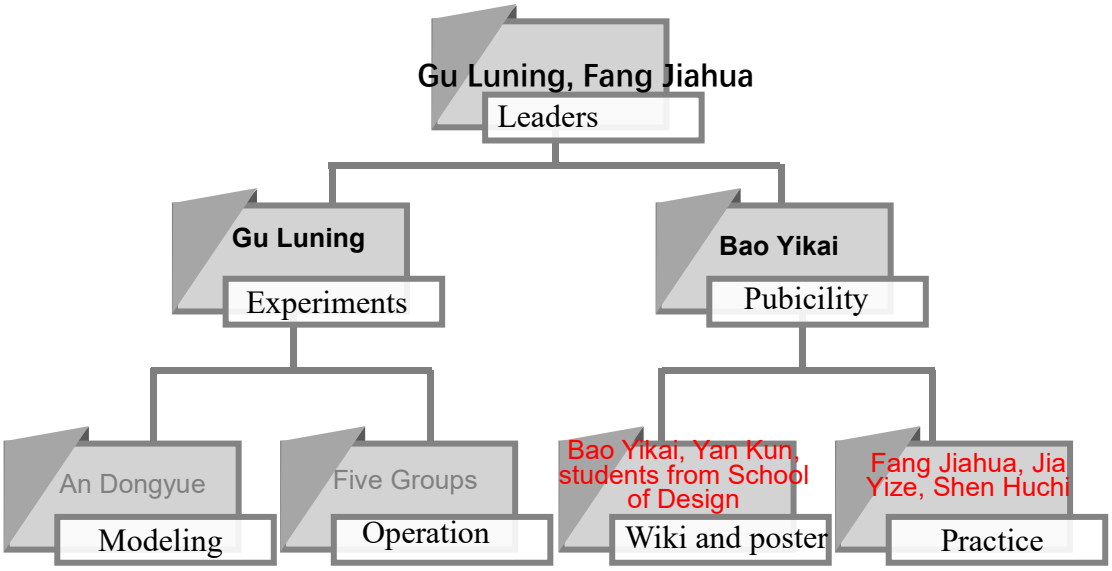
11

TEAM

Team Formation

We found that γ-GPA is an essential polymer with large utilization potentiality. So we want to build a team to conduct more study on it. In this month, we invited many outstanding students from various majors and formed our iGEM team. Meanwhile, we sincerely invited Teacher Xu Guoqiang, Teacher Zhang Xiaomei, and Teacher Zhang xiaojuan as our leading teachers. In this month, we also divided the work to every team member. To improve students' knowledge and share their understanding about this project, we also arranged different people to report a literature that they read on the group conferences.



Five groups of operation:

Gu Luning, Zhang Jianan, Jia Yize	(Directed by Cao Rong)
Fang Jiahua, Bao Yikai	(Directed by Cheng Hui)
Chi Kaijun, Gao Haixin	(Directed by Zhu Yaxin)
Cao Yu, Tao Zhenyan	(Directed by Duan Yanting)
Ma Qiaoqiao	(Directed by Teacher Qian)

The Arrangement of Literature Report on IGEM Group Conference	
3.17 Week 5th	Bao Yikai, An Dongyue

3.24 Week 6th	Shen Huchi
3.31 Week 7th	Tao Zhenyan
4.14 Week 8th	Gao Haixin
4.21 Week 10th	Gu Luning
4.28 Week 11th	Fang Jiahua
5.5 Week 12th	Yan Kun
5.12 Week 13th	Jia Yize
5.19 Week 14th	Ma Qiaoqiao
5.26 Week 15th	Zhang Jianan
6.2 Week 16th	Chi Kaijun

12

HP

12.14 Factory field Investigation

Through the literature research, we knew that the different ratios of L-glutamic acid and D-glutamic acid residues in polyglutamic acid will affect its properties. At the same time, we also found many advantages of L-glutamate-rich γ-PGA, but literature research showed that the production of L-glutamate-rich γ-PGA was low. Therefore, we hoped to conduct a field survey on the production plant of polyglutamic acid to obtain more information about the production and market of polyglutamic acid, so as to promote the progress of the project.

1

Winter Holiday, Yeah!

2

EXPERIMENT

Chose two sources of γ-PGA synthase

2.5-2.15
After lots of literature investigation and factory field investigation in winter holiday, we found that Bacillus Subtilis is a bacterial strain mainly used to producing D-polyglutamic acid with glutamic acid added while the *Corynebacterium glutamicum* can synthesis L-polyglutamic acid directly from glucose. So, we decided to use the *Corynebacterium glutamicum* as chassis microorganism.
We referred to the papers about the synthesis of γ-PGA and found that in Bacillus licheniformis and Bacillus subtilis, the key genes of which are *capB, capC, capA* and *pgsB,pgsC* and *pgsA* respectively. We decided to construct plasmids with their genes and wanted to figure out which source of genes performed better.

