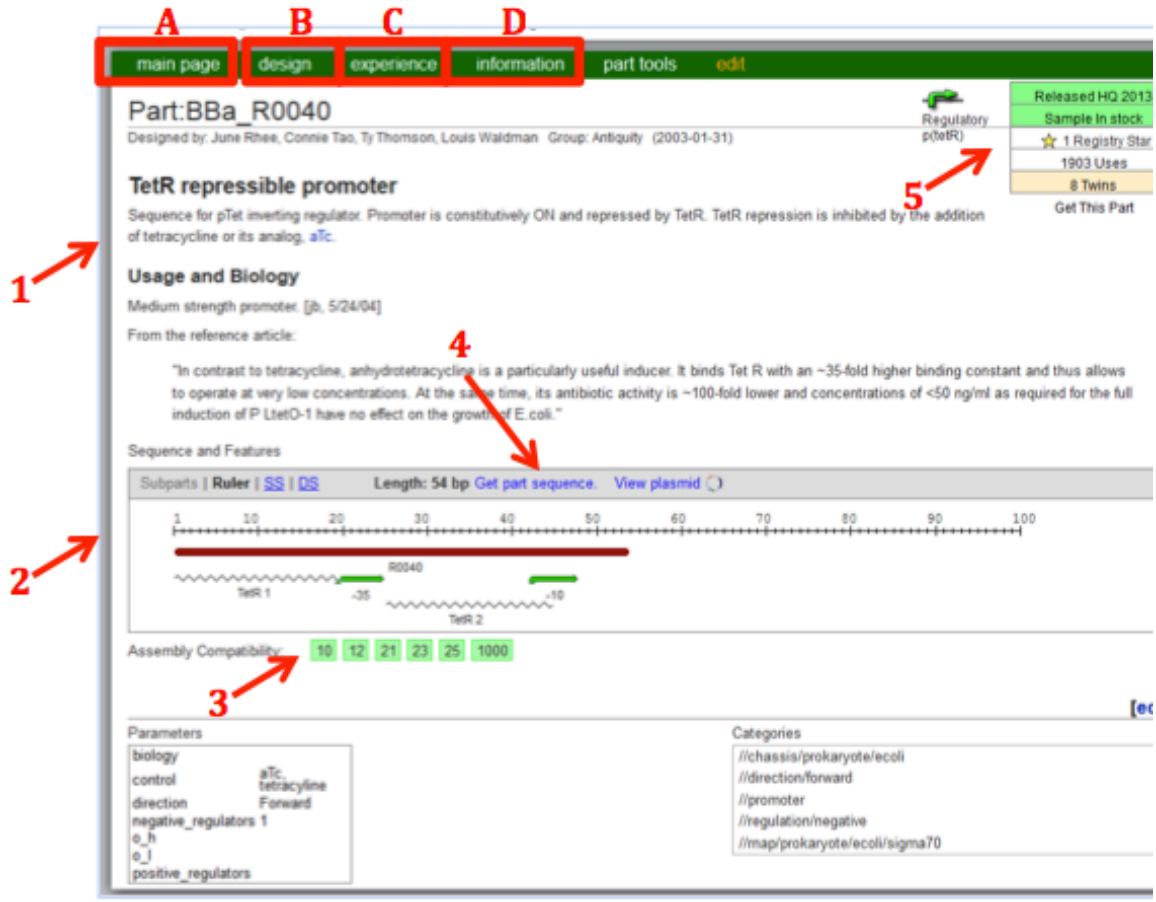
Contents of a kit plate will be displayed by which well in the plate they are found in, the part name, if the sequence of the part has been confirmed (is the part what it is supposed to be?- note that long parts will look like they have bad sequences here even if they are accurate), which backbone it is in, which strain it has been shown to grow in, and additional information about how that part works (is it toxic to the cells?). In the plasmid backbone, you will find a letter which tells you the resistance: commonly it will be chloramphenicol, as PSB1C3 is the standard backbone, however occasionally you may find a part with PSB1A3 (ampicilin), PSB1K3 (kanamycin), or one with multiple resistances (PSB1AK2- ampicilin and kanamycin). This will tell you what type of plate you should grow the bacteria you transform with this DNA on. Clicking on a part name will then take you to the main page for that part.



The search box will allow you to search for a part by number, if you know it. By clicking the arrow next to the part search box, an extended menu will be brought up. In this menu, if you know a particular part name, you can search 1) for larger constructs that contain this particular BioBrick, 2) parts in the same number range (generally submitted by the same team that submitted the original part in the same project, and therefore sometimes containing or used with the part you are searching), or 3) containing a certain keyword. You can also search keywords for a part in the entire registry (4). This will allow you to search for a particular part or a part that does a particular function without necessarily knowing the part name. Here, we will try to search by saying we want a tetracycline repressible promoter, and searching the entire registry (this will give you the widest range of results).

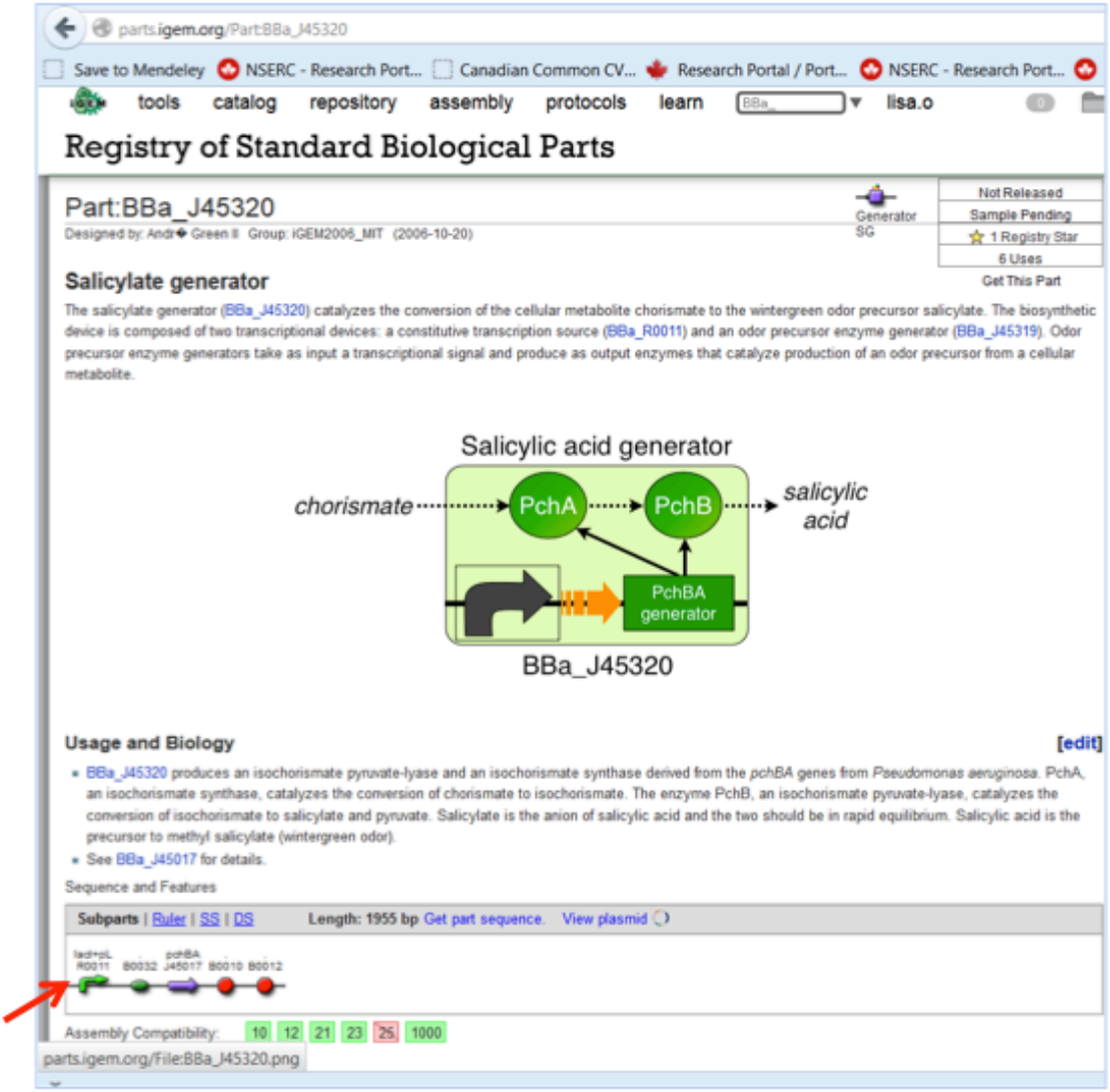


The search engine will give you results that match the text that you have entered. Alternatively to this, you may also use Google directly to find a part by typing the same search terms followed by "parts registry".

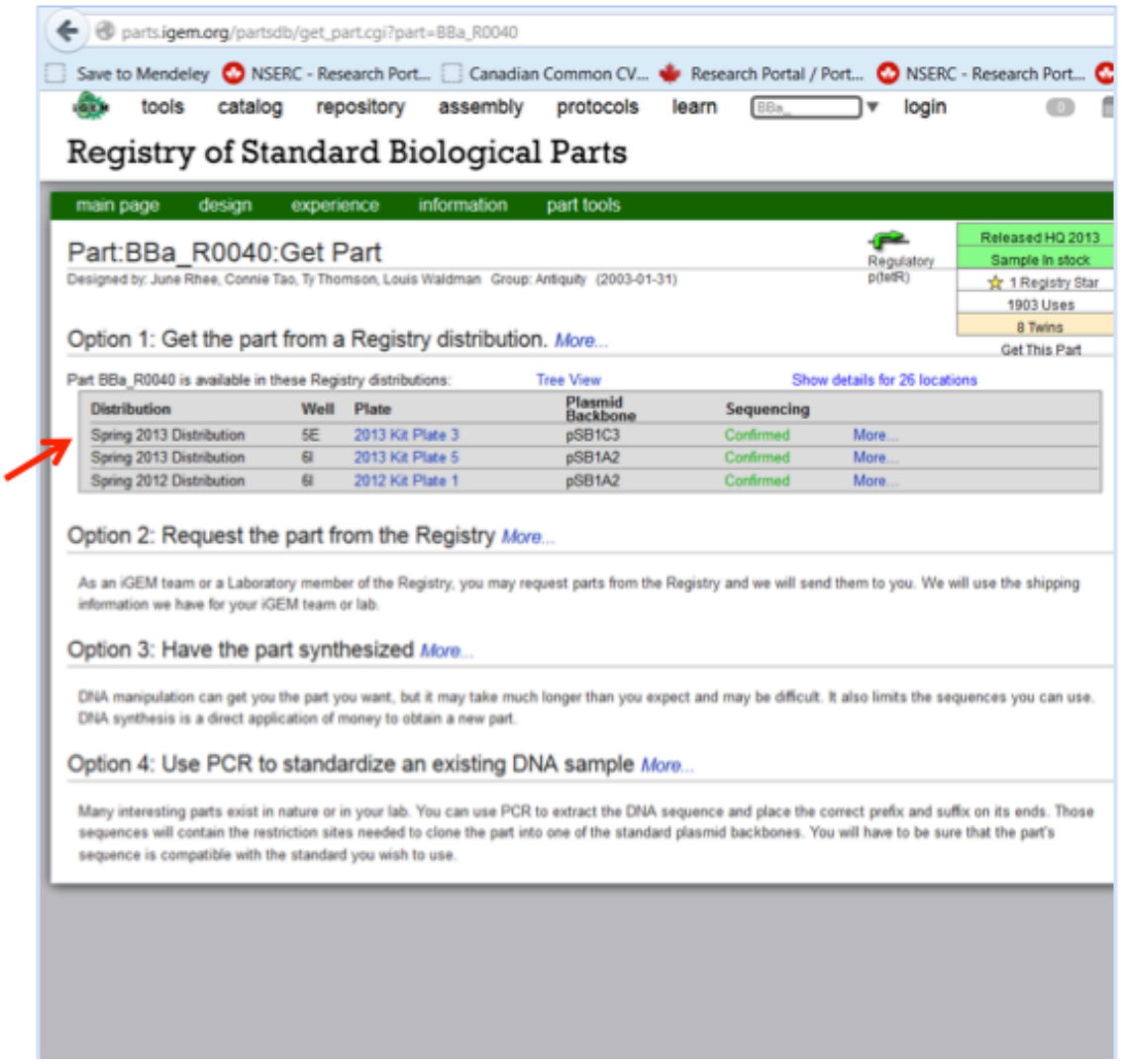


Clicking on this will bring you to the main page for the part (A). On this main page, you will find all the information on a particular part. There will be the number of the part, its name, and description of what it is/does (1). You will also find information on the composition of the part, certain features present in the sequence that have been annotated, and the length of the sequence (2). There will also be information about its compatibility with assembly standards (3). By clicking on get part sequence, you will be given the DNA sequence of the part immediately after the BioBrick prefix, and ending right before the BioBrick suffix (4). Information about the part can be found in the top right corner (5) including if the part was released in a distribution plate, whether the sample is in stock at the registry, if the part has any registry stars associated with it, the number of times it has been "used" (mentioned), if there are any twin parts with the same sequence, and a link in order to get the part. Clicking on this link will take you to a page which will allow you to request the part, or tell you where it is located in a kit plate from a distribution.

The design page (B) will give you more information about how the part was designed, experience (C) will give you information about the function of the part and how other iGEM teams have used it, and the information section (D) will give you more hard information about the part itself.



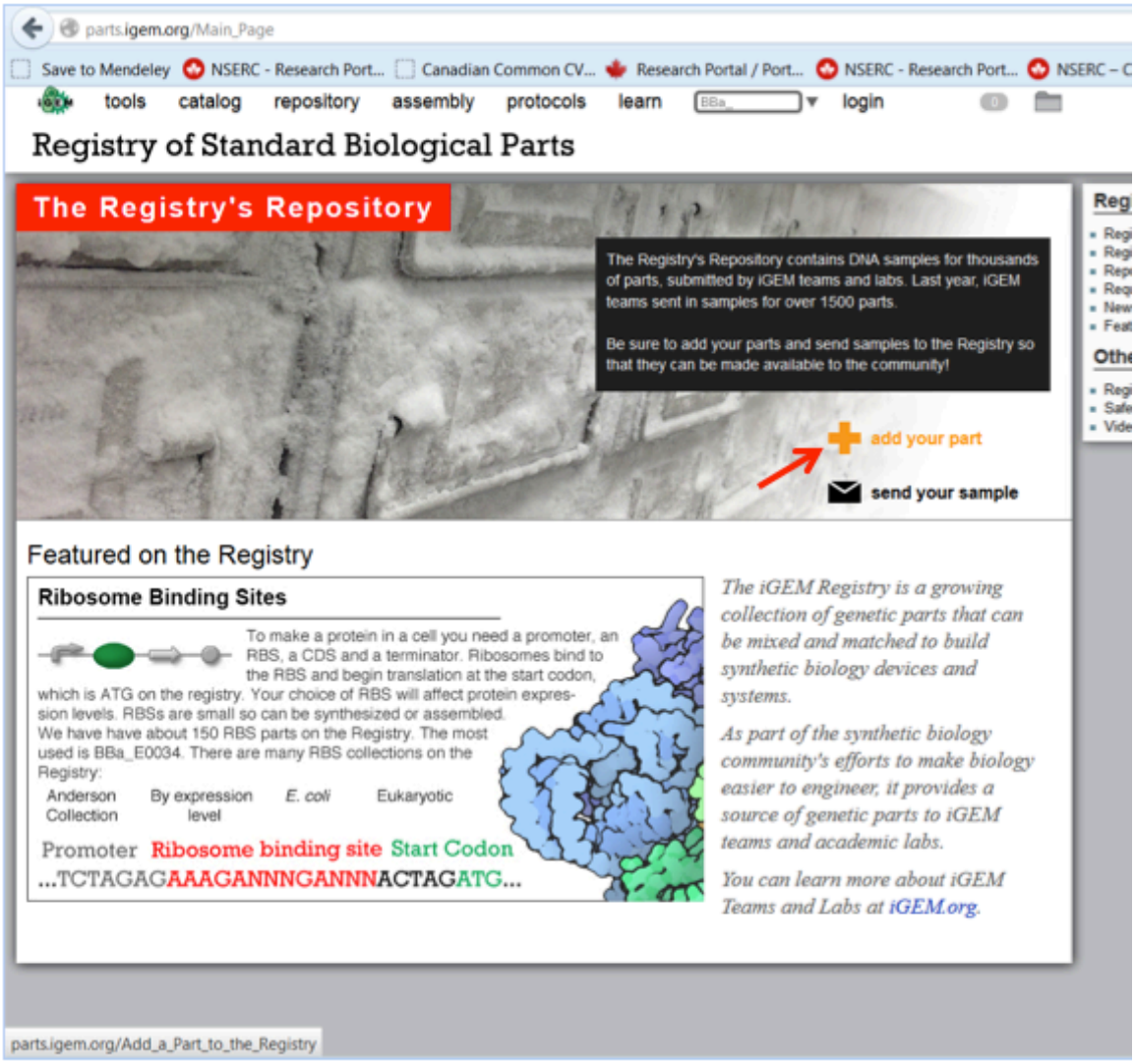
For a composite part composed of other BioBricks, the part information display will show you subparts that it is composed of, and the symbol of what type of part it is. By clicking any of these subparts you will be redirected to the main part page for that subpart.



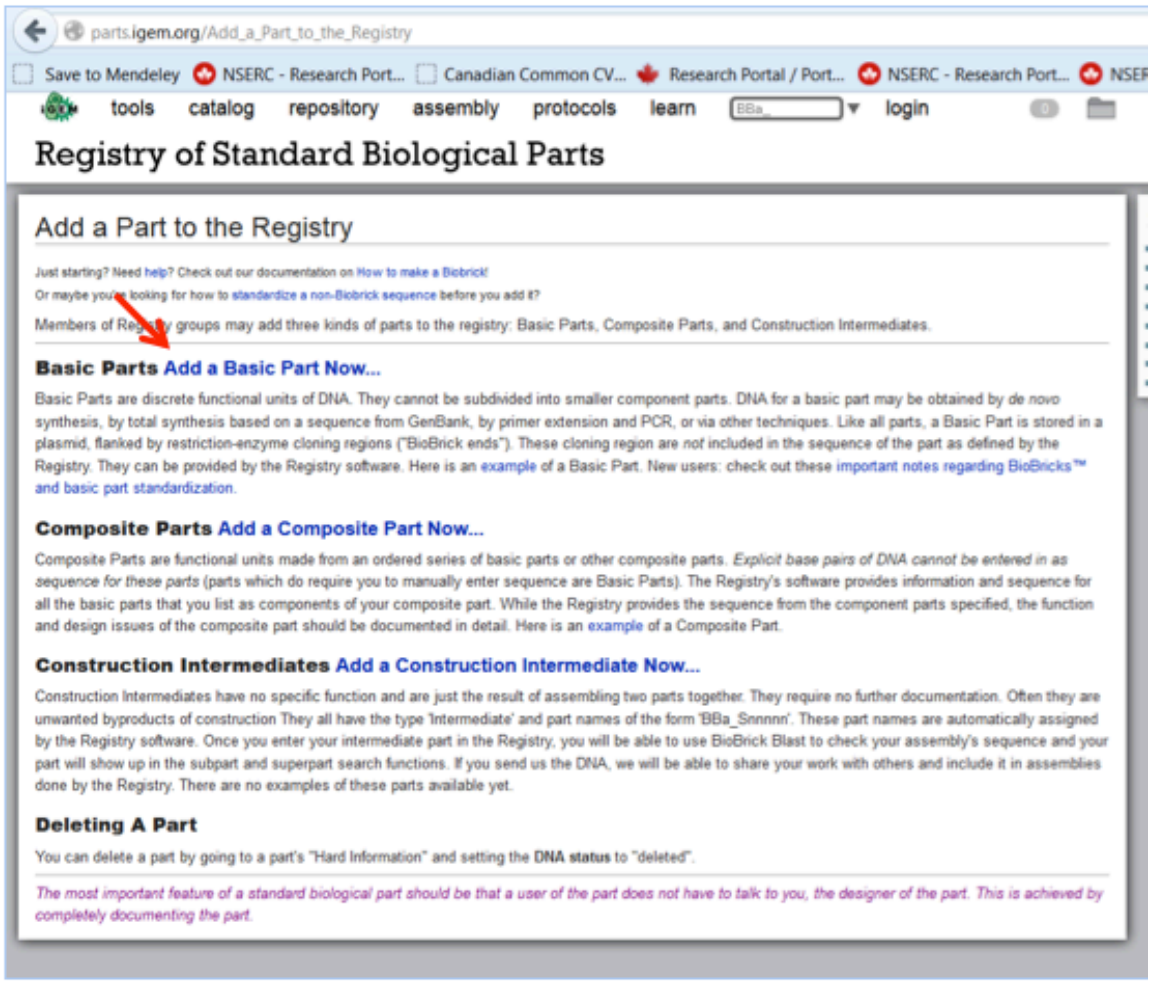
By clicking the "Get This Part" link, you will be brought to a page that lists if the part has been released in a distribution, which well and plate it is in, what the backbone is (and therefore what resistance), and if sequencing has been confirmed. In addition, if a part is not available in your distribution, you can use this page to request the specific part from the registry. Other options are given as well, if the part is not available from the registry.

4. How do we submit our own Biobrick parts to the registry?

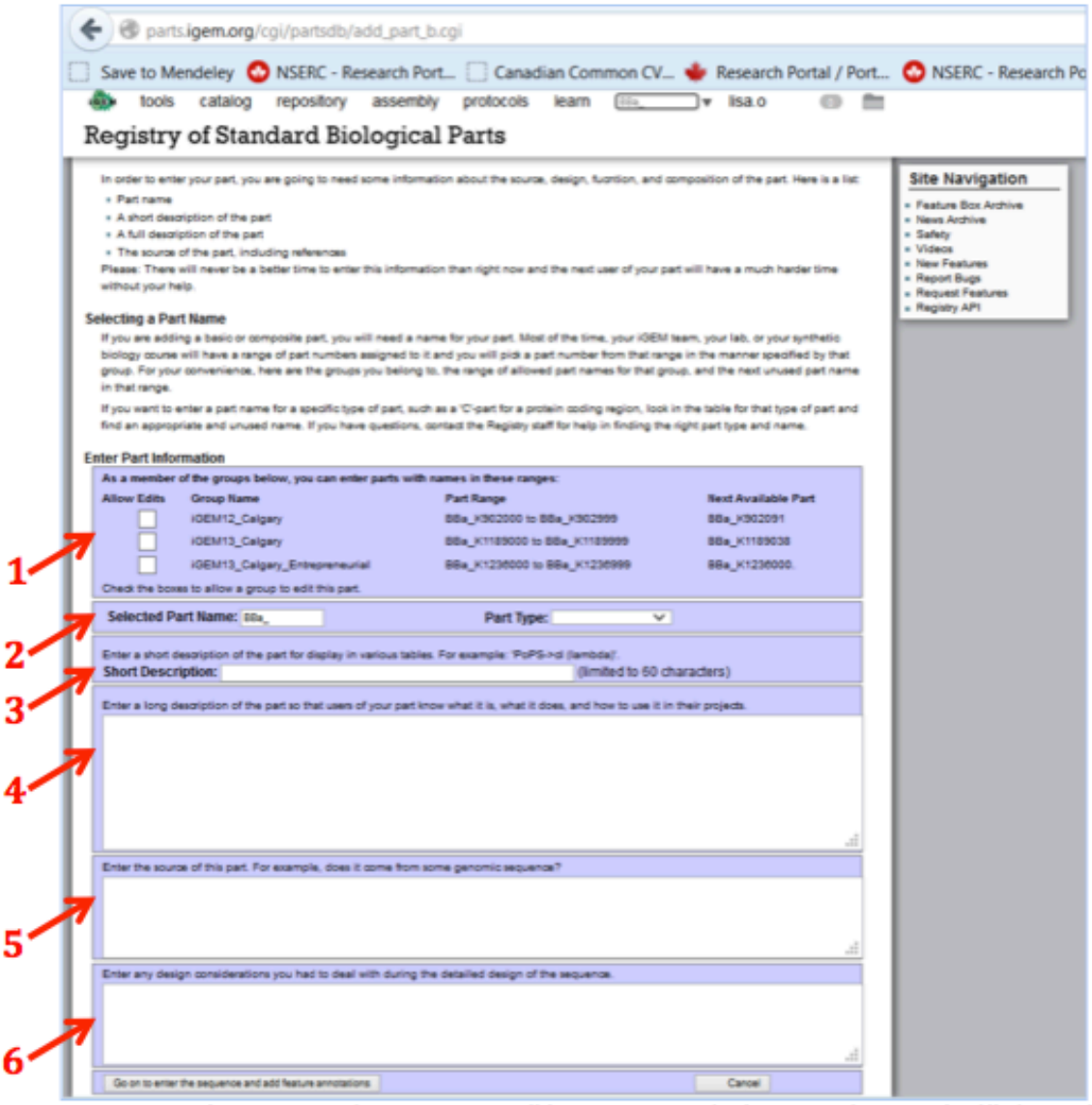
During your project, you will be building a variety of BioBricks. These parts will need to be submitted to the parts registry by a certain deadline, both through creation of a page describing your part as well as through sending DNA samples to the registry.



The first step of this process is to create a parts page for your particular part, through the "add your part" tool. In order to do this, make sure you are logged in.

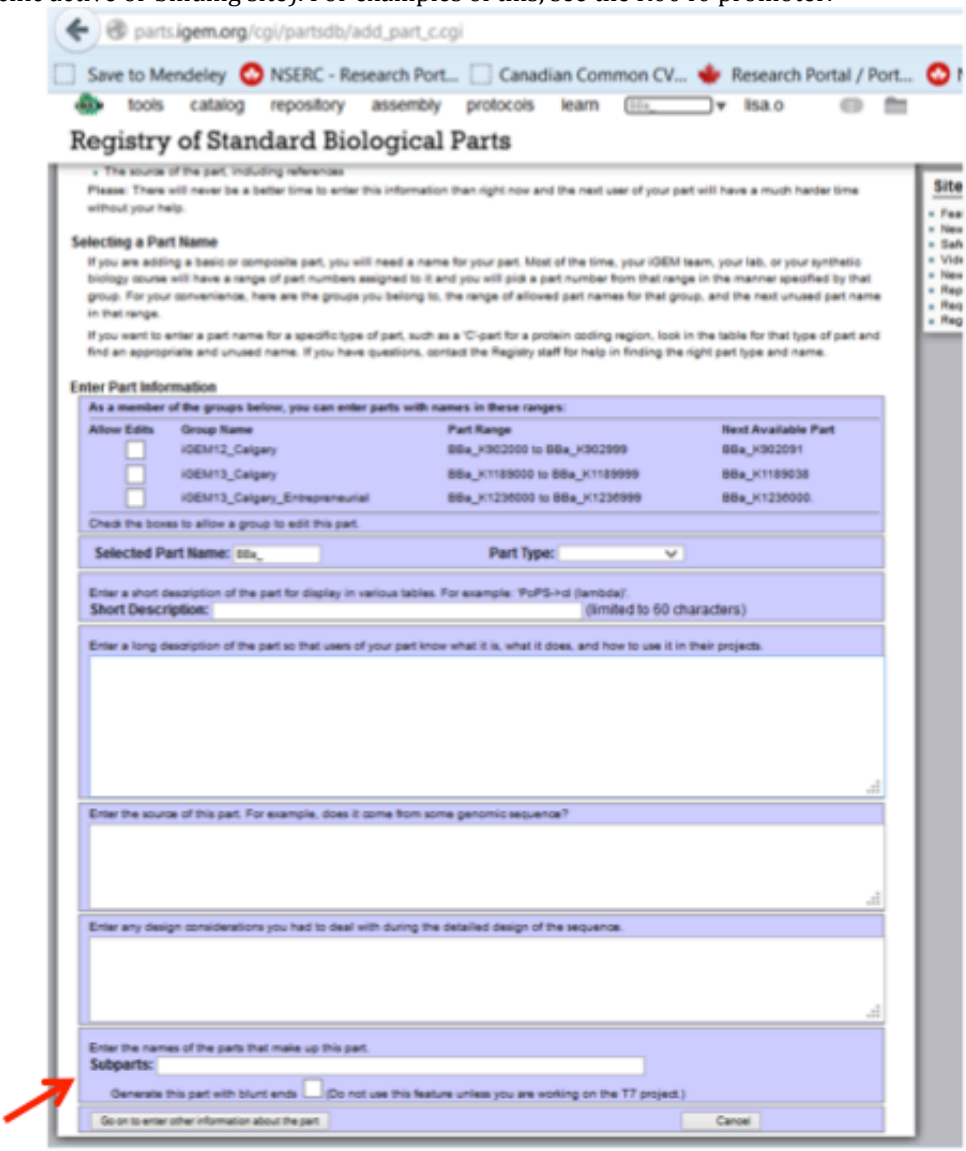


There are a few different types of parts that you can submit. A basic part usually consists of a single gene/part. A composite part is a part built out of other BioBricks as subcomponents, and usually contains more than one functional unit. A construction intermediate is a part that you have created in the process of building a functional composite part, but that on its own has no specific purpose or function. Generally, the first parts you will need to create are basic parts.

