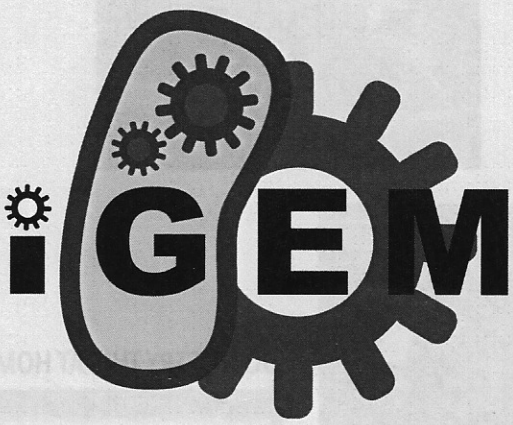
iGEM High School Handbook:

A Primer on Synthetic Biology



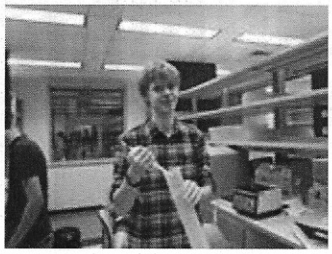
The 2012 University of Alberta
Collegiate iGEM Team

Introduction

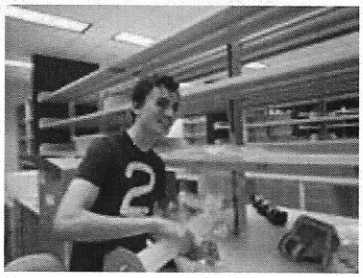
A disruptive technology is one that changes how society functions. Computers allowed us to process and store vast amounts of information. Cell phones allow us to be constantly connected to each other and the world. Genetic engineering has the potential to become the next big technology, but where are we in the field right now?

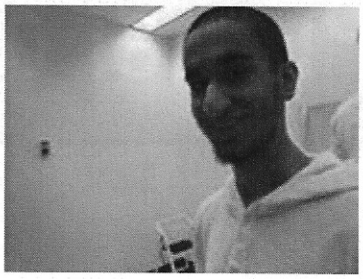
We can read and write DNA at ever greater speeds for lower costs. We can take that DNA, multiply it , and put it into cells. We can see these cells express the genes we've created. We're aiming for great things, altering life at the level of its most basic instructions, and learning how to control those instructions, and use them.

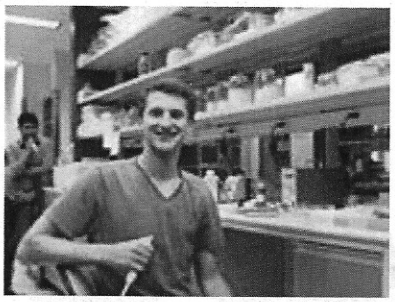
A great deal of current research being done is using *Escherichia coli*, a type of bacteria that has been greatly standardized and takes up new DNA well. This handbook looks at the protocols, concepts, and potential uses of altering DNA and adding it to *E. coli* in a simple, but comprehensive way, to assist high school students in understanding these concepts so that they can better carry them out within a lab.

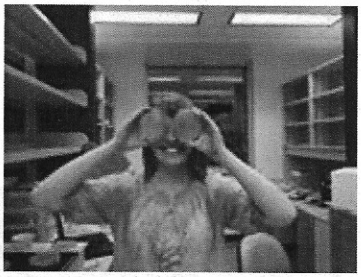


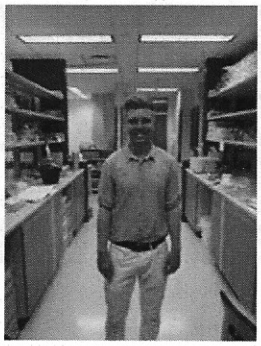
The Team

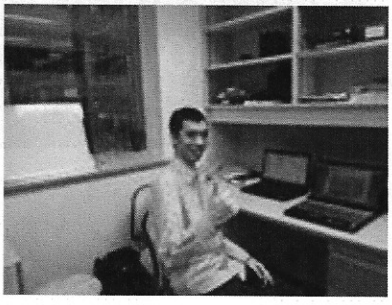


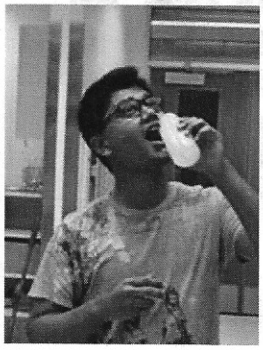












DO NOT TRY THIS AT HOME!

Basic Knowledge

DNA

You are probably super excited to start hacking your bacterium and produce innovative synthetic biology creations, but before we start, there is some background information that is important to know.

The starting point, of course, is at the molecule of heredity, used by all living things on this planet, DNA! Deoxyribonucleic acid (DNA) is made up of a phosphate backbone, a deoxyribose sugar, and nitrogenous bases. The four bases in DNA are adenine, guanine, thymine, and cytosine. These four bases, called nucleotides, are arranged in different formations and create the genetic code. But what secret does this "code" actually hold?

The "code" of DNA takes us to a general concept in molecular biology called the "central dogma of molecular genetics". This, despite it's philosophical-sounding name, is simple: one gene=one mRNA=one protein. This means that for any one gene, there is a single protein produced as a final product. Proteins, or polypeptides, are made up of amino acids. These specific amino acids come from specific arrangement of triplets of nucleotides. For example, methionine, an amino acid, is produced from the combination ATG. There are often different combinations of nucleotides that produce a single amino acid. However, the idea here is that one gene=one protein.

The intermediary step to the production of a protein is the formation of mRNA. For the cell to achieve maximum efficiency, it doesn't make sense to remove the DNA from the nucleus every time a protein needs to be translated. Instead, a copy is produced in the form of messenger RNA. RNA, or ribonucleic acid, is single-stranded rather than double stranded, and so it is able to bind to strands of DNA that have been unzipped and separated. RNA also contains A, C, and G, but instead of thymine (T) a uracil (U) takes its place in code.

