Experimental procedures

20/07/2019
Background:
Primers are expected to arrive today, if so, then process it.
PCR amplify afterwards

Procedure:

Check if we are receiving six tubes of primer.
Dilute primer to 100mM

-Centrifuge at 5000rpm for 15s
-uncap the tubes carefully, place them on the ice box
-add ddH2O following the instructions on the side of the tubes to make it 100mM

Prepare PCR mix
Do the whole thing on ice and make sure it is always cool enough.

-take a new tube, name it “rdfz igem trans-pcr mix” note the date on its side.

5*reaction buffer	40μl
10mM dNTPs	20μl
DNA Polymerase	4μl
ddH20	36μl
	Total 100μl

-note to add water before DNA polymerase
-place it on the centrifuge with another tube with 100μl water as balance and centrifuge at 5000rpm for 15s

PCR amplify
Do the whole process on ice and make sure it is always cool enough.

-Amplify K2034000, K1322231, and K2656025 as template in three tube
-For each tube, add

PCR mix	25μl
F primer	1μl 10mM
R primer	1μl 10mM
Template	1μl
ddH2O	22μl

-centrifuge them at 5000rpm for 10s

-Place the three tubes in the PCR machine, and run file pcrvector with sample volume 50μl
-place the three tubes in the 4 degree Celsius

26/07/2019
Background:
Dilute to 10mM if primer bsmt limst k257303 arrives
Check if primer vf vr works, amplify it and see the results.

Procedure:

Check if we are receiving six tubes of primer (bsmt limst k257303).
Dilute primer to 10mM

-Centrifuge at 5000rpm for 15s
-for each tube: uncap the tubes carefully, place them on the ice box
-add 10x of ddH2O suggested by the instructions on the side of the tubes to make it 10mM

PCR amplify

20μl system:

Taq PCR mix-20	10μl
F primer (VF&VR)	1μl 10mM
R primer (VF&VR)	1μl 10mM
Template pSB3K3	1μl
ddH2O	7μl

-centrifuge them at 5000rpm for 10s
-place tubes in the PCR machine

Temp	
96	5min
98	30s
58	30s
72	90s
72	5min
4	forever
2-4 30cycle	

-place the six tubes in the 4 degree Celsius

Gel electrophoresis
-make gel with 30ml TAE buffer, 0.3g agarose 1μl DNA dye
-insert products from PCR along with 2kb DNA marker
-run at 140V for 20min
-check the results

28/07/2019
PCR amplify

Primer: Seq VF Seq VR, and new primer (f&r)
Template: Gibson product bsmt limst 2753003

Six tubes in total
20µl system:

Taq PCR mix-20	10µl
F primer	1µl 10mM
R primer	1µl 10mM
Template	1µl
ddH2O	7µl

-centrifuge them at 5000rpm for 10s
-place tubes in the PCR machine

Temp	
96	5min
98	30s
58	30s
72	90s
72	5min
4	forever

2-4 30cycle

-place the six tubes in the 4 degree Celsius

Gel electrophoresis
-make gel with 30ml TAE buffer, 0.3g agarose 1µl DNA dye
-insert products from PCR along with 2kb DNA marker
-run at 140V for 20min
-check the results

Tracks

New primer 275300	New primer lsmt	New primer bsmt	Seq vf Seq vr 2753003 gibson product	Seq vf Seq vr limst gibson product	Seq vf Seq vr Bsmt gibson product	Marker 4µl

01/08/2019
Background:
Examine the reactivity of our Gibson assembly mix
BNDS will mail us our positive control

Check if the things have arrived, which contains a tube of carrier pSB4C5 and a tube of positive control.
Label pSB4C5 and place it in the plasmid kit in -20°C
Place positive control on ice

Gibson assembly:
Do all procedures on ice

Material:
-PCR tube
-Gibson assembly Mix
-Gibson assembly Mix positive control
-Backbone(18.0ng/μl）
-bsmt(29.8ng/μl ）
-ddH2O

Two tubes in total

Label mix as 1
Label positive control as 2

Prepare reaction system(10μl)
For each tube:
-Mix/control 5μl
-Backbone 2μl
-BSMT 1μl
-ddH2O 2μl

Note:
place ddH2O before mix
unfreeze backbone and target genes prior

Temp:
50°Cx2hrs
12°Cxforever

Run gibsonassem

PCR amplify:

Primer:
-Seq VF Seq VR, for target gene(f&r)
-Vector for carrier (f&r)
Template: Gibson product bsmt limst 2753003

Three primers, two gibson product
Six tubes in total
20μl system:

Taq PCR mix-20	10μl
F primer	1μl 10mM

R primer 1µl 10mM
Template 1µl
ddH2O 7µl

-centrifuge them at 5000rpm for 10s
-place tubes in the PCR machine

Temp
96 5min
98 30s
58 30s
72 90s
72 5min
4 forever
2-4 30cycle

-place the six tubes in the 4 degree Celsius

Gel electrophoresis
-make gel with 30ml TAE buffer, 0.3g agarose 1µl DNA dye
-insert products from PCR along with 2kb DNA marker
-run at 140V for 20min
-check the results

2 bsmt primer	2 vector	2 seq v	1 Bsmt primer	1 vector	1 seq v	Marker

04/08/2019
Background:
We done the positive control yesterday. Check whether our medium have bacteria grown on it. pSB3K3 backbones in the kit is amplified unsuccessfully, the stripes are diffused, the annealing temperature could be wrong, redo it today.
Primers for pSB4C5 is expected to arrive today

Check if there is two tubes of new primer
Dilute primer to 10mM

-Centrifuge at 5000rpm for 15s
-for each tube: uncap the tubes carefully, place them on the ice box
-add 10x of ddH2O suggested by the instructions on the side of the tubes to make it 10mM
-place them in a new kit labeled as RDFZ-China pSB4c5 10mM+

PCR Amplify:

T5 per mix
Primer: VF VR, and new pSB4C5 primer (f&r)
Template: pSB3K3 and pSB4C5

-For each tube, add

PCR mix	25μl
F primer	1μl 10mM
R primer	1μl 10mM
Template	1μl
ddH2O	22μl

-centrifuge them at 5000rpm for 10s
-place tubes in the PCR machine

Temp

96	5min
98	30s
58	30s
72	90s
72	5min
4	forever

2-4 30cycle

-place the six tubes in the 4 degree Celsius

Gel electrophoresis
-make gel with 30ml TAE buffer, 0.3g agarose 1μl DNA dye
-insert products from PCR along with 2kb DNA marker
-run at 140V for 20min
-check the results

15/08/2019
Background:
No PCR experiment today
Examine Gibson assembly positive control
Linkage and Transformation
Culture substrate for Ampicillin resistance selection is depleted, need to prepare more.

