Notebook June

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DATE 6.12 Transformation of plasmid pSB1C3 containing MtrCAB and CysDes Recorder: Yonghao Liang

DATE 6.13 Colony picking of plasmid pSB1C3 containing CysDes Transformation of Mtr CAB failed.

Transformation of plasmid pSB1C3 containing MtrCAB and CysDes Recorder: Yawei Wu, Menglong Jin, Ziyi Wang, Shihan Zhu NOTE:Generally, competent bacteria are restrored in -70 degree centrigrade environment. 1.Take the competent bacteria from refrigerator and incubate them into ice about 5 mins until it is dissolved 2.Absorb 100pg to 10 ng plasmid(normally 1 to 2 uL, DO NOT add more than 5% volumn of bacteria solution) and mix it with bacteria solution thoroughly. ATTENTION: Please operate this step tenderly!!! 3.Put the tubes on the ice about 30 mins.(Time SHOULD BE ACCURATE) 4.Make a heat shock at 42 degree centigrade about 30 sec(TIME SHOULD BE ACCURATE) 5.Put the tubes on the ice about 2 to 3 mins again. 6.Add 900 ul LB medium into EP tubes and cultivate the bacteria at 37 degree centrigrade about 40 to 60 min. 7.Centrifuge them at 12,000xg about 15 sec and we will see sediment in the tubes. 8.Discard the supernatant liquid and leave about 220 ul medium. 9.Coat plate: add 200 ul solution in a large plate while add 20 ul solution in a small plate. 10.Cultivate these bacteria overnight for further use.

A negative-control Coating to check whether the LB solid culture we used can select the chloromycetin resistent colony

Transformation of part BBa_J04450 Recorder: Yonghao Liang NOTE:Generally, competent bacteria are restrored in -70 degree centrigrade environment. 1.Take the competent bacteria from refrigerator and incubate them into ice about 5 mins until it is dissolved 2.Absorb 100pg to 10 ng plasmid(normally 1 to 2 uL, DO NOT add more than 5% volumn of bacteria solution) and mix it with bacteria solution thoroughly. ATTENTION: Please operate this step tenderly!!! 3.Put the tubes on the ice about 30 mins.(Time SHOULD BE ACCURATE) 4.Make a heat shock at 42 degree centigrade about 1 min (TIME SHOULD BE ACCURATE) 5.Put the tubes on the ice about 5 mins again. 6.Add 900 ul LB medium into EP tubes and cultivate the bacteria at 37 degree centrigrade about 2 hours . 7.Centrifuge them at 4000 rpm about 2 mins and we will see sediment in the tubes. 8.Discard the supernatant liquid and leave about 220 ul medium. 9.Coat plate: add 200 ul solution in a large plate while add 20 ul solution in a small plate. 10.Cultivate these bacteria overnight for further use.

We find that the strain(XL 10 Gold) we use come s with Chloramphenicol resistance, that's why all the transformation experiments we have done are failed!!!!!!!!

DATE 6.14 We get plasmid pET22 from Pro.Liu's lab, and have it amplified.

streak plating of Top 10 Recorder: Chenyang Li

DATE 6.15 Plasmid Extraction of PET22. Recorder: Yonghao Liang, Ziyi Wang, Jingyu Wang, Linhang Li Procedure:

1. Centifuge 1.5 mL bacterium solution at 11000 rpm, few sediment getted. Remove the supernatant. Repeat twice.
2. Add 250 μL Buffer P1, resuspend cells.
3. Add 250 μL Buffer P2, mix well, 3 min's standing.
4. Add 350 μL Buffer P3, mix well.
5. 13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate.

6. Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate.
7. Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once.
8. 12000 rpm centifuge 1 min.
9. Lying for 10 min.
10. Put the adsorption column in a new EP tube. Add 40 μL 50°C elusion buffer, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with elusion buffer at 4 degree Celsius.

After extraction, we measured the OD A260/A280 and the density of plasmids.

	1	2	3	4
260/280	2.08	2.33	2.06	1.94
Concentration(ng/μl)	62.5	70	82.5	87.5

Preparation of Competent Cell(E.coli) Recoreder: Yonghao Liang Procedure 1.Use inoculating loop to pick somebacteria solution and use streak plate method to inoculate it on the LB plate(without any antibiotics). 2.Cultivate the plates at 37°C overnight. 3.Pick the colonies in the test tubes (5mL LB liquid medium). 4.Cultivate the bacteria at 37°C and shockat 250 rpm/min for 6h. 5.Transfer the bacteria medium into the 200mL LB fluidmedium and cultivate the at 37°C and shock at 250rpm/min till OD reach 0.4.(Our OD is 3.896) 6.Transfer the medium into the 50 mL centrifuge tube(precool in ice) and then incubate on ice for 30 minutes. 7.Centrifuge the tube at 4000 rpm for 15min at 4°C. 8.Discard supernatant liquid and add 30 mL80 mM CaCl2-20 mM MgCl2 per tube. 9.Resuspend the bacteria and incubate onice for 30 minutes. 10.Centrifuge the tube at 2700 rpm for 15min at 4°C. 11.Discard supernatant liquid and add 2 mL100 mM CaCl2 -18% glycerin per tube. 12.Resuspend the bacteria and subpackage100 uL per tube(1.5 mL). 13.Store the tube at -80°C.

Plasmid Extraction of PET22. Recorder: Menglong Jin，Bijunyao Zhong，Mengxue Sun Procedure:

1. Centifuge 1.5 mL bacterium solution at 11000 rpm, few sediment getted. Remove the supernatant. Repeat twice.
2. Add 250 μL Buffer P1, resuspend cells.
3. Add 250 μL Buffer P2, mix well, 3 min's standing.
4. Add 350 μL Buffer P3, mix well.
5. 13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate.
6. Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate.
7. Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once.
8. 12000 rpm centifuge 1 min.
9. Lying for 10 min.
10. Put the adsorption column in a new EP tube. Add 40 μL 50°C elusion buffer, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with elusion buffer at 4 degree Celsius.

After extraction, we measured the OD A260/A280 and the density of plasmids.

	1	2	3	4
260/280	2.0	1.5	3.0	2.0
Concentration(ng/μl)	10.0	15.0	7.5	15

Unfortunately，the Wash Solution we used didn't contain ethanol，so we failed to get the plasmid.We should be more careful next time.

Transformation of plasmid pSB1C3 containing MtrCAB and CysDes，PSB1C3 and PET22 Recorder: Yawei Wu, Menglong Jin, Yitian Zhou, Mengxue Sun NOTE:Generally, competent bacteria are restrored in -70 degree centrigrade environment. 1.Take the competent bacteria from refrigerator and incubate them into ice about 5 mins until it is dissolved 2.Absorb 100pg to 10 ng plasmid(normally 1 to 2 uL, DO NOT add more than 5% volumn of bacteria solution) and mix it with bacteria solution thoroughly. ATTENTION: Please operate this step tenderly!!! 3.Put the tubes

on the ice about 30 mins.(Time SHOULD BE ACCURATE) 4.Make a heat shock at 42 degree centigrade about 60 sec(TIME SHOULD BE ACCURATE) 5.Put the tubes on the ice about 2 to 3 mins again. 6.Add 900 ul LB medium into EP tubes and cultivate the bacteria at 37 degree centrigrade about 40 to 60 min. 7.Centrifuge them at 4,000 rpm about 2 min and we will see sediment in the tubes. 8.Discard the supernatant liquid and leave about 220 ul medium. 9.Coat plate: add 200 ul solution in a large plate while add 20 ul solution in a small plate. 10.Cultivate these bacteria overnight for further use. **But we only have one flat plate（PET22 in AP plate）grows better. We can't get single colony in other plates. May be the heat-shock time we used isn't probable.**

DATE 6.15 Plasmid Extraction of PET22. **Recorder: Jianjian Guo, Xiaoya Zhang, Yawei Wu, Yunjing Wu** Procedure:

1. Centifuge 1.5 mL bacterium solution at 11000 rpm, few sediment getted. Remove the supernatant. Repeat twice.
2. Add 250 μL Buffer P1, resuspend cells.
3. Add 250 μL Buffer P2, mix well, 3 min's standing.
4. Add 350 μL Buffer P3, mix well.
5. 13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate.
6. Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate.
7. Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once.
8. 12000 rpm centifuge 1 min.
9. Lying for 10 min.
10. Put the adsorption column in a new EP tube. Add 40 μL 50°C elusion buffer, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with elusion buffer at 4 degree Celsius. After extraction, we measured the OD A260/A280 and the density of plasmids.

	1	**2**	**3**	**4**
260/280	2.5	2.286	2.33	2.111
Concentration(ng/μl)	37.5	40.0	17.5	47.5

DATE 6.16 Transformation of plasmid pSB1C3 containing CysDes Recorder: Shihan Zhu, Dongdong Jiang, Xingwei Yang NOTE:Generally, competent bacteria are restrored in -70 degree centrigrade environment. 1.Take the competent bacteria from refrigerator and incubate them into ice about 5 mins until it is dissolved 2.Absorb 100pg to 10 ng plasmid(normally 1 to 2 uL, DO NOT add more than 5% volumn of bacteria solution) and mix it with bacteria solution thoroughly. ATTENTION: Please operate this step tenderly!!! 3.Put the tubes on the ice about 30 mins.(Time SHOULD BE ACCURATE) 4.Make a heat shock at 42 degree centigrade about 90 sec(TIME SHOULD BE ACCURATE) 5.Put the tubes on the ice about 2 to 3 mins again. 6.Add 900 ul LB medium into EP tubes and cultivate the bacteria at 37 degree centrigrade about 40 to 60 min. 7.Centrifuge them at 12000 rpm about 30 minutes and we will see sediment in the tubes. 8.Discard the supernatant liquid and leave about 220 ul medium. 9.Coat plate: add 100 ul solution in a large plate while add 20 ul solution in a small plate. 10.Cultivate these bacteria overnight for further use.

Unfortunately, we did not get any single colony in our 5 plates. Maybe the concentration of our bacteria is too high.

DATE 6.16 Transformation of plasmid pET22 Recorder: Yunjing Wu NOTE:Generally, competent bacteria are restrored in -70 degree centrigrade environment. 1.Take the competent bacteria from refrigerator and incubate them into ice about 5 mins until it is dissolved 2.Absorb 100pg to 10 ng plasmid(normally 1 to 2 uL, DO NOT add more than 5% volumn of bacteria solution) and mix it with bacteria solution thoroughly. ATTENTION: Please operate this step tenderly!!! 3.Put the tubes on the ice about 30 mins.(Time SHOULD BE ACCURATE) 4.Make a heat shock at 42 degree centigrade about 90 sec(TIME SHOULD BE ACCURATE) 5.Put the tubes on the ice about 2 to 3 mins again. 6.Add 900 ul LB medium into EP tubes and cultivate the bacteria at 37 degree centrigrade about 60 min. 7.Centrifuge them at 4000 rpm for 2 minutes and we will see sediment in the tubes. 8.Discard the supernatant liquid and leave about 220 ul

medium. 9.Coat plate: add 100 ul solution in a large plate while add 20 ul solution in a small plate. 10.Cultivate these bacteria overnight for further use.

This time we get 7 colonies on the plate !!!!!!

DATE 6.16 Colony picking of plasmid pET22 Recorder: Yan Shi & Yonghao Liang

DATE 6.17 Plasmid Extraction of PET22. **Recorder: Yonghao Liang, Menglong Jin** Procedure:

1. Centifuge 1.5 mL bacterium solution at 11000 rpm, few sediment getted. Remove the supernatant. Repeat twice.
2. Add 250 μL Buffer P1, resuspend cells.
3. Add 250 μL Buffer P2, mix well, 3 min's standing.
4. Add 350 μL Buffer P3, mix well.
5. 13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 45 s, discard filtrate.
6. Add 500 μL Buffer DW1, 12000 rpm centifuge 45 s, discard filtrate.
7. Add 500 μL Wash Solution, 12000 rpm centifuge 45 s, discard filtrate. Repeat once.
8. 12000 rpm centifuge 1 min.
9. Lying for 10 min.
10. Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with elusion buffer at 4 degree Celsius.

After extraction, we measured the OD A260/A280 and the density of plasmids.

origin:

	1	**2**	**3**	**4**
260/280	2.222	2.571	2.5	2.091
Concentration(ng/μl)	50	45	50	57.5

transform:

	1	**2**	**3**	**4**	**5**	**6**	**7**
260/280	1.846	2.083	1.778	2	2.667	2.365	2.111
Concentration(ng/μl)	60	62.5	40	90	40	65	95

**As we can see, the outcome of the transform group is quiet good!! which means our transformation succeeded.

DATE 6.17 Transformation of plasmid pSB1A3 Recorder: Shihan Zhu NOTE:Generally, competent bacteria are restrored in -70 degree centrigrade environment. 1.Take the competent bacteria from refrigerator and incubate them into ice about 5 mins until it is dissolved 2.Absorb 100pg to 10 ng plasmid(normally 1 to 2 uL, DO NOT add more than 5% volumn of bacteria solution) and mix it with bacteria solution thoroughly. ATTENTION: Please operate this step tenderly!!! 3.Put the tubes on the ice about 30 mins.(Time SHOULD BE ACCURATE) 4.Make a heat shock at 42 degree centigrade about 90 sec(TIME SHOULD BE ACCURATE) 5.Put the tubes on the ice about 2 to 3 mins again. 6.Add 900 ul LB medium into EP tubes and cultivate the bacteria at 37 degree centigrade about 40 to 60 min. 7.Coat plate: add 200 ul solution in a large plate while add 20 ul solution in a small plate. 8.Cultivate these bacteria overnight for further use.

DATE 6.17 Preparation of Glycerin bacteria containing plasmid pET22 Recorder: Yonghao Liang Procedure: Add 500 μL glycerin and 1000 μL Bacteria liquid into a tube and store it at -40 degree centigrade. The bacteria is from the colony we picked on 6.16. we cultivated the bacteria for 4 hours and 38 minutes before we made the glycerin bacteria.

DATE 6.17 Plasmid Extraction of PET22. **Recorder: Yan Shi** Procedure:

1. Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice.
2. Add 250 μL Buffer P1, resuspend cells.
3. Add 250 μL Buffer P2, mix well, 3 min's standing.

4. Add 350 μL Buffer P3, mix well.
5. 13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate.
6. Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate.
7. Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once.
8. 12000 rpm centifuge 1 min.
9. Lying for 10 min.
10. Put the adsorption column in a new EP tube. Add 40 μL 50°C elusion buffer, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with elusion buffer at 4 degree Celsius. After extraction, we measured the OD A260/A280 and the density of plasmids.

	1	**2**	**3**	**4**	**5**	**6**	**7**
Concentration(ng/μl)	32.4	36.0	35.4	35.0	30.1	35.5	34.2
260/280	1.05	1.64	1.08	1.63	1.66	1.74	1.57
260/230	1.28	1.14	1.15	1.14	1.18	1.44	1.29

DATE 6.17 Colony picking of plasmid pSB1C3 containing CysDes

DATE 6.18 Plasmid Extraction of pSB1C3 containing CysDes. **Recorder: Shihan Zhu**
Procedure:

1. Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice.
2. Add 250 μL Buffer P1, resuspend cells.
3. Add 250 μL Buffer P2, mix well, 3 min's standing.
4. Add 350 μL Buffer P3, mix well.
5. 13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate.
6. Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate.
7. Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once.
8. 12000 rpm centifuge 1 min.
9. Lying for 10 min.
10. Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius. After extraction, we measured the OD A260/A280 and the density of plasmids.

	1	**2**	**3**	**4**
Concentration(ng/μl)	38.8	29.3	25.4	9.3
260/280	1.80	1.90	1.83	1.71
260/230	1.28	1.89	1.32	1.30

Then we did Agarose gel electrophoresis

1. Add 0.4 g agarose to 40 mL TAE buffer.
2. Dissolved by heating.
3. Cool down.
4. Pour into electrophoresis tank.
5. Mix 50 μL PCR product and 10 μL loading buffer.
6. Loading：DNA marker 1 μL, PCR productrs: 5 μL : Plasmid extraction products (pET22) from yesterday 1-7,8-13, plasmid extraction products(pSB1C3 containing CysDes) 14-16.
7. Electrophoresis gel：90 V 30 min.
8. Autoradiography(UV).

