Notebook July

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DATE 7.1 Recorder: Shihan Zhu Purification of PCR product of mtr We have 50 μL solution in each tube, from which we use 5 μL of them to do electrophoresis (mixed with 6× DNA loading buffer;110 V, 30 min). And the other 45 μL is purified by PCR purification kit (bought from Sangon Biotech). And the procedure is showed below:

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

PCR purification kit:

sample	MTR1	MTR2	MTR3	MTR4	MTR5	MTR6
Concentration(ng/ul)	159.9	236.2	225.0	205.6	147.4	186.6
260/280	1.81	1.83	1.87	1.85	1.84	1.86
260/230	1.58	1.93	2.24	2.20	2.17	2.00

Plasmid Extraction of the pET28 and pSB1C3 containing mtr Recorder: Shihan Zhu Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

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

sample	mtr	mtr2	mtr3	pET28-1	pET28-2
Concentration(ng/ul)	435.6	330.8	420.5	49.6	47.3
260/280	1.88	1.86	1.88	1.95	1.93
260/230	2.29	2.33	2.33	1.91	2.20

PCR and Agarose gel electrophoresis of CysDes and pLUX Recorder: Jianjian Guo,Yawei Wu 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×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

sample	1	2	3	4	5	6	7	8
XbaI(10 μM)	1 μL	1 μL	0 μL	0 μL	0 μL	0 μL	0 μL	0 μL
PstI(10 μM)	1 μL	1 μL	0 μL	0 μL	0 μL	0 μL	0 μL	0 μL
VF2(10 μM)	0 μL	0 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
VR(10 μM)	0 μL	0 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

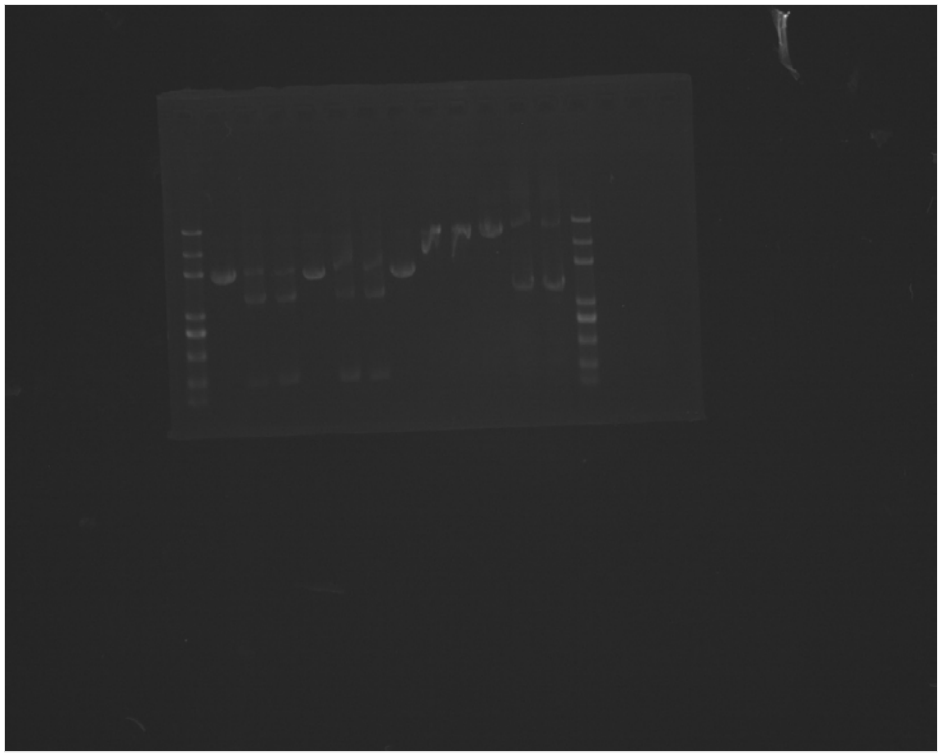
*sample 1-6 are CysDes from 3 plasmid extraction tube,while sample 7-8 are pLux from one plasmid extraction tube.

2.PCR reaction Parameters setting：

stage	temperature	time
step 1	95	10 min
step 2	95	20 s
step 3	55	20 s
step 4	72	1 min 30 s for sample 1,2,7,8;2 min 30 s for the rest
step 5	72	10 min
step 6	4	--

30 cycles

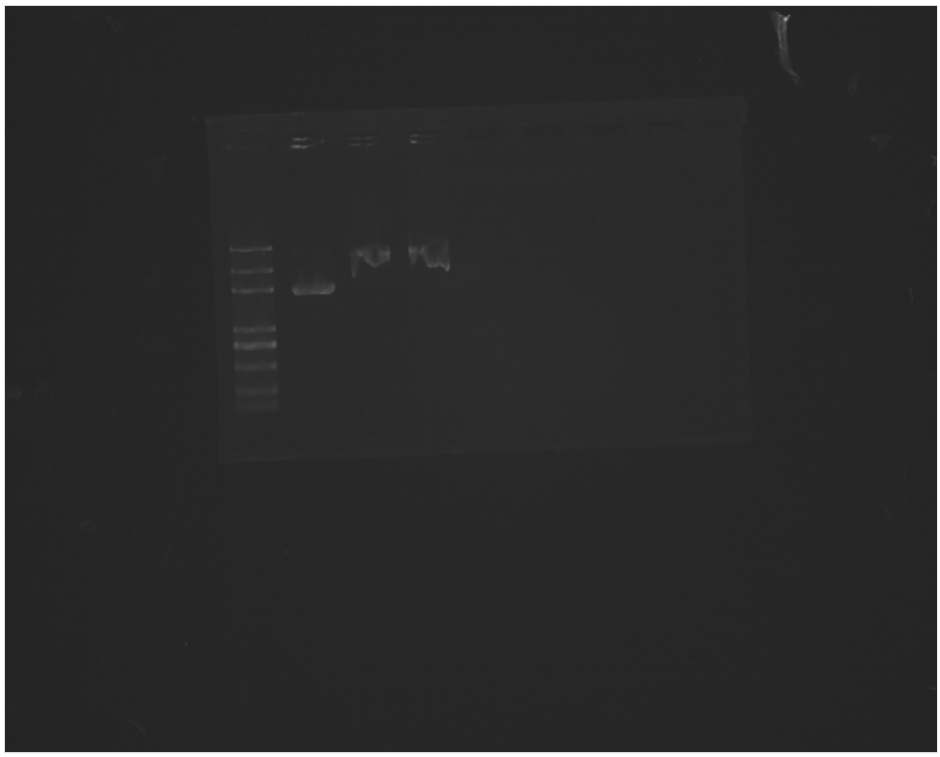
3.Agarose gel electrophoresis mixed with 1 μL 6× DNA loading buffer each 5 μL sample;110 V, 30 min Result:



lane1-12: plasmid1,sample3,sample4,plasmid2,sample5,sample6,plasmid3,sample1,sample2,plasmid4,sample7,sample8.

To futher ensure the result of sample 1,2 are not due to sample loading, we did Agarose gel electrophoresis again for these 3 groups.

Result:



lane1-3: plasmid3,sample1,sample2

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

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

sample	pBAD-1	pBAD-2	pBAD-3	pBAD-4
Concentration(ng/ul)	240.3	249.3	212.4	244.3
260/280	1.86	1.86	1.86	1.86
260/230	2.09	2.07	2.23	2.09

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

Colony pick up of pTB Recorder: Yonghao Liang

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

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

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

sample	**Cys 1**	**Cys 1'**	**Cys 2**	**Cys 3**	**Cys 4**
Concentration(ng/ul)	379.2	451.4	396.8	331.6	504.4
260/280	1.87	1.88	1.88	1.87	1.77
260/230	2.27	2.28	2.27	2.29	1.29

sample	**pluxR 1**	**pluxR 1'**	**pluxR 1''**	**pluxR 2**	**pluxR 3**
Concentration(ng/ul)	509.3	529.1	533.3	464.0	364.1
260/280	1.84	1.86	1.78	1.89	1.86
260/230	1.94	2.30	1.28	2.36	2.11

DATE 7.2 Colony pick up of pTB Recorder:Tong Xiao

PCR of pTB Recorder: Yonghao Liang

Experimental materials

1. Template: plasmids extracted from pTB's colonies;
2. Primer: VF2, VR. synthesized by Sangon Biotech;
3. Sterilized ddH2O, 2×Taq.

Procedure:

1.Prepare 6 PCR tubes and sequentially add：

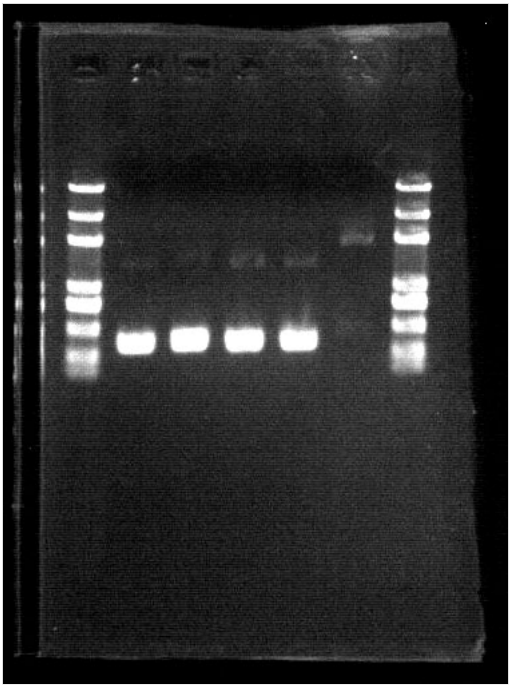
sample	**1**	**3**	**5**	**6**	**8**
Sterilized ddH2O	7 μL	7 μL	7 μL	7 μL	7 μL
2×Primer Star	10 μL	10 μL	10 μL	10 μL	10 μL
template	1 μL	1 μL	1 μL	1 μL	1 μL
VF2(10 μM)	1 μL	1 μL	1 μL	1 μL	1 μL
VR(10 μM)	1 μL	1 μL	1 μL	1 μL	1 μL
total	20 μL	20 μL	20 μL	20 μL	20 μL

2.PCR reaction Parameters setting：

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

30 cycles(step 2 ~ step 4)

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



As you can see, we got a band about 300 bp long, which perfectly met our anticipation. So we are going to have these four plasmids sequenced, to prove the ligation is successful or not.

Date 7.3

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

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

sample	**pET28-1**	**pET28-2**	**pET28-3**	**pET28-4**	**pET28-5**	**pET28-6**	**pET28-7**	**pET28-8**
Concentration(ng/μL)	43.5	49.2	37.6	33.9	34.2	111.6	41.3	34.1
260/280	1.91	1.92	1.82	1.97	2.06	1.50	2.01	2.04
260/230	1.31	1.69	0.49	1.33	2.44	0.63	1.65	2.43

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

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

sample	**pBAD 5**	**pBAD 4**	**pBAD 3**	**pBAD 2**	**pBAD 1**
Concentration(ng/μL)	137.0	146.3	79.1	164.8	88.6

sample	pBAD 5	pBAD 4	pBAD 3	pBAD 2	pBAD 1
260/280	1.74	1.62	1.87	1.63	1.81
260/230	1.18	0.79	1.97	0.82	1.48

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sample	pTB 6	pTB 5	pTB 3	pTB 1
Concentration(ng/μL)	209.9	152.4	179.9	210.2
260/280	1.79	1.85	1.85	1.78
260/230	1.40	2.11	1.92	1.40

PCR of NapC and Ccm A-H from BL21(DE3)'s genome Recorder: Yan Shi, Yonghao Liang

Experimental materials

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

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

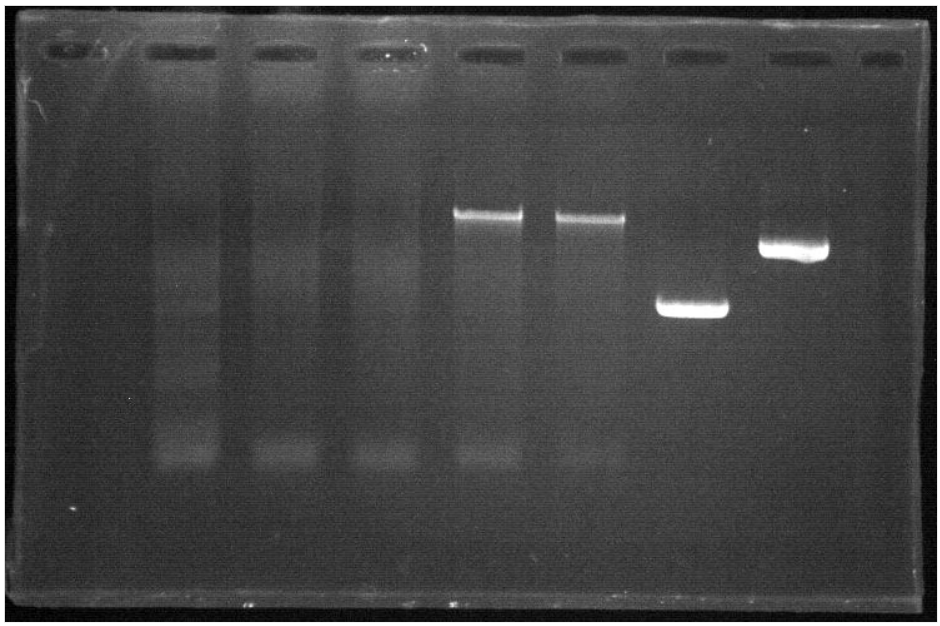
sample	1	2	3	4
Sterilized ddH2O	7 μL	7 μL	7 μL	7 μL
2×Taq-PCR Master	10 μL	10 μL	10 μL	10 μL
Genome	1 μL	1 μL	1 μL	1 μL
ccm-res-f(10 μM)	1 μL	1 μL	0	0
ccm-mid-r(10 μM)	1 μL	1 μL	0	0
ccm-mid-f(10 μM)	0	0	1 μL	1 μL
ccm-res-r(10 μM)	0	0	1 μL	1 μL
total	20 μL	20 μL	20 μL	20 μL

2.PCR reaction Parameters setting：

stage	temperature(°C)	time
step 1	95	10 min
step 2	95	45 s
step 3	59	45 s
step 4	72	4 min
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:



(lane 2 to 8: PCR of ccm 4,1,2,3-1,3-2,pBAD,pTB)

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

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

sample	pLUX R 1	pLUX R 2	Cys 1	Cys 2	Cys 3	Cys 4
Concentration(ng/μL)	277.7	230.9	319.5	275.4	385.2	350.0
260/280	1.84	1.84	1.86	1.85	1.86	1.84
260/230	2.07	2.12	2.11	2.14	2.21	2.18

sample	pBAD 1	pBAD 2	pBAD 3	pBAD 4	pBAD 5
Concentration(ng/μL)	60.6	49.5	64.0	115.8	109.4
260/280	1.85	1.83	1.73	1.62	1.62

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

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

sample	pLUX R 1	pLUX R 2	Cys 1	Cys 2	Cys 3	Cys 4

sample	pLUX R 1	pLUX R 2	Cys 1	Cys 2	Cys 3	Cys 4
Concentration(ng/μL)	502.8	542.9	525.4	573.5	531.2	536.3
260/280	1.83	1.80	1.80	1.80	1.81	1.82
260/230	1.76	1.61	1.48	1.69	1.52	1.69

sample	pLUX R 1'	pLUX R 2'	Cys 1'	Cys 2'	Cys 3'	Cys 4'
Concentration(ng/μL)	359.7	443.5	274.3	377.7	332.9	349.7
260/280	1.86	1.84	1.80	1.82	1.81	1.80
260/230	2.25	1.81	1.37	1.70	1.55	1.45

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

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

sample	pBAD 1	pBAD 2	pBAD 3	pBAD 4	pBAD 5
Concentration(ng/μL)	174.9	106.5	111.9	96.1	109.7
260/280	1.77	1.77	1.83	1.91	1.88
260/230	1.21	1.26	1.66	2.26	2.09

sample	pTB 1	pTB 3	pTB 5	pTB 6
Concentration(ng/μL)	173.8	188.1	144.9	170.4
260/280	1.78	1.87	1.82	1.85
260/230	1.39	2.04	1.63	1.78

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

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

sample	pBAD 1	pBAD 2	pBAD 3	pBAD 4	pBAD 5
Concentration(ng/μL)	72.4	91.8	94.1	77.3	105.3
260/280	1.78	1.81	1.82	1.80	1.77
260/230	1.57	1.97	2.16	1.82	1.55

sample	pTB 1	pTB 3	pTB 5	pTB 6
Concentration(ng/μL)	230.9	229.9	243.2	192.3
260/280	1.83	1.85	1.85	1.82

sample	pTB 1	pTB 3	pTB 5	pTB 6
260/230	1.93	2.14	1.87	1.74

Date 7.4

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PCR of MtrCAB Recorder: Chenyang Li

Experimental materials

1. Template 1: 1:5 dilution of plasmid MtrCAB (Mtr5 XT 6.28 500.1,1.88), 100ng/μL;
2. Primer: mtr-res-f, mtr-res-r. Designed by ourselves, synthesized by Sangon Biotech;
3. Sterilized ddH2O, 2×Prime Star.

Procedure: 1.Prepare 1 EP tubes and sequentially add：

sample	1
Sterilized ddH2O	66 μL
template	3 μL
mtr-res-f(10 μM)	3 μL
mtr-res-r(10 μM)	3 μL
2×Primer Star	75 μL
total	150 μL

1. Subpackage it to 3 PCR tubes.

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

2.PCR reaction Parameters setting：

stage	temperature(°C)	time
step 1	98	10 min
step 2	98	10 s
step 3	56	5 s
step 4	72	5 min 30 s
step 5	72	10 min
step 6	4	--

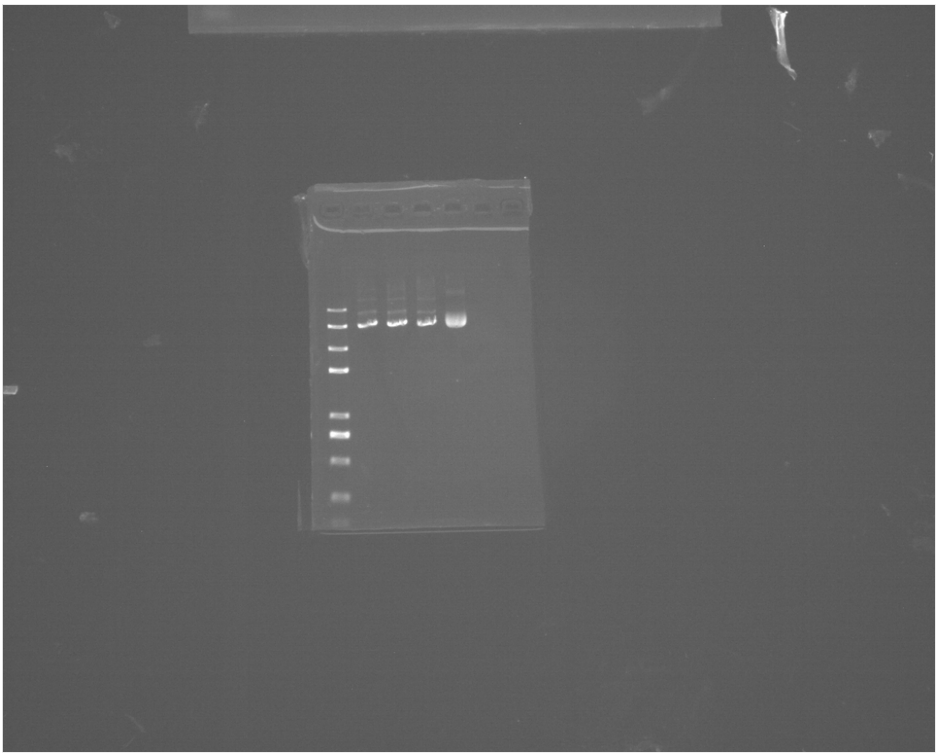
30 cycles(step 2 ~ step 4)

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

OD measurement result:

sample	Mtr PCR pur 1-1	Mtr PCR pur 1-2	Mtr PCR pur 1-3
Concentration(ng/μL)	148.4	157.0	175.2
260/280	1.82	1.85	1.82
260/230	1.59	2.16	1.82

4.Agarose gel electrophoresis mixed with 1 μL 6× DNA loading buffer each 5 μL 1:5 dilution of PCR product; mixed 1 μL 6× DNA loading buffer with 5μL template;120 V,30 min Result:



(from left to right: Trans 2K plusII(contain Gelred), Mtr PCR pur 1-1, Mtr PCR pur 1-2, Mtr PCR pur 1-3, template 1)

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

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

sample	**pBAD 1**	**pBAD 2**	**pBAD 3**	**pBAD 4**	**pBAD 5**
Concentration(ng/μL)	92.7	60.7	90.3	77.2	88.1
260/280	1.74	1.74	1.71	1.81	1.75
260/230	1.41	1.50	1.26	1.43	1.42

sample	**pTB 1**	**pTB 3**	**pTB 5**	**pTB 6**
Concentration(ng/μL)	161.8	48.7	119.3	167.6
260/280	1.78	1.75	1.82	1.75
260/230	1.57	1.40	1.91	1.23

Plasmid Extraction of pET28 and Mtr Recorder: Yitian Zhou, 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 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

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

sample	pET28-1	pET28-2	pET28-3	pET28-4	pET28-5	pET28-6	pET28-7	pET28-8	pET28-9	pET28-10	pET28-11
Concentration(ng/μL)	72.0	30.7	49.5	75.1	455.0	46.9	52.7	61.7	51.0	75.7	43.8
260/280	1.80	2.00	2.01	1.84	1.87	1.96	1.96	1.94	1.93	1.75	1.99
260/230	1.11	2.03	2.56	1.20	2.29	2.21	2.00	1.89	1.93	1.04	2.05

sample	Mtr-1	Mtr-2	Mtr-3
Concentration(ng/μL)	382.6	239.2	181.9
260/280	1.88	1.87	1.88
260/230	2.34	2.40	2.49

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

Experimental materials

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

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

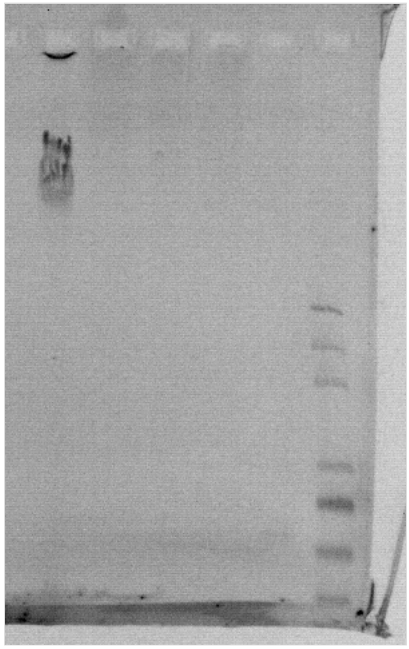
sample	1	2	3	4
Sterilized ddH2O	7 μL	7 μL	7 μL	7 μL
2×Taq-PCR Master	10 μL	10 μL	10 μL	10 μL
Genome	1 μL	1 μL	1 μL	1 μL
ccm-res-f(10 μM)	1 μL	1 μL	0	0
ccm-mid-r(10 μM)	1 μL	1 μL	0	0
ccm-mid-f(10 μM)	0	0	1 μL	1 μL
ccm-res-r(10 μM)	0	0	1 μL	1 μL
total	20 μL	20 μL	20 μL	20 μL

2.PCR reaction Parameters setting：

stage	temperature(°C)	time
step 1	95	10 min
step 2	95	40 s
step 3	59	40 s
step 4	72	4 min
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

帮助

3.Agarose gel electrophoresis Result:  (lane 1 to 6: genome of BL21(DE3), PCR of first half, PCR of second half-1,2,3,marker)

As you can see, we failed to amplify the ccm gene this time. TAT.

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

Experimental materials

1. pTB ligated from pTET and RBS by ourselves.
2. FastDigest restriction enzyme HindIII, EcoRI, PstI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water
4. marker:Trans 2K Plus

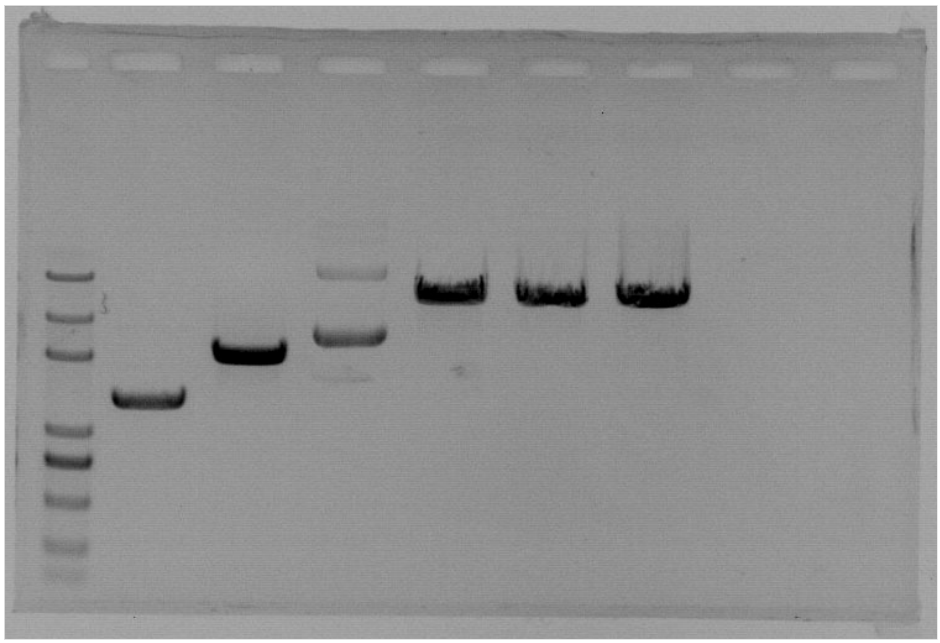
	pTB	**pBAD**
Concentration(ng/μL)	179.9	249.3
260/280	1.85	1.86
260/230	1.92	2.07

Reaction system:

Sample	**1**	**2**	**3**	**4**
pTB(μL)	7	0	0	0
pBAD(μL)	0	5	5	5
nuclease-free water(μL)	10	11	12	12
fastdigest green buffer(μL)	2	2	2	2
PstI(μL)	1	0	0	0
EcoRI(μL)	0	1	1	0
HindIII(μL)	0	1	0	1
total(μL)	20	20	20	20

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

Agarose gel electrophoresis Result:



(lane 1 to 7: marker, pTB, dd of pTB, pBAD, double digestion of pBAD 5,6,7)

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

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

Plasmid Extraction of pET28 and Mtr Recorder: Yitian Zhou,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 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

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

sample	Cys-1	Cys-2	Cys-3	Cys-4	plux-1	plux-2
Concentration(ng/μL)	220.7	334.1	351.1	386.3	549.9	495.0
260/280	1.84	1.83	1.81	1.84	1.81	1.82
260/230	1.92	1.63	1.53	1.50	2.08	1.94

sample	Cys-1	Cys-2	Cys-3	Cys-4	plux-1	plux-2
Concentration(ng/μL)	182.6	319.1	248.8	270.6	390.4	355.3
260/280	1.83	1.83	1.85	1.86	1.84	1.85
260/230	1.88	1.64	2.11	2.05	1.91	1.94

Plasmid Extraction of pBAD and pTB Recorder: Xingwei Yang Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350

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

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

sample	**pBAD 1**	**pBAD 2**	**pBAD 3**	**pBAD 4**	**pBAD 5**
Concentration(ng/μL)	95.6	87.9	90.3	77.3	111.7
260/280	1.74	1.77	1.78	1.80	1.79
260/230	1.52	1.80	1.90	2.04	1.68

sample	**pTB 1**	**pTB 3**	**pTB 5**	**pTB 6**
Concentration(ng/μL)	216.9	253.6	286.3	230.6
260/280	1.82	1.77	1.81	1.85
260/230	1.80	1.32	1.69	2.17

Date 7.5

PCR of MtrCAB Recorder: Chenyang Li

Experimental materials

1. Template 2: 1:50 dilution of plasmid MtrCAB (Mtr5 XT 6.28 500.1,1.88), 10ng/μL; Template 3: 1:500 dilution of plasmid MtrCAB (Mtr5 XT 6.28 500.1,1.88), 1ng/μL.
2. Primer: mtr-res-f, mtr-res-r. Designed by ourselves, synthesized by Sangon Biotech;
3. Sterilized ddH2O, 2×Prime Star.

Procedure: 1.Prepare 2 EP tubes and sequentially add：

sample	**2**	**3**
Sterilized ddH2O	66 μL	66 μL
template	3 μL(10ng/μL)	3 μL(1ng/μL)
mtr-res-f(10 μM)	3 μL	3 μL
mtr-res-r(10 μM)	3 μL	3 μL
2×Primer Star	75 μL	75 μL
total	150 μL	150 μL

1. Subpackage it to 3 PCR tubes.

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

2.PCR reaction Parameters setting：

stage	**temperature(°C)**	**time**
step 1	98	10 min
step 2	98	10 s
step 3	56	5 s
step 4	72	5 min 30 s

stage	temperature(°C)	time
step 5	72	10 min
step 6	4	--

帮助

30 cycles(step 2 ~ step 4)

OD measurement result:

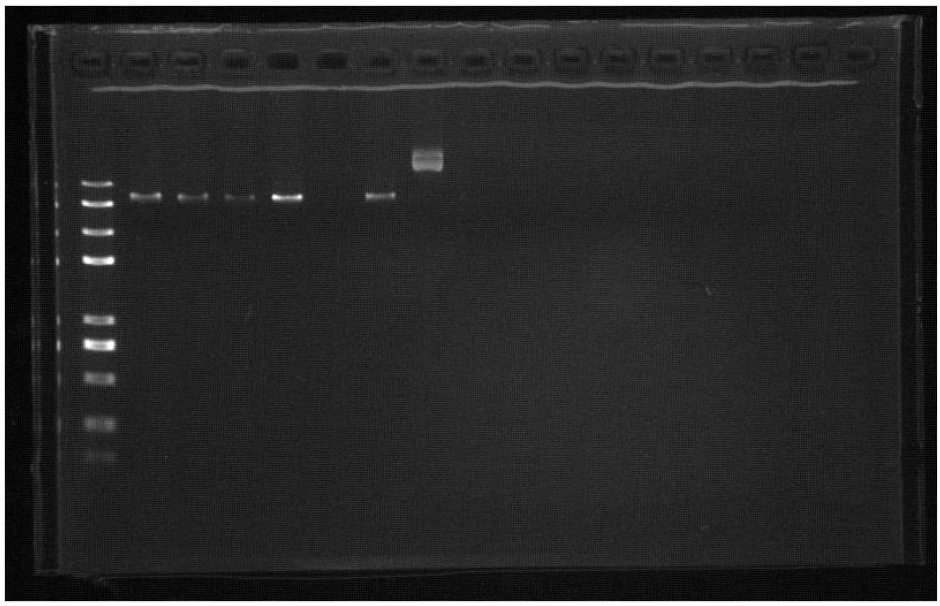
sample	Mtr PCR 2-1	Mtr PCR 2-2	Mtr PCR 2-3	Mtr PCR 3-1	Mtr PCR 3-2	Mtr PCR 3-3
Concentration(ng/μL)	441.9	451.1	467.5	462.8	473.9	465.3
260/280	1.82	1.81	1.77	1.82	1.83	1.82
260/230	1.85	1.77	1.89	1.87	1.84	1.87

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

OD measurement result:

sample	Mtr PCR pur 2-1	Mtr PCR pur 2-2	Mtr PCR pur 2-3	Mtr PCR pur 3-1	Mtr PCR pur 3-2	Mtr PCR pur 3-3
Concentration(ng/μL)	62.6	30.2	43.6	13.6	16.9	12.9
260/280	1.79	1.54	1.82	1.77	1.70	1.78
260/230	1.77	0.61	1.93	1.81	1.25	1.21

4.Agarose gel electrophoresis mixed with 1 μL 6× DNA loading buffer each 5 μL 1:5 dilution of PCR product; mixed 1 μL 6× DNA loading buffer with 5μL template;120 V,30 min Result:



(from left to right: Trans 2K plusII(contain Gelred), Mtr PCR 2-1, Mtr PCR 2-2, Mtr PCR 2-3, Mtr PCR pur 2-1, Mtr PCR pur 2-2, Mtr PCR pur 2-3, template 2, Mtr PCR 3-1, Mtr PCR 3-2, Mtr PCR 3-3, Mtr PCR pur 3-1, Mtr PCR pur 3-2, Mtr PCR pur 3-3, template 3)

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

Experimental materials

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

帮助

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

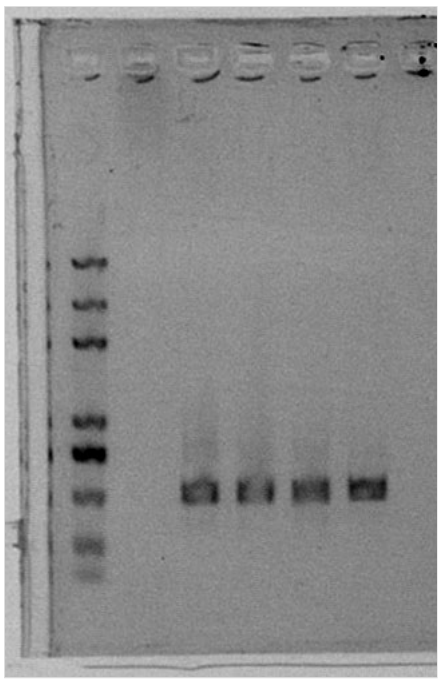
sample	**1**	**2**	**3**	**4**
Sterilized ddH2O	7 μL	7 μL	7 μL	7 μL
2×Taq-PCR Master	10 μL	10 μL	10 μL	10 μL
Genome	1 μL	1 μL	1 μL	1 μL
ccm-res-f(10 μM)	1 μL	1 μL	0	0
ccm-mid-r(10 μM)	1 μL	1 μL	0	0
ccm-mid-f(10 μM)	0	0	1 μL	1 μL
ccm-res-r(10 μM)	0	0	1 μL	1 μL
total	20 μL	20 μL	20 μL	20 μL

2.PCR reaction Parameters setting：

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

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:

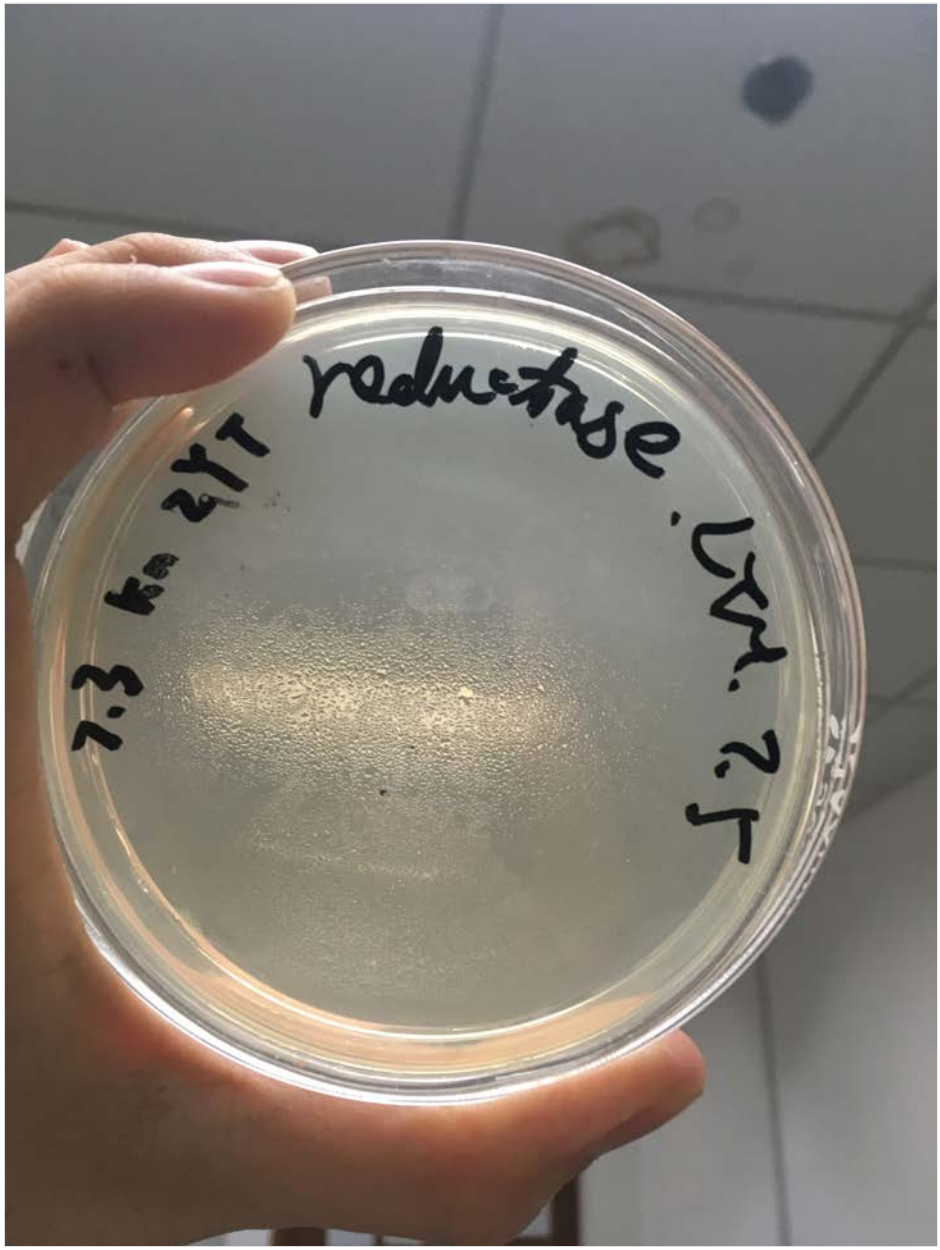


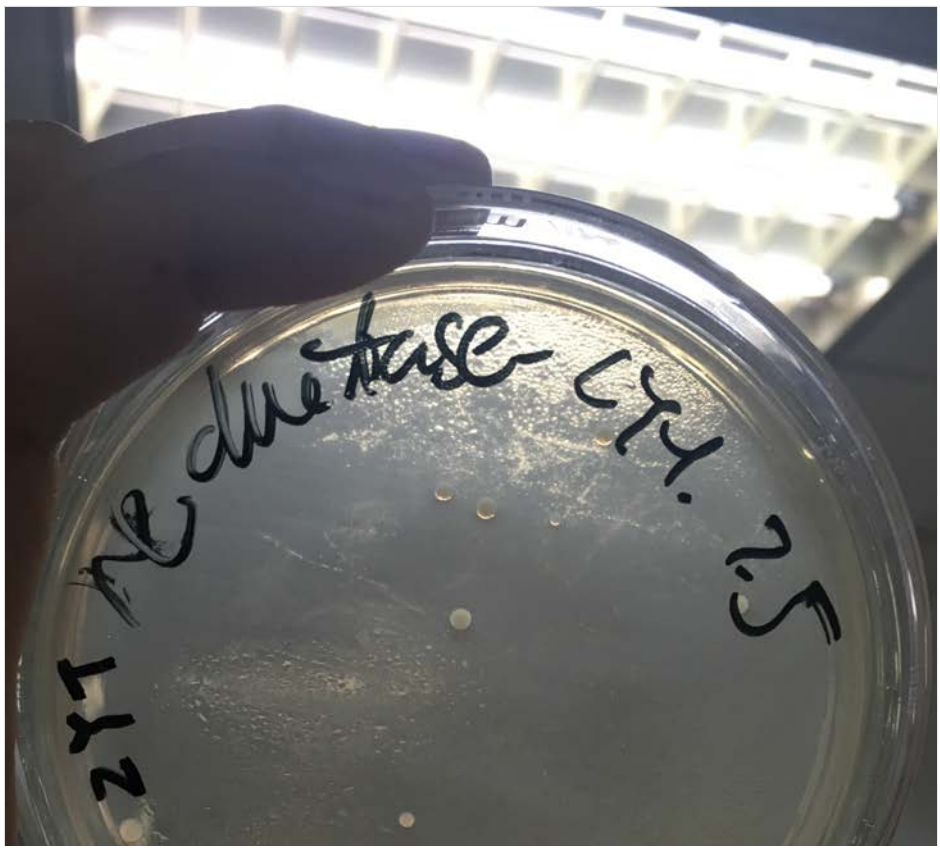
We failed to amplify the ccm gene again.TAT

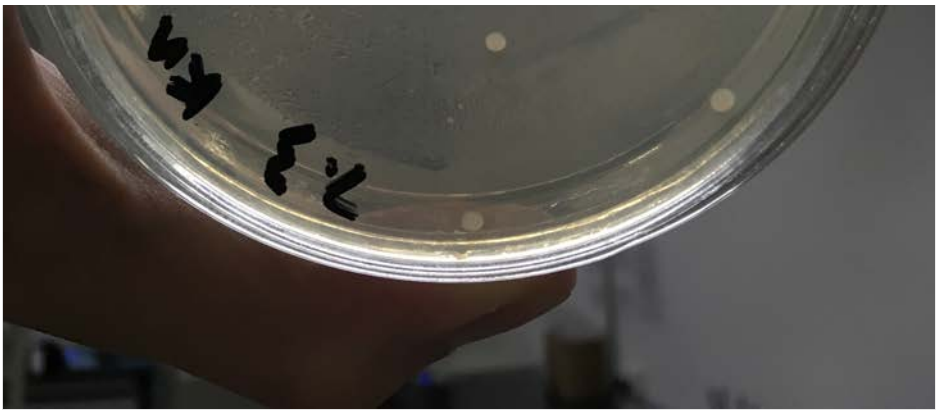
Transformation of Reductase S1 Recorder: Yonghao Liang NOTE:Generally, competent bacteria are restrored in -70 degree centrigrade environment. 1.Take the competent bacteria from refrigerator and incubate them into ice about 5 mins until it is dissolved 2.Absorb 100pg to 10 ng plasmid(normally 1 to 2 μL, DO NOT add more than 5% volumn of bacteria solution) and mix it with bacteria solution thoroughly. ATTENTION: Please operate this step tenderly!!! 3.Put the tubes on the ice about 30 mins.(Time SHOULD BE ACCURATE) 4.Make a heat shock at 42 degree centigrade about 90 sec(TIME SHOULD BE ACCURATE) 5.Put the tubes on the ice about 5 mins again. 6.Add 900 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 kanamycin. 10.Cultivate these bacteria overnight for further use.

The plating result is as following:

帮助







As you can see on the plate, there're only a few colonies on the plate, which makes me wonder the plasmid the reductase is on does NOT have the Kanamycin resistance factor, but has the Ampicillin resistance factor...

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

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

sample	pET28-1	pET28-2	pTB-1	pTB-2	pTB-3	pTB-4	pTB-5	pTB-6
Concentration(ng/μL)	38.6	35.6	173.9	164.2	138.3	175.6	173.2	186.1
260/280	1.81	1.87	1.86	1.78	1.86	1.82	1.81	1.82
260/230	1.58	1.90	2.16	1.70	2.25	1.61	1.71	1.85

Date 7.6

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

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

sample	pBAD 1	pBAD 2	pBAD 3	pBAD 4
Concentration(ng/μL)	173.5	236.6	257.7	142.8
260/280	1.89	1.82	1.87	1.83
260/230	2.12	1.64	1.97	1.74

sample	pTB 1	pTB 2	pTB 3	pTB 4
Concentration(ng/μL)	227.3	223.9	264.0	204.3
260/280	1.80	1.85	1.77	1.77
260/230	1.44	2.11	1.30	1.30

帮助

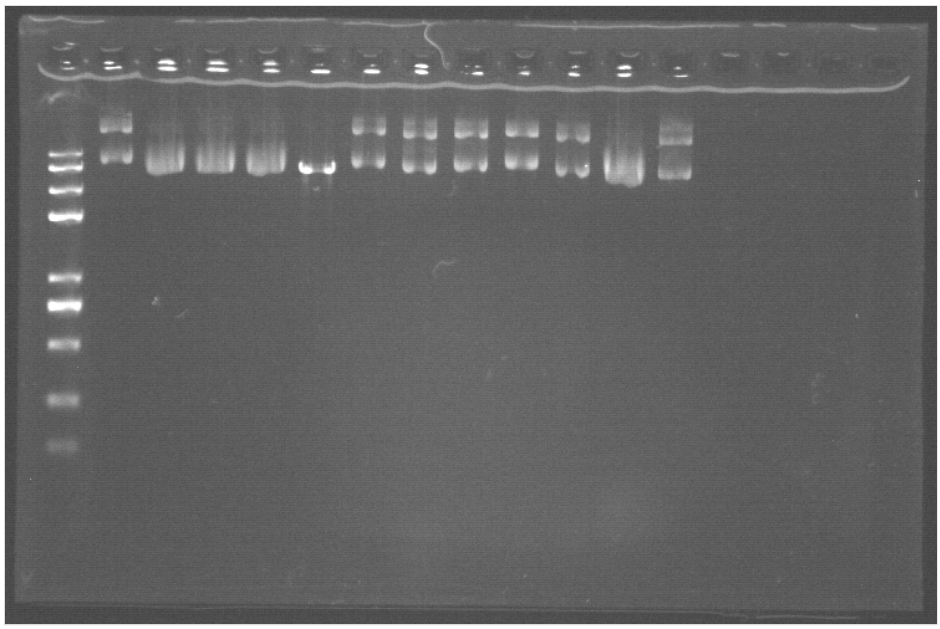
Plasmid Extraction of pET28 Recorder: Shihan Zhu， Wenfei Yu, Yijun Chen Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3,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

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

sample	pET28-1	pET28-2	pET28-3	pET28-4	pET28-5	pET28-6	pET28-7	pET28-8
Concentration(ng/μL)	76.0	188.7	187.1	96.0	124.8	67.4	109.3	151.8
260/280	1.80	1.50	1.55	1.75	1.57	1.79	1.62	1.59
260/230	1.29	0.54	0.64	1.20	0.67	1.60	0.90	0.68

sample	Mtr1	Mtr2	Mtr3	Mtr4
Concentration(ng/μL)	645.6	703.1	757.6	745.9
260/280	1.79	1.80	1.80	1.73
260/230	1.73	1.84	1.61	1.18

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



PCR of MtrCAB Recorder: Chenyang Li

Experimental materials

1. Template 2: 1:50 dilution of plasmid MtrCAB (Mtr5 XT 6.28 500.1,1.88)， 10ng/μL;
2. Primer: mtr-res-f， mtr-res-r. Designed by ourselves, synthesized by Sangon Biotech;
3. Sterilized ddH2O, 2×Prime Star.

Procedure: 1.Prepare 2 EP tubes and sequentially add：

sample	2
Sterilized ddH2O	132 μL
template	6 μL(10ng/μL)
mtr-res-f(10 μM)	6 μL

sample	2
mtr-res-r(10 μM)	6 μL
2×Primer Star	150 μL
total	300 μL

帮助

1. Subpackage it to 3 PCR tubes.

sample	2-4,2-5,2-6	2-7,2-8,2-9
Sterilized ddH2O	22 μL	22 μL
template	1 μL (10ng/μL)	1 μL (1ng/μL)
mtr-res-f(10 μM)	1 μL	1 μL
mtr-res-r(10 μM)	1 μL	1 μL
2×Primer Star	25 μL	25 μL
total	50 μL	50 μL

2.PCR reaction sample2-4,2-5,2-6 parameters setting：

stage	temperature(°C)	time
step 1	98	10 min
step 2	98	10 s
step 3	56	5 s
step 4	72	5 min 30 s
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

sample2-7,2-8,2-9 parameters setting：

stage	temperature(°C)	time
step 1	94	10 min
step 2	94	15 s
step 3	56	5 s
step 4	72	5 min 30 s
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

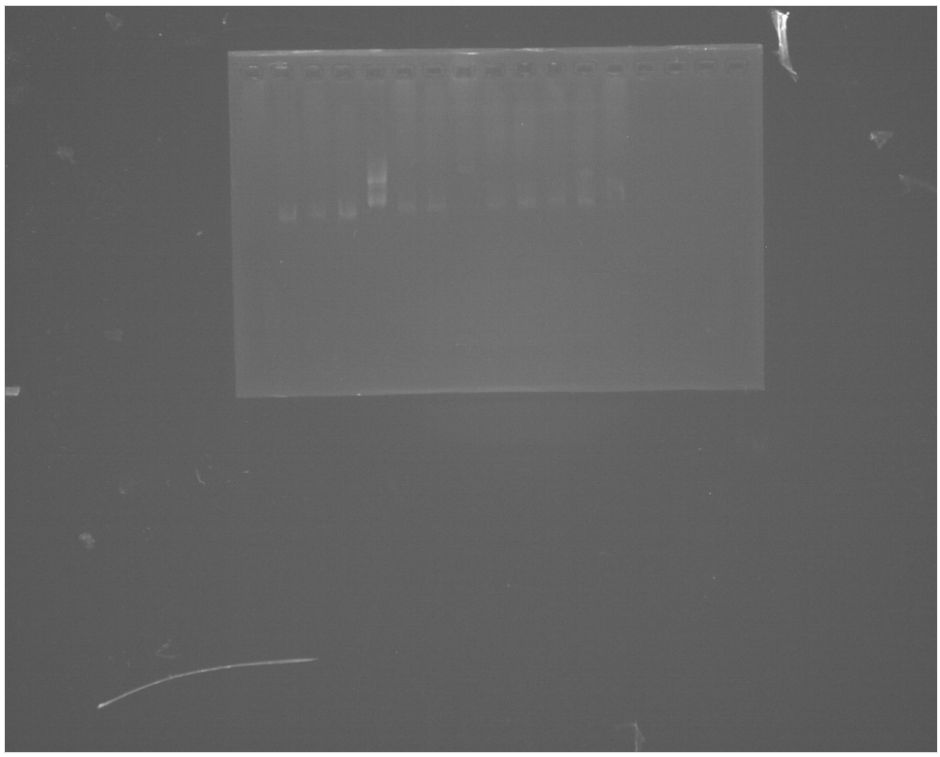
3.Purification of PCR product (Done by Shihan Zhu) (1). Add 225 μL Buffer B3 to the 25 μL solution and mix it up. Add it to an adsorption column. (2). 11,000 rpm centrifuge for 30 s, and then discard the filtrate. (3). Add 500 μL Wash Solution, 12,000 rpm centrifuge for 30 s, and then discard the filtrate.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

OD measurement result:

sample	Mtr PCR pur 2-4	Mtr PCR pur 2-5	Mtr PCR pur 2-6	Mtr PCR pur 2-7	Mtr PCR pur 2-8	Mtr PCR pur 2-9
Concentration(ng/μL)	181.2	263.9	90.1	307.8	155.6	207.3
260/280	1.59	1.47	1.68	1.44	1.50	1.51
260/230	0.63	0.53	0.92	0.50	0.54	0.55

4.Agarose gel electrophoresis(Done by Shihan Zhu) mixed with 1 μL 6× DNA loading buffer each 5 μL 1:5 dilution of PCR product; mixed 1 μL 6× DNA loading buffer with 5μL template;120 V,30 min

Result:



(from left to right: Trans 2K plusII(contain Gelred), Mtr PCR pur 2-4, Mtr PCR pur 2-5, Mtr PCR pur 2-6, temeplate 2, Mtr PCR pur 2-7, Mtr PCR pur 2-8, Mtr PCR pur 2-9, Mtr PCR 2-4, Mtr PCR 2-5, Mtr PCR 2-6, Mtr PCR 2-7, Mtr PCR 2-8, Mtr 2-9)

Single and double digestion of plasmid pET28 and Mtr Recorder: Tong Xiao,Meiying Cui

Experimental materials

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

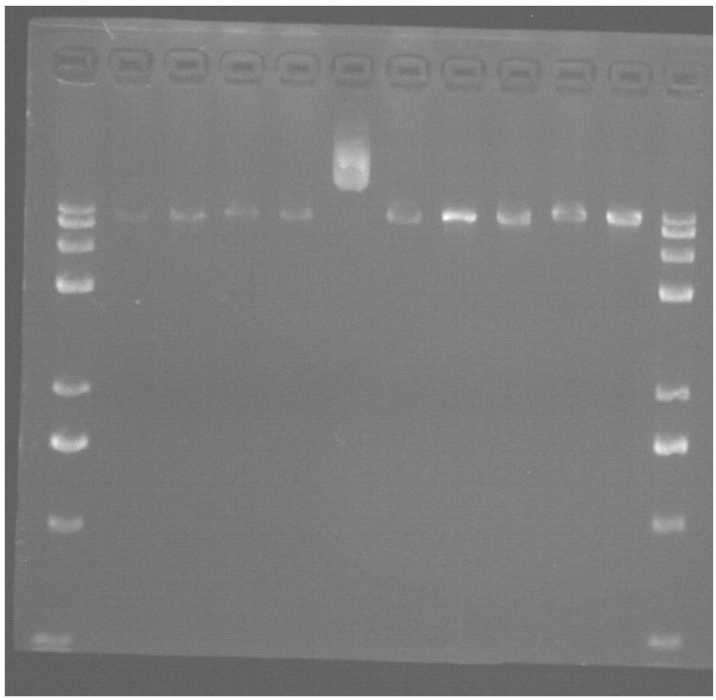
Reaction system:

Sample	**1**	**2**	**3**	**4**	**5**
pET28(μL)	17	17	17	17	16
fastdigest green buffer(μL)	2	2	2	2	2
NdeI(μL)	1	1	0	0	1
XhoI(μL)	0	0	1	1	1
total(μL)	20	20	20	20	20

Sample	**1**	**2**	**3**	**4**
Mtr(μL)	1	1	1	1
nuclease-free water(μL)	10	10	10	10
fastdigest green buffer(μL)	2	2	2	2
NdeI(μL)	1	1	0	0
XhoI(μL)	0	0	1	1
total(μL)	20	20	20	20

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

Agarose gel electrophoresis Result:



(lane1:marker 2k plus II lane2:mtr-N1 lane3: mtr-N2 lane4:mtr-X1 lane5:mtr-X2 lane6:pSB1C3 lane7:mtr-N-X lane8:pET28-N1 lane9:pET28-N2 lane10:pET28-X1 lane11:pET28-X2 lane12:marker 2k plus II)

Date:7.7

Single and double digestion of plasmid pET28 and Mtr Recorder: Shihan Zhu

Experimental materials

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

Reaction system:

Sample	**1**	**2**	**3**	**4**	**5**	**6**
pET28(μL)	8	8	8	8	8	8
fastdigest green buffer(μL)	2	2	2	2	2	2
NdeI(μL)	1	1	0	0	1	1
XhoI(μL)	0	0	1	1	1	1
Sterilized water(μL)	9	9	9	9	8	8
total(μL)	20	20	20	20	20	20

Sample	**1**	**2**	**3**	**4**
Mtr(μL)	1	1	1	1
nuclease-free water(μL)	16	16	16	16
fastdigest green buffer(μL)	2	2	2	2
NdeI(μL)	1	1	0	0
XhoI(μL)	0	0	1	1
total(μL)	20	20	20	20

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

Agarose gel electrophoresis Result: 图片名称 (lane1:marker 2k plus II lane2:mtr-N1 lane3: mtr-N2 lane4:mtr-X1 lane5:mtr-X2 lane6:mtr on pSB1C3 lane7:marker 2k plus lane8:marker 2k plus II lane9:pET28-N1 lane10:pET28-N2 lane11:pET28-X1 lane12:pET28-X2 lane13:dd_pET28-1 lane14:dd_pET28-2 lane15:pET28 lane16:marker 2k plus)

帮助

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

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

sample	**pBAD 1**	**pBAD 2**	**pBAD 3**
Concentration(ng/μL)	219.5	273.7	195.4
260/280	1.87	1.78	1.81
260/230	1.64	1.24	1.60

sample	**pTB 1**	**pTB 2**
Concentration(ng/μL)	229.6	240.9
260/280	1.81	1.72
260/230	1.60	1.06

Plasmid Extraction of pET28 and Mtr Recorder: Wenfei Yu, 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 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

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

sample	**Mtr1**	**Mtr2**	**Mtr3**	**Mtr4**	**pET28-1,2**	**pET28-3,4**	**pET28-5,6**	**pET28-7,8**
Concentration(ng/μL)	1029.1	563.0	778.8	656.5	116.0	157.5	170.3	133.6
260/280	1.81	1.84	1.89	1.83	1.86	1.63	1.71	1.78
260/230	1.61	2.31	2.35	2.18	1.96	0.65	0.86	1.45

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

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	Cys1	Cys 2	Cys 3	Cys 4	Cys new	pLUX 1	pLUX 2
Concentration(ng/μL)	324.6	296.8	192.9	199.0	370.5	431.7	385.3
260/280	1.82	1.88	1.88	1.88	1.88	1.87	1.87
260/230	1.62	2.31	2.35	2.29	2.33	2.21	2.25

帮助

Plasmid Extraction of Red and pBAD Recorder: Xingwei Yang, 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 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

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

sample	pBAD 1	pBAD 2	pBAD 3	pBAD 4
Concentration(ng/μL)	188.9	96.0	120.7	111.4
260/280	1.63	1.77	1.66	1.70
260/230	0.76	1.47	0.86	0.97

sample	red 1	red 2	red 3	red 4	red 5	red 6	red 7	red 8
Concentration(ng/μL)	131.1	70.0	122.4	28.5	126.4	60.6	30.6	33.7
260/280	1.60	1.61	1.61	1.87	1.63	1.72	1.96	1.97
260/230	0.71	0.80	0.69	1.80	0.73	1.00	2.13	1.74

Digestion of pET28 and Mtr on pSB1C3 Recorder: Shihan Zhu

Experimental materials

1. Mtr on pSB1C3,pET28
2. FastDigest restriction enzyme NdeI, XhoI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water
4. marker:Trans 2K PlusII and Trans 2K Plus

Reaction system:

Sample	1	1'	2	2'	3	3'
XhoI(μL)	1	1	0	0	1	1
NdeI(μL)	0	0	1	1	1	1
nuclease-free water(μL)	9	9	9	9	8	8
fastdigest green buffer(μL)	2	2	2	2	2	2
pET28(μL)	8	8	8	8	8	8
total(μL)	20	20	20	20	20	20

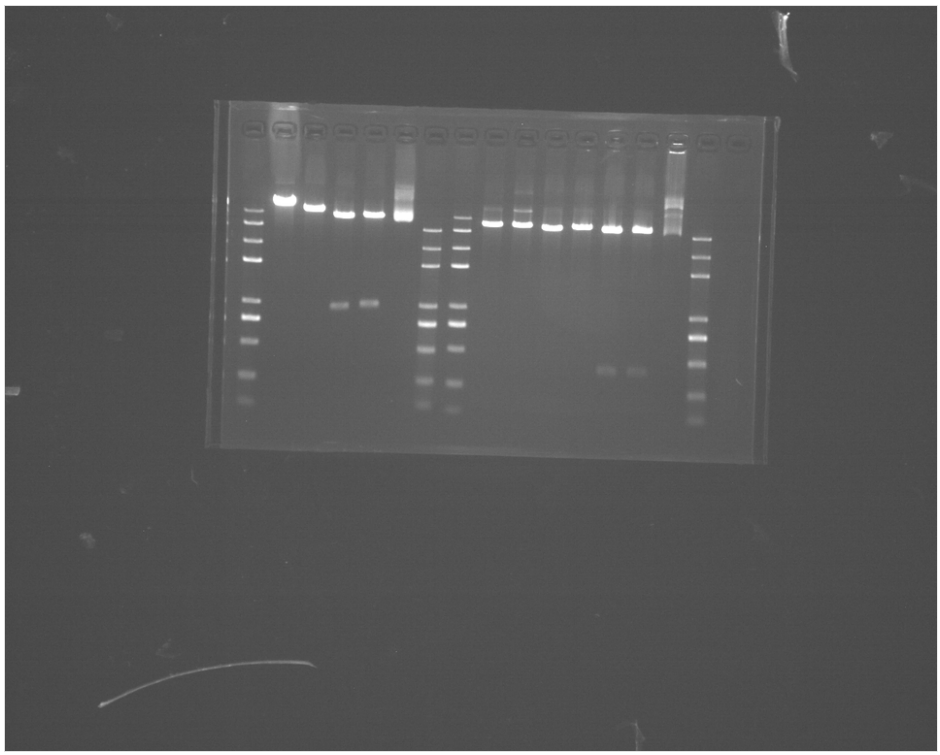
Sample	1	1'	2	2'
XhoI(μL)	1	1	0	0
NdeI(μL)	0	0	1	1

帮助

Sample	1	1'	2	2'
nuclease-free water(μL)	16	16	16	16
fastdigest green buffer(μL)	2	2	2	2
Mtr(μL)	1	1	1	1
total(μL)	20	20	20	20

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

Agarose gel electrophoresis Result:

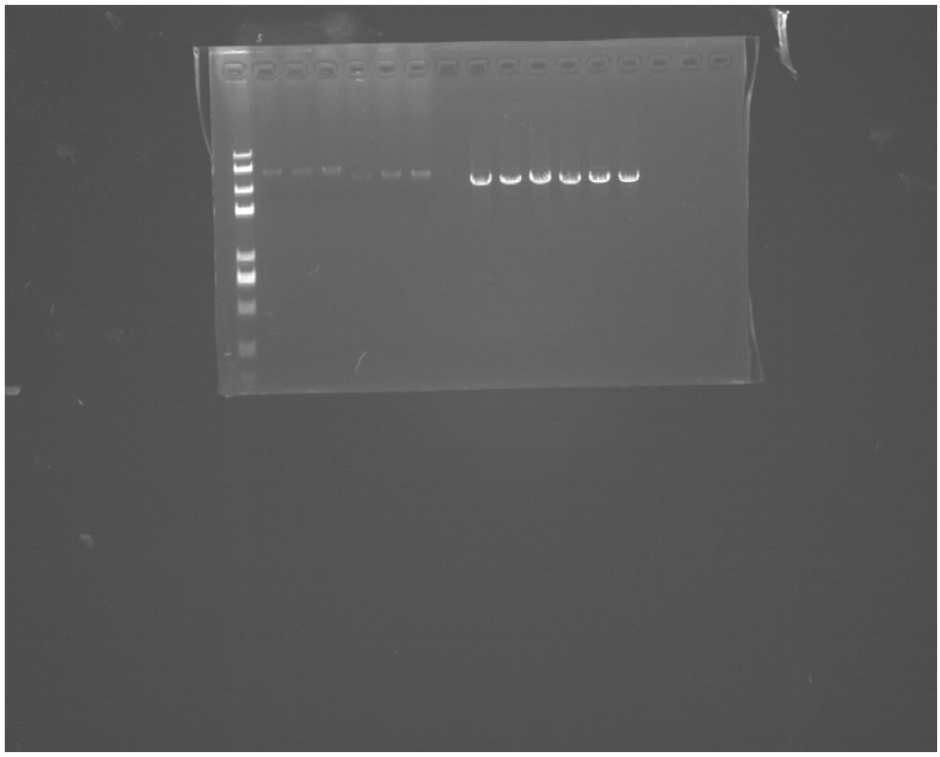


(lane left to right: marker 2K PlusII, sdmtr-1, sdmtr-1', sdmtr-2, sdmtr-2', mtr, marker 2K Plus, marker 2K PlusII, ddpET28-1, ddpET28-1', ddpET28-2, ddpET28-2', ddpET28-3, ddpET28-3', pET28, marker 2K Plus)

Date:7.8

帮助

Agarose gel electrophoresis Recorder: Shihan Zhu 110 V 30 min



(lane left to right: marker 2K PlusII, mtr-3-GE, mtr-2-GE, mtr-1-GE, pET28-1-GE, pET28-2-GE, pET28-3-GE, Mtr PCR pur 2-4, Mtr PCR pur 2-5, Mtr PCR pur 2-6, Mtr PCR pur 2-9, Mtr PCR pur 2-7, Mtr PCR pur 2-8)

Double digestion of pET28 and Mtr on pSB1C3 Recorder: Shihan Zhu

Experimental materials

1. Mtr on pSB1C3,pET28
2. FastDigest restriction enzyme NdeI, XhoI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water
4. marker:Trans 2K Plus II

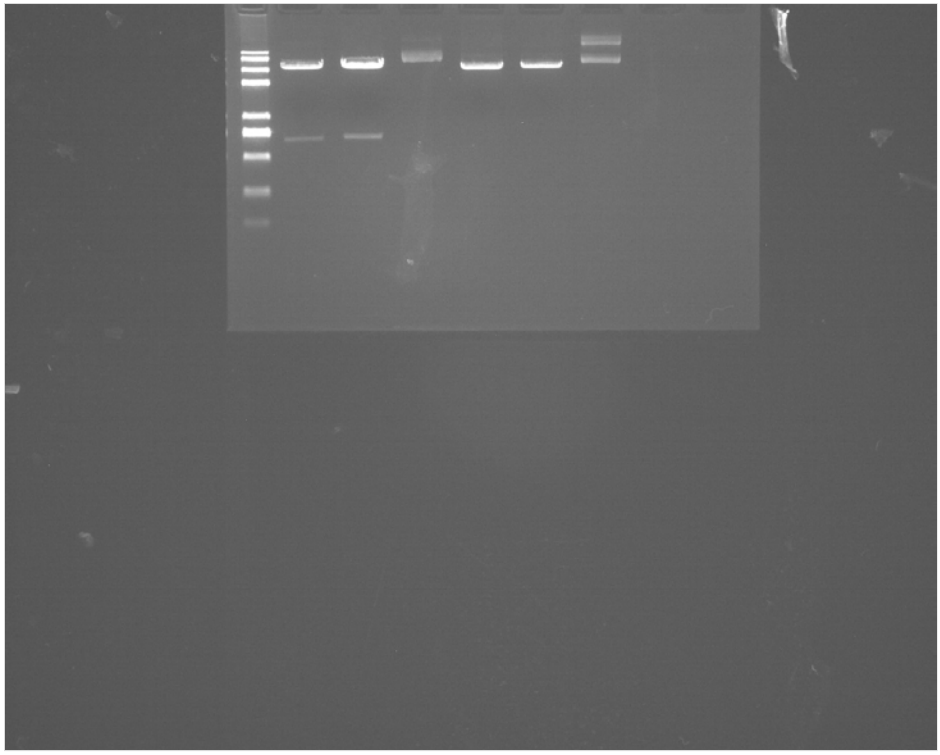
Reaction system:

Sample	**1**	**2**
XhoI(μL)	1	1
NdeI(μL)	1	1
nuclease-free water(μL)	0	0
fastdigest green buffer(μL)	2	2
PET28(μL)	16	16
total(μL)	20	20

Sample	**1**	**2**
XhoI(μL)	1	1
NdeI(μL)	1	1
nuclease-free water(μL)	14	14
fastdigest green buffer(μL)	2	2
Mtr(μL)	2	2
total(μL)	20	20

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

Agarose gel electrophoresis Result:



(lane 1 to 9: marker,ddmtr-1,ddmtr-2,mtr,ddpET28-1,ddpET28-2,pET28)

Double digestion of PCR products of Mtr Recorder: Chenyang Li

Experiment Materials

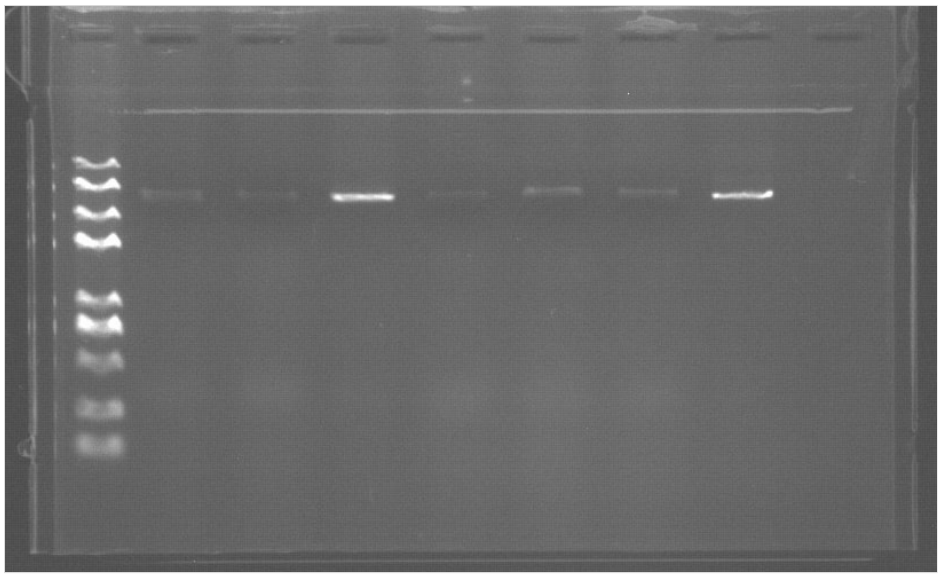
1. mtr PCR products(Mtr PCR pur 2-4, Mtr PCR pur 2-5, Mtr PCR pur 2-6, Mtr PCR pur 2-7, Mtr PCR pur 2-8, Mtr PCR pur 2-9,)
2. FastDigest restriction enzyme NdeI, XhoI and 10×FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water
4. marker:Trans 2K Plus

Reaction system:

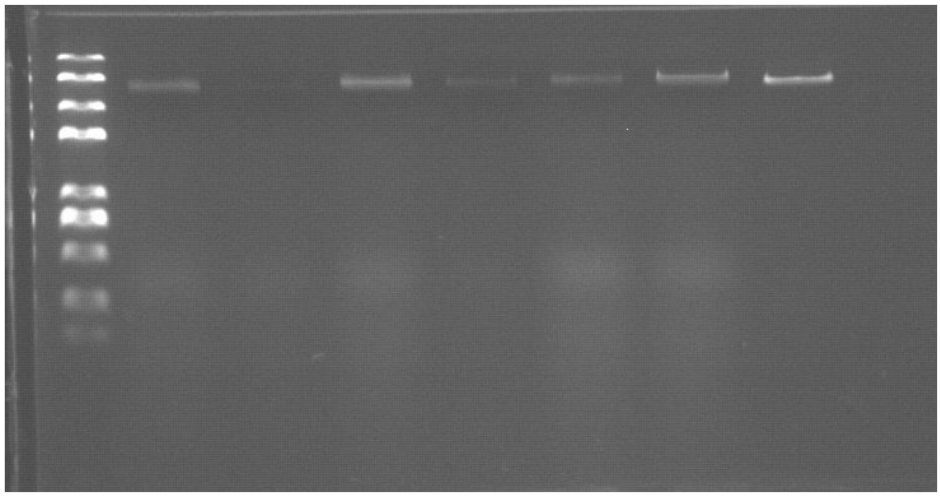
Sample	**2-4,2-4'**	**2-5,2-5'**	**2-6,2-6'**	**2-7,2-7'**	**2-8,2-8'**	**2-9,2-9'**
mtr PCR products(μL)	Mtr PCR pur 2-4	Mtr PCR pur 2-5	Mtr PCR pur 2-6	Mtr PCR pur 2-7	Mtr PCR pur 2-8	Mtr PCR pur 2-9
mtr(μL)	2	1.5	4.5	1.3	2.6	2
nuclease-free water(μL)	14	14.5	11.5	14.7	13.4	14
fastdigest green buffer(μL)	2	2	2	2	2	2
NdeI(μL)	1	1	1	1	1	1
XhoI(μL)	1	1	1	1	1	1
total(μL)	20	20	20	20	20	20

Mix gently and incubate at 37 degree Celsius for 60 mins .

Agarose gel electrophoresis Result:



(lane 1 to 8: Trans 2K plus(contain Gelred), 2-4, 2-5, 2-6, 2-7, 2-8, 2-9, Mtr PCR pur 2-5)



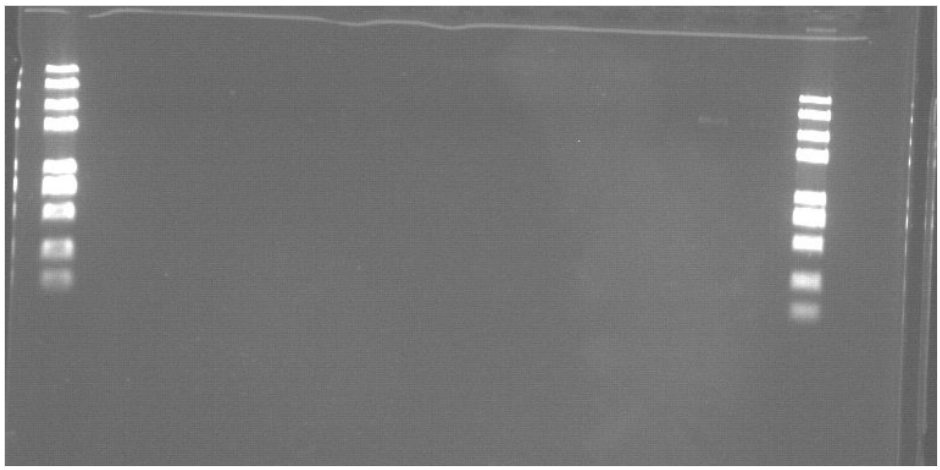
(lane 1 to 8: Trans 2K plus(contain Gelred), 2-4', 2-5', 2-6', 2-7', 2-8', 2-9', Mtr PCR pur 2-7)

Gel Extraction of ddpET28 and ddMtr Recorder: Chenyang Li, Jianjian Guo, Yawei Wu
Procedure:

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

sample	**ddMtr 2-4**	**ddMtr 2-4'**	**ddMtr 2-5**	**ddMtr 2-5'**	**ddMtr 2-6**	**ddMtr 2-6'**	**ddMtr 2-7**	**ddMtr 2-7'**	**ddMtr 2-8**	**ddMtr 2-8'**	**ddMtr 2-9**	**ddMtr 2-9'**	**ddpET28**	**ddpET28**
Concentration(ng/μL)	3.6	4.0	2.9	3.6	4.1	4.1	5.0	12.0	13.3	5.1	4.9	3.4	92.6	4.0
260/280	1.57	1.48	1.49	1.17	1.57	1.59	1.34	1.89	1.51	1.16	1.68	1.53	1.51	1.41
260/230	0.05	0.27	0.02	0.21	0.14	0.11	0.07	0.07	0.29	0.60	0.13	0.02	0.24	0.49

Agarose gel electrophoresis Result:



(lane 1 to 16: Trans 2K plus(contain Gelred), ddMtr 2-4, ddMtr 2-4', ddMtr 2-5, ddMtr 2-5', ddMtr 2-6, ddMtr 2-6', ddMtr 2-7, ddMtr 2-7', ddMtr 2-8, ddMtr 2-8', ddMtr 2-9, ddMtr 2-9', ddpET28, ddpET28, Trans 2K plus(contain Gelred))

There was nothing!

Ligation of pET28 and yeast Mtr PCR product Recorder: Chenyang　Li，Jianjian Guo

Experimental materials

1. double digestion product of pET28 1（92.6 ng/μL）
2. double digestion product of Mtr PCR product 2-7'（12.0 ng/μL）
3. 10× T4 DNA ligase buffer,T4 DNA ligase(bought from Thermo Fisher Scientific)

Procedure: Add to either of samples: 1 μL ddpET28 1 16.6 μL ddMtr PCR product 2-7' 2 μL 10* T4 DNA Ligase Buffer 0.4 μL T4 DNA Ligase

Mix gently and incubate at 16 degree Celsius for 8 hours.

Single digestion of plasmid containing RED Recorder: Xingwei Yang, Dongdong Jiang

Experimental materials

1. RED .
2. FastDigest restriction enzyme NcoI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. marker:Trans 2K Plus II

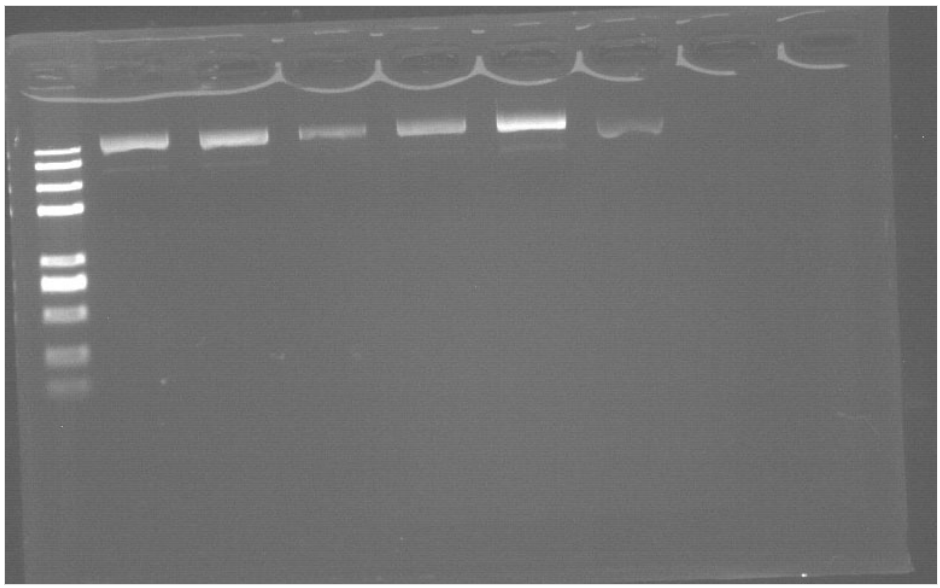
Sample	**RED 7**	**RED 8**
Concentration(ng/μL)	30.6	33.7
260/280	1.96	1.97
260/230	2.13	1.74

Reaction system:

Sample	**1**	**2**	**3**	**4**
RED(μL)	17	17	17	17
fastdigest green buffer(μL)	2	2	2	2
NcoI(μL)	1	1	1	1
total(μL)	20	20	20	20

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

Agarose gel electrophoresis Result:



(lane 1 to 7 : marker 2k plus II, RED-7.1-sd, RED-7.2-sd, RED-7, RED-8.1-sd, RED-8.2-sd, RED-8)

Double digestion of pET28 and Mtr Recorder:Meiying Cui， Wenfei Yu

Experimental materials

1. Mtr,PET28
2. FastDigest restriction enzyme NdeI, XhoI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water
4. marker:Trans 2K PlusII

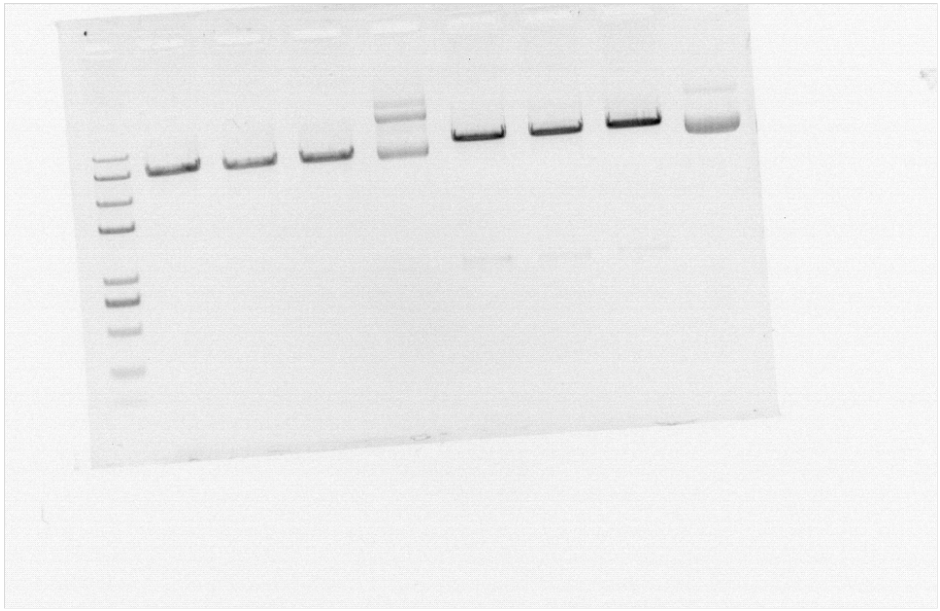
Reaction system:

Sample	**1**	**2**	**3**
XhoI(μL)	1	1	1
NdeI(μL)	1	1	1
nuclease-free water(μL)	8	8	8
fastdigest green buffer(μL)	2	2	2
PET28(μL)	8	8	8
total(μL)	20	20	20

Sample	**1**	**2**	**3**
XhoI(μL)	1	1	1
NdeI(μL)	1	1	1
nuclease-free water(μL)	15	15	15
fastdigest green buffer(μL)	2	2	2
Mtr(μL)	1	1	1
total(μL)	20	20	20

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

Agarose gel electrophoresis Result:



(lane 1 to 9: marker,pET283, pET282, pET281, PET28,Mtr2, Mtr1, Mtr3, Mtr)

Gel Extraction of Mtr PCR products Recorder: Meiying Cui, Wenfei Yu Procedure:

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

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

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

Single digestion of plasmid containing RED Recorder: Xingwei Yang,Shihan Zhu

Experimental materials

1. RED .
2. FastDigest restriction enzyme pst 1 and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. marker:Trans 2K Plus II

As the spectrophotometer didn't function well this noon,we haven't got the od and the concentrations of RED we made this morning,so we use the same procedures as we did this morning only changing the enzyme from NcoI to pst 1. Reaction system:

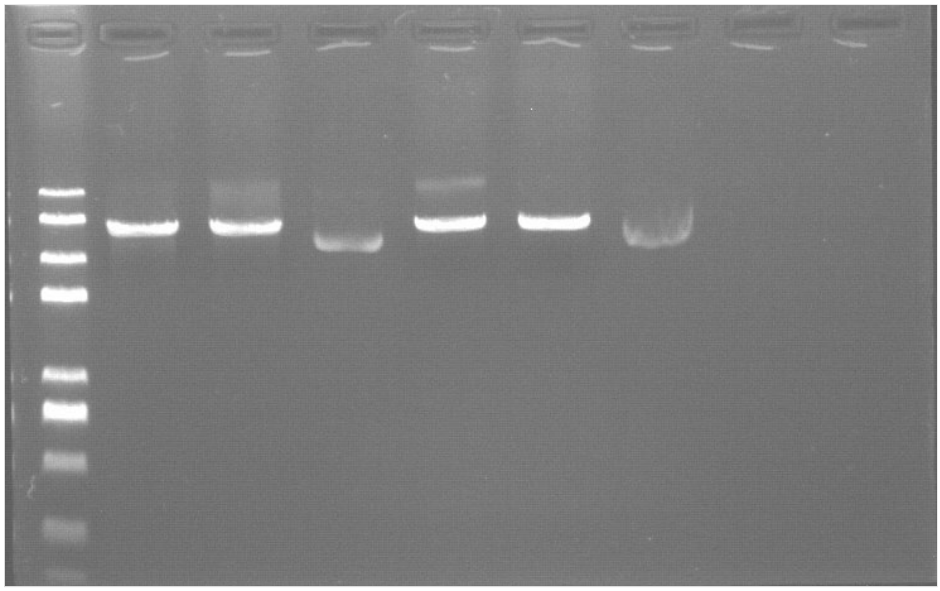
Sample	**1**	**2**	**3**	**4**
RED(μL)	17	17	17	17

Sample	1	2	3	4
fastdigest green buffer(μL)	2	2	2	2
pst 1(μL)	1	1	1	1
total(μL)	20	20	20	20

帮助

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

Agarose gel electrophoresis Result:



(lane 1 to 7 :marker 2k plus II, RED-7.1-sd, RED-7.2-sd, RED-7, RED-8.1-sd, RED-8.2-sd, RED-8)

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

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

sample	pBAD 1	pBAD 2	pBAD 3	pBAD 4
Concentration(ng/μL)	140.7	179.5	135.4	155.9
260/280	1.79	1.81	1.82	1.84
260/230	1.73	1.98	1.93	1.96

sample	RED 4	RED 7	RED 8	RED 9
Concentration(ng/μL)	48.1	41.2	53.7	45.9
260/280	1.87	1.87	1.84	1.89
260/230	1.65	1.70	1.84	1.67

Plasmid Extraction of the Red，pBAD Recorder: Zhenyu Jiang, Xingwei Yang Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the

adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

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

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sample	pBAD 2	pBAD 3	pBAD 4
Concentration(ng/μL)	123.1	130.6	141.5
260/280	1.67	1.64	1.66
260/230	1.06	0.98	1.00

sample	RED 4	RED 7	RED 8	RED 9
Concentration(ng/μL)	48.8	63.6	43.6	34.3
260/280	1.82	1.87	1.94	1.98
260/230	1.37	1.67	2.30	2.77

Plasmid Extraction of pET28 and Mtr Recorder: Wenfei Yu, 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, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

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

sample	Mtr1	Mtr2	Mtr4	pET28-1,2	pET28-3,4	pET28-5,6	pET28-7,8
Concentration(ng/μL)	523.8	682.6	615.5	71.8	95.0	134.0	97.4
260/280	1.84	1.93	1.85	1.76	1.83	1.82	1.74
260/230	2.25	2.68	2.41	1.38	1.84	1.86	1.38

PCR of Mtr Recorder: Wenfei Yu, Meiying Cui

Experimental materials

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

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

sample	1	2	3	4	5	6
sample	1	2	3	4	5	6
Sterilized ddH2O(μL)	22	22	22	22	22	22
2×Taq-PCR Master(μL)	25	25	25	25	25	25
Mtr(μL)	1	1	1	1	1	1
mtr-res-f(μL)	1	1	1	1	1	1
mtr-res-r(μL)	1	1	1	1	1	1
total(μL)	50	50	50	50	50	50

2.PCR reaction 1,2,3 Parameters setting：

stage	temperature(°C)	time
step 1	98	10 min

stage	temperature(°C)	time
step 2	98	10 s
step 3	56	5 s
step 4	72	5 min 30 s
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

4,5,6 Parameters setting：

stage	temperature	time
step 1	94	10 min
step 2	94	15 s
step 3	56	5 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: The picture shows nothing....