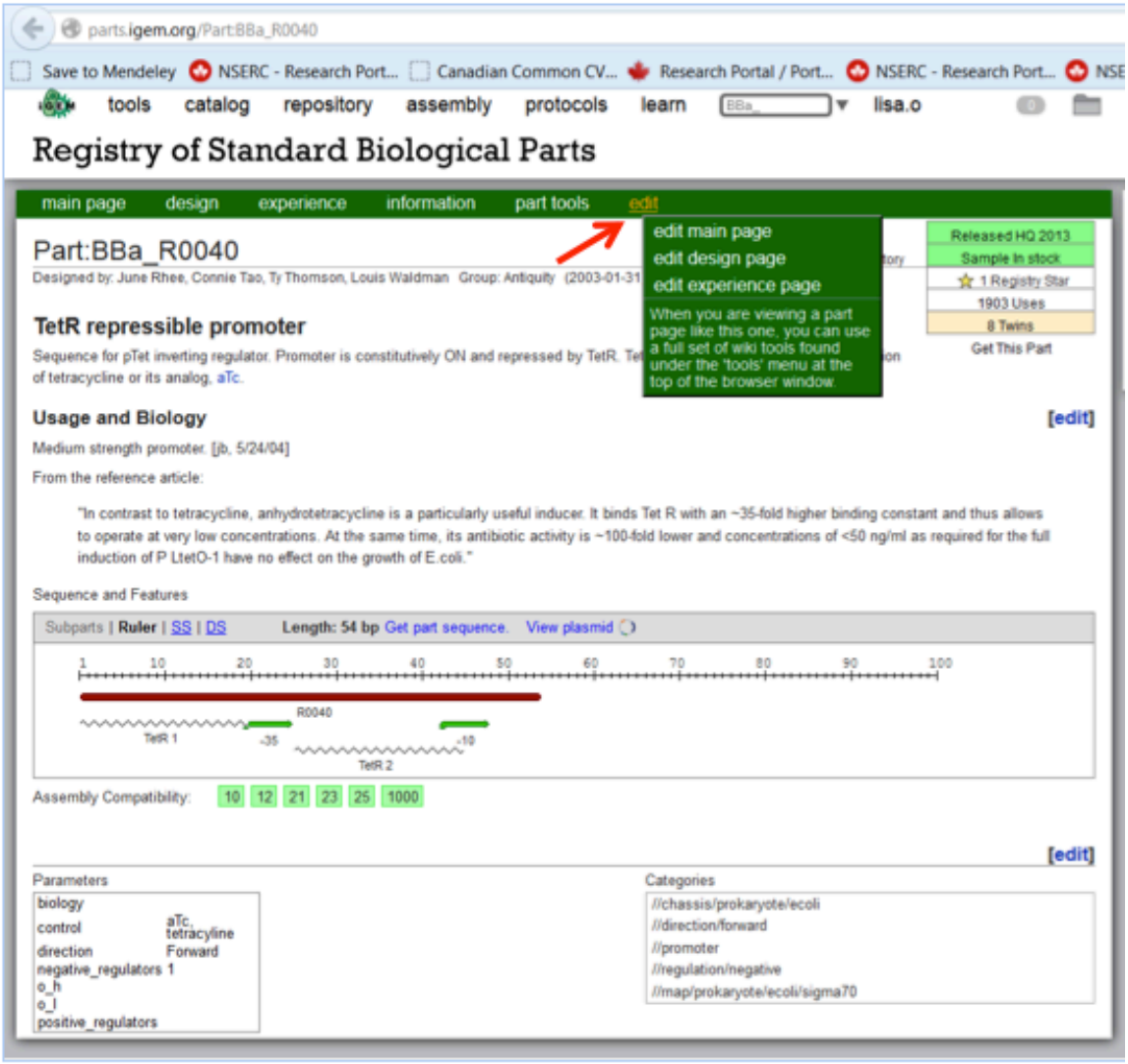


When creating a basic part, you will be given areas of information that must be filled out. The first box (1) indicates which team you belong to, as well as the range of part numbers you are designated to use. If your team has created other parts pages already, it will also tell you what the next available number you could use for a part. Here, you must check a box for which team you are submitting the part for. The next area (2) is where you input the number for the part that you are creating, based on the range that you were given above. Also, this is where you select what type of part it is- A promoter, coding sequence for a gene, ect. Next (3), you provide a short description of the part, which will essentially become its name. Be descriptive but concise (i.e. don't just put the gene name, put the gene name, then what that protein is in full). This cannot be changed easily, so make sure what you name it is finalized. The next box (4) will be where you type the description of what the part is and how it works which will show up on the main page. This can be edited later, but must be present. Following this (5) you need to describe the source of the part (did you PCR it from an organism, or synthesize it? What organism is it from?). The final section (6) allows you to put in any design considerations (i.e. did you add a purification tag, or a useful restriction site, or did you have to point-mutate an illegal cut site from the sequence and where was the change made?). Clicking "go on" will then bring you to an area where you can enter the DNA sequence for your part (from immediately after the BioBrick prefix [usually the start codon] to immediately before the BioBrick suffix). As well,

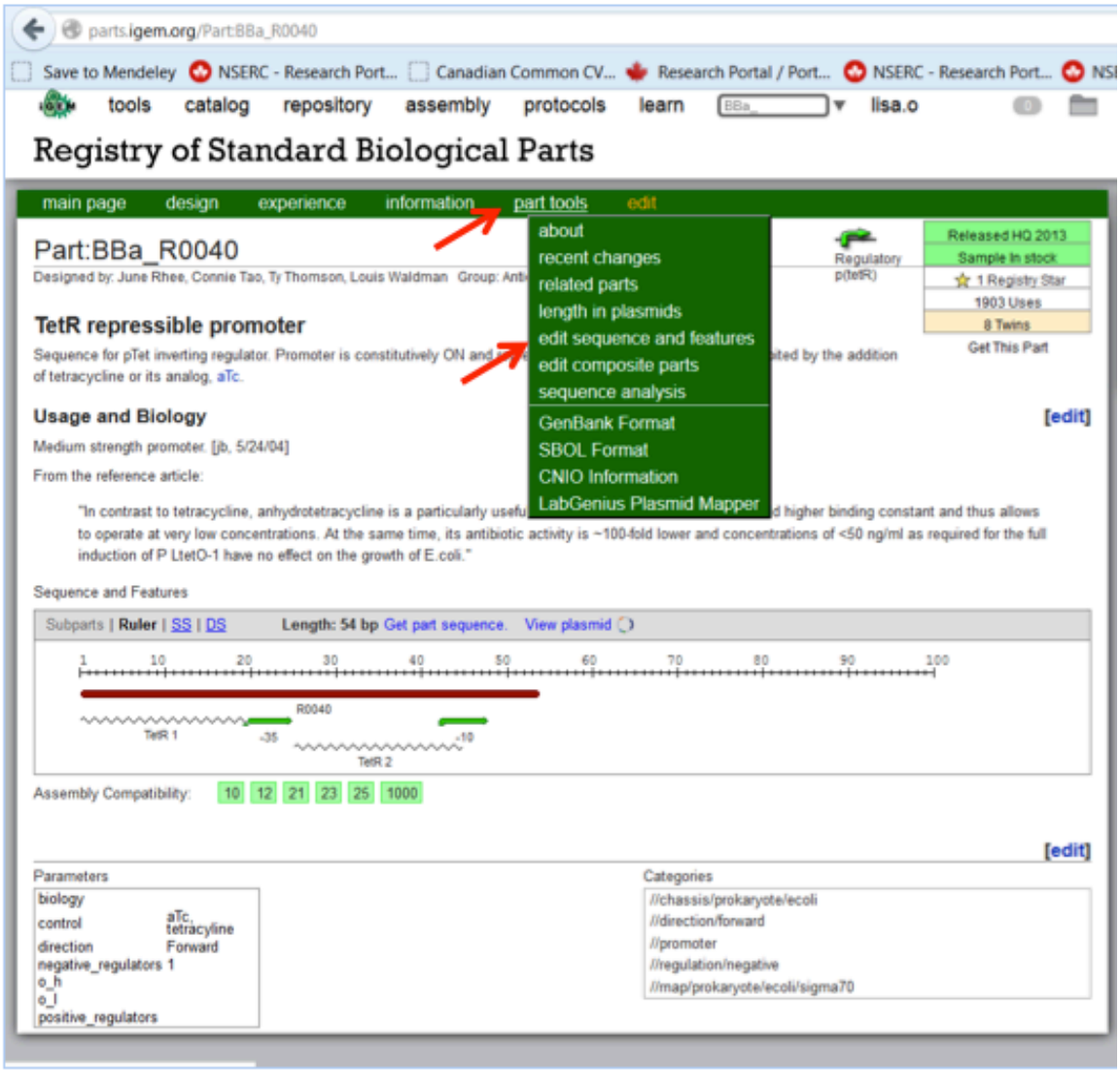
this next section will allow you to annotate features in specific regions of your sequence (such as a specific active or binding site). For examples of this, see the R0040 promoter.



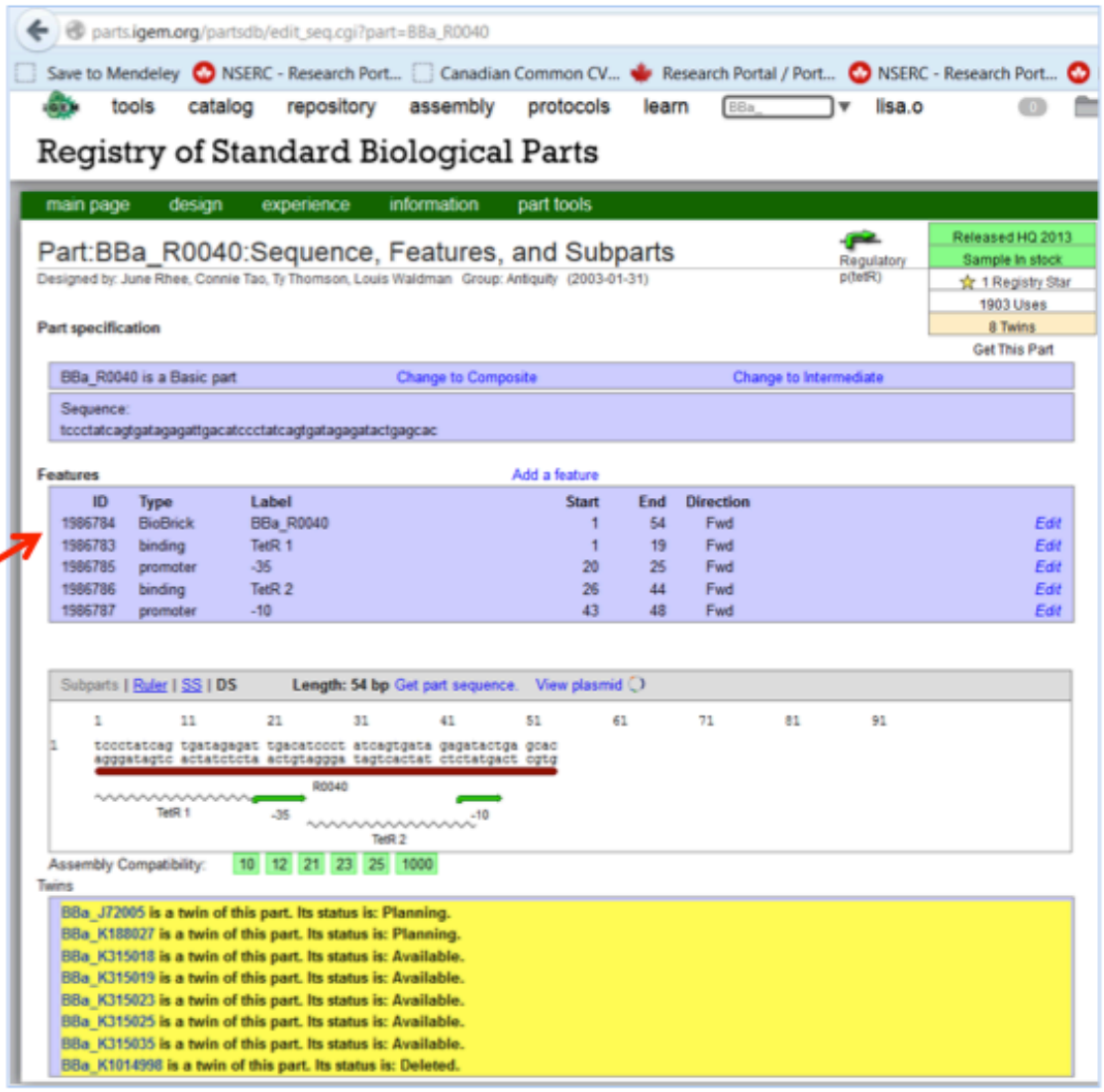
When creating a composite part as opposed to a basic part, the form is nearly the same, however an area at the bottom will allow you to enter the BioBrick numbers for the subparts that the composite is made up of. Enter them in the order that they are found in the sequence of the part. This will also automatically generate a DNA sequence for your part using the sequences of the subparts.



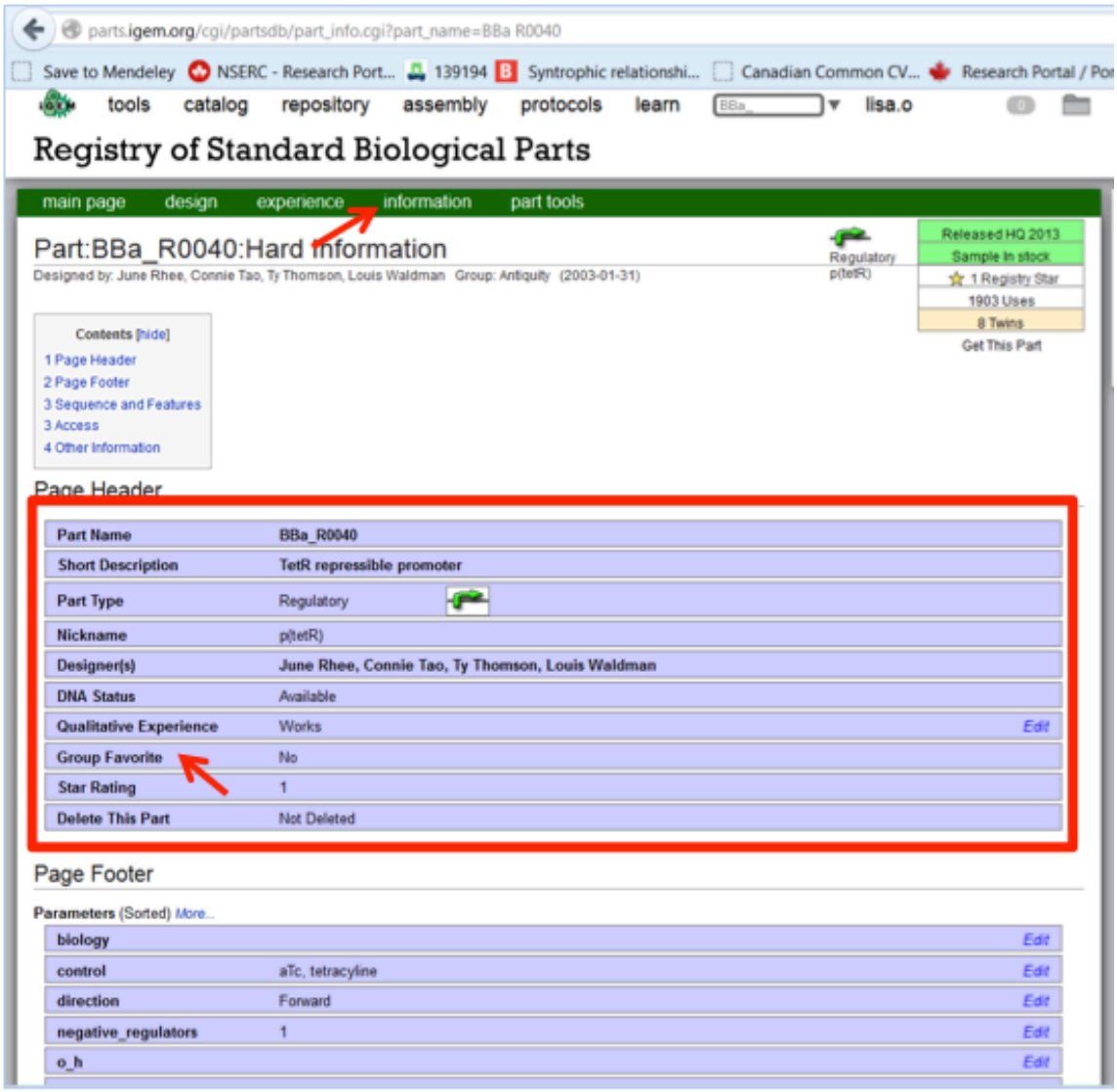
Once you have finished submitting a part page, and entering in information, the part will now have its own page in the Registry. It is important that you properly document all of your parts. In order to do this once a page has been created, you can use the edit menu. The links here will allow you to edit the main, design, and experience pages. The main page should have a description of your part and what it does, as well as how it is used. Also, if you have any characterization data, it should go here as well. Characterization data also must be put onto the experience page. The design page will have information about where the part came from, any design considerations, as well as a required reference to where you found the information/idea/sequence of the part you are submitting and any citations you use in describing your parts. Make sure you reference at least one source. These pages are coded in a similar fashion to the wiki, however if you decide to code in html, you must make sure that the <html> tag comes before what you are typing, and the </html> tag comes after.



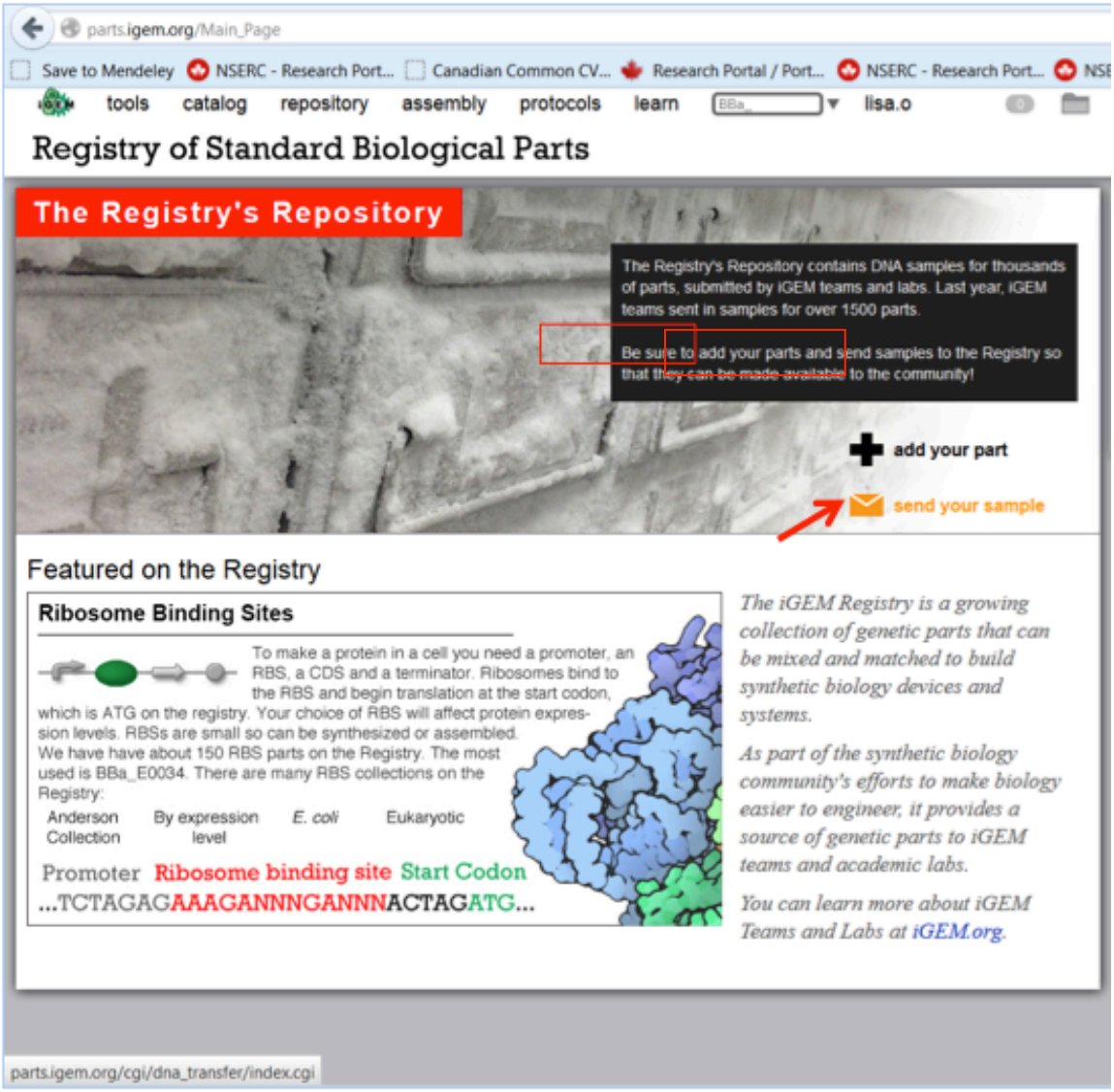
Additional pages that need to be edited are found under the part tools menu. The edit sequence and features area will allow you to edit the DNA sequence you entered for that part, as well as allow you to annotate features of that sequence.



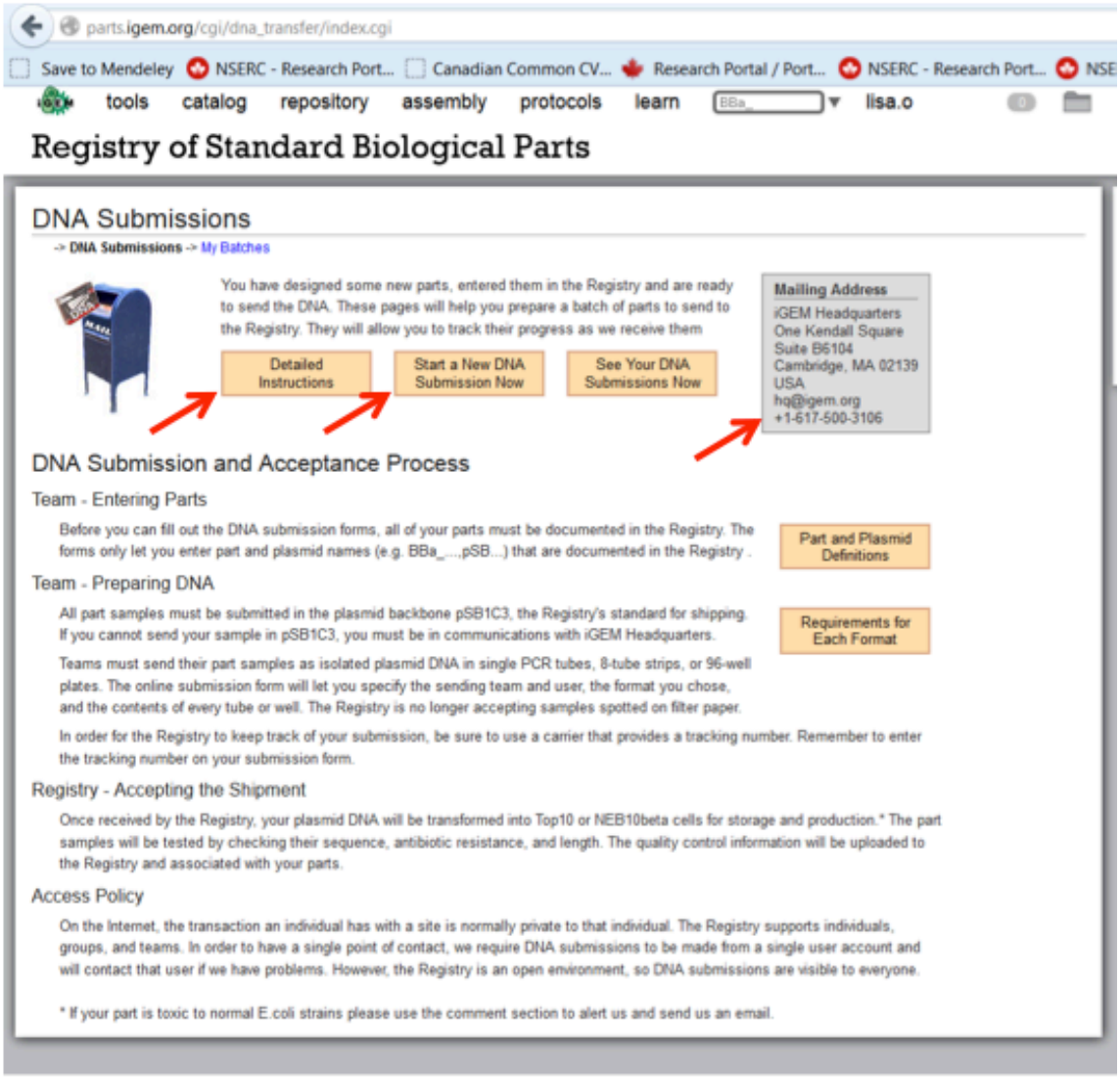
In order to add a feature, you must select what type of feature it is, name it, as well as put in the nucleotide number that it begins and ends at in the full sequence.



The information page will allow you to edit the hard description of the part, such as its name, the type of part, who on your team created it, and whether the part works or not. This is also where you can delete a part if you have made a mistake creating a page if necessary. On this page, you can also rate a part as a group favorite- this should be done for the parts that worked the best and had the most data for your project, as this will flag this part for close inspection by the judges.



Now that you have created a part page for all the parts you are submitting, you can move on to actually sending those samples to the registry. Be mindful of the deadline and the time zone- the parts must be AT THE REGISTRY by that date and time. All parts pages must have been created for the parts you want to submit and sequences must be imputed before you can fill out the form, however the information you put in can be expanded on at a later date (but should be completed as thoroughly as possible before Wiki-Freeze, as that is when judging begins). All parts submitted to the registry MUST be in the PSB1C3 backbone.



On this page, you will find all the necessary information you need to submit a part. The "Detailed Instructions" section will walk you through the submission process and how to actually submit your DNA. "Starting a new submission" will allow you to actually fill out the form. The mailing address of iGEM HQ is found on the right. Before submitting parts, talk to your post office or mailroom in order to get all the required forms in order to send the DNA. Mention that it is DNA you are sending so you are given the proper forms, and make sure you send it so that it will make the deadline. On all forms when asked what you are shipping, make sure to write "Plasmid DNA, non toxic, non hazardous, for research purposes only". Don't ever write the words E.coli, or bacteria, as this will result in customs holds and charges. You will also need to assign some kind of value to your samples- generally use $1.00/tube.

5. A Word on Quality

Although the registry is a fantastic toolkit for iGEM teams, it should be noted that although efforts are made to ensure quality, this cannot be guaranteed. It is always advisable to sequence parts obtained from the registry, especially if they have not been used by multiple teams. In addition, it is advisable to try to select commonly used parts whenever possible. As you search, you will find out that there are a variety of parts that have 'twins', where the same part has been submitted by multiple teams. When this has happened, if you want to use this part in your project, try to pick the one that has the most data for it (found on the experience page for the part), or the one that has a green 'W' for working. This will increase the chances that it is a good quality part. In the case where a part doesn't have a twin, be cautious about using parts that have little to no data, or do not have a 'W' when you search them. This could mean that they are not good quality and may impede your progress. Finally, you may also become frustarted that some parts don't seem to have a lot of information entered about them. Try to use parts that do have the information you're looking for. In addition, when you create your own parts, ensure that what you enter has a ton of meaningful, carefully written information and that the sequence is correct and entered. This will help other teams in the future and will help tp build more 'good quality parts' in the Registry.

BIOSAFETY

David Lloyd

Why Biosafety?

Working in a lab comes with it's own unique set of experiences some of which can be harmful if you do not know what you are doing! This section of the guidebook will focus on safe practices for performing molecular biology within your high school space.

Please note: If you have ever are unsure of anything that you are doing in your lab space, talk to your mentors! It is incredibly important to keep you safe from your bacteria and your bacteria safe from you (we don't want to get our cultures contaminated)!

Also this section will define a more broad definition of what biosafety really is in the context of the competition and what the judges will be looking for when evaluating this specific component of the Human Practices elements of your project.

Step 1: Review the iGEM Biosafety Information Sheets

If you have not already, be sure to check out the biosafety resources available to you as part of the iGEM 2014 information page which can be found here: http://2014hs.igem.org/Safety

Also feel free to check out parts registry information pertaining to biosafety and biosecurity here: http://parts.igem.org/Biosafety

Step 2: Introducing Biosafety into Your iGEM Project

Hopefully these pages will give you an idea that biosafety is an important issue that must always be reflected in your project. When designing your idea be sure to think about questions pertaining to how your ideas will affect the world:

1. Who or what will be affected by developing our organism? (Environment, populations, animals, etc.)
2. How will these groups be affected?
3. What are the potential negative consequences of what we plan to do, no matter how rare or unlikely?
4. What can we do to minimize the risk of such negative consequences?
5. How will our organism be viewed by the public and people in our community?
6. What is the ethical way that we can use our organism to better our world?

It is important to consider all of these types of questions into the design of your system early to ensure that your final organism would be able to be adopted by society or not be seen negatively due to any potential risk.
This is an incredibly important element of the competition that will be judged in your **human practices** section. Human practices refers to the social, legal, ethical, and safety elements of your project. Do not be fooled, judges take this component of projects quite seriously as it is critical that we as individuals who perform synthetic biology think about the effects that our actions have on the world around us.

