Experiment Name: pET-30a plasmid extraction

Date：2019/07/09 **Operators: Shi Jing Bai Ning Yu**

Detailed Steps	Remarks
Extraction of pET-30a plasmid (8 test tubes) **Strictly follow the steps of the plasmid extraction kit.** Note: Receive bacteria twice (3-4 ml bacterial solution) 50 μL of eluate was adde.	

Experiment Name： CenA temperature gradient amplication

Date：2019/07/10 **Operators: Shi Jing Bai Ning Yu**

Detailed Steps	Remarks
The optimal annealing temperature of the primers was determined by PCR temperature gradient amplification of CenA gene and DNA gel electrophoresis. (1) DdH _ 2O, polymerase Buffer, primer, dNTPs and plasmid were added to each PCR system according to the ratio given by protocol. Three tubes were designed according to different primers and temperature gradient. (2) The temperature gradient was set from 55-58.5oC, and only 2, 6, 10 slots were used, which were 56.1, 57.6, 59.0oC respectively. (3) The agarose gel was prepared at 0.8% agarose concentration and 50 ml agarose gel was prepared.	This column indicates special phenomena or unexpected errors in operation.

Date：2019/07/10 **Operators: Shi Jing Bai Ning Yu**

Detailed Steps	Remarks
The optimal annealing temperature of the primers was determined by PCR temperature gradient amplification of CenA gene and DNA gel electrophoresis. (4) After PCR was finished, 2 μl samples were taken and mixed with about 1 μl L loading buffer, then the samples were electrophoresised at 150 V for 20 min, and the results were observed under ultraviolet light. (5) The remaining samples were stored at -20oC.	This column indicates special phenomena or unexpected errors in operation.

PCR Special Form

Gene Name：CenA

Primer sequence：F1: GGAATTCCATATGATGAAGTACCTGCTGCCG

F2: GCTCTAGATCACACAAGAAGGTACTAGATGAAG

R: CGGGATCCTTACCAACGCGCGTTACG

Date：2019/07/10 **Operators: Shi Jing Bai Ning Yu**

Reagent	Volume	Total Volume
4x buffer	10 μL	50 μL
10 mM dNTPs	1 μL	
5 '-primer/3' -primer	2.5 μL	
Phusion DNA polymerase	0.5 μL	
DEIONIZED WATER	31.5 μL	
Template plasmid	2 μL	

PCR Program

First unspin	Unspin	Annealing	Extension	Cycle		
98ºC	98ºC	57.5ºC	72ºC	cycle	72ºC	4ºC

30 s	10 s	30 s	1min	30×	5 min	∞

Experiment Results

To be supplemented.

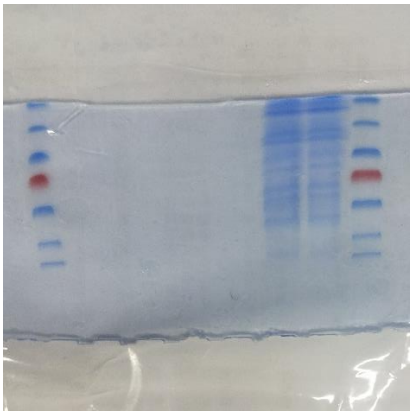
Detection of Proteins in Concentrated Bacterial Solution by Experiment Name: TCA

Date：2019/07/11 **Operators: Shi Jing Bai Ning Yu**

Detailed Steps	Remarks
Please specify the operation name, reagent name, reagent dosage, temperature, reaction time and instrument setting parameters in detail; when adding steps, please stop the cursor at the top of the lower left corner of each line, and click after the plus sign appears.	This column indicates special phenomena or unexpected errors in operation.
(6) 200 μl of overnight shaking culture solution was added to liquid LB culture medium for about 3 hours, and the OD600 was 0.82.	
(7) Take 1.5ml (1) of bacterial fluid, centrifuge at 10000rpm for 1min, take 1ml of supernatant in the Ep tube, and collect the remaining bacteria in the Ep tube.	
(8) 250 μl of precooled 100% TCA was added to the supernatant, and the supernatant was discarded after 30 min of ice bath, 13 000 rpm of centrifugation at 4 ℃ for 10 min.	
(9) Add 200 μl precooled acetone washing precipitation, 13 000 rpm, centrifuge at 4 ℃ for 10 min, and discard the supernatant.	
(10) Repeat step (4).	
(11) Place the precipitate in the fume hood for 5 to 10 minutes, and dry the precipitate.	Not to be too dry
(12) 50. Mu. L of 0.1 m Naoh solution, 25. Mu. L of ddh2o and 25. Mu. L of 4 X SDS loading buffer (2% mercaptoethanol) were adde to that clear solution, and 75. Mu. L of ddh2o and 25. Mu. L of 4 X SDS loading buffer (2% mercaptoethanol) were adde to the mycelium in turn.	Open the cap at 2 min to prevent the cap of Ep tube from bursting
(13) 14 000 rpm centrifugation for 10 min. (Supernatant cannot be centrifuged)	

Date：2019/07/11	Operators: Shi Jing Bai Ning Yu
Detailed Steps	**Remarks**
Please specify the operation name, reagent name, reagent dosage, temperature, reaction time and instrument setting parameters in detail; when adding steps, please stop the cursor at the top of the lower left corner of each line, and click after the plus sign appears. (14) Take 30 μl sample, 80 V for 20 min, 120 V for 50 min. (15) Coomassie brilliant blue staining for 30 minutes, decolorizing solution elution for 6 hours, the results were observed.	This column indicates special phenomena or unexpected errors in operation.

Experiment Results



Results of Figure SEQ Figure Gel electrophoresis

The size of the target protein is 46.7 kD. Only the bacterial band appears, because the army weight also contains a large number of other proteins, more heterozygous bands, and can not determine whether the target protein corresponding to the target band, we need to change the conditions to continue the experiment.

Experiment Name: endoglucanase PCR and glue recovery

Date：2019/07/16	Operators: Bai Ning Yu Liang Si Wen

Detailed Steps	Remarks
(1) PCR: One group did the temperature gradient of the PFU mix enzyme, and the other group used phusion enzyme (to change the condition). (2) Phusion enzyme change condition: HF buffer or GC buffer (3) 3% DMSO or not (4) Plus 0.5 mM Mg2 + or not (5) (Mixing and packing of all components except DMSO and Mg2 +) (6) No.1 to 8:1234 (7) HF HF+Mg2+ HF+DMSO HF+Mg2++DMSO (8) 5 6 7 8 (9) GC GC+Mg2+ GC+DMSO GC+Mg2++DMSO (2) setting the temperature gradient on the PCR apparatus from 53 to 63 DEG C 1 2 3 4 5 6 7 8 9 10 11 12 53 53.4 54.3 55.5 56.6 57.5 58.3 59.2 60.3 61.7 62.6 63 Pfu mix hole 2 ~ 11; phusion hole 6 (the position is not enough, the No.1 hole is 5). (3) Agar gel was prepared in the course of waiting for the amplified fragment, and the agarose was mixed with 0.8% agarose concentration. And preparing 100 ml of agarose gel. (4) after PCR, take 2 μl samples, add about 1 μl L loading buffer to mix the samples, 170 V electrophoresis for 20 minutes, and then observe the results under ultraviolet. The remaining samples were stored at -20 ℃.	

