SynBio workshop with Prof. Camenares and the Alma iGEM team

Special thanks to Alma iGEM team members Connor Arens and Rhianna Haynes, and others that have assisted in the creation of this workshop. A thanks to form team member Whitney Miller for logo design

Handbook and Protocols

Name: _____

Introduction

Welcome to the Synthetic Biology / iGEM research week workshop! This workshop is designed to give you the student a glimpse into the world of synthetic biology. We hope that this experience helps to both illuminate this subject for you, and inspire you to continue a journey in the field.

This document is a guidebook for your experience this week – feel free to mark it up as needed, but keep in mind that you may be asked to provide a copy of some of your notes here to help keep track of the results of the experiments.

You will also be required to follow the safety procedures that will be outlined by Professor Camenares at the beginning of the week. The biochemistry lab in which we will work is generally a safe place, but it is important to be aware of some dangerous that are present, both to yourself and to the outside world. In general, if you pay attention and use common sense, you will have a great experience!

Several students on the iGEM team will be conducting research alongside you. While they might be working on different aspects of the project, they are experienced in the lab and are here to help you when available.

One of the great aspects of synthetic biology is that it is designed to be as welcoming to the uninitiated as possible – an advanced degree is not a requirement to begin to create your own genetic engineering designs! However, some basic background is useful. This document will help provide this background, and also gives you step by step guide to some of the protocols you will use. There are variations on these available, and changes may be made if necessary (i.e. depending on experimental results).

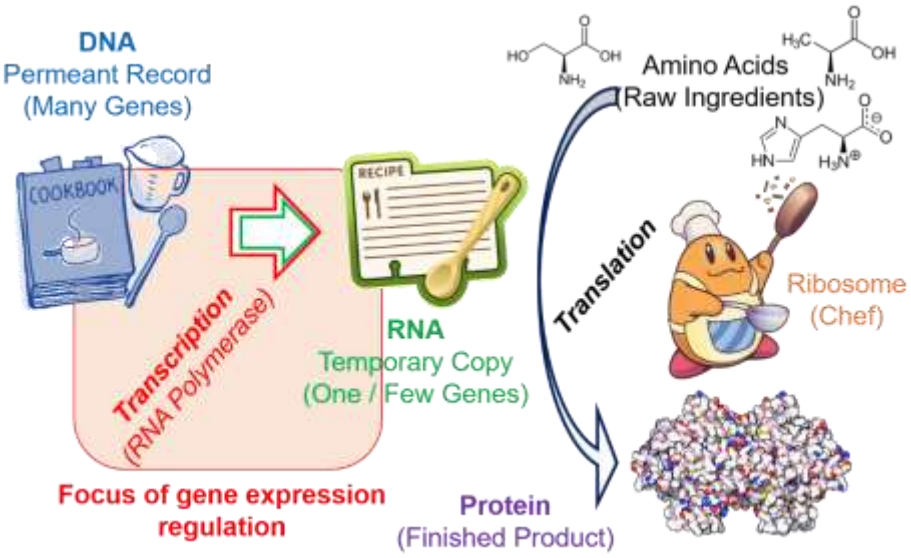
Central Dogma of Molecular Biology

In this workshop, you will be attempting to change the DNA of an organism. DNA is the heritable genetic material for every living organism on the planet. But what does DNA actually do?

My favorite way of thinking about how DNA functions, a process collectively known as the central dogma of molecular biology, is to think about a cookbook filled with recipes. The pages of the cookbook themselves are not useful – the paper the words are printed on are not tasty. However, the information that it contains encodes instructions on how to make some delicious dishes! The information can be copied into a form that can be more easily utilized in the kitchen (a recipe card, for example) and then a trained chef can decode the information and use it to assemble ingredients in the correct order to make a functional or useful plate of food. This process involved two steps: transcribing or copying the information, and decoding or translating it.

Much the same thing happens inside a cell – the DNA is first transcribed into a similar molecule known as RNA, but the basic structure of the information is intact. This information is a code that contains instructions on how to make another type of molecule: a protein. Proteins are build from ‘ingredients’ known as amino acids and come in all different arrangements and shapes, with each carrying out a different function. Proteins are responsible for most of the functions and behaviors in living things – it is proteins in your muscles that allow you to move, proteins in your neurons that help conduct salt ions while you are reading this. There are proteins such as insulin that act as metabolic signals in your body, while other proteins are responsible for actually carrying out the chemical reactions

that keep you alive. Even Keratin, the substance that forms your hair, nails, and components of your skin, are proteins!