Many people confuse human practices to just be outreach. Numerous teams have gone to high schools, middle schools, or different community groups to teach them more about synthetic biology and educate them in regards to this new field of research. However, human practices is much more than simply sharing knowledge about synthetic biology. Judges will be looking for how your human practices elements have benefitted your project, how they have influenced other decisions you have made in regards to building your organism, and how your project specifically fits into the world around us. I would highly recommend that you start with a comprehensive view of how biosafety fits into your lab, not just what you are developing but also how you are developing it for the competition.

You will be required to submit some safety information to the competition and it is highly recommended that you consider these elements.

Step 3: Designing Your Space to Be Safe To Work In

Now that we have an idea of what to include for biosafety it is important to ensure that you prepare your lab space for molecular biology work that will be going on.

If your school or organization has some kind of safety committee, please ensure that you have communicated with them in regards to the types of experiments and the work that will be done in the classroom. Also ensure that your safety standards are in line with their expectations of the students.

Basic Requirements of a space:

1. All surfaces should be clean and disinfected, and it is recommended that you work on a surface that is non-absorbant and non-flammable. Coat the area in benchcoat paper to make any messes made easier to clean and to contain any contamination.
2. Separate chemicals and store them away from any gas or flame in the area that you will be working in. We will touch more on proper handling of chemicals later in this section.
3. Ensure that all students have appropriate personal protective equipment for each of the activities they will be performing and are aware of personal hygiene rules while in the lab. Gloves, lab coats, eye protection should be worn when dealing with any chemicals or organisms in the lab. Hair should be tied back, glasses should be worn instead of contacts, and no food or drink should be allowed in the area.
4. Bags, books, etc. should be in a "work free zone" away from any potential source of contamination.
5. All students should be supervised when performing lab work.
6. Mouth pipetting should NEVER be allowed.
7. Emergency procedures in case of fire, gas leak, chemical spill, or injury should be posted and available to the students.
8. Ensure a fully stocked first aid kit is available in the lab and the location of which is known
9. The lab should be equipped with an eye wash station and shower station in case of a chemical spill.
10. A spill kit should be included in case of a chemical agent spilled onto the floor. This should contain:
    a. Paper towel
    b. Kitty litter (or some kind of other material to absorb the spill)
    c. Gloves
    d. Waste bags
    e. lab coats
    f. Goggles
    g. A sealable container
    h. Sodium Bicarbonate (for acid spill)
    i. Citric Acid (for base spill)

    j. Dust pan
    k. Brush
11. All students should be able to read the appropriate WHMIS labels that are contained on chemicals
12. Biosafety manual should be developed with appropriate safety information an example follows: https://www.ucalgary.ca/safety/biosafety_manual

Chemical Safety:

Every chemical that you order must be securely dealt with. Some of those chemicals can be extremely toxic or dangerous to use, so each chemical should be dealt with care. Each chemical will come with a Material Safety Data Sheet (MSDS) which will detail all of the information regarding the safety of the chemical.

Keep all MSDS sheets for reference and put them into a place that will be easily accessible in case of emergency.

Some of the more harsh chemicals you will deal with follow:

1. Sodium Dodecyl Sulfate (SDS) – Very strong detergent. If breathed in a powdered form may cause lung damage.
2. SyberGreen – Used to illuminate DNA. Generally is regarded as safe, but because it is a molecule which insert's itself into DNA it would be recommended to treat it carefully.
3. HCl – Strong acid which could burn skin if spilled
4. NaOH – Strong base which could burn skin if spilled

There are numerous other chemical compounds that you may find within the laboratory but these are just some of the more toxin ones you may find.

When storing chemicals, some may be prone to oxidation or may become reactive when mixed with other chemicals. Always follow the MSDS guidelines for where certain chemicals should be stored. For example some solvents like chloroform or hexane, should be stored within a vented fumehood.

Step 4: Aseptic Technique and Sterility

When it comes to biosafety, things work as a two way street. We do not want you to become contaminated or exposed to anything in the lab, but we also need to ensure that you do not expose anything to your cultures. The mechanism by which we do this is called aseptic technique and ensures the sterility of you the researcher, and the experiments that you will be doing.

Here are some examples of how to maintain good aseptic technique:

1. Anything that will or could be in contact with anything that is living (i.e. your bacteria) needs to be sterile. This sterility can come from numerous sources:
    a. 70% Ethanol – A great decontaminating agent that will kill most bacteria, fungus, and other organisms that you don't want to contaminate your cultures
    b. Detergents/Cleaning Agents – Good for also cleaning areas
    c. High Temperature and Pressure – Heating solutions to boiling or using an autoclave are great ways of sterilizing materials. Most molecular biology solutions reagents will need to be autoclaved to ensure sterility
    d. Bleach – A bleach solutions of approximately 5% is very efficient at killing most living material. Do not the concerns with this being corrosive and take care when using these materials.

   e. Gloves/lab coat/eye protection – Make sure to follow regular safety protocols as this will protect any of your experiment getting onto you. On the other side it is important to ensure that you do not touch any of the cultures as you could contaminate them with bacteria naturally found on your skin.
2. When you are working in a lab make sure to follow some basic asceptic techniques before starting any experiment involving live organisms:
   a. Maintain a clean and sterile area, whipe down surfaces with ethanol or with a detergent product (Lysol for example).
   b. Only use clean, sterile glassware. This includes tips, tubes, solutions, anything that will be in contact with your bacteria.
   c. Whenever you open up a container you expose it to the air which is non-sterile (there may be particulates or bacteria that could contaminate your solutions). To keep these solutions and materials clean, pass the opening through a flame from a bunsen burner. This will kill off ay organisms which could contaminate your solutions during pouring from one bottle to another.

Step 5: Disposing of Bacterially Contaminated Solutions and Materials

All solutions or materials that have come into contact with bacteria must be separated from other waste materials. The bacteria must be killed prior to disposal and this can be done by either of two methods. The materials can be autoclaved, or they can be bleached with a 5% solution. You may find it easier for liquid solutions to soak it in bleach for an extended period of time, and autoclave dry materials. Once disinfected, these materials can be disposed of as you would any other type of materials. Please note: If there were any chemical compounds that should be disposed of separately, this chemical waste should be disinfected and then disposed of in the correct fashion, depending on the type of chemical waste involved.

Always consult a researcher, mentor, or the appropriate guidelines when dealing with biosafety. If at any point you have questions or concerns about the safety or proper handling of a piece of equipment, chemical, or protocol do not attempt it without help from a researcher.

STARTING A TEAM

TEACHER ADMINISTRATION

Robert Mayall

1. Permissions

Starting a Team

Starting an iGEM team has requirements both at the level of the organizing body (iGEM), as well as at the level of your institution. The first and foremost objective is to engage the stakeholders within your school, as without their support it may prove challenging to start a team. The first step would be to discuss the idea with the principal of your school to ensure that entering into a competition such as this is within the purview of your school. An additional follow up step that may be required would be to approach the school board for your district to seek their approval. As well as securing support for the initiation of an iGEM team, this could lead to potential sponsors in terms of contacts or funding for your team. Other key stakeholders that should be brought into discussion could include other teachers at your school, alumni from your school that might be interested in supporting the team, and parents of students who have expressed interest in being involved in a team such as this.

There are also requirements imposed by the iGEM foundation, the body that organizes the competition. A full list of these requirements can be found here: http://2013hs.igem.org/Requirements. These requirements may change in subsequent years, so it would be prudent to look at the requirements for the year of the competition you are seeking to enter. In brief, the requirements are as follows:

1) The team must be made of high school students with a teacher acting as a supervisor.
2) The team can be from more than one high school if desired.
3) Consent forms must be completed (the following section describes this).
4) The team must register with iGEM and pay a registration fee.
5) The teams are encouraged to attend the high school iGEM jamboree.
6) The project must be documented on a wiki.
7) All work must be attributed to the person who completed it.
8) Teams must fill out a safety form (detailed below).
9) The project must be presented (oral and poster) at the jamboree.
10) The team members should enjoy themselves.

As well as the requirements laid out above, there will be significant time requirements on both the parts of the team leader, and of the students. While the students should be primarily responsible for the laboratory work, they will also need to create the poster, presentation, and wiki for the team. Team meetings will also help to keep the project on track, with most teams setting aside time once per week to discuss what they are working on and to help each other out. The time needed per week for these items can vary greatly depending upon the progress of the project and the number of team members, but be prepared for many long nights and weekends closer to the competition deadlines.

Forms

There are a number of forms that have to be completed for the iGEM competition. The official forms can be found here: http://2013hs.igem.org/Consent_Forms. There are four forms to be found from this link. The first is the Competition Information Sheet, which describes the competition and can be

handed out to students and parents who are interested in learning about iGEM. The second is the Participant Consent Form, which testifies to the student's and instructors' desire to be involved in the iGEM team. This form also outlines some information that may be of concern to parents (such as the fact that their children will be given an online identity). The third form is the Principal Consent Form, which address liability at the competition. The final form is the Department Head Consent Form, which is only applicable if the work is being undertaken at a post-secondary institution. This form is to signify that the head of the department that runs the laboratory being used understands that he/she is responsible for the safety of the students using the laboratory.

An additional form that must be completed is called the Safety Page, and is mandatory for all iGEM teams. The information for this form can be found here: http://2013hs.igem.org/Safety. This form primarily addresses the safety of your project and how you will be conducting it. Examples of the potential hazards that would necessitate explanation in this form would be the use of hazardous organisms or potentially harmful genes/proteins. The judges at the iGEM competition are more than willing to provide advice with this form, as everyone wants to see teams compete in the competition and have fun.

As well as the forms required for iGEM, your school and/or district might impose additional requirements. The best way to determine this would be through discussions with the stakeholders mentioned in the previous subsection. These additional requirements could include out-of-country travel forms, biological organism usage forms, or forms required for the purchase of certain equipment or reagents necessary for your project.

Lab Safety & Supervision

Before working in any lab students need to receive the proper safety training. The requirements for each lab will vary, so the administrator for the space you are using will need to be contacted. Almost every lab will require students to have the workplace hazardous materials information system (WHMIS) training at the bare minimum. After this course, which is a simple lesson followed by a quick test, courses such as biosafety training may be needed. The level of biosafety training required will depend on the lab and which organisms your project will be using, with more training required for bacteria with higher risk levels.

As well as the training required for the students, they will likely require constant supervision in the laboratory. This is primarily for safety, but also for insurance and liability purposes in most laboratories. The supervisor does not necessarily have to be the primary teacher though, as any professor, graduate student, or senior undergraduate student associated with your team may also be appropriate for this purpose. It is highly recommended to discuss supervision with the administrator of the laboratory space you will be using before setting up a supervision schedule.

2. Finances

Budgeting

An essential part of a team revolves around the management of the funds needed to operate the laboratory. Without money it may prove difficult to obtain reagents, pay the necessary fees, or attend the competitions. One key to making the money stretch as far as possible is to keep track of it in a detailed budget. This budget should include every expense that can be planned for, erring on the side of caution with price estimates. An example budget for an iGEM team is shown below. The excel file for this can be produced upon request to act as a template for your team.

<*Insert picture of example HS budget here*>

Fundraising

Fundraising is an important part of leading a team. Without raising money it is difficult to obtain the equipment and reagents necessary to perform experiments. There are many places to look for funding, and the project that you choose may open additional sources of funding. In Alberta the government has invested in training students in the area of genomics through the geekStarter initiative, run by AITF. Granting opportunities are available for high school teams on a competitive basis, with more information available on their website: http://www.albertatechfutures.ca/AcademicProgramsiCORE/geekStarter.aspx.

As well as AITF, many companies directly related to synthetic biology may be interested in sponsoring your teams. In the past companies such as VWR and NEB (two major suppliers for biological laboratories) have sponsored teams for up to $2,500 each! Gene synthesis and sequencing companies have also donated their skills and expertise for free or reduced costs too. Practically any company that you may be interested in purchasing supplies from will be interested in discussing educational sponsorship at the very least. Do not get disheartened if one company turns you down or cannot give you enough of a discount on their supplies for your needs; there are hundreds of other companies to choose from.

There are also large opportunities that can be applied for based on the applications of your project. If your work is geared towards cattle for example, approaching the Alberta Livestock and Meat Association (ALMA) may present you with the chance to apply for additional grants. Sometimes these opportunities request a detailed breakdown of all funds gathered to date and how you plan to use their funding (they may have certain restrictions, such as travel). The example budget in the previous subsection is geared towards a mock application to Genome Alberta; a company that sponsors research geared towards genomics based solutions to real world problems. The column titled Genome Alberta Request is a detailed breakdown of the use of the funds being requested from the organization.

Fundraising can be challenging at first, especially if you have no contacts in place with the suppliers whom you would wish to talk to. Asking previous iGEM teams or members of your local university is a good place to start or seek advice. Never give up! The worst anyone could do is just say no.

FUNDRAISING

Iain George

Fundraising for iGEM

This can seem like an enormous task but fundraising for your team is one of the most important aspects of a successful run. As this outline will discuss in detail there are numerous places where any funds your team can raise will be used (from equipment, supplies to travel expenses). But don't fret; there are already considerable resources and experience you can build off of as a platform for raising funds and resources for your team.

Who to approach?

Just as there are many types of restriction enzymes for cutting DNA there are many ways you can get prospective supporters to help fund your team. In short, this can include financial grants, financial donations, studentships, in-kind donations of supplies and promotional rates for services. Most iGEM teams rely on a combination of these pieces of support in their overall financial plan each year. Now that we have an idea of the major pieces of the fundraising puzzle we will discuss each in detail below.

1. Grants – often from major funding agencies in your Province or Federally. Can vary significantly in size from a few thousand to tens of thousands of dollars.

Generally, have a process of application for the grant followed by review of the agency and finally awarding. Major research agencies in Canada and Alberta for these grants are NSERC, CIHR, AIHS, AITF, and Genome Alberta.
These grants could also encompass government agencies whose jobs it is to support specific industries or disperse funds raised from specific industries. For example: if your team is developing a system that could solve a specific problem facing the oil and gas industry, then you could target the industry associations such as COSIA to raise funds.

2. Donations – teams may rely on donations from friends, family, businesses and non-profit groups in their surrounding communities. These are usually smaller denomination funds but can quickly add up.
Many local businesses and non-profits have specific funds dedicated to supporting local groups in their programs. Teams in the past have put together one-page sponsorship documents that summarize who they are, what iGEM is, how a potential donation could help the team, and how the team can recognize the donation.
iGEM teams have previously run bake sales, gala style dinners and Science Café like events to get their friends, families and the general public out to help support the team and also build out reach opportunities for the team.

3. Studentships – While not as typical for high school teams, the vast majority of collegiate teams rely on summer studentships to help support their students work on an iGEM team during the competition season. These scholarships typically are in the range of $4000-$6000 and cover a period of approximately 4 months. Major granting agencies (NSERC, Genome Canada/Alberta, CIHR, AIHS, Markin) or Universities usually administer such studentships.
4. In-Kind Donations & Rebates – Many biotechnology companies offer large discounts or free access to their products and services to iGEM teams. Some of these companies have formal program through iGEM headquarters such as AutoDesk, MathWorks and IDT. Other companies are simply

waiting for you to email them and ask. Major biotechnology suppliers such as BioBasic, Eurofins, GenScript, New England Biolabs, and VWR have supported teams in the past.

The Approach

Approaching groups for funding and support will vary significantly between who your team is looking to raise support from. For example, a pitch with a corporate donor to get their support could occur over a single meeting, while approaching a granting agency could involve a thorough application and review process, whereas approaching friends, family and colleagues could be as routine as a bake-sale to support your teams travel budget. What has been shown year over year by iGEM teams in both the collegiate and high school realms is that there is no single way to raising funds for a team.

It is usually very beneficial to dedicate the coordination of fundraising organizational work with one individual – ideally this would be a student, but will vary with each teams internal structure. A coordinator position can provide continuity and ensures that this component of the project is continuously meeting milestones.

A major initial milestone for the fundraising group is to identify who the group is, what they are hoping to accomplish and what funds will be necessary to achieve those goals (these can be very rough).

HUMAN PRACTICES

Kayla Baretta

1. Why do we do human practices?

Human practices is the part of our projects that allows us to relate what we are doing to everyday life and the advancement of the field. As a scientist, we want what we are doing in the lab to eventual result in a practical application that is accepted by the society – whether this is research relating to small mutations that cause cancer or the creation of new genes – we need to be able to see and explain the impact of our work. Human practice projects allow you to explain to society and to justify why your project is not only valuable but also safe and legal.
There are four main reasons why we do human practices in iGEM:

1. Ethics
   We need to make sure that our projects are ethical and are compiling with the regulations set by our institutions and governments. This can be as simple as researching the legal requirements of releasing a genetically modified organism or as complex as engineering your organism to contain a safe switch gene. A safe switch gene is a gene that can be turned on with the addition of another reagent that will cause the organism to die.
2. Advancement of Synthetic Biology
   Is what I am doing useful to the field of synthetic biology? This idea functions in two ways. One way is the education of society about synthetic biology whether this is other students, young people, or even governmental entities. Education about synthetic biology doesn't need to be a lecture of a workshop. It can be creative and even artistic. A great example of this is the movie created by the University of Lethbridge team in 2011. Projects like this increase the accessibility of synthetic biology.
   The other way to look at this is the creation of parts, techniques and the submission of new organisms to be used in the future. How is your project advancing the way we do synthetic biology? A great example of this is the project from the University of Alberta in 2010 where they aimed to make a kit to build DNA using minimal equipment in junior high or high school labs.
3. Ensuring your research relates to society and is practical
   Does my project have an impact on society? For example, if you are making a biofuel, it would be important to consider the cost of creating your biofuel for an average family and how much the family would save. Or if you are creating a pen from E. Coli, knowing how the manufacturing and sustainability of your pen would work, becomes an important aspect.

2. Choosing a human practices project

Choosing a human practices project should not be a last minute endeavor. If you are having trouble thinking of a human practices project, think about how your project is ethical and how it relates to society. Does your project have day-to-day functions? How will your project impact the field? How will your project be perceived in society?
Once you have decided on some ideas for a human practices project, make sure to evaluate the usefulness of your idea with these questions:

- How does it relate to your project?
- Why do you want to do it?

- Does it help us think about, and act differently in, the practice of synthetic biology? How?
- How will you communicate this (on your wiki, in your presentation, to others etc)?

3. Examples of Human Practices Projects

1. Workshop Based Education
   In 2010, the Imperial College of London created on outline for workshops on how to teach younger students about synthetic biology. Their explanation of how they did it and resources to create your own workshops can be found here: http://2010.igem.org/Team:Imperial_College_London/School_Workshops
2. A well-rounded approach to human practices in a project.
   In 2009, Edinburgh created an extremely well-rounded approach to human practices. They surveyed the public view of synthetic biology in their area and created statistics to support their findings about public perception. They composed a report on the legislation issues and possible problems releasing their product would have within the UK society. Lastly, they created a game that could both educate and cause discussion around the ethical issues of human practices.
   http://2009.igem.org/Team:Edinburgh/ethics%28publicperception%29
3. Comprehensive Project Relating to the Oil Sands
   In 2012, Cornell focussed on addressing human practices in multi stages. They assessed environmental risks associated with their project in relation to the actual environment their product would operate in. They evaluated the applicability of their device. Lastly, they worked to educate the public about synthetic biology.
   http://2012.igem.org/Team:Cornell/project/hprac

Wiki

Patrick Wu

1. The Wiki

What is a wiki? Why is it so important? This chapter will cover the basics of creating your team's wiki and hopefully provide some stepping stones for you to create the Best Wiki at the iGEM competition. We will cover some basic concepts in designing a good wiki, and I will describe HTML and CSS techniques you can use to help circumvent the iGEM wiki's strange behaviours.

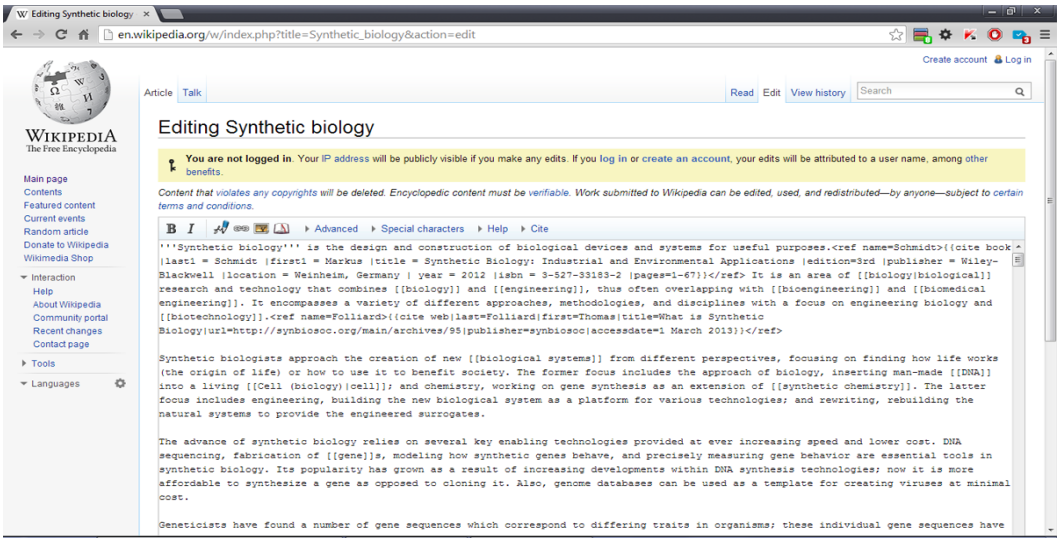
The term *wiki* was coined by Ward Cunningham, the inventor of the first wiki, and comes from a Hawaiian word meaning "quick". A wiki is a tool that allows **collaborative edits from multiple people** on a website. This means any member of the team is able to add, modify, or delete content as they see fit. This is ideal for the iGEM competition because this allows the entire team to contribute data to the site without burdening a small team of web developers. (The developers have a lot of work of their own to worry about, as you'll soon see!)

The most popular wiki is likely Wikipedia, where you can see this kind of collaboration in action. Many contributors can modify information on any page of Wikipedia. On a smaller scale, this is what your team will be doing, too.

If you have ever worked with Wikipedia, you may notice another feature of wikis. *Wiki markup* is the simplified language used to add content to pages. The iGEM wikis run off a similar engine (MediaWiki) and you can use similar code in order to edit pages on the iGEM wikis.

Syntax refers to the "grammar" that lets the browser **unambiguously** understand what you want it to display. For example, in wiki markup, square brackets are used to denote links to other pages within the same wiki.

The iGEM wiki is capable of interpreting HTML and CSS syntax, which is a much more powerful set of languages that allows finer and more sophisticated control over the design of your wiki. Wiki markup is a simplified template that is translated into HTML when your browser loads the page, so anything



Wikipedia is a very well-known wiki. The markup used has special formatting to denote features such as links, images, tables, and bolded, italicized, or underlined text.

wiki markup can do can theoretically be accomplished with HTML and CSS. Nowadays, almost all Collegiate-level teams will have their wikis coded in HTML and CSS, and forgoes wiki markup almost entirely (with a few exceptions). While more complicated, learning HTML and CSS will ultimately give you greater rewards as you will have much better control over what you are building.

2. Why Should I Care About the Wiki?

The simple answer is because the wiki is a powerful tool that allows you to communicate what your project is to the judges, other iGEMmers, and the rest of the world. Judges at the competition are very busy people who have to watch a large number of presentations throughout the day. The wiki is your opportunity to remind the judges what your project is, and to highlight what your major accomplishments are.

Remember that the judges aren't necessarily scientists trained in synthetic biology. It's recommended that you write the wiki so "anybody with a high school level of education" can understand it. In other words, you should be writing your wiki so that if your friends and family read it, they will understand the basic idea behind your project. Therefore, try to avoid using too much scientific jargon, and if you do, make sure you explain what it means.

This is your opportunity to show off everything you've learned over the summer not only to the judges, but also your family, friends, and the rest of the world.

Also, your wiki is being judged along with your project, and there is an award for Best Wiki at the competition. You can keep that in mind, if you wish.

3. What's in a Wiki?

The official requirements about what can and cannot be put on the wiki can be found here: http://2013hs.igem.org/Requirements/Wiki. There should be a similar page each year (as of this writing, the 2014 site does not have guidelines just yet). In summary, these are your main requirements:

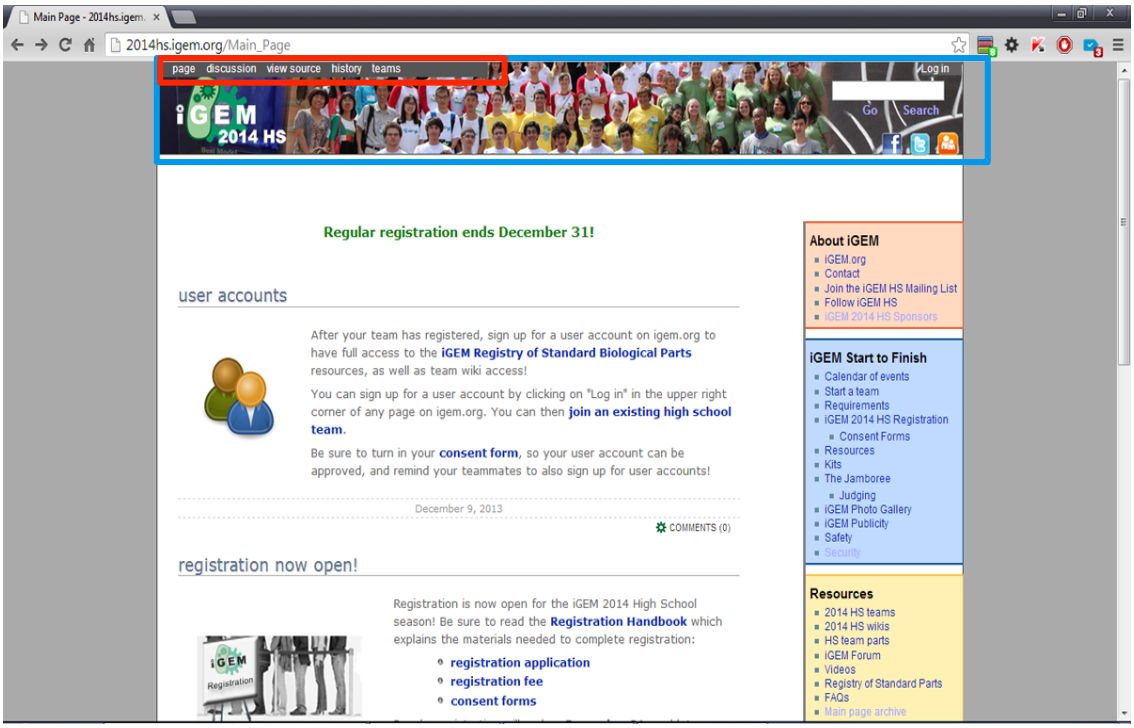
- **You must have your project documented by the deadlines** – there are a few major deadlines that are outlined on iGEM's Calendar of Events. One of your most critical dates is the **wiki freeze**, where you must have everything on your wiki finalized before the servers are locked down. After the wiki freeze, you will not be able to make any more changes to your wiki until after the iGEM competition. You should be working on your wiki throughout the competition, but be ready to set aside a few days prior to the wiki freeze deadline to polish up any last details.
- **All content must be uploaded to the iGEM server of your respective year** – there are a number of different domains that iGEM runs off of. Make sure you upload all your photos, images, and wiki-building content (like buttons and icons) on the proper server: *20XXhs.igem.org*, where *20XX* is the year of your competition
    - **Do not** confuse *20XX**hs**.igem.org* with *20XX.igem.org* since the latter is the Collegiate server of the same year!
- **Flash is not permitted** – with the current trends in tablets and smartphones, this shouldn't be a major loss. Adobe Flash, while it produces very visually appealing sites, is not a good crutch to be building a website on. Also, iGEM strives to make all wiki code open-source (in other words, you are free to take any code from any wiki to use on your own—as long as you credit them, of course.) Flash hides this code into an embedded movie and therefore goes against this open-source philosophy.
- **Your wiki must remain within your own namespace** – for example, if your team is called *Example_Canada*, your homepage on the iGEM servers will be on

  20XXhs.igem.org/Team:Example_Canada.

This is your *namespace*. Any new pages you create must have *Team:Example_Canada* before it. For example:

20XXhs.igem.org/Team:Example_Canada/Team
20XXhs.igem.org/Team:Example_Canada/Project
20XXhs.igem.org/Team:Example_Canada/Project/SubProject

- **Your wiki must contain a safety and attributions page** – the Safety page is a questionnaire that you must answer in order to demonstrate to iGEM and the judges that you are protecting yourselves and others as you are working over the year. The safety page also allows you to demonstrate a thorough understanding of the potential safety issues your project might create. How are you handling them? How are you mitigating risk? The attributions page is important for showing that this project is done primarily by yourselves as students (as opposed to having a teacher or university mentor doing all the work for you). The attributions page must list any external help you have received, such as labs which have given you materials, web designers that have designed the wiki for you, and so forth.
- **You must have a link back to the iGEM home page** – this is an iGEM competition, after all. By default, you have a large photographic banner at the top of your wiki which is sufficient to lead viewers to iGEM's main site. However, if you find this banner rather unsightly and decide to remove it through your code, you must provide a link back to iGEM's site somewhere else. **You cannot erase the top control which lets you edit pages and log in**. But iGEM teams in the past have pieced together CSS code which can erase the photo banner while preserving the control bar. You can find a code snippet in Appendix A.