2.16-2.27
We designed our experiments and made the schedule for experiments.

3

EXPERIMENT

Plasmid construction

3.1-3.7
We bought primers. With the genomes of Bacillus licheniformis and Bacillus subtilis as models, we augmented the fragments of *capB, capc*, and *capA* via PCR. To improve the concentration, we repeated the experiments for many times and several of them failed.
Applying the technique of isoenzyme technology, we conducted the double enzyme digestion on the target genes and then linking the fragments to the plasmids. We transformed the plasmids into the competent cells of Escherichia ColiJM109 and selected the transformants on the LB culture medium. But we failed.

3.8-3.19
Repeated the experiments we did in the last week and we got the transformants. Then we conducted PCR and digestion to confirm the correctness.
With the genomes of *Bacillus subtilis* as models, the target genes of *pgsB,pgsC*, and *pgsA*. We repeated the experiments for many times.
At the same time, we tried to link the genes of *capB, capC*, and *capA* to one carrier pZM1, but failed.

3.20-3.31
We conducted the double enzyme digestion on the target genes and then linking the fragments to the plasmids. We transformed the plasmids into the competent cells of Escherichia Coli, JM109 and selected the transformants on the LB culture medium. After the confirm of PCR and digestion, we got the right transformations.

4

EXPERIMENT

4.1-4.7
We tried to link *pgsB, pgsC*, and *pgsA* to the plasmid pZM1. We did not finish.
Our partner studied some models in the last year's composition and built some simple constructive models.

4.8-4.14
We succeeded in constructing the plasmid and transformed it to the Escherichia Coli. After augmenting, we extracted the plasmids to conduct the digestion of enzymes to confirm. We also sent the plasmids to sequencing but haven't received the results. We failed to conduct the plasmids of *capBCA,* but we tried again.

MODEL: We consult the mechanism and the mathematic models of the lac operon and built the regulation and control model.

4.14-4.21
The result came out. We got the recombinant plasmid pZM1(P_{tac})-*capBCA*.
We succeeded in linking *pgsB, pgsC,* and *pgsA* to pZM1 and transformed them into *Escherichia Coli* for augmentation. After that, we extracted the plasmids to conduct double digests for confirm. The gene was sent for sequencing and the result was unknown.

pZM1(P_{tac})-*capBCA*-Corynebacterium glutamicum 13032

4.22-4.30
We transferred the plasmid pZM1(P_{tac})-*capBCA* into the competent cells of Corynebacterium glutamicum 13032. The cells were coated to the culture medium with Kan. But none bacteria stain was available.
We received the result that we got the recombinant plasmid pZM1(P_{tac})-*pgsBCA*.
The transformation was successful. Single bacteria strains were inoculated to the seed medium. We cultivated the single bacteria strains and took samples according to times.
Besides, we transferred the plasmid pZM1(P_{tac})-*capBCA* into the competent cells of Corynebacterium glutamicum 13032. The cells were coated to the culture medium with Kan. Single bacteria strains were inoculated to the seed medium. We cultivated the single bacteria strains and took samples according to times.

MODEL: We decided to conduct regulation and control experiments with IPTG introducing. Before this, we decided to do some forecast.

We consult the mechanism and the mathematic models of the lac operon and built the regulation and control model.

HP

4.7 Popularization of Synthetic Biology in Vanke City Garden

Our team members visited the community Vanke City Garden, which is near Jiangnan University. We showed people there about γ-PGA and how γ-PGA help forming a kind of materials called hydrogel.

4.13 iGEM Shanghai Regional Conference

Members of totally twenty old or new teams which are from various provinces gathered together in Shanghai New York University. We had a heated discussion about our teams' projects. New teams learned some experience from old teams, such as how to promote projects. It was a fruitful trip to Shanghai.

4.19-20 Popularization of Synthetic Biology Sports Meeting

During the sports days in our school, members of JNU-China built several panels in the vacancy areas among spectator seats and showed some knowledge about synthetic biology and iGEM, as well as γ- polyglutamic acid and its synthesis.

Time	People	Tasks
Before the opening ceremony（8:30）	Gao Haixin, Gu Luning, Jia Yize, Fang Jiahua	All goods and materials in place beforehand 1. Jia Yize is responsible for posters and other pubilicity materials. 2. Gao Haixin and Gu Luning should guarantee panels in place. 3. Jiahua shoulders for taking facial masks.
9:35-10;00	Everyone, take photos.	Carry out the activity and take some photos.
10:00-11:00	Everyone.	1. Yan Kun ia responsible

	11:00，Group photo.	for collecting photos. 2. All people should conduct publicity. 3. Girls for guidance as boys for explanation.
14:00-15:30		Publicity, chatting and eating.
15:30		Pick up things. Hand all materials for publicity to Bao Yikai and Yan Kun for reservation. Others help carrying things.