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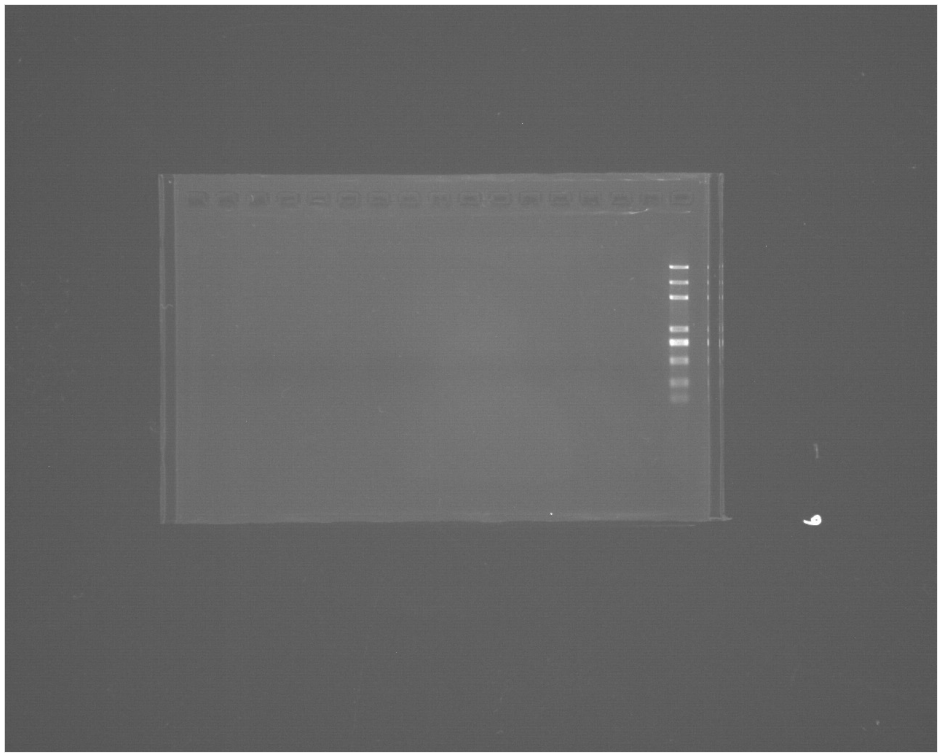
Result



As we can see in the picture abouve, there are no band on the gel, which means what we extracted from the bacteria is not plasmid, even though we had already gotten a positive result when we tested the OD of the extraction products. It makes us wonder whether the ddH2O we use for elution has been contaminated, or what we extracted from the bacteria was part of the genome of the E.coli. These reasons may lead to the false positive result of the OD testing.

DATE 6.18 Recorder: Yonghao Liang We got another copy of TOP 10 strain, so we did two streak plating on two solid culture with no antibiotics, and two pour plating on a solid culture with chloromycetin and another with kanamycin. In addition, we inoculated some in the tube of liquid culture with no antibiotic in it.**6.19 Recorder: Yonghao Liang Colony picking of strain Top 10**

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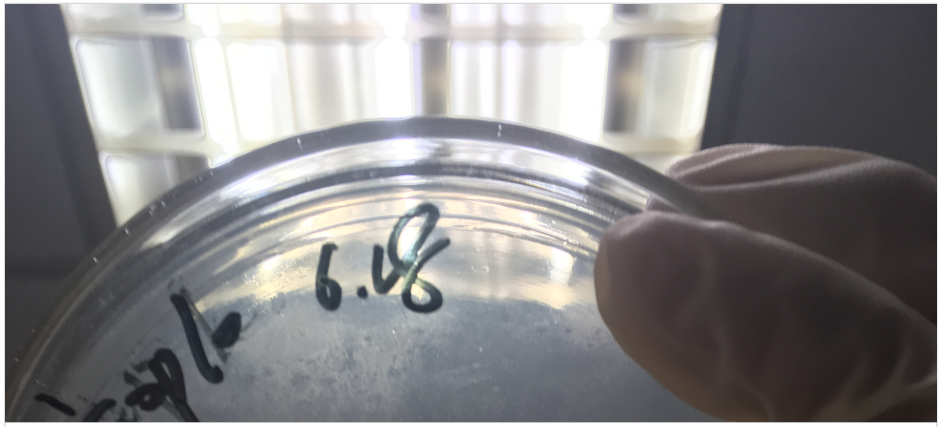
PS:The outcomes of last night's plating:


6.18

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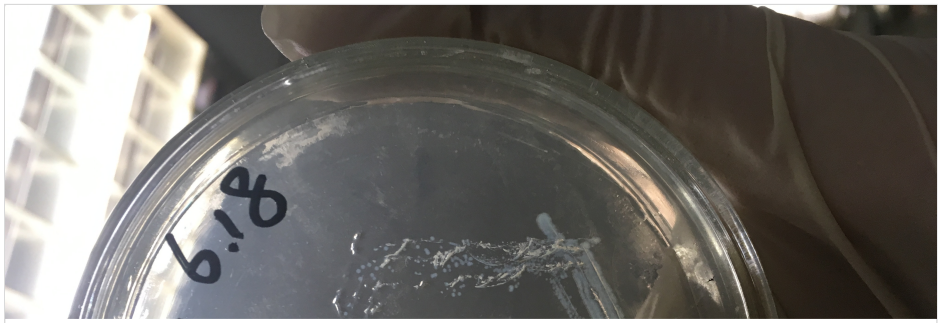

To double check whether the strain we use has any resistance or not, we are going to do this experiment again when preparing the competent cell.

Preparation of Glycerin bacteria of Top 10 Recorder: Yonghao Liang Procedure: Add 500 μL glycerin and 1000 μL Bacteria liquid into a tube and store it at -40 degree centigrade. The bacteria is from the colony we picked on 6.18. we cultivated the bacteria for 4 hours and 38 minutes before we made the glycerin bacteria.

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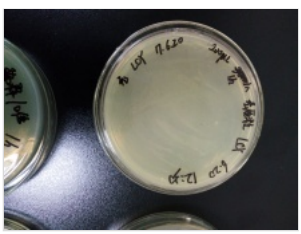
Recorder: Yonghao Liang To double check whether the antibiotic we use has its effect on the bacteria, we did another pour-plating experiment. The results shows that all the antibiotic we use is GREAT!! **We need to pay more attention to contamination!!!**

DATE 6.20 Recorder: Chenyang LI To find out the proper parameter for our transformation experiment, we did a series of experiments about transformation of plasmid pSB1C3. Except the variables we set listed as follows: 1.Take the competent bacteria from refrigerator and incubate them into ice about 5 mins until it is dissolved 2.Absorb 10pg，1pg plasmid(1 μL) and 1μL sterilized distilled water respectively, marked "10h"，"100h"，"0h" relatively.And mix it tenderly with bacteria solution thoroughly. 3.Put the tubes on the ice 30 mins.(Time SHOULD BE ACCURATE) 4.Make a heat shock at 42 degree centigrade about 90 sec(TIME SHOULD BE ACCURATE) 5.Put the tubes on the ice about 5 mins again. 6.Add 900 μl LB medium into EP tubes and cultivate the bacteria at 37 degree Celsius about 60 min. 7.Coat plate: add 200 μl solution of "10h" in a plate with chloromycetin while add 20 μl of "10h" solution in another plate with chloromycetin. Add 200 μl solution of "100h" in a plate with chloromycetin while add 20 μl of "100h" solution in another plate with chloromycetin. Add 200 μl solution of "0h" in a plate without antibiotics. Add 200 μl solution of "0h" in a plate with ampicillin. Add 200 μl solution of "0h" in a plate with kanamycin. 8.Centrifuge 10h" and "100h" at 4000 rpm for 2 minutes and we will see sediment in the tubes. 9.Discard the supernatant liquid and leave about 220 μl medium. 10.Streak plate with "10h"and another streak plate with"100h". 11.Coat plate: add 200 μl solution of "10h" in a plate with chloromycetin while add 20 μl of "10h" solution in another plate with chloromycetin. Add 200 μl solution of "100h" in a plate with chloromycetin while add 20 μl of "100h" solution in another plate with chloromycetin. 10.Cultivate these bacteria overnight at 37 degree Celsius for further use.

Recorder: Xiaoyu Zhang To find out the proper parameter for our transformation experiment, we did a series of experiments about transformation of plasmid pSB1C3. Except the variables we set listed as follows: 1.Take the competent bacteria from refrigerator and incubate them into ice about 5 mins until it is dissolved 2.Absorb 100pg,10pg,1pg plasmid(1 μL) and 1μL sterilized distilled water respectively, marked “1m”,"10m","100m","0m" relatively.And mix it tenderly with bacteria solution thoroughly. 3.Put the tubes on the ice 30 mins.(Time SHOULD BE ACCURATE) 4.Make a heat shock at 42 degree centigrade about 90 sec(TIME SHOULD BE ACCURATE) 5.Put the tubes on the ice about 5 mins again. 6.Add 900 μl LB medium into EP tubes and cultivate the bacteria at 37 degree Celsius about 60 min. 7.Coat plate: add 200 μl solution of "1m" in a plate with chloromycetin while add 20 μl of "1m" solution in another plate with chloromycetin.add 200 μl solution of "10m" in a plate with chloromycetin while add 20 μl of "10m" solution in another plate with chloromycetin. Add 200 μl solution of "100m" in a plate with chloromycetin while add 20 μl of "100m" solution in another plate with chloromycetin. Add 200 μl solution of "0m" in a plate without antibiotics. Add 200 μl solution of "0m" in a plate with ampicillin. Add 200 μl solution of "0m" in a plate with kanamycin. 8.Centrifuge “1m”,“10m" and "100m" at 4000 rpm for 2 minutes and we will see sediment in the tubes. 9.Discard the supernatant liquid and leave about 250 μl medium. 10.Streak plate with "1m",one with “10m” and another streak plate with"100m". 11.Coat plate: add 200 μl solution of "1m" in a plate with chloromycetin while add 20 μl of "1m" solution in another plate with chloromycetin. add 200 μl solution of "10m" in a plate with chloromycetin while add 20 μl of "10m" solution in another plate with chloromycetin. Add 200 μl solution of "100m" in a plate with chloromycetin while add 20 μl of "100m" solution in another plate with chloromycetin. 10.Cultivate these bacteria overnight at 37 degree Celsius for further use.

DATE 6.21 Recorder: Chenyang LI The outcomes of last night's plating: The blank control group is normal.  There are many bacterial colonies on the other control plate that we added 200 μl solution of "0h" in a plate with kanamycin. It is confusing. May be

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there are some thing wrong with our competent cells.  There are no bacterial colonies on the rest plates.

DATE 6.21 Recorder: Xiaoyu Zhang The outcomes of last night's plating: The blank control group is normal.

There are many bacterial colonies on the other control plate that we added 200 μl solution of "0m" in a plate with kanamycin.

It is confusing. May be there are some thing wrong with our competent cells. There are no bacterial colonies on the rest plates.

DATE 6.21 Recorder: Jianjian Guo Operator: Chenyang Li, Yonghao Liang 1.Take the competent bacteria from refrigerator and incubate them into ice about 5 mins until it is dissolved. 2.Absorb 1 μL plasmid containing CysDes and plasimid containing PUC19(0.1ng/μL),absorb 100 μL plasmid respectively and mix it tenderly with bacteria thoroughly. 3.Put the tubes on the ice 30 mins.(Time SHOULD BE ACCURATE) 4.Make a heat shock at 42 degree centigrade about 90 sec(TIME SHOULD BE ACCURATE) 5.Put the tubes on the ice about 5 mins again. 6.Add 900 μl LB medium into EP tubes and cultivate the bacteria at 37 degree Celsius about 60 min. 7.Coat plate: for cells possibly containing PUC19,add 100 μL solution in a plate with ampicyllin.For cells possibly containing CysDes,add 100 μL solution/200 μL solution from 2-minute centrifugation at

4000 rpm of the original solution/20μL solution from 2-minute centrifugation at 4000 rpm of the original solution respectively to three plates with chloromycetin. 8.Cultivate these bacteria overnight at 37 degree Celsius for further use.

DATE 6.22 Recorder: Jianjian Guo Pick 4 colonies from the 100 μL CysDes plate and cultivate them in LB media with chloromycetin respectively.Three of them grow well and marked "1","2","4".

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DATE 6.22 Recorder: Jianjian Guo Operator: Chenyang LI Pick 3 other colonies from the 100 μL CysDes plate and cultivate them in LB media with chloromycetin respectively.All of them grow well and marked "5","6","7".

DATE 6.22 Recorder: CHenyang LI Result of transformation efficiency test：There are about 1439 Colonies on the plate. So the transformation efficiency of the Top10 competent cell is about 1.439*10^8 cfu/μg DNA. This transformation efficiency is enough for future experiments.

DATE 6.22 Recorder: Jianjian Guo 1.Plasmid Extraction of the colonies picked in the same day.All of the tubes are pink, differing only in the extent of the color.Done by Jianjian Guo,Tong Xiao,Yu Han and Mengxue Sun. Procedure: Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. Add 250 μL Buffer P1, resuspend cells. Add 250 μL Buffer P2, mix well, 3 min's standing. Add 350 μL Buffer P3, mix well. 13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 12000 rpm centifuge 1 min. Lying for 10 min. Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

2.PCR of the same tubes marked as "PCR1-1" "PCR1-2""PCR2-1""PCR2-2". 1 μL Primer1 1 μL Primer2 1 μL solution 10 μL 2*Taq 7 μL sterilized distilled water Parameters:30 cycle

DATE 6.23 Recorder: Chenyang LI Agarose gel electrophoresis of the PCR product and plasmid extraction product. Procedure: Add 0.4 g agarose to 40 mL TAE buffer. Dissolved by heating. Cool down. Pour into 1 electrophoresis tank in equal amount. Mix 10 μL each product with 2 μL 6*loading buffer each to form 7 loading groups. Loading：DNA marker 5 μL. Electrophoresis gel：110 V 30 min. Autoradiography(UV).

(from left to right: Trans 2K plus(contain Gelred), CysDes plamisd 1, CysDes plamisd 2, PCR1-1, PCR1-2, PCR2-1, PCR2-2.) There are nothing.Maybe there are something wrong with Gelred in the loading buffer.

DATE 6.23 Recorder: Chenyang LI PCR of CysDes plamisd 1.Prepare 2 PCR tubes and sequentially add：

sample	**1,2**
Sterilized ddH2O	7μL
2×Taq	10 μL
Template(CysDes plamisd)	1.5 μL
Primer VR	1 μL
Primer VF2	1 μL
total	20 μL

2.PCR reaction Parameters setting：

stage	**temperature(°C)**	**time**
Pre-Duration	95	10 min
Duration	95	15 sec
Anneal	55	20 sec
Extend	72	2 min
Post-Extend	72	10 min
Final	4	--

30 cycles(Duration ~ Extend)

2.Agarose gel electrophoresis of the PCR product and plasmid extraction product.Done by Jianjian Guo and Mengxue Sun. Procedure: Add 0.7 g agarose to 70 mL TAE buffer. Dissolved by heating. Cool down. Pour into 2 electrophoresis tank in equal amount. Mix 10 μL each product with 2 μL 6*loading buffer each to form 12 loading groups. Loading： DNA marker 6 μL,12 groups listed above. Electrophoresis gel： 120 V 30 min. Autoradiography(UV).

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The upper part are PCR groups,from left to right marker,'1','2','4','5','6','7',Other groups are unwanted. The lower part are plasmid extraction groups,from left to right marker,'1','2','4','5','6','7',Other groups are unwanted. These prove that the plasmids transformed contain RFP instead of CysDes.

6.23 Recorder: Chenyang LI Colony picking of strain mTRCAB We picked three colonies of strain mTRCAB , and cultivated them overnight (from 5 p.m. to 8:30 a.m.) at 37 degree Celsius, 250 rpm.

6.23 Recorder: Yan Shi Colony picking of strain pTeT & RBS We picked four colonies of strain pTeT & four of RBS , and cultivated them overnight (from 5 p.m. to 8:30 a.m.) at 37 degree Celsius, 250 rpm.

DATE 6.23 Preparation of Glycerin bacteria of Top 10 & BL21(DE3) Recorder: Jianjian Guo Procedure: Add 500 μL glycerin and 1000 μL Bacteria liquid into a tube and store it at -40 degree centigrade. The bacteria is from the strain we bought from Transgen. We cultivated the bacteria for 1 hours to raise the competent cells before we made the glycerin bacteria.

DATE 6.24 Preparation of Glycerin bacteria of Top 10 & BL21(DE3) Recorder: Jianjian Guo Picked 4 colonies of pLuxR at 9:30 am. Picked 2 colonies of TOP10 at 10:52 am, cultivated them in 200 mL LB media respectively at 5:30 pm after 6 hours of tube-cultivation respectively. Prepared competent TOP10 cell. Procedure: 1.Transport the LB media containing TOP10 to 2 pre-cooled 50 mL-centrifuge tubes and bath them in ice for 30 minutes. 2.Centrifugation at 4000 rpm for 15 minutes. 3.Discard the supernatent, add 30 mL 80mM $CaCl_2$-20mM $MgCl_2$ per tube to dissolve the cells, then bath in ice for 30 minutes.

1. Centrifugation at 2700 rpm for 15 minutes. 5.Discard the supernatent, add 2 mL 100mM $CaCl_2$-50% glycerol per tube to dissolve the cells, then sub-package into EP tubes(100 μL per tube). 6.Store at -80°C.

DATE 6.24 Plasmid Extraction of pSB1C3 containing pTeT and pSB1C3 containing RBS. **Recorder: Yonghao Liang Yan Shi** Procedure:

1. Centifuge 1.5 mL bacterium solution at 11000 rpm, few sediment getted. Remove the supernatant. Repeat twice.
2. Add 250 μL Buffer P1, resuspend cells.
3. Add 250 μL Buffer P2, mix well, 3 min's standing.
4. Add 350 μL Buffer P3, mix well.
5. 13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate.
6. Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate.
7. Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once.
8. 12000 rpm centifuge 1 min.
9. Lying for 10 min.
10. Put the adsorption column in a new EP tube. Add 40 μL 50°C elusion buffer, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with elusion buffer at 4 degree Celsius.