The top banner (blue) appears by default on all iGEM pages, and links to the main iGEM page. The menu area (red) only appears if you roll your mouse cursor over it. It does not easily appear on touch-based devices such as tablets. The CSS snippet provided in Appendix A removes the banner but preserves the menu. The snippet also removes the mouseover behaviour of the menu and instead keeps it visible at all times.

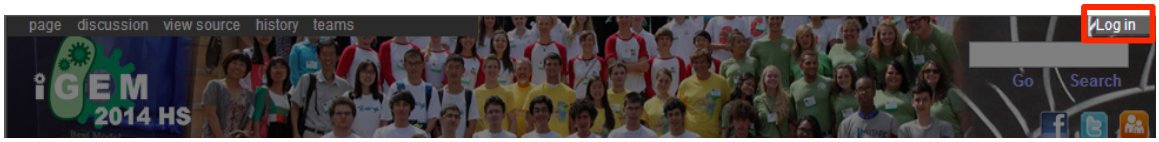
Some other key components of the wiki include:

- A **project page** –this is where the description of everything you have done over the year will be found. Be sure to explain what the problem you were tackling was, and what kind of solution you had proposed. What were your experiments? Why did you do them? What were you hoping to see, and do your results reflect this? What can you conclude from your results? *Can* you conclude something from your results (incidentally, something that is very common in science and should not be considered a failure)? What needs to be done in the future? This will likely be the largest section of your wiki, so you should be ready to break this into a number of different pages (such as your introduction, methods, results, and conclusions).
- A **notebook page** – this is where you document the day-to-day experiments that you have done. This is critical in science, as this allows peers to review your experiments and your thought processes. You do not need to repetitively mention the amount of reagents that you're using, but you should be mentioning which parts you are using and what constructs you are trying to build. The 2013 Lethbridge high school team (http://2013hs.igem.org/Team:Lethbridge_Canada) has a good example of a notebook (and of a wiki in general).
- A **team page** – who are you? This section lets you briefly talk about yourselves as students. This page can be as simple or as elaborate as you want it to be. Take a look at other iGEM teams for inspiration.
- A **protocols section** – a lot of teams place this within the Notebook section of their wiki. This is a summary of all the molecular biology techniques and experiments you have done over the year. Others who read these protocols should be able to replicate your experiments and get similar results.
- A **human practices section** – this is where you discuss your Human Practices component of the project.
- A **sponsors section** – thank the people who have helped you along the way! You can also put your required attributions here.

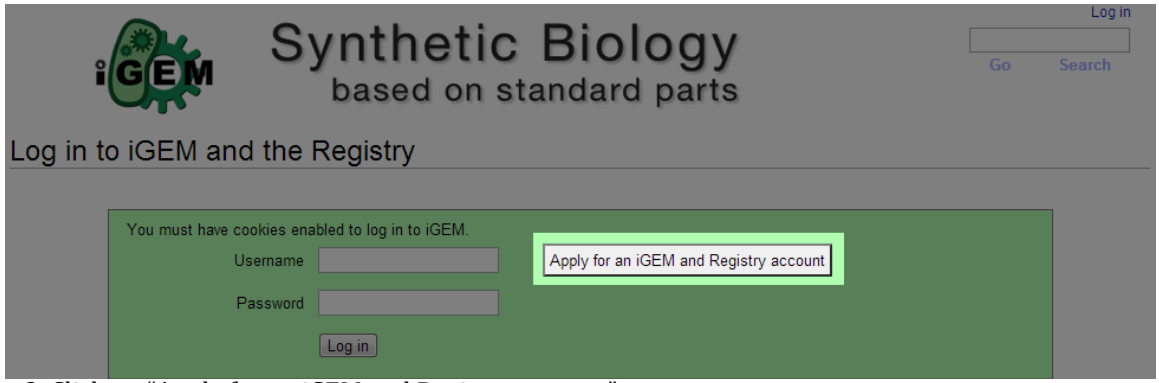
By no means is this list exhaustive. Feel free to add or omit any sections you feel are necessary in order to get the best parts of your project across to the judges.

4. Accessing the iGEM Wiki

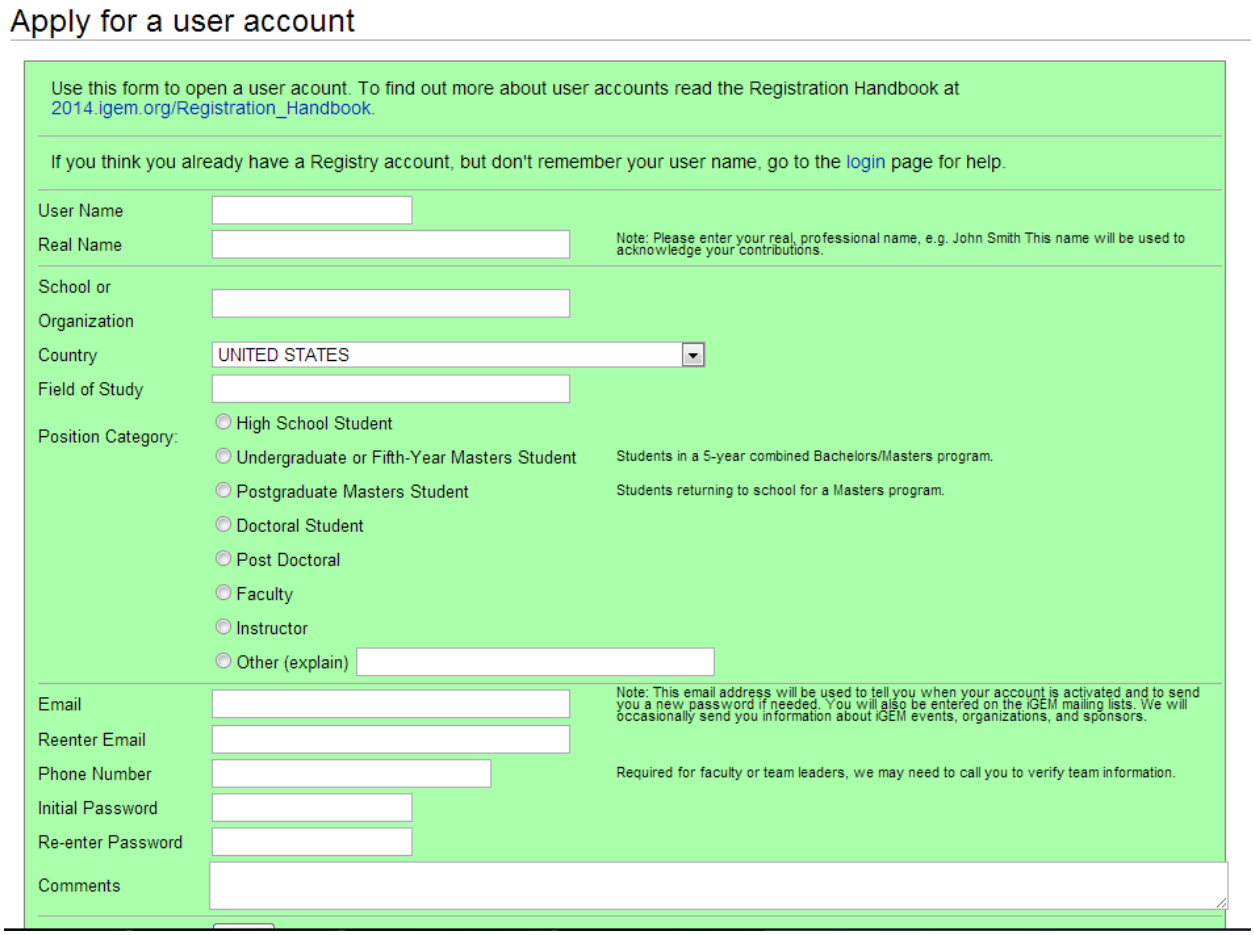
Once your team is signed up and registered in the High School competition, you will be asked to create an account with iGEM. This is what will allow you to edit the wiki.



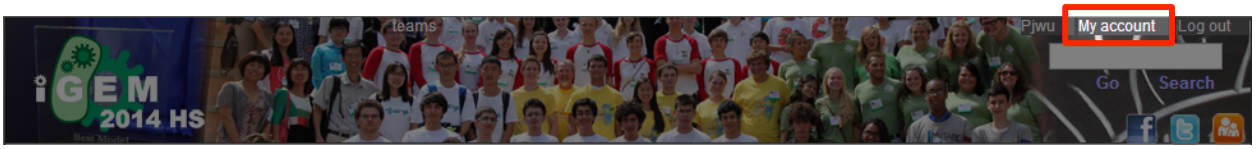
1. Click on "Log in" on the top-right hand corner of the iGEM banner.



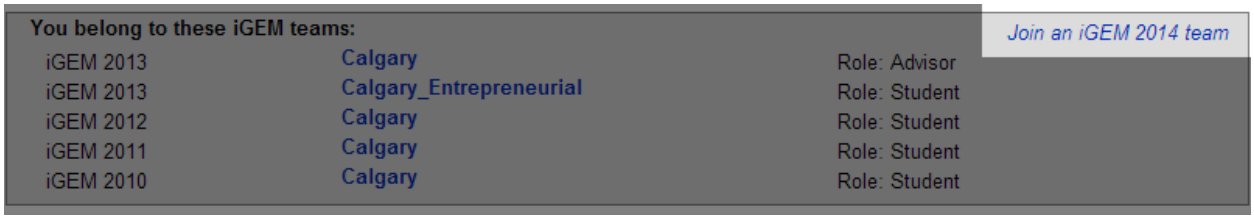
2. Click on "Apply for an iGEM and Registry account".



3. Fill in your information and click "Apply"



4. After logging in, click "My account" in the upper left-hand corner

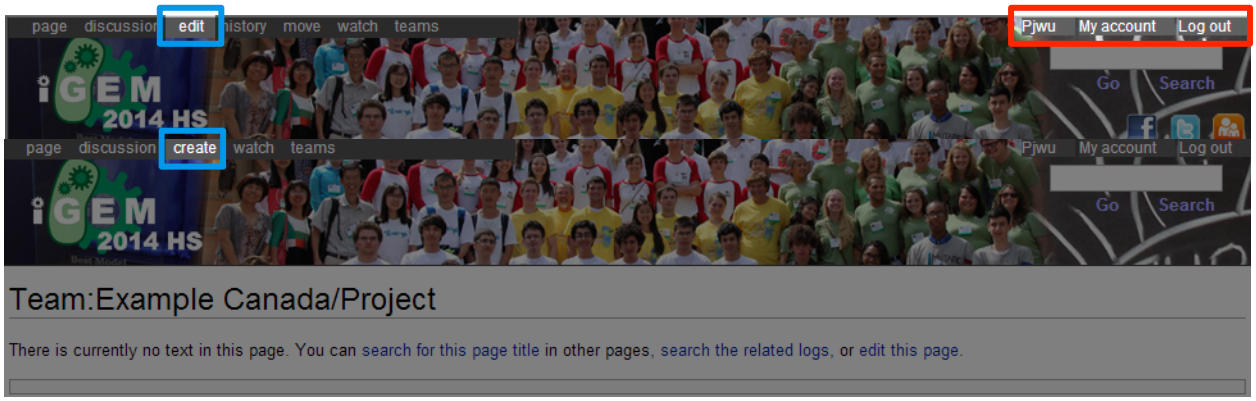


5. There will be a section which lists all of the teams you participate in. As a new user, you will not have anything here. Click "Join an iGEM 2014 team" and follow the instructions. Your team leader will add you to the roster.

Make sure your instructors/advisors have added you to the proper team. You will not be able to edit any wikis on the High School server until you are part of a team.

5. Editing the Wiki

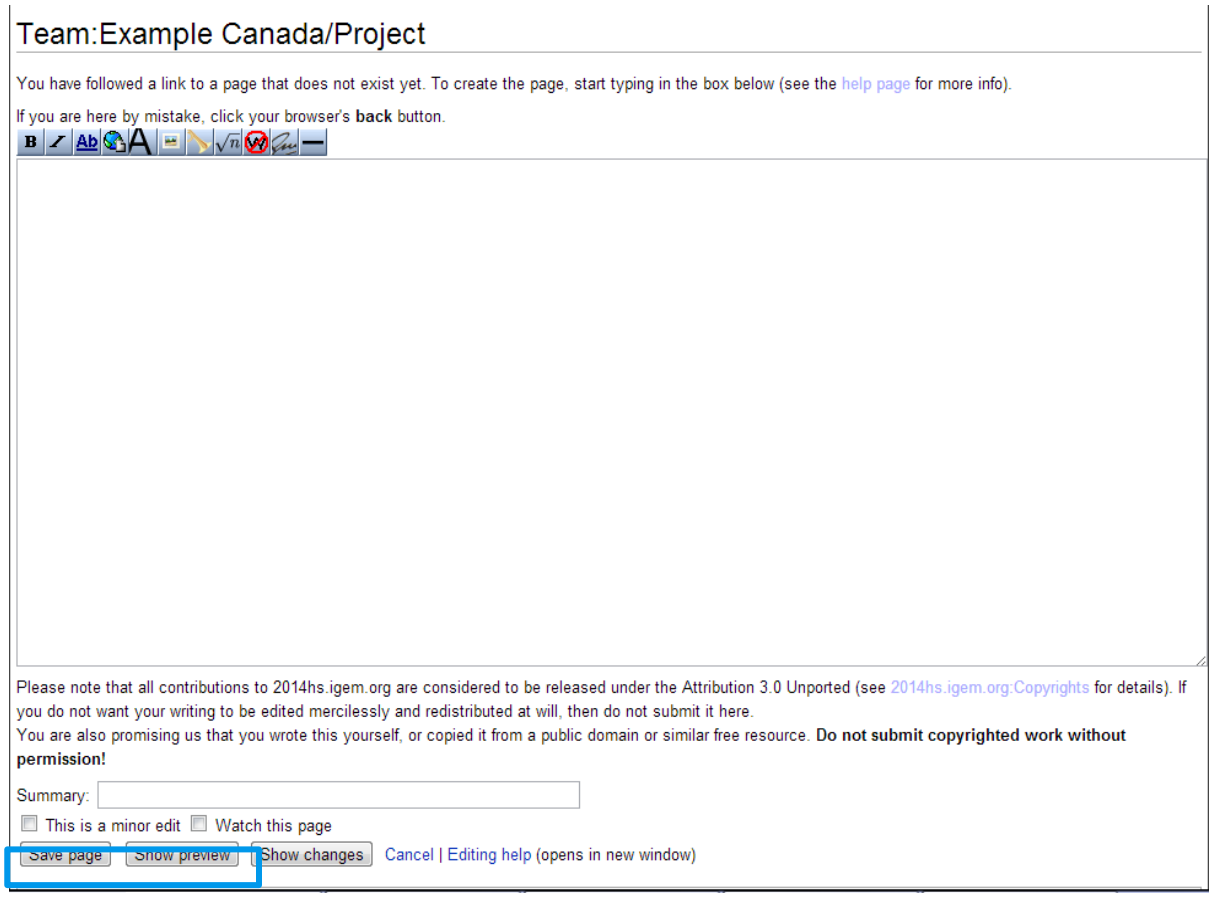
Editing the wiki is as simple as logging in and clicking "Edit" in the menu at the top of the banner. If you see "View source" instead, you have not logged in properly or the iGEM servers have kicked you off. Try to log in again.



For example, if the page to be created is a project page in the namespace *Example_Canada*, you would first navigate to *http://2014hs.igem.org/Example_Canada/Project*. You should then see this message. Click "Create" to make a page.

To create a new page, type the URL of the future page into the address box. You should see a message telling you that this page does not exist. Click "Create" on the top menu and you will be brought to the editing window.

From here, you can type all of your code into the box and then click either "Show preview" to see how it impacts the final page or "Save page" to commit it to public viewing. Remember that in order to have changes visible to others, you must **save** the page. However, it is a good habit to **preview** pages first, in order to confirm you have not made any flagrant mistakes prior to publishing the page for the public to see.



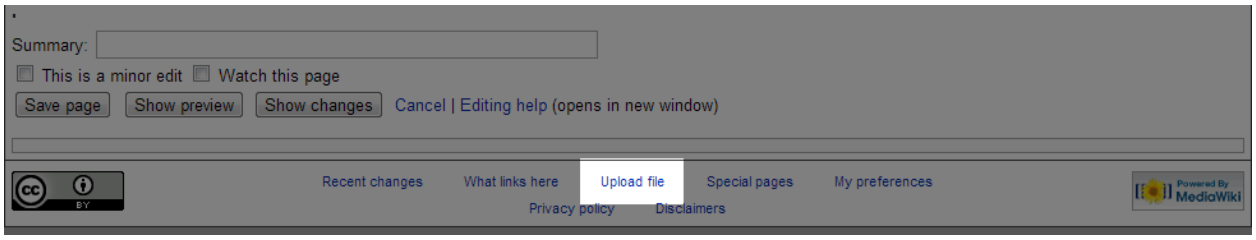
The large white area is where markup will go. The "Save page" and "Show preview" buttons are near the bottom (blue).

Feel free to play around with a wiki page on your own namespace by clicking "Edit" at the top of the banner (preferably a page within your own namespace to minimize damage). Historically I have always liked to create a page called *Sandbox* in the namespace so that I can test out code without the public seeing any changes until it is fully done. Then I would copy and paste the markup onto its destination page. It is good practice to build pages elsewhere on the wiki and avoid publicly launching a wiki page until you are comfortable with how it looks. This also saves the viewers from trying to work with a buggy wiki.

6. Uploading and Using Images (and Other Media)

In order to build a successful wiki, you will need to be able to upload images on to the iGEM server so that you can utilize them in your wiki. Remember that one of the official rules for the wiki is that all content must be hosted on the wiki server of that year.

At the bottom of the page (if you are logged in), click the "Upload file" link. If you cannot see it, ensure that you are logged in and that the iGEM server has not kicked you off.



The "Upload file" link only appears if you are logged in.

Choose a file by clicking on the "Choose file" button (blue). Then, give the file a name which will be used to find the file again on the server (red). Finally, upload the file by clicking the "Upload file" button (purple). The destination filename cannot have spaces, but can contain underscore characters (_) to represent spaces.

The remaining steps should be intuitive. The only point I must stress is that all images **share the same namespace** on the iGEM server, meaning any image your team uploads can be overwritten by another team if they upload a file of the same name. Therefore, **you cannot upload an image with**

simple names like "Image1.jpg" because there is a high risk that somebody on a different team can overwrite your photo.

My recommendation is that you prefix the file with something strictly for your own team, such as LethbridgeHS2014_ or ConsortHS2014_ so that the file is less likely to be mistaken for another team's file.

After you click "Upload file", you will be brought to a screen which will describe details about the file you have just uploaded.

There are three ways to find this image you have just uploaded. You can find it again either through the URL (red), the wiki server filename derived from the URL (blue), and the direct link to the image you have uploaded (if you click the link in purple). The browser URL on this page cannot be used for HTML and CSS purposes. Only the direct link can be used for HTML and CSS markup.

The wiki server filename is only needed when dealing with wiki markup. The direct URL link provides a link to the image for HTML coding purposes. You will require that if you decide to use <img> tags since wiki markup cannot be used in HTML and CSS.

In summary, you have the browser URL, the wiki server filename, and the direct URL to refer to this image you have uploaded. **Ensure that you keep and save at least one of these addresses so you are able to find this file again if need be.**

7. User-Centered Design and User Experience Design

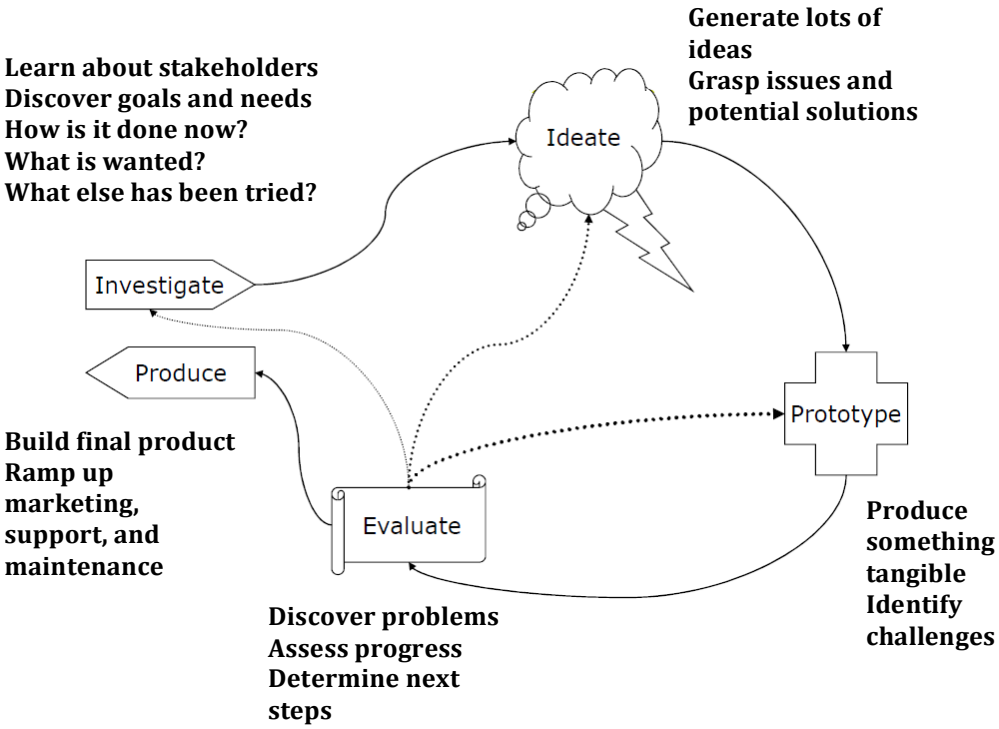
Think about the websites, apps, and computer programs that you use often. Do you enjoy using them? Do you find some features on some of them rather frustrating? Do you find yourself lost when you're using some apps? Keep these questions in mind as you go about your daily web browsing today.

There is plenty of very careful thought that is put behind designing a good user interface. The process behind good design is known as **user-centered design (UCD)**, and usually well-designed interfaces

have many hours, weeks, or even months of design, prototyping, and testing behind them. UCD is part of a much larger field of **user experience design (UX)**. UX is in turn part of **human-computer interaction (HCI)**, a field of computer science that intersects graphic and industrial design, behavioural sciences, and communication theory, amongst many others. Many papers and journals for UX exist, and it continues to be a very heavily researched field.

UCD is an iterative process that focuses on building an interface that facilitates the needs and desires of the **user**—in this case, whoever will be viewing your wiki. UCD cycles through four main steps:

- **Investigating** what the user does and wants to have
- **Ideating** or brainstorming ideas that can be used to solve the problem at hand
- **Prototyping** a few of these ideas and develop them into potential products, and
- **Evaluating** the prototypes with the users to see if they are effective and usable solutions



The UCD cycle begins with investigation and proceeds to ideation, prototyping, and evaluation. Note that traditionally, if something fails in any of the latter steps, there is a path which leads back to earlier steps and the cycle repeats itself. Only when the evaluation succeeds does the product go into production.

Why is this relevant? UX is about making the users **happy** and **satisfied** with using the interface. The easier you make it for the user (namely, the judges) to access all the information you have on your wiki, and the more pleasant the experience is, the less frustrated the judges will be with you. And in a competition like iGEM, annoying the judges is likely not high on your list of priorities.

UX is difficult—there wouldn't be Ph.D. degrees in the field otherwise—but you are taking a major step by simply considering who might be using this interface/product in the natural conditions that they will be using them. For example, will the users be viewing your wiki on laptops? (The answer is "yes"). Are they going to be viewing them on tablets? (The answer is also "yes"). Are they going to view them on phones? (The answer is… well, you know.)

What does this imply? What do you need to do in order to make it easier for these users? (Hint: tablets do not have a mouse-over action. All you have is a thumb that taps. Try to edit the wiki on an iPad, for example, and you will find it rather difficult to access the mouse-over control bar on the default templates.)

As you're building your wiki, or as you're drawing and sketching your ideas for potential interfaces and making prototypes, let other members of your team test them out. As the designer, **you know your interface too intimately to be unbiased** about how to use it, so your judgement alone is not necessarily the best judgement. Watch as your user tries to navigate your interface. Don't help them. Note where they seem to be confused or struggling, and note where they are easily finding the information they need. Use this information to build on new iterations of your idea. Be prepared to make alterations to your interface as necessary in order to accommodate for usability challenges that people find.

8. Nielsen's Usability Heuristics

One of the more well-known individuals in web usability is Dr. Jakob Nielsen, one of the co-founders of the Nielsen Norman Group and has been researching and consulting in web usability since 1998. Nielsen has a Ph.D. in human-computer interaction and has published many articles, patents, and books on how to improve web usability.

Heuristics are general principles, or rules of thumb. For interaction design, Nielsen published ten particular usability heuristics, which can be found on the Nielsen Norman Group website (http://www.nngroup.com/articles/ten-usability-heuristics/). Each one will be covered in some detail here, but remember that these are general guidelines and may not necessarily apply to all situations:

- **Visibility of system status—***The system should always keep users informed about what is going on, through appropriate feedback within reasonable time.*

  If you have ever installed a program before, you will have likely seen small animations like a spinning wheel or a progress bar. These help the user recognize that the computer is, in fact, doing something with the information provided to it. Providing feedback ensures the user doesn't get too lost in what the system is doing.
  On a website, this can even be as simple as highlighting the section of the site on the navigation menu. This way, users can remember what part of the website they are reading. Here's a simple tutorial: http://hicksdesign.co.uk/journal/highlighting-current-page-with-css
- **Match between system and the real world—***The system should speak the users' language, with words, phrases and concepts familiar to the user, rather than system-oriented terms. Follow real-world conventions, making information appear in a natural and logical order.*

  Say you were using a desktop application and suddenly this appeared:

  A runtime error has occurred.
  0x49cfb8
  Error: Syntax error.
  Do you wish to debug? Y/N

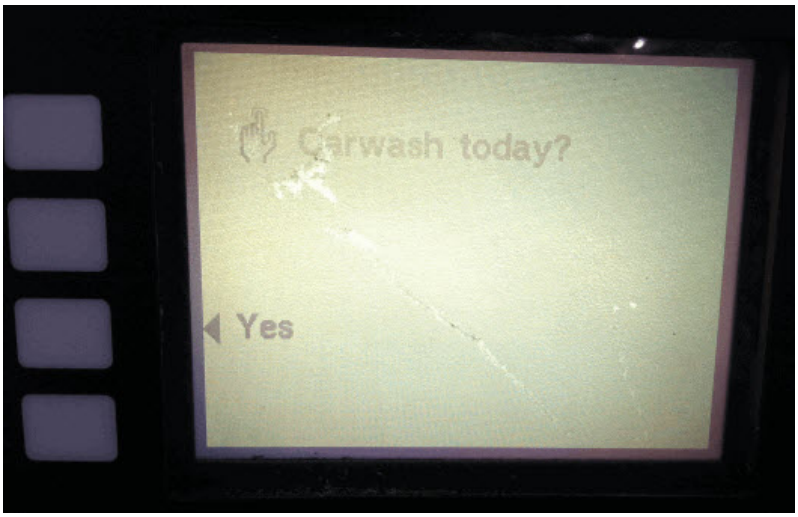
  Does an error message like that mean anything to you as a user? Likely not, since a message like that was more intended for the developers. These system-oriented words are meaningful only to a small subset of the end users.

  In web design, there are particular words that people are used to seeing and have an implied meaning. "Home" brings the user to the front page of the site. Esoteric naming of links

confuses users (one example being the official website for the movie *Space Jam*—a site that incidentally never left the 90's for many other reasons). For iGEM, judges understand terms such as "Notebook", "Safety", and "Attributions", and have expectations as to what can be found on those sorts of pages. Therefore, we recommend that you keep pages with those names.

- **User control and freedom—***Users often choose system functions by mistake and will need a clearly marked "emergency exit" to leave the unwanted state without having to go through an extended dialogue. Support undo and redo.*
-

In essence, allow escape routes so that users do not feel trapped when going through a process. You should offer a way out (even something like a red X on the top corner of the window to abort the whole process is still considered a way out).
People learn interfaces by exploring. Exploring means to poke around the interface to see



You *will* get a car wash. There is no escape.

what particular actions do. People make mistakes, so you shouldn't punish them for attempting to do something. For example, shopping websites will let you review your information before finally committing to a payment. In the worst case, many websites have a "Home" button that simply brings the user back to a safe, reset area where they can try to explore again.

- **Consistency and standards—***Users should not have to wonder whether different words, situations, or actions mean the same thing. Follow platform conventions.*

It may sound really nitpicky and almost unnecessary, but consistency in fonts, colours, shapes, and wording reduces the chance for users to be surprised. Having *internal consistency* means that an interface is consistent with itself, such as having system buttons collected together. *External consistency* is where the interface is consistent with similar applications on similar platforms. This is where "muscle memory" problems between different programs can cause frustrations. For example, one of Adobe Illustrator's undo functions (where one can undo multiple steps) has Ctrl+Z as its shortcut, while Adobe Photoshop's same undo function is Ctrl+Alt+Z. This is where external consistency breaks down.