When a particular cell or part of your body needs a particular protein, it opens up its cookbook – the genomic DNA – and transcribes a gene into RNA. This RNA is then translated by the ribosome – a complex of RNA and protein itself, the ribosome is like a molecular chef, and is the site where the protein that is encoded for in the gene being accessed is built.

Synthetic Biology and the International Genetically Engineered Machine competition (iGEM)

Each year, teams of students compete in iGEM – they do so by designing and developing synthetic biology solutions to real world problems. In this context, machine does not refer to a piece of lab equipment. In the new field of synthetic biology, living systems are reprogrammed to act as machines; to reliably produce a useful behavior, designed to address some need that conventional technology cannot.

BioBricks

The Synthetic Biology and iGEM community creates, characterizes, uses and shares a set of standardized biological parts known as BioBricks. Each BioBrick could be an individual component of a genetic circuit, like a protein coding region, a promoter, ribosome binding site, or other DNA sequence. Some BioBricks in the registry are composite parts, consisting of one or more of these strung together. Ideally, each BioBrick should be well characterized, so that synthetic biologists can use them in a predictable fashion for creating new behaviors in the cell. For promoters, this characterization might include how much transcription it directs, and under what conditions. For enzymes, it is important to know the characteristic parameters of kinetics such as V_{max}, K_m, and K_{cat}, as well as stability in the cell. All of these variables can influence how useful a particular BioBrick is in a specific genetic circuit.

The registry of standard biological parts hosts a database website, with pages devoted to each BioBrick in the collection. These pages will present the sequence and any user submitted information and data on the part.

BioBricks Featured in this workshop

The iGEM registry contains over 20,000 BioBricks; the distribution kit, available in the lab, contains several thousand. However, the activities you will carry out in this workshop will feature just four BioBricks – chosen because they give an easily detectable visual output. The same approach you use to assemble these BioBricks together can be used for any in the collection, and can (theoretically) be used iteratively, so that you can create complex genetic circuits where the only limit is your own imagination!

The BioBricks below are available in the distribution kit and were chosen by the iGEM team students. They helped to transform these parts into bacteria and isolate the plasmid DNA in a form that can be used in PCR. Each part described below has a page on the registry that can be accessed using the ID (the "BBa_" name).

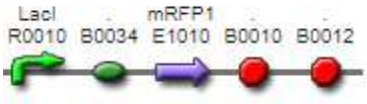
Each description below contains a diagram which highlights the subparts that form the BioBrick; the green arrows represent promoter sequences. Together with the green circles, which are ribosome binding sites, these act as the switch or signal necessary for producing the protein, which is shown as a purple arrow or other symbol. Some, but not all, of the parts contain red stop signals that prevent the promoter in the part from activating downstream or connected parts.

BBa_J364001: GFP

Constitutive promoter + GFP generator (Plate 1, Well 13C)

Produces a green fluorescent protein

BBa_J04450: mRFP



RFP coding device (Plate 6, Well 4A)

Produces a red fluorescent protein

BBa_K1073026: cjBlue

Constitutively expressed chromoprotein cjBlue (Plate 6, Well 13D)

Produces a green-blue protein

BBa_K1033903: tsPurple

Purple chromoprotein incl RBS, J23110 (Plate 6, Well 5P)

