Week1 2019.03.25-2019.03.31

We recruited members from different schools and different specialties and established our team. We learnt about synthetic biology by reading papers and wikis, and we started to look for the PI of our project.

Week2 2019.04.01-2019.04.07

The principles of all projects had been further explored. We sorted out the previous projects to have an overview of the competition and we initially decided the direction of our research.

Week3 2019.04.08-2019.04.14

We held a meeting and did brainstorms. The college instructor and other professors participated in our report, and we finalized the topic after the meeting. Through many active discussions and literature review, we had learnt about that Branchiostoma digest tract epithelial cells can effectively degrade harmful substances such as algal toxins and achieve the purpose of directly converting algae into absorbable and non-toxic nutrients. But because of the rareness of this creature, we planned to use genetic engineering method to amplify the proteome of its internal digestive function.

Week4 2019.04.15-2019.04.21

In this week, we asked two experts, Dr. Tao Li and Dr. Gongliang Yu, for some advice. Dr. Gongliang Yu, Associate Researcher of Institute of Hydrobiology, Chinese Academy of Sciences, said that the complex species, outbreak mechanism and interaction with water environment of algae make cyanobacteria blooms control a challenge all over the world. We told him our core idea, trying to develop cyanobacteria resources through reverse thinking. He gave us many constructive advices. For example, he suggested that we can combine with remote sensing monitoring technology of water environment, such as UAV remote sensing technology, and dynamically track the source of cyanobacteria outbreak, it will play a greater role in promoting the control of algae bloom. Li Tao, who works for the International Water Association (IWA), is currently the Director of Greater China. He highly praised our project especially for the idea of combining with wastewater treatment equipment for large-scale application. He said how to treat wastewater containing algae is an important and our project is innovative and of great significance on this issue. After exchanging ideas with the two experts, we were greatly encouraged!

Week5 2019.04.22-2019.04.28

We held a meeting with our advisor discussing the feasibility of our project in school competition and drafted a simple plan. We knew more details about our project and how to start it! At the same time, we design our team logo, T-shirts, team flag and other creative props.

Week6 2019.04.29-2019.05.05

We started a deep exploration! We divided all the members into two groups to explore more useful information. We all actively seek out relevant literatures about algae degradation and involved gene to make the ideas clearer.

Week7 2019.05.06-2019.05.12

With the introduction of our instructor, we got in touch with Dr. Jianhong Li, Professor of School of Life Sciences, Nanjing Normal University, and Dr. Renhui Li, Director of Algae Research Center of Institute of Hydrobiology, Chinese Academy of Sciences. Dr. Jianhong Li was impressed by our discovery of the enzymes developed from amphioxus that directly degrade algale toxins. He thought our ideas were worth trying and likely to succeed. He believed that our idea of using biotechnology to solve the problem of cyanobacteria is a great innovation. It is technically feasible and has the potential to make breakthroughs. On Sunday, we met with Siyu Wu. As the founder and partner of the investment company, Siyu Wu has abundant practical experience in entrepreneurship. At the same time, he also serves as an entrepreneurship mentor to provide advice and guidance to young teams. Therefore, we consult him from the point of view of product design and business model, hoping to get constructive suggestions.

Week8 2019.05.13-2019.05.19

We began to learn microbial experiment operation and carried out the test of experiment operation.
Unfortunately, most experiments this week ended in failure. We still had a long way to go!

Week9 2019.05.20-2019.05.26

Our teacher instructed us to carry out the experiment. During this period, all student members learned laboratory fundamental qualities and received molecular cloning operation training. Everything seemed to be going smoothly.

Week10 2019.05.27-2019.06.02

We started to study genomics and bioinformatics analysis. Such as differential expression analysis, gene annotation, and how to use some relevant software. Thank our instructor and many warmhearted seniors for giving us so much help and guidance!

Week11 2019.06.03-2019.06.09

The full-length functional proteomic Library of amphioxus cecum epithelial cells constructed in vitro was sequenced by Pacbio third-generation sequencing technology. We got these datas from our instructor, and analyze the result using databases including Blastn, Blastx, SWISSPROT, KEGG, COG, Interpro and GO.