In the wiki's case, this rule overlaps with using real-world words. Again, judges expect to see a Notebook section of the wiki, since it is conventional for all teams to have one. However, other website conventions exist, too. For example, you expect to have a navigation menu

somewhere on the top or on the side. It would be rather unconventional and confusing to have the navigation menu at the bottom of the page. Take time to make sure your images and text are aligned and well-organized. An uncluttered website is much more pleasant to navigate than a disorganized mess.

- **Error prevention—***Even better than good error messages is a careful design which prevents a problem from occurring in the first place. Either eliminate error-prone conditions or check for them and present users with a confirmation option before they commit to the action.*

  This is a good opportunity to watch your users test out a prototype. Where do they make mistakes? What can you do to help reduce the chances of that mistake happening again? Many desktop applications have menu items on dropdowns that are greyed-out in order to prevent users from performing actions that are impossible.

  When building your wiki, be sure to test out all the links and all potential areas where you think an error can happen. It would be tragic post-wiki freeze to realize one of your major links doesn't actually lead to a page you wanted it to because somebody had copy-pasted the code from another page.

- **Recognition rather than recall—***Minimize the user's memory load by making objects, actions, and options visible. The user should not have to remember information from one part of the dialogue to another. Instructions for use of the system should be visible or easily retrievable whenever appropriate.*

  Recognition is in general much easier for people than recall. For example, if I show you this image, you can immediately recognize what it is:

"It's a jackal, right?"

You may have a harder time with *recall*, for example, if I ask you what colour the shirt you wore was two weeks ago. In general, recognition of an item is much easier than having the user attempt to recall the information for themselves.

As another example, is a multiple-choice exam easier than a short-answer exam? Sometimes you can pick out what you were looking for when the option is presented to you. It is generally easier to pick an item out from a list instead of having to write it out yourself, because seeing that item in the list can help remind you of what the choice is.

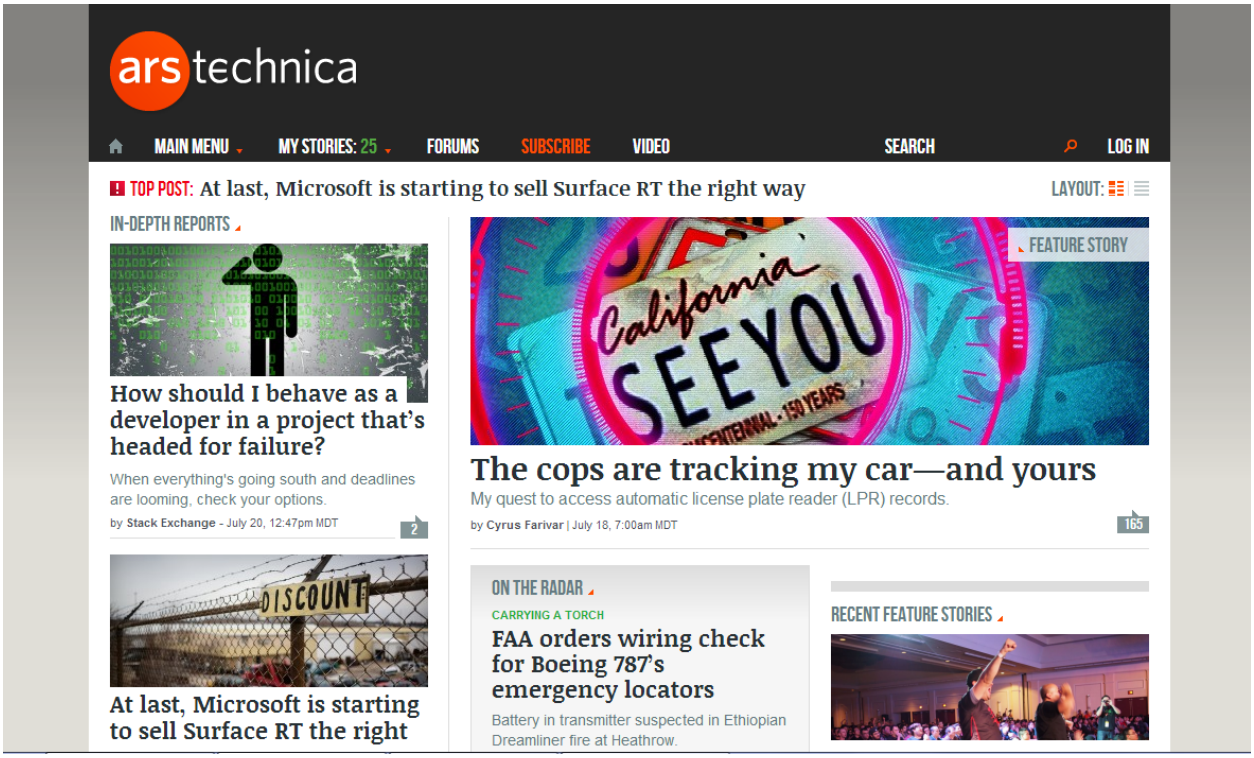
- **Flexibility and efficiency of use—**Accelerators – *unseen by the novice user – may often speed up the interaction for the expert user such that the system can cater to both inexperienced and experienced users. Allow users to tailor frequent actions.*

  Accelerators are essentially shortcuts that optimize your workflow. Do you right-click items to copy and paste? Or do you use the Ctrl+C and Ctrl+V shortcuts on your keyboard? (Or are you smug with your Macs and use ⌘+C and ⌘+V instead?) Do you hit "Enter" when you submit a form, or are you forced by the interface to click "submit"? This is more commonly a consideration for more complex web applications such as email or shopping sites. One easy way to improve efficiency is to add hyperlinks in your main text. If you're talking about another aspect of a project in one of your paragraphs, provide a link to it so the user can simply jump to that aspect of your project and then return later. Keeping your site structure simple will be a major step in preventing users from getting lost. The general rule of thumb is that all pages should be accessible within three to four clicks of the home page (but this has been debated: http://uxmyths.com/post/654026581/myth-all-pages-should-be-accessible-in-3-clicks)

- **Aesthetic and minimalist design—***Dialogues should not contain information which is irrelevant or rarely needed. Every extra unit of information in a dialogue competes with the relevant units of information and diminishes their relative visibility.*

  This concept was popularized by a number of technology companies in recent history. Apple, Google, and Microsoft have interface design languages. Content is king, and should be brought to the forefront. Keeping your design clutter-free helps users find the information they do need in an easy manner.

Clutter prevents people from finding what they want to find. Whitespace can be more effectively utilized (along with a substantial number of colour and font changes) in order to space out and delineate sections better.



Minimalist design doesn't mean minimal content. Sites such as ArsTechnica are very effective at presenting a large amount of information in a clean, organized manner. Note how whitespace is utilized to prevent text and images from being too close to each other.

However, you should be cognizant of the concept of minimalism even as you are writing content for your wiki. How can you concisely convey everything you need to tell your audience without losing important information? Are you rambling? Can you cut this paragraph down and express yourself more concisely?

Minimalism is deceptively difficult. If too much is removed, the wiki may look incomplete or bland. There is a fine line that separates a clean wiki from a barren one. This is where you will need to exercise some graphic design and artistic skill.

- **Help users recognize, diagnose, and recover from errors—***Error messages should be expressed in plain language (no codes), precisely indicate the problem, and constructively suggest a solution.*

  It's not always sufficient to provide error messages such as "An error has occurred" since that does not give information to the user about how to fix it. Granted, it is sometimes extremely difficult to pinpoint the cause of the error, but if you are able to, you should provide that kind of information.

  For example, if you are signing up for a new account, you may be asked to provide a password. Which of these three error messages would be the most helpful for you?

  1. Sorry, the request was unsuccessful.
  2. Email address is improperly formatted or contains invalid characters.
  3. The password has an incorrect number of characters. Please provide a password between 6-16 characters using any combination of upper-case letters, lower-case letters, and numbers.

The third choice offers a suggestion to the user as to how to fix the error they have created.

For the wiki, you may end up using JavaScript to accomplish some aspect of your design (JavaScript will not be covered in the scope of this chapter, however, so go out there and take a look at various HTML tutorials). While less common now, there will be a few users who have disabled JavaScript on their browser. What I like to do is to show a box by default stating "JavaScript should be enabled for an optimal browsing experience" and then using JavaScript to hide it when the page loads. This way, the box is hidden when JavaScript is enabled and is shown by default when it is not. This may be something that you can consider (though again, based on the popularity of many JavaScript-dependent sites, this may be a non-issue).

- **Help and documentation—***Even though it is better if the system can be used without documentation, it may be necessary to provide help and documentation. Any such information should be easy to search, focused on the user's task, list concrete steps to be carried out, and not be too large.*

  Supplement your interface with information, even simple instructions such as "click here to see what our project is about". This way, users are reassured that what they are about to do will accomplish what they want.

  iGEM wikis sometimes have a "tour" feature, where they lead the user through the various components highlights of the project without having the user look for the sections themselves. This may be something for you to consider as you build your own, but remember that a lot of other wikis do not do this.

9. HTML and CSS

HTML stands for *Hypertext Markup Language*, and is the markup language which all internet browsers understand. Plain, pure HTML only specifies **what content appears**, including text, images, links, tables, and divisions (known as *divs*). HTML by itself does not specify **how the content looks**—that is what CSS is for. CSS stands for *Cascading Style Sheets* and is a separate language (with its own separate syntax) that manipulates the positioning, colour, font, line spacing, and all visual aspects of an HTML document.

In a nutshell, internet browsers work by downloading an HTML document (and all the supplementary data such as images, CSS, and JavaScript) from the internet, interpreting it, and then graphically rendering it for the user to read. The iGEM wiki server is no different—the HTML is there, but it is wrapped in a layer of wiki markup. When you edit in wiki markup, the server translates the markup into HTML before sending it to the user's browser (the *client*). The person editing the wiki doesn't need to worry about scary-looking angle brackets and instead can focus on the content.

Editing the iGEM wiki in HTML is not as difficult as it may first appear. It is a matter of understanding what each individual component of the language does and how it affects what is displayed on the browser. Web development is a lot of trial-and-error in that respect.

10. But Wait, You're Not Teaching Me HTML?

There are many resources online for learning HTML and CSS, and they do a much better job than any attempt I can make to teach you the actual syntax of the languages. For this reason, this chapter will not be teaching you anything about the HTML and CSS syntax. **Instead** (before you scoff and throw the guidebook away in anger and disappointment), I am going to focus on how to utilize HTML and CSS in the iGEM wikis, since the server behaves differently from a traditional blank web page. Almost all tutorials that you will encounter online assume you are building a website on your own domain

with minimal clutter. The iGEM server space provided is a shell that surrounds your wiki and therefore adds code that makes it a little harder to manipulate the page into something you want.

Before we go into that, I recommend that you take a look at the following tutorials that guide you through aspects of the languages step-by-step. These tutorials should not take you any longer than a few days of practice before you at least grasp the vocabulary and understand what the syntax looks like, as the final section ("HTML and the iGEM Wiki") will become rather technical:

http://htmldog.com/guides/html/beginner/
http://www.htmldog.com/guides/css/beginner/
http://html.net/tutorials/html/
http://www.csstutorial.net/

Required reading after finishing a CSS tutorial:
http://css-tricks.com/the-css-box-model/

Remember that Google is your friend. In this day and age of web design, there are many sites that can provide tutorials and solutions for almost any problem you encounter as you are building your wiki.

11. HTML and the iGEM Wiki

This section will assume that you understand vocabulary such as HTML *tags*, *elements*, *attributes*, *divs*, *ids*, *classes*, and the CSS *Box Model*, *selectors*, and *properties*. In this section, I will discuss some of the tricks that I have discovered as I continued developing wikis for the past few years. Unfortunately, a few of these workarounds are not going to be considered good web design practices but are sufficient to complete what you need to complete. If you do find solutions that are more effective than the ones I have described, congratulations (and please let me know about them)!

Tools Required

The major tools used in wiki building are a text editor and some kind of image editing software (Adobe Photoshop or Illustrator is a common standard, but if you are short on cash you can always find open-source software such as GIMP and Inkscape). For text editing, I recommend that you get an editor such as TextWrangler (for Macs) or Notepad++ (for Windows) as they can perform *syntax highlighting*—essentially, colouring in tags and keywords in your code to make reading easier. From here, you can copy and paste code you write onto the iGEM wiki.

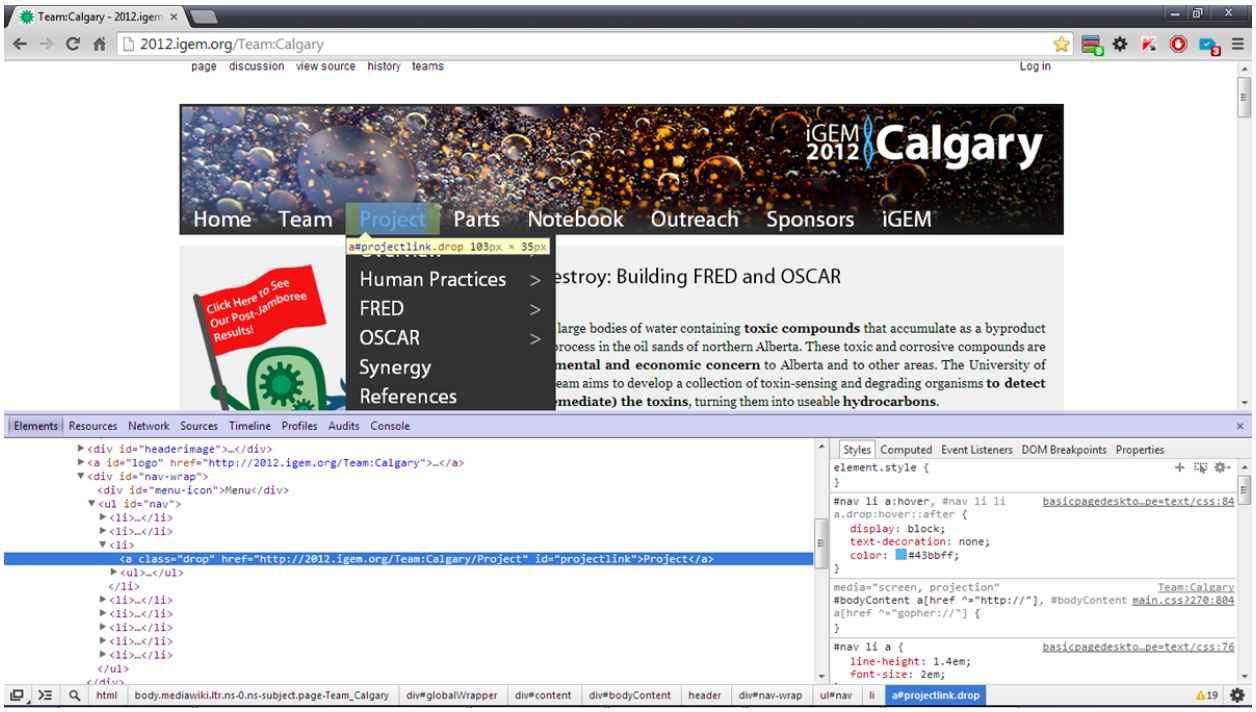
The Element Inspector

Your finest weapon during this process will be the ability to right-click on a webpage element and seeing exactly what part of the HTML and CSS creates it. Internet Explorer does not do this, but you are able to "Inspect Element" on both Chrome and Firefox (and potentially Safari).

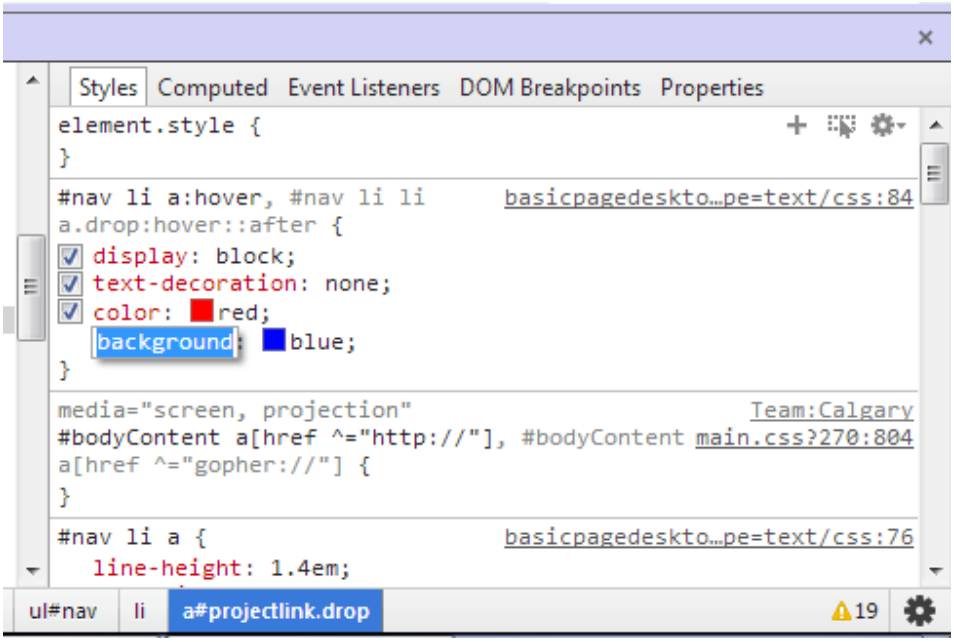
One of the benefits of the Inspect Element tool is to edit the CSS on the page itself to see how it can impact the layout of the page (temporarily—the changes are erased on refresh). For Firefox, you will require a plugin called Firebug, but it is native on Chrome.

Therefore, if you find an element on a wiki that you are curious about, you can right-click it, use "Inspect Element", and play with the CSS rules displayed to see how it is affected, or create new CSS rules altogether.

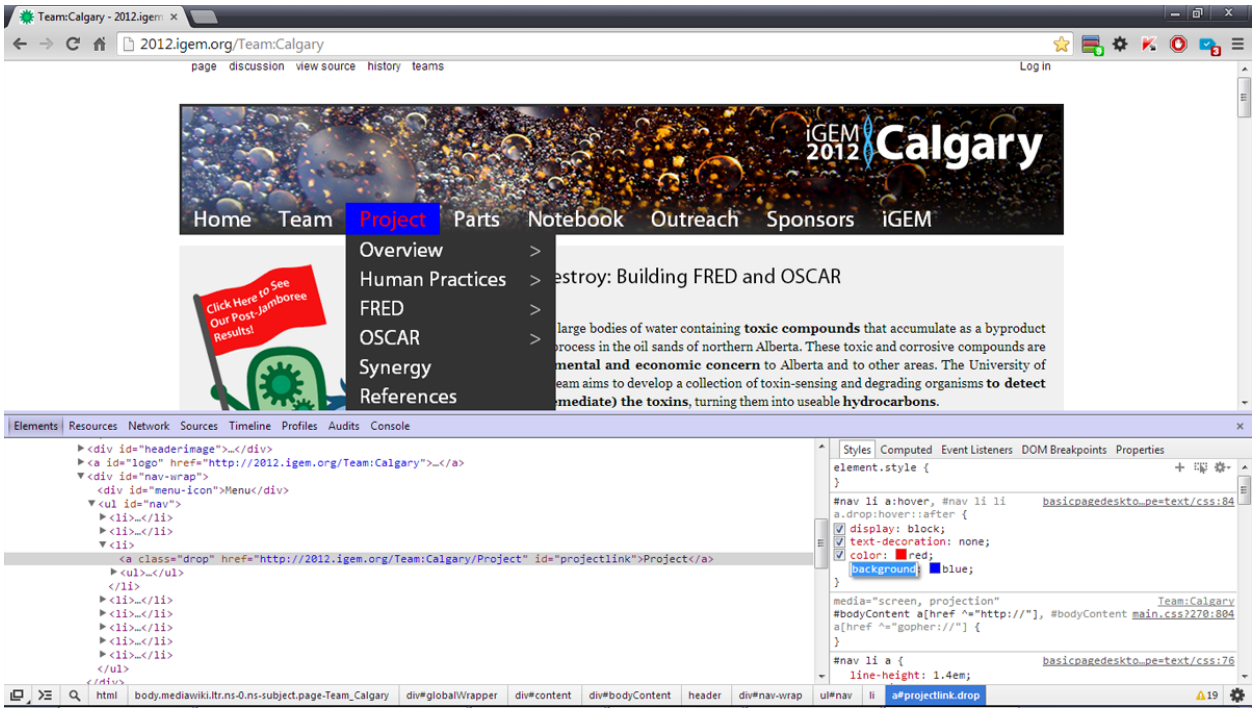
Right-clicking on an element and clicking "Inspect Element" allows you to examine it in further detail.



Mousing over particular lines on the HTML side shows you the element's location, as well as parts of the Box Model such as padding, margins, and borders.



Clicking on an empty area in the CSS area creates a new CSS rule. You can also change existing properties and values of the element. Pay attention to the selectors. If you want a rule for just the element you are highlighting, use the `element.style{}` selector at the top instead.



You can see that by changing font colour to red and background to blue, this particular element has now adopted new temporary CSS properties.

Switching Between HTML and Wiki Markup

In order for your browser to understand your HTML code, you can simply put everything in the iGEM wiki editor between two <html> tags. CSS and JavaScript go between <head> tags, and the rest of the content goes into <body> tags. Rather straightforward and, in general, not an issue.

However, if you are using snippets of wiki markup (such as when using templates), you have to close the <html> tag you are working with, enter the wiki markup, and then reopen the <html> tag again. This tells the browser to stop interpreting in HTML and begin to interpret in wiki markup.
For example, the following snippet adds a thumbnail image in a wiki image box:

```
<html>
<body>

<p>Paragraph of text.</p>

</html>[[Image:test.png|400px|thumb|caption]]<html>

</body>
</html>
```

Templates

One of the nice aspects of the iGEM wiki is the ability to utilize wiki markup to create templates. Templates can hold stylistic elements, CSS, and JavaScript which can be reused for all pages of a wiki. The benefit is that if there are changes to be made to the template, such as adding a new item to a

navigation menu, it will automatically apply to every page that utilizes that template, without requiring you to find and test every link.

Templates are the exception to the rule that you must have all wiki pages within your own wiki namespace. Templates do not work if you create one under your own namespace. They must come directly from the main iGEM site.

http://2014hs.igem.org/Template:TemplateName

Note how there is no indication of whose team this template belongs to. Theoretically you could use any team's template from that year (but why would you?) If you want to be very specific about whose team the template belongs to, you are able to use colons and spaces after the *Template:* keyword.

http://2014hs.igem.org/Template:Team:Example_Canada/TemplateName

Template pages are created and edited like any other wiki page, but it comes with an extra feature. In wiki markup, if you use triple curly braces, {{{ }}}, you can create *parameters* in the template. When the template is used in your wiki, you can then put content into those parameters without affecting the template's CSS and other elements. Therefore, you can hide the HTML and CSS of things such as sidebars, fonts, and stylesheets in your main wiki and make it easier for the rest of your team to edit. (They will thank you for it. And you will thank them since there will be less risk of them accidentally editing important structural elements on the page.)

Further details on how to use parameters in templates can be found here: http://meta.wikimedia.org/wiki/Help:Template

Remember that since templates are **not HTML**, but rather are specific to the wiki itself, it will not be recognized by the browser if parameters or other features are within <html> tags.

In order to use a template on a normal page, you use two curly braces and then specify the name of the template without the word *Template:* at the beginning. Parameter values can then be entered, separated by pipe characters, |, and then closed with two curly braces.

For example, if you were using a template with the location *http://2014hs.igem.org/Template:Team:Example_Canada/MainTemplate* with the following:

```
<html>
        <body>
                <p>This is text that appears on all pages.</p>
                </html>{{{TEXT|This is placeholder text}}}<html>
        </body>
</html>
```

You can then use it on any other page like so:

```
 {{Team:Example_Canada/MainTemplate|
TEXT=
        <html>
                <p>This overrides the placeholder text.</p>
        </html>
}}
```

Note how you need to have the <html> tags again when you're filling in replacement HTML content. Without them, the browser would interpret the <p> tags inside as wiki markup and show them. Also note how there is no *Template:* present when specifying the template—this is already implied when you utilize the double curly braces.

The link provided will have more examples on how to properly use a template. If a template is successfully used, you will see it listed on the bottom of the editing window of the normal page when you edit it again. If you like, you can start modularly stacking templates together—templates can use other templates and pass on parameters by making it their own! Take a look at some of the collegiate wikis of years past to see how they used their templates.

External Stylesheets and JavaScript

To minimize errors in redundancy and to save the amount of data that a browser needs to download, many sites will have their CSS and JavaScript externally, in a separate file from the HTML document. When a browser downloads an HTML document for the first time, it downloads a copy of the CSS and JavaScript (if it is external) and stores it locally. This speeds up the download of all other pages that use the same CSS. If CSS rules are found on every page, the browser has no choice but to download it each time.

Therefore, it is good practice to have your CSS stylesheets uploaded separately on the iGEM wiki and referenced within your own CSS as an external file.

In order to do this, you simply create a new wiki page and paste the CSS into it, with no extra HTML tags. However, you will discover that the page will look rather strange, as it interprets the CSS as wiki markup and formats it accordingly.

Take this CSS sheet for example:
http://2013.igem.org/Team:Calgary_Entrepreneurial/Stylesheets/global.css. If you click "View source" or "Edit", you will see that it is in fact properly formatted CSS. The wiki interprets it otherwise, however.

The solution is to add the snippet ?action=raw&ctype=text/css to the end of the page's web address. This forces the wiki server to show the file as raw css. Observe the difference between the link above and this one:
http://2013.igem.org/Team:Calgary_Entrepreneurial/Stylesheets/global.css?action=raw&ctype=text/css

The second link can be properly interpreted when you use it in a <link> tag on your pages:

```
<link rel="stylesheet" href="http://2013.igem.org/Team:Calgary_Entrepreneurial/Stylesheets/global.css?action=raw&ctype=text/css" type="text/css"/>
```

As for JavaScript, I have not been able to determine what the ctype can be changed to that lets the browser interpret it as external JavaScript. For my own purposes, since JavaScript is used lightly in the wikis I have built, they remained inline in the templates that I utilized. If there is a snippet that can be used for external JavaScript, by all means use it.

Images

Remember that there are three links that you can use to find an image file you have uploaded. For <img> tags, you **must** use the direct, full-resolution link in the src attribute. The other two do not work since they are wiki pages with their own markup. If you are not using <img> tags and are instead using wiki markup to put up figures, you must use the wiki server filename (with all spaces replaced by underscores, _).

Responsive Web Design and Media Queries

You may see a lot of articles online talking about responsive web design. This may not be of any concern to you (there is, in fact, a lot of extra work involved in making a website truly responsive),

but if you are brave and committed, one of the easiest ways to create responsiveness is through media queries. However, while a lot of sites will say that you should have the media queries within the <link> tags and have multiple external CSS files, it does not work on the wiki. Instead, you must have **all media queries within a single CSS** file that is then linked externally.

An example can be found on the 2013 Calgary Entrepreneurial wiki's CSS: http://2013.igem.org/Team:Calgary_Entrepreneurial/Stylesheets/main.css?action=raw&ctype=text/css. Note how the media queries are present in this one file.

The !important Statement

CSS is known as "cascading" because rules written later in the document override rules earlier in the document. This allows very general CSS rules to be established at the beginning of the document and very specific rules later on in the document.

However, sometimes a CSS rule doesn't stick no matter what you try. This may be due to the presence of !important, a rule appended to the end of a CSS statement that causes it to override all other rules regardless of where it is in the document. The iGEM wiki may have !important statements in its own CSS that overrides a rule you are trying to implement. !important statements cascade with other !important statements, so this might help.

Use this technique sparingly. It can cause you severe headaches down the road if you abuse this feature.

12. Conclusion

Hopefully this chapter has given you some groundwork on beginning to build your own wiki. Remember to keep the end user in mind as you continue building your wiki. Look for examples of Best Wikis in both the High School and Collegiate divisions for inspiration on how to further improve your own.

HTML and CSS are rather tricky to learn, but you will begin to grasp the behaviour of the code as your continue to practice. Don't be afraid to experiment and don't be discouraged if something doesn't seem to work quite right. The internet is a great resource—it's very likely that if you are having a problem, a lot of other web developers have had similar ones. You can always find tutorials online that teach you how to build specific website features, like a fixed sidebar or a dropdown navigation menu. Tutorials are an excellent way to see how HTML, CSS, and JavaScript can work together in order to build something tangible.

If this chapter has sparked some interest in UX and web design/development, there are literally millions of UX resources online that you can Google. Keep in mind that since UX is rapidly changing with rapidly changing technology, there are lots of heated debates about whether or not specific user interface elements are good UX (dropdown menus, for example, remain a very big point of contention.) The tips and tricks shown here, and even the Nielsen heuristics, are all rules of thumb that can be broken if it ultimately improves usability.

Remember that while your end goal is achieving usability, it isn't everything. UX is about **providing a good experience** for those who visit your wiki, and if your users are able to use your interface and design effectively with minimal frustration, the design has not failed.

With that, I wish you all the best of luck in your projects, and I look forward to seeing how your wikis turn out!