Produces a bright purple protein

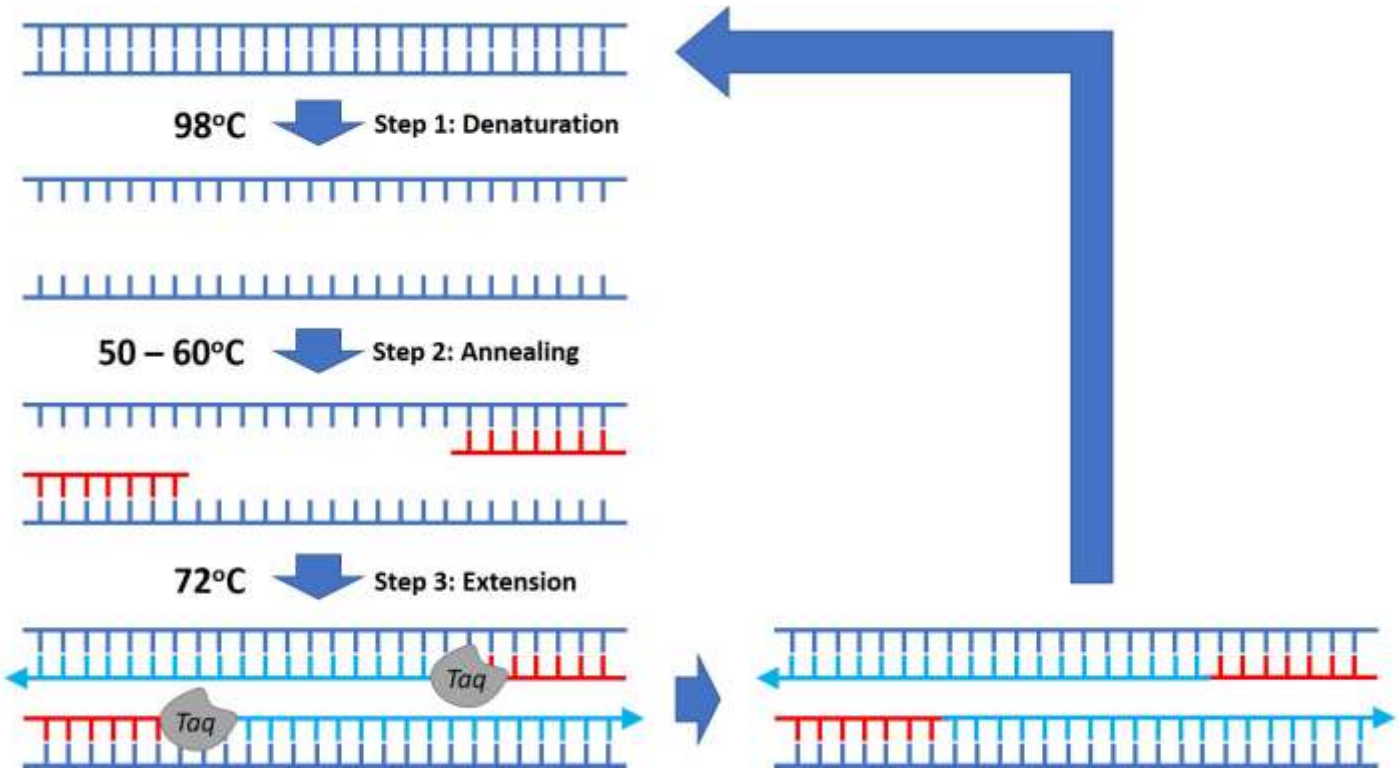
Figure shows bacteria in tubes containing, clockwise from the left, RFP, GFP, cjBlue, tsPurple, and a 'regular' *E. coli* strain.

Polymerase Chain Reaction (PCR)

There is no more utilized, useful, and adapted technique in molecular biology and life science research than PCR – and it plays a powerful role in synthetic biology as well. At its most basic level, PCR is used to make many copies of a specific region of DNA, sometimes in ways that incorporates changes to that DNA sequence.

PCR can be used in a diagnostic or preparative way. It is often responsible for DNA fingerprinting in forensics, even when only minuscule amounts of DNA are present in the crime scene. You will use it to prepare BioBricks for assembly into a larger, multi-part piece.

PCR works by carrying out many cycles of three basic steps: denaturation at 95C, primer or oligo annealing/binding at a temperature of 55-65C, and extension of the primers at 72C. The reaction must contain the primers, and these must be designed to bind DNA at specific sequences (**by binding complementary bases –A binds T, G binds C**). A heat-stable polymerase is responsible for the extension, and most reactions contain nucleotides, buffers and salts necessary for this enzyme. The diagram below illustrates this process:



Each PCR cycle, the region flanked by the primers is doubled (if the reaction is 100% efficient). Thus, after the 1st cycle, there is twice as much, after 2 cycles, 4X, after 3 cycles, 8X, and so on. After about 30 cycles, you can generate over a billion molecules of DNA from a single copy of the template!

The trickiest part of PCR is proper primer design and annealing temperature selection. You must achieve a temperature in which your primer will bind the target region, but not participate in any other binding!

In this workshop, you will be using sets of primers that have been designed and tested in some preliminary studies. They bind the targets, but also contain overhanging sequences on the 5' end that match one of the other fragments. This will enable you to create different color combinations:

Primer Combinations

The following describes mixes of different primers and their sequences. The primer pairs are named for the overhang they contain – the capital letter indicates which BioBrick is an appropriate template.