After extraction, we measured the OD A260/A280 and the density of plasmids.

pTeT	**1**	**2**	**3**	**4**
Concentration(ng/μl)	252.6	112.0	188.1	189.3
260/280	1.86	1.85	1.84	1.85
260/230	2.20	2.06	2.10	2.26

RBS	**1**	**2**	**3**	**4**

RBS	1	2	3	4
Concentration(ng/μl)	119.1	132.6	130.7	127.4
260/280	1.89	1.87	1.85	1.88
260/230	2.26	2.21	2.21	2.30

DATE 6.24 Plasmid Extraction of the colonies(pLuxR) picked yesterday. Recorder: Jianjian Guo，Jingyu Wang Procedure: Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. Add 250 μL Buffer P1, resuspend cells. Add 250 μL Buffer P2, mix well, 3 min's standing. Add 350 μL Buffer P3, mix well. 13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 12000 rpm centifuge 1 min. Lying for 10 min. Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

6.24 Transformation of plasmid pSB1C3 containing CysDes Recorder: Yawei Wu NOTE:Generally, competent bacteria are restrored in -70 degree centrigrade environment. 1.Take the competent bacteria from refrigerator and incubate them into ice about 5 mins until it is dissolved 2.Absorb 100pg to 10 ng plasmid(normally 1 to 2 uL, DO NOT add more than 5% volumn of bacteria solution) and mix it with bacteria solution thoroughly. ATTENTION: Please operate this step tenderly!!! 3.Put the tubes on the ice about 30 mins.(Time SHOULD BE ACCURATE) 4.Make a heat shock at 42 degree centigrade about 30 sec(TIME SHOULD BE ACCURATE) 5.Put the tubes on the ice about 2 to 3 mins again. 6.Add 900 ul LB medium into EP tubes and cultivate the bacteria at 37 degree centrigrade about 40 to 60 min. 7.Centrifuge them at 12,000xg about 15 sec and we will see sediment in the tubes. 8.Discard the supernatant liquid and leave about 220 ul medium. 9.Coat plate: add 200 ul solution in a large plate while add 20 ul solution in a small plate. 10.Cultivate these bacteria overnight for further use.

DATE 6.24 Plasmid Extraction of the colonies(Mtr) Recorder: Shihan Zhu Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

After extraction, we measured the OD A260/A280 and the density of plasmids.

Mtr	1(1)	1(2)	2(1)	2(2)	3(1)	3(2)
Concentration(ng/μl)	772.9	730.18	652.7	813.8	809.6	925.3
260/280	1.83	1.84	1.85	1.86	1.83	1.86
260/230	2.10	2.26	2.35	2.38	2.13	2.19

PCR of Mtr colonies Recorder: Shihan Zhu 1 μL Primer1 VR 1 μL Primer2 VF2 1 μL template 10 μL 2*Taq 7 μL sterilized distilled water template: Mtr1 Mtr2 Mtr3 Parameters:30 cycle

Plasmid Extraction of pSB1C3 containing pTeT and pSB1C3 containing RBS. **Recorder: Yonghao Liang Yan Shi** Procedure:

1. Centifuge 1.5 mL bacterium solution at 11000 rpm, few sediment getted. Remove the supernatant. Repeat twice.
2. Add 250 μL Buffer P1, resuspend cells.
3. Add 250 μL Buffer P2, mix well, 3 min's standing.
4. Add 350 μL Buffer P3, mix well.
5. 13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate.
6. Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate.
7. Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once.

8. 12000 rpm centifuge 1 min.
9. Lying for 10 min.
10. Put the adsorption column in a new EP tube. Add 40 μL 50°C elusion buffer, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with elusion buffer at 4 degree Celsius.

After extraction, we measured the OD A260/A280 and the density of plasmids.

pTeT	**1**	**2**	**3**	**4**
Concentration(ng/μl)	121.1	107.8	93.8	88.8
260/280	1.83	1.84	1.75	1.87
260/230	1.75	2.23	1.18	2.17

RBS	**1**	**2**	**3**	**4**
Concentration(ng/μl)	71.3	97.8	82.2	171.3
260/280	1.91	1.85	1.88	1.64
260/230	2.23	1.76	1.79	0.84? ? ?

6.25 Recorder: Bijunyao Zhong Colony picking of strain pSB1C3 containing pTeT and pSB1C3 containing RBS

DATE 6.25 Preparation competent BL21 cells Recorder: Jianjian Guo Procedure: 1.coat the original BL21 cells we bought to an LB plate overnight,then pick a single colony into 200mL LB media,cultivate at 37°C for 2.5 hours, and the OD600 measures 0.76. 2.Transport the LB media containing BL21 to 2 pre-cooled 50 mL-centrifuge tubes and bath them in ice for 30 minutes. 2.Centrifugation at 4000 rpm for 15 minutes. 3.Discard the supernatent, add 30 mL 80mM CaCl2-20mM MgCl2 per tube to dissolve the cells, then bath in ice for 30 minutes.

1. Centrifugation at 2700 rpm for 15 minutes. 5.Discard the supernatent, add 2 mL 100mM CaCl2-50% glycerol per tube to dissolve the cells, then sub-package into EP tubes(100 μL per tube). 6.Store at -80°C.

6.25 Recorder: Bijunyao Zhong Colony picking of strain pSB1C3 containing pTeT and pSB1C3 containing RBS

DATE 6.25 Transformation of pUC19 and RFP to test the competent BL21 cells made the same day Recorder: Jianjian Guo result:RFP didn't grow any colony while pUC19 has plenty single colonies.Repeat transfomation on RFP, this time there're 3 or so small colonies.

DATE 6.25 Plasmid Extraction of the colonies(pLuxR) Recorder: Jianjian Guo，Jingyu Wang Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

pLuxR	**1**	**2**	**3**
Concentration(ng/μl)	437.2	267.1	308.7
260/280	1.88	1.87	1.84
260/230	2.32	2.30	2.23

DATE 6.25 Plasmid Extraction of the colonies(Mtr) Recorder: Tong Xiao，Mengxue Sun，Menglong Jin Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution,

12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius. PS:Only two out of three tubes of colonies were extracted successfully because of abandoning one tube of supernate in the extracting process mistakenly.

After extraction, we measured the OD A260/A280 and the density of plasmids.

Mtr	**1**	**3**
Concentration(ng/μl)	140.9	121.5
260/280	1.88	1.85
260/230	2.34	2.30

DATE 6.25 Agarose gel electrophoresis of the plasmid extraction product of pLuxR 1-4 Recorder: Jingyu Wang Procedure: Add 0.4 g agarose to 40 mL TAE buffer. Dissolved by heating. Cool down. Pour into 1 electrophoresis tank. Mix 5 μL each product with 1 μL 6*loading buffer each to form 4 loading groups. Loading：DNA marker 6 μL,4 groups listed above. Electrophoresis gel：110 V 30 min. Autoradiography(UV).

DATE 6.25 Agarose gel electrophoresis of plasmid extraction product Recorder: Mengxue Sun,Tong Xiao,Jianjian Guo Procedure: Add 0.4 g agarose to 40 mL TAE buffer. Dissolved by heating. Cool down. Pour into 1 electrophoresis tank. Mix 5 μL each product with 1 μL 6*loading buffer each to form 4 loading groups. Loading：DNA marker 6 μL,4 groups listed above. Electrophoresis gel：110 V 30 min. Autoradiography(UV). The result is showed below:

The left part are Mtr groups,from left to right marker,'1','3','1'',3''.Because we couldn't distinguish which was the right loading buffer from two tubes,we added those two tubes of loading buffer apart to two groups of plasmid extraction product of the same composition('1','3'and'1'',3''). The result shows that only one tube of loading buffer is right. The right part are plasmid extraction product of pLuxR 1-4,from left to right'1','2','3','4',marker.Because of adding wrong tube of loading buffer,'3'and'4'get no result.

DATE 6.25 Agarose gel electrophoresis of plasmid extraction product Recorder: Yitian Zhou Procedure: Add 0.4 g agarose to 40 mL TAE buffer. Dissolved by heating. Cool down. Pour into 1 electrophoresis tank. Mix 5 μL each product with 1 μL 6*loading buffer each to form 9 loading groups. Loading：DNA marker 6 μL, 9 groups listed above. Electrophoresis gel：90 V 30 min. Autoradiography(UV). The result is showed below:

from left to right (4-13) lanes are **M1(1), M1(2), M2(1), M2(2), M3(1), M3(2), PCR_M1, PCR_M2, PCR_M3, Marker**

Marker we choose are *Trans*2K plus. The size of PCR result is about 5000 bp, which confirm our prediction.

PS. We ran a gel yesterday with the same product, but no result showed in autoradiography. We change another loading buffer today and show the result of both plasmid and PCR result.

PCR of Mtr plasmids Recorder: Shihan Zhu 1 μL Primer1 Mtr-res-r 1 μL Primer2 Mtr-res-f 1 μL template 10 μL 2*Taq 7 μL sterilized distilled water template: Mtr1(1) Mtr1(2) Mtr2(1) Mtr2(2) Mtr3(1) Mtr3(2) Mtr1-XT Mtr3-JML parameters:30 cycle

Sequence of primers Mtr-res-r:ccg ctc gag tta gag ttt gta act cat gc Mtr-res-f:gga att cca tat ggc ctc ttc atg aac gc

DATE 6.25 Preparation of Glycerin bacteria of pSB1C3 containing pTeT , pSB1C3 containing RBS and pSB1C3 containing MTR Recorder: Yan Shi Procedure: Add 500 μL glycerin and 1000 μL Bacteria liquid into a tube and store it at -40 degree centigrade. The bacteria is from the colony we picked on 6.18. we cultivated the bacteria for 4 hours and 38 minutes before we made the glycerin bacteria.

DATE 6.25 Plasmid Extraction of the colonies(Cys) Recorder: Jianjian Guo，Jingyu Wang Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all

supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

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CysDes	**1**	**2**
Concentration(ng/μl)	181.9	262.7
260/280	1.74	1.79
260/230	1.04	1.38

DATE 6.26 Recorder: Jianjian Guo,Yu Han Plasmid Extraction of the colonies picked yesterday (CysDes1-4) All of the tubes are pink, differing only in the extent of the color. Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

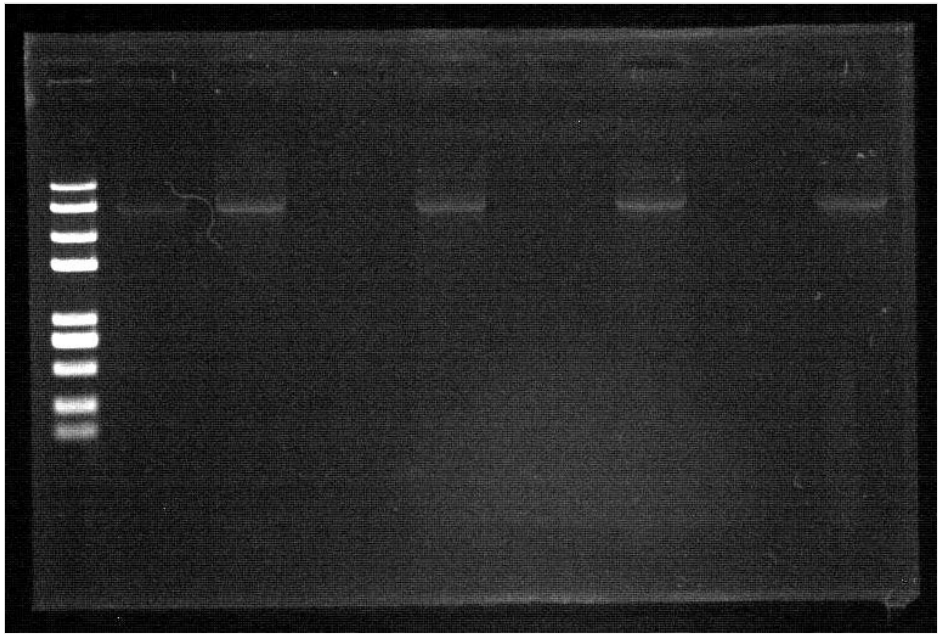
CysDes	**1**	**2**	**3**	**4**
Concentration(ng/ul)	360.6	335.7	285.8	199.9
260/280	1.85	1.87	1.73	1.83
260/230	2.06	2.44	1.02	1.90

Recorder: Tong Xiao,Mengxue Sun Plasmid Extraction of the colonies (Mtr1-3). Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

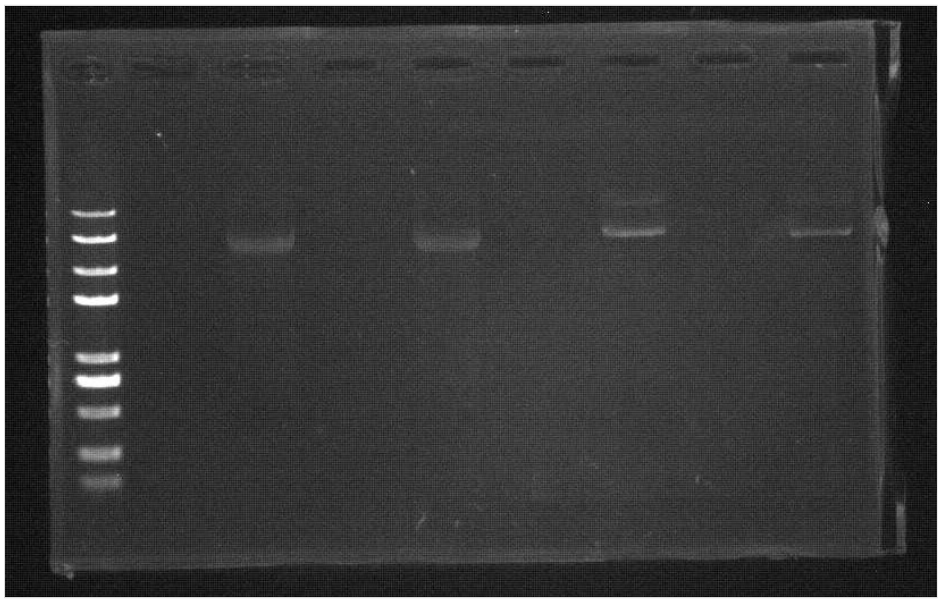
After extraction, we measured the OD A260/A280 and the density of plasmids，done by Mengxue Sun.

Mtr	**1**	**2**	**3**
Concentration(ng/ul)	383.8	351.3	453.2
260/280	1.86	1.88	1.87
260/230	2.32	2.26	2.09

DATE 6.26 Agarose gel electrophoresis of mtr gene PCR products Recorder: Shihan Zhu Procedure: Add 0.8 g agarose to 80 mL 1*TAE buffer. Dissolved by heating. Cool down. Pour into 2 electrophoresis tanks. Mix 20 μL each product with 4 μL 6*loading buffer each to form 7 loading groups. Loading：DNA marker 6 μL, 7 groups listed above. DNA marker: 2K plus II Electrophoresis gel：110 V 30 min. Autoradiography(UV). The result is showed below:



picture 1: lane1 marker, lane3 Mtr1(2), lane5 Mtr3(2), lane7 Mtr3(1), lane2&9 Mtr2(2)



picture 2: lane1 marker, lane3 Mtr3-JML, lane5 Mtr1-XT, lane7&9 Mtr2(1)

Recorder: Shihan Zhu Gel Extraction of Mtr PCR products

Procedure:

1. Cut off the Gel part containing the target band. Get 6 tubes of AD and 5 tubes of BD.
2. Weigh the cut off Gel part.
3. Add Buffer B2 which 300 times the weight of the Gel.
4. Add isopropanol which is 1/3 volume of the Buffer B2.
5. Move the solution to adsorption column, 11000 rpm centifuge 30 s, discard filtrate.
6. Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once.
7. 12000 rpm centifuge 1 min.
8. Lying for 10 min.
9. Put the adsorption column in a new EP tube. Add 20 μL ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

After extraction, we measured the OD A260/A280, OD A260/A230 and the density of plasmids. According to OD data of our products, we confirm that there are few DNA in our gel extraction products. So we only measure the OD of three tubes and exact data will not be shown here.