PCR Special Form

Gene Name：endoglucanase（CenA）

Primer sequence：R1: CGGGATCCTTACCAACGCGCGTTACG

F1: GGAATTCCATATGATGAAGTACCTGCTGCCG

Date：2019/07/16	Operators: Bai Ning Yu Liang Si Wen

Reagent	Volume	Total Volume

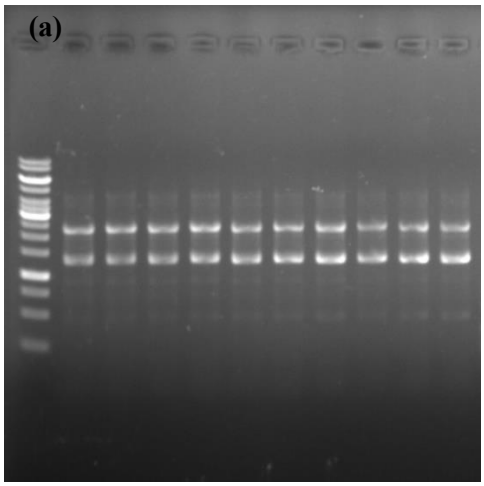
ddH2O	12.4 μL	20
5x PCR buff (HF or GC)	4 μ L	
10 mM dNTPs	0.4 μ L	
5 '-primer/3' -primer	1+1 μ L	
Template DNA (UESTC plasmid)	1 μL	
Phusion DNA Polymerase	0.2 μL	
DMSO (optional)	0.6 μL	
Mg2 + (optional)	0.2 μL	

Reagent	Volume	Total Volume
Pfu mix	10μL	20 μ L
5 '-primer/3' -primer	1+1μL	
DEIONIZED WATER	7μL	
Template DNA	1μL	

PCR Program

First unspin	Unspin	Annealing	Extension	Cycle		
98ºC	98ºC	57.5ºC	72ºC	cycle	72ºC	4ºC
30 s	10 s	30 s	1min	30×	5 min	∞

Experiment Results



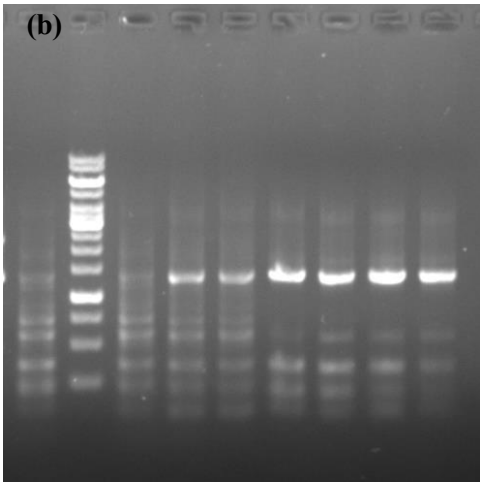


Figure SEQ Figure 1 (a) Pfu mix enzyme-temperature gradient; (B) Phusion enzyme-change condition.

The size of the target band is about 1400 BP.

Pfu mix enzyme could produce the target band, but it also produced a band larger than 2000 BP.

The optimum condition of PCR with Phusion enzyme was GC buffer, annealing temperature was 57.5 ℃.

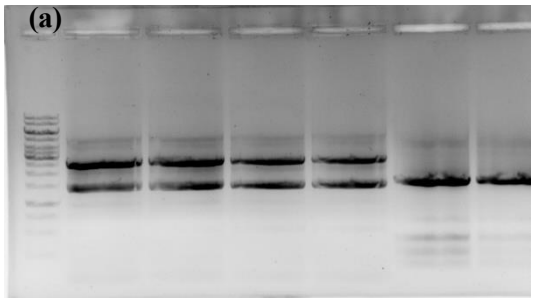


Figure 2 (a) Recycling of destination tape adhesive

Reagent preparation

Reagent name: YEME medium 1L (Streptomyces cereus)

Date：2019/07/16 **Operators: Bai Ning Yu Liang Si Wen**

Reagent	Volume （300mL）
Sucrose	340g（102g）
Glucose	10g（3g）
Yeast Paste	3g（0.9g）
Bacterial peptone	5g（1.5g）
Malt Paste	3g（0.9g）
MgCl2·6 H2O（203.3g/mol）	0.005mol（0.0015mol）

100 mL solid (add 2 G agar) + 100 mL X 2 liquid

Sterilization

Experiment Name: endo-his (ligated pET-30a) PCR recovery

Date：2019/07/19 **Operators: Liang Siwen**

Detailed Steps	Remarks
(16) Endo-his PCR (two primers) First do the F3R2, but the marker added the wrong (blue lid 1kb DNA ladder). F3R2, F4R2 together to do again; Rubber recycling; (17) Disposition Gao's No.1 medium to activate Streptomyces coelicolor (lineation) (18) UESTC new plasmid shake bacteria (100 μL, shake two tubes)	

PCR Special Form

Gene Name: endo-his (ligated pET30a vector)

Primer sequence：R:endo-his R2：acg cgt ccc aac gcg cgt tac

F: endo-his F3：gga att cca tat gat gag cac ccg tcg tac
endo-his F4：cgc gga tcc atg agc acc cgt cgt ac

Date：2019/07/19 **Operators: Liang Siwen**

Reagent	Volume	Total Volume
5 × PCR buffer (GC)	4μL	20 μL
10 mM dNTPs	0.4μL	
5 '-primer/3' -primer	1+1μL	
Phusion enzyme	0.2μL	
DEIONIZED WATER	12.4μL	
Template DNA	1μL	

Mix 4 parts of the mixture and subassemble into 20 μL.

PCR Program

First unspin	Unspin	Annealing	Extension	Cycle	Deactivation	
98ºC	98ºC	57.5ºC	72ºC	cycle	72ºC	4ºC
30s	10 s	30 s	min	30×	5 min	∞