Date：7.9 Plasmid Extraction of Red and pBAD Recorder: Zhenyu Jiang, 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 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

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

sample	pBAD 1	pBAD 2	pBAD 3	pBAD 4
Concentration(ng/μL)	213.6	208.4	240.8	206.8
260/280	1.85	1.84	1.83	1.82
260/230	2.14	2.07	1.87	1.84

sample	RED 4	RED 7	RED 8	RED 9
Concentration(ng/μL)	113.8	79.9	84.7	100.3
260/280	1.74	1.81	1.81	1.82
260/230	1.20	1.57	1.49	1.65

Plasmid Extraction of the Red，pBAD Recorder: Zhenyu Jiang, 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	pBAD 1	pBAD 2	pBAD 3	pBAD 4

sample	pBAD 1	pBAD 2	pBAD 3	pBAD 4
Concentration(ng/μL)	165.1	101.3	119.8	121.0
260/280	1.74	1.81	1.77	1.77
260/230	1.54	2.07	1.28	1.57

sample	RED 4	RED 7	RED 8	RED 9
Concentration(ng/μL)	75.1	63.0	66.4	102.0
260/280	1.89	1.90	1.85	1.77
260/230	1.85	2.17	1.73	1.49

Plasmid Extraction of pET28 Recorder: Wenfei Yu, 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, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

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

sample	pET28-1	pET28-2	pET28-3	pET28-4	pET28-5	pET28-6	pET28-7	pET28-8
Concentration(ng/μL)	195.2	179.6	230.9	111.3	167.4	232.7	117.3	97.5
260/280	1.82	1.85	1.83	1.81	1.88	1.76	1.76	1.83
260/230	1.82	2.01	1.69	1.19	2.24	1.23	1.39	1.96

PCR of Mtr Recorder: Wenfei Yu, Meiying Cui

Experimental materials

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

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

sample	1	2	3	4	5	6
Sterilized ddH2O	22 μL	22 μL	22 μL	22 μL	22 μL	22 μL
2×Taq-PCR Master Mix	25 μL	25 μL	25 μL	25 μL	25 μL	25 μL
Mtr	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
sample	1	2	3	4	5	6
mtr-res-r	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
total	50 μL	50 μL	50 μL	50 μL	50 μL	50 μL

2.PCR reaction 1,2,3 Parameters setting：

stage	temperature(°C)	time
step 1	98	10 min
step 2	98	10 s
step 3	56	5 s
step 4	72	5 min 30 s

stage	temperature(°C)	time
step 5	72	10 min
step 6	4	--

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30 cycles(step 2 ~ step 4)

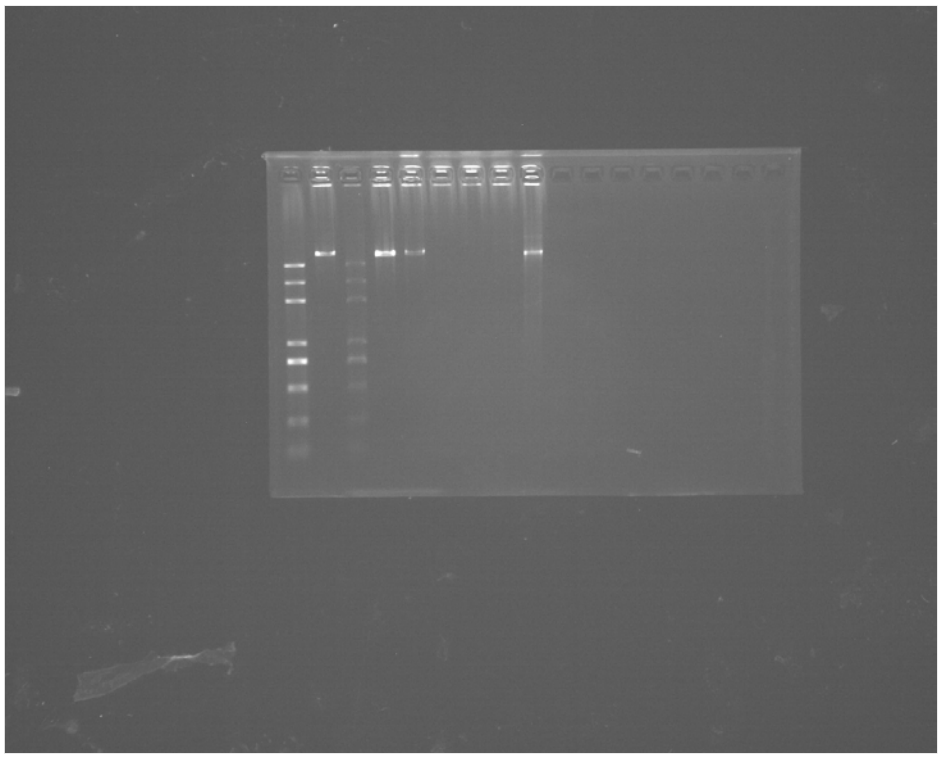
4,5,6 Parameters setting：

stage	temperature	time
step 1	94	10 min
step 2	94	15 s
step 3	56	5 s
step 4	72	5 min 30 s
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

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

4.Agarose gel electrophoresis mixed with 1 μL 6× DNA loading buffer each 5 μL 1:5 dilution of PCR product; mixed 1 μL 6× DNA loading buffer with 5μL template;120 V,30 min Result:



Recorder: Shihan Zhu Double digestion of pET28

Experimental materials

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

Reaction system:

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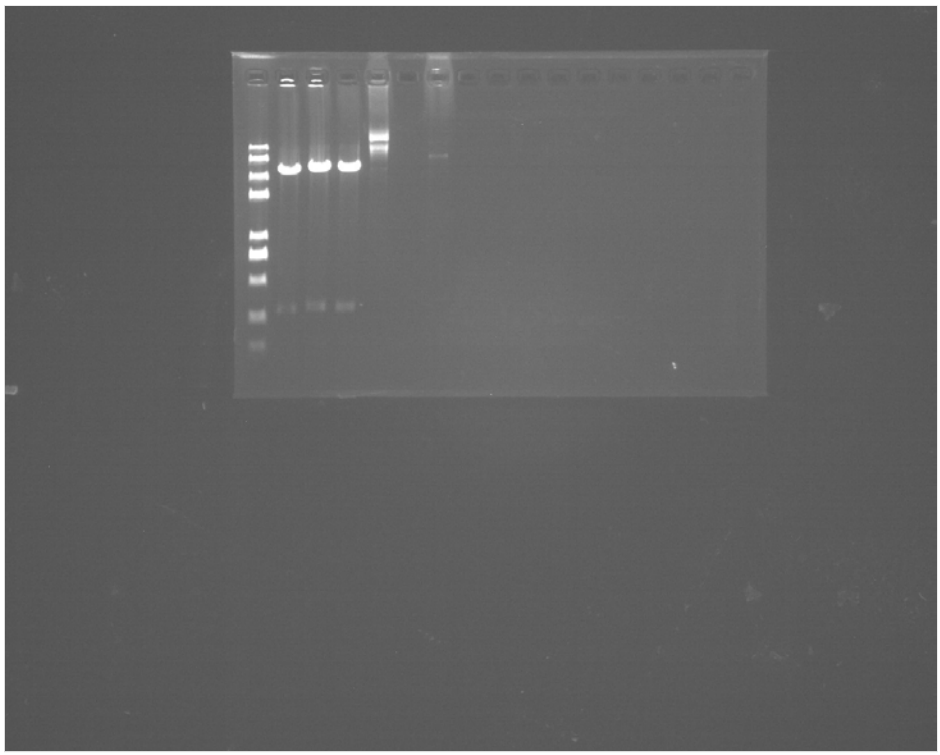
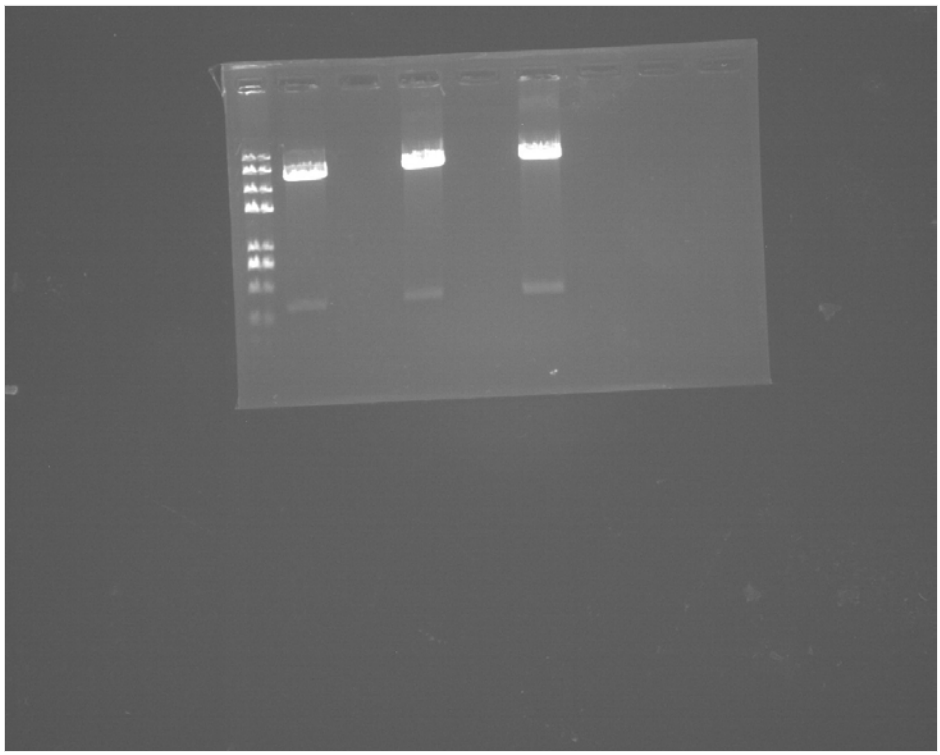
Sample	1	2	3
pET28(μL)	10	10	10
nuclease-free water(μL)	6	6	6
fastdigest green buffer(μL)	2	2	2
NdeI(μL)	1	1	1
XhoI(μL)	1	1	1
total(μL)	20	20	20

Mix gently and incubate at 37 degree Celsius for 60 mins .

Agarose gel electrophoresis Result:



(lane 1 to 6 : Trans 2K plus II(contain Gelred), 1, 2, 3, pET28, ligation products)

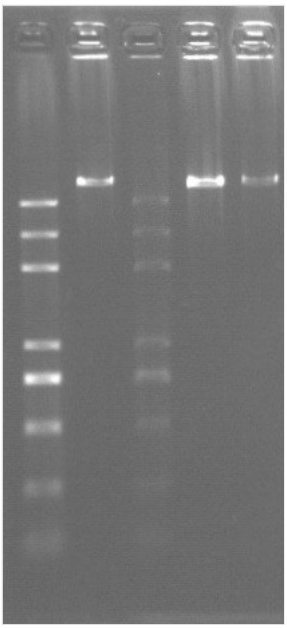


(lane 1 to 4 : Trans 2K plus II(contain Gelred), 1, 2, 3)

Gel Extraction of ddpET28 Recorder: Shihan Zhu Procedure:

1. Cut off the Gel part containing the target band. Get 6 tubes of AD and 5 tubes of BD.
2. Weigh the cut off Gel part.
3. Add Buffer B2 which 300 times the weight of the Gel.
4. Add isopropanol which is 1/3 volume of the Buffer B2.
5. Move the solution to adsorption column, 11000 rpm centifuge 30 s, discard filtrate.
6. Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once.
7. 12000 rpm centifuge 1 min.
8. Lying for 10 min.
9. Put the adsorption column in a new EP tube. Add 20 μL ddH2O, 10 min's standing, 12000 rpm centifuge 1.5 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

sample	**dd-pET28-1**	**dd-pET28-2**	**dd-pET28-3**
Concentration(ng/μL)	8.8	31.5	16.1
260/280	1.95	1.67	1.71
260/230	0.35	0.38	0.52

Agarose gel electrophoresis Result:  (lane 1 to 5 : Trans 2K plus(contain Gelred), ddpET28-1, Trans 2K plus(contain Gelred), ddpET28-2, ddpET28-3)

Date:7.7

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

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

sample	**pBAD 1**	**pBAD 2**	**pBAD 3**
Concentration(ng/ul)	219.5	273.7	195.4
260/280	1.87	1.78	1.81
260/230	1.64	1.24	1.60

sample	**pTB 1**	**pTB 2**
Concentration(ng/ul)	229.6	240.9
260/280	1.81	1.72
260/230	1.60	1.06

Plasmid Extraction of pET28 and Mtr Recorder: Wenfei Yu, 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 30 s, discard filtrate. 6.Add 500 µL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 µL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 µL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

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

sample	Mtr1	Mtr2	Mtr3	Mtr4	pET28-1,2	pET28-3,4	pET28-5,6	pET28-7,8
Concentration(ng/ul)	1029.1	565.0	776.8	656.5	110.6	157.6	170.8	153.6
260/280	1.81	1.84	1.89	1.83	1.86	1.63	1.71	1.78
260/230	1.61	2.31	2.35	2.18	1.96	0.65	0.86	1.45

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

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	Cys1	Cys 2	Cys 3	Cys 4	Cys new	pLUX 1	pLUX 2
Concentration(ng/ul)	324.6	296.8	192.9	199.0	370.5	431.7	385.3
260/280	1.82	1.88	1.88	1.88	1.88	1.87	1.87
260/230	1.62	2.31	2.35	2.29	2.33	2.21	2.25

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

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

sample	pBAD 1	pBAD 2	pBAD 3	pBAD 4
Concentration(ng/ul)	188.9	96.0	120.7	111.4
260/280	1.63	1.77	1.66	1.70
260/230	0.76	1.47	0.86	0.97

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

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sample	red 1	red 2	red 3	red 4	red 5	red 6	red 7	red 8
Concentration(ng/ul)	131.1	70.0	122.4	28.5	126.4	60.6	30.6	33.7
260/280	1.60	1.61	1.61	1.87	1.63	1.72	1.96	1.97
260/230	0.71	0.80	0.69	1.80	0.73	1.00	2.13	1.74

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Digestion of pET28 and Mtr on pSB1C3 Recorder: Shihan Zhu Materials:

1. Mtr on pSB1C3,pET28
2. FastDigest restriction enzyme NdeI, XhoI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water
4. marker:Trans 2K PlusII and Trans 2K Plus

Reaction system:

Sample	1	1'	2	2'	3	3'
XhoI(μL)	1	1	0	0	1	1
NdeI(μL)	0	0	1	1	1	1
nuclease-free water(μL)	9	9	9	9	8	8
fastdigest green buffer(μL)	2	2	2	2	2	2
pET28(μL)	8	8	8	8	8	8
total(μL)	20	20	20	20	20	20

Sample	1	1'	2	2'
XhoI(μL)	1	1	0	0
NdeI(μL)	0	0	1	1
nuclease-free water(μL)	16	16	16	16
fastdigest green buffer(μL)	2	2	2	2
Mtr(μL)	1	1	1	1
total(μL)	20	20	20	20

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

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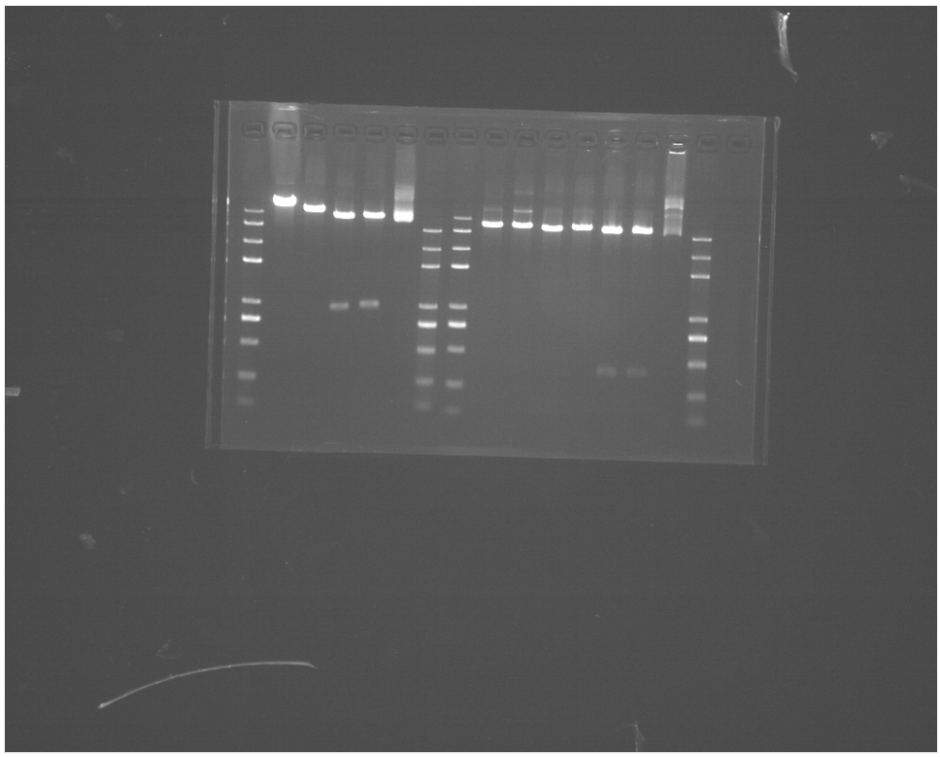
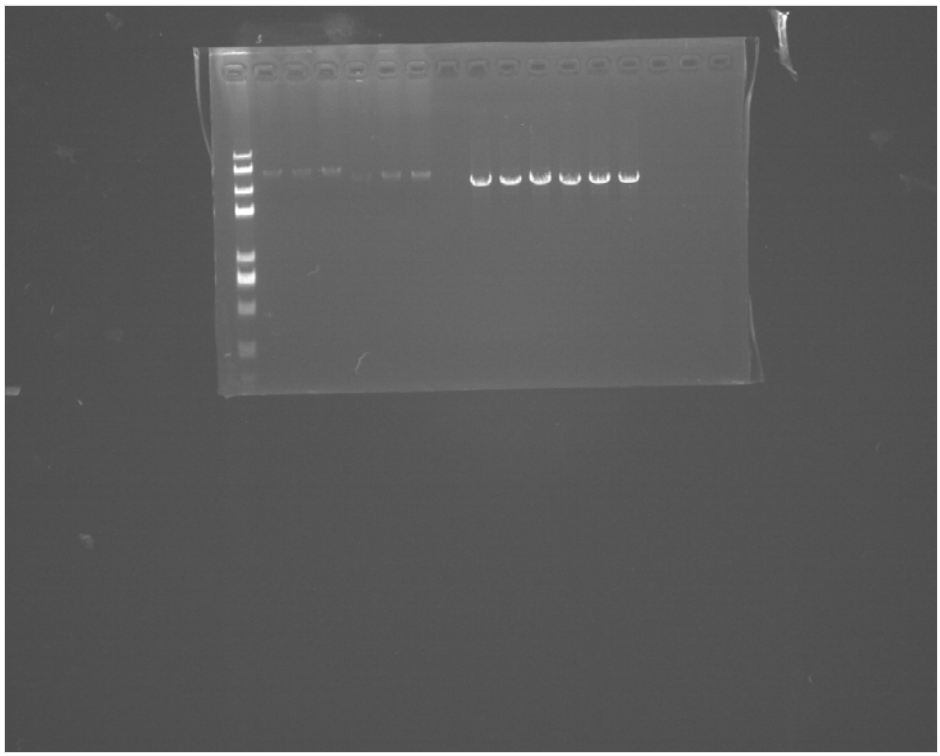
Agarose gel electrophoresis Result:



(lane left to right: marker 2K PlusII, sdmtr-1, sdmtr-1', sdmtr-2, sdmtr-2', mtr, marker 2K Plus, marker 2K PlusII, ddpET28-1, ddpET28-1', ddpET28-2, ddpET28-2', ddpET28-3, ddpET28-3', pET28, marker 2K Plus)

Date:7.8 Agarose gel electrophoresis Recorder: Shihan Zhu 110 V 30 min



(lane left to right: marker 2K PlusII, mtr-3-GE, mtr-2-GE, mtr-1-GE, pET28-1-GE, pET28-2-GE, pET28-3-GE, Mtr PCR pur 2-4, Mtr PCR pur 2-5, Mtr PCR pur 2-6, Mtr PCR pur 2-9, Mtr PCR pur 2-7, Mtr PCR pur 2-8)

Double digestion of pET28 and Mtr on pSB1C3 Recorder: Shihan Zhu Materials:

1. Mtr on pSB1C3,pET28
2. FastDigest restriction enzyme NdeI, XhoI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water
4. marker:Trans 2K Plus II

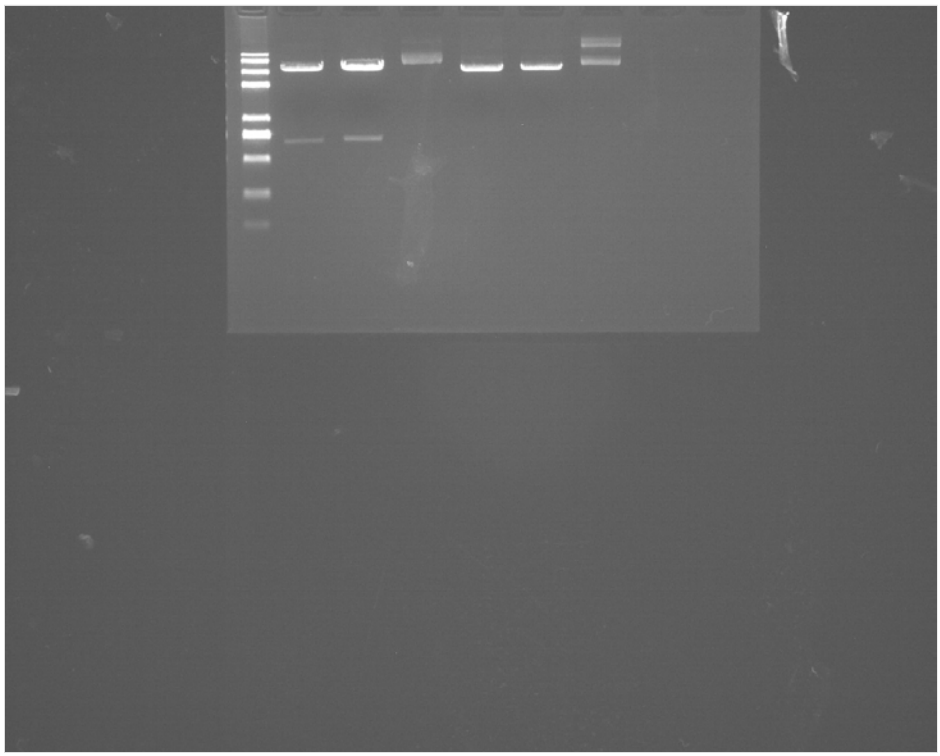
Reaction system:

Sample	**1**	**2**
XhoI(μL)	1	1
NdeI(μL)	1	1
nuclease-free water(μL)	0	0
fastdigest green buffer(μL)	2	2
PET28(μL)	16	16
total(μL)	20	20

Sample	**1**	**2**
XhoI(μL)	1	1
NdeI(μL)	1	1
nuclease-free water(μL)	14	14
fastdigest green buffer(μL)	2	2
Mtr(μL)	2	2
total(μL)	20	20

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

Agarose gel electrophoresis Result:



(lane 1 to 9: marker,ddmtr-1,ddmtr-2,mtr,ddpET28-1,ddpET28-2,pET28)

Recorder: Chenyang LI Double digestion of PCR products of Mtr Materials:

1. mtr PCR products(Mtr PCR pur 2-4, Mtr PCR pur 2-5, Mtr PCR pur 2-6, Mtr PCR pur 2-7, Mtr PCR pur 2-8, Mtr PCR pur 2-9,)
2. FastDigest restriction enzyme NdeI, XhoI and 10×FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water
4. marker:Trans 2K Plus

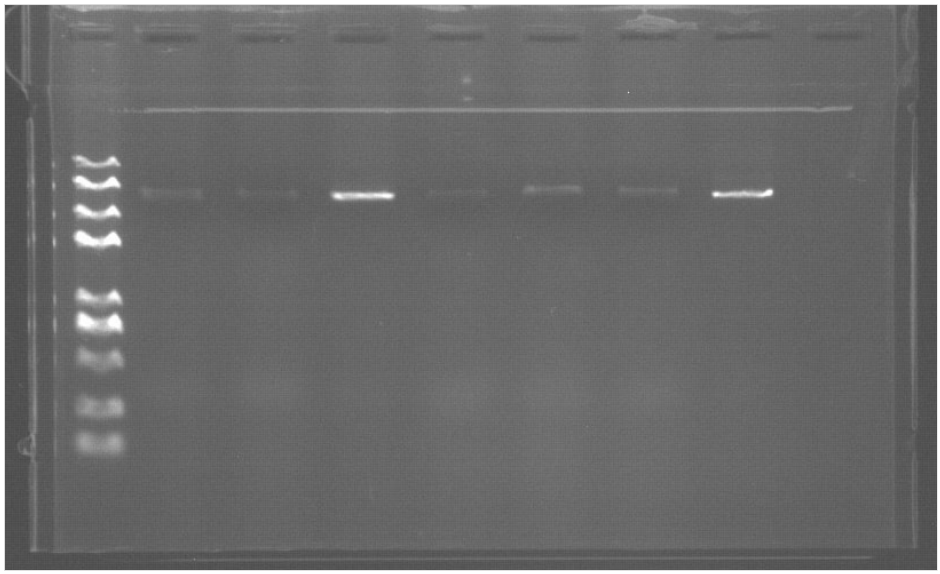
Reaction system:

Sample	**2-4,2-4'**	**2-5,2-5'**	**2-6,2-6'**	**2-7,2-7'**	**2-8,2-8'**	**2-9,2-9'**
mtr PCR products(μL)	Mtr PCR pur 2-4	Mtr PCR pur 2-5	Mtr PCR pur 2-6	Mtr PCR pur 2-7	Mtr PCR pur 2-8	Mtr PCR pur 2-9
mtr(μL)	2	1.5	4.5	1.3	2.6	2

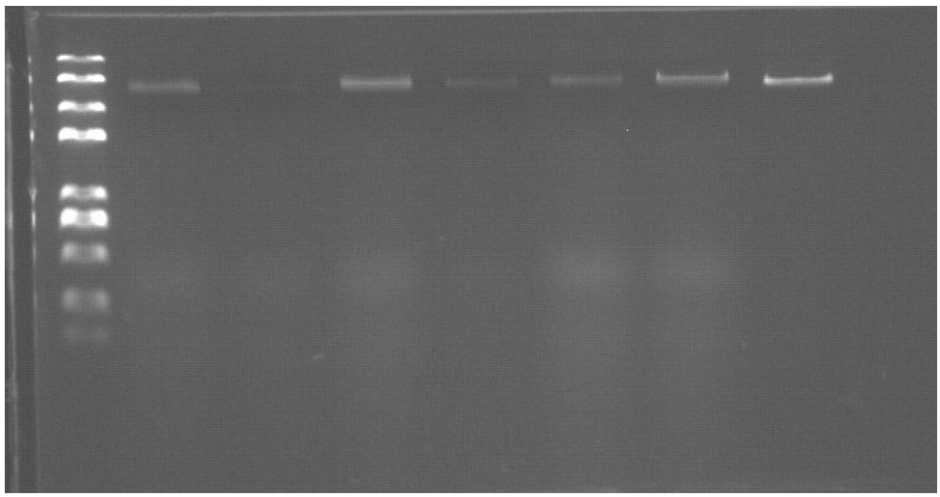
Sample	2-4,2-4'	2-5,2-5'	2-6,2-6'	2-7,2-7'	2-8,2-8'	2-9,2-9'
nuclease-free water(μL)	14	14.5	11.5	14.7	13.4	14
fastdigest green buffer(μL)	2	2	2	2	2	2
NdeI(μL)	1	1	1	1	1	1
XhoI(μL)	1	1	1	1	1	1
total(μL)	20	20	20	20	20	20

Mix gently and incubate at 37 degree Celsius for 60 mins .

Agarose gel electrophoresis Result:



(from left to right: Trans 2K plus(contain Gelred), 2-4, 2-5, 2-6, 2-7, 2-8, 2-9, Mtr PCR pur 2-5)



(from left to right: Trans 2K plus(contain Gelred), 2-4', 2-5', 2-6', 2-7', 2-8', 2-9', Mtr PCR pur 2-7)

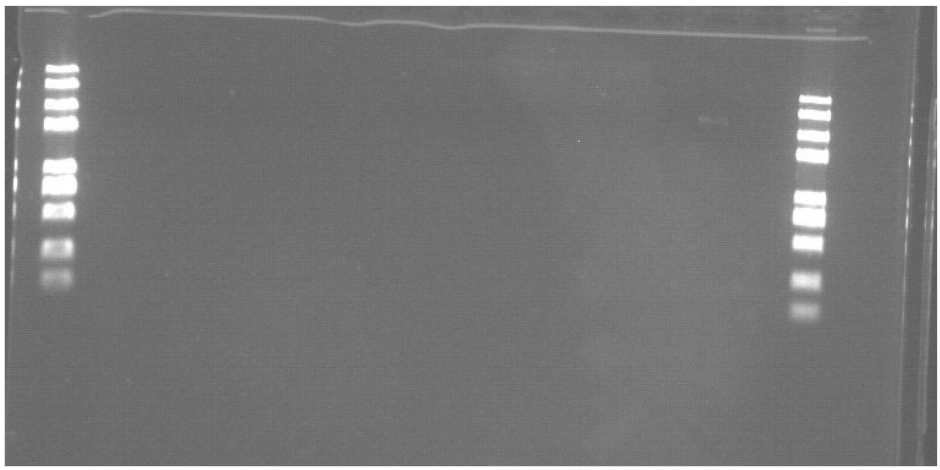
Gel Extraction of ddpET28 and ddMtr (Done by Chenyang LI, Jianjian Guo, Yawei Wu)
Procedure:

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

9. Put the adsorption column in a new EP tube. Add 20 μL ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

sample	ddMtr 2-4	ddMtr 2-4'	ddMtr 2-5	ddMtr 2-5'	ddMtr 2-6	ddMtr 2-6'	ddMtr 2-7	ddMtr 2-7'	ddMtr 2-8	ddMtr 2-8'	ddMtr 2-9	ddMtr 2-9'	ddpET28	ddpET28
Concentration(ng/ul)	3.6	4.0	2.9	3.6	4.1	4.1	5.0	12.0	13.3	5.1	4.9	3.4	92.6	4.0
260/280	1.57	1.48	1.49	1.17	1.57	1.59	1.34	1.89	1.51	1.16	1.68	1.53	1.51	1.41
260/230	0.05	0.27	0.02	0.21	0.14	0.11	0.07	0.07	0.29	0.60	0.13	0.02	0.24	0.49

Agarose gel electrophoresis Result:



(from left to right: Trans 2K plus(contain Gelred), ddMtr 2-4, ddMtr 2-4', ddMtr 2-5, ddMtr 2-5', ddMtr 2-6, ddMtr 2-6', ddMtr 2-7, ddMtr 2-7', ddMtr 2-8, ddMtr 2-8', ddMtr 2-9, ddMtr 2-9', ddpET28, ddpET28, Trans 2K plus(contain Gelred))

There was nothing!

Recorder: Chenyang LI, Jianjian GUO Ligation of pET28 and yeast Mtr PCR product

Material:

1. double digestion product of pET28 1（92.6 ng/μL）
2. double digestion product of Mtr PCR product 2-7'（12.0 ng/μL）
3. 10× T4 DNA ligase buffer,T4 DNA ligase(bought from Thermo Fisher Scientific)

Procedure: Add to either of samples: 1 μL ddpET28 1 16.6 μL ddMtr PCR product 2-7' 2 μL 10* T4 DNA Ligase Buffer 0.4 μL T4 DNA Ligase

Mix gently and incubate at 16 degree Celsius for 8 hour.

Single digestion of plasmid containing RED Recorder: Xingwei Yang,Dongdong Jiang
Materials:

1. RED .
2. FastDigest restriction enzyme NcoI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. marker:Trans 2K PlusII

	RED 7	RED 8
Concentration(ng/ul)	20.6	23.7
260/280	1.96	1.97
260/230	2.13	1.74

RED 7 RED 8

Reaction system:

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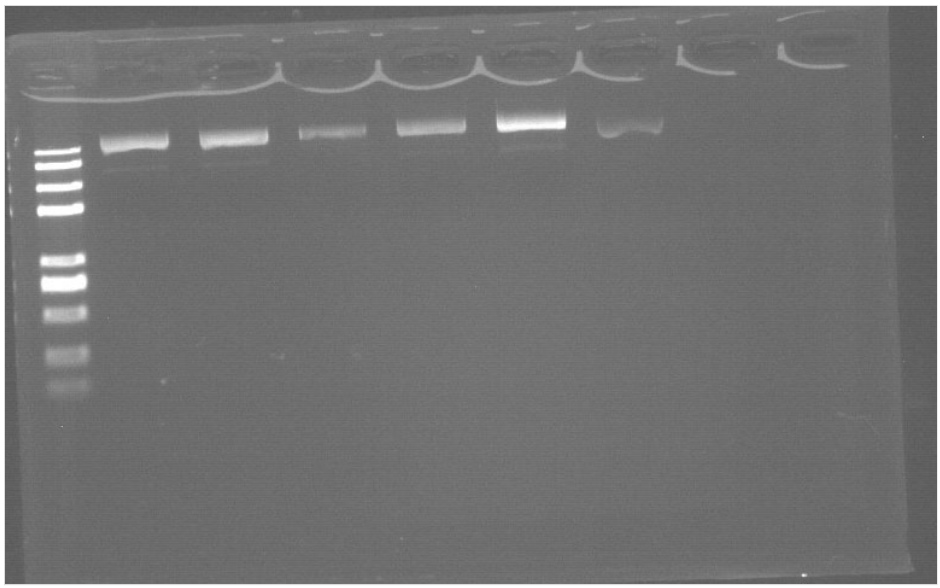
Sample	1	2	3	4
RED(μL)	17	17	17	17
fastdigest green buffer(μL)	2	2	2	2
NcoI(μL)	1	1	1	1
total(μL)	20	20	20	20

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

Agarose gel electrophoresis Result:



(lane1:marker 2k plus II lane2:RED-7.1-sd lane3: RED-7.2-sd lane4:RED-7 lane5:RED-8.1-sd lane6:RED-8.2-sd lane7:RED-8)

Double digestion of pET28 and Mtr Recorder:Meiying Cui， Wenfei Yu Materials:

1. Mtr,PET28
2. FastDigest restriction enzyme NdeI, XhoI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water
4. marker:Trans 2K PlusII

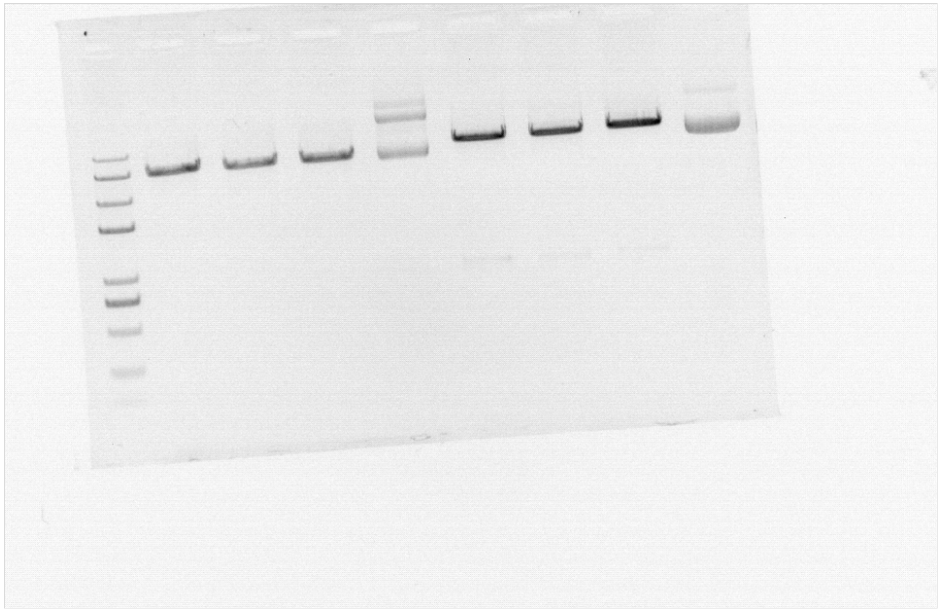
Reaction system:

Sample	1	2	3
XhoI(μL)	1	1	1
NdeI(μL)	1	1	1
nuclease-free water(μL)	8	8	8
fastdigest green buffer(μL)	2	2	2
PET28(μL)	8	8	8
total(μL)	20	20	20

Sample	1	2	3
XhoI(μL)	1	1	1
NdeI(μL)	1	1	1
nuclease-free water(μL)	15	15	15
fastdigest green buffer(μL)	2	2	2
Mtr(μL)	1	1	1
total(μL)	20	20	20

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

Agarose gel electrophoresis Result:



(lane 1 to 9: marker,PET283,2,1,PET28,Mtr2,1,3,Mtr)

Gel Extraction of Mtr PCR products Recorder: Meiying Cui, Wenfei Yu

Procedure:

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

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

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

Single digestion of plasmid containing RED Recorder: Xingwei Yang,Shihan Zhu Materials:

1. RED .
2. FastDigest restriction enzyme pst 1 and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. marker:Trans 2K PlusII

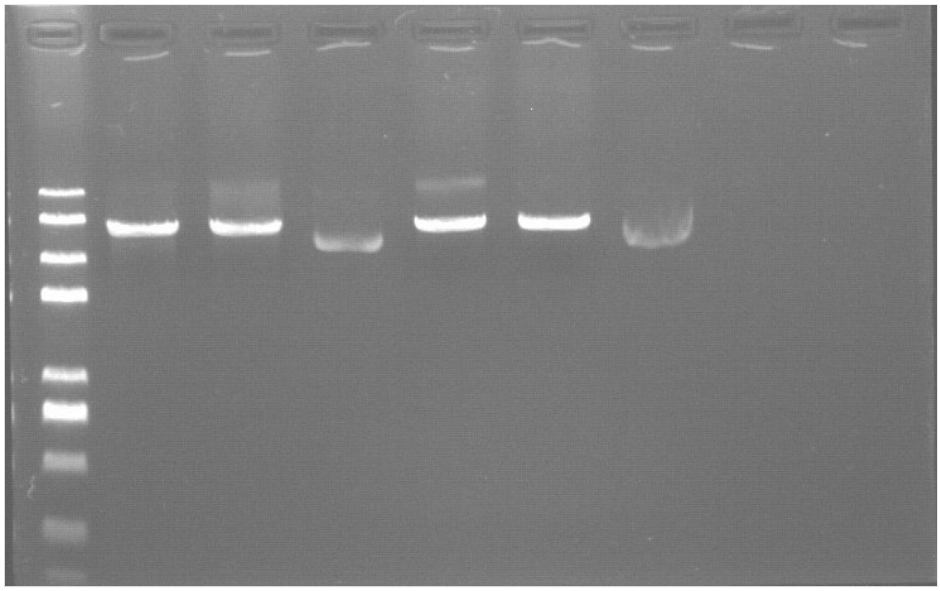
As the spectrophotometer didn't function well this noon,we haven't got the od and the concentrations of RED we made this morning,so we use the same procedures as we did this morning only changing the enzyme from NcoI to pst 1. Reaction system:

Sample	**1**	**2**	**3**	**4**
RED(μL)	17	17	17	17

Sample	1	2	3	4
fastdigest green buffer(μL)	2	2	2	2
pst 1(μL)	1	1	1	1
total(μL)	20	20	20	20

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

Agarose gel electrophoresis Result:



(lane1:marker 2k plus II lane2:RED-7.1-sd lane3: RED-7.2-sd lane4:RED-7 lane5:RED-8.1-sd lane6:RED-8.2-sd lane7:RED-8)

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

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

sample	pBAD 1	pBAD 2	pBAD 3	pBAD 4
Concentration(ng/ul)	140.7	179.5	135.4	155.9
260/280	1.79	1.81	1.82	1.84
260/230	1.73	1.98	1.93	1.96

sample	RED 4	RED 7	RED 8	RED 9
Concentration(ng/ul)	48.1	41.2	53.7	45.9
260/280	1.87	1.87	1.84	1.89
260/230	1.65	1.70	1.84	1.67

Plasmid Extraction of the Red，pBAD Recorder: Zhenyu Jiang,Xingwei Yang Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the

adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

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

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sample	pBAD 2	pBAD 3	pBAD 4
Concentration(ng/ul)	123.1	130.6	141.5
260/280	1.67	1.64	1.66
260/230	1.06	0.98	1.00

sample	RED 4	RED 7	RED 8	RED 9
Concentration(ng/ul)	48.8	63.6	43.6	34.3
260/280	1.82	1.87	1.94	1.98
260/230	1.37	1.67	2.30	2.77

Plasmid Extraction of pET28 and Mtr Recorder: Wenfei Yu, 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, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

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

sample	Mtr1	Mtr2	Mtr4	pET28-1,2	pET28-3,4	pET28-5,6	pET28-7,8
Concentration(ng/ul)	523.8	682.6	615.5	71.8	95.0	134.0	97.4
260/280	1.84	1.93	1.85	1.76	1.83	1.82	1.74
260/230	2.25	2.68	2.41	1.38	1.84	1.86	1.38

PCR of Mtr Recorder: Wenfei Yu, Meiying Cui

Experimental materials

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

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

sample	1	2	3	4	5	6
Sterilized ddH2O	22 μL	22 μL	22 μL	22 μL	22 μL	22 μL
2×Taq-PCR Master	25 μL	25 μL	25 μL	25 μL	25 μL	25 μL
Mtr	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
mtr-res-r	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
total	50 μL	50 μL	50 μL	50 μL	50 μL	50 μL

2.PCR reaction 1,2,3 Parameters setting：

stage	temperature	time
step 1	98	10 min

stage	temperature	time
step 2	98	10 s
step 3	56	5 s
step 4	72	5 min 30 s
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

4,5,6 Parameters setting：

stage	temperature	time
step 1	94	10 min
step 2	94	15 s
step 3	56	5 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: The picture shows nothing....

Date：7.9 Plasmid Extraction of the Red，pBAD Recorder: Zhenyu Jiang,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 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

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

sample	pBAD 1	pBAD 2	pBAD 3	pBAD 4
Concentration(ng/ul)	213.6	208.4	240.8	206.8
260/280	1.85	1.84	1.83	1.82
260/230	2.14	2.07	1.87	1.84

sample	RED 4	RED 7	RED 8	RED 9
Concentration(ng/ul)	113.8	79.9	84.7	100.3
260/280	1.74	1.81	1.81	1.82
260/230	1.20	1.57	1.49	1.65

Plasmid Extraction of the Red，pBAD Recorder: Zhenyu Jiang,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	pBAD 1	pBAD 2	pBAD 3	pBAD 4

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sample	pBAD 1	pBAD 2	pBAD 3	pBAD 4
Concentration(ng/ul)	165.1	101.3	119.8	121.0
260/280	1.74	1.81	1.77	1.77
260/230	1.54	2.07	1.28	1.57

sample	RED 4	RED 7	RED 8	RED 9
Concentration(ng/ul)	75.1	63.0	66.4	102.0
260/280	1.89	1.90	1.85	1.77
260/230	1.85	2.17	1.73	1.49

Plasmid Extraction of pET28 and Mtr Recorder: Wenfei Yu, 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, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

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

sample	pET28-1	pET28-2	pET28-3	pET28-4	pET28-5	pET28-6	pET28-7	pET28-8
Concentration(ng/ul)	195.2	179.6	230.9	111.3	167.4	232.7	117.3	97.5
260/280	1.82	1.85	1.83	1.81	1.88	1.76	1.76	1.83
260/230	1.82	2.01	1.69	1.19	2.24	1.23	1.39	1.96

PCR of pET28 Recorder: Wenfei Yu, Meiying Cui

Experimental materials

1. Template: pET28;
2. Primer: mtr-res-f, mtr-res-r. Designed by ourselves, synthesized by Sangon Biotech;
3. Sterilized ddH2O, 2×Taq-PCR Master, bought from Sangon Biotech.

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

sample	1	2	3	4	5	6
Sterilized ddH2O	22 μL	22 μL	22 μL	22 μL	22 μL	22 μL
2×Taq-PCR Master Mix	25 μL	25 μL	25 μL	25 μL	25 μL	25 μL
Mtr	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
mtr-res-r	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
total	50 μL	50 μL	50 μL	50 μL	50 μL	50 μL

2.PCR reaction 1,2,3 Parameters setting：

stage	temperature	time
step 1	98	10 min
step 2	98	10 s
step 3	56	5 s
step 4	72	5 min 30 s

stage	temperature	time
step 5	72	10 min
step 6	4	--

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30 cycles(step 2 ~ step 4)

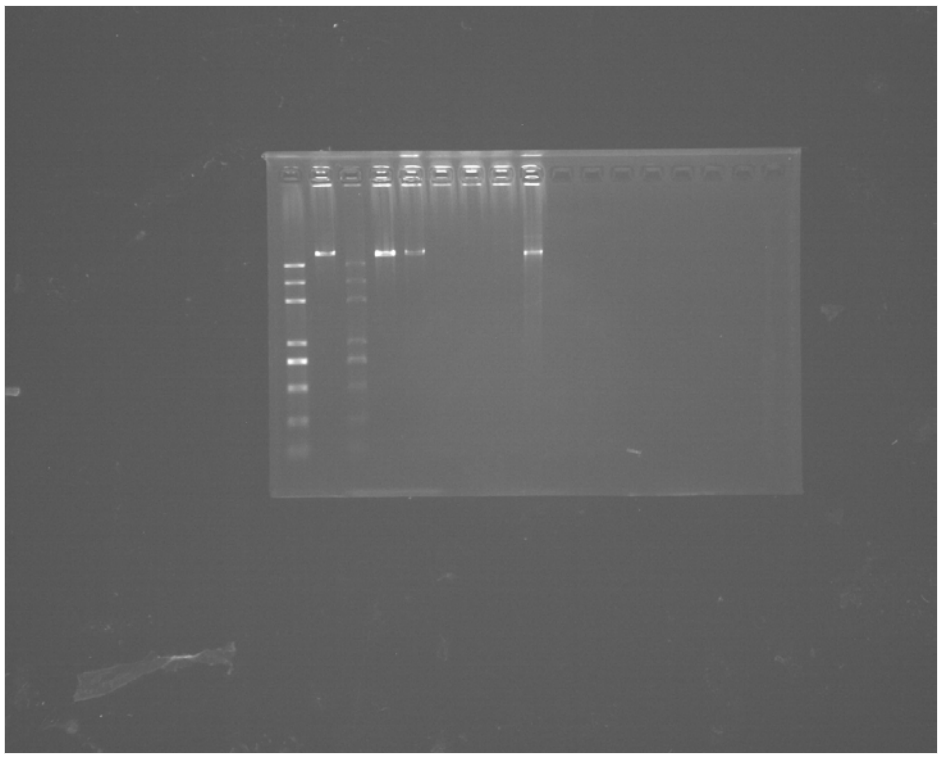
4,5,6 Parameters setting：

stage	temperature	time
step 1	94	10 min
step 2	94	15 s
step 3	56	5 s
step 4	72	5 min 30 s
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

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

4.Agarose gel electrophoresis mixed with 1 μL 6× DNA loading buffer each 5 μL 1:5 dilution of PCR product; mixed 1 μL 6× DNA loading buffer with 5μL template;120 V,30 min Result:



Recorder: Shihan Zhu Double digestion of pET28 Materials:

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

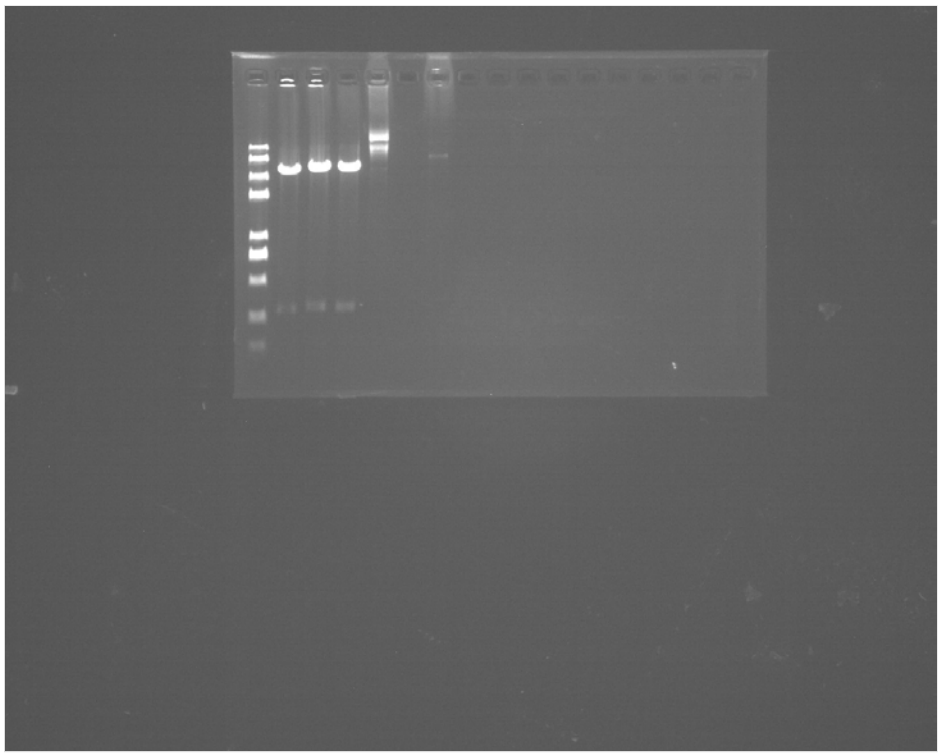
Reaction system:

Sample	1	2	3
pET28(μL)	10	10	10

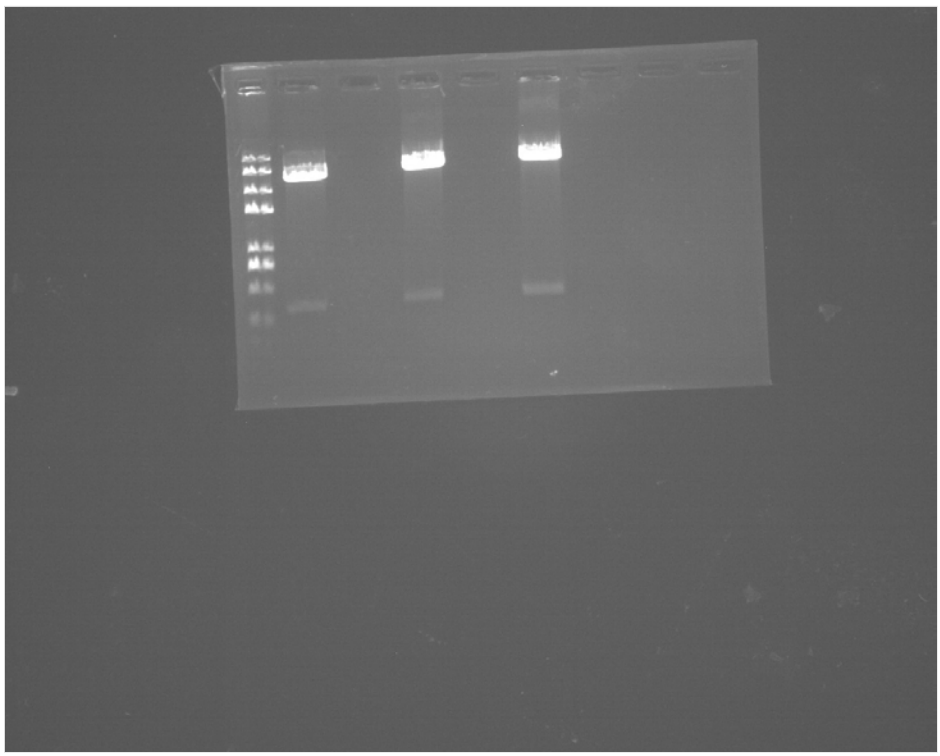
Sample	1	2	3
nuclease-free water(μL)	6	6	6
fastdigest green buffer(μL)	2	2	2
NdeI(μL)	1	1	1
XhoI(μL)	1	1	1
total(μL)	20	20	20

Mix gently and incubate at 37 degree Celsius for 60 mins .

Agarose gel electrophoresis Result:



(from left to right: Trans 2K plus II(contain Gelred), 1, 2, 3, pET28, ligation products)



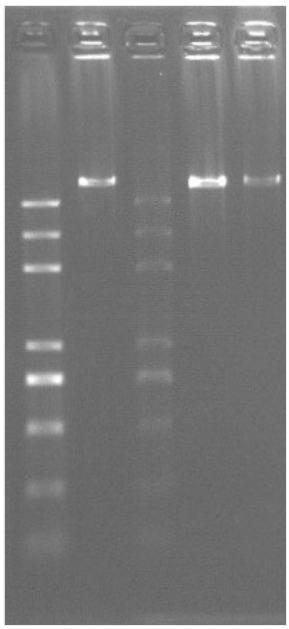
(from left to right: Trans 2K plus II(contain Gelred), 1, 2, 3)

Gel Extraction of ddpET28 Recorder: Shihan Zhu Procedure:

1. Cut off the Gel part containing the target band. Get 6 tubes of AD and 5 tubes of BD.

2. Weigh the cut off Gel part.
3. Add Buffer B2 which 300 times the weight of the Gel.
4. Add isopropanol which is 1/3 volume of the Buffer B2.
5. Move the solution to adsorption column, 11000 rpm centifuge 30 s, discard filtrate.
6. Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once.
7. 12000 rpm centifuge 1 min.
8. Lying for 10 min.
9. Put the adsorption column in a new EP tube. Add 20 μL ddH2O, 10 min's standing, 12000 rpm centifuge 1.5 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

sample	**dd-pET28-1**	**dd-pET28-2**	**dd-pET28-3**
Concentration(ng/ul)	8.8	31.5	16.1
260/280	1.95	1.67	1.71
260/230	0.35	0.38	0.52

Agarose gel electrophoresis Result:  (from left to right: Trans 2K plus(contain Gelred), ddpET28-1, Trans 2K plus(contain Gelred), ddpET28-2, ddpET28-3) **Date 7.7**

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

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

sample	**pBAD 1**	**pBAD 2**	**pBAD 3**
Concentration(ng/ul)	219.5	273.7	195.4
260/280	1.87	1.78	1.81
260/230	1.64	1.24	1.60

sample	**pTB 1**	**pTB 2**
Concentration(ng/ul)	229.6	240.9
260/280	1.81	1.72
260/230	1.60	1.06

帮助

Plasmid Extraction of pET28 and Mtr Recorder: Wenfei Yu, 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 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

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

sample	Mtr1	Mtr2	Mtr3	Mtr4	pET28-1,2	pET28-3,4	pET28-5,6	pET28-7,8
Concentration(ng/ul)	1029.1	563.0	778.8	656.5	116.0	157.5	170.3	133.6
260/280	1.81	1.84	1.89	1.83	1.86	1.63	1.71	1.78
260/230	1.61	2.31	2.35	2.18	1.96	0.65	0.86	1.45

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

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	Cys1	Cys 2	Cys 3	Cys 4	Cys new	pLUX 1	pLUX 2
Concentration(ng/ul)	324.6	296.8	192.9	199.0	370.5	431.7	385.3
260/280	1.82	1.88	1.88	1.88	1.88	1.87	1.87
260/230	1.62	2.31	2.35	2.29	2.33	2.21	2.25

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

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

sample	pBAD 1	pBAD 2	pBAD 3	pBAD 4
Concentration(ng/ul)	188.9	96.0	120.7	111.4
260/280	1.63	1.77	1.66	1.70
260/230	0.76	1.47	0.86	0.97

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

sample	red 1	red 2	red 3	red 4	red 5	red 6	red 7	red 8
Concentration(ng/ul)	131.1	70.0	122.4	28.5	126.4	60.6	30.6	33.7
260/280	1.60	1.61	1.61	1.87	1.63	1.72	1.96	1.97
260/230	0.71	0.80	0.69	1.80	0.73	1.00	2.13	1.74

帮助

Digestion of pET28 and Mtr on pSB1C3 Recorder: Shihan Zhu Materials:

1. Mtr on pSB1C3,pET28
2. FastDigest restriction enzyme NdeI, XhoI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water
4. marker:Trans 2K PlusII and Trans 2K Plus

Reaction system:

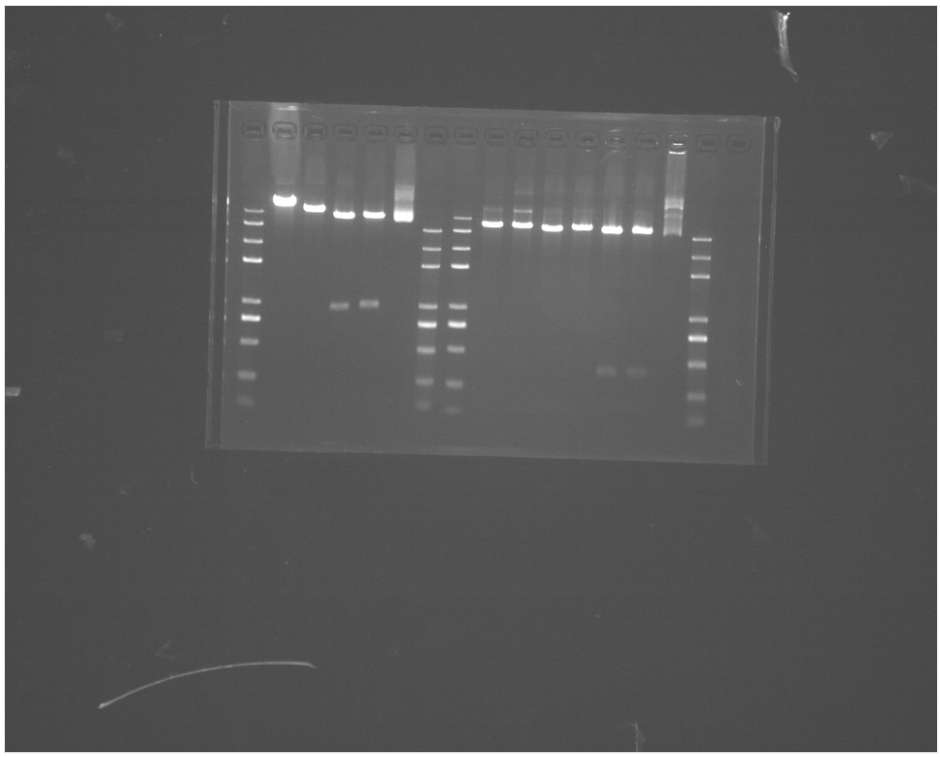
Sample	1	1'	2	2'	3	3'
XhoI(μL)	1	1	0	0	1	1
NdeI(μL)	0	0	1	1	1	1
nuclease-free water(μL)	9	9	9	9	8	8
fastdigest green buffer(μL)	2	2	2	2	2	2
pET28(μL)	8	8	8	8	8	8
total(μL)	20	20	20	20	20	20

Sample	1	1'	2	2'
XhoI(μL)	1	1	0	0
NdeI(μL)	0	0	1	1
nuclease-free water(μL)	16	16	16	16
fastdigest green buffer(μL)	2	2	2	2
Mtr(μL)	1	1	1	1
total(μL)	20	20	20	20

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

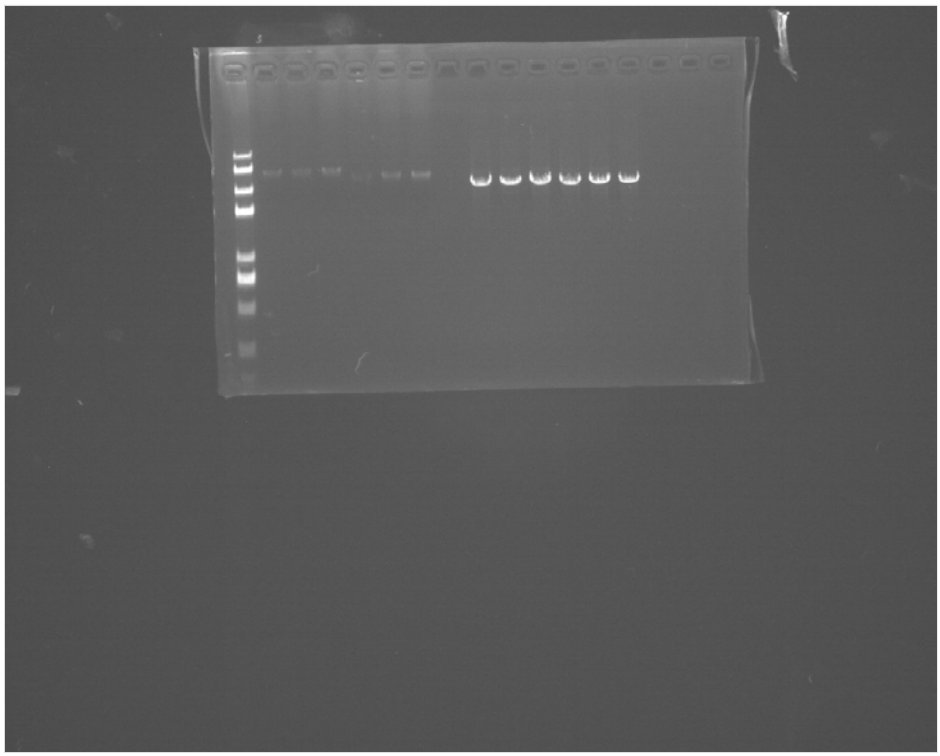
帮助

Agarose gel electrophoresis Result:



(lane left to right: marker 2K PlusII, sdmtr-1, sdmtr-1', sdmtr-2, sdmtr-2', mtr, marker 2K Plus, marker 2K PlusII, ddpET28-1, ddpET28-1', ddpET28-2, ddpET28-2', ddpET28-3, ddpET28-3', pET28, marker 2K Plus)

Date 7.8 Agarose gel electrophoresis Recorder: Shihan Zhu 110 V 30 min



(lane left to right: marker 2K PlusII, mtr-3-GE, mtr-2-GE, mtr-1-GE, pET28-1-GE, pET28-2-GE, pET28-3-GE, Mtr PCR pur 2-4, Mtr PCR pur 2-5, Mtr PCR pur 2-6, Mtr PCR pur 2-9, Mtr PCR pur 2-7, Mtr PCR pur 2-8)

Double digestion of pET28 and Mtr on pSB1C3 Recorder: Shihan Zhu Materials:

1. Mtr on pSB1C3,pET28
2. FastDigest restriction enzyme NdeI, XhoI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water
4. marker:Trans 2K Plus II

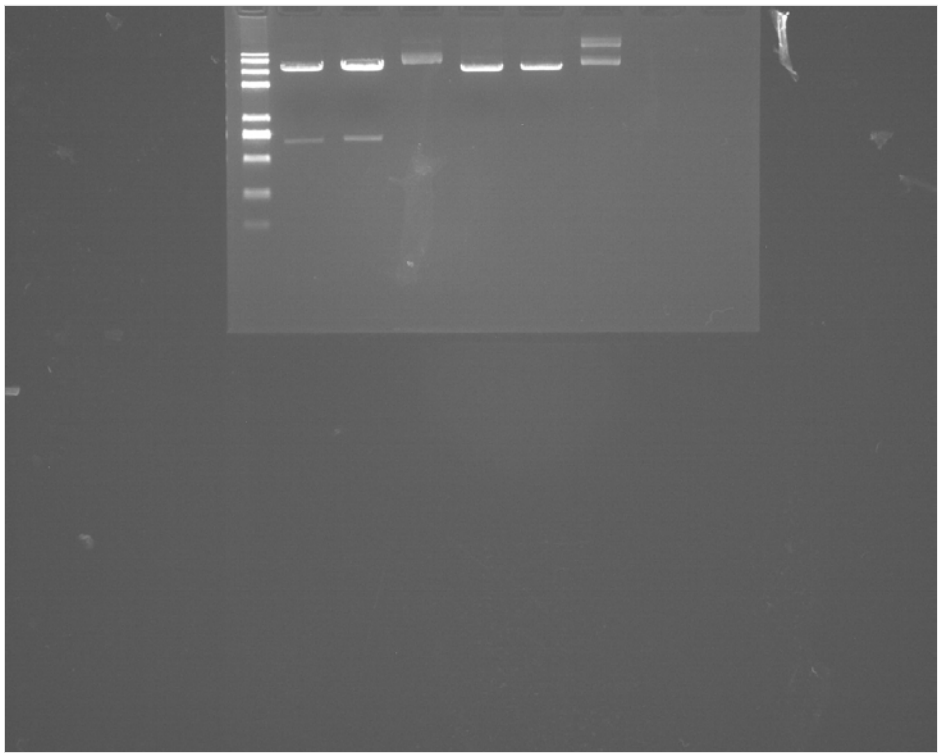
Reaction system:

Sample	**1**	**2**
XhoI(μL)	1	1
NdeI(μL)	1	1
nuclease-free water(μL)	0	0
fastdigest green buffer(μL)	2	2
PET28(μL)	16	16
total(μL)	20	20

Sample	**1**	**2**
XhoI(μL)	1	1
NdeI(μL)	1	1
nuclease-free water(μL)	14	14
fastdigest green buffer(μL)	2	2
Mtr(μL)	2	2
total(μL)	20	20

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

Agarose gel electrophoresis Result:



(lane 1 to 9: marker,ddmtr-1,ddmtr-2,mtr,ddpET28-1,ddpET28-2,pET28)

Recorder: Chenyang LI Double digestion of PCR products of Mtr Materials:

1. mtr PCR products(Mtr PCR pur 2-4, Mtr PCR pur 2-5, Mtr PCR pur 2-6, Mtr PCR pur 2-7, Mtr PCR pur 2-8, Mtr PCR pur 2-9,)
2. FastDigest restriction enzyme NdeI, XhoI and 10×FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water
4. marker:Trans 2K Plus

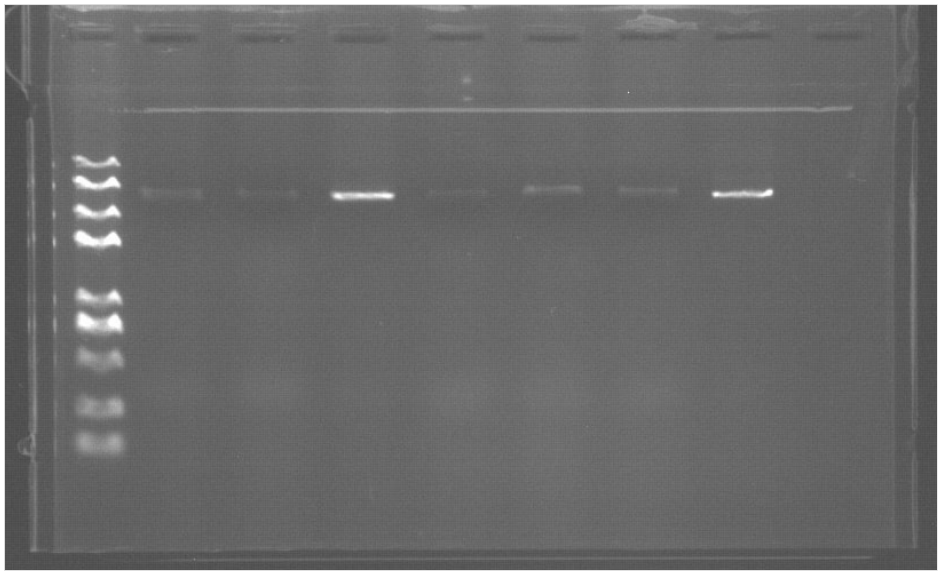
Reaction system:

Sample	**2-4,2-4'**	**2-5,2-5'**	**2-6,2-6'**	**2-7,2-7'**	**2-8,2-8'**	**2-9,2-9'**
mtr PCR products(μL)	Mtr PCR pur 2-4	Mtr PCR pur 2-5	Mtr PCR pur 2-6	Mtr PCR pur 2-7	Mtr PCR pur 2-8	Mtr PCR pur 2-9
mtr(μL)	2	1.5	4.5	1.3	2.6	2

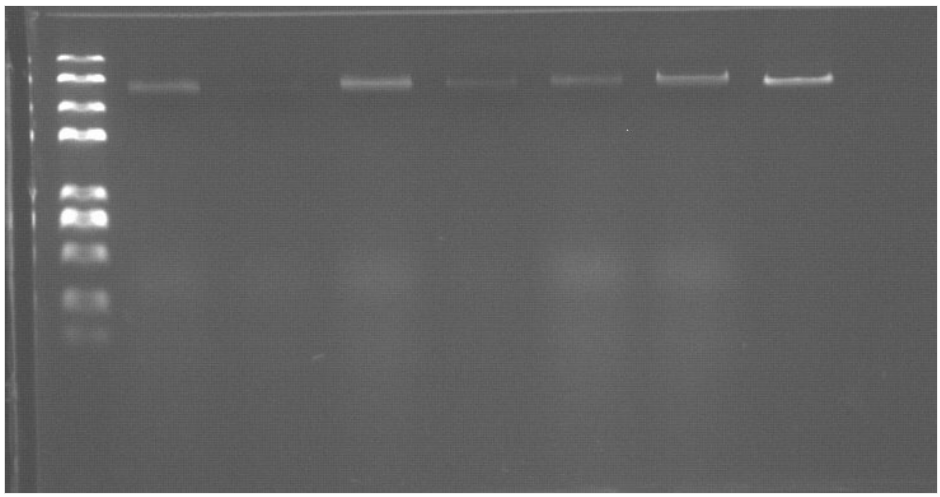
Sample	2-4,2-4'	2-5,2-5'	2-6,2-6'	2-7,2-7'	2-8,2-8'	2-9,2-9'
nuclease-free water(μL)	14	14.5	11.5	14.7	13.4	14
fastdigest green buffer(μL)	2	2	2	2	2	2
NdeI(μL)	1	1	1	1	1	1
XhoI(μL)	1	1	1	1	1	1
total(μL)	20	20	20	20	20	20

Mix gently and incubate at 37 degree Celsius for 60 mins .

Agarose gel electrophoresis Result:



(from left to right: Trans 2K plus(contain Gelred), 2-4, 2-5, 2-6, 2-7, 2-8, 2-9, Mtr PCR pur 2-5)



(from left to right: Trans 2K plus(contain Gelred), 2-4', 2-5', 2-6', 2-7', 2-8', 2-9', Mtr PCR pur 2-7)

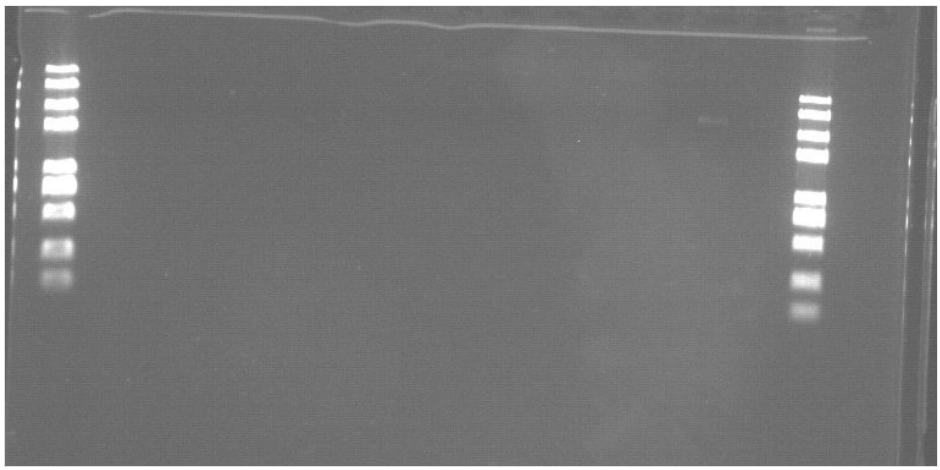
Gel Extraction of ddpET28 and ddMtr (Done by Chenyang LI, Jianjian Guo, Yawei Wu)
Procedure:

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

9. Put the adsorption column in a new EP tube. Add 20 μL ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

sample	ddMtr 2-4	ddMtr 2-4'	ddMtr 2-5	ddMtr 2-5'	ddMtr 2-6	ddMtr 2-6'	ddMtr 2-7	ddMtr 2-7'	ddMtr 2-8	ddMtr 2-8'	ddMtr 2-9	ddMtr 2-9'	ddpET28	ddpET28
Concentration(ng/ul)	3.6	4.0	2.9	3.6	4.1	4.1	5.0	12.0	13.3	5.1	4.9	3.4	92.6	4.0
260/280	1.57	1.48	1.49	1.17	1.57	1.59	1.34	1.89	1.51	1.16	1.68	1.53	1.51	1.41
260/230	0.05	0.27	0.02	0.21	0.14	0.11	0.07	0.07	0.29	0.60	0.13	0.02	0.24	0.49

Agarose gel electrophoresis Result:



(from left to right: Trans 2K plus(contain Gelred), ddMtr 2-4, ddMtr 2-4', ddMtr 2-5, ddMtr 2-5', ddMtr 2-6, ddMtr 2-6', ddMtr 2-7, ddMtr 2-7', ddMtr 2-8, ddMtr 2-8', ddMtr 2-9, ddMtr 2-9', ddpET28, ddpET28, Trans 2K plus(contain Gelred))

There was nothing!

Recorder: Chenyang LI, Jianjian GUO Ligation of pET28 and yeast Mtr PCR product

Material:

1. double digestion product of pET28 1（92.6 ng/μL）
2. double digestion product of Mtr PCR product 2-7'（12.0 ng/μL）
3. 10× T4 DNA ligase buffer,T4 DNA ligase(bought from Thermo Fisher Scientific)

Procedure: Add to either of samples: 1 μL ddpET28 1 16.6 μL ddMtr PCR product 2-7' 2 μL 10* T4 DNA Ligase Buffer 0.4 μL T4 DNA Ligase

Mix gently and incubate at 16 degree Celsius for 8 hour.

Single digestion of plasmid containing RED Recorder: Xingwei Yang,Dongdong Jiang
Materials:

1. RED .
2. FastDigest restriction enzyme NcoI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. marker:Trans 2K PlusII

	RED 7	RED 8
Concentration(ng/ul)	20.6	23.7
260/280	1.96	1.97
260/230	2.13	1.74

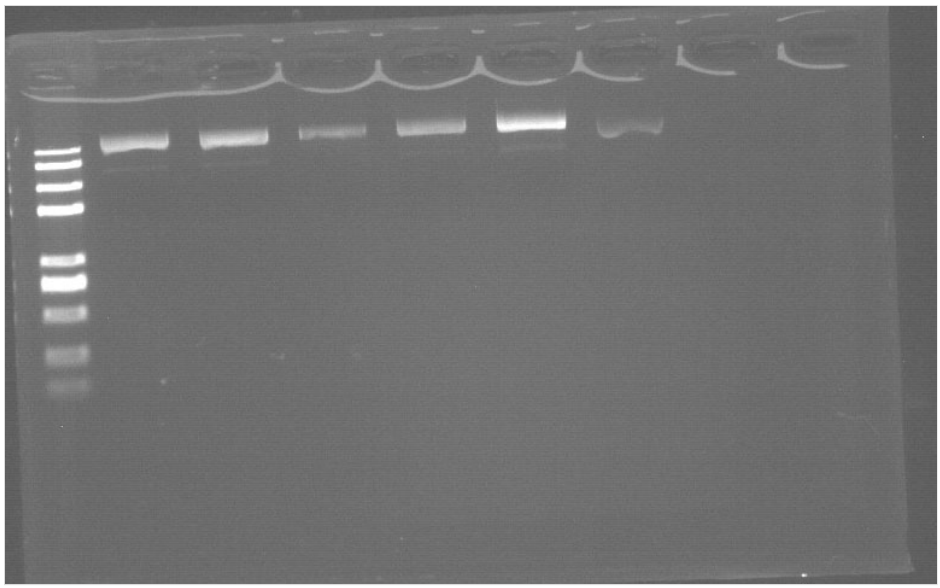
RED 7 RED 8

Reaction system:

Sample	1	2	3	4
RED(μL)	17	17	17	17
fastdigest green buffer(μL)	2	2	2	2
NcoI(μL)	1	1	1	1
total(μL)	20	20	20	20

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

Agarose gel electrophoresis Result:



(lane1:marker 2k plus II lane2:RED-7.1-sd lane3: RED-7.2-sd lane4:RED-7 lane5:RED-8.1-sd lane6:RED-8.2-sd lane7:RED-8)

Double digestion of pET28 and Mtr Recorder:Meiying Cui， Wenfei Yu Materials:

1. Mtr,PET28
2. FastDigest restriction enzyme NdeI, XhoI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water
4. marker:Trans 2K PlusII

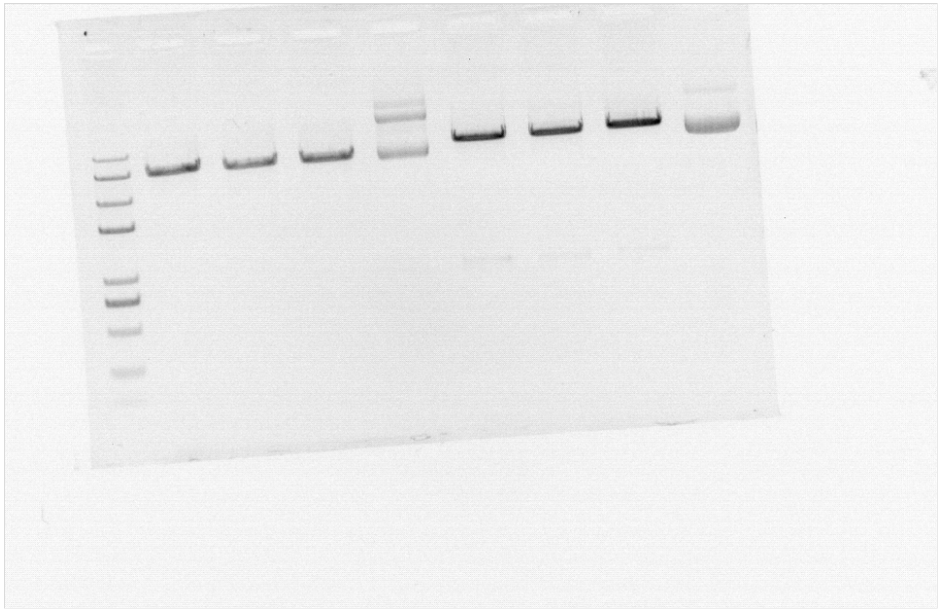
Reaction system:

Sample	1	2	3
XhoI(μL)	1	1	1
NdeI(μL)	1	1	1
nuclease-free water(μL)	8	8	8
fastdigest green buffer(μL)	2	2	2
PET28(μL)	8	8	8
total(μL)	20	20	20

Sample	1	2	3
XhoI(μL)	1	1	1
NdeI(μL)	1	1	1
nuclease-free water(μL)	15	15	15
fastdigest green buffer(μL)	2	2	2
Mtr(μL)	1	1	1
total(μL)	20	20	20

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

Agarose gel electrophoresis Result:



(lane 1 to 9: marker,PET283,2,1,PET28,Mtr2,1,3,Mtr)

Gel Extraction of Mtr PCR products Recorder: Meiying Cui, Wenfei Yu

Procedure:

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

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

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

Single digestion of plasmid containing RED Recorder: Xingwei Yang,Shihan Zhu Materials:

1. RED .
2. FastDigest restriction enzyme pst 1 and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. marker:Trans 2K PlusII

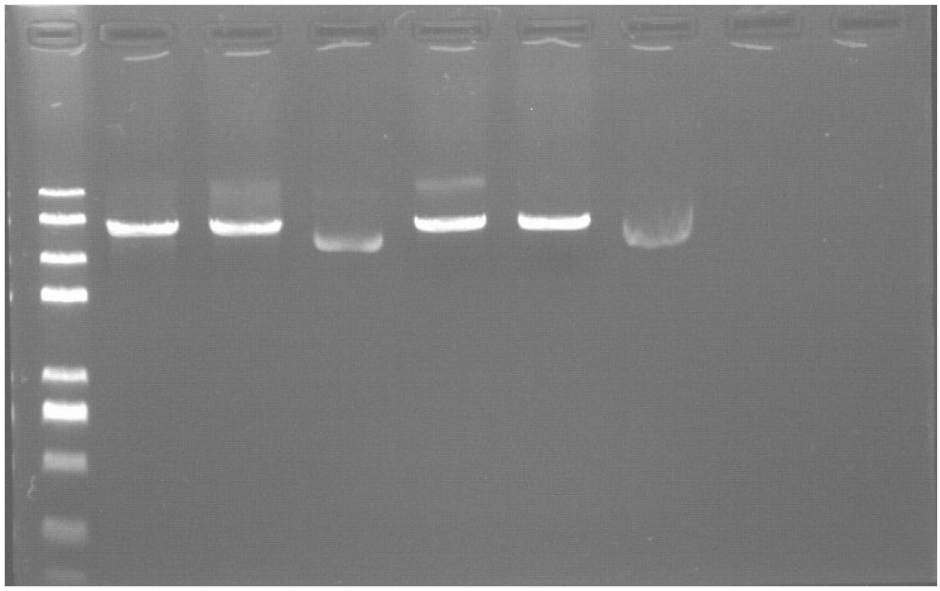
As the spectrophotometer didn't function well this noon,we haven't got the od and the concentrations of RED we made this morning,so we use the same procedures as we did this morning only changing the enzyme from NcoI to pst 1. Reaction system:

Sample	**1**	**2**	**3**	**4**
RED(μL)	17	17	17	17

Sample	1	2	3	4
fastdigest green buffer(μL)	2	2	2	2
pst 1(μL)	1	1	1	1
total(μL)	20	20	20	20

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

Agarose gel electrophoresis Result:



(lane1:marker 2k plus II lane2:RED-7.1-sd lane3: RED-7.2-sd lane4:RED-7 lane5:RED-8.1-sd lane6:RED-8.2-sd lane7:RED-8)

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

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

sample	pBAD 1	pBAD 2	pBAD 3	pBAD 4
Concentration(ng/ul)	140.7	179.5	135.4	155.9
260/280	1.79	1.81	1.82	1.84
260/230	1.73	1.98	1.93	1.96

sample	RED 4	RED 7	RED 8	RED 9
Concentration(ng/ul)	48.1	41.2	53.7	45.9
260/280	1.87	1.87	1.84	1.89
260/230	1.65	1.70	1.84	1.67

Plasmid Extraction of the Red，pBAD Recorder: Zhenyu Jiang,Xingwei Yang Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the

adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

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

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sample	pBAD 2	pBAD 3	pBAD 4
Concentration(ng/ul)	123.1	130.6	141.5
260/280	1.67	1.64	1.66
260/230	1.06	0.98	1.00

sample	RED 4	RED 7	RED 8	RED 9
Concentration(ng/ul)	48.8	63.6	43.6	34.3
260/280	1.82	1.87	1.94	1.98
260/230	1.37	1.67	2.30	2.77

Plasmid Extraction of pET28 and Mtr Recorder: Wenfei Yu, 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, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

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

sample	Mtr1	Mtr2	Mtr4	pET28-1,2	pET28-3,4	pET28-5,6	pET28-7,8
Concentration(ng/ul)	523.8	682.6	615.5	71.8	95.0	134.0	97.4
260/280	1.84	1.93	1.85	1.76	1.83	1.82	1.74
260/230	2.25	2.68	2.41	1.38	1.84	1.86	1.38

PCR of Mtr Recorder: Wenfei Yu, Meiying Cui

Experimental materials

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

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

sample	1	2	3	4	5	6
Sterilized ddH2O	22 μL	22 μL	22 μL	22 μL	22 μL	22 μL
2×Taq-PCR Master	25 μL	25 μL	25 μL	25 μL	25 μL	25 μL
Mtr	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
mtr-res-r	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
total	50 μL	50 μL	50 μL	50 μL	50 μL	50 μL

2.PCR reaction 1,2,3 Parameters setting：

stage	temperature	time
step 1	98	10 min

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stage	temperature	time
step 2	98	10 s
step 3	56	5 s
step 4	72	5 min 30 s
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

4,5,6 Parameters setting：

stage	temperature	time
step 1	94	10 min
step 2	94	15 s
step 3	56	5 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: The picture shows nothing....