DATE 6.27 PCR of mtr gene Recorder: Shihan Zhu

Experimental materials

1. Template: pSB1C3 containing mtr gene(m11,m12,m21,m22,m31,m32);
2. Primer: mtr-res-f, mtr-res-r.synthesized by Sangon Biotech;
3. Sterilized ddH2O, 2×Primer Star

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Procedure:

1.Prepare 6 PCR tubes and sequentially add：

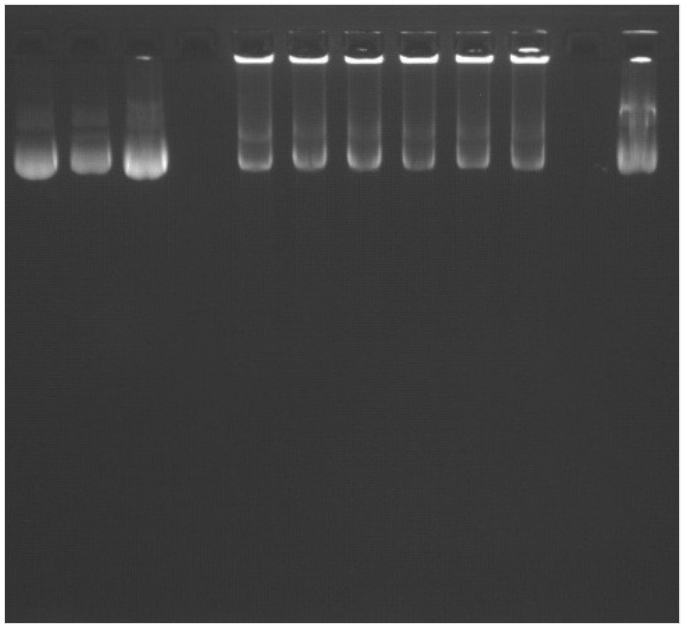
sample	**1**	**2**	**3**	**4**	**5**	**6**
Sterilized ddH2O	22 μL	22 μL	22 μL	22 μL	22 μL	22 μL
2×Primer Star	25 μL	25 μL	25 μL	25 μL	25 μL	25 μL
template	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
mtr-res-f(10 μM)	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
mtr-res-r(10 μM)	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
total	50 μL	50 μL	50 μL	50 μL	50 μL	50 μL

2.PCR reaction Parameters setting：

stage	**temperature**	**time**
step 1	95	10 min
step 2	95	20 s
step 3	55	20 s
step 4	72	5 min 30 s
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis mixed with 6× DNA loading buffer;110 V, 30 min Agarose gel electrophoresis Result:



lane1-10: Mtr1-XT Mtr2-XT Mtr3-XT m11 m12 m21 m22 m31 m32 Mtr2-XT

Recorder: Shihan Zhu Purification of PCR product of mtr We have 50 μL solution in each tube, from which we use 5 μL of them to do electrophoresis (mixed with 6× DNA loading buffer;110 V, 30 min). And the other 45 μL is purified by PCR purification kit (bought from Sangon Biotech). And the procedure is showed below:

1. Add 225 μL Buffer B3 to the 25 μL solution and mix it up. Add it to an adsorption column.
2. 11,000 rpm centrifuge for 30 s, and then discard the filtrate.
3. Add 500 μL Wash Solution, 12,000 rpm centrifuge for 30 s, and then discard the filtrate.

4. Repeat last process.
5. Centrifuge the empty column at 12,000 rpm for 1 min.
6. Lie the column still for 10 min.
7. Put the column to an 1.5 ml EP tube, add 20 μL ddH2O, stand them still for 10 min. Centrifuge at 12,000 rpm for 1 min.

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PCR purification kit:

sample	z11	m11	m12	m21	m22	m31	m32	MTR1	MTR2	MTR3
Concentration(ng/ul)	25.6	129.3	165.1	190.3	183.2	151.5	286.0	32.5	23.3	20.6
260/280	1.80	1.59	1.82	1.81	1.85	1.82	1.55	1.60	1.67	1.44
260/230	1.53	0.67	1.91	1.82	2.13	2.07	0.55	0.59	1.01	0.02

Recorder: Jianjian Guo,Yu Han Plasmid Extraction of the colonies persisted yesterday (CysDes1-4、1'-4') All of the tubes are pink, differing only in the extent of the color. Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

After extraction, we measured the OD A260/A280, OD A260/A230 and the density of plasmids. The results are shown in the chart below.

CysDes	1	2	3	4
Concentration(ng/ul)	280.3	269.1	344.8	281.2
260/280	1.84	1.78	1.83	1.81
260/230	1.97	1.35	1.83	1.56

CysDes	1'	2'	3'	4'
Concentration(ng/ul)	36.9	41.2	52.9	71.4
260/280	1.93	1.90	1.90	1.85
260/230	2.87	3.06	2.91	2.44

The density of CysDes 1'-4' is relatively low because these four colonies are only cultivated for about 7 hours.

DATE 6.27 Plasmid Extraction of the colonies persisted yesterday(pTET,RBS) Recorder: Liudong Luo,Xingwei Yang Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

RBS	1	2	3	4
Concentration(ng/ul)	33.3	100.1	34.7	36.1
260/280	1.82	1.85	1.84	1.84

RBS	1	2	3	4
260/230	0.72	0.45	0.50	0.54

pTET	1	2	3	4
Concentration(ng/ul)	34.1	15.0	17.2	13.2
260/280	1.58	1.91	1.67	1.82
260/230	0.75	0.52	0.58	0.97

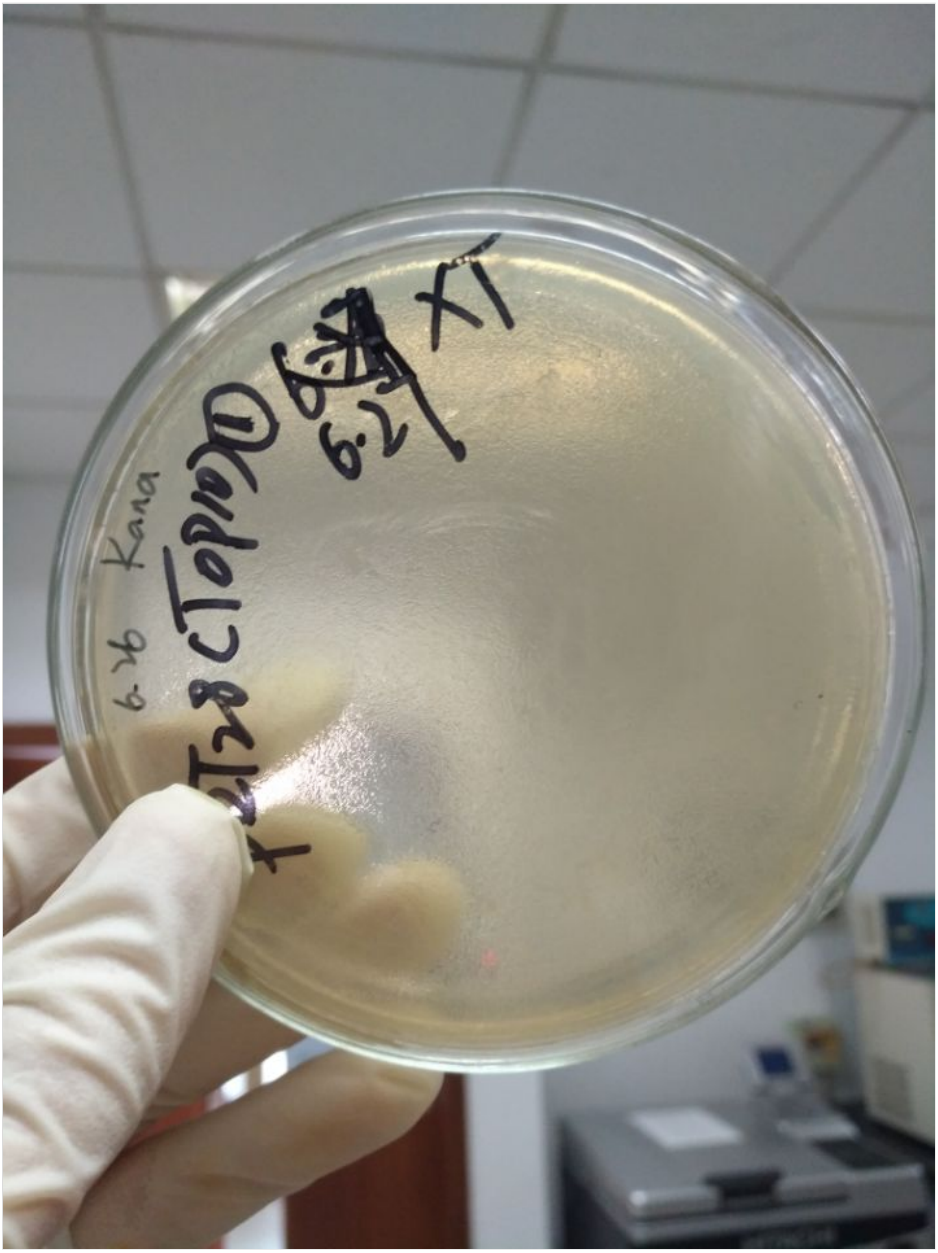
Cause we both did this the first time,we failed.

Transformation of plasmid pET28 Recorder:Tong Xiao,Mengxue Sun NOTE:Generally, competent bacteria are restrored in -70 degree centrigrade environment. 1.Take the competent bacteria from refrigerator and incubate them into ice about 5 mins until it is dissolved 2.Absorb 100pg to 10 ng plasmid(normally 1 to 2 uL, DO NOT add more than 5% volumn of bacteria solution) and mix it with bacteria solution thoroughly. ATTENTION: Please operate this step tenderly!!! 3.Put the tubes on the ice about 30 mins.(Time SHOULD BE ACCURATE) 4.Make a heat shock at 42 degree centigrade about 90 sec(TIME SHOULD BE ACCURATE) 5.Put the tubes on the ice about 5 mins again. 6.Add 900 ul LB medium into EP tubes and cultivate the bacteria at 37 degree centrigrade about 60 min. 7.Centrifuge them at 4000 rpm for 2 minutes and we will see sediment in the tubes. 8.Discard the supernatant liquid and leave about 200 ul medium. 9.Coat plate: Add 200 ul solution in a plate with kanamycin. 10.Cultivate these bacteria overnight for further use.

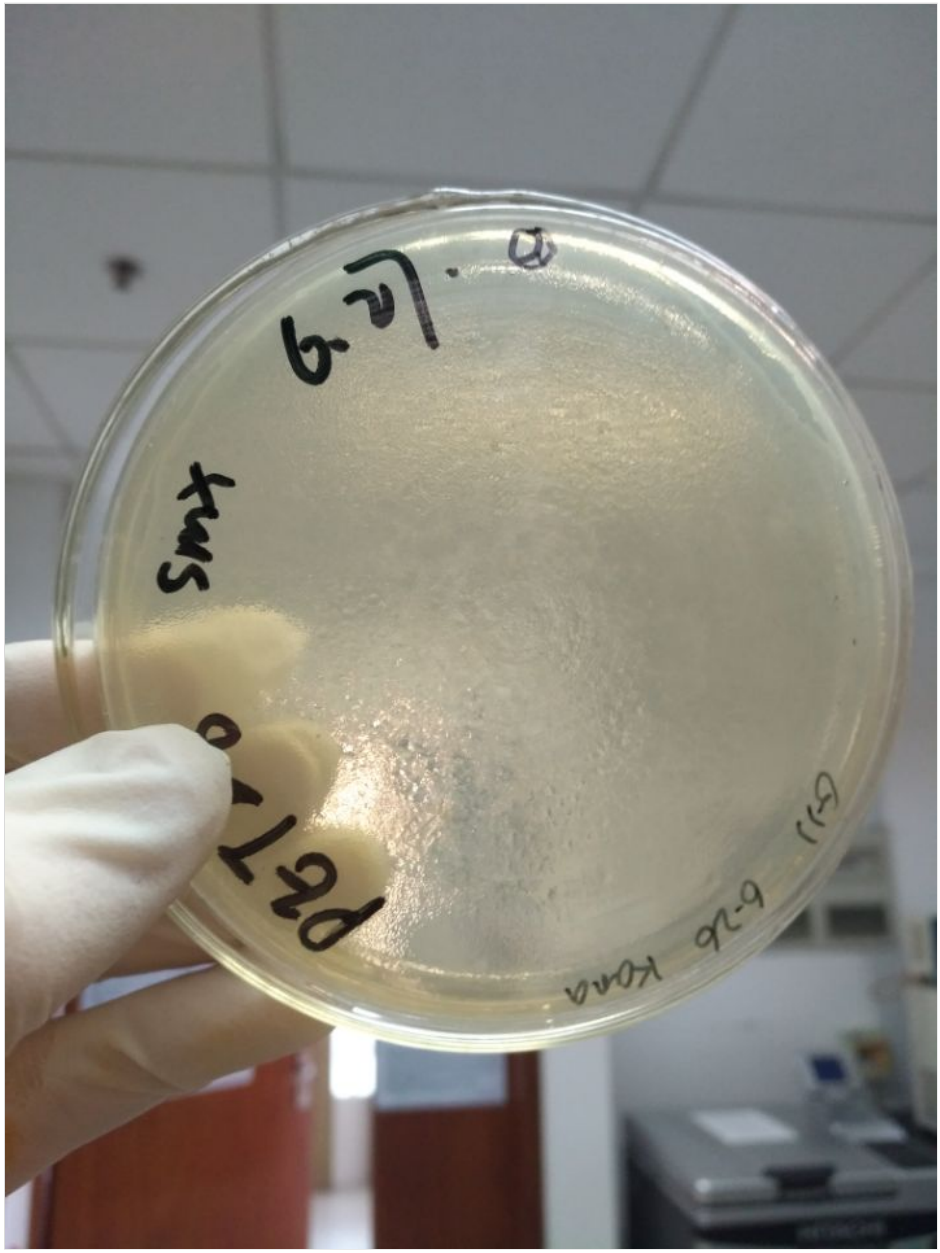
PS:The temperature of the water bath has not reached 42 degree centigrade until we make a heat shock.It’s about 41 degree centigrade.

Colony pick up of this morning's transformation and plating Recorder:Tong Xiao PS:The outcomes of this morning's plating.

帮助



帮助



Through the picture we can see that these two plates are completely covered by bacterial colonies. The plates we use has been added kanamycin and pET28 has a kanamycin resistance gene on it. According to our protocol of transformation,we discard the supernatant liquid and leave about 200 ul medium which we used for plating.We think that maybe 200ul is too much,resulting in high concentration of bacterial strain.So,we decide to delete the step of centrifuging and just use 100ul medium for plating.Follow-up experiment is going to be done by Yitian Zhou.

Yonghao Liang Double digestion of plasmid containing pTET and RBS Materials:

1. pTET gene in plasmid pSB1C3(from iGEM kit plate)
2. FastDigest restriction enzyme SpeI, EcoRI, PstI and 10×FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water
4. marker:Trans 2K Plus

	RBS	**pTET**
Concentration(ng/ul)	130.7	252.6
260/280	1.85	1.86
260/230	2.21	2.20

Reaction system:

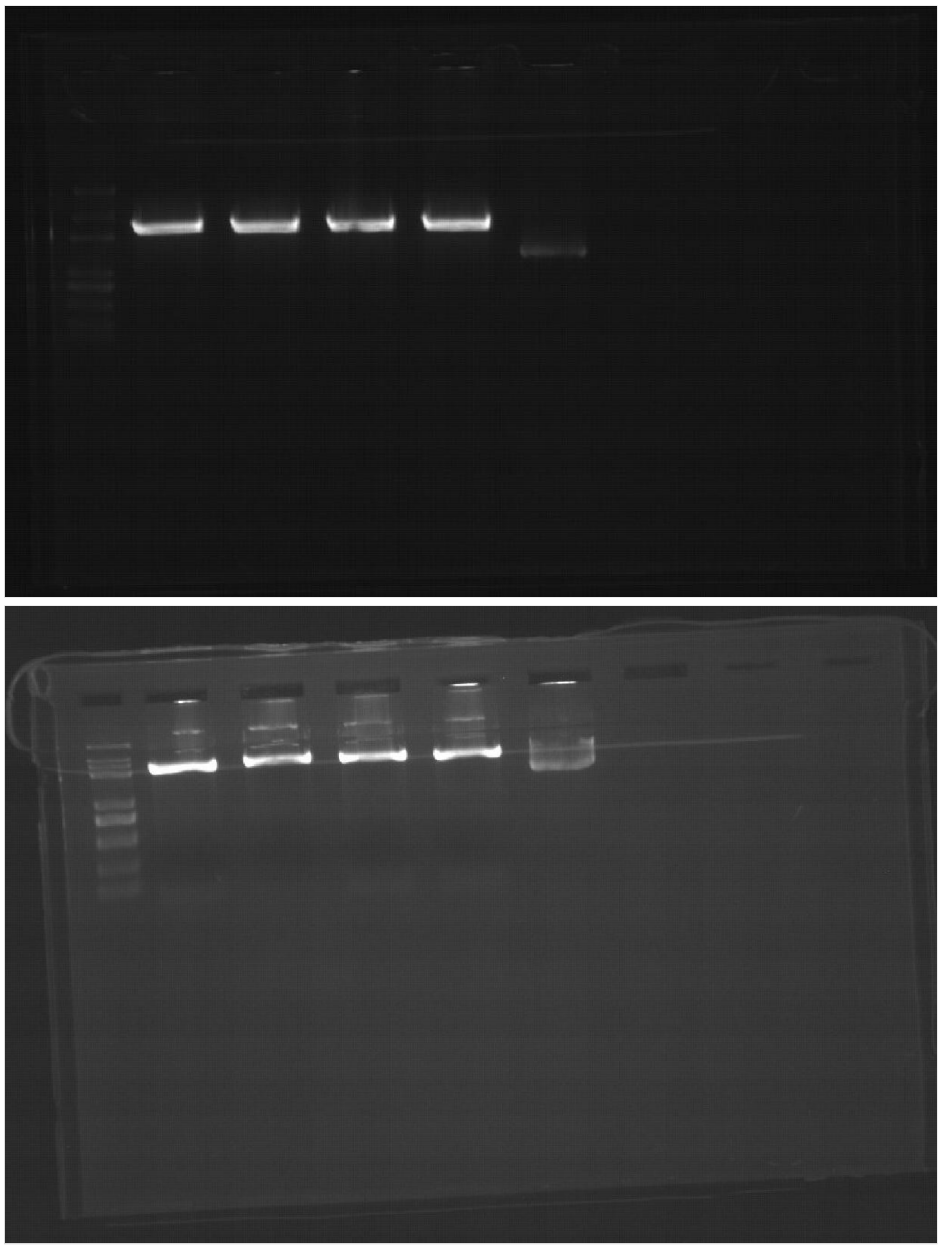
Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**

帮助

Sample	1	2	3	4	5	6	7	8
pSB1C3 containing RBS(μL)	7	7	7	7	0	0	0	0
pSB1C3 containing pTET(μL)	0	0	0	0	4	4	4	4
nuclease-free water(μL)	9	9	9	9	12	12	12	12
fastdigest green buffer(μL)	2	2	2	2	2	2	2	2
PstI(μL)	1	1	1	1	1	1	1	1
XbaI(μL)	1	1	1	1	0	0	0	0
SpeI(μL)	0	0	0	0	1	1	1	1
total(μL)	20	20	20	20	20	20	20	20

Mix gently and incubate at 37 degree Celsius for 30 mins .