Experiment Results

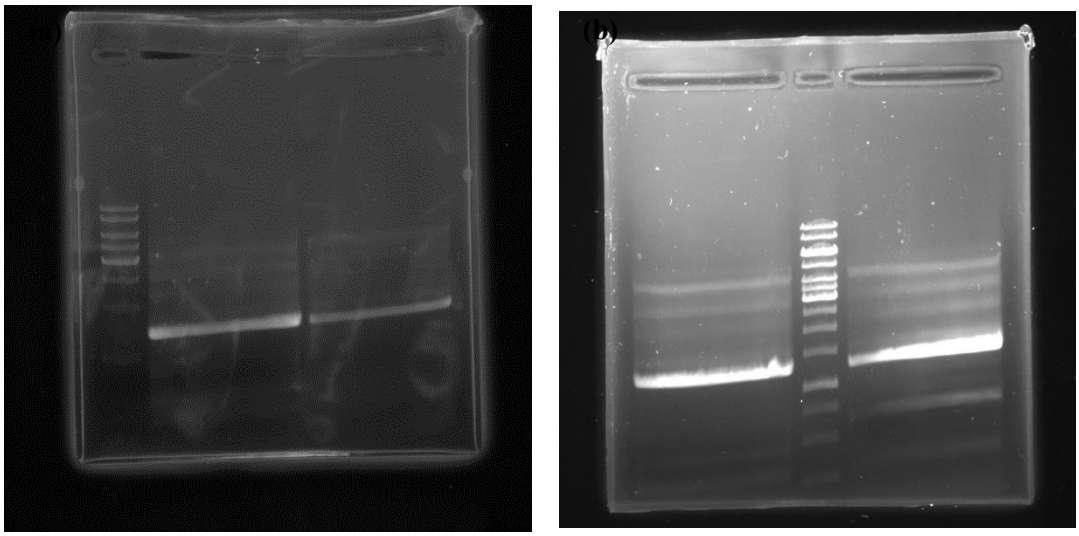


Figure 1 (a) endo-his F3R2 Gel recovery electrophoresis; (B) endo-his F3R2 & F4R2 Gel recovery electrophoresis.

a figure: First made a primer PCR (run in two lanes), but the marker added the wrong, visual size correct.
b figure: Two primers, sequencing identification can be digested, ligated vector.

Reagent preparation

Reagent name: Gao's 1 medium (*Streptomyces coelicolor*)

Date：2019/07/19 **Operators: Liang Siwen**

Reagent	Volume（300mL）
Soluble Starch	20.0g（6g）
KNO3	1.00g（0.3g）

NaCl	0.50g（0.15g）
K2HPO4 • 3H2O	0.50g（0.15g）
MgSO4 • 7H2O	0.50g（0.15g）
FeSO4 • 7H2O	0.01g（0.003g）
Agar	20.0g
Distilled Water	1000mL
pH	7.2~7.4

First, the soluble starch is mixed into paste with a small amount of cold water, heated gently, then water and other drugs are added, and sterilized at 121 ℃ for 20 minutes.

Invert plate, activate *Streptomyces ceruloides* (line, 2 plates), culture at 30℃.

Experiment Name: endo digestion

Date：2019/07/20 **Operators: Liang Siwen & Bai Ningyu**

Detailed Steps	Remarks
(19) Double enzyme digestion of two endo PCR products (20 μL system, two in each group) **Endo（F3R2）** Nde1 0.4 μL Sal1 0.4 μL ddH2O 13.2 μL PCR product 4 μL Buffer 2μL **Endo（F4R2）** BamH1 0.4 μL Sal1 0.4 μL ddH2O 13.2 μL PCR product 4 μL Buffer 2μL 37 ℃ for 1 h Run the glue, recycle	**This enzyme cutting system is the system of enzyme cutting check, which is not quite right, and then cut again in the afternoon.**

Date：2019/07/20 **Operators: Liang Siwen & Bai Ningyu**

Detailed Steps	Remarks

(20) UESTC plasmid extraction (1 tube)
Run glue for size verification.

(21) Endo & pET-30a digestion (50 μL system)

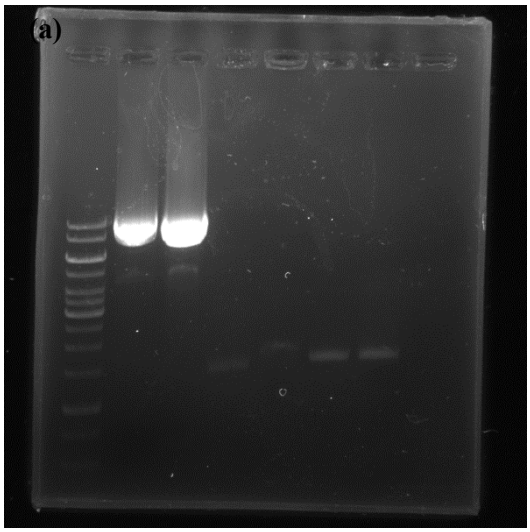
Endo（F3R2）		**pET-30a**	
Nde1	1 μL	Nde1	1 μL
Sal1	1 μL	Sal1	1 μL
ddH2O	21 μL	ddH2O	28 μL
PCR product	22 μL	Plasmid	15 μL
Buffer	5μL	Buffer	5μL

Endo（F4R2）		**pET-30a**	
BamH1	1 μL	BamH1	1 μL
Sal1	1 μL	Sal1	1 μL
ddH2O	21 μL	ddH2O	28 μL
PCR product	22 μL	Plasmid	15 μL
Buffer	5μL	Buffer	5μL

Enzymatic digestion in water bath at 37 ℃ for 2 h, electrophoresis and gel recovery

(22) UESTC bacteria were cultured overnight by shaking 4 tubes (plasmids were extracted the next day).

Experiment Results



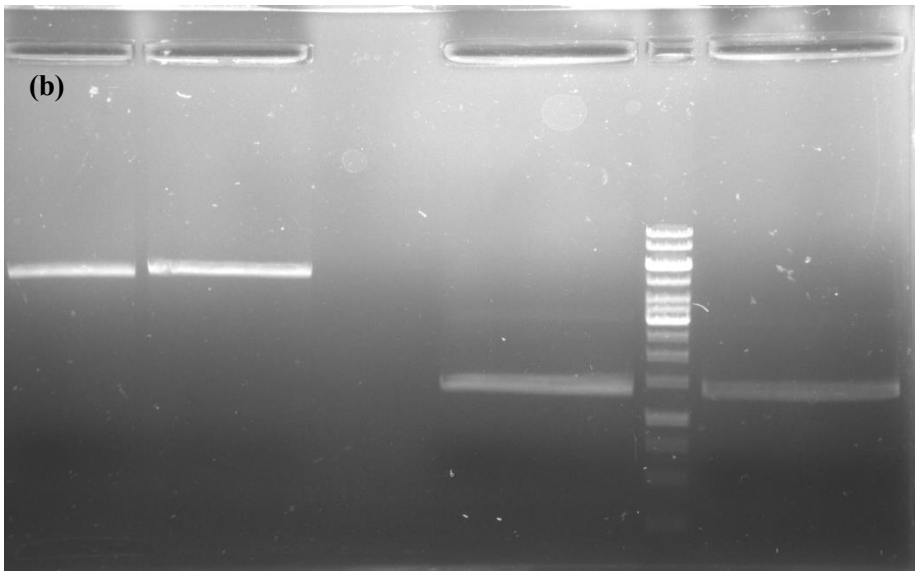


Figure 1 (a) UESTC plasmid and Endo digestion electrophoresis assay; (b) Endo and pet30a digestion electrophoresis assay.

a: The plasmid size is correct, combined with the sequencing results provided by UESTC, the plasmid is correct; endo restriction enzyme digestion only adds 8 μL PCR product, the band is not too bright; F3R2 primers cut out two sizes, it is not clear why; in the afternoon, the enzyme digestion of 50 micron system was performed again.
b: From left to right, the sequence is pET30a F3R2, pET30a F4R2, endo F3R2, endo F4R2.

