Notebook August

吴昀荆 20分钟前保存
查看编辑历史

Date 7.31

**PCR of full Ccm **

Recorder: Liudong Luo, Zhenyu Jiang

Experimental materials

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

2. Primer: ccm-res-f,ccm-res-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O, 2×PrimeStar(Premix), bought from Takara.

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|9|10|11|12|13|14|15|16|

|-|

|Sterilized ddH₂O|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|

|2×Prime Star(premix)|25 μL|25 μL|25 μL|25 μL|25 μL|25 μL|25 μL|25 μL|25 μL|25 μL|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|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|ccm-res-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|ccm-res-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|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|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|

2.PCR reaction

1,2,3,4,5,6,7,8 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|10 min|

|step 2|98|10 s|

|step 3|59|5 s|

|step 4|72|7 min|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

9,10,11,12,13,14,15,16 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|10 min|

|step 2|98|10 s|

|step 3|55|15 s|

帮助

|step 4|72|7 min|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

Purification of full ccm PCR product

Recorder: Zhenyu Jiang, Liudong Luo

(1). Add 900 μL Buffer B3 to the 180 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 25 μL ddH₂O, stand them still for 10 min. Centrifuge at 12,000 rpm for 1 min.

OD measurement result:

|sample|ccm PCR pur 1+2+3+4+5+6+7+8|

|-|

|Concentration(ng/ul)|216.0|

|260/280|1.85|

|260/230|2.23|

|sample|ccm PCR pur 9+10+11+12+13+14+15+16|

|-|

|Concentration(ng/ul)|137.4|

|260/280|1.84|

|260/230|2.40|

Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/7a34ccf9f07a49338b5900aa58d7b9ce?filename=7.31+lld+jzy+ddccm%2Cpcr+of+ccm%2Cddpuc%2Cpuc%2CddpTB%2CpTB.JPG)

(lane left to right: marker 2K Plus II, ddccm-1, ddccm-2, ccm-pcr-1, ccm-pcr-2,ddpuc, puc, ddpTB, pTB)

**Double digestion of ccm **

**Recorder: Liudong Luo, Zhenyu Jiang **

Materials:

1. full ccm

2. FastDigest restriction enzyme XbaI, pstI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)

3. Nuclease-free water

Reaction system:

帮助

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

|-|

|XbaI(μL)|1|1|1|1|1|1|1|1|

|pstI(μL)|1|1|1|1|1|1|1|1|

|nuclease-free water(μL)|25|25|25|25|25|25|25|25|

|fastdigest green buffer(μL)|2|2|2|2|2|2|2|2|

|ccm(μL)|1|1|1|1|1|1|1|1|

|total(μL)|30|30|30|30|30|30|30|30|

Reaction system:

|Sample|9|10|11|12|13|14|15|16|

|-|

|XbaI(μL)|1|1|1|1|1|1|1|1|

|pstI(μL)|1|1|1|1|1|1|1|1|

|nuclease-free water(μL)|25|25|25|25|25|25|25|25|

|fastdigest green buffer(μL)|2|2|2|2|2|2|2|2|

|ccm(μL)|1|1|1|1|1|1|1|1|

|total(μL)|30|30|30|30|30|30|30|30|

Reaction system:

|Sample|17|18|19|20|21|22|23|24|

|-|

|XbaI(μL)|1|1|1|1|1|1|1|1|

|pstI(μL)|1|1|1|1|1|1|1|1|

|nuclease-free water(μL)|25|25|25|25|25|25|25|25|

|fastdigest green buffer(μL)|2|2|2|2|2|2|2|2|

|ccm(μL)|1|1|1|1|1|1|1|1|

|total(μL)|30|30|30|30|30|30|30|30|

Reaction system:

|Sample|25|26|27|28|29|30|31|32|

|-|

|XbaI(μL)|1|1|1|1|1|1|1|1|

|pstI(μL)|1|1|1|1|1|1|1|1|

|nuclease-free water(μL)|25|25|25|25|25|25|25|25|

|fastdigest green buffer(μL)|2|2|2|2|2|2|2|2|

|ccm(μL)|1|1|1|1|1|1|1|1|

|total(μL)|30|30|30|30|30|30|30|30|

Mix gently and incubate at 37 degree Celsius for 20 hours .

Then we did the purification of the fragments we got.

The results are as following:

|sample|ccmXPdd 1+2+3+4+5+6+7+8+9+10+11+12+13+14+15+16|

|-|

|Concentration(ng/ul)|94.5|

|260/280|1.87|

|260/230|2.20|

|sample|ccmXPdd 17+18+19+20+21+22+23+24+25+26+27+28+29+30+31+32|

|-|

|Concentration(ng/ul)|65.5|

|260/280|1.81|

|260/230|1.82|

Double digestion of puc & PTB

Recorder: Liudong Luo, Zhenyu Jiang

Materials:

1. puc,PTB

2. FastDigest restriction enzyme XbaI, pstI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)

3. Nuclease-free water

Reaction system:

|Sample|1|2|3|4|

|-|

|XbaI(μL)|1|1|1|1|

|pstI(μL)|1|1|1|1|

|nuclease-free water(μL)|11|11|11|11|

|fastdigest green buffer(μL)|2|2|2|2|

|fast AP(μL)|1|1|1|1|

|puc(μL)|5|5|5|5|

|total(μL)|20|20|20|20|

|Sample|1|2|3|4|

|-|

|XbaI(μL)|1|1|1|1|

|pstI(μL)|1|1|1|1|

|nuclease-free water(μL)|9|9|9|9|

|fastdigest green buffer(μL)|2|2|2|2|

|fast AP(μL)|1|1|1|1|

|pTB(μL)|6|6|6|6|

|total(μL)|20|20|20|20|

Mix gently and incubate at 37 degree Celsius for 1 hours .

|sample|pucXPdd 1+2+3+4|

|-|

|Concentration(ng/ul)|118.3|

|260/280|1.81|

|260/230|2.09|

|sample|pTBXPdd 1+2+3+4|

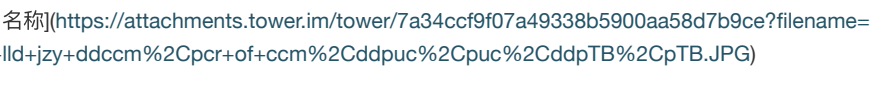
|-|

|Concentration(ng/ul)|95.2|

|260/280|1.80|

|260/230|1.99|

Agarose gel electrophoresis and purification Result:



(lane left to right: marker 2K Plus II, ddccm-1, ddccm-2, ccm-pcr-1, ccm-pcr-2,ddpuc, puc, ddpTB, pTB)

Plasmid Extraction of the RED

Recorder: 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 60 s, discard filtrate.

6.Add 500 μL Buffer DW1, 12000 rpm centifuge 60 s, discard filtrate.

7.Add 500 μL Wash Solution, 12000 rpm centifuge 60 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|RED-1|RED-2|RED-3|RED-4|RED-5|RED-6|RED-7|RED-8|

|-|

|Concentration(ng/ul)|87.3|132.6|155.3|232.7|101.9|167.8|293.1|278.0|

|260/280|1.86|1.84|1.87|1.86|1.86|1.85|1.87|1.86|

|260/230|2.34|2.26|2.36|2.29|2.33|2.25|2.31|2.12|

Plasmid Extraction of the mtr+PET28

Recorder: Meiying Cui, Liwen Zhang

Procedure:

1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice.

帮助

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 60 s, discard filtrate.

6.Add 500 μL Buffer DW1, 12000 rpm centifuge 60 s, discard filtrate.

7.Add 500 μL Wash Solution, 12000 rpm centifuge 60 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|9-2|9-4|10-1|9-3|15-1|15-3|10-4|12-2|15-2|12-4|

|-|

|Concentration(ng/ul)|99.7|99.4|113.5|100.4|91.2|105.3|80.8|101.6|79.9|92.2|

|260/280|1.86|1.79|1.78|1.87|1.81|1.74|1.88|1.83|1.91|1.77|

|260/230|2.18|1.16|1.59|2.25|1.77|2.28|2.38|1.99|2.48|1.51|

Plasmid Extraction of the PYYDT, PYRED

Recorder: Xiaoyu Zhang, Yawei Wu

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 60 s, discard filtrate.

6.Add 500 μL Buffer DW1, 12000 rpm centifuge 60 s, discard filtrate.

7.Add 500 μL Wash Solution, 12000 rpm centifuge 60 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|PYYDT-1|PYYDT-2|PYYDT-3|PYYDT-4|PYYDT-5|PYYDT-6|PYYDT-7|PYYDT-8|

|-|

|Concentration(ng/ul)|50.7|63.5|51.0|41.6|49.8|46.7|46.7|30.0|

|260/280|1.83|1.75|1.87|2.05|1.94|1.95|2.01|1.86|

|260/230|1.40|1.26|2.36|2.83|1.92|2.31|2.01|2.74|

|sample|PYRED-1|PYRED-2|PYRED-3|

|-|

|Concentration(ng/ul)|34.5|39.3|40.1|

|260/280|1.96|1.96|1.91|

|260/230|2.28|2.06|1.56|

Double digestion of RED and PYYDT

**Recorder: Yawei Wu, Menglong Jin **

Materials:

1. RED and PYYDT:

|sample|RED1|RED2|PYYDT1|PYYDT2|

|-|

|Concentration(ng/μl)|167.8|101.9|166.9|273.8|

|260/280|1.85|1.86|1.84|1.85|

|260/230|2.25|2.33|2.20|2.20|

2. FastDigest restriction enzyme XhoI, NdeI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)

3. Nuclease-free water

Reaction system:

|Sample|RED-1|RED-2|RED-3|RED-4|RED-5|RED-6|RED-7|RED-8|

|-|

|XhoI(μL)|1|1|1|1|1|1|1|1|

|NdeI(μL)|1|1|1|1|1|1|1|1|

|nuclease-free water(μL)|10|10|10|10|7|7|7|7|

|fastdigest green buffer(μL)|2|2|2|2|2|2|2|2|

|RED1(μL)|6|6|6|6|0|0|0|0|

|RED2(μL)|0|0|0|0|9|9|9|9|

|total(μL)|20|20|20|20|20|20|20|20|

Reaction system:

|Sample|PYYDT-1|PYYDT-2|PYYDT-3|PYYDT-4|PYYDT-5|PYYDT-6|PYYDT-7|PYYDT-8|

|-|

|XhoI(μL)|1|1|1|1|1|1|1|1|

|NdeI(μL)|1|1|1|1|1|1|1|1|

|nuclease-free water(μL)|10|10|10|10|12.5|12.5|12.5|12.5|

|fastdigest green buffer(μL)|2|2|2|2|2|2|2|2|

|RED1(μL)|6|6|6|6|0|0|0|0|

|RED2(μL)|0|0|0|0|3.5|3.5|3.5|3.5|

|total(μL)|20|20|20|20|20|20|20|20|

Mix gently and incubate at 37 degree Celsius for 20 hours .

Agarose gel electrophoresis and purification Result:

帮助

(https://attachments.tower.im/tower/46acc6a285734480b8f30ab96cfd357c?filename=7.31+WYW+ddPYYDT+PYRED.JPG)

(From left to right: marker 2K plusII, sample PYYDT1-8, plasmid PYRED1-7.)

(https://attachments.tower.im/tower/c567ee1d085d412983a65719796a01bc?filename=7.31+WYW+dd+Red+on+pet21.JPG)

(From left to right: marker 2K plusII, sample RED1-8.)

Then we did the purification and Glue recycling of the fragments we got.

Date 8.1

Plasmid Extraction of the RED

Recorder: Menglong Jin, 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 60 s, discard filtrate.

6.Add 500 μL Buffer DW1, 12000 rpm centifuge 60 s, discard filtrate.

7.Add 500 μL Wash Solution, 12000 rpm centifuge 60 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|RED-1|RED-2|RED-3|RED-4|RED-5|RED-6|RED-7|RED-8|

|-|

|Concentration(ng/ul)|52.2|54.0|70.0|100.1|37.7|63.8|68.4|65.9|

|260/280|1.99|2.01|1.97|1.84|2.00|1.95|1.86|1.93|

|260/230|2.72|2.20|2.42|1.46|2.02|2.31|2.19|2.27|

Transformation of PYRED123 into Top10

Recorder: Menglong Jin

NOTE:Generally, competent bacteria are restrored in -80 degree centrigrade environment.

1.Take the competent bacteria from refrigerator and incubate them into ice about 5 mins until it is dissolved

2.Absorb 1μL PUC19 and mix it wμith 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)

帮助

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 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 μL medium.

9.Coat plate: Add 200 μL solution in a plate with Kana.

10.Cultivate these bacteria overnight for further use.

Results:

![图片名称](https://attachments.tower.im/tower/454c6e9772d742f4b55cc7622253d7c8?filename=IMG_20170802_130549.jpg)

![图片名称](https://attachments.tower.im/tower/fc82e9a2c15140589e199ebd442b2f2b?filename=IMG_20170802_130614.jpg)

![图片名称](https://attachments.tower.im/tower/2ca8a48b51dc4af5a3cd2dcff8aa948c?filename=IMG_20170802_130532.jpg)

**Double digestion of ccm **

**Recorder: Liudong Luo, Zhenyu Jiang **

Materials:

1. full ccm

2. FastDigest restriction enzyme XbaI, pstI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)

3. Nuclease-free water

Reaction system:

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

|-|

|XbaI(μL)|1|1|1|1|1|1|1|1|

|pstI(μL)|1|1|1|1|1|1|1|1|

|nuclease-free water(μL)|25|25|25|25|25|25|25|25|

|fastdigest green buffer(μL)|2|2|2|2|2|2|2|2|

|ccm(μL)|1|1|1|1|1|1|1|1|

|total(μL)|30|30|30|30|30|30|30|30|

Reaction system:

|Sample|9|10|11|12|13|14|15|16|

|-|

|XbaI(μL)|1|1|1|1|1|1|1|1|

|pstI(μL)|1|1|1|1|1|1|1|1|

|nuclease-free water(μL)|25|25|25|25|25|25|25|25|

|fastdigest green buffer(μL)|2|2|2|2|2|2|2|2|

|ccm(μL)|1|1|1|1|1|1|1|1|

|total(μL)|30|30|30|30|30|30|30|30|

Reaction system:

帮助

|Sample|17|18|19|20|21|22|23|24|

|-|

|XbaI(μL)|1|1|1|1|1|1|1|1|

|pstI(μL)|1|1|1|1|1|1|1|1|

|nuclease-free water(μL)|25|25|25|25|25|25|25|25|

|fastdigest green buffer(μL)|2|2|2|2|2|2|2|2|

|ccm(μL)|1|1|1|1|1|1|1|1|

|total(μL)|30|30|30|30|30|30|30|30|

Reaction system:

|Sample|25|26|27|28|29|30|31|32|

|-|

|XbaI(μL)|1|1|1|1|1|1|1|1|

|pstI(μL)|1|1|1|1|1|1|1|1|

|nuclease-free water(μL)|25|25|25|25|25|25|25|25|

|fastdigest green buffer(μL)|2|2|2|2|2|2|2|2|

|ccm(μL)|1|1|1|1|1|1|1|1|

|total(μL)|30|30|30|30|30|30|30|30|

Mix gently and incubate at 37 degree Celsius for 20 hours .

Then we did the purification of the fragments we got.

The results are as following:

|sample|ccmXPdd 1+2+3+4+5+6+7+8+9+10+11+12+13+14+15+16+17+18+19+20+21+22+23+24+25+26+27+28+29+30+31+32|

|-|

|Concentration(ng/ul)|101.3|

|260/280|1.83|

|260/230|2.22|

Double digestion of puc & PTB

Recorder: Liudong Luo, Zhenyu Jiang

Materials:

1. puc,PTB

2. FastDigest restriction enzyme XbaI, pstI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)

3. Nuclease-free water

Reaction system:

|Sample|1|2|

|-|

|XbaI(μL)|1|1|

|pstI(μL)|1|1|

|nuclease-free water(μL)|11|11|

|fastdigest green buffer(μL)|2|2|

|fast AP(μL)|1|1|

|puc(μL)|5|5|

|total(μL)|20|20|

|Sample|1|2|

|-|

|XbaI(μL)|1|1|

|pstI(μL)|1|1|

|nuclease-free water(μL)|9|9|

|fastdigest green buffer(μL)|2|2|

|fast AP(μL)|1|1|

|pTB(μL)|6|6|

|total(μL)|20|20|

Mix gently and incubate at 37 degree Celsius for 1 hours .

Our Buffer B3 was used up, so we only did the purification of pTBXPdd.

|sample|pTBXPdd 1+2|

|-|

|Concentration(ng/ul)|35.8|

|260/280|1.90|

|260/230|2.27|

Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/e0b1369282ac4a5a92068a1c7eb17d2d?filename=8.1+lld+jzy+pcr+of+ccm+%26ddccm%26ddpTB%26pTB.JPG)

(lane left to right: marker 2K Plus II, ccm-pcr-1, ccm-pcr-2, ccm-pcr-3, ccm-pcr-4, ccm-pcr-5, ccm-pcr-6, ccm-pcr-7, ccm-pcr-8, ccm-pcr-9, ccm-pcr-10, ccm-pcr-11, ccm-pcr-12, ccm-pcr-13,ddccm, ddpTB, pTB)

**PCR of full Ccm **

Recorder: Liudong Luo, Zhenyu Jiang

Experimental materials

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

2. Primer: ccm-res-f,ccm-res-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O, 2×PrimeStar(Premix), bought from Takara.