Agarose gel electrophoresis Result:



(from left to right:marker(Trans plus 2K),sample1,,sample2,sample3,control-

1,sample4,sample5,sample6,control-2) (For RBS, we use 2% gel and run for 20 mins at 90 V. For pTET, we use 1% gel and run for 30 mins at 90 V.)

We did gel extration. The result are as follows: (For RBS: we put 4 fragments into one column. For pTET: we put 2 fragments into one column, so we have two columns for pTET.)

sample	**RBS**	**pTET-1**	**pTET-2**
concentration(ng/μL)	27.2	78.7	59.9
A260/A280	1.45	1.59	1.40
A260/A230	0.26	0.10	0.27

As you can see, we failed to extract the DNA from the gel. I think it was because the column we used had been contaminate.

Transformation of plasmid pET28 containg reductase S1 Recorder: Yitian Zhou
NOTE:Generally, competent bacteria are restrored in -70 degree centrigrade environment. 1.Take the competent bacteria from refrigerator and incubate them into ice about 5 mins until it is dissolved 2.Absorb 100pg to 10 ng plasmid(normally 1 to 2 uL, DO NOT add more than 5% volumn of bacteria solution) and mix it with bacteria solution thoroughly. ATTENTION: Please operate this step tenderly!!! 3.Put the tubes on the ice about 30 mins.(Time SHOULD BE ACCURATE) 4.Make a heat shock at 42 degree centigrade about 90 sec(TIME SHOULD BE ACCURATE) 5.Put the tubes on the ice about 5 mins again. 6.Add 900 ul LB medium into EP tubes and cultivate the bacteria at 37 degree centrigrade about 60 min. 7.Centrifuge them at 4000 rpm for 2 minutes and we will see sediment in the tubes. 8.Discard the supernatant liquid and leave about 200 ul medium. 9.Coat plate: Add 200 ul solution in a plate with kanamycin. 10.Cultivate these bacteria overnight for further use.

Awake the Glycerin bacteria BL21(DE3) and Top 10 containing pTET and RBS on the plasmid pSB1C3 Recorder: Shihan Zhu Add 200 μL of bacteria into a 5 mL LB culture and cultivate these bacteria overnight for extraction at 37 degree centigrade, 250 rpm.

DATE 6.28 Recorder: Shihan Zhu Double digestion of plasmid pET28 and mtr PCR products
Materials:

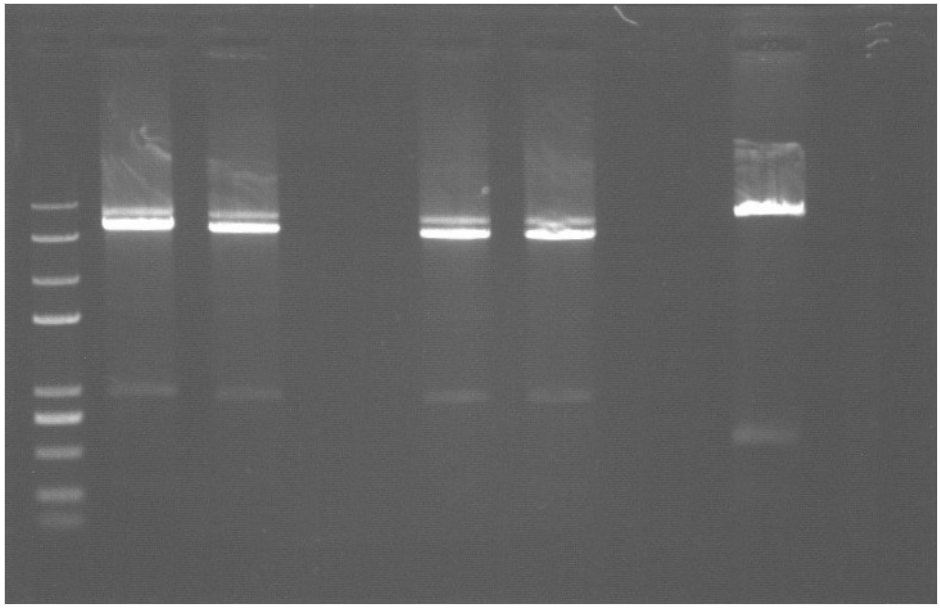
1. plasmid pET28, mtr PCR products
2. FastDigest restriction enzyme NdeI, XhoI and 10×FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water
4. marker:Trans 2K Plus

Reaction system:

Sample	**1**	**2**	**3**
pET28(μL)	5	0	0
mtr(μL)	0	5	5
nuclease-free water(μL)	11	20	20
fastdigest green buffer(μL)	2	3	3
NdeI(μL)	1	1	1
XhoI(μL)	1	1	1
total(μL)	20	30	30

Mix gently and incubate at 37 degree Celsius for 30 mins .

Agarose gel electrophoresis Result:



We did gel extration. The result are as follows: (For mtr: we put 2 fragments into one column. For pET28: we put 1 fragments into one column.)

sample	mtr12	mtr31	pET28
concentration(ng/μL)	6.4	12.2	0.5
A260/A280	2.20	2.15	-0.55
A260/A230	0.22	0.06	0.07

Recorder: Jianjian Guo Transformation of CysDes from the 2017 Kit Plate 6, Well K4
Precedure: NOTE:Generally, competent bacteria are restrored in -70 degree centrigrade environment. 1.Take the competent bacteria from refrigerator and incubate them into ice about 5 mins until it is dissolved 2.Absorb 100pg to 10 ng plasmid(normally 1 to 2 uL, DO NOT add more than 5% volumn of bacteria solution) and mix it with bacteria solution thoroughly. 3.Put the tubes on the ice about 30 mins.(Time SHOULD BE ACCURATE) 4.Make a heat shock at 42 degree centigrade about 90 sec(TIME SHOULD BE ACCURATE) 5.Put the tubes on the ice about 5 mins again. 6.Add 900 ul LB medium into EP tubes and cultivate the bacteria at 37 degree centrigrade about 60 min. 7.Centrifuge them at 4000 rpm for 2 minutes and we will see sediment in the tubes. 8.Discard the supernatant liquid and leave about 200 ul medium. 9.Coat plate: Add 200 ul solution in a plate with kanamycin. 10.Cultivate these bacteria overnight for further use.

Recorder: Yonghao Liang Plasmid Extraction of the pTET and RBS Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

After extraction, we measured the OD A260/A280, OD A260/A230 and the density of plasmids. The results are shown in the chart below.

sample	RBS-1	RBS-2	RBS-3	pTET-1	pTET-2	pTET-3
Concentration(ng/ul)	129.7	124.4	91.3	95.1	98.9	115.1
260/280	1.83	1.81	1.84	1.79	1.82	1.82
260/230	2.10	2.21	2.09	1.95	2.00	2.07

Recorder: Tong Xiao Plasmid Extraction of the Mtr1-8 and TOP10 transformed with pET28.
Done by Tong Xiao,Shihan Zhu,Mengxue Sun. Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix

well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

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After extraction, we measured the OD A260/A280, OD A260/A230 and the density of plasmids. The results are shown in the chart below.

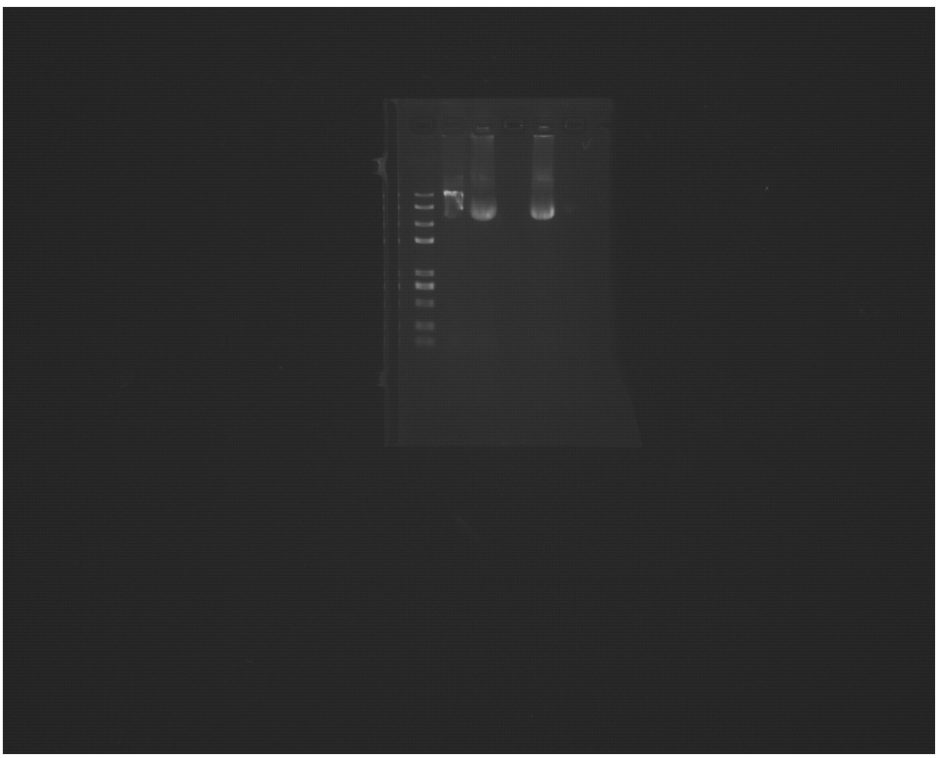
Mtr	**1**	**2**	**3**	**4**
Concentration(ng/ul)	557.7	515.2	634.7	554.7
260/280	1.83	1.88	1.86	1.84
260/230	2.06	2.47	2.45	2.32

Mtr	**5**	**6**	**7**	**8**
Concentration(ng/ul)	500.1	525.8	552.4	512.1
260/280	1.88	1.89	1.80	1.87
260/230	2.52	2.52	1.78	2.49

sample	**pET28**
Concentration(ng/ul)	241.1
260/280	1.85
260/230	2.49

Agarose gel electrophoresis of Mtr and pET28 Done by Tong Xiao. Mix 5 μL each product with 1 μL 6*loading buffer each to form 3 loading groups. Loading：DNA marker 6 μL, 3 groups listed above. DNA marker: 2K plus II Electrophoresis gel：90 V 30 min.

Agarose gel electrophoresis Result:



From left to right: lane1: marker, lane2:pET28, lane3:Mtr3, lane5:Mtr7

DATE 6.28 Recorder: Shihan Zhu Purification of PCR product of mtr We have 50 μL solution in each tube, from which we use 5 μL of them to do electrophoresis (mixed with 6× DNA loading buffer;110 V, 30 min). And the other 45 μL is purified by PCR purification kit (bought from Sangon Biotech). And the procedure is showed below:

1. Add 225 μL Buffer B3 to the 25 μL solution and mix it up. Add it to an adsorption column.
2. 11,000 rpm centrifuge for 30 s, and then discard the filtrate.
3. Add 500 μL Wash Solution, 12,000 rpm centrifuge for 30 s, and then discard the filtrate.
4. Repeat last process.
5. Centrifuge the empty column at 12,000 rpm for 1 min.
6. Lie the column still for 10 min.
7. Put the column to an 1.5 ml EP tube, add 20 μL ddH2O, stand them still for 10 min. Centrifuge at 12,000 rpm for 1 min.

PCR purification kit:

sample	**mtr-pcr1**	**mtr-pcr2**	**mtr-pcr3**	**mtr-pcr4**
Concentration(ng/ul)	83.6	148.0	34.2	134.8
260/280	1.80	1.82	1.71	1.84
260/230	1.67	1.97	0.81	2.07

Recorder: Yawei Wu, Yu Han, Ziyi Wang Plasmid Extraction of the colonies picked yesterday (CysDes1-4, CysDes1'-4') All of the tubes are pink, differing only in the extent of the color. Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

CysDes	**1**	**2**	**3**	**4**
Concentration(ng/ul)	279.0	258.8	126.6	210.0
260/280	1.83	1.82	1.85	1.85
260/230	2.21	4.51???	2.14	2.16

| **CysDes** | **1** | **2** | **3** | **4** |

CysDes	**1'**	**2'**	**3'**	**4'**
Concentration(ng/ul)	150.5	170.0	201.7	210.0
260/280	1.84	1.80	1.83	1.84
260/230	2.27	1.59	2.08	2.30

DATE 6.28 Recorder: Liudong Luo,Xingwei Yang,Dongdong Jiang Plasmid Extraction of the colonies persisted this morning(pTET,RBS) Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

RBS	**1**	**2**	**3**	**4**
Concentration(ng/ul)	47.1	52.4	54.0	46.3
260/280	1.87	1.89	1.84	1.79
260/230	1.74	1.47	1.36	1.41

pTET	**1**	**2**	**3**
Concentration(ng/ul)	79.9	28.9	238.8

pTET	1	2	3
260/280	1.60	1.90	1.44
260/230	0.61	1.38	0.48

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We are looking forward to doing better next time.

DATE 6.28 Menglong Jin Double digestion of plasmid containing pTET and RBS Materials:

1. pTET gene in plasmid pSB1C3(from iGEM kit plate)
2. FastDigest restriction enzyme SpeI, EcoRI, PstI and 10×FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water
4. marker:Trans 2K Plus

	RBS	pTET
Concentration(ng/ul)	119.1	188.1
260/280	1.89	1.84
260/230	2.26	2.10

Reaction system:

Sample	1	2	3	4	5	6	7	8
pSB1C3 containing RBS(μL)	8	8	8	8	0	0	0	0
pSB1C3 containing pTET(μL)	0	0	0	0	6	6	6	6
nuclease-free water(μL)	8	8	8	8	10	10	10	10
fastdigest green buffer(μL)	2	2	2	2	2	2	2	2
PstI(μL)	1	1	1	1	1	1	1	1
XbaI(μL)	1	1	1	1	0	0	0	0
SpeI(μL)	0	0	0	0	1	1	1	1
total(μL)	20	20	20	20	20	20	20	20

Mix gently and incubate at 37 degree Celsius for 30 mins .

Recorder: Shihan Zhu PCR of mtr

Experimental materials

1. Template: pSB1C3 containing mtr gene;
2. Primer: mtr-res-f, mtr-res-r;
3. Sterilized ddH2O, 2×PrimeSTAR.

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

sample	1	2	3	4
Sterilized ddH2O	22 μL	22 μL	22 μL	22 μL
2×PrimeSTAR	25 μL	25 μL	25 μL	25 μL
pSB1C3	1 μL	1 μL	1 μL	1 μL
mtr-res-f(10 μM)	1 μL	1 μL	1 μL	1 μL
mtr-res-r(10 μM)	1 μL	1 μL	1 μL	1 μL

sample	1	2	3	4
total	50 μL	50 μL	50 μL	50 μL

2.PCR reaction Parameters setting：

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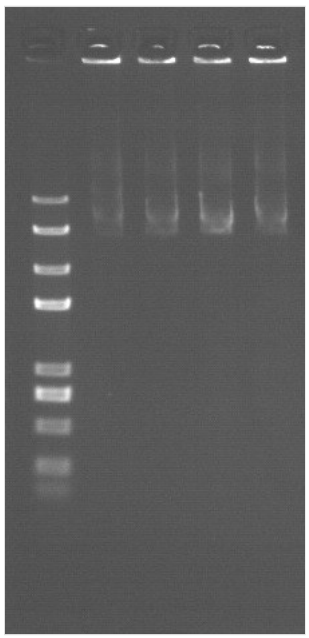
stage	temperature	time
step 1	95	10 min
step 2	95	20 s
step 3	55	40 s
step 4	72	5 min 30 s
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result: (90V,40min)  From left to right: lane1:marker(trans2K plus); lane2:sample1; lane3:sample2; lane4:sample3; lane5:sample4.