Culture substrate preparation:
Do whole operation on Clean Bench
200ml water
6.6g nutrient agarose

Autoclaving

Split to 150ml and 50 ml
Add chloramphenicol to the 150ml substrate
Add Ampicillin to the 50ml substrate
Place them on culture plates, approximately 25ml for each, wait for 20-30min label afterwards.

Linking:
System
5μl positive control
5μl Gibson assembly mix
All operations on ice

Temp:
50ºC 60min
12ºC forever

Transform:
Follow the booklet
Place bacteria on Ampicillin substrate containing plates.
Place them in the incubation device upside-down

Experimental results and notes

2019.7.11

Goal:

Do PCR of the backbone pSB3K3, look at the strip after finishing electrophoresis and cut the gel.

Background:
Because the amount of PCR product in the third lane was small, the color of the strip was too light, indicating that the fragments were too small, so we did not do the gel extraction today, and stored it.

Procedure:
For each tube, add

PCR mix	25μl
F primer	1μl 10mM
R primer	1μl 10mM
Template	1μl

ddH2O 22μl
-centrifuge them at 5000rpm for 10s

-Place the three tubes in the PCR machine, and run file pcrvector with sample volume 50μl
-place the three tubes in the 4 degree Celsius

Note:
• Be careful when taking the PCR products out, and try to hold the top of the vessel
• Do not touch the white part of the pipette tip
• Dispose of the pipette to the maximum volume
• Wear gloves in contaminated areas when making gel
• Wear gloves when writing the experiment log. When waiting, you can take out the blue notebook in the first drawer on the right-hand side and write the paper version (with a pencil) of the log.
• After placing the gel, you should make sure the water surface has passed the gel surface.
• When weighing agarose, pay attention to whether it starts with 0 or not.
• Be careful when removing the heated flask.
• Be careful when cutting the gel: don't use too much force
• There is a mobile phone bag placed in the place where the experimental notebook is placed. When using a mobile phone with gloves, you should put it in the mobile phone bag.

2019.7.13

Goal:

1. Complete the PCR that failed in the previous day and the PCR that is scheduled to be completed today
2. Making gel, electrophoresis, checking results
3. Do gel extraction for successful PCR products (gel extraction)
4. Nanodrop (measuring DNA concentration)
5. Complete the experiment record, and perfectly hand over

Target genes today: bmst, lmst, gds, k1322231, k2034000 (labelled 1 on small centrifuge tube), k2753003 (labeled 2 on small centrifuge tube), 2598059 (labeled 3 on small centrifuge tube)

Procedure:
For each tube, add
PCR mix 25μl
F primer 1μl 10mM
R primer 1μl 10mM
Template 1μl
ddH2O 22μl
-centrifuge them at 5000rpm for 10s

-Place the three tubes in the PCR machine, and run file PCR vector with sample volume 50μl
-place three tubes in the 4 degree Celsius refrigerator

Gel electrophoresis
-make gel with 30ml TAE buffer, 0.3g agarose 1μl DNA dye
-insert products from PCR along with 2kb DNA marker
-run at 140V for 20min
-check the results

Results:
In today's results, we conducted a total of 7 PCRs, but only four were successful by electrophoresis, namely bmst, lmst, gds, and k2753003. The failed ones were not matched by the number of base pairs (bp).
We did not do Nanodrop (no time for quq)
Unsuccessful PCR for three additional target genes

2019.7.14
Goal:
Use Nanodrop to measure concentration (preparing for subsequent connections)
PCR (delaying the unsuccessful ones on previous day and starting doing today's ones)
Making medium (preparing for subsequent coating plates)
Completed today:
Use Nanodrop to measure concentration (measured on July 12th and 13th, today's not measured)
PCR (target genes) J45003, K1129005, K2656025 (the name on the centrifuge tube is the same)

Procedure:
Three tubes in total
50μl system
-For each tube, add
PCR mix 25μl
F primer 1μl 10mM
R primer 1μl 10mM
Template 1μl
ddH2O 22μl
-centrifuge them at 5000rpm for 10s
-place tubes in the PCR machine

Temp
96 5min
98 30s
58 30s
72 90s
72 5min
4 forever
2-4 30cycle

-place three tubes in the 4 degree Celsius refrigerator.

Gel electrophoresis
-make gel with 30ml TAE buffer, 0.3g agarose 1μl DNA dye
-insert products from PCR along with 2kb DNA marker
-run at 140V for 20min
-check the results

Nanodrop * This process is abbreviated
Purpose: To measure the concentration of DNA in (1 μl)
process:
1) Calibration: Use paper towel (equipped at the instrument side) + ddH2O (first drop and then wipe) * to do it twice
2) Use the instrument and then, add a drop on the platform
3) Test the next solution after each use
reading:
There are three values from left to right

Left side: concentration (for what you want to record on the centrifuge tube)
Middle/Right: Other values

Preparation of the culture medium
The process involves: the proportion of the material in the preparation medium + sterilization pot sterilization
1) Proportion of medium material * Adjust the quantity according to the amount of medium (material ratio is constant)
The following is the amount of related materials when formulating 1L
Trptone (protein jelly) --10g
Yeastier extract -- 5 g
NaCl -- 10g
Agar -- 20 ~ 22g
2) Put the material into the Erlenmeyer Bottle and place it in the sterilizer

Use of sterilizer
* Find a senior at the time of use. Do not operate by yourself
* Generally wait for 1 hour and maintain sterilization near the small red circle