Experiment Name: endo & pET-30a Gum Recovery from

Enzyme Digestion

Date：2019/07/21 **Operators: Bai Ningyu**

Detailed Steps	Remarks
Recycle with kit glue Run the glue and find it has not been recycled. Cause: New glue recovery kit used, W2 no ethanol added	

Experiment Name: UESTC plasmid extraction

Date：2019/07/21 **Operators: Yan Xueshan**

Detailed Steps	Remarks
Extraction of plasmids by a kit, 4 tubes Run the glue, the size is correct, merge into a tube, put 4 ℃.	

Experiment Name：endoglucanase PCR（F3R2/F4R2）

Date：2019/07/22	Operators: Bai Ningyu

Detailed Steps	Remarks
(1) Phusion: 25 μL system F3R2 * 2 F4R2 * 2 GC buffer (2) Annealing at 57.5 ℃ for 1 min. (3) Agar gel was prepared in the course of waiting for the amplified fragment, and the agarose was mixed with 0.8% agarose concentration. And preparing 100 ml of agarose gel. (4) After PCR is completed, the glue is recycled directly.	

PCR Special Form

Gene Name：endoglucanase

Primer sequence：endo-F3-Nde1 + endo-R2-Sal1
endo-F4-BamH1 +endo-R2-Sal1

Date：2019/07/22	Operators: Bai Ning Yu	
Reagent	**Volume**	**Total Volume**
ddH2O	14.75 μL	
5X GC PCR buffer	5 μL	
10 mM dNTPs	0.5 μL	
5 '-primer/3' -primer	1.25+1.25 μL	25
Template DNA (UESTC plasmid)	2 μL	
Phusion DNA Polymerase	0.25 μL	

Note: Template DNA (UESTC plasmid) is generally added to 1 μL.

PCR Program

First unspin	Unspin	Annealing	Extension	Cycle		
98ºC	98ºC	57.5ºC	72ºC	cycle	72ºC	4ºC
30 s	10 s	30 s	1min	30×	5 min	∞

Experiment Results

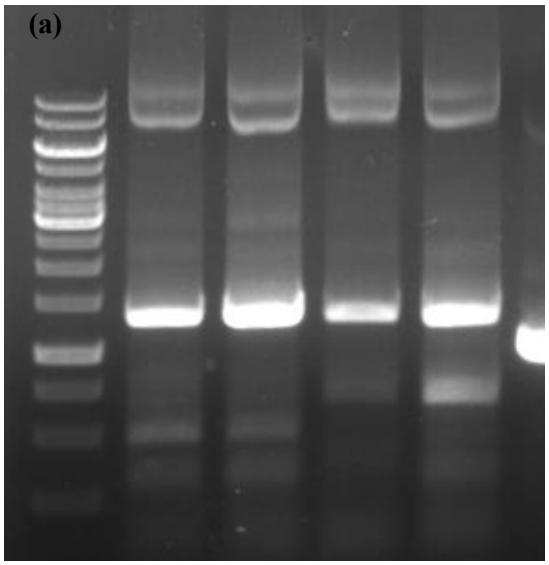


Figure 1 (a) 1-2: endo F3 + R2 3-4: endo F4 + R2

The size of the target band is 1350 bP.

Experiment Name: exoglucanase PCR (F1R1) test

Date：2019/07/22 **Operators: Liang Siwen**

Detailed Steps	Remarks
(10) Phusion enzyme change condition: HF buffer or GC buffer 3% DMSO or not Plus 0.5 mM Mg2 + or not (Mixing and packing of all components except DMSO and Mg2 +) No.1 ~ 8:8765 HF HF+Mg2+ HF+DMSO HF+Mg2++DMSO 4 3 2 1 GC GC+Mg2+ GC+DMSO GC+Mg2++DMSO (2) Annealing at 56 ℃ for 1 min. (3) Agar gel was prepared in the course of waiting for the amplified fragment, and the agarose was mixed with 0.8% agarose concentration. And preparing 100 ml of agarose gel. (4) after PCR, take 2 μl samples, add about 1 μl L loading buffer to mix the samples, 130 V electrophoresis for 30 minutes, and then observe the results under ultraviolet.	

PCR Special Form

Gene Name：exoglucanase

Primer sequence：exo-F1-Nde1：ggaattccatatgATGCCGCGTACCACCCC

exo-R1-Sal1：acgcgtcgacACCCACGGTGCACGG

Date：2019/07/22 **Operators: Liang Siwen**

Reagent	Volume	Total Volume
ddH2O	12.4 μL	
5x PCR buff (HF or GC)	4 μ L	
10 mM dNTPs	0.4 μ L	20
5 '-primer/3' -primer	1+1 μ L	
Template DNA (UESTC plasmid)	1 μL	

Phusion DNA Polymerase	0.2 μL
DMSO (optional)	0.6 μL
Mg^{2+} (optional)	0.2 μL

PCR Program

First unspin	Unspin	Annealing	Extension	Cycle		
98ºC	98ºC	57.5ºC	72ºC	cycle	72ºC	4ºC
30 s	10 s	30 s	1min	30×	5 min	∞

Experiment Results

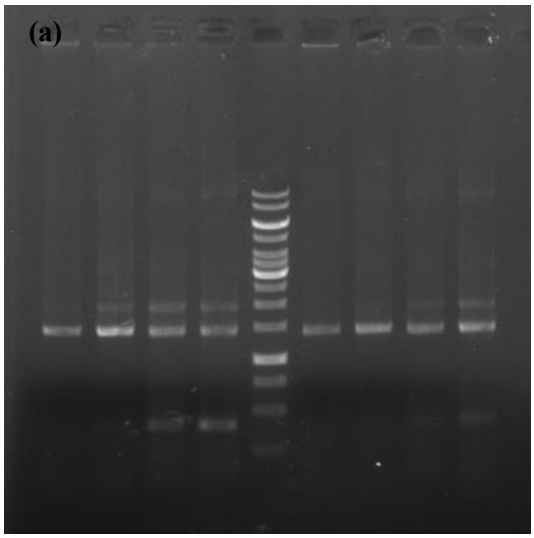


Figure 1 (a) 1-8:

8	7	6	5
HF	HF+Mg^{2+}	HF+DMSO	HF+Mg^{2+}+DMSO
4	3	2	1
GC	GC+Mg^{2+}	GC+DMSO	GC+Mg^{2+}+DMSO

The size of the target band is about 1450 bP.

Experiment Name： exoglucanase PCR(F2R1)

Date：2019/07/22 **Operators: Bai Ning Yu**

Detailed Steps	Remarks
(1) Phusion enzyme: 25 μL system * 6 tubes HF buffer 3% DMSO (2) Annealing at 56 ℃ for 1 min. (3) Agar gel was prepared in the course of waiting for the amplified fragment, and the agarose was mixed with 0.8% agarose concentration. And preparing 100 ml of agarose gel. (4) After PCR is completed, the glue is recycled directly.	