For example, the sequence "ATG" in DNA corresponds to "AUG" in the RNA molecule. This molecule then, which contains the complementary sequence of the DNA molecule, carries the genetic information to the ribosome, which identifies the sequence and produces amino acids based on the code relayed by the mRNA.

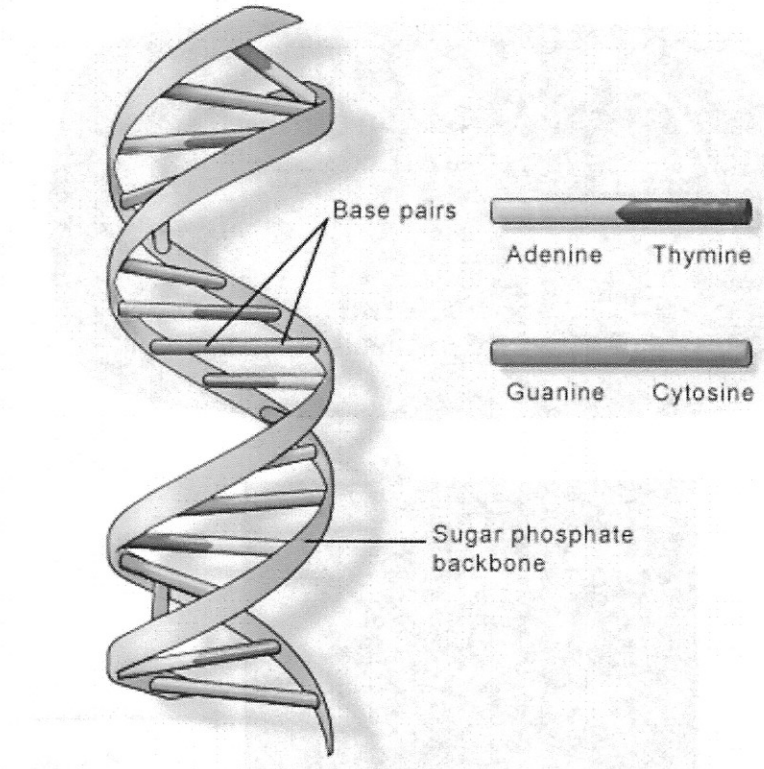
However, when the cell replicates, it can't just mass produce RNA molecules from the first cell, because as the next cell replicates, there will be very little amounts of the messenger RNA left. How does the cell deal with this problem?

The cell copies DNA before replication, producing a full copy of all the DNA in the cell for the daughter cells. In order to do this, several enzymes work to first untangle the DNA complex, after which initiator proteins, which initiate replication, separate the DNA strands and bind to the origin of replication of the

sequence. These origins are usually adenine-thymine rich, which results in them being more easily broken apart, compared to guanine-cytosine rich sequences, which are generally more thermodynamically stable.

After the initiator proteins separate the strands of DNA, an enzyme called primase (nope, it's not an ape relative) binds to the sequence. This enzyme synthesizes a short ribonucleic acid (RNA) primer, or small segment of DNA. This segment, or primer, allows another enzyme, known as DNA polymerase, to bind to the primer and complete the sequence. We call this semi-conservative replication; replication occurs using an old strand of DNA and produces another strand of DNA complementary to that. So, the DNA molecule that has been replicated actually contains one strand that is from the "template".

DNA is awesome! There are so many cool steps that your cells undergo to modify DNA, replicate it, and use it as a code to produce proteins. In your body, your entire sequence of DNA makes up your genome. We call this DNA genomic DNA. This genomic DNA contains the biological instructions that make your cells "yours".



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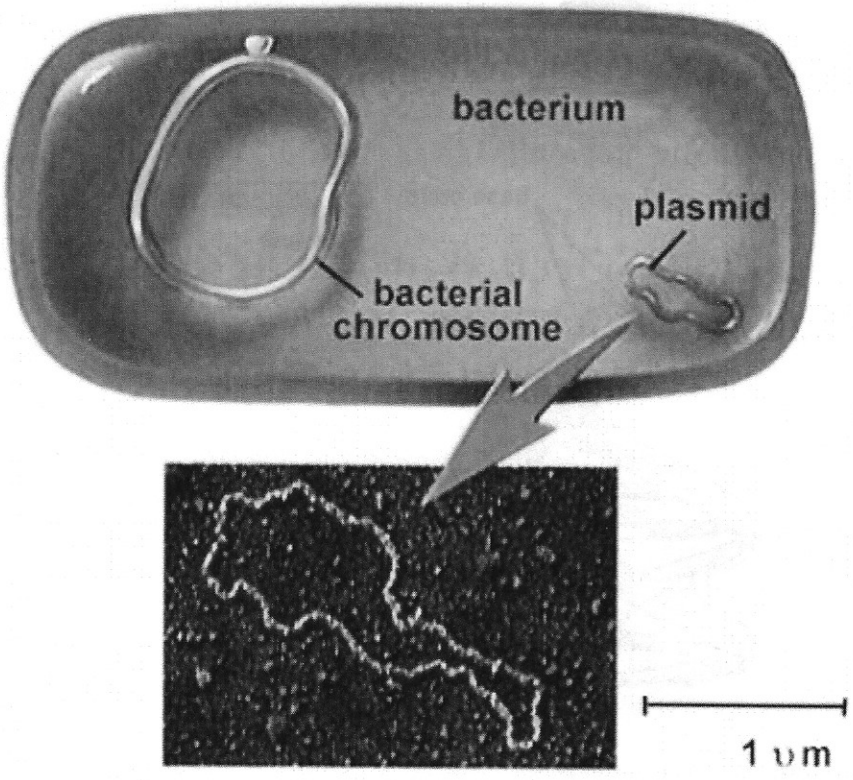
Plasmids

Bacteria, which are the organisms we normally use in synthetic biology, also have genomic DNA. However, they also have what are called "plasmids"; which are basically circular pieces of DNA that are able to replicate independently of the genomic DNA. Thus, the plasmid DNA is an easier piece of genetic information for us to make modifications to, as we don't have to replicate all of the cell's DNA in order to produce our gene of interest in high amounts.