Recorder:Yan Shi Double digestion of plasmid containing pTET and RBS Materials:

1. pTET gene in plasmid pSB1C3(from iGEM kit plate)
2. FastDigest restriction enzyme SpeI, EcoRI, PstI and 10×FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water
4. marker:Trans 2K Plus

	RBS	pTET
Concentration(ng/ul)	119.1	188.1
260/280	1.89	1.84
260/230	2.26	2.10

Reaction system:

Sample	1	2	3	4	5	6	7	8
pSB1C3 containing RBS(μL)	8	8	8	8	0	0	0	0
pSB1C3 containing pTET(μL)	0	0	0	0	6	6	6	6

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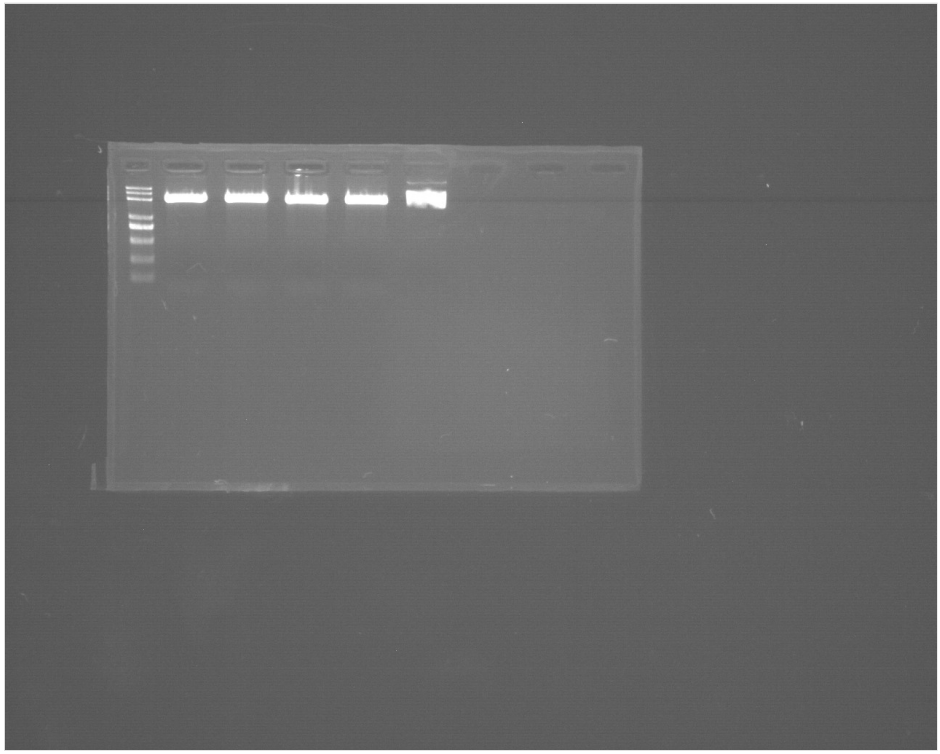
Sample	1	2	3	4	5	6	7	8
nuclease-free water(μL)	8	8	8	8	10	10	10	10
fastdigest green buffer(μL)	2	2	2	2	2	2	2	2
PstI(μL)	1	1	1	1	1	1	1	1
XbaI(μL)	1	1	1	1	0	0	0	0
SpeI(μL)	0	0	0	0	1	1	1	1
total(μL)	20	20	20	20	20	20	20	20

Mix gently and incubate at 37 degree Celsius for 30 mins .

Agarose gel electrophoresis of products of double digestion of pTeT and RBS Recorder: **Yan Shi** Procedure: Add 0.8 g agarose to 80 mL 1*TAE buffer. Dissolved by heating. Cool down. Pour into 2 electrophoresis tanks. Mix 20 μL each product with 4 μL 6*loading buffer each to form 7 loading groups. Loading：DNA marker 6 μL, 7 groups listed above. DNA marker: 2K plus II Electrophoresis gel：2% for product of double digestion of RBS 1% for product of double digestion of pTeT Autoradiography(UV): 90V 30min for product of double digestion of RBS 90V 20min for product of double digestion of pTeT The result is showed below:



Recorder: Yan Shi Gel Extraction of pTeT and RBS products

Procedure:

1. Cut off the Gel part containing the target band. Get 6 tubes of AD and 5 tubes of BD.
2. Weigh the cut off Gel part.
3. Add Buffer B2 which 300 times the weight of the Gel.
4. Add isopropanol which is 1/3 volume of the Buffer B2.
5. Move the solution to adsorption column, 11000 rpm centifuge 30 s, discard filtrate.
6. Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once.
7. 12000 rpm centifuge 1 min.
8. Lying for 10 min.
9. Put the adsorption column in a new EP tube. Add 20 μL ddH2O, 10 min's standing, 12000 rpm centifuge 1 min.

After extraction, we measured the OD A260/A280, OD A260/A230 and the density of plasmids. The data is showed below:

	RBS	pTET1	pTET2
Concentration(ng/ul)	fail	38.9	22.3
260/280	fail	1.86	1.86
260/230	fail	0.48	0.18

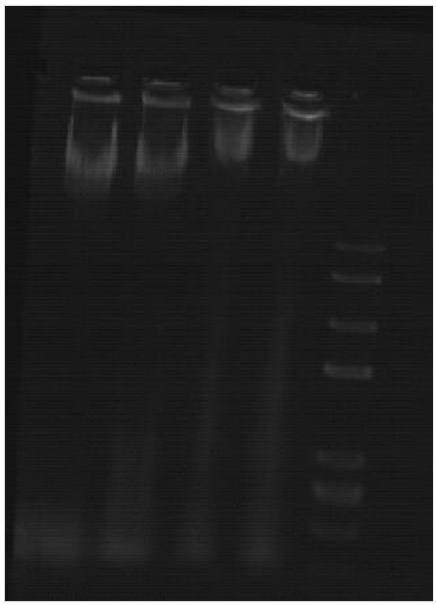
Colony pick up of Reductase S1 and pET 28 Recorder: Mengxue Sun We picked 4 colonies from each plate, so get 12 tubes (4 Reductase S1 and 8 pET 28).

Genome extraction of BL21(DE3) Recorder: Yonghao Liang We use the extraction kit bought from Sangon Biotech.

Procedure:

1. Cultivate bacteria overnight;
2. Absorb 1.5 to 5 mL bacteria solution in to EP tubes, centrifuge them at 8000 rpm for 2 mins and then discard the culture medium.
3. Add 400 □L Buffer Digestion into the tubes and overturn the tubes for 5 to 10 times;
4. Incubate the EP tubes in thermostat water bath about 65 degree centigrade about 1 hour; (overturn the tube every 10 mins)
5. Add 200 □L Buffer PB and overturn thoroughly;
6. Put the tubes at -20°C for 5 mins;
7. Centrifuge the tubes at 10,000 rpm for 5 mins, retrieve the supernatant to a new tube;
8. Add same volume of isopropyl alcohol and overturn 5-8 times thoroughly;
9. Put the tubes at room temperature for 2-3 mins;
10. Centrifuge the tubes at 10,000 rpm for 5 mins, discard the supernatant;
11. Add 1 mL 75% ethanol into each tube and wash the sediment for 1-3 mins;
12. Centrifuge at 10,000 rpm for 2 mins, discard the supernatant;
13. Repeat step 11 and 12 once;
14. Open the EP tube and air it about 5-10 mins;
15. Solubilize the sediment with 50-100 □L TE Buffer for future experiments.

Results:



As you can see, the DNA we get from genome extraction are very very very long!! which means our extraction is successful!!

Recorder: Yonghao Liang PCR of nNapC and Ccm A-H from BL21(DE3)'s genome

Experimental materials

1. Template: Genome extraction from BL21(DE3);

2. Primer: ccm-res-f, ccm-mid-r, ccm-mid-f, ccm-res-r. Designed by ourselves, synthesized by Sangon Biotech;
3. Sterilized ddH2O, 2×Taq-PCR Master, bought from Sangon Biotech.

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Procedure:

1.Prepare 4 PCR tubes and sequentially add：

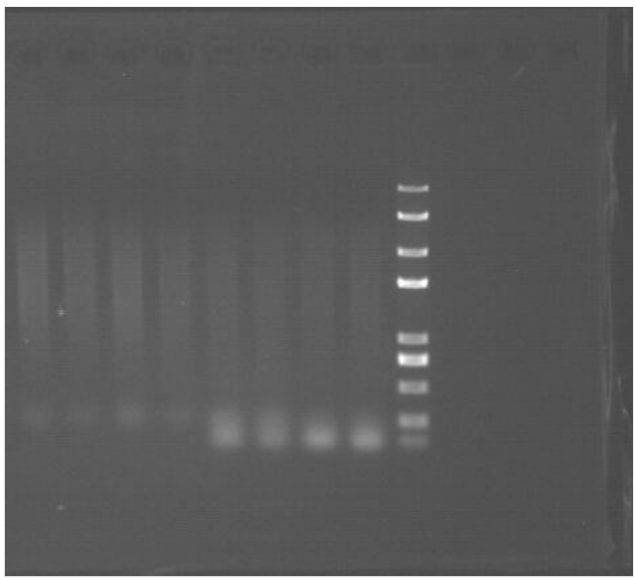
sample	**1**	**2**	**3**	**4**
Sterilized ddH2O	7 μL	7 μL	7 μL	7 μL
2×Taq-PCR Master	10 μL	10 μL	10 μL	10 μL
Genome	1 μL	1 μL	1 μL	1 μL
ccm-res-f(10 μM)	1 μL	1 μL	0	0
ccm-mid-r(10 μM)	1 μL	1 μL	0	0
ccm-mid-f(10 μM)	0	0	1 μL	1 μL
ccm-res-r(10 μM)	0	0	1 μL	1 μL
total	20 μL	20 μL	20 μL	20 μL

2.PCR reaction Parameters setting：

stage	**temperature**	**time**
step 1	95	10 min
step 2	95	40 s
step 3	59	20 s
step 4	72	7 min 30 s
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:



As you can see, we failed to get the target band, which should be about 3000 bp long. We still need to try more to find out the little tricks of Genome PCR.

Awake the Glycerin bacteria Top 10 containing pTET and RBS on the plasmid pSB1C3 Recorder: Menglong Jin Add 200 μL of bacteria into a 5 mL LB culture and cultivate these bacteria overnight for extraction at 37 degree centigrade, 250 rpm.

DATE 6.29 Recorder: Yan Shi,Liudong Luo,Xingwei Yang Plasmid Extraction of the colonies persisted yesterday(pTET,RBS,RED) Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix

well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

帮助

RBS	**1**	**2**	**3**	**4**
Concentration(ng/ul)	97.2	93.4	99.7	101.8
260/280	1.84	1.90	1.86	1.89
260/230	1.44	2.03	1.65	1.97

pTET	**1**	**2**	**3**	**4**
Concentration(ng/ul)	44.2	59.7	37.1	48
260/280	1.88	1.89	1.98	1.88
260/230	1.05	1.18	1.69	1.05

RED	**1**	**2**
Concentration(ng/ul)	14.7	18.1
260/280	1.25	1.65
260/230	0.29	0.54

Date：6.29 Recorder: Shihan Zhu Double digestion of mtr gene and pET28 Materials:

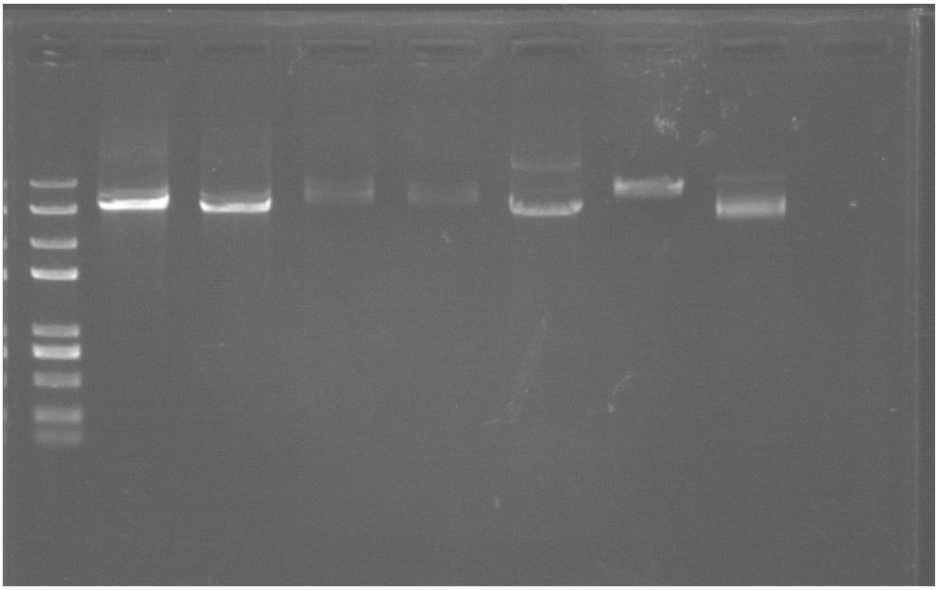
1. mtr gene (get from pcr products).
2. FastDigest restriction enzyme NdeI, XhoI, and 10×FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water
4. marker:Trans 2K Plus II

Reaction system:

Sample	**1**	**2**	**3**	**4**	**5**			
mtr pcr purification products(μL)	5	5	5	5	0			
pET28(μL)	0	0	0	0	5			
nuclease-free water(μL)	20	20	20	20	11			
fastdigest green buffer(μL)	3	3	3	3	2			
NdeI(μL)	1	1	1	1	1	1	1	1
XhoI(μL)	1	1	1	1	1	1	1	1
total(μL)	30	30	30	30	20			

Mix gently and incubate at 37 degree Celsius for 30 mins .

Agarose gel electrophoresis Result: (90V,40min)



From left to right: lane1:marker(trans2K plus); lane2:sample1; lane3:sample2; lane4:sample3; lane5:sample4; lane6:mtr gene; lane7:sample5; lane8:pET28.

Recorder: Menglong Jin PCR of nNapC and Ccm A-H from BL21(DE3)'s genome

Experimental materials

1. Template: Genome extraction from BL21(DE3);
2. Primer: ccm-res-f, ccm-mid-r, ccm-mid-f, ccm-res-r. Designed by ourselves, synthesized by Sangon Biotech;
3. Sterilized ddH2O, 2×Taq-PCR Master, bought from Sangon Biotech.

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

sample	**1**	**2**	**3**	**4**
Sterilized ddH2O	7 μL	7 μL	7 μL	7 μL
2×Taq-PCR Master	10 μL	10 μL	10 μL	10 μL
Genome	1 μL	1 μL	1 μL	1 μL
ccm-res-f(10 μM)	1 μL	1 μL	0	0
ccm-mid-r(10 μM)	1 μL	1 μL	0	0
ccm-mid-f(10 μM)	0	0	1 μL	1 μL
ccm-res-r(10 μM)	0	0	1 μL	1 μL
total	20 μL	20 μL	20 μL	20 μL

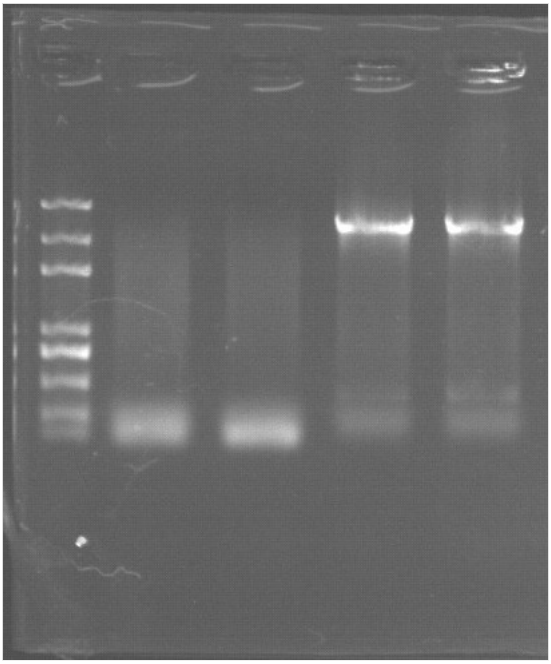
sample **1** **2** **3** **4**

2.PCR reaction Parameters setting：

stage	**temperature**	**time**
step 1	95	10 min
step 2	95	40 s
step 3	59	40 s
step 4	72	4 min
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result: (90V,40min)



From left to right: lane1:marker(trans2K plus); lane2:sample1; lane3:sample2; lane4:sample3; lane5:sample4.

As you can see, we succeeded in getting the second half of the gene(sample3 and 4, which were about 3000bp long), but failed to get the upper half. We still have to try more.

Recorder: Tong Xiao,Mengxue Sun Plasmid Extraction of the colonies picked and cultured yesterday(mtr and) Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

Recorder: Bijunyao Zhong, Yitian Zhou Gel Extraction of pTeT and RBS products

Procedure:

1. Cut off the Gel part containing the target band. Get 6 tubes of AD and 5 tubes of BD.
2. Weigh the cut off Gel part.
3. Add Buffer B2 which 300 times the weight of the Gel.
4. Add isopropanol which is 1/3 volume of the Buffer B2.
5. Move the solution to adsorption column, 11000 rpm centifuge 30 s, discard filtrate.
6. Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once.
7. 12000 rpm centifuge 1 min.
8. Lying for 10 min.
9. Put the adsorption column in a new EP tube. Add 20 μL ddH2O, 10 min's standing, 12000 rpm centifuge 1 min.

