Notebook May

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DATE 5.14 Transformation of plasmid pSB1C3 containing MtrCAB Recorder: Yonghao Liang

DATE 5.15 Colony picking of plasmid pSB1C3 containing MtrCAB Recorder: Yonghao Liang

We did colony picking of plasmid containing MtrCAB. After colony picking,we cultivate the bacteria at 37°C and shock at 250 rpm/min for 8 hours.

Plasmid Extraction of MtrCAB Recorder: Yonghao Liang 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.

PCR of bacteria containing MtrCAB expression plasmid (tube 1-8) Recorder: Yonghao Liang

About primers

primer	**1**	**2**
sequence	5'-TGCCACCTGACGTCTAAGAA-3'	5'-ATTACCGCCTTTGAGTGAGC-3'

Procedure:

1.Prepare 8 PCR tubes and sequentially add：

sample	**1,2,3,4,5,6,7,8**
Sterilized ddH2O	22 μL
2×PrimeSTAR MAX Premix	25 μL
Template(bacteria)	1 μL
VF2	1 μL
VR	1 μL
total	50 μL

2.PCR reaction Parameters setting：

stage	**temperature**	**time**
step 1	95	10 min

stage	temperature	time
step 2	95	10 s
step 3	56	20 s
step 4	72	5 min 18 s
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

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 10 μL, PCR productrs: 50 μL : sample1-1,1-2,1-3,1-4,2-1,2-2,2-3,2-4.
7. Electrophoresis gel：120 V 30 min.
8. Autoradiography(UV).

Result

DATE 5.16 Streak-plates for BL21 and XL GOLD Recorder: Chenyagn Li, Jianjian Guo 1 for BL21 and 2 for XL GOLD

Plasmid Extraction of MtrCAB Recorder: Yonghao Liang 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.

DATE 5.17

Selection for single colony and cultivation Recorder: Chenyagn Li

We picked single colonies, 2 from XL GOLD, 2 from XL GOLD(1) and 2 from BL21, and cultivated the bacteria sperately at 37°C and shock at 250 rpm/min for 8 hours.

Plasmid Extraction of MtrCAB Recorder: Jianjian Guo

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.

帮助

DATE 5.18 Recorder: Yitian Zhou & Shihan Zhu PCR of bacteria containing MtrCAB expression plasmid (tube 1-8)

About primers

primer	**1**	**2**
sequence	5'-TGCCACCTGACGTCTAAGAA-3'	5'-ATTACCGCCTTTGAGTGAGC-3'

Procedure:

1.Prepare 8 PCR tubes and sequentially add：

sample	**1,2,3,4,5,6,7,8**
Sterilized ddH2O	22 μL
2×Taq MAX Premix	25 μL
Template(bacteria)	1 μL
VF2	1 μL
VR	1 μL
total	50 μL

2.PCR reaction Parameters setting：

stage	**temperature**	**time**
step 1	95	10 min
step 2	95	40 s
step 3	56	20 s
step 4	72	5 min 18 s
step 5	72	10 min
step 6	4	--

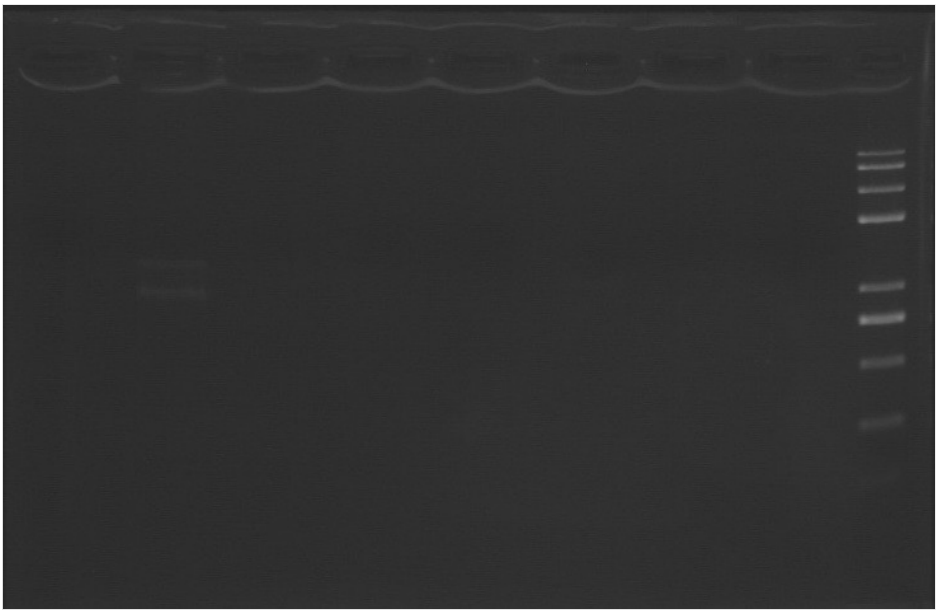
30 cycles(step 2 ~ step 4)