Plasmids are much easier to manipulate, take in, and out of cells than genomic DNA. Genomic DNA resides in the nucleus, whereas plasmids are much easier to access in the cytoplasm. *E. coli* does not have a nucleus, so much of the genetic information is encoded onto plasmids. The rest is contained in genomic DNA floating around in the cytoplasm.



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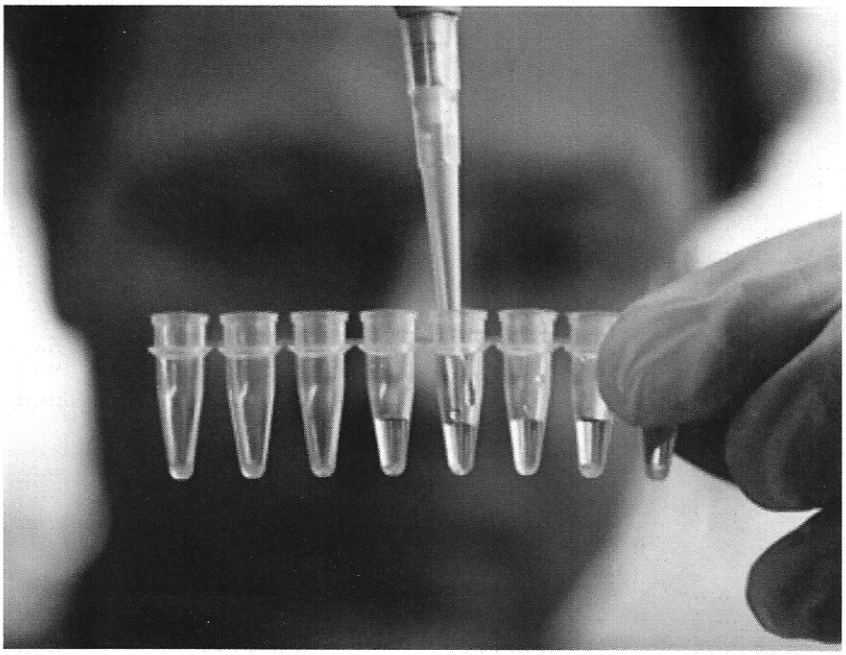
Tools of Molecular Biologists

As synthetic biologists, we try to look at biology from an engineering perspective. In order to engineer something, you need to have a set of tools. Take construction. You need to have a saw, a drill, a measuring tape, and of course, some way to obtain lots and lots of wood. In biology, we need to have methods to cut DNA (restriction enzymatic digest), glue it back together (ligation reaction), measure it (gel electrophoresis), make copies of it (polymerase chain reaction, put it into cells (transformation), and extract it out of cells (miniprep)! These are the basic tools that you, as a high school student, can use in order to engineer simple biological systems. All of the methods described above will be covered in this handbook.

Now that you have a better understanding of how DNA works, and how bacteria are easily engineerable, let's take a look at some methods for how to engineer plasmids into bacteria. What you do with these tools is up to you. Whether you work on a biological oscillator, memory storage, cancer-killing bacteria, or glowing cheese, remember that with great power comes great responsibility. Just because we can do something it does not necessarily mean we should. Always consult with a professor or advisor before carrying out an experiment, to ensure that unintended dangerous consequences don't occur.

With that note, let's get started!



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Procedures

Overnight Cultures

You can make an overnight culture in order to grow a single colony of desired cells into several hundred million cells. These cells will all contain the identical DNA as from the colony that was chosen, giving you many copies of the desired cell to work with. These cells can then be put to a wide number of purposes, including re-plating or minipreps (see later).

What you need:

- 5 mL sterile LB broth, which provides nutrients for the cells
- Autoclaved 20 mL culture tube
- 1 colony of desired cells
- Antibiotics, if necessary; these inhibit the growth of contaminant cells, allowing only the desired, antibiotic-resistant cells to grow
    - Kanamycin
    - Chloramphenicol
    - Ampicillin
    - Tetracycline
- Incubator Shaker

Procedure:

1. Add 5 mL of sterile LB medium to autoclaved 20 mL culture tube
2. Mix one or more of the following antibiotic concentrations, if needed (it should be noted that these values may vary from lab to lab, depending on what their use is)

    - Kanamycin- stock solution=50 mg/mL (use 1 µL/mL)
    - Chloramphenicol- stock solution=42.5 mg/mL (use 0.6 µL/mL)
    - Ampicillin- stock solution=100mg/mL (use 1 µL/mL)
    - Tetracycline- stock solution=12.5mg/mL (use 1 µL/mL)

3. Pick a colony with a sterile pipette tip and eject tip into culture tube, being careful not to contaminate the culture
4. Incubate in Incubator Shaker at 37°C for a minimum of 12 hours

Plasmid Purification (Miniprep)

A miniprep is used to take plasmid DNA out of your cell. This is done by breaking the cell membrane, and separating out everything except for the plasmid DNA. Once you have separated these plasmids, you can do a variety of things with them, such as modification or sequencing.