After extraction, we measured the OD A260/A280, OD A260/A230 and the density of plasmids. The data is showed below:

	pTeT	**RBS**
Concentration(ng/ul)	51.4	5.4
260/280	1.90	1.65
260/230	0.41	0.19

Ligation of pTET and RBS Recorder: Yan Shi

Material:

1. double digestion product of pTET
2. double digestion product of RBS
3. 10× T4 DNA ligase buffer,T4 DNA ligase (bought from Thermo Fisher Scientific)
4. nuclease-free water

帮助

Materials	pTeT	RBS
Concentration(ng/ul)	105.6	5.4
260/280	1.85	1.65
260/230	0.30	0.19

Procedure:

Sample	pTET	RBS	10* T4 DNA Ligase Buffer	T4 DNA Ligase
Volume(μL)	2	16	2	0.4

Mix gently and incubate at 16 degree Celsius overnight.(2200-0900)

Recorder: Bijunyao Zhong, Dongdong Jang Plasmid Extraction of the colonies (pTeT). Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

After extraction, we measured the OD A260/A280 and the density of plasmids， Then we show it below.

	pTeT1	pTeT2	pTeT3	pTeT4
Concentration(ng/ul)	148.4	166.3	165.8	171.9
260/280	1.81	1.83	1.84	1.81
260/230	1.73	1.87	2.13	1.50

	RBS2	RBS3	RBS4
Concentration(ng/ul)	120.8	118.4	148.9
260/280	1.85	1.86	1.79
260/230	1.88	2.20	1.47

RBS2 RBS3 RBS4

Recorder:Yan Shi Double digestion of plasmid containing pTET and RBS Materials:

1. pTET gene in plasmid pSB1C3(from iGEM kit plate)
2. FastDigest restriction enzyme SpeI, EcoRI, PstI and 10×FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water
4. marker:Trans 2K Plus

	RBS	pTET1	pTET2	pTET3
Concentration(ng/ul)	129.7	252.6	188.1	112.0
260/280	1.83	1.86	1.84	1.85
260/230	2.10	2.20	2.10	2.01

Reaction system:

Sample	1	2	3	4	5	6	7	8

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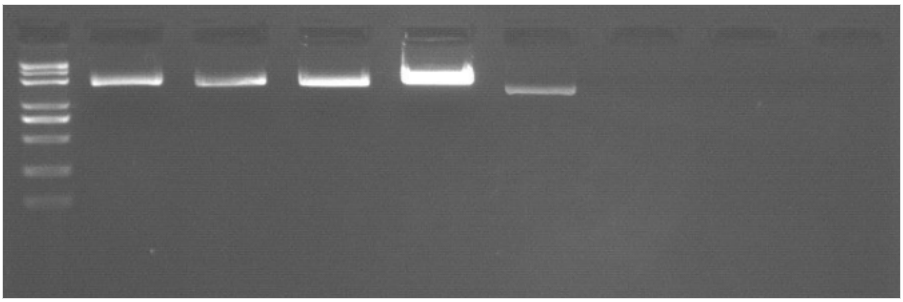
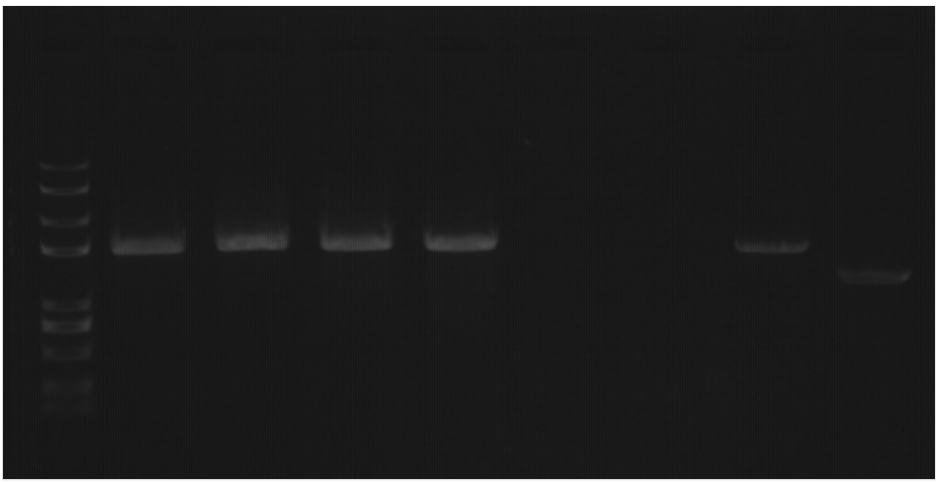
Sample	1	2	3	4	5	6	7	8
pSB1C3 containing RBS(μL)	8	8	8	8	0	0	0	0
pSB1C3 containing pTET(μL)	0	0	0	0	4	4	6	10
nuclease-free water(μL)	8	8	8	8	12	12	10	6
fastdigest green buffer(μL)	2	2	2	2	2	2	2	2
PstI(μL)	1	1	1	1	1	1	1	1
XbaI(μL)	1	1	1	1	0	0	0	0
SpeI(μL)	0	0	0	0	1	1	1	1
total(μL)	20	20	20	20	20	20	20	20

Mix gently and incubate at 37 degree Celsius for 30 mins.

Agarose gel electrophoresis of products of double digestion of pTeT and RBS Recorder: Yan Shi, Bijunyao Zhong Procedure: Add 0.8 g agarose to 80 mL 1*TAE buffer. Dissolved by heating. Cool down. Pour into 2 electrophoresis tanks. Mix 20 μL each product with 4 μL 6*loading buffer each to form 7 loading groups. Loading：DNA marker 6 μL, 7 groups listed above. DNA marker: 2K plus II Electrophoresis gel：2% for product of double digestion of RBS 1% for product of double digestion of pTeT Autoradiography(UV): 90V 30min for product of double digestion of RBS 90V 20min for product of double digestion of pTeT The result is showed below:



From left to right: lane1:marker(trans2K plus); lane2:sample1; lane3:sample2; lane4:sample3; lane5:sample4;lane6:plasmid



From left to right: lane1:marker(trans2K plus); lane2:sample1; lane3:sample2; lane4:sample3; lane5:sample4;lane6:plasmid

Recorder:Yan Shi Double digestion of plasmid containing RBS Materials:

1. RBS gene in plasmid pSB1C3(from iGEM kit plate)
2. FastDigest restriction enzyme SpeI, EcoRI, PstI and 10×FastDigest Green Buffer(from Thermo Fisher Scientific)

3. Nuclease-free water
4. marker:Trans 2K Plus

	RBS
Concentration(ng/ul)	127.4
260/280	1.88
260/230	2.30

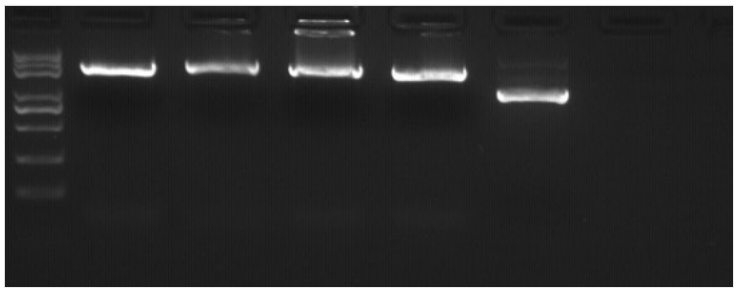
Reaction system:

Sample	**1**	**2**	**3**	**4**
pSB1C3 containing RBS(μL)	8	8	8	8
nuclease-free water(μL)	8	8	8	8
fastdigest green buffer(μL)	2	2	2	2
PstI(μL)	1	1	1	1
Xbal(μL)	1	1	1	1
total(μL)	20	20	20	20

Mix gently and incubate at 37 degree Celsius for 30 mins.

Agarose gel electrophoresis of products of double digestion of RBS Recorder: Yan Shi
Procedure: Add 0.8 g agarose to 40 mL 1*TAE buffer. Dissolved by heating.Then add 4ul gelred. Cool down. Pour into 1 electrophoresis tanks. Mix 20 μL each product with 4 μL 6*loading buffer each to form 7 loading groups. Loading：DNA marker 6 μL, 7 groups listed above. DNA marker: 2K plus II Electrophoresis gel：2% for product of double digestion of RBS Autoradiography(UV): 90V 30min for product of double digestion of RBS

The result is showed below:



From left to right:
lane1:marker(trans2K plus); lane2:sample1; lane3:sample2; lane4:sample3; lane5:sample4;lane6:plasmid

PCR of nNapC and Ccm A-H from BL21(DE3)'s genome Recorder: Menglong Jin

Experimental materials

1. Template: Genome extraction from BL21(DE3);
2. Primer: ccm-res-f, ccm-mid-r, ccm-mid-f, ccm-res-r. Designed by ourselves, synthesized by Sangon Biotech;
3. Sterilized ddH2O, 2×Taq-PCR Master, bought from Sangon Biotech.

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

sample	**1**	**2**	**3**	**4**
Sterilized ddH2O	7 μL	7 μL	7 μL	7 μL
2×Taq-PCR Master	10 μL	10 μL	10 μL	10 μL
Genome	1 μL	1 μL	1 μL	1 μL

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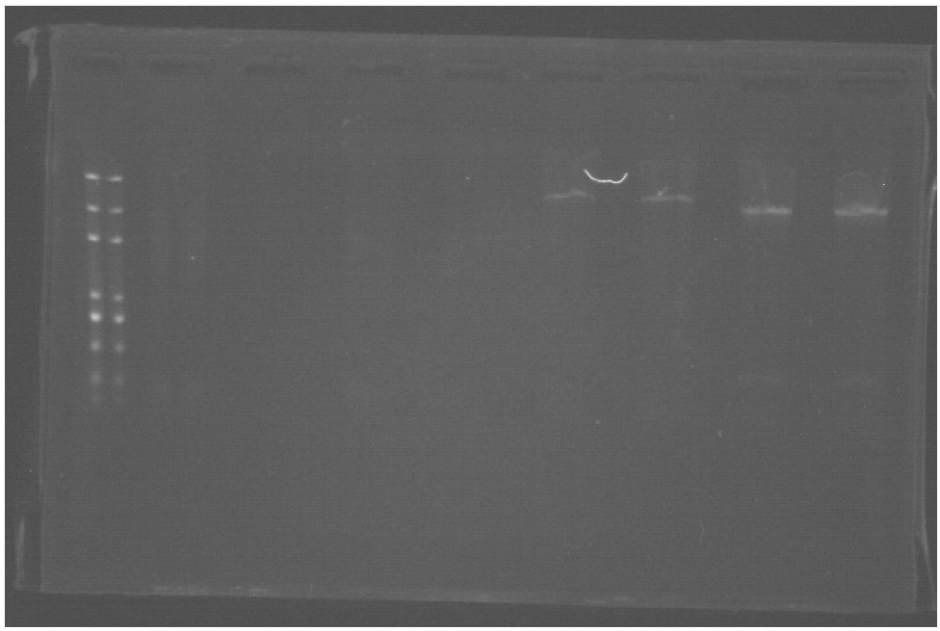
sample	1	2	3	4
ccm-res-f(10 μM, new)	1 μL	1 μL	0	0
ccm-mid-r(10 μM)	1 μL	1 μL	0	0
ccm-mid-f(10 μM)	0	0	1 μL	1 μL
ccm-res-r(10 μM, new)	0	0	1 μL	1 μL
total	20 μL	20 μL	20 μL	20 μL

2.PCR reaction Parameters setting：

stage	temperature	time
step 1	95	10 min
step 2	95	40 s
step 3	58	40 s
step 4	72	4 min
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4) React over night.

3.Agarose gel electrophoresis mixed with 6× DNA loading buffer;110 V, 30 min Agarose gel electrophoresis Result:

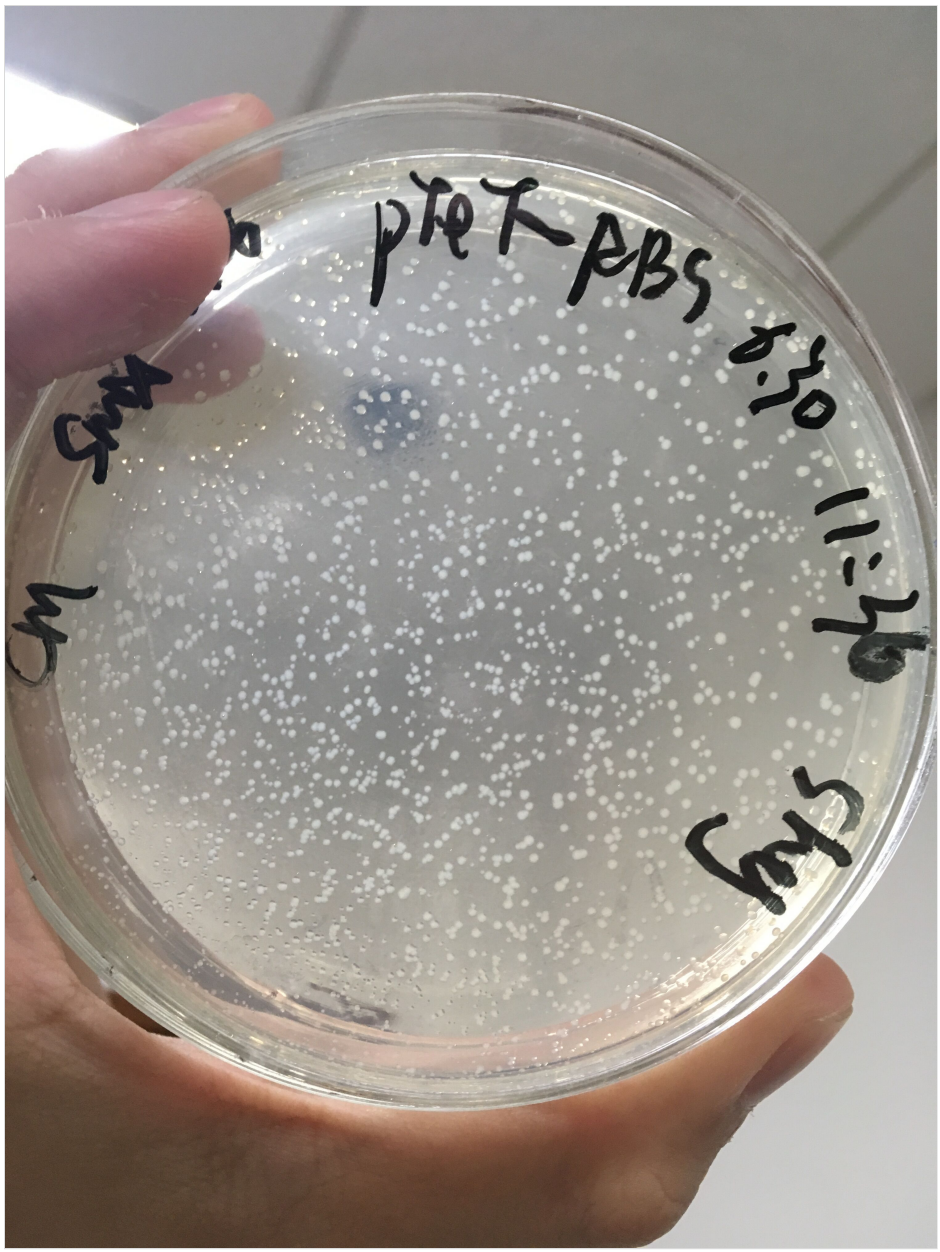


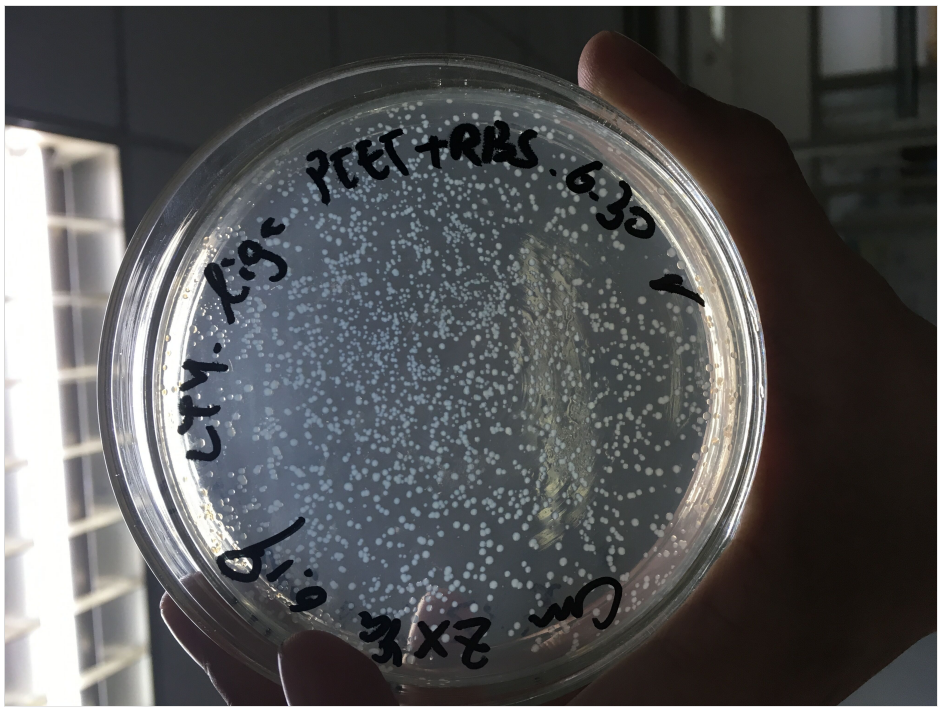
Lane	0	1	2	3	4	5	6	7	8
Sample	Marker 2K Plus	first half part of ccm-1	first half part of ccm-2	first half part of ccm-3	first half part of ccm-4	second half part of ccm-1	second half part of ccm-2	second half part of ccm-3	second half part of ccm-4

DATE 6.30 Transformation of ligation product (pTET + RBS) NOTE:Generally, competent bacteria are restrored in -70 degree centrigrade environment. 1.Take the competent bacteria from refrigerator and incubate them into ice about 5 mins until it is dissolved 2.Absorb 100pg to 10 ng plasmid(normally 1 to 2 uL, DO NOT add more than 5% volumn of bacteria solution) and mix it with bacteria solution thoroughly. ATTENTION: Please operate this step tenderly!!! 3.Put the tubes on the ice about 30 mins.(Time SHOULD BE ACCURATE) 4.Make a heat shock at 42 degree centigrade about 90 sec(TIME SHOULD BE ACCURATE) 5.Put the tubes on the ice about 5 mins again. 6.Add 900 ul LB medium into EP tubes and cultivate the bacteria at 37 degree centrigrade about 60 min. 7.Centrifuge them at 4000 rpm for 2 minutes and we will see sediment in the tubes. 8.Discard the supernatant liquid and leave about 200 ul medium. 9.Coat plate: Add 200 ul solution in a plate with kanamycin. 10.Cultivate these bacteria overnight for further use.