Date 7.9 Plasmid Extraction of the Red，pBAD Recorder: Zhenyu Jiang,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 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

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

sample	pBAD 1	pBAD 2	pBAD 3	pBAD 4
Concentration(ng/ul)	213.6	208.4	240.8	206.8
260/280	1.85	1.84	1.83	1.82
260/230	2.14	2.07	1.87	1.84

sample	RED 4	RED 7	RED 8	RED 9
Concentration(ng/ul)	113.8	79.9	84.7	100.3
260/280	1.74	1.81	1.81	1.82
260/230	1.20	1.57	1.49	1.65

Plasmid Extraction of the Red，pBAD Recorder: Zhenyu Jiang,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	pBAD 1	pBAD 2	pBAD 3	pBAD 4

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sample	pBAD 1	pBAD 2	pBAD 3	pBAD 4
Concentration(ng/ul)	165.1	101.3	119.8	121.0
260/280	1.74	1.81	1.77	1.77
260/230	1.54	2.07	1.28	1.57

sample	RED 4	RED 7	RED 8	RED 9
Concentration(ng/ul)	75.1	63.0	66.4	102.0
260/280	1.89	1.90	1.85	1.77
260/230	1.85	2.17	1.73	1.49

Plasmid Extraction of pET28 and Mtr Recorder: Wenfei Yu, 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, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 30 s, discard filtrate. 6.Add 500 μL Buffer DW1, 12000 rpm centifuge 30 s, discard filtrate. 7.Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once. 8.12000 rpm centifuge 1 min. 9.Lying for 10 min. 10.Put the adsorption column in a new EP tube. Add 50 μL 50°C ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

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

sample	pET28-1	pET28-2	pET28-3	pET28-4	pET28-5	pET28-6	pET28-7	pET28-8
Concentration(ng/ul)	195.2	179.6	230.9	111.3	167.4	232.7	117.3	97.5
260/280	1.82	1.85	1.83	1.81	1.88	1.76	1.76	1.83
260/230	1.82	2.01	1.69	1.19	2.24	1.23	1.39	1.96

PCR of pET28 Recorder: Wenfei Yu, Meiying Cui

Experimental materials

1. Template: pET28;
2. Primer: mtr-res-f, mtr-res-r. Designed by ourselves, synthesized by Sangon Biotech;
3. Sterilized ddH2O, 2×Taq-PCR Master, bought from Sangon Biotech.

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

sample	1	2	3	4	5	6
Sterilized ddH2O	22 μL	22 μL	22 μL	22 μL	22 μL	22 μL
2×Taq-PCR Master Mix	25 μL	25 μL	25 μL	25 μL	25 μL	25 μL
Mtr	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
mtr-res-r	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
total	50 μL	50 μL	50 μL	50 μL	50 μL	50 μL

2.PCR reaction 1,2,3 Parameters setting：

stage	temperature	time
step 1	98	10 min
step 2	98	10 s
step 3	56	5 s
step 4	72	5 min 30 s

stage	temperature	time
step 5	72	10 min
step 6	4	--

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30 cycles(step 2 ~ step 4)

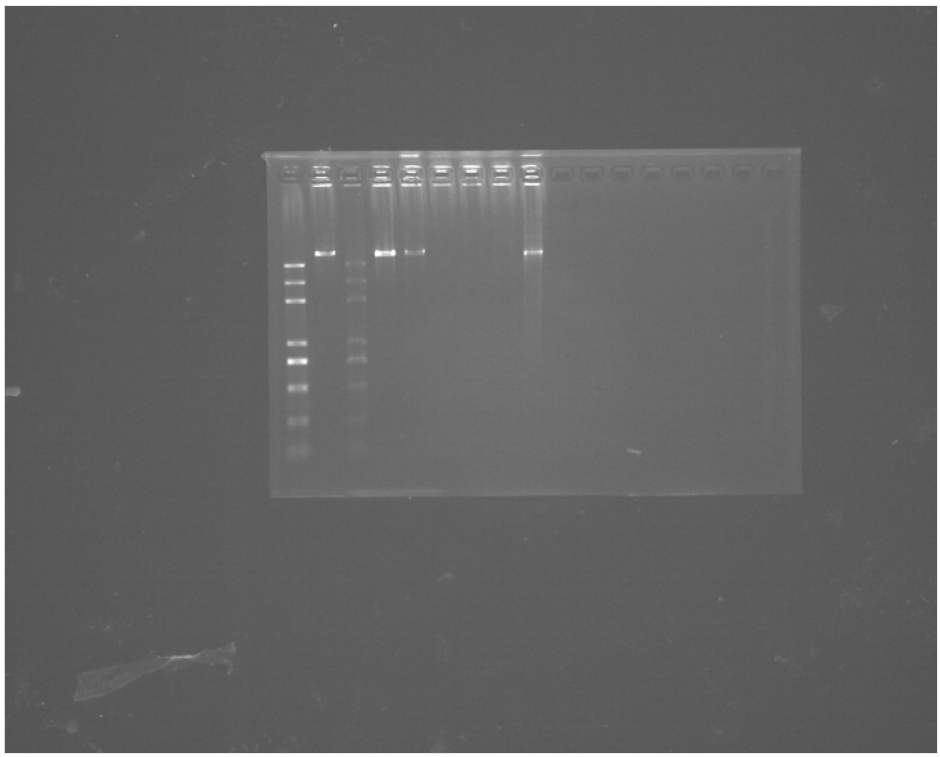
4,5,6 Parameters setting：

stage	temperature	time
step 1	94	10 min
step 2	94	15 s
step 3	56	5 s
step 4	72	5 min 30 s
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

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

4.Agarose gel electrophoresis mixed with 1 μL 6× DNA loading buffer each 5 μL 1:5 dilution of PCR product; mixed 1 μL 6× DNA loading buffer with 5μL template;120 V,30 min Result:



Recorder: Shihan Zhu Double digestion of pET28 Materials:

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

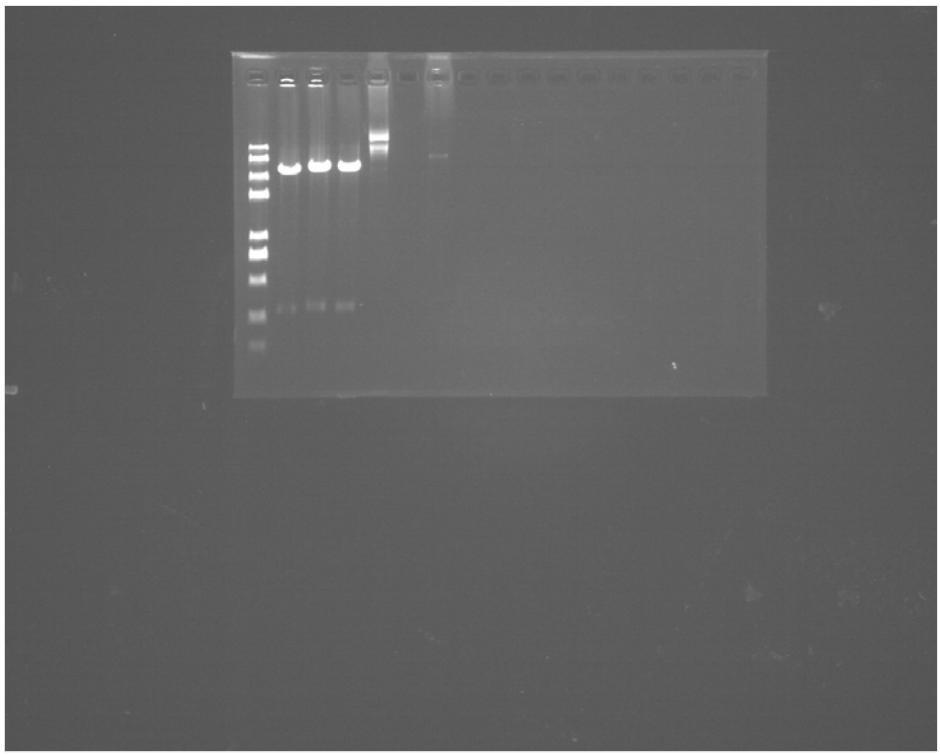
Reaction system:

Sample	1	2	3
pET28(μL)	10	10	10

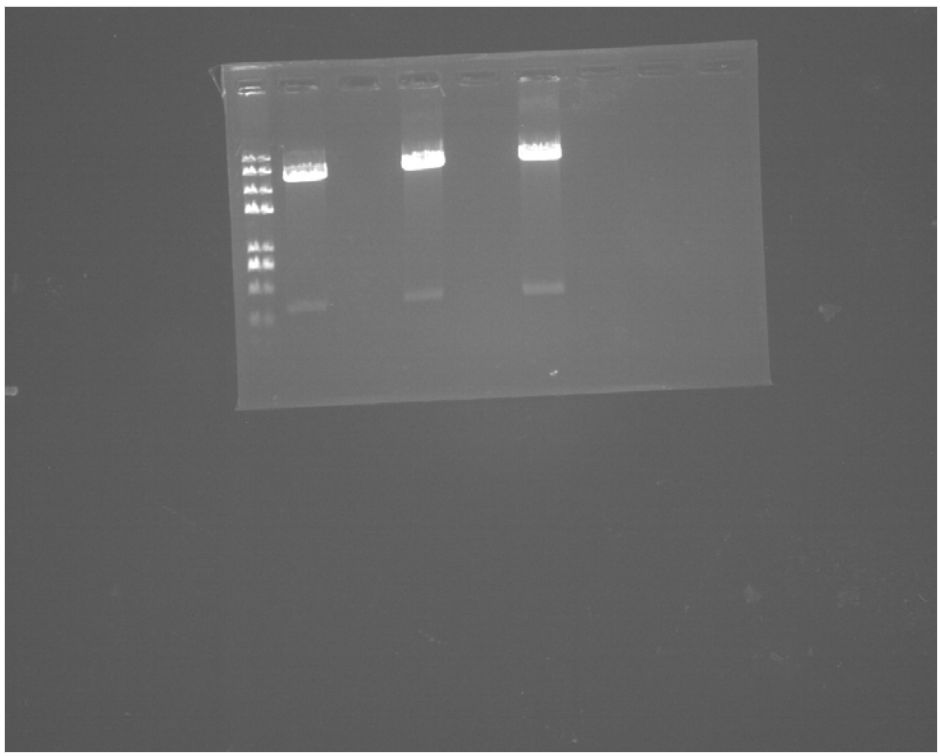
Sample	1	2	3
nuclease-free water(μL)	6	6	6
fastdigest green buffer(μL)	2	2	2
NdeI(μL)	1	1	1
XhoI(μL)	1	1	1
total(μL)	20	20	20

Mix gently and incubate at 37 degree Celsius for 60 mins .

Agarose gel electrophoresis Result:



(from left to right: Trans 2K plus II(contain Gelred), 1, 2, 3, pET28, ligation products)



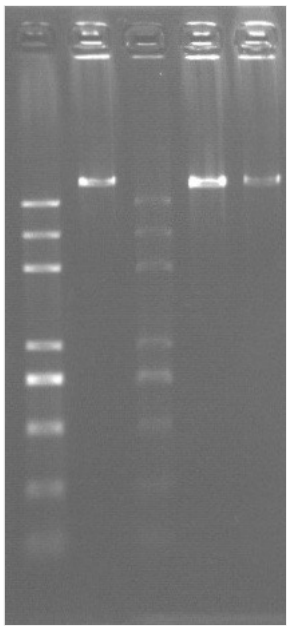
(from left to right: Trans 2K plus II(contain Gelred), 1, 2, 3)

Gel Extraction of ddpET28 Recorder: Shihan Zhu Procedure:

1. Cut off the Gel part containing the target band. Get 6 tubes of AD and 5 tubes of BD.

2. Weigh the cut off Gel part.
3. Add Buffer B2 which 300 times the weight of the Gel.
4. Add isopropanol which is 1/3 volume of the Buffer B2.
5. Move the solution to adsorption column, 11000 rpm centifuge 30 s, discard filtrate.
6. Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once.
7. 12000 rpm centifuge 1 min.
8. Lying for 10 min.
9. Put the adsorption column in a new EP tube. Add 20 μL ddH2O, 10 min's standing, 12000 rpm centifuge 1.5 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

sample	**dd-pET28-1**	**dd-pET28-2**	**dd-pET28-3**
Concentration(ng/ul)	8.8	31.5	16.1
260/280	1.95	1.67	1.71
260/230	0.35	0.38	0.52

Agarose gel electrophoresis Result:  (from left to right: Trans 2K plus(contain Gelred), ddpET28-1, Trans 2K plus(contain Gelred), ddpET28-2, ddpET28-3) **Date 7.10**

PCR of MtrCAB Recorder: Chenyang Li

Experimental materials 1.Template 4: 1:6 dilution of plasmid MtrCAB (Mtr2 YWF 7.8 680.8, 1.93, 2.68), 113 ng/μL;
Template 5: 1:30 dilution of plasmid MtrCAB (Mtr2 YWF 7.8 680.8, 1.93, 2.68), 11 ng/μL;
Template 6: 1:12 dilution of plasmid MtrCAB (Mtr5 XT 6.28 500.1,1.88), 56 ng/μL;

1. Primer: mtr-res-f, mtr-res-r. Designed by ourselves, synthesized by Sangon Biotech;
2. Sterilized ddH2O, 2×Prime Star.

Procedure: 1.Prepare 2 EP tubes and sequentially add：

sample	**5**	**6**
Sterilized ddH2O	66 μL	
template	3 μL Template 5	3 μL Template 6
mtr-res-f(10 μM)	3 μL	3 μL
mtr-res-r(10 μM)	3 μL	3 μL
2×Primer Star	75 μL	75 μL
total	150 μL	150 μL

1. Subpackage it to 3 PCR tubes.

sample	**5-1,5-2,5-3**	**6-1,6-2,6-3**
Sterilized ddH2O	22 μL	22 μL

sample	5-1,5-2,5-3	6-1,6-2,6-3
template	1 μL (Template 5)	1 μL (Template 6)
mtr-res-f(10 μM)	1 μL	1 μL
mtr-res-r(10 μM)	1 μL	1 μL
2×Primer Star	25 μL	25 μL
total	50 μL	50 μL

2.PCR reaction sample 5-1,5-2,5-3,6-1,6-2,6-3 parameters setting：

stage	temperature(°C)	time
Pre-Duration	98	10 min
Duration	98	10 s
Anneal	50	5 s
Extend	72	5 min 30 s
Post-Extend	72	10 min
Final	4	--

30 cycles(Duration ~ Extend)

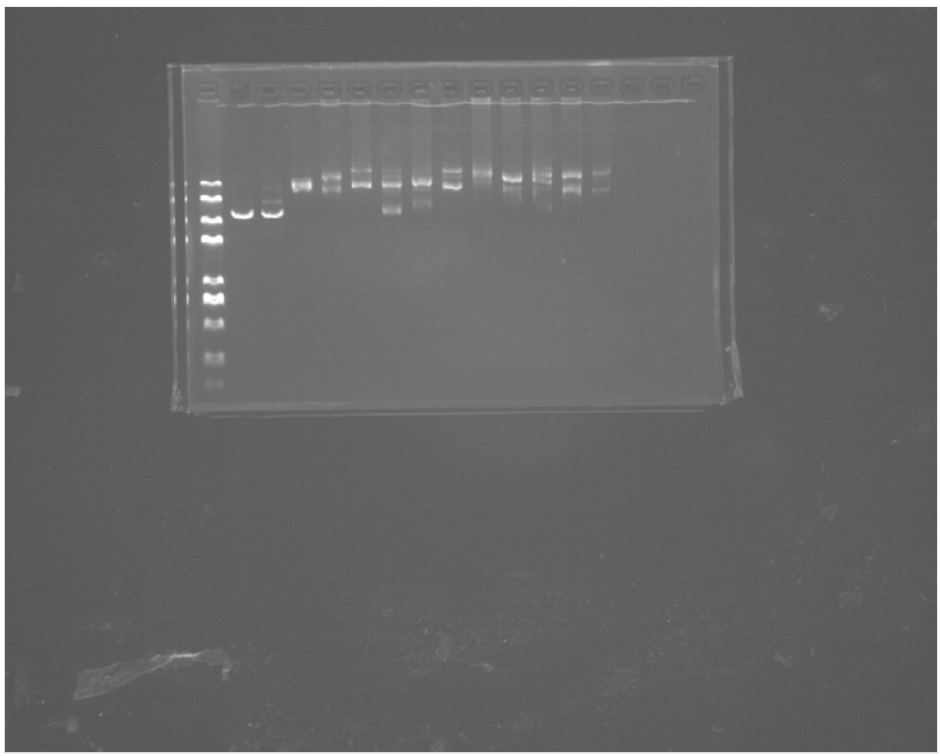
3.Purification of PCR product (1). Add 225 μL Buffer B3 to the 25 μL solution and mix it up. Add it to an adsorption column. (2). 11,000 rpm centrifuge for 30 s, and then discard the filtrate. (3). Add 500 μL Wash Solution, 12,000 rpm centrifuge for 30 s, and then discard the filtrate. (4). Repeat last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

OD measurement result:

sample	Mtr PCR pur 5-1,2,3	Mtr PCR pur 6-1,2,3
Concentration(ng/μL)	277.3	271.7
260/280	1.85	1.85
260/230	2.23	2.18

4.Agarose gel electrophoresis(Done by Shihan ZHU) mixed with 1 μL 6× DNA loading buffer each 5 μL 1:5 dilution of PCR product; mixed 1 μL 6× DNA loading buffer with 5μL template;120 V,30 min

Result:



(lane 1 to 14 : Trans 2K plusII(contain Gelred), Mtr PCR pur 5, Mtr PCR pur 26, template 5, pET28 8(7.9), pET28 YWF 1 (7.10), pET28 YWF 2 (7.10), pET28 YWF 3 (7.10), pET28 YWF 4 (7.10), pET28 YWF 5 (7.10), pET28 YWF 6 (7.10), pET28 YWF 7 (7.10), pET28 YWF 8 (7.10), pET28 YWF 12 (7.8))

PCR of MtrCAB Recorder: Chenyang Li

Experimental materials

1. Template 5: 1:30 dilution of plasmid MtrCAB (Mtr2 YWF 7.8 680.8, 1.93, 2.68), 11 ng/μL;
2. Primer: mtr-res-f, mtr-res-r. Designed by ourselves, synthesized by Sangon Biotech;
3. Sterilized ddH2O, 2×Prime Star.

Procedure: 1.Prepare 2 EP tubes and sequentially add：

sample	**5**	**6**
Sterilized ddH2O	66 μL	
template	3 μL Template 5	3 μL Template 6
mtr-res-f(10 μM)	3 μL	3 μL
mtr-res-r(10 μM)	3 μL	3 μL
2×Primer Star	75 μL	75 μL
total	150 μL	150 μL

1. Subpackage it to 3 PCR tubes.

sample	**5-4,5-5,5-6**	**5-7,5-8,5-9**
Sterilized ddH2O	22 μL	22 μL
template	1 μL (Template 5)	1 μL (Template 6)
mtr-res-f(10 μM)	1 μL	1 μL
mtr-res-r(10 μM)	1 μL	1 μL
2×Primer Star	25 μL	25 μL
total	50 μL	50 μL

3.PCR reaction sample 5-4,5-5,5-6,5-7,5-8,5-9 parameters setting：

stage	**temperature(°C)**	**time**
Pre-Duration	98	10 min
Duration	98	10 s
Anneal	56	5 s
Extend	72	5 min 30 s
Post-Extend	72	10 min
Final	4	--

30 cycles(Duration ~ Extend)

Transformation of Mtr-pET28 Recorder: Chenyang Li NOTE:Generally, competent bacteria are restrored in -70 degree centrigrade environment. 1.Take the competent bacteria from refrigerator and incubate them into ice about 5 mins until it is dissolved 2.Absorb 10μL Mtr-pET28 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 kanamycin. 10.Cultivate these bacteria overnight for further use.

Plasmid Extraction of Red，pBAD Recorder: Xingwei Yang Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 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.

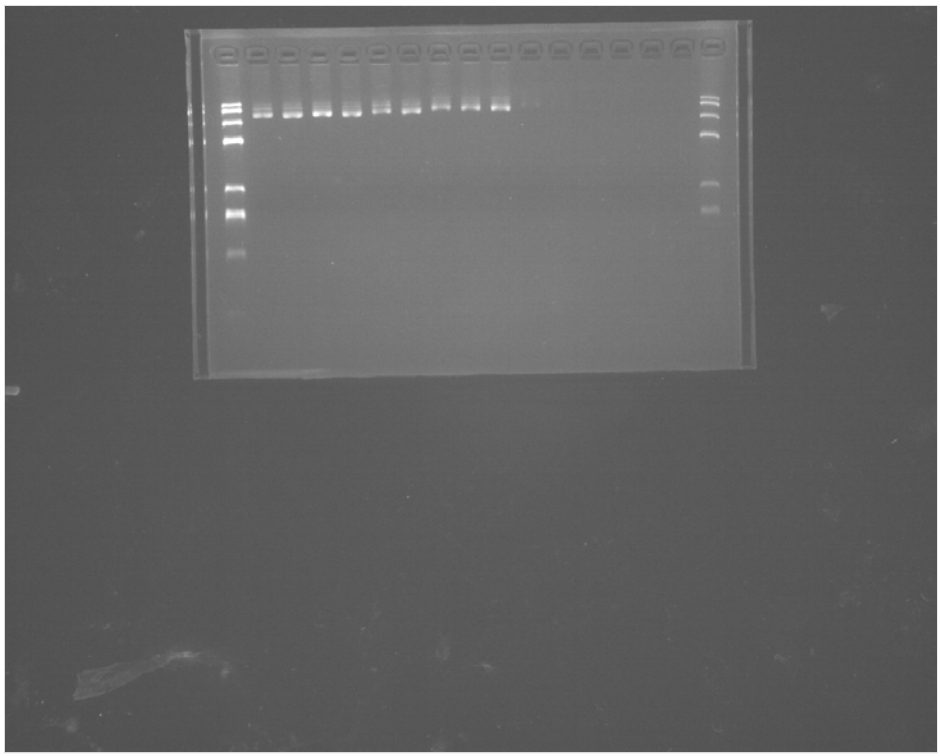
After extraction, we measured the OD A260/A280, OD A260/A230 and the density of plasmids. The results are shown in the chart below.

sample	**pBAD 1**	**pBAD 2**	**pBAD 3**	**pBAD 4**
Concentration(ng/μL)	193.4	196.5	223.2	204.8
260/280	1.87	1.85	1.87	1.78
260/230	2.29	2.13	2.31	1.45

sample	**RED 4**	**RED 7**	**RED 8**	**RED 9**
Concentration(ng/μL)	92.5	68.8	113.6	229.3
260/280	1.77	1.85	1.65	1.83
260/230	1.36	1.69	0.87	1.76

There may be something wrong with the plasmid RED 9 and we will find it out soon.

Agarose gel electrophoresis Recorder: Shihan Zhu 1% agarose gel 120 V 45 min



(lane 1 to 17 : marker 2K PlusII, Mtr1-pcr-pur, Mtr2-pcr-pur, Mtr3-pcr-pur, Mtr4-pcr-pur, Mtr5-pcr-pur, Mtr6-pcr-pur, Mtr-pcr-pur-1-1, Mtr-pcr-pur-1-2, Mtr-pcr-pur-1-3, Mtr-pcr-pur-2-1, Mtr-pcr-pur-2-3, Mtr-pcr-pur-2-2, Mtr-pcr-pur-3-1, Mtr-pcr-pur-3-2, Mtr-pcr-pur-3-3, marker 2K PlusII)

Plasmid Extraction of the pET28 Recorder: Wenfei Yu, 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	pET28-1	pET28-2	pET28-3	pET28-4	pET28-5	pET28-6	pET28-7	pET28-8
Concentration(ng/μL)	205.7	219.2	196.0	211.3	173.8	186.0	187.8	152.7
260/280	1.87	1.88	1.87	1.87	1.85	1.84	1.87	1.84
260/230	2.16	2.16	2.11	2.16	2.01	2.11	2.22	2.10

帮助

Date: 7.11

PCR of Ccm A-H Recorder: Yonghao Liang

Experimental materials

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

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

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

2.PCR reaction

stage	temperature	time
step 1	98	10 min
step 2	98	10 s
step 3	56	5 s
step 4	72	5 min 30 s
step 5	72	10 min
step 6	4	--

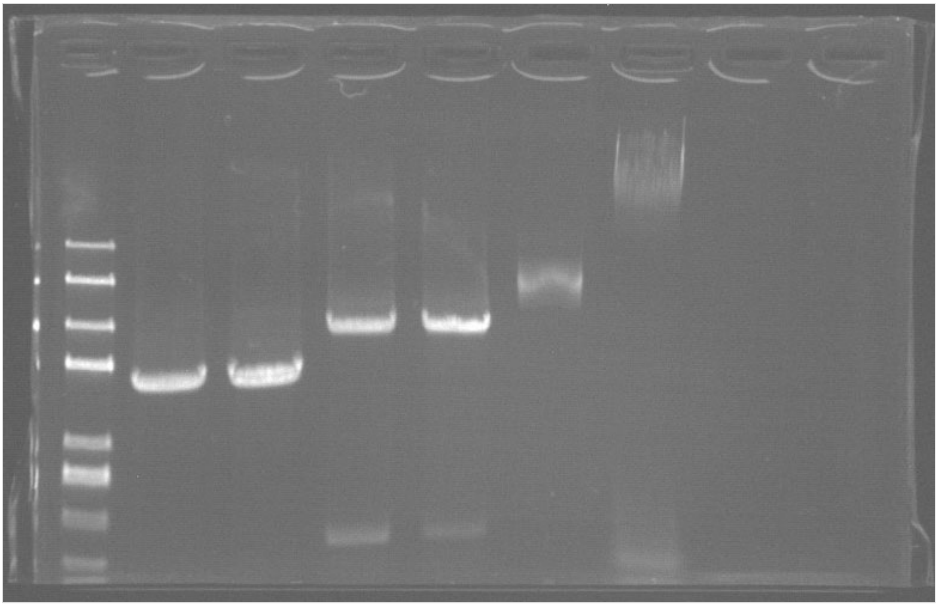
30 cycles(step 2 ~ step 4)

4,5,6 Parameters setting：

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

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:



(lane 1 to 7: marker 2K plus 2; first half of ccm; first half of ccm; second half of ccm; second half of ccm; plasmid pET21 containing Red; genome of BL21(DE3))

Plasmid Extraction of Red and pBAD 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	**pBAD 1**	**pBAD 2**	**pBAD 3**	**pBAD 4**
Concentration(ng/μL)	127.9	70.7	196.1	161.6
260/280	1.84	1.87	1.83	1.85
260/230	1.94	2.04	1.93	2.10

sample	**RED 4**	**RED 7**	**RED 8**
Concentration(ng/μL)	80.1	74.3	71.4
260/280	1.80	1.87	1.87
260/230	1.44	1.73	1.87

Plasmid Extraction of Red Recorder: Yitian Zhou 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 12**	**RED 34**	**RED 56**	**RED 78**
Concentration(ng/μL)	98.9	86.5	185.8	98.1

sample	RED 12	RED 34	RED 56	RED 78
260/280	1.82	1.87	1.67	1.86
260/230	1.73	2.06	0.87	2.03

帮助

As you can see, there are something wrong with the plasmid RED 56. Maybe it was polluted.

PCR of Ccm A-H Recorder: Xingwei Yang, Liudong Luo, Zhenyu Jiang

Experimental materials

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

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

sample	1	2	3	4	5	6	7	8	9	10	11	12
Sterilized ddH2O(μL)	22	22	22	22	22	22	22	22	22	22	22	22
2×PrimeStar(premix)(μL)	25	25	25	25	25	25	25	25	25	25	25	25
template(μL)	1	1	1	1	1	1	1	1	1	1	1	1
ccm-res-f(μL)	1	1	0	0	1	1	0	0	1	1	0	0
ccm-mid-r(μL)	1	1	0	0	1	1	0	0	1	1	0	0
ccm-mid-f(μL)	0	0	1	1	0	0	1	1	0	0	1	1
ccm-res-r(μL)	0	0	1	1	0	0	1	1	0	0	1	1
total(μL)	50	50	50	50	50	50	50	50	50	50	50	50

2.PCR reaction 1,2,3,4,9,12 Parameters setting：

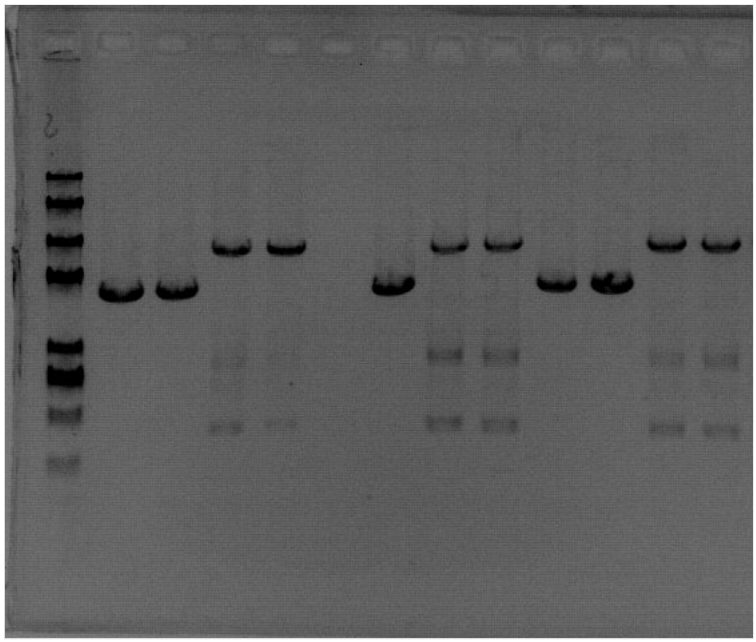
stage	temperature	time
step 1	95	10 min
step 2	98	10 s
step 3	59	5 s
step 4	72	4 min
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

5,6,7,8,10,11 Parameters setting：

stage	temperature	time
step 1	95	10 min
step 2	98	10 s
step 3	55	15 s
step 4	72	4 min
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4) 3.Agarose gel electrophoresis Result:



(lane 1 to 13 : marker 2K PlusII, ccm(f)-pcr-1, ccm(f)-pcr-2,ccm(s)-pcr-3,ccm(s)-pcr-4,ccm(f)-pcr-5,ccm(f)-pcr-6,ccm(s)-pcr-7,ccm(s)-pcr-8,ccm(f)-pcr-9,ccm(f)-pcr-10,ccm(s)-pcr-11,ccm(s)-pcr-12)

4.Purification of PCR product (Done by Liudong Luo, Zhenyu Jiang) (1). Add 400 μL Buffer B3 to the 80 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(f) PCR pur 1,2	ccm(s) PCR pur 3,4	ccm(f) PCR pur 5,6	ccm(s) PCR pur 7,8	ccm(f) PCR pur 9,10	ccm(s) PCR pur 11,12
Concentration(ng/μL)	303.3	140.8	163.2	440.1	302.0	293.7
260/280	1.86	1.82	1.84	1.86	1.86	1.85
260/230	2.08	1.90	1.92	2.14	2.11	2.00

Plasmid Extraction of pET28 Recorder: Wenfei Yu, 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.

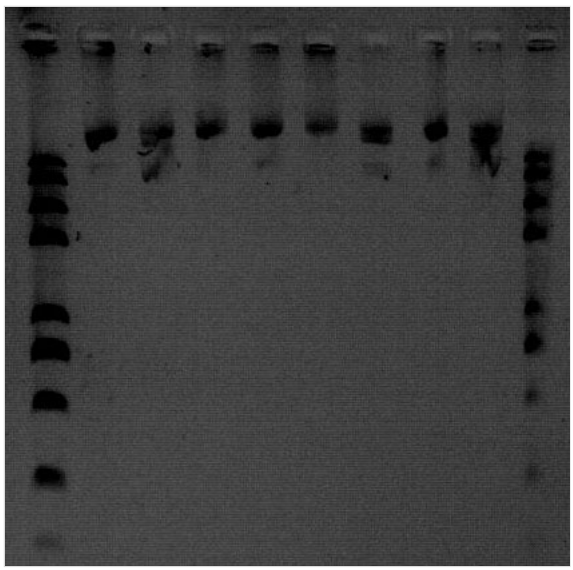
After extraction, we measured the OD A260/A280, OD A260/A230 and the density of plasmids. The results are shown in the chart below.

sample	pET28-1	pET28-2	pET28-3	pET28-4	pET28-5	pET28-6	pET28-7	pET28-8

帮助

sample	pET28-1	pET28-2	pET28-3	pET28-4	pET28-5	pET28-6	pET28-7	pET28-8
Concentration(ng/μL)	142.6	186.0	136.0	173.3	119.5	173.8	192.9	197.7
260/280	1.85	1.84	1.83	1.84	1.85	1.86	1.85	1.84
260/230	2.18	2.13	2.14	2.03	2.12	2.25	2.16	1.98

Agarose gel electrophoresis Result:



(Lane 1 to 10 : Marker 2K plus II,pET28-1,pET28-2,pET28-3,pET28-4,pET28-5,pET28-6,pET28-7,pET28-8,Marker 2K plus II)

Digestion of and Mtr PCR products Recorder: Shihan Zhu

Experiment Materials

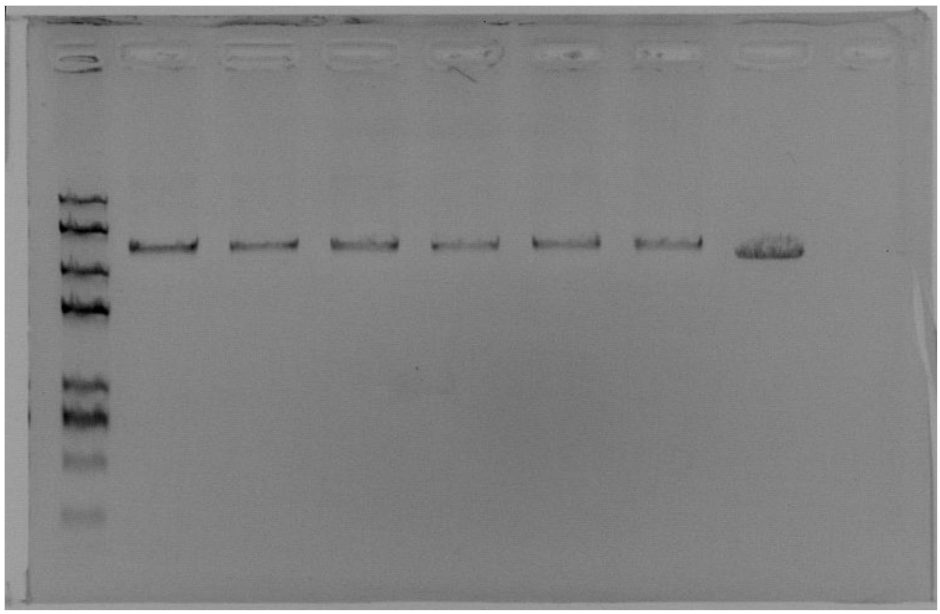
1. Mtr PCR purication products
2. FastDigest restriction enzyme NdeI, XhoI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water
4. marker:Trans 2K PlusII

Reaction system:

Sample	1	1'	2	2'	3	4	5
Mtr PCR purication products(μL)	1	1	1	1	1.5	1.5	1.5
XhoI(μL)	0	0	1	1	1	1	1
NdeI(μL)	1	1	0	0	1	1	1
nuclease-free water(μL)	26	26	26	26	24.5	24.5	24.5
fastdigest green buffer(μL)	2	2	2	2	2	2	2
pET28(μL)	8	8	8	8	8	8	8
Sample	1	1'	2	2'	3	4	5
total(μL)	30	30	30	30	30	30	30

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

Agarose gel electrophoresis Result:

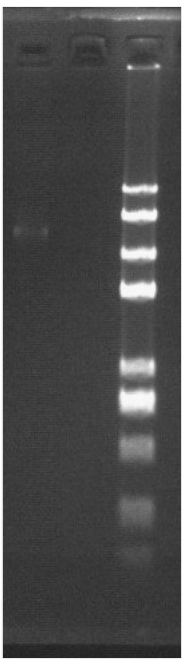


(lane 1 to 8: marker 2K PlusII, 3, 3, 4, 4, 5, 5, Mtr PCR purication products)

Gel Extraction of ddMtr Recorder: Shihan Zhu Procedure:

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

sample	**ddMtr 1**	**ddMtr 2**
Concentration(ng/μL)	21.0	8.7
260/280	1.43	1.25
260/230	0.23	0.17

Agarose gel electrophoresis Result:  (lane 1 to 3: ddMtr 1, ddMtr 2, Trans 2K plusII(contain Gelred)) (Done by Yijun Chen and Meiying Cui)

PCR of Mtr Recorder: Meiying Cui，Yijun Chen

帮助

Experimental materials

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

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

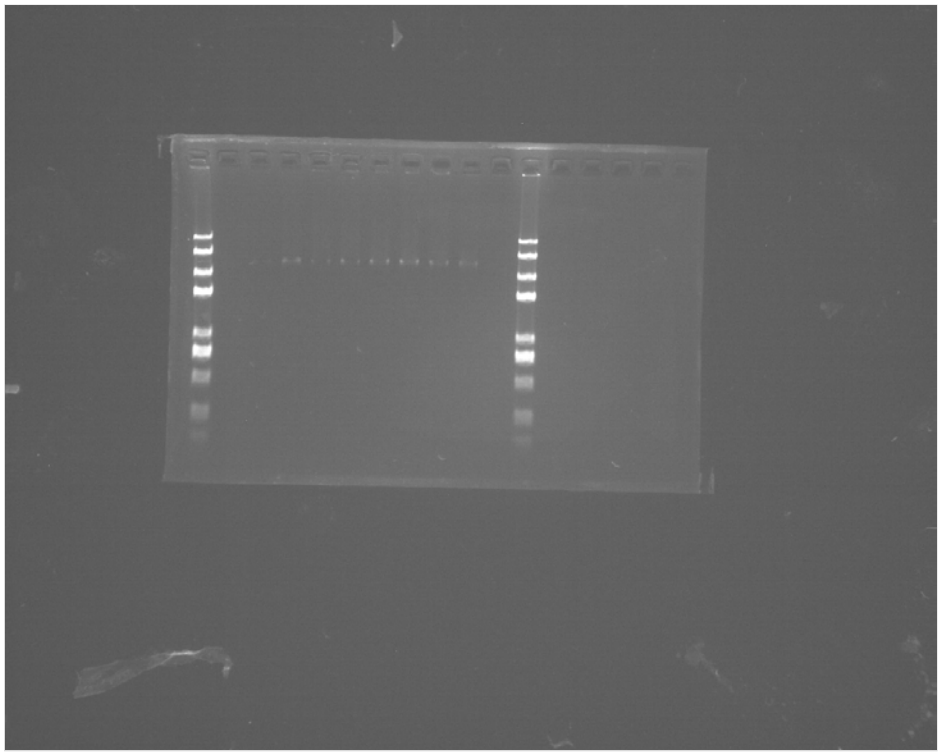
sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Sterilized ddH2O(μL)	22	22	22	22	22	22	22	22
2×Taq-PCR Master(μL)	25	25	25	25	25	25	25	25
Mtr(μL)	1	1	1	1	1	1	1	1
mtr-res-f(μL)	1	1	1	1	1	1	1	1
mtr-res-r(μL)	1	1	1	1	1	1	1	1
total(μL)	50	50	50	50	50	50	50	50

2.PCR reaction 1,2,3 Parameters setting：

stage	**temperature**	**time**
step 1	98	10 min
step 2	98	10 s
step 3	56	5 s
step 4	72	5 min 30 s
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

Agarose gel electrophoresis Result:



(lane 1 to 12: Trans 2K plusII(contain Gelred，PCR8，PCR7，PCR6，PCR5，PCR1，PCR2，

PCR3，PCR4，ddMtr 1, ddMtr 2,Trans 2K plusII(contain Gelred)) (Done by Yijun Chen and Meiying Cui)

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	PCR pur1+2	PCR pur3+4	PCR pur5+6	PCR pur 7+8
Concentration(ng/μL)	90.7	94.8	67.7	47.7
260/280	1.85	1.83	1.91	1.88
260/230	1.96	1.66	2.14	1.87

Ligation of pET28 and Mtr PCR product Recorder: Shihan Zhu Experiment Materials

1. double digestion product of pET28 1（31.0 ng/μL）
2. double digestion product of Mtr PCR product (21.0 ng/μL）
3. 10× T4 DNA ligase buffer,T4 DNA ligase(bought from Thermo Fisher Scientific)

Procedure: Add to either of samples: 3 μL ddpET28 15 μL ddMtr PCR product 2 μL 10* T4 DNA Ligase Buffer 0.4 μL T4 DNA Ligase

Mix gently and incubate at 16 degree Celsius for 11 hour.

Plasmid Extraction of Red Recorder: Yitian Zhou 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 12	RED 34	RED 56	RED 78
Concentration(ng/μL)	87.7	87.7	85.4	192.1
260/280	1.88	1.79	1.89	1.68
260/230	2.07	1.41	2.23	0.96

Genome Extraction of BL21(DE3) Recorder: Yonghao Liang We use the Genome Extraction Kit bought from Shanghai Sangon Biotech.

Procedure: To be continued..

Date 7.12

Pick a single clone of Red Recorder: Menglong Jin Procedure:

1. Prepare 4 test tubes(with 5 ml LB), each adding 2.5μl Amp;
2. Pick single clones from Red plate and put them in the tubes;
3. Shaking culture.

PCR of full ccmA-H Recorder: Menglong Jin

Experimental materials 1.Templates: ccm f, ccm r, PCR by ourselves;

Sample	ccm f	ccm r
Concentration(ng/μL)	303.3	440.1
260/280	1.86	1.86
260/230	2.08	2.14

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

Procedure:

1. Dilution of the templates Prepare 2 PCR tubes and sequentially add:

sample	**ccm f(diluted)**	**ccm r(diluted)**
PCR of ccm f(μL)	4	0
PCR of ccm r(μL)	0	4
Sterilized ddH2O(μL)	12	12
Total(μL)	16	16

2.Prepare 4 PCR tubes and sequentially add：

sample	**1**	**2**	**3**	**4**
Sterilized ddH2O(μL)	6	6	6	6
2×Taq-PCR Master(μL)	10	10	10	10
ccm f(diluted)(μL)	1	1	1	1
ccm r(diluted)(μL)	1	1	1	1
ccm-res-f(μL)	1	1	0	0
ccm-res-r(μL)	1	1	0	0
total(μL)	20	20	18	18

3.PCR reaction 1,2,3 Parameters setting： *sample 1 & 2*

stage	**temperature**	**time**
step 1	95	10 min
step 2	95	40 s
step 3	58	40 s
step 4	72	7 min
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

sample 3 & 4

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

10 cycles(step 2 ~ step 4)

4.After the reaction of sample 3 & 4, add 1μl ccm-res-f and 1μl ccm-res-r to sample 3 & 4. PCR again. 1,2,3 Parameters setting：

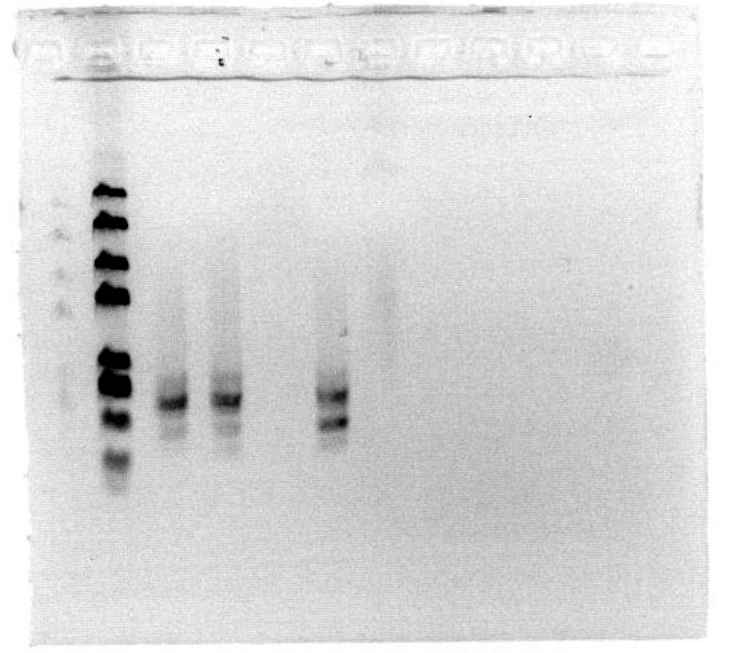
stage	**temperature**	**time**
step 1	95	10 min
step 2	95	40 s
step 3	58	40 s
step 4	72	7 min
step 5	72	10 min

stage	temperature	time
step 6	4	--

25 cycles(step 2 ~ step 4)

帮助

Agarose gel electrophoresis Result:



(From left to right: marker, sample 1, sample 2, sample 3, sample 4.) As you can see, we failed to get the full sequence of ccm, which should be about 70000bp long. We will try more.

PCR of Ccm A-H Recorder: Yonghao Liang

Experimental materials

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

Procedure:

1.Prepare 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(μL)	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22
2×PrimeStar (premix) (μL)	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
template(μL)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
ccm-res-f(μL)	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0

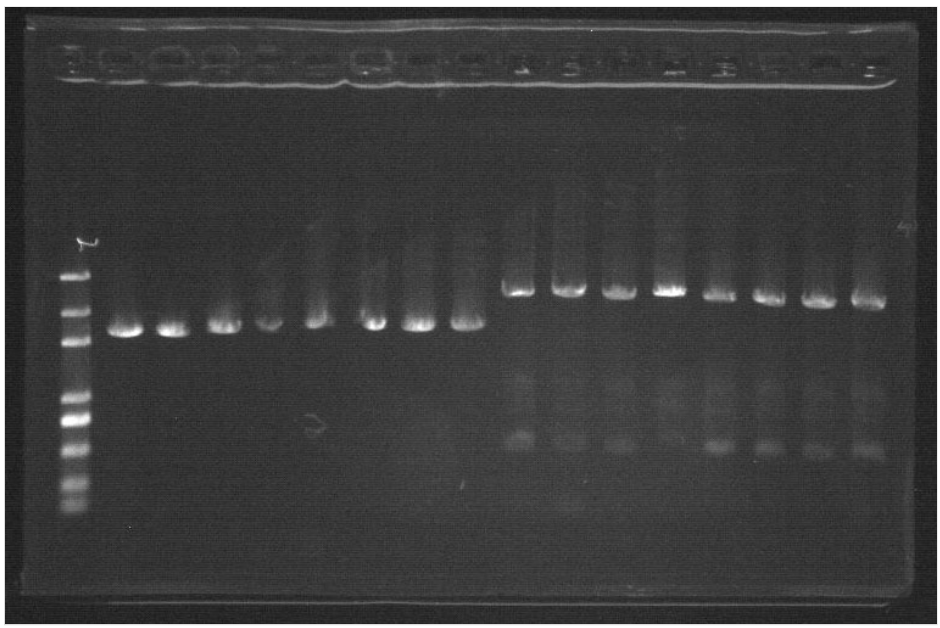
sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
ccm-mid-r(μL)	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0
ccm-mid-f(μL)	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
ccm-res-r(μL)	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
total (μL)	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50

2.PCR reaction

stage	temperature	time
step 1	95	10 min
step 2	98	10 s
step 3	59	5 s
step 4	72	4 min
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:



4.Purification of PCR product (Done by Xingwei Yang, Zhenyu Jiang) (1). Add 400 μL Buffer B3 to the 80 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(f) PCR pur 1,2	ccm(f) PCR pur 3,4	ccm(f) PCR pur 5,6	ccm(f) PCR pur 7,8	ccm(s) PCR pur 1,2	ccm(s) PCR pur 3,4	ccm(s) PCR pur 5,6	ccm(s) PCR pur 7,8

sample	ccm(f) PCR pur 1,2	ccm(f) PCR pur 3,4	ccm(f) PCR pur 5,6	ccm(f) PCR pur 7,8	ccm(s) PCR pur 1,2	ccm(s) PCR pur 3,4	ccm(s) PCR pur 5,6	ccm(s) PCR pur 7,8
Concentration(ng/μL)	325.7	314.8	324.0	257.0	201.5	185.0	158.4	178.8
260/280	1.85	1.84	1.85	1.84	1.84	1.85	1.84	1.83
260/230	2.16	1.88	2.08	1.88	2.10	2.21	2.06	2.02

PCR of mtr Recorder: Shihan Zhu

Experimental materials

1. Template: Mtr gene on pSB1C3;
2. Primer: mtr-res-f, mtr-res-r. Designed by ourselves, synthesized by Sangon Biotech;
3. Sterilized ddH2O, 2×PrimeSTAR, bought from Sangon Biotech.

Procedure:

1.Prepare 6 PCR tubes and sequentially add：

sample	1	1'	2	2'	3	3'
Sterilized ddH2O(μL)	22	22	22	22	22	22
2×PrimeSTAR(μL)	25	25	25	25	25	25
template(μL)	1 (10ng/μL)	1 (10ng/μL)	1 (1ng/μL)	1 (1ng/μL)	1 (0.5ng/μL)	1 (0.5ng/μL)
mtr-res-f(μL)	1	1	1	1	1	1
mtr-res-r(μL)	1	1	1	1	1	1
total(μL)	50	50	50	50	50	50

2.PCR reaction

stage	temperature	time
step 1	98	10 min
step 2	98	10 s
step 3	56	5 s
step 4	72	5 min 30 s
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

Purification of mtr PCR product Recorder: Liudong Luo (1). Add 500 μL Buffer B3 to the 100 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	mtr PCR pur 0.5	mtr PCR pur 1	mtr PCR pur 10
Concentration(ng/μL)	43.3	29.6	118.8
260/280	1.87	1.69	1.67
260/230	4.30	1.44	1.13

Double digestion of pTB Recorder: Liudong Luo, Zhenyu Jiang

Experiment Materials

1. pTB

2. FastDigest restriction enzyme BcuI, pstI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water

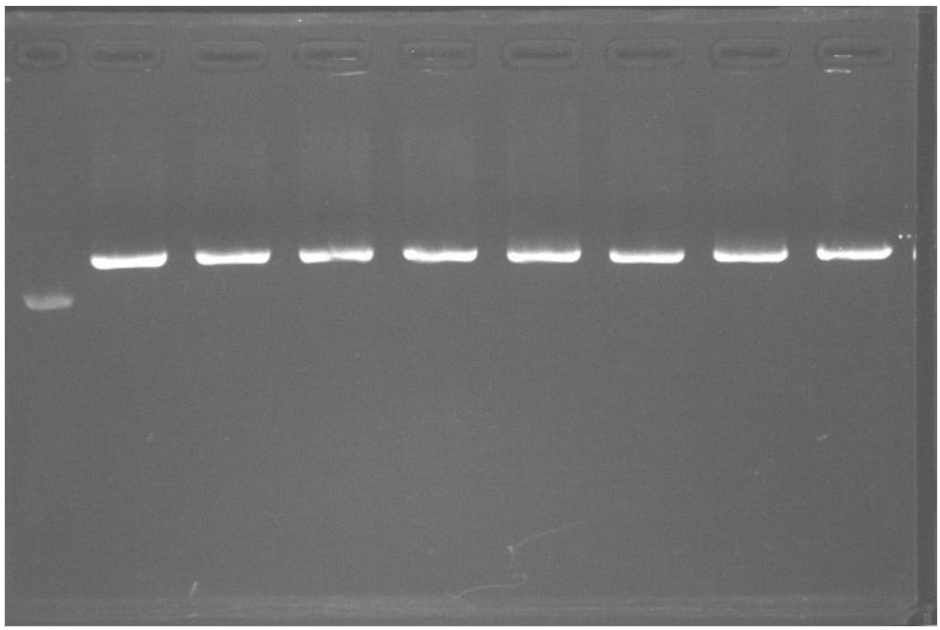
Reaction system:

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)	5	5	5	5
total(μL)	20	20	20	20

Sample	**5**	**6**	**7**	**8**
BcuI(μL)	1	1	1	1
pstI(μL)	1	1	1	1
nuclease-free water(μL)	10	10	10	10
fastdigest green buffer(μL)	2	2	2	2
pTB(μL)	6	6	6	6
total(μL)	20	20	20	20

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

Agarose gel electrophoresis Result:



(lane 1 to 9: pTB,ddpTB-1,ddpTB-2,ddpTB-3,ddpTB-4,ddpTB-5,ddpTB-6,ddpTB-7,ddpTB-8)

Then we did the gel extraction of the fragments we got.

The results are as following:

sample	**PTBSPdd 1**	**PTBSPdd 2**	**PTBSPdd 3**	**PTBSPdd 4**
Concentration(ng/μL)	28.6	186.5	25.1	30.0
260/280	1.1.76	1.33	1.76	1.87
260/230	0.59	0.41	0.23	0.04

Double digestion of PCR products of Mtr Recorder: Wenfei Yu

Experiment Materials

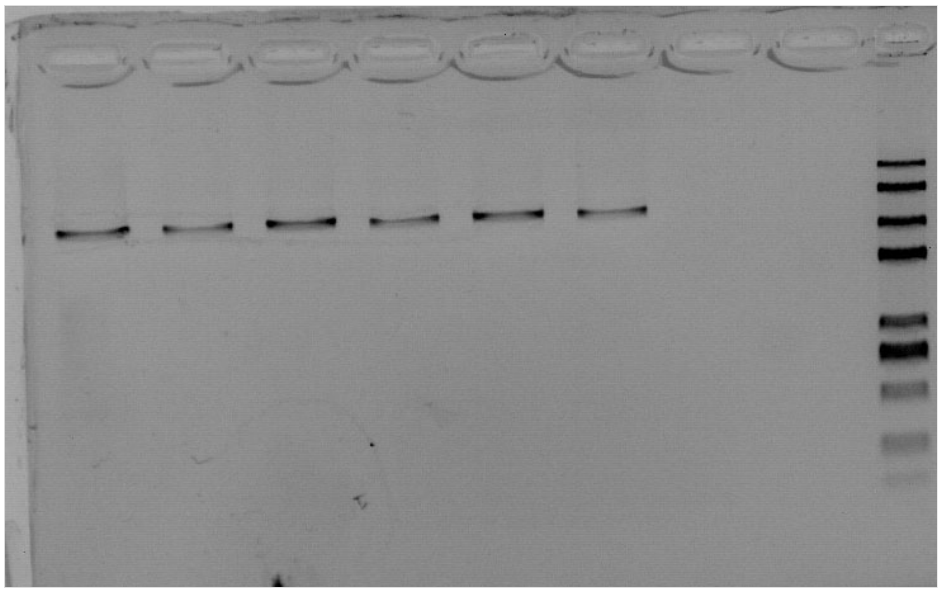
1. Mtr PCR products
2. FastDigest restriction enzyme NdeI, XhoI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water
4. marker:Trans 2K PlusII

Reaction system:

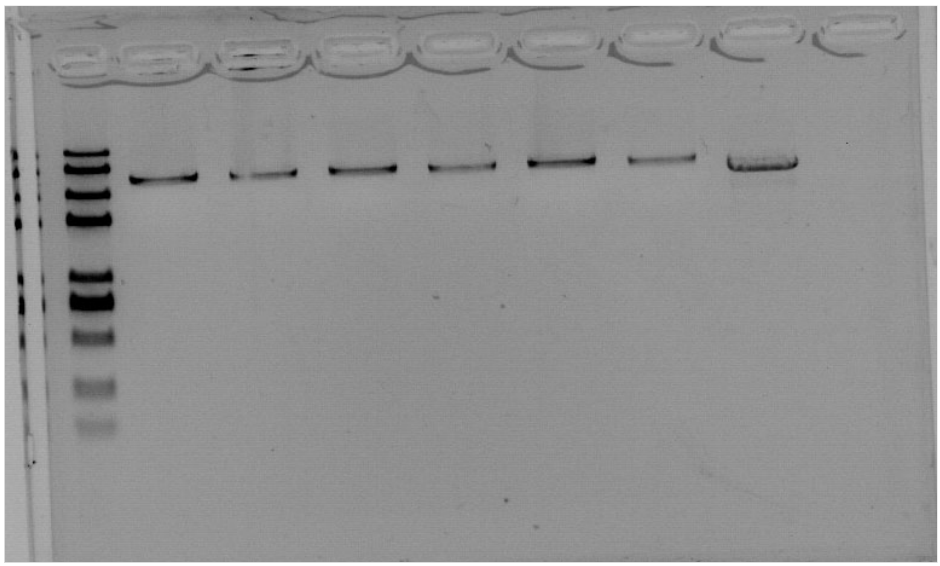
Sample	**1**	**2**	**3**
XhoI(μL)	1	1	1
NdeI(μL)	1	1	1
nuclease-free water(μL)	22	22	22
fastdigest green buffer(μL)	2	2	2
Mtr(μL)	4	4	4
total(μL)	30	30	30

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

Agarose gel electrophoresis Result:



(lane 1 to 9:Mtr1-1, Mtr1-1, Mtr1-2, Mtr1-2, Mtr1-3, Mtr1-3, empty, empty, marker Trans 2K Plus II)



(lane 1 to 9:marker Trans 2K Plus II, Mtr2-1, Mtr2-1, Mtr2-2, Mtr2-2, Mtr2-3, Mtr2-3, Mtr PCR pur, empty)

Gel Extraction of Double digestion of PCR products of Mtr Recorder: Wenfei Yu

Procedure:

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

After extraction, we measured the OD A260/A280, OD A260/A230 and the density of plasmids.
|sample|dd Mtr1-1|dd Mtr1-2|dd Mtr1-3|dd Mtr2-1|dd Mtr2-2|dd Mtr2-3| |-| |Concentration(ng/μL)|8.7|6.6|98.8|129.8|97.7|8.8| |260/280|1.64|1.69|1.37|1.38|1.34|1.55| |260/230|0.07|0.09|0.35|0.46|0.22|0.22|

Double digestion of PET28 Recorder: Meiying Cui

Experiment Materials

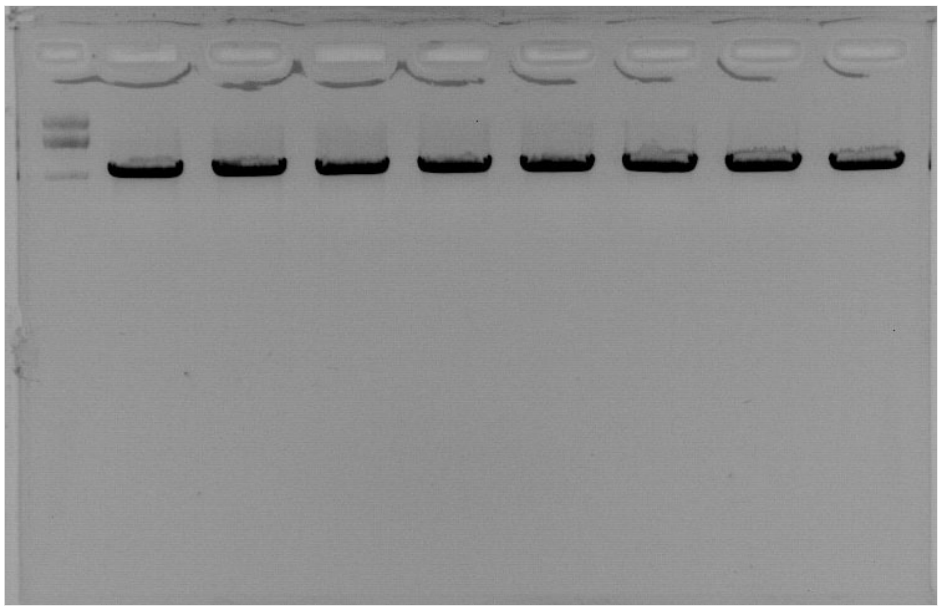
1. PET28 plasmid
2. FastDigest restriction enzyme NdeI, XhoI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water

Reaction system:

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**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)	8	8	8	8	8	8	8	8
fastdigest green buffer(μL)	2	2	2	2	2	2	2	2
Mtr(μL)	8	8	8	8	8	8	8	8
total(μL)	20	20	20	20	20	20	20	20

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

Agarose gel electrophoresis Result:



(lane 1 to 9:PET28 plasmid，dd product1，2，3，4，5，6，7，8)

Gel Extraction of Double digestion of PCR products of PET28 Recorder: Meiying Cui

Procedure:

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

After extraction, we measured the OD A260/A280, OD A260/A230 and the density of plasmids.

sample	**dd PET28-1**	**dd PET28-2**
Concentration(ng/μL)	240.3	215.5
260/280	1.35	1.36
260/230	0.47	0.45

Ligation of pET28 and Mtr PCR product Recorder: Meiying Cui

Experiment Materials

1. double digestion product of pET28 1（240.3ng/μL）
2. double digestion product of Mtr PCR product (129.8 ng/μL)
3. 10× T4 DNA ligase buffer,T4 DNA ligase(bought from Thermo Fisher Scientific)

Procedure: Add to either of samples: dilute 5μL ddpET28 to 10μL and add 1μL 12 μL ddMtr PCR product 2 μL 10* T4 DNA Ligase Buffer 0.4 μL T4 DNA Ligase

Mix gently and incubate at 16 degree Celsius and stay overnight.

Plasmid Extraction of the pET28 Recorder: Xiaoyu Zhang, 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	**pET28-1**	**pET28-2**	**pET28-3**	**pET28-4**	**pET28-5**	**pET28-6**	**pET28-7**	**pET28-8**	**pET28-9**	**pET28-10**	**pET28-11**
Concentration(ng/μL)	104.0	71.9	108.7	89.6	111.1	114.1	96.7	126.7	85.2	106.0	48.4
260/280	1.83	1.90	1.73	1.83	1.88	1.72	1.81	1.69	1.84	1.82	1.81
260/230	2.09	2.30	1.32	1.91	2.64	1.28	1.81	1.24	2.10	2.04	1.36

Double digestion of pET28 Recorder: Tong Xiao

Experiment Materials

1. Plasmid extraction product of pET28
2. FastDigest restriction enzyme NdeI, XhoI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water
4. marker:Trans 2K PlusII

帮助

sample data:

sample	**pET28-1**	**pET28-2**
Concentration(ng/μL)	211.3	196.0
260/280	1.87	1.87
260/230	2.16	2.11

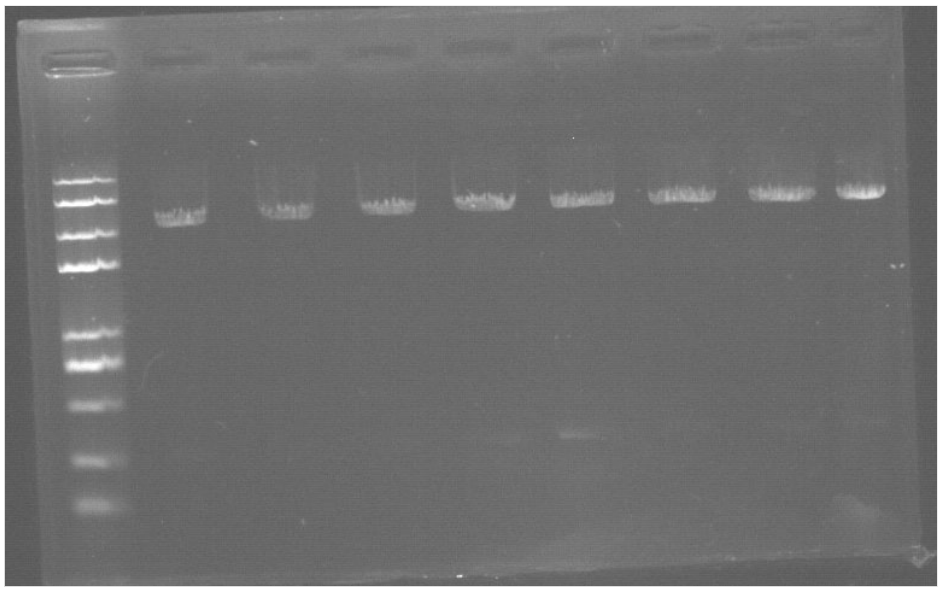
(sample1-4 uses pET28-1,sample5-8 uses pET28-2)

Reaction system:

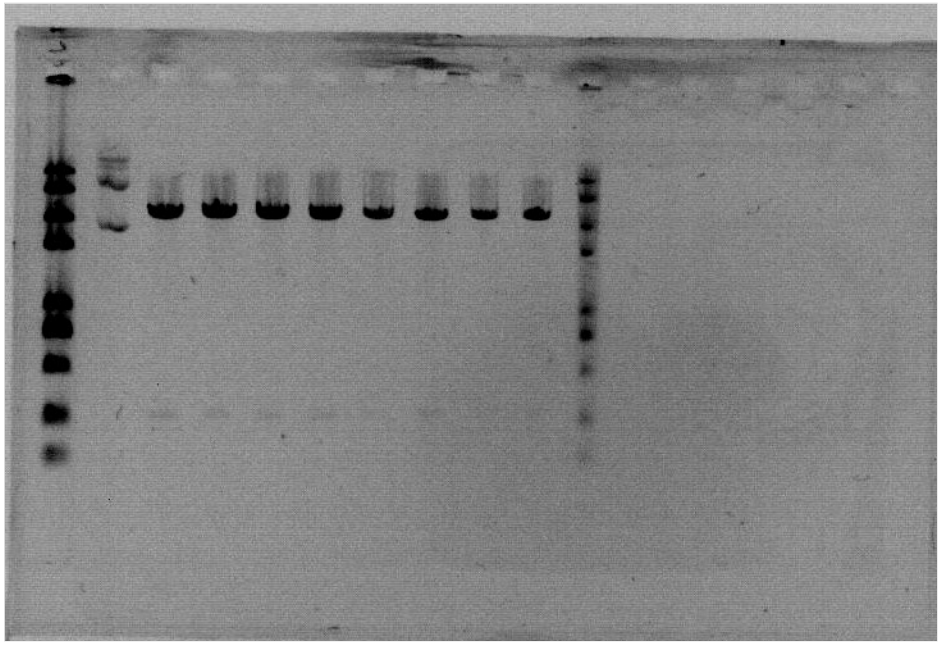
Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
pET28 products(μL)	10	10	10	10	8	8	8	8
XhoI(μL)	1	1	1	1	1	1	1	1
NdeI(μL)	1	1	1	1	1	1	1	1
nuclease-free water(μL)	6	6	6	6	8	8	8	8
fastdigest green buffer(μL)	2	2	2	2	2	2	2	2
total(μL)	20	20	20	20	20	20	20	20

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

Agarose gel electrophoresis Result:



(lane 1 to 9: marker 2K PlusII, pET28 1, pET28 2, pET28 3, pET28 4, pET28 5, pET28 6, pET28 7, pET28 8)



(lane 1 to 11: marker 2K PlusII, plasmid extraction product of pET28, pET28 1 to 8, marker 2K PlusII)

Date 7.13

PCR of full Ccm Recorder: Yonghao Liang, Yitian Zhou, Menglong Jin, Xingwei Yang, 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**
Sterilized ddH2O(μL)	22	22	22	22	22	22	22	22	22	22	22	22
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
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
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
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 Parameters setting：

stage	**temperature**	**time**
step 1	95	10 min
step 2	98	10 s
step 3	59	5 s
step 4	72	4 min

stage	temperature	time
step 5	72	10 min
step 6	4	--

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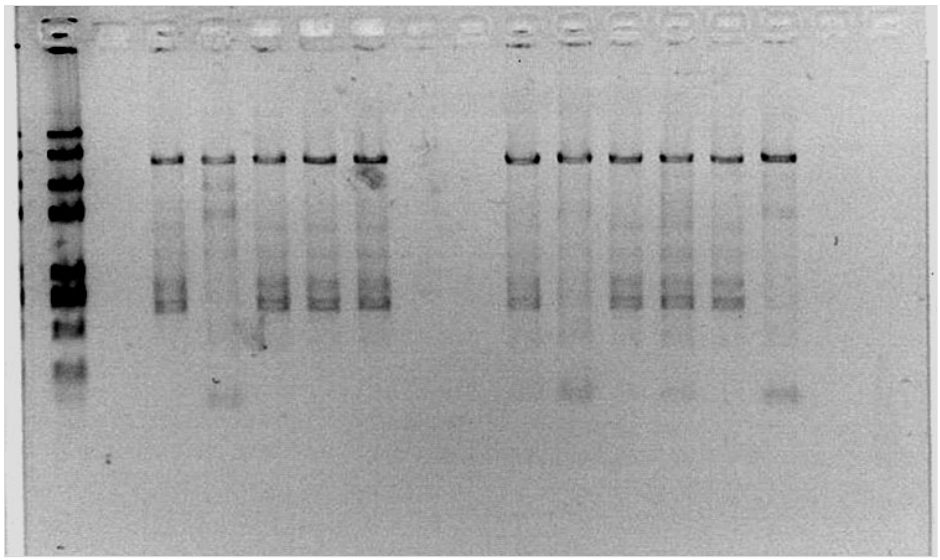
30 cycles(step 2 ~ step 4)

7,8,9,10,11,12 Parameters setting：

stage	temperature	time
step 1	95	10 min
step 2	98	10 s
step 3	55	15 s
step 4	72	4 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 2K PlusII, 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)

Purification of full ccm PCR product Recorder: Liudong Luo, Menglong Jin, Zhenyu Jiang (1). Add 450 μL Buffer B3 to the 90 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	ccm PCR pur 3+4	ccm PCR pur 5+6
Concentration(ng/μL)	107.5	114.8	96.5
260/280	1.82	1.83	1.84
260/230	2.06	2.06	2.22

sample	ccm PCR pur 7+8	ccm PCR pur 9+10	ccm PCR pur 11+12
Concentration(ng/μL)	113.4	164.6	150.3
260/280	1.82	1.82	1.81
260/230	1.94	2.00	1.89

Transformation of Mtr-pET28 Recorder: Wenfei Yu NOTE:Generally, competent bacteria are restrored in -70 degree centrigrade environment. 1.Take the competent bacteria from refrigerator and incubate them into ice about 5 mins until it is dissolved 2.Absorb 100pg to 10 ng plasmid(normally 1 to 2 μL, DO NOT add more than 5% volumn of bacteria solution) and mix it

with bacteria solution thoroughly. ATTENTION: Please operate this step tenderly!!! 3.Put the tubes on the ice about 30 mins.(Time SHOULD BE ACCURATE) 4.Make a heat shock at 42 degree centigrade about 90 sec(TIME SHOULD BE ACCURATE) 5.Put the tubes on the ice about 5 mins again. 6.Add 900 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 kanamycin. 10.Cultivate these bacteria overnight for further use.

The plating result is as following:

图片名称 图片名称

PCR of Mtr Recorder: Wenfei Yu

Experimental materials

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

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
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	22 μL	22 μL
2× PrimeStar(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	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	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	1 μL
mtr-resr	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	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	50 μL

2.PCR reaction 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18 Parameters setting：

stage	**temperature**	**time**
step 1	98	10 min
step 2	98	10 s
step 3	56	5 s
step 4	72	5 min 30s
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

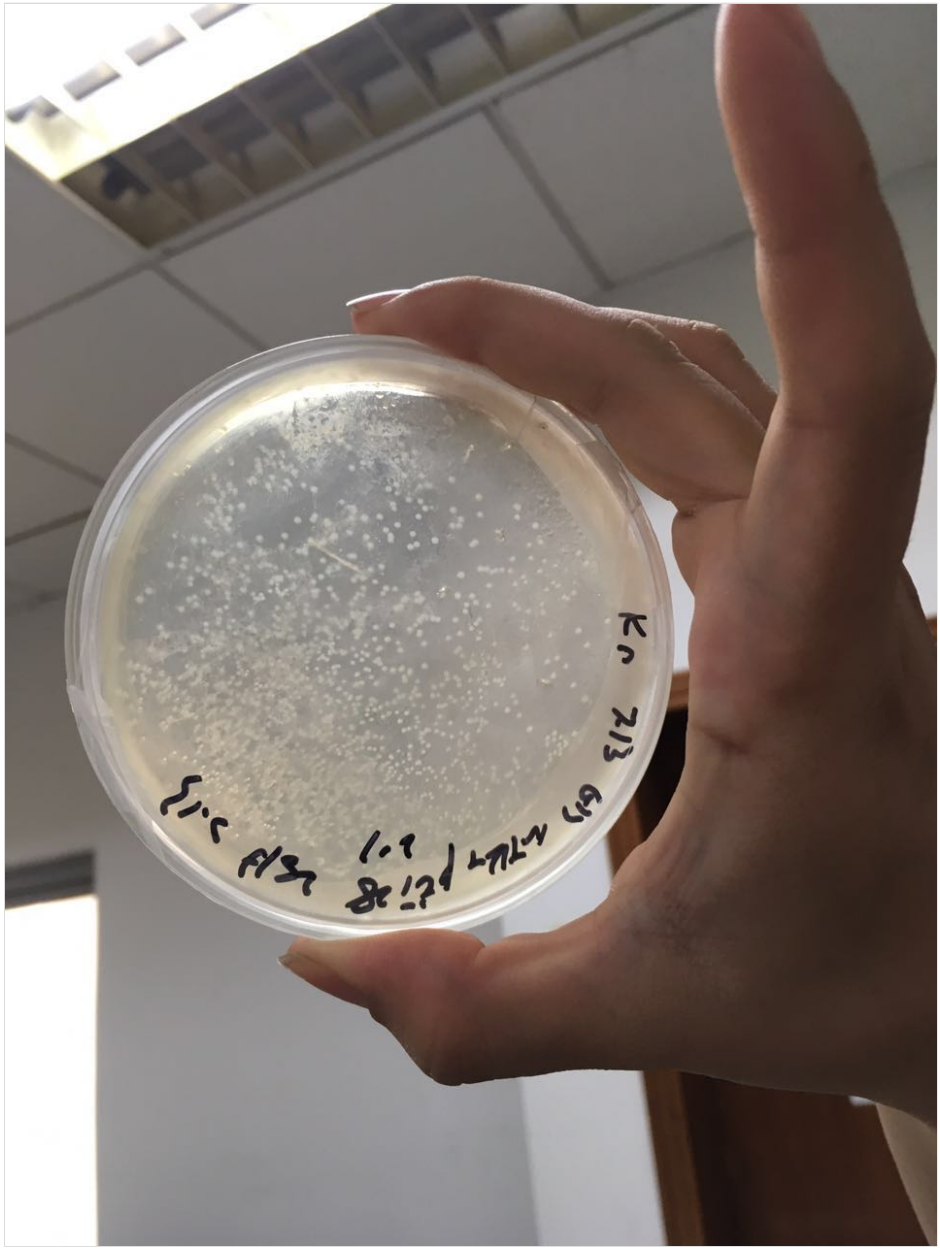
Purification of Mtr PCR product Recorder: Wenfei Yu (1). Add 450 μL Buffer B3 to the 90 μL solution and mix it up. Add it to an adsorption column. (2). 11,000 rpm centrifuge for 30 s, and then discard the filtrate. (3). Add 500 μL Wash Solution, 12,000 rpm centrifuge for 30 s, and then discard the filtrate. (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.

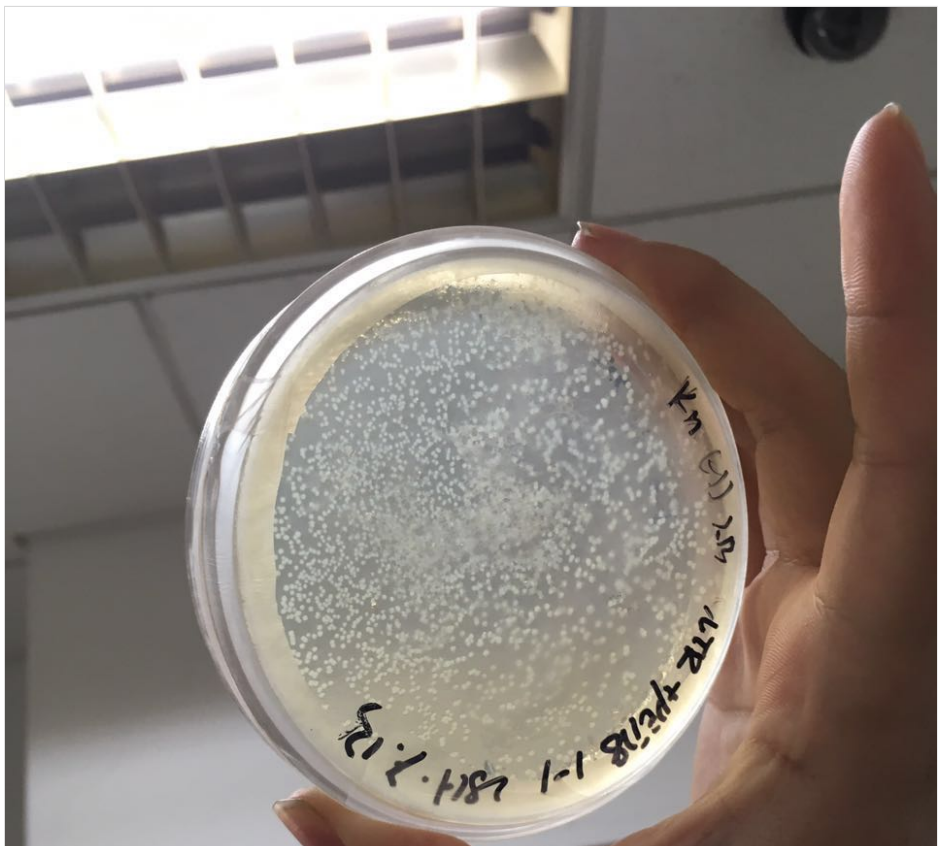
OD measurement result:

sample	**Mtr PCR pur-10 1+2+3**	**Mtr PCR pur-10 4+5+6**	**Mtr PCR pur-100 1+2+3**	**Mtr PCR pur-100 4+5+6**	**Mtr PCR pur-200 1+2+3**	**Mtr PCR pur-200 4+5+6**
Concentration(ng/μL)	224.3	153.8	93.1	276.9	570.2	464.3
260/280	1.88	1.82	1.79	1.87	1.84	1.87
260/230	2.17	1.90	1.61	2.27	2.30	2.21

Transformation of Mtr-pET28 Recorder: Meiying Cui NOTE:Generally, competent bacteria are restrored in -70 degree centrigrade environment. 1.Take the competent bacteria from refrigerator and incubate them into ice about 5 mins until it is dissolved 2.Absorb 10 μL product and mix it with bacteria solution thoroughly. ATTENTION: Please operate this step tenderly!!! 3.Put the tubes on the ice about 30 mins.(Time SHOULD BE ACCURATE) 4.Make a heat shock at 42 degree centigrade about 90 sec(TIME SHOULD BE ACCURATE) 5.Put the tubes on the ice about 5 mins again. 6.Add 900 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 kanamycin. 10.Cultivate these bacteria overnight for further use.

The plating result is as following:1st-4th is the experimental group,5th is the control group.