Result:
1) nanodrop
Backbone1: 18.0 ng/ml
Backbone0: 5.0 ng/ml
Backbone2: 2.4ng/ml
Gds : 8.9bg/ml
lmst: 9.7ng/ml
1454000-1: 36.1ng/ml
1454000-2: 31.7ng/ml
bmst: 29.8 ng/ml
2753003: 25.7ng/ml
2) Electrophoresis
J45003&K1129005 Succeeded
K2656025 failed
(The second from left to right is J45003, the third is K1129005)
3) Cutting gel
J45003 successfully cut
K1129005 failed

2019.7.15

Objectives:
1. Make 5 culture media
2. ligation — synthesis of the desired plasmid by Gibson Assembly

Preparation of the culture medium
The process involves: the proportion of the material in the preparation medium + sterilization pot sterilization
1) Proportion of medium material * Adjust the quantity according to the amount of medium (material ratio is constant)
The following is the amount of related materials when formulating 1L
Trptone (protein jelly) --10g
Yeastier extract -- 5 g
NaCl -- 10g
Agar -- 20 ~ 22g
2) Put the material into the Erlenmeyer Bottle and place it in the sterilizer

Linking:
System
5μl positive control
5μl gibson assembly mix
All operations on ice

Temp:
50°C 60min
12°C forever

Transform:
Follow the booklet
Place bacteria on Ampicillin substrate containing plates.
Place them in the incubation device upside-down

Result: gel electrophoresis failed

2019.7.16

Background:
Yesterday, we did five Gibson of target genes (although we did not verify the results). lmst and bmst were transformed. If the bacteria grow up, pick up the bacteria and send them to sequencing. If the bacteria do not grow up, we will not do experiments today and dilute the remaining primers.
The bacteria are grown, but they are still relatively small, so today we will convert gds, 1454000, 2773003, and do experiments again.

Transformation:
Follow the booklet
Place bacteria on Ampicillin substrate containing plates.
Place them in the incubation device upside-down

2019.7.17

Experiment task: pick bacteria, send samples to a company in Beijing for sequence testing
Note: when inoculating the bacteria, lightly sweep the medium

Procedure:
1. First, two transformed monoclonal strains are picked up with small tips.
2. Inject the selected bacteria in the corresponding position in the new culture dish (draw board)
3. Put the small gun head into the shaking tube
4. Add 3000μl of LB medium to each shaker tube.
5. Add kanamycin to each shaker tube at a ratio of 1:1000 (3 microliters of antibiotic should be added to each shaker tube)
6. Packaging and label

2019.7.19

Background:

The sequencing of strains transformed in the past few days and the registration of10 kb gene fragments from Twist Bioscience
Outlook:
If the sequencing results come out, then carry out the bacteria preservation work.
If the primers arrive tomorrow, dilute the primers.
Three sets of PCR experiments will be conducted tomorrow

2019.7.20

Background:
We are supposed to do bacteria preservation on 20th, but the sequencing results do not come out. We don't know which one to process.
The primers arrive, so first process the primers
Then do another PCR

2019.7.21

The work to be done today:
Do PCRs for 4 target genes. Look at the strips after electrophoresis and cut the gel
Run the gel of the three target genes made yesterday and recycle the gel.
Configure LB medium.

Procedure:
For each tube, add
PCR mix 25μl
F primer 1μl 10mM
R primer 1μl 10mM
Template 1μl
ddH2O 22μl
-centrifuge them at 5000rpm for 10s

-Place the three tubes in the PCR machine, and run file pcrvector with sample volume 50μl
2.PCR setting：98°C---1 min
98°C---30s
68°C---30s
72°C---30s
30 cycles
72°C---5min
4°C---∞
-place the three tubes in the 4 degree Celsius

Gel electrophoresis
-make gel with 30ml TAE buffer, 0.3g agarose 1μl DNA dye
-insert products from PCR along with 2kb DNA marker
-run at 140V for 20min
-check the results

First lane: marker
Second lane: K2034000
Fourth lane: K1322231
Sixth lane: K256025
We only recycled the second lane because it had about a thousand and formed a strip.

First lane: marker
Second lane: K1403016

Third lane: K1129005
Fourth lane: K1129041
Sixth lane: K1129042
We recycled gel from second, third, and fourth lane because there were corresponding bands. The second band is not obvious (about 700bp). The third and fourth lane (about 1500bp and above)

Notes:
Do not bring nucleic acid dyes to contaminated area.
Maximize the range of the pipette.
Gloves should be worn in contaminated areas when you making gel.
Gel should first be put on an empty bottle, weighed the tare, then put the centrifuge tube with the strip.
When doing the eighth step of the gel extraction, which is to drop in ddH2O, for more than 0.2g, drop about 50 microliters; for 0.15g or more, drop 40 microliters; and for the smaller one, 30 microliters can be used.

Unfinished work:
What we have done: nano drop, Gibson ligation and transformation, bacterial PCR
Outlook: I hope person who will come tomorrow will do nano drop first.

2019.7.22

Experiments:
1.use Nanodrop to measure the concentration of gel extraction yesterday
2.make PCR system
3.run four results of bacteria PCR products
4.Gibson assembly
5.transform and then coat

Procedure:
1.nano drop result:
K1129005 55.2 ng/μl
K2034000 10.3 ng/μl
K1129041 18.5 ng / microliter
K1403016 8.5 ng/μl

2. Bacterial system: LMST: Mix---10 μl
Primer-F---1 microliter
Primer-R---1 microliter
ddH2O---8 microliters
20 microliters
BMST: Mix---10 microliters
Primer-F---1 microliter
Primer-R---1 microliter
Primer-M---1 microliter
ddH2O---7 microliters
20 microliters

2.PCR setting：96°C---10 min
96°C---30s
58°C---30s
72°C---90s
25 cycles
72°C---5min
4°C---∞
Gel electrophoresis

-make gel with 30ml TAE buffer, 0.3g agarose 1μl DNA dye
-insert products from PCR along with 2kb DNA marker
-run at 140V for 20min
-check the results

Gibson Assembly:
(see details in the booklet)

Transformation:
Step: (The whole process is operated in the ultra-clean station)
It must be carried out in strict accordance with the parameters and the specified time!