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 10 μL, PCR productrs: 50 μL : sample1-1,1-2,1-3,1-4,2-1,2-2,2-3,2-4.
7. Electrophoresis gel：120 V 30 min.
8. Autoradiography(UV).

帮助

Result



DATE 5.19 Recorder: Chenyagn Li, Jianjian Guo Selection for single colony and cultivation

We picked single colonies, 2 from XL GOLD(2) and 2 from BL21, and cultivated the bacteria sperately at 37°C and shock at 250 rpm/min for 8 hours.

DATE 5.20 Preparation of Competent Cell(E.coli) Recoreder: Shihan Zhu & Yonghao Liang Procedure 1.Pick out the simple colony(DH5α) and cultivate bacteria in 2 to 3 mL LB media at 37 degree centigrade about 12 to 16 hours with shocking. 2.Take 0.05mL nutrient solution into 50 mL media and cultivate bacteria at 37 degree centigrade about 2 to 3 hours with shocking till the OD550 reaches 0.2 to 0.4. 3.Absorb 1.5mL nutrient solution into EP tubes with ice-bath about 10 mins. 4.Centrifuge the tubes at 4000xg at 4 degree centigrade about 10mins and discard the supernatant liquid. 5.Utilize 0.5 to 1 mL 0.1M calcium chloride with 15% glycerol to suspend bacteria.(ATTENTION:USE SPEARHEAD TO SUSPEND BACTERIA TENDERLY INSTEAD OF VIBRATOR) 6.Centrifuge the tubes at 4000xg at 4 degree centigrade about 8 mins and discard the supernatant liquid. 7.Utilize 100 ul 0.1M calcium chloride with 15% glycerol to suspend bacteria and then ready for transformation quickly or restore at -70 degree centigrade.(WITH ATTENTION AGAIN) TIPS: EXPERIENCE SAYS MODERATE MAGNESIUM ION HELPS DEVELOPMENT OF COMPETENT CELL.

DATE 5.21 Transformation(E.coli) Recorder: Yonghao Liang Procedure 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 5.22 Recorder: Yan Shi & Shihan Zhu & Yonghao Liang Selection for single colony and cultivation

We picked 4 single colonies from the plate we cultivated that bacteria that has been transformed with Mtr CAB, and cultivated the bacteria sperately at 37°C and shock at 250 rpm/min for 8 hours.

Recorder: Shihan Zhu Plasmid Extraction of MtrCAB 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**
Concentration(ng/μl)	25.0	20.0	0.25	12.5

Recorder: Yan Shi & Yonghao Liang PCR of bacteria containing MtrCAB expression plasmid (tube 1-4)

About primers

primer	**1**	**2**
sequence	5'-TGCCACCTGACGTCTAAGAA-3'	5'-ATTACCGCCTTTGAGTGAGC-3'

Procedure:

1.Prepare 8 PCR tubes and sequentially add：

sample	**1,2,3,4,5,6,7,8**
Sterilized ddH2O	22 μL
2×Taq MAX Premix	25 μL
Template(bacteria)	1 μL
VF2	1 μL
VR	1 μL
total	50 μL

