GUIDE ON HIGH SCHOOL DIVISION

iGEM 2012

Hong Kong – CUHK

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Preface

The iGEM High School Division first started in 2010 and we have observed that there are relatively few Asian teams participating in this competition. We conducted questionnaires to investigate the reasons for this, and this guidebook was drafted on the basis of the first round of our findings.

Survey findings showed that a majority of Hong Kong high school students still do not know about Synthetic Biology. Therefore the primary aim of this guide is to further promote Synthetic Biology and enable high school students to understand its prevalence in our society. One teacher responded, “Our students are not so creative. They are highly dependent on teachers' guidance”. Thus our second aim is to inspire students, help them comprehend and apply an abstract set of concepts in different situations and aid in the design and implementation.

Another major concern is a lack of laboratory facilities and instructors. So with this guidebook, we offer checklists of requirements and policies that need to be met to form a team and jumpstart an innovative project, so high school students may articulate, document and legitimize their creations. It also includes step-by-step instructions and examples on BioBrick design and construction to illustrate meaningful links of BioBrick constructs to various fields of expertise and industries.

We believe this publication will be of great value to potential project innovators and entrepreneurs, and encourage them to be involved in the design, creation and execution of Synthetic Biology projects.

It is also important to state the limitations of this guidebook. The examples included are not intended to dictate project design or methodologies. There are several approaches that may be used in project design, construct and execution. One method used in one project may not be compatible and fail in another.

Acknowledgements

We, the Hong Kong – CUHK iGEM 2012 team, draw on the experience and support of many individuals and institutions, and wish to acknowledge it publicly. Their support and contribution made our project, *Light of No Return,* and human practise activities possible, leading to the preparation of this guidebook.

We would first like to give acknowledgement to our professors and instructors whose advice and dedication encouraged us to strive for gold. We would like to thank:

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Chapter 1 – What is Synthetic Biology?

"I have never heard of Synthetic Biology before. What are BioBricks? What is iGEM about?"

In a city of about 7 million, there are over 500 high schools with nearly half a million students. And with only 3 out of 8 universities in Hong Kong, namely The Hong Kong University of Science and Technology (HKUST), The Hong Kong University (HKU), and The Chinese University of Hong Kong (CUHK), participating in iGEM, a majority of Hong Kong high school students still do not know much about Synthetic Biology, the concept of BioBricks and iGEM. This shows that more effort can be focused on promoting Synthetic Biology. Our previous high school outreach which included around 200 students was only the tip of the iceberg, therefore in this secondary investigation, we would like to outreach to high school students, as well as the general public, at a larger scale. Hence, the first component of this book is a brief introduction to these topics.

Synthetic Biology is the combination of systems biology, engineering, computer science and molecular biology. These are collectively applied in the redesigning and re-engineering of a living system to manufacture products that do not occur in the natural environment. Various products of Synthetic Biology have also contributed to improvements and discoveries in science and our everyday surroundings.

The BioBrick Part Registry is a collection of genetic parts which, like Lego parts, can be arranged in numerous ways to build Synthetic Biology devices and systems. The registry serves as an open source catalogue and database, making a wide array of genetic part available for Synthetic Biology labs, academic labs and perspective project innovators and entrepreneurs. BioBricks are the basic units and components of Synthetic Biology. Like blocks of Lego, they can be mixed and matched to obtain desired gene products as the cornerstone of a system or device.

iGEM embodies the use of BioBricks and the advancement of Synthetic Biology through hands on laboratory experience and community outreach. It also provides a platform for international convening of young aspiring scientists interested in experiencing this branch of science outside of the classroom.

Chapter 2 – How do I get started? – Team formation and Brainstorming

2.1. Forming a Team

"I am interested in creating a Synthetic Biology project, but where do I begin?"

Coming together is a beginning.To get started you first need to form a team. The structure of iGEM teams is flexible. You can choose to form a several teams with students from different grades and classes in your school. You can also choose to form a regional team and collaborate with high school students and instructors from different schools.

"Who do we need in our team and how do we find them?"

Your team will need to comprise of at least 1 student and 1 instructor, but why have only 2 people when you can make a bigger team and have more fun? You may first consult with any of Hong Kong's existing collegiate teams. We may offer you guidance regarding recruitment of team members.

There are various ways you can recruit students and instructors for your team such as poster advertisements and brochures around your school campus. You can also make an online forum to find students from other schools who would be interested in joining your team. Take advantage of the many social networking platforms such as Facebook.