What you need:

- Your selected plasmid
- 2mL microfuge tube
- Microcentrifuge
- The following buffers:
  - P1, which is used to resuspend plasmid DNA, and remove RNA.
  - P2, which is used to break the cell membrane
  - N3, which is used to destroy lysozymes, which break down the cell membrane
  - PB, binds DNA to the silica of the spin column
  - PE, used to wash out anything that is not plasmid DNA
  - TE (tris-EDTA, for long-term storage of plasmid DNA) OR EB (elution buffer, for immediate use of plasmid DNA); both used to unstick and resuspend plasmid DNA
- Spin Column (contains a silica which attracts the plasmid) and its corresponding column bottom
- 1.5 mL microfuge tube
- Vacuum manifold
- Spectrophotometer, used to find the mass of DNA per volume at the end of a miniprep

Procedure:

(The following protocol is taken from the instructions provided by Qiagen's QIAprep Spin Miniprep Kit. We changed the rpm of centrifuge from 13,000 to 14,000, and used a vacuum manifold in the place of a centrifuge for certain steps.)

2. Prepare overnight cultures of *E. coli* in 5 mL LB medium (optional: chemical selection)
3. Transfer culture to 2 mL microfuge tube
4. Spin for 1 minute at 14,000 rpm at room temperature, and then pour out the liquid (composed of LB media) on top. Be sure to keep the solid pellet of cells at the bottom of the tube.
5. Repeat steps 2 and 3 again, if needed
6. Resuspend bacterial pellet in 250 µL buffer P1 (which contains RNase A)
7. Add 250 µL P2 buffer and invert 4-6 times
8. Add 350 µL N3 buffer and invert 4-6 times immediately

9. Centrifuge for 10 minutes
10. Pipette supernatant into spin column attached to a manifold vacuum
11. Turn on vacuum
12. Wash spin column with 500 µL of PB buffer
13. After PB buffer is removed, wash spin column with 750 µL of PE buffer
14. Remove spin column from vacuum after buffer is fully removed, and attach it to column bottom
15. Centrifuge for 1 minute to remove residual buffer
16. Discard column bottom, and attach spin column to 1.5 mL Eppendorf tube
17. Add 50 µL of EB/TE or any low salt buffer to center of spin column to elute DNA
18. After resting for 1 minute, centrifuge for 1 minute
19. Use a spectrophotometer to measure purity and concentration for storage and for further experiments

Making LB Plates

LB agar plates are a nutrient-rich medium on which cell colonies are grown. It is a gel-like, yellow substance that turns liquid when heated. This property allows us to pour it into petri dishes, let it resolidify, and then have a thin layer for cells to grow on.

PART 1: Making LB Agar

What you need:

- Erlenmeyer Flask
- Stir bar
- Stir plate
- 4x 500mL Autoclavable bottles
- 10g Tryptone
- 5g Yeast Extract
- 10g NaCl
- 15g Agar powder
- 1L deionized water

Making LB Agar:

1. Combine 10g Tryptone, 5g yeast extract, and 10g NaCl in an Erlenmeyer flask, then add 1L deionized water
2. Center stir bar in flask, then place flask on stir plate
3. Mix contents on high speed and medium heat
4. Wait ~10 minutes until mixture is fully dissolved
5. Add 3.75 g agar into each of four 500 mL autoclave-safe bottles, and pour 250 mL of the LB mixture into each bottle
6. Autoclave bottles, and ensure that the bottle caps are screwed just loose enough to lift the bottle by grabbing the cap. This will prevent the bottles from exploding due to internal pressure, and will also prevent the formation of a vacuum

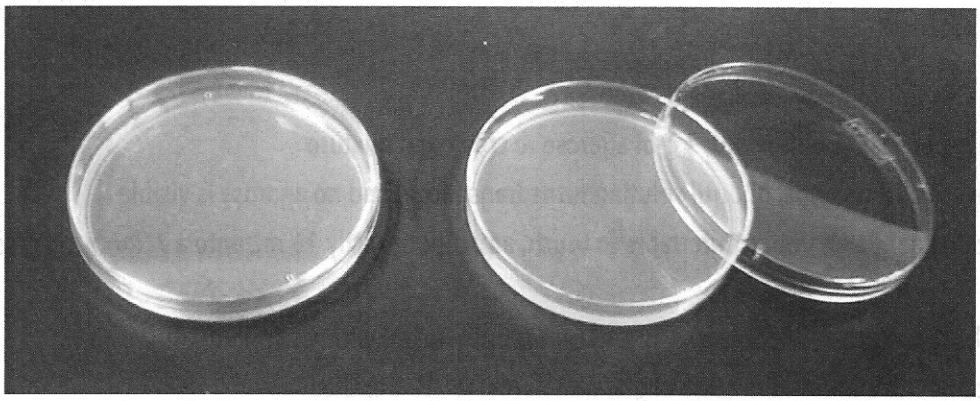
PART 2: Making LB Agar Plates

What you need:

- 25 mL of liquefied LB agar
- Petri dish
- Antibiotics, if necessary; these inhibit the growth of contaminant cells, allowing only the desired, antibiotic-resistant cells to grow
  - Kanamycin
  - Chloramphenicol
  - Ampicillin
  - Tetracycline
- Selected cells in cell broth

How to pour an LB agar plate:

1. Pour 25 mL of molten agar onto a sterile petri dish
2. Antibiotics and other chemicals may be mixed in at this stage
   a. Kanamycin= 1 μL/mL
   b. Chloramphenicol= 0.6 μL/mL
   c. Ampicillin= 1 μL/mL
   d. Tetracycline= 1 μL/mL
3. Let petri dishes cool down until agar solidifies, then it can be used immediately, or stored in fridge
4. If stored, let agar warm to room temperature before plating, so bacteria are not shocked by rapid temperature decrease
5. Streak cells onto plate or use ball bearings to make an even lawn



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Gel Electrophoresis

DNA, being negatively charged, will move through the agarose gel in the TAE buffer, to the positively charged side. The agarose creates a matrix for the DNA to move through. Long DNA moves slowly through the matrix, and shorter strands move quicker, meaning that DNA fragments of the similar size will form bands. The gel is stained in ethidium bromide, a molecule that fluoresces orange in a hydrophobic environment, allowing the DNA bands to appear under UV light. A 1-kilobase ladder is used along with DNA fragment samples in order to provide a relative size scale.