After the publicity, Shen Huchi is responsible for the drafts and Yan Kun should provide pictures for Shen Huchi. All these things should be checked by Teacher Zhang Xiaojuan.

TEAM

Our members designed a series of posters to make more people know about our project.

5

EXPERIMENT

Fermentation

The bacterial concentrations and the molecular weights of the samples were measured. Via H-NMP, the product of the fermentation was γ-PGA.
We continued fermentation. After fermentation, the bacterial concentrations and the molecular weights of the samples were measured. Via H-NMP, the product of the fermentation was γ-PGA. Besides, we transformed the plasmid pZM1(P_{tac})-*capBCA* and pZM1(Ptac)-*pgsBCA* seperately into *Corynebacterium glutamicum* F343. But we failed for several times and succeeded finally.

MODEL：
We consulted the literature and found some data.

HP

5.7 Poly Experimental Kindergarten

The HP team of JNU-China of Jiangnan University came to Baoli Kindergarten in Wuxi City. As the keynote speaker, Cao Yu prepared a "biology class" for the kids and we learned a more flexible and relevant science popularization method with the help of students and teachers.

5.15 Wuxi People's Hospital

Members of JNU-China paid a visit to Wuxi People's hospital and consulted the doctors about the clinic problems of using MTX, which showed the direction of the following experiments.

5.17-18 Public Science Day

School of Bioengineering in Jiangnan University held the Science public day for three days and accepted the undergraduates in our school, students from primary schools in Wuxi and their parents, which was totally more than 200 people. Volunteers from JNU-China introduced them into the amazing world of micro-biology, showed them the mystery and interest of micro-biology, and presented the latest achievements in Bioengineering via presentations, visit to plants and the museum of liquor, creative experiments and drawing about science dreams. Meanwhile,

volunteers talked with freshmen who attended this activity, showing our project to them and encouraged them to take part in iGEM next time.

5.23 Xin'an Street Community Health Service Center

We found that L-glutamic-rich γ-PGA may be applied to treat the rheumatoid arthritis (RA) after viewing literature. We connected with the doctors in Xin’an Street Community Health Service Center for further investigation. Learning that the Xin’an Street Community Health Service Center is experimenting with MTX drugs, and they also need L-glutamic-rich γ-PGA samples. So we decided to cooperate with them and provide them with L-glutamic-rich γ-PGA.

5.26 Wuxi Museum Biotechnology Zone

Under the guidance of Teacher Xu and Teacher Zhang, we, JNU-China, with Wuxi museum, conducted a bio-technology popularization to the youth from the whole city. All the members of our team came to the museum. They introduced the basic knowledge of Biology to the children and helped them practicing to feel the charm of Biology.

5.29 Wuxi Mini Meet-up

We invited the iGEMers of ECUST-CHINA and Teacher Li, the adviser of BIT-CHINA coming to Jiangnan University for a deep discussion on projects and details of iGEM.

TEAM

Do a lot of preparatory work to help HP operate better.

6

EXPERIMENT

Fermentation optimization

We selected some singe bacterial colonies and inoculated them to the culture medium for fermentation. We took samples according to the schedule. Besides, we repeat the process for many times.
We treated samples and determinate D / L ratios, molecular weight and the yield.
Via comparing data, we found that the *Corynebacterium glutamicum* F343 transformed with the plasmid pZM1(P_{tac})-*capBCA* has the highest productivity. Therefore, we selected this bacterial strain for the following research.

MODEL：We chose the *Corynebacterium glutamicum* F343 transformed with the plasmid pZM1(P_{tac})-*capBCA* as chassis microorganism. We design the scheme for regulation and control experiments of IPTG introducing. At the same time, we built a model about how the amount of enzyme influenced by the times of adding IPTG and did some forecast. We want to do some experiments to confirm.
Besides, We chose the *Corynebacterium glutamicum* F343 transformed with the plasmid pZM1(P_{tac})-*capBCA* as chassis microorganism. We designed the scheme for regulation and control experiments of IPTG introducing.

HP

6.3 Wuxi Big Bridge Academy

As the speaker, Jiahua Fang, the captain of JNU-China, prepared an activity called “The Class of Biology” for the students of the fifth grade and International Department. Starting from the story of the Spiderman, Jiahua Fang explain the structure, the functions and the applications of gemes to the students of the fifth grade. With the beginning of Mendel’s story, she introduced the structure and the applications of the geme to the International Department of the high school, emphasizing on the development of Genetic Engineering and Synthetic Biology. Finally, we encouraged them to take part in iGEM in the future.