Dispense 50 μl of competent cells from the -80 ° C freezer and place in an ice box.
(The whole state of the competing state is on the ice, and the high temperature will make it invalid)
Add 2 μl of Gibson's product to the competent state and flick the bottle to make it evenly mixed.
(Do not touch the bottle or inner wall of the bottle to prevent the bacteria from being introduced)
Place the above mixed liquid in a -20 ° C refrigerator and let stand for 20 min.
Heat in a water bath at 42 ° C for 90 s, install with float, put
(When waiting for the water bath to 42 ° C, you can put it at -20 ° C for a while)
Place in a refrigerator at -20 ° C for 2 min.
Add 400 μl of LB medium to each tube in a clean bench
Shake at 37 °C for 40 min
Scrape the bacteria on the board and observe the surface without any obvious liquid under the light.
(The inoculated rod after burning should be used at room temperature, otherwise it will kill our bacteria)
Inoculate the inoculation stick with alcohol lamp between inoculation of different bacteria (remember to turn off the alcohol lamp in time)
Cover the Petri dish with a lid, place it in a PE glove, and place it in the incubator.

First lane: marker
Second lane: BMST 1
Third lane: BMST 2
Fifth lane: LMST 1
Seventh lanes: LMST 2
All: no results

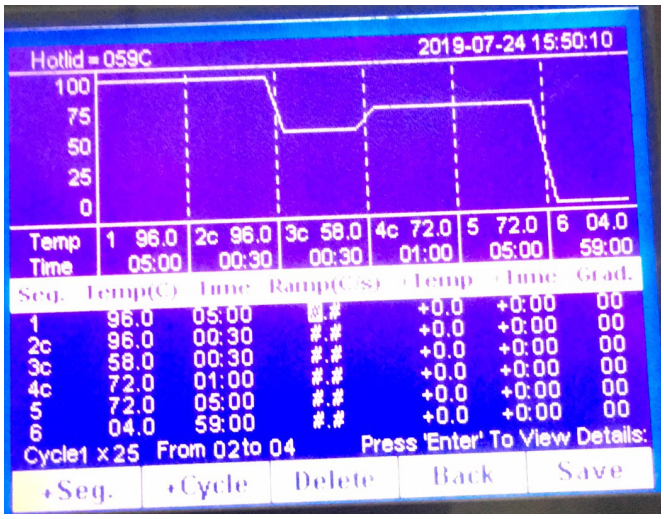
Note:
• Do not touch the inner wall and tube of the competent body with gloves when doing transformation
• Be careful with sterile objects

Jul. 24th
experimenter: Zeyuan Li Michael Jiang
Background:
Debug
Check if primer vf vr works, amplify it and see the results.

Procedure:

PCR amplify
Using two sets of enzyme in PCR mix, one of which is proven active, the other is ours
20μl system:
Taq PCR mix 10μl

F primer (VF&VR) 1μl 10mM
R primer (VF&VR) 1μl 10mM
Template pSB3K3 1μl
ddH2O 7μl
-centrifuge them at 5000rpm for 10s



-place tubes in the PCR machine
Temp
96 5min
96 30s
58 30s
72 60s
72 5min
4 forever
2-4 30cycle

Gel electrophoresis
-make gel with 30ml TAE buffer, 0.3g agarose 1μl DNA dye
-insert products from PCR along with 2kb DNA marker
-run at 140V for 20min
-check the results
Results:

It seems that our DNA polymerase can amplify target gene, therefore it's not where the problem is occurring.

Jul. 26th
experimenter: Mingyang Li

Objectives
PCR Amplifying and Gel electrophoresis for pSB3K3
Dilute Primers

Check if we are receiving six tubes of primer (bsmt-c limst-c k257 (F&R)).
Dilute primer to 10mM

-Centrifuge at 5000rpm for 15s
-for each tube: uncap the tubes carefully, place them on the ice box
-add 10x of ddH2O suggested by the instructions on the side of the tubes to make it 10mM

PCR amplify

20μl system:

Taq PCR mix-20	10μl
F primer (VF&VR)	1μl 10mM
R primer (VF&VR)	1μl 10mM
Template pSB3K3	1μl
ddH2O	7μl

-centrifuge them at 5000rpm for 10s
-place tubes in the PCR machine

Temp	
96	5min
98	30s
58	30s
72	90s
72	5min
4	forever

2-4 30cycle
Gel electrophoresis
-make gel with 30ml TAE buffer, 0.3g agarose 1μl DNA dye
-insert products from PCR along with 2kb DNA marker
-run at 140V for 20min
-check the results

Track 1：marker
Track 2：old VR,VF
Track 4：Seq VR,VF
Track 6：Seq VR, VF

Positive results for track #6 seq for track4&6 is for target gene, so the primers are fine

Jul 27th
Experimenter: Mingyang Li

Objections:
Determine whether target gene and backbones exist in bacterias (They are not, so keep incubate and determine whether the transformation is correct and whether the antibiotics are denatured)

PCR amplify

20μl system:

Taq PCR mix	10μl
F primer	1μl 10mM
R primer	1μl 10mM
ddH2O	8μl

Add bacteria

And

20μl system:

Taq PCR mix	10μl
F primer-vector	1μl 10mM
R primer-vector	1μl 10mM
ddH2O	8μl

Add bacteria

24 tubes in total

Temp	
96	10min
96	30s
58	30s
60	90s
72	5min
4	forever

2-4 25cycle

Gel electrophoresis
-make gel with 30ml TAE buffer, 0.3g agarose 1μl DNA dye
-insert products from PCR along with 2kb DNA marker
-run at 140V for 20min
-check the results

Incubation:
60ml LB medium+ 6μl Kanamycin
3ml mixture above for each tube
Select bacteria from single colony to the tubes
Note that number 7,9,12 may be different from actual bacteria
Shake for 24h

Jul 28th
Experimenter: Jingwen Li

PCR amplify
Objective:
Determine whether the gibson assembly is correct
Determine whether Kanamycin is denatured

Primer: Seq VF Seq VR, and new primer (f&r)
Template: Gibson product bsmt, limst, 2753003