It is recommended you find team members with from various disciplines, backgrounds, and levels of expertise. You will also need members with strengths in public speaking who are confident in giving presentations to a large audience, graphic design,

Below is a checklist of who you may ask for help with recruitment or to be part of the first iGEM HS Hong Kong team, as well as a checklist of the different positions and responsibilities different members can take within the team.

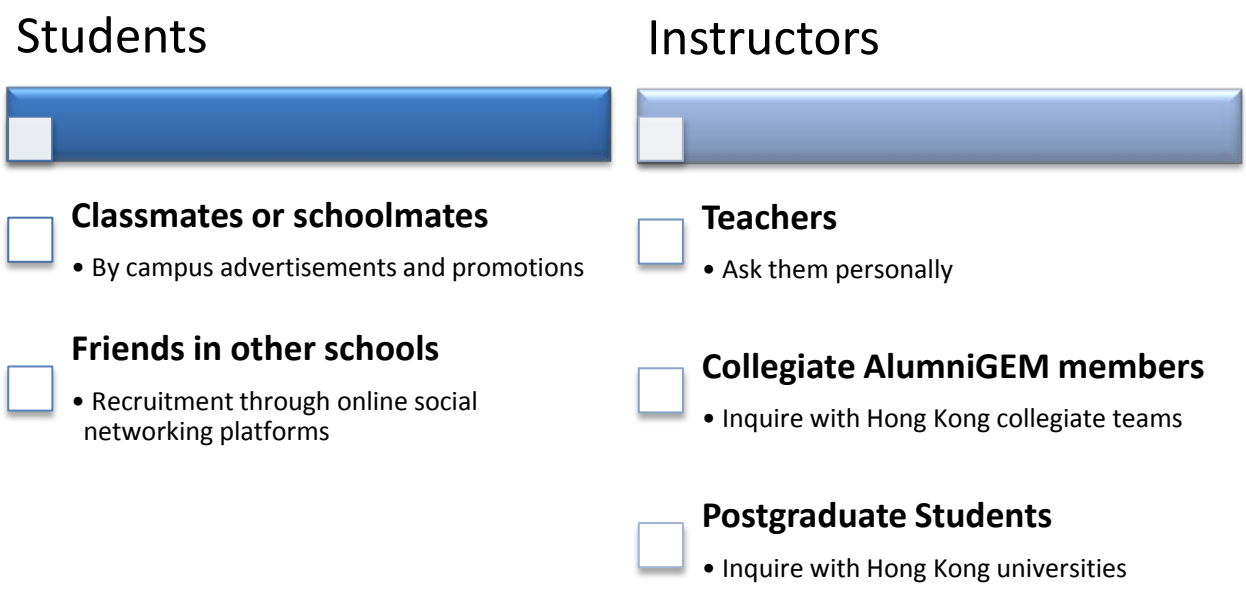

Figure 1 – Checklist of potential team members

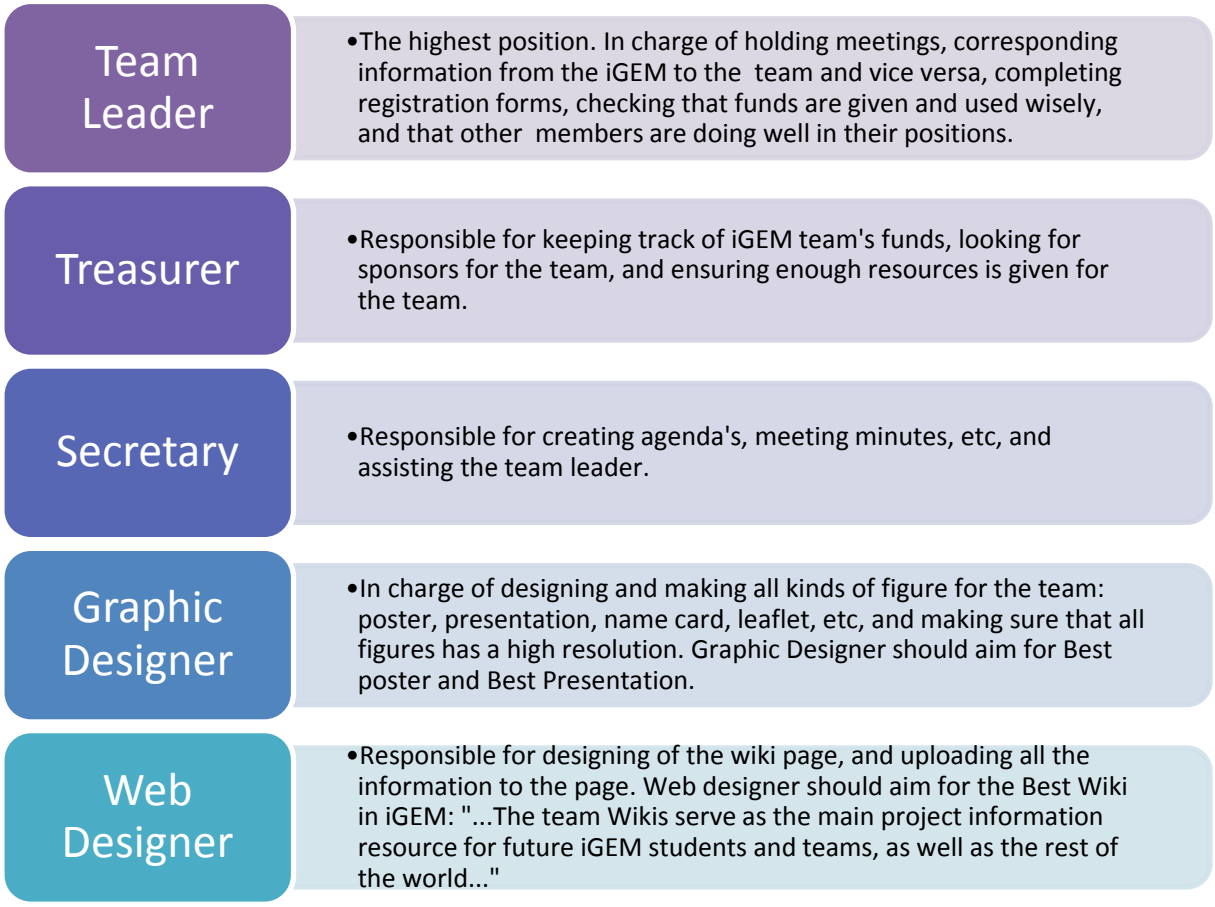

Figure 2 – List of Team Responsibilities

2.2. Brainstorming

"I have formed a team, what should we do now?"

Now you have to put on your thinking caps. You can brainstorm for projects from different points of view or strategies. One ways is known as the "bottom-up" strategy where you think of a BioBrick construction and incorporate it into a potential system or device. Another tactic is the "top-down" strategy where you look for an inconvenience, inadequacy or a novel application, then you device a BioBrick to resolve the situation. Synthetic Biology has great potential for applications in different fields such as food and energy, information processing and health and medicine. Listed here are some examples of different industries, also known as tracks.

Choosing Tracks

Here are the tracks with the descriptions from iGEM criteria, and one project from previous iGEM team is given as an example for each tract.

Each tract is arranged in page as shown below:

"Tract"

"Tract description from iGEM Criteria"

They are mostly in the question form which makes you to think more

All are quotes from: *http://2012.igem.org/Judging/Judging_Criteria*

"Example"

"Problem is stated and how Synthetic Biology is applied and solution is created by the previous teams"

"Human Practice of the project" *(if any)*