PCR Special Form

Gene Name： exoglucanase

Primer sequence： exoF2-BamH1： cgcggatccATGCCGCGTACCACCCC

exo-R1-Sal1： acgcgtcgacACCCACGGTGCACGG

Date：2019/07/22 **Operators: Bai Ningyu**

Reagent	Volume	Total Volume
ddH2O	15.15 μL	
5X HF PCR buffer	5 μL	25
10 mM dNTPs	0.5 μL	

5 '-primer/3' -primer	1.25+1.25 μ L
Template DNA (UESTC plasmid)	1 μL
Phusion DNA Polymerase	0.25 μL
DMSO	0.6 μL

PCR Program

First unspin	Unspin	Annealing	Extension	Cycle		
98ºC	98ºC	57.5ºC	72ºC	cycle	72ºC	4ºC
30 s	10 s	30 s	1min	30×	5 min	∞

Experiment Results

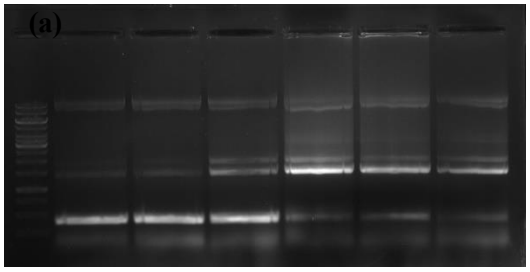


Figure 1 (a) 1-3: exo F2R1 4-6: exo F1R1

The PCR condition (HF + DMSO) of exo F2R1 is not good, the non-specific band is too bright, next time change GC buffer and Mg^{2+}.

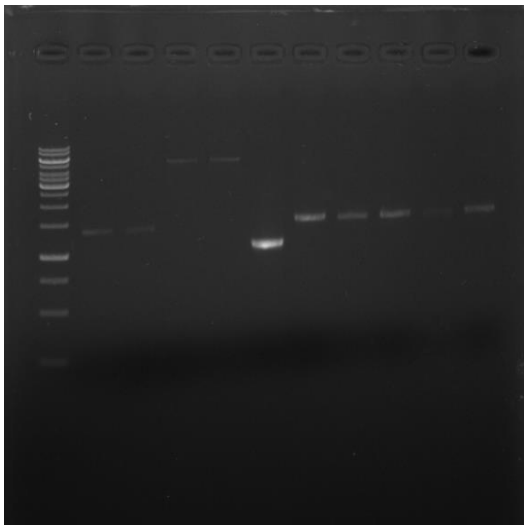


Figure 2 1: endo F3R2 Nde1/Sal1/ 2: endo F4R2 BamH1/Sal1

3: pET-30a Nde1/Sal1 Channel 4: pET-30a BamH1/Sal1

5-7: exo F1R1 8-9: exo F2R2

Experiment Name: endoglucanase PCR production & pET-30a enzyme cutting and glue recovery

Date：2019/07/22 **Operators: Liang Siwen & Bai Ningyu**

Detailed Steps	Remarks
(1) Preparation of 50 μL enzyme cutting system (2) Water bath at 37 ℃ for 2 H (3) Rubber recovery	

Enzyme cutting system

Date：2019/07/22

Operators: Liang Siwen

Reagent	Volume	Total Volume

ddH2O	15 μL（28 μL）	
CutSmart buffer	5 μL	
PCR production（vector）	28 μL（15 μL）	50
Restriction endonuclease	1+1 μL	

Experiment Results

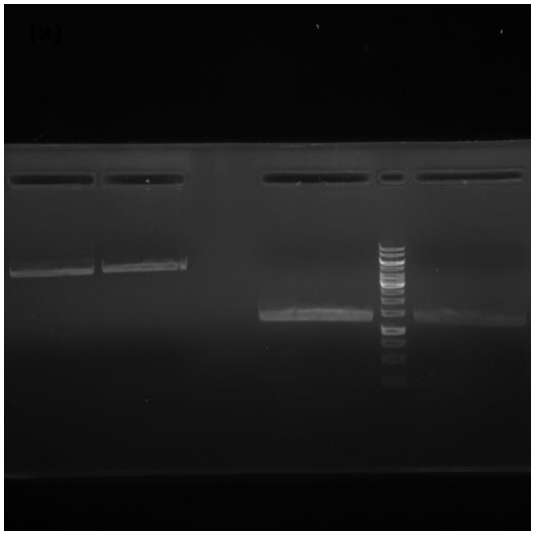


Figure 1 (a) 1: PET-30A NDE1/SAL1 2: PET-30A BAMH1/SAL1

3: endo F3R2 Nde1/Sal1 4: endo F4R2 BamH1/Sal1

Experiment Name: endoglucanase PCR production & pET-30a link

Date：2019/07/22 **Operators: Liang Siwen & Bai Ningyu**

Detailed Steps	Remarks
(1) Preparation of 10 μL enzyme-linked system (2) Metal bath at 16 ℃, overnight	

Enzymatic system

Date：2019/07/22 **Operators: Liang Siwen & Bai Ningyu**

Reagent	Volume	Total Volume
T4 ligase buffer	1 μL	10
PCR production F3R2 (F4R2)	5.5 μL（4 μL）	

Vector	2.5 μL（4 μL）
T4 ligase	1 μL

F3R2（1350 bp）：5.5 μL*3.4 ng/μL =18.7 ng
Vector（5420 bp）： 2.5 μL*4 ng/μL =10 ng

F4R2（1350 bp）：4 μL*10.4 ng/μL = 41.6 ng
Vector（5420 bp）： 4 μL* 6.9 ng/μL =27.6 ng

Reagent preparation

Reagent name: LB medium 250 ml

Date：2019/07/22 **Operators: Yan Xueshan**

Reagent	Volume 1L（250mL）
Peptone	10.0 g（2.5 g）
Yeast Extract	5.0 g（1.25 g）
NaCl	10.0 g（2.5 g）
Agar	5g（1.25 g）

Sterilization

Experiment Name: endoglucanase PCR (F3R2/F4R2) & restriction enzyme & ligation to vector

Date：2019/07/24

Operators: Liang Siwen & Bai Ningyu & Yan Xueshan

Detailed Steps

Remarks

LINKAGE PRODUCTS IN COLON TRANSFORMATION

（1） Ligation products: pET30a-endo-his & pET30a-his-endo-his (each 10 μL);

（2） Adding the ligated product to the sensed state (10. Mu. L);

（3） Ice for 30 min, 42 ℃ heat shock for 90 s;

（4） Ice for 2 min, LB liquid for 1 h at 37 ℃;

（5） Coating: Kan resistance, overnight culture at 37 ℃.