Primer Mix Name	Forward	Reverse	Template BioBrick
vRx	Rw21_V-R_F	Rw21_R_R	Red
vGx	Rw21_V-G_F	Rw21_G_R	Green
vBx	Rw21_V-B_F	Rw21_B_R	Blue
vPx	Rw21_V-P_F	Rw21_P_R	Purple
gRv	Rw21_G-R_F	Rw21_V-R_R	Red
bRv	Rw21_B-R_F	Rw21_V-R_R	Red
pRv	Rw21_P-R_F	Rw21_V-R_R	Red
rGv	Rw21_R-G_F	Rw21_V-G_R	Green
bGv	Rw21_B-G_F	Rw21_V-G_R	Green
pGv	Rw21_P-G_F	Rw21_V-G_R	Green
rBv	Rw21_R-B_F	Rw21_V-B_R	Blue
gBv	Rw21_G-B_F	Rw21_V-B_R	Blue
pBv	Rw21_P-B_F	Rw21_V-B_R	Blue
rPv	Rw21_R-P_F	Rw21_V-P_R	Purple
gPv	Rw21_G-P_F	Rw21_V-P_R	Purple
bPv	Rw21_B-P_F	Rw21_V-P_R	Purple

Example Combinations

In exercise 1-B (pg 12), you will setup a few different PCR reactions, 2 of which you will choose the BioBricks and primers to mix. In the table below, some possible combinations are indicated.

End Result (Fusion)	Part 1	Mix-A	Part 2	Mix-B
Red-Green	Red	vRx	Green	rGv
Red-Blue	Red	vRx	Blue	rBv
Red-Purple	Red	vRx	Purple	rPv
Green-Red	Green	vGx	Red	gRv
Green-Blue	Green	vGx	Blue	gBv
Green-Purple	Green	vGx	Purple	gPv
Blue-Red	Blue	vBx	Red	bRv
Blue-Green	Blue	vBx	Green	bGv
Blue-Purple	Blue	vBx	Purple	bPv
Purple-Red	Purple	vPx	Red	pRv
Purple-Green	Purple	vPx	Green	pGv
Purple-Blue	Purple	vPx	Blue	pBv

Primer Sequences

For reference, here are the individual primer sequences (annealing sequences in bold):