This could be another Biobrick or a method in using the Biobrick for the safety measure of the project. Although human practice include both ethical and safety issues, the later one is being described here. More information for human practice please see the Chapter: [add]

For more information ***"Link to Wiki- page of the team"***

2.2.1 Food & Energy:

"Can biotechnology be responsibly used to produce food or energy without causing widespread shortages of either, and without harming the environment that future generations will inherit?"

Example for food:

iGarden is a toolkit that lets one to grow a personalized garden:

1. Knockdown of plant allergens to make safer foods
   Two types of RNA were designed to knockdown the protein that is similar to *Arabidopsis* in pollen causing seasonal allergy. Other RNAs were made to delete the allergens in other common plants in home gardening.

2. Novel flavors for nutritious foods to be more favorable
   Sour flavor can be received as sweet as miraculin binds to tongue receptor. Also the protein, Brazzein originally provides sweetness in fruits can be given to plants by inserting the corresponding genes into the vector and transforming them to seed through bacteria and the flower. Aside from the flavor, banana and mint scents could be added to plants through plant-specific promoter to make the scent-altering system for a particular plant.

3. Modification of plant metabolism to color the white petals
   White flower can be colored into red or orange through accumulation of the pigment, carotenoids via gene knockdown to enzymes that catalyse the related metabolism and specific promoter for expression and localization.

Plants are modified through several steps. All constructs were made in bacteria, and once complete, they were being cloned into the BioBrick agrobacterium vectors. Bacteria were then transformed with the constructs via electroporation, and flowers were dipped into the transgenic

bacteria. In the final step the transformed seeds were harvested and screened for the designed constructs using antibiotic selection.