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|9|10|11|12|13|14|15|16|

|-|

|Sterilized ddH₂O|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|

|2×Prime Star(premix)|25 μL|25 μL|25 μL|25 μL|25 μL|25 μL|25 μL|25 μL|25 μL|25 μL|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|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|ccm-res-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|ccm-res-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|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|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|

2.PCR reaction

1,2,3,4,5,6,7,8 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|10 min|

|step 2|98|10 s|

|step 3|59|5 s|

|step 4|72|7 min|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

9,10,11,12,13,14,15,16 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|10 min|

|step 2|98|10 s|

|step 3|55|15 s|

|step 4|72|7 min|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/e0b1369282ac4a5a92068a1c7eb17d2d?filename=8.1+lld+jzy+pcr+of+ccm+%26ddccm%26ddpTB%26pTB.JPG)

(lane left to right: marker 2K Plus II, ccm-pcr-1, ccm-pcr-2, ccm-pcr-3, ccm-pcr-4, ccm-pcr-5, ccm-pcr-6, ccm-pcr-7, ccm-pcr-8, ccm-pcr-9, ccm-pcr-10, ccm-pcr-11, ccm-pcr-12, ccm-pcr-13,ddccm, ddpTB, pTB)

Our Buffer B3 was used up, so we didn't do the purification .

**Bacteria PCR **

Recorder: Yonghao Liang

Experimental materials

1. Template: bacteria containing ccm+pTB recombined plasmid picked from the plate ;

2. Primer: VF2, VR. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O;

4. 2X Taq DNA polymerase.

Procedure:

1.Prepare 30 PCR tubes and sequentially add：

帮助

|sample|1|2|3|4|5|6|7|8|9|10|11|12|13|14|15|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Taq DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|VF2|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|VR|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

|sample|16|17|18|19|20|21|22|23|24|25|26|27|28|29|30|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Taq DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|VF2|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|VR|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

3.PCR reaction

1-9 Parameters setting：

|stage|temperature|time|

|-|

|step 1|94|10 min|

|step 2|94|30 s|

|step 3|58|30 s|

|step 4|72|7 min|

|step 5|72|10 min|

|step 6|4|--

25 cycles(step 2 ~ step 4)

Result:

![图片名称](https://attachments.tower.im/tower/5db5e016f97440afb49accacc12a929a?filename=8.1+lyh+bac+pcr+of+ccm%2BpTB.JPG)

**Bacteria PCR **

Recorder: Liudong Luo, Zhenyu Jiang

Experimental materials

1. Template: bacteria containing ccm+pTB recombined plasmid picked from the plate ;

2. Primer: VF2, VR,ccm-r, ccm-f. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O;

4. 2X Taq DNA polymerase.

帮助

Procedure:

1.Prepare 60 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|9|10|11|12|13|14|15|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Taq DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|VF2|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|VR|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

|sample|16|17|18|19|20|21|22|23|24|25|26|27|28|29|30|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Taq DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|VF2|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|VR|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

|sample|31|32|33|34|35|36|37|38|39|40|41|42|43|44|45|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Taq DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|ccm-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|ccm-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

|sample|46|47|48|49|50|51|52|53|54|55|56|57|58|59|60|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Taq DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|ccm-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|ccm-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

帮助

3.PCR reaction

1-9 Parameters setting：

|stage|temperature|time|

|-|

|step 1|94|10 min|

|step 2|94|30 s|

|step 3|58|30 s|

|step 4|72|7 min|

|step 5|72|10 min|

|step 6|4|--

25 cycles(step 2 ~ step 4)

Result:

![图片名称](https://attachments.tower.im/tower/8d0406ae99944b05839cbf44ff95bc61?filename=8.1+bac+pcr+of+ccm%2BpTB+1.JPG)

![图片名称](https://attachments.tower.im/tower/7a868c6fad6541b7ba06581e2057bfc5?filename=8.1+bac+pcr+of+ccm%2BpTB+2.JPG)

![图片名称](https://attachments.tower.im/tower/482821eaad7840a8a7e6c109e2aeee11?filename=8.1+bac+pcr+of+ccm%2BpTB+3.JPG)

![图片名称](https://attachments.tower.im/tower/bd7fbb8c3bcc41dbbbb7e13cd915eef8?filename=8.1+bac+pcr+of+ccm%2BpTB+4.JPG)

Date 8.2

**PCR of full CysDes **

Recorder: Menglong Jin

Experimental materials

1. Template: Gene Fragments of CysDes, synthesized by Sangon Biotech;

2. Primer: Cys-Xba-f, Cys-Pst-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O, 2×PrimeStar(Premix), bought from Takara.

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

|sample|1|2|3|4|

|-|

|Sterilized ddH₂O|9.5 μL|9.5 μL|9.5 μL|9.5 μL|

|2×Prime Star(premix)|12.5 μL|12.5 μL|12.5 μL|12.5 μL|

|template|1 μL|1 μL|1 μL|1 μL|

|Cys-Xba-f|1 μL|1 μL|1 μL|1 μL|

|Cys-Pst-r|1 μL|1 μL|1 μL|1 μL|

|total|25 μL|25 μL|25 μL|25 μL|

2.PCR reaction

帮助

1,2,3,4,5,6,7,8 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|10 min|

|step 2|98|10 s|

|step 3|54|5 s|

|step 4|72|1 min 12 sec|

|step 5|72|10 min|

|step 6|4|--

100 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:

Date: 8.2

Plasmid Extraction of the PYRED

Recorder: Menglong Jin, Yawei Wu

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 60 s, discard filtrate.

6.Add 500 μL Buffer DW1, 12000 rpm centifuge 60 s, discard filtrate.

7.Add 500 μL Wash Solution, 12000 rpm centifuge 60 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|PYRED-1|PYRED-2|PYRED-3|PYRED-1d|PYRED-2b|PYRED-2c|PYRED-3c|PYRED-3d|

|-|

|Concentration(ng/ul)|25.4|37.9|26.0|27.5|24.5|24.1|31.3|17.7|

|260/280|1.75|1.85|1.87|1.88|1.94|1.90|1.93|1.97|

|260/230|1.90|1.82|1.82|1.53|2.39|1.82|1.86|3.24|

Result:

![图片名称](https://attachments.tower.im/tower/687d008f38324af981cd5c87377ca89c?filename=8.2+PYRED+test.JPG)

(lane left to right: Marker, PYRED1d, 2b, 2c, 3c, 3d, PYRED1-3)

The mysterious band between 1000bp-2000bp disappear in both purified PYRED and the original ones. We need do more proving experiments.

帮助

**Bacteria PCR **

Recorder: Shihan Zhu

Experimental materials

1. Template: bacteria containing mtr+pET28 recombined plasmid picked from the plate ;

2. Primer: T7, T7-TER.

3. Sterilized ddH₂O;

4. 2X Taq DNA polymerase.

Procedure:

1.Prepare 30 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|9|10|11|12|13|14|15|16|

|-|

|Sterilized ddH₂O|6 μL|6 μL|6 μL|6 μL|6 μL|6 μL|6 μL|6 μL|6 μL|6 μL|6 μL|6 μL|6 μL|6 μL|6 μL|6 μL|6 μL|6 μL|

|2X Taq DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|2 μL|2 μL|2 μL|2 μL|2 μL|2 μL|2 μL|2 μL|2 μL|2 μL|2 μL|2 μL|2 μL|2 μL|2 μL|2 μL|

|T7|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|T7-TER|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

3.PCR reaction

1-9 Parameters setting：

|stage|temperature|time|

|-|

|step 1|94|10 min|

|step 2|94|30 s|

|step 3|47|30 s|

|step 4|72|5 min 30 s|

|step 5|72|10 min|

|step 6|4|--

25 cycles(step 2 ~ step 4)

Result:

![图片名称](https://attachments.tower.im/tower/afe202d00df64413b5ad45e1249aac4d?filename=8.2+bac+pcr+of+pET28.JPG)

(lane: marker, 10-4-1, 10-4-2, 10-4-3, 10-4-4, 12-2-1, 12-2-2, 12-2-3, 12-2-4, 12-4-1, 12-4-2, 12-4-3, 12-4-4, 15-2-1, 15-2-2, 15-2-3, 15-2-4)

**Bacteria PCR **

Recorder:Zhenyu Jiang

Experimental materials

1. Template: bacteria containing ccm+pTB recombined plasmid picked from the plate ;

2. Primer: VF2, VR. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O;

4. 2X Taq DNA polymerase.

Procedure:

1.Prepare 37 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|9|10|11|12|13|14|15|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Taq DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|VF2|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|VR|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

|sample|16|17|18|19|20|21|22|23|24|25|26|27|28|29|30|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Taq DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|VF2|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|VR|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

|sample|31|32|33|34|35|36|37|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Taq DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|ccm-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|ccm-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

3.PCR reaction

1-9 Parameters setting：

|stage|temperature|time|

|-|

|step 1|94|10 min|

|step 2|94|30 s|

|step 3|58|30 s|

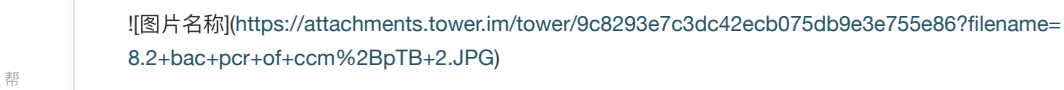
|step 4|72|7 min|

|step 5|72|10 min|

|step 6|4|--

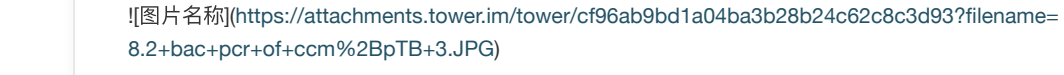
25 cycles(step 2 ~ step 4)

Result:



![图片名称](https://attachments.tower.im/tower/9c8293e7c3dc42ecb075db9e3e755e86?filename=8.2+bac+pcr+of+ccm%2BpTB+2.JPG)

![图片名称](https://attachments.tower.im/tower/9c8293e7c3dc42ecb075db9e3e755e86?filename=8.2+bac+pcr+of+ccm%2BpTB+2.JPG)


![图片名称](https://attachments.tower.im/tower/cf96ab9bd1a04ba3b28b24c62c8c3d93?filename=8.2+bac+pcr+of+ccm%2BpTB+3.JPG)

**PCR of M28 **

Recorder:Wenfei Yu

Experimental materials

1. Template: M28;

2. Primer: T7, T7-TER. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O, 2×PrimeStar(Premix), bought from Takara.

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

|sample| 12-2-3| 12-2-4| 15-2-4| 10-4-3| 12-4-4| 12-4-3| 10-4-2| 10-4-4| 12-4-1| 12-2-1| 15-2-2|

|-|

|Sterilized ddH₂O|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|

|2×Prime Star(premix)|25 μL|25 μL|25 μL|25 μL|25 μL|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|1 μL|1 μL|1 μL|1 μL|1 μL|

|T7|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|T7-TER|1 μL|1 μL|1 μL|1 μL|1 μL|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|50 μL|50 μL|50 μL|50 μL|50 μL|

2.PCR reaction

1,2,3,4,5,6,7,8,9,10,11 Parameters setting：

|stage|temperature|time|

|-|

|step 1|98|10 min|

|step 2|98|10 s|

|step 3|47|15 s|

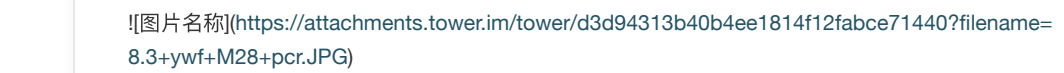
|step 4|72|5 min 30 s|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:


![图片名称](https://attachments.tower.im/tower/d3d94313b40b4ee1814f12fabce71440?filename=8.3+ywf+M28+pcr.JPG)

Date 8.3

Plasmid Extraction of the PYRED

帮助

Recorder: 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 60 s, discard filtrate.

6.Add 500 μL Buffer DW1, 12000 rpm centifuge 60 s, discard filtrate.

7.Add 500 μL Wash Solution, 12000 rpm centifuge 60 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|RED-1a|RED-1b|RED-1c|RED-1d|RED-2a|RED-2b|RED-2c|RED-2d|RED-3a|RED-3b|RED-3c|RED-3d|

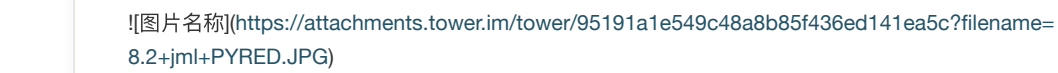
|-|

|Concentration(ng/ul)|31.9|81.4|37.5|36.4|33.4|64.0|37.0|32.5|26.6|31.8|31.1|32.2|

|260/280|1.90|1.54|1.85|1.86|1.87|1.65|1.84|1.91|1.93|1.89|1.91|1.93|

|260/230|2.71|0.74|2.01|1.56|2.87|0.83|1.96|2.53|3.23|2.73|2.44|2.78|

Agarose gel electrophoresis Result:



(From left to right: marker 1K; sample PYRED1a-d; sample PYRED2a-d; sample PYRED3a-d; PYRED extracted before; PYRED extracted after Gel recycle.)

Double digestion of PYRED

**Recorder: Yawei Wu, Menglong Jin **

Materials:

1. PYRED:

|sample|PYRED1d|PYRED2b|PYRED2c|PYRED3c|PYRED3d|PYRED2|

|-|

|Concentration(ng/μl)|27.5|24.5|24.1|31.3|17.7|37.9|

|260/280|1.88|1.94|1.90|1.93|1.97|1.85|

|260/230|1.53|2.39|1.82|1.86|3.24|1.82|

2. FastDigest restriction enzyme NdeI, XhoI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)

3. Nuclease-free water

Reaction system:

|Sample|PYRED1d|PYRED2b|PYRED2c|PYRED3c|PYRED3d|PYRED2|

帮助

|-|

|XhoI(μL)|1|1|1|1|1|1|

|NdeI(μL)|1|1|1|1|1|1|

|fastdigest green buffer(μL)|2|2|2|2|2|2|

|RED1(μL)|16|16|16|16|16|16|

|total(μL)|20|20|20|20|20|20|

Mix gently and incubate at 37 degree Celsius for 20 hours .

Agarose gel electrophoresis and purification Result:

![图片名称](https://attachments.tower.im/tower/36024a50ef754d34840dbeae5651ac28?filename=8.2+wyw+pcr+%26+dd+of+PYRED.JPG)

(left to right: Marker 2K plusII, PCR of RED, ddPYRED)

PCR of RED

Recorder: Yawei Wu

Experimental materials

1. Template: PYRED;

2. Primer: Red-f, Red-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O;

4. 2X Taq DNA polymerase.

Procedure:

1.Prepare 16 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Taq DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Red-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Red-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

3.PCR reaction

1-9 Parameters setting：

|stage|temperature|time|

|-|

|step 1|94|10 min|

|step 2|94|30 s|

|step 3|67|30 s|

|step 4|72|50 S|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

Agarose gel electrophoresis and purification Result:

帮助

(left to right: Marker 2K plusII, PCR of RED, ddPYRED)

According to the PCR and double digestion results, we pick PYRED1d, PYRED2b, PYRED2c. It is a pity we still cannot figure out the mysterious band.

Plasmid Extraction of the pYYDT and PYRED

Recorder: 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 60 s, discard filtrate.

6.Add 500 μL Buffer DW1, 12000 rpm centifuge 60 s, discard filtrate.

7.Add 500 μL Wash Solution, 12000 rpm centifuge 60 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|PYYDT-1|PYYDT-2|PYYDT-3|PYYDT-4|PYYDT-5|PYYDT-6|PYYDT-7|PYYDT-8|

|-|

|Concentration(ng/ul)|52.2|43.0|39.1|46.1|152.1|36.2|32.3|41.8|

|260/280|1.86|1.86|1.91|1.76|1.68|1.82|1.92|1.87|

|260/230|2.24|2.24|2.13|1.32|0.85|1.45|2.98|1.75|

|sample|PYRED-1|PYRED-2|PYRED-3|

|-|

|Concentration(ng/ul)|27.3|28.4|32.9|

|260/280|1.84|1.71|1.71|

|260/230|1.49|1.13|0.99|

**PCR of full CysDes **

Recorder:Liudong Luo, Zhenyu Jiang

Experimental materials

1. Template: Gene Fragments of CysDes, synthesized by Sangon Biotech;

2. Primer: Cys-Xba-f, Cys-Pst-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O, 2×PrimeStar(Premix), bought from Takara.

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

帮助

|sample|1|2|3|4|5|6|7|8|9|10|11|12|

|-|

|Sterilized ddH₂O|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|

|2×Prime Star(premix)|25 μL|25 μL|25 μL|25 μL|25 μL|25 μL|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|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Cys-Xba-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Cys-Pst-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|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|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|

2.PCR reaction

1,2,3,4,5,6,7,8,9,10,11,12 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|5 min|

|step 2|98|5 s|

|step 3|55|5 s|

|step 4|72|1 min 12 sec|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/628e8f6d3e4f42ad8d006a4b8d416394?filename=8.3+lld+jzy+pcr+of+cysdes.JPG)

![图片名称](https://attachments.tower.im/tower/4b452549640d4839b911ab48c9497984?filename=8.3+lld+jzy+pcr+of+cysdes+2.JPG)

Purification of cysdes PCR product

Recorder: Zhenyu Jiang, Liudong Luo

(1). Add 900 μL Buffer B3 to the 180 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 25 μL ddH₂O, stand them still for 10 min. Centrifuge at 12,000 rpm for 1 min.

OD measurement result:

|sample|cysdes PCR pur 1+2+3+4|

|-|

|Concentration(ng/ul)|444.7|

|260/280|1.88|

|260/230|2.36|

帮助

|sample|cysdes PCR pur 5+6+7+8|

|-|

|Concentration(ng/ul)|461.9|

|260/280|1.87|

|260/230|2.34|

|sample|ccm PCR pur 9+10+11+12|

|-|

|Concentration(ng/ul)|404.3|

|260/280|1.88|

|260/230|2.39|

|sample|ccm PCR pur 13+14+15+16|

|-|

|Concentration(ng/ul)|148.1|

|260/280|1.67|

|260/230|1.35|

Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/3658350d48b7427d9bd4798fda22999f?filename=8.3+lld+jzy+pcr+of+cysdes+after+pur.JPG)

(lane left to right: marker-Q, pcr-cys-1, pcr-cys-2, pcr-cys-3, pcr-cys-4)

**Double digestion of **Mtr-pet28

Recorder: Meiying Cui

Materials:

1. full Mtr-PET28

2. FastDigest restriction enzyme NdeI, XhoI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)

3. Nuclease-free water

Reaction system:

|Sample|12-4-2|12-4-2'|12-2-2|12-2-2'|15-2-4|15-2-4'|10-4-3|10-4-3'|

|-|

|XhoI(μL)|1|1|1|1|1|1|1|1|

|NdeI(μL)|1|1|1|1|1|1|1|1|

|nuclease-free water(μL)|0|6|10|10|0|0|0|8|

|fastdigest green buffer(μL)|2|2|2|2|2|2|2|2|

|sample(μL)|16|10|6|6|16|16|16|8|

|total(μL)|20|20|20|20|20|20|20|20|

Mix gently and incubate at 37 degree Celsius for 1 hour .

Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/cd2df977a8f5430f96602a5b65c58141?filename=8.3+CMY+dd-pET28.JPG)

(lane left to right: marker Q, 12-4-2,12-4-2,12-2-2,12-2-2,10-4-3,10-4-3,15-2-4,15-2-4,12-4-2',12-4-2',12-2-2',12-2-2',10-4-3',10-4-3',15-2-4',15-2-4')

Purification of M28 PCR product

Recorder: Wenfei Yu

(1). Add 225 μL Buffer B3 to the 45 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 25 μL ddH₂O, stand them still for 10 min. Centrifuge at 12,000 rpm for 1 min.

OD measurement result:

|sample| 12-2-3| 12-2-4| 15-2-4| 10-4-3| 12-4-4| 12-4-3| 10-4-2| 10-4-4| 12-4-1| 12-2-1| 15-2-2|

|-|

|Concentration(ng/ul)|149.3|165.4|194.8|151.4|94.8|157.4|147.4|118.0|152.1|143.5|114.5|

|260/280|1.84|1.79|1.82|1.78|1.77|1.78|1.73|1.76|1.81|1.81|1.76|

|260/230|2.26|1.73|1.81|1.55|1.57|1.68|1.26|1.49|1.85|1.92|1.41|

Plasmid Extraction of the RED

Recorder: Menglong Jin, 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 60 s, discard filtrate.

6.Add 500 μL Buffer DW1, 12000 rpm centifuge 60 s, discard filtrate.

7.Add 500 μL Wash Solution, 12000 rpm centifuge 60 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|RED-1|RED-2|RED-3|RED-4|RED-5|RED-6|RED-7|RED-8|

|-|

|Concentration(ng/ul)|52.2|54.0|70.0|100.1|37.7|63.8|68.4|65.9|

|260/280|1.99|2.01|1.97|1.84|2.00|1.95|1.86|1.93|

|260/230|2.72|2.20|2.42|1.46|2.02|2.31|2.19|2.27|

帮助

Transformation of PYRED123 into Top10

Recorder: Menglong Jin

NOTE:Generally, competent bacteria are restrored in -80 degree centrigrade environment.

1.Take the competent bacteria from refrigerator and incubate them into ice about 5 mins until it is dissolved

2.Absorb 1μL PUC19 and mix it wμith 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 μL 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 μL medium.

9.Coat plate: Add 200 μL solution in a plate with Kana.

10.Cultivate these bacteria overnight for further use.

Results:

![图片名称](https://attachments.tower.im/tower/454c6e9772d742f4b55cc7622253d7c8?filename=IMG_20170802_130549.jpg)

![图片名称](https://attachments.tower.im/tower/fc82e9a2c15140589e199ebd442b2f2b?filename=IMG_20170802_130614.jpg)

![图片名称](https://attachments.tower.im/tower/2ca8a48b51dc4af5a3cd2dcff8aa948c?filename=IMG_20170802_130532.jpg)

**Double digestion of ccm **

**Recorder: Liudong Luo, Zhenyu Jiang **

Materials:

1. full ccm

2. FastDigest restriction enzyme XbaI, pstI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)

3. Nuclease-free water

Reaction system:

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

|-|

|XbaI(μL)|1|1|1|1|1|1|1|1|

|pstI(μL)|1|1|1|1|1|1|1|1|

|nuclease-free water(μL)|25|25|25|25|25|25|25|25|

|fastdigest green buffer(μL)|2|2|2|2|2|2|2|2|

|ccm(μL)|1|1|1|1|1|1|1|1|

|total(μL)|30|30|30|30|30|30|30|30|

Reaction system:

帮助

|Sample|9|10|11|12|13|14|15|16|

|-|

|XbaI(μL)|1|1|1|1|1|1|1|1|

|pstI(μL)|1|1|1|1|1|1|1|1|

|nuclease-free water(μL)|25|25|25|25|25|25|25|25|

|fastdigest green buffer(μL)|2|2|2|2|2|2|2|2|

|ccm(μL)|1|1|1|1|1|1|1|1|

|total(μL)|30|30|30|30|30|30|30|30|

Reaction system:

|Sample|17|18|19|20|21|22|23|24|

|-|

|XbaI(μL)|1|1|1|1|1|1|1|1|

|pstI(μL)|1|1|1|1|1|1|1|1|

|nuclease-free water(μL)|25|25|25|25|25|25|25|25|

|fastdigest green buffer(μL)|2|2|2|2|2|2|2|2|

|ccm(μL)|1|1|1|1|1|1|1|1|

|total(μL)|30|30|30|30|30|30|30|30|

Reaction system:

|Sample|25|26|27|28|29|30|31|32|

|-|

|XbaI(μL)|1|1|1|1|1|1|1|1|

|pstI(μL)|1|1|1|1|1|1|1|1|

|nuclease-free water(μL)|25|25|25|25|25|25|25|25|

|fastdigest green buffer(μL)|2|2|2|2|2|2|2|2|

|ccm(μL)|1|1|1|1|1|1|1|1|

|total(μL)|30|30|30|30|30|30|30|30|

Mix gently and incubate at 37 degree Celsius for 20 hours .

Then we did the purification of the fragments we got.

The results are as following:

|sample|ccmXPdd 1+2+3+4+5+6+7+8+9+10+11+12+13+14+15+16+17+18+19+20+21+22+23+24+25+26+27+28+29+30+31+32|

|-|

|Concentration(ng/ul)|101.3|

|260/280|1.83|

|260/230|2.22|

Double digestion of puc & PTB

Recorder: Liudong Luo, Zhenyu Jiang

Materials:

1. puc,PTB

2. FastDigest restriction enzyme XbaI, pstI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)

3. Nuclease-free water

Reaction system:

|Sample|1|2|

|-|

|XbaI(μL)|1|1|

|pstI(μL)|1|1|

|nuclease-free water(μL)|11|11|

|fastdigest green buffer(μL)|2|2|

|fast AP(μL)|1|1|

|puc(μL)|5|5|

|total(μL)|20|20|

|Sample|1|2|

|-|

|XbaI(μL)|1|1|

|pstI(μL)|1|1|

|nuclease-free water(μL)|9|9|

|fastdigest green buffer(μL)|2|2|

|fast AP(μL)|1|1|

|pTB(μL)|6|6|

|total(μL)|20|20|

Mix gently and incubate at 37 degree Celsius for 1 hours .

Our Buffer B3 was used up, so we only did the purification of pTBXPdd.

|sample|pTBXPdd 1+2|

|-|

|Concentration(ng/ul)|35.8|

|260/280|1.90|

|260/230|2.27|

Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/e0b1369282ac4a5a92068a1c7eb17d2d?filename=8.1+lld+jzy+pcr+of+ccm+%26ddccm%26ddpTB%26pTB.JPG)

(lane left to right: marker 2K Plus II, ccm-pcr-1, ccm-pcr-2, ccm-pcr-3, ccm-pcr-4, ccm-pcr-5, ccm-pcr-6, ccm-pcr-7, ccm-pcr-8, ccm-pcr-9, ccm-pcr-10, ccm-pcr-11, ccm-pcr-12, ccm-pcr-13,ddccm, ddpTB, pTB)

**PCR of full Ccm **

Recorder: Liudong Luo, Zhenyu Jiang

Experimental materials

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

2. Primer: ccm-res-f,ccm-res-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O, 2×PrimeStar(Premix), bought from Takara.

Procedure:

帮助

1.Prepare 4 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|9|10|11|12|13|14|15|16|

|-|

|Sterilized ddH₂O|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|

|2×Prime Star(premix)|25 μL|25 μL|25 μL|25 μL|25 μL|25 μL|25 μL|25 μL|25 μL|25 μL|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|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|ccm-res-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|ccm-res-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|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|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|

2.PCR reaction

1,2,3,4,5,6,7,8 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|10 min|

|step 2|98|10 s|

|step 3|59|5 s|

|step 4|72|7 min|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

9,10,11,12,13,14,15,16 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|10 min|

|step 2|98|10 s|

|step 3|55|15 s|

|step 4|72|7 min|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/e0b1369282ac4a5a92068a1c7eb17d2d?filename=8.1+lld+jzy+pcr+of+ccm+%26ddccm%26ddpTB%26pTB.JPG)

(lane left to right: marker 2K Plus II, ccm-pcr-1, ccm-pcr-2, ccm-pcr-3, ccm-pcr-4, ccm-pcr-5, ccm-pcr-6, ccm-pcr-7, ccm-pcr-8, ccm-pcr-9, ccm-pcr-10, ccm-pcr-11, ccm-pcr-12, ccm-pcr-

13,ddccm, ddpTB, pTB)

Our Buffer B3 was used up, so we didn't do the purification .

**Bacteria PCR **

帮助

Recorder: Yonghao Liang

Experimental materials

1. Template: bacteria containing ccm+pTB recombined plasmid picked from the plate ;

2. Primer: VF2, VR. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O;

4. 2X Taq DNA polymerase.

Procedure:

1.Prepare 30 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|9|10|11|12|13|14|15|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Taq DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|VF2|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|VR|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

|sample|16|17|18|19|20|21|22|23|24|25|26|27|28|29|30|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Taq DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|VF2|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|VR|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

3.PCR reaction

1-9 Parameters setting：

|stage|temperature|time|

|-|

|step 1|94|10 min|

|step 2|94|30 s|

|step 3|58|30 s|

|step 4|72|7 min|

|step 5|72|10 min|

|step 6|4|--

25 cycles(step 2 ~ step 4)

Result:

帮助

**Bacteria PCR **

Recorder: Liudong Luo, Zhenyu Jiang

Experimental materials

1. Template: bacteria containing ccm+pTB recombined plasmid picked from the plate ;

2. Primer: VF2, VR,ccm-r, ccm-f. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O;

4. 2X Taq DNA polymerase.

Procedure:

1.Prepare 60 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|9|10|11|12|13|14|15|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Taq DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|VF2|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|VR|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

|sample|16|17|18|19|20|21|22|23|24|25|26|27|28|29|30|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Taq DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|VF2|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|VR|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

|sample|31|32|33|34|35|36|37|38|39|40|41|42|43|44|45|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Taq DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|ccm-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|ccm-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

|sample|46|47|48|49|50|51|52|53|54|55|56|57|58|59|60|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Taq DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|ccm-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|ccm-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

3.PCR reaction

1-9 Parameters setting：

|stage|temperature|time|

|-|

|step 1|94|10 min|

|step 2|94|30 s|

|step 3|58|30 s|

|step 4|72|7 min|

|step 5|72|10 min|

|step 6|4|--

25 cycles(step 2 ~ step 4)

Result:

![图片名称](https://attachments.tower.im/tower/8d0406ae99944b05839cbf44ff95bc61?filename=8.1+bac+pcr+of+ccm%2BpTB+1.JPG)

![图片名称](https://attachments.tower.im/tower/7a868c6fad6541b7ba06581e2057bfc5?filename=8.1+bac+pcr+of+ccm%2BpTB+2.JPG)

![图片名称](https://attachments.tower.im/tower/482821eaad7840a8a7e6c109e2aeee11?filename=8.1+bac+pcr+of+ccm%2BpTB+3.JPG)

![图片名称](https://attachments.tower.im/tower/bd7fbb8c3bcc41dbbbb7e13cd915eef8?filename=8.1+bac+pcr+of+ccm%2BpTB+4.JPG)

Date 8.2

**PCR of full CysDes **

Recorder: Menglong Jin

Experimental materials

1. Template: Gene Fragments of CysDes, synthesized by Sangon Biotech;

2. Primer: Cys-Xba-f, Cys-Pst-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O, 2×PrimeStar(Premix), bought from Takara.

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

|sample|1|2|3|4|

|-|

|Sterilized ddH₂O|9.5 μL|9.5 μL|9.5 μL|9.5 μL|

|2×Prime Star(premix)|12.5 μL|12.5 μL|12.5 μL|12.5 μL|

|template|1 μL|1 μL|1 μL|1 μL|

|Cys-Xba-f|1 μL|1 μL|1 μL|1 μL|

|Cys-Pst-r|1 μL|1 μL|1 μL|1 μL|

|total|25 μL|25 μL|25 μL|25 μL|

2.PCR reaction

1,2,3,4,5,6,7,8 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|10 min|

|step 2|98|10 s|

|step 3|54|5 s|

|step 4|72|1 min 12 sec|

|step 5|72|10 min|

|step 6|4|--

100 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:

Date: 8.2

Plasmid Extraction of the PYRED

Recorder: Menglong Jin, Yawei Wu

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 60 s, discard filtrate.

6.Add 500 μL Buffer DW1, 12000 rpm centifuge 60 s, discard filtrate.

7.Add 500 μL Wash Solution, 12000 rpm centifuge 60 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|PYRED-1|PYRED-2|PYRED-3|PYRED-1d|PYRED-2b|PYRED-2c|PYRED-3c|PYRED-3d|

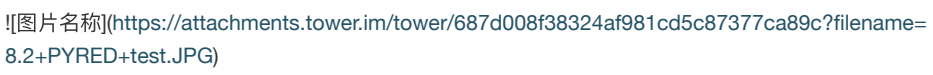
|-|

|Concentration(ng/ul)|25.4|37.9|26.0|27.5|24.5|24.1|31.3|17.7|

|260/280|1.75|1.85|1.87|1.88|1.94|1.90|1.93|1.97|

|260/230|1.90|1.82|1.82|1.53|2.39|1.82|1.86|3.24|

Result:



(lane left to right: Marker, PYRED1d, 2b, 2c, 3c, 3d, PYRED1-3)

The mysterious band between 1000bp-2000bp disappear in both purified PYRED and the original ones. We need do more proving experiments.

**Bacteria PCR **

Recorder: Shihan Zhu

Experimental materials

1. Template: bacteria containing mtr+pET28 recombined plasmid picked from the plate ;

2. Primer: T7, T7-TER.

3. Sterilized ddH₂O;

4. 2X Taq DNA polymerase.

Procedure:

1.Prepare 30 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|9|10|11|12|13|14|15|16|

|-|

|Sterilized ddH₂O|6 μL|6 μL|6 μL|6 μL|6 μL|6 μL|6 μL|6 μL|6 μL|6 μL|6 μL|6 μL|6 μL|6 μL|6 μL|6 μL|

|2X Taq DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|2 μL|2 μL|2 μL|2 μL|2 μL|2 μL|2 μL|2 μL|2 μL|2 μL|2 μL|2 μL|2 μL|2 μL|2 μL|2 μL|

|T7|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|T7-TER|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

3.PCR reaction

1-9 Parameters setting：

|stage|temperature|time|

|-|

|step 1|94|10 min|

|step 2|94|30 s|

|step 3|47|30 s|

|step 4|72|5 min 30 s|

|step 5|72|10 min|

|step 6|4|--

25 cycles(step 2 ~ step 4)

Result:

帮助

(https://attachments.tower.im/tower/afe202d00df64413b5ad45e1249aac4d?filename=8.2+bac+pcr+of+pET28.JPG)

(lane: marker, 10-4-1, 10-4-2, 10-4-3, 10-4-4, 12-2-1, 12-2-2, 12-2-3, 12-2-4, 12-4-1, 12-4-2, 12-4-3, 12-4-4, 15-2-1, 15-2-2, 15-2-3, 15-2-4)

**Bacteria PCR **

Recorder:Zhenyu Jiang

Experimental materials

1. Template: bacteria containing ccm+pTB recombined plasmid picked from the plate ;

2. Primer: VF2, VR. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O;

4. 2X Taq DNA polymerase.

Procedure:

1.Prepare 37 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|9|10|11|12|13|14|15|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Taq DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|VF2|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|VR|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

|sample|16|17|18|19|20|21|22|23|24|25|26|27|28|29|30|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Taq DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|VF2|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|VR|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

|sample|31|32|33|34|35|36|37|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Taq DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|ccm-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|ccm-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

帮助

3.PCR reaction

1-9 Parameters setting：

|stage|temperature|time|

|-|

|step 1|94|10 min|

|step 2|94|30 s|

|step 3|58|30 s|

|step 4|72|7 min|

|step 5|72|10 min|

|step 6|4|--

25 cycles(step 2 ~ step 4)

Result:

![图片名称](https://attachments.tower.im/tower/9c8293e7c3dc42ecb075db9e3e755e86?filename=8.2+bac+pcr+of+ccm%2BpTB+2.JPG)

![图片名称](https://attachments.tower.im/tower/9c8293e7c3dc42ecb075db9e3e755e86?filename=8.2+bac+pcr+of+ccm%2BpTB+2.JPG)

![图片名称](https://attachments.tower.im/tower/cf96ab9bd1a04ba3b28b24c62c8c3d93?filename=8.2+bac+pcr+of+ccm%2BpTB+3.JPG)

**PCR of M28 **

Recorder:Wenfei Yu

Experimental materials

1. Template: M28;

2. Primer: T7, T7-TER. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O, 2×PrimeStar(Premix), bought from Takara.

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

|sample| 12-2-3| 12-2-4| 15-2-4| 10-4-3| 12-4-4| 12-4-3| 10-4-2| 10-4-4| 12-4-1| 12-2-1| 15-2-2|

|-|

|Sterilized ddH₂O|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|

|2×Prime Star(premix)|25 μL|25 μL|25 μL|25 μL|25 μL|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|1 μL|1 μL|1 μL|1 μL|1 μL|

|T7|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|T7-TER|1 μL|1 μL|1 μL|1 μL|1 μL|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|50 μL|50 μL|50 μL|50 μL|50 μL|

2.PCR reaction

1,2,3,4,5,6,7,8,9,10,11 Parameters setting：

|stage|temperature|time|

|-|

|step 1|98|10 min|

|step 2|98|10 s|

|step 3|47|15 s|

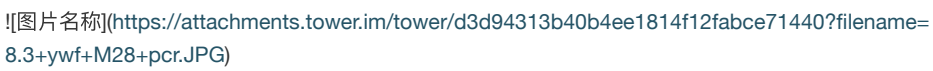
|step 4|72|5 min 30 s|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:



Date 8.3

Plasmid Extraction of the PYRED

Recorder: 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 60 s, discard filtrate.