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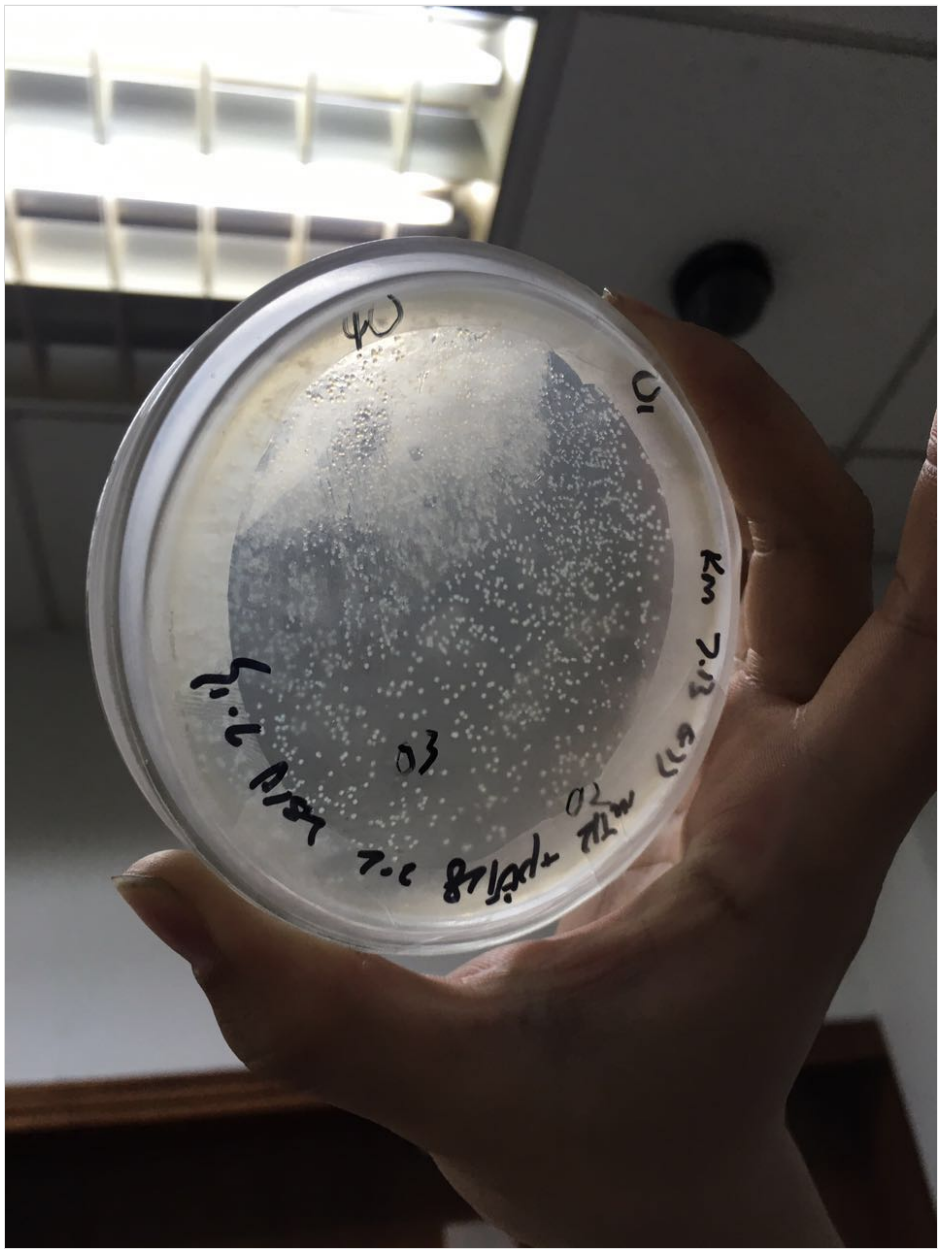
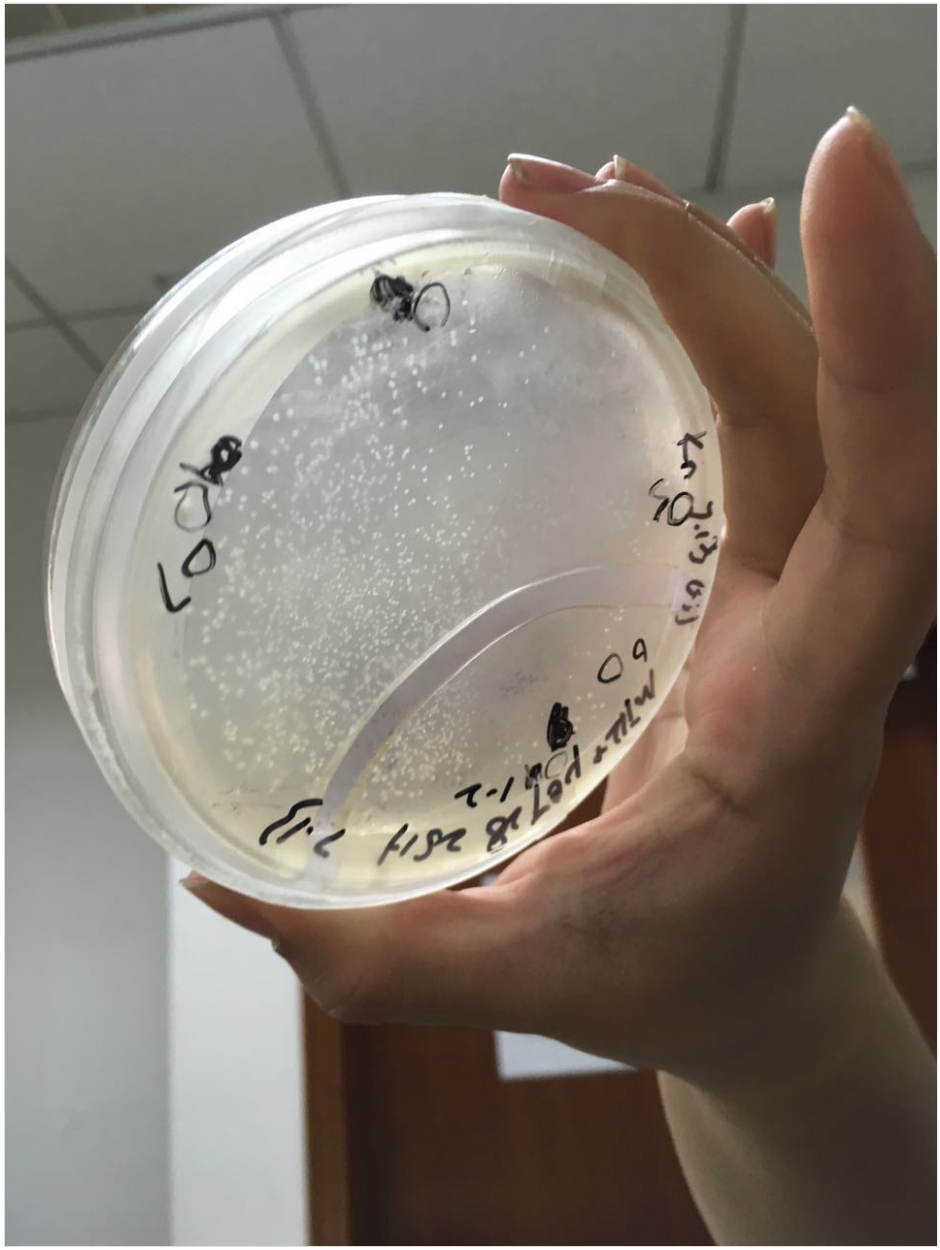
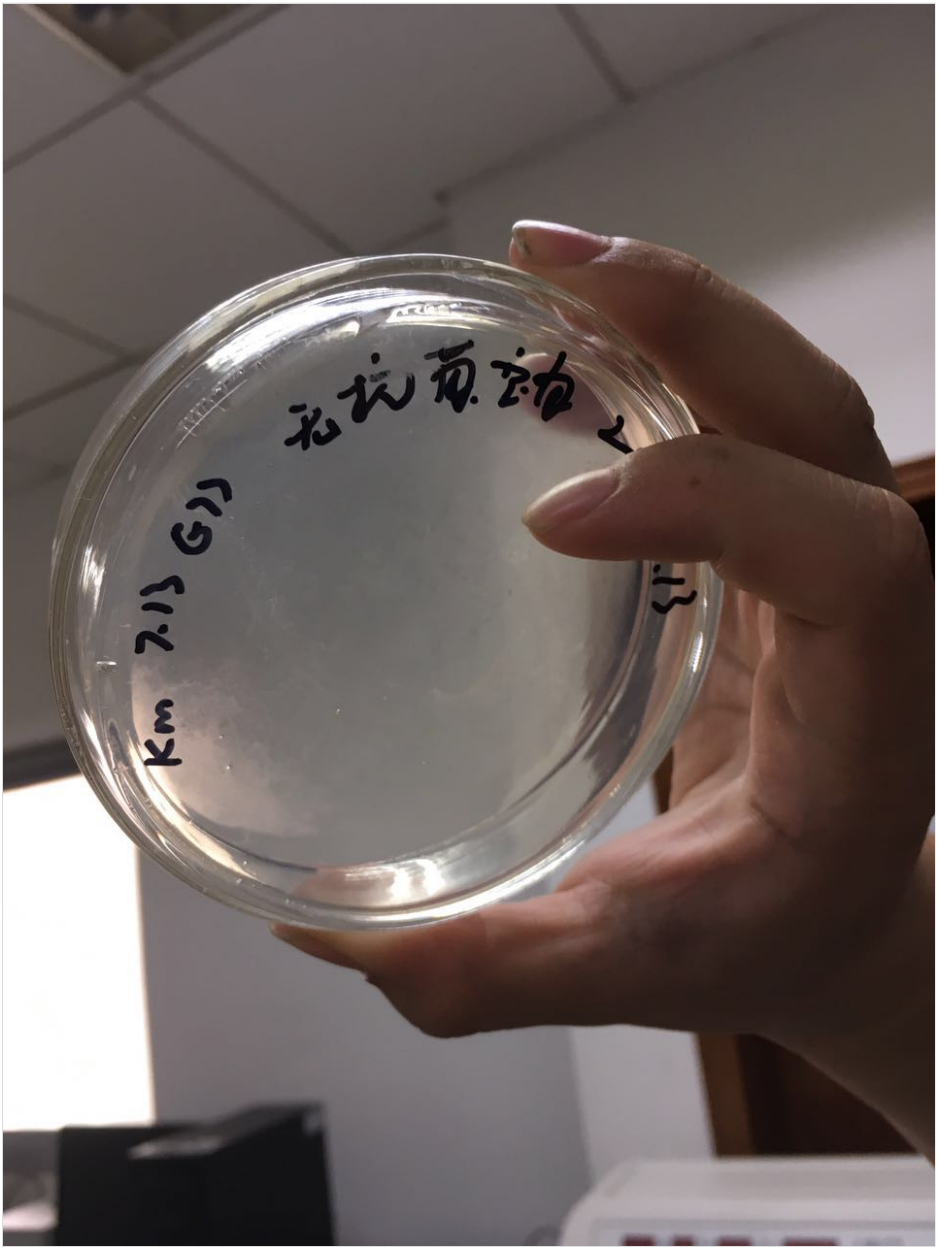


帮助



帮助



PCR of Mtr Recorder: Meiying Cui，Wenfei Yu

Experimental materials

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

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
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	22 μL	22 μL

sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
2× PrimeStar(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	25 μL	25 μL
template (20ng/μL)	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	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	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	20 μL

2.PCR reaction 1,2,3,4,5,6 Parameters setting：

stage	temperature	time
step 1	98	10 min
step 2	98	10 s
step 3	56	10 s
step 4	72	5 min 30s
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

7,8,9,10,11,12 Parameters setting：

stage	temperature	time
step 1	95	10 min
step 2	98	10 s
step 3	55	15 s
step 4	72	4 min
step 5	72	10 min
step 6	4	--

| stage | temperature | time |

30 cycles(step 2 ~ step 4)

Plasmid Extraction of the pET28 Recorder: Wenfei Yu, Xiaoyu Zhang 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	pET28-1	pET28-2	pET28-3	pET28-4	pET28-5	pET28-6	pET28-7	pET28-8
Concentration(ng/μL)	249.3	247.1	267.3	221.5	140.3	161.7	223.4	170.2
260/280	1.85	1.84	1.85	1.80	1.59	1.84	1.82	1.88
260/230	1.74	1.95	1.99	1.46	0.73	1.85	1.64	2.18

Recorder: Shihan Zhu Ligation of pET28 and Mtr PCR product

Material:

1. double digestion product of pET28 1（212 ng/μL）
2. double digestion product of Mtr PCR product (98 ng/μL)
3. 10× T4 DNA ligase buffer,T4 DNA ligase(bought from Thermo Fisher Scientific)

Procedure: Add to either of samples: 1 μL ddpET28 10 μL ddMtr PCR product 2 μL 10* T4 DNA Ligase Buffer 0.4 μL T4 DNA Ligase 7 μL sterilized water

Mix gently and incubate at 16 degree Celsius for 11 hour.

PCR of full Ccm Recorder: Yonghao Liang

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：

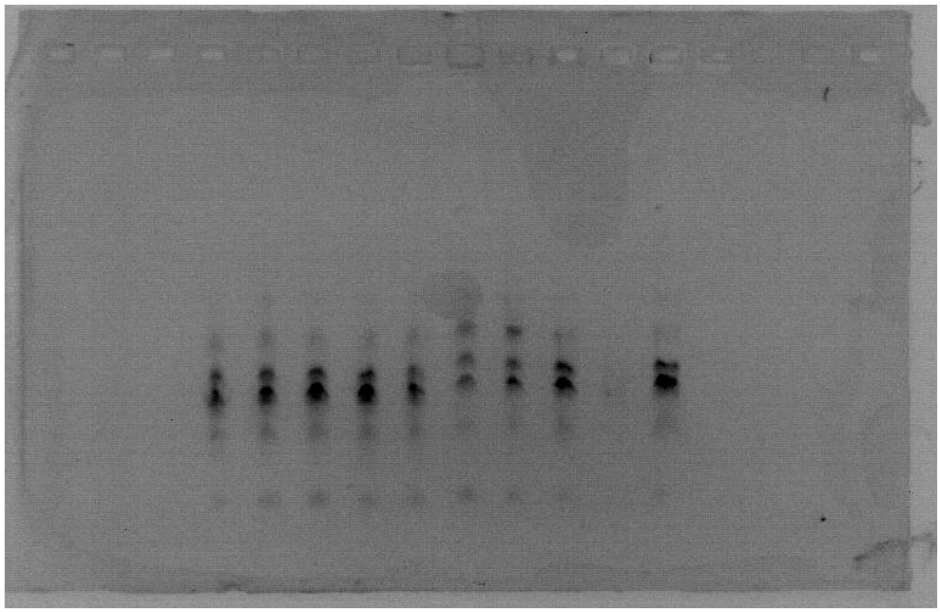
sample	1	2	3	4	5	6	7	8	9	10	11	12
Sterilized ddH2O	22 μL	22 μL	22 μL	22 μL	22 μL	22 μL	22 μL	22 μL	22 μL	22 μL	22 μL	22 μL
2×Prime Star(premix)	25 μL	25 μL	25 μL	25 μL	25 μL	25 μL	25 μL	25 μL	25 μL	25 μL	25 μL	25 μL
template	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
ccm-res-f	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
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
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

stage	temperature	time
step 1	95	10 min
step 2	98	10 s
step 3	55	15 s
step 4	72	4 min
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:



Purification of Mtr PCR product Recorder: Meiying Cui (1). Add 100 μL Buffer B2 to the product 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	**Mtr PCR pur(20ng/μL) 1+2+3**	**Mtr PCR pur-10(20ng/μL) 4+5+6**	**Mtr PCR pur(20ng/μL) 7+8+9**	**Mtr PCR pur (10ng/μL) 1'+2'+3'**	**Mtr PCR pur (10ng/μL)4'+5'+6'**	**Mtr PCR pur (10ng/μL)7'+8'+9'**
Concentration(ng/μL)	48.2	162.4	208.5	215.3	69.4	14.7
260/280	1.90	1.86	1.87	1.86	1.83	1.80
260/230	1.75	2.24	2.28	2.15	1.63	2.45

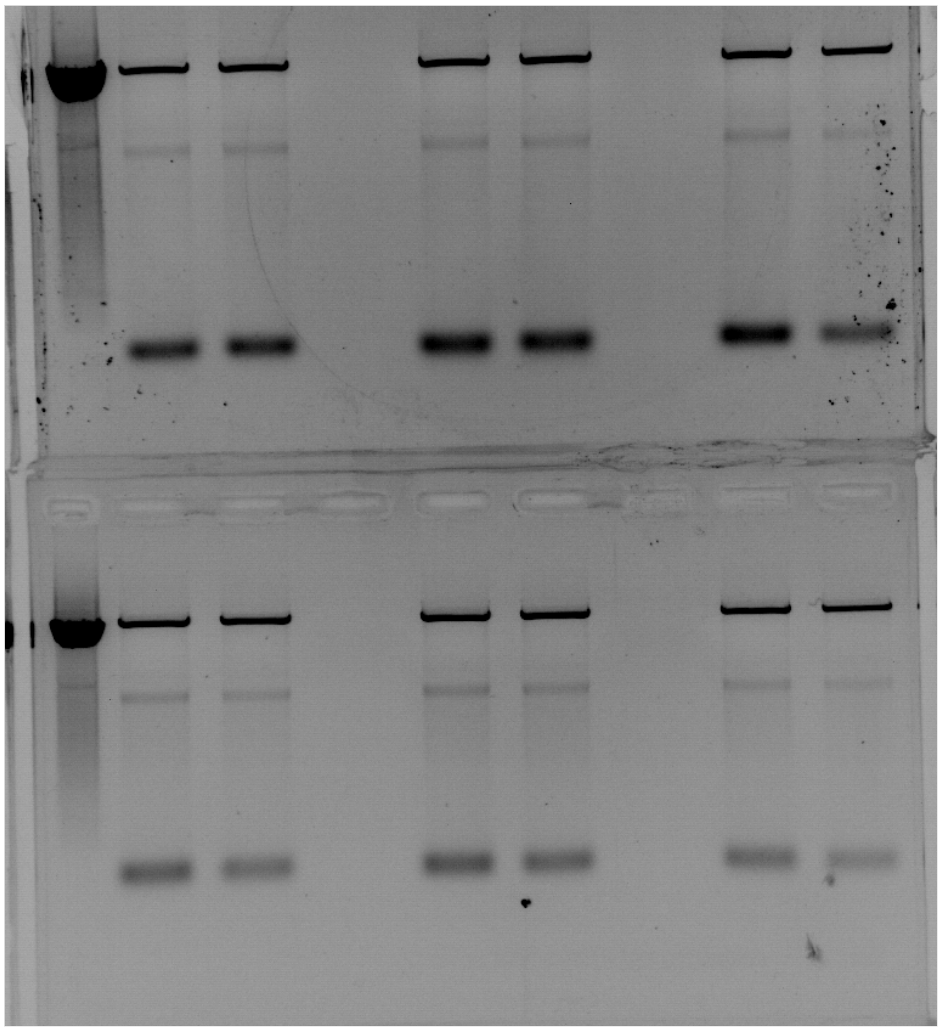
Gel Extraction of ddMtr (Done by Wenfei Yu) Procedure:

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

sample	**ddMtr 1**	**ddMtr 2**	**ddMtr 3**	**ddMtr 4**	**ddMtr 5**	**ddMtr 6**
Concentration(ng/μL)	3.3	2.6	2.4	10.6	9.6	5.8
260/280	2.41	3.05	2.33	1.80	1.71	2.09
260/230	0.55	0.12	1.17	0.19	0.46	0.04

帮助

Agarose gel electrophoresis Result:



(from left to right: I: Mtr, ddMtr 1-1, ddMtr 1-2, empty, ddMtr 2-1, ddMtr 2-2, empty, ddMtr 3-1, ddMtr 3-2; II: Mtr, ddMtr 4-1, ddMtr 4-2, empty, ddMtr 5-1, ddMtr 5-2, empty, ddMtr 6-1, ddMtr 6-2)

Double digestion of ccm Recorder: Xingwei Yang,Liudong Luo, Zhenyu Jiang Materials:

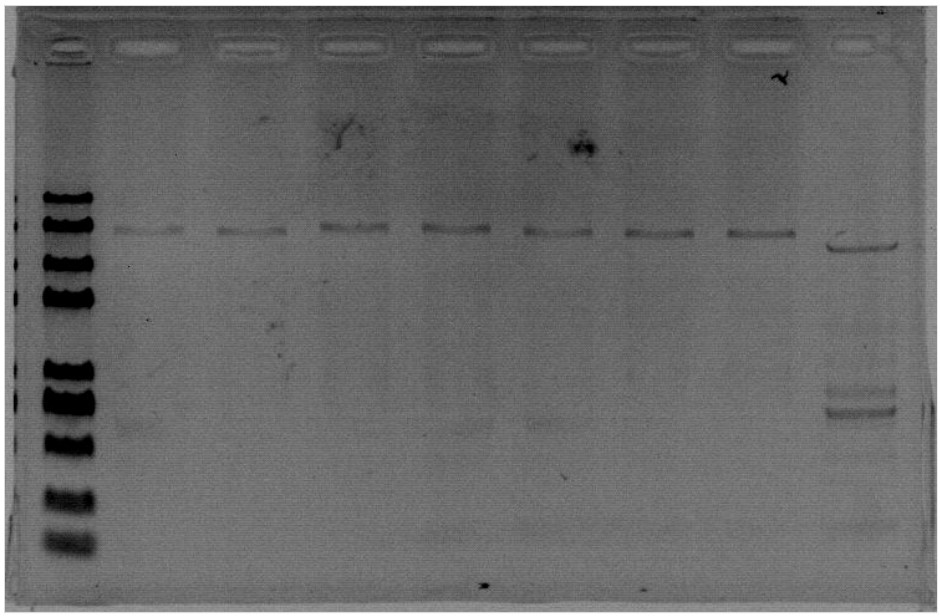
1. full ccm
2. FastDigest restriction enzyme XbaI, pstI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water

Reaction system:

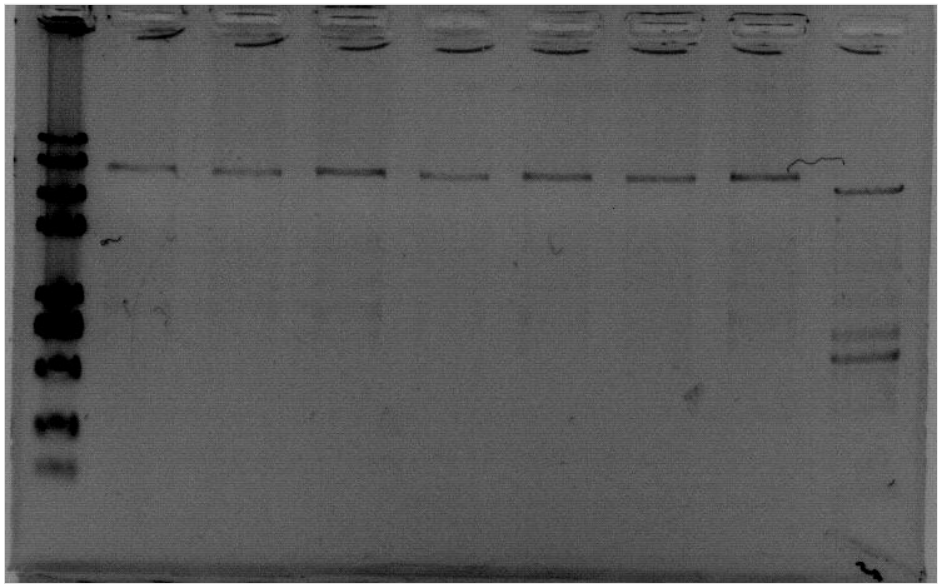
Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
XbaI(μL)	1	1	1	1	1	1	1
pstI(μL)	1	1	1	1	1	1	1
nuclease-free water(μL)	24	24	24	24	24	24	24
fastdigest green buffer(μL)	2	2	2	2	2	2	2
pTB(μL)	2	2	2	2	2	2	2
total(μL)	30	30	30	30	30	30	30

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

Agarose gel electrophoresis Result:



(lane 1 to 9: 2K plus 2,ddccm-1-1,ddccm-1-2,ddccm-2-1,ddccm-2-2,ddccm-3-1,ddccm-3-2,ddccm-7-1,ccm-1)



(lane 1 to 9: 2K plus 2,ddccm-4-1,ddccm-4-2,ddccm-5-1,ddccm-5-2,ddccm-6-1,ddccm-6-2,ddccm-7-2,ccm-2)

Then we did the gel extraction of the fragments we got.

The results are as following:

sample	**ccmXPdd 1**	**ccmXPdd 2**	**ccmXPdd 3**	**ccmXPdd 4**
Concentration(ng/μL)	7.5	16.2	16.5	14.7
260/280	1.27	1.18	1.52	1.65
260/230	0.03	0.03	0.16	0.09

Double digestion of ccm Recorder: Menglong Jin,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

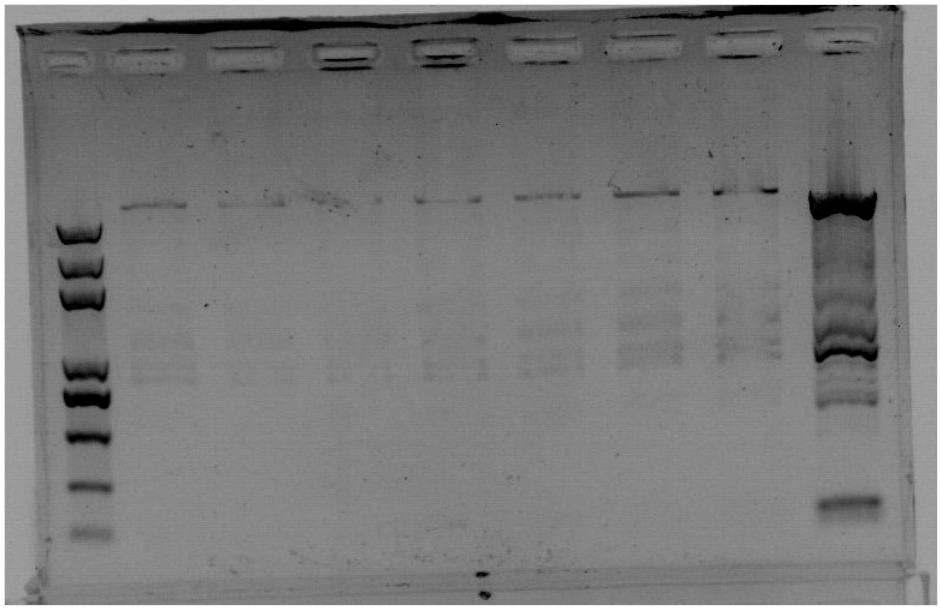
Reaction system:

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
XbaI(μL)	1	1	1	1	1	1	1

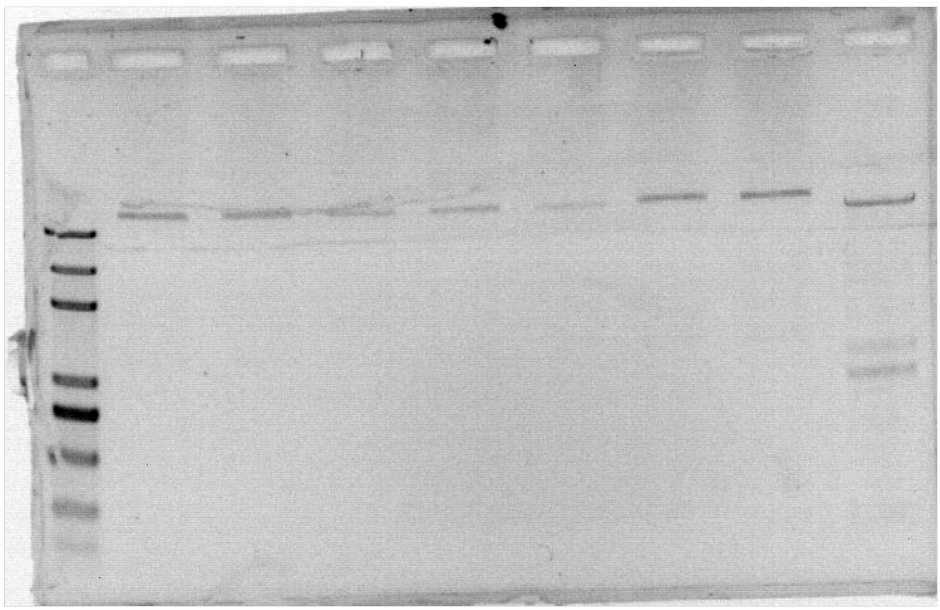
Sample	1	2	3	4	5	6	7
pstI(μL)	1	1	1	1	1	1	1
nuclease-free water(μL)	24	24	24	24	24	24	24
fastdigest green buffer(μL)	2	2	2	2	2	2	2
pTB(μL)	2	2	2	2	2	2	2
total(μL)	30	30	30	30	30	30	30

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

Agarose gel electrophoresis Result:



(lane 1 to 9: 2K plus 2,ddccm-1-1,ddccm-1-2,ddccm-4-1,ddccm-4-2,ddccm-5-1,ddccm-5-2,ddccm-7-1,ccm-1)



(lane 1 to 9: 2K plus 2,ddccm-2-1,ddccm-2-2,ddccm-3-1,ddccm-3-2,ddccm-6-1,ddccm-6-2,ddccm-7-2,ccm-2)

Then we did the gel extraction of the fragments we got.

The results are as following:

sample	ccmXPdd 1	ccmXPdd 2
Concentration(ng/μL)	5.0	8.9
260/280	1.37	1.57

sample	ccmXPdd 1	ccmXPdd 2
260/230	0.11	0.13

Double digestion of pET28 Recorder: Tong Xiao Materials:

1. Plasmid extraction product of pET28
2. FastDigest restriction enzyme NdeI, XhoI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water
4. marker:Trans 2K PlusII

sample data:

sample	pET28-1	pET28-2
Concentration(ng/μL)	85.2	71.9
260/280	1.84	1.90
260/230	2.10	2.30

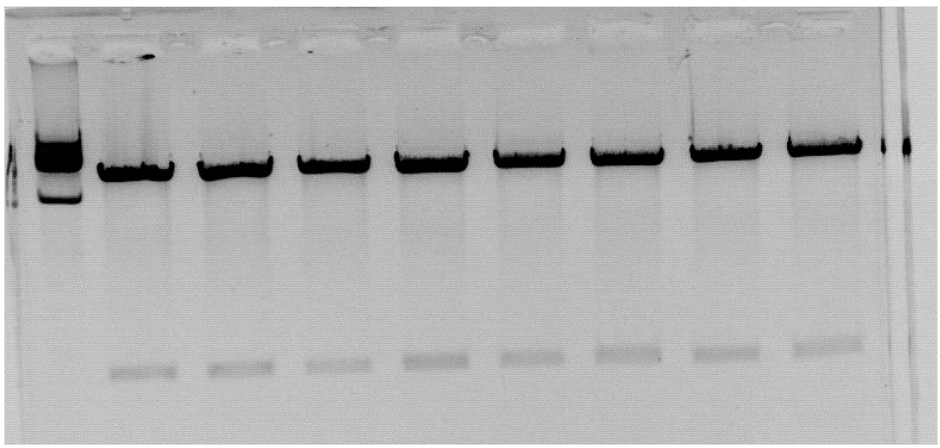
(sample1-4 uses pET28-1,sample5-8 uses pET28-2)

Reaction system:

Sample	1	2	3	4	5	6	7	8
pET28 products(μL)	8	8	8	8	8	8	8	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)	8	8	8	8	8	8	8	8
fastdigest green buffer(μL)	2	2	2	2	2	2	2	2
total(μL)	20	20	20	20	20	20	20	20
Sample	1	2	3	4	5	6	7	8

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

Agarose gel electrophoresis Result:



(lane left to right: plasmid extraction product of pET28, pET28 1-8)

Bacteria PCR of Mtr and pET28 Recorder: Chenyang LI Procedure: 1.Prepare 9 PCR tubes and sequentially add：

sample	1	2	3	4	5	6	7	8	9
Sterilized ddH2O	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL

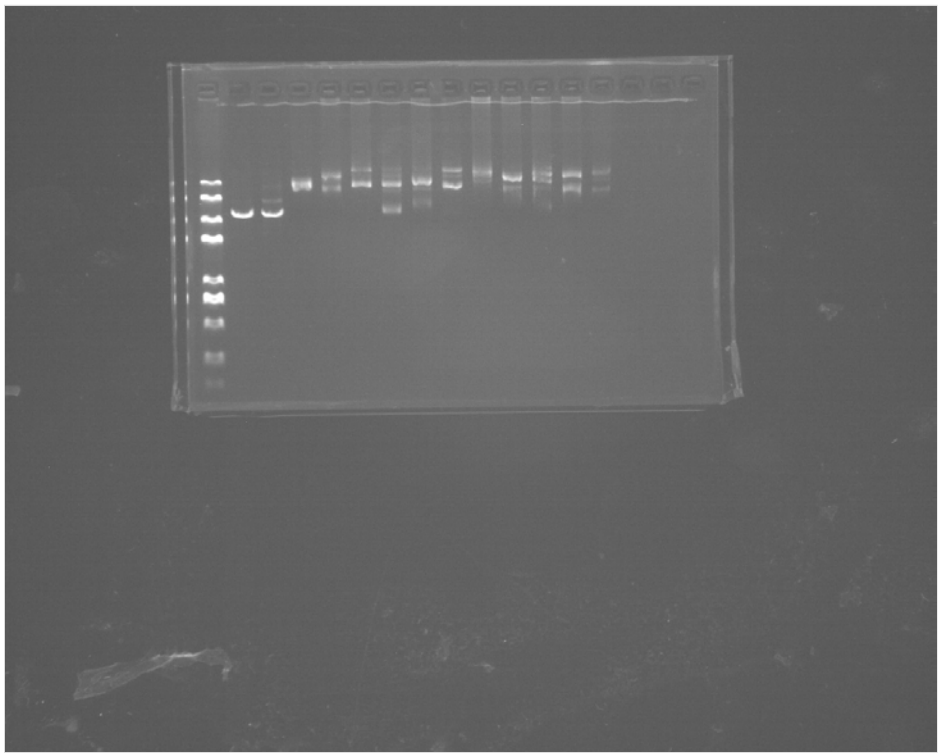
sample	1	2	3	4	5	6	7	8	9
template	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
mtr-res-f(10 μM)	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
mtr-res-r(10 μM)	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
2×Taq	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL
total	20 μL	20 μL	20 μL	20 μL	20 μL	20 μL	20 μL	20 μL	20 μL

2.PCR reaction parameters setting：

stage	temperature(°C)	time
Pre-Duration	95	10 min
Duration	95	40 s
Anneal	56	40 s
Extend	72	5 min 30 s
Post-Extend	72	10 min
Final	4	--

30 cycles(Duration ~ Extend)

3.Agarose gel electrophoresis results:



(from left to right: Trans 2K plusII(contain Gelred), Mtr PCR pur 5, Mtr PCR pur 26, template 5, pET28 8(7.9), pET28 YWF 1 (7.10), pET28 YWF 2 (7.10), pET28 YWF 3 (7.10), pET28 YWF 4 (7.10), pET28 YWF 5 (7.10), pET28 YWF 6 (7.10), pET28 YWF 7 (7.10), pET28 YWF 8 (7.10), pET28 YWF 12 (7.8))

Date 7.14

Purification of full ccm PCR product Recorder: Xingwei Yang, Zhenyu Jiang (1). Add 450 μL Buffer B3 to the 90 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.

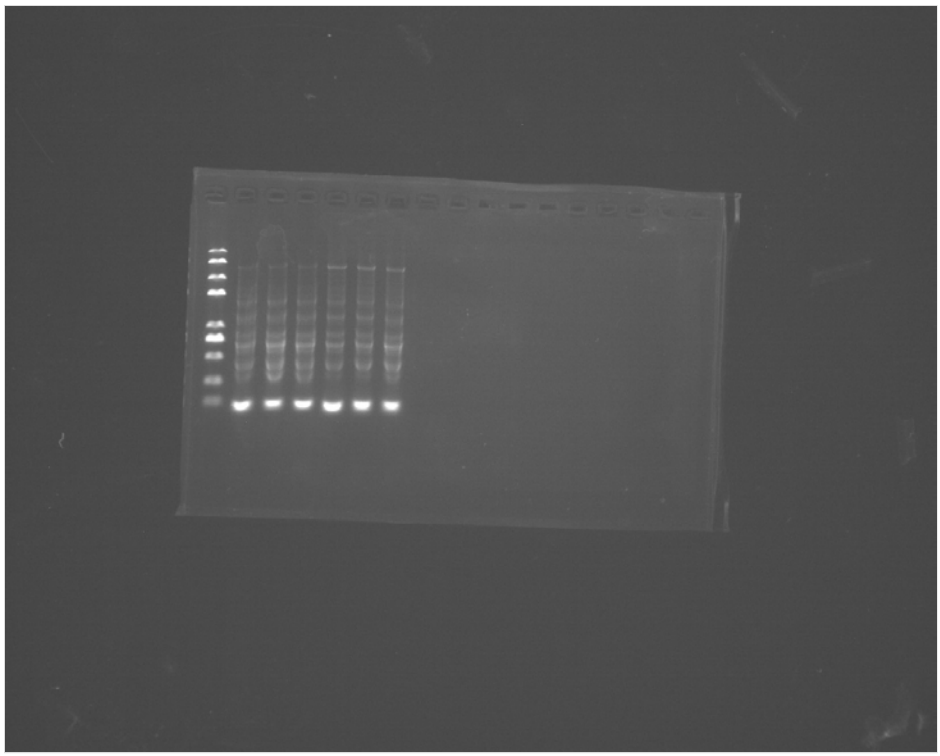
OD measurement result:

sample	ccm PCR pur 1+2	ccm PCR pur 3+4	ccm PCR pur 5+6

sample	ccm PCR pur 1+2	ccm PCR pur 3+4	ccm PCR pur 5+6
Concentration(ng/μL)	312.7	202.6	316.2
260/280	1.86	1.85	1.86
260/230	2.06	2.13	2.05

sample	ccm PCR pur 7+8	ccm PCR pur 9+10	ccm PCR pur 11+12
Concentration(ng/μL)	203.3	175.2	286.3
260/280	1.84	1.84	1.86
260/230	2.06	2.03	2.01

Agarose gel electrophoresis Result:



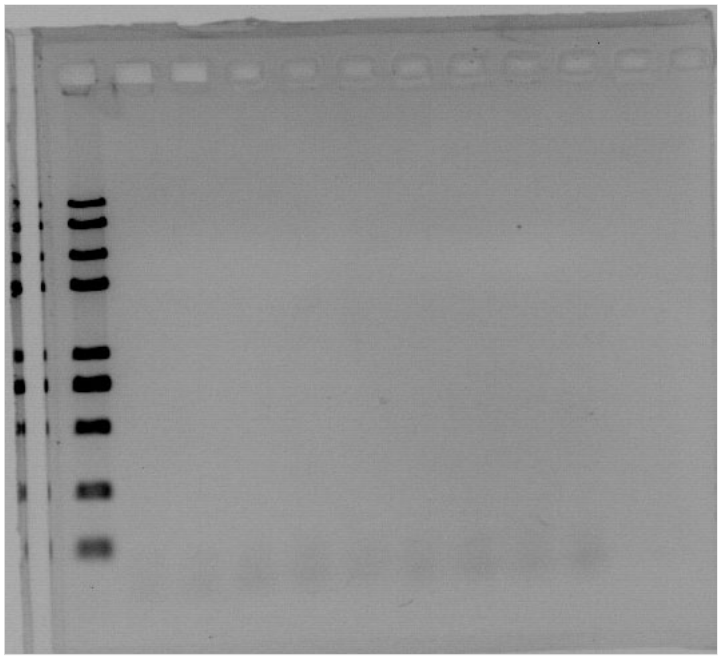
(lane left to right:2k plus 2 ,PCR of ccm 1,PCR of ccm 2,PCR of ccm 3,PCR of ccm 4,PCR of ccm 5,PCR of ccm 6)

Transformation of plasmid pTB containing CcmA-H and pET28 containing MTR 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 10μl plasmid and mix it with bacteria solution thoroughly. ATTENTION: Please operate this step tenderly!!! 3.Put the tubes on the ice about 30 mins.(Time SHOULD BE ACCURATE) 4.Make a heat shock at 42 degree centigrade about 30 sec(TIME SHOULD BE ACCURATE) 5.Put the tubes on the ice about 2 to 3 mins again. 6.Add 900 μL LB medium into EP tubes and cultivate the bacteria at 37 degree centrigrade about 40 to 60 min. 7.Centrifuge them at 12,000xg about 15 sec and we will see sediment in the tubes. 8.Discard the supernatant liquid and leave about 220 μL medium. 9.Coat plate: add 200 μL solution in a large plate while add 20 μL solution in a small plate. 10.Cultivate these bacteria overnight for further use.

帮助

Agarose gel electrophoresis of bacteria PCR of Mtr and pET28 Recorder:Wenfei Yu



(from left to right: marker, ba' PCR-1, ba' PCR-2, ba' PCR-3, ba' PCR-4, ba' PCR-5, ba' PCR-6, ba' PCR-7, ba' PCR-8, ba' PCR-9)

PCR of Mtr Recorder: Tong Xiao

Experimental materials

1. Template: Genome extracted from BL21(DE3);
2. Primer: mtr-res-f, mtr-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
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	50 μL	50 μL	50 μL	50 μL	50 μL	50 μL	50 μL	50 μL

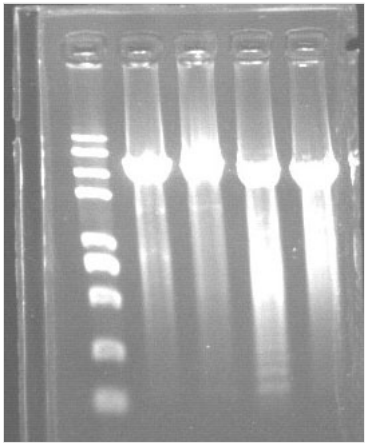
2.PCR reaction 1,2,3,4,5,6,7,8 Parameters setting：

stage	**temperature**	**time**
step 1	98	10 min
step 2	98	10 s
step 3	56	10 s
step 4	72	5 min 30s
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

帮助

The result of agarose gel electrophoresis of PCR of Mtr:



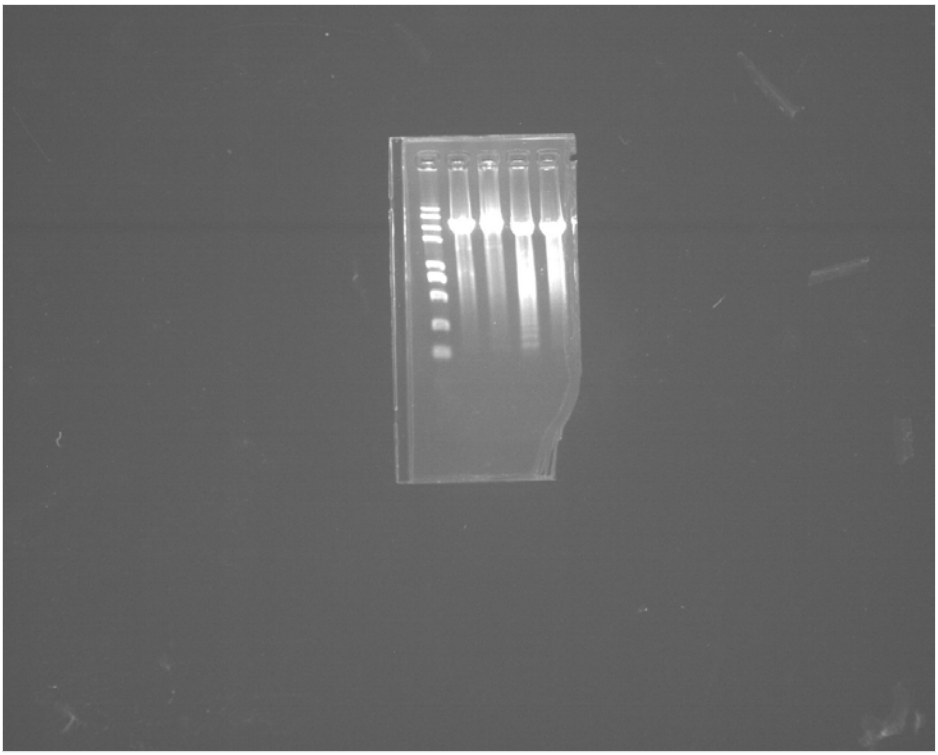
(from left to right: marker 2K plus II, PCR-1, PCR-2, PCR-3, PCR-4)

Purification of mtr PCR product Recorder: Shihan Zhu (1). Add 250 μL Buffer B3 to the 50 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 30 μL ddH2O, stand them still for 10 min. Centrifuge at 12,000 rpm for 1 min.

OD measurement result:

sample	**mtr-pcr-pur-1**	**mtr-pcr-pur-2**	**mtr-pcr-pur-3**	**mtr-pcr-pur-4**
Concentration(ng/μL)	144.1	422.8	626.5	216.4
260/280	1.85	1.87	1.81	1.88
260/230	2.18	2.20	2.15	2.29

Agarose gel electrophoresis of pur product of PCR of Mtr Recorder:Meiying Cui



(from left to right: marker, Mtr PCR pur3,2,1,4)

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.

帮助

Procedure:

1.Prepare 4 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 1,2,3,4,5,6 Parameters setting：

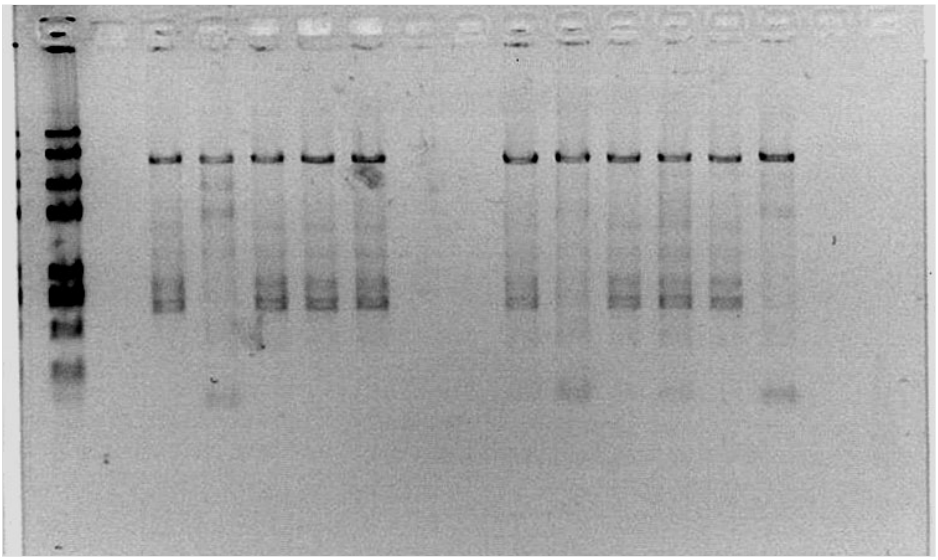
stage	**temperature**	**time**
step 1	95	10 min
step 2	98	10 s
step 3	59	5 s
step 4	72	4 min
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

7,8,9,10, Parameters setting：

stage	**temperature**	**time**
step 1	95	10 min
step 2	98	10 s
step 3	55	15 s
step 4	72	4 min
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4) 3.Agarose gel electrophoresis Result:



(lane left to right: marker 2K PlusII, 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)

Purification of full ccm PCR product Recorder: Liudong Luo, Zhenyu Jiang (1). Add 450 μL Buffer B3 to the 90 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**	**ccm PCR pur 3+4**	**ccm PCR pur 5+6**
Concentration(ng/μL)	83.9	72.2	76.6
260/280	1.80	1.82	1.80
260/230	1.85	1.88	1.80

sample	**ccm PCR pur 7+8**	**ccm PCR pur 9+10**
Concentration(ng/μL)	36.0	53.4
260/280	1.70	1.66
260/230	1.03	1.08

bacteria PCR of M28(Mtr+pET28) and pET28 Recorder: Wenfei Yu

Experimental materials

1. Template: Genome extracted from BL21(DE3);
2. Primer: T7, T7-TER. Designed by ourselves, synthesized by Sangon Biotech;
3. Sterilized ddH2O, 2×PrimeStar(Premix), bought from Takara.

Procedure:

1.Prepare 24 PCR tubes and sequentially add：

sample	**M28 1-1**	**M28 1-2**	**M28 2-1**	**M28 2-2**	**M28 3-1**	**M28 3-2**	**M28 4-1**	**M28 4-2**	**M28 5-1**	**M28 5-2**	**M28 6-1**	**M28 6-2**	**M28 7-1**	**M28 7-2**	**M28 8-1**	**M28 8-2**	**M28 9-1**	**M28 9-2**	**pET28 45°C-1**	**pET28 45°C-2**	**pET28 47°C-1**	**pET28 47°C-2**	**pET28 50°C-1**	**pET28 50°C-2**
Sterilized dH2O	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	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL
2X 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	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	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	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL

sample	M281-1	M281-2	M282-1	M282-2	M283-1	M283-2	M284-1	M284-2	M285-1	M285-2	M286-1	M286-2	M287-1	M287-2	M288-1	M288-2	M289-1	M289-2	pET28 45°C-1	pET28 45°C-2	pET28 47°C-1	pET28 47°C-2	pET28 50°C-1	pET28 50°C-2
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	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	20 μL	20 μL	20 μL	20 μL	20 μL	20 μL	20 μL

2.PCR reaction M28 1-1~9-1, pET28 45°C-1, pET28 45°C-2 Parameters setting：

stage	temperature	time
step 1	94	4 min
step 2	94	30 s
step 3	45	30 s
step 4	72	5 min 30s
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

M28 1-2~9-2, pET28 50°C-1, pET28 50°C-2 Parameters setting：

stage	temperature	time
step 1	94	4 min
step 2	94	30 s
step 3	50	30 s
step 4	72	5 min 30s
step 5	72	10 min
step 6	4	--

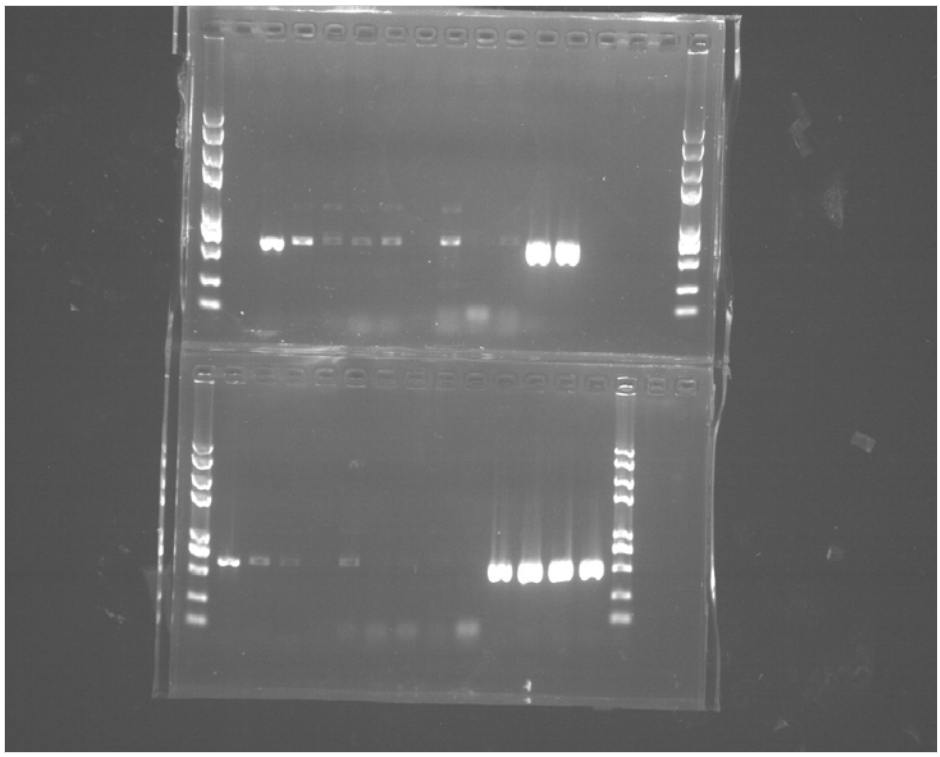
30 cycles(step 2 ~ step 4)

pET28 47°C-1, pET28 47°C-2 Parameters setting：

stage	temperature	time
step 1	94	4 min
step 2	94	30 s
step 3	47	30 s
step 4	72	5 min 30s
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:



(I: From left to right: marker, empty, 1-1, 2-1, 3-1, 4-1, 5-1, 6-1, 7-1, 8-1, 9-1, pET28 45°C-1, pET28 45°C-2, empty, empty, empty, marker II: From left to right: marker, 1-2, 2-2, 3-2, 4-2, 5-2, 6-2, 7-2, 8-2, 9-2, pET28 50°C-1, pET28 50°C-2, pET28 47°C-1, pET28 47°C-2, marker, empty, empty)

Double digestion of PET28 Recorder:Meiying Cui Materials:

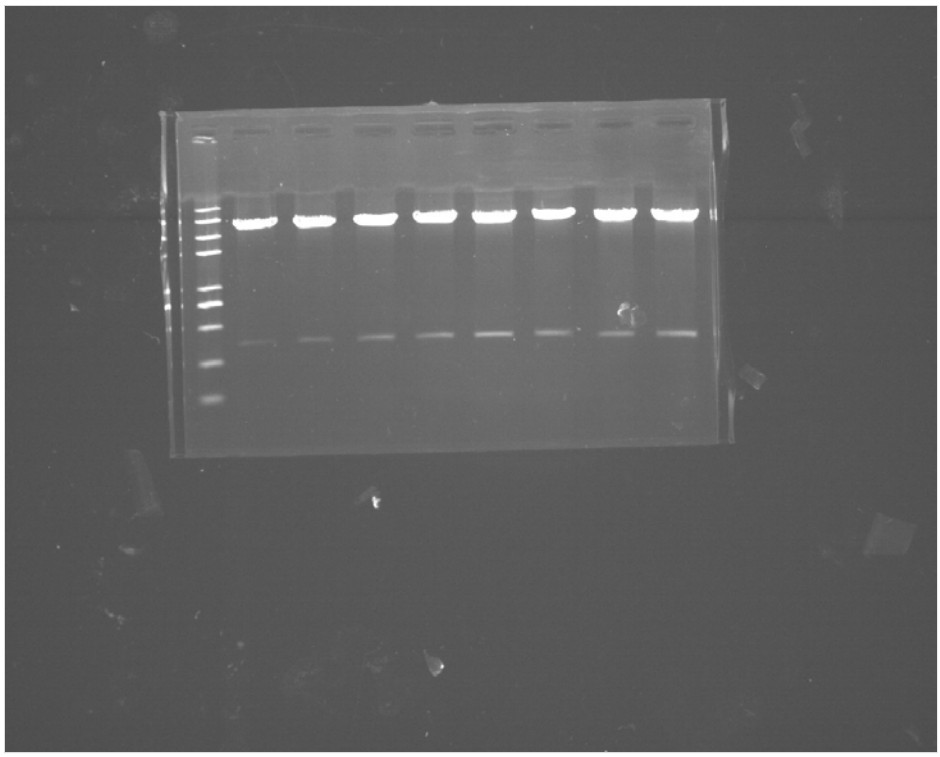
1. PET28 plasmid
2. FastDigest restriction enzyme XhoI, NdeI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water

Reaction system:

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**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)	6	6	6	8	0	5	0	0
fastdigest green buffer(μL)	2	2	2	2	2	2	2	2
PET28(μL)	10	10	10	8	16	11	16	16
total(μL)	20	20	20	20	20	20	20	20

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

Agarose gel electrophoresis Result:



(lane 1 to 9: 2K plus 2,PET28 1,2,3,4,5,6,7,8)

Then we did the gel extraction of the fragments we got.

The results are as following:

sample	**ddPET28 GE 1-4**	**ddPET28 GE 5-8**
Concentration(ng/μL)	19.8	24.2
260/280	1.86	2.01
260/230	0.12	0.11

Double digestion of PET28 Recorder: Wenfei Yu Materials:

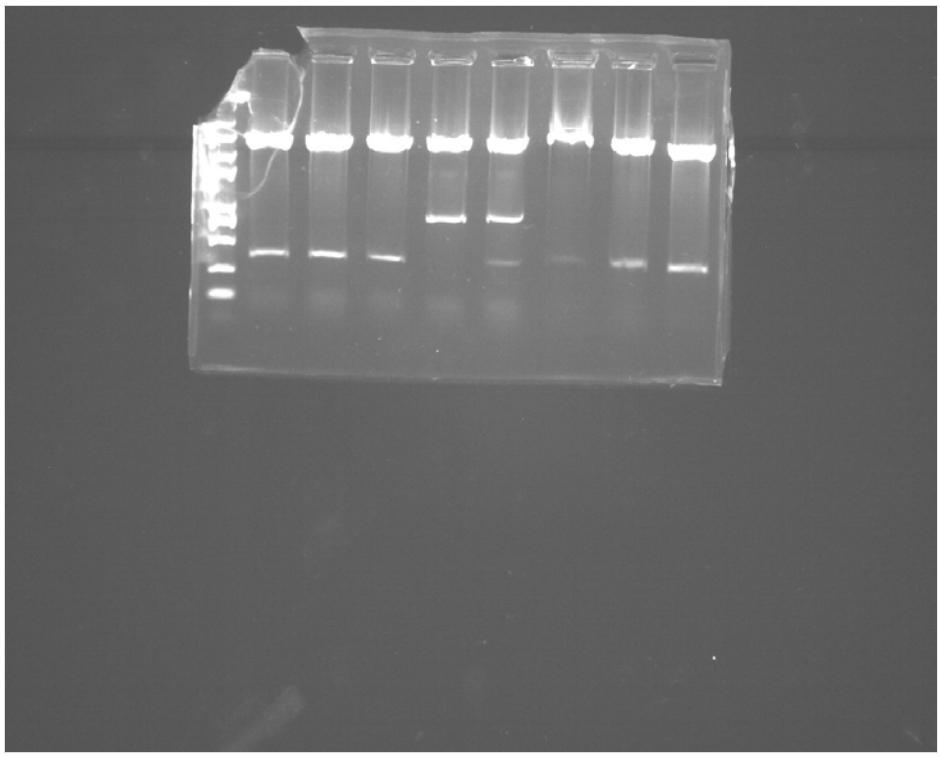
1. PET28 plasmid
2. FastDigest restriction enzyme XhoI, NdeI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water

Reaction system:

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**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)	3	3	3	0	4	6	6	6
fastdigest green buffer(μL)	2	2	2	2	2	2	2	2
PET28(μL)	13	13	13	16	12	10	10	10
total(μL)	20	20	20	20	20	20	20	20

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

Agarose gel electrophoresis Result:



(lane 1 to 9: 2K plus 2,PET28 1,2,3,4,5,6,7,8)

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

stage	**temperature**	**time**
step 1	95	10 min
step 2	98	10 s
step 3	59	5 s
step 4	72	4 min
step 5	72	10 min
step 6	4	--

stage	temperature	time

30 cycles(step 2 ~ step 4)

7,8,9,10, Parameters setting：

stage	temperature	time
step 1	95	10 min
step 2	98	10 s
step 3	55	15 s
step 4	72	4 min
step 5	72	10 min
step 6	4	--

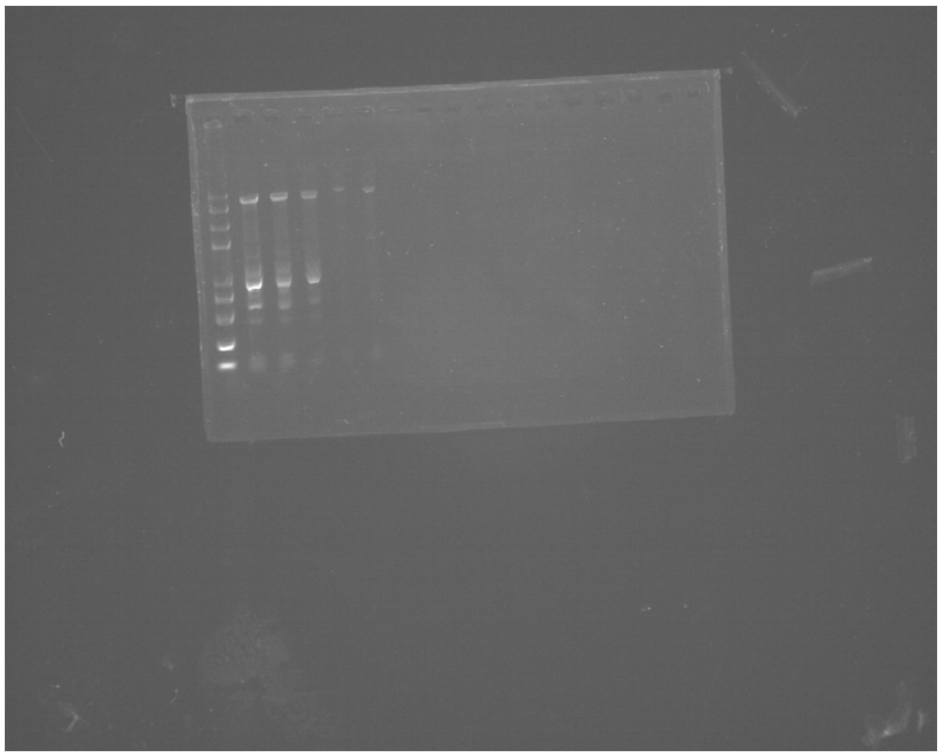
30 cycles(step 2 ~ step 4) **Purification of full ccm PCR product Recorder: Liudong Luo, Zhenyu Jiang** (1). Add 450 μL Buffer B3 to the 90 μL solution and mix it up. Add it to an adsorption column. (2). 11,000 rpm centrifuge for 30 s, and then discard the filtrate. (3). Add 500 μL Wash Solution, 12,000 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	ccm PCR pur 3+4	ccm PCR pur 5+6
Concentration(ng/μL)	83.9	72.2	76.6
260/280	1.80	1.82	1.80
260/230	1.85	1.88	1.80

sample	ccm PCR pur 7+8	ccm PCR pur 9+10
Concentration(ng/μL)	36.0	53.4
260/280	1.70	1.66
260/230	1.03	1.08

Agarose gel electrophoresis Result:



(lane left to right: marker 2K PlusII, ccm-pcr-1, ccm-pcr-2,ccm-pcr-3,ccm-pcr-4,ccm-pcr-5)

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

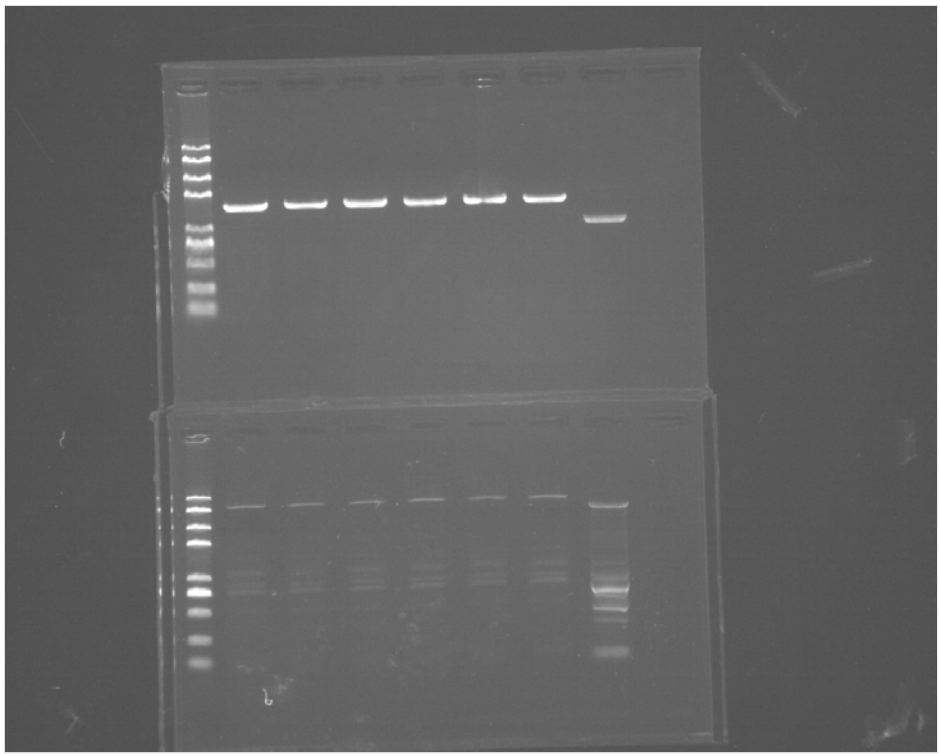
帮助

Reaction system:

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
XbaI(μL)	1	1	1	1	1	1	1
pstI(μL)	1	1	1	1	1	1	1
nuclease-free water(μL)	24	24	24	24	24	24	24
fastdigest green buffer(μL)	2	2	2	2	2	2	2
pTB(μL)	2	2	2	2	2	2	2
total(μL)	30	30	30	30	30	30	30

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

Agarose gel electrophoresis Result:



(lane 1 to 9: 2K plus 2,ddccm-1-1,ddccm-1-2,ddccm-4-1,ddccm-4-2,ddccm-5-1,ddccm-5-2,ddccm-7-1,ccm-1)

Then we did the gel extraction of the fragments we got.

The results are as following:

sample	**ccmXPdd 1**	**ccmXPdd 2**
Concentration(ng/μL)	48.9	41.3
260/280	1.37	1.47
260/230	0.11	0.07

Double digestion of pTB Recorder: Liudong Luo, Zhenyu Jiang Materials:

1. pTB
2. FastDigest restriction enzyme BcuI, pstI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water

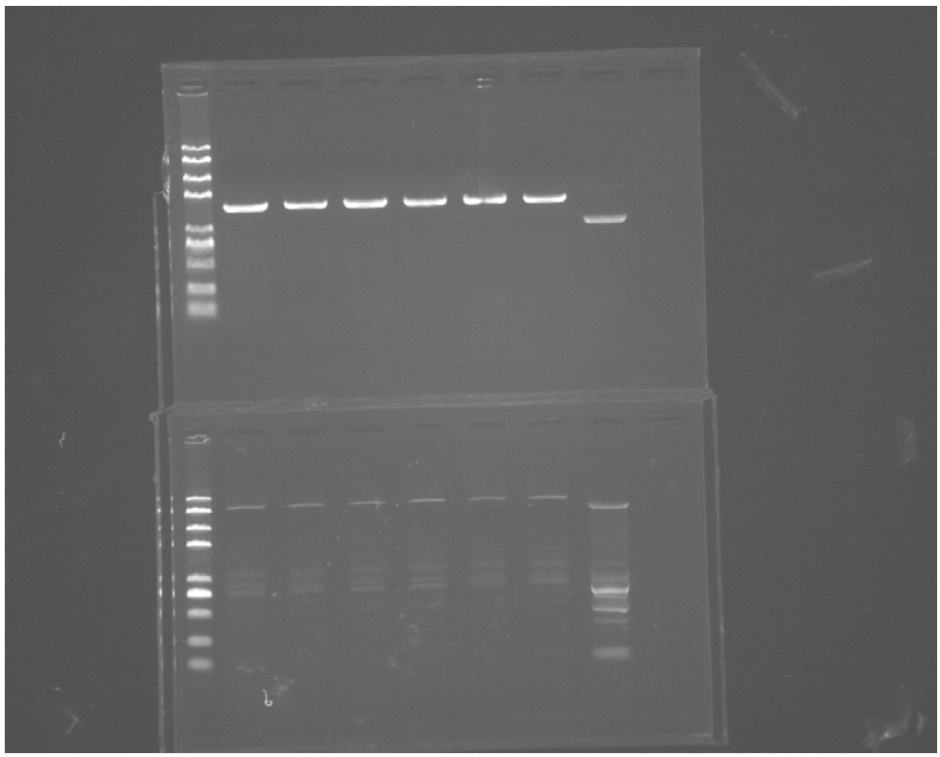
Reaction system:

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)	5	5	5	5
total(μL)	20	20	20	20

Sample	**5**	**6**	**7**	**8**
BcuI(μL)	1	1	1	1
pstI(μL)	1	1	1	1
nuclease-free water(μL)	10	10	10	10
fastdigest green buffer(μL)	2	2	2	2
pTB(μL)	6	6	6	6
total(μL)	20	20	20	20

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

Agarose gel electrophoresis Result:



(lane 1 to 9: 2k plus 2,ddpTB-1,ddpTB-2,ddpTB-3,ddpTB-4,ddpTB-5,ddpTB-6,ddpTB-7,pTB)

Then we did the gel extraction of the fragments we got.

The results are as following:

sample	**PTBSPdd 1**	**PTBSPdd 2**	**PTBSPdd 3**
Concentration(ng/μL)	25.7	22.9	28.7
260/280	1.91	1.96	1.91
260/230	0.54	1.05	0.78

Date 7.15

PCR of full Ccm Recorder: Xingwei Yang

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.

帮助

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		
2×PrimeStar(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
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
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
sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
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 Parameters setting：

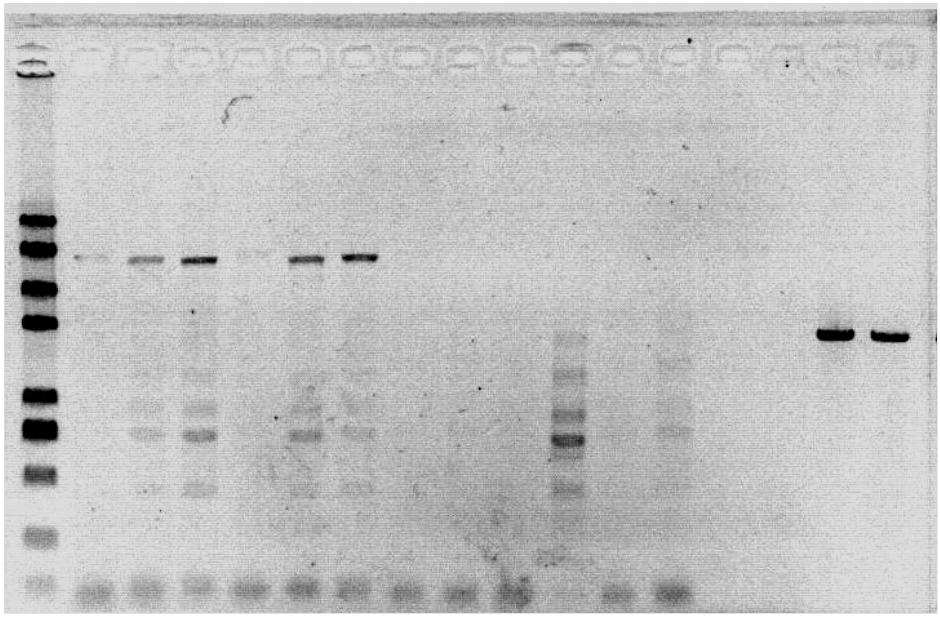
stage	**temperature**	**time**
step 1	95	10 min
step 2	98	10 s
step 3	59	5 s
step 4	72	4 min
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

7,8,9,10,11,12 Parameters setting：

stage	**temperature**	**time**
step 1	95	10 min
step 2	98	10 s
step 3	55	15 s
step 4	72	4 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 2K PlusII, 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,ddccm-1.ddccm-2,ddpTB-1,ddpTB-2) **Purification of full ccm PCR product Recorder: Zhenyu Jiang** (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 2+3+5+6**
Concentration(ng/μL)	103.9
260/280	1.84
260/230	1.89

Double digestion of mtr and pET28 Recorder: Shihan Zhu Materials:

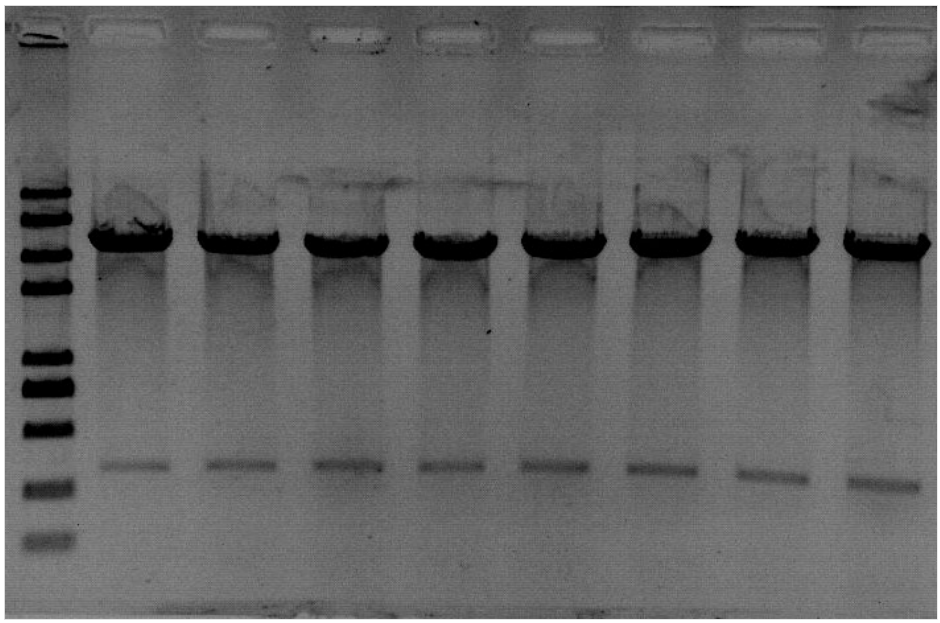
1. pcr purification products of mtr, pET28
2. FastDigest restriction enzyme NdeI, XhoI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water

Reaction system:

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**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)	6	6	6	6	6	6	6	6
fastdigest green buffer(μL)	2	2	2	2	2	2	2	2
pET28(μL)	10	10	10	10	10	10	10	10
total(μL)	20	20	20	20	20	20	20	20

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

Agarose gel electrophoresis Result:

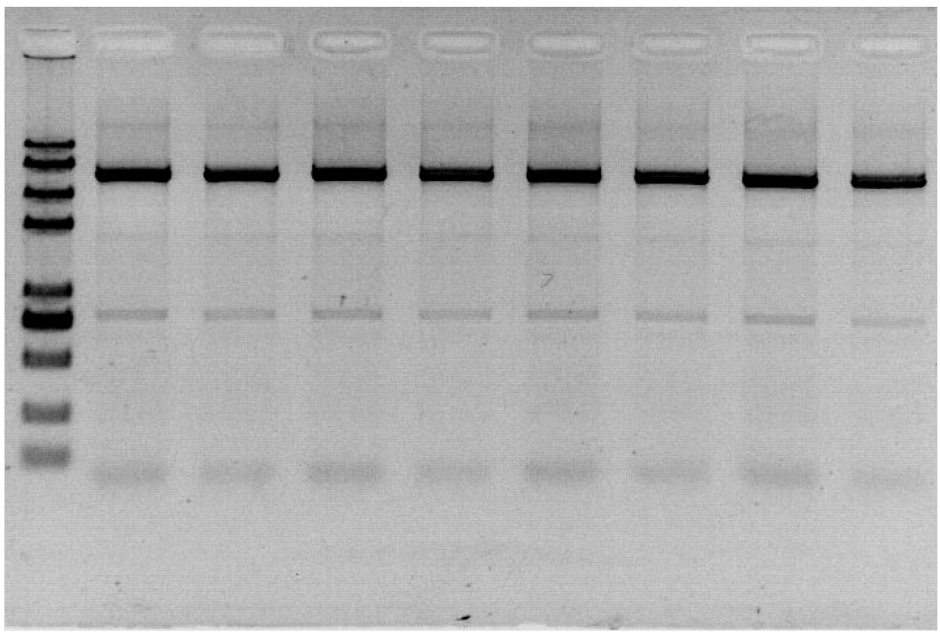


(lane 1 to 9: 2K plus II,dd-pET28-1,dd-pET28-2,dd-pET28-3,dd-pET28-4,dd-pET28-5,dd-pET28-6,dd-pET28-7,dd-pET28-8)

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**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)	25	25	25	25	25	25	25	25
fastdigest green buffer(μL)	2	2	2	2	2	2	2	2
mtr(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 30 mins .

Agarose gel electrophoresis Result:



(lane 1 to 9: 2K plus II,dd-mtr-1,dd-mtr-2,dd-mtr-3,dd-mtr-4,dd-mtr-5,dd-mtr-6,dd-mtr-7,dd-mtr-8)

Gel Extraction of dd-Mtr and dd-pET28 **Recorder: Shihan Zhu Procedure:

1. Cut off the Gel part containing the target band. Get 6 tubes of AD and 5 tubes of BD.

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

sample	**dd-Mtr-1**	**dd-Mtr-2**	**dd-Mtr-3**	**dd-Mtr-4**	**dd-pET28-1**	**dd-pET28-2**
Concentration(ng/μL)	9.4	7.3	2.6	21.7	41.7	54.5
260/280	1.50	1.55	1.43	1.40	1.64	1.68
260/230	0.35	0.21	0.09	0.33	0.68	0.72

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)	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

Date 7.17

Double digestion of pTB Recorder: Menglong Jin, Bijunyao Zhong Materials: 1.pTB, produced by ourselves:

Sample	**1**	**2**	**3**	**4**	**5**	**6**
concentration(ng/μl)	223.9	223.9	223.9	138.3	138.3	138.3
260/280	1.85	1.85	1.85	1.86	1.86	1.86
260/230	2.11	2.11	2.11	2.25	2.25	2.25

2.FastDigest restriction enzyme SpeI, PstI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific) 3.Nuclease-free water

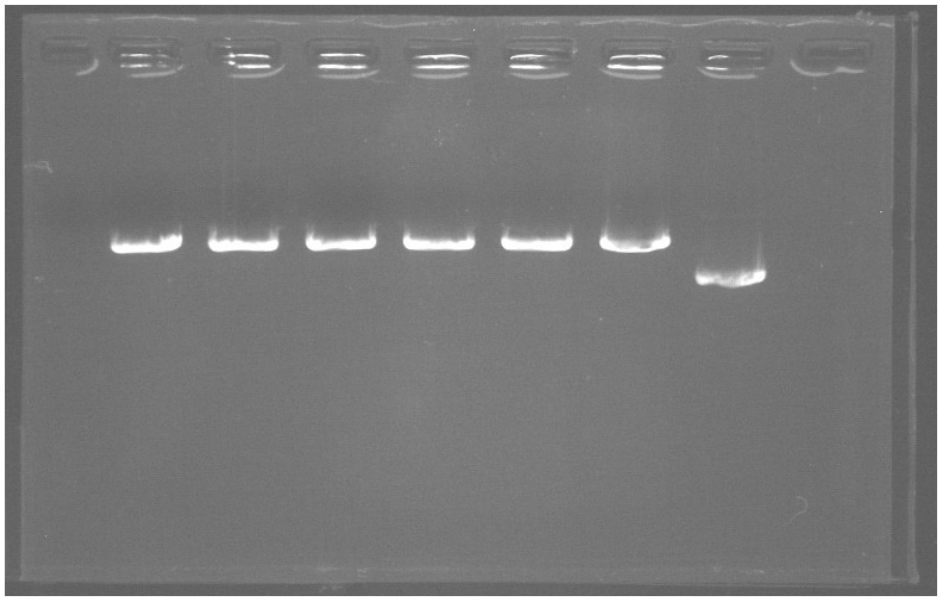
Reaction system:

Sample	**1**	**2**	**3**	**4**	**5**	**6**
XhoI(μL)	1	1	1	1	1	1
NdeI(μL)	1	1	1	1	1	1
nuclease-free water(μL)	11	11	11	8	8	8

Sample	1	2	3	4	5	6
fastdigest green buffer(μL)	2	2	2	2	2	2
pET28(μL)	5	5	5	8	8	8
total(μL)	20	20	20	20	20	20

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

Agarose gel electrophoresis Result(110V, 30min):



The marker may not contain coloring agent. (lane 1 to 8: 2K plus I, dd-pTB-1, dd-pTB-2, dd-pTB-3, dd-pTB-4, dd-pTB-5, dd-pTB-6, pTB)

After that, we did Gel Extraction of pTB(This time, we did not use acetone)：

Sample	1	2	3	4	5	6
concentration(ng/μl)	24.0	21.2	97.0	27.2	11.1	17.6
260/280	1.84	1.82	7.08	1.75	1.82	1.89
260/230	0.57	0.49	1.43	0.41	0.64	0.16

There is something wrong with sample 3. May be it was polluted.

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 16 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×PrimeStar(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

帮助

sample	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
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 1,2,3,4,5,6,7,8 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	--

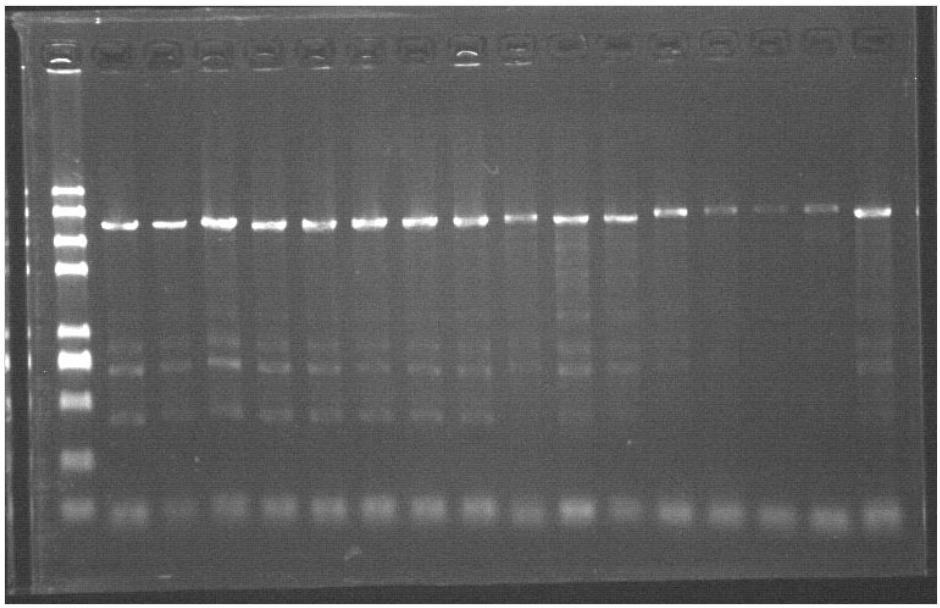
30 cycles(step 2 ~ step 4)

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:



(lane left to right: marker 2K PlusII, 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)

Date 7.18

帮助

Transformation of PUC19 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 kanamycin. 10.Cultivate these bacteria overnight for further use.

PCR of MtrCAB Recorder: Chenyang LI Experimental materials 1.Template 5: 1:30 dilution of plasmid MtrCAB (Mtr2 YWF 7.8 680.8, 1.93, 2.68), 11 ng/μL;

1. Primer: mtr-res-f, mtr-res-r. Designed by ourselves, synthesized by Sangon Biotech;
2. Sterilized ddH2O, 2×Prime Star.

Procedure: 1.PCR Mtr from pSB1c3 with 2×Primer Star Prepare 4 PCR tubes and sequentially add：

sample	**p1，p2，p3，p4**
Sterilized ddH2O	22 μL
template	1 μL
mtr-res-r(10 μM)	1 μL
mtr-res-f(10 μM)	1 μL
2×Primer Star	25 μL
total	50 μL

PCR reaction parameters setting：

stage	**temperature(°C)**	**time**
Pre-Duration	98	10 min
Duration	98	10 s
Anneal	56	5 s
Extend	72	5 min 30 s
Post-Extend	72	10 min
Final	4	--

30 cycles(Duration ~ Extend)

2.PCR Mtr from pSB1c3 with 2×Taq for TA cloning Prepare 4 PCR tubes and sequentially add：

sample	**t1，t2，t3，t4**
Sterilized ddH2O	20 μL
template	1 μL
mtr-res-r(10 μM)	2 μL
mtr-res-f(10 μM)	2 μL
2×Taq	25 μL
total	50 μL

PCR reaction parameters setting：

stage	**temperature(°C)**	**time**
Pre-Duration	94	4 min
Duration	94	30 s
Anneal	56	30 s
Extend	72	5 min 30 s
Post-Extend	72	10 min
Final	4	--

35 cycles(Duration ~ Extend)

Purification of mtr PCR product Recorder: Shihan Zhu (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 30 μL ddH2O, stand them still for 10 min. Centrifuge at 12,000 rpm for 1 min.

OD measurement result:

sample	**mtr-PCR-pur-taq**	**mtr-PCR-pur-PrimeSTAR**
Concentration(ng/ul)	386.7	152.3
260/280	1.87	1.87
260/230	2.16	2.18

TA cloning of MtrCAB Recorder: Chenyang LI Procedure: 1.Prepare 2 PCR tubes and sequentially add：

sample	**1**	**2**
mtr-PCR-pur-taq	3 μL	4 μL
pMDTM 19-T Vector	1 μL	1 μL
Sterilized ddH2O	1 μL	0 μL
Solution I	5 μL	5 μL
total	10 μL	10 μL

2.Mix gently and incubate at 16 degree Celsius for 6 hour.

Transformation of plasmid pMDTM 19-T Vector containg mtr-PCR-pur-taq Recorder: Chenyang LI 1.Take the competent bacteria from -80 degree centigrade refrigerator and incubate them into ice about 5 mins until it is dissolved 2.Absorb 10μL plasmid and mix it with bacteria solution thoroughly. ATTENTION: Please operate this step tenderly!!! 3.Put the tubes on the ice about 30 mins.(Time SHOULD BE ACCURATE) 4.Make a heat shock at 42 degree centigrade about 90 sec(TIME SHOULD BE ACCURATE) 5.Put the tubes on the ice about 5 mins again. 6.Add 900 ul LB medium into EP tubes and cultivate the bacteria at 37 degree centrigrade about 60 min. 7.Centrifuge them at 4000 rpm for 2 minutes and we will see sediment in the tubes. 8.Discard the supernatant liquid and leave about 200 ul medium. 9.Coat plate: Add 200 ul solution in a plate with kanamycin. 10.Cultivate these bacteria overnight for further use.