rw21_V-B_F gaattcgcggccgcttctag**ttgacggctagctcagtcct**

rw21_V-R_F gaattcgcggccgcttctag**caatacgcaaaccgcctctc**

rw21_V-P_F gaattcgcggccgcttctag**tttacggctagctcag**

rw21_V-G_F gaattcgcggccgcttctag**tttacagctagctcagt**

rw21_R-G_F gcctttctgcgtttata**tttacagctagctcagt**

rw21_B-G_F ttggtcataattaataa**tttacagctagctcagt**

rw21_P-G_F aaaaagcgacgtaataa**tttacagctagctcagt**

rw21_G-R_F gcctttctgcgtttata**caatacgcaaaccgcctctc**

rw21_B-R_F ttggtcataattaataa**caatacgcaaaccgcctct**c

rw21_P-R_F aaaaagcgacgtaataa**caatacgcaaaccgcctctc**

rw21_G-B_F gcctttctgcgtttata**ttgacggctagctcagtcct**

rw21_R-B_F gcctttctgcgtttata**ttgacggctagctcagtcct**

rw21_P-B_F aaaaagcgacgtaataa**ttgacggctagctcagtcct**

rw21_G-P_F gcctttctgcgtttata**tttacggctagctcag**

rw21_B-P_F ttggtcataattaataa**tttacggctagctcag**

rw21_R-P_F gcctttctgcgtttata**tttacggctagctcag**

rw21_V-B_R ctgcagcggccgctactag**tattattaattatgaccaagtttgctc**

rw21_V-R_R ctgcagcggccgctactag**taccggaaagacgca**

rw21_V-P_R ctgcagcggccgctactag**tagccttttcgctgca**

rw21_V-G_R ctgcagcggccgctactag**tagcctttctgcgtttata**

rw21_B_R **ttattaattatgaccaagtttgctc**

rw21_R_R **ccggaaagacgca**

rw21_P_R **gccttttcgctgca**

rw21_G_R **gcctttctgcgtttata**

Gibson Assembly

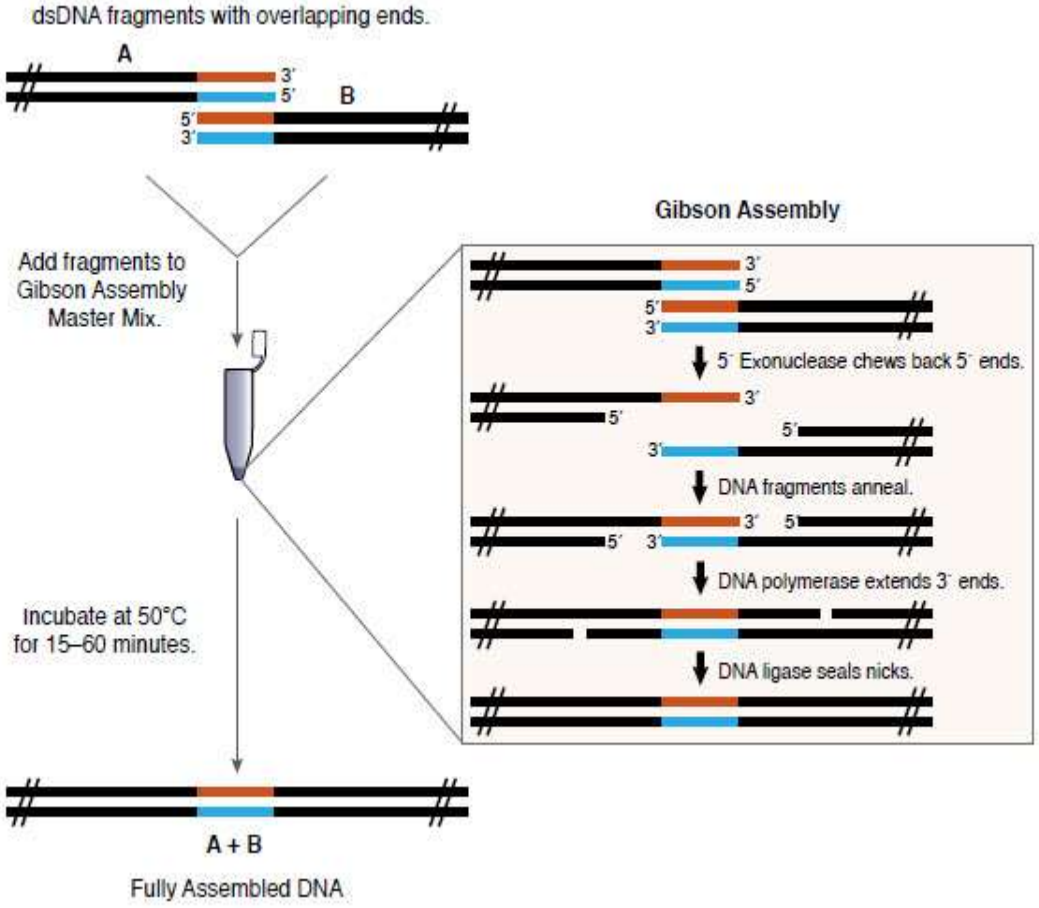
Recombinant DNA is a DNA sequence and molecule created by combing DNA from two or more different sources – it is in essence a new sequence and can have new properties. There are a multitude of strategies that can be used to create recombinant DNA, and most require the DNA from each source to be cut in some way, exposing ends that can be stitched together.

Gibson assembly is a particular way of going about doing this. In Gibson assembly, we mix compatible DNA fragments together with three enzymes: an exonuclease, a DNA polymerase, and a DNA ligase.

First, the exonuclease will 'chew-back' the exposed ends of the DNA fragments, leaving regions of DNA that are single stranded. If the ends of the DNA fragments are compatible – if they are homologous or complementary to each other – they will base pair.

When the ends of two fragments base pair together, they will act as a starting point for DNA polymerase to fill back in the sequence removed by the exonuclease. These two enzymes will be in a game of tug-of-war – one removing sequences, the other filling them back in.

If the DNA polymerase successfully fills back in enough of the sequence, the DNA ligase will step in and seal the fragments together. Since the finished product is a circular DNA molecule, there will be no 'ends' for the exonuclease to remove, and the product will be completed.



Step by Step Protocols

Schedule in Brief, Summary

Day 1: Setup a series Polymerase Chain Reactions to amplify BioBricks of your choice.

Day 2: Run the PCR on DNA gel to analyze the results; Setup Gibson assembly.

Day 3: Transformation of cloning reactions into competent bacteria.

Day 4: Selecting colonies in liquid culture, clean up, discussion with iGEM team

Day 5: Assay for bacteria color, Mini-prep and sequencing prep, preparing presentation

Protocols for Days 1-3 are below; instructions for Day 4 and 5 will be provided as needed, based upon experimental results.