PRESENTATION AND POSTER

Lisa Oberding and Zak Stinson

1. Introduction

At the Jamboree, in order to present the work that you have done over the summer, your team will be required to give a 20 minute presentation as well as a presentation of a poster explaining the project. In addition to the Wiki, these are the other criteria upon which your project is judged.

2. The Presentation

During the Jamboree, each team will give a 20 minute presentation to both judges as well as other iGEM participants. Each team has 20 minutes of presentation time, 5 minutes for questions and answers, and 5 minutes to switch with the next presenters. Please be sure to bring the necessary equipment for your presentation, such as your laptop, cables/adaptors, and power supply, as iGEM will not provide these. You should also have a PDF version of your PowerPoint on a flash drive ready to give to the iGEM staff before you present. The purpose of the presentation is to explain your project to the audience in a way that will allow them to understand what you have done, why you have done it, what was involved, and what results you obtained. Presentations are oral with a PowerPoint presentation on screen behind the presenters, and must be given by student members of the team. There is a strict 20 minute time limit to keep in mind, as speakers will be cut off if this limit is passed. Though there is no set template for your presentation, there are certain guidelines to keep in mind as to what should be said.

Components

1) Title: In order to start off the presentation, it is not advisable to launch directly into the story of your project without a brief introduction. First, your presenters should introduce themselves as well as the team they are from, and then introduce the project. It helps if you can come up with a creative name for your project, as well as possibly a catchphrase describing what it was in a few words. For an idea of this, browse through past projects: Having a memorable name, catchphrase, and/or logo can help judges to remember you and gets the audience interested. Your title slide should have thought put into it, even if it is just your team or project name. Remember; this is the first slide that the judges and the audience will see of your presentation, and it sets the tone for what they are about to watch.
2) Background: Your project likely seems super interesting to your team, because it is what you have dedicated all your time and energy to in the past few months. Your team will know why it is interesting and useful, but it is important to remember that other teams or judges aren't as familiar with your project- it is your job to make them as interested in what you did as you are. The introduction to your project is important- you have to set some kind of a background to your story before you launch into all of the things you did, otherwise you will lose your audience and nobody will have any clue what you are talking about or why they should care. You must explain what it is you are doing, and also very importantly, why you chose to do this. Your team picked this particular idea or project for a reason- what was it? What was interesting about it? What was the problem you were trying to solve or the thing you were trying to accomplish, and why was it important? Remember you are presenting to other people from other fields of study from around the world- it is important to explain the background of your project to them, both what it is as well as why you chose it/why it is

important to look at. You also want to make sure that this introduction into your project is not only interesting, but that it is also understandable by someone who does not have any background knowledge. This is one of the most crucial, if not the most crucial, parts of your presentation as it is the beginning of your story- you want to convince your audience that what you are about to tell them is interesting, and grab their attention. This section sets the tone for your presentation, and determines whether the audience is interested and understands your project.

3) Your Project: Following the explanation of what problem you were solving and why, you can begin talking about how you solved it- your project. This is where you explain your experiments, results, and everything that you did over the summer. From here on in, the layout of your presentation is far more flexible, and how you explain your project will depend entirely on what it is. Some projects have sub-project components to them and are most easily explained in sections, with a conclusion as to how everything ties together. Other projects only have a single component, where it makes more sense to explain how each step came together to lead to the final result. This will be the longest section of your presentation, and there are some easy mistakes to avoid.
    a. It is not necessary to discuss every little experiment you did and every method involved in this section. Rushing through to show all your data will be stressful to your presenters and hard for the judges to understand. You should discuss what you did and how you did it in general terms, and highlight the most important experiments that gave you a result that was significant in showing your project working. Judges will have access to you Wiki where you will have everything documented- in your presentation you want to highlight the experiments that best show your project worked.
    b. When discussing experimental results from parts that you have constructed and tested, make sure you highlight the fact that these were parts that your team had built during the summer.
    c. Generally explain how you did your experiments, but don't give exact protocols - explain only what people need to know to understand what you did. Keep your audience in mind- they will be familiar general procedures like restriction digests and cloning, so you don't need to go into the procedural details unless you have done something unusual.
    d. Make sure to explain what an experimental result meant, and what it showed (not just "there was a band on the gel", but "the band on the gel when we cut the plasmid was the size of our gene, meaning that our part contained out gene of interest". Not just "the cells turned green", but "the cells turned green when we transformed them, indicating that they must have our part in them and that our part is working/expressing".).
    e. Show your data in a way that people can read: they will likely be seeing a slide for only a minute- a color coded graph with a clear legend or pictures of your cells will be more effective in communicating your point than a table of numbers any day.
4) Conclusion: Here, you want to bring everything together. What was the final system you came up with, and how did you show it worked? How do the components or subprojects you have come together? Whether you have a full system or only the components completed, go back to the big picture of what you said you were trying to accomplish and explain what you did achieve. Explain the implications of what you created, and how it can be used- both in relation to your chosen problem and in other future applications. What future directions does your project have that can be researched next? How is your system the solution to the

problem you were trying to solve? This is your last chance in your presentation to really sell what you accomplished and that your project worked- really make sure this is obvious, and relates back to your original purpose.

5) Attributions: iGEM teams rely heavily on help from sponsors as well as other people. At the conclusion of your presentation, it is important to thank them for all their help. Do not forget to do this- you will lose points without it in addition to damaging future relationships with sponsors and support networks. Generally, this is done through a slide or two with the names of everyone who helped you, and the logos of your sponsors. You don't have to read every single one of them out loud- just a general thank you will do, as long as you acknowledge everyone. Following this, you can wrap up your presentation by letting the audience know you will take any questions or comments they may have, and thanking them for their attention.
6) Q&A: During the question session, you will be asked questions not only on what you presented but also on what is on your Wiki. The best preparation for this is to practice, practice, practice. Have mock question sessions with your team beforehand in order to test your knowledge, and get everyone to read the team Wiki. Not everyone needs to have 100% knowledge of every detail, but everyone on the team should know the general details of what was done in every aspect of the project. Here, it helps to have a team member or advisor direct questions to students to keep things organized, and it is also good to limit the number of answering team members for a single question to one only (two if someone has clearly missed an important point).

Tips

1) Avoid, at all costs, a wall of text. Your slides should have figures and pictures, with key bullet points to convey the main points- do not write a full paragraph or page on a PowerPoint slide. The slides are there to highlight what you are saying, and to show figures and graphs of your results as well as key images and points that you are trying to emphasize. They should not be a script you are reading directly off of, and having too many words on a slide is distracting and unpleasant for the audience- they will be trying to read instead of listening to you.
2) Keep your fonts big enough to read (25pt or more for fonts like Arial). Also, though decorative fonts might seem nice, they are very hard to read on a presentation. Keep the fonts simple, just like you would for your Wiki. In fact, following your wiki design guidelines for your PowerPoint will help you make an awesome slide deck to go with your oral presentation.
3) Figures should be clear, crisp, and easy to see- not pixelated. They should also be simple: if they are complex, highlight and point out the part that the audience should care about. If a figure of data is used, clearly label it and make sure your labels are big enough to be seen by your audience. Tables should be kept simple, if used at all. Explain your data figures during your presentation and what they are supposed to show (don't just put them up and expect people to understand the significance of the result).
4) Pictures are good, but in moderation. Don't cover your slides in random images- it is very distracting and looks messy. Pick ones which convey your point best, and if you must have a lot of images, separate them onto separate slides.
5) Color schemes: You want your presentation to be readable, and visually attractive. Don't insert a crazy background- though it might look fancy, it will be hard for your audience to read. Stick to simple color schemes that are not super bright.

6) Animation- keep it minimal. Having text appear on a slide is fine- if well rehearsed, it helps the audience follow the presenter, who can click to bring up the next point for discussion. DON'T have it spin and twirl and get bigger before coming in- remember you want something that is simple and easy for your audience to read.
7) Have titles on your slides- short, but reminding people what you are talking about. Similarly, if you are jumping to a different section (say, experimental design to experimental results, or from subsection to subsection), have a transition title slide where you can explain that you are now moving into this new section ("now that I have shown you X, I will talk about Y").
8) Your presentation is not the PowerPoint! The PowerPoint is there to back up the presenters. Try not to read directly off of your slides- use the key points on them to remind you of what you had to say. Rehearse the presentation multiple times- as many times as you can, giving feedback after each round. When presenting, try to make eye contact with the audience, smile, move around a little bit. Remember- <u>you know what you are talking about better than anyone else in the world when it comes to talking about your own work.</u> The audience is not there to tear you apart- they are very interested to hear what you have done and what you have to say. Just talk to them like you would a classmate.
9) It's usually easiest to have a few presenters only; 2 people, maximum 4. When trying to hand the microphone off to every member of the team, it can get hectic. However, you are free to do so, and many teams do choose to. Go with whatever works best for your team and your story.
10) **MOST IMPORTANTLY: Practice, Practice, Practice.** Start in advance. Start working on your presentation as soon as possible. The more time you have to work on the presentation, the better it will get, and the easier presenting it will be.

3. The Poster

Each High School team is required to present a poster at the Jamboree. In your team package you pick up at the desk at the Jamboree, there will be a list of locations for where team posters are assigned. Guidelines for when posters must be up and when the poster sessions are will be posted in the information for the Jamboree on the iGEM site. The poster must be no larger than 48 x 48 in (1.22m x 1.22m, 4ft by 4ft), however keep in mind this size tends to be quite large, and it may be advantageous to make a slightly smaller poster if possible. Keep in mind as well you will need some type of tubing in order to transport your poster to and from the competition. In addition to submitting a PDF of your presentation before you present, you will also turn in a PDF of your poster.
All teams will hang their posters up in a designated area, and during a designated time period (normally 2 hours), there will be a poster presentation session. During this time, members from other teams as well as judges will circulate through all of the team's posters, and ask questions to team members about their projects. There will be two types of judges; poster-specific judges as well as judges which also mark presentations and Wiki's.
Each student on the team should be able to explain the project using the poster- the point of this is to explain the story of your project to anyone who asks in a short 5-10 minute talk, using the poster as a guideline in order to point out the most important components. Questions may also be asked about any component of the project during this period, so it is important to be prepared.
<u>Components</u>
In general, the layout of a scientific poster will have the same components as the project presentation:

1) <u>Title:</u> This should be large enough to see from far away, and possibly have some kind of hook or phrase with it in order to draw people in closer to view the poster.
2) <u>Authors:</u> This is where the names of your team members and advisors are listed. Generally this is in alphabetical order by last name, with student members before advisors. The format is usually "Last Name, First Initial., Last Name, First Initial."

3) Introduction: Just the same as the presentation, this should tell the story of what you are trying to do, the background to your problem, and why it is important.
4) Project: The components of the project can be split up differently depending on the structure of the project. Some teams split sections for each sub-project, containing methods and results for each. Some will have separate methods and results sections. Some projects use their Human Practices component in their introduction, whereas some have a separate section on the poster. How this is laid out will depend on your project, and the best way to figure out how to lay out a poster is to look at other posters (see the examples section at the end of this section).
5) Conclusion: Just the same as the presentation, this should wrap up your project, explaining how the components come together, or what the further applications of the project are, or show the results of testing the final system. Again, this section will depend very much on the structure of your project, and viewing other posters for examples will be helpful.
6) Attributions: There should be a section on your poster for attributions and sponsor logos- again, points can be lost if this is not present.

Tips

1) Design guidelines are similar to that for the presentation and Wiki: Keep colors simple, make sure the font is large and easy to read (generally ~19pt minimum, no fancy swirly fonts, dark on a very light background or vice versa.), and keep a good balance of text to pictures: you should have short sentences maximum, bullet points at best, with images of results and concepts if possible.
2) Sections on the poster should be clearly separated somehow, in order to make the poster easy to read (boxes, borders of some kind, lighter backgrounds). There should also be clear titles for each section.
3) Sections should be ordered logically- remember that people will be reading the poster, and that people will either read left to right, or top to bottom. Make your sections in order in rows/columns accordingly in order to make your story on your poster easy to follow.
4) The content on your poster should again highlight only the most important results- you have very limited space, and you want to tell the story of your project as short and sweet as possible while hitting the most impressive and important points.
5) Figures should match the color scheme of the poster, and be as simplified as possible. They must also be clearly labeled (axis, legends, ect.), as well as have a figure caption (above tables, below figures). They should be numbered in the order that they appear on the poster from where the audience would start reading to where they would end (note: sponsor logos don't count).
6) There should be around 3 students at your poster at all times during the poster session (those that can present it best), but no more: you don't want to crowd the poster with your team, have team members mingle and hang back, and trade off if necessary. It is best to have team members with detailed knowledge of each section close to the poster so that questions can be directed accordingly if someone wants more detail about that area, however all team members (including those NOT near your poster) can be asked questions and should be able to tell the story of the project. Again, practice as much as possible.
7) Ask people approaching the poster what it is they would like to hear: do they have questions, or do they want to hear the overall story of the project? This way, you can direct what you say accordingly.
8) Be active in approaching people. If someone is lingering near your poster, approach them. Have other team members mingle throughout the area; some teams create flyers or have

other things like key chains to hand out and draw people in to their posters. You want to get as many people to come see your poster as possible and be as memorable in the crowd as possible (bright shirts, costumes, flyers) in order to get the judges to remember you and to come see your poster.

9) Remember you are telling a story- you want to explain your project like you would in the presentation; what you are trying to do, why it is important, how you did it, and what results you obtained.
10) Have fun. Meet people, ask them questions about their projects, make new friends- poster sessions can be stressful, but they are also your opportunity to meet other people and make new friends.

APPENDIX 1
PROTOCOLS

Himika Dastidar

1. Aseptic technique

Theory:
Tips:

2. Rehydration of Registry DNA

Biobrick parts are shipped from the registry in a dehydrated form. As such they must be rehydrated before they can be used.

1. Puncture a hole through the foil with a pipette tip into the well that corresponds to the Biobrick - standard part that you want
2. Add 10 µL of ddH_2O (deionized water)
3. Let the water incubate at room temperature for 10 minutes
4. Take 1 µL DNA and transform into your desired competent cells, plate out onto a plate with the correct antibiotic and grow overnight. Your goal here is to obtain single colonies.

3. Making Chemically competent *E. coli* cells

Materials:
- Top10 *E. coli* cells
- LB Broth
- 50mM $CaCl_2$ solution (store at 4°C)
- 50% Glycerol

Protocol:

Note: It is extremely important to make sure everything in this procedure is done under aseptic conditions.

1. Inoculate 5-10 mL LB at 37°C while shaking with *E. coli* cells. **Do not** add antibiotics into this culture.
2. Subculture 1 mL of bacteria solution into 50 mL LB broth (without antibiotics) at 37°C; while shaking until OD600 is 0.4-0.6 (This step should require approximately 2.5 hours)
3. Centrifuge the subculture at 3750 rpm and 4°C for 20 minutes or until you see a clear pellet forming
4. Re-suspend pellet in 12.5 mL of cold $CaCl_2$ solution (50 mM). Leave on ice for 10 minutes
5. Centrifuge the bacteria at 3750 rpm and 4°C for 20 minutes. Re-suspend in 2 mL of cold $CaCl_2$ (50 mM, 15% glycerol solution)
6. Leave on ice for at least 30 minutes and then distribute in 50 µL aliquots and freeze at -80°C

4. Bacterial Transformation

Theory:
Transformation is the process by which bacteria can take up foreign DNA. For this process to occur, competent cells are necessary. Transformation allows bacteria to express traits/genotypes that are not native to the strain, for example: antibiotic resistance, GFP etc.

Heat Shock method
- In this method the competent cells are thawed and heat shocked for 5 minutes in the presence of the foreign DNA plasmid. Compentent cells are frozen with pores in their cell walls/membranes. Icing the cells for 30 minutes allows the plasmid to adhere to the cell wall/membrane. Heat shocking allows the plasmid to get in by enlarging the pores, and then the cooling of the cells right after allows the pores to become smaller. The SOC treatment allows the bacteria to activate itself and "heal the wounds". Although, it is believed that heat shock works this way it is not proven in the literature.

Electroporation
- Electroporation essentially uses the same principles but engages in a differnt method of inserting the DNA. Instead of a heat shock, electroporation applies an electric field across the cells, which creates pores in their walls/membranes. This allows the plasmid to be taken in by the cells. These cells are then recovered and allowed to grow.

Tips:
You may leave the cells shaking for longer than an hour (2-4 hours) but not overnight. Leaving the cells in the shaker for about 2-4 hours allows the cells to grow and recover well. This gives high transformation efficieny with lots of colonies.

Materials:
- Competent cells
- DNA to transform
- 37°C water bath
- Ice

1. Thaw 100 µL of competent cells (per transformation) on ice just before they are needed
2. Add DNA (1µl up to max 20µl) to the thawed cells and mix by flicking the side of the tube. Leave on ice for 30 minutes
3. Heat shock 5 minutes in the 37°C water bath
4. Place on ice for 5 minutes
5. Add 250 µL SOC medium to each tube
6. Incubate for 30 to 60 minutes with shaking at 37°C. (Note that for Kanamycin containing plasmids always use one hour)
7. Spin down to remove all supernatant (top layer) except approximately 100 µL
8. Plate approximately 50 µL on each of two antibiotic plates
9. Grow overnight at 37°C

For this protocol we used a couple of controls
- *Positive Control* - TOP10 competent cells transformed with pBluescript plasmid and plated on Ampicillin plates
- *Negative Control* - TOP10 competent cells grown on Ampicillin plates

5. Making LB broth

Materials:
- LB broth powder
- Glass bottle

Protocol

1. Weigh out 20 g of LB broth powder and mix with 1L of water. Scale up or down as desired. Autoclave (sterilize) the broth at 121°C for 15 minutes.

Note: The amount of powder you add will differ based on the manufacturer. Please read the manufacturer's instruction before making the LB broth.

6. Making LB agar plates

Materials:

- LB-agar powder mix
- Plates

Protocol:

Note: It is extremely important to make sure everything in this procedure is done under aseptic conditions.

1. Weigh out 35 g of LB-agar powder mix per litre of media desired. One litre makes 40-50 plates. Ensure that the mixture volume does not exceed half of the volume of the flask/container used, otherwise it will boil over in the autoclave.

- DIY protocol:

2. Dissolve the LB-agar, using water from one of the wall mounted Nanopure filters. Add a stir bar and use a magnetic stirrer to facilitate mixing.
3. Cover the flask with aluminum foil, and secure the foil with autoclave tape. The foil should be somewhat loose (to avoid building pressure in the flask while sterilizing), but not so loose that lots of liquid can escape
4. Put the flask in a plastic autoclave tray, load into the autoclave, and sterilize using the 20 minute liquid program.
5. Allow the media to cool until it can be handled.
6. Once media is cool, add other desired ingredients. Use the magnetic stirrer to mix, but do NOT add a stir bar now, or the media will be contaminated. (If one wasn't added before, you must do without.)
7. Add the antibiotic that will select for your plasmid. The following antibiotic concentrations are used commonly in iGEM: **Ampicillin** (stock 100mg/ml, final 100µg/ml); **Kanamycin** (stock 50mg/ml, final 50µg/ml); **Chloramphenicol** (stock 50 mg/ml, final 10µg/ml)

Note: To achieve final concentrations, add 1mL of stock per 1L of culture media, except for chloramphenicol, where 0.6mL stock per 1L of media is added instead.

8. Pour the sterile, antibiotic-containing broth directly from the flask into the sterile Petri plates carefully to avoid trapping air bubbles.
9. Allow the plates to stand right side up overnight, and then store them at 4°C (in fridge).

7. M9 Minimal Media Preparation

Protocol:

1. Dissolve the following salts in about 500 mL of ddH2O:
   - 12.8 grams anhydrous Na2HPO4
   - 3.0 grams KH2PO4
   - 0.5 grams NaCl
   - 1.0 gram NH4Cl
2. Bring the media to approximately 950 ml with ddH2O and pH to 7.4 with NaOH.
3. Autoclave (sterilize) for 20 minutes on slow exhaust. Store at 4°C when finished.
4. Separately the following salts were dissolved in 50 mL of water and the solution was sterilized cold with a sterilized 0.22 micron filter:
   - 10.8 grams glucose
   - 0.19g of MgCl2

- 0.0152 grams CaCl2.2H2O
- 0.0100 grams Thiamine
- 0.007g of FeCl2.4H2O

5. Add the filter sterilized solution to the autoclaved media, along with any other desired ingredient (e.g. antibiotic). Store at 4°C.

8. Making Glycerol stocks

Materials:

- Overnight culture of the bacterial strain of interest
- Screw cap tubes
- 50% Glycerol (Sterile)

Protocol:

	**Note:** It is extremely important to make sure everything in this procedure is done under aseptic conditions.

1. Make a 5 mL overnight culture with your bacterial clone of interest and with the appropriate antibiotics.
2. In a sterile screw cap tube mix 50% glycerol and bacterial culture at a ratio of 1:1. Ex: mix 0.5 mL culture and 0.5 mL 50% glycerol into the tube.
3. Mix by pipetting up and down gently
4. Freeze at -80 °C for long term storage

9. Overnight Cultures

Materials (per culture prepared):

- 10 mL culture tube. Use 16mm x 160mm or 16mm x 125mm
- 5 mL LB
- 5 µL 1000X stock of antibiotic solution
- Single colonies from a plate (avoid starting an overnight culture from a glycerol stock)

Protocol:

1. Add 5 mL sterile/autoclaved LB broth in a 10 mL culture tube.
2. Add 5µL of the 1000X antibiotic solution to the LB.
3. Select a single colony using a sterile toothpick, wire loop, or pipette tip.
4. Place toothpick or pipette tip in the culture tube and stir.
5. Place culture tube in incubator at 37°C overnight with vigorous shaking (250 RPM)

10. Agarose Gel Electrophoresis

Materials:

- 1x TAE buffer
- Graduated cylinder
- 125 mL flask
- Agarose powder
- Gel Pouring Tray
- Tape
- Gel rig
- SYBR Safe

Protocol:

1. Measure out 100 mL of buffer
2. Pour the buffer into 125 mL flask
3. Weigh out enough agarose to make a 1% gel (in our case 1.0 g of agarose was the right amount)

4. Transfer the agarose to the 125 mL flask
5. Melt agarose in microwave until solution is almost boiling, stirring every 15-20 seconds (should take around 2 minutes) Keep an eye on it at all times as it can unexpectedly boil over!
6. Allow the agarose to cool down but do not let it cool too much to the point where it becomes hard
7. Add 4 µL of SYBR Safe to the cooled agarose
8. Assemble the gel pouring apparatus by inserting gate into slots.
9. Allow agarose to cool until flask can be handled comfortably.
10. Place comb in the gel rig.
11. Pour agarose into gel tray.
12. Allow to solidify. While the gel is solidifying prepare the DNA samples. Prepare the samples by mixing 1 µL 10x Loading Dye, 4 µL of DNA and 5 µL of sterile water
13. Pour 1x TAE buffer over gel so that there is a layer of 3-5 mm buffer on top of the gel
14. Load DNA samples into wells/slots (Don't forget to load a 1kb+ ladder into one of the wells !)
15. Hook electrodes to gel apparatus.
16. Run the apparatus at 100 V for 30 - 45 minutes (Make sure to watch that the loading dye does not run off the gel)
17. Visualize the gel and record the results

11. Bacterial Genomic DNA Extraction

DNA extraction from unknown bacterial colonies from biofilms and planktonic cultures grown on a variety of media types. This protocol is based on protocols from Monika Schwering and ***MP Bio's DNA Spin Kit*** *extraction protocol.*

Materials:
- MP Bio FastPrep bead beater
- Micro-centrifuge
- Heating Block
- MP Bio FastDNA SPIN Kit (cat # 6540-600)
- Lysing Matrix A Tubes
- DNA catch tubes
- 2.0 mL eppendorf tubes
- 1.5 mL eppendorf tubes
- CLS-TC buffer
- DNA binding matrix
- SEWS-M diluted with ethanol
- DES water
- Lysozyme (EC 3.2.1.17)
- Lysis buffer (20 mM Tris, 2 mM EDTA, 1% Triton X-100, adjusted to pH 8.0)
- Proteinase K

Protocol:
1. Prepare 5mL overnight cultures of each organism to be extracted in a 15mL Falcon tube. Incubate overnight in a shaker at the required growth temperature and rotation speed for the organism of interest.
2. Centrifuge the overnight cultures at 3000rpm for 10 minutes. Dump the supernatant and use a 200 µL pipette tip to remove any excess supernatant remaining in the tube.
3. Prepare the lysis buffer () with 20 mg/mL concentration of lysozyme.
   a. First, calculate the total volume of lysis buffer needed for the experiment:
      i. (# of samples + 0.5 for pipetting error) * 0.200 mL of lysis buffer per sample
      ii. ie: (12 samples + 0.5) * 0.200 mL = 2.5 mL of lysis buffer
   b. Next, calculate the quantity of lysozyme required to achieve a concentration of 20 mg/mL of lysozyme in the lysis buffer:
      i. Total volume of lysis buffer * 20 mg/mL of lysozyme
      ii. ie: 2.5 mL * 20 mg/mL = 50 mg of lysozyme

4. Add 200 µL of lysis buffer to each samples tube and vortex each sample to re-suspend the mixture. Place the tubes on a shaker in a 37ºC incubator for 30 minutes.
5. Assemble one lysing matrix A tube for each sample and place 880 µL of CLS-TC and 20 µL of proteinase K in each tube.
6. Set a heating block to 55ºC.
7. Remove the samples from the shaker in the 37ºC incubator. Vortex each samples to re-suspend any precipitate and pipette all of the sample (using a pipette set at 300 µL) into a separate lysing matrix A tube.
8. Bead beat the samples for 40 seconds at speed 6.0.
9. Place the samples on the 56ºC heating block for 30 minutes.
10. Spin the samples for 10 minutes at 14,000xg.
11. Prepare one 2.0 mL eppendorf tube for each sample and pipette 800 µL of DNA binding matrix into each tube. Then pipette, 800 µL of the supernatant from each lysing matrix A tube into the corresponding tube. Ensure that the precipitate is not disturbed during this process.
12. Invert the tubes for 5 minutes by hand.
13. Gently invert each sample tube to ensure that there is no precipitate and pipette 800 µL (1/2 of the sample) into a catch tube.
14. Spin the catch tubes for 1 minute at 14,000xg
15. Empty each catch tube and transfer the remaining ~800 µL of sample into the corresponding catch tubes.
16. Spin the catch tubes for 1 minute at 14,000xg. Empty the catch tubes.
17. Add 500 µL of SEWS-M/ethanol to each catch tube and pipetting up and down to re-suspend the pellet
18. Spin the catch tubes for 1 minute at 14,000xg. Empty the catch tubes.
19. Spin the catch tubes again for 2 minutes at 14,000xg to dry out the pellets.
20. Replace the recovery tubes with fresh 2.0 mL eppendorf tubes with final storage labeling.
21. Add 100 µL of DES water and re-suspend the pellets by pipetting up and down. Incubate the tubes at 55ºC for 30 minutes in a heating block.
22. Spin down the tubes for 2 minutes at 14,000xg. Keep the liquid and tube. Dispose of the filter column.
23. Storage: place the tubes into a -20ºC freezer.

12. Plasmid Purification from *E. coli*

Theory:

Plasmid purification or mini-prepping allows one to extract plasmid DNA from bacteria. The principle behind this includes alkaline lysis to open the bacterial cells followed by a silica column adhesion and elusion using water/ respective buffer.

The procedure consists of three basic steps:

Firstly, the cells are cultured overnight and then spun down and resuspended to a concentrated volume. The resuspension solution contains RNAase, which is an enzyme that degrades the RNA. to minimize the contamination and possible interaction of RNA with the plasmids. Subsequently the cells are lysed using an alkaline lysis buffer. This breaks open the cell wall and membrane to spill out the inner composition of the cell. Following the lysis, the cell is neutralized using an acidic solution which also serves the purpose of creating a high salt environment with ions. Following this step, the cells are transferred to a silica column which binds DNA along with some endonucleases but allows everything else to pass through. A final wash step is done to ensure that all trace of endonuclease is washed off leaving mostly the DNA in the column. However, this can have some RNA contamination left in it. Then the column is washed with 50-100 µL and speced to measure the concentration.

Tips:

- When you are spec-ing your DNA, make sure you use the elution water/ buffer as your blank.
- When spinning your DNA after lysing and neutralization you can spin for 15-20 minutes. This allows the debris to stick to the side of the tube allowing transferring the supernatant with the plasmid much easier and contaminant free.