Six tubes in total
20μl system:

Taq PCR mix-20	10μl
F primer	1μl 10mM
R primer	1μl 10mM
Template	1μl
ddH2O	7μl

-centrifuge them at 5000rpm for 10s

-place tubes in the PCR machine

Temp
96 5min
98 30s
58 30s
72 90s
72 5min
4 forever
2-4 30cycle

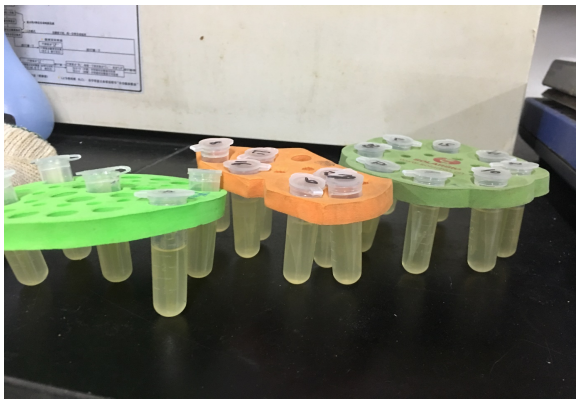
-place the six tubes in the 4 degree Celsius

Gel electrophoresis
-make gel with 30ml TAE buffer, 0.3g agarose 1μl DNA dye
-insert products from PCR along with 2kb DNA marker
-run at 140V for 20min
-check the results

Tracks

New primer 275300	New primer lsmt	New primer bsmt	Seq vf Seq vr 2753003 gibson product	Seq vf Seq vr limst gibson product	Seq vf Seq vr Bsmt gibson product	Marker 4μl

Incubation results:



It's very dilute, they suggest us to send them for DNA sequencing

Jul 30th
Experimenter: Zeyuan Li Michael Jiang

Background:
We determined that the assemble is wrong.
Now our goal is to determine whether our carrier is not right. Use pSB3K3 in the distribution kit to do so.
Get ready for transformation tomorrow.

Get plasmids from 18A 18B 18C of the distribution kit, place them in three separate EP tubes and label.

Three tubes in total
50μl system
-For each tube, add

PCR mix	25μl
F primer	1μl 10mM
R primer	1μl 10mM
Template	1μl
ddH2O	22μl

-centrifuge them at 5000rpm for 10s
-place tubes in the PCR machine

Temp	
96	5min
98	30s
58	30s
72	90s
72	5min
4	forever
2-4	30cycle

-place the three tubes in the 4 degree Celsius

Gel electrophoresis
-make gel with 30ml TAE buffer, 0.3g agarose 1μl DNA dye
-insert products from PCR along with 2kb DNA marker
-run at 140V for 20min
-check the results

Results:
No target stripes

Use raw plasmid for the same gel electrophoresis process
Results: no target stripes.

Conclusion:
The plasmids are all problematic, transformation for tomorrow is impossible.
We got new polymerase which can amplify longer sequences.

Team RDFZ-China
Experiment Record 01/08/19
Operators: Zeyuan Li, Yilin Shen
Background:
Today we receive the Gibson assembly positive control from our collaborator, team BNDS_China. Thus, we can do the BMST Gibson assembly. We also receive the plasmid pSB4c5 from Beijing no.4 school. Because of the division of those materials were late, thus today we can only do a Gibson. The teammates who conduct the experiment tomorrow can finish my work by do the PCR and gel electrophoresis.
Experiment:
Gibson assembly:
Do all procedures on ice

Material:
-PCR tube
-Gibson assembly Mix
-Gibson assembly Mix positive control
-Backbone(18.0ng/μl)
-bsmt(29.8ng/μl)
-ddH2O

Two tubes in total

Label mix as 1
Label positive control as 2

Prepare reaction system(10μl)
For each tube:
-Mix/control 5μl
-Backbone 2μl
-BSMT 1μl
-ddH2O 2μl

Note:
place ddH2O before mix
unfreeze backbone and target genes prior

Temp:
50°Cx2hrs
12°Cxforever

Run gibsonassem
Item placement:

Gibson's products had been placed into the 4-degree Celsius refrigerator.
The plasmid sponsor by Beijing N0.4 school had been placed in -20-degree Celsius refrigerator.
The positive control sponsor from team BNDS_China, had been place in a box labeled with "reagents".
The LB growth medium we make today is on the top shelf.

Expectation:
The teammates who will come tomorrow please finish PCR and gel electrophoresis.
According to a reliable source, our 2019 distribution kit will reach at Aug 3 to our campus, and I will send it to the lab. Please do not use the kit from 2018, it's already one year ago.
Tomorrow please get the antibiotic and the Glycerin bacteria from BNDS_China.

Precautions:

1. If you don't place the ice box reversely, then it will deform.
2. T5mix should be used when making p2500 or more base segments.
3. The PCR experiment steps of 20190802 should be written in the experiment record book. If you don't understand, please take a look.

Team RDFZ-China
Experiment Record 02/08/19
Operators: Haoran Xin, Yilin Xin
background: today we receive the sponsorship from BNDS_China (pSB4c5, which has been put into the plasma box in the – 20-degree Celsius refrigerator)

Experiment:
PCR& gel electrophoresis
Material:
Taq PCR mix-20 (colored)
T5 mix-20 (colored)
Seq VF and seq VR primers (in a rectangular 10mm box)
Vector F and vector r primers (in rectangular 10mm box)
Bmst F and bmst r primers (in a rectangular 10mm box)
Template: Gibson products 1 and 2 of 4-degree Celsius refrigerator (mix label 1; control characterization 2)
Ultra pure water ddH2O
6 PCR tubules
Three primers and two Gibson products

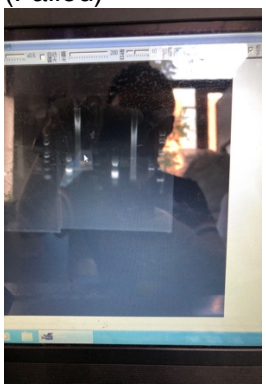
Taq PCR mix-20 10μl
F primer 1μl 10mM
R primer 1μl 10mM
Template 1μl
ddH2O 7μl
there should be six PCR tube templates should be packed separately, which are two Gibson products respectively (the specific formula for them is written in the record book)

Gel electrophoresis
-make gel with 30ml TAE buffer, 0.3g agarose 1μl DNA dye
-insert products from PCR along with 2kb DNA marker
-run at 140V for 20min
-check the results

2 bmst primer	2 vectors	2 seq-v	1 bmst primer	1 vector	1 seq-v	marker

Ps: 1(gibson1: mix)
2(Gibson 2: control)

Result:
(Failed)



Item placement:
Gibson products is in the 4-degree Celsius refrigerator
pSB4c5 sponsored by BNDS_China is in the plasma box
Today's new LB medium is on the top of the glove shelf, where there are a bunch of conical bottles. The biggest one is ours (marked with RDFZ iGEM).