Gel Electrophoresis is done after PCR, ligation, and digestion in order observe the size of the resulting DNA fragments, and therefore often reports if your experiment has been performed correctly. For example, if you have done a ligation of 200 base pair fragments and 300 base pair fragments, you would expect a yield of 500 base pair fragments. Gel electrophoresis can be used to observe whether or not this is the case.

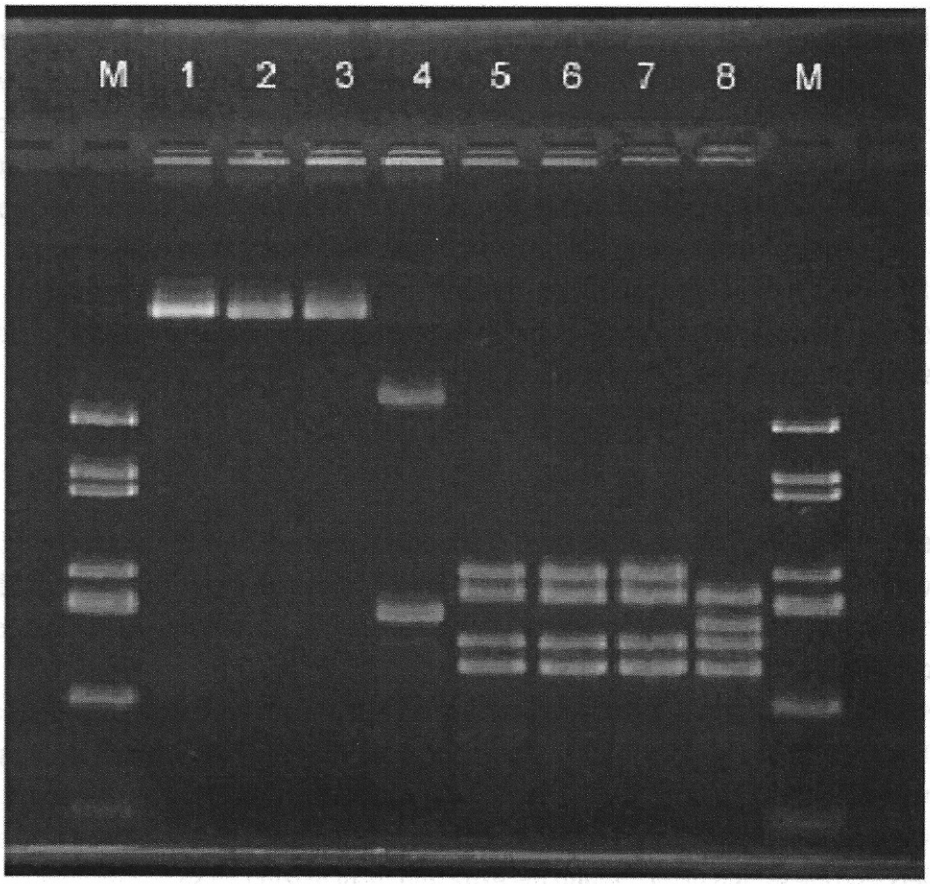
What you need:

- A 7.7x6.5 cm glass plate
- One comb
- Two comb supports
- 1x TAE buffer
- Agarose
- Microwave
- Pipette
- Ethidium Bromide
- DNA
- DNA Ladder (standard pieces of DNA that provide a comparison scale for DNA fragment size)
- Loading Dye (provides colour for referencing distance DNA has travelled)

Procedure:

1. Mix 100 mL of 1X TAE buffer with 1 g of agarose to create gel mixture
2. Microwave for 45 seconds, or until solution turns transparent and no agarose is visible
3. Let solution cool down until comfortable to touch, and pipet exactly 16 mL onto a 7.7x6.5 cm glass plate
4. Ensure that gel mixture spreads evenly and covers entire plate without spilling off
5. Place a comb 1.5 cm from the edge of glass, then rest for 10 minutes
6. Squirt few drops of milliQ water along the interface of comb and gel, then gently remove comb

7. MilliQ water will prevent vacuum from distorting the lanes, and allows easy removal of comb
8. Place solidified gel plate in gel apparatus, and fill with enough 1X TAE to fully submerge gel
9. Create loading solutions of DNA and loading dye, then insert into gel lanes alongside the DNA ladder
10. Run gel using 150 V, then turn off machine when DNA bands reach 2 cm from end (~20 minutes)
11. Make an ethidium bromide solution with 2.5 μL ethidium bromide and 50 mL 1X TAE
12. Remove the gel from the plate, and transfer only gel to ethidium bromide solution
13. After soaking gel for 10 minutes, view banding patterns using UV machine



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Transformation

So you've learned how DNA encodes all of your cellular functions, by translation into proteins. You learned specifically about bacterial DNA, and those incredibly useful circular pieces of DNA that are not part of the main chromosome of bacteria, PLASMIDS! You learned about how plasmids replicate independently of the main chromosome, and how they are able to move between cells through conjugation. Through this, you understood why plasmids are much easier to engineer compared to direct engineering of the chromosome, due to the fact that regulatory parts can be assembled fairly easily on one plasmid and many copies of a specific gene can be replicated.

When we talk about using plasmids for engineering, it is important to understand that with conventional tools in synthetic biology, this plasmid DNA has to be modified *in vitro*, or "in a test tube". After we modify our plasmid, we have to have some way of putting that modified DNA back into the cell. This is so that we can see how our modified gene affects the traits, or phenotype, of the bacteria. Perhaps we are adding an antibiotic resistance gene to our plasmid so that it can survive in the presence of a certain antibiotic. Perhaps you are working on engineering a biosensor that can detect and remove environmental toxins. The method for adding plasmids to a specific cell through chemical or electrical means is called transformation. This is a really simple protocol, but its wide usage makes it an essential for you to know how to do, as someone new to the field of synthetic biology.