6.Add 500 μL Buffer DW1, 12000 rpm centifuge 60 s, discard filtrate.

7.Add 500 μL Wash Solution, 12000 rpm centifuge 60 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|RED-1a|RED-1b|RED-1c|RED-1d|RED-2a|RED-2b|RED-2c|RED-2d|RED-3a|RED-3b|RED-3c|RED-3d|

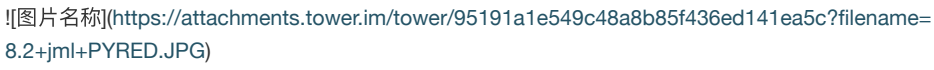
|-|

|Concentration(ng/ul)|31.9|81.4|37.5|36.4|33.4|64.0|37.0|32.5|26.6|31.8|31.1|32.2|

|260/280|1.90|1.54|1.85|1.86|1.87|1.65|1.84|1.91|1.93|1.89|1.91|1.93|

|260/230|2.71|0.74|2.01|1.56|2.87|0.83|1.96|2.53|3.23|2.73|2.44|2.78|

Agarose gel electrophoresis Result:



(From left to right: marker 1K; sample PYRED1a-d; sample PYRED2a-d; sample PYRED3a-d; PYRED extracted before; PYRED extracted after Gel recycle.)

Double digestion of PYRED

**Recorder: Yawei Wu, Menglong Jin **

Materials:

1. PYRED:

|sample|PYRED1d|PYRED2b|PYRED2c|PYRED3c|PYRED3d|PYRED2|

|-|

|Concentration(ng/μl)|27.5|24.5|24.1|31.3|17.7|37.9|

|260/280|1.88|1.94|1.90|1.93|1.97|1.85|

|260/230|1.53|2.39|1.82|1.86|3.24|1.82|

2. FastDigest restriction enzyme NdeI, XhoI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)

3. Nuclease-free water

Reaction system:

|Sample|PYRED1d|PYRED2b|PYRED2c|PYRED3c|PYRED3d|PYRED2|

|-|

|XhoI(μL)|1|1|1|1|1|1|

|NdeI(μL)|1|1|1|1|1|1|

|fastdigest green buffer(μL)|2|2|2|2|2|2|

|RED1(μL)|16|16|16|16|16|16|

|total(μL)|20|20|20|20|20|20|

Mix gently and incubate at 37 degree Celsius for 20 hours .

Agarose gel electrophoresis and purification Result:

![图片名称](https://attachments.tower.im/tower/36024a50ef754d34840dbeae5651ac28?filename=8.2+wyw+pcr+%26+dd+of+PYRED.JPG)

(left to right: Marker 2K plusII, PCR of RED, ddPYRED)

PCR of RED

Recorder: Yawei Wu

Experimental materials

1. Template: PYRED;

2. Primer: Red-f, Red-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O;

4. 2X Taq DNA polymerase.

Procedure:

1.Prepare 16 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Taq DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Red-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Red-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

3.PCR reaction

1-9 Parameters setting：

帮助

|stage|temperature|time|

|-|

|step 1|94|10 min|

|step 2|94|30 s|

|step 3|67|30 s|

|step 4|72|50 S|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

Agarose gel electrophoresis and purification Result:

![图片名称](https://attachments.tower.im/tower/36024a50ef754d34840dbeae5651ac28?filename=8.2+wyw+pcr+%26+dd+of+PYRED.JPG)

(left to right: Marker 2K plusII, PCR of RED, ddPYRED)

According to the PCR and double digestion results, we pick PYRED1d, PYRED2b, PYRED2c. It is a pity we still cannot figure out the mysterious band.

Plasmid Extraction of the pYYDT and PYRED

Recorder: 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 60 s, discard filtrate.

6.Add 500 μL Buffer DW1, 12000 rpm centifuge 60 s, discard filtrate.

7.Add 500 μL Wash Solution, 12000 rpm centifuge 60 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|PYYDT-1|PYYDT-2|PYYDT-3|PYYDT-4|PYYDT-5|PYYDT-6|PYYDT-7|PYYDT-8|

|-|

|Concentration(ng/ul)|52.2|43.0|39.1|46.1|152.1|36.2|32.3|41.8|

|260/280|1.86|1.86|1.91|1.76|1.68|1.82|1.92|1.87|

|260/230|2.24|2.24|2.13|1.32|0.85|1.45|2.98|1.75|

|sample|PYRED-1|PYRED-2|PYRED-3|

|-|

|Concentration(ng/ul)|27.3|28.4|32.9|

|260/280|1.84|1.71|1.71|

|260/230|1.49|1.13|0.99|

**PCR of full CysDes **

帮助

Recorder:Liudong Luo, Zhenyu Jiang

Experimental materials

1. Template: Gene Fragments of CysDes, synthesized by Sangon Biotech;

2. Primer: Cys-Xba-f, Cys-Pst-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O, 2×PrimeStar(Premix), bought from Takara.

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|9|10|11|12|

|-|

|Sterilized ddH₂O|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|

|2×Prime Star(premix)|25 μL|25 μL|25 μL|25 μL|25 μL|25 μL|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|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Cys-Xba-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Cys-Pst-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|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|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|

2.PCR reaction

1,2,3,4,5,6,7,8,9,10,11,12 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|5 min|

|step 2|98|5 s|

|step 3|55|5 s|

|step 4|72|1 min 12 sec|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/628e8f6d3e4f42ad8d006a4b8d416394?filename=8.3+lld+jzy+pcr+of+cysdes.JPG)

![图片名称](https://attachments.tower.im/tower/4b452549640d4839b911ab48c9497984?filename=8.3+lld+jzy+pcr+of+cysdes+2.JPG)

Purification of cysdes PCR product

Recorder: Zhenyu Jiang, Liudong Luo

(1). Add 900 μL Buffer B3 to the 180 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.

帮助

(7). Put the column to an 1.5 ml EP tube, add 25 μL ddH₂O, stand them still for 10 min. Centrifuge at 12,000 rpm for 1 min.

OD measurement result:

|sample|cysdes PCR pur 1+2+3+4|

|-|

|Concentration(ng/ul)|444.7|

|260/280|1.88|

|260/230|2.36|

|sample|cysdes PCR pur 5+6+7+8|

|-|

|Concentration(ng/ul)|461.9|

|260/280|1.87|

|260/230|2.34|

|sample|ccm PCR pur 9+10+11+12|

|-|

|Concentration(ng/ul)|404.3|

|260/280|1.88|

|260/230|2.39|

|sample|ccm PCR pur 13+14+15+16|

|-|

|Concentration(ng/ul)|148.1|

|260/280|1.67|

|260/230|1.35|

Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/3658350d48b7427d9bd4798fda22999f?filename=8.3+lld+jzy+pcr+of+cysdes+after+pur.JPG)

(lane left to right: marker-Q, pcr-cys-1, pcr-cys-2, pcr-cys-3, pcr-cys-4)

**Double digestion of **Mtr-pet28

Recorder: Meiying Cui

Materials:

1. full Mtr-PET28

2. FastDigest restriction enzyme NdeI, XhoI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)

3. Nuclease-free water

Reaction system:

|Sample|12-4-2|12-4-2'|12-2-2|12-2-2'|15-2-4|15-2-4'|10-4-3|10-4-3'|

|-|

|XhoI(μL)|1|1|1|1|1|1|1|1|

|NdeI(μL)|1|1|1|1|1|1|1|1|

|nuclease-free water(μL)|0|6|10|10|0|0|0|8|

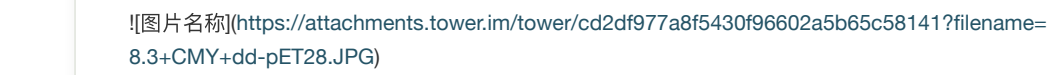
|fastdigest green buffer(μL)|2|2|2|2|2|2|2|2|

|sample(μL)|16|10|6|6|16|16|16|8|

|total(μL)|20|20|20|20|20|20|20|20|

Mix gently and incubate at 37 degree Celsius for 1 hour .

Agarose gel electrophoresis Result:



(lane left to right: marker Q, 12-4-2,12-4-2,12-2-2,12-2-2,10-4-3,10-4-3,15-2-4,15-2-4,12-4-2',12-4-2',12-2-2',12-2-2',10-4-3',10-4-3',15-2-4',15-2-4')

Purification of M28 PCR product

Recorder: Wenfei Yu

(1). Add 225 μL Buffer B3 to the 45 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 25 μL ddH₂O, stand them still for 10 min. Centrifuge at 12,000 rpm for 1 min.

OD measurement result:

|sample| 12-2-3| 12-2-4| 15-2-4| 10-4-3| 12-4-4| 12-4-3| 10-4-2| 10-4-4| 12-4-1| 12-2-1| 15-2-2|

|-|

|Concentration(ng/ul)|149.3|165.4|194.8|151.4|94.8|157.4|147.4|118.0|152.1|143.5|114.5|

|260/280|1.84|1.79|1.82|1.78|1.77|1.78|1.73|1.76|1.81|1.81|1.76|

|260/230|2.26|1.73|1.81|1.55|1.57|1.68|1.26|1.49|1.85|1.92|1.41|

Date 8.4

Plasmid Extraction of the PYRED

Recorder: Menglong Jin, Yawei Wu

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, 6 and 2 min's standing(We extend the time for sample PYRED2a & 2b and shorten the time for sample PYRED1a & 1b & 1c!).

4.Add 350 μL Buffer P3, mix well.

5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 60 s, discard filtrate.

6.Add 500 μL Buffer DW1, 12000 rpm centifuge 60 s, discard filtrate.

7.Add 500 μL Wash Solution, 12000 rpm centifuge 60 s, discard filtrate. Repeat once.

8.12000 rpm centifuge 1 min.

9.Lying for 10 min.

帮助

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|PYRED1-a|PYRED1-b|PYRED1-c|PYRED2-a|PYRED2-d|

|-|

|Concentration(ng/ul)|99.3|76.5|30.9|32.5|41.6|

|260/280|1.57|1.64|1.98|1.97|1.92|

|260/230|0.72|0.90|2.69|2.42|1.97|

**PCR of full Ccm **

Recorder: Liudong Luo, Zhenyu Jiang

Experimental materials

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

2. Primer: ccm-res-f,ccm-res-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O, 2×PrimeStar(Premix), bought from Takara.

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|9|10|11|12|13|14|15|16|

|-|

|Sterilized ddH₂O|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|

|2×Prime Star(premix)|25 μL|25 μL|25 μL|25 μL|25 μL|25 μL|25 μL|25 μL|25 μL|25 μL|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|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|ccm-res-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|ccm-res-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|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|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|

2.PCR reaction

1,2,3,4,5,6,7,8 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|10 min|

|step 2|98|10 s|

|step 3|59|5 s|

|step 4|72|7 min|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

9,10,11,12,13,14,15,16 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|10 min|

|step 2|98|10 s|

|step 3|55|15 s|

|step 4|72|7 min|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/9058e4e39d994ec5bcb2522c3767ca51?filename=8.4+jzy+lld+pcr+of+ccm.JPG)

(lane left to right: marker Q, ccm-pcr-1, ccm-pcr-2,ccm-pcr-3,ccm-pcr-4,ccm-pcr-5, ccm-pcr-6, ccm-pcr-7,ccm-pcr-8,ccm-pcr-9, ccm-pcr-10,ccm-pcr-11,ccm-pcr-12,ccm-pcr-13, ccm-pcr-14, ccm-pcr-15,ccm-pcr-16)

Purification of full ccm PCR product

Recorder: Zhenyu Jiang, Liudong Luo

(1). Add 900 μL Buffer B3 to the 180 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 25 μL ddH₂O, stand them still for 10 min. Centrifuge at 12,000 rpm for 1 min.

OD measurement result:

|sample|ccm PCR pur 1+2+3+4+5+6+7+8|ccm PCR pur 9+10+11+112+13+14+15+16|

|-|

|Concentration(ng/ul)|274.4|289.3|

|260/280|14.85|9.86|

|260/230|13.45|11.77|

maybe something wrong when we did the purification

![图片名称](https://attachments.tower.im/tower/c669a312f10647f98cdf1c286e6df2c1?filename=8.4+jzy+lld+pcr+of+ccm%26cys+after+pur.JPG)

(lane left to right: 2K plus II, 2K plus II, 2K plus II, 2K plus II, 2K plus II, 2K plus II(test), pur-pcr-ccm-1, pur-pcr-ccm-2, pur-pcr-cys-1, pur-pcr-cys-2, Marker Q)

**PCR of full CysDes **

帮助

Recorder:Liudong Luo, Zhenyu Jiang

Experimental materials

1. Template: Gene Fragments of CysDes, synthesized by Sangon Biotech;

2. Primer: Cys-Xba-f, Cys-Pst-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O, 2×PrimeStar(Premix), bought from Takara.

Procedure:

1.Prepare 8 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|

|-|

|Sterilized ddH₂O|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|

|2×Prime Star(premix)|25 μL|25 μL|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|1 μL|1 μL|

|Cys-Xba-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Cys-Pst-r|1 μL|1 μL|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|50 μL|50 μL|

2.PCR reaction

1,2,3,4,5,6,7,8 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|5 min|

|step 2|98|5 s|

|step 3|55|5 s|

|step 4|72|1 min 12 sec|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/a8cbe530d5a44cf5b9da1c97d0a48638?filename=8.4+jzy+lld+pcr+of+cysdes+1.JPG)

(lane left to right: marker-Q, pcr-cys-1-1, pcr-cys-1-2, pcr-cys-2-1, pcr-cys-2-2, pcr-cys-3-1, pcr-cys-3-2, pcr-cys-4-1, pcr-cys-4-2)

![图片名称](https://attachments.tower.im/tower/b6077b88f0ee47879128b36f5aacf82b?filename=8.4+jzy+lld+pcr+of+cysdes+2.JPG)

(lane left to right: marker-Q, pcr-cys-5-1, pcr-cys-5-2, pcr-cys-6-1, pcr-cys-6-2, pcr-cys-7-1, pcr-cys-7-2, pcr-cys-8-1, pcr-cys-8-2)

OD measurement result:

|sample|cysdes PCR pur 1+2+3+4|cysdes PCR pur 5+6+7+8|

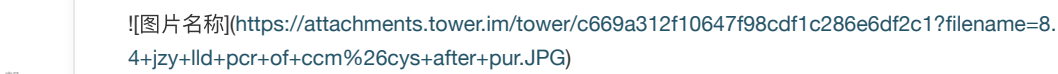
|-|

|Concentration(ng/ul)|28.6|31.8|

|260/280|1.73|1.80|

|260/230|0.25|0.25|

Agarose gel electrophoresis Result:



![图片名称](https://attachments.tower.im/tower/c669a312f10647f98cdf1c286e6df2c1?filename=8.4+jzy+lld+pcr+of+ccm%26cys+after+pur.JPG)

(lane left to right: 2K plus II, 2K plus II, 2K plus II, 2K plus II, 2K plus II, 2K plus II(test), pur-pcr-ccm-1, pur-pcr-ccm-2, pur-pcr-cys-1, pur-pcr-cys-2, Marker Q)

Double digestion of Cys&pluxR

**Recorder: Liudong Luo, Zhenyu Jiang **

Materials:

1. full Cys

2. FastDigest restriction enzyme XbaI, pstIand 10× FastDigest Green Buffer(from Thermo Fisher Scientific)

3. Nuclease-free water

Reaction system:

|Sample|1|2|3|4|

|-|

|XbaI(μL)|1|1|1|1|

|pstI(μL)|1|1|1|1|

|nuclease-free water(μL)|19|19|19|19|

|fastdigest green buffer(μL)|2|2|2|2|

|Cys(μL)|7|7|7|7|

|total(μL)|30|30|30|30|

Reaction system:

|Sample|1|2|3|4|

|-|

|speI(μL)|1|1|1|1|

|pstI(μL)|1|1|1|1|

|nuclease-free water(μL)|20|20|20|20|

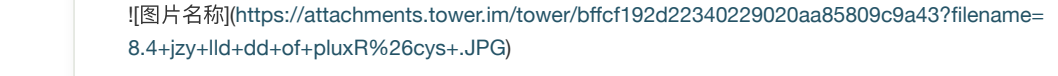
|fastdigest green buffer(μL)|2|2|2|2|

|RED(μL)|1|1|1|1|

|total(μL)|30|30|30|30|

Mix gently and incubate at 37 degree Celsius for 20 hours .

Agarose gel electrophoresis Result:



![图片名称](https://attachments.tower.im/tower/bffcf192d22340229020aa85809c9a43?filename=8.4+jzy+lld+dd+of+pluxR%26cys+.JPG)

(lane 1 to 10: Marker Q ,dd-cys-1,dd-cys-2,dd-cys-3,dd-cys-4,dd-pluxR-1,dd-pluxR-2,dd-pluxR-3,dd-pluxR-4,pluxR)

Then we did the purification of the fragments we got.