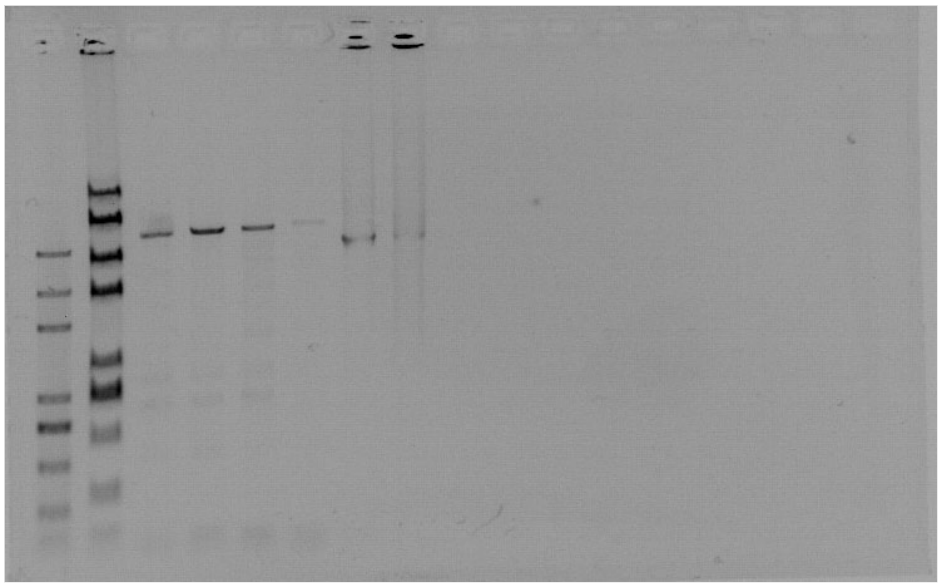
Results There was nothing on the plates because of the failure competent bacteria

Purification of full ccm PCR product Recorder: Xingwei Yang (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**	**ccm PCR pur 5+6+7+8**	**ccm PCR pur 9+10+11+12**	**ccm PCR pur 13+14+15+16**
Concentration(ng/ul)	169.2	152.3	139.9	74.9
260/280	1.84	1.82	1.80	1.81
260/230	2.39	2.47	2.20	2.08

Agarose gel electrophoresis Result:



(lane left to right: marker 5K, marker 2K PlusII, ccm PCR pur 1+2+3+4,ccm PCR pur 5+6+7+8,ccm PCR pur 9+10+11+12,ccm PCR pur 13+14+15+16, mtr PCR pur taq, mtr pcr pur PrimeSTAR)

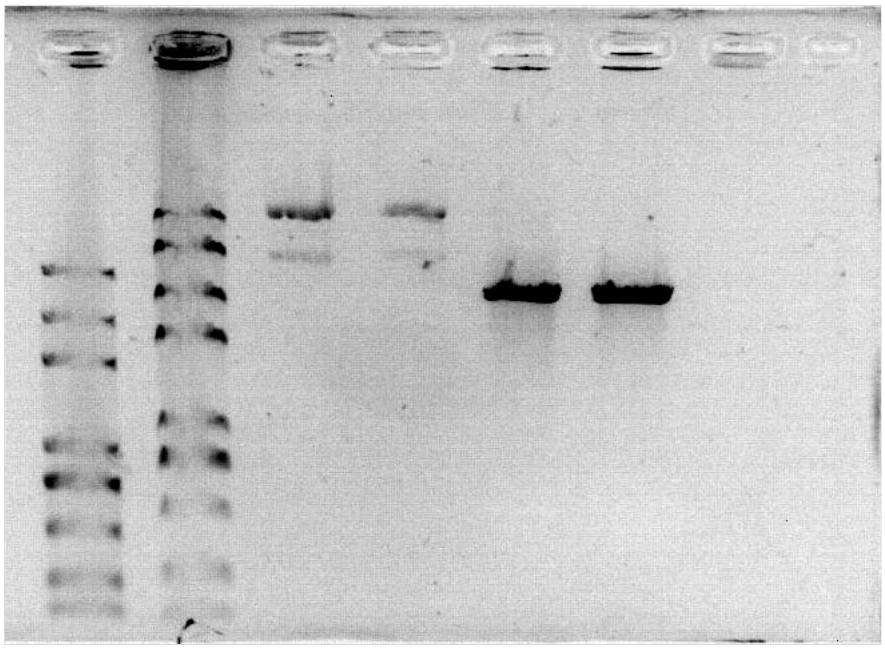
Double digestion of PBAD Recorder:Xingwei Yang Materials: 1.pBAD, produced by ourselves: 2.FastDigest restriction enzyme XhoI, HindIII and 10× FastDigest Green Buffer(from Thermo Fisher Scientific) 3.Nuclease-free water

Reaction system:

Sample	**1**	**2**
XhoI(μL)	1	1
HindIII(μL)	1	1
nuclease-free water(μL)	11	11
fastdigest green buffer(μL)	2	2
PBAD(μL)	5	5
total(μL)	20	20

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

Agarose gel electrophoresis Result(120V, 30min):



(lane 1 to 6: 2K plus , 2K plus II , PBAD-1,PBAD-2,dd-PBAD-1, dd-PBAD-2)

Double digestion of PBAD Recorder: Zhenyu Jiang, Liudong Luo Materials: 1.pBAD, produced by ourselves:

Sample	1	2
concentration(ng/μl)	236.6	253.4
260/280	1.82	1.90
260/230	1.64	2.32

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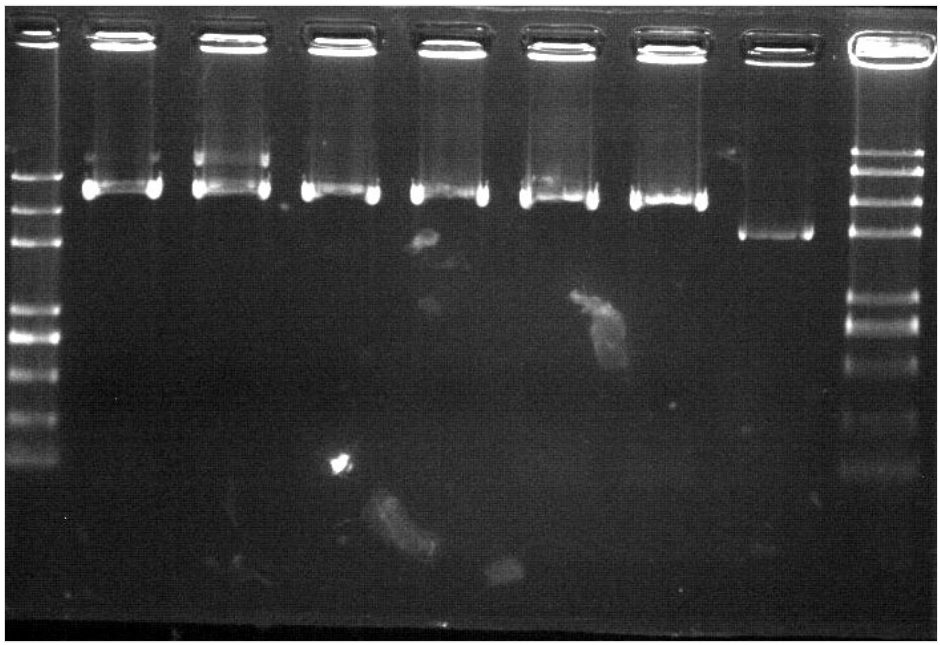
2.FastDigest restriction enzyme XhoI, HindIII and 10× FastDigest Green Buffer(from Thermo Fisher Scientific) 3.Nuclease-free water

Reaction system:

Sample	1	2	3	4	5	6
XhoI(μL)	1	1	0	0	1	1
HindIII(μL)	0	0	1	1	1	1
nuclease-free water(μL)	12	12	12	12	11	11
fastdigest green buffer(μL)	2	2	2	2	2	2
PBAD(μL)	5	5	5	5	5	5
total(μL)	20	20	20	20	20	20

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

Agarose gel electrophoresis Result(120V, 30min):



(lane 1 to 8: 2K plus , sd-PBAD-1, sd-PBAD-2, sd-PBAD-3, sd-PBAD-4, dd-PBAD-5, dd-PBAD-6, PBAD,2K plus II)

Gel Extraction of ddMtr Recorder: Wenfei Yu, Liwen Zhang Procedure:

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

sample	ge ddMtr 1	ge ddMtr 2	ge ddMtr 3	ge ddMtr 4

sample	ge ddMtr 1	ge ddMtr 2	ge ddMtr 3	ge ddMtr 4
Concentration(ng/ul)	13.1	18.7	18.2	15.1
260/280	1.76	1.77	1.83	1.88
260/230	0.31	0.38	0.57	0.35

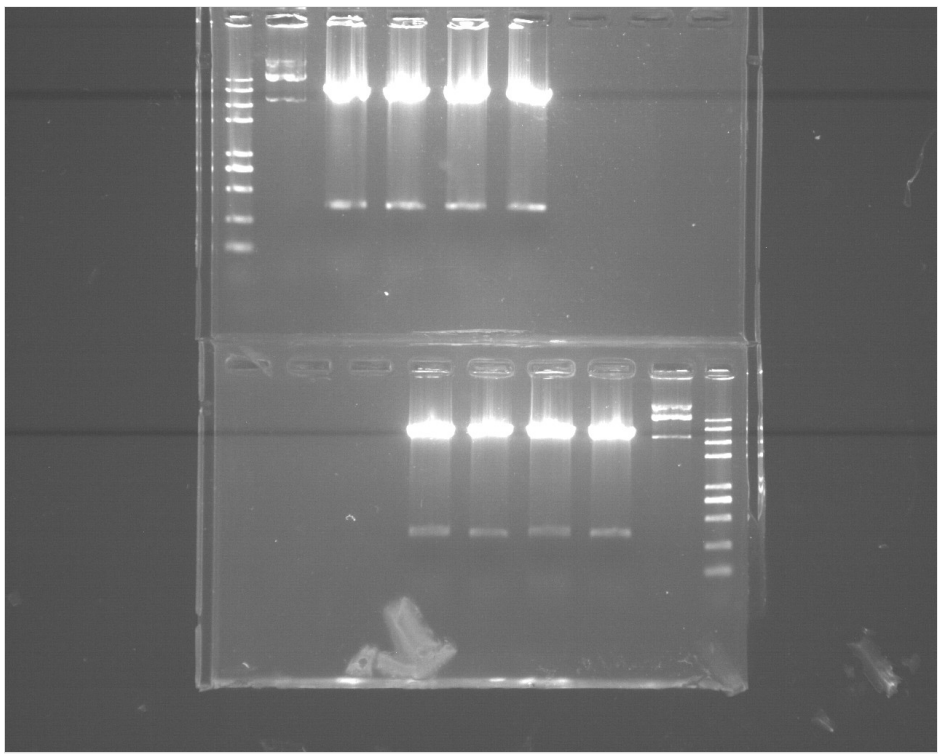
Double digestion of pET28 Recorder: Wenfei Yu Materials: 1.pET28 PCR pur: 2.FastDigest restriction enzyme XhoI, HindIII and 10× FastDigest Green Buffer(from Thermo Fisher Scientific) 3.Nuclease-free water

Reaction system:

Sample	1	2	3	4	5	6	7	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)	6	6	6	3	3	6	7	4
Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
fastdigest green buffer(μL)	2	2	2	2	2	2		
PBAD(μL)	10	10	10	13	13	10	9	12
total(μL)	20	20	20	20	20	20	20	20

Mix gently and incubate at 37 degree Celsius for 30 mins / 45 mins.

Agarose gel electrophoresis Result(120V, 30min)(Done by Meiying Cui, Liwen Zhang):



(I: lane 1 to 6: marker, pET28, dd pET28 1, dd pET28 2, dd pET28 3, dd pET28 4 II: lane 9 to 4: marker, pET28, dd pET28 5, dd pET28 6, dd pET28 7, dd pET28 8)

Gel Extraction of ddpET28 Recorder: Wenfei Yu, Liwen Zhang Procedure:

1. Cut off the Gel part containing the target band. Get 6 tubes of AD and 5 tubes of BD.
2. Weigh the cut off Gel part.
3. Add Buffer B2 which 300 times the weight of the Gel.
4. Add isopropanol which is 1/3 volume of the Buffer B2.
5. Move the solution to adsorption column, 11000 rpm centifuge 30 s, discard filtrate.
6. Add 500 μL Wash Solution, 12000 rpm centifuge 30 s, discard filtrate. Repeat once.
7. 12000 rpm centifuge 1 min.

8. Lying for 10 min.
9. Put the adsorption column in a new EP tube. Add 20 μL ddH2O, 10 min's standing, 12000 rpm centifuge 1 min. Preserve in the EP tube with ddH2O at 4 degree Celsius.

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sample	ge ddpET28 1	ge ddpET28 2
Concentration(ng/ul)	58.6	55.1
260/280	1.86	1.89
260/230	0.54	0.86

Double digestion of pSB1C3 carrying Mtr Recorder: Tong Xiao Materials: 1.pSB1C3 extracted by ourselves:

Sample	1
concentration(ng/μl)	554.9
260/280	1.87
260/230	2.28

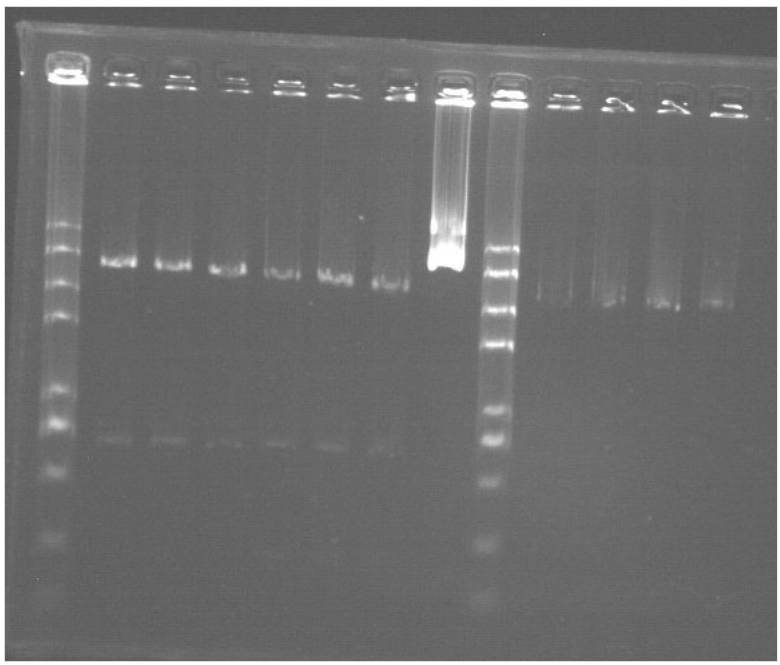
2.FastDigest restriction enzyme XhoI, NdeI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific) 3.Nuclease-free water

Reaction system:

Sample	1	2	3	4	5	6
XhoI(μL)	1	1	1	1	1	1
NdeI(μL)	0	0	0	1	1	1
nuclease-free water(μL)	15	15	15	13	13	13
fastdigest green buffer(μL)	2	2	2	2	2	2
PBAD(μL)	2	2	2	3	3	3
total(μL)	20	20	20	20	20	20

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

Agarose gel electrophoresis Result(120V, 30min):



(lane 1 to 9:Marker 2K plus , sd-Mtr-1, sd-Mtr-2, sd-Mtr-3, dd-Mtr-1, dd-Mtr-2, dd-Mtr-3, pSB1C3, Marker 2K plus II)

Transformation of PUC19 and MTR+PET28 into BL21 Recorder: Menglong Jin
NOTE:Generally, competent bacteria are restrored in -80 degree centrigrade environment. 1.Take

帮助

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 kanamycin. 10.Cultivate these bacteria overnight for further use.

Date 7.19

Double digestion of ccm Recorder: Liudong Luo, Zhenyu Jiang Materials:

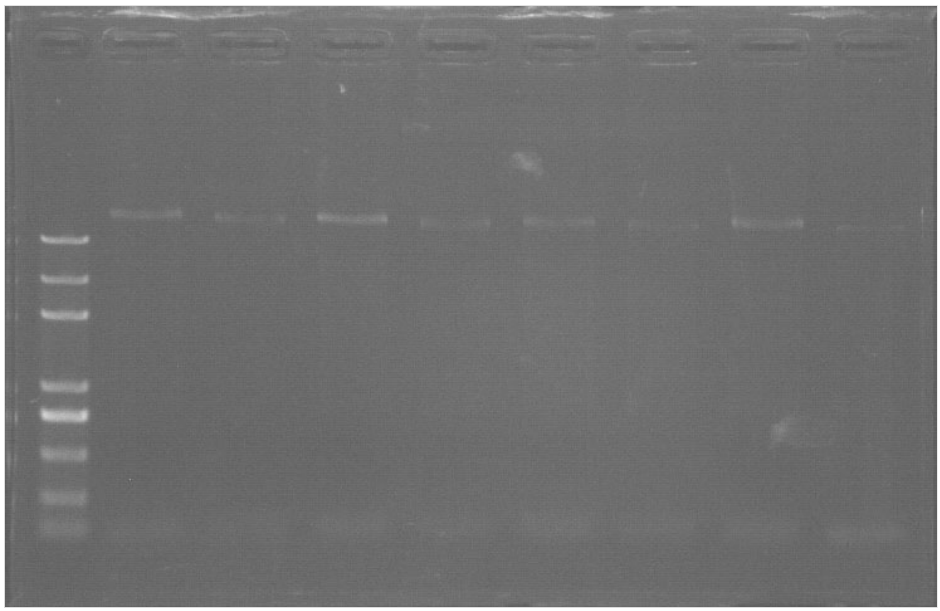
1. full ccm
2. FastDigest restriction enzyme XbaI, pstI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water

Reaction system:

Sample	**1**	**2**	**3**	**4**
XbaI(μL)	1	1	1	1
pstI(μL)	1	1	1	1
nuclease-free water(μL)	24	24	24	24
fastdigest green buffer(μL)	2	2	2	2
ccm(μL)	2	2	2	2
total(μL)	30	30	30	30

Mix gently and incubate at 37 degree Celsius for 12 hours .

Agarose gel electrophoresis Result:



(lane 1 to 9: 2K plus ,ddccm-1-1,ddccm-1-2,ddccm-2-1,ddccm-2-2,ddccm-3-1,ddccm-3-2,ddccm-4-1,ccm)

Then we did the gel extraction of the fragments we got.

The results are as following:

sample	**ccmXPdd 1+2+3+4**
Concentration(ng/ul)	9.6
260/280	1.65
260/230	0.12

帮助

Date 7.20

Transformation of PUC19 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 Amp. 10.Cultivate these bacteria overnight for further use.

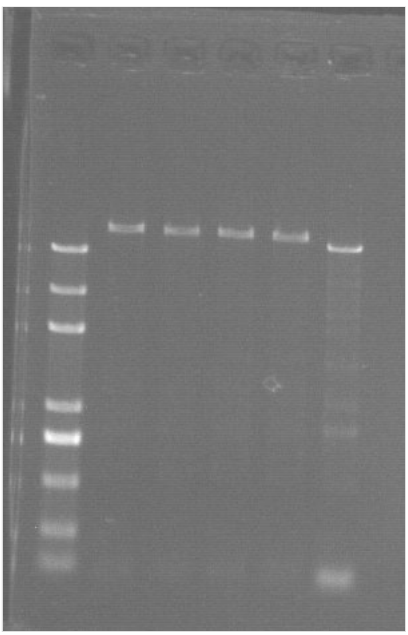
Double digestion of ccm Recorder: Liudong Luo, Zhenyu Jiang Materials:

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**
XbaI(μL)	1	1	1	1
pstI(μL)	1	1	1	1
nuclease-free water(μL)	24	24	24	24
fastdigest green buffer(μL)	2	2	2	2
ccm(μL)	2	2	2	2
total(μL)	30	30	30	30

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

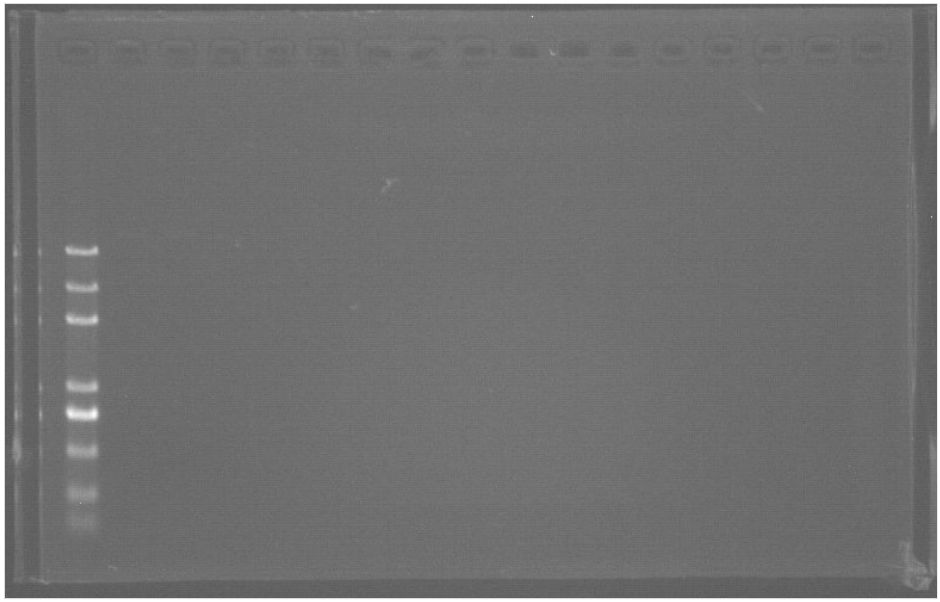
Agarose gel electrophoresis Result:  (lane 1 to 6: 2K plus ,ddccm-1,ddccm-2,ddccm-3,ddccm-4,ccm)

Then we did the purification of the fragments we got.

The results are as following:

sample	**ccmXPdd 1+2**	**ccmXPdd3+4**
Concentration(ng/ul)	12.0	7.8
260/280	1.52	1.65
260/230	0.18	0.39

Agarose gel electrophoresis Result:



(lane 1 to 3: 2K plus ,ddccm-1+2,ddccm-3+4)

Plasmid Extraction of the puc19 Recorder: Xingwei Yang Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 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	**puc19-1**	**puc19-2**	**puc19-3**	**puc19-4**	**puc19-5**	**puc19-6**	**puc19-7**	**puc19-8**
Concentration(ng/ul)	199.2	220.0	198.4	163.1	185.5	161.7	178.1	183.3
260/280	1.85	1.77	1.87	1.85	1.83	1.84	1.82	1.79
260/230	1.82	1.17	2.03	1.82	1.68	1.80	1.64	1.40

Double digestion of puc19 Recorder: Liudong Luo, Zhenyu Jiang Materials:

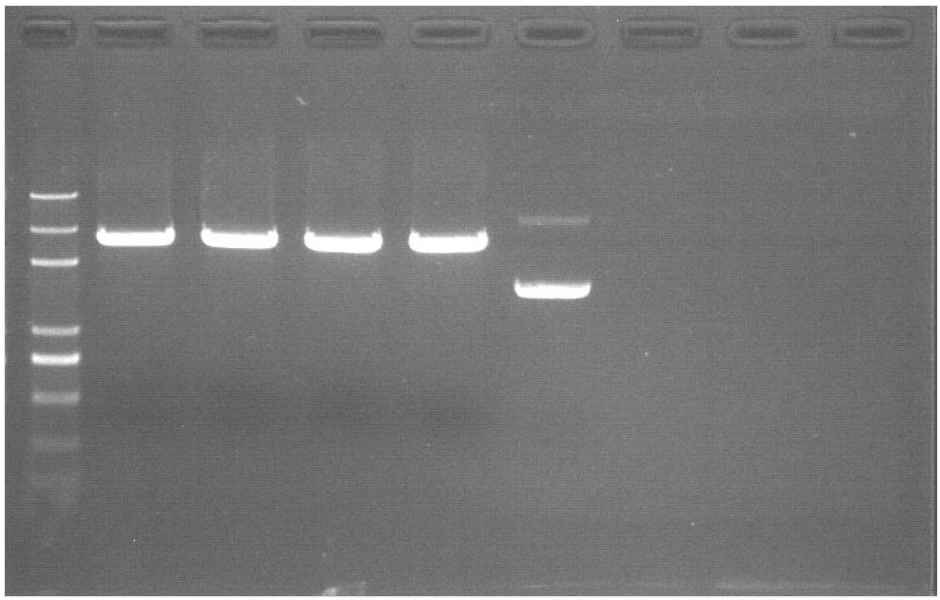
1. puc19
2. FastDigest restriction enzyme XbaI, pstI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water

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
puc19(μL)	5	5	5	5
total(μL)	20	20	20	20

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

Agarose gel electrophoresis Result:



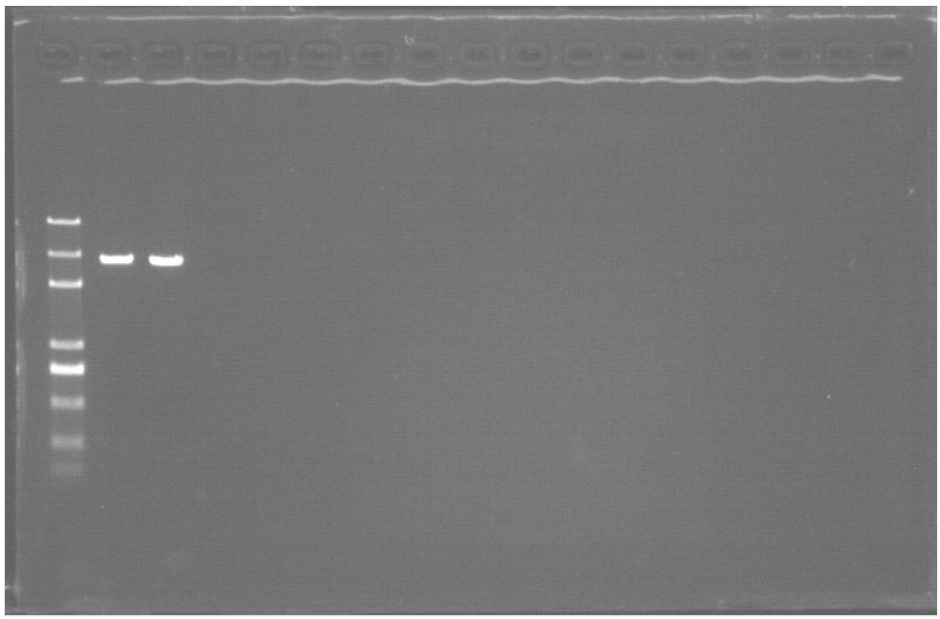
(lane 1 to 6: 2K plus ,ddpuc-1,ddpuc-2,ddpuc-3,ddpuc-4,puc)

Then we did the Gel Extraction of the fragments we got.

The results are as following:

sample	**pucXPdd 1+2**	**pucXPdd3+4**
Concentration(ng/ul)	25.2	23.9
260/280	1.77	1.84
260/230	0.74	0.17

Agarose gel electrophoresis Result:



(lane 1 to 3: 2K plus ,ddpuc-1+2,ddpuc-3+4)

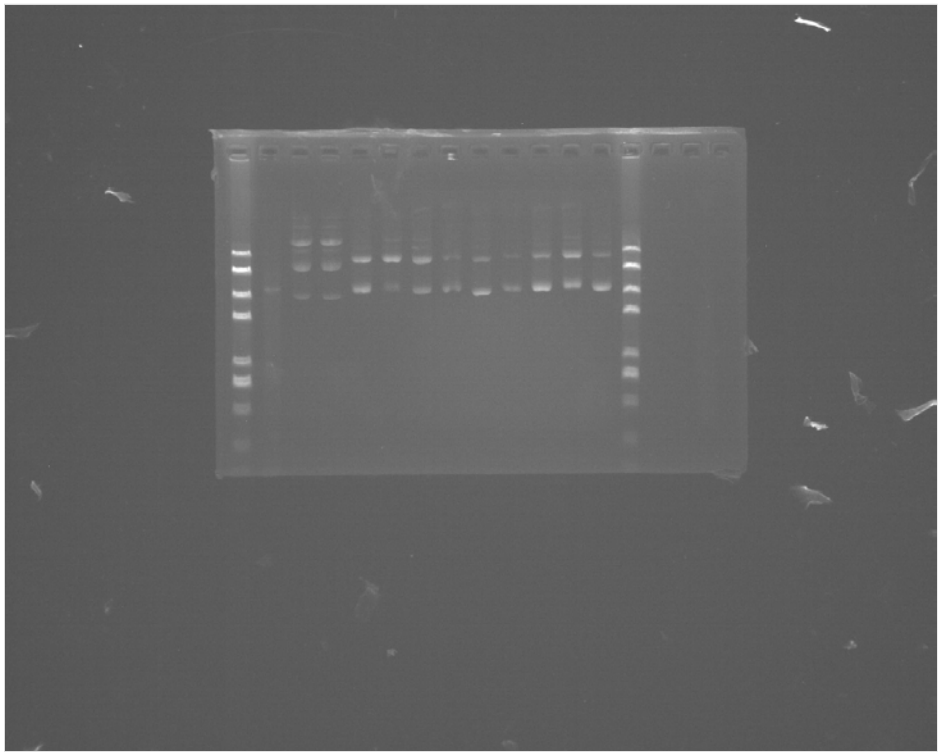
Plasmid Extraction of the pET28 and Mtr-pET28 Recorder: Wenfei Yu, Meiying Cui
Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer 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.

帮助

sample	pET28-1	pET28-2	M28-1	M28-2	M28-3	M28-4	pET28 0-1	pET28 0-2	pET28 0-3	pET28 0-4
Concentration(ng/ul)	252.9	230.5	79.3	60.4	125.6	93.8	70.7	68.4	65.0	101.6
260/280	1.85	1.87	1.83	1.91	1.69	1.74	1.88	1.91	1.76	1.67
260/230	1.90	2.11	1.35	1.76	0.90	1.04	1.77	1.55	0.95	0.83

Agarose gel electrophoresis Result:



(lane 1 to 14: 2K plus II, Mtr PCR pur, pET28-1, pET28-2, pET28 0-3, pET28 0-4, pET28 0-1, pET28 0-2, M28-1, accident, M28-2, M28-3, M28-4, 2K plus II)

Plasmid Extraction of the PYYDT Recorder: Wenfei Yu 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
Concentration(ng/ul)	49.6	38.5	37.1	38.2
260/280	1.67	1.83	1.93	1.81
260/230	1.08	1.54	1.89	1.57

PCR of MtrCAB Recorder: Chenyang LI Experimental materials 1.Template 5: 1:30 dilution of plasmid MtrCAB (Mtr2 YWF 7.8 680.8, 1.93, 2.68), 11 ng/μL;

1. Primer: mtr-res-f, mtr-res-r. Designed by ourselves, synthesized by Sangon Biotech;
2. Sterilized ddH2O, Taq.

Procedure: 1.Prepare 4 PCR tubes and sequentially add：

帮助

sample	10-1,10-2,10-3,10-4	50-1,50-2
Sterilized ddH2O	35 μL	31 μL
template	1 μL	5 μL
mtr-res-r(10 μM)	2 μL	2 μL
mtr-res-f(10 μM)	2 μL	2 μL
10×PCR Buffer	5 μL	5 μL
Mg ion(25mM)	3 μL	3 μL
dNTP	1 μL	1 μL
Taq	1 μL	1 μL
total	50 μL	50 μL

2.PCR reaction parameters setting：

stage	temperature(°C)	time
Pre-Duration	95	5 min
Duration	95	30 s
Anneal	56	30 s
Extend	72	5 min 30 s
Post-Extend	72	10 min
Final	4	--

30 cycles(Duration ~ Extend)

Purification of mtr PCR product Recorder: Shihan Zhu (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 30 μL ddH2O, stand them still for 10 min. Centrifuge at 12,000 rpm for 1 min.

OD measurement result:

sample	mtr-PCR-pur-10	mtr-PCR-pur-50
Concentration(ng/ul)	251.6	113.0
260/280	1.84	1.79
260/230	1.86	1.69

Plasmid Extraction of the puc19 Recorder: Zhenyu Jiang, 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. 8.13400 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	puc19-1	puc19-2	puc19-3	puc19-4	puc19-5	puc19-6	puc19-7	puc19-8
Concentration(ng/ul)	278.8	272.8	216.8	217.6	229.7	241.8	234.1	202.7
260/280	1.88	1.86	1.87	1.87	1.78	1.87	1.89	1.87
260/230	2.22	2.07	2.24	2.04	1.31	2.23	2.25	2.10

Date 7.21

帮助

Transformation of PUC19 containing ccmA-H 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 10μL PUC19 + ccmA-H 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.

Single digestion of PYYDT Recorder: Zhenyu Jiang, Liudong Luo Materials: 1.PYYDT, produced by ourselves:

Sample	**1**	**2**
concentration(ng/μl)	38.5	38.2
260/280	1.83	1.81
260/230	1.54	1.57

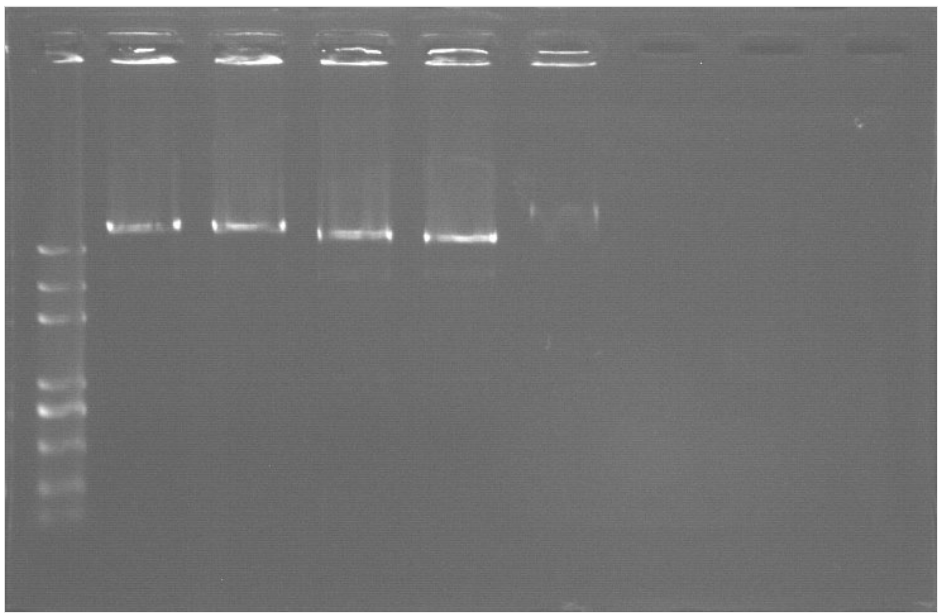
2.FastDigest restriction enzyme XbaII, pstI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific) 3.Nuclease-free water

Reaction system:

Sample	**1**	**2**	**3**	**4**
XbaII(μL)	1	1	0	0
pstI(μL)	0	0	1	1
nuclease-free water(μL)	0	0	0	0
fastdigest green buffer(μL)	2	2	2	2
PYYDT(μL)	17	17	17	17
total(μL)	20	20	20	20

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

Agarose gel electrophoresis Result(110V, 30min):



(lane 1 to 6: 2K plus , sd-PYYDT-1, sd-PYYDT-2, sd-PYYDT-3, sd-PYYDT-4,PYYDT)

Transformation of PUC19 containing ccmA-H 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 10μL PUC19 + ccmA-H 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 and X-Gal. 10.Cultivate these bacteria overnight for further use.

Plasmid Extraction of the puc19 Recorder: Xingwei Yang Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 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	puc19-1	puc19-2	puc19-3	puc19-4	puc19-5	puc19-6	puc19-7	puc19-8
Concentration(ng/ul)	215.0	309.0	204.4	179.2	266.3	291.9	227.3	196.5
260/280	1.82	1.71	1.85	1.86	1.78	1.76	1.80	1.84
260/230	1.75	0.96	1.94	2.09	1.30	1.17	1.47	1.88

Plasmid Extraction of the puc19 Recorder: Xingwei Yang Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 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	puc19-1	puc19-2	puc19-3	puc19-4	puc19-5	puc19-6	puc19-7	puc19-8
Concentration(ng/ul)	270.9	344.5	242.3	221.9	303	265.6	332.9	327.8
260/280	1.86	1.79	1.88	1.84	1.86	1.86	1.80	1.74
260/230	2.10	1.33	2.12	1.96	2.08	2.25	2.18	1.07

Plasmid Extraction of the PYYDT and pET28 Recorder: Tong Xiao, Liwen Zhang 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|PYYDT1|PYYDT2|pET28|

|-| |Concentration(ng/ul)|58.0|57.9|3.0| |260/280|1.41|1.77|2.8| |260/230|2.07|1.23|1.58|

PCR of Mtr Recorder: Shihan Zhu, Tong Xiao, Xiaoyu Zhang, Wenfei Yu

Experimental materials

1. Template: extraction product of pSB1C3 carrying Mtr;
2. Primer: mtr-res-f,mtr-res-r. Designed by ourselves, synthesized by Sangon Biotech;
3. Sterilized ddH2O, Taq DNA polymerase.
4. 10X PCR Buffer.
5. Mg2+(25mM).
6. dNTP.

Procedure:

1.Prepare 16 PCR tubes and sequentially add：

sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Sterilized ddH2O	31 μL	31 μL	31 μL	31 μL	31 μL	31 μL	31 μL	31 μL
Taq DNA polymerase	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
template	5 μL	5 μL	5 μL	5 μL	5 μL	5 μL	5 μL	5 μL
mtr-res-f	2 μL	2 μL	2 μL	2 μL	2 μL	2 μL	2 μL	2 μL
mtr-res-r	2 μL	2 μL	2 μL	2 μL	2 μL	2 μL	2 μL	2 μL
10X DNA Buffer	5 μL	5 μL	5 μL	5 μL	5 μL	5 μL	5 μL	5 μL
Mg2+ (25mM)	3 μL	3 μL	3 μL	3 μL	3 μL	3 μL	3 μL	3 μL
dNTP	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

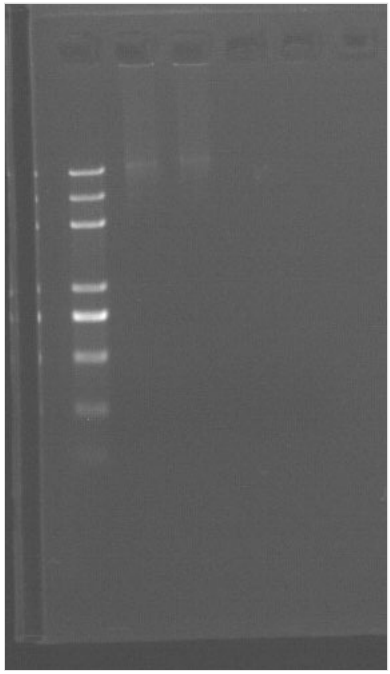
sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Sterilized ddH2O	34.4 μL	34.4 μL	34.4 μL	34.4 μL	34.4 μL	34.4 μL	34.4 μL	34.4 μL
Taq DNA polymerase	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
template	1.6 μL	1.6μL	1.6 μL	1.6 μL	1.6 μL	1.6 μL	1.6 μL	1.6 μL
mtr-res-f	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
mtr-res-r	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
10X DNA Buffer	5 μL	5 μL	5 μL	5 μL	5 μL	5 μL	5 μL	5 μL
Mg2+ (25mM)	3 μL	3 μL	3 μL	3 μL	3 μL	3 μL	3 μL	3 μL
dNTP	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 1-16 Parameters setting：

stage	**temperature**	**time**
step 1	95	5 min
step 2	95	30 s
step 3	56	30 s
step 4	72	5 min 30s
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

帮助

3.Agarose gel electrophoresis Result:  (lane left to right: marker 2K Plus, Mtr-1, Mtr-2,Mtr-3,Mtr-4)

Date 7.22

PCR of Mtr Recorder: Tong Xiao, Xiaoyu Zhang

Experimental materials

1. Template: extraction product of pSB1C3 carrying Mtr;
2. Primer: mtr-res-f,mtr-res-r. Designed by ourselves, synthesized by Sangon Biotech;
3. Sterilized ddH2O, Taq DNA polymerase.
4. 10X PCR Buffer.
5. Mg2+(25mM).
6. dNTP.

Procedure:

1.Prepare 8 PCR tubes and sequentially add：

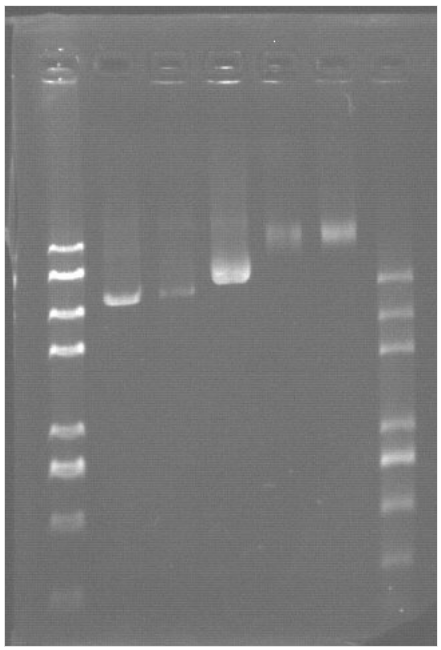
sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Sterilized ddH2O	35 μL	35 μL	35 μL	35 μL	35 μL	35 μL	35 μL	35 μL
Taq DNA polymerase	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
template	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
mtr-res-f	2 μL	2 μL	2 μL	2 μL	2 μL	2 μL	2 μL	2 μL
mtr-res-r	2 μL	2 μL	2 μL	2 μL	2 μL	2 μL	2 μL	2 μL
10X DNA Buffer	5 μL	5 μL	5 μL	5 μL	5 μL	5 μL	5 μL	5 μL
Mg2+ (25mM)	3 μL	3 μL	3 μL	3 μL	3 μL	3 μL	3 μL	3 μL
dNTP	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 1-16 Parameters setting：

stage	**temperature**	**time**
step 1	95	5 min
step 2	95	30 s
step 3	56	30 s
step 4	72	5 min 30s

stage	temperature	time
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:  (lane left to right: marker 2K Plus II, Mtr-1, Mtr-2,template,PYYDT1,PYYDT2,marker 2K Plus)

Plasmid Extraction of the puc19 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	puc19-1	puc19-2	puc19-3	puc19-4	puc19-5	puc19-6	puc19-7	puc19-8
Concentration(ng/ul)	283.7	301.9	276.4	275.7	290.7	268.7	277.9	286.2
260/280	1.86	1.84	1.91	1.88	1.87	1.83	1.79	1.89
260/230	1.86	1.74	2.23	1.98	2.01	1.73	1.48	2.02

Purification of mtr PCR product Recorder: Yitian Zhou (1). Add 975 μL Buffer B3 to the 195 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 13 min. (7). Put the column to an 1.5 ml EP tube, add 30 μL ddH2O, stand them still for 10 min. Centrifuge at 12,000 rpm for 1 min.

OD measurement result:

sample	mtr-PCR-pur-50[1]	mtr-PCR-pur-50[2]
Concentration ng/ul	16.4	15.3
260/280	1.65	1.76
260/230	1.67	1.38

Bacteria PCR of pUC19+ccm Recorder: Yonghao Liang

Experimental materials

1. Template: Bacteria picked from the plate containing pUC19+ccm;
2. Primer: ccm-res-f, ccm-res-r. Designed by ourselves, synthesized by Sangon Biotech;
3. Sterilized ddH2O, Taq DNA polymerase.
4. 2X Taq DNA polymerase.

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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
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	20 μL	20 μL	20 μL	20 μL	20 μL	20 μL	20 μL	20 μL

2.PCR reaction 1-16 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	--

20 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:

Bacteria PCR of Reductase Recorder: Yonghao Liang

Experimental materials

1. Template: pET21 containing Reductase;
2. Primer: red-res-f, red-res-r. Designed by ourselves, synthesized by Sangon Biotech;

3. Sterilized ddH2O, Taq DNA polymerase.
4. 2X Taq DNA polymerase.

Procedure:

帮助

1.Prepare 16 PCR tubes and sequentially add：

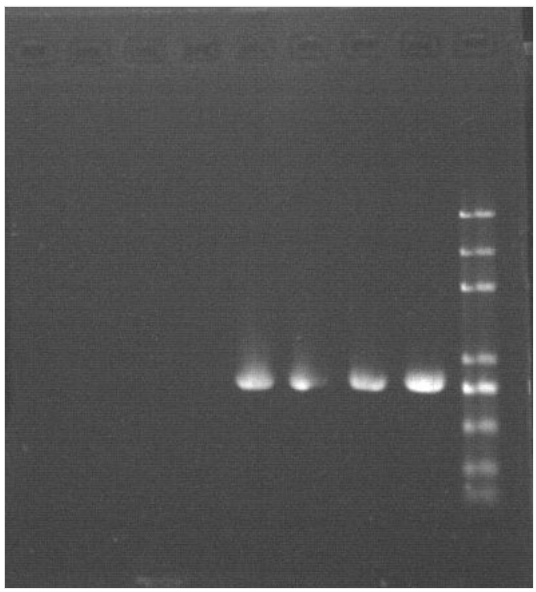
sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Sterilized ddH2O	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL
2X Taq DNA polymerase	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL
template	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
red-res-f	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
red-res-r	1 μL	1 μL	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

2.PCR reaction 1-16 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)

3.Agarose gel electrophoresis Result:



Plasmid Extraction of the puc19 Recorder: Xingwei Yang Procedure: 1.Centifuge 1.5 mL bacterium solution at 11000 rpm for 2 minutes. Remove the supernatant. Repeat twice. 2.Add 250 μL Buffer P1, resuspend cells. 3.Add 250 μL Buffer P2, mix well, 3 min's standing. 4.Add 350 μL Buffer P3, mix well. 5.13400 rpm centifuge 10 min, move all supernatant to adsorption column, 11000 rpm centifuge 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	puc19-1	puc19-2	puc19-3	puc19-4	puc19-5	puc19-6	puc19-7	puc19-8
Concentration(ng/ul)	179.4	189.7	191.4	175.7	153.1	190.1	174.9	285.5
260/280	1.84	1.82	1.82	1.79	1.83	1.77	1.79	1.72
260/230	1.96	1.79	1.76	1.64	1.92	1.38	1.55	1.12

Plasmid Extraction of the puc+ccm 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	puc+ccm-1	puc+ccm-2	puc+ccm-3	puc+ccm-4	puc+ccm-5	puc+ccm-6	puc+ccm-7	puc+ccm-8
Concentration(ng/ul)	26.3	48.8	35.1	35.5	27.1	31.9	26.4	15.7
260/280	1.75	1.80	1.93	1.99	1.88	2.01	1.90	1.73
260/230	1.50	1.42	1.68	1.32	1.28	1.73	1.29	0.81

Double digestion of puc+ccm Recorder: Liudong Luo, Zhenyu Jiang Materials:

1. puc+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)	6	6	6	6	6	6	6	6
fastdigest green buffer(μL)	2	2	2	2	2	2	2	2
ccm(μL)	10	10	10	10	10	10	10	10
total(μL)	20	20	20	20	20	20	20	20

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

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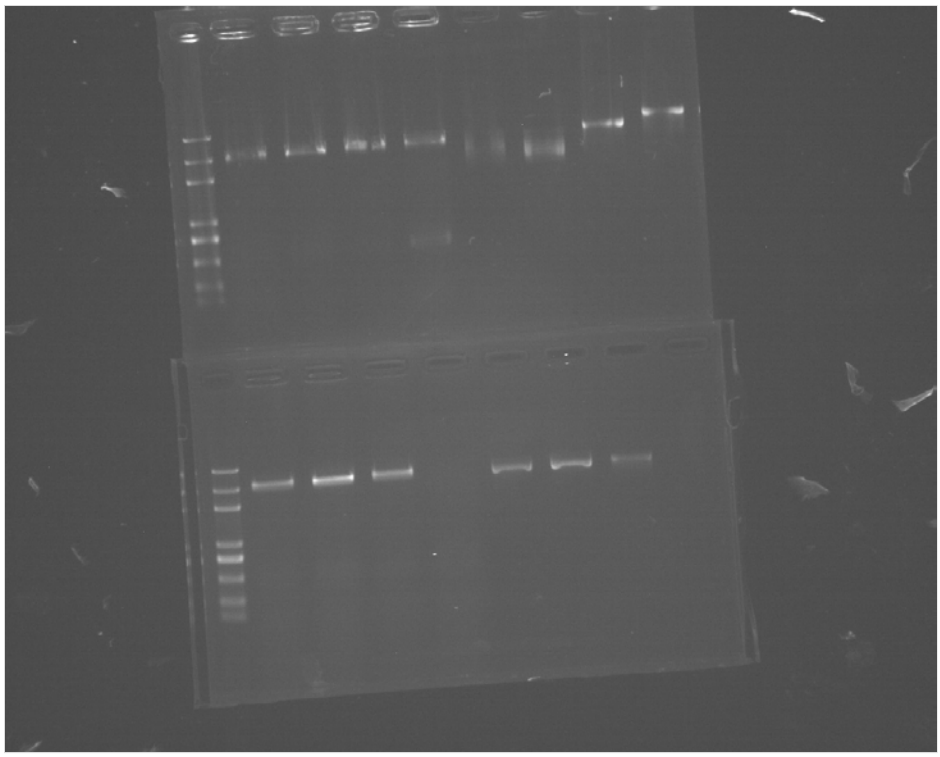
Agarose gel electrophoresis Result:



(lane 1 to 9: 2K plus ,dd-puc+ccm-1,dd-puc+ccm-2,dd-puc+ccm-3,dd-puc+ccm-4,puc+ccm-1,puc+ccm-2,puc+ccm-3,puc+ccm-4,) (lane 1 to 9: 2K plus ,dd-puc+ccm-5,dd-puc+ccm-6,dd-puc+ccm-7,dd-puc+ccm-8,puc+ccm-5,puc+ccm-6,puc+ccm-7,puc+ccm-8,)

Date 7.23

Transformation of Mtr into T-Vetor Recorder: Shihan Zhu,Liwen Zhang 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 Amp. 10.Cultivate these bacteria overnight for further use.

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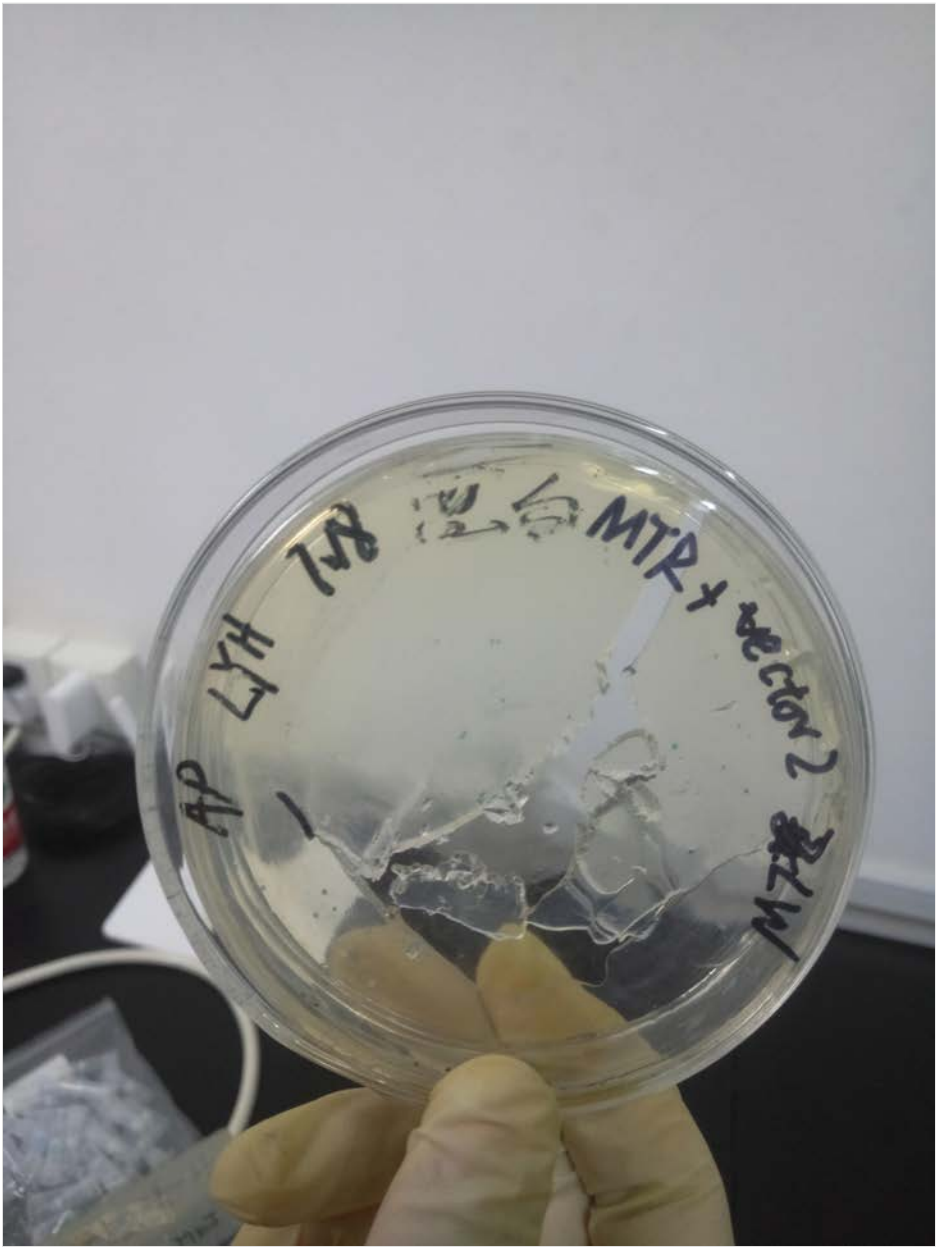

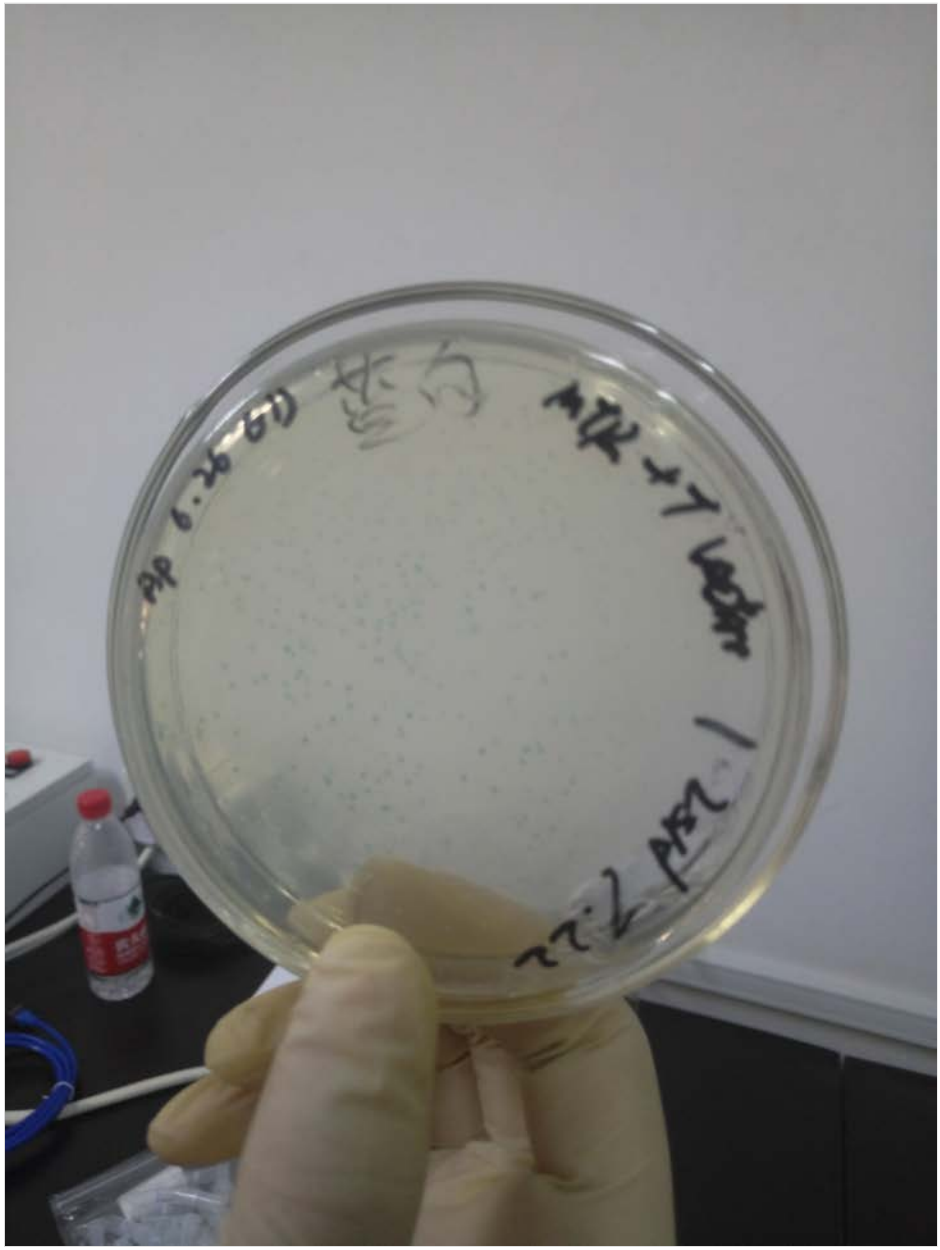

Colony PCR of PUC19 containing ccmA-H Recorder: Menglong Jin

Experimental materials

1. Template: E.coli Top 10 with PUC19 plasmid containing ccmA-H;
2. Primer: PUC-res-f,PUC-res-r. Designed by ourselves, synthesized by Sangon Biotech;
3. Sterilized ddH2O, 2×Taq PCR master mix.

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×Taq PCR master mix	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
PUC-res-f	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
PUC-res-r	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL

sample	1	2	3	4	5	6	7	8
total	20 μL	20 μL	20 μL	20 μL	20 μL	20 μL	20 μL	20 μL

2.PCR reaction 1-16 Parameters setting：

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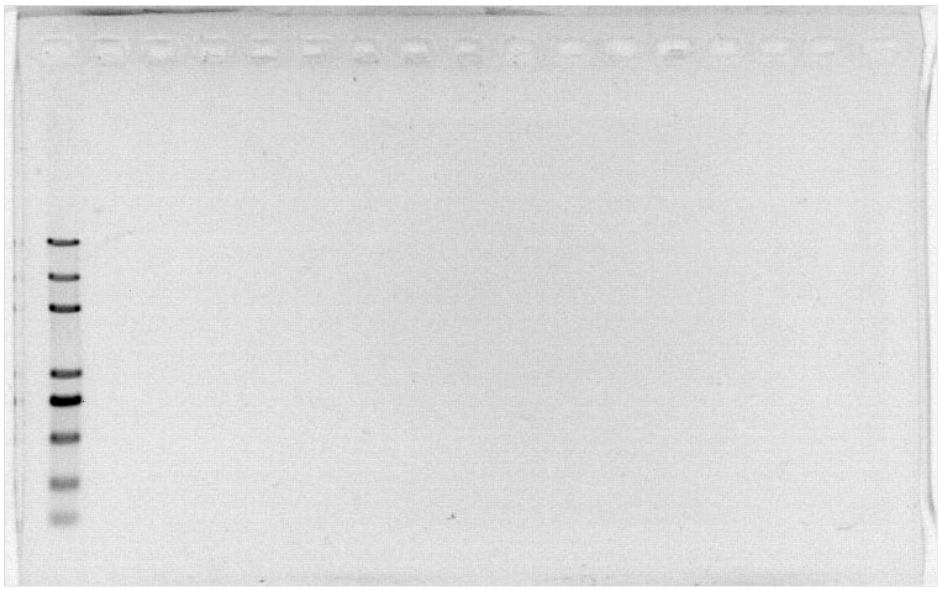
stage	temperature	time
step 1	94	10 min
step 2	94	30 s
step 3	67	30 s
step 4	72	3 min
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:



(From left to right: marker, sample1-8) As you can see, we did not get anything from the PCR.

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×PrimeStar (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

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

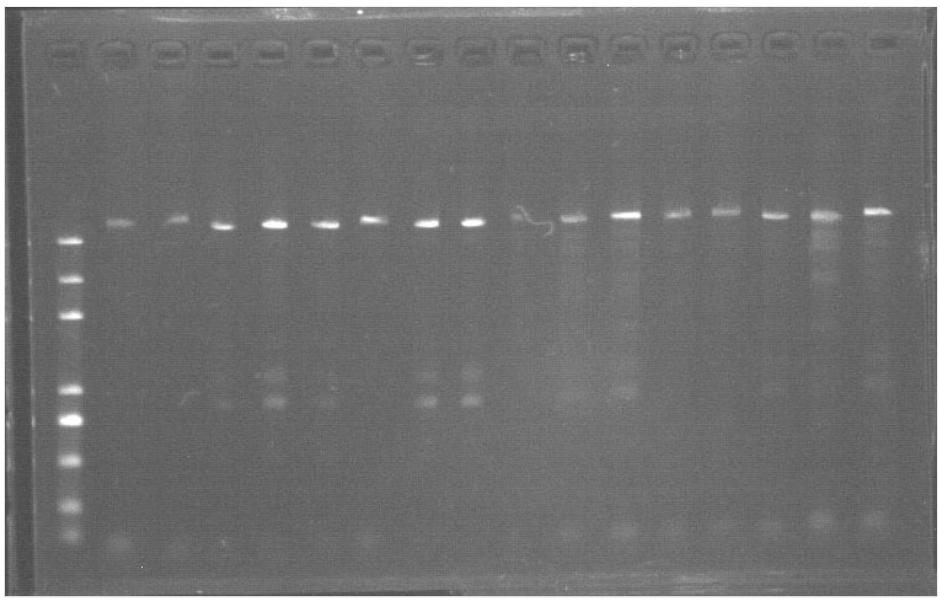
30 cycles(step 2 ~ step 4)

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:



(lane left to right: marker 2K Plus, 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+12+13+14+15+16**
Concentration(ng/ul)	293.0	277.2
260/280	1.85	1.84
260/230	2.14	2.13

PCR of RED Recorder: Liudong Luo, Zhenyu Jiang

Experimental materials

1. Template:RED;
2. Primer: RED-res-f,RED-res-r. Designed by ourselves, synthesized by Sangon Biotech;
3. Sterilized ddH2O, taq.

Procedure:

1.Prepare 4 PCR tubes and sequentially add：

sample	**1**	**2**	**3**	**4**
Sterilized ddH2O	7 μL	7 μL	7 μL	7 μL
taq	10 μL	10 μL	10 μL	10 μL
template	1 μL	1 μL	1 μL	1 μL
RED-res-f	1 μL	1 μL	1 μL	1 μL
RED-res-r	1 μL	1 μL	1 μL	1 μL
total	20 μL	20 μL	20 μL	20 μL

2.PCR reaction 1,2,3,4 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)

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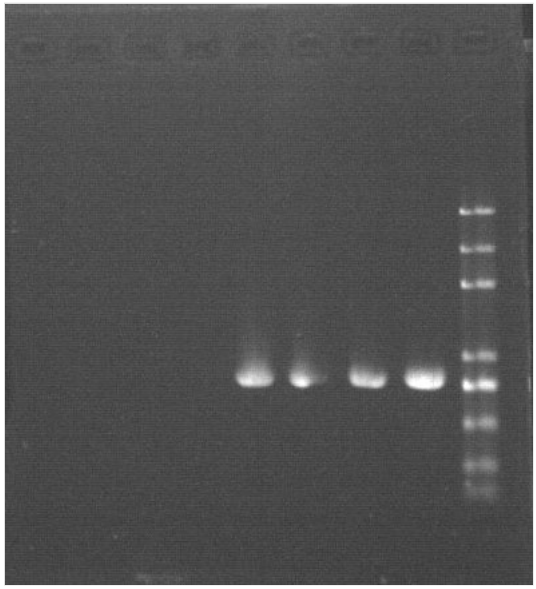
3.Agarose gel electrophoresis Result:



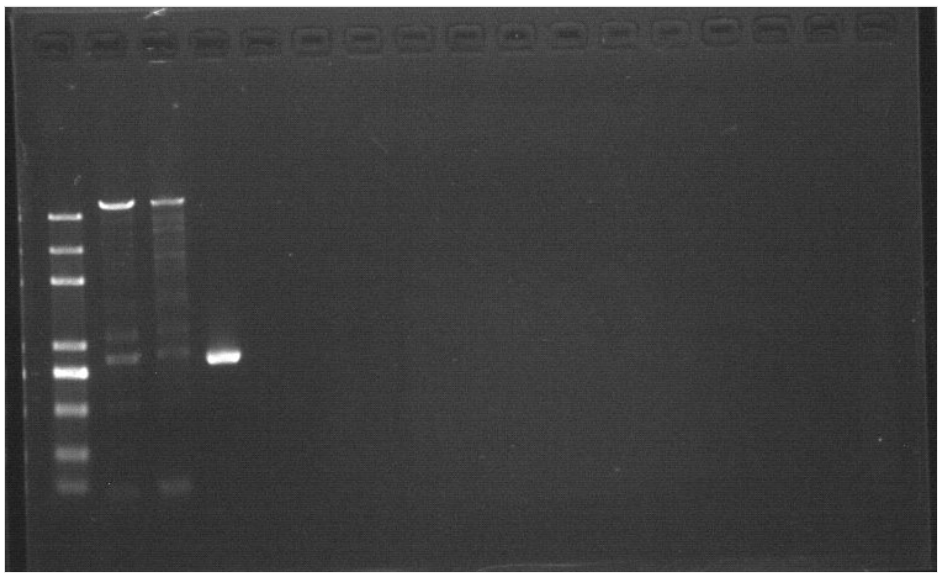
(lane right to left: marker 2K Plus, RED-pcr-1, RED-pcr-2,RED-pcr-3,RED-pcr-4)

Purification of RED PCR product Recorder: Zhenyu Jiang, Liudong Luo (1). Add 240 μL Buffer B3 to the 48 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	**RED PCR pur 1+2+3+4**
Concentration(ng/ul)	342.2
260/280	1.86
260/230	1.95

Agarose gel electrophoresis Result:



(lane left to right: marker 2K Plus, ccm-pcr-pur-1,ccm-pcr-pur-2, RED-pcr-pur-1)

PCR of Mtr Recorder: Tong Xiao, Wenfei Yu

Experimental materials

1. Template: extraction product of pSB1C3 carrying Mtr;
2. Primer: mtr-res-f,mtr-res-r. Designed by ourselves, synthesized by Sangon Biotech;
3. Sterilized ddH2O, Taq DNA polymerase.
4. 10X PCR Buffer.
5. Mg2+(25mM).

6. dNTP.

Procedure:

1.Prepare 16 PCR tubes and sequentially add：

sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Sterilized ddH2O	35 μL	35 μL	35 μL	35 μL	35 μL	35 μL	35 μL	35 μL
Taq DNA polymerase	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
template	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
mtr-res-f	2 μL	2 μL	2 μL	2 μL	2 μL	2 μL	2 μL	2 μL
mtr-res-r	2 μL	2 μL	2 μL	2 μL	2 μL	2 μL	2 μL	2 μL
10X DNA Buffer	5 μL	5 μL	5 μL	5 μL	5 μL	5 μL	5 μL	5 μL
Mg2+ (25mM)	3 μL	3 μL	3 μL	3 μL	3 μL	3 μL	3 μL	3 μL
dNTP	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.Prepare 16 PCR tubes and sequentially add：

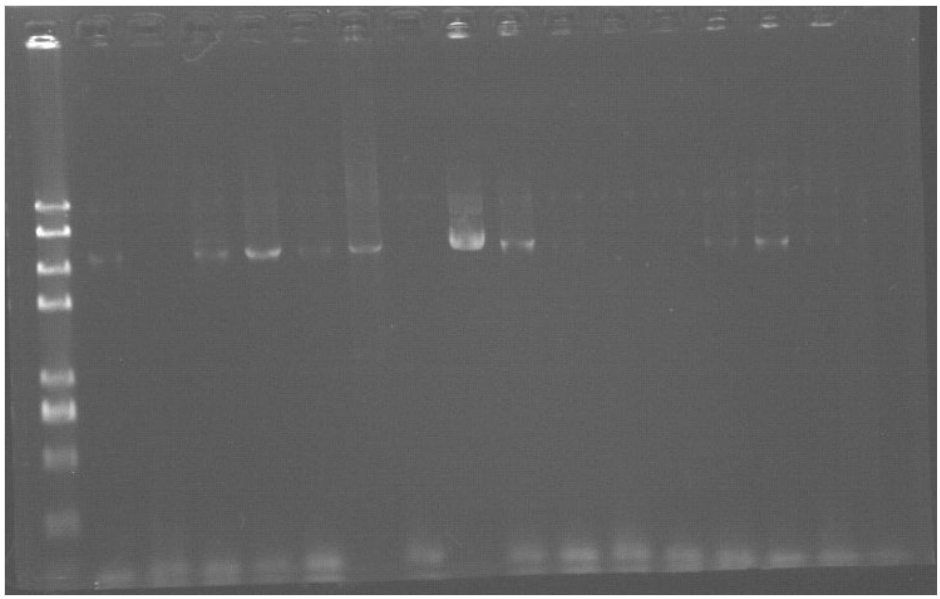
sample	**1'**	**2'**	**3'**	**4'**	**5'**	**6'**	**7'**	**8'**
Sterilized ddH2O	35 μL	35 μL	35 μL	35 μL	35 μL	35 μL	35 μL	35 μL
Taq DNA polymerase	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
template	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
mtr-res-f	2 μL	2 μL	2 μL	2 μL	2 μL	2 μL	2 μL	2 μL
sample	**1'**	**2'**	**3'**	**4'**	**5'**	**6'**	**7'**	**8'**
mtr-res-r	2 μL	2 μL	2 μL	2 μL	2 μL	2 μL	2 μL	2 μL
10X DNA Buffer	5 μL	5 μL	5 μL	5 μL	5 μL	5 μL	5 μL	5 μL
Mg2+ (25mM)	3 μL	3 μL	3 μL	3 μL	3 μL	3 μL	3 μL	3 μL
dNTP	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

3.PCR reaction 1-16 Parameters setting：

stage	**temperature**	**time**
step 1	95	5 min
step 2	95	30 s
step 3	56	30 s
step 4	72	5 min 30s
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:



(lane 1-17: marker 2K Plus II, Mtr-1-7,template,Mtr-1'-8') **Date 7.24 PCR of Reductase**
Recorder: Yonghao Liang

Experimental materials

1. Template: extraction product of pET21 carrying Reductase;
2. Primer: red-res-f,red-res-r. Designed by ourselves, synthesized by Sangon Biotech;
3. Sterilized ddH2O;
4. 2X PrimeStar DNA polymerase.

Procedure:

1.Prepare 16 PCR tubes and sequentially add：

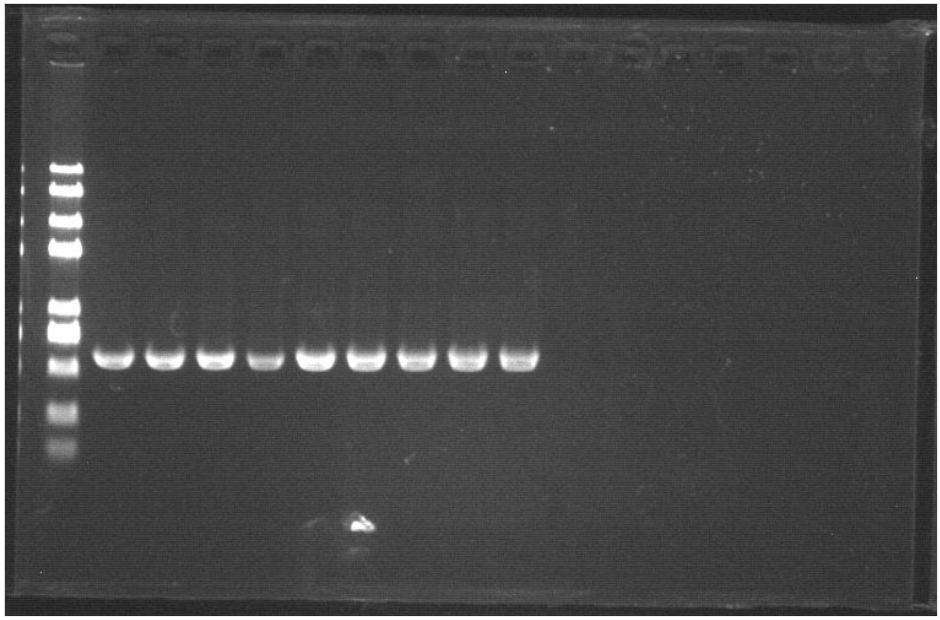
sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Sterilized ddH2O	22 μL	22 μL	22 μL	22 μL	22 μL	22 μL	22 μL	22 μL	22 μL
2X PrimeStar DNA polymerase	25 μL	25 μL	25 μL	25 μL	25 μL	25 μL	25 μL	25 μL	25 μL
template	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
red-res-f	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
red-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

3.PCR reaction 1-9 Parameters setting：

stage	**temperature**	**time**
step 1	95	10 min
step 2	98	10 s
step 3	55	5 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:



(lane 1-10: Marker 2K plus II, sample 1-9)

Bacteria PCR of pUC+ccm Recorder: Yonghao Liang

Experimental materials

1. Template: bacteria picked from the plate containing pUC+ccm;
2. Primer: pUC-f, pUC-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**	**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 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	1 μL
pUC-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
pUC-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

3.PCR reaction 1-9 Parameters setting：

stage	**temperature**	**time**
step 1	94	10 min
step 2	94	30 s
step 3	56	30 s

stage	temperature	time
step 4	72	7 min
step 5	72	10 min
step 6	4	--

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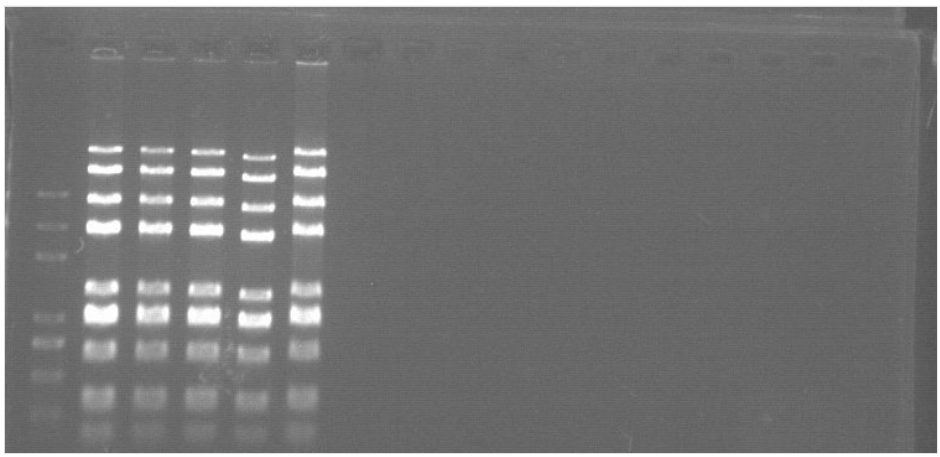
25 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result: (lane 1-17:)

Check for Marker 2K plus II Recorder: Yonghao Liang We doubted that the marker 2K plus II we bought had quality problem, so we did a Agarose gel electrophoresis for it.



Double digestion of ccm & RED Recorder: Liudong Luo, Zhenyu Jiang Materials:

1. full ccm,RED
2. FastDigest restriction enzyme XbaI, pstI,XhoI,HindIII and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water

Reaction system:

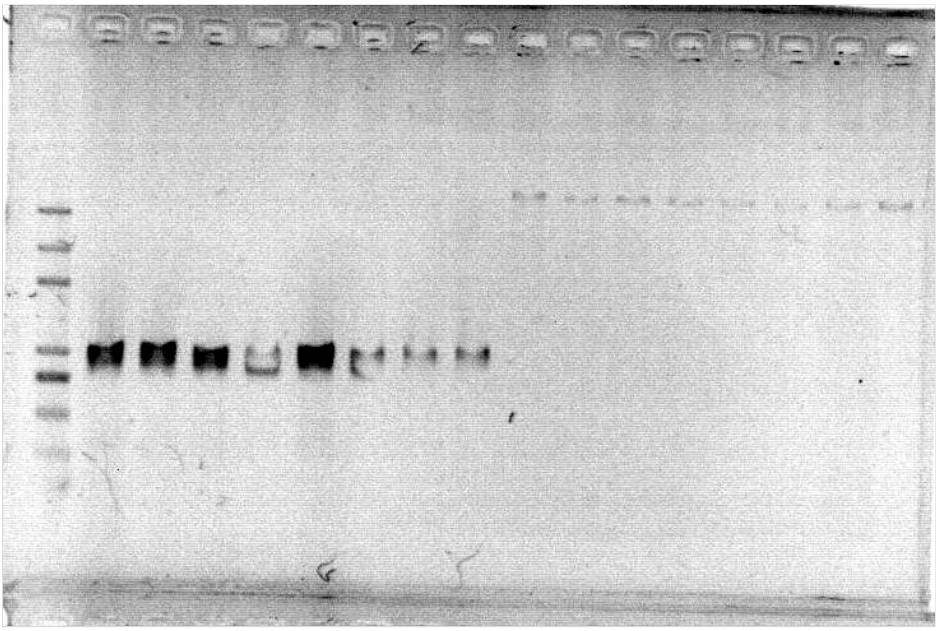
Sample	1	2	3	4	5	6	7	8
XbaI(μL)	1	1	1	1	1	1	1	1
pstI(μL)	1	1	1	1	1	1	1	1
nuclease-free water(μL)	25	25	25	25	25	25	25	25
fastdigest green buffer(μL)	2	2	2	2	2	2	2	2
ccm(μL)	1	1	1	1	1	1	1	1
total(μL)	30	30	30	30	30	30	30	30

Reaction system:

Sample	1	2	3	4	5	6	7	8
XhoI(μL)	1	1	1	1	1	1	1	1
HindIII(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
RED(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 .

Agarose gel electrophoresis Result:



(lane 1 to 17: 2K plus ,ddccm-1,ddccm-2,ddccm-3,ddccm-4,ddccm-5,ddccm-6,ddccm-7,ddccm-8,ddRED-1,ddRED-2,ddRED-3,ddRED-4,ddRED-5,ddRED-6,ddRED-7,ddRED-8)

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**
Concentration(ng/ul)	49.3
260/280	1.68
260/230	1.17

sample	**RedXHdd 1+2+3+4**	**RedXHdd5+6+7+8**
Concentration(ng/ul)	45.5	42.6
260/280	1.67	1.68
260/230	1.03	1.04

Double digestion of puc & PBAD Recorder: Liudong Luo, Zhenyu Jiang Materials:

1. puc,PBAD
2. FastDigest restriction enzyme XbaI, pstI,XhoI,HindIII and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water

Reaction system:

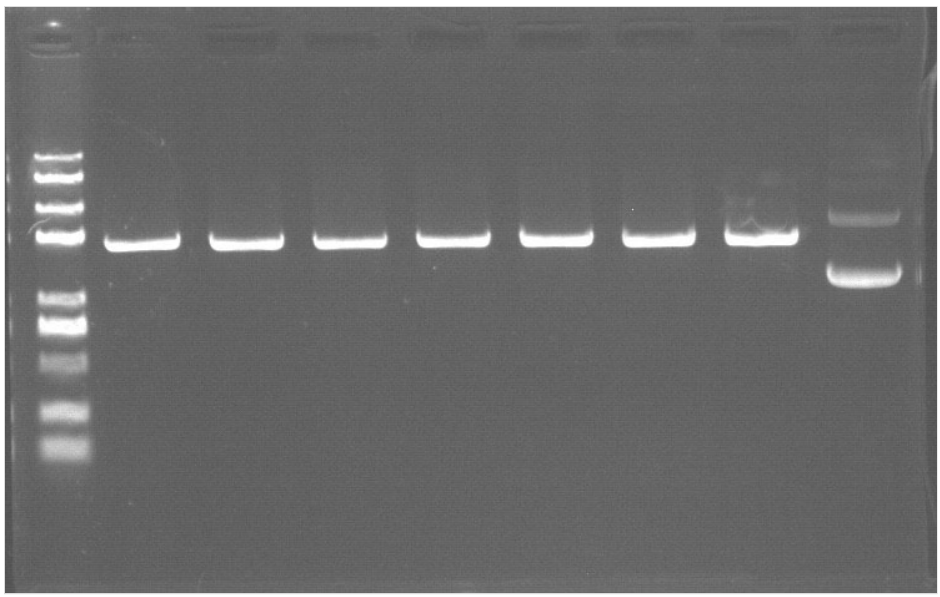
Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
XbaI(μL)	1	1	1	1	1	1	1
pstI(μL)	1	1	1	1	1	1	1
nuclease-free water(μL)	13	13	13	13	13	13	13
fastdigest green buffer(μL)	2	2	2	2	2	2	2
puc(μL)	3	3	3	3	3	3	3
total(μL)	20	20	20	20	20	20	20

Reaction system:

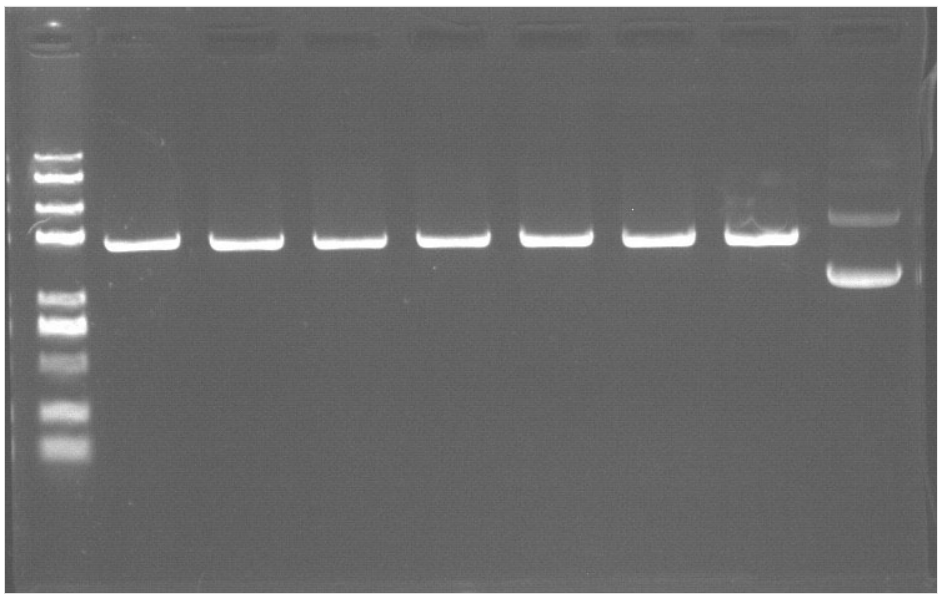
Sample	1	2	3	4	5	6	7
XhoI(μL)	1	1	1	1	1	1	1
HindIII(μL)	1	1	1	1	1	1	1
nuclease-free water(μL)	11	11	11	11	11	11	11
fastdigest green buffer(μL)	2	2	2	2	2	2	2
RED(μL)	5	5	5	5	5	5	5
total(μL)	20	20	20	20	20	20	20

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

Agarose gel electrophoresis Result:



(lane 1 to 9: 2K plus II ,dd-puc-1,dd-puc-2,dd-puc-3,dd-puc-4,dd-puc-5,dd-puc-6,dd-puc-7,puc)



(lane 1 to 9: 2K plus II ,dd-PBAD-1,dd-PBAD-2,dd-PBAD-3,dd-PBAD-4,dd-PBAD-5,dd-PBAD-6,dd-PBAD-7,PBAD)

Then we did the purification of the fragments we got.

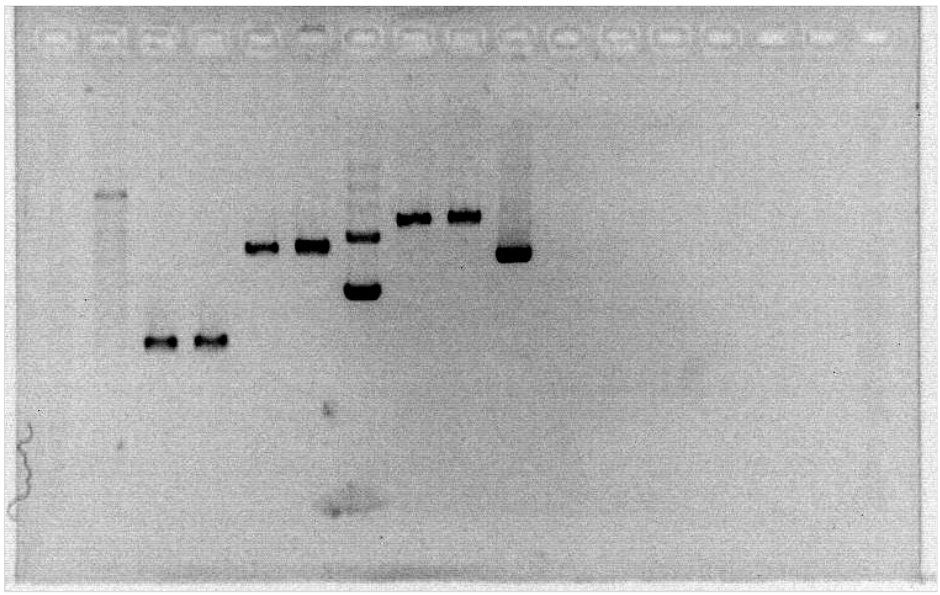
The results are as following:

sample	pucXPdd 1+2+3+4	pucXPdd 5+6+7+8
Concentration(ng/ul)	74.7	38.0
260/280	1.59	1.72

sample	pucXPdd 1+2+3+4	pucXPdd 5+6+7+8
260/230	0.63	0.67

sample	PBADXHdd 1+2+3+4	PBADXHdd5+6+7+8
Concentration(ng/ul)	45.5	38.4
260/280	1.68	1.68
260/230	0.65	0.70

Agarose gel electrophoresis and purification Result:



(lane 1 to 10: loading Buffer,pur-dd-ccm,pur-dd-RED-1,pur-dd-RED-2,pur-dd-puc-1,pur-dd-puc-2,puc,pur-dd-PBAD-1,pur-dd-PBAD-2,PBAD)

Colony PCR of Mtr-T Recorder: Wenfei Yu

Experimental materials

1. Template: E.coli Top 10 with T plasmid containing Mtr;
2. Primer: mtr-res-f,mtr-res-r. Designed by ourselves, synthesized by Sangon Biotech;
3. Sterilized ddH2O, 2×Taq PCR master mix.