Specific Human Practice of *iGarden*:

"Genetic fence" is designed specifically for these plants to grow within the area the gardener prefers. Plants can only grow inside the genetic fence but not outside of the fence. Hence, the genetically modified seeds will not be released and neither its modified genes.

Source: http://2010.igem.org/Team:Harvard/vectors

Example for Energy:

Several kinds of renewable resources have been developed to generate electricity, but the efficiency of the energy production from these resources is very low compared to the non-renewable ones. During cheese production, a by-product called whey is produced, and whey disposal is a problem to creameries as it causes water pollution.
Even though microorganisms do not naturally have an efficient pathway of both lactose-cleaving and ethanol-producing, Synthetic biologists designed a novel pathway from the nature, selected the powerful enzymes and combined the genes of each essential component to produce a new microorganism that gives the highest yield of ethanol from lactose

Source: http://2009.igem.org/Team:UNIPV-Pavia

2.2.2 Environment:

"Can biotechnology be used to help clean the air, provide fresh drinking water, restore or enhance soil quality, terraform a near-Earth asteroid, or protect, preserve, or enhance natural biological diversity?"

Example:

Auxin is a solution for desertification

A worldwide problem, densification causes loss of resources and lands and leads to soil erosion. Auxin was designed to accelerate plant root development via its contracted genomic system. As seeds are coated with this type of bacteria and germinate in soil, the bacteria can move toward the roots via additional chemoreceptor. Later, as they enter or taken by the root, their modified pathways leads to secretion of the hormone, auxin that promotes growth and thus prevent soil erosion. The long term vision of this project is to revitalize dry land and make soil fertile again for vegetation

Specific Human Practice of Auxin:

Gene guard is a containment device to allow nonviable transfer of incomplete genome during the horizontal gene transfer which minimizes the risk of accidental release of modified genomes to the environment.

Source: http://2011.igem.org/Team:Imperial_College_London/Tour

2.2.3 Health & Medicine:

"Many health and medical problems might best be addressed by improved biological technologies. What can Synthetic Biology do?"

Example:

There are not enough organ donor for patients with organ failure, and with the shortage, the donated organs are expensive. Organs are formed by the various combinations of 4 different kinds of tissues: connective, muscle, nervous, and epithelial; and they are groups of cells.

The project, Tissue-by-Design was an exploitation of this basic structure of human body. The mechanisms of the tissue and organ formation could be engineered in different aspects: cell-cell communication pathways, intracellular information processing circuits, and cell-cell adhesion, thus the undifferentiated cells are control to synthesize desire organs by the respect pattern.

Source: http://2011.igem.org/Team:MIT

2.2.4 Manufacturing:

"Have you ever heard of nanotechnology? Well, biology is a nanotechnology that already exists, and that actually works. The ribosome is a programmable nanoassembler embedded within a reproducing machine. Could we responsibly use biology to manufacture useful products, from the nanoscale (atoms) to the decascale (buildings and bridges)? What can biology be programmed to manufacture?"

Example:

BioFactory aims for efficiency

The current methods for purification of biomolecule from cellular lysate are expensive and time consuming.

BioFactory are modular microfluidic chips that modified enzymes attached onto the surface of corresponding chip, and when combined in series, these chips operate as a linear biochemical pathway for continuous flow reactions. To reduce the undesired reaction and lower the costs of producing bio-pharmaceuticals in the future, E. coli was engineered with the mechanism for light-induced apoptosis, so they only flow through the through the microfluidic channels, coating them with the desired enzyme, so the traditional protocol is unnecessary.

Source: http://2011.igem.org/Team:Cornell

2.2.5 New Application:

"We're guessing that you have great ideas that nobody has ever thought about, or if they have they forgot to tell somebody else. Can you imagine an entirely new application area for biological technology?"

Example:

In the body, the enzymes are not directed to the substrates, and catalysis only occurs when they meet each other after randomly moving around.

Like shortening the distance of each working section during the industrial revolution for a higher efficiency of the entire production, the stable DNA chain can be used as an assembling line for catalysis. Each enzyme can be fused onto DNA china and line up in order, so they can carry out the catalysis one after another. This application shortens the distance and increases the efficiency of the reactions.

Source: http://2010.igem.org/Team:Slovenia

2.2.6 Foundational Advance:

"Just thirty-five years ago, scientists could not cut and paste pre-existing fragments of genetic material like we can today. The discovery and application of DNA recombination allowed us to assemble new genes. The Synthetic Biology community needs other enabling technologies that help to make new accomplishments possible. What are other types of basic tricks does nature use? Have you discovered and applied one that could revolutionize Synthetic Biology?"