The results are as following:

|sample|dd Cys 1+2+3+4|

|-|

|Concentration(ng/ul)|15.5|

|260/280|1.73|

|260/230|0.98|

|sample|dd pluxR 1+2+3+4|

|-|

|Concentration(ng/ul)|74.9|

|260/280|1.75|

|260/230|1.70|

Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/e435656c9c124cf6a9eb5259c3879cf5?filename=8.4+jzy+lld+dd+of+pluxR%26cys+after+pur+.JPG)

(lane 1 to 4: Marker Q ,pur-dd-cys,pur-dd-pluxR,pluxR)

Plasmid Extraction of the Mtr-Pet28

**Recorder: Meiying Cui **

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, 6 and 2 min's standing(We extend the time for sample PYRED2a & 2b and shorten the time for sample PYRED1a & 1b & 1c!).

4.Add 350 μL Buffer P3, mix well.

5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 60 s, discard filtrate.

6.Add 500 μL Buffer DW1, 12000 rpm centifuge 60 s, discard filtrate.

7.Add 500 μL Wash Solution, 12000 rpm centifuge 60 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|10-4-2-1|10-4-2-2|10-4-2-3|10-4-2-4|

|-|

|Concentration(ng/ul)|102.4|62.1|93.3|81.4|

|260/280|188|1.94|1.78|1.86|

|260/230|2.54|2.64|1.60|1.82|

Plasmid Extraction of the pYRED

Recorder: Yu Han

Procedure:

1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice.

2.Add 270 μL Buffer P1, resuspend cells.(Because of the larger amount of supernatant than before, we enlarge the amount of all of the solution we use in the experiment)

帮助

3.Add 270 μL Buffer P2, mix well, 3 min's standing.

4.Add 370 μL Buffer P3, mix well.

5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 60 s, discard filtrate.

6.Add 520 μL Buffer DW1, 12000 rpm centifuge 60 s, discard filtrate.

7.Add 520 μL Wash Solution, 12000 rpm centifuge 60 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|pYRED-1|pYRED-2|pYRED-3|pYRED-4|pYRED-5|pYRED-6|

|-|

|Concentration(ng/ul)|32.8|35.0|36.7|102.5|42.5|24.7|

|260/280|1.94|1.93|1.83|1.64|1.95|1.96|

|260/230|2.25|1.42|0.76|0.78|1.77|2.13|

Transformation of pBAR into BL21

Recorder: Menglong Jin

NOTE:Generally, competent bacteria are restrored in -80 degree centrigrade environment.

1.Take the competent bacteria from refrigerator and incubate them into ice about 5 mins until it is dissolved

2.Absorb 2μl pBAR and mix it wμith 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 μL 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 μL medium.

9.Coat plate: Add 200 μL solution in a plate with Amp.

10.Cultivate these bacteria overnight for further use.

Date 8.4

Plasmid Extraction of the PYRED

Recorder: Menglong Jin, Yawei Wu

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, 6 and 2 min's standing(We extend the time for sample PYRED2a & 2b and shorten the time for sample PYRED1a & 1b & 1c!).

4.Add 350 μL Buffer P3, mix well.

5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 60 s, discard filtrate.

6.Add 500 μL Buffer DW1, 12000 rpm centifuge 60 s, discard filtrate.

7.Add 500 μL Wash Solution, 12000 rpm centifuge 60 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|PYRED1-a|PYRED1-b|PYRED1-c|PYRED2-a|PYRED2-d|

|-|

|Concentration(ng/ul)|99.3|76.5|30.9|32.5|41.6|

|260/280|1.57|1.64|1.98|1.97|1.92|

|260/230|0.72|0.90|2.69|2.42|1.97|

**PCR of full Ccm **

Recorder: Liudong Luo, Zhenyu Jiang

Experimental materials

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

2. Primer: ccm-res-f,ccm-res-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O, 2×PrimeStar(Premix), bought from Takara.

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|9|10|11|12|13|14|15|16|

|-|

|Sterilized ddH₂O|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|

|2×Prime Star(premix)|25 μL|25 μL|25 μL|25 μL|25 μL|25 μL|25 μL|25 μL|25 μL|25 μL|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|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|ccm-res-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|ccm-res-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|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|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|

帮助

2.PCR reaction

1,2,3,4,5,6,7,8 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|10 min|

|step 2|98|10 s|

|step 3|59|5 s|

|step 4|72|7 min|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

9,10,11,12,13,14,15,16 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|10 min|

|step 2|98|10 s|

|step 3|55|15 s|

|step 4|72|7 min|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/9058e4e39d994ec5bcb2522c3767ca51?filename=8.4+jzy+lld+pcr+of+ccm.JPG)

(lane left to right: marker Q, ccm-pcr-1, ccm-pcr-2,ccm-pcr-3,ccm-pcr-4,ccm-pcr-5, ccm-pcr-6, ccm-pcr-7,ccm-pcr-8,ccm-pcr-9, ccm-pcr-10,ccm-pcr-11,ccm-pcr-12,ccm-pcr-13, ccm-pcr-14, ccm-pcr-15,ccm-pcr-16)

Purification of full ccm PCR product

Recorder: Zhenyu Jiang, Liudong Luo

(1). Add 900 μL Buffer B3 to the 180 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 25 μL ddH₂O, stand them still for 10 min. Centrifuge at 12,000 rpm for 1 min.

OD measurement result:

|sample|ccm PCR pur 1+2+3+4+5+6+7+8|ccm PCR pur 9+10+11+112+13+14+15+16|

|-|

帮助

|Concentration(ng/ul)|274.4|289.3|

|260/280|14.85|9.86|

|260/230|13.45|11.77|

maybe something wrong when we did the purification

![图片名称](https://attachments.tower.im/tower/c669a312f10647f98cdf1c286e6df2c1?filename=8.4+jzy+lld+pcr+of+ccm%26cys+after+pur.JPG)

(lane left to right: 2K plus II, 2K plus II, 2K plus II, 2K plus II, 2K plus II, 2K plus II(test), pur-pcr-ccm-1, pur-pcr-ccm-2, pur-pcr-cys-1, pur-pcr-cys-2, Marker Q)

**PCR of full CysDes **

Recorder:Liudong Luo, Zhenyu Jiang

Experimental materials

1. Template: Gene Fragments of CysDes, synthesized by Sangon Biotech;

2. Primer: Cys-Xba-f, Cys-Pst-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O, 2×PrimeStar(Premix), bought from Takara.

Procedure:

1.Prepare 8 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|

|-|

|Sterilized ddH₂O|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|

|2×Prime Star(premix)|25 μL|25 μL|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|1 μL|1 μL|

|Cys-Xba-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Cys-Pst-r|1 μL|1 μL|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|50 μL|50 μL|

2.PCR reaction

1,2,3,4,5,6,7,8 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|5 min|

|step 2|98|5 s|

|step 3|55|5 s|

|step 4|72|1 min 12 sec|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/a8cbe530d5a44cf5b9da1c97d0a48638?filename=8.4+jzy+lld+pcr+of+cysdes+1.JPG)

(lane left to right: marker-Q, pcr-cys-1-1, pcr-cys-1-2, pcr-cys-2-1, pcr-cys-2-2, pcr-cys-3-1, pcr-cys-3-2, pcr-cys-4-1, pcr-cys-4-2)

帮助

(lane left to right: marker-Q, pcr-cys-5-1, pcr-cys-5-2, pcr-cys-6-1, pcr-cys-6-2, pcr-cys-7-1, pcr-cys-7-2, pcr-cys-8-1, pcr-cys-8-2)

OD measurement result:

|sample|cysdes PCR pur 1+2+3+4|cysdes PCR pur 5+6+7+8|

|-|

|Concentration(ng/ul)|28.6|31.8|

|260/280|1.73|1.80|

|260/230|0.25|0.25|

Agarose gel electrophoresis Result:

(lane left to right: 2K plus II, 2K plus II, 2K plus II, 2K plus II, 2K plus II, 2K plus II(test), pur-pcr-ccm-1, pur-pcr-ccm-2, pur-pcr-cys-1, pur-pcr-cys-2, Marker Q)

Double digestion of Cys&pluxR

**Recorder: Liudong Luo, Zhenyu Jiang **

Materials:

1. full Cys

2. FastDigest restriction enzyme XbaI, pstIand 10× FastDigest Green Buffer(from Thermo Fisher Scientific)

3. Nuclease-free water

Reaction system:

|Sample|1|2|3|4|

|-|

|XbaI(μL)|1|1|1|1|

|pstI(μL)|1|1|1|1|

|nuclease-free water(μL)|19|19|19|19|

|fastdigest green buffer(μL)|2|2|2|2|

|Cys(μL)|7|7|7|7|

|total(μL)|30|30|30|30|

Reaction system:

|Sample|1|2|3|4|

|-|

|speI(μL)|1|1|1|1|

|pstI(μL)|1|1|1|1|

|nuclease-free water(μL)|20|20|20|20|

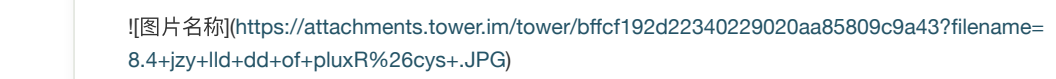
|fastdigest green buffer(μL)|2|2|2|2|

|RED(μL)|1|1|1|1|

|total(μL)|30|30|30|30|

Mix gently and incubate at 37 degree Celsius for 20 hours .

Agarose gel electrophoresis Result:



(lane 1 to 10: Marker Q ,dd-cys-1,dd-cys-2,dd-cys-3,dd-cys-4,dd-pluxR-1,dd-pluxR-2,dd-pluxR-3,dd-pluxR-4,pluxR)

Then we did the purification of the fragments we got.

The results are as following:

|sample|dd Cys 1+2+3+4|

|-|

|Concentration(ng/ul)|15.5|

|260/280|1.73|

|260/230|0.98|

|sample|dd pluxR 1+2+3+4|

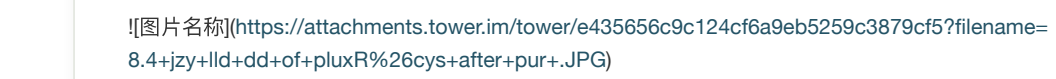
|-|

|Concentration(ng/ul)|74.9|

|260/280|1.75|

|260/230|1.70|

Agarose gel electrophoresis Result:



(lane 1 to 4: Marker Q ,pur-dd-cys,pur-dd-pluxR,pluxR)

Plasmid Extraction of the Mtr-Pet28

**Recorder: Meiying Cui **

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, 6 and 2 min's standing(We extend the time for sample PYRED2a & 2b and shorten the time for sample PYRED1a & 1b & 1c!).

4.Add 350 μL Buffer P3, mix well.

5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 60 s, discard filtrate.

6.Add 500 μL Buffer DW1, 12000 rpm centifuge 60 s, discard filtrate.

7.Add 500 μL Wash Solution, 12000 rpm centifuge 60 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|10-4-2-1|10-4-2-2|10-4-2-3|10-4-2-4|

帮助

|-|

|Concentration(ng/ul)|102.4|62.1|93.3|81.4|

|260/280|188|1.94|1.78|1.86|

|260/230|2.54|2.64|1.60|1.82|

Plasmid Extraction of the pYRED

Recorder: Yu Han

Procedure:

1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice.

2.Add 270 μL Buffer P1, resuspend cells.(Because of the larger amount of supernatant than before, we enlarge the amount of all of the solution we use in the experiment)

3.Add 270 μL Buffer P2, mix well, 3 min's standing.

4.Add 370 μL Buffer P3, mix well.

5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 60 s, discard filtrate.

6.Add 520 μL Buffer DW1, 12000 rpm centifuge 60 s, discard filtrate.

7.Add 520 μL Wash Solution, 12000 rpm centifuge 60 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|pYRED-1|pYRED-2|pYRED-3|pYRED-4|pYRED-5|pYRED-6|

|-|

|Concentration(ng/ul)|32.8|35.0|36.7|102.5|42.5|24.7|

|260/280|1.94|1.93|1.83|1.64|1.95|1.96|

|260/230|2.25|1.42|0.76|0.78|1.77|2.13|

Transformation of pBAR into BL21

Recorder: Menglong Jin

NOTE:Generally, competent bacteria are restrored in -80 degree centrigrade environment.

1.Take the competent bacteria from refrigerator and incubate them into ice about 5 mins until it is dissolved

2.Absorb 2μl pBAR and mix it wμith 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 μL 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 μL medium.

9.Coat plate: Add 200 μL solution in a plate with Amp.

帮助

10.Cultivate these bacteria overnight for further use.

Date 8.5

SDS-PAGE of cell lysate

Materials:

1. BL21(DE3) induced to express Mtr CAB.

2. Many different solutions.

Procedure:

1.

2.

3.

Results:

![图片名称](https://attachments.tower.im/tower/b22e143c517145f4bc44728af128667f?version=large&filename=8.5+mtrSDS+1.Tif)

Date 8.5

SDS-PAGE of cell lysate

Materials:

1. BL21(DE3) induced to express Mtr CAB.

2. Many different solutions.

Procedure:

1. Cultivate the induced bacteria overnight;

2. Centrifuge at 8000 rpm, 4°C, for 10 min, then remove the supernatant；

3. Resuspend the bacteria with 50 mL TBS;

4. Centrifuge at 8000 rpm, 4°C, for 10 min, then remove the supernatant;

5. Resuspend the bacteria with 15 mL lysis buffer;

6. The cells were disrupted via ultrasonication (Power 30%, 30 min. Total duty in

cycles of 1s on, 2s off);

7. Centrifuge at 14000 rpm, 4°C, for 20 min;

8. Retrieve the supernatant for SDS-PAGE.

Results:

![图片名称](https://attachments.tower.im/tower/b22e143c517145f4bc44728af128667f?version=large&filename=8.5+mtrSDS+1.Tif)

![图片名称](https://attachments.tower.im/tower/ddac146fd9cd4e25b1aec6d99962d0a1?filename=8.6+SDS+of+mtr.JPG)

(Lane 1 to 4: WT,-IPTG, +1mM IPTG, marker)

After heat treatment:

![图片名称](https://attachments.tower.im/tower/2aa5f6090fa046738ea630e5d77b0489?filename=8.6+SDS+of+mtr+after+heat+treatment.JPG)

帮助

Date 8.6

SDS-PAGE of cell lysate

Materials:

1. BL21(DE3) induced to express Mtr CAB.

2. Many different solutions.

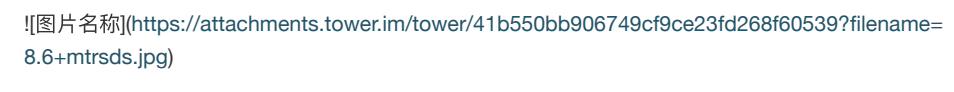
Procedure:

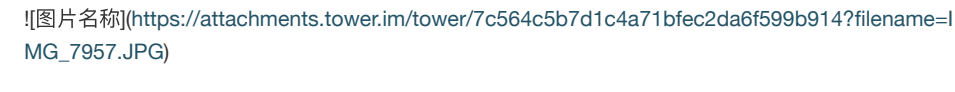
1.

2.

3.

Results:





Plasmid Extraction of the pBAR

Recorder: 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 60 s, discard filtrate.

6.Add 500 μL Buffer DW1, 12000 rpm centifuge 60 s, discard filtrate.

7.Add 500 μL Wash Solution, 12000 rpm centifuge 60 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|pBAR1|pBAR2|pBAR3|pBAR4|pBAR5|pBAR6|

|-|

|Concentration(ng/ul)|127.4|117.5|139.4|140.8|146.8|119.0|

|260/280|1.85|1.79|1.87|1.85|1.83|1.86|

|260/230|2.23|1.54|2.03|1.89|1.77|2.12|

Plasmid Extraction of the Cys+pluxR

Recorder: Liudong Luo, Zhenyu Jiang

Procedure:

帮助

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, 6 and 2 min's standing(We extend the time for sample PYRED2a & 2b and shorten the time for sample PYRED1a & 1b & 1c!).

4.Add 350 μL Buffer P3, mix well.

5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 60 s, discard filtrate.

6.Add 500 μL Buffer DW1, 12000 rpm centifuge 60 s, discard filtrate.