Protocol:

*We used two plasmid miniprep protocols. The first protocol is taken from the **GenElute Miniprep Plasmid Preparation Kits** distributed by Sigma Aldrich. We modified the elution portion of the procedure by using double distilled water to elute rather than using TE buffer. We also skipped the step with the optional wash solution. Instead, the step with the addition of Wash Solution in the Column Tube was done twice.*

1. Make overnight cultures from an LB agar plate (The protocol for the making of overnight cultures can be found as a separate protocol).
2. Centrifuge the cells for 20 minutes at a speed of 4000 rpm and 4°C.
3. Discard the supernatant (liquid on top of cells) being careful not to discard any of the cell pellet.
4. Resuspend the cell pellet in 200 µL of Resuspension Solution (with RNase A added) provided in the kit. In case of *Rhodococcus* plasmid purification, 20 µL of lysozyme with a concentration of 20 mg/mL was added and the tube was incubated at 37°C for 30 minutes.
5. Transfer the solution to a 1.5 µL (must be mL?) microcentrifuge tube.
6. Add 200 µL of Lysis Solution and invert gently to mix. Allow the mixture to clear for less than 5 minutes.
7. Add 350 µL of Neutralization Solution and invert the tube 4-6 times to mix.
8. Pellet the microcentrifuge tubes at 14000 rpm for 10 minutes. The resulting solution is the lysate.
9. Add 500 µL of Column Preparation Solution to a binding column inside a collection tube. Centrifuge this tube for 1 minute at 14 000 rpm and discard the liquid underneath the binding tube.
10. Transfer the lysate into the binding column, being careful not to transfer any solid. Discard the tube with the leftover solid.
11. Centrifuge the collection tube at 14 000 rpm for 1 minute. Discard whatever liquid flowed through the binding column into the collection tube.
12. Add 750 µL of Wash Solution (with added concentrated ethanol) to the column and centrifuge at 14 000 rpm for 1 minute. Discard the liquid that flowed through into the collection tube.
13. Repeat Step 12 a second time with the same quantity of Wash Solution.
14. Centrifuge the tube for 1 minute at 14 000 rpm to dry the column
15. Transfer the column to a new 1.5 mL microcentrifuge tube.
16. Add 50 µL of double distilled water to the column and spin for 1 minute at 14 000 rpm.
17. Use a spectrophotometer to measure the concentration and purity of your plasmid DNA.

DIY (Do It Yourself) protocol:

This protocol uses in-house reagents and ethanol precipitation of plasmid DNA. The required buffer solutions are:

- P1 : 50 mM Tris-HCl (pH 8.0), 10 mM EDTA, 100 µg/ml RNAse A (store at 4°C)
- P2 : 200 mM NaOH, 1% SDS
- P3 : 3 M KAc (pH 5.5) (store at 4°C) (KAc = Potassium Acetate)

1. Grow a 2.5 mL culture overnight. Use 2 mL for isolating plasmid DNA and 0.5 mL for glycerol stock.
2. Pellet culture into 2 mL microfuge tube.
3. Aspire supernatant; Repeat if necessary.
4. Resuspend cell pellet in 300 µL P1 (Keep on ice).
5. Perform the following quickly i.e. in less than 1 min!: Add 300 µL P2 → Invert → Add 300 µL P3
6. Centrifuge at 14000 rpm and room temperature for 10 minutes.
7. Aliquot the supernatant into 1.5 mL microfuge tube (should be about 600-800 µL).
8. Add 650 µL isopropanol (at room temperature) → Invert → Incubate for 10 min at room temperature.
9. Centrifuge at 14000 rpm and 4°C for 10 minutes → Aspirate.
10. Wash pellet with approx. 500 µL 70% cold (-20°C) EtOH (Ethanol).
11. Centrifuge at 14000 rpm and 4°C for 5 minutes → Aspirate.

12. Air-dry the pellet by placing the tubes upside down - this pellet contains the DNA! Alternatively, you can dry the pellet using a SpeedVac for 10 min.
13. Resuspend the pellet in 30 µL double distilled water. Flick the tube to facilitate resuspension.
14. Leave the tube at room temperature for a few minutes to ensure that all plasmid DNA has dissolved.
15. Run 3-4 µL of the DNA solution on an agarose gel to check quality AND/OR Measure DNA concentration with a spectrophotometer (A260/A280).

13. Polymerase chain reaction (PCR)

This protocol is for Taq DNA polymerase and biobrick primers that bind to the following plasmid backbones: pSB1C3, pSB1A3, pSB1K3 and pSB1AC3.

Theory:
PCR amplifies a portion of DNA, as flanked by specific primers. The process mimics the replication of DNA in a regular cell.

1. First, the two strands of the template DNA are separated (**denatured**) at 95°C, which is a high enough temperature to break the hydrogen bonds between the base pairs.
2. Next comes the **annealing** of the primers. The annealing temperature is extremely specific to the primers being used. There are many programs out there that can help you determine the annealing temperature to use with your primers.
3. Then, at 72°C, the *Taq* DNA polymerase **extends** the annealed primers to create a copy of the DNA template. The process repeats. Note: Instead of Taq you can use Pfu or other polymerases with exonuclease activity to ensure that no mutations are added.
   Primers are short pieces of DNA (approximately 20-30 bases long) that are complementary to base strand. Since the DNA polymerase can only attach an incoming 5' nucleotide to an existing 3' end, a starting point for the polymerase to "attach to" has to exist. This is the 3' end of the primer As a result, the portion of DNA between the two primers is amplified.
   ***Taq* DNA polymerase** is harvested from bacteria that live in hot springs, and it is useful because it functions at relatively high temperatures. Its optimal temperature is 72°C. If a regular DNA Polymerase would be used it would be destroyed during the denaturation step at 95°C, and the PCR reaction would fail, the denaturation temperatures () will destroy the polymerase and cause the PCR to fail. $MgCl_2$ is a salt needed by *Taq* DNA polymerase to function properly.
   To ensure that polymerization is complete and each strand is copied in full before the cycle repeats, you should allow approximately 1 minute for every 1000 base pairs to be amplified. Step 5 is the **final extension** step, which ensures that all strands are extended completely. Then the PCR product can be held at 4°C for as long as you need.

Materials:

- 10 x PCR buffer (10 x means 10 times higher than the final concentration)
- 10 mM dNTP mixture
- 50 mM MgCl2 stock solution
- Primer F and Primer R (10mM)
  - Bbk_CP_F: CACCTGACGTCTAAGAAACC
  - Bbk_CP_R: AGGAAGCCTGCATAACGCG
    (Forward and Reverse Primer solutions)
- Taq DNA polymerase
- ddH2O

Protocol:

Reagent	**1x Mix (µL)**	**5x Mix (µL)**
ddH_2O	36	180

10x PCR Buffer	5	25
2 mM dNTP	5	25
Forward Primer (10 mM)	1	5
Reverse Primer (10 mM)	1	5
50 mM $MgCl_2$	1.5	7.5
Taq DNA Polymerase	0.5	2.5
Total	**50**	**250**

PCR program:

STEP	**TEMP**	**TIME**
Initial Denaturation	95°C	30 seconds
30 Cycles	denaturation: 95°C annealing: 55°C* extension: 72°C**	30 seconds 30 seconds 1 minute/kb**
Final Extension	72°C	5 minutes
Hold	4-10°C	

*depending on the primers, the annealing temperature will have to be optimized.
**depending on the polymerase, the extension temperature and time will change.

14. PCR Purification

Theory:
PCR purification is done in order to remove excess debris from the PCR reactions such as enzymes, salts, RNA and primers. Such contaminants can interfere with the correct functioning of the PCR-amplified DNA later on. For example, salts from the PCR reaction could prevent restriction enzymes from cutting the DNA. The presence of salts could also inhibit transformation of bacteria with the PCR product.
Salts that are present in the 10x DNA buffer can interfere with a future restriction enzyme digest that might be done. It can also interfere with transformation and reduce transformation efficieny.

*This protocol is optimized for **the Qiagen PCR purification kit**. Please follow manufacturers specification if you choose to use a different kit.*
Comment: Will teams be using Qiagen kits??

Materials:
- Qiagen Kit

Protocol:
1. Add 5 volumes of Buffer PB to 1 volume of PCR product and mix (for example, add 250 µL Buffer to 50 µL PCR product).
2. Place a Qiagen spin column in a 2 mL collection tube.
3. Apply the mixture from step 1 onto the column and centrifuge for 1 minute.
4. Discard the flow-through and place the column back in the tube.
5. Add 750 µL buffer PE to the column and centrifuge for 1 min.
6. Discard the flow-through and place the column back into the tube.

7. Centrifuge the column again to get rid of any liquid trapped inside.
8. Place the column into a clean microcentrifuge tube and recover the DNA by washing the column with buffer EB.

15. Gel Extraction

Theory:
This method allows selection for specific bands. Gel extracting allows disregarding non-specific bands and product that might have been a part of a procedure such as PCR, Restriction digest etc. This ensures selection of the right product for cloning experiments so that the product obtained is the product that is needed.
I would replace the above paragraph with the following:
This is a method for purifying a specific DNA product and separating it from contaminants, such as enzymes, salts, as well as other DNA.

Protocol:
This protocol is utilized in accordance to the manufacturer's protocol from ***Omega E.Z.N.A (EaZy Nucleic Acid Isolation****). If you choose to use another protocol please follow manufacturer's instructions.*
1. Place agarose gel from gel electrophoresis on top of UV box
2. Carefully extract the DNA product of interest from the gel using a clean scalpel
3. Mass gel fragments
4. Place fragment into a 1.5 mL tube and add 4 µL of H_2O
5. Volume of water added to volume of gel is 200% however if fragment it small 1 mL of water will suffice
6. Remove H_2O
7. Add equal amounts of H2O and Binding Buffer (XP2) to the gel
8. Incubate mixture at 55 °C for 7 mins
9. Mix with vortex for 2 mins
10. Place mixture in the HiBind DNA Mini Column held in a 2 mL centrifuge tube
11. Add 700 µL and centrifuge at 10,000xg for 1 min
12. Discard liquid
13. Add 300 µL Binding Buffer (XP2) to the HiBind DNA Mini Column and spin down at 10,000xg for 1 min
14. Discard liquid
15. Wash the column with 700 µL of SPW buffer with added ethanol and spin down at 10,000xg for 1 min
16. Discard liquid
17. Wash the column with 700 µL of SPW buffer again and spin down at 10,000xg for 1 min
18. Discard the liquid
19. Spin down at 13,000xg for 1 min to dry the column - At this stage the column contains the DNA product of interest
20. Elute in 50 µL of H_2O to the column and wait 1 min
21. Spin down at 13,000xg for 1 min to dry the column. The liquid collected at the bottom of the tube contains the DNA product.
22. Use a spectrophotometer to measure the concentration and purity of the DNA.

16. Constructing two parts together using BioBrick assembly:

Materials:
- EcoRI restriction enzyme stock solution
- XbaI restriction enzyme stock solution
- SpeI restriction enzyme stock solution
- PstI restriction enzyme stock solution

- Restriction Buffer (depending on the reaction and enzyme company you will have to look up the buffer that is compatible to the restriction enzymes you are using)
- ddH2O
- Antarctic Phosphatase
- Phosphatase buffer
- Ligase
- Ligase buffer
- Competent cells

Protocol:

Restriction Digests:

Add to the Insert Tube (in this tube restriction enzymes will cut an insert/part, eg. a promoter, out of a plasmid)
- 600 ng of DNA (Calculate the volume you need from the concentration of plasmid DNA)
- 3.5 µL of 10x Restriction Buffer
- 0.5 µL of each restriction enzyme used (a combination of two enzymes from among EcoRI, XbaI, SpeI, or PstI, eg. 0.5 µL EcoRI and 0.5 µL SpeI)
- ddH_2O such that the volume of water and DNA in the tube is 30.5 µL and the total volume in each tube is 35 µL

Add to the Vector Tube (in this tube restriction enzymes will cut open a plasmid vector containing another DNA part, eg. an RBS-Coding Region-Terminator construct):
- 250 ng of DNA (Calculate the volume you need to add from the concentration of plasmid DNA)
- 3.5 µL of appropriate 10x Restriction Buffer
- 0.5 µL of each restriction enzyme used (a combination of two enzymes from among EcoRI, XbaI, SpeI, or PstI, eg. 0.5 µL EcoRI and 0.5 µL XbaI)
- ddH_2O up to a total volume of 35 µL

1. Mix two tubes as indicated above
2. Put the tubes into a 37°C water bath for one hour
3. Place the tubes into an 80°C heating block for 20 minutes to heat-kill the enzymes
4. Freeze the parts at -20°C until they are needed. Label the tubes.

Ligation Protocol with Antarctic Phosphatase:
1. In a new tube, mix 5 µL of the insert digest with 5 µL of the vector digest.
2. Clearly label the tubes with the remainder of digests (mark as unligated, and date) and freeze them at -20°C. You will need them again if transformation does not work at first trial.
3. Add 10 µL of 2x Quick Ligase Buffer and 1 µL of Quick Ligase to the tube from step 1.
4. Let the tube sit at room temperature for 5 minutes
5. Add 5 µL of 10x Antarctic Phosphatase Buffer, 4 µL of ddH_2O, and 1 µL of Antarctic Phosphatase to the Vector while freezing the insert. Put the tube into a 37°C water bath for 30 minutes and then place into the 65 °C heating block for 10 minutes
6. Transform this mix (all 21 µL into Top10 Competent Cells

APPENDIX 2
DO IT YOURSELF (DIY) BIO

David Lloyd

1. What is DIY Biology?

Do it Yourself (DIY) biology is a new movement in the molecular biology world aimed at making molecular biology and genetic engineering more accessible.. This idea was immediately embraced by the synthetic biology community for multiple reasons, the foremost being that it enabled access to people from outside the field. The DIY biology movement makes it possible for advanced technical skills in molecular biology to be effectively transferred into the hands of people who may have little to no specialty background.

Since high school students will typically enter the iGEM program with little specialty background knowledge, lessons from the DIY movement will likely come in handy when teaching students how to conduct molecular biology work. This section of the guidebook aims to teach you how to build molecular biology equipment, which you can use in your classroom to facilitate much of your iGEM wetlab (organism creation) work.

Please note: Many of these protocols may involve some work with electricity, power tools, or other potentially dangerous items. Please always follow strict safety guidelines, and if you feel uncomfortable performing any of the work described here please inform your IGEM mentors and they will be happy to advise you.

2. What is going on in the world surrounding DIY Bio?

DIY biology is already happening in many places around the world (see links below for some of the locations). For example Genspace, located in New York, (and) is one of the largest DIY biology centres in the world. It has been functional for many years and acted as a powerful force in developing this movement. Another example is BioCurious, based out of the San Francisco area in California - a centre that opens up their doors to anyone interested in learning about synthetic biology. Be sure to look them up online if you are interested in learning more about these type of endeavours.

3. What kind of things do you need in your lab space?

Below is a list of equipment that your lab will need in order to get started with synthetic biology. It is very important to note that many of these materials may change depending on the specific needs and scope of your project. Use this list as a starting point and be sure to contact your mentors to help you identify the exact needs that your project will have.

Also noteworthy is that the links are not provided to tell you what you should actually buy; they are only supposed to give you an idea of the different distributors that exist for these products. (and what they look like.) A later section will indicate where you can (actually) buy some of this equipment.

Equipment List - Large Items		
Item Name	**Description**	**Link**
Centrifuge - Mini	Used to pellet (force to the bottom of the tube) cells or to separate mixtures	https://uk.vwr.com/app/catalog/Product?article_number=521-1647
Vortexer	An agitator for tubes and solutions	https://uk.vwr.com/app/catalog/Product?article_number=444-2791
Pipettes	Used to move solutions from one tube to another. You will need at least three types, a P10, P200, and P1000.	http://www.google.ca/imgres?imgurl=&imgrefurl=http%3A%2F%2Fupcycleus.blogspot.com%2F2011%2F02%2Fclock-made-with-bottle-and-pendulum-for.html&h=0&w=0&sz=1&tbnid=iZA1Nu9I4KSySM&tbnh=225&tbnw=225&zoom=1&docid=wyAdGJUXeY0zoM&ei=ZJ-4UrL7E9TsoASC6IGAAw&ved=0CAQQsCUoAQ
Dry Block Heater	A block which can be used to increase the temperature of tubes from 20 to 100 degrees celsius.	http://www.thermoscientific.com/en/select-country.html?comeFromUrl=%2Fen%2Fproduct%2Fdry-block-heaters.html
Computer	Used to access important programs that will be used in molecular biology	
Water Bath	Used to keep solutions at given temperatures (to increase or decrease the temperature of solutions) for long periods of time	http://www.thermoscientific.com/en/search-results.html?keyword=water+bath&countryCode=US&matchDim=Y
Autoclave	Used to sterilize equipment and solutions which will be used to grow your bacteria.	http://www.ebay.com/bhp/mini-autoclave

Dish Washer	While not completely necessary, something to clean your equipment in may prove useful.	
Scale	Used to measure reagents for making solutions. It should read to at least one decimal place per gram (~0.1)	http://www.globalindustrial.ca/p/packaging/scales/lab-balance/lseriesdigital-lab-scale?infoParam.campaignId=T9A&gclid=CJv_pJGfx7sCFcxAMgod3SgA5A&gclsrc=aw.ds
pH Meter	Used to pH solutions to the correct level	https://ca.vwr.com/store/catalog/product.jsp?product_id=8932759
Fridge	Must be able to hold a temperature of 4 degrees Celsius to keep solutions cold	
Freezer	Must be able to hold a temperature of -20 degrees Celsius to keep solutions cold	
PCR Machine	Used to selectively amplify a sequence of DNA.	http://loci.wisc.edu/equipment/polymerase-chain-reaction-pcr-machine
Power supply	Important for gel electrophoresis	http://www.bio-rad.com/en-us/product/power-supplies
Gel Box	Used to run DNA gel electrophoresis	www.bio-rad.com/en-ca/category/nucleic-acid-electrophoresis-blotting
Ice maker (supply of ice)	You will require a way to keep some tubes at around 4 degrees	http://www.kmart.com/search=portable%20crushed%20ice%20cube%20maker?storeId=10151&catalogId=10104&viewItems=50&levels=Appliances&vDropDown=defaultOpt&sLevel=0&redirectType=SKIP_LEVEL
Fumehood	Some chemicals may be dangerous, these should be kept in a fume hood to prevent exposure	http://en.wikipedia.org/wiki/Fume_hood

Water filtration system	You will need some clean water in order to make most of your solutions. The water should be distilled for making solutions, and double distilled when working with DNA	http://www.ebay.com/bhp/water-distillation-system
Gas line (bunsen burner)	Gas will be required to sterilize some equipment and for aseptic technique with bacteria	
Centrifuge - large	If you will be spinning down (pelleting) cultures of 15 mL or more, you may want to invest in a larger centrifuge that is amenable to these volumes	https://ca.vwr.com/store/catalog/product.jsp?catalog_number=51000-030
Gel imager	A light generator (usually blue or UV light) that will allow you to visualize DNA from gel electrophoresis	http://www.lifetechnologies.com/us/en/home/life-science/dna-rna-purification-analysis/nucleic-acid-gel-electrophoresis/e-gel-electrophoresis-system/e-gel-imager-system.html
Magnetic stir plate/heater	Used to mix solutions or heat them	http://www.ebay.com/bhp/magnetic-stir-plate
Microwave	Used to dissolve the agarose used in gel electrophoresis and to warm/semi-sterilize some solutions	

For a complete list of all chemicals, small equipment, and large equipment pieces please see the appendix for more information.

4. What pieces of equipment can you build instead of buying?

Because of limited funding and budgets, you may find it useful to try and develop some of your own pieces of equipment rather than buying them. Below are some DIY projects that you and your students can undergo. Please communicate with your mentors prior to starting these projects as they may provide you with alternative solutions.

Please note that if you intend to do any of these projects yourselves, please make safety a priority. Some of the projects involve building electrical devices or using other potentially dangerous components. Please follow all necessary safety precautions when building or using these devices.

Potential DIY Devices:

1) Build your own Gel Electrophoresis set up
    a. Building a Power Source
    b. Building an Electrophoresis Gel Running Apparatus
    c. Gel Box solutions

2) Building Your Own Centrifuge
3) Building Your Own Incubator
4) Building Your Own Water Bath
5) Building Your Own Shaking Platform/Vortexer

Please note that the above list of equipment is a starting point, to show you some DIY opportunities that have been developed by other individuals. It is recommended that you search the internet for alternative ways to build these pieces of equipment prior to deciding on the most suitable way for you to do it

5. DIY Building Projects

Building a power source

The power source is the device, which will supply a current to your gel box, forcing the DNA into the agarose gel and separating it. The device is basically a transformer, which will allow for a powerful voltage (usually between 80-100 V) to be applied to the solution. If you feel uncomfortable building with developing this unit, ask the mentors if there is any power source available to borrow. (there may be some available from your experts. Be sure to get into contact with them.)

One way to build a power source can be found here: **http://www.instructables.com/id/Gel-electrophoresis-power-supply/step1/Materials/**http://www.instructables.com/id/Gel-electrophoresis-power-supply/step1/Materials/

http://www.instructables.com/id/Gel-electrophoresis-power-supply/step1/Materials/

An alternative way to build a power source is described below. The procedure was developed for a graduate student thesis at the University of Calgary. The goal of the thesis was to design a way to build a power supply, which could be constructed for use in third world countries. The details are listed below:

Please note that all of this protocol was adapted from Dr. Leif Prebeau-Menezes Thesis with his permission:

Materials List:

Hammond Manufacturing Transformer [115 VAC 100VCT 0.5A]
PadBoard [~75 mm L x ~75mm W]
Male Pin Connector Receptacle [AC Inlet 2.8 mm]
Banana Jack (Red/Black) Female Plugs [2mm Sheathed]
Switch ON/ON [3- Way Receptacle Switch Rocker]
Low-Density Polyethylene(LDPE) or Polypropylene (PP) Non- Conducting Plastic Container [~220 mm L x ~130 mm W x ~120mm H]
Switch ON/OFF [2-Way Receptacle Switch Rocker]
Through Hole Resistors [RES 10.0K OHM 7W]
Capacitor [160V 47µF]
General Purpose Diodes
Standard Digital Multimeter [Volt/Ampere/Ohm meter]
Standard Glue Gun and Glue Sticks [10W Dual-Temperature]
Standard Soldering Iron [60W Pencil Tip] and Solder [63/37 20 Gauge 1/2 lb]
Receptacle, Female Pin Connector with 3 Pin Electrical cord
[6 Inches in length]
Hook-up Wire (Red/Black) [300 V]
Retractable Utility Knife with Snap off Blades

Procedure:

1) In the clear, non-conducting, LDPE/PP plastic container four holes of approximately 6.0 mm in diameter were cut with a sharp retractable utility knife at approximately 30 mm from the corners of the container. The top two holes on the container were used for installing the black female banana jack plug (negative outlet lead) and the red female banana jack plug (positive outlet lead), identified as "A" in Figure 1.

The bottom left hole, identified as "B", in Figure 1, was used for mounting the toggle switch between 70V and 140V (ON/ON 3- Way Receptacle Switch Rocker). The bottom right hole, identified as "C", in Figure 1, was used to install the main ON/OFF power switch (2-Way Receptacle Switch Rocker). Two green peg-boards, recycled from discarded pipette boxes, were used as reinforcement for the container.

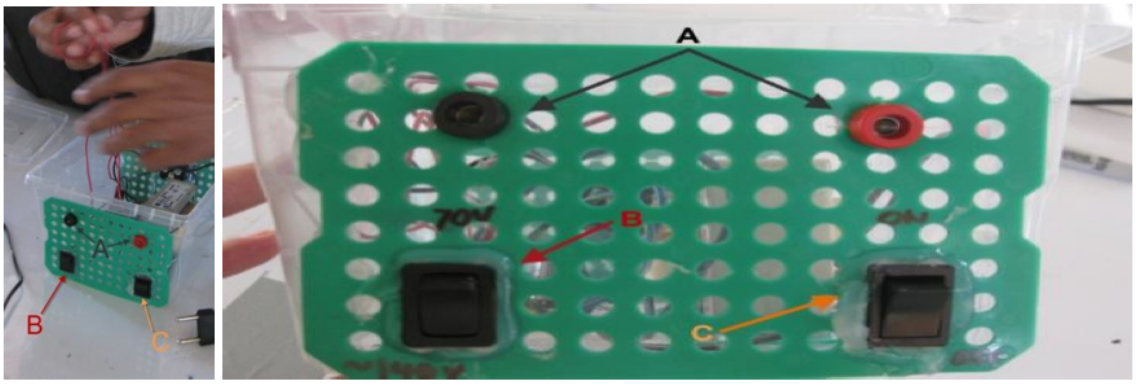


Figure 1: Illustrates the location of: A) banana jack red/black female plug set; the black negative plug on the left and the red positive plug on the right, B) toggle switch for switching between 70V and 140V, and C) main ON/OFF power switch

2) On the opposite side of the container, a single hole approximately 30mm L x 15mm W was cut with a retractable utility knife and was prepared for the installation of the male pin connector receptacle (AC Inlet 2.8 mm) identified as "A", in Figure 2.

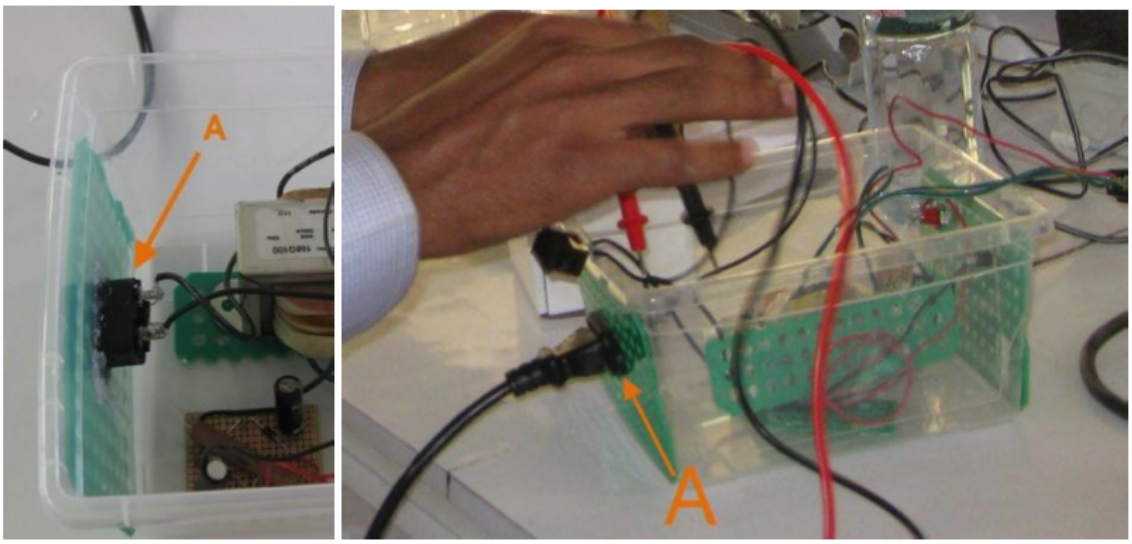


Figure 2: Illustrates the location of: A) the male pin connector receptacle attached to the clear LDPE non-conducting container

3) The Hammond Manufacturing Transformer (115 VAC 100VCT 0.5A) and electronic components were configured to the Padboard according to the circuit diagram (Figure 3). All electronic components, wiring and circuit board connections were soldered using a standard soldering iron (Standard Soldering Iron 60W Pencil Tip) and solder. With this current configuration it was imperative that only 110V AC inlet power be supplied to the transformer. Any current above this threshold would damage the electrical components, the transformer, and become a potential fire hazard rendering the SBPSU inoperable.

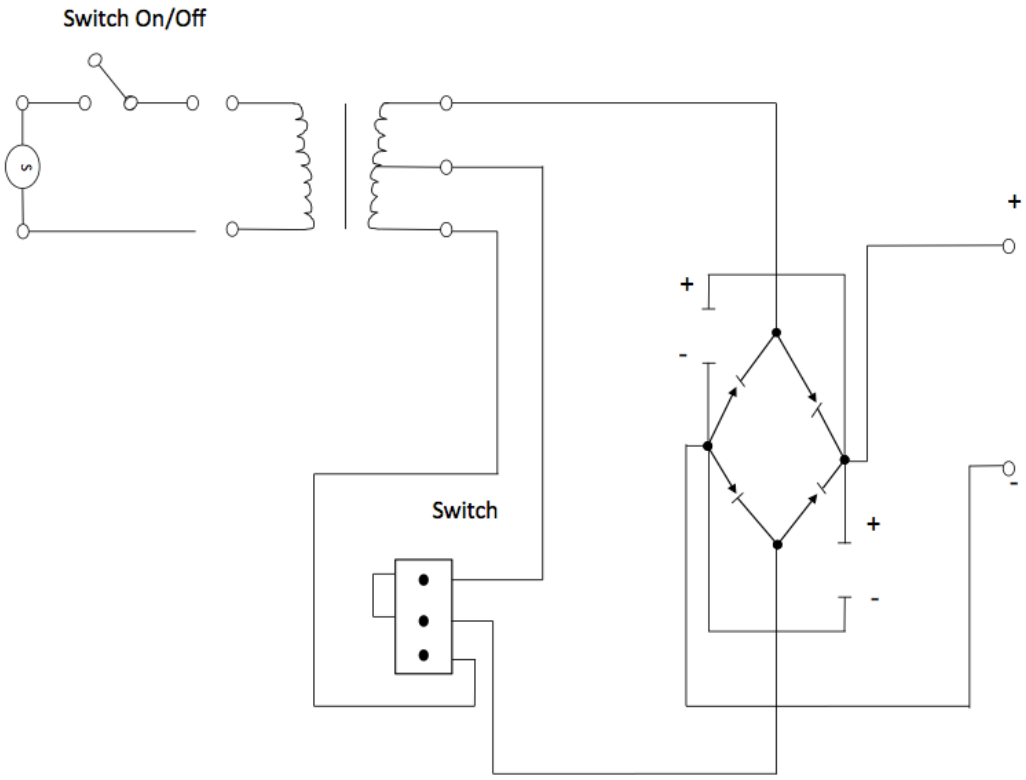


Figure 3: Circuit Diagram

4) The transformer, padboard with its electronic components, positive and negative leads, switches and male pin connector were securely mounted onto the LDPE/PP 30 container as shown in Figure 4, using the non conductive plastic adhesive from the glue gun (Standard Glue Gun 10W Dual-Temperature with glue sticks).