What you need:

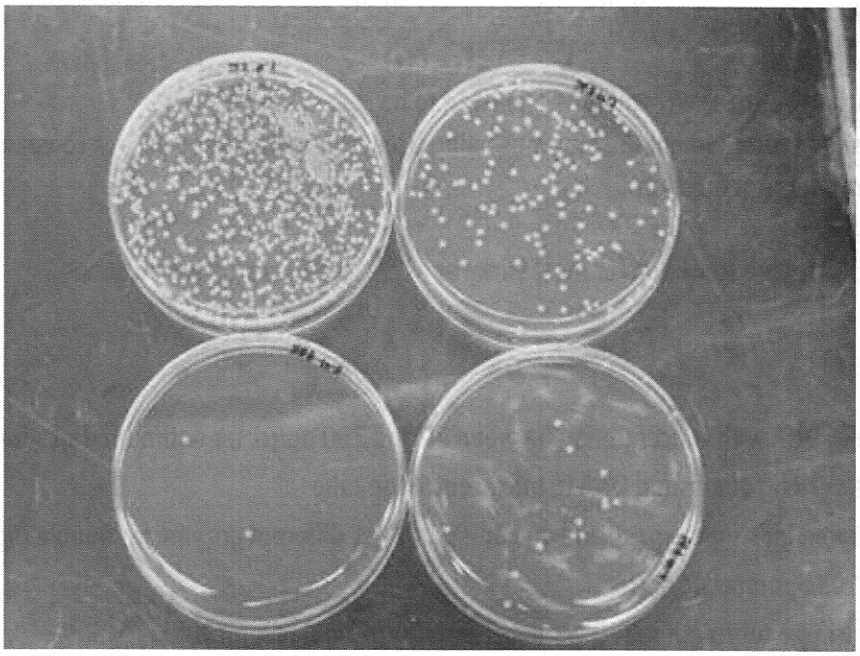
1. Modified plasmid DNA: This should be clean of any enzymes (you'll learn the protocol for cleanup later) and shouldn't be kept outside of the fridge for too long. You want to measure how much DNA you have in your solution by using a nifty tool called a spectrophotometer. The amount of DNA that you add to your cells should never exceed 10%.
2. Competent cells: These are cells that have been treated with various buffers and chemicals and then flash frozen in liquid nitrogen in order to make them permeable, or easily penetrable, to the plasmid DNA. When you are working with E.coli, these cells have to be kept frozen in a -80°C freezer. We aren't going to go into the details of how to make these competent cells, but we hope that you have some hard-working senior members who are willing to go through the extra trouble of making these.
3. Ice bucket: This is very important in ensuring that the competent cells do not lose their permeability. It is also important when performing heat shocking of the cells.
4. Water bath: This is used for the heat shocking of the cells, allowing the uptake of DNA. This should be set to 42°C.
5. A 37°C incubator: This is essential for growing up the cells that you are adding the plasmid DNA to.
6. Plates containing specific antibiotic: When building your plasmid, you always want to include some kind of selection marker, or a gene that will allow you to know whether or not your cells have retained the

plasmid. In order to ensure that those cells are selected for, you need to have agar plates containing the appropriate antibiotic.

7. Pipette
8. Luria-Bertani (LB) Broth: This is the broth in which your newly transformed cells recover in.
9. Centrifuge (optional): You can use this to concentrate your newly transformed cells before adding them to the antibiotic plate (plating a lawn).

Procedure:

1. Remove competent cells from -80°C freezer, and immediately thaw on ice
2. Wait until cells fully thaw (~10 minutes) before adding DNA; do not add more than 10% of cell volume
3. Rest on ice for 30 minutes
4. Put into 42°C water bath for exactly 90 seconds, then immediately transfer to ice for 2 minutes
5. Add 1 mL of LB broth to microcentrifuge tube, and incubate for 1 hour at 37°C
6. Plate 200 µL of culture on selective plates, such as chloramphenicol, kanamycin, or any combination with appropriate concentrations (see LB Agar Plates for our concentration)



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Digestion and Ligation

Digestion and ligation are useful tools in the manipulation of plasmids and DNA. It occurs as a two-step process. First, the section of DNA must be cut at a particular point. To do this, we use restriction enzymes. Each restriction enzyme has a particular sequence of around 4-8 base pairs, which it will recognize and attach to. Once attached to the strand of DNA, the enzyme will proceed to cut its section in in a specific way, leaving two "sticky" ends. These ends can be reattached to any other sticky end with a matching DNA sequence.

The second step in the process is ligation. This is where two ends of DNA are "glued" back together to create one continuous piece of DNA. Ligase works in a similar way to restriction enzymes, except that it will not recognize specific sites on the DNA. Instead, ligase will glue back together any matching sticky ends that it can find. In this way, molecular biologists can cut out and put in pieces of DNA as they choose. Digestion and ligation are two essential tools in a molecular biologist's toolkit.