ExoF1&F2 digestion

（1） Preparation of 50 μL enzyme digestion system (same as 7 _ 22)

（2） Water bath at 37 ℃ for 2 h;

（3） Rubber Recovery

Exo F2R1 PCR condition test

（1） Phusion enzyme change condition: HF buffer or GC buffer

3% DMSO or not

Plus 0.5mM Mg2 + or not

(Except DMSO and Mg2 +, the other components are mixed first and then packed separately)

No.1 to 8:1234

GC+Mg^{2+}+DMSO	GC+DMSO	GC+Mg^{2+}	GC
5	6	7	8
HF+Mg^{2+}+DMSO	HF+DMSO	HF+Mg^{2+}	HF

(2) annealing at 56 ℃ for 1 min;

(3) preparing the agarose gel during waiting for the amplified fragment, and preparing the agarose gel with 0.8% agarose concentration (100 mL);

(4) after PCR, take 2 μl samples, add about 1 μl L loading buffer to mix the samples, electrophoresis at 130 V for 30 min, and then observe the results under ultraviolet light.

Exo and pET connection (16 ℃ overnight)

(1) The concentration of restriction products of exo F _ 1-F _ 2 fragments was measured and the ligating system was calculated; (2) System: 1 μL T4 ligase 1 μL T4 connection buffer 2 μL gene fragment 6 μL carrier fragment	

PCR Special Form

Gene Name：exo F2R1

Primer sequence：exoF2-BamH1：cgcggatccATGCCGCGTACCACCCC

exo-R1-Sal1：acgcgtcgacACCCACGGTGCACGG

Operators: Liang Siwen Bai Ning Yu Yan Xueshan

Date：2019/07/24

Reagent	Volume	Total Volume
See above.		

PCR Program

First unspin	Unspin	Annealing	Extension	Cycle		
98ºC	98ºC	57.5ºC	72ºC	cycle	72ºC	4ºC
30 s	10 s	30 s	1min	30×	5 min	∞

Experiment Results

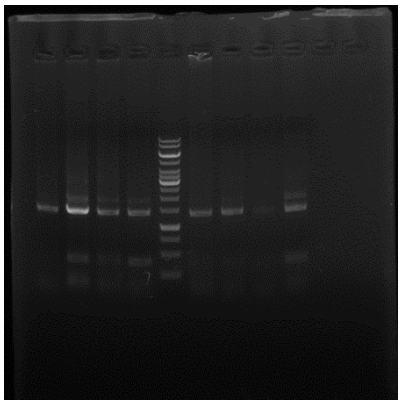
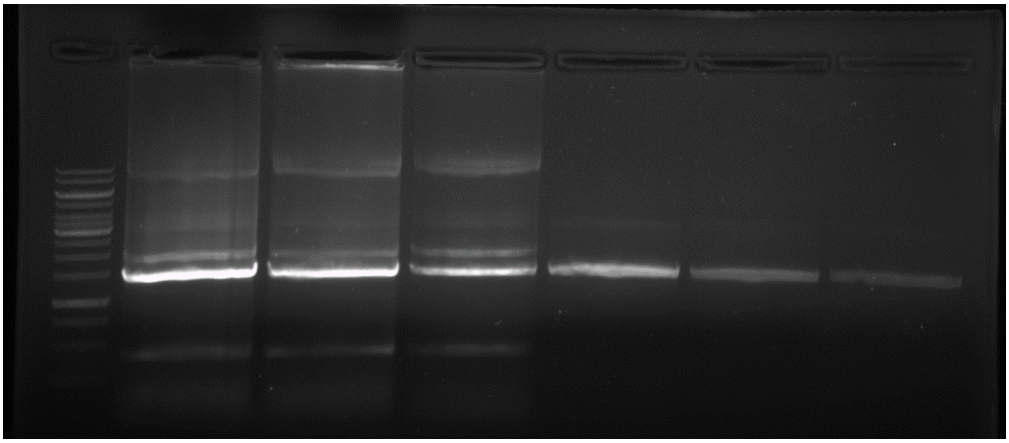


Figure 1 (a) exo F2 PCR condition test; (B) exo enzyme digestion recovery.



（a） Fig.: Exo F2 PCR digestion condition: GC buffer + DMSO

（b） The brightness can also be estimated and the concentration can be connected.

Experiment Name： E.coli transformation with UESTC plasmid

Date：2019/07/23 **Operator name: Yan Xueshan**

Protocol	Notes
Prepare the ice box.	
Take competent E.coli cells from –80 ℃freezer（Strain：DH5α）and thaw on ice	2 tubes
Add 2ul of the plasmid into the competent cells	
Incubate the mixture on ice for 30 minutes. Do not mix	
Heat shock at 42°C for 30 seconds. Do not mix.	
Chill the mixture on ice for 5 minutes.	
Add 800 µl of room temperature LB liquid media (with no antibiotic added) to each tube. (Conduct in the laminar flow cabinet)	
Incubate tubes for 45 minutes at 37 ℃ shaker.	
Centrifugate the mixture and remove a partial supernatant	

Date：2019/07/23	Operator name: Yan Xueshan

Protocol	Notes
Prepare the ice box. Resuspend the cells and spread the rest mixture onto the Amp+ LB plate. Incubate the plates at 37 ℃ overnight	

Experiment Name：

Date：2019/07/25	Operators: Liang Siwen Bai Ning Yu Yan Xueshan

Detailed Steps	Remarks
Endo plasmid extraction (1) According to the results of 24 days, 15, 17 of F3 and 1, 2, 9 of F4 were selected to shake the bacteria overnight (24 nights), and the plasmid was extracted on the morning of 25 days. (2) Plasmid extraction protocol is the same as before; (3) Electrophoresis showed that the plasmids were put forward and sent for sequencing in the afternoon. **Exo linker PCR** （1） In the super-clean platform, 13 colonies were picked up from the growing positive streaks and P. F _ 1, and 20 colonies were picked up from the growing positive streaks and P; （2） PCR mix (20 μL system) Taq enzyme mix 10 μL Primer 1μL+1μL ddH2O 8μL	

Date：2019/07/25	Operators: Liang Siwen Bai Ning Yu Yan Xueshan

Detailed Steps	Remarks
Endo plasmid extraction (Ignoring template DNA volume) （3） PCR & electrophoresis detection **Agrobacterium** （1） According to the result of PCR electrophoresis, 1, 5, 9 of F1 and 4, 5, 9 of F2 were inoculated in LB liquid medium (Kana-resistance); （2） 37 ℃, shake overnight	

PCR Special Form

Gene Name：exo

Primer sequence：exoR1

exoF1&F2

Date：2019/07/25	Operators: Liangsiwen

Reagent	Volume	Total Volume
5 '-primer/3' -primer	1μL+1μL	
Taq enzyme mix	10μL	
Deionized water	8μL	

PCR Program

First unspin	Unspin	Annealing	Extension	Cycle	Deactivation	
95ºC	95ºC	55ºC	72ºC	cycle	72ºC	12ºC
5 min	30 s	30 s	90s	30	5 min	∞