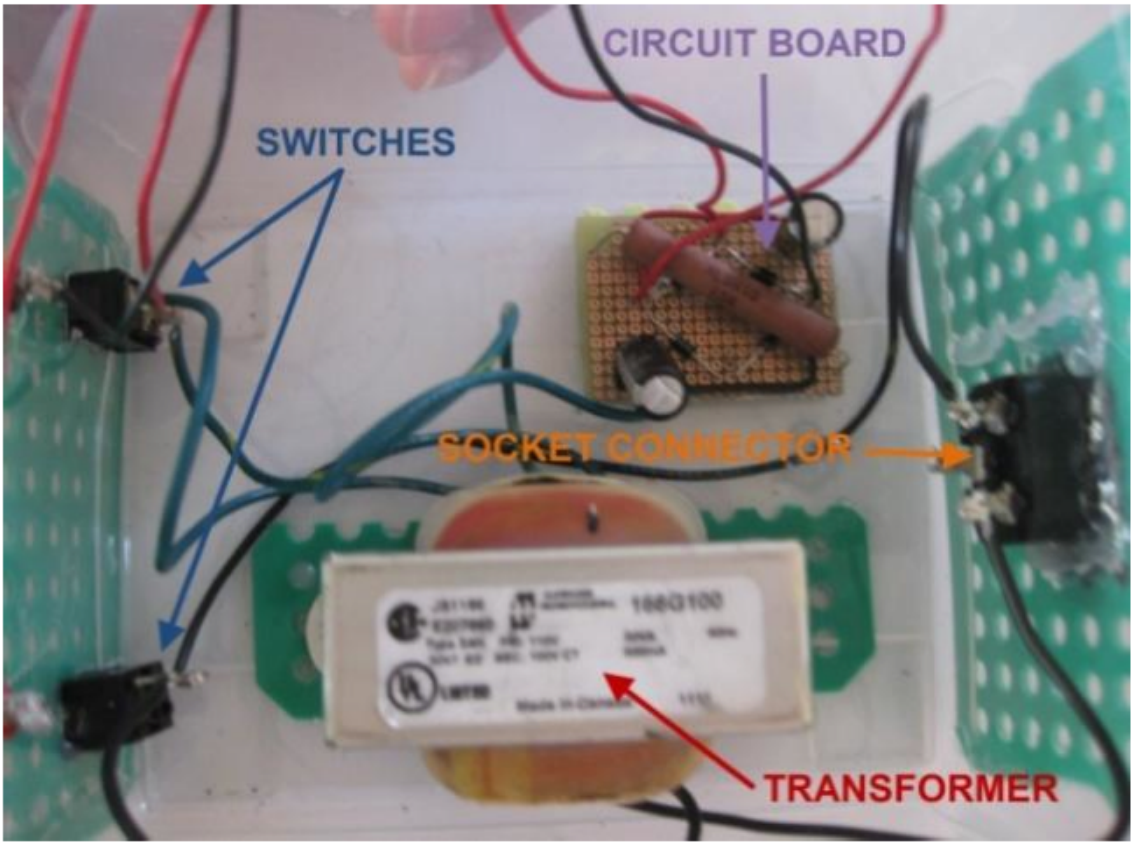


Figure 4: Illustrates the electronic components, positive and negative leads, switches and male pin connector receptacle mounted to the container securely using non conductive plastic adhesive

5) The cover of the container was placed securely to prevent accidental opening or tampering. The outside of the container was labelled PLUG IN TO 110V ONLY to prevent accidental plug-in to the lab-electrical input connection of 220V AC (Figure 5).

Figure 5: SBPSU with secured cover and warning label indicating only 110V AC input

Construction Safety Protocols:

The safety protocols observed during the construction of the SBPSU were:

1) Ensuring that the equipment chassis and cabinets were grounded.
2) Disconnecting power and unplugging all unnecessary electric equipment before working on the electronic circuits.
3) Avoiding any contact with moisture or water during construction. Never placing containers of liquid on/near the SBPSU.
4) Removing metal ornaments such as jewelry, watches, rings, etc., before working on electrical circuits.
5) Ensuring to never overload circuits or leave unprotected (charged) SBPSU's unattended.
6) Securing the SBPSU properly for extended absences/storage.
7) Safely discharging capacitors in the SBPSU before working on its circuit board.
8) Wearing proper personnel protective equipment before doing any soldering or working alongside an open flame.

Building a gel electrophoresis tank

Once you have a power supply, you can now set this up with the gel electrophoresis tank to perform your experiments. You can make a gel electrophoresis tank fairly easily. However, because of the safety considerations involved, if you do not feel absolutely comfortable with building it yourself, it is recommended that you purchase a professionally built electrophoresis unit instead.

Protocol 1: This is a very crude design, but it gives a great overview of how the technique works and what you will need for building one of these devices. Note that it is also much safer than some of the other types of constructions. If you already have a voltage pack it is not as important that you use this kind of design:

http://www.sciencebuddies.org/science-fair-projects/project_ideas/BioChem_p028.shtml#procedurehttp://www.sciencebuddies.org/science-fair-projects/project_ideas/BioChem_p028.shtml - procedure

http://www.sciencebuddies.org/science-fair-projects/project_ideas/BioChem_p028.shtml - procedure

Protocol 2: This contains very clear instructions on how to build a gel box. Note that the wires used are bare and therefore they pose more of a safety concern. If you can find plastic coated wires instead, this will decrease the risk associated with using the device:

http://citizensciencequarterly.com/?p=3084&preview=truehttp://citizensciencequarterly.com/?p=3084&preview=true

http://citizensciencequarterly.com/?p=3084&preview=true

Once you have the power supply and the gel tank you can run a DNA agarose gel. After running the gel you will need to visualize the DNA inside of the gel. One of the easiest ways of doing this is with SyberStain, which will allow low UV or blue light to visualize the DNA very well. A blue light pen available for purchase from BioRad will allow you to visualize the DNA :

Long-Wave UV Pen Light #166-0530EDU

http://www.bio-rad.com/en-us/sku/166-0530edu-long-wave-uv-pen-light

Building Your Own Centrifuge:

The following excerpt and procedure were taken from:

http://www.thelabworldgroup.com/blogs/build-your-own-centrifuge-under-fifty-dollars-diy-centrifuge-project

< The 3-D–printed wheel looks deceptively simple, with six slots for standard 1.5-microliter Eppendorf tubes, oriented horizontally. Garvey says his early prototypes often deformed, which cracked and shot off tubes like oversize plastic bullets. "People said I was mad," Garvey says. Yet he prevailed, reshaping the wheel's slots to safely cradle the tubes' thick rims. "I've never had a tube eject since," he says. Still, Garvey advises using extreme caution—and proper eye protection—when running what he's dubbed the Dremelfuge.>

The procedure will require access to a 3D printer. You may have to do a bit of searching to see if you can find a 3D printer in your area. There is one available at the University of Calgary.

1) Download schematics for the Dremelfuge wheel here (https://gitorious.org/dremelfuge). Two designs are available; pick the one that fits a Dremel 300 (not a standard drill).

2) 3-D–print the wheel in ABS plastic, using hexagonal infill to strengthen it. (PLA is another common printing material, but Garvey says it tends to shatter under high centrifugal G-forces.) If you lack a 3-D printer, order the wheel from Shapeways.com for about $50.
3) Screw a rotary-tool disc holder into the wheel's axis, and attach it to a Dremel.
4) Fit tubes containing biological samples into the wheel, making sure that each tube has a counterweight for balance. This means that there is always another tube on the opposite side holding an equal amount of liquid. Failure to do this will lead to damage of the centrifuge and a big mess.
5) Place the Dremelfuge in a Styrofoam cooler, and let 'er rip! The foam will absorb the impact if a tube pops off.

***WARNING:* Use shatterproof eye protection, and operate the Dremelfuge inside a sturdy container, as the device can throw off hunks of plastic at dangerously high speeds.**

TEST RUN of your newly built centrifuge

The cells lining your inner cheeks constantly slough off into saliva but are too small to see individually—This makes them perfect candidates for testing the Dremelfuge.

1) Swab a Q-tip inside your mouth for about 10 seconds to grab some inner cheek cells.

2) Dip the cotton tip repeatedly in a tube filled with isotonic saline solution to dislodge the cells. (Make your own 0.90 % w/v solution from table salt and distilled water, or buy wound wash at a pharmacy.)

3) Fill a second tube with water to the same level as the one containing the cells. Insert the tubes in opposite slots in the 3-D–printed wheel.

4) Run for one minute at the Dremel's second setting (about 10,000 rpm). You'll see a whitish cell pellet at the bottom of the tubes. Voilà! Isolated cheek cells.

Building an Incubator:

Again there are multiple strategies for how to build your own incubator. One of the methods is detailed below. You can also use any of the set-ups for chicken eggs incubators available online. Your incubator must hold the temperature constant at or near 37 degrees Celsius for growing *E. coli*. The closer you keep it to that temperature the better your bacteria are going to grow.

The following is taken from "1999 Science in the Real World: Microbes in Action". The material may be duplicated by teachers for use in the classroom; however any other use is prohibited.

Materials

- 20 gallon aquarium (does not need to be watertight)
- Heavyweight clear plastic
- Strong tape
- Small lamp that can use up to 75-watt bulb
- Thermometer (0-100º C). (preferably in a clear plastic thermometer case)

Instructions

1. Turn the aquarium so that the opening faces the front instead of the top.
2. Cut the plastic slightly wider than the opening and about 2 inches longer than the height of the opening.
3. Tape the plastic to the top of the aquarium, so that the plastic falls over the opening at the front. This is your "door."
4. Place the lamp in the aquarium, letting the cord come out the front under the plastic covering.

5. Place the thermometer in the aquarium so that you can take readings through the glass without opening the plastic "door."
6. Try different bulbs until you find one that gives you the temperature you need for your incubator.

You can be really fancy and attach a dimmer to the lamp so that you can regulate the temperature by using the dimmer switch.

A genetic engineering experiment using E. coli grown inside this kind of incubator is described by John Iovine in "The Amateur Scientist" in Scientific American, June, 1994 pp. 108-111

Building a Water Bath:

Only the link to how to build one of your own water bath units will be provided here. The online instructions are great but do require some ability to solder your own electronics. The water bath described controls temperature accurately and is quite cheap. If you do not presently have a water bath unit, this may be a cheap viable option for you.

http://seattlefoodgeek.com/2010/02/diy-sous-vide-heating-immersion-circulator-for-about-75/http://seattlefoodgeek.com/2010/02/diy-sous-vide-heating-immersion-circulator-for-about-75/

http://seattlefoodgeek.com/2010/02/diy-sous-vide-heating-immersion-circulator-for-about-75/

Building a Shaker and Vortex Mixer:

Vortex mixer units are important for mixing solutions rapidly. A vortex can be purchased at a fairly cheap price, and it can be adapted for mixing multiple solutions at the same time using the following protocol. It could also be used in conjunction with an incubator to heat solutions at low speed and 37 degrees Celsius.

http://citizensciencequarterly.com/2012/01/cheapass-science-diy-vortex-mixer-tube-holder/http://citizensciencequarterly.com/2012/01/cheapass-science-diy-vortex-mixer-tube-holder/

http://citizensciencequarterly.com/2012/01/cheapass-science-diy-vortex-mixer-tube-holder/

6. Other Pieces of Equipment that Can be Modified

Solutions to an autoclave:
Autoclaving is a process by which you heat a solution or substance at a high temperature and pressure. Normally, it uses steam at about 121 degrees Celsius for 20-45 minutes, which is enough to destroy all the organic contaminants in a solution. Autoclaving is needed primarily to destroy spores that are otherwise hard to kill off using regular antibiotics or cleaning reagents.

If obtaining an autoclave is difficult for you, here are some tricks to get around this issue. An important thing to remember is that you need about 30 psi - pounds per square inch of pressure - in order to achieve proper sterilization. One potential option is to use a **pressure cooker in order to sterilize media and other components you will be working** with. Remember that the pressure cooker must reach a psi level of 30.

Whenever you autoclave something, be sure to place a piece of autoclave tape onto the item. This special tape will turn black if sufficiently high temperature and pressure is reached to kill off bacteria and other organic contamination.

One component that you will need to prepare often is the nutrient solution (media) that your cells can grow in. Since this will be one of the most common solutions that you will use, it is convenient to know ways to get around having to autoclave it. Here is a possible protocol to use:

1. Add 32 grams of LB Agar powder to a 1 L autoclaved/sterile bottle.
2. Using a clean and autoclaved/bleached graduated cylinder, fill up bottle with 900 mL of distilled water (if you use a graduated bottle you can pour the distilled water directly inside the bottle).
3. Close the cap and shake vigorously.
4. Remove cap (!) and microwave on High for 1 min. Repeat this several times, stirring occasionally with a sterile pipette until the LB Agar is dark yellow and slightly viscous and there are no lumps left. Be sure to check the bottle often as it may boil over!
5. Place bottle in 50 degree Celsius water bath with the cap loosely fastened. Stir gently after 30 minutes.
6. The LB Agar is ready to pour into plates when the bottle is slightly warm and easily handled.
7. Thaw out a tube of Ampicillin.
8. Add the Ampicillin to your bottle of LB Agar (see protocols section for amount of antibiotics to add). Stir with a sterile pipette.
9. Lay out 50 petri dishes (2 sleeves) on the bench top; keep the plastic sleeves. You can choose to either use a pipette aid to pipette your LB Agar, or you can pour directly into the plate. Pour about ¼ inch of LB Agar per plate.
10. Cover petri dish with the petri lid; be sure to leave the lid slightly off center until Agar is fully cooled and hardened.
11. After the plates have cooled and solidified, replace them back in to the plastic sleeve and close with some tape. Be sure to label the tape with "LB Agar + Amp" and the date the plates were made.

NOTE: THIS PROTOCOL IS NOT FOR MAKING LB AGAR PLATES WITHOUT ANTIBIOTICS!!! If you need LB agar plates without antibiotics, make them the old fashion way using the autoclave. (trust me, you'll be sorry if you don't)

Solutions to a -80 degrees Celsius Freezer:

In order to store cells, certain solutions, and other chemicals for long periods of time you require a freezer, which can reach -80 degrees Celsius. These are expensive and difficult to obtain. Therefore you may need some solutions to getting around using this piece of equipment.

One of the major requirements you will need to use your -80 freezer for is to store competent cells. These cells are ready to be used for transformation, which is the process by which cells take up foreign DNA. The procedure below describes a way to prepare competent cells fresh every time you need them, such that you do not need a freezer to store them.

Materials Needed:

- LB Broth (made from Yeast Extract, Tryptone, and NaCl. See Protocols section)
- Sterile Falcon Tubes of 15 mL and 50 mL
- Plate incubator to store the plates
- Ice
- Water bath at 42 degrees Celsius
- $CaCl_2$ (Calcium Chloride) 100 mM liquid solution, sterile!

1. Grow up your bacteria overnight in 5 mL LB media. Be sure it is sterile because there is no antibiotic selection. Incubate with shaking overnight at 37 degrees Celsius.
2. Next morning dilute the bacteria 1:100 eg. 0.1 mL or 100 uL bacterial culture into 9.9 mL LB media and allow them to grow for another 3-4 hours at 37 degrees Celsius with shaking.

3. Take 0.5 mL or 500 uL of the bacteria you have grown up, spin down in a centrifuge at 7,000 RPM and remove the supernatant.
4. Add 0.5 mL or 500 uL of $CaCl_2$ solution and resuspend the bacteria. Spin the solution down in the centrifuge again, remove supernatant and add 0.1 mL or 100 uL of $CaCl_2$ solution. You now have a fresh suspension of competent cells ready to be transformed with DNA.
5. Add 1 uL (or any amount that you need) of DNA to the competent bacteria and pipette up and down to mix. **Note: this part of the procedure is the transformation of your DNA; see the protocols section for more. Please note that this procedure is a bit different than the one in the Protocols. Both procedures will work depending on the reagents you presently have available to you.**
6. Place the mixture of cells and DNA on ice for 5 minutes
7. Heat at 42 degrees Celsius for 50 seconds and return to ice immediately
8. Ice for 2 minutes and add 0.250 mL or 250 uL LB medium
9. Incubate 30 minutes at 37 degrees Celsius and plate 100 uL per plate

The bacteria successfully transformed with new DNA constructs must be placed in long-term storage. This is done by preparing a glycerol stock of these bacteria. A **glycerol stock** is a suspension of the cells in LB media with 30% glycerol, which allows them to stay alive at temperatures as low as -80 degrees Celsius.

If you have no access to a -80 degrees Celsius freezer you have several alternative options:

1. Create an agar stab
   (This is a relatively easy protocol.) Take a 15 mL Falcon tube (sterile) and pour in liquid LB Agar with the appropriate antibiotic selection for your construct about half way. Take some of the bacterial culture that you wish to preserve and stab it into the LB Agar once it has solidified and cooled. Store this in a fridge with the cap tightened. These cells will usually be good to use for a fairly long time.

2. Store you glycerol stocks at -20 Degrees Celsius i.e. in a regular freezer
   Follow the procedure in the protocols section but store the tube in the freezer. These cells will not be as stable as when stored at -80 degrees (This will not be as stable for the cells) and they (you) can lose the (your) new construct (plasmid) over time. This method is only recommended as a backup way of storing your cells.

3. Alternative Room Temperature Storage Solutions
   There are companies that offer cryo-solutions using new and innovative ways of storing cells at room temperature. You can check them out online by searching for "room temperature cryo solutions for bacterial long term storage".

Ways of Measuring Number of Cells

One important and common technique that you may require is a way to measure the number of cells in a solution. This is usually accomplished with a spectrophotometer by measuring the optical density (OD) of the suspension at a wavelength of 600 nm. You can do a google search to learn more about what the spectrophotometer does. The problem is that a spectrophotometer can be expensive! An inexpensive way of getting around (using) a spectrophotometer (one of these devices) is by using (through) a mini-spectrophotometer on (that uses) your iPhone or Android device!

http://shop.breadpig.com/products/mini-fold-up-spectrometerhttp://shop.breadpig.com/products/mini-fold-up-spectrometer

http://shop.breadpig.com/products/mini-fold-up-spectrometer

7. Where to Buy Equipment That You Cannot Make

Most equipment can be purchased from large biotechnology companies that make top of the line equipment for premium prices. With a limited budget you may not be able to afford all of the top end equipment that you need. Some of the main companies that sell biotechnology equipment are:

Fisher Scientific
Thermo
Bio-Rad
VWR
Invitrogen/Life Technology
Roche
Rose Scientific
Sarstedt
Sigma Aldrich – For Chemicals
NEB Biosciences
Cedar Lane
Gold Biotechnology

Be sure to check out these companies' websites to get an idea of the types of high-end equipment that are available. You will notice that many of these companies do not list prices for their equipment as the selling price will vary depending on the institution that you are with. If you are thinking of buying something from one of the companies listed above please contact the mentors as they may be able to get you a discounted price by using university pricing.

Some of these companies will service high schools, particularly NEB Biosciences, which is a great resource for enzymes that you will need, as well as Sigma, from which you can purchase many of the chemicals you will need.

VWR, Fisher, and Cedar Lane are distribution companies that sell products from various other biotechnology groups and are great resources for alternative products available on the market.

Alternative vendors that you can use are second hand refurbished providers such as www.southwestscience.com. Such companies sell biotechnology products at a lower price compared to other biotechnology companies; however, the advantage is that they guarantee the quality of the machinery that they are selling to you. You never have to worry if the machine you just purchased will be in good working order. This is just one provider and there are numerous others in North America. It is highly recommended that you shop around (on the internet) with as many providers as possible to find the best fit (that is best) for you.

8. Other References

a. diybio.org/
b. https://groups.google.com/forum/#!forum/diybiohttps://groups.google.com/forum/ - !forum/diybio
c. http://openwetware.org/wiki/Protocolshttp://openwetware.org/wiki/Protocols
d. http://openwetware.orghttp://openwetware.org
e. http://openwetware.org/wiki/Materialshttp://openwetware.org/wiki/Materials
f. http://openwetware.org/wiki/Tk:lab_stuffhttp://openwetware.org/wiki/Tk:lab_stuff
g. http://www.mlo-online.com/articles/201112/the-quest-for-the-500-home-molecular-biology-laboratory.phphttp://www.mlo-online.com/articles/201112/the-quest-for-the-500-home-molecular-biology-laboratory.php
h. http://www.popsci.com/diy/article/2013-07/how-build-your-own-diy-centrifuge

APPENDIX 3

WIKI: MINIMAL HEADER CSS

Patrick Wu

Minimal Header CSS

Copy and paste this to your global CSS file and it should remove the photo banner while retaining the menu bar (aka 'div#top-section').

```
/***
Minimal header: removes the search bar and header image and readjusts
font colours in the menus.

Header compiled by Patrick Wu, iGEM Calgary 2011-2013.

Thanks a lot to the 2011 Brown-Stanford and 2012 Lethbridge iGEM teams
for snippets of their code!
Check out their wikis at:
http://2011.igem.org/Team:Brown-Stanford
http://2012.igem.org/Team:Lethbridge
***/

/*hides the search bar and photos*/
#content h1.firstHeading, #contentSub{
      display: none;
}
#p-logo {
      display: none;
}
#searchform {
    display: none;
}

.left-menu {
      background-color: #555;

}
.left-menu a {
    color: #000;
}

div#top-section{        /*the div containing the entire top bar*/
      background: #DEDEDE;
      border: none;
      height: 1.5em;
      margin-bottom: 0px !important;
      position: fixed;
      width: 100%;
      z-index: 3;
}
```

```
#content{
	border: 0;
	margin: 0;
	padding: 0;
	position: static;
	width: 100%;
	z-index: 1;
}


#search-controls {
	overflow:hidden;
	display:none;
	background: none;
	position: absolute;
	top: 170px;
	right: 40px;
}


div#header {
	width: 975px;
	text-align: left;
	margin-left: auto;
	margin-right: auto;
	margin-bottom: 0px !important;
}

#menubar {
	position: absolute;
	background: none;
	color: black;
	font-family: Roboto, Sans-Serif;
}

.left-menu, .right-menu{
	position: absolute;
	background: none;
	color: black;
}

.left-menu li a, .right-menu li a {
	color: #000 !important;
	font-size: 0.8rem;
	padding: 0 0.6em 0 0;
}


.left-menu ul li, .right-menu ul li a{
	background: none;
	color: #000 !important;
	font-size: 0.8rem;
}

.left-menu li a:hover, .right-menu li a:hover, .right-menu li
a:visited, .right-menu li a:active {
```

```
    color: #000 !important;
      font-size: 0.8rem;
}

div.right-menu{
      z-index: 0;
}

#catlinks{
      display:none;
}

#footer-box{
      background: #DEDEDE;
      width: 100%;
      margin: 0;
      padding: 0;
      border: none;
}

#f-list li a{
      color: #555555;
      font-family: Roboto, Sans-Serif;
      font-size: 0.8rem;
      text-decoration: none;
}

      #f-list li a:hover{
            text-decoration: none;
      }

/*important for background colours*/
.mediawiki{
      background: #ffffff;
}

/***End minimal header***/
```

APPENDIX 4

LEARNING OUTCOMES

Magdalena Pop

iGEM Project – Skills/Process Assessment /100

Skills	Criteria	Max Point value/Weight
Initiating & Planning	• Uses the iGEM website/resources independently and effectively – e.g. shows initiative and drive to learn and discover. • Seeks feedback regularly to learn and clarify - e.g. paraphrases ideas, asks questions, takes notes. • Contributes insightfully to project idea development – e.g. comes up with viable ideas, is willing and able to adjust to more realistic project ideas. • Makes significant and valuable contributions to the design and planning of the project – e.g. identifies concrete steps and needs, evaluates options.	20
Performing & Recording	• Conducts experimental procedures systematically and reliably – e.g. follows attentively all protocols and safety rules, including the use of all lab equipment. • Adjusts and repeats experiments to improve results and advance the project – e.g. shows dedication and resilience when faced with poor or unexpected results. • Keeps detailed records of all experiments and any related pertinent information – e.g. keeps activity log with detailed protocols, changes thereof, as well as current ideas. • Collects and records results and data clearly and completely – e.g. gathers all results and observations in a systematic way.	30
Analysing & Interpreting	• Contributes meaningfully to troubleshooting and the redesigning of experiments – e.g. is able to identify problems and come up with realistic and feasible solutions. • Evaluates experiments and results competently – e.g. uses valid criteria to assess outcomes and to adjust course as needed • Draws valid and pertinent conclusions from experimental data – e.g. is able to select the best quality results, and to draw out the full meaning • Summarizes experimental outcomes effectively and comes up with novel interpretations and ideas – e.g. is able to extract the relevance and meaning in the data; maps out potential future developments	20

Collaborating & Communicating	• Interacts productively with other team members in designing and evaluating ideas and experiments – e.g. provides useful feedback, listens actively, backs up opinions, helps and supports others, seeks help • Completes assigned tasks effectively and on time – e.g. has jobs done by deadline, always tries their best • Communicates clearly in a well structured and organized way and according to requirements – e.g. presents content, ideas and visuals in such a way as to deliver a most clear and complete message • Presents information in a manner appropriate for the target audience – e.g. uses adequate means of communication, including multiple media, in a competent fashion	30

APPENDIX 5
CURRICULUM MAPPING

Magdalena Pop

iGEM for High-School Science – Mapping to Programs of Study

***By participating in the iGEM project Science 10 students will work towards meeting learning outcomes outlined in the Science 10 Program of Study
http://education.alberta.ca/media/654833/science10.pdf

Sc10 – Unit C: Cycling of Matter in Living Systems (Nature of Science Emphasis)

Outcomes for Science, Technology and Society (STS) and Knowledge
Will identify areas of cell research at the molecular level (e.g., DNA and gene mapping, transport across cell membranes)
Will describe the function of cell organelles and structures in a cell, in terms of life processes, and use models to explain these processes and their applications

Skill Outcomes (focus on scientific inquiry)
Initiating and Planning – will ask questions about observed relationships, and plan investigations of questions, ideas, problems and issues
Performing and Recording - will conduct investigations into relationships between and among observable variables, and use a broad range of tools and techniques to gather and record data and information
Analyzing and Interpreting – will analyze data and apply mathematical and conceptual models to develop and assess possible solutions
Communication and Teamwork – will work as members of a team in addressing problems, and apply the skills and conventions of science in communicating information and ideas and in assessing results

Attitude Outcomes
Interest in Science – will show interest in science-related questions and issues, and confidently pursue personal interests and career possibilities within science-related fields
Mutual Respect – will appreciate that scientific understanding evolves from the interaction of ideas involving people with different views and backgrounds
Scientific Inquiry – will seek and apply evidence when evaluating alternative approaches to investigations, problems and issues
Collaboration – will work collaboratively in planning and carrying out investigations, as well as in generating and evaluating ideas
Stewardship – will demonstrate sensitivity and responsibility in pursuing a balance between the needs of humans and a sustainable environment
Safety – will show concern for safety in planning, carrying out and reviewing activities

***By participating in the iGEM project Bio 20-30 students will work towards meeting learning outcomes outlined in the Bio 20-30 Program of study
http://education.alberta.ca/media/654841/bio203007.pdf

Bio30 – Unit C: Cell Division, Genetics, and Molecular Biology

Specific outcomes for Knowledge
30–C3.2k will describe, in general, how genetic information is contained in the sequence of bases in DNA molecules in chromosomes and how the DNA molecules replicate themselves
30–C3.3k will describe, in general, how genetic information is transcribed into sequences of bases in RNA molecules and is finally translated into sequences of amino acids in proteins
30–C3.4k will explain, in general, how restriction enzymes cut DNA molecules into smaller fragments and how ligases reassemble them
30–C3.5k will explain, in general, how cells may be transformed by inserting new DNA sequences into their genomes
30–C3.6k will explain how a random change (mutation) in the sequence of bases results in abnormalities or provides a source of genetic variability

Specific Outcomes for Science, Technology and Society (STS) (Social and Environmental Contexts Emphasis)

30–C3.1sts will explain that science and technology have both intended and unintended consequences for humans and the environment
30–C3.2sts will explain that scientific research and technological development help achieve a sustainable society, economy and environment

Specific Outcomes for Skills (Nature of Science Emphasis)

Initiating and Planning (30–C3.1s) will formulate questions about observed relationships and plan investigations of questions, ideas, problems and issues
Performing and Recording (30–C3.2s) will conduct investigations into relationships between and among observable variables and use a broad range of tools and techniques to gather and record data and information
• will construct models of DNA to demonstrate the general structure and base arrangement (PR–ST2) [ICT C6–4.4]
• will perform simulations to demonstrate the replication of DNA and the transcription and translation of its information (PR–NS2, PR–NS4)
• will perform simulations to demonstrate the use of restriction enzymes and ligases (PR–NS3, PR–NS4)
Analyzing and Interpreting (30–C3.3s) will analyze data and apply mathematical and conceptual models to develop and assess possible solutions
• will analyze, from published data, relationships between human activities and changes in genetic information that lead to heritable mutations and cancer (AI–NS2)
Communication and Teamwork (30–C3.4s) will work collaboratively in addressing problems and apply the skills and conventions of science in communicating information and ideas and in assessing results