Materials Needed for Each Student:

5uL Fixed Volume Pipette (To be shared), Pipette Tips, and 1mL Syringe Pipette

4uM Primer Mixes and 0.1ng/uL of BioBrick Templates (To be shared) – >30uL each.

PCR Tubes and 0.5mL Tubes

(3x) 10uL Aliquots of Q5

>20uL Aliquot of NEB HiFi Assembly Mix

>15uL of 2X Loading Dye

(5x) 100uL competent cells

>5uL of pACYC control plasmid, 10pg/uL

~5mL of TB, SOC, or SOB media

1mL of dH20

Day 1-A; Practice Pipetting (Optional)

Proper use of the digital pipettors (Pipetmen) is necessary for success in molecular biology labs. This week, you will not need to use the adjustable pipettes – you will used fixed volume pipettes and a syringe. However, the below instructions are valuable and useful if you proceed to work in similar labs or need to use the adjustable pipettes at some point. Although you may have prior experience, please read the following carefully!

1. Arrange the pipets from largest to smallest.

   Note the plunger labels: P-1000, P-200, P-20, and P-10

2. You should have three boxes of disposable tips on your bench.

   Large blue tips match the P-1000, and the tiny tips work on the P-10.

   ***The mid-sized tips fit both the P-200 and P-20, and this frequently causes confusion over which device is actually being used. Be alert!

3. Study the digital window of the P-1000, holding it vertically.

   Notice the top number is RED?! This is followed by two black numbers.

   This color change is to indicate the existence of a decimal point!!!

   So a reading of 073 really means 0.73, and the units are mL.

   Likewise a reading of 100 is *not* 100 µL, but rather 1.00 mL, or 1000 µL!!

   The maximum setting of the P-1000 is for 1000 µL. Never **never** exceed it.

   The minimum setting should be for 200 µL (0.20), because at and below this you should employ the P-200.

4. Now study the window for the P-200. All numbers are black, thus signifying no decimal points between the numbers. A reading of 128 means 128 µL.

   Never never never exceed a setting of 200 µL with this pipet!

   The minimum setting should be for 20 µL, because smaller volumes are best delivered using the P-20 device.

5. Now carefully examine the P-20 window. Notice the colors?!

   A setting of 128 on this device is 12.8 µL (twelve-*point*-eight!!!).

   Never never never exceed a setting of 20 µL with this pipet!

   Consider using the P-10 pipet for volumes less than 10 µL.

6. Always double-check that you are using the P-200 vs. P-20 appropriately.

7. A nice way to pipet 1.2 mL is to set the P-1000 to 0.60 (600 µL), and then pipet twice. If you are careful, a new tip may not be necessary.

8. Now hold a pipettor squarely in your hand, with your thumb on the plunger knob. This is the correct and most secure grippage. Slowly depress the plunger to the first stop, and then press a bit harder to the final stop. The first stop will load the tip with the calibrated volume. The distance from the first to the second stop is to achieve "blow out". Sometimes when delivering, some liquid wicks back into the tip, or does not flow out completely. The "blow out" facilitates complete delivery of the last bit.

9. Slow down! Make sure the tip is secure!!

10. Withdraw fluids from the top of the solution, thus preventing coating the outside of the tip with excess solution.
11. Take notice of where the fluid level arrives within a tip. With practice, this will give a visual check for correct loading.

12. Deliver the fluid deliberately, but not with such force as to cause splashing or spillage. If working with small volumes, pipet into the bottoms of the vials. Watch for complete volume delivery!

You should make an attempt to ensure the pipettes you are using are accurate and precise. You can do this by calibrating them on a balance. Since 1mL of pure water should weigh 1 gram, you can pipette pure water into a dish and measure the weight. To do this, you will need to place a weighing dish on the balance, hit zero or tare to calibrate the weight to zero, and pipette pure water onto the balance. You should repeat this 5x for each volume tested on a pipette. You do not need to clean the weighing dish each time (simply hit zero or tare again), but should change your tip as best practice.

Use the table below to record your results.

Pipette (Circle One): [P10] [P20] [P200] [P1000] **Color Label: _____**

Target Volume	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 4**	**Trial 5**	**Average**

Day 1-B; Setting up PCR reaction

Starting from the plasmid DNA you purified, you will setup three PCR reactions – one for each BioBrick you isolated, and a third from either, which is intended to copy just the backbone region. For these reactions you will need the **Q5 PCR master mix**, the **Primer Mixes**, and **Template DNA**.