Experiment Results

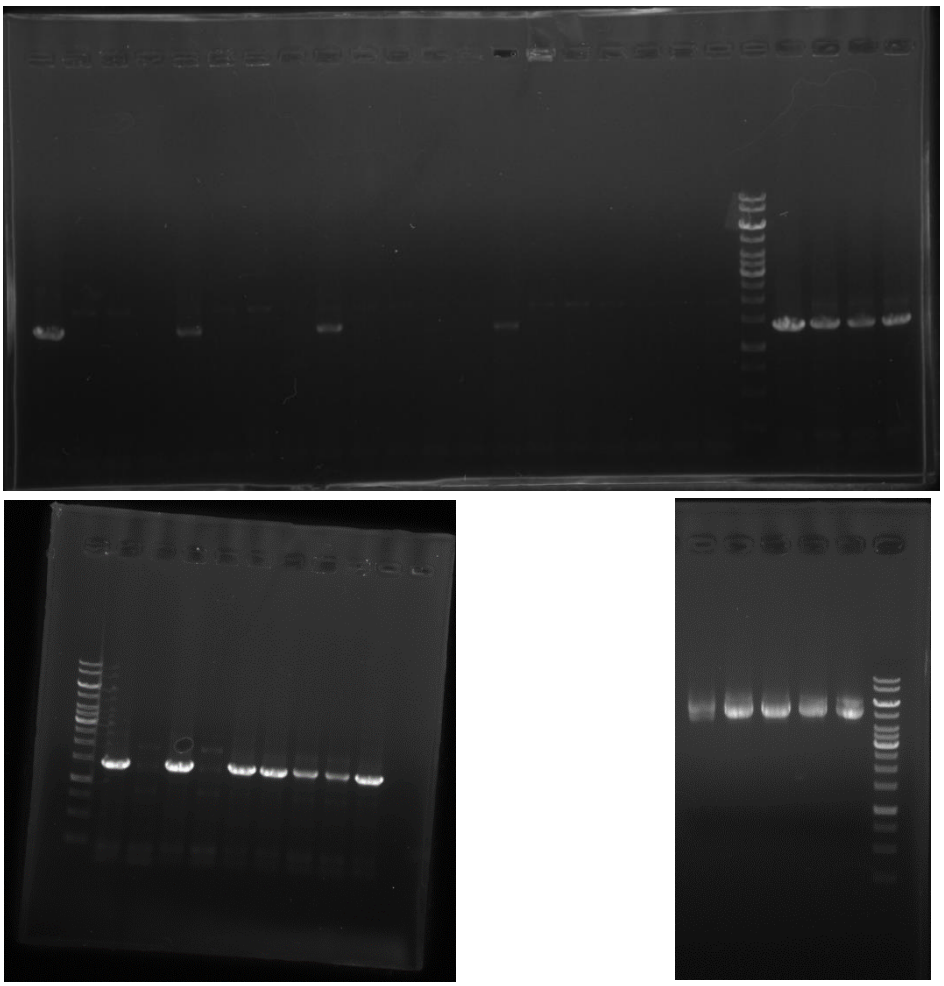


Figure 1 (a) exo F1-PET connection check; (b) exo F2-PET connection check; (C) endo plasma detection

（a） On the left side of marker, 20 holes were used to link exo F1-pET, and only 4 samples had bands;

（b） (A) the right side of marker in the figure and (B) the exoF2-pET connecting products in the figure, with more bands;

All have bands, indicating that the plasmid has been put forward (sample).

Experiment Name: exo Plasmid Extraction & Transfer of *Streptomyces coelicolor* & Activation of *Escherichia coli* Containing Xylanase Plasmid

Date：2019/07/26	Operators: Liang Siwen Bai Ning Yu Yan Xueshan

Detailed Steps	Remarks
Extraction of Exo Plasmid (4) According to the result of strain PCR on the 25_{th}, the strains of F1 (1,5,9) and F2 (4,5,9) were selected to be shaken overnight (25 nights). The plasmid was extracted on the morning of 26_{th}. (5) Plasmid extraction protocol is the same as before; (6) Electrophoresis showed that the plasmids were put forward and sent for sequencing in the afternoon. **Transfer of Streptomyces coelicolor** （1） Streptomyces coelicolor was scraped off the medium with a gun and inoculated into liquid Goss 1 medium (100 mL); （2） Put in a shaker at 30 ℃ and culture. **Activation of Escherichia coli containing xylanase plasmid** （1） The plasmid containing xylanase (-80 ℃) was obtained from brother Miao Ting and was bleomycin-resistant; （2） Inverted plates (4, bleomycin-resistant); （3） Dip a bit of bacterial liquid on the gun head, draw a line on the plate, and culture at 37 ℃.	

Experiment Results

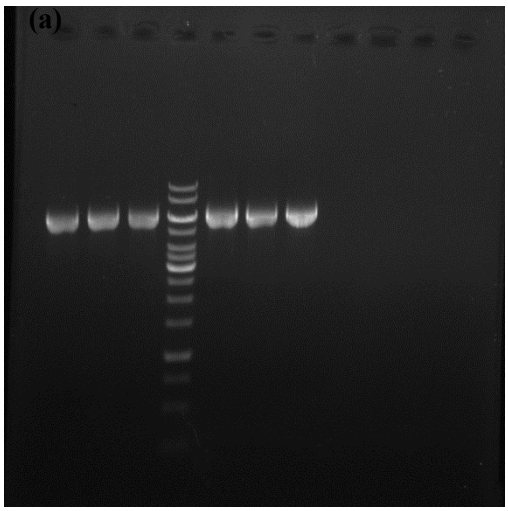


Figure 1 (a) exo-extracted plasmid detection electrophoresis;

The band is clear and shows that the plasmid has been proposed.

Reagent preparation

Reagent name: LB medium (solid, 200 mL × 2 + 100 mL)

Date：2019/07/26 **Operators: Liangsiwen**

Reagent	Volume
Tryptone	10g（5g）
Yeast Extract	5g（2.5g）
NaCl	10g（5g）
Agar	10g（2g＋2g＋1g）

Experiment Name: exo, pET-30a Plasmid BL21 *E.coli* Transformation & *Streptomyces coelicolor* Exchange Medium & *E. coli* streaks with xylanase plasmid & endo、UESTC inoculated plasmid

Date：2019/07/29

Operators: Liang Siwen Bai Ning Yu Yan Xueshan

Detailed Steps	Remarks

Plasmid BL21 of exo, pET-30a was transformed into large intestine.

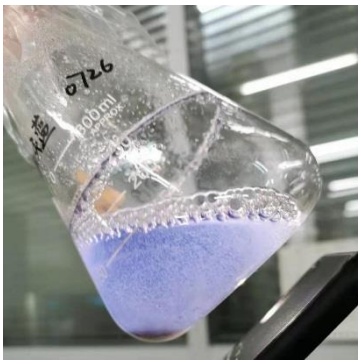
(3) 5 (pET-30a-exo-his) of exo F1R1 and 4 (pET-30a-his-exo-his) of exoF2R1 were selected for protein expression test, and a negative control plasmid (pET-30a) was added and transferred into BL21 (DE3) competent *Escherichia coli.*

(4) The BL21 (DE3) competent Escherichia coli was removed from the refrigerator at -80 ℃ and put on the ice to be melted.