What you need:

- Plasmid DNA
- Microcentrifuge tube
- Enzymes (chosen for the DNA segments that need to be cut)
- Buffers (chosen to complement the enzymes being used, the buffers keep pH at a constant range where the enzymes can function
- 37°C incubator
- T4 DNA Ligase enzyme (common for most sticky ends created by restriction enzymes

Procedure:

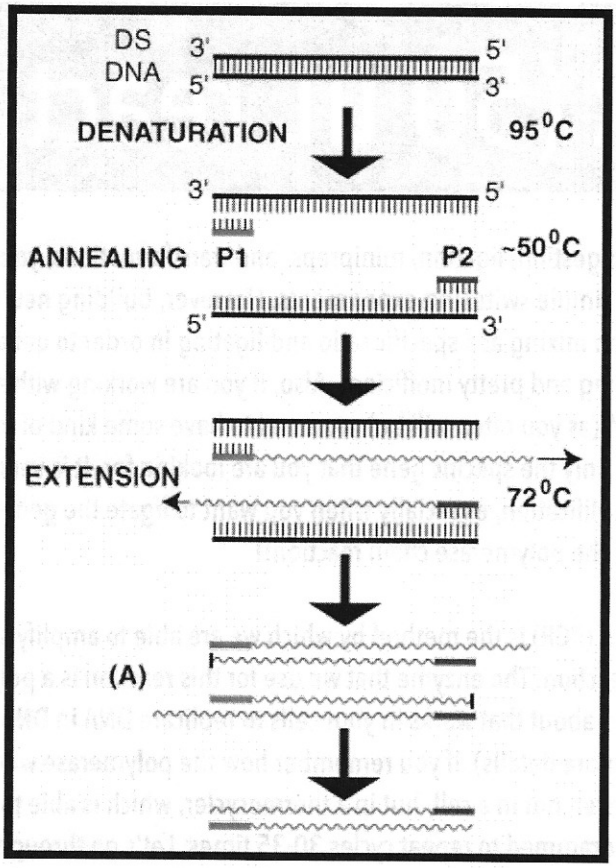
1. Create plasmid DNA with concentration between 20 and 200 ng/µL for optimum digestion
2. Transfer 200 ± 10 ng of plasmid DNA to microcentrifuge tube
3. Add 1 µL enzyme for single digest, and an additional 1 µL different enzyme for double digest
4. Add 2 µL of 10X appropriate buffer
5. Fill microcentrifuge tube with milliQ water to reach 20 µL final volume
6. Incubate for 1 hour at 37°C, or longer if appropriate buffer cannot be used
7. Refer PCR/Digestion/Ligation Clean-up for purifying digested DNA in preparation of ligation
8. Add 3 µL T4 DNA Ligase 10X buffer to 30 µL eluted DNA, then add 1 µL T4 DNA Ligase
9. Keep mixture at room temperature for 1 hour
10. If transforming, use 10 µL of ligated DNA for transformation
11. Refer PCR/Digestion/Ligation Clean-up if further experiments will be performed

PCR (50 uL reaction)

Now that you can do simple digestion, ligation, minipreps, and transformations, you have the tools to work with *E.coli* and perform simple switching experiments. However, building new plasmids with this method (cutting the DNA, then mixing at a specific ratio and ligating in order to get a higher concentration of what you want) is painstaking and pretty inefficient. Also, if you are working with a very low concentration of plasmid DNA (as you often will be) you need to have some kind of tool to create a large number of copies, or amplify only the specific gene that you are looking for. It is very important that you are able to do this kind of amplification, especially when you want to ligate the gene into a plasmid with an increased efficiency. Enter the polymerase chain reaction!!

The polymerase chain reaction (PCR) is the method by which we are able to amplify specific sections of DNA through an enzymatic reaction. The enzyme that we use for this reaction is a polymerase, similar to the polymerase that we talked about that works in your cells to replicate DNA in DNA replication (see Background Information for more details). If you remember how the polymerase worked, PCR is very similar. It is performed, however, not in a cell, but in a thermocycler, which is able to change to different temperatures and can be programmed to repeat cycles 30-35 times. Let's go through the different steps of how PCR works first.

First, the DNA is denatured by heat at a high temperature. After that, the temperature is lowered to allow the primers to bind. If you remember DNA replication (see Background Information) you will recall that short sequences of RNA bind to the separated DNA and allow for mRNA transcription. In PCR, however, this short sequence (called a primer) made of chemically synthesized single-stranded DNA, binds specifically to a certain region of DNA, and has a specific melting point based on its guanine-thymine content. These primers are essential to the process, as they must bind specifically to the separated strands of DNA at specific points in the sequence. After this, the primers are extended with the dNTPs, and two molecules of DNA are produced, but only of the specific region of interest. This process is then repeated again and again, with the number of DNA molecules doubling each time. Here is a diagram that will simplify the process:



http://www.flmnh.ufl.edu/cowries/PCR.gif

What you need:

- Template DNA containing the region of interest. This is just normal plasmid DNA.
- DNA polymerase (Taq, Pfu, and Phusion are some commonly used polymerases): This is a high efficiency polymerase that can withstand the high temperatures reached in this reaction.
- dNTPs: These are the nucleotides added to the strand of DNA by the polymerase.
- Buffer: This maintains optimal reaction conditions in terms of pH, and also contains magnesium chloride, $MgCl_2$.
- Primers: There must be one primer that binds to the 5' strand, and one that binds to the initial 3' strand. The polymerase always works in the 5' to 3' direction, so you have to have a "+" primer and a "-" primer.
- A thermocycler: The thermocycler is the main apparatus in this experiment. It is used to change the temperature of the reaction in order to amplify the DNA.
- A PCR program: This program should be fairly simple to design, depending on the interface of the thermocycler used. Your program has to be specifically tailored to your primers and gene fragment.