You are able to choose from the different combinations of BioBricks and primer mixes that are listed on page 7. For the rest of the document, I will refer to the choice you make here as Part 1 or 2, and Mix A or B. Please record the BioBrick ID for each part below, and the corresponding primer mix:

Part 1: _____ **Part 2: _____**

Mix A: _____ **Mix B: _____**

In small 0.2mL tubes, you will setup the PCR reactions as follows:

Reaction	**Q5 Mix (uL)**	**Primer Mix (5uL) – 4uM each**	**Template DNA (5ul) – 1pg each**
1	10*	Mix A	Part 1
2	10	Mix B	Part 2
3	10	SB Prep Mix	pSB1C3 Empty
4	10	Positive Control Mix	Part 1 or 2
5	10	Negative Control Mix	Part 1 or 2

**To pipette 10uL, use the 5uL pipettor twice. Alternatively, an aliquot of 10uL may already be prepared for you.*

Additional controls are possible to include – this would be using a control mix of primers, or leaving the primers out entirely. Since this is just a control for the PCR reaction, it doesn't matter if you select part 1 or 2. You should coordinate with the other students in the lab to make sure your controls are not redundant.

These PCR reactions will be incubated in program 26 of our thermal cycler, which is programmed as follows:

95C 5 min
32 cycles of : 98C for 20 sec, 58C for 30 Sec, 72C for 1.5 mins
72C 6 min
4C Hold

Following this incubation, 0.5uL of DpnI will be added to each reaction, and incubated for at least 1 hour at 37C. This step is intended to remove any background vectors (template) from the reactions. Your instructor or Alma college student aids will carry out this step.

Day 2-A; Running an Agarose Gel

Your instructor and/or student aids will prepare a 0.8% agarose gel for you the preceding day. This is done by mixing 0.8g of agarose powder with 100mL of the appropriate running buffer (1X Lithium Acetate – Boric acid buffer, or LAB buffer). This mixture is heated to dissolve the agarose, mixed with a small amount of ethidium bromide, and poured into a gel casting tray to allow to solidify.

Once the gel has cooled / solidified, and the digest DNA has been column purified (optional), you can load the PCR reactions on the gel. The order of samples to be loaded on the gel will depend upon how many gels were cast, how many samples are present, and will be determined by your instructor. Carefully remove the comb and make sure the gel is oriented appropriately. It is also standard practice to submerge the gel in 1X running buffer (prepared in the earlier step) before loading each well, but you may load the gel dry and carefully add the running buffer afterwards if more convenient.

For each well, you will load 5uL of DNA from either a control (a molecular weight marker or ladder), the plasmid mini-prep, and/or the (un)purified PCR reactions. For each 5ul of sample, it must first be mixed with 5uL of 2X dye. The recommended way to do this is as follows: take a piece of wax paper / parafilm, upon which you will place several spots containing 5uL of dye. To each spot, you will add 5uL of the appropriate DNA sample, mix by pipetting up and down, and then load the entire mixture (up to 10uL) to the appropriate well on the gel.

The recommended loading order is:

Lane	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Sample	Ctrl	Rxn1	2	3	4	5	*Ctrl*	*Rxn1*	*2*	*3*	*4*	5

In the above table, Ctrl represents the ladder, Rxn1-5 are the PCR reactions. The Italic version indicate other students loading their reactions, since you will share a gel with at least one other student.

Once the gel is loaded, connect the electrodes to the power supply (make sure the negative electrode is near the end of the gel that has the DNA – the DNA will migrate towards the positive end). The gel should be run at a voltage of 120 – 200 volts (the higher, the fast the gel runs) for about 30 to 60 mins. Be careful to make sure that the dye front moves in the correct direction and has travelled at least halfway down the gel. Running at too high a voltage will cause the gel to heat up, and excessive temperatures will melt the agarose and ruin the experiment. Periodically checking the temperature if running at a high voltage is a good idea.