2.PCR reaction Parameters setting：

stage	**temperature**	**time**
step 1	95	10 min
step 2	95	40 s
step 3	56	25 s
step 4	72	5 min 20 s
step 5	72	10 min
step 6	4	--

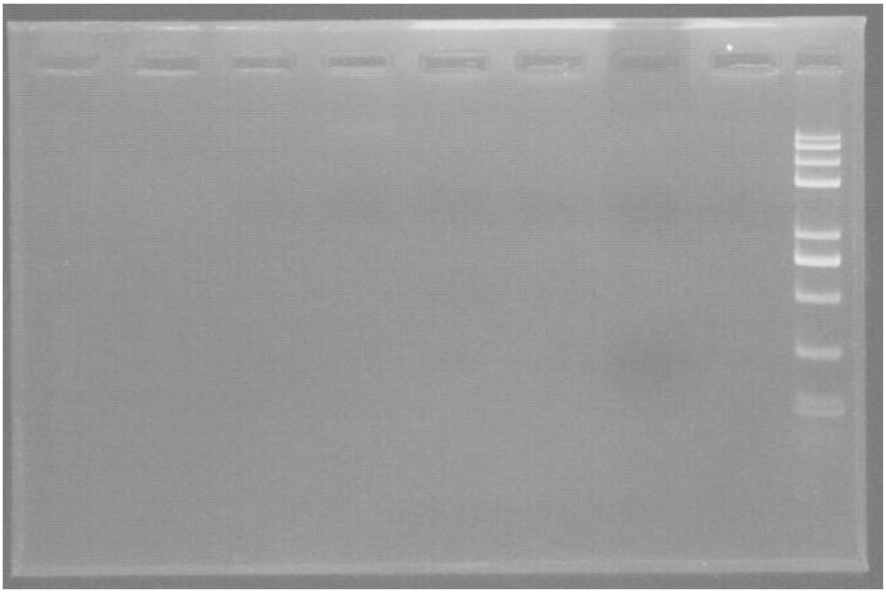
30 cycles(step 2 ~ step 4)

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 10 μL, PCR products: 50 μL with 10 μL new loading buffer.(from right to left: marker, PCR product 1, PCR product 2, PCR product 3, PCR product 4, plasmid with old loading buffer, plasmid with new loading buffer)

7. Electrophoresis gel：120 V 30 min.
8. Autoradiography(UV).

Result



We can see there is a very light band in the fourth track, so we think the new loading buffer is unable to indicate the DNA.

So we borrow Prof Hong 's lab's loading buffer to do another PCR, and do a PCR to check whether the primer is working or not.

Recorder: Yan Shi & Yonghao Liang Plasmid Extraction of MtrCAB 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**
Concentration(ng/μl)				

Recorder: Yan Shi & Yonghao Liang PCR of MtrCAB

About primers

primer	**1**	**2**
sequence	5'-TGCCACCTGACGTCTAAGAA-3'	5'-ATTACCGCCTTTGAGTGAGC-3'

Procedure:

1.Prepare 8 PCR tubes and sequentially add：

sample	1,2,3,4,5,6,7,8
Sterilized ddH2O	22 μL
2×Taq MAX Premix	25 μL
Template(bacteria)	1 μL
VF2	1 μL
VR	1 μL
total	50 μL