Procedure:

1.Prepare 8 PCR tubes and sequentially add：

sample	1	2	3	4	5	6	7	8	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	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL
2×Taq PCR master mix	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	1 μL	1 μL

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

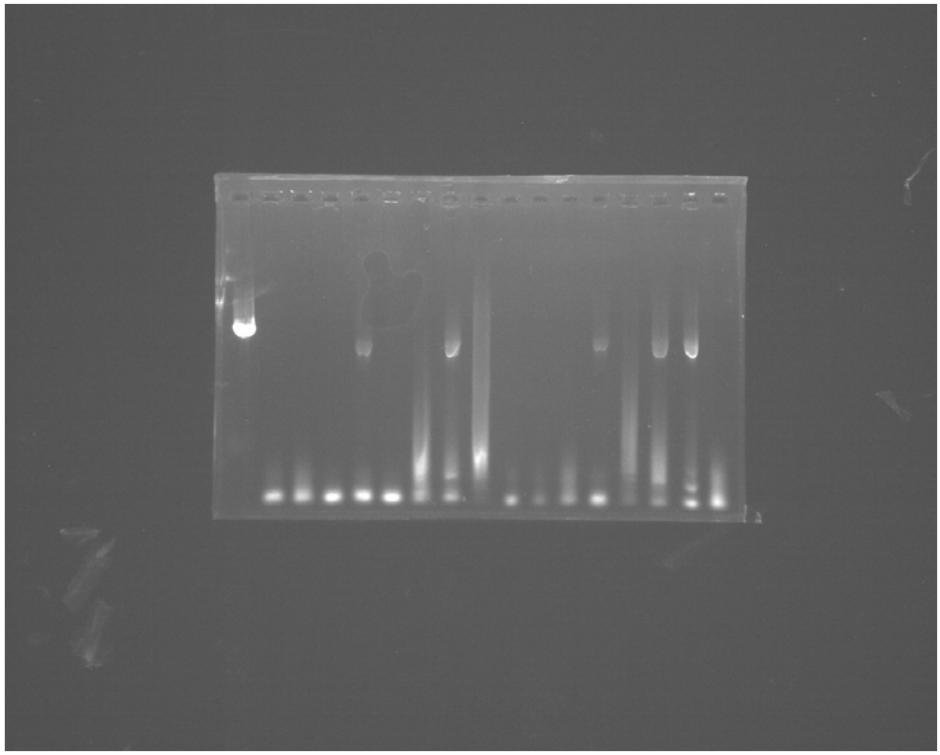
帮助

2.PCR reaction 1-16 Parameters setting：

stage	temperature	time
step 1	94	10 min
step 2	94	30 s
step 3	56	30 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:



(From left to right: Marker, 1, 2, 3, 4, 5, 6, 7, 8, 1', 2', 3', 4', 8', 6', 7', 5') No.4 and No.7 are ok, No.6 is not sure

PCR of MtrCAB Recorder: Chenyang LI Experimental materials 1.Template 5: 1:30 dilution of plasmid MtrCAB (Mtr2 YWF 7.8 680.8, 1.93, 2.68), 11 ng/μL;

1. Primer: mtr-res-f, mtr-res-r. Designed by ourselves, synthesized by Sangon Biotech;
2. Sterilized ddH2O, Taq.

Procedure: 1.Prepare 4 PCR tubes and sequentially add：

sample	1,2,3,4
Sterilized ddH2O	35 μL
template	1 μL
mtr-res-r(10 μM)	2 μL
mtr-res-f(10 μM)	2 μL
10×PCR Buffer	5 μL
Mg ion(25mM)	3 μL
dNTP	1 μL
Taq	1 μL

sample	1,2,3,4
total	50 μL

2.PCR reaction parameters setting：

帮助

stage	temperature(°C)	time
Pre-Duration	95	5 min
Duration	95	30 s
Anneal	56	30 s
Extend	72	5 min 30 s
Post-Extend	72	10 min
Final	4	--

30 cycles(Duration ~ Extend)

Purification of Mtr PCR product Recorder: Wenfei Yu (1). Add 250 μL Buffer B3 to the 50 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	Mtr PCR pur
Concentration(ng/ul)	351.8
260/280	1.87
260/230	2.19

Double digestion of pET28 Recorder: Shihan Zhu Materials:

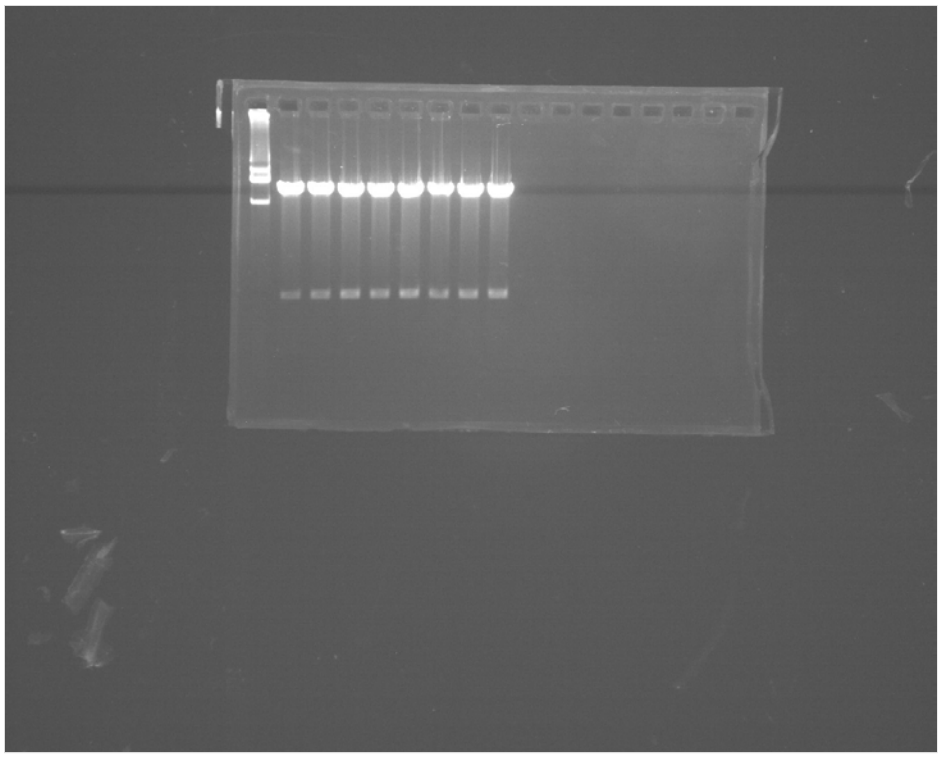
1. pET28
2. FastDigest restriction enzyme XhoI, NdeI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water

Reaction system:

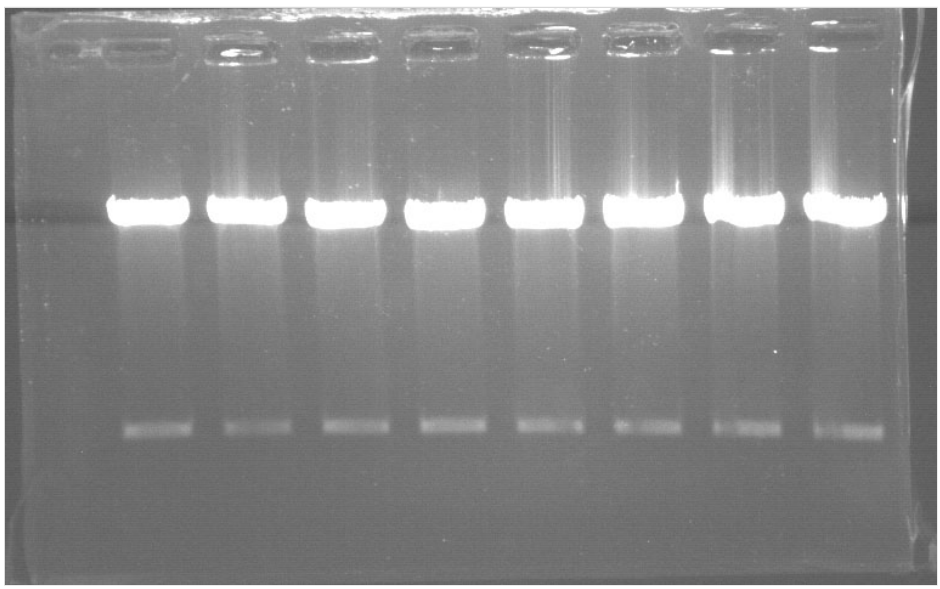
Sample	1	2	3	4	5	6	7	8
XbaI(μL)	1	1	1	1	1	1	1	1
pstI(μL)	1	1	1	1	1	1	1	1
nuclease-free water(μL)	8	8	8	8	8	8	8	8
fastdigest green buffer(μL)	2	2	2	2	2	2	2	2
ccm(μL)	8	8	8	8	8	8	8	8
total(μL)	20	20	20	20	20	20	20	20

Mix gently and incubate at 37 degree Celsius for 0.5h(1,2), 1h(3,4), 1.5h(5,6), 2h(7,8) .

Agarose gel electrophoresis Result:



(lane 1 to 17: pET28, dd-pET28-1, dd-pET28-2, dd-pET28-3, dd-pET28-4, dd-pET28-5, dd-pET28-6, dd-pET28-7, dd-pET28-8)



(lane 1 to 8: dd-pET28-1, dd-pET28-2, dd-pET28-3, dd-pET28-4, dd-pET28-5, dd-pET28-6, dd-pET28-7, dd-pET28-8)

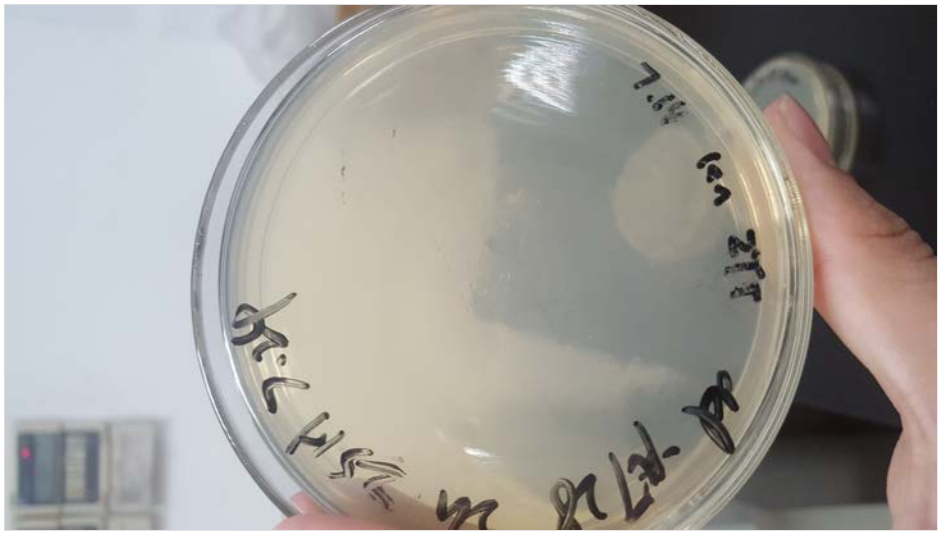
Then we did the purification of the fragments we got.

The results are as following:

sample	**dd-pET28-3&4**	**dd-pET28-5&6**	**dd-pET28-7&8**
Concentration(ng/ul)	45.4	24.0	28.9
260/280	1.71	1.73	1.75
260/230	0.49	0.43	0.33

Transformation of dd-pET28 Recorder: Shihan Zhu,Yonghao Liang 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 10μL dd-pET28 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.



Plasmid Extraction of the mtr-T Recorder: Tong Xiao, Liwen Zhang 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	mtr-t-1	mtr-T-2	mtr-T-3	mtr-T-4	mtr-T-5	mtr-T-6	mtr-T-7	mtr-T-8
Concentration(ng/ul)	315.8	313.9	516.2	486.1	158.0	413.0	144.1	374.0
260/280	1.88	1.84	1.87	1.84	1.80	1.87	1.77	1.84
260/230	2.33	1.83	2.24	2.01	1.48	2.08	1.17	1.75

self-Ligation of pET28 Recorder: Wenfei Yu Material:

1. double digestion product of pET28
2. 10× T4 DNA ligase buffer,T4 DNA ligase(bought from Thermo Fisher Scientific)

Procedure: Add to either of samples: 100ng ddpET28 2 μL 10* T4 DNA Ligase Buffer 0.4 μL T4 DNA Ligase sterilized water

Mix gently and incubate at 16 degree Celsius for 11 hour.

帮助

Ligation of ccm + PUC19, RED + pBAD and self-ligation of PUC19 and pBAD Recorder: Menglong Jin, Zhenyu Jiang, Liudong Luo Material:

1. double digestion product of ccmA-H, PUC19, RED and pBAD;
2. 10× T4 DNA ligase buffer,T4 DNA ligase(bought from Thermo Fisher Scientific).

Procedure: Add to either of samples:

sample	CP	RP1	RP2	PP	BB
ddccmA-H	16μl	0μl	0μl	0μl	0μl
ddRED	0μl	2μl	2μl	0μl	0μl
ddPUC19	2μl	0μl	0μl	2μl	0μl
ddpBAD	0μl	2μl	2μl	0μl	2μl
10* T4 DNA Ligase Buffer	2μl	2μl	2μl	2μl	2μl
T4 DNA Ligase	0.4μl	0.4μl	0.4μl	0.4μl	0.4μl
sterilized water	0μl	14μl	14μl	16μl	16μl
Total	20μl	20μl	20μl	20μl	20μl

Mix gently and incubate at 16 degree Celsius for about 16 hour.

Date 7.25

Transformation of ccmA-H+PUC19, RED+pBAD and PP, BB 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(RED+pBAD; BB) in a plate with Amp; add 200 μL solution (ccmA-H+PUC19; PP) in a plate with Amp and X-Gal. 10.Cultivate these bacteria for about 10h for further use.

PCR of Mtr Recorder: Wenfei Yu

Experimental materials

1. Template: extraction product of Mtr;
2. Primer: mtr-res-f, mtr-res-r. Designed by ourselves, synthesized by Sangon Biotech;
3. Sterilized ddH2O;
4. 2X PrimeStar DNA polymerase.

Procedure:

1.Prepare 16 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
2X PrimeStar DNA polymerase	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
red-res-f	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL

sample	1	2	3	4	5	6	7	8
red-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

帮助

3.PCR reaction 1-8 Parameters setting：

stage	temperature	time
step 1	98	10 min
step 2	98	10 s
step 3	56	5 s
step 4	72	5 min 30 s
step 5	72	10 min
step 6	4	--

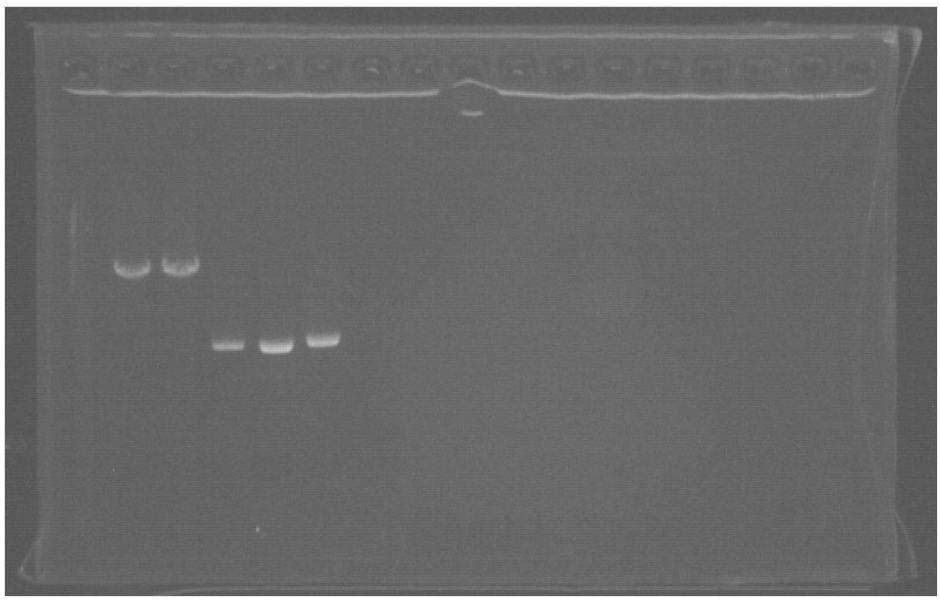
30 cycles(step 2 ~ step 4)

Then we did the purification of the fragments we got.

The results are as following:

sample	Mtr PCR pur 1	Mtr PCR pur 2
Concentration(ng/ul)	464.6	456.1
260/280	1.89	1.88
260/230	2.26	2.14

3.Agarose gel electrophoresis Result:



(lane 1 to 6: Marker, Mtr PCR pur 2, Mtr PCR pur 2, Mtr-T 7, Mtr-T 4, Mtr-T 6)

Double digestion of Mtr Recorder: Meiying Cui Materials:

1. Mtr PCR
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	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)	23.5	23.5	23.5	23.5	23.5	23.5	23.5	23.5	23.5

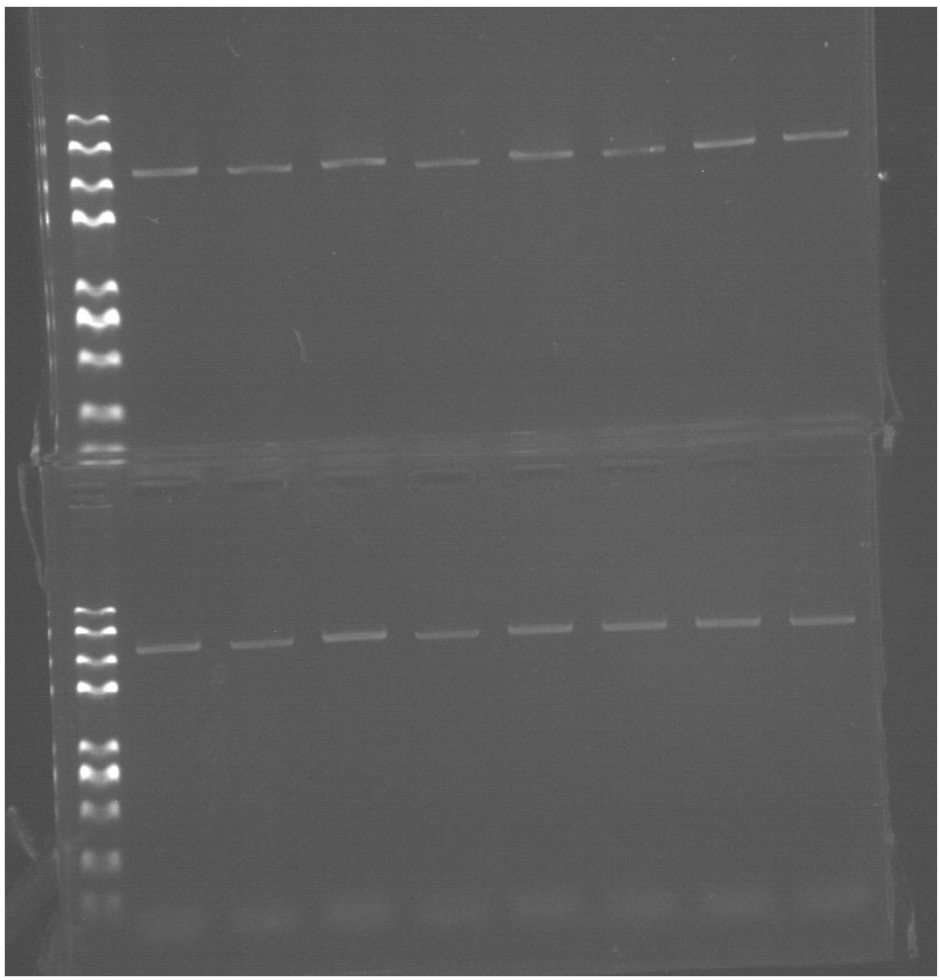
Sample	1	2	3	4	5	6	7	8
fastdigest green buffer(μL)	3	3	3	3	3	3	3	3
total(μL)	30	30	30	30	30	30	30	30

(1,2was treated for 2h,3,4,7,8 were 5h,5,6 were 7h)

Gel Extraction of ddMtr (Done by Meiying Cui) Procedure:

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

|sample|ddMtr 1|ddMtr 2|ddMtr 3|ddMtr 4|ddMtr 5|ddMtr 6|ddMtr 7|ddMtr 8| Agarose gel electrophoresis Result:



(from left to right: I: Marker, ddMtr 1-1, ddMtr 1-2, empty, ddMtr 2-1, ddMtr 2-2, empty, ddMtr 3-1, ddMtr 3-2; II: Marker, ddMtr 4-1, ddMtr 4-2, empty, ddMtr 5-1, ddMtr 5-2, empty, ddMtr 6-1, ddMtr 6-2)

The OD results are as following:

sample	ddMtr GE(7h 1+2+5+6)	ddMtr GE(5h 3+4)
Concentration(ng/ul)	8.6	31.1
260/280	1.53	1.33

sample	ddMtr GE(7h 1+2+5+6)	ddMtr GE(5h 3+4)
260/230	0.36	0.34

Colony PCR of Mtr-T Recorder: Wenfei Yu

帮助

Experimental materials

1. Template: E.coli Top 10 with T plasmid containing Mtr;
2. Primer: PUC-f, PUC-r. Designed by ourselves, synthesized by Sangon Biotech;
3. Sterilized ddH2O, 2×Taq PCR master mix.

Procedure:

1.Prepare 8 PCR tubes and sequentially add：

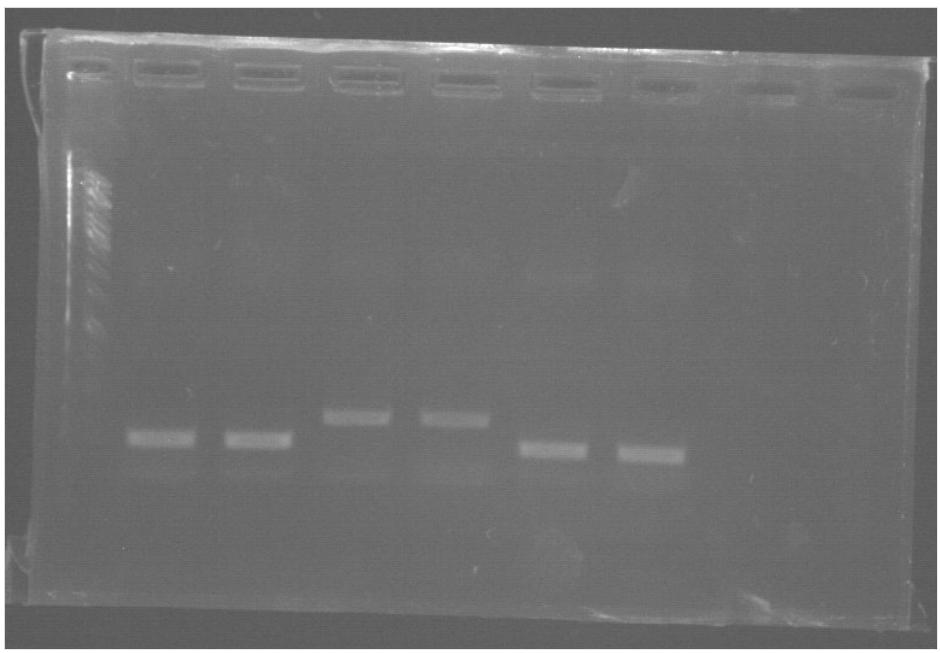
sample	1	2	3	4	5	6
Sterilized ddH2O	7 μL	7 μL	7 μL	7μL	7 μL	7 μL
2×Taq PCR master mix	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL
template	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
PUC-f	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
PUC-r	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
total	20 μL	20 μL	20 μL	20 μL	20 μL	20 μL

2.PCR reaction 1-6 Parameters setting：

stage	temperature	time
step 1	94	10 min
step 2	94	30 s
step 3	56	30 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:



Ligation of pET28 and mtr Recorder: Shihan Zhu Material:

1. double digestion product of pET28 and mtr-pcr products
2. 10× T4 DNA ligase buffer,T4 DNA ligase(bought from Thermo Fisher Scientific)

Procedure: Add to either of samples: 2 μL dd-pET28 10 μL dd-mtr 2 μL 10* T4 DNA Ligase Buffer 0.4 μL T4 DNA Ligase 8 μL sterilized water

Mix gently and incubate at 16 degree Celsius for 11 hour.

Plasmid Extraction of the mtr-T Recorder: Liwen Zhang 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	mtr-t-4	mtr-T-6	mtr-T-7
Concentration(ng/ul)	350.6	412.2	159.1
260/280	1.75	1.75	1.84
260/230	1.16	1.18	2.1

Transformation of self-ligation of dd-pET28 Recorder: Shihan Zhu 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 Amp. 10.Cultivate these bacteria overnight for further use.

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:

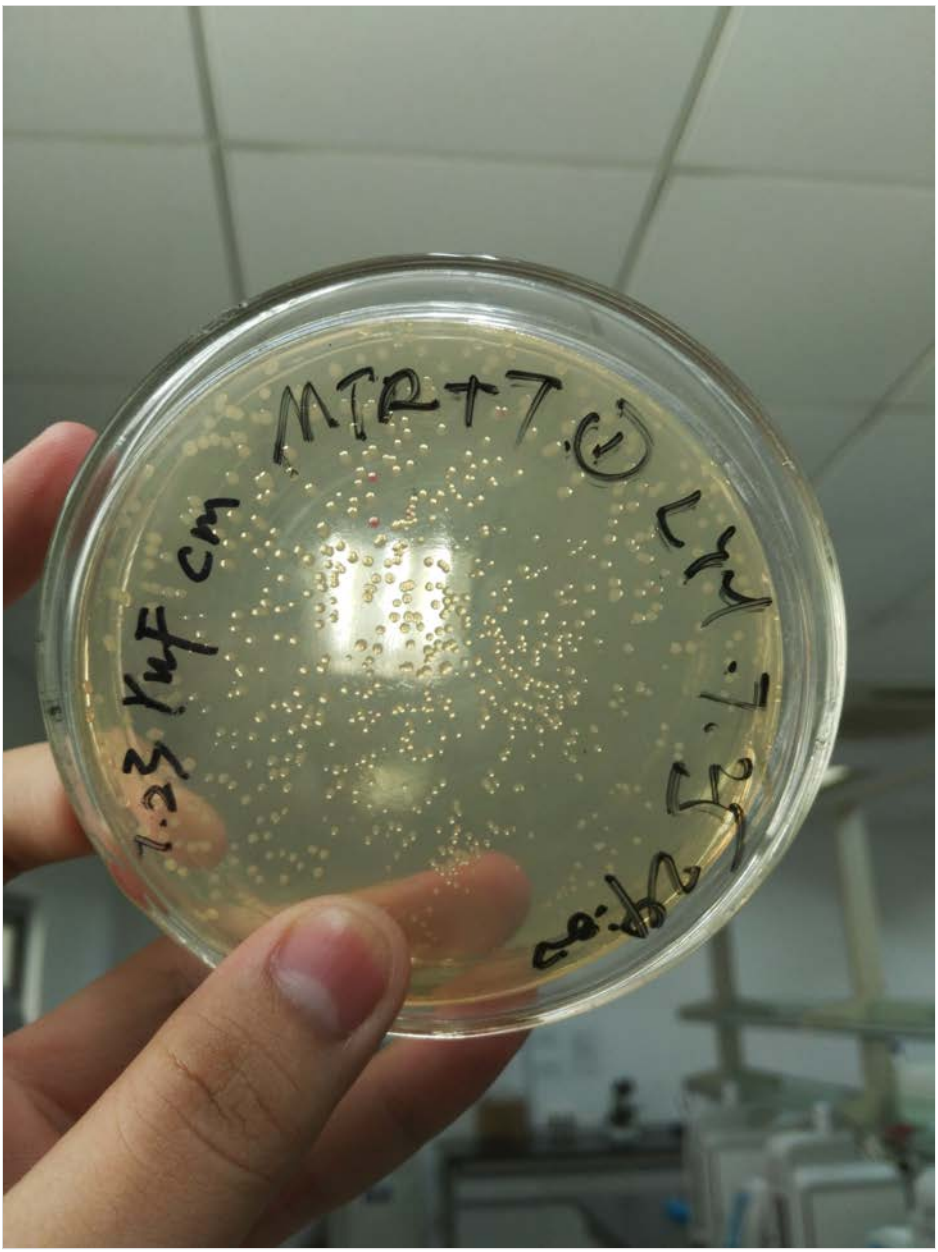
sample	**ccm PCR pur 1+2+3+4+5+6+7+8**
Concentration(ng/ul)	136.6
260/280	1.81
260/230	2.10

Date 7.26 Transformation of Mtr+T vector Recorder: Wenfei Yu, Yonghao Liang, Shihan Zhu, Meiying Cui

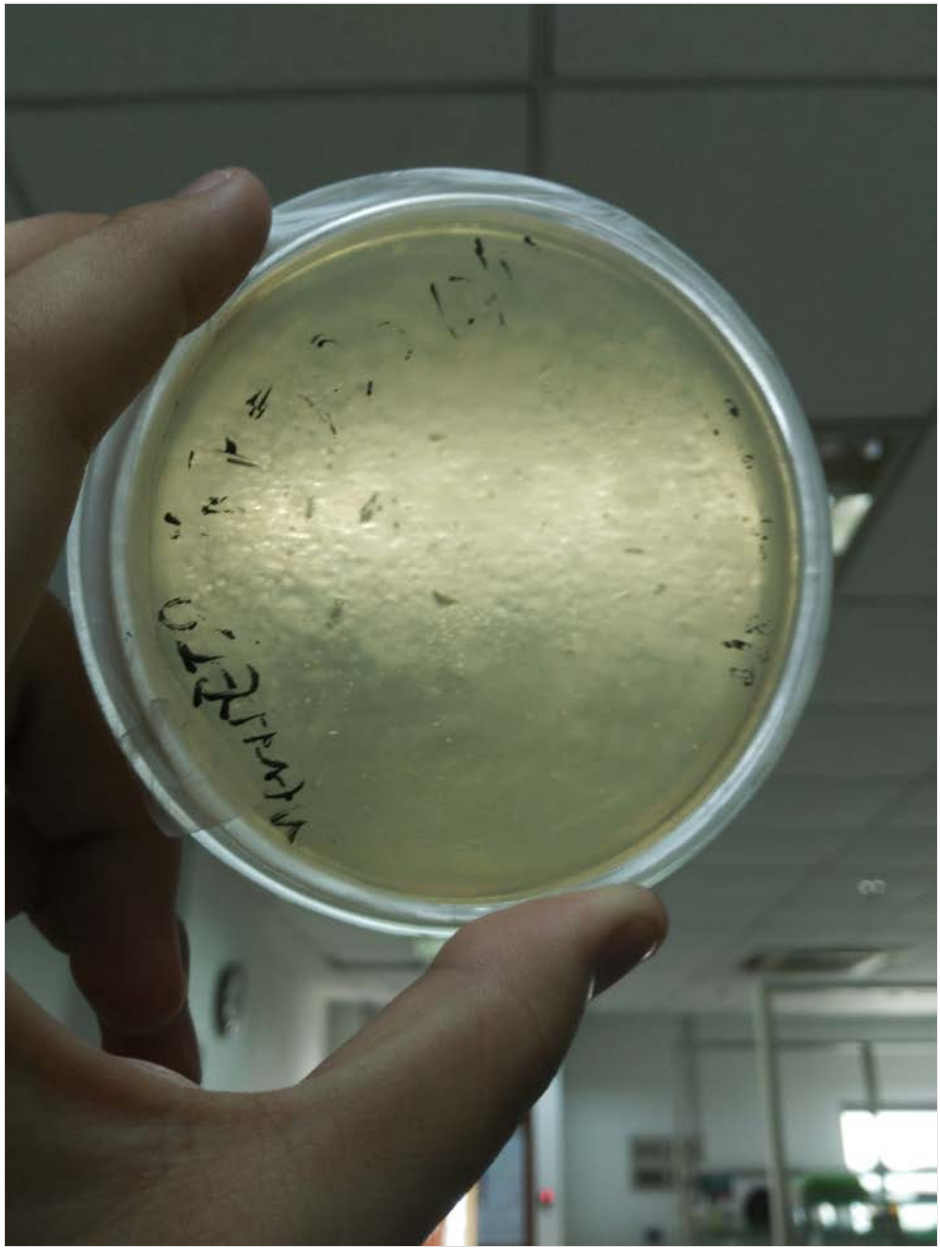
NOTE:Generally, competent bacteria are restrored in -80 degree centrigrade environment. 1.Take the competent bacteria from refrigerator and incubate them into ice about 5 mins until it is dissolved 2.Absorb 1μL PUC19 and mix it wμith bacteria solution thoroughly. ATTENTION: Please operate this step tenderly!!! 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 Amp. 10.Cultivate these bacteria overnight for further use.

帮助

Result:



帮助



Double digestion of pET28 Recorder: Shihan Zhu Materials:

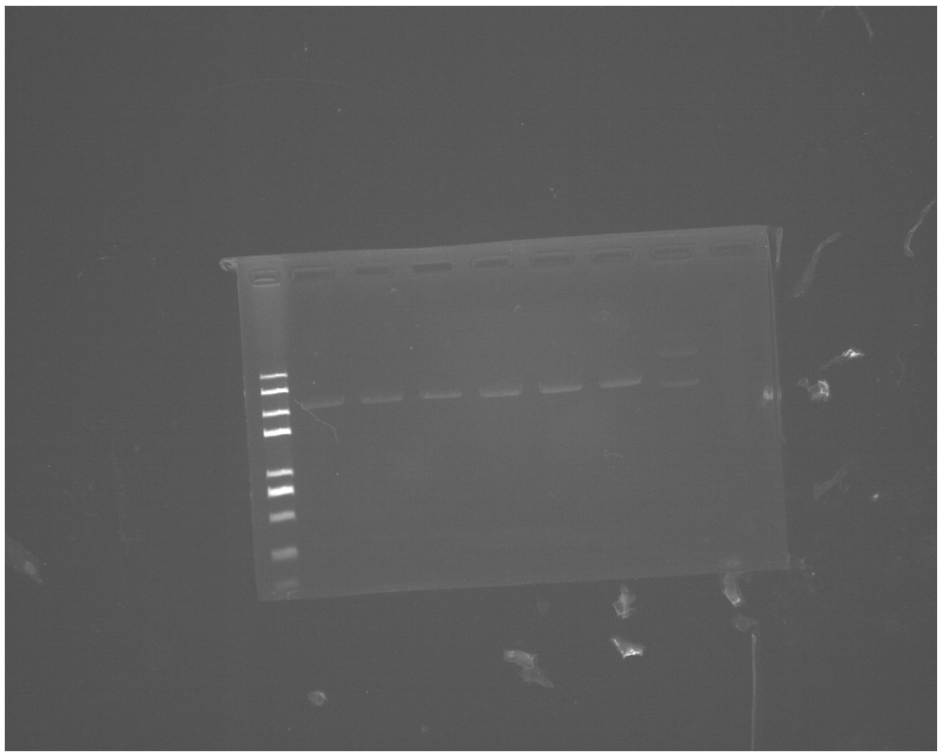
1. pET28
2. FastDigest restriction enzyme XhoI, NdeI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific) and Fast AP.
3. Nuclease-free water

Reaction system:

Sample	1	2	3	4	5	6
NdeI(μL)	1	1	1	1	1	1
XhoI(μL)	1	1	1	1	1	1
nuclease-free water(μL)	10	10	10	10	10	10
fastdigest green buffer(μL)	2	2	2	2	2	2
pET28(μL)	5	5	5	5	5	5
Fast AP(μL)	1	1	1	1	1	1
total(μL)	20	20	20	20	20	20

Mix gently and incubate at 37 degree Celsius for 45 hours .

Agarose gel electrophoresis Result:



(lane 1 to 9: 2K plus II,dd-pET28-1,dd-pET28-2,dd-pET28-3,dd-pET28-4,dd-pET28-5,dd-pET28-6,pET28)

Then we did the purification of the fragments we got.

Double digestion of ccm & RED Recorder: Liudong Luo, Zhenyu Jiang Materials:

1. full ccm,RED
2. FastDigest restriction enzyme XbaI, pstI,XhoI,HindIII 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

Sample	1	2	3	4	5	6	7	8
ccm(μL)	1	1	1	1	1	1	1	1
total(μL)	30	30	30	30	30	30	30	30

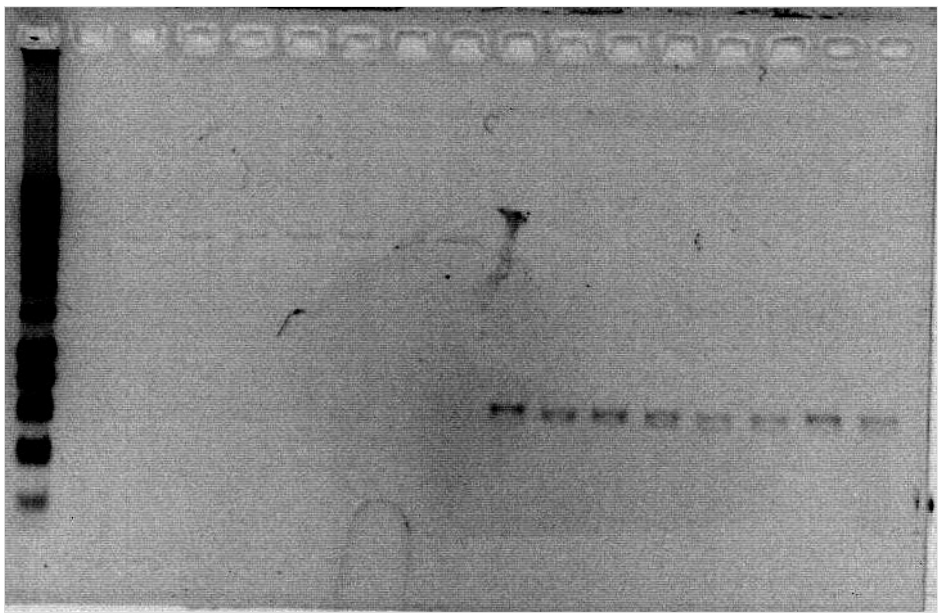
帮助

Reaction system:

Sample	1	2	3	4	5	6	7	8
XhoI(μL)	1	1	1	1	1	1	1	1
HindIII(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
RED(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 .

Agarose gel electrophoresis Result:



(lane 1 to 17: 2K plus ,ddccm-1,ddccm-2,ddccm-3,ddccm-4,ddccm-5,ddccm-6,ddccm-7,ddccm-8,ddRED-1,ddRED-2,ddRED-3,ddRED-4,ddRED-5,ddRED-6,ddRED-7,ddRED-8)

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
Concentration(ng/ul)	22.7
260/280	1.72
260/230	1.08

sample	RedXHdd 1+2+3+4+5+6+7+8
Concentration(ng/ul)	26.6
260/280	1.83
260/230	1.31

Double digestion of puc & PBAD Recorder: Liudong Luo, Zhenyu Jiang Materials:

1. puc,PBAD

2. FastDigest restriction enzyme XbaI, pstI,XhoI,HindIII and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water

帮助

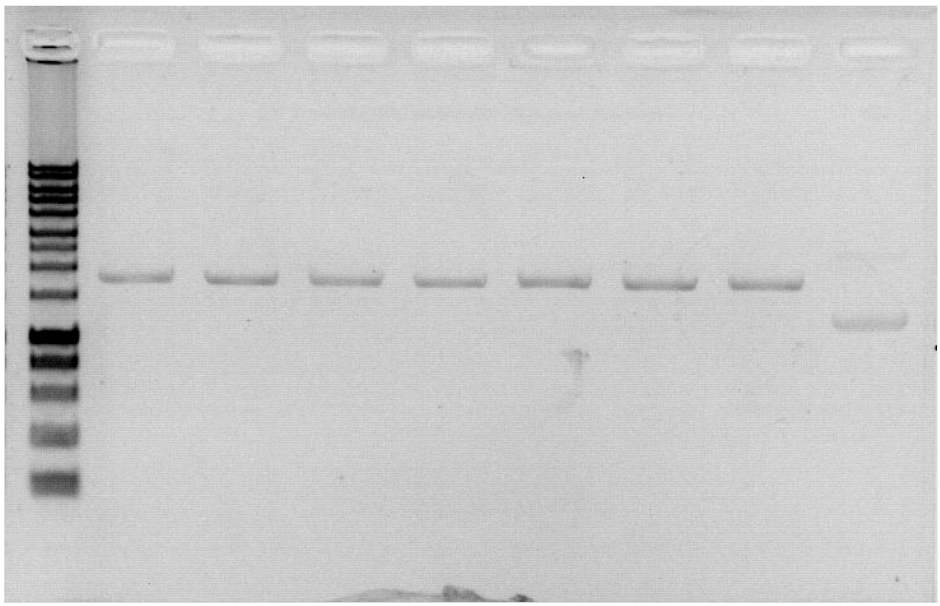
Reaction system:

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
XbaI(μL)	1	1	1	1	1	1	1
pstI(μL)	1	1	1	1	1	1	1
nuclease-free water(μL)	11	11	11	11	11	11	11
fastdigest green buffer(μL)	2	2	2	2	2	2	2
puc(μL)	5	5	5	5	5	5	5
total(μL)	20	20	20	20	20	20	20

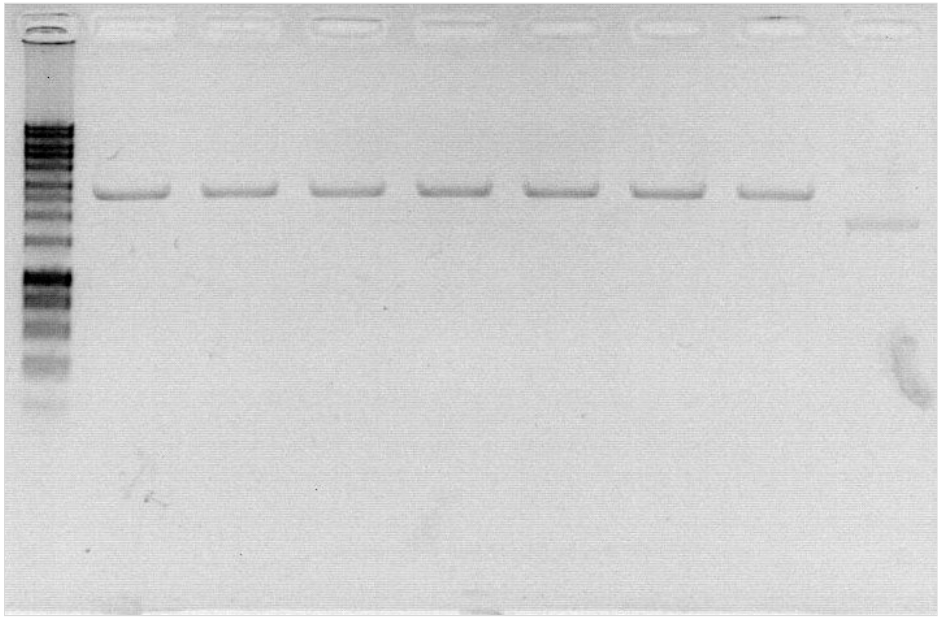
Reaction system:

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
XhoI(μL)	1	1	1	1	1	1	1
HindIII(μL)	1	1	1	1	1	1	1
Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
nuclease-free water(μL)	11	11	11	11	11	11	11
fastdigest green buffer(μL)	2	2	2	2	2	2	2
RED(μL)	5	5	5	5	5	5	5
total(μL)	20	20	20	20	20	20	20

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



(lane 1 to 9: Marker-Q ,dd-PBAD-1,dd-PBAD-2,dd-PBAD-3,dd-PBAD-4,dd-PBAD-5,dd-PBAD-6,dd-PBAD-7,PBAD)



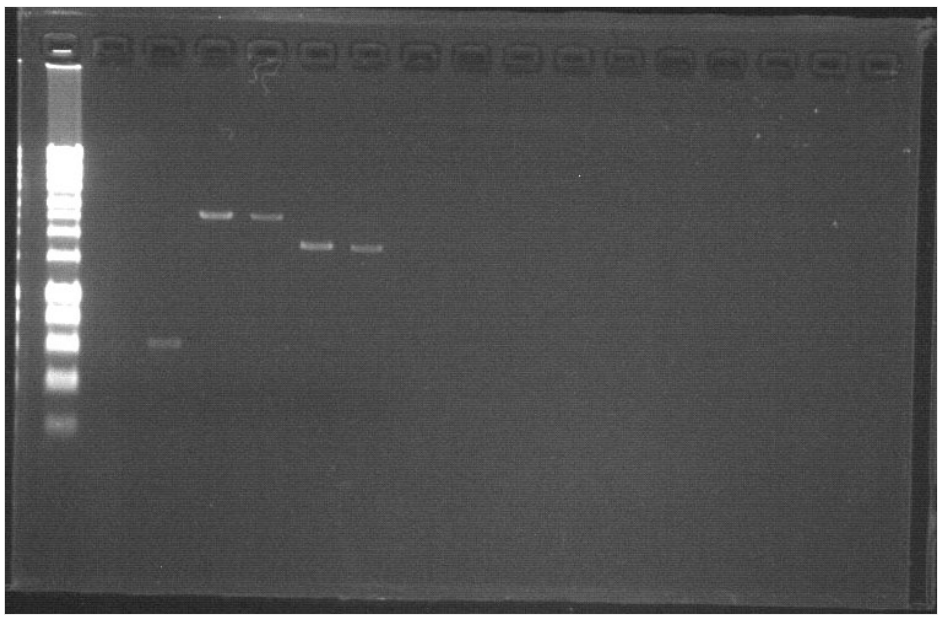
(lane 1 to 9: Marker-Q ,dd-PBAD-1,dd-PBAD-2,dd-PBAD-3,dd-PBAD-4,dd-PBAD-5,dd-PBAD-6,dd-PBAD-7,PBAD)

Agarose gel electrophoresis Result:

sample	**pucXPdd 1+2+3+4**	**pucXPdd 5+6+7+8**
Concentration(ng/ul)	28.4	94.0
260/280	1.81	1.54
260/230	0.70	0.56

sample	**PBADXHdd 1+2+3+4**	**PBADXHdd5+6+7+8**
Concentration(ng/ul)	34.9	55.4
260/280	1.72	1.63
260/230	0.64	0.58

Agarose gel electrophoresis and purification Result:



(lane 1 to 7: Marker—Q,pur-dd-ccm,pur-dd-RED,pur-dd-puc-1,pur-dd-puc-2,puc,pur-dd-PBAD-1,pur-dd-PBAD-2)

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**
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
stage	**temperature**	**time**
step 4	72	7 min
step 5	72	10 min
step 6	4	--

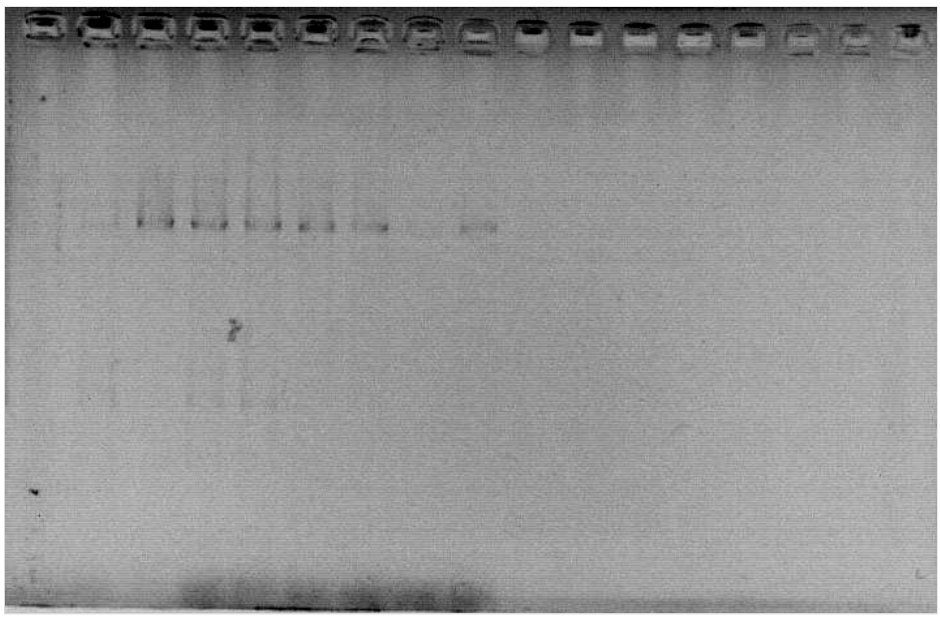
30 cycles(step 2 ~ step 4)

5,6,7,8 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:



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

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)	241.6
260/280	1.85
260/230	2.21

Plasmid Extraction of the PBar Recorder: Tong Xiao, Liwen Zhang 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	**PBar-2**	**PBar-5**	**PBar-6**	**PBar-7**	**PBar-8**	**PBar-9**	**PBar-10**	**PBar-11**	**PBar-12**	**PBar-13**
Concentration(ng/ul)	27.9	42.6	55.7	39.1	33.9	58.6	95.6	62.4	81.9	42.3
260/280	1.82	1.84	1.65	1.76	1.73	1.66	1.58	1.56	1.69	1.79
260/230	1.04	1.59	0.77	0.86	0.79	0.86	0.75	0.64	1.02	1.15

Double digestion of ccm & RED Recorder: Menglong Jin Materials:

1. full ccm,RED

sample	**ccmA-H**	**RED**
concentration(ng/μl)	241.6	807.8
260/280	1.85	1.87
260/230	2.21	2.23

Dilute the RED: add 2μl RED into 7μl sH2O.

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

Sample	1	2	3	4	5	6	7	8
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	1	2	3	4	5	6	7	8
XhoI(μL)	1	1	1	1	1	1	1	1
HindIII(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
RED(μL)	1	1	1	1	1	1	1	1
Sample total(μL)	1 30	2 30	3 30	4 30	5 30	6 30	7 30	8 30

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

Agarose gel electrophoresis Result:

Bacteria PCR Recorder: Meiying Cui,Yonghao Liang

Experimental materials

1. Template: bacteria picked from the plate ;
2. Primer: pUC-f, pUC-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：

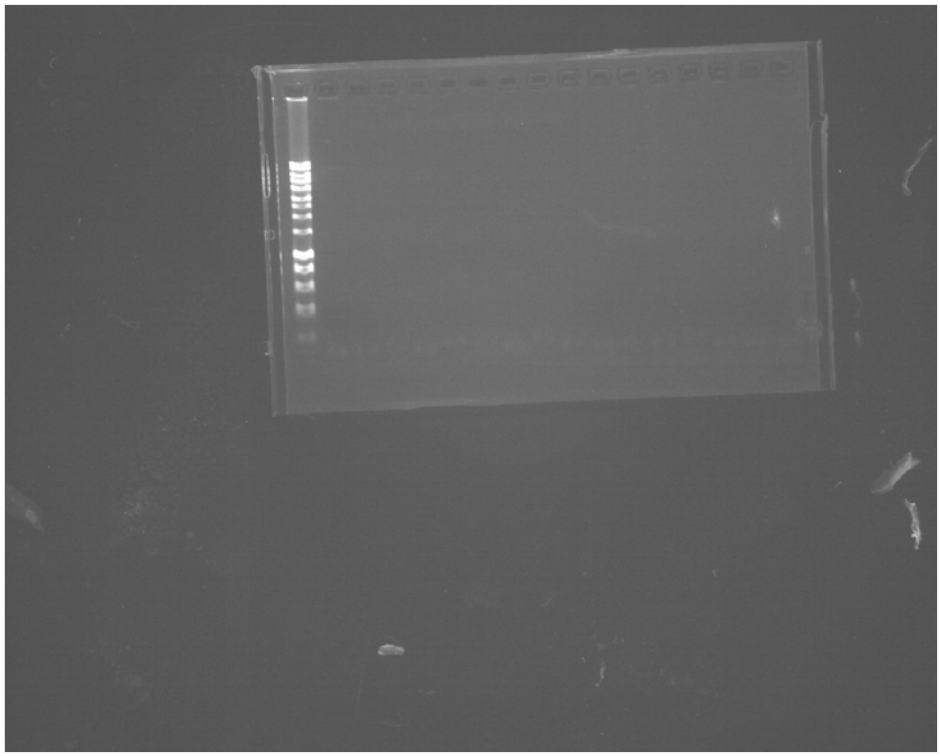
sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Sterilized ddH2O	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL
2X Taq DNA polymerase	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL
template	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
pUC-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
pUC-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

3.PCR reaction 1-9 Parameters setting：

帮助

stage	temperature	time
step 1	94	10 min
step 2	94	30 s
step 3	56	30 s
step 4	72	7 min
step 5	72	10 min
step 6	4	--

25 cycles(step 2 ~ step 4) Result:



Double digestion of RED & PYYDT Recorder: Xiaoyu Zhang,Yawei wu Materials:

1. RED,PYYDT
2. FastDigest restriction enzyme NdeI，XhoI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water

Reaction system:

Sample	1	2	3	4	5	6
XbaI(μL)	1	1	1	1	1	1
pstI(μL)	1	1	1	1	1	1
nuclease-free water(μL)	4.6	4.6	4.6	4.3	4.3	4.3
fastdigest green buffer(μL)	2	2	2	2	2	2
RED(μL)	11.4	11.4	11.4	11.7	11.7	11.7
total(μL)	20	20	20	20	20	20

Reaction system:

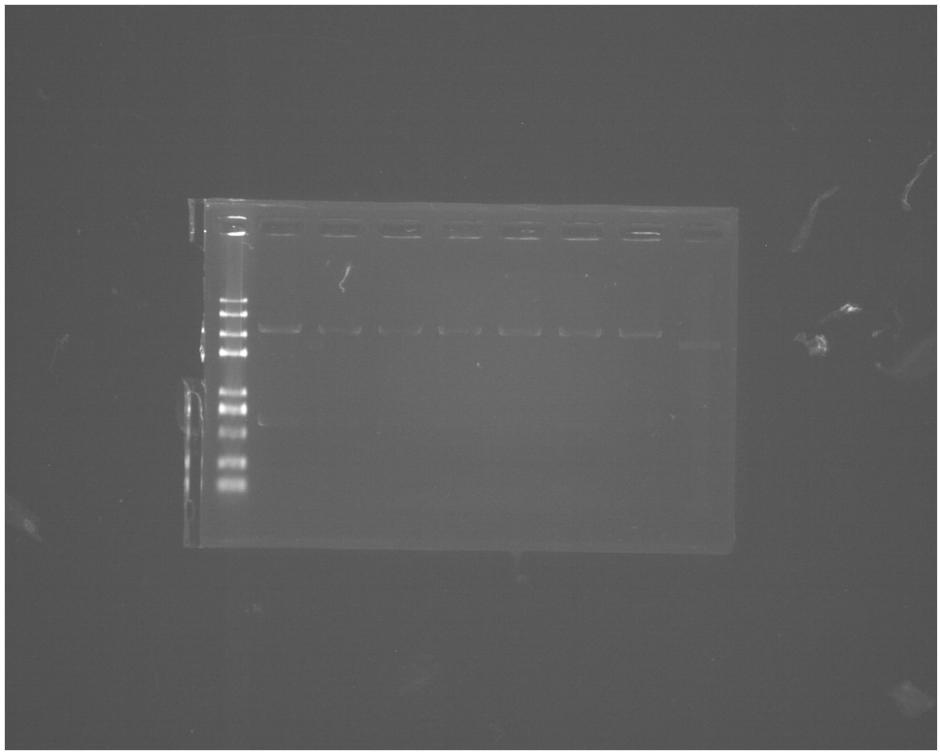
Sample	1	2	3	4	5	6
XhoI(μL)	1	1	1	1	1	1
HindIII(μL)	1	1	1	1	1	1

帮助

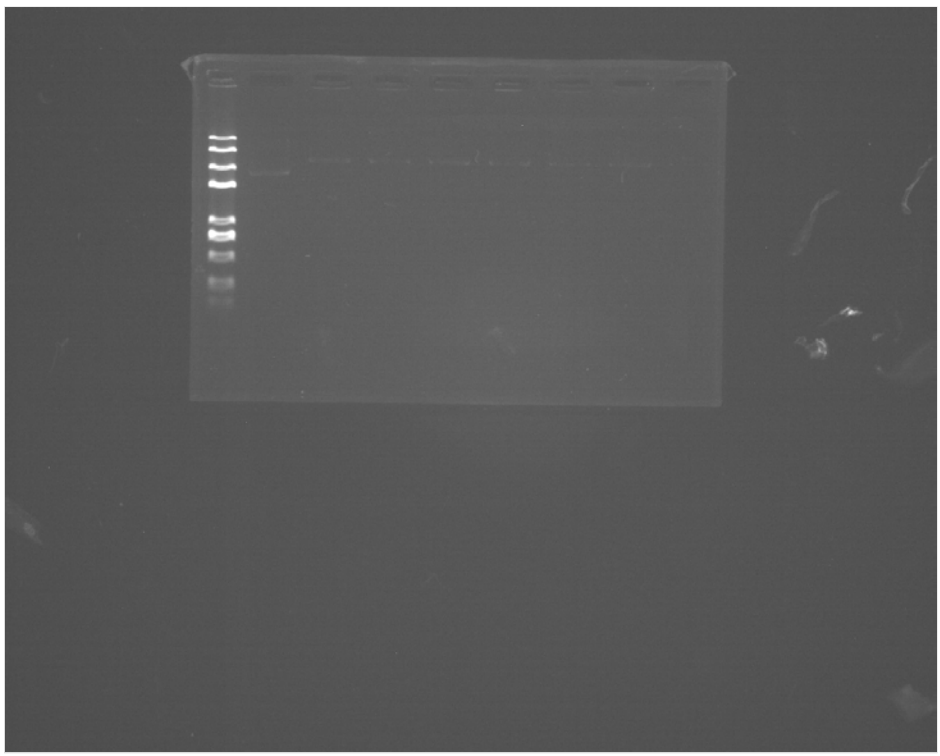
Sample	1	2	3	4	5	6
nuclease-free water(μL)	10.8	10.8	10.8	12.1	12.1	12.1
fastdigest green buffer(μL)	2	2	2	2	2	2
PYYDT(μL)	5.2	5.2	5.2	3.9	3.9	3.9
total(μL)	20	20	20	20	20	20

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

Agarose gel electrophoresis Result:



(lane left to right: Marker,ddRED1-7, RED)



(lane left to right: Marker, PYYDT, ddPYYDT1-6)

Then we did the purification of the fragments we got.

Double digestion of Mtr Recorder: Wenfei Yu Materials:

1. Mtr
2. FastDigest restriction enzyme XbaI, pstI,XhoI,HindIII and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water

Reaction system:

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**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)	25	25	25	25	25	25	25	25
fastdigest green buffer(μL)	2	2	2	2	2	2	2	2
Mtr(μL)	1	1	1	1	1	1	1	1
total(μL)	30	30	30	30	30	30	30	30

Reaction system:

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
XhoI(μL)	1	1	1	1	1	1	1
HindIII(μL)	1	1	1	1	1	1	1
nuclease-free water(μL)	11	11	11	11	11	11	11
fastdigest green buffer(μL)	2	2	2	2	2	2	2
RED(μL)	5	5	5	5	5	5	5
total(μL)	20	20	20	20	20	20	20
Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**

Mix gently and incubate at 37 degree Celsius for 5 hours .

Then we did the purification of the fragments we got.

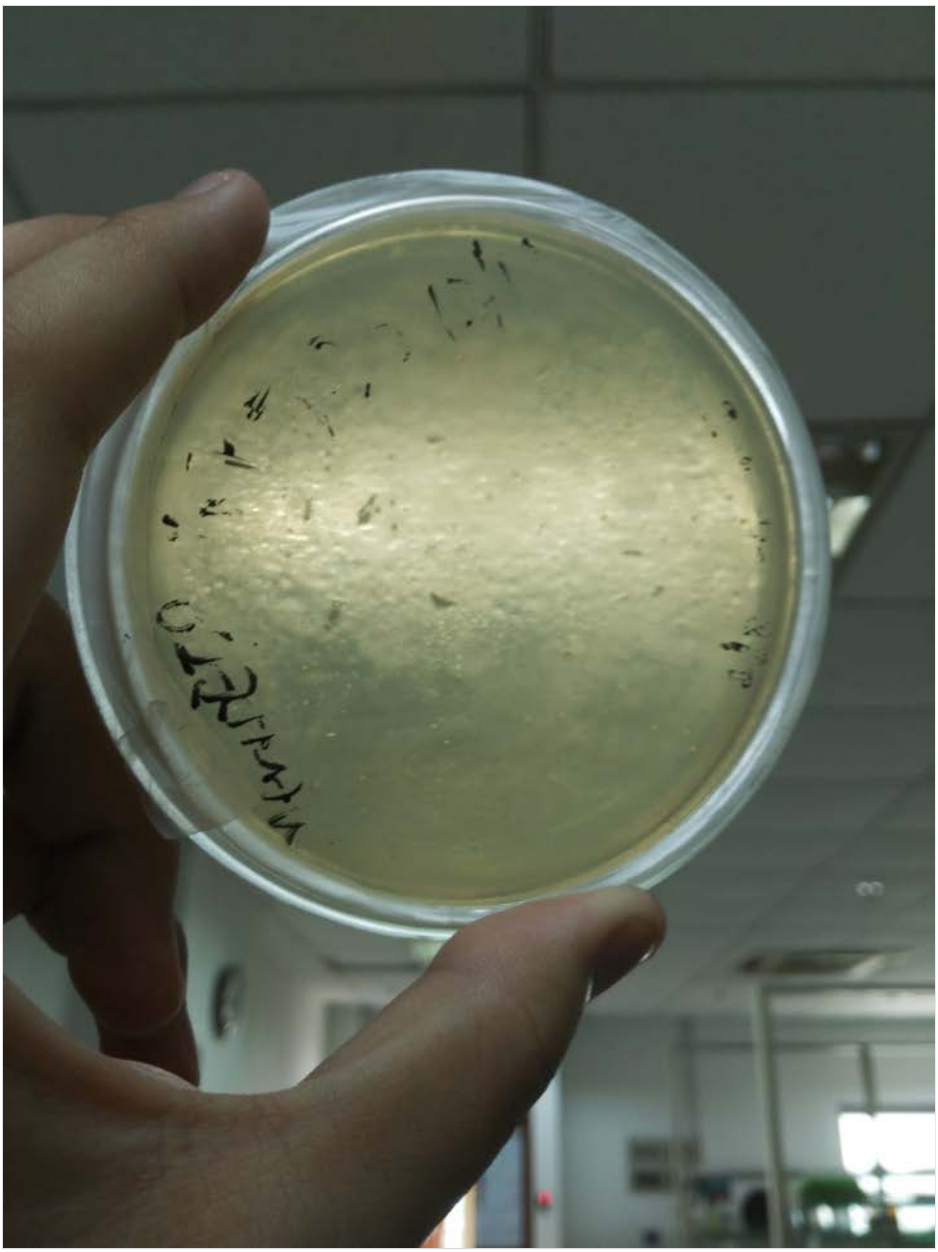
The results are as following:

sample	**ddMtr 1+2+3+4**	**ddMtr 5+6+7+8**
Concentration(ng/ul)	56.1	47.0
260/280	--	--
260/230	--	--

Transformation of Mtr into pET28 Recorder: Wenfei Yu

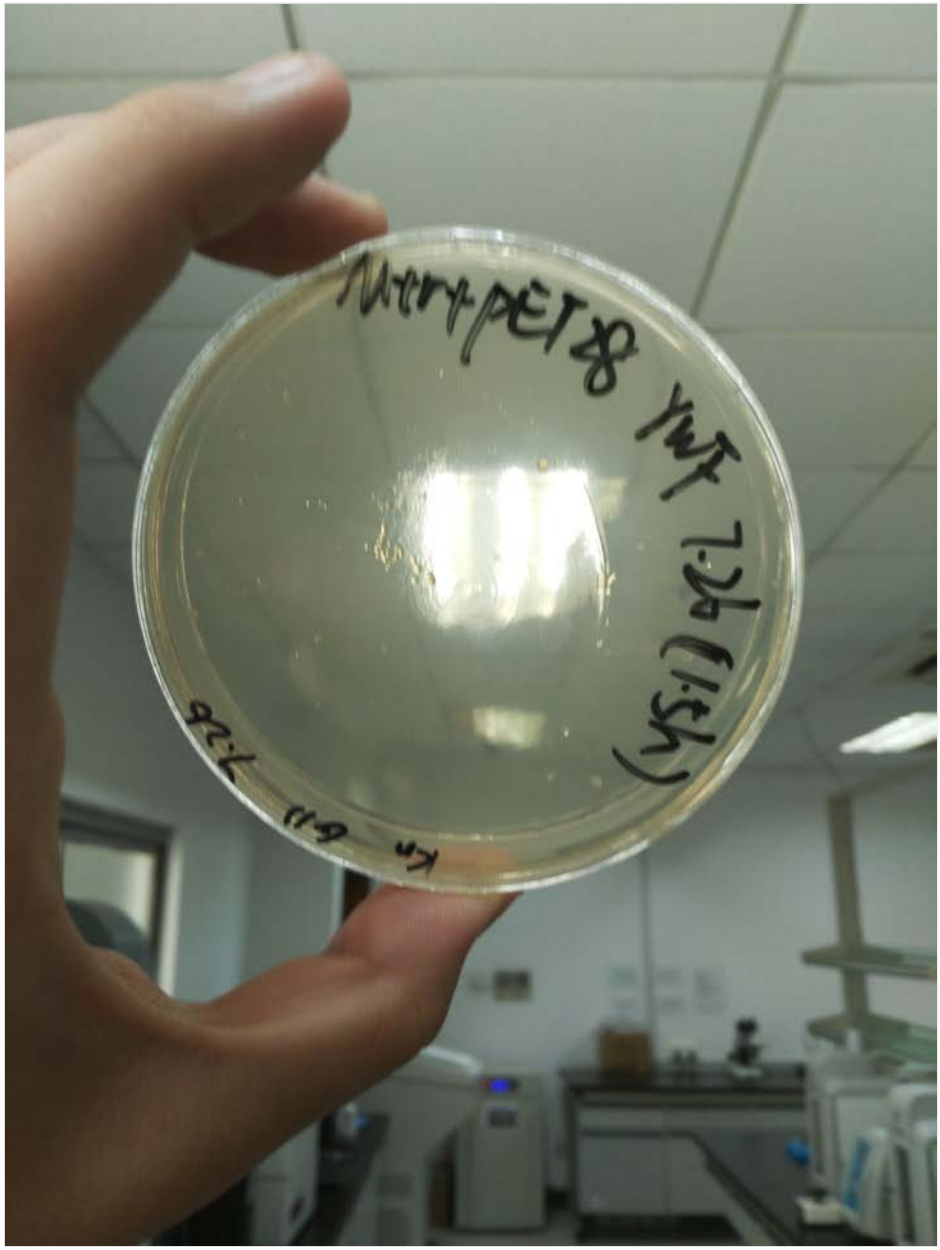
NOTE:Generally, competent bacteria are restrored in -80 degree centrigrade environment. 1.Take the competent bacteria from refrigerator and incubate them into ice about 5 mins until it is dissolved 2.Absorb 1μL PUC19 and mix it wμith bacteria solution thoroughly. ATTENTION: Please operate this step tenderly!!! 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 Amp. 10.Cultivate these bacteria overnight for further use.

Result:



帮助

帮助



Bacteria PCR Recorder: Yonghao Liang

Experimental materials

1. Template: bacteria containing ccm+pUC19 recombined plasmid picked from the plate ;
2. Primer: pUC-f, pUC-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**	**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 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

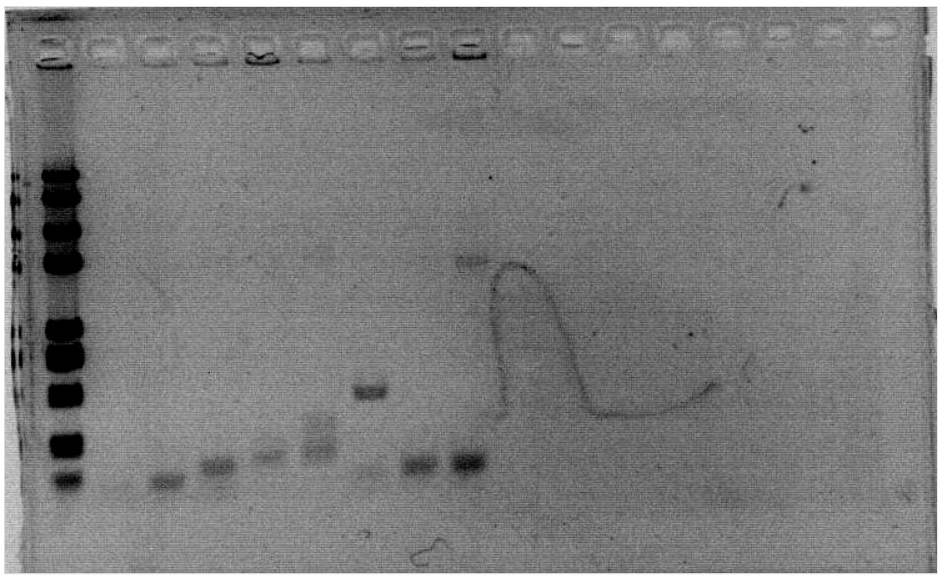
帮助

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

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:



Bacteria PCR Recorder: Yonghao Liang

Experimental materials

1. Template: bacteria containing Red+pBAD recombined plasmid picked from the plate ;
2. Primer: pBAD-f, pBAD-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	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

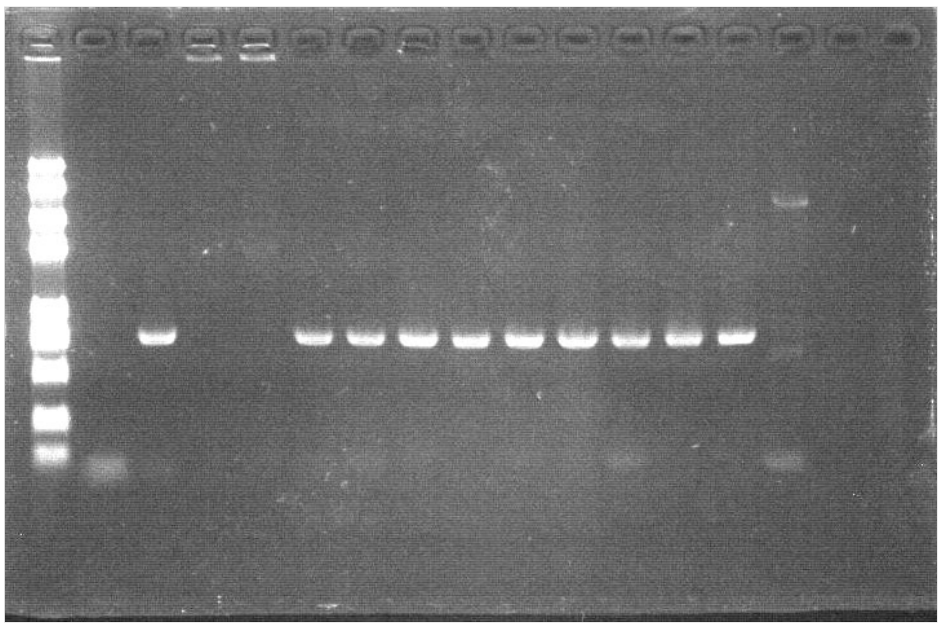
帮助

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

3.PCR reaction 1-9 Parameters setting：

stage	temperature	time
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:



Ligation of pET28 and Mtr Recorder: Wenfei Yu Material:

1. double digestion product of pET28 and Mtr
2. 10× T4 DNA ligase buffer,T4 DNA ligase(bought from Thermo Fisher Scientific)

Procedure: Add to either of samples: 100ng ddpET28 300~500ng ddMtr 2 μL 10* T4 DNA Ligase Buffer 0.4 μL T4 DNA Ligase sterilized water

Mix gently and incubate at 16 degree Celsius for 11 hour.

Date 7.27

Transformation of RED+pBAD 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 Amp. 10.Cultivate these bacteria overnight for further use.

帮助

Transformation of Red+PYYDT into Top10 Recorder: Xiaoyu Zhang, Jingyu Wang
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 Red+PYYDT and mix it with bacteria solution thoroughly. ATTENTION: Please operate this step tenderly!!! 3.Put the tubes on the ice about 30 mins.(Time SHOULD BE ACCURATE) 4.Make a heat shock at 42 degree centigrade about 90 sec(TIME SHOULD BE ACCURATE) 5.Put the tubes on the ice about 5 mins again. 6.Add 900 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.

Result:

帮助

Double digestion of ccm & RED Recorder: Liudong Luo, Zhenyu Jiang Materials:

1. full ccm,RED
2. FastDigest restriction enzyme XbaI, pstI,XhoI,HindIII 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

帮助

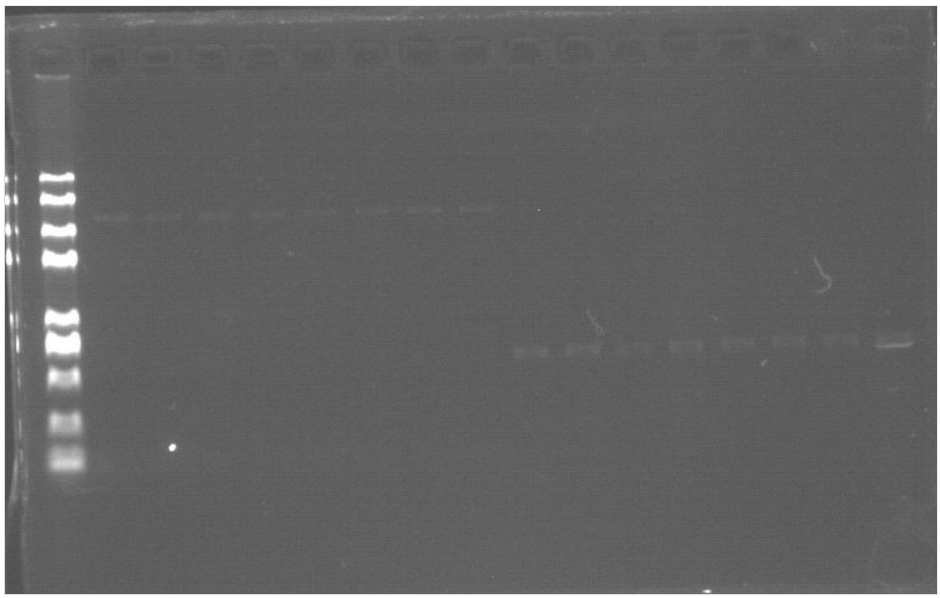
Sample	1	2	3	4	5	6	7	8
nuclease-free water(μL)	25	25	25	25	25	25	25	25
fastdigest green buffer(μL)	2	2	2	2	2	2	2	2
ccm(μL)	1	1	1	1	1	1	1	1
total(μL)	30	30	30	30	30	30	30	30

Reaction system:

Sample	1	2	3	4	5	6	7	8
XhoI(μL)	1	1	1	1	1	1	1	1
HindIII(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
RED(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 .

Agarose gel electrophoresis Result:



(lane 1 to 17: 2K plus II ,ddccm-1,ddccm-2,ddccm-3,ddccm-4,ddccm-5,ddccm-6,ddccm-7,ddccm-8,ddRED-1,ddRED-2,ddRED-3,ddRED-4,ddRED-5,ddRED-6,ddRED-7,ddRED-8)

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
Concentration(ng/ul)	50.7
260/280	1.72
260/230	1.08

sample	RedXHdd 1+2+3+4+5+6+7+8
Concentration(ng/ul)	46.0
260/280	1.64

sample	RedXHdd 1+2+3+4+5+6+7+8
260/230	0.85

Double digestion of puc & PBAD Recorder: Liudong Luo, Zhenyu Jiang Materials:

1. puc,PBAD
2. FastDigest restriction enzyme XbaI, pstI,XhoI,HindIII and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water

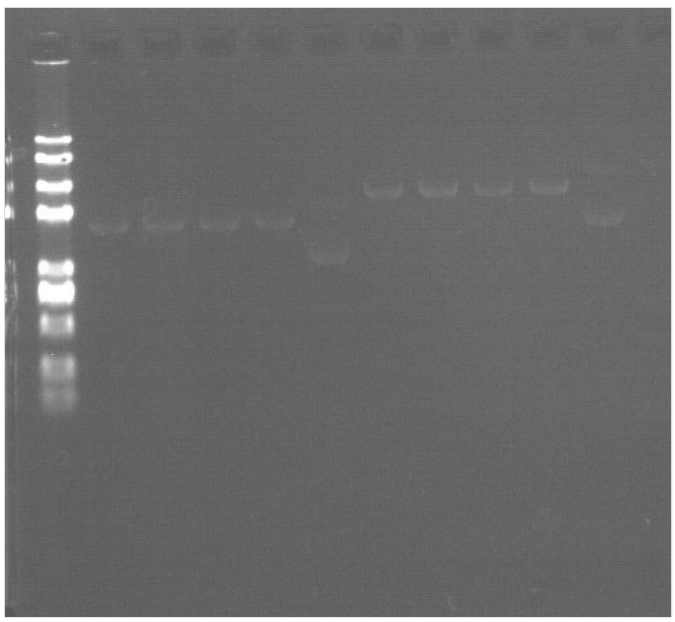
Reaction system:

Sample	1	2	3	4	5	6	7
XbaI(μL)	1	1	1	1	1	1	1
pstI(μL)	1	1	1	1	1	1	1
nuclease-free water(μL)	11	11	11	11	11	11	11
fastdigest green buffer(μL)	2	2	2	2	2	2	2
puc(μL)	5	5	5	5	5	5	5
total(μL)	20	20	20	20	20	20	20

Reaction system:

Sample	1	2	3	4	5	6	7
XhoI(μL)	1	1	1	1	1	1	1
HindIII(μL)	1	1	1	1	1	1	1
nuclease-free water(μL)	11	11	11	11	11	11	11
fastdigest green buffer(μL)	2	2	2	2	2	2	2
RED(μL)	5	5	5	5	5	5	5
total(μL)	20	20	20	20	20	20	20

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



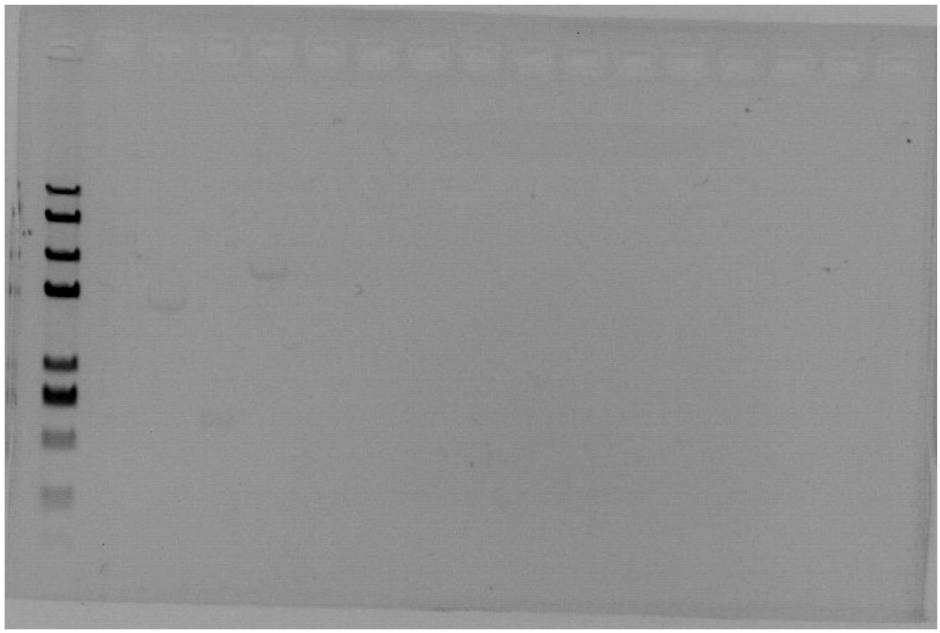
(lane 1 to 9: Marker 2k plus II ,dd-puc-1,dd-puc-2,dd-puc-3,dd-puc-4,puc,dd-PBAD-1,dd-PBAD-2,dd-PBAD-3,dd-PBAD-4,PBAD)

Agarose gel electrophoresis Result:

sample	pucXPdd 1+2+3+4+5+6+7+8
Concentration(ng/ul)	115.0
260/280	1.70
260/230	1.17

sample	PBADXHdd 1+2+3+4+5+6+7+8
Concentration(ng/ul)	60.6
260/280	1.76
260/230	1.43

Agarose gel electrophoresis and purification Result:



(lane 1 to 7: Marker 2k plus II,pur-dd-ccm,pur-dd-RED,pur-dd-puc,pur-dd-PBAD)

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
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

stage	temperature	time
step 3	59	5 s
step 4	72	7 min
step 5	72	10 min
step 6	4	--

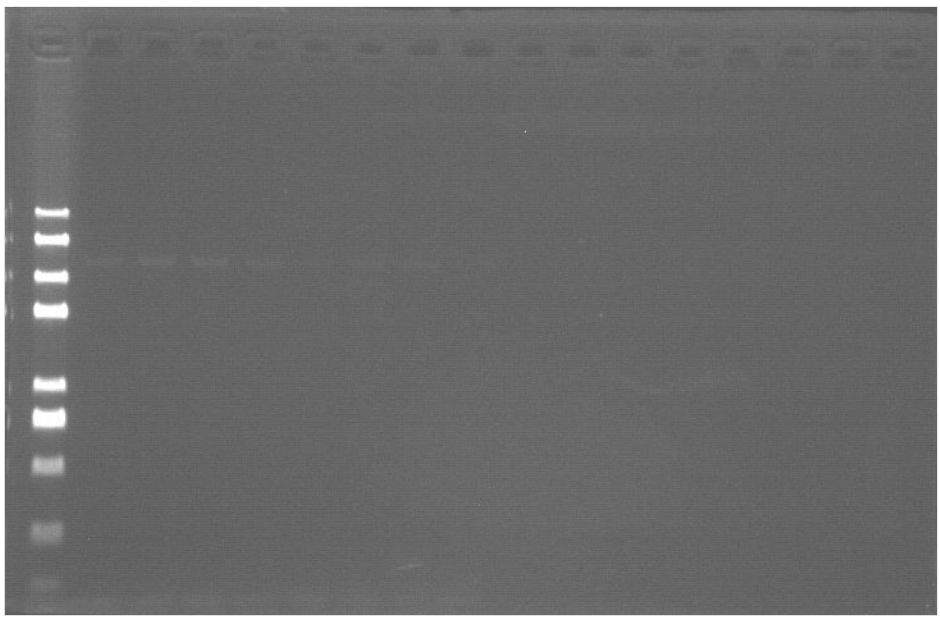
30 cycles(step 2 ~ step 4)

5,6,7,8 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:

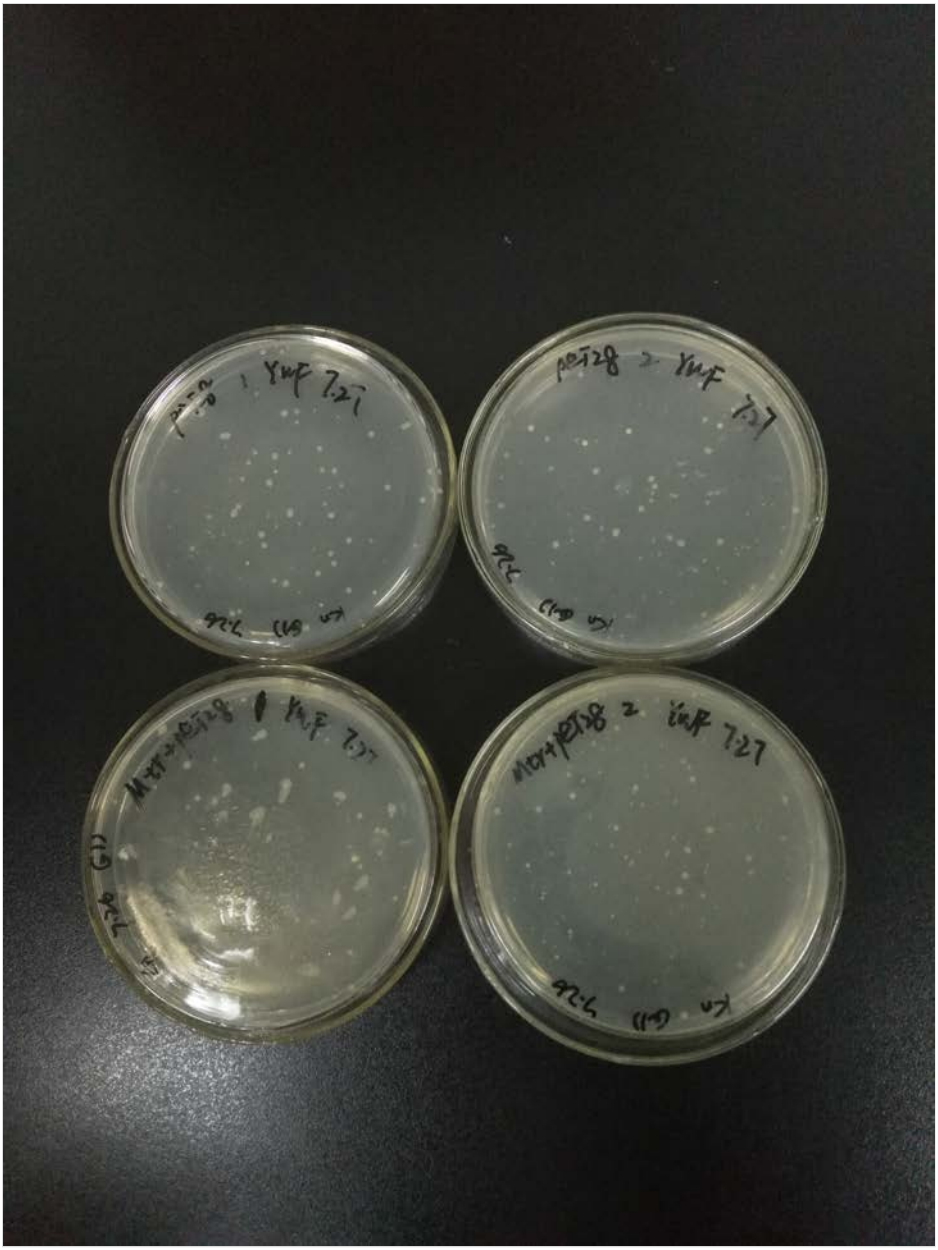


(lane left to right: marker 2K Plus, ccm-pcr-1, ccm-pcr-2,ccm-pcr-3,ccm-pcr-4,ccm-pcr-5, ccm-pcr-6, ccm-pcr-7,ccm-pcr-8)

Transformation of Mtr into pET28 Recorder: Wenfei Yu

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 10μL Mtr+pET28 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.