7.Add 500 μL Wash Solution, 12000 rpm centifuge 60 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+pluxR 1|Cys+pluxR 2|Cys+pluxR 3|Cys+pluxR 4|Cys+pluxR 5|Cys+pluxR 6|Cys+pluxR 7|Cys+pluxR 8|

|-|

|Concentration(ng/ul)|42.6|87.4|105.6|97.7|57.4|60.3|34.8|65.7|

|260/280|1.66|1.87|1.71|1.77|1.72|1.87|1.93|1.67|

|260/230|0.90|2.59|1.18|1.47|1.18|1.89|2.11|1.03|

Double digestion of Cys+pluxR & pluxR

**Recorder: Liudong Luo, Zhenyu Jiang **

Materials:

1. Cys+pluxR

2. FastDigest restriction enzyme EcoI, pstI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)

3. Nuclease-free water

Reaction system:

|Sample|1|2|3|4|5|6|7|8|9|10|11|12|13|14|15|16|

|-|

|EcoI(μL)|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|

|pstI(μL)|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|

|nuclease-free water(μL)|7|7|7|7|7|7|7|7|7|7|7|7|7|7|7|7|

|fastdigest green buffer(μL)|2|2|2|2|2|2|2|2|2|2|2|2|2|2|2|2|

|Cys+pluxR(μL)|10|10|10|10|10|10|10|10|10|10|10|10|10|10|10|10|

|total(μL)|20|20|20|20|20|20|20|20|20|20|20|20|20|20|20|20|

Reaction system:

|Sample|1|2|3|4|

|-|

|spel(μL)|1|1|1|1|

|pstI(μL)|1|1|1|1|

|nuclease-free water(μL)|20|20|20|20|

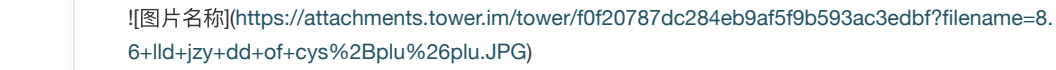
|fastdigest green buffer(μL)|2|2|2|2|

|RED(μL)|1|1|1|1|

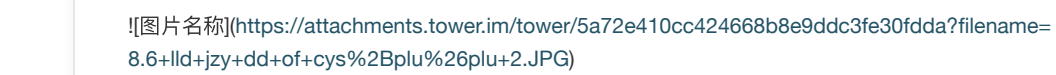
|total(μL)|30|30|30|30|

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

Agarose gel electrophoresis Result:



(lane 1 to 15: 2K plusII ,dd-cys+pluxR-1-1,dd-cys+pluxR-1-2,dd-cys+pluxR-2-1,dd-cys+pluxR-2-2,dd-cys+pluxR-3-1,dd-cys+pluxR-3-2,dd-cys+pluxR-4-1,dd-cys+pluxR-4-2,dd-pluxR,cys+pluxR-1,cys+pluxR-2,cys+pluxR-3,cys+pluxR-4,pluxR)



(lane 1 to 15: 2K plusII ,dd-cys+pluxR-5-1,dd-cys+pluxR-5-2,dd-cys+pluxR-6-1,dd-cys+pluxR-6-2,dd-cys+pluxR-7-1,dd-cys+pluxR-7-2,dd-cys+pluxR-8-1,dd-cys+pluxR-8-2,dd-pluxR,cys+pluxR-5,cys+pluxR-6,cys+pluxR-7,cys+pluxR-8,pluxR)

Date 8.6

SDS-PAGE of cell lysate

Materials:

1. BL21(DE3) induced to express Mtr CAB.

2. Many different solutions.

Procedure:

1. Cultivate the induced bacteria overnight;

2. Centrifuge at 8000 rpm, 4°C, for 10 min, then remove the supernatant；

3. Resuspend the bacteria with 50 mL TBS;

4. Centrifuge at 8000 rpm, 4°C, for 10 min, then remove the supernatant;

5. Resuspend the bacteria with 15 mL lysis buffer;

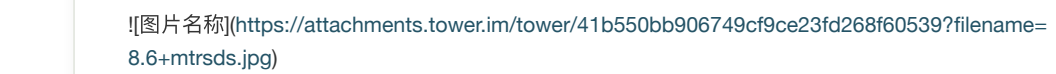
6. The cells were disrupted via ultrasonication (Power 30%, 30 min. Total duty in

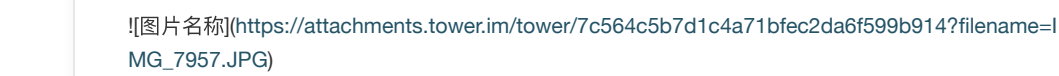
cycles of 1s on, 2s off);

7. Centrifuge at 14000 rpm, 4°C, for 20 min;

8. Retrieve the supernatant for SDS-PAGE.

Results:





Plasmid Extraction of the pBAR

Recorder: Menglong Jin

Procedure:

帮助

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 60 s, discard filtrate.

6.Add 500 μL Buffer DW1, 12000 rpm centifuge 60 s, discard filtrate.

7.Add 500 μL Wash Solution, 12000 rpm centifuge 60 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|pBAR1|pBAR2|pBAR3|pBAR4|pBAR5|pBAR6|

|-|

|Concentration(ng/ul)|127.4|117.5|139.4|140.8|146.8|119.0|

|260/280|1.85|1.79|1.87|1.85|1.83|1.86|

|260/230|2.23|1.54|2.03|1.89|1.77|2.12|

Plasmid Extraction of the Cys+pluxR

Recorder: Liudong Luo, Zhenyu Jiang

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, 6 and 2 min's standing(We extend the time for sample PYRED2a & 2b and shorten the time for sample PYRED1a & 1b & 1c!).

4.Add 350 μL Buffer P3, mix well.

5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 60 s, discard filtrate.

6.Add 500 μL Buffer DW1, 12000 rpm centifuge 60 s, discard filtrate.

7.Add 500 μL Wash Solution, 12000 rpm centifuge 60 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+pluxR 1|Cys+pluxR 2|Cys+pluxR 3|Cys+pluxR 4|Cys+pluxR 5|Cys+pluxR 6|Cys+pluxR 7|Cys+pluxR 8|

|-|

帮助

|Concentration(ng/ul)|42.6|87.4|105.6|97.7|57.4|60.3|34.8|65.7|

|260/280|1.66|1.87|1.71|1.77|1.72|1.87|1.93|1.67|

|260/230|0.90|2.59|1.18|1.47|1.18|1.89|2.11|1.03|

Double digestion of Cys+pluxR & pluxR

**Recorder: Liudong Luo, Zhenyu Jiang **

Materials:

1. Cys+pluxR

2. FastDigest restriction enzyme EcoI, pstI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)

3. Nuclease-free water

Reaction system:

|Sample|1|2|3|4|5|6|7|8|9|10|11|12|13|14|15|16|

|-|

|EcoI(μL)|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|

|pstI(μL)|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|0.5|

|nuclease-free water(μL)|7|7|7|7|7|7|7|7|7|7|7|7|7|7|7|7|

|fastdigest green buffer(μL)|2|2|2|2|2|2|2|2|2|2|2|2|2|2|2|2|

|Cys+pluxR(μL)|10|10|10|10|10|10|10|10|10|10|10|10|10|10|10|10|

|total(μL)|20|20|20|20|20|20|20|20|20|20|20|20|20|20|20|20|

Reaction system:

|Sample|1|2|3|4|

|-|

|speI(μL)|1|1|1|1|

|pstI(μL)|1|1|1|1|

|nuclease-free water(μL)|20|20|20|20|

|fastdigest green buffer(μL)|2|2|2|2|

|RED(μL)|1|1|1|1|

|total(μL)|30|30|30|30|

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

Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/f0f20787dc284eb9af5f9b593ac3edbf?filename=8.6+lld+jzy+dd+of+cys%2Bplu%26plu.JPG)

(lane 1 to 15: 2K plusII ,dd-cys+pluxR-1-1,dd-cys+pluxR-1-2,dd-cys+pluxR-2-1,dd-cys+pluxR-2-2,dd-cys+pluxR-3-1,dd-cys+pluxR-3-2,dd-cys+pluxR-4-1,dd-cys+pluxR-4-2,dd-pluxR,cys+pluxR-1,cys+pluxR-2,cys+pluxR-3,cys+pluxR-4,pluxR)

![图片名称](https://attachments.tower.im/tower/5a72e410cc424668b8e9ddc3fe30fdda?filename=8.6+lld+jzy+dd+of+cys%2Bplu%26plu+2.JPG)

(lane 1 to 15: 2K plusII ,dd-cys+pluxR-5-1,dd-cys+pluxR-5-2,dd-cys+pluxR-6-1,dd-cys+pluxR-6-2,dd-cys+pluxR-7-1,dd-cys+pluxR-7-2,dd-cys+pluxR-8-1,dd-cys+pluxR-8-2,dd-pluxR,cys+pluxR-5,cys+pluxR-6,cys+pluxR-7,cys+pluxR-8,pluxR)

Periplasmic Fractionation

Recorder: Zhu Shihan, Yu Wenfei & Liang Yonghao

Procedure:

1. Cultivate cells in a 100 mL culture overnight;

2. 4000 rcf, 15 mins, 4°C；

3. Slowly resuspend the resulting cell pellet with 3 mL of ice-cold Tris-HCl, pH 8.0;

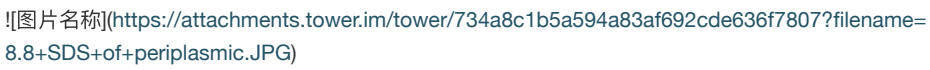
4. Add chicken egg white lysozyme to a final concentration of 0.5 mg/mL (3mL) and incubate at RT for 15 mins;

5. Add 3 mL of ice-cold water, shake the suspension horizontally on ice for 15 mins at 100 rpm;

6. 12000 rcf, 20 mins, 4°C；

7. Get the supernatant for future experiments.

Result:



(Lane 1 to 6: WT, -IPTG, 0.01 mM IPTG, 0.1 mM IPTG, 1 mM IPTG, Marker)

Date 8.7

Plasmid Extraction of the PYRED

Recorder: Ziyi 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 60 s, discard filtrate.

6.Add 500 μL Buffer DW1, 12000 rpm centifuge 60 s, discard filtrate.

7.Add 500 μL Wash Solution, 12000 rpm centifuge 60 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|PYRED1|PYRED2|PYRED3|PYRED4|

|-|

|Concentration(ng/ul)|36.7|42.7|50.0|43.8|

|260/280|1.92|1.89|1.83|1.88|

|260/230|1.62|1.61|1.39|1.49|

Plasmid Extraction of the plcys

Recorder: Liudong Luo, Zhenyu Jiang

Procedure:

帮助

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 60 s, discard filtrate.

6.Add 500 μL Buffer DW1, 12000 rpm centifuge 60 s, discard filtrate.

7.Add 500 μL Wash Solution, 12000 rpm centifuge 60 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|plcys 1|plcys 2|plcys 5|plcys 7|plcys 8|

|-|

|Concentration(ng/ul)|506.4|443.4|534.0|508.5|566.6|

|260/280|1.78|1.88|1.85|1.86|1.80|

|260/230|1.96|2.32|2.01|2.12|1.93|

Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/d2a5496fe1b84abca8aaa6fa98d7b2c8?filename=8.7+lld+jzy+plcys.JPG)

(lane from left to right: Mark-Q, plcys-1, plcys-2, plcys-3,plcys-4, plcys-5, pluxR)

Plasmid Extraction of the plcys

Recorder: Liudong Luo

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 60 s, discard filtrate.

6.Add 500 μL Buffer DW1, 12000 rpm centifuge 60 s, discard filtrate.

7.Add 500 μL Wash Solution, 12000 rpm centifuge 60 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|plcys 1|plcys 2|plcys 5|plcys 7|plcys 8|

|-|

|Concentration(ng/ul)|193.7|258.4|204.2|166.0|232.9|

|260/280|1.82|1.75|1.77|1.84|1.81|

|260/230|1.69|1.10|1.81|1.89|1.65|

** Bac PCR of RED in DAP **

Recorder: Menglong Jin

Experimental materials

1. Template: PYRED in DAP Defective Escherichia coli;

2. Primer: RED-f, RED-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O, 2×PrimeStar(Premix), bought from Takara.

Procedure:

1.Prepare 8 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7μL|7 μL|7 μL|7 μL|7 μL|

|2×Prime Star(premix)|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|RED-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|RED-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

2.PCR reaction

1,2,3,4,5,6,7,8 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|10 min|

|step 2|98|5 s|

|step 3|55|5 s|

|step 4|72|1 min|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/649a3c75bff74e98a5ed159de0fbf654?filename=8.7+jml+pcr+of+RED.JPG)

(From left to right: marker 2K plusII; sample 1-8; PYRED. As you can see, there is something in sample 3, which we may get what we want.)

**PCR of full Ccm **

帮助

Recorder: Liudong Luo, Zhenyu Jiang

Experimental materials

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

2. Primer: ccm-res-f,ccm-res-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O, 2×PrimeStar(Premix), bought from Takara.

Procedure:

1.Prepare 10 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|9|10|

|-|

|Sterilized ddH₂O|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|

|2×Prime Star(premix)|25 μL|25 μL|25 μL|25 μL|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|1 μL|1 μL|1 μL|1 μL|

|ccm-res-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|ccm-res-r|1 μL|1 μL|1 μL|1 μL|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|50 μL|50 μL|50 μL|50 μL|

2.PCR reaction

1,3，5，7，9 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|10 min|

|step 2|98|10 s|

|step 3|59|5 s|

|step 4|72|7 min|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

2，4，6，8，10 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|10 min|

|step 2|98|10 s|

|step 3|55|15 s|

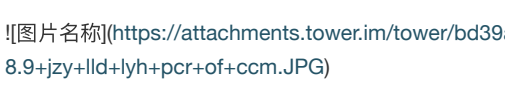
|step 4|72|7 min|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:



(lane left to right: marker Q, ccm-pcr-1, ccm-pcr-2,ccm-pcr-3,ccm-pcr-4,ccm-pcr-5, ccm-pcr-6, ccm-pcr-7,ccm-pcr-8,ccm-pcr-9,ccm-pcr-10,ccm)

Purification of full ccm PCR product

Recorder: Zhenyu Jiang, Liudong Luo

(1). Add 900 μL Buffer B3 to the 180 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 25 μL ddH₂O, stand them still for 10 min. Centrifuge at 12,000 rpm for 1 min.

OD measurement result:

|sample|ccm PCR pur 1+4+8+9+10|

|-|

|Concentration(ng/ul)|208.8|

|260/280|1.83|

|260/230|2.08|

3.Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/77ef830671e34384a6c5634e28b829cd?filename=8.9+jzy+lld+lyh+pcr+of+ccm+afterpur.JPG)

(lane: marker Q, pur-pcr-ccm)

Double digestion of ccm

Recorder: Liudong Luo, Zhenyu Jiang

Materials:

1. full ccm

|sample|ccm PCR pur 1+4+8+9+10|

|-|

|Concentration(ng/ul)|208.8|

|260/280|1.83|

|260/230|2.08|

2. FastDigest restriction enzyme XbaI, pstIand 10× FastDigest Green Buffer(from Thermo Fisher Scientific)

3. Nuclease-free water

Reaction system:

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

|-|

|XbaI(μL)|1|1|1|1|1|1|1|1|

|pstI(μL)|1|1|1|1|1|1|1|1|

|nuclease-free water(μL)|24|24|24|24|24|24|24|24|

|fastdigest green buffer(μL)|2|2|2|2|2|2|2|2|

|ccm(μL)|2|2|2|2|2|2|2|2|

|total(μL)|30|30|30|30|30|30|30|30|

Mix gently and incubate at 37 degree Celsius for 3 hours .

帮助

Double digestion of pTB

Recorder: Menglong Jin

Materials:

1. pTB

|sample|pTB|

|-|

|Concentration(ng/ul)|243.2|

|260/280|1.85|

|260/230|1.57|

2. FastDigest restriction enzyme bcuI, pstI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)

3. Nuclease-free water

Reaction system:

|Sample|1|2|3|4|

|-|

|BcuI(μL)|1|1|1|1|

|pstI(μL)|1|1|1|1|

|nuclease-free water(μL)|11|11|11|11|

|fastdigest green buffer(μL)|2|2|2|2|

|pTB(μL)|4|4|4|4|

|total(μL)|20|20|20|20|

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

then we do the purification.

OD measurement result:

|sample|dd ccm |dd pTB|

|-|

|Concentration(ng/ul)|34.5|81.8|

|260/280|1.80|1.79|

|260/230|1.16|1.75|

3.Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/22d58cf2c43f4785a5d0afae84f8dd26?filename=8.9+jzy+pcr+ccm+%26+dd+ccm+%26+dd+pTB+%26+pTB.JPG)

(lane: marker Q, genome 1 ,genome 2 ,genome 3 ,genome 4 ,dd ccm , dd pTB, pTB, 2K plus II)

Date 8.7

Plasmid Extraction of the PYRED

Recorder: Ziyi Wang

帮助

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 60 s, discard filtrate.

6.Add 500 μL Buffer DW1, 12000 rpm centifuge 60 s, discard filtrate.

7.Add 500 μL Wash Solution, 12000 rpm centifuge 60 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|PYRED1|PYRED2|PYRED3|PYRED4|

|-|

|Concentration(ng/ul)|36.7|42.7|50.0|43.8|

|260/280|1.92|1.89|1.83|1.88|

|260/230|1.62|1.61|1.39|1.49|

Plasmid Extraction of the plcys

Recorder: Liudong Luo, Zhenyu Jiang

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 60 s, discard filtrate.

6.Add 500 μL Buffer DW1, 12000 rpm centifuge 60 s, discard filtrate.

7.Add 500 μL Wash Solution, 12000 rpm centifuge 60 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|plcys 1|plcys 2|plcys 5|plcys 7|plcys 8|

|-|

|Concentration(ng/ul)|506.4|443.4|534.0|508.5|566.6|

|260/280|1.78|1.88|1.85|1.86|1.80|

|260/230|1.96|2.32|2.01|2.12|1.93|

Agarose gel electrophoresis Result:

(lane from left to right: Mark-Q, plcys-1, plcys-2, plcys-3,plcys-4, plcys-5, pluxR)

Plasmid Extraction of the plcys

Recorder: Liudong Luo

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 60 s, discard filtrate.

6.Add 500 μL Buffer DW1, 12000 rpm centifuge 60 s, discard filtrate.

7.Add 500 μL Wash Solution, 12000 rpm centifuge 60 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|plcys 1|plcys 2|plcys 5|plcys 7|plcys 8|

|-|

|Concentration(ng/ul)|193.7|258.4|204.2|166.0|232.9|

|260/280|1.82|1.75|1.77|1.84|1.81|

|260/230|1.69|1.10|1.81|1.89|1.65|

** Bac PCR of RED in DAP **

Recorder: Menglong Jin

Experimental materials

1. Template: PYRED in DAP Defective Escherichia coli;

2. Primer: RED-f, RED-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O, 2×PrimeStar(Premix), bought from Takara.

Procedure:

1.Prepare 8 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|

|-|

帮助

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7μL|7 μL|7 μL|7 μL|

|2×Prime Star(premix)|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|RED-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|RED-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

2.PCR reaction

1,2,3,4,5,6,7,8 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|10 min|

|step 2|98|5 s|

|step 3|55|5 s|

|step 4|72|1 min|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/649a3c75bff74e98a5ed159de0fbf654?filename=8.7+jml+pcr+of+RED.JPG)

(From left to right: marker 2K plusII; sample 1-8; PYRED. As you can see, there is something in sample 3, which we may get what we want.)

**PCR of full Ccm **

Recorder: Liudong Luo, Zhenyu Jiang

Experimental materials

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

2. Primer: ccm-res-f,ccm-res-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O, 2×PrimeStar(Premix), bought from Takara.