Here are the steps of a PCR program:

Step 1: 95 C for 2 minutes-This is the initial denaturation of the DNA. As well, the Phusion and Taq polymerases require a "hot start" for the enzyme to become active.
Step 2: 95 C for 1 minute- this is the second denaturation of the DNA.
Step 3: 50-60 for 30 seconds- this is the annealing step, when the temperature is lowered so that the primers can bind to their sites in the DNA. It is important that you hit the right temperature; if the temperature is too high, you may hit the melting point of your primers, preventing the primers from binding, and if the temperature is too low, you will get non-specific binding of the primers, and you may get unwanted PCR products at low yields. There are plenty of websites you can use to calculate the annealing temperature for your primer. However, you will probably have to verify these values experimentally or by talking to your supervisor.
Step 4: 72 C for 1 min/kb- this is the extension time, where the polymerase adds the dNTPs to the strands of DNA and extends them down the length of the gene of interest. The time for this step depends upon how long the sequence is. The rule of thumb is 1 minute of extension time for every kilobase of DNA. However, it is usually better to increase the time by a little bit, as [suggestion: delete "as" and make a new sentence] if the time happens to be too little, there will be incomplete formation of the PCR product.
Step 5: Repeat steps 2-5, between 30-35 times in order to properly amplify your sequence of interest.
Step 6: 72 C for 10 minutes: This is done in order to ensure that the product is complete, and the sequences are fully extended.
Step 7: 4.0 C indefinitely- once the sample has cooled down to 4 degrees, you can remove it or let it stay in thermocycler overnight. You should probably check that the correct product has formed using gel electrophoresis.

Here are the specific steps of setting up the reaction:

1. Create a working stock (5 ng/μL) of template DNA, and add 1 μL to PCR tube
2. Add 2.5 μL each of 10 μM forward and reverse primers to PCR tube
3. Add 1 μL of 25 μM dNTPs
4. Add 10 μL of 5X HF Phusion buffer along with 0.5 μL Phusion enzyme
5. Adjust final tube volume to 50 μL with highly purified water
6. Try to minimize bubble formation, as this can hinder Phusion from functioning optimally
7. Set the PCR machine with appropriate denature, annealing, and extension temperatures, as well as appropriate durations and cycles

PCR/Digestion/Ligation Cleanup

In between digestion and ligation, there needs to be an intermediate step called cleanup. This process entails the removal of enzymes still in the mixture. If these enzymes are left in the mixture, they will continue to cut up the newly ligated DNA, and the whole process becomes useless. To prevent this, everything except the plasmid DNA must be filtered out. The cleanup process is similar to the last stages of a miniprep, precipitating the plasmid DNA, and then putting it back into solution. Once the cleanup process is done, ligation can occur unhindered by excess enzymes.

Cleanups also have other applications. Any process which requires enzymes at a certain stage, but which need to be removed later on, uses a cleanup. For example, after a PCR, the product mixture will still contain various enzymes. To use the product elsewhere, the enzymes need to be filtered out. This is done using a cleanup.

What you need:

- Mixture containing plasmid DNA and various unwanted enzymes
- Buffer PB
- Buffer PE
- Vacuum apparatus
- Spin columns with corresponding column bottoms
- 1.5mL microcentrifuge tubes
- Buffer EB or TE
- Spectrophotometer

Procedure:

1. Add PB buffer of volume five times the desired cleanup DNA volume (i.e., a 20 µL digest will be mixed with 100 µL PB buffer)
2. Transfer mixture to spin column attached to a vacuum apparatus, then turn on vacuum
3. Wash with 750 µL PE buffer
4. Remove spin column from vacuum after buffer is fully removed, and attach it to column bottom
5. Centrifuge for 1 minute to remove residual buffer
6. Discard column bottom, and attach spin column to 1.5 mL microcentrifuge tube
7. Add 50 µL of EB/TE or any low salt buffer to center of spin column to elute DNA
8. After resting for 1 minute, centrifuge for 1 minute
9. Use a spectrophotometer to measure purity and concentration for storage and for further experiments

In Practice

Advantages/Disadvantages

Having high school students working in the lab has both its advantages and disadvantages. Below, we list some o f the problems we encountered over the summer, as well as some of the ways where having high school students was helpful.

Advantages:

- Enthusiasm and willingness to learn
- Provide a different perspective on the project
- Prepares students for participation in future iGEM teams
- Provides a route to explore biological research at a young age
- Gives hands-on lab experience that would be otherwise unavailable
- High school students have more spare time
- Introduces high school students to the world of academia
- Reach out to a wider and younger audience among the local community

Disadvantages:

- It takes time for high school students to learn the ways of the lab (this handbook will help lower that time)
- Steeper learning curve
- Supervision required
- Often, students cannot drive themselves or do not live close to campus.
- High school students have class until the end of June, so their contributions are limited before then
- Scheduling difficulties

Ethics

Now you know the steps you need to make *E. coli* work with genetic engineering, and why you carry out each step, so now it's time to take a step back, and ask a bigger 'why?' If you're reading this handbook, you're probably working on a lab, or you're planning on working in a lab, but what project is that lab working on? What's the expected end result of the project? How will that end product benefit the world? What are the dangers in reaching it. Whenever you step into the lab, you must ask yourself 'why am I here?' If you don't know, then find out. There's a reason genetic engineering is a disrupting technology: it has a huge amount of potential. How will you turn that potential into something good, and, more importantly, how will you prevent it from being something bad for the world?

Tips and Tricks

Here are some helpful hints to making your participation in an iGEM team successful:

- **Communication is key**- The more senior members of your team are your most valuable resources
- **Rely on each other**- It is much more difficult to make a mistake when everybody is constantly checking each others' work and providing constructive criticism
- **Don't get discouraged**- Not everything will work the first time for you. Don't fret. This is part of the scientific process. Try to identify any mistakes you made and correct them.
- **There are multiple paths to pass every obstacle**- If one method is not working, don't be afraid to try several others
- **Write down everything**- You never know what you will need to refer back to later
- **Ask for help**- Don't try to do everything yourself
- **Be careful**- There are dangers in every lab. Safety is the number one priority
- **Take initiative**- If you are available, ask somebody what you can do to help. There's always something to do
- **Admit your failures**- Keeping quiet about something can set the whole team back.
- **ASK QUESTIONS!** Never be afraid to clarify something you are sure of.
- **Work hard and have fun**- iGEM is an amazing program to participate in. Do your best and have the experience of a lifetime