Example:

In Synthetic Biology, standard parts are registered, and the information of each part is uploaded onto the iGEM websites. Synthetic biologists either modify the previous parts or create a new one, but in either ways, there are difficulties.

One way to deal with them is to create different mutants of the standard parts which have various characteristics and recorded them as a "Mutant library". One of the mutants may be suitable for the future modification or creation with its appropriate protein-binding ability, satisfactory expression strength, and peculiar sensitivity to the chemical used.

Source: http://2011.igem.org/Team:UC_Davis/Project

2.2.7 Information Processing:

"The diversity and abundance of biological properties, behaviors, and parts presents a huge information processing challenge. Has your project led to an innovative system that allows us to navigate and use lots of information quickly and effectively?"

Example:

Bioencryption and Bacterial USB:

Data encryption is weakened due to the advance in mathematics, and we need larger storage size in a smaller device.

Similar to binary number system (0 and 1) in the electronic devices, the basic DNA code (A, T, C, G) in bacteria can be used as a quaternary number system to convert message for storage, and in this case, 'Hi' is converted into DNA code 'TCCATCCT' for the storage in genome of DNA. As for the encryption system, a built-in (intrinsic) system is self-synthesized enzyme that recognizes specific DNA sequence to encrypt the messages in bacteria.

Bacteria have a much larger capacity of storage than electronic data storage. The storage capacity of 1 g of living bacteria, E. coli, is equivalent to 450 hard drives with the capacity of 2 TB, given that 1 g of hard disk carries 1 to 4 GB only. Electronic data can hardly be repaired when the storage components are damaged, but bacterial storage is a living system which can better maintain the data integrity. Bacteria can replicate extensively to give abundant copies of the information. Therefore, one does not need to worry about the data loss even when some of them are dead.

Source: http://2010.igem.org/Team:Hong_Kong-CUHK

Chapter 3 – What do we need to create our project?

"We have some good project ideas, but we do not have adequate laboratory facilities"

One other major concern among students and teachers alike is that high school labs in Hong Kong are fully equipped with the necessary apparatus and tools to make BioBricks. This chapter will cover how you can acquire a laboratory, the equipment and materials you will need in your laboratory, as well as the necessary safety precautions you need to take when handling microorganisms.

3.1 Acquire a Lab

The laboratory will be the home and witness to the progress and development of your project. Furthermore you will need to acquire a lab and register it to gain access to the BioBrick Parts Registry from which you can obtain the necessary parts for your project.

Most high schools in Hong Kong have their own laboratory facilities that simply need additional equipment. However, if yours does not, there is no need to worry. There are several ways you can acquire fully equipped laboratory facilities.

(1) The Hong Kong Science Park has fully equipped laboratory spaces and engineering services available at an hourly basis or on project basis. They can also provide equipment that tailor to your project needs.

(2) If your instructors are university students, or members of the alumni, you can request to use a university laboratory during the course of your project.

3.2 Laboratory Equipment Checklist

When you have acquired a laboratory, you may need to check if you have all the necessary apparatus. Each apparatus plays an important and specific role in the construction of your BioBricks.

Equipment

Autoclave Machine

- for aseptic technique: to kill all the microorganism and their spores

Burner

- for aseptic technique: to create the aseptic zone as your working area

Incubator

- where you grow your bacteria in plate

Oven

- to dry the autoclaved materials and to warm up solution like elution buffer

PCR Machine or Thermocycler

- capable of setting the required cycling condition (temperature and time) for DNA amplification (PCR)

Spectrophotometer

- to find out the concentration of a certain substance through a specific wavelength

Shaker

- where you grow your bacteria in LB broth

Water Bath or Shaking Water Bath

- mainly for transformation

Weighing Machine

- to weigh accurately to make the required solution with exact concentration

Lab Supply

Bottles with cover

- to contain liquids

Cuvette

- to be used in spectrophotometer

Flask

- to make gel

Glove

- protection wear
- XS, S, M, L, and XL

Micropipette & Tip

- Blue 1000 µl
- Yellow 200 µl
- White 10 µl

Plate

Tube

- Centrifuge tube with conical bottom
  - small & big
- Conical or Snap cap
- Eppendorf
  - 1.5 µl and 2.0 µl
- PCR tube

Figure 3 – Checklist of necessary laboratory equipment and materials

3.3 Laboratory Safety and Useful Techniques

iGEM pays special attention your safety preparation about your BioBrick, lab, and team in relation to the community, environment and safety issue of Synthetic Biology.