Week12 2019.06.10-2019.06.16

This is a huge project！ We still continued doing bioinformatics analysis. 28 proteins had been screened out as the main effective ingredients for degrading cyanobacteria. However, we thought some of these proteins might be useless. Many properties of proteins can be obtained by sequence, so we compared the similarities between our unknown proteins' sequences and known protein sequences by searching database, and predict the function of proteins using BLAST. Methods of bioinformatics are used to further analyze and simplify the proteome. Comparing with other digestive functional proteomes, such as cathepsin, in vitro digestion of near-source organisms, the specific domain was analyzed and the specific proteins were screened.

Week13 2019.06.17-2019.06.23

Based on our sequenced proteins and combined with relevant literature, we use nucleic acid (NCBI, EMBL, DDBJ) and protein (SWISSPROT) databases to identify homologous genes or proteins of the corresponding proteins and analyze their evolutionary history. In the process of database mining, we analyze the potential information in other people's data, find out the characteristic sequences of homologous proteins, and assist the experimental design. We used the *BLAST* tool of web page version to obtain homologous sequence data, and used ClustalW in Mega for multi-sequence alignment.

Week14 2019.06.24-2019.06.30

We submitted the sequence to company, and they synthesized 28 proteases. At the same time, we got the plasmid from the company and planned to transform it into E. coli for expression.
According to the results of bioinformatics analysis, we chose to express cathepsin B.

Week15 2019.07.01-2019.07.07

Uploaded our safety form and project inspiration/description!
We constructed our own part. The vector we used is pET28b expression vector. The plasmid map and polyclonal site information are shown below. Target gene was cloned E.coli DH5 α and expressed in E. coli BL21 (DE3). For more details, please refer to Parts.

Week16 2019.07.08-2019.07.14

This week we visited MetaBD Biotechnology Co. Ltd. in Wuxi, Jiangsu province on 13rd July. MetaBD Biotechnology Co., Ltd. was established in Wuxi, Jiangsu province in April 2016, focusing on industrialized applications of high-throughput sequencing technologies of Genomics, Metagenomics, Metatranscriptomics and Metaproteomics in microbe, human health and life sciences. Our PI, Professor Lu holds the post of expert advisor, providing technical guidance and help for the company. We also learned about Algae-Hub (www.algaehub.cn), a large data platform that gathers information about algae species to promote the study and popularization of algae. After returning to school, we collected water samples from Taihu Lake in Wuxi and Jiulonghu Lake in Nanjing, and analyzed the species of algae. We uploaded their information, and shared our research results online.

Week18 2019.07.15-2019.07.21

We visited the Wuxi Environment Monitor Center to get the picture of monitor system of the lake's aquatic ecosystem. There, officers mainly introduced the methods to us that they used to decide the eco-conditions. We visited the ecology monitor center, which mainly focuses on water property and met engineer Song, who is responsible for processing data sent by satellites. Which imprinted us most is the exchange and corporation between different sections, the integrates utilization of data and information makes it possible for researchers to spend less time and energy to gain higher efficiency. Plus, the way automatic monitor combined with manual operation also inspires us the advance of science and technology not only can emancipate human sources, but also devote to be a man Friday in the field of environmental and human safety. On Friday, we went to another campus Jiulonghu campus, where the Jiulonghu lake is also polluted by algae. We communicated with the manager and provided our special algae-controlling methods in this project.

Week19 2019.07.22-2019.07.28

We received the proteins and microcystin LR and RR. We diluted all the proteins and algal toxin to 50mg/L. Each one of the 28 proteins and algal toxin was mixed together with equal quality and the total volume was up to 200ul by adding PSB. After reacting at room temperature for 24h, the mixture was sent to do analysis of HPLC -MS/MS.

Week20 2019.07.29-2019.08.04

We analyzed the result of HPLC-MS/MS. For microcystin LR, cathepsin B has a significant effect while for microcystin RR, the combination of subtilisin-like protease and carboxypeptidase Z/N is most effective.

Week21 2019.08.05-2019.08.11

We designed a questionnaire survey. Through it we hope to learn about the public's views on the outbreak of cyanobacteria and genetic engineering.