帮助

PS: The result is as follow:





Plasmid Extraction of the pLuxR perserved in glycerol Recorder: Jianjian Guo,Yu Han

Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the

supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius. OD measurement resut:

sample	pLuxR-1.1	pLuxR-2.1	pLuxR-3.1	pLuxR-1.2	pLuxR-2.2	pLuxR-4.2
Concentration(ng/ul)	472.4	531.5	430.9	522.7	576.6	499.0
260/280	1.86	1.78	1.87	1.79	1.86	1.83
260/230	2.14	1.67	2.28	1.95	2.17	2.38

Plasmid Extraction of the pTET，RBS and PBAD Recorder:Menglong Jin, Liudong Luo, Bijunyao Zhong Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

After extraction, we measured the OD A260/A280 and the density of plasmids.

RBS	1	2	3	4
Concentration(ng/ul)	172.6	128.9	134.1	151.9
260/280	1.80	1.88	1.81	1.82
260/230	1.56	2.25	1.57	1.60

pTET	1	2	3	4
Concentration(ng/ul)	164	204.6	218	245.2
260/280	1.85	1.81	1.86	1.86
260/230	2.25	1.67	2.12	2.30

PBAD	1	2	3	4
Concentration(ng/ul)	97.6	199.2	208.8	200.8
260/280	1.88	1.87	1.86	1.87
260/230	2.14	2.27	2.04	2.22

Plasmid Extraction of the Mtr，pET28,Mtr(wrongly transformed) Recorder:Tong Xiao, Mengxue Sun Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

After extraction, we measured the OD A260/A280 and the density of plasmids.

Mtr	1	5	6	
Concentration(ng/ul)	516.1	552.4	525.3	
260/280	1.87	1.88	1.87	1.93

Mtr	1	5	6	
260/230	2.29	2.37	2.31	2.15

Mtr(wrong)	1	2	3	4	2'	3'
Concentration(ng/ul)	12.3	61.8	13.2	12.7	13.5	11.0
260/280	2.05	1.64	2.09	2.21	2.21	2.04
260/230	1.05	2.18	2.35	2.11	2.27	1.26

pET28	1
Concentration(ng/ul)	63.6
260/280	1.93
260/230	2.15

Because of transforming the wrong plasmid,the strain we picked from the plate is false positive(Due to the low concentration of Kanamycin of the plate,it could grow into some colonies).So the transformation efficiency is very low according to the data.

Recorder:Tong Xiao Double digestion of plasmid containing Mtr gene and pET28 Materials:

1. Mtr gene in plasmid pSB1C3(from iGEM kit plate)
2. pET28 plasmid.
3. FastDigest restriction enzyme XhoI, NedI and 10×FastDigest Green Buffer(from Thermo Fisher Scientific)
4. Nuclease-free water
5. marker:Trans 2K Plus

Reaction system:

Mtr Sample	1	2
pSB1C3 containing RBS(μL)	5	5
nuclease-free water(μL)	20	20
fastdigest green buffer(μL)	3	3
XhoI(μL)	1	1
NdeI(μL)	1	1
total(μL)	30	30

pET28	1
pET28(μL)	5
nuclease-free water(μL)	11
fastdigest green buffer(μL)	2
XhoI(μL)	1
NdeI(μL)	1
total(μL)	20

Mix gently and incubate at 37 degree Celsius for 30 mins.

Recorder: Yawei Wu,Jingyu Wang Plasmid Extraction of CysDes(2017 kit) Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. *Two EP tubes for every cell tube before removing to one adsorbing tube 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

After extraction, we measured the OD A260/A280, OD A260/A230 and the density of plasmids. The results are shown in the chart below.

sample	Cys-1	Cys-2	Cys-3	Cys-1	Cys-4
Concentration(ng/ul)	38.2	64.8	26.9	47.4	
260/280	1.88	1.86	1.90	1.79	1.88
260/230	1.15	1.66	1.21	1.95	1.39

PCR of Mtr Recorder: Tong Xiao 1.Prepare 6 PCR tubes and sequentially add：

sample	1	1'	2	2'	3	3'
Sterilized ddH2O	22 μL	22 μL	22 μL	22 μL		
template	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
mtr633(10 μM)	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
mtr634(10 μM)	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
2×Primer Star(or Taq)	25 μL	25 μL	25 μL	25 μL	25 μL	25 μL
total	50 μL	50 μL	50 μL	50 μL	50 μL	50 μL

(1'and 2'used Taq,the rest used 2xPrimer Star)

2.Prepare 2 PCR tubes and sequentially add：

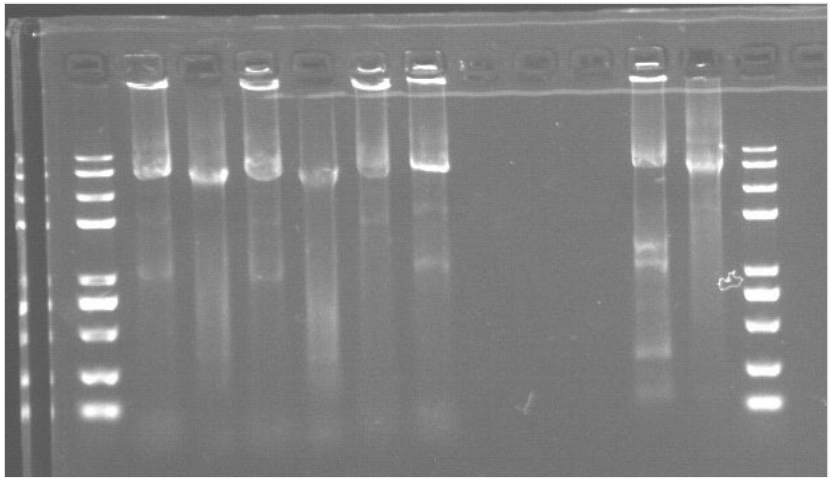
sample	4	4'
Sterilized ddH2O	22 μL	22 μL
template	1 μL	1 μL
mtrVR(10 μM)	1 μL	1 μL
mtrVF2(10 μM)	1 μL	1 μL
2×Primer Star	25 μL	25 μL
total	50 μL	50 μL

3.PCR reaction Parameters setting：

stage	temperature	time
step 1	95	10 min
step 2	95	20 s
step 3	55	20 s
step 4	72	5 min 30 s
step 5	72	10 min
step 6	4	--

30 cycles

3.Agarose gel electrophoresis mixed with 1 μL 6× DNA loading buffer each 5 μL sample;90 V, 30 min Result:



lane1-8:

Mtr1 Mtr1' Mtr2 Mtr2' Mtr3 Mtr3' Mtr4 Mtr4'(1,1',2,2',3,3'used primer 633 and 634,4 and 4' used primer VR and VF2)

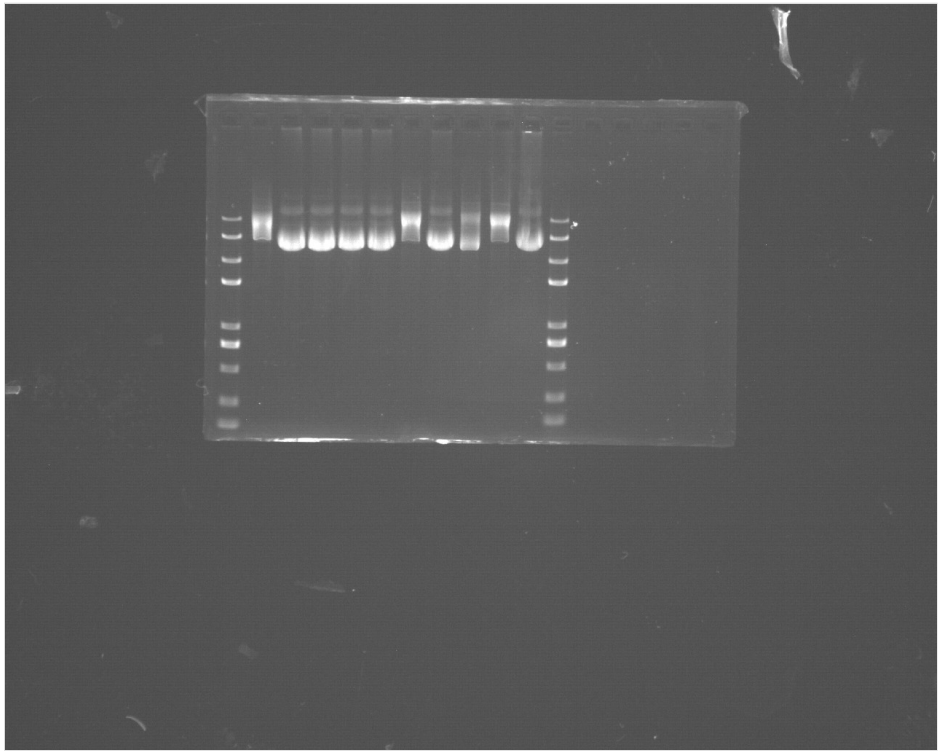
Plasmid Extraction of the Mtr，pET28 Recorder:Xingwei Yang, Yijun Chen, Shihan Zhu
Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

After extraction, we measured the OD A260/A280 and the density of plasmids.

Mtr	**1**	**5**	**6_3**	**6_6**	**2_1**	**2_2**
Concentration(ng/ul)	556.5	571.3	557.7	554.9	560.5	434.3
260/280	1.83	1.86	1.85	1.87	1.86	1.88
260/230	2.30	2.28	2.35	2.28	2.28	2.33

pET28	**z_1**	**z_2**	**c**	**y**
Concentration(ng/ul)	53.7	48.2	63.3	60.0
260/280	1.97	1.97	1.97	1.97
260/230	2.14	2.09	2.13	2.14

Agarose gel electrophoresis mixed with 1 μL 6× DNA loading buffer each 5 μL sample;110 V, 30 min Result:



lane1-12: marker pET28-Z_1 Mtr1 Mtr2_1 Mtr2_2 Mtr5 pET28-z_2 Mtr6_3 pET28-c pET28-y Mtr6_6 marker

Plasmid Extraction of the pTET and RBS Recorder: Yonghao Liang & Xingwei Yang
Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

After extraction, we measured the OD A260/A280, OD A260/A230 and the density of plasmids. The results are shown in the chart below.

sample	RBS-1	RBS-2	RBS-3	RBS-4	pTET-1	pTET-2	pTET-3	pTET-4
Concentration(ng/ul)	104.2	148.7	117.8	75.1	159.2	145.5	171.7	189.7
260/280	1.83	1.77	1.84	1.87	1.85	1.84	1.85	1.86
260/230	2.40	1.29	2.41	2.22	2.39	2.23	2.31	2.30

sample	pBAD-1	pBAD-2	pBAD-3	pBAD-4
Concentration(ng/ul)	165.7	185.1	172.3	180.6
260/280	1.86	1.86	1.88	1.87
260/230	2.48	2.37	2.43	2.45

PCR of NapC and Ccm A-H from BL21(DE3)'s genome Recorder: Shihan Zhu

Experimental materials

1. Template: Genome extraction from BL21(DE3);
2. Primer: ccm-res-f, ccm-mid-r, ccm-mid-f, ccm-res-r. Designed by ourselves, synthesized by Sangon Biotech;
3. Sterilized ddH2O, 2×Taq-PCR Master, bought from Sangon Biotech.

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

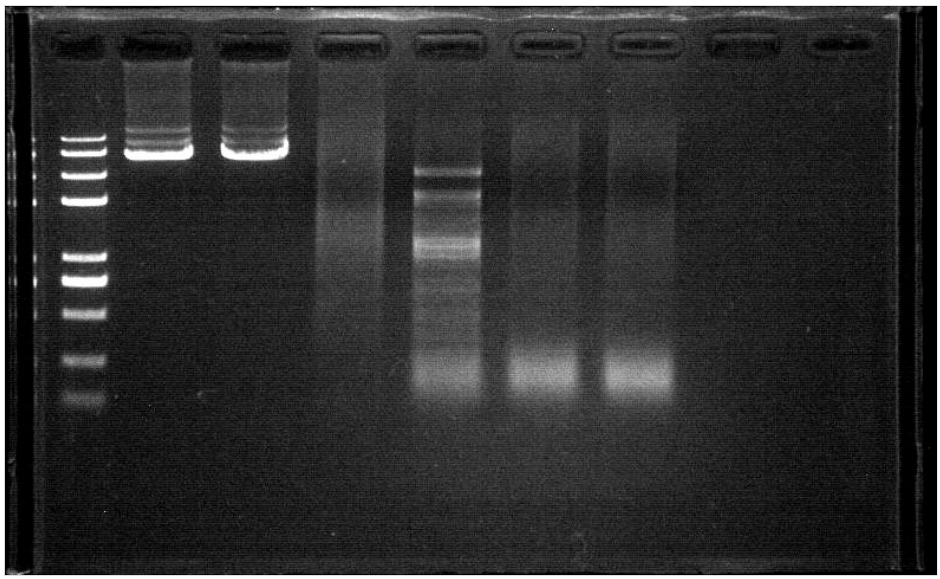
sample	**1**	**2**	**3**	**4**
Sterilized ddH2O	7 μL	7 μL	7 μL	7 μL
2×Taq-PCR Master	10 μL	10 μL	10 μL	10 μL
Genome	1 μL	1 μL	1 μL	1 μL
ccm-res-f(10 μM, new)	1 μL	1 μL	0	0
ccm-mid-r(10 μM)	1 μL	1 μL	0	0
ccm-mid-f(10 μM)	0	0	1 μL	1 μL
ccm-res-r(10 μM, new)	0	0	1 μL	1 μL
total	20 μL	20 μL	20 μL	20 μL

2.PCR reaction Parameters setting：

stage	**temperature**	**time**
step 1	95	10 min
step 2	95	40 s
step 3	58	40 s
step 4	72	4 min
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4) React over night.

The result is as follow:



(Lane 3 to 6 is the PCR result. As you can see, we failed to amplified Ccm A-H this time)

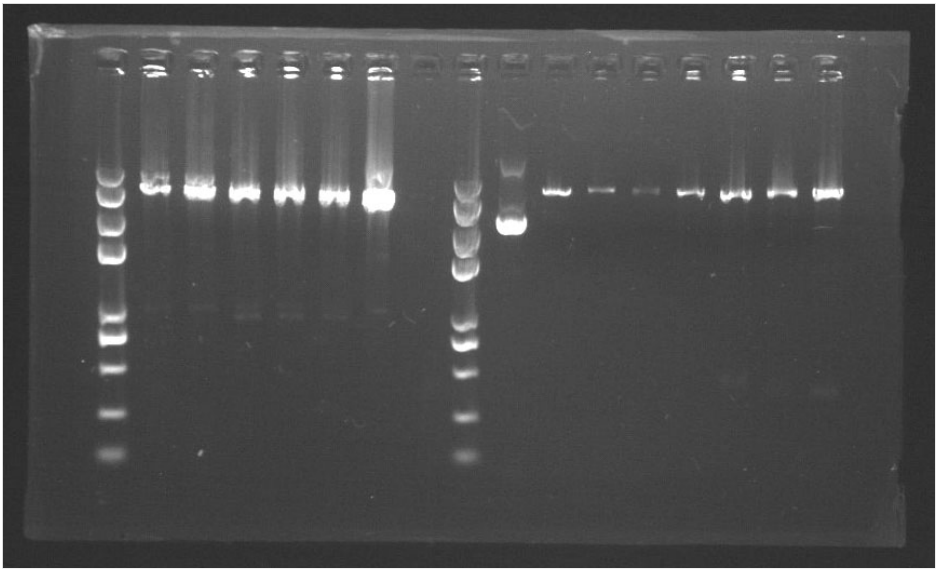
Single and double digestion of pET28 and mtr pcr products Recorder: Yitian Zhou, Shihan Zhu

Materials:

1. pET28 plasmid (Transformed and extracted before), mtr pcr products
2. FastDigest restriction enzyme NdeI, XhoI, Bgl II and 10×FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water
4. marker:Trans 2K Plus

帮助

The result is as follow:



Lane	Sample	Note
1	Marker	5μL
2	mtr NdeI digestion	
3	NdeI digestion	same as 2
4	mtr XhoI digestion	
5	XhoI digestion	same as 4
6	mtr double digestion	NdeI & XhoI
7	mtr pcr products	control
9	Marker	5μL
10	pET28 plasmid	control
11	NdeI digestion	
12	NdeI digestion	same as 11
13	XhoI digestion	
14	XhoI digestion	same as 13
15	double digestion	NdeI & XhoI
16	Bgl II digestion	
17	Bgl II digestion	same as 16

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