To be qualified and meet the iGEM standard for safety, you need to:

1. Follow the iGEM safety requirement in all steps
   - Getting started: http://partsregistry.org/Help:Distribution_Kits
   - Requesting part: http://partsregistry.org/Help:Requesting_Parts
   - Safety concern: http://igem.org/Safety and http://2012.igem.org/Safety
2. Obtain a certificate for Safety Level 1 from an educational institution
3. Work in a qualified lab examined by a local biosafety group
4. Research the safety proposal of related standard parts on the iGEM page called "Registry of Standard Biological Parts".
5. Read the safety manual from WHO to understand biosafety and biosecurity
6. Search for the safety information about your project
7. Check previous team's safety approach for more information
8. Answer the safety questions from iGEM in details
9. Try to create and design new safety approach.

These 3 links are provided from 2012 iGEM as reference, and Step 4, 5 and 6 are important and helpful in designing Biobrick and constructing the protocol of the project.

Below are some basic laboratory rules and techniques which you may refer to and apply in your laboratory preparation.

Basic Laboratory Rules

1. Wash your hands with detergent and dry them with paper towel upon entering and prior to leaving the laboratory.
2. Tie back long hair to minimize the exposure to the flames.

3. Wear long trousers, lab coat and closed shoes to protect you from contamination of bacteria or accidental spill of chemicals.
4. Do not drink or eat in the laboratory.
5. Take off gloves and lab coat before touching any fixtures in the lab
6. Wear glasses instead of contact lenses whenever possible.
7. Leave only essential items only on the bench.
8. Understand the protocol and know all of the steps before the star. Sometimes preparation should be done ahead but is not always mentioned in the beginning of the protocol.
9. Label all the containers and materials to avoid confusion.
10. Add date and name of the person who made the materials in labelling the solutions and bacterial culture.

Aseptic Technique

Aseptic means the absence of the significant contamination, and aseptic technique is the performance to process laboratory work under a sterile condition by using flame, autoclaved equipment, and disinfectants. It is to keep the sample from contamination as well as to limit the risk of infection to our body.

1. Autoclave the necessary equipment and material prior to use.
    - Pipette tips, centrifuge tubes, eppendorf tubes, and other containers
2. Use 70% of ethanol in water as disinfectant to wrap the table before start.
3. Never take culture out of the laboratory.
4. Light up a burner set an aseptic zone while working with microorganism.
5. Work within the aseptic zone and flame the equipment before and after use.
6. All laboratory wastes should be autoclaved prior to disposal.

Chapter 4 – How do we make a BioBrick?

Before starting any BioBrick construction you need to prepare several components beforehand. This section offers a step-by-step instruction to those necessary components.

4.1 Stage 1 – Preparation of Buffers

1) TAE and TBE Buffer

5X TAE Buffer	
Chemical	**Amount/Volume**
Tris base	24.2 g
Glacial acetic acid	5.71 ml
0.5M EDTA (pH 8.0)	10 ml
ddH_2O	Up to 1 ml
Total Volume	1 ml

5X TBE Buffer	
Chemical	**Amount/Volume**
Tris base	54 g
Boric Acid	27.5 g
0.5M EDTA (pH 8.0)	20 ml
ddH_2O	Up to 1 ml
Total Volume	1 ml

This is used in Agarose electrophoresis typically for the separation of nucleic acid such as DNA and RNA.

2) PBS Buffer

10 X PBS	
Chemical	**Amount/Volume**
NaCl	40 g
KCl	1 g
Na_2HPO_4	7 g
KH_2PO_4	1 g
ddH_2O	Up to 500 ml
Total Volume	500 ml pH7.4

3) Antibiotics:

10 X PBS	
Chemical	**Amount/Volume**
Antibiotic powder	0.5 g
ddH2O	Up to 10 ml
Total Volume	10 ml, filter

Source: Loo J. 2012. iGEM Hong_Kong-CUHK 2012 Wet-lab Training Manual. The Chinese University of Hong Kong School of Life Science. Version 2.2.