Procedure:

1.Prepare 10 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|9|10|

|-|

|Sterilized ddH₂O|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|

|2×Prime Star(premix)|25 μL|25 μL|25 μL|25 μL|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|1 μL|1 μL|1 μL|1 μL|

|ccm-res-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|ccm-res-r|1 μL|1 μL|1 μL|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|50 μL|50 μL|50 μL|50 μL|

2.PCR reaction

1,3，5，7，9 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|10 min|

|step 2|98|10 s|

|step 3|59|5 s|

|step 4|72|7 min|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

2，4，6，8，10 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|10 min|

|step 2|98|10 s|

|step 3|55|15 s|

|step 4|72|7 min|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/bd39a2ead6334b109d82ba3e63cf6cf4?filename=8.9+jzy+lld+lyh+pcr+of+ccm.JPG)

(lane left to right: marker Q, ccm-pcr-1, ccm-pcr-2,ccm-pcr-3,ccm-pcr-4,ccm-pcr-5, ccm-pcr-6, ccm-pcr-7,ccm-pcr-8,ccm-pcr-9,ccm-pcr-10,ccm)

Purification of full ccm PCR product

Recorder: Zhenyu Jiang, Liudong Luo

(1). Add 900 μL Buffer B3 to the 180 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 25 μL ddH₂O, stand them still for 10 min. Centrifuge at 12,000 rpm for 1 min.

OD measurement result:

|sample|ccm PCR pur 1+4+8+9+10|

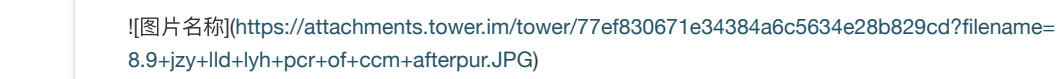
|-|

|Concentration(ng/ul)|208.8|

|260/280|1.83|

|260/230|2.08|

3.Agarose gel electrophoresis Result:



(lane: marker Q, pur-pcr-ccm)

Double digestion of ccm

Recorder: Liudong Luo, Zhenyu Jiang

Materials:

1. full ccm

|sample|ccm PCR pur 1+4+8+9+10|

|-|

|Concentration(ng/ul)|208.8|

|260/280|1.83|

|260/230|2.08|

2. FastDigest restriction enzyme XbaI, pstIand 10× FastDigest Green Buffer(from Thermo Fisher Scientific)

3. Nuclease-free water

Reaction system:

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

|-|

|XbaI(μL)|1|1|1|1|1|1|1|1|

|pstI(μL)|1|1|1|1|1|1|1|1|

|nuclease-free water(μL)|24|24|24|24|24|24|24|24|

|fastdigest green buffer(μL)|2|2|2|2|2|2|2|2|

|ccm(μL)|2|2|2|2|2|2|2|2|

|total(μL)|30|30|30|30|30|30|30|30|

Mix gently and incubate at 37 degree Celsius for 3 hours .

Double digestion of pTB

Recorder: Menglong Jin

Materials:

1. pTB

|sample|pTB|

|-|

|Concentration(ng/ul)|243.2|

|260/280|1.85|

|260/230|1.57|

2. FastDigest restriction enzyme bcuI, pstI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)

3. Nuclease-free water

Reaction system:

|Sample|1|2|3|4|

帮助

|-|

|BcuI(μL)|1|1|1|1|

|pstI(μL)|1|1|1|1|

|nuclease-free water(μL)|11|11|11|11|

|fastdigest green buffer(μL)|2|2|2|2|

|pTB(μL)|4|4|4|4|

|total(μL)|20|20|20|20|

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

then we do the purification.

OD measurement result:

|sample|dd ccm |dd pTB|

|-|

|Concentration(ng/ul)|34.5|81.8|

|260/280|1.80|1.79|

|260/230|1.16|1.75|

3.Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/22d58cf2c43f4785a5d0afae84f8dd26?filename=8.9+jzy+pcr+ccm+%26+dd+ccm+%26+dd+pTB+%26+pTB.JPG)

(lane: marker Q, genome 1 ,genome 2 ,genome 3 ,genome 4 ,dd ccm , dd pTB, pTB, 2K plus II)

Date 8.10

**Bac PCR of full RED of Shewanella **

Recorder: Menglong Jin, Yawei Wu

Experimental materials

1. Template: PYRED in Shewanella;

2. Primer: RED-f, RED-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O, 2×Taq.

Procedure:

1.Prepare 8 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2×Taq|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|RED-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|RED-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

2.PCR reaction

|stage|temperature|time|

|-|

|step 1|94|10 min|

帮助

|step 2|94|30 s|

|step 3|67|30 s|

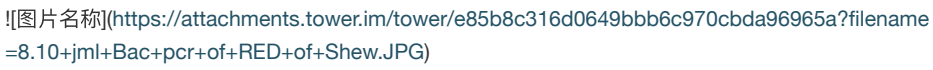
|step 4|72|50 s|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:



(From left to right: marker 2K plusII; sample 1-8; pcr of RED)

As you can see, we did not get anything from the PCR.

**PCR of full Ccm **

Recorder: Liudong Luo

Experimental materials

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

2. Primer: ccm-res-f,ccm-res-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O, 2×PrimeStar(Premix), bought from Takara.

Procedure:

1.Prepare 8 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|

|-|

|Sterilized ddH₂O|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|

|2×Prime Star(premix)|25 μL|25 μL|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|1 μL|1 μL|

|ccm-res-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|ccm-res-r|1 μL|1 μL|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|50 μL|50 μL|

2.PCR reaction

1,3，5，7 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|10 min|

|step 2|98|10 s|

|step 3|59|5 s|

|step 4|72|7 min|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

2，4，6，8 Parameters setting：

|stage|temperature|time|

帮助

|-|

|step 1|95|10 min|

|step 2|98|10 s|

|step 3|55|15 s|

|step 4|72|7 min|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/84ae504b4f164aebb1d6b23394936397?filename=8.11+lld+pcr+of+ccm.JPG)

(lane left to right: marker Q, ccm-pcr-1, ccm-pcr-2,ccm-pcr-3,ccm-pcr-4,ccm-pcr-5, ccm-pcr-6, ccm-pcr-7,ccm-pcr-8,ccm)

Purification of full ccm PCR product

Recorder: Liudong Luo

(1). Add 675 μL Buffer B3 to the 135 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 25 μL ddH₂O, stand them still for 10 min. Centrifuge at 12,000 rpm for 1 min.

OD measurement result:

|sample|ccm PCR pur 1+3+5|

|-|

|Concentration(ng/ul)|177.9|

|260/280|1.83|

|260/230|2.14|

3.Agarose gel electrophoresis Result:

![图片名称](未跑)

(lane:)

Date 8.10

**Bac PCR of full RED of Shewanella **

Recorder: Menglong Jin, Yawei Wu

Experimental materials

1. Template: PYRED in Shewanella;

2. Primer: RED-f, RED-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O, 2×Taq.

Procedure:

1.Prepare 8 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2×Taq|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|RED-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|RED-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

2.PCR reaction

|stage|temperature|time|

|-|

|step 1|94|10 min|

|step 2|94|30 s|

|step 3|67|30 s|

|step 4|72|50 s|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/e85b8c316d0649bbb6c970cbda96965a?filename=8.10+jml+Bac+pcr+of+RED+of+Shew.JPG)

(From left to right: marker 2K plusII; sample 1-8; pcr of RED)

As you can see, we did not get anything from the PCR.

**PCR of full Ccm **

Recorder: Liudong Luo

Experimental materials

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

2. Primer: ccm-res-f,ccm-res-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O, 2×PrimeStar(Premix), bought from Takara.

Procedure:

1.Prepare 8 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|

|-|

|Sterilized ddH₂O|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|

|2×Prime Star(premix)|25 μL|25 μL|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|1 μL|1 μL|

|ccm-res-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|ccm-res-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

帮助

|total|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|50 μL|

2.PCR reaction

1,3，5，7 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|10 min|

|step 2|98|10 s|

|step 3|59|5 s|

|step 4|72|7 min|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

2，4，6，8 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|10 min|

|step 2|98|10 s|

|step 3|55|15 s|

|step 4|72|7 min|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/84ae504b4f164aebb1d6b23394936397?filename=8.11+lld+pcr+of+ccm.JPG)

(lane left to right: marker Q, ccm-pcr-1, ccm-pcr-2,ccm-pcr-3,ccm-pcr-4,ccm-pcr-5, ccm-pcr-6, ccm-pcr-7,ccm-pcr-8,ccm)

Purification of full ccm PCR product

Recorder: Liudong Luo

(1). Add 675 μL Buffer B3 to the 135 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 25 μL ddH₂O, stand them still for 10 min. Centrifuge at 12,000 rpm for 1 min.

OD measurement result:

|sample|ccm PCR pur 1+3+5|

|-|

帮助

|Concentration(ng/ul)|177.9|

|260/280|1.83|

|260/230|2.14|

3.Agarose gel electrophoresis Result:

![图片名称](未跑)

(lane:)

Date 8.13

Joint transformation of Shewanella and E.coli

Recorder: Menglong Jin

1. Cultivate Shewanella and E.coli(deficiency type) overnight.

2. Add 500μl Shewanella and 500μl E.coli into an EP tube. Centrifuge it at 12000 rpm for 3 minutes.

3. Remove the supernatant. Add 1000μl ddH2O, resuspend cells. Centrifuge it at 12000 rpm for 3 minutes. Remove the supernatant.

4. Add 1000μl ddH2O, resuspend cells. Add 200μl solution in a plate with DAP.

5. Cultivate these bacteria overnight.

6. Scrape these bacteria down and use 1000μl ddH2O to resuspend them in a new EP tube.

7. Add 200μl solution in a plate with kanamycin.

8. Cultivate these bacteria overnight for further use.

Results:

![图片名称](https://attachments.tower.im/tower/d02819203acb4b6db25160847432c94e?filename=IMG20170814145256.jpg)

![图片名称](https://attachments.tower.im/tower/d02dde148e8246aaa117a734b04932f6?filename=IMG20170814145446.jpg)

Date 8.13

Joint transformation of Shewanella and E.coli

Recorder: Menglong Jin

1. Cultivate Shewanella and E.coli(deficiency type) overnight.

2. Add 500μl Shewanella and 500μl E.coli into an EP tube. Centrifuge it at 12000 rpm for 3 minutes.

3. Remove the supernatant. Add 1000μl ddH2O, resuspend cells. Centrifuge it at 12000 rpm for 3 minutes. Remove the supernatant.

4. Add 1000μl ddH2O, resuspend cells. Add 200μl solution in a plate with DAP.

5. Cultivate these bacteria overnight.

6. Scrape these bacteria down and use 1000μl ddH2O to resuspend them in a new EP tube.

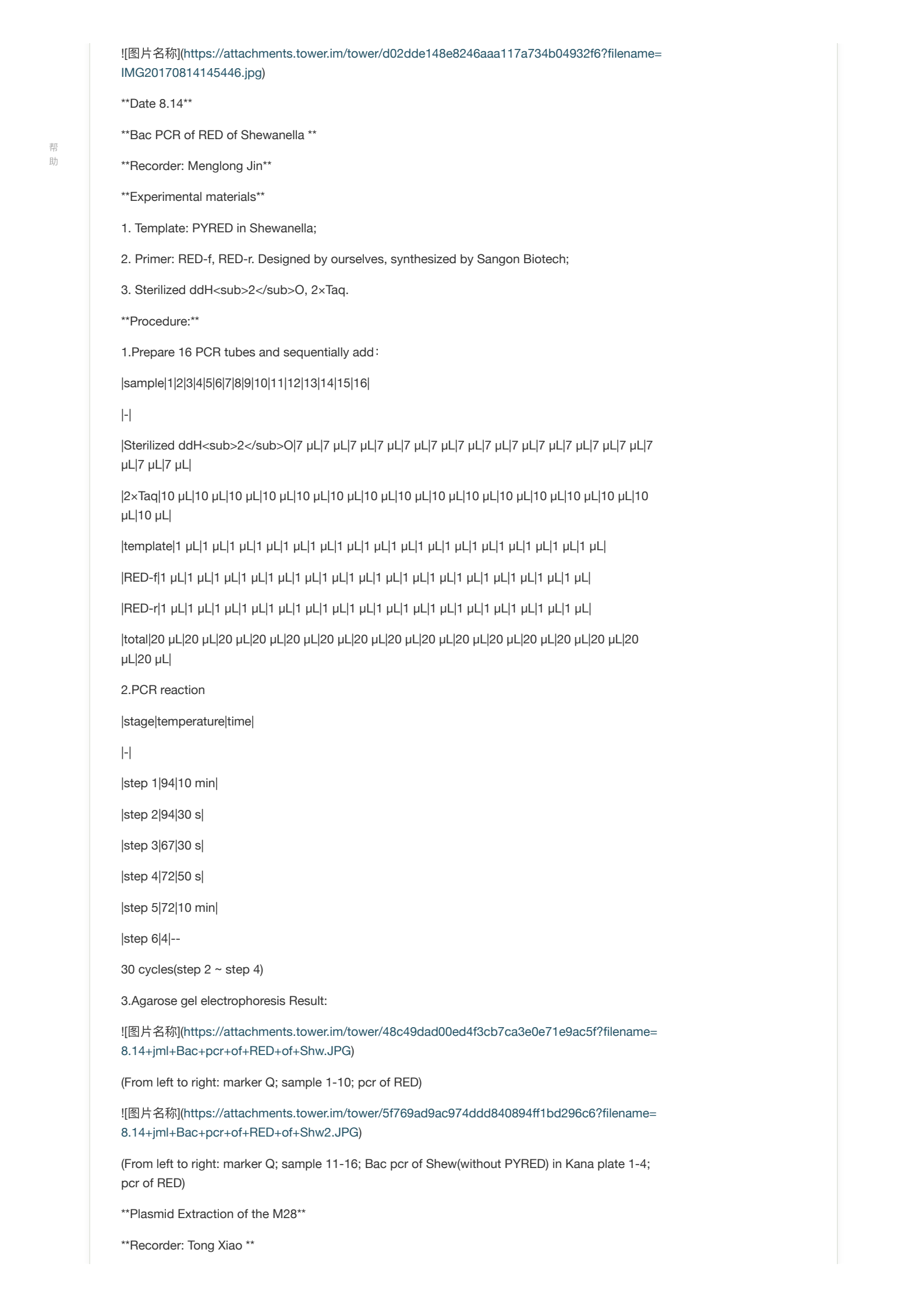
7. Add 200μl solution in a plate with kanamycin.

8. Cultivate these bacteria overnight for further use.

Results:

![图片名称](https://attachments.tower.im/tower/d02819203acb4b6db25160847432c94e?filename=IMG20170814145256.jpg)

帮助



Date 8.14

**Bac PCR of RED of Shewanella **

Recorder: Menglong Jin

Experimental materials

1. Template: PYRED in Shewanella;

2. Primer: RED-f, RED-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O, 2×Taq.

Procedure:

1.Prepare 16 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|9|10|11|12|13|14|15|16|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2×Taq|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|RED-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|RED-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

2.PCR reaction

|stage|temperature|time|

|-|

|step 1|94|10 min|

|step 2|94|30 s|

|step 3|67|30 s|

|step 4|72|50 s|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:

(From left to right: marker Q; sample 1-10; pcr of RED)

(From left to right: marker Q; sample 11-16; Bac pcr of Shew(without PYRED) in Kana plate 1-4; pcr of RED)

Plasmid Extraction of the M28

**Recorder: Tong Xiao **

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 60 s, discard filtrate.

6.Add 500 μL Buffer DW1, 12000 rpm centifuge 60 s, discard filtrate.

7.Add 500 μL Wash Solution, 12000 rpm centifuge 60 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|PYRED1|PYRED2|PYRED3|PYRED4|

|-|

|Concentration(ng/ul)|171.4|94.6|

|260/280|1.89|1.88|

|260/230|2.35|2.50|

Date 8.14

**Bac PCR of RED of Shewanella **

Recorder: Menglong Jin

Experimental materials

1. Template: PYRED in Shewanella;

2. Primer: RED-f, RED-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O, 2×Taq.

Procedure:

1.Prepare 16 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|9|10|11|12|13|14|15|16|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2×Taq|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|RED-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|RED-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

2.PCR reaction

|stage|temperature|time|

|-|

|step 1|94|10 min|

|step 2|94|30 s|

|step 3|67|30 s|

|step 4|72|50 s|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/48c49dad00ed4f3cb7ca3e0e71e9ac5f?filename=8.14+jml+Bac+pcr+of+RED+of+Shw.JPG)

(From left to right: marker Q; sample 1-10; pcr of RED)

![图片名称](https://attachments.tower.im/tower/5f769ad9ac974ddd840894ff1bd296c6?filename=8.14+jml+Bac+pcr+of+RED+of+Shw2.JPG)

(From left to right: marker Q; sample 11-16; Bac pcr of Shew(without PYRED) in Kana plate 1-4; pcr of RED)

Date 8.15

SDS of Mtr

**Recorder:Shihan Zhu **

Procedure

1.Inoculate the bacteria from the glycerinum stock and cultivate it overnight, 37℃, 250 rpm.

2.Delute the bacteria into a 5 mL medium and cultivate for 6 hours, 37℃, 250 rpm.

3.Dulute 2 mL of the bacteria above into a 200 mL medium and cultivate it till its OD reach 0.4-0.6.

4.Add appropriate concentration of inducer into the system and induce the expression for 1-3 hours.

5.Take certain volume of bacteria out of the medium, mix with appropriate volume of protein loading buffe, and heat it at 100 ℃ for 20 mins.

6.Use the heated sample for SDS-PAGE.

![图片名称](https://attachments.tower.im/tower/3d3429d7136149bf874553a9c06481f9?filename=8.15+zsh+SDS+of+mtr.JPG)

(From left to right: ？？)

**PCR of full Ccm **

Recorder: Liudong Luo, Zhenyu Jiang

Experimental materials

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

2. Primer: ccm-res-f,ccm-res-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O, 2×PrimeStar(Premix), bought from Takara.