Result:



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)	180.2
260/280	1.83
260/230	1.96

Bacteria PCR Recorder: Meiying Cui

Experimental materials

1. Template: bacteria picked from the plate ;
2. Primer: pUC-f, pUC-r. Designed by ourselves, synthesized by Sangon Biotech;
3. Sterilized ddH2O;
4. 2X Taq DNA polymerase.

Procedure:

帮助

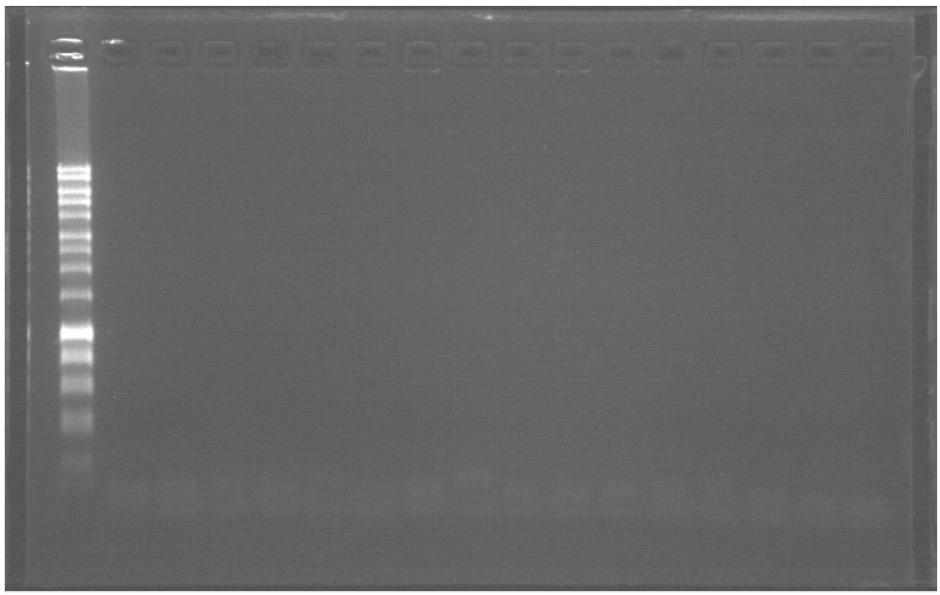
1.Prepare 16 PCR tubes and sequentially add：

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 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	1 μL
pUC-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
pUC-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

3.PCR reaction 1-9 Parameters setting：

stage	temperature	time
step 1	94	10 min
step 2	94	30 s
step 3	56	30 s
step 4	72	5 min 30s
step 5	72	10 min
step 6	4	--

25 cycles(step 2 ~ step 4) Result:



Bacteria PCR Recorder: Shihan Zhu, Chenyang Li

Experimental materials

1. Template: bacteria picked from the plate ;
2. Primer: pUC-f, pUC-r. Designed by ourselves, synthesized by Sangon Biotech;
3. Sterilized ddH2O;
4. 2X Taq DNA polymerase.

Procedure:

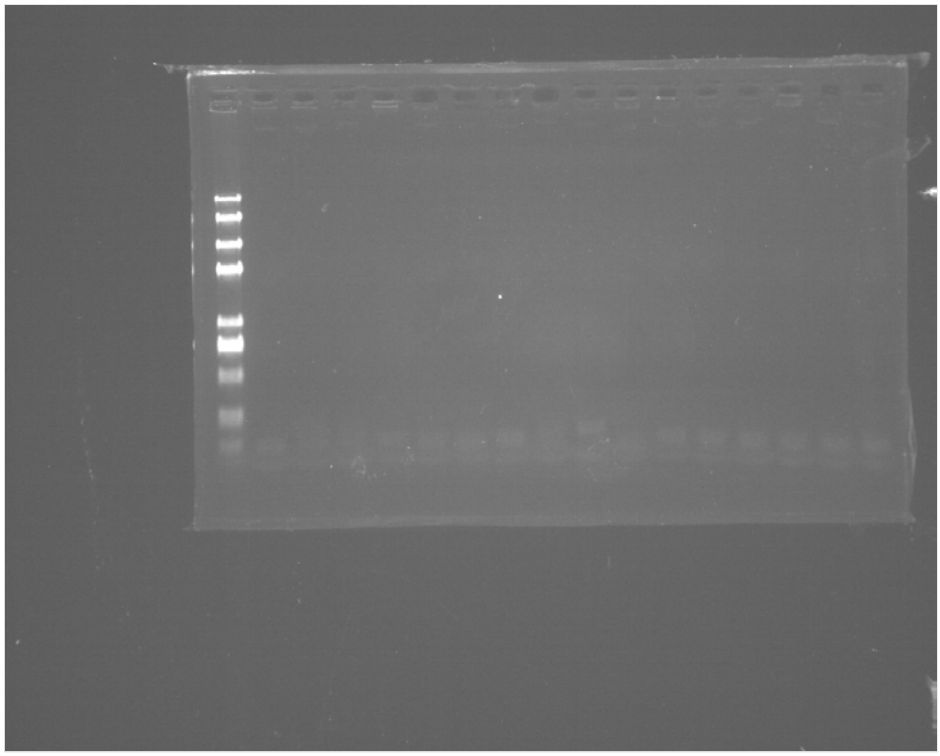
1.Prepare 16 PCR tubes and sequentially add：

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

3.PCR reaction 1-9 Parameters setting：

stage	temperature	time
step 1	94	10 min
step 2	94	30 s
step 3	56	30 s
step 4	72	5 min 30 s
step 5	72	10 min
step 6	4	--

25 cycles(step 2 ~ step 4) Result:



Plasmid Extraction of the PBar *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, 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	**PBar-9**	**PBar-5**	**PBar-7**	**PBar-6**	**PBar-8**	**PBar-11**	**PBar-13**	**PBar-10**	**PBar-2**	**PBar-12**
Concentration(ng/ul)	93.9	59.4	92.8	101.3	92.1	79.2	103.0	140.4	45.6	139.3
260/280	1.89	1.96	1.91	1.91	1.89	1.92	1.88	1.76	1.94	1.70
260/230	2.17	2.10	2.26	2.06	2.11	2.13	1.85	1.30	1.61	0.96

Double digestion of pBAR Recorder: Yonghao Liang Materials:

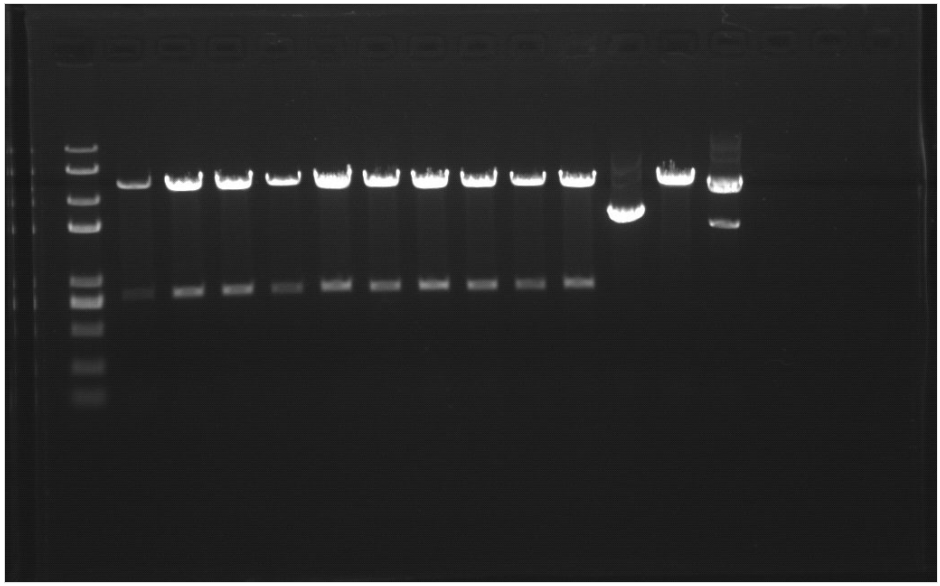
1. pBAR
2. FastDigest restriction enzyme XhoI,HindIII and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water

Reaction system:

Sample	**2**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**con-pBAD**
XhoI(μL)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
HindIII(μL)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
fastdigest green buffer(μL)	2	2	2	2	2	2	2	2	2	2	2
pBAR(μL)	17	10	10	5	10	10	10	10	8	12	10
nuclease-free water(μL)	0	7	7	12	7	7	7	7	9	5	7
total(μL)	20	20	20	20	20	20	20	20	20	20	20

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

Agarose gel electrophoresis Result:



PS: The first ten lanes are pBAR, and the last two is control group.

Plasmid Extraction of the pBAR Recorder: Yonghao Liang 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	pBAR-2	pBAR-5	pBAR-6	pBAR-7	pBAR-8	pBAR-9	pBAR-10	pBAR-11	pBAR-12	pBAR-13
Concentration(ng/ul)	30.8	90.3	92.9	22.93	86.3	100.1	90.9	95.0	120.6	70.1
260/280	1.85	1.81	1.82	1.64	1.84	1.73	1.83	1.77	1.67	1.82
260/230	1.39	1.74	1.84	0.83	2.15	1.35	1.94	1.53	0.94	1.76

Transformation of plasmid PYRED into Top10 Recorder: Xiaoyu Zhang 1.Take the competent bacteria from -80 degree centigrade refrigerator and incubate them into ice about 5 mins until it is dissolved 2.Absorb 10uL plasmid(normally 1 to 2 uL, DO NOT add more than 5% volumn of bacteria solution) and mix it with bacteria solution thoroughly. ATTENTION: Please operate this step tenderly!!! 3.Put the tubes on the ice about 30 mins.(Time SHOULD BE ACCURATE) 4.Make a heat shock at 42 degree centigrade about 90 sec(TIME SHOULD BE ACCURATE) 5.Put the tubes on the ice about 5 mins again. 6.Add 900 ul LB medium into EP tubes and cultivate the bacteria at 37 degree centrigrade about 60 min. 7.Centrifuge them at 4000 rpm for 2 minutes and we will see sediment in the tubes. 8.Discard the supernatant liquid and leave about 200 ul medium. 9.Coat plate: Add 200 ul solution in a plate with kanamycin. 10.Cultivate these bacteria overnight for further use.

Awaken of the Glycerin bacteria Top10 containing RED on the plasmid pET21 Recorder: Xiaoyu Zhang Add 200 μL of bacteria into a 5 mL LB culture and cultivate these bacteria overnight for extraction at 37 degree centigrade, 250 rpm.

Date 7.28

Transformation of RED+pBAD and ccmA-H+PUC19 int5o 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 Amp. 10.Cultivate these bacteria overnight for further use.

Plasmid Extraction of the pBAR Recorder: 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	**pBAR-2**	**pBAR-5**	**pBAR-6**	**pBAR-7**	**pBAR-8**	**pBAR-9**	**pBAR-10**	**pBAR-11**	**pBAR-12**	**pBAR-13**
Concentration(ng/ul)	68.6	131.3	112.8	93.8	156.5	98.7	100.9	83.2	83.8	79.8
260/280	1.66	1.70	1.79	1.86	1.70	1.74	1.75	1.87	1.76	1.82
260/230	0.95	1.03	1.67	2.24	1.90	1.30	1.31	2.21	1.52	1.80

Double digestion of ccm Recorder: Liudong Luo, Zhenyu Jiang, Menglong Jin 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

Sample	9	10	11	12	13	14	15	16
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
Xbal(μL)	1	1	1	1	1	1	1	1
pstl(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
Xbal(μL)	1	1	1	1	1	1	1	1
pstl(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)	76.5
260/280	1.80
260/230	1.71

sample	ccmXPdd 17+18+19+20+21+22+23+24+25+26+27+28+29+30+31+32
Concentration(ng/ul)	87.1
260/280	1.84
260/230	1.93

Double digestion of puc & PTB Recorder: Liudong Luo, Zhenyu Jiang, Menglong Jin
Materials:

1. puc,PTB
2. FastDigest restriction enzyme Xbal, pstl and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)

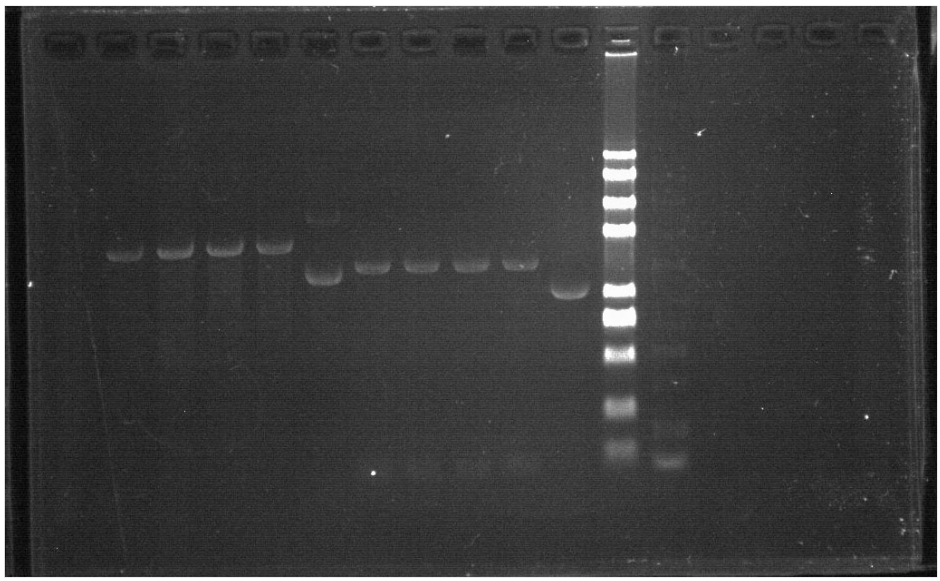
3. Nuclease-free water

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 .

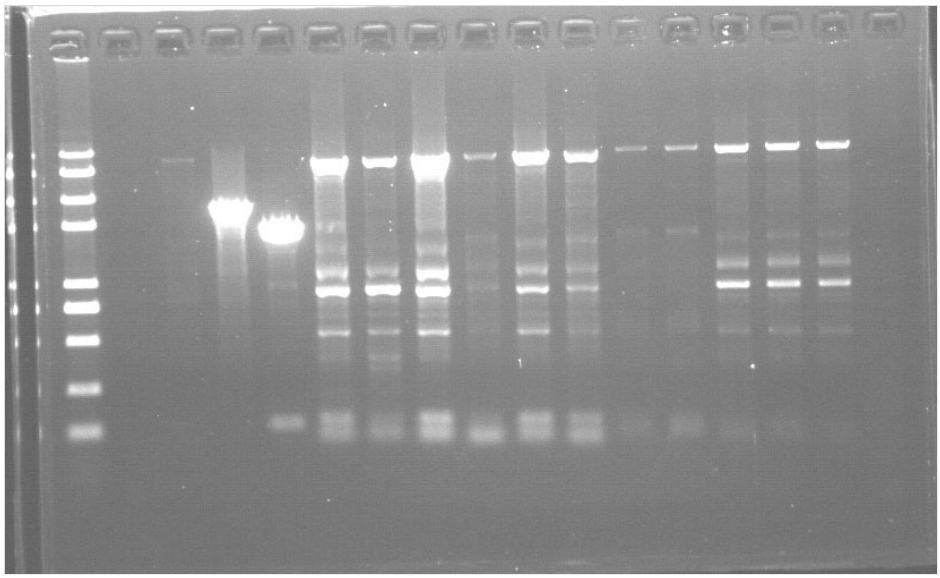


(lane 1 to 9: dd-puc-1,dd-puc-2,dd-puc-3,dd-puc-4,puc,dd-PTB-1,dd-PTB-2,dd-PTB-3,dd-PTB-4,PTB,Marker 2k plus II)

sample	**pucXPdd 1+2+3+4**
Concentration(ng/ul)	71.0
260/280	1.73
260/230	1.17

sample	**pTBXPdd 1+2+3+4**
Concentration(ng/ul)	76.3
260/280	1.79
260/230	1.73

Agarose gel electrophoresis and purification Result:



(lane 1 to 7: Marker 2k plus II,pur-dd-ccm-1,pur-dd-ccm-2,pur-dd-puc,pur-dd-PTB,pcr-ccm-1,pcr-ccm-2,pcr-ccm-3,pcr-ccm-4,pcr-ccm-5,pcr-ccm-6,pcr-ccm-7,pcr-ccm-8,pcr-ccm-9,,pcr-ccm-10,pcr-ccm-11)

PCR of full Ccm Recorder: Liudong Luo, Zhenyu Jiang,Menglong Jin

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**
Sterilized ddH2O	22 μL	22 μL	22 μL	22 μL	22 μL	22 μL	22 μL	22 μL
2×PrimeStar(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) 3.Agarose gel electrophoresis Result:

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

Colony PCR of pET28 containing Mtr Recorder: Wenfei Yu

Experimental materials

1. Template: E.coli Top 10 with pET28 plasmid containing Mtr;
2. Primer: T7,T7-TER. Designed by ourselves, synthesized by Sangon Biotech;
3. Sterilized ddH2O, 2×Taq PCR master mix.

Procedure:

1.Prepare 16 PCR tubes and sequentially add：

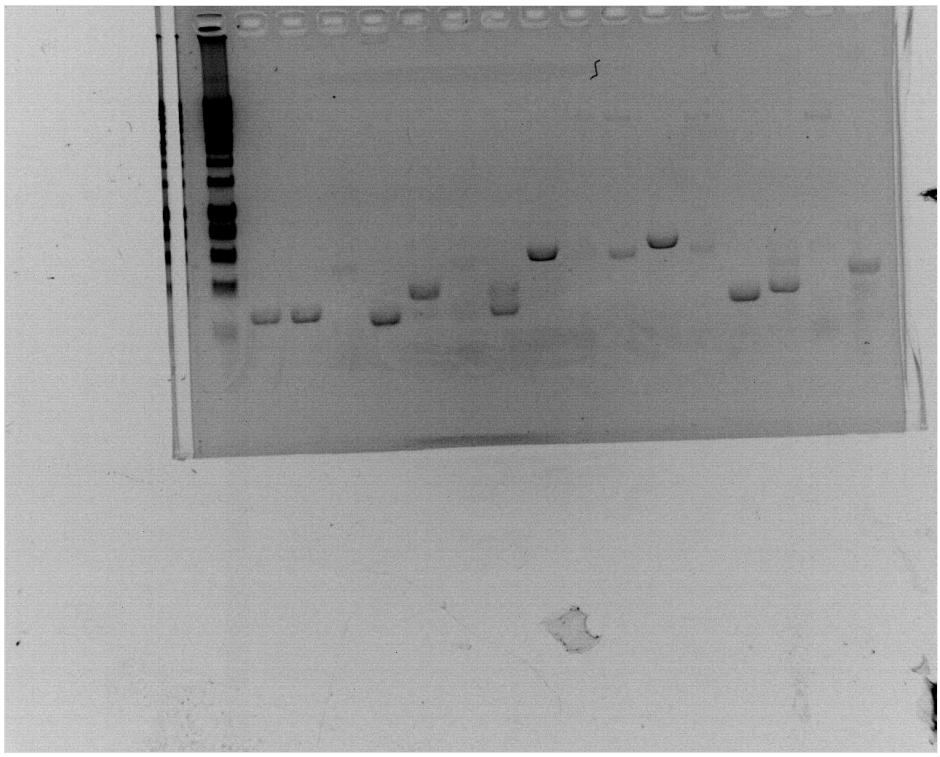
sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Sterilized ddH2O	7 μL	7 μL	7 μL	7μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7μL	7 μL	7 μL	7 μL	7 μL
2×Taq PCR master mix	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 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
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

2.PCR reaction 1-16 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	--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:



(From left to right: marker, sample1-16)

Bacteria PCR Recorder: Yawei Wu

Experimental materials

1. Template: bacteria containing Red+PYYDT recombined plasmid picked from the plate ;
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：

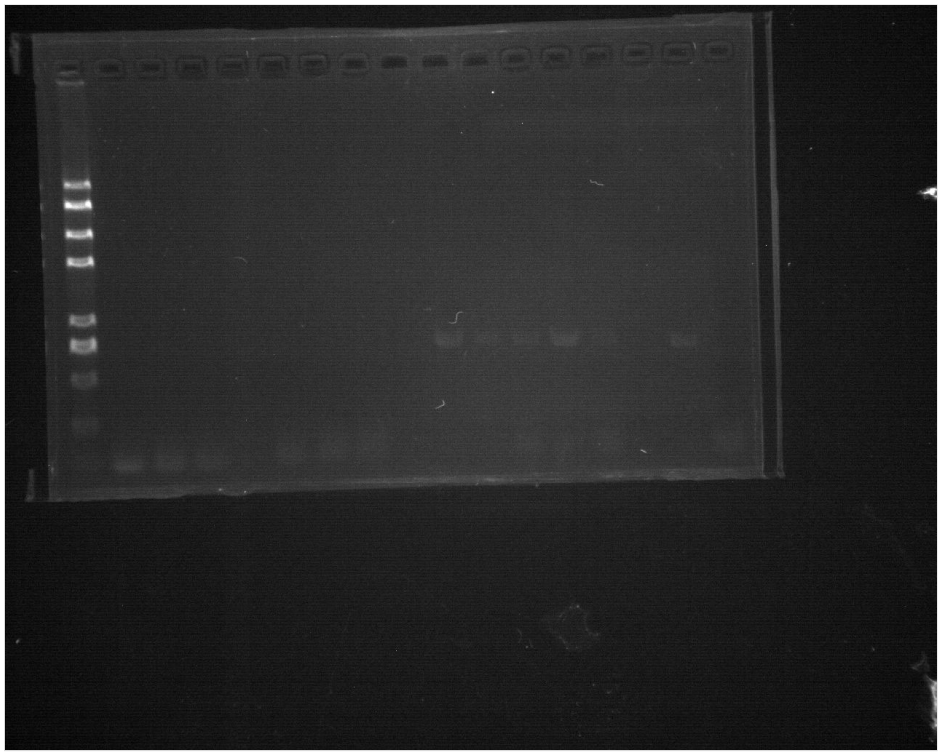
sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Sterilized ddH2O	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL
2X Taq DNA polymerase	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	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	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

3.PCR reaction 1-9 Parameters setting：

stage	**temperature**	**time**

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) Result:



(lane left to right: Marker, PYDDT+Red1-16)

Pick PYYDT+Red9, PYYDT+Red12, PYYDT+Red15, rename them as PYRed1-3 and cultivate these bacteria in medium with Kana for further use.

Date 7.29

Transformation of ccmA-H+PUC19 and ccmA-H+pTB into Top10 Recorder: Menglong Jin

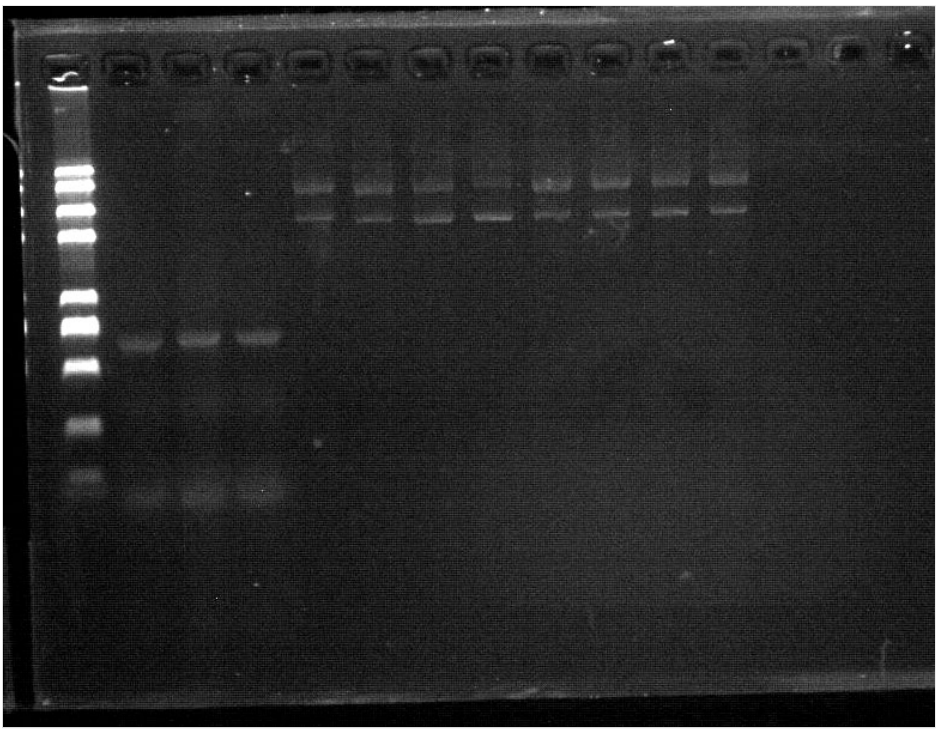
NOTE:Generally, competent bacteria are restrored in -80 degree centrigrade environment. 1.Take the competent bacteria from refrigerator and incubate them into ice about 5 mins until it is dissolved 2.Absorb 1μL PUC19 and mix it wμith bacteria solution thoroughly. ATTENTION: Please operate this step tenderly!!! 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 Amp(ccm+pTB); add 200 μL solution in a plate with Amp, IPTG and X-Gal(ccm+pTB). 10.Cultivate these bacteria overnight for further use.

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)	65.1	67.0	176.8	175.4	189.8	161.5	255.5	240.9
260/280	1.86	1.86	1.67	1.67	1.64	1.65	1.61	1.64
260/230	2.19	2.16	0.85	0.84	0.80	0.71	0.72	0.76
sample	RED-1	RED-2	RED-3	RED-4	RED-5	RED-6	RED-7	RED-8

We did agarose gel electrophoresis then:



(From left to right: line1: marker 2K plusII; line 2-4: pcr of RED; line 5-12: sample RED1-8.) As you can see, all of the eight samples contain the plasmid we need, but sample 3-8 contain more impurities. We will do better next time.

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
Sterilized ddH2O	22 μL	22 μL	22 μL	22 μL	22 μL	22 μL	22 μL	22 μL
2×PrimeStar(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

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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	--

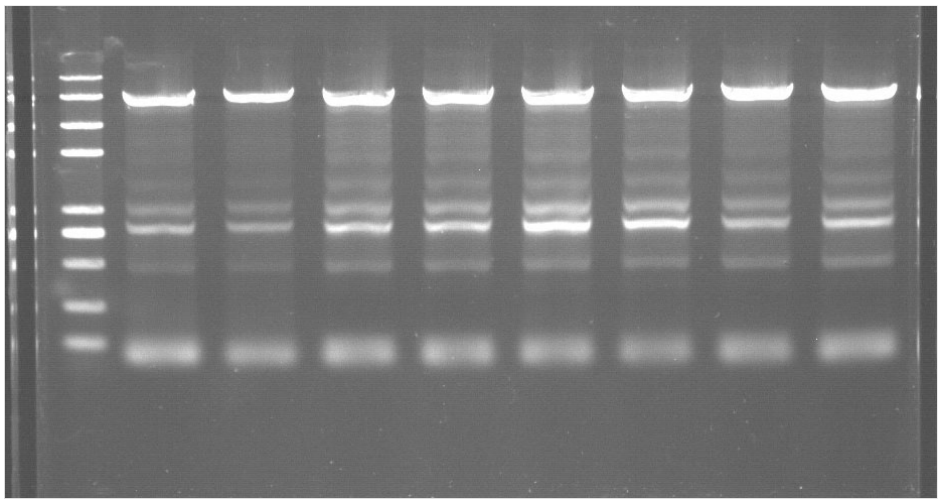
30 cycles(step 2 ~ step 4)

5,6,7,8 Parameters setting：

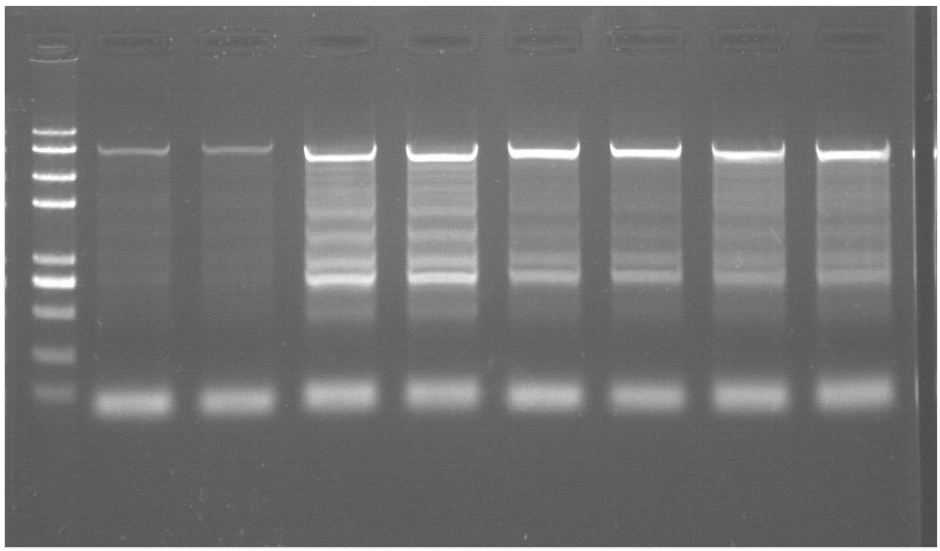
stage	temperature	time
step 1	95	10 min
step 2	98	10 s
step 3	55	15 s
step 4	72	7 min
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:



(lane left to right: marker 2K Plus, ccm-pcr-1-1, ccm-pcr-1-2,ccm-pcr-2-1,ccm-pcr-2-2,ccm-pcr-3-1, ccm-pcr-3-2, ccm-pcr-4-1,ccm-pcr-4-2)



(lane left to right: marker 2K Plus, ccm-pcr-5-1, ccm-pcr-5-2,ccm-pcr-6-1,ccm-pcr-6-2,ccm-pcr-7-1, ccm-pcr-7-2, ccm-pcr-8-1,ccm-pcr-8-2)

4.Gel Extraction

The results are as following:

sample	ccmXPdd 1+2+3+4+5+6+7+8
Concentration(ng/ul)	22.9
260/280	1.82
260/230	1.57

Double digestion of ccm Recorder: Liudong Luo, Zhenyu Jiang, Menglong Jin 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:

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

Sample	25	26	27	28	29	30	31	32
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)	64.4
260/280	1.74
260/230	1.21

sample	ccmXPdd 17+18+19+20+21+22+23+24+25+26+27+28+29+30+31+32
Concentration(ng/ul)	87.5
260/280	1.74
260/230	1.38

Double digestion of puc & PTB Recorder: Liudong Luo, Zhenyu Jiang, Menglong Jin
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

Sample	1	2	3	4
pTB(μL)	6	6	6	6
total(μL)	20	20	20	20

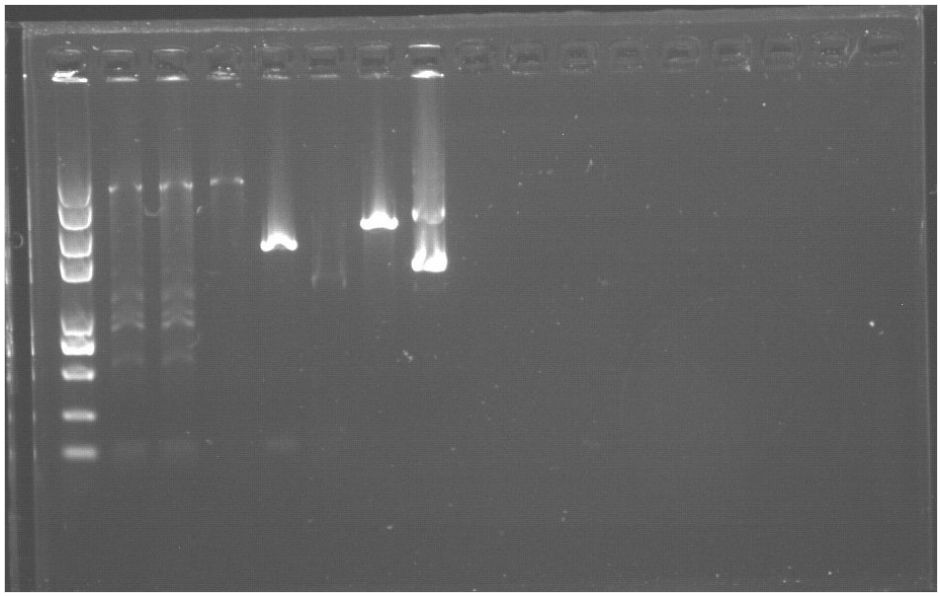
帮助

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

sample	pucXPdd 1+2+3+4
Concentration(ng/ul)	80.9
260/280	1.84
260/230	2.31

sample	pTBXPdd 1+2+3+4
Concentration(ng/ul)	143.7
260/280	1.61
260/230	0.52

Agarose gel electrophoresis and purification Result:



(lane 1 to 7: Marker 2k plus II,pur-dd-ccm-1,pur-dd-ccm-2,ccm,pur-dd-PTB,pTB,pur-dd-puc,puc)

Double digestion of PYRed Recorder: Yawei Wu, Xiaoyu Zhang Materials:

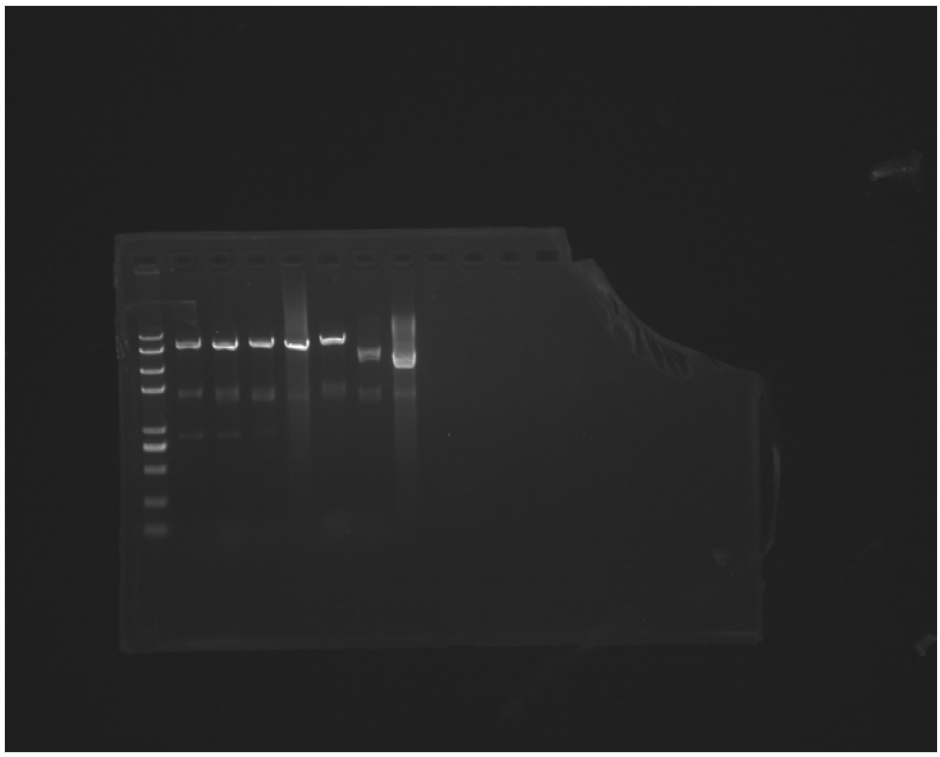
1. PYRed, PYYDT
2. FastDigest restriction enzyme XhoI, NdeI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water

Reaction system:

Sample	1	2	3	4	5
XhoI(μL)	1	1	1	1	1
NdeI(μL)	1	1	1	0	1
fastdigest green buffer(μL)	2	2	2	2	2
Nuclease-free water(μL)	0	0	0	0	7.3
plasmid(μL)	16	16	16	17	8.7
total(μL)	20	20	20	20	

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

Result:



(lane left to right: Marker, ddPYRed1-3, ddPYYDT, sdPYRed, PYRed, PYYDT)

Construction of PYRed succeeded!

Plasmid Extraction of the pBAR Recorder: Zhenyu Jiang, 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	pBAR-2	pBAR-5	pBAR-6	pBAR-7	pBAR-8	pBAR-9	pBAR-10	pBAR-11	pBAR-12	pBAR-13
Concentration(ng/ul)	43.5	88.1	83.8	118.6	100.7	58.8	94.3	82.7	98.5	74.9
260/280	1.93	1.80	1.84	1.79	1.86	1.88	1.88	1.82	1.83	1.80
260/230	1.62	1.59	1.76	1.60	1.98	1.90	1.72	1.64	1.65	1.48
sample	**pBAR-2**	**pBAR-5**	**pBAR-6**	**pBAR-7**	**pBAR-8**	**pBAR-9**	**pBAR-10**	**pBAR-11**	**pBAR-12**	**pBAR-13**

Date 7.30

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) **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.

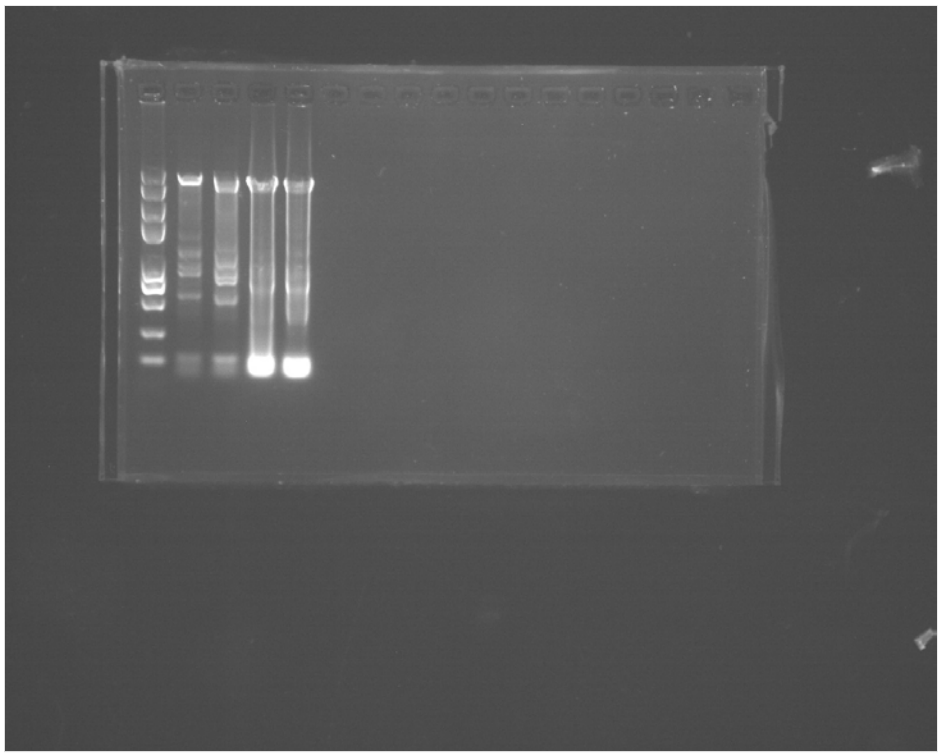
OD measurement result:

sample	ccm PCR pur 1+2+3+4+5+6+7+8
Concentration(ng/ul)	531.8
260/280	1.88
260/230	2.17

sample	ccm PCR pur 9+10+11+12+13+14+15+16
Concentration(ng/ul)	365.2
260/280	1.85
260/230	2.17

帮助

Agarose gel electrophoresis Result:



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

Plasmid Extraction of PYRED Recorder: 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	RED-1	RED-2	RED-3
Concentration(ng/ul)	24.2	26.2	22.1
260/280	1.97	1.95	1.86
260/230	3.50	3.26	2.15

Double digestion of ccm Recorder: Liudong Luo, Zhenyu Jiang, Menglong Jin 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

帮助

Sample	1	2	3	4	5	6	7	8
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
Xbal(μL)	1	1	1	1	1	1	1	1
pstl(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
Xbal(μL)	1	1	1	1	1	1	1	1
pstl(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
Xbal(μL)	1	1	1	1	1	1	1	1
pstl(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:

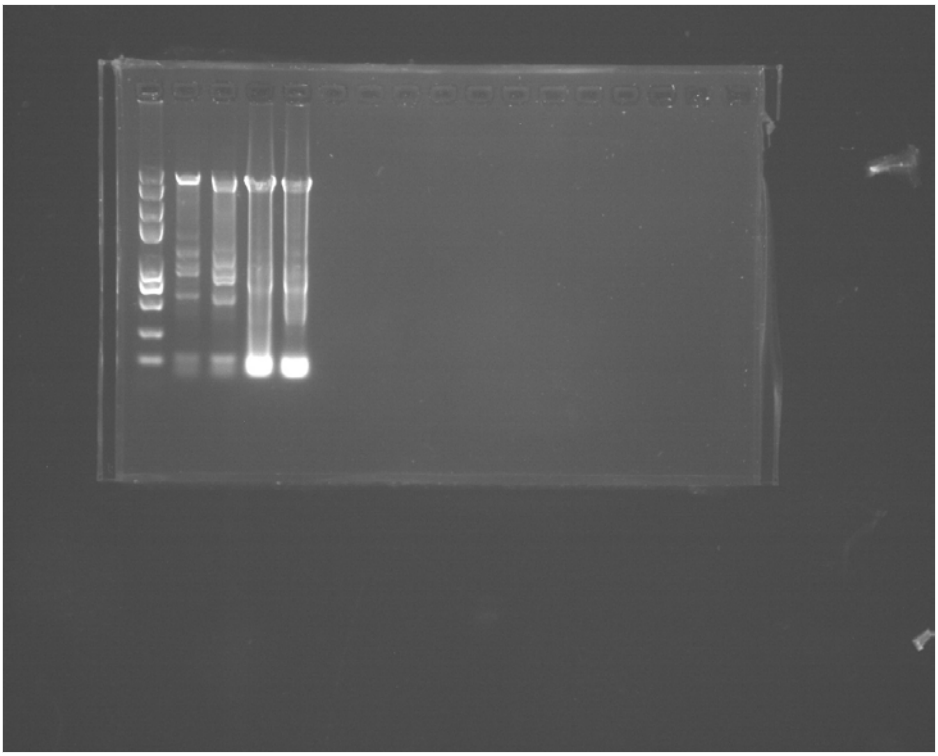
sample	ccmXPdd 1+2+3+4+5+6+7+8+9+10+11+12+13+14+15+16

帮助

sample	ccmXPdd 1+2+3+4+5+6+7+8+9+10+11+12+13+14+15+16
Concentration(ng/ul)	64.5
260/280	1.80
260/230	1.59

sample	ccmXPdd 17+18+19+20+21+22+23+24+25+26+27+28+29 +30+31+32
Concentration(ng/ul)	55.3
260/280	1.85
260/230	1.63

Agarose gel electrophoresis Result:



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

Bacteria PCR Recorder: Meiying Cui，Chenyang Li

Experimental materials

1. Template: bacteria picked from the plate ;
2. Primer: , T7,T7-TEER,Mtr-res-f,Mtr-res-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	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

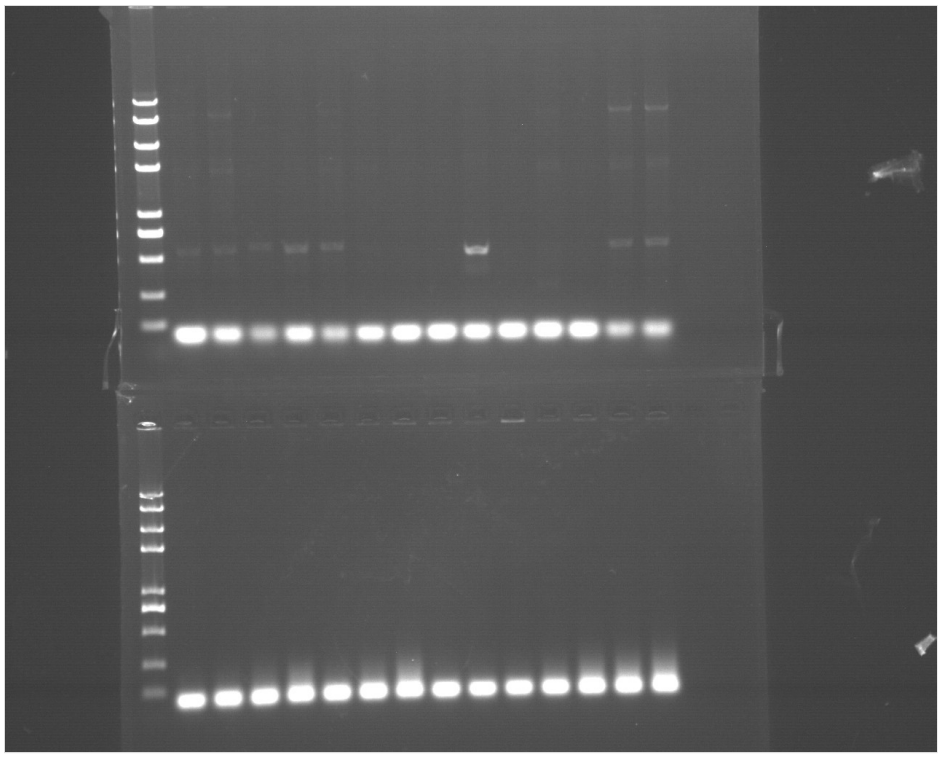
sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
2X Taq DNA polymerase	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL
template	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
T7	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	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

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 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	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	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	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:



Bacteria PCR Recorder: Meiying Cui

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 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	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	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	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

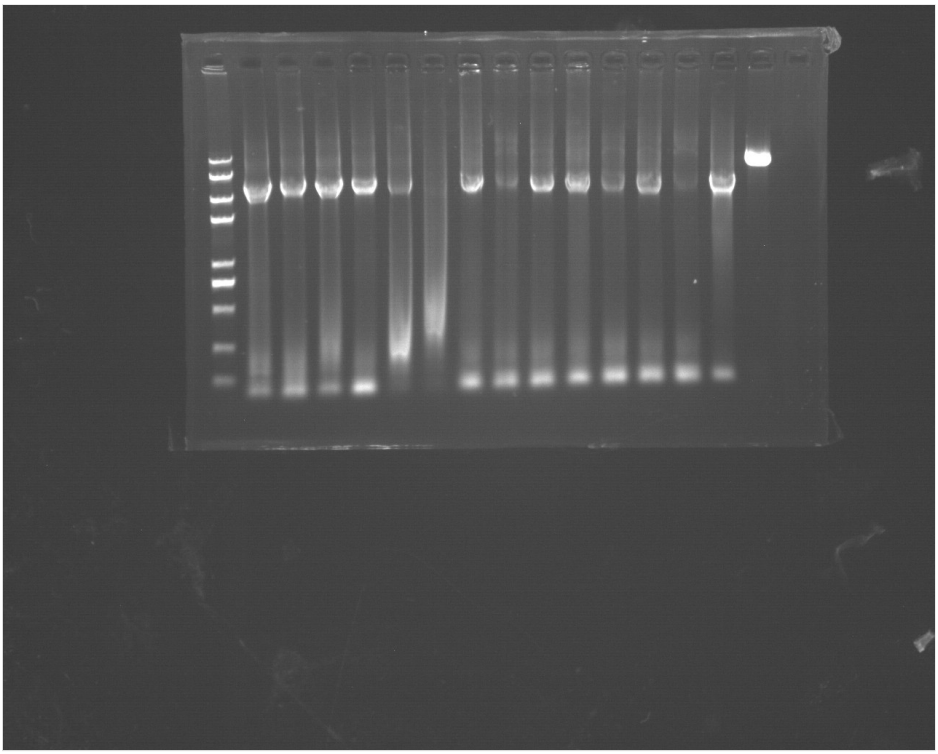
PCR reaction 1-9 Parameters setting：

stage	**temperature**	**time**
step 1	94	4 min

帮助

stage	temperature	time
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:

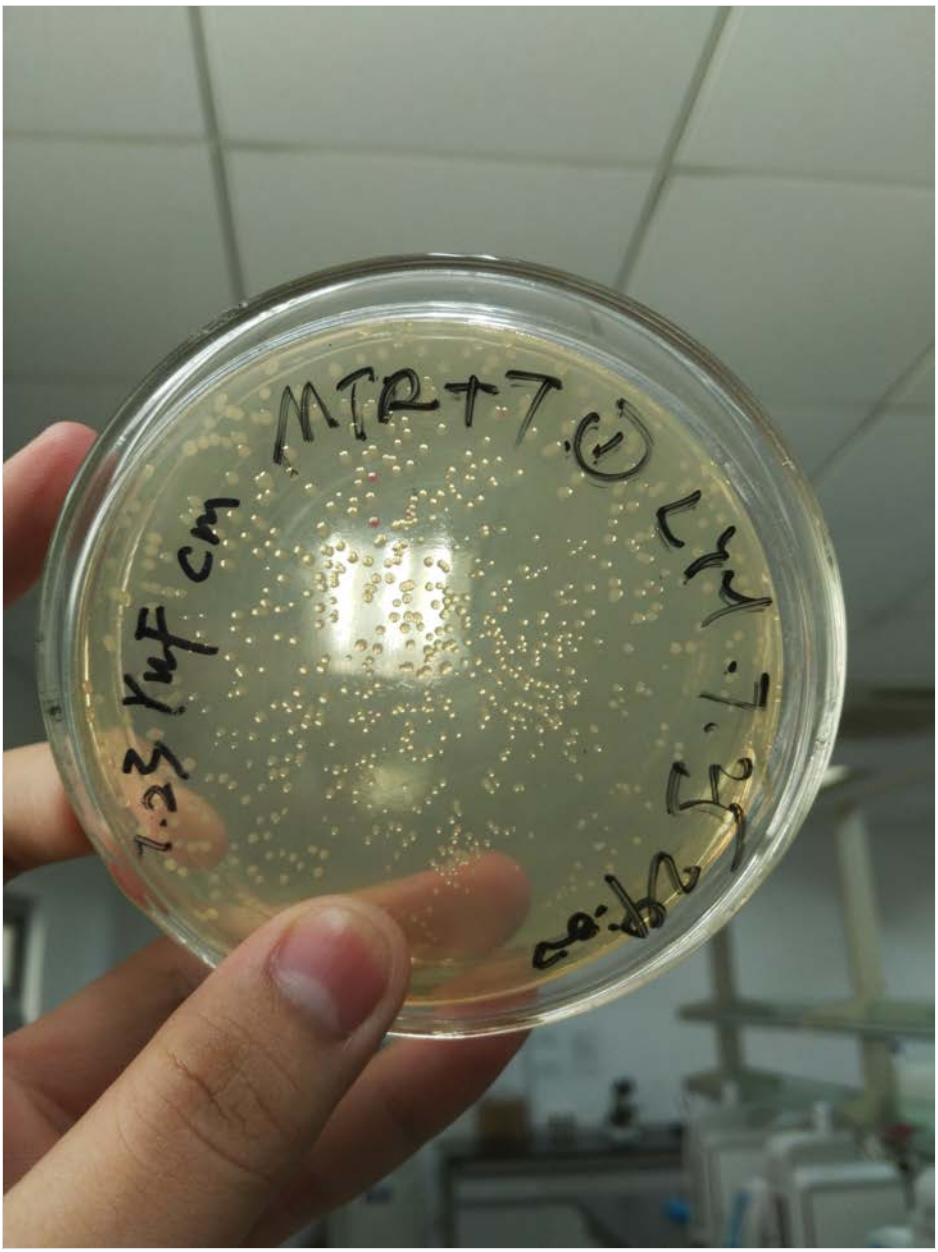


Date 7.26 Transformation of Mtr+T vector Recorder: Wenfei Yu, Yonghao Liang, Shihan Zhu, Meiying Cui

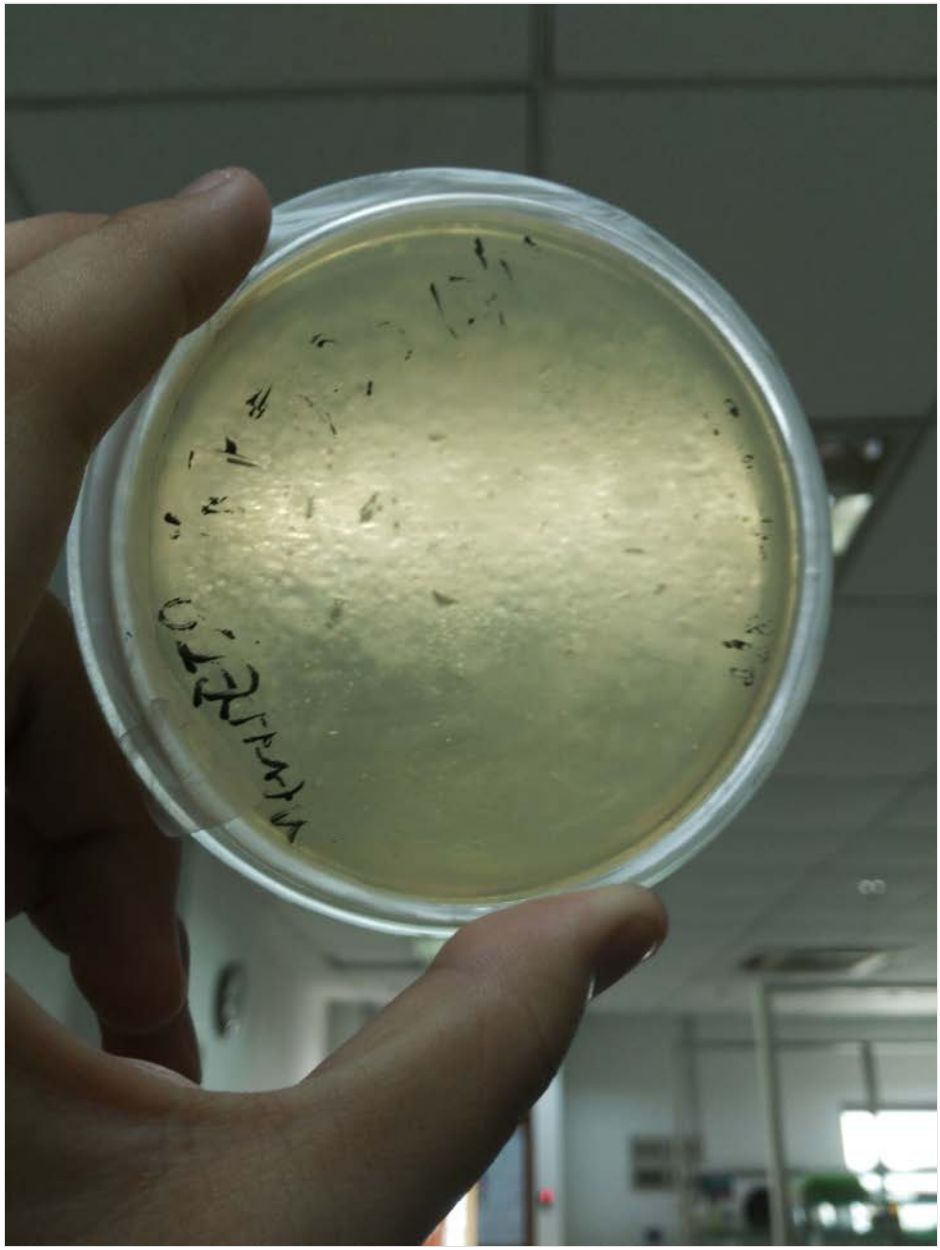
NOTE:Generally, competent bacteria are restrored in -80 degree centrigrade environment. 1.Take the competent bacteria from refrigerator and incubate them into ice about 5 mins until it is dissolved 2.Absorb 1μL PUC19 and mix it wμith bacteria solution thoroughly. ATTENTION: Please operate this step tenderly!!! 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 Amp. 10.Cultivate these bacteria overnight for further use.

帮助

Result:



帮助



Double digestion of pET28 Recorder: Shihan Zhu Materials:

1. pET28
2. FastDigest restriction enzyme XhoI, NdeI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific) and Fast AP.
3. Nuclease-free water

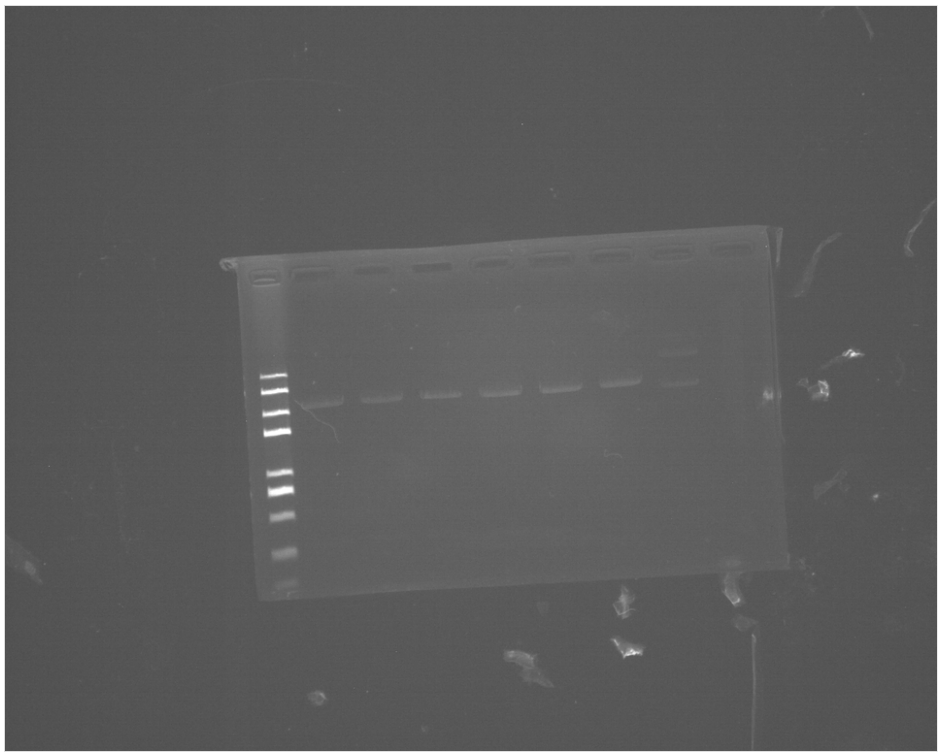
Reaction system:

帮助

Sample	1	2	3	4	5	6
NdeI(μL)	1	1	1	1	1	1
XhoI(μL)	1	1	1	1	1	1
nuclease-free water(μL)	10	10	10	10	10	10
fastdigest green buffer(μL)	2	2	2	2	2	2
pET28(μL)	5	5	5	5	5	5
Fast AP(μL)	1	1	1	1	1	1
total(μL)	20	20	20	20	20	20

Mix gently and incubate at 37 degree Celsius for 45 hours .

Agarose gel electrophoresis Result:



(lane 1 to 9: 2K plus II,dd-pET28-1,dd-pET28-2,dd-pET28-3,dd-pET28-4,dd-pET28-5,dd-pET28-6,pET28)

Then we did the purification of the fragments we got.

Double digestion of ccm & RED Recorder: Liudong Luo, Zhenyu Jiang Materials:

1. full ccm,RED
2. FastDigest restriction enzyme XbaI, pstI,XhoI,HindIII 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

Sample	1	2	3	4	5	6	7	8
ccm(μL)	1	1	1	1	1	1	1	1
total(μL)	30	30	30	30	30	30	30	30

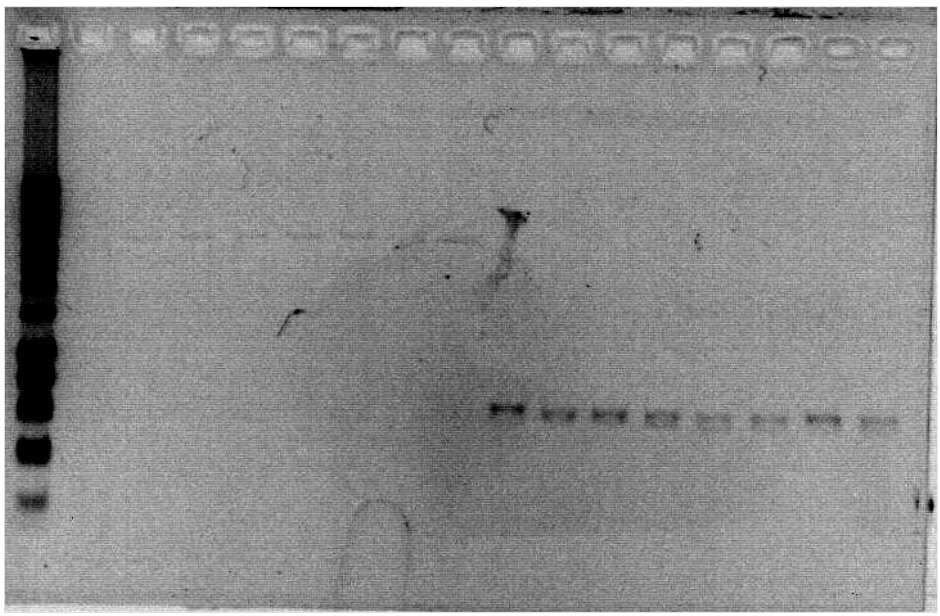
帮助

Reaction system:

Sample	1	2	3	4	5	6	7	8
XhoI(μL)	1	1	1	1	1	1	1	1
HindIII(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
RED(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 .

Agarose gel electrophoresis Result:



(lane 1 to 17: 2K plus ,ddccm-1,ddccm-2,ddccm-3,ddccm-4,ddccm-5,ddccm-6,ddccm-7,ddccm-8,ddRED-1,ddRED-2,ddRED-3,ddRED-4,ddRED-5,ddRED-6,ddRED-7,ddRED-8)

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
Concentration(ng/ul)	22.7
260/280	1.72
260/230	1.08

sample	RedXHdd 1+2+3+4+5+6+7+8
Concentration(ng/ul)	26.6
260/280	1.83
260/230	1.31

Double digestion of puc & PBAD Recorder: Liudong Luo, Zhenyu Jiang Materials:

1. puc,PBAD

2. FastDigest restriction enzyme XbaI, pstI,XhoI,HindIII and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water

帮助

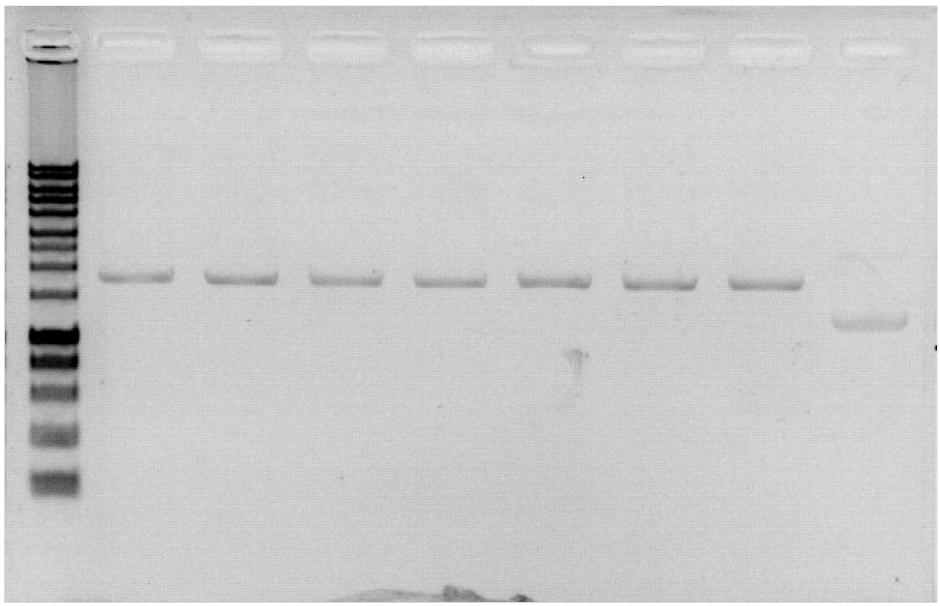
Reaction system:

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
XbaI(μL)	1	1	1	1	1	1	1
pstI(μL)	1	1	1	1	1	1	1
nuclease-free water(μL)	11	11	11	11	11	11	11
fastdigest green buffer(μL)	2	2	2	2	2	2	2
puc(μL)	5	5	5	5	5	5	5
total(μL)	20	20	20	20	20	20	20

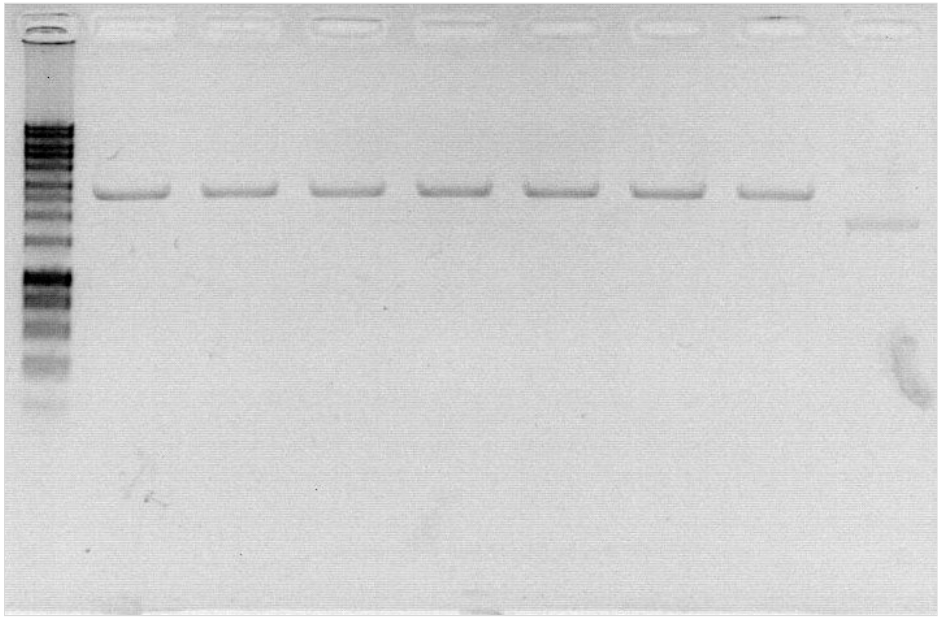
Reaction system:

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
XhoI(μL)	1	1	1	1	1	1	1
HindIII(μL)	1	1	1	1	1	1	1
Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
nuclease-free water(μL)	11	11	11	11	11	11	11
fastdigest green buffer(μL)	2	2	2	2	2	2	2
RED(μL)	5	5	5	5	5	5	5
total(μL)	20	20	20	20	20	20	20

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



(lane 1 to 9: Marker-Q ,dd-PBAD-1,dd-PBAD-2,dd-PBAD-3,dd-PBAD-4,dd-PBAD-5,dd-PBAD-6,dd-PBAD-7,PBAD)



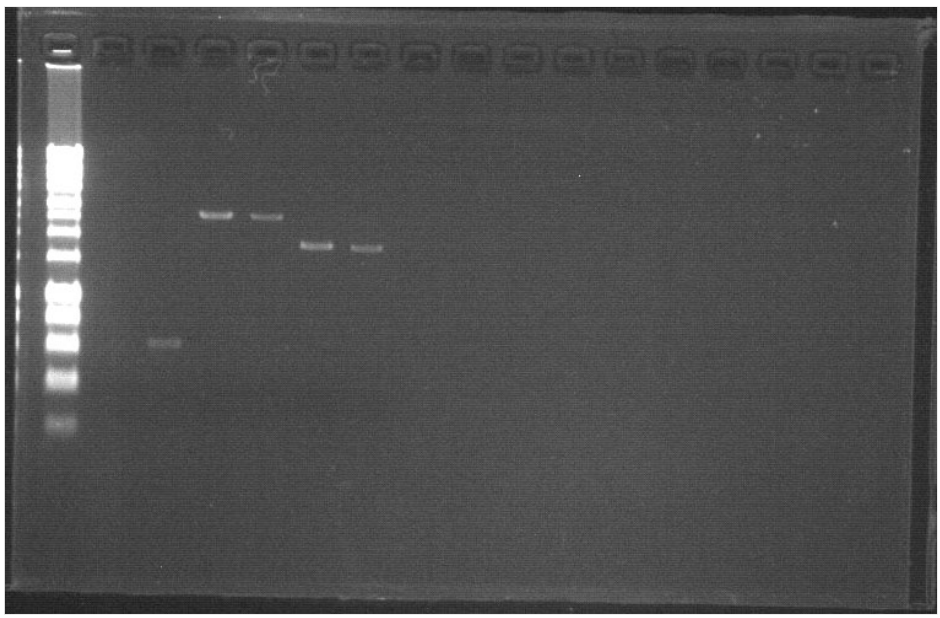
(lane 1 to 9: Marker-Q ,dd-PBAD-1,dd-PBAD-2,dd-PBAD-3,dd-PBAD-4,dd-PBAD-5,dd-PBAD-6,dd-PBAD-7,PBAD)

Agarose gel electrophoresis Result:

sample	**pucXPdd 1+2+3+4**	**pucXPdd 5+6+7+8**
Concentration(ng/ul)	28.4	94.0
260/280	1.81	1.54
260/230	0.70	0.56

sample	**PBADXHdd 1+2+3+4**	**PBADXHdd5+6+7+8**
Concentration(ng/ul)	34.9	55.4
260/280	1.72	1.63
260/230	0.64	0.58

Agarose gel electrophoresis and purification Result:



(lane 1 to 7: Marker—Q,pur-dd-ccm,pur-dd-RED,pur-dd-puc-1,pur-dd-puc-2,puc,pur-dd-PBAD-1,pur-dd-PBAD-2)

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**
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
stage	**temperature**	**time**
step 4	72	7 min
step 5	72	10 min
step 6	4	--

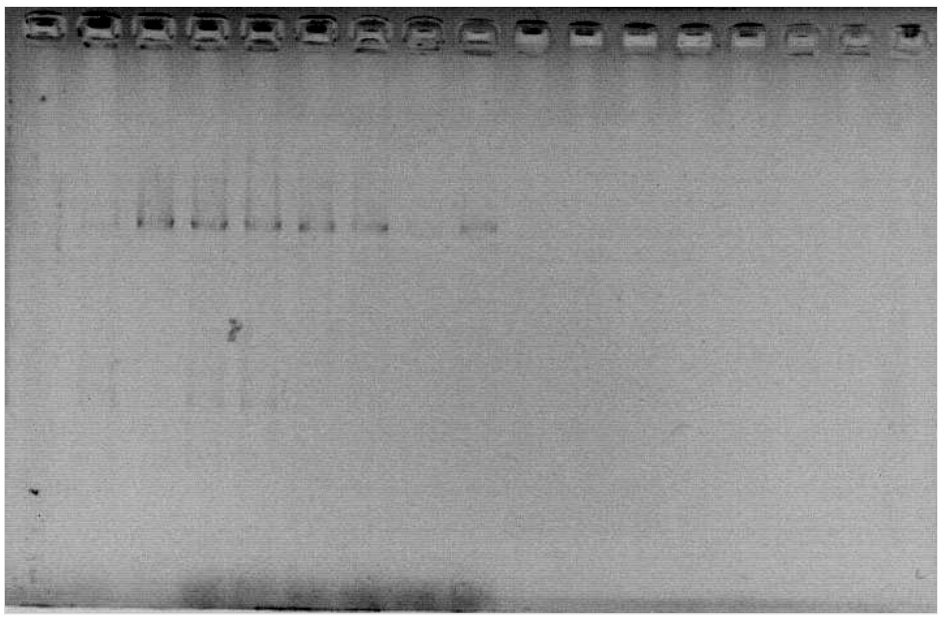
30 cycles(step 2 ~ step 4)

5,6,7,8 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:



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

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)	241.6
260/280	1.85
260/230	2.21

Plasmid Extraction of the PBar Recorder: Tong Xiao, Liwen Zhang 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	**PBar-2**	**PBar-5**	**PBar-6**	**PBar-7**	**PBar-8**	**PBar-9**	**PBar-10**	**PBar-11**	**PBar-12**	**PBar-13**
Concentration(ng/ul)	27.9	42.6	55.7	39.1	33.9	58.6	95.6	62.4	81.9	42.3
260/280	1.82	1.84	1.65	1.76	1.73	1.66	1.58	1.56	1.69	1.79
260/230	1.04	1.59	0.77	0.86	0.79	0.86	0.75	0.64	1.02	1.15

Double digestion of ccm & RED Recorder: Menglong Jin Materials:

1. full ccm,RED

sample	**ccmA-H**	**RED**
concentration(ng/μl)	241.6	807.8
260/280	1.85	1.87
260/230	2.21	2.23

Dilute the RED: add 2μl RED into 7μl sH2O.

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

Sample	1	2	3	4	5	6	7	8
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	1	2	3	4	5	6	7	8
XhoI(μL)	1	1	1	1	1	1	1	1
HindIII(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
RED(μL)	1	1	1	1	1	1	1	1
Sample total(μL)	1 30	2 30	3 30	4 30	5 30	6 30	7 30	8 30

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

Agarose gel electrophoresis Result:

Bacteria PCR Recorder: Meiying Cui,Yonghao Liang

Experimental materials

1. Template: bacteria picked from the plate ;
2. Primer: pUC-f, pUC-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	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 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	1 μL
pUC-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
pUC-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

3.PCR reaction 1-9 Parameters setting：

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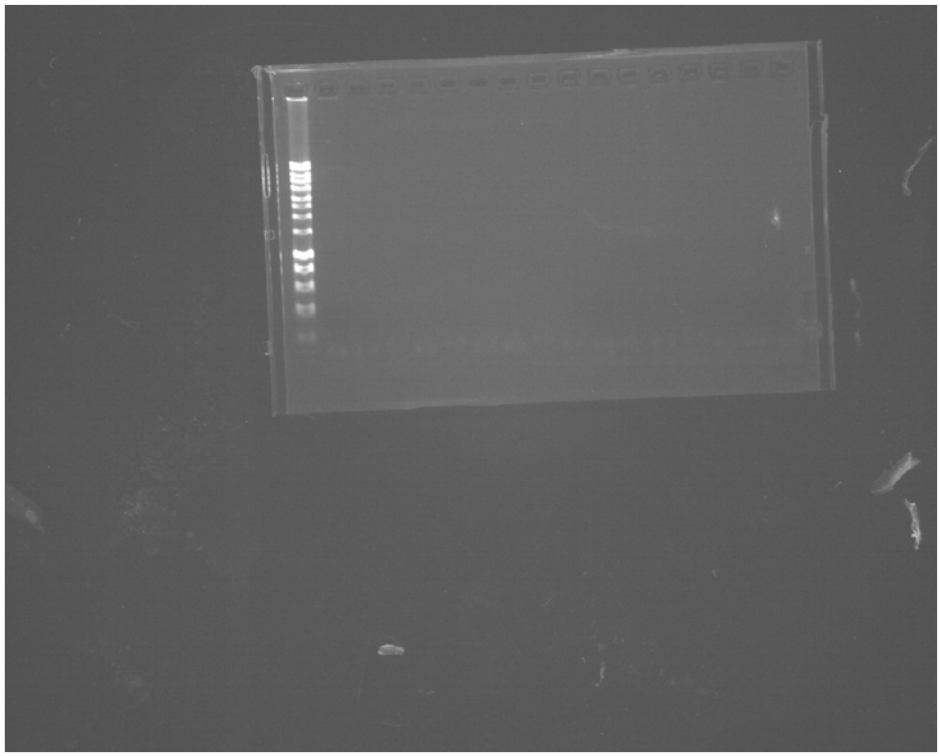
stage	temperature	time
step 1	94	10 min
step 2	94	30 s
step 3	56	30 s
step 4	72	7 min
step 5	72	10 min
step 6	4	--

25 cycles(step 2 ~ step 4) Result:



Double digestion of RED & PYYDT Recorder: Xiaoyu Zhang,Yawei wu Materials:

1. RED,PYYDT
2. FastDigest restriction enzyme NdeI，XhoI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water

Reaction system:

Sample	1	2	3	4	5	6
XbaI(μL)	1	1	1	1	1	1
pstI(μL)	1	1	1	1	1	1
nuclease-free water(μL)	4.6	4.6	4.6	4.3	4.3	4.3
fastdigest green buffer(μL)	2	2	2	2	2	2
RED(μL)	11.4	11.4	11.4	11.7	11.7	11.7
total(μL)	20	20	20	20	20	20

Reaction system:

Sample	1	2	3	4	5	6
XhoI(μL)	1	1	1	1	1	1
HindIII(μL)	1	1	1	1	1	1

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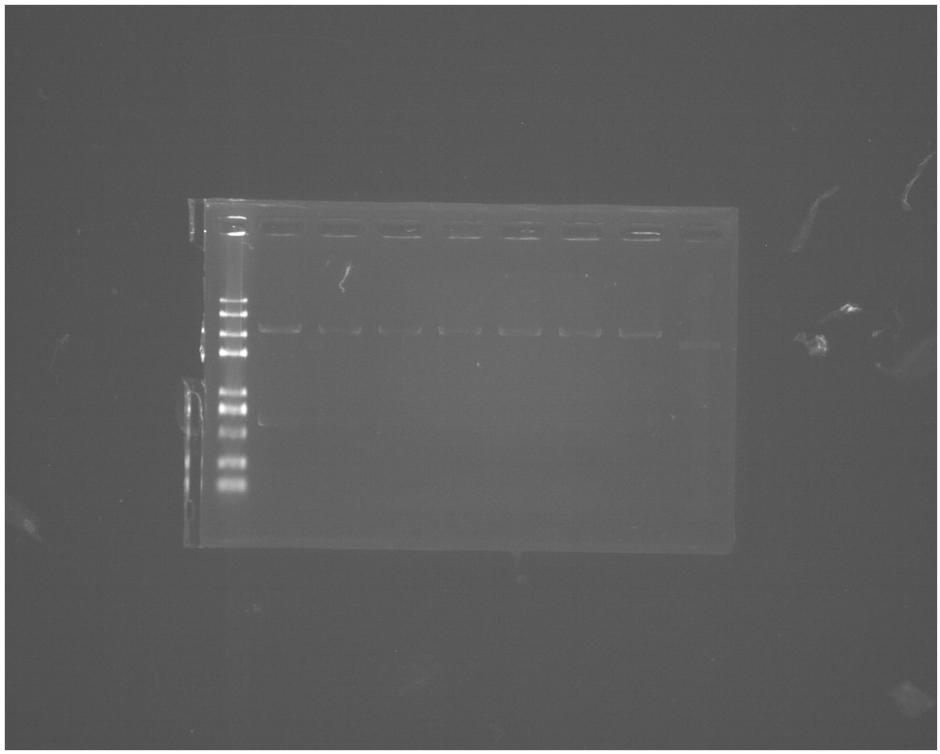
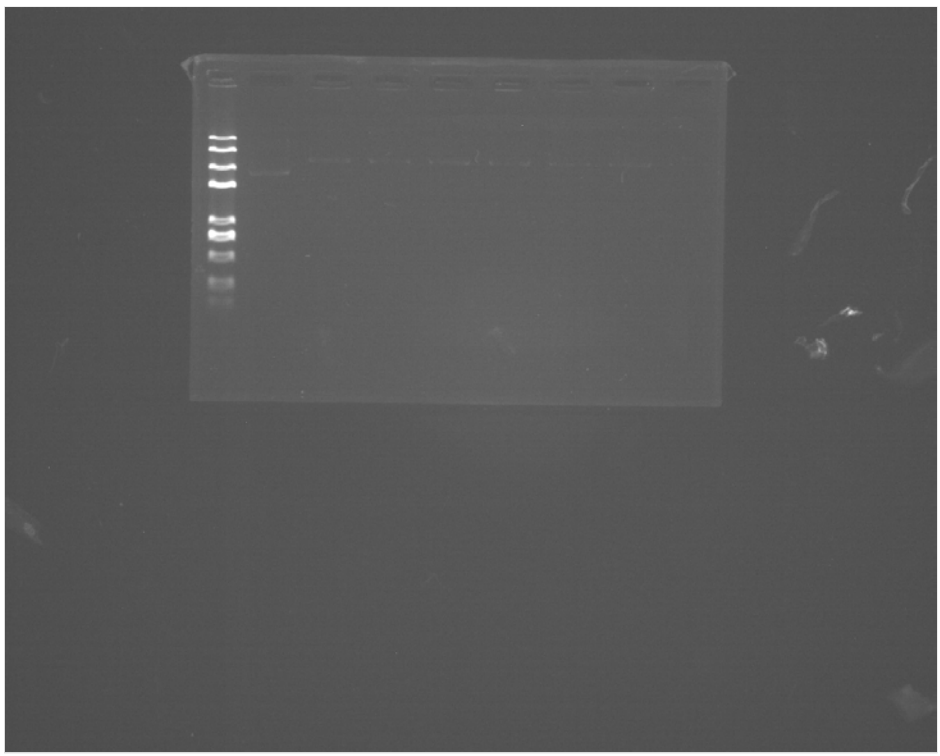
Sample	1	2	3	4	5	6
nuclease-free water(μL)	10.8	10.8	10.8	12.1	12.1	12.1
fastdigest green buffer(μL)	2	2	2	2	2	2
PYYDT(μL)	5.2	5.2	5.2	3.9	3.9	3.9
total(μL)	20	20	20	20	20	20

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

Agarose gel electrophoresis Result:



(lane left to right: Marker,ddRED1-7, RED)



(lane left to right: Marker, PYYDT, ddPYYDT1-6)

Then we did the purification of the fragments we got.

Double digestion of Mtr Recorder: Wenfei Yu Materials:

1. Mtr
2. FastDigest restriction enzyme XbaI, pstI,XhoI,HindIII and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water

Reaction system:

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**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)	25	25	25	25	25	25	25	25
fastdigest green buffer(μL)	2	2	2	2	2	2	2	2
Mtr(μL)	1	1	1	1	1	1	1	1
total(μL)	30	30	30	30	30	30	30	30

Reaction system:

Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**
XhoI(μL)	1	1	1	1	1	1	1
HindIII(μL)	1	1	1	1	1	1	1
nuclease-free water(μL)	11	11	11	11	11	11	11
fastdigest green buffer(μL)	2	2	2	2	2	2	2
RED(μL)	5	5	5	5	5	5	5
total(μL)	20	20	20	20	20	20	20
Sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**

Mix gently and incubate at 37 degree Celsius for 5 hours .

Then we did the purification of the fragments we got.