4.2 Stage 2 – Preparation of Bacterial Culture Media

1) LB Broth and Plate

LB Broth	
Chemical	**Amount/Volume**
LB powder	20 g
ddH_2O	Up to 1 L
Total Volume	1 L, autoclaved

LB Plate	
Chemical	**Amount/Volume**
LB powder	20 g
Agar powder	15 g
ddH_2O	Up to 1 L
Total Volume	1 L, autoclaved

2) SOB and SOC Medium

SOB Medium	
Chemical	**Amount/Volume**
SOB powder	25.5g
ddH_2O	Up to 1 L
Total Volume	1 L, autoclaved

SOC Medium	
Chemical	**Amount/Volume**
Autoclaved SOB medium	980 ml
1M Glucose (Fitered)	20ml
Total Volume	1 L

Source: Loo J. 2012. iGEM Hong_Kong-CUHK 2012 Wet-lab Training Manual. The Chinese University of Hong Kong School of Life Science.Version 2.2.

4.3 Stage 3 – Make your BioBrick

Here are the descriptions of one of the traditional way to make a BioBrick. General materials are listed to be prepared before the lab starts, and some points should be noted in the process.

This session can be used as your check list before you are familiar with the actual protocol from different kits, but please take note that variation always exists and understanding the protocol before start is helpful as well as necessary for the desired result.

Do not be upset with negative result. Protocols vary among the different designs of the BioBrick, and asides from the one described above, there are other ways to make a BioBrick.

➔	**Polymerase Chain Reaction (PCR)**
	DAN Digestion by Restriction Enzyme (Restriction Cut)
	DNA Purification / **DNA Purification from Gel**
	T4 DNA Ligation
	Bacterial Transformation
	Biobrick Amplification in the Plasmid Form
	Plasmid DNA Extraction

Figure 4a - Outline of Wet Lab Part 1: BioBrick Construction

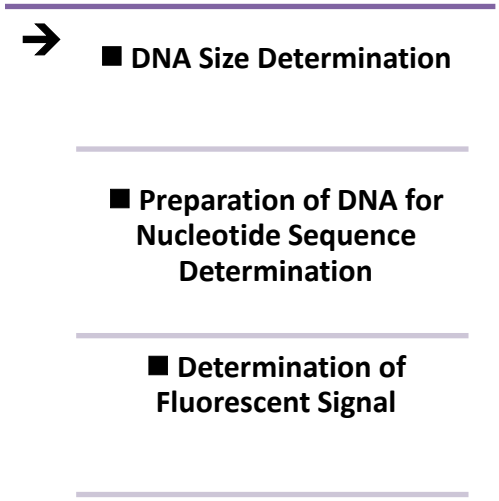

Figure 4b – Outline of Wet Lab Part 2: BioBrick Characterization

Part 1: BioBrick Construction

Step 1: Polymerase Chain Reaction (PCR)

A DNA amplification process to make the target sequence into linear DNA copies

Material: dNTPs, water, DNA templates, forward and reverse primers, heat stable DNA polymerase and its corresponding buffer

Note:

- DNA templates should be added at last, and thermocycle should start immediately after adding all the materials

Step 2: DAN Digestion by Restriction Enzyme (Restriction Cut)

Restriction cut can be used and repeated for different purposes: create a sticky end of the DNA, make blunt end of DNA, or make linear plasmid into circular form.

Material: water, DNA template, restriction enzyme, 100X BSA buffer, 10X NEWB buffer, and 37 ◦ C incubator or dry bath

Note:

- Water is added first
- Buffer is added before enzyme
- Ensure the pipette tip touches the surface of solution
- Glycerol can be added to prevent further undesirable DNA digestion

Step 3: DNA Purification

Removing excess nucleotides, enzymes and ions in the solution with desired 100 bp - 10 kb DNA

Material: use PCR Purification Kit

Note:

- Elution efficiency is pH dependent, and the optimal pH for maximum efficiency is in between pH 7 and 8.5
- DNA should be stored at 20 ° C to prevent degradation
- EDTA buffer may inhibit subsequent enzyme reactions
- Recovery can be maximized by warm elution buffer

Source: Loo J. 2012. iGEM Hong_Kong-CUHK 2012 Wet-lab Training Manual.
The Chinese University of Hong Kong School of Life Science.Version 2.2.

Step 4: DNA Purification from Gel

After checking the band size through gel electrophoresis, enzymes, ions, nucleotides, needs to be removed from solution for the target DNA (70 bp - 10 kb)

Material: use gel clean kit, distilled water, and 60 ◦ C dry bath

Note:

- Both DNA purifications give high possibility of correct ligation later. But gel purification allows the selection of target DNA sequence from the other DNA fragments while PCR purification only lowers the contamination.