Day 2-B; Purification of DNA samples (Optional)

In this step, you will purify the DNA from the PCR reaction, removing any buffering salts and the restriction enzymes. This step is a necessary precursor to mixing the DNA from each Golden Gate assembly reaction. Continued presence of the salts and enzymes from PCR may interfere with this step. This step is very similar to the last half of the mini-prep protocol you may use later in the week, and follows exactly the protocol provided by QIAgen. Here is a brief summary, with volumes adjusted for this workshop:

- Add ~100uL of Buffer **PB** to the PCR mixture. Pipette up and down several times to mix.
- Transfer the entire volume of the digestion / binding buffer mixture (~200uL) to the provided spin columns. Centrifuge 1' at max speed (12500 rpm is OK) and discard flow through.
- Wash the column with ~750uL of Wash solution containing Ethanol. Centrifuge 1' at max speed and discard flow through.
- Centrifuge the column an additional 1' to remove any residual Ethanol.
- Place the spin column in a fresh 1.5mL micro-centrifuge. Add 30uL (5uL 6 times) of dH20 to the spin column, incubate 1', and centrifuge for 1'. Remove the spin column – the flow through contains your DNA, and the micro-centrifuge tube should be labelled appropriately.

Day 2-C; Setting up Gibson Assembly Reactions

If your gel provided adequate results, and you have finished purifying the DNA samples (optional), you can then proceed to mix these together in a Gibson assembly cloning reaction. In the event that your gel did not work as intended, you will use the PCR reactions already prepared by Prof. C or successful reactions setup by your classmates. You will pre-mix your fragments together to achieve a proper mix or

dilution for each subsequent reaction (this helps to save on the amount of enzyme mix that you need to use.

Pre-Mix your Fragments:

Pre-Mix 1: 5uL PCR-1, 5uL PCR-2, 5uL PCR-3, 5uL dH20

Pre-Mix 2: 5uL PCR-1, 5uL PCR-2, 10uL dH20

Pre-Mix 3: 5uL PCR-3, 15uL dH20

Reaction	**dH20**	**Pre-Mix (5uL)**	**Enzyme Mix**
A) Assembly		#1	5uL
B) No backbone		#2	5uL
C) No insert		#3	5uL
D) No Rxn	5uL	#1	

Only reaction "A" should yield colonies with the assembled plasmid. The other reactions are controls to help determine what has occurred in your reaction.

Incubate the reactions for ~60 minutes at 50C, save the reaction for the transformation the following day.

Day 3-A; Bacterial Transformation

In this step, you will use chemically competent cells, prepared by the instructor, to transform the five assembly reactions setup on Day 2.

- Before beginning, have ready an ice bucket and set a heating block to 42C. Also, obtain 10 LB plates with chloramphenicol, and label them with the date and an indicator matching the transformation it will be used for A through E.

- Add 5uL of the assembly reactions A through D to the 100uL competent cell mixture. You will also add 5uL of the transformation control, pACYC, to 100uL of cells for transformation "E".

- Incubate on ice for 30 minutes. It is critically important that the competent cells are kept cold until the next step – be careful when handling them!

- Heat shock at 42C for 45 seconds

- Incubate on ice for an additional 5 minutes

- Add ~900uL of fresh sterile TB, SOC or SOB solution – use the syringe pipette for this.

- Incubate for at least an hour at 37C, shaking if possible. Alternatively, shaking at room temperature is acceptable, especially if additional time can be allotted (~30 mins). This recovery step is necessary to allow the bacteria time to express genes located on the plasmid (such as the selectable marker) before they are applied to a selective media.

- Pipette bacteria (100uL – use the syringe pipette) onto LB agar petri dish with the appropriate antibiotic, spread evenly using a sterile spreading tool. Allow the plate to dry and place at 37C overnight.

As stated above, it is imperative in this protocol that you keep the bacteria on ice when indicated! Pre-mature thawing of the bacteria can greatly reduce the efficiency at which it accepts foreign DNA. In addition, the final hour incubation is necessary so that the bacteria have time to express the antibiotic resistance gene.

You should take care and remember that you are dealing with live cells, and recombinant DNA. Avoid contamination and prevent escape of this bacteria from the lab!

Day 4 and Beyond

To determine how well your transformation worked, you will need to count the number of colonies on your plate "E". This represents about 1/10 of the total amount of bacteria transformed in that experiment (since only 100uL was plated). Multiply this count by 10 – this is the total number of transformed bacteria that could have formed colonies – normally referred to as 'colony forming units' or CFUs.

Transformation efficiency is normally reported in CFUs per ug of DNA used. In this experiment, you transformed the cells with 50pg of DNA, which is the same as 0.00005 ug (In scientific notation, that is 5×10^{-5} ug). So divide your total CFU count by that number to find your transformation efficiency. A typical transformation efficiency is between 1 to 10 million.

You will also select different colored colonies and re-streak them on another plate, and/or setup a liquid culture. You can also use them (and the original strains) to 'paint' an agar petri dish.