Week22 2019.08.12-2019.08.18

On August 13, we went to the China Taihu algae water separation station for further survey. Through this investigation, we understand that most of the algae treatment schemes today are based on incineration, which is not harmless and can create favorable social value. The results of this study prove that our project is of great significance. Afterwards, we went to Water Bureau at Wuxi. In order to understand the former progress and ideas of Wuxi Municipal Government on the control of cyanobacteria pollution in Taihu Lake Basin, we interviewed Zhang Zhenghui, deputy director of the cyanobacteria Control Office of Wuxi Water Bureau. During the interview, Director Zhang introduced to us the history and achievements of cyanobacteria control in Wuxi City, the way and scale of cyanobacteria salvage, the methods of follow-up treatment as well as the attitude and expectation of innovative treatment and utilization prospects, which made the project team have a more concrete and realistic understanding of the demand and prospects for the future engineering application of our project in the control of cyanobacteria pollution.

In this week, we received 2019 DNA Distribution Kit Plates! The part we chose is BBa_K398331, from 2010 iGEM team iGEM10_TU_Delft. Our aim is to characterize the pCaiF promoter. We are very interested in this promoter. According to the description of this part, pCaiF is a natural promoter found in E. coli K12, which regulates the expression of genes involved in the degradation of non-glucose carbon sources. The promoter is regulated by the level of cAMP. At low glucose level, the level of cAMP increases. CRP binds to cAMP to form complex cAMP-CRP. cAMP-CRP binds to pCaiF and activates the transcription of downstream components. Therefore, we hope to observe the experimental results: the expression of GFP in high glucose should be lower than that in low glucose.

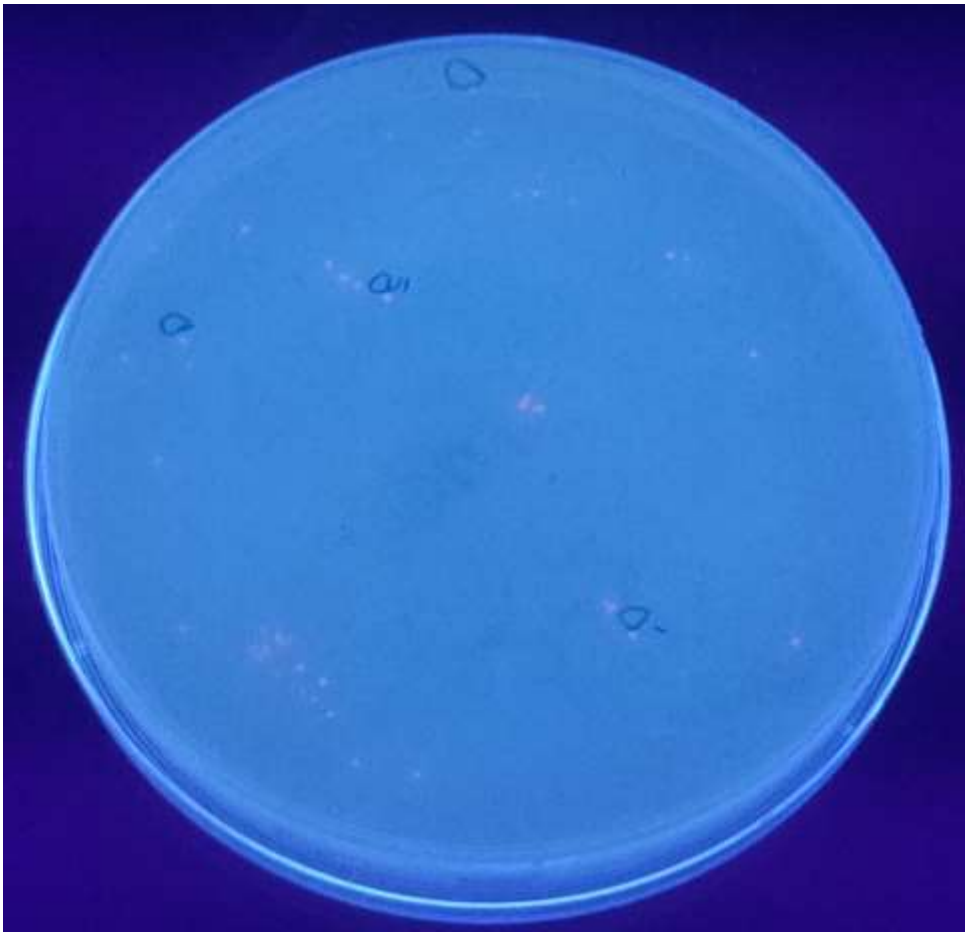
Week23 2019.08.19-2019.08.25

The 6th Conference of China iGEMer Community (CCiC) was held in Shenzhen from August 19th to 23rd, 2019. At the venue of CCiC, we made a presentation on our project. Our display had achieved good results on the spot. The judges and the students at the scene were very interested in the content of our project and put forward a series of related questions and suggestions. The judges' questions focused on the cost and goals of our projects. The judges believed that the cost of our project may be high, and the goal of our project should be to degrade algal toxins. Based on the opinions of the judges, we had made some adjustments to the subsequent experimental programs.