Step 5: T4 DNA Ligation

Linking the sticky ends or blunt end of DNA segments after digestion to make linear or circular plasmid

Materials: T4 DNA ligase, 10X T4 DNA buffer, ddH2O, restriction enzyme cut, 16 ◦ C dry bath or - 4 ◦ C fridge

Note:

- Reagent should be placed on ice
- Reaction mixture should be prepare in a fixed series of steps
- Insert to mass of vector should be added with one of the following ratio: 1:1; 1:3; 3:1; Or you can use the Bio-math calculator like this one: https://www.promega.com/techserv/tools/biomath/calc06.htm to find out the amount to be added. Moreover the total amount should be > 100 ng to prevent self-ligation or inhibition of ligation.
- Reaction takes place over night at - 4 or 16 ◦ C or for 2 hours at 22 to 25 ◦ C

Step 6: Bacterial Transformation

Opening pores on the membrane of cell by heat shock or chemical to allow circular DNA plasmid to enter

Material: aseptic technique, cell, LB plates, plasmid DNA, LB media or SOC media, incubator

Notes:

- Timing for heat shock or use of chemical use on competent cell should be accurately measured with timer to prevent killing the cell
- Spreader should be cool after flaming to spread bacteria onto the plate

Source: Loo J. 2012. iGEM Hong_Kong-CUHK 2012 Wet-lab Training Manual. The Chinese University of Hong Kong School of Life Science.Version 2.2.

Step 7: Biobrick Amplification in Plasmid Form

Amplifying the desire DNA plasmid by growing the colonies from the spread plates with antibiotic to select the expect bacteria which contains the backbone making the bacteria to be resistant to a particular antibiotic.

Material: autoclaved tooth pick, LB media, antibiotic, snap cap, and 37 ◦ C shaker

Notes:

- Require aseptic technique for picking clone
- Mark the clone you picked
- Storage of the bacteria cell with desired plasmid: 800 µl of cell + 200 µl of 80% sterile glycerol, and they should be stored in -80 ◦ C freezer.

Step 8: Plasmid DNA Extraction

Material: miniprep plasmid DNA extraction kit

Notes:

- Be careful when transferring supernatants from centrifuge tubes into the spin column to avoid contamination of genomic DNA
- To increase the concentration of plasmid, increase the incubation time while the Elution buffer stains in a 60 ◦ C dry bath

Source: Loo J. 2012. iGEM Hong_Kong-CUHK 2012 Wet-lab Training Manual. The Chinese University of Hong Kong School of Life Science.Version 2.2.

Part 2: Characterization of BioBrick

Step 1: DNA Size Determination

Through comparing DNA ladder as the reference with your DNA or plasmid, you can know whether you have the desired DNA or plasmid. This step is normally done to ensure the quality of DNA after PCR, restriction enzyme cut, ligation, and extraction.

Material: agarose gel, TAE buffer, gel imager, computer, DNA ladder, 6X or 10X DNA loading dye, DNA staining dye.

Notes:

- The higher the voltage, the lesser the time to run a gel electrophoresis
- Never run a gel with > 200 V because the heat generated from the machine may melt the gel and leads to electric leakage and inaccurate result of the DNA bands
- 120 to 180 V is the acceptable range
- 100ng of DNA is needed to be loaded and appeared in the image of the gel

Step 2: Preparation of DNA for Nucleotide Sequence Determination

Material: backward and forward primers sequence the DNA and result is analysis by sequencing machine and shown in a computer with the software. This is the genotypic method to identify the bacteria, and in this case, to see whether it matches your BioBrick design.

Notes:

- This can be sent and done by some biotechnology companies around your area.

Step 3: Determination of Fluorescent Signal

Fluorescent plate reader is used to measure and calculate the fluorescent signal expressed from fluorescence-fusion protein

Material: 96-well plate, standard fluorescence, and micro-plate reader

Notes:

- 488 nm for excitement wavelength and 509 nm emission wavelngth
- The standard for one 96-well plate cannot be used to another plate.

Source: Loo J. 2012. iGEM Hong_Kong-CUHK 2012 Wet-lab Training Manual. The Chinese University of Hong Kong School of Life Science.Version 2.2.

Afterword

Each team starts as a small group of people with the pure interest and little curiosity over the truth and potential of the new science. We then become determined to continue in the endless space of the game for different potential abilities. We may find ourselves immersed in challenges, surrounded by questions, yet stoop deeply into where students can impossibly reach without such participation. Like the old saying goes “from small beginning come great things.” Each team turns the abstract idea into a concrete project to redefine the limitation of human in conquering difficulty, solving problems, or even dealing global issues. We hope this book is not only the guidance but also an inspiration for the high school students, for them to experience what we have earned throughout the progress in the competition.