Precaution:
If you don't place the ice box reversely, then it will deform.
T5mix should be used when making p2500 or more base segments.
The PCR experiment steps of 20190802 should be written in the experiment record book. If you don't understand, please take a look.

Team RDFZ-China
Experiment Record 03/08/19
Operators: Mingyi Wan, Haoran Xin, Ruochen Liu
background:
today we receive the official distribution kit
we plan to do positive control's conversion

experiment:
Gibson conversion
PCR
Today we use the backbone in the distribution kit.

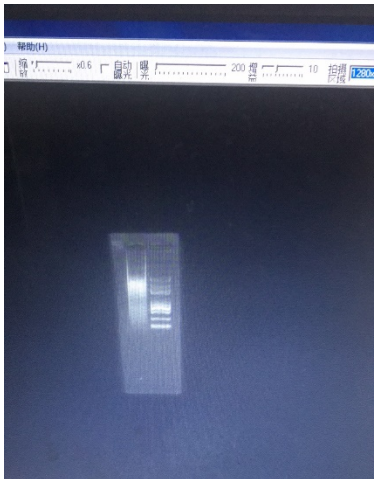
Primer: v-f, v-r, each 1μl
Template: backbone,1μl
Mix: t5 mix,25μl
ddH2O,22μl

Procedure:
You can see in our lab booklet

Item placement:
-The coated amp board is in the incubator, and the teammate who come to the experiment tomorrow can do further processing.

Precaution:
Do use the LB medium in the lab, please make it up yourselves.

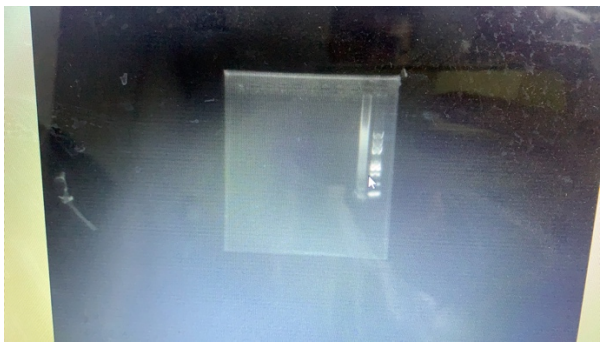
Result:
PCR



(right: 2Kmarker, left backbone

Team RDFZ-China
Experiment Record 04/08/19
Operators: Yiqiao Wang, Menghan Wang
background:
No pSB4c5 was received today, only pSB3k3 PCR was done.
Experiment:
PCR:
System (50 μ L):
DdH2O 22 L
pSB3k3 1 L
Vf 1 L
Vr 1 L
PCR mix 25 μ L

Temperature:
96 degrees 5min
98 degrees 30s
68 degrees 30s
72 degrees 90s
72 degrees 5min
4 degrees forever

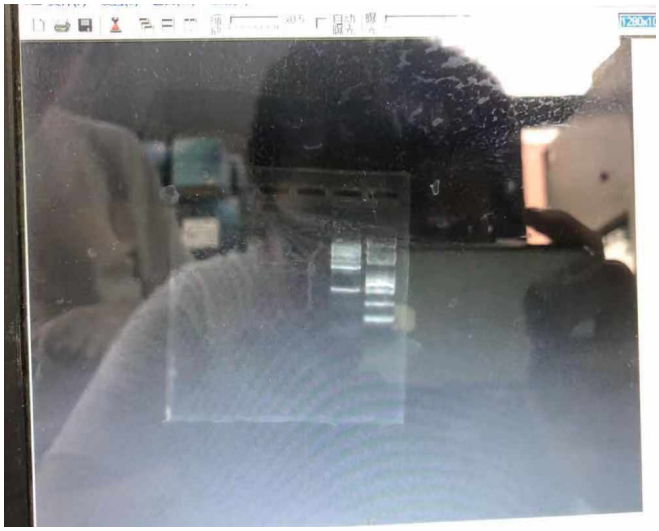


Team RDFZ-China
Experiment Record 05/08/19
Operators: Yiqiao Wang, Menghan Wang
Background:
Today, we received two new primers and made PCR of pSB 4C 5.
The bacteria didn't grow, so we only do the conversion.

Experiment:
PCR:
System (50 μ L):
DdH2O 22 L
pSB4c5 1 L
VF 1 μ L (diluted to 10mm)
Vr 1 L
PCR mix 25 μ L

Conversion
The details are all on the booklet.

Result:



Right: 2kb DNA marker

Item placement:
The gel was wrapped with PE gloves, and DNA marker and loading buffer were collected in the second 4-degree refrigerator on the right side of the door.

Team RDFZ-China
Experiment Record 06/08/19

Operator: Yilin Shen Menghan Wang

background:
continue the PCR of pSB4c5
There was no growth on the medium of AMP 8.5

Experiment:
PCR
Gel electrophoresis

Procedure:
You can see it on the booklet.