2.PCR reaction Parameters setting：

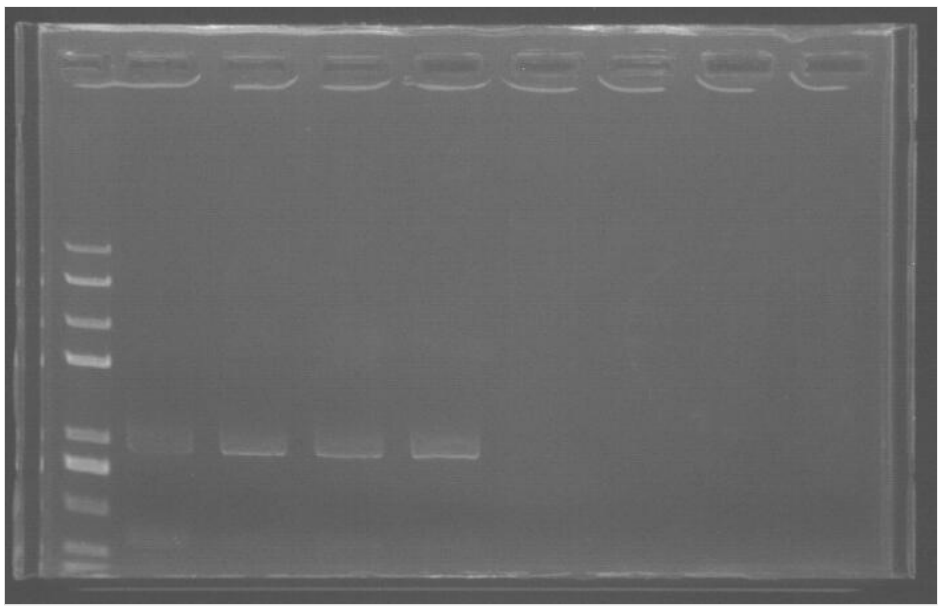
stage	temperature	time
step 1	95	10 min
step 2	95	40 s
step 3	56	25 s
step 4	72	5 min 20 s
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

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 10 μL, PCR products: 50 μL with 10 μL new loading buffer.(from left to right: marker, PCR product 1, PCR product 2, PCR product 3, PCR product 4, plasmid 1, plasmid 2, plasmid 3, plasmid 4)
7. Electrophoresis gel：110 V 30 min.
8. Autoradiography(UV).

Result



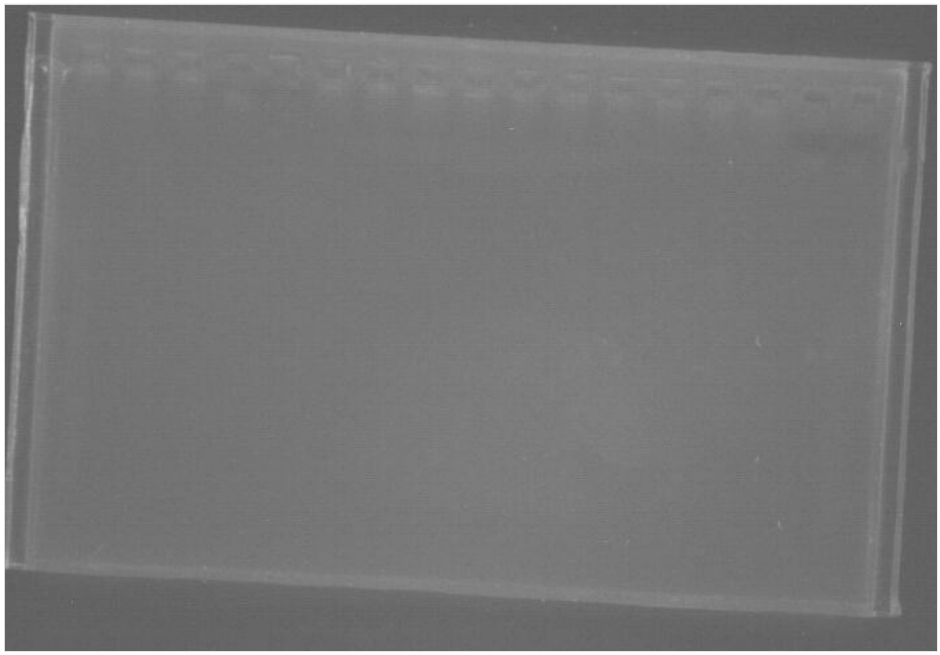
This time the loading buffer we use is from Prof Hong' lab, which means the loading buffer we used before did NOT work.

But the band is not in the right place, the fragment we get after PCR should be 5000 bp long, but not about 1000 bp.

Moreover, the plasmids we extract did NOT show up.

In addition, we did an Agarose gel electrophoresis for the primers we use.

Result



It means maybe the primers we use have degraded already.

05-23 22:50 **梁永浩** 创建了文档

10-22 19:45 **梁永浩** 编辑了文档

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