6.19-6.21 Summer Program for Our Graduates on Synthetic Biology

School of Bioengineering in Jiangnan University invited nine famous scholars from international colleges and universities as teachers of Summer Program for Our Graduates on Synthetic Biology. These scholars introduced the theoretical knowledge and the latest achievement of Biology. The content varied from glycobiology to the process optimization and controlling. The members of JNU-China were organized to attend the classes and study further.

6.20 International scholars face to face

At this time, we still have a lot of questions about the expression and modeling of the dissolved oxygen circuit. Therefore, we are fortunate to have invited Professor Yajun Yan and Professor Peng Xu, who have returned from the United States to Jiangnan University for academic reporting. Professor Peng Xu explained the dissolved oxygen circuit to us. The opening conditions are not clear and are not suitable for use in our experiments, so we gave up this circuit. Regarding modeling, the professor suggested that we establish a model for the IPTG-induced expression circuit to predict the highest yield of γ-PGA. In addition, the professor also suggested that we add the oscillating expression circuit and consider the population effects of the strain.

7

EXPERIMENT

Tailored D/L ratios

After chatting with the experts in China-Japan Friendship Hospital, we decided to conduct the regulation and control experiments about the ratios between D-glutamic acid and L-glutamic acid in the γ-PGA. We consulted the literature and made the plan of experiments.
We consulted the literature for some data.
We found some data and built the model, which shows that the ratios of D-glutamic acid and L-glutamic acid in the γ-PGA could be changed via regulating the gene expression of synthetase and racemase.

MODEL：We conducted the fermentation and took samples. We treated the samples and sent the samples for examination. We got the results.

HP

7.5 China-Japan Friendship Hospital

Team members of JNU-China consulted the pharmacists from Beijing China-Japan Friendship Hospital about the clinic application of MTX. The experts answered some questions and gave some guiding suggestions.

7.6 Beijing Normal University Meetup

We had a heated discussion about projects with teams from Pecking University and Beijing Normal University. During the conference, teams showed their own projects, promoted questions and gave advice.

7.7 iGEM Meetup - Beijing District

We interacted with teams from high schools in Beijing and several undergraduate teams about tasks. We got some guidance about the CCiC and presentations.

7.9-7.12 Conference in Shaanxi Province

Members travelled to Hanzhong and Xi’an, doing the presentations and exchanging ideas with the teachers and students in Shaanxi University of Technology and Shaanxi University of Science and Technology. Starting from Rheumatoid Arthritis, Jiahua Fang gave a speech about Synthetic Biology and iGEM. Yu Cao introduced the meaning of Synthetic Biology lively from standardization and modulization to decoupling.

7.22 Collaboretion with Xin'an Street Community Health Service Center(1)

We provided the L-glutamate-rich γ-PGA that we had produced for the related department in Xin'an Street Community Health Service Center to conduct follow-up studies.

TEAM

8

EXPERIMENT

Roughly regulation

We constructed the plasmid pZM1-*capBCA*-Ptac-*racE* and the plasmid was right.
We chose the successfully-constructed bacterial strains for fermentation and took samples.
We dealt with the samples and sent them for examinations. After getting the data, we treated them. We conducted the real time PCR to compare the transcriptional level of *racE* gene in the bacterial strain fc1 and fc2. The result showed that the series connection of two *lacO* genes can lower the expression level of glutamic acid racemase gene.
We used the bacterial strain FC2 for fermentation and took samples.

HP

8.19-8.24 6th Conference of China iGEMer Community

JNU-China arrived in Shenzhen, participating in the 6th CCiC whose topic was Synbiopunk. Seventy teams from over sixty high schools across the county showed their own projects. Hundreds of students from different majors showing their teams' unusual but wonderful ideas.

TEAM

9

EXPERIMENT

We purified the γ-PGA in different times and made them hydrolytic derivative. Then we used HPLC to exam ratios of D-glutamic acids and L-glutamic acids.
We regulated the level of BBa_K2963006 on the RBS Calculator, and then designed the primer according to the new RBS.
We tried to construct the plasmid pZM1-capBCA-Ptac- RL/RH -*racE*, but failed.
We tried again and finally got the recombinant plasmid. We transformed the plasmid into the Escherichia coli and confirmed it via PCR. After examined, it was sent for sequencing.
The plasmid was transferred into the *Corynebacterium glutamicum* F343. We chose the right bacterial colony for fermentation and took samples.
We dealt with the samples and sent them for examination. After we got the data, we dealt with them.

TEAM

Assigned the task of text draft, summarized the preliminary work and built the web page.

10

EXPERIMENT

The experimental work is basically completed, and the data of bicistron is obtained.

TEAM

Stay up late to provide drafts for webpage construction, make various designs and presentations