Result:

Left to right

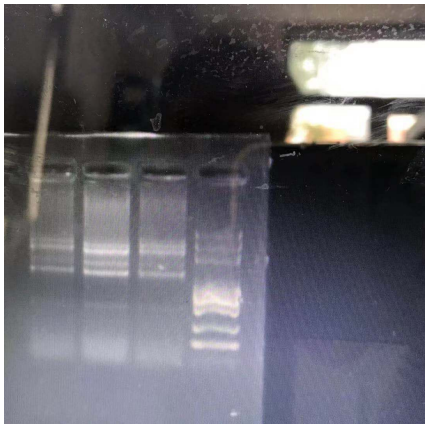
pSB4C5	pSB4C5	pSB4C5	pSB4C5	pSB4C5	marker

Team RDFZ-China
Experiment Record 08/08/19
Experimenter: Zhuolang Li and Menghan Wang
Background:
Today, we received 11 tubes of primers. We are going to conduct PCR of pSB4c5.

Experiment Process:
1. pSB4c5 PCR
2. Gel electrophoresis
-make gel with 30ml TAE buffer, 0.3g agarose 1μl DNA dye
-insert products from PCR along with 2kb DNA marker
-run at 140V for 20min
-check the results

From left to right:

pSB4c5 pSB4c5 pSB4c5 marker



3. Dilute the new primers
We got 11 tubes of new primers. We diluted all of them according to the information labelled on the tubes and put them into the refrigerator.

Note:
The primers we used today, pSB4c5-v-f and pSB4c5-v-r, are not the primers we should have used. Instead, we should use new primers 010A10 and 010B10. Thus, we have made PCR setup and kept it in the 4°C refrigerator. Tomorrow, we will conduct the same experiment again.

Team RDFZ-China
Experiment Record
Experimenter: Zhuolang Li and Hang Xu
Objective: new primers PCR: 010A10 and 010B10

Experiment Process：

1. PCR
2. Gel electrophoresis
   -make gel with 30ml TAE buffer, 0.3g agarose 1μl DNA dye
   -insert products from PCR along with 2kb DNA marker
   -run at 140V for 20min

Results：

Errors:
1. DNA marker is not clear.
2. When adding PCR products, the track labelled by the yellow arrow was damaged.

What to do tomorrow:
Make setup again and conduct the experiment with no mistakes.

Team RDFZ-China
Experiment Record 10/08/2019
Experimenter: Zhuolang Li and Hang Xu
Objectives: Finish the experiment which we failed to conduct, that is to PCR 010A10 and 010B10.
Experiment Process:
1. PCR
2. Gel electrophoresis
    -make gel with 30ml TAE buffer, 0.3g agarose 1μl DNA dye
    -insert products from PCR along with 2kb DNA marker
    -run at 140V for 20min
Results:
From left to right:
O1OA10 O1OB10 *6 marker

Note:
After a few days of experiments, we found that some PCR tubes and tubes that contains liquid in the lab are subjected to contamination. Also, we are not sure if some tubes are sterilized. These factors could lead to invalid results, so we think the problems need to be solved. For example, we can put the sterilized tubes into a specific drawer.

Experiment Record 11/08/2019
Operator: Hang Xu, Mingyi Wan, Ruochen Liu
Experiment:
PCR1:
Ddh2o 22ul
Plate4 7E 1ul
Primer 8 1ul
Primer9 1ul
Tac Mix 25ul

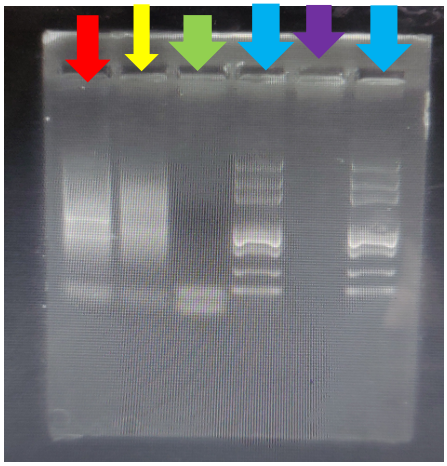
PCR2:
Ddh20 22ul
Plate6 4E 1ul
Primer1 1ul
Primer2 1ul
T5 Mix 25ul
(Centrifuge the remaining solution of the two Plates and keep them in -20 refrigerator)

PCR3:
Ddh2o 22ul
pSB4c5 1ul
Primer1 1ul
Primer2 1ul
T5 Mix 25ul

Part two: Settings for today's PCR

96	5min
96	30s
68	30s
72	1min30s
72	5min
4	forever

（file named as pcrtsvecto3）
Results:



Red: pcr3
Yellow:pcr2
Green:pcr1
Blue: 2k DNA marker
Purple: failed DNA marker
(the gel has been put into a PE glove and kept in the-20 fridge)

Team RDFZ-China
Experiment Record 12/08/2019
Goal:
Exam whether T5 Mix is still usable

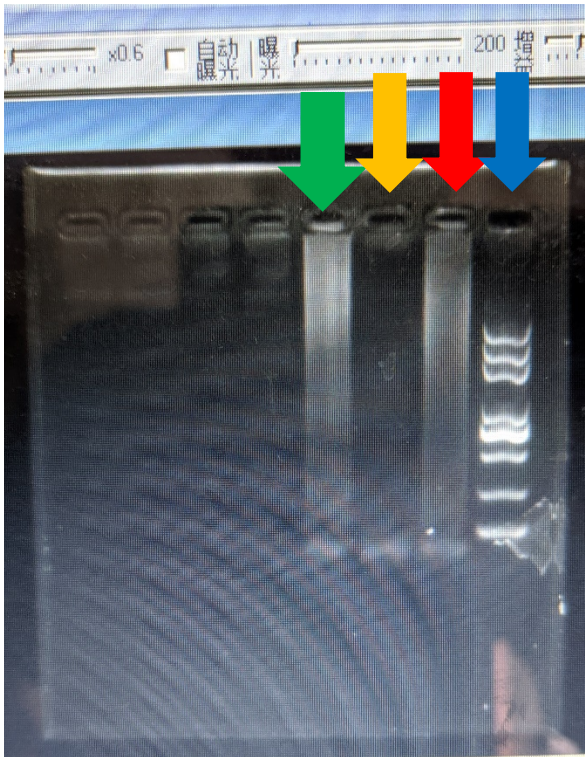
Part one: preparation
Pcr1:
Ddh2o 7ul
Backbone 1ul (2.4ng/ul)
v-f 1ul
v-r 1ul
T5 Mix 25ul

Pcr2:
Ddh2o 7ul
Backbone 1ul (5.0ng/ul)
v-f 1ul
v-r 1ul
T5 Mix 25ul

Pcr3:
Ddh2o 7ul
Backbone 1ul (18ng/ul)
v-f 1ul
v-r 1ul
T5 Mix 25ul

Part two: settings for today's PCR
96 5min
96 30s
68 30s
72 1min30s
72 5min
4 forever

Part three: preparing and running for standard agarose DNA gels
Results:



Green: pcr3
Yellow: pcr2
Red: pcr1
Blue: DNA marker

NOTE: got the recycled DNA (previously in gel) from Zhao. It was kept inside the “plasmid” box in -20fridge.