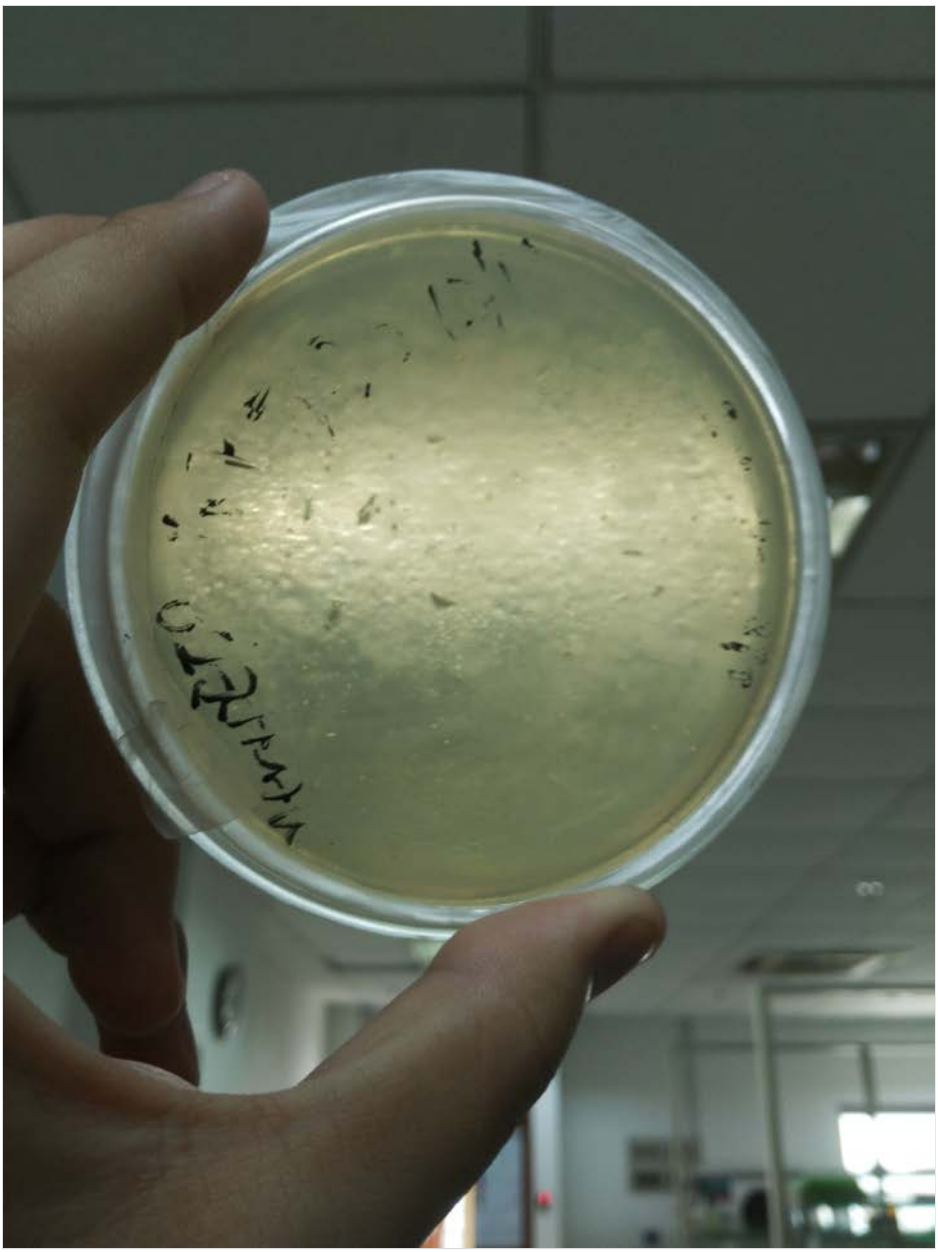
The results are as following:

sample	**ddMtr 1+2+3+4**	**ddMtr 5+6+7+8**
Concentration(ng/ul)	56.1	47.0
260/280	--	--
260/230	--	--

Transformation of Mtr into pET28 Recorder: Wenfei Yu

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 Amp. 10.Cultivate these bacteria overnight for further use.

Result:



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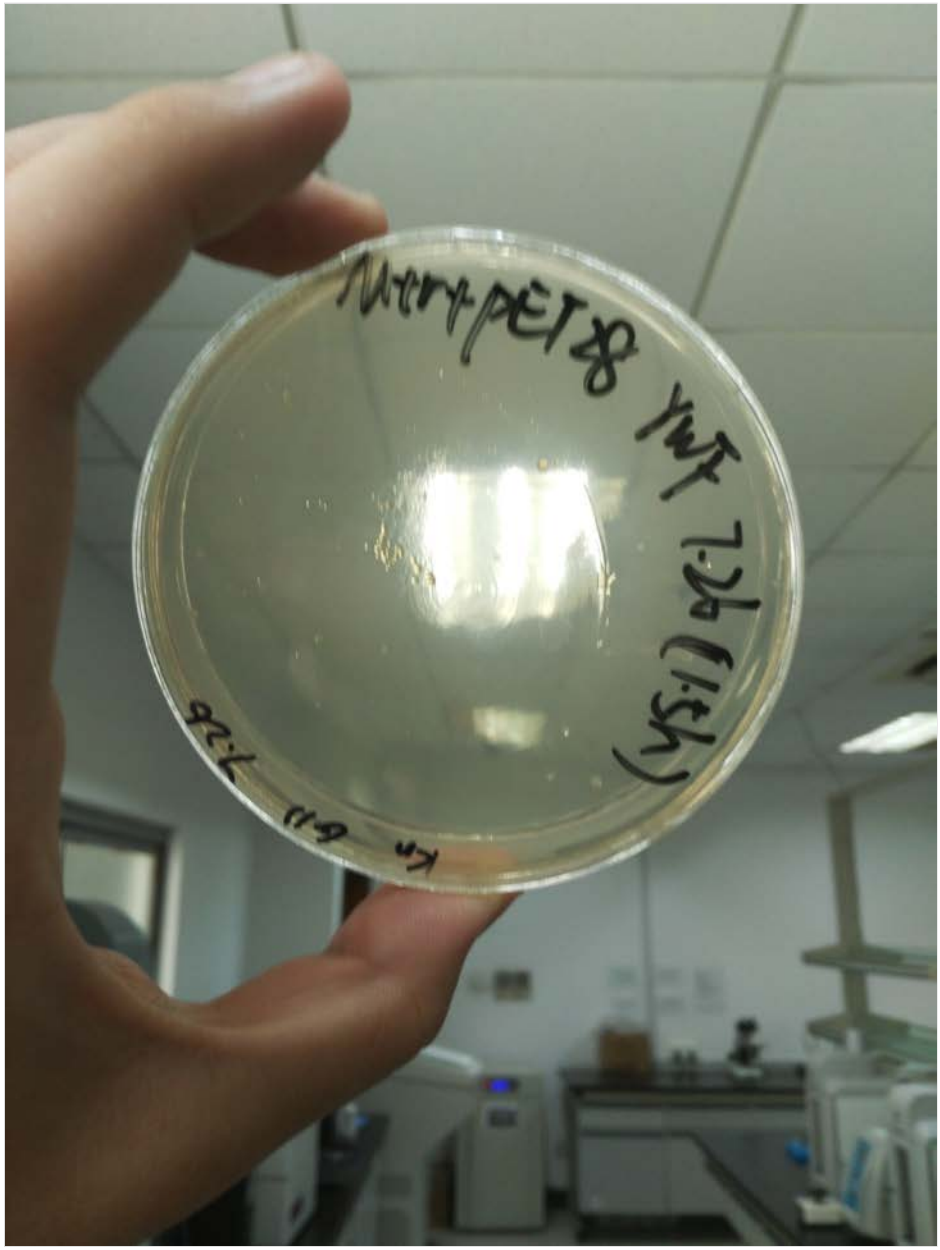
帮助



Bacteria PCR Recorder: Yonghao Liang

Experimental materials

1. Template: bacteria containing ccm+pUC19 recombined plasmid picked from the plate ;
2. Primer: pUC-f, pUC-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**	**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 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

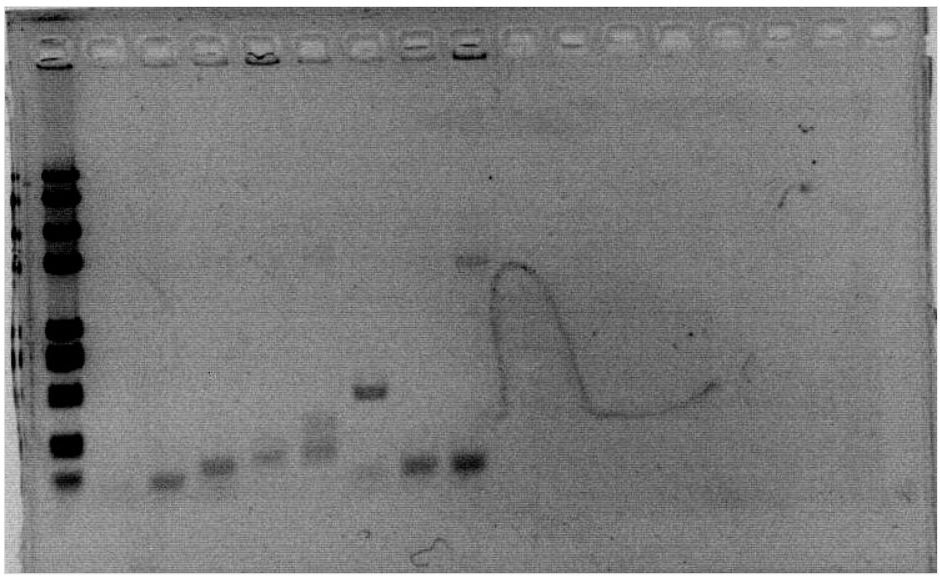
帮助

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

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:



Bacteria PCR Recorder: Yonghao Liang

Experimental materials

1. Template: bacteria containing Red+pBAD recombined plasmid picked from the plate ;
2. Primer: pBAD-f, pBAD-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：

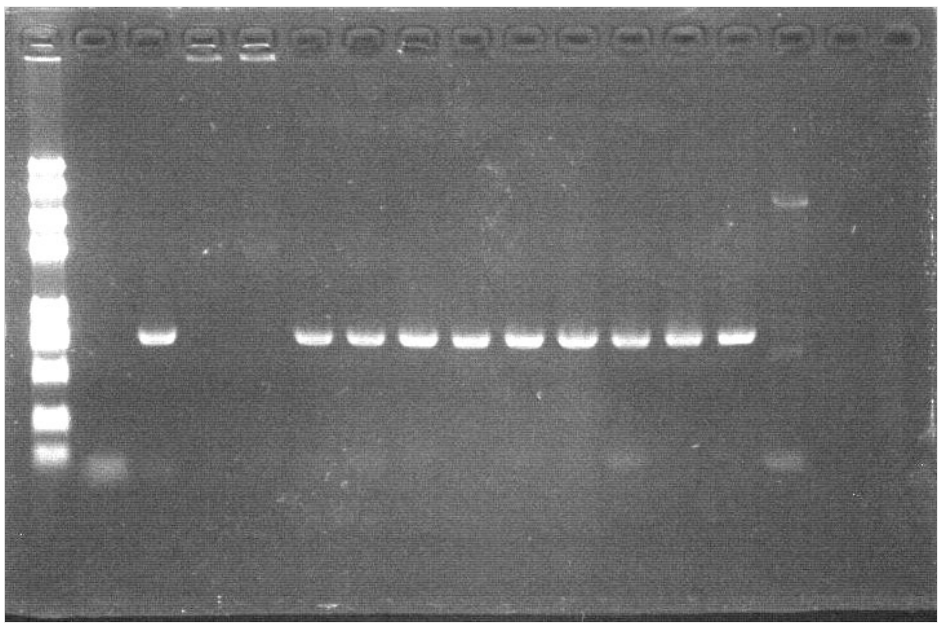
sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Sterilized ddH2O	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL

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

3.PCR reaction 1-9 Parameters setting：

stage	temperature	time
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:



Ligation of pET28 and Mtr Recorder: Wenfei Yu Material:

1. double digestion product of pET28 and Mtr
2. 10× T4 DNA ligase buffer,T4 DNA ligase(bought from Thermo Fisher Scientific)

Procedure: Add to either of samples: 100ng ddpET28 300~500ng ddMtr 2 μL 10* T4 DNA Ligase Buffer 0.4 μL T4 DNA Ligase sterilized water

Mix gently and incubate at 16 degree Celsius for 11 hour.

Date 7.27

Transformation of RED+pBAD 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 Amp. 10.Cultivate these bacteria overnight for further use.

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Transformation of Red+PYYDT into Top10 Recorder: Xiaoyu Zhang, Jingyu Wang
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 Red+PYYDT and mix it with bacteria solution thoroughly. ATTENTION: Please operate this step tenderly!!! 3.Put the tubes on the ice about 30 mins.(Time SHOULD BE ACCURATE) 4.Make a heat shock at 42 degree centigrade about 90 sec(TIME SHOULD BE ACCURATE) 5.Put the tubes on the ice about 5 mins again. 6.Add 900 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.

帮助

Result:

Double digestion of ccm & RED Recorder: Liudong Luo, Zhenyu Jiang Materials:

1. full ccm,RED
2. FastDigest restriction enzyme XbaI, pstI,XhoI,HindIII 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

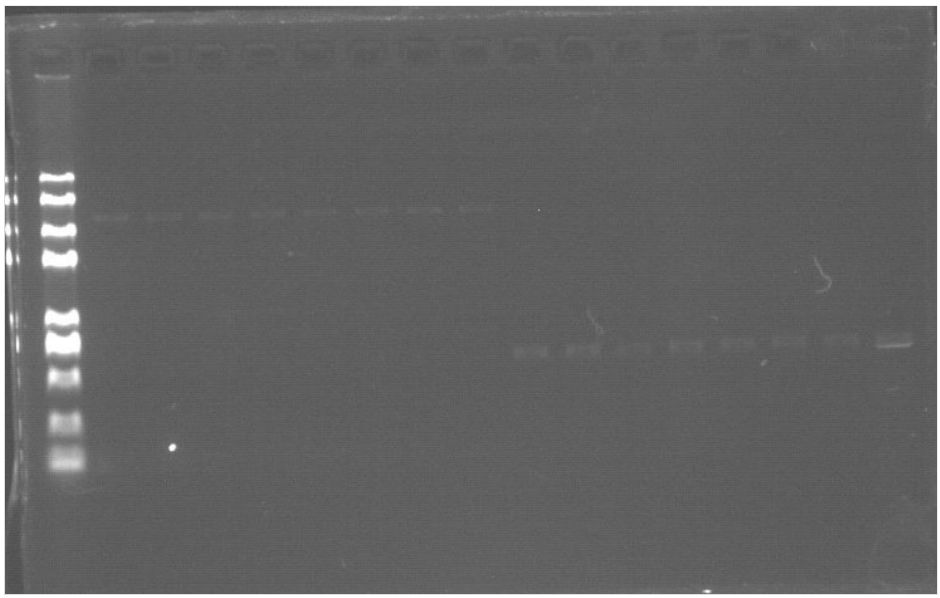
Sample	1	2	3	4	5	6	7	8
nuclease-free water(μL)	25	25	25	25	25	25	25	25
fastdigest green buffer(μL)	2	2	2	2	2	2	2	2
ccm(μL)	1	1	1	1	1	1	1	1
total(μL)	30	30	30	30	30	30	30	30

Reaction system:

Sample	1	2	3	4	5	6	7	8
XhoI(μL)	1	1	1	1	1	1	1	1
HindIII(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
RED(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 .

Agarose gel electrophoresis Result:



(lane 1 to 17: 2K plus II ,ddccm-1,ddccm-2,ddccm-3,ddccm-4,ddccm-5,ddccm-6,ddccm-7,ddccm-8,ddRED-1,ddRED-2,ddRED-3,ddRED-4,ddRED-5,ddRED-6,ddRED-7,ddRED-8)

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
Concentration(ng/ul)	50.7
260/280	1.72
260/230	1.08

sample	RedXHdd 1+2+3+4+5+6+7+8
Concentration(ng/ul)	46.0
260/280	1.64

sample	RedXHdd 1+2+3+4+5+6+7+8
260/230	0.85

Double digestion of puc & PBAD Recorder: Liudong Luo, Zhenyu Jiang Materials:

1. puc,PBAD
2. FastDigest restriction enzyme XbaI, pstI,XhoI,HindIII and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water

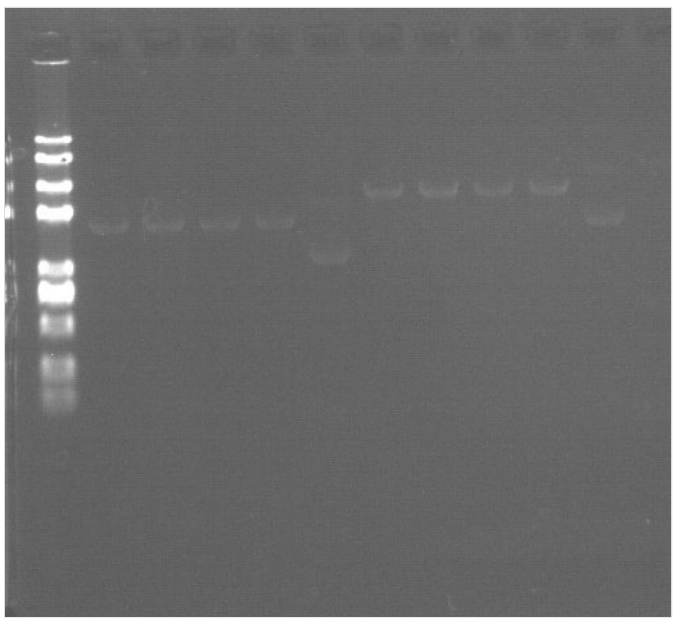
Reaction system:

Sample	1	2	3	4	5	6	7
XbaI(μL)	1	1	1	1	1	1	1
pstI(μL)	1	1	1	1	1	1	1
nuclease-free water(μL)	11	11	11	11	11	11	11
fastdigest green buffer(μL)	2	2	2	2	2	2	2
puc(μL)	5	5	5	5	5	5	5
total(μL)	20	20	20	20	20	20	20

Reaction system:

Sample	1	2	3	4	5	6	7
XhoI(μL)	1	1	1	1	1	1	1
HindIII(μL)	1	1	1	1	1	1	1
nuclease-free water(μL)	11	11	11	11	11	11	11
fastdigest green buffer(μL)	2	2	2	2	2	2	2
RED(μL)	5	5	5	5	5	5	5
total(μL)	20	20	20	20	20	20	20

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



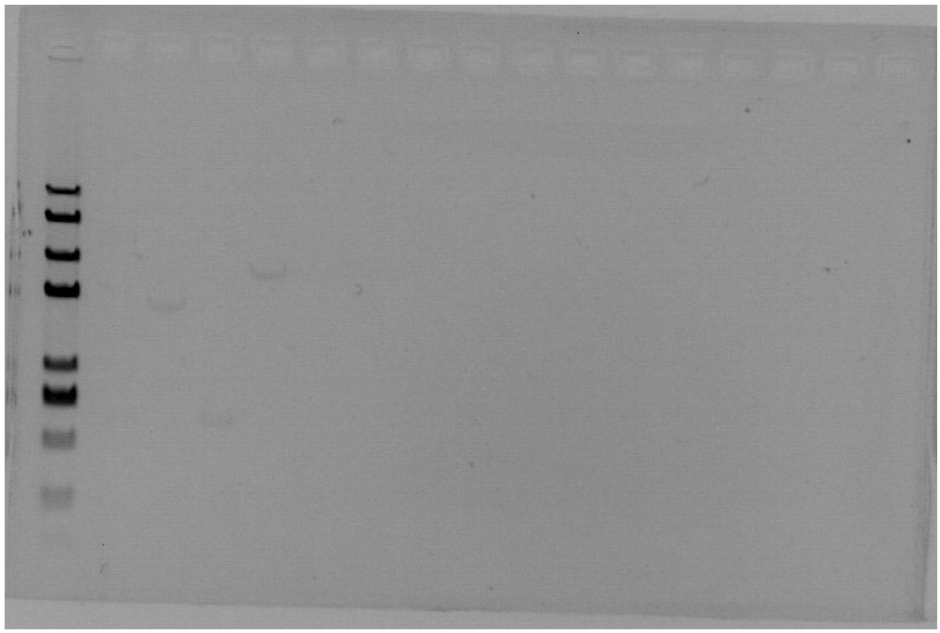
(lane 1 to 9: Marker 2k plus II ,dd-puc-1,dd-puc-2,dd-puc-3,dd-puc-4,puc,dd-PBAD-1,dd-PBAD-2,dd-PBAD-3,dd-PBAD-4,PBAD)

Agarose gel electrophoresis Result:

sample	pucXPdd 1+2+3+4+5+6+7+8
Concentration(ng/ul)	115.0
260/280	1.70
260/230	1.17

sample	PBADXHdd 1+2+3+4+5+6+7+8
Concentration(ng/ul)	60.6
260/280	1.76
260/230	1.43

Agarose gel electrophoresis and purification Result:



(lane 1 to 7: Marker 2k plus II,pur-dd-ccm,pur-dd-RED,pur-dd-puc,pur-dd-PBAD)

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
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

stage	temperature	time
step 3	59	5 s
step 4	72	7 min
step 5	72	10 min
step 6	4	--

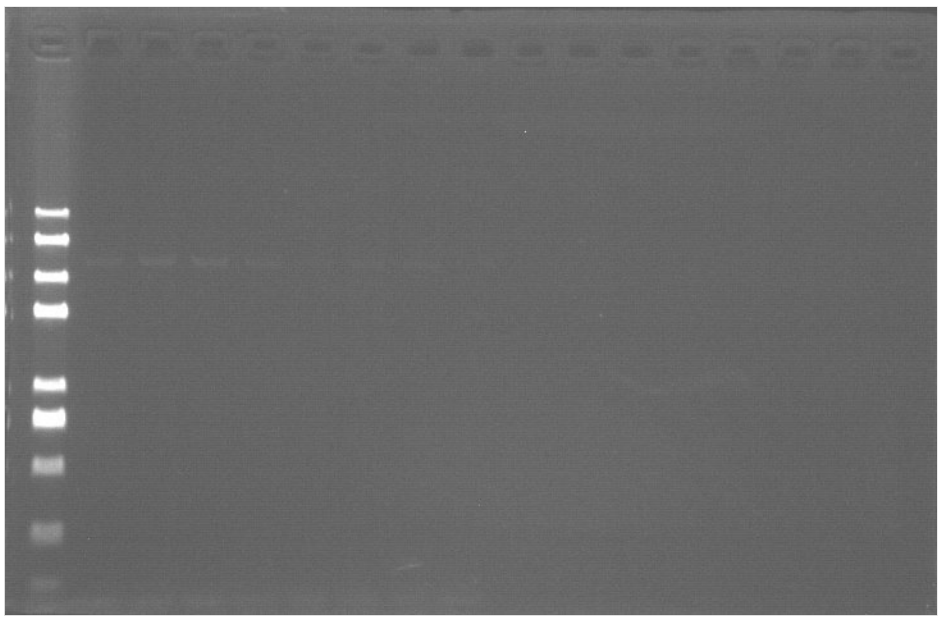
30 cycles(step 2 ~ step 4)

5,6,7,8 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:



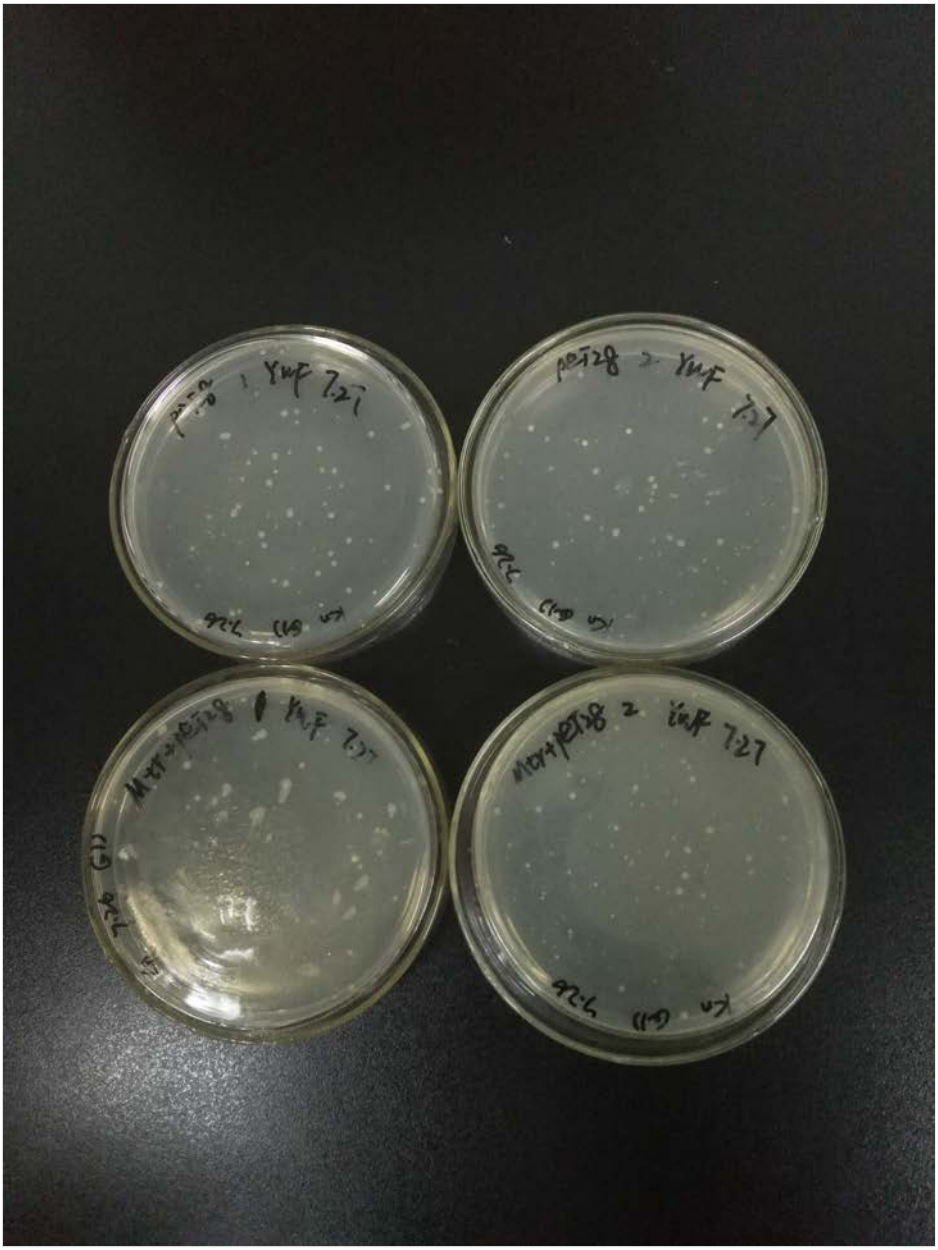
(lane left to right: marker 2K Plus, ccm-pcr-1, ccm-pcr-2,ccm-pcr-3,ccm-pcr-4,ccm-pcr-5, ccm-pcr-6, ccm-pcr-7,ccm-pcr-8)

Transformation of Mtr into pET28 Recorder: Wenfei Yu

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 10μL Mtr+pET28 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.

帮助

Result:



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)	180.2
260/280	1.83
260/230	1.96

Bacteria PCR Recorder: Meiying Cui

Experimental materials

1. Template: bacteria picked from the plate ;
2. Primer: pUC-f, pUC-r. Designed by ourselves, synthesized by Sangon Biotech;
3. Sterilized ddH2O;
4. 2X Taq DNA polymerase.

Procedure:

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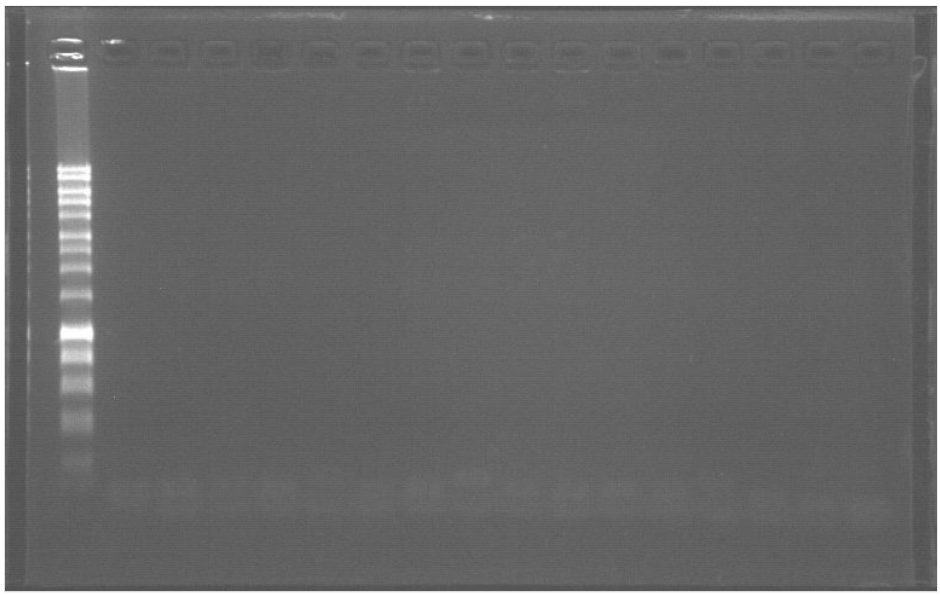
1.Prepare 16 PCR tubes and sequentially add：

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 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	1 μL
pUC-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
pUC-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

3.PCR reaction 1-9 Parameters setting：

stage	temperature	time
step 1	94	10 min
step 2	94	30 s
step 3	56	30 s
step 4	72	5 min 30s
step 5	72	10 min
step 6	4	--

25 cycles(step 2 ~ step 4) Result:



Bacteria PCR Recorder: Shihan Zhu, Chenyang Li

Experimental materials

1. Template: bacteria picked from the plate ;
2. Primer: pUC-f, pUC-r. Designed by ourselves, synthesized by Sangon Biotech;
3. Sterilized ddH2O;
4. 2X Taq DNA polymerase.

Procedure:

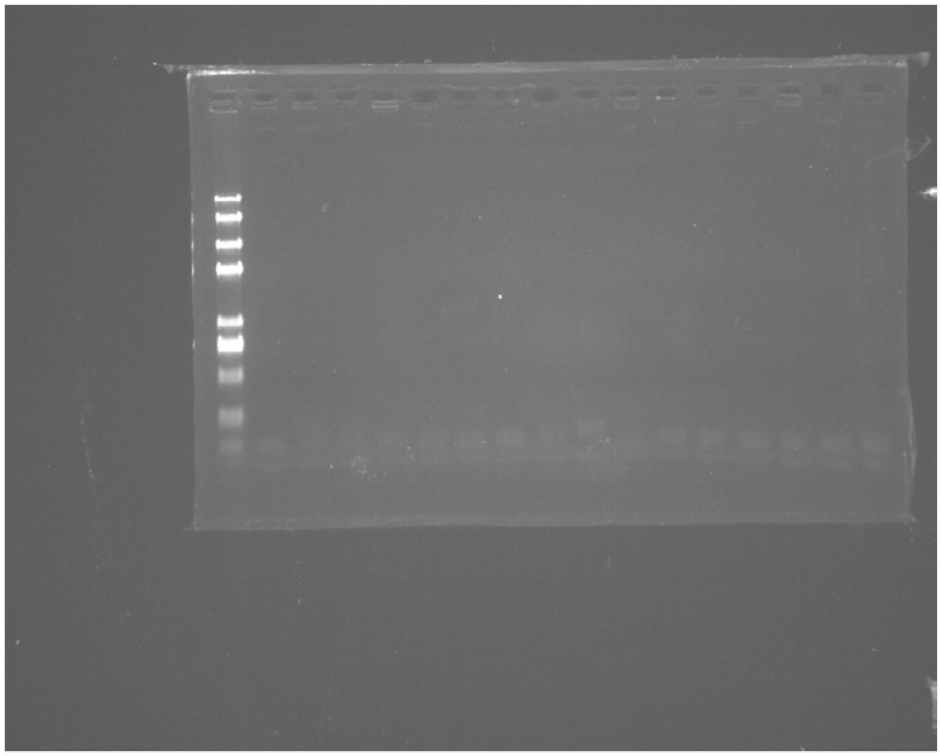
1.Prepare 16 PCR tubes and sequentially add：

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

3.PCR reaction 1-9 Parameters setting：

stage	temperature	time
step 1	94	10 min
step 2	94	30 s
step 3	56	30 s
step 4	72	5 min 30 s
step 5	72	10 min
step 6	4	--

25 cycles(step 2 ~ step 4) Result:



Plasmid Extraction of the PBar *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, 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	**PBar-9**	**PBar-5**	**PBar-7**	**PBar-6**	**PBar-8**	**PBar-11**	**PBar-13**	**PBar-10**	**PBar-2**	**PBar-12**
Concentration(ng/ul)	93.9	59.4	92.8	101.3	92.1	79.2	103.0	140.4	45.6	139.3
260/280	1.89	1.96	1.91	1.91	1.89	1.92	1.88	1.76	1.94	1.70
260/230	2.17	2.10	2.26	2.06	2.11	2.13	1.85	1.30	1.61	0.96

Double digestion of pBAR Recorder: Yonghao Liang Materials:

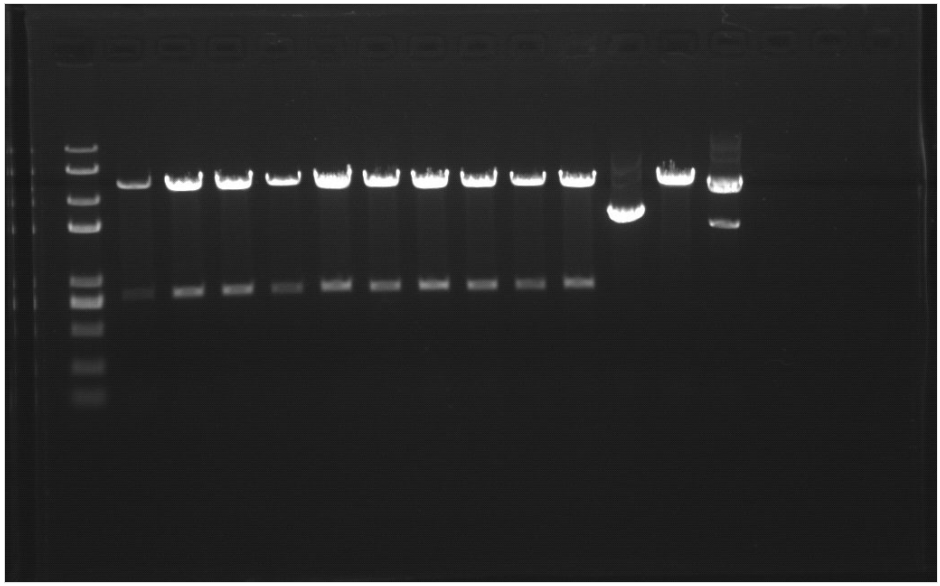
1. pBAR
2. FastDigest restriction enzyme XhoI,HindIII and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water

Reaction system:

Sample	**2**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**con-pBAD**
XhoI(μL)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
HindIII(μL)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
fastdigest green buffer(μL)	2	2	2	2	2	2	2	2	2	2	2
pBAR(μL)	17	10	10	5	10	10	10	10	8	12	10
nuclease-free water(μL)	0	7	7	12	7	7	7	7	9	5	7
total(μL)	20	20	20	20	20	20	20	20	20	20	20

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

Agarose gel electrophoresis Result:



PS: The first ten lanes are pBAR, and the last two is control group.

Plasmid Extraction of the pBAR Recorder: Yonghao Liang 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	pBAR-2	pBAR-5	pBAR-6	pBAR-7	pBAR-8	pBAR-9	pBAR-10	pBAR-11	pBAR-12	pBAR-13
Concentration(ng/ul)	30.8	90.3	92.9	22.93	86.3	100.1	90.9	95.0	120.6	70.1
260/280	1.85	1.81	1.82	1.64	1.84	1.73	1.83	1.77	1.67	1.82
260/230	1.39	1.74	1.84	0.83	2.15	1.35	1.94	1.53	0.94	1.76

Transformation of plasmid PYRED into Top10 Recorder: Xiaoyu Zhang 1.Take the competent bacteria from -80 degree centigrade refrigerator and incubate them into ice about 5 mins until it is dissolved 2.Absorb 10uL plasmid(normally 1 to 2 uL, DO NOT add more than 5% volumn of bacteria solution) and mix it with bacteria solution thoroughly. ATTENTION: Please operate this step tenderly!!! 3.Put the tubes on the ice about 30 mins.(Time SHOULD BE ACCURATE) 4.Make a heat shock at 42 degree centigrade about 90 sec(TIME SHOULD BE ACCURATE) 5.Put the tubes on the ice about 5 mins again. 6.Add 900 ul LB medium into EP tubes and cultivate the bacteria at 37 degree centrigrade about 60 min. 7.Centrifuge them at 4000 rpm for 2 minutes and we will see sediment in the tubes. 8.Discard the supernatant liquid and leave about 200 ul medium. 9.Coat plate: Add 200 ul solution in a plate with kanamycin. 10.Cultivate these bacteria overnight for further use.

Awaken of the Glycerin bacteria Top10 containing RED on the plasmid pET21 Recorder: Xiaoyu Zhang Add 200 μL of bacteria into a 5 mL LB culture and cultivate these bacteria overnight for extraction at 37 degree centigrade, 250 rpm.

Date 7.28

Transformation of RED+pBAD and ccmA-H+PUC19 int5o 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 Amp. 10.Cultivate these bacteria overnight for further use.

Plasmid Extraction of the pBAR Recorder: 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	**pBAR-2**	**pBAR-5**	**pBAR-6**	**pBAR-7**	**pBAR-8**	**pBAR-9**	**pBAR-10**	**pBAR-11**	**pBAR-12**	**pBAR-13**
Concentration(ng/ul)	68.6	131.3	112.8	93.8	156.5	98.7	100.9	83.2	83.8	79.8
260/280	1.66	1.70	1.79	1.86	1.70	1.74	1.75	1.87	1.76	1.82
260/230	0.95	1.03	1.67	2.24	1.90	1.30	1.31	2.21	1.52	1.80

Double digestion of ccm Recorder: Liudong Luo, Zhenyu Jiang, Menglong Jin 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

Sample	9	10	11	12	13	14	15	16
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
Xbal(μL)	1	1	1	1	1	1	1	1
pstl(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
Xbal(μL)	1	1	1	1	1	1	1	1
pstl(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)	76.5
260/280	1.80
260/230	1.71

sample	ccmXPdd 17+18+19+20+21+22+23+24+25+26+27+28+29+30+31+32
Concentration(ng/ul)	87.1
260/280	1.84
260/230	1.93

Double digestion of puc & PTB Recorder: Liudong Luo, Zhenyu Jiang, Menglong Jin
Materials:

1. puc,PTB
2. FastDigest restriction enzyme Xbal, pstl and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)

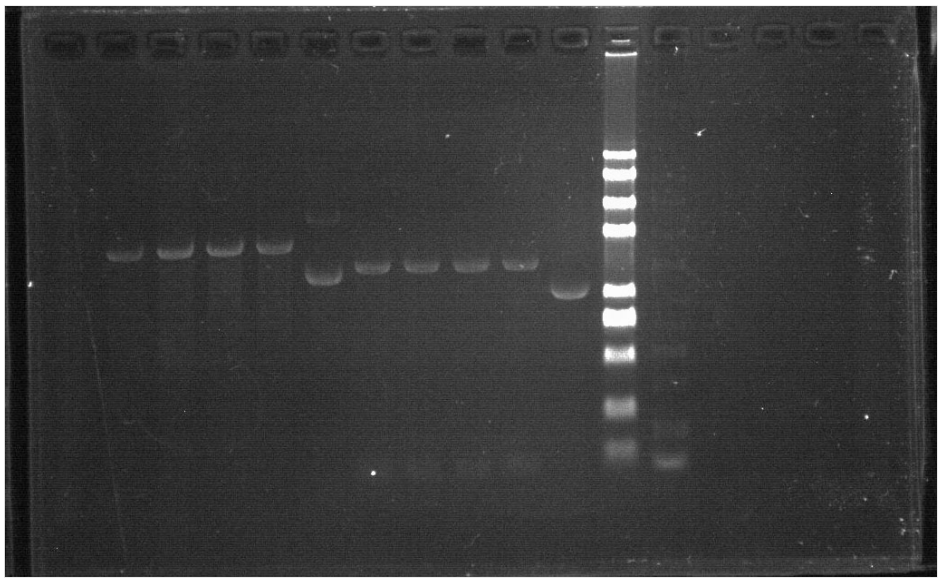
3. Nuclease-free water

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 .

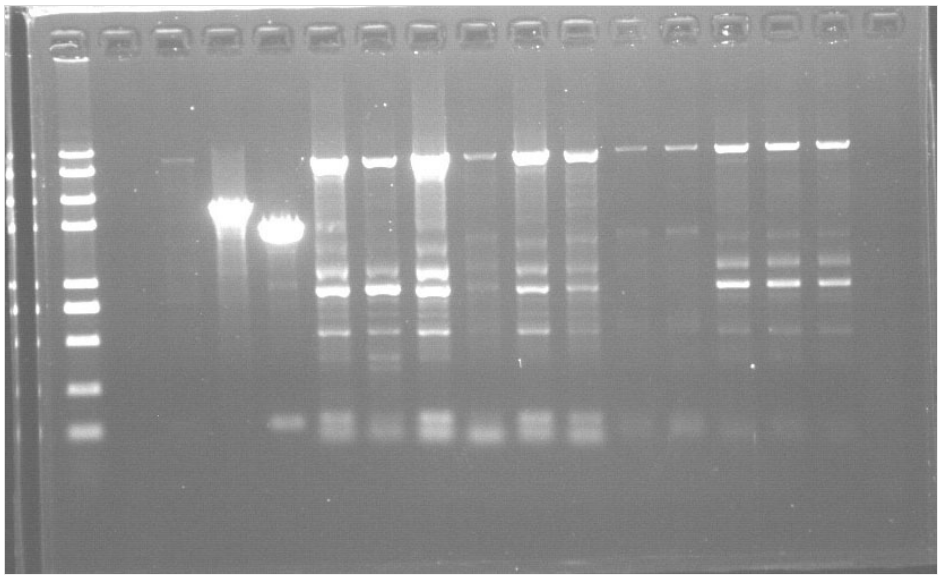


(lane 1 to 9: dd-puc-1,dd-puc-2,dd-puc-3,dd-puc-4,puc,dd-PTB-1,dd-PTB-2,dd-PTB-3,dd-PTB-4,PTB,Marker 2k plus II)

sample	**pucXPdd 1+2+3+4**
Concentration(ng/ul)	71.0
260/280	1.73
260/230	1.17

sample	**pTBXPdd 1+2+3+4**
Concentration(ng/ul)	76.3
260/280	1.79
260/230	1.73

Agarose gel electrophoresis and purification Result:



(lane 1 to 7: Marker 2k plus II,pur-dd-ccm-1,pur-dd-ccm-2,pur-dd-puc,pur-dd-PTB,pcr-ccm-1,pcr-ccm-2,pcr-ccm-3,pcr-ccm-4,pcr-ccm-5,pcr-ccm-6,pcr-ccm-7,pcr-ccm-8,pcr-ccm-9,,pcr-ccm-10,pcr-ccm-11)

PCR of full Ccm Recorder: Liudong Luo, Zhenyu Jiang,Menglong Jin

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**
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) 3.Agarose gel electrophoresis Result:

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

Colony PCR of pET28 containing Mtr Recorder: Wenfei Yu

Experimental materials

1. Template: E.coli Top 10 with pET28 plasmid containing Mtr;
2. Primer: T7,T7-TER. Designed by ourselves, synthesized by Sangon Biotech;
3. Sterilized ddH2O, 2×Taq PCR master mix.

Procedure:

1.Prepare 16 PCR tubes and sequentially add：

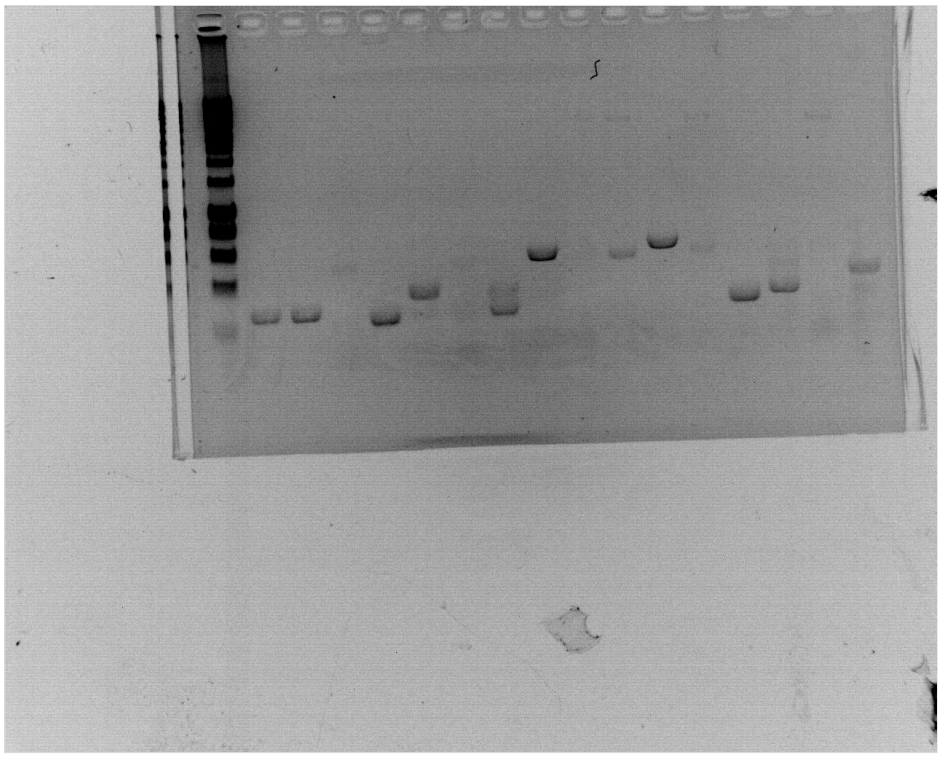
sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Sterilized ddH2O	7 μL	7 μL	7 μL	7μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7μL	7 μL	7 μL	7 μL	7 μL
2×Taq PCR master mix	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 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
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

2.PCR reaction 1-16 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	--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:



(From left to right: marker, sample1-16)

Bacteria PCR Recorder: Yawei Wu

Experimental materials

1. Template: bacteria containing Red+PYYDT recombined plasmid picked from the plate ;
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：

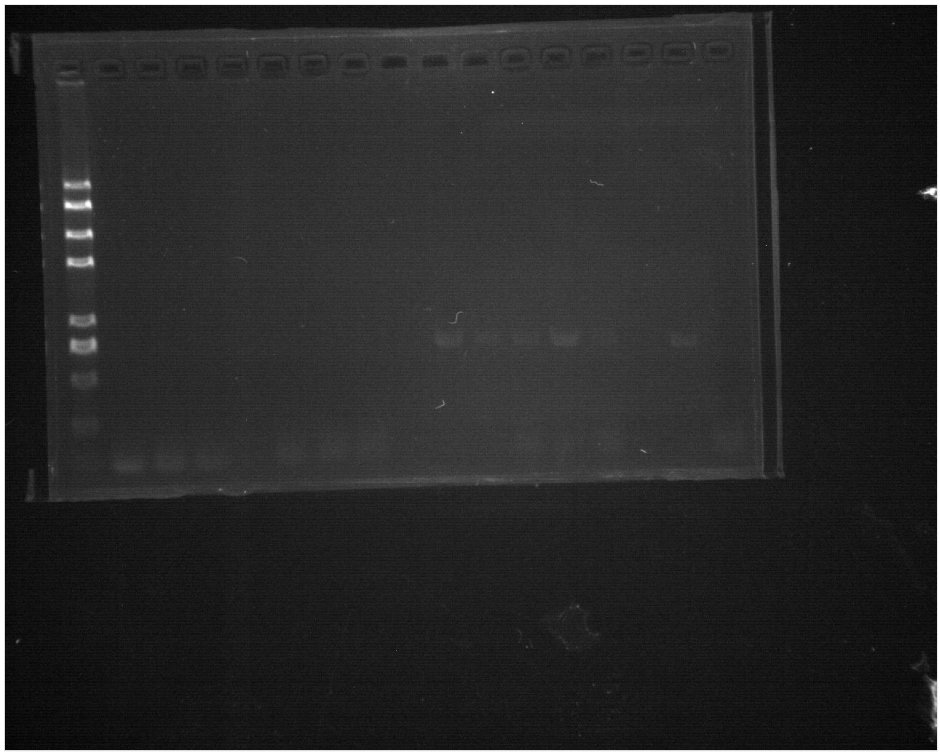
sample	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Sterilized ddH2O	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL	7 μL
2X Taq DNA polymerase	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	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	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

3.PCR reaction 1-9 Parameters setting：

stage	**temperature**	**time**

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) Result:



(lane left to right: Marker, PYDDT+Red1-16)

Pick PYYDT+Red9, PYYDT+Red12, PYYDT+Red15, rename them as PYRed1-3 and cultivate these bacteria in medium with Kana for further use.

Date 7.29

Transformation of ccmA-H+PUC19 and ccmA-H+pTB into Top10 Recorder: Menglong Jin

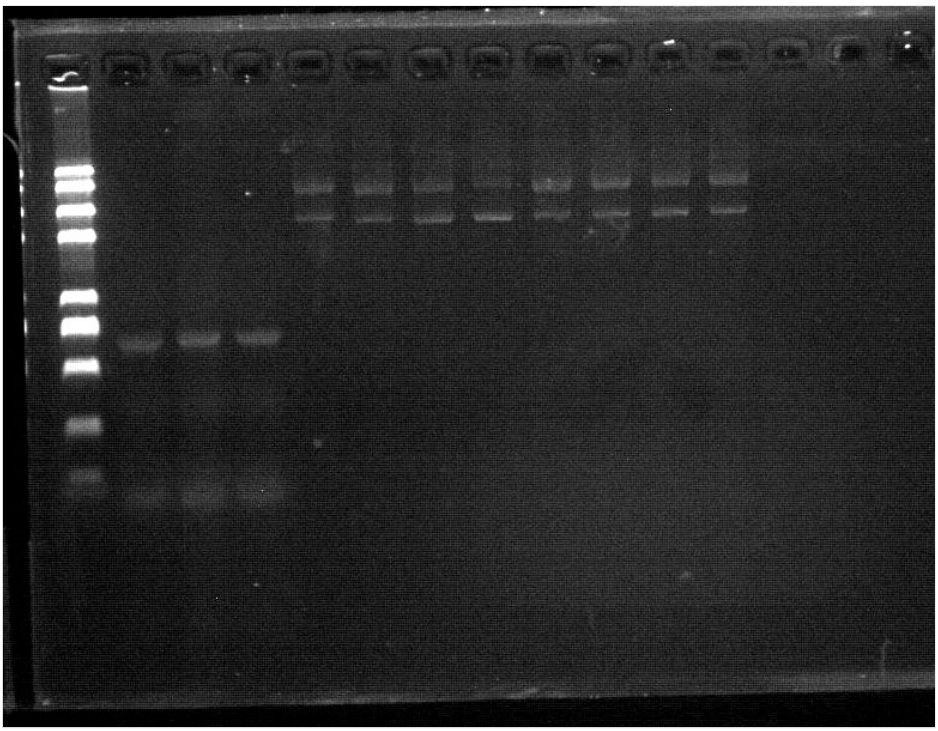
NOTE:Generally, competent bacteria are restrored in -80 degree centrigrade environment. 1.Take the competent bacteria from refrigerator and incubate them into ice about 5 mins until it is dissolved 2.Absorb 1μL PUC19 and mix it wμith bacteria solution thoroughly. ATTENTION: Please operate this step tenderly!!! 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 Amp(ccm+pTB); add 200 μL solution in a plate with Amp, IPTG and X-Gal(ccm+pTB). 10.Cultivate these bacteria overnight for further use.

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)	65.1	67.0	176.8	175.4	189.8	161.5	255.5	240.9
260/280	1.86	1.86	1.67	1.67	1.64	1.65	1.61	1.64
260/230	2.19	2.16	0.85	0.84	0.80	0.71	0.72	0.76
sample	RED-1	RED-2	RED-3	RED-4	RED-5	RED-6	RED-7	RED-8

We did agarose gel electrophoresis then:



(From left to right: line1: marker 2K plusII; line 2-4: pcr of RED; line 5-12: sample RED1-8.) As you can see, all of the eight samples contain the plasmid we need, but sample 3-8 contain more impurities. We will do better next time.

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
Sterilized ddH2O	22 μL	22 μL	22 μL	22 μL	22 μL	22 μL	22 μL	22 μL
2×PrimeStar(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

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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	--

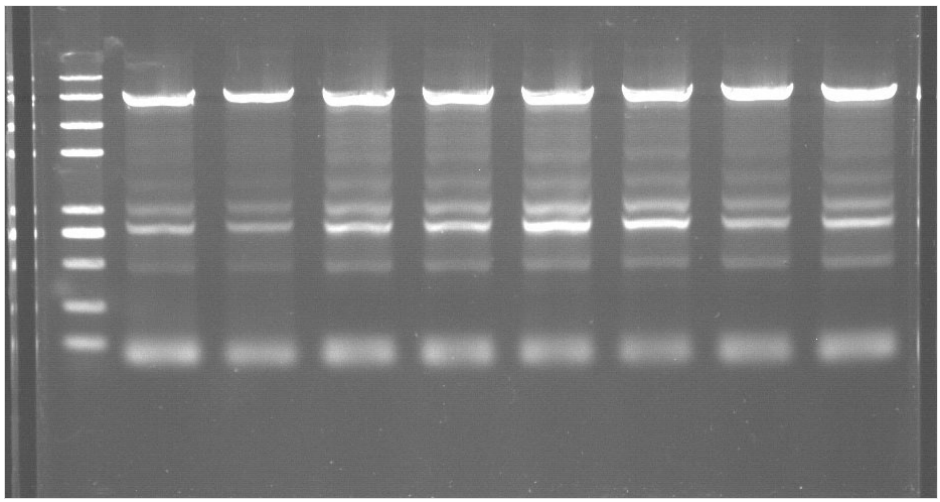
30 cycles(step 2 ~ step 4)

5,6,7,8 Parameters setting：

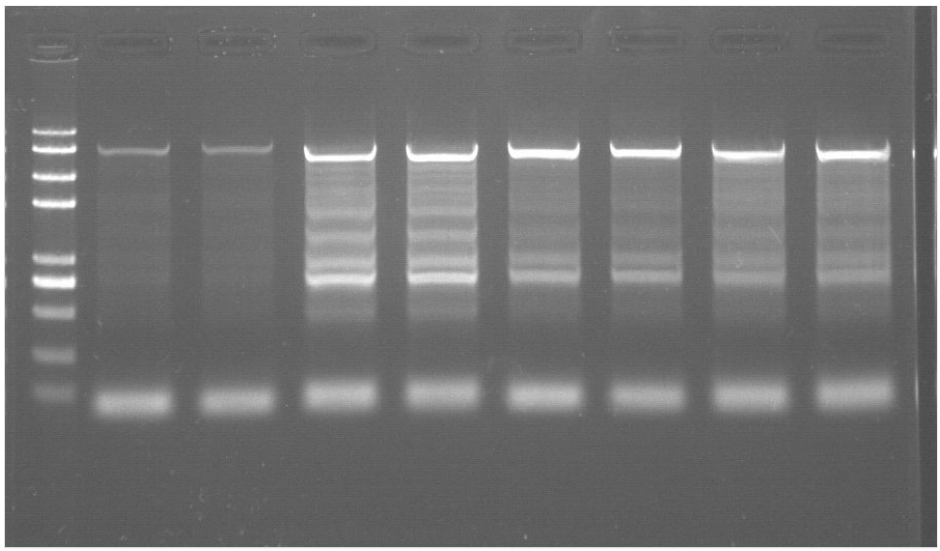
stage	temperature	time
step 1	95	10 min
step 2	98	10 s
step 3	55	15 s
step 4	72	7 min
step 5	72	10 min
step 6	4	--

30 cycles(step 2 ~ step 4)

3.Agarose gel electrophoresis Result:



(lane left to right: marker 2K Plus, ccm-pcr-1-1, ccm-pcr-1-2,ccm-pcr-2-1,ccm-pcr-2-2,ccm-pcr-3-1, ccm-pcr-3-2, ccm-pcr-4-1,ccm-pcr-4-2)



(lane left to right: marker 2K Plus, ccm-pcr-5-1, ccm-pcr-5-2,ccm-pcr-6-1,ccm-pcr-6-2,ccm-pcr-7-1, ccm-pcr-7-2, ccm-pcr-8-1,ccm-pcr-8-2)

4.Gel Extraction

The results are as following:

sample	ccmXPdd 1+2+3+4+5+6+7+8
Concentration(ng/ul)	22.9
260/280	1.82
260/230	1.57

Double digestion of ccm Recorder: Liudong Luo, Zhenyu Jiang, Menglong Jin 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:

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

Sample	25	26	27	28	29	30	31	32
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)	64.4
260/280	1.74
260/230	1.21

sample	ccmXPdd 17+18+19+20+21+22+23+24+25+26+27+28+29+30+31+32
Concentration(ng/ul)	87.5
260/280	1.74
260/230	1.38

Double digestion of puc & PTB Recorder: Liudong Luo, Zhenyu Jiang, Menglong Jin
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

Sample	1	2	3	4
pTB(μL)	6	6	6	6
total(μL)	20	20	20	20

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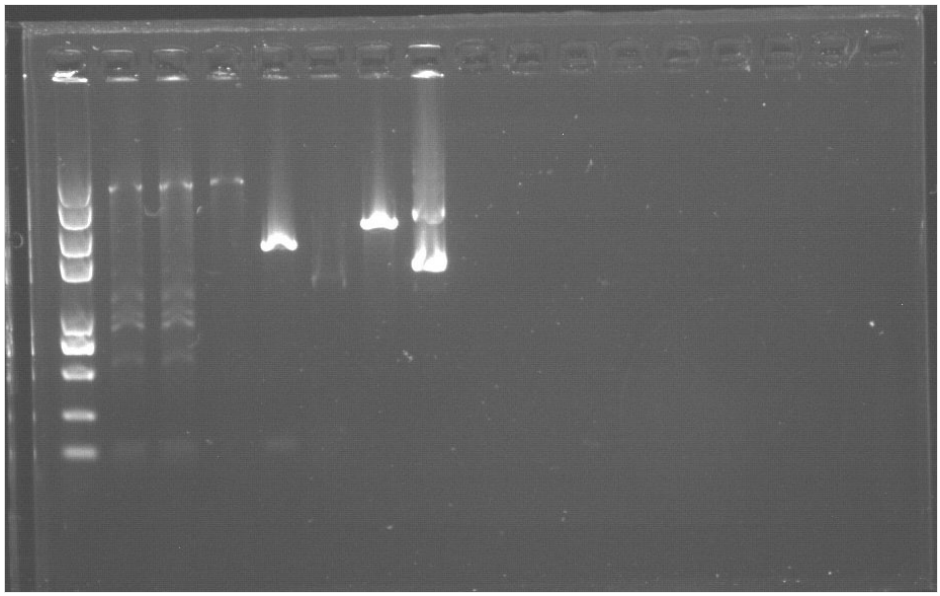
Mix gently and incubate at 37 degree Celsius for 1 hours .

sample	pucXPdd 1+2+3+4
Concentration(ng/ul)	80.9
260/280	1.84
260/230	2.31

sample	pTBXPdd 1+2+3+4
Concentration(ng/ul)	143.7
260/280	1.61
260/230	0.52

Agarose gel electrophoresis and purification Result:



(lane 1 to 7: Marker 2k plus II,pur-dd-ccm-1,pur-dd-ccm-2,ccm,pur-dd-PTB,pTB,pur-dd-puc,puc)

Double digestion of PYRed Recorder: Yawei Wu, Xiaoyu Zhang Materials:

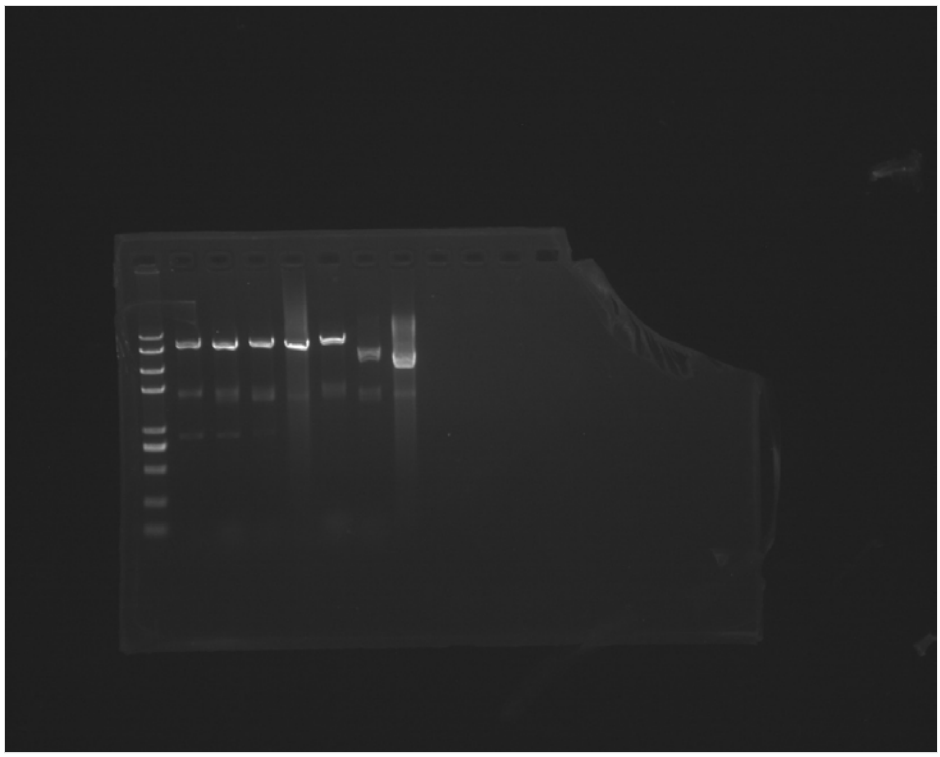
1. PYRed, PYYDT
2. FastDigest restriction enzyme XhoI, NdeI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water

Reaction system:

Sample	1	2	3	4	5
XhoI(μL)	1	1	1	1	1
NdeI(μL)	1	1	1	0	1
fastdigest green buffer(μL)	2	2	2	2	2
Nuclease-free water(μL)	0	0	0	0	7.3
plasmid(μL)	16	16	16	17	8.7
total(μL)	20	20	20	20	

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

Result:



(lane left to right: Marker, ddPYRed1-3, ddPYYDT, sdPYRed, PYRed, PYYDT)

Construction of PYRed succeeded!

Plasmid Extraction of the pBAR Recorder: Zhenyu Jiang, 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	**pBAR-2**	**pBAR-5**	**pBAR-6**	**pBAR-7**	**pBAR-8**	**pBAR-9**	**pBAR-10**	**pBAR-11**	**pBAR-12**	**pBAR-13**
Concentration(ng/ul)	43.5	88.1	83.8	118.6	100.7	58.8	94.3	82.7	98.5	74.9
260/280	1.93	1.80	1.84	1.79	1.86	1.88	1.88	1.82	1.83	1.80
260/230	1.62	1.59	1.76	1.60	1.98	1.90	1.72	1.64	1.65	1.48
sample	**pBAR-2**	**pBAR-5**	**pBAR-6**	**pBAR-7**	**pBAR-8**	**pBAR-9**	**pBAR-10**	**pBAR-11**	**pBAR-12**	**pBAR-13**

Date 7.30

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) **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.

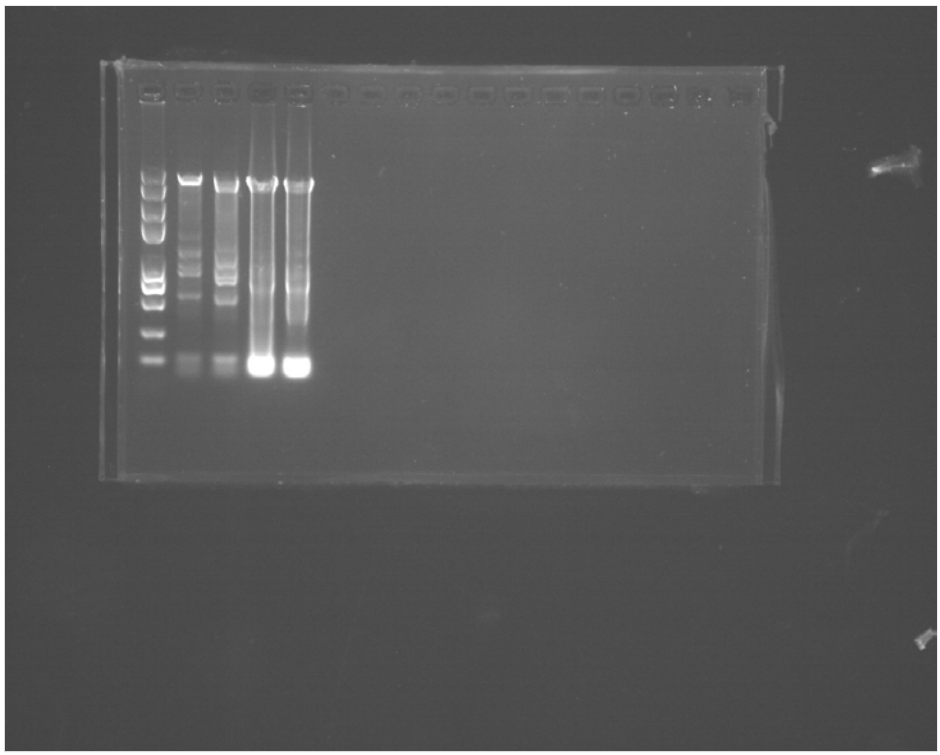
OD measurement result:

sample	ccm PCR pur 1+2+3+4+5+6+7+8
Concentration(ng/ul)	531.8
260/280	1.88
260/230	2.17

sample	ccm PCR pur 9+10+11+12+13+14+15+16
Concentration(ng/ul)	365.2
260/280	1.85
260/230	2.17

帮助

Agarose gel electrophoresis Result:



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

Plasmid Extraction of PYRED Recorder: 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	RED-1	RED-2	RED-3
Concentration(ng/ul)	24.2	26.2	22.1
260/280	1.97	1.95	1.86
260/230	3.50	3.26	2.15

Double digestion of ccm Recorder: Liudong Luo, Zhenyu Jiang, Menglong Jin 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

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Sample	1	2	3	4	5	6	7	8
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
Xbal(μL)	1	1	1	1	1	1	1	1
pstl(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
Xbal(μL)	1	1	1	1	1	1	1	1
pstl(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
Xbal(μL)	1	1	1	1	1	1	1	1
pstl(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:

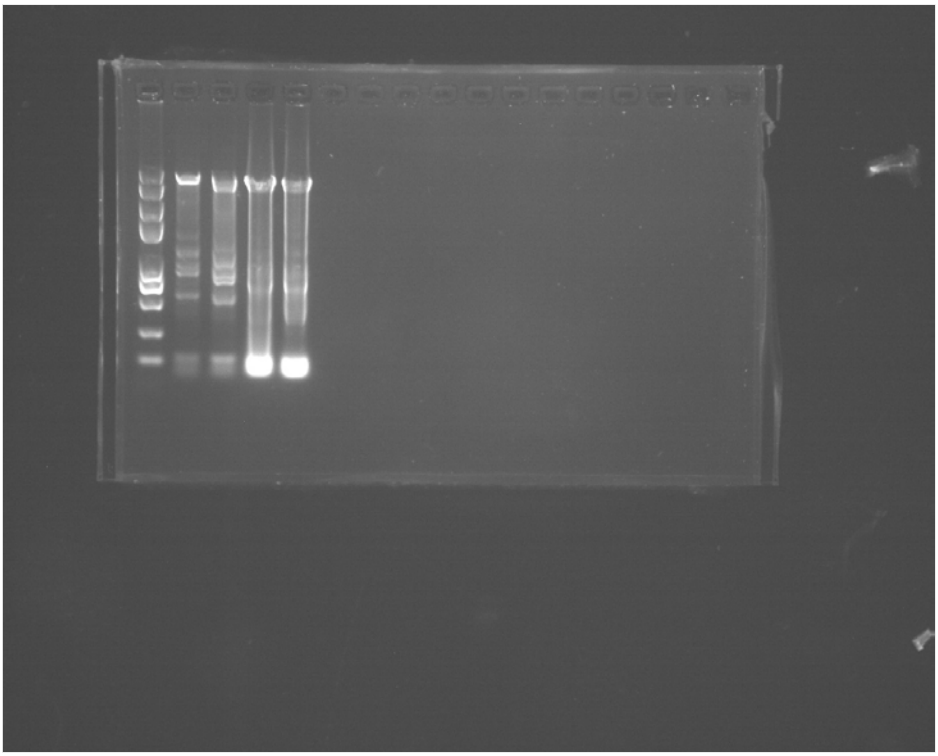
sample	ccmXPdd 1+2+3+4+5+6+7+8+9+10+11+12+13+14+15+16

帮助

sample	ccmXPdd 1+2+3+4+5+6+7+8+9+10+11+12+13+14+15+16
Concentration(ng/ul)	64.5
260/280	1.80
260/230	1.59

sample	ccmXPdd 17+18+19+20+21+22+23+24+25+26+27+28+29 +30+31+32
Concentration(ng/ul)	55.3
260/280	1.85
260/230	1.63

Agarose gel electrophoresis Result:



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

Bacteria PCR Recorder: Meiying Cui，Chenyang Li

Experimental materials

1. Template: bacteria picked from the plate ;
2. Primer: , T7,T7-TEER,Mtr-res-f,Mtr-res-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	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

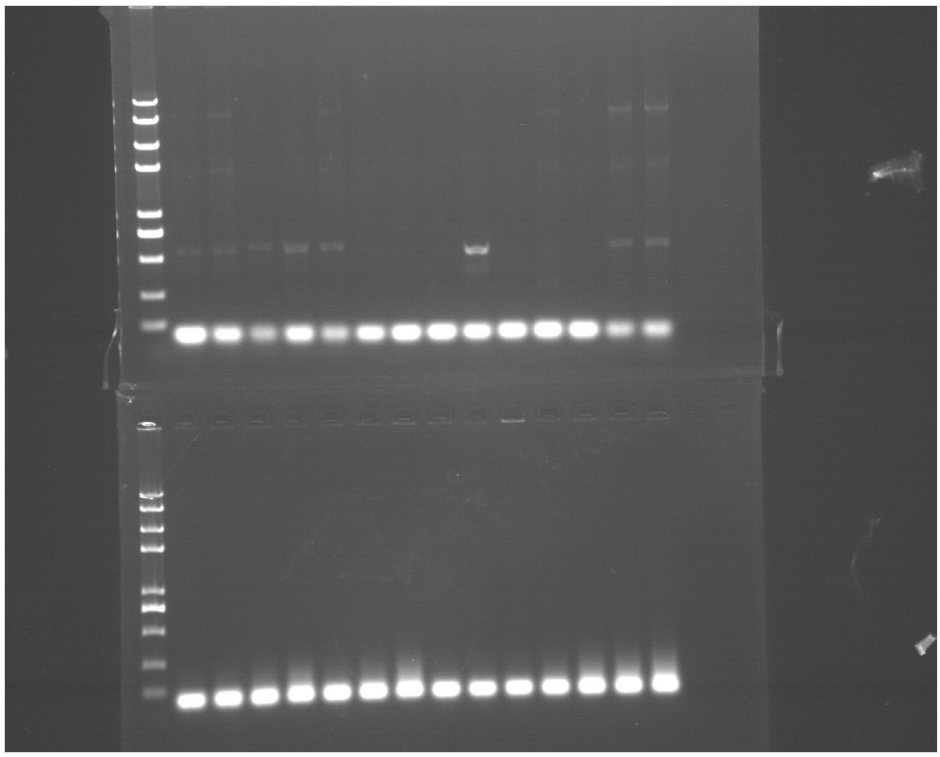
sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
2X Taq DNA polymerase	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL	10 μL
template	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL
T7	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	1 μL	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

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 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	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	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	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

Bacteria PCR Recorder: Meiying Cui

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 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	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	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	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

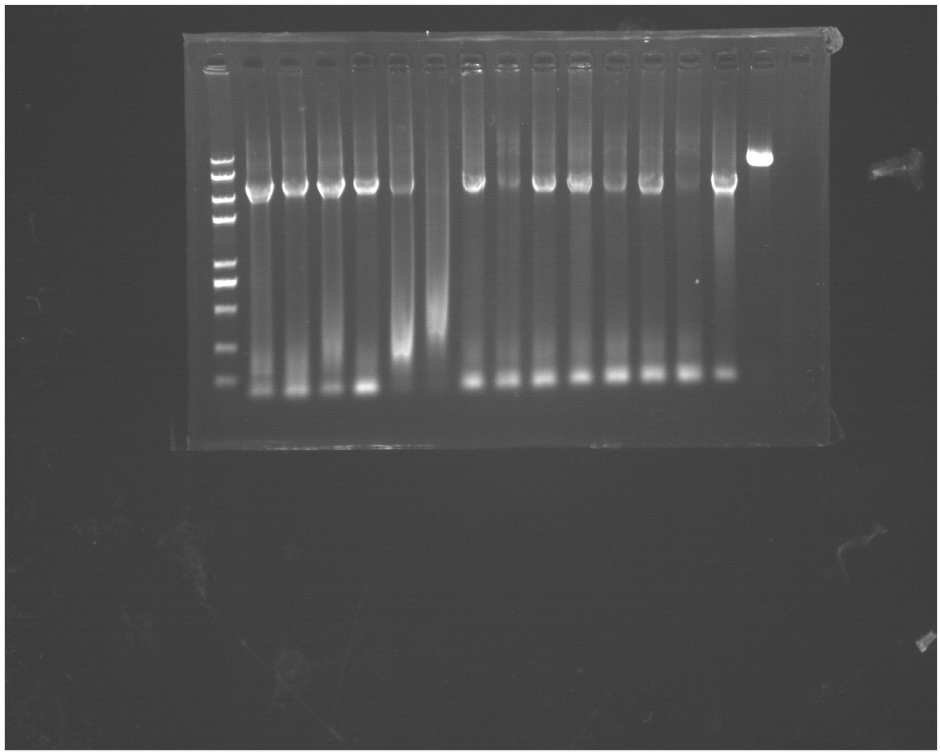
PCR reaction 1-9 Parameters setting：

stage	**temperature**	**time**
step 1	94	4 min

帮助

stage	temperature	time
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 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×PrimeStar (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

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

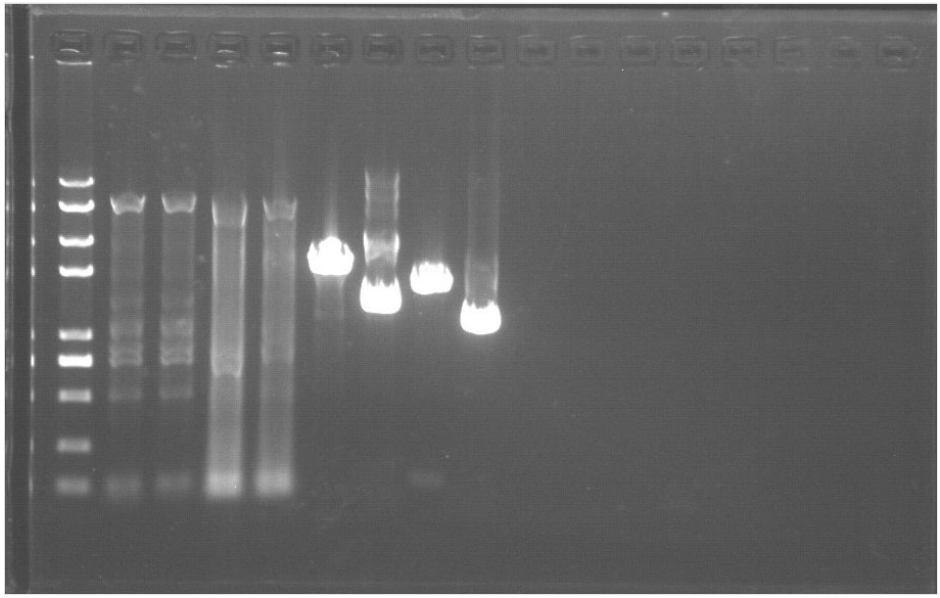
30 cycles(step 2 ~ step 4) **Purification of full ccm PCR product Recorder: Zhenyu Jiang, Liudong Luo** (1). Add 900 μL Buffer B3 to the 180 μL solution and mix it up. Add it to an adsorption column. (2). 11,000 rpm centrifuge for 30 s, and then discard the filtrate. (3). Add 500 μL Wash Solution, 12,000 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:



(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:

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

Sample	17	18	19	20	21	22	23	24
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
Xbal(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 Xbal, pstI and 10× FastDigest Green Buffer(from Thermo Fisher Scientific)
3. Nuclease-free water

Reaction system:

Sample	1	2	3	4
Xbal(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

Sample	1	2	3	4
puc(μL)	5	5	5	5
total(μL)	20	20	20	20

帮助

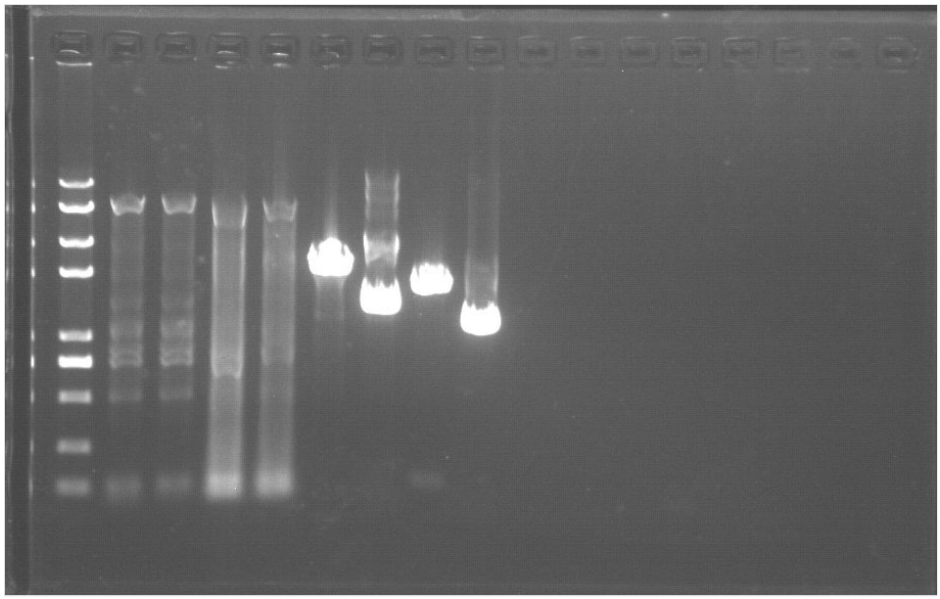
Sample	1	2	3	4
XbaI(μL)	1	1	1	1
pstI(μL)	1	1	1	1
nuclease-free water(μL)	9	9	9	9
fastdigest green buffer(μL)	2	2	2	2
fast AP(μL)	1	1	1	1
pTB(μL)	6	6	6	6
total(μL)	20	20	20	20

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. 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

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

帮助

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

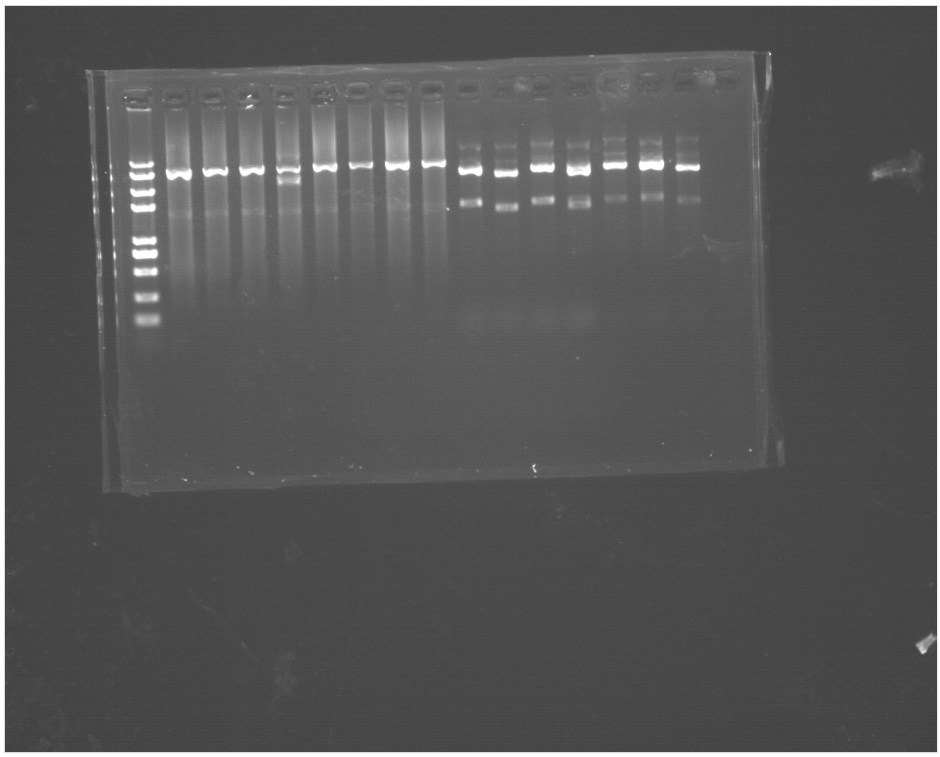
Reaction system:

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

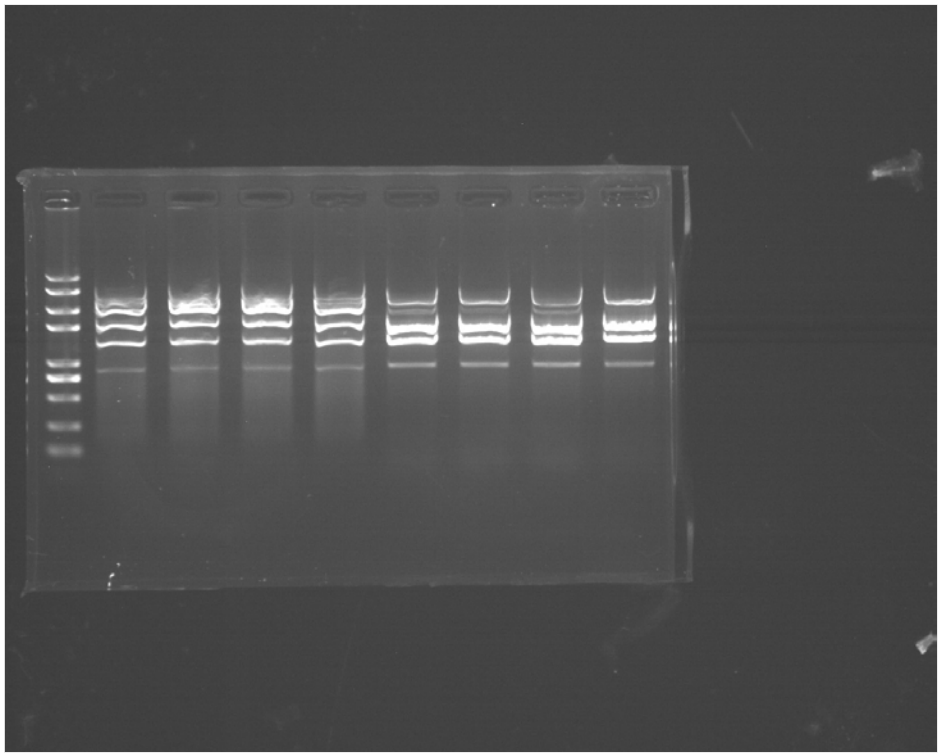
Mix gently and incubate at 37 degree Celsius for 20 hours .

帮助

Agarose gel electrophoresis and purification Result:



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



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

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

10-22 20:43 **梁永浩** 创建了文档

10-22 20:47 **梁永浩** 编辑了文档

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创建讨论后，这些人会收到通知：

停止接收该内容的通知　编辑通知成员

帮助