Procedure:

1.Prepare 8 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|

|-|

|Sterilized ddH₂O|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|22 μL|

|2×Prime Star(premix)|25 μL|25 μL|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|1 μL|1 μL|

|ccm-res-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|ccm-res-r|1 μL|1 μL|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|50 μL|50 μL|

2.PCR reaction

1,2,3,4 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|10 min|

|step 2|98|10 s|

|step 3|59|5 s|

|step 4|72|7 min|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

9,10,11,12 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|10 min|

|step 2|98|10 s|

|step 3|55|15 s|

|step 4|72|7 min|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:

(lane left to right: marker Q, ccm-pcr-1, ccm-pcr-2,ccm-pcr-3,ccm-pcr-4,ccm-pcr-5, ccm-pcr-6, ccm-pcr-7,ccm-pcr-8,ccm)

Purification of full ccm PCR product

Recorder: Zhenyu Jiang, Liudong Luo

(1). Add 900 μL Buffer B3 to the 180 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.

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(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 25 μL ddH₂O, stand them still for 10 min. Centrifuge at 12,000 rpm for 1 min.

Bacteria PCR of Final-Reductase

Recorder: Yonghao Liang

Experimental materials

1. Template: bacteria containing Red+pSB1C3 recombined plasmid picked from the plate ;

2. Primer: VF2, VR. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O;

4. 2X Taq DNA polymerase.

Procedure:

1.Prepare 16 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|9|10|11|12|13|14|15|16

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Taq DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|pBAD-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|pBAD-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

3.PCR reaction

1-9 Parameters setting：

|stage|temperature|time|

|-|

|step 1|94|10 min|

|step 2|94|30 s|

|step 3|67|30 s|

|step 4|72|50 S|

|step 5|72|10 min|

|step 6|4|--

25 cycles(step 2 ~ step 4)

Result:

![图片名称](https://attachments.tower.im/tower/83c568d7097c47358e66a4435dd34780?filename=8%2C15+lld+bac+pcr+of+fin-Red.JPG)

(lane left to right: marker Q, fin-red-1, fin-red-2, fin-red-3, fin-red-4, fin-red-5, fin-red-6, fin-red-7, fin-red-8,red)

Date 8.18

PCR of Mtr ptar pSB1C3

**Recorder:Yonghao Liang **

Experimental materials

1. Template: ;

2. Primer: ;

3. Sterilized ddH₂O, 2×Taq.

1.Prepare 8 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2×Taq|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|mtr-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|mtr-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

2.PCR reaction

|stage|temperature|time|

|-|

|step 1|94|10 min|

|step 2|94|30 s|

|step 3|67|30 s|

|step 4|72|50 s|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/fcf964ec1e04424b92e6a45eb94a32a6?filename=8.18+lyh+pcr+of+mtr+ptar+psb1c3.JPG)

(From left to right:)

Date 8.19

Double digestion of Mtr and pET28

**Recorder:Yonghao Liang **

Materials:

1. Mtr on pET28

2. FastDigest restriction enzyme XhoI,NdeI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)

3. Nuclease-free water

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Reaction system:

|Sample|1|2|3|4|

|-|

|NdeI(μL)|1|1|1|1|

|nuclease-free water(μL)|26|26|26|26|

|fastdigest green buffer(μL)|2|2|2|2|

|Mtr(μL)|1|1|1|1|

|total(μL)|30|30|30|30|

Reaction system:

|Sample|1|2|3|

|-|

|NdeI(μL)|1|1|1|

|XhoI(μL)|1|1|1|

|nuclease-free water(μL)|25|25|25|

|fastdigest green buffer(μL)|2|2|2|

|pET28(μL)|1|1|1|

|total(μL)|30|30|30|

Mix gently and incubate at 37 degree Celsius for 20 hours .

Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/fd987a1912ab426a9fbd03695dfb465e?filename=8.19lyh+dd+of+mtr+%26pet28.JPG)

(From left to right:)

Date 8.21

Bac PCR of M28

Recorder: Tong Xiao

Experimental materials

1. Template: bacteria picked from the plate ;

2. Primer: Mtr-res-f,Mtr-res-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O;

4. 2X Primerstar DNA polymerase.

Procedure:

1.Prepare 16 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|9|10|11|12|13|14|15|16|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Primerstar DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Mtr-res-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Mtr-res-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

2.Prepare 16 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|9|10|11|12|13|14|15|16|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Primerstar DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Mtr-res-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Mtr-res-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

3.Prepare 16 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|9|10|11|12|13|14|15|16|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Primerstar DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Mtr-res-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Mtr-res-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

4.Prepare 16 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|9|10|11|12|13|14|15|16|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Primerstar DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Mtr-res-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Mtr-res-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

5.Prepare 16 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|9|10|11|12|13|14|15|16|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Primerstar DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Mtr-res-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Mtr-res-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

6.Prepare 16 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|9|10|11|12|13|14|15|16|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Primerstar DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Mtr-res-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Mtr-res-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

7.Prepare 16 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Primerstar DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Mtr-res-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Mtr-res-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

PCR reaction

1-9 Parameters setting：

|stage|temperature|time|

|-|

|step 1|94|4 min|

|step 2|94|30 s|

|step 3|56|30 s|

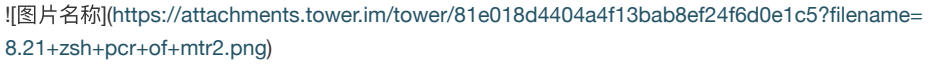
|step 4|72|5min 30s|

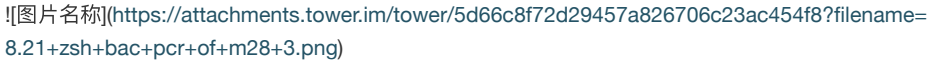
|step 5|72|10 min|

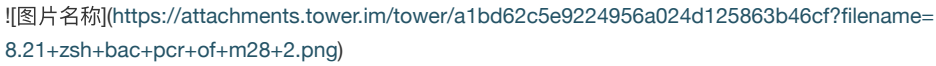
|step 6|4|--

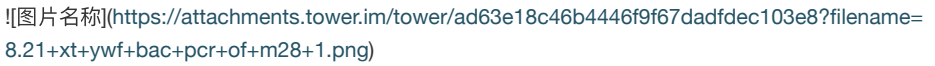
25 cycles(step 2 ~ step 4)

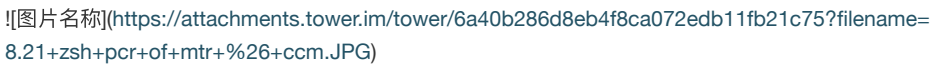
Result:











Date 8.22

Double digestion of M28

Recorder: Wenfei Yu

Experimental materials

1. Mtr on pET28

2. FastDigest restriction enzyme XhoI,NdeI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)

3. Nuclease-free water

Reaction system:

|Sample|1|2|3|4|5|6|7|

|-|

|NdeI(μL)|1|1|1|1|1|1|1|

|XhoI(μL)|1|1|1|1|1|1|1|

|nuclease-free water(μL)|25|25|25|25|25|25|25|

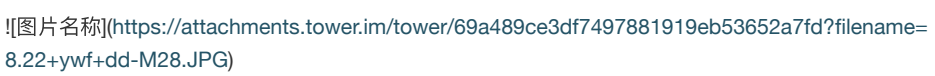
|fastdigest green buffer(μL)|2|2|2|2|2|2|2|

|pET28(μL)|1|1|1|1|1|1|1|

|total(μL)|30|30|30|30|30|30|30|

Mix gently and incubate at 37 degree Celsius for 20 hours .

Agarose gel electrophoresis Result:



(From left to right:)

Date 8.23

PCR of plcys

Recorder: Liudong Luo

Experimental materials

1. Template: plcys ;

2. Primer: fin-plcys-f,fin-plcys-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O, 2×PrimeStar(Premix), bought from Takara.

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

帮助

|sample|1|2|3|4|

|-|

|Sterilized ddH₂O|22 μL|22 μL|22 μL|22 μL|

|2×Prime Star(premix)|25 μL|25 μL|25 μL|25 μL|

|template|1 μL|1 μL|1 μL|1 μL|

|fin-plcys-f|1 μL|1 μL|1 μL|1 μL|

|fin-plcys-r|1 μL|1 μL|1 μL|1 μL|

|total|50 μL|50 μL|50 μL|50 μL|

2.PCR reaction

1,2,3,4 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|10 min|

|step 2|98|5 s|

|step 3|56|5 s|

|step 4|72|2 min 30s|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/529e138db070401d9e01fca8122fa7a5?filename=8.23+lld+pcr+of+plcys.JPG)

(lane left to right: marker 2K Plus II, pcr-plcys-1,pcr-plcys-2,pcr-plcys-3,pcr-plcys-4)

Date 8.24

Bac PCR of M28

Recorder: Shihan Zhu

Experimental materials

1. Template: bacteria picked from the plate ;

2. Primer: Mtr-res-f,Mtr-res-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O;

4. 2X Primerstar DNA polymerase.

Procedure:

1.Prepare 16 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|9|10|11|12|13|14|15|16|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Primerstar DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Mtr-res-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Mtr-res-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

PCR reaction

1-9 Parameters setting：

|stage|temperature|time|

|-|

|step 1|94|4 min|

|step 2|94|30 s|

|step 3|56|30 s|

|step 4|72|5min 30s|

|step 5|72|10 min|

|step 6|4|--

25 cycles(step 2 ~ step 4)

Result:

![图片名称](https://attachments.tower.im/tower/35ac59abd1a8408e837e70e2e904239f?filename=8.24+zsh+bac+pcr+of+m28.png)

lane1-17:2K plus II marker, m28-1~m28-16

Purification of plcys PCR product

Recorder: Liudong Luo

Procedure:

(1). Add 480 μL Buffer B3 to the 100 μL solution and mix it up. Add it to an adsorption column.

(2). 11,000 rpm centrifuge for 60 s, and then discard the filtrate.

(3). Add 500 μL Wash Solution, 12,000 rpm centrifuge for 60 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 25 μL ddH₂O, stand them still for 10 min. Centrifuge at 12,000 rpm for 1 min.

Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/a5b2f995744648e58152c0f1d6f58918?filename=8.23+lld+pcr+of+plcys+2.JPG)

(lane left to right: marker Q, pur-pcr-plcys--1-1,pur-pcr-plcys-1-2,pur-pcr-plcys--2-1,pur-pcr-plcys--2-2)

anaerobic cultivation of Mtr

Recorder: Wenfei Yu

Procedure:

1. Inoculate 2uL culture of BL21 and BL21 with pET28 plasmid containing Mtr into 2 test tubes with 5mL liquid LB culture medium, cultivate for overnight at 37℃

2. Inoculate 2mL overnight culture of each test tubes into 2 conical flasks, cultivate for till OD600 reaches 0.4-0.6

3. Add 200uL 1mol/L IPTG, cultivate for 3 hours.

4. Take out the conical flasks, preserve 200 uL culture of each conical flask and keep it in 4 EP tubes, each EP tubes contains 100uL.

5. Use syringe to inoculate 500 uL culture with Mtr+pET28 and 50 uL kanamycin into an anaerobic bottle, cultivate for at 37 ℃ for 21 hours.

6. Take out the EP tubes, 12000 rpm centrifuge 2 min, remove the supernatant, add 100uL 2X SDS PAGE Sample Loading Buffer, resuspend, transfer into PCR tubes, put them in sample boiling apparatus, 100 degree centigrade, 20min.

7. Run SDS PAGE, western blot.

8. Take out the anaerobic bottle, preserve 5mL bacterium solution to 5 EP tubes, each contains 1mL, 4000 rpm centrifuge 2 min, remove the supernatant, add 1mL 2X PBS Buffer, resuspend, put in 4 degree centigrade, continue cultivating.

9. Add 45 uL 1mol/L IPTG, cultivate for 3h.

10. Repeat step 8.

11. Cultivate for 4 hours, repeat step 8.

12. Cultivate for 59 hours, repeat step 8.

13. Take EP tubes from step 8, 10, 11, 12, and BL21, BL21 with Mtr+pET28 from step 4, repeat step 6

14. Run SDS GAGE.

Result: There is no difference between anaerobic cultivated samples.The anaerobic cultivation experiment has failed.

Date 8.26

**Bacteria PCR of fin-plcys **

Recorder: Liudong Luo, Xingwei Yang

Experimental materials

1. Template: fin-plcys;

2. Primer: f-plcys-f,f-plcys-r. Designed by ourselves, synthesized by Genneral biosystems

;

3. Sterilized ddH₂O, 2×PrimeStar(Premix), bought from Takara.

Procedure:

1.Prepare 14 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|9|10|11|12|13|14|

|-|

|Sterilized ddH₂O|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|2×Prime Star(premix)|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|f-plcys-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|f-plcys-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

2.PCR reaction

1,2,3,4,5,6,7,8,9,10,11,12,13,14 Parameters setting：

|stage|temperature|time|

|-|

|step 1|95|10 min|

|step 2|98|5 s|

帮助

|step 3|56|5 s|

|step 4|72|2 min 30 s|

|step 5|72|10 min|

|step 6|4|--

30 cycles(step 2 ~ step 4)

Agarose gel electrophoresis Result:

![图片名称](https://attachments.tower.im/tower/5518a7d8ca954a4ab3cde0beb7f1692b?filename=8.26+yxw+bac+pcr+of+fin-plcys.JPG)

(lane left to right: 2K plus II, pcr-fin-plcys-1, pcr-fin-plcys-2, pcr-fin-plcys-3, pcr-fin-plcys-4, pcr-fin-plcys-5, pcr-fin-plcys-6, pcr-fin-plcys-7, pcr-fin-plcys-8, pcr-fin-plcys-9, pcr-fin-plcys-10, pcr-fin-plcys-11, pcr-fin-plcys-12, pcr-fin-plcys-13, pcr-fin-plcys-14)

Bac PCR of M28

Recorder: Tong Xiao

Experimental materials

1. Template: bacteria picked from the plate ;

2. Primer: Mtr-res-f,Mtr-res-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O;

4. 2X Primerstar DNA polymerase.

Procedure:

1.Prepare 8 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Primerstar DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Mtr-res-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Mtr-res-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

PCR reaction

1-9 Parameters setting：

|stage|temperature|time|

|-|

|step 1|94|4 min|

|step 2|94|30 s|

|step 3|56|30 s|

|step 4|72|5min 30s|

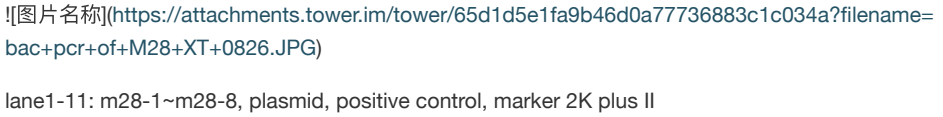
|step 5|72|10 min|

|step 6|4|--

25 cycles(step 2 ~ step 4)

Result:

帮助



lane1-11: m28-1~m28-8, plasmid, positive control, marker 2K plus II

Bac PCR of M28

Recorder: Wenfei Yu

Experimental materials

1. Template: bacteria picked from the plate ;

2. Primer: Mtr-res-f,Mtr-res-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH₂O;

4. 2X Primerstar DNA polymerase.

Procedure:

1.Prepare 8 PCR tubes and sequentially add：

|sample|1|2|3|4|5|6|7|8|

|-|

|Sterilized ddH₂O|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|7 μL|

|2X Primerstar DNA polymerase|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|10 μL|

|template|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Mtr-res-f|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|Mtr-res-r|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|1 μL|

|total|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|20 μL|

PCR reaction

1-9 Parameters setting：

|stage|temperature|time|

|-|

|step 1|94|4 min|

|step 2|94|30 s|

|step 3|56|30 s|

|step 4|72|5min 30s|

|step 5|72|10 min|

|step 6|4|--

25 cycles(step 2 ~ step 4)

Result:

lane1-9: marker 2K plus II, m28-1~m28-8

Date 9.24

** Expression of Mtr, SDS-PAGE and Western blot**

帮助

Recorder: Tong Xiao & Chenyang Li & Wenfei Yu

SDS-PAGE

Procedure:

1. Inoculate the bacteria from the glycerinum stock and cultivate it overnight, 37℃, 250 rpm;

2. Delute the bacteria into a 5 mL medium and cultivate for 6 hours, 37℃, 250 rpm;

3. Dulute 2 mL of the bacteria above into a 200 mL medium and cultivate it till its OD reach 0.4-0.6;

4. Add appropriate concentration of inducer into the system and induce the expression for 1-3 hours;

5. Take certain volume of bacteria out of the medium, mix with appropriate volume of protein loading buffe, and heat it at 100 ℃ for 20 mins;

6. Use the heated sample for SDS-PAGE.

![图片名称](https://attachments.tower.im/tower/3612b170340d4164b824f101cd890a1c?filename=+8.25+SDS-PAGE.png)

(lane left to right: wt wt mtr mtr ladder)

Western blot--anti-His tag

Procedure:

1. Block the membrane for 1 h at room temperature or overnight at 4°C using blocking buffer.

2. Incubate the membrane with appropriate dilutions of primary antibody in blocking buffer. We recommend overnight incubation at 4°C; other conditions can be optimized.

3. Wash the membrane in three washes of TBST, 5 min each.

4. Incubate the membrane with the recommended dilution of conjugated secondary antibody in blocking buffer at room temperature for 1 h.

5. Wash the membrane in three washes of TBST, 5 min each.

6. For signal development, follow the kit manufacturer's recommendations. Remove excess reagent and cover the membrane in transparent plastic wrap.

7. Acquire image using darkroom development techniques for chemiluminescence, or normal image scanning methods for colorimetric detection.

(lane left to right: wt wt mtr mtr ladder)

10-22 21:06 **吴昀荆** 创建了文档

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