Team RDFZ-China
Experiment Record 17/08/2019
Laboratorian: Mingjia Pan, Ruochen Liu

Background:
The primer of 4c5 arrived, 4 tubes in total. Yesterday’s bacteria successfully grew, but since we did not check it on time, we doubt that they are infectious microbes, and quite unobvious.

Today we plan to:
1. Dilute the primer
2. PCR
3. electrophoresis

Dilute the primer:
1. 5000rmp in centrifugal machine, 15s

Note: the primers are powder, so do not open the lid until centrifuging.

2. Dilute: use the constants and notices written on the side of the tubes to add substances into the tube.

PCR
1. Making the solution
   50ul

pcr mix	25ul
F primer	1ul 10mM
R primer	1ul 10mM

Template	1ul
ddH2O	22ul

template：Plate 4 M8/lmst/

operation should be made on ice, especially when adding mix
mix is the last, water is the first
5000rpm 10s centrifuging after adding all the substances

PRC temperature:

98	5min
98	30sec
68	30sec
72	90sec
72	5min
4	forever

2-4 30 cycles

Electrophoresis
Material:
30ml TAE
Agarose
1ul DNA stain
Models

Process:
30ml TAE in conical flask + 0.3g agar, shaking up
Heating 2min in microwave oven
1ul DNA stain, sake up
Put in model
Wait 20-30min for condensing

Running the gel:
Material:
Gel just made
equipment
PCR products
DNA marker

Outcome:
PCR only Lmst has obvious dimer. Plate4 m8 totally fails
Cutting the gel: we only cut the successful one, the band is very thin.
Super unlucky, Ruochen Liu discarded all the things by mistake

Notice:
Ask teammate before you throw something

PS:
All the primers today are put into a box full of primer
PCR star mix two tubes are empty
Up - end the ice box
Image of the gel is in computer

2019/8/22
Recorder: Zeyuan Li, Jingwen Li

Background: today we received new PCR mix, so we can combine our primers (No.4, 5, 6, 7). Also, we can use the combined primers with the product of ptaq which our instructor gave us to perform a SOE PCR, gel electrophoresis, extraction purification and testing the concentration of the product.

Experiment:
#1 combine the primers
Add each primer (4,5,6,7) to make the 8-microliter system
Use the black PCR machine to set the temperature for this experiment (temperatures are: 98, 93, 88, 83, 78, 73, 68, 63, 58, 53, 48, 43, 37) (each temperature lasts for 90 seconds). Finally set the temperature to 16 degrees Celsius and the time for that temperature is forever.
Set the sample volume to 10 microliters(this is the minimum sample volume of the black PCR machine)

#2 SOE PCR
Make the combined primers 10 times dilute: 1 microliter primers + 9 microliters of ddH2O
PCR system: sample volume is 50 microliters
(25 microliters of PCR mix + 1 microliter of dilute combined primers + 1 microliter of the product of ptaq + 1 microliter of #3 primer + 1 microliter of #9 primer + 21 microliter of ddH2O)
[temperature for setting the PCR machine is in 2019 RDFZ-China lab booklet]

#3 gel electrophoresis
Gel making process:
Add 30 ml of TAE solution and mix that with 0.3 grams of regular argarlose.
Then use the microwave oven to heat the mixture for 2min.
After heating, add 1 microliter of DNA dye to the mixture
Then pour the mixture in the solidify box and use the big electrophoresis lane mold
Wait for 20mins until the gel solidified
Lane distribution: 2k DNA marker, 2k DNA marker, product with loading buffer
Note: add 10 microliters of loading buffer to the SOE PCR product
Gel electrophoresis machine coefficients setting: electric potential 140 volts, please don't set up the current

#4 cut the gel + extraction purification
Gel cutting process:
Use the knife to cut the part with valid strips. (note: please don't cut too much invalid parts, and you should wear a lab coat when cutting the gel)

Reminders:
Please come to the lab early
The ice box should be placed upside down, or it will deform
Up to now, we have a lot of reagents, so please be patient when finding certain reagents. Please put the reagents back as it were after using them.
When the petite nozzles are used up, we need to load them by ourselves and ask seniors in the lab to help us sterilize them.
Please take photos when you are doing the experiment; if you don't have a lab partner, you can selfie.

Message for tomorrow's lab: we need to finish extraction purification and test the concentration of the product.

Team RDFZ-China
Experiment Record 25/08/2019
Laboratorian: Mingjia Pan Mingyi Wan

Process:
Transformation
Get competent cell from -80 degrees refrigerator(top 10)
Use super clean bench, add 2ul Gibson product(bmst) into competent cell, blend them
Put competent cell into -20 degree refrigerator, wait 20min
Set the water bath as 42 degree
Set the tube in buoy, water bathing for 90s
Put it into -20 degree, wait 2min
Add 400ul LB culture solution
37 degree bacteria shaking 40 min
Decant the liquid into the substrate, use disinfected and room-temperature glass rob to apply the liquid uniformly.

Making the culture
In super clean bench
Conical flask: 200ml water, 6.6g agar, then seal it
Put it into sterilizer to kill bacteria
Decant into petri dish, 25ml per dish, cover the lid
Wait 20-30 min till the culture condenses, then upend the dish

Outcome:
Today's petri dishes(with competent cells) are in incubator, marking RDFZ-China c 8.15

Today we made 8 anti-c culture. They are in 4 degree refrigerator. (btw: there are too many things in the refrigerator, please organize them tomorrow)