The students of other teams are very interested in our project. After our presentation, many teams have contacted us and hoped to further exchange the project content. After our presentation, we also discussed with other teams' members. Our discussion focused on academic integrity and project sustainability.

Week24 2019.08.26-2019.09.01

We made competent cells. Before characterizing, first we tested our competent cell efficiency. We tried different concentrations of cells plated. When diluted 10000 times, countable colonies can be obtained. Here is one example:



We collated the results of last week's experiment.

DNA concentration	Colonies(100ng/ul)	Colonies(10ng/ul)
Eg.1	321	47
Eg.2	350	31
Eg.3	319	42
Average	330	40

The measurement "Amount of DNA plated" refers to how much DNA was plated onto each agar plate, not the total amount of DNA used per transformation. You can calculate this number using the following equation:

Amount of DNA plated (ng) = Volume DNA added (1 µL) x concentration of DNA (refer to vial, convert to ng/µL) x [volume plated (100 µL) / total reaction volume (1000 µL)]

Efficiency (in cfu/µg) = [colonies on plate (cfu) / Amount of DNA plated (ng)] x 1000 (ng/µg)

DNA concentration	100ng/ul	10ng/ul
Efficiency (in cfu/µg)	$3.3*10^8$	$4*10^8$

Now we started to characterize the Part BBa_K398331. The experimental characterization was documented on the main page of BBa_K398331(这个 part 的链接).

Week25 2019.09.02-2019.09.08

On Monday (2019.09.02), we went to near-by residential area, commercial streets, primary school and parks. We interviewed some passers-by. We held a poster board that displays our cartoon character, algae terminator, and another one designed like a frame so people can take photos with it. Another day we went to Chenxianjie Street Community, a nearby children's activity center, where we wanted to promote the knowledge related to experiment safety through games and interaction. We introduced what iGEM is, what our project is about and more knowledge on lab security.

Week26 2019.09.09-2019.09.15

We consulted two companies, Wecomput Tech and modekeji, learning about the principle and technology of Molecular Docking and Homology Modeling. Molecular docking is a technical method to simulate the interaction between ligand small molecules and receptor biological macromolecules based on the "lock-key principle" of ligand-receptor interaction.

Week27 2019.09.16-2019.09.22

We completed the Homology Modelling. It is well known that a protein embodies its activity and biological functions only when its linear sequence of amino acids folds into specific spatial conformation, which is crucial for biology research. In order to delve into the proteins, we are researching, the detailed information of their three-dimensional structure is required. But it's difficult and time-consuming to determinate protein structure by X-ray and NMR. So, we chose computer modeling to obtaine predictive models of three-dimensional protein structures. In our project, proteins developed from natural creatures, amphioxus, are shown to have high homology with known protein sequences. Thus, we conduct modeling process mainly using SWISS-MODEL to further predict the three-dimensional structure of our gained proteins.

Week28 2019.09.23-2019.09.29

We did protein–ligand docking this week. It predicts the position of a ligand when it is bound to its receptor molecule, the protein. The interaction between ligand and receptor is a process of molecular recognition, including electrostatic interaction, hydrogen bonding interaction, hydrophobic interaction, van der Waals interaction and so on. The binding mode and affinity between them can be predicted by calculation.
Design our team banner and members' cartoon creative photos.

Week29 2019.09.30-2019.10.06

We bought the Cathepsin L Activity Fluorometric Assay Kit, Cathepsin D Activity Fluorometric Assay Kit, Cathepsin B Activity Fluorometric Assay Kit, Lipase Activity Assay Kit, Lysozyme Activity Assay Kit, to test the corresponding proteins' activity.

Week30 2019.10.07-2019.10.13

Summarize and report all the work we have done and working hard on Wiki.

Week31 2019.10.14-2019.10.20

Optimize some details on Wiki.