(5) Add 2 μl plasmid, ice bath for 30 min.
(6) 42 ℃ heat shock 90 s.
(7) Ice bath 3 ~ 5 min.
(8) 800 μl LB medium was added and cultured at 37 ℃ for 45 min.
(9) Coat the plate (Kan +) and put it in an incubator at 37 ℃.

***Streptomyces coelicolor* exchange medium**

(1) A large number of mycelial balls were produced after 72 hours of cultivation at 30 ℃ on Gaoshi No.1 liquid medium.



After consulting Ms. Wen Ying's sister in the laboratory, she learned that *Streptomyces azurus* could be cultured in solid medium of imoson, and the bacteria grew slowly, then boiled in sterile water, and the template genome for PCR could be obtained.

Date：2019/07/29	Operators: Liang Siwen Bai Ning Yu Yan Xueshan

Detailed Steps	Remarks
(2) Preparation of imoson solid culture medium, sterilization, inverted plate. (3) The spores of Streptomyces coelicolor were dipped in sterilized cotton swabs, smeared on Imoson plate and placed in an incubator at 30 ℃. **E. coli strain containing xylanase plasmid** (4) The activated *Escherichia coli* containing the xylanase plasmid was placed on a new bleomycin-resistant plate and placed in a 37 ℃ incubator. **Endo, UESTC inoculated plasmids** (1) The selected bacteria were inoculated to the endo of P check: Endo F3R2 (pET-30a-endo-his) 9, 18 Endo F4R2 (pET-30a-hig-endo-his) 6, 11 A total of 4 tubes (Kan +) were connected. (2) *E. coli* containing UESTC plasmid was connected with 4 tubes (Amp +).	

Reagent preparation

Reagent name: Imoson medium (solid, 100 mL)

Date：2019/07/29	Operators: Liang Siwen Bai Ning Yu
Reagent	**Volume 1L(100mL)**
Glucose	10g（1g）

Tryptone	4g（0.4g）
Beef Paste	4g（0.4g）
Yeast Extract	10g（1g）
NaCl	2.5g（0.25g）
Agar	18g（1.8g）

Adjust pH to 7.0 and sterilize at 115 ℃ for 20 min.

Reagent name: 0.2M phosphate buffer (liquid, 300mL)

Date：2019/07/29 **Operators: Liangsiwen**

Reagent	Volume
Na_2HPO_4	20.414g
NaH_2PO_4	0.468g
ddH_2O	Up to 300 ml

Experiment Name：

Date：2019/07/30 **Operators: Liang Siwen Bai Ning Yu Yan Xueshan**

Detailed Steps	Remarks
Extracted plasmids: endo F3 (9-18), endo F4 (6-12), UESTC (7) Extracting plasmids according to protocol; (8) The plasmids were successfully obtained by electrophoresis detection; (9) 9 & 18 & 6 & 12 & UESTC were sent for sequencing.	

Date：2019/07/30	Operators: Liang Siwen Bai Ning Yu Yan Xueshan

Detailed Steps	Remarks
Extracted plasmids: endo F3 (9-18), endo F4 (6-12), UESTC Agaricus (in preparation for IPTG induction tomorrow) Three clones of exoF1.5, exoF2.4 and pET30a empty plasmids were selected and one of them was transferred into 5 mL liquid LB medium (kana-resistant) at 37 ℃ for overnight shaking culture.	

Experiment Results

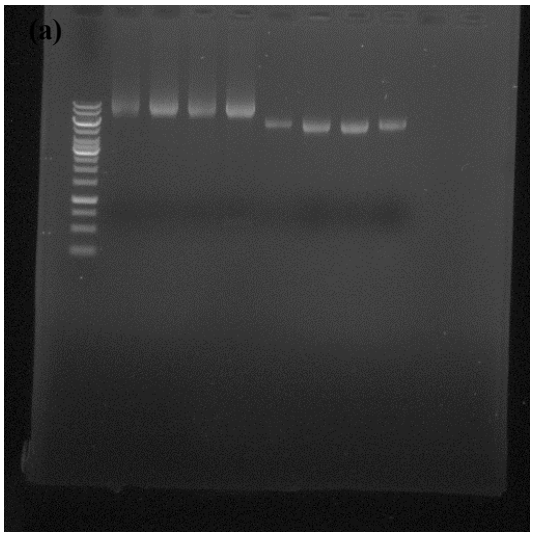


Figure 1 (a) Plasmid extraction and detection

The swimming lanes were: UESTC X 4, F 39 9 & 18, F 46 & 12. There were obvious bands, which indicated that the plasmid was put forward.

Reagent preparation

Reagent name: 400mL 1.5M NaCl mother liquor

Date：2019/07/30	Operators: Liangsiwen
Reagent	**Volume**

0.6mol NaCl（35.06g）
Dissolve to 400 mL of water

Reagent name: 250 mL liquid LB formulation

Date：2019/07/30 **Operators: Liangsiwen**

Reagent	Volume
Tryptone	2.5g
Yeast extract	1.25g
NaCl	2.5g
ddH2O	250mL

In test tubes (5 mL per tube), sterilize

Experiment Name： Exo F1.5, exoF2.4 IPTG small amount of induction conditions

Date： 2019/07/31

Operators： Liang siwen Bai ningyu Yan xueshan

Detailed Steps	Remarks
① Absorb 50 mu L overnight culture (press 1:100) and transfer it to 5mL fresh culture medium. When OD600 is 0.6-0.8 (about two and a half hours), set an IPGT 0 as uninduced (before induction).Inducible IPTG was added and the inducible gradient was set at 0,0.2, 0.4, 0.6, 0.8, 1.0mm, 37℃, 220rpm for induction for 3h. ② Calculation method of IPTG addition: $V(IPTG) = \frac{IPTG\ \text{final concentration}c \times \text{Bacteria liquid product}v}{IPTG\text{original concentration}}$ IPTG reservoir： 0.8 mol/L So add 0, 1.25, 2.5, 3.75, 5, and 6.25 respectively ③ OD_{600}=1.26 (IPTG gradient 0) was measured. 795 micron bacterial solution (1 OD) was taken as the solution for each group. After induction, the solution was centrifuged at 12000rpm for 1min to remove the supernatant. ④ Add 1× supernatant buffer 100 mul (50 mul 2X supernatant buffer + ddH2O) to the bacterial precipitate. Boil at 100℃ for 10min, boil and centrifuge at 12,000 RPM for 5min. ⑤ Sds-page electrophoresis detection, sample loading volume calculation: 20 mul (according to the bacteria solution OD600 before and after induction, OD_{600} was converted into 0.2 sample loading). 8% separation glue. ⑥ dyeing for 30min, decolorization	Cook for 2 minutes and then remove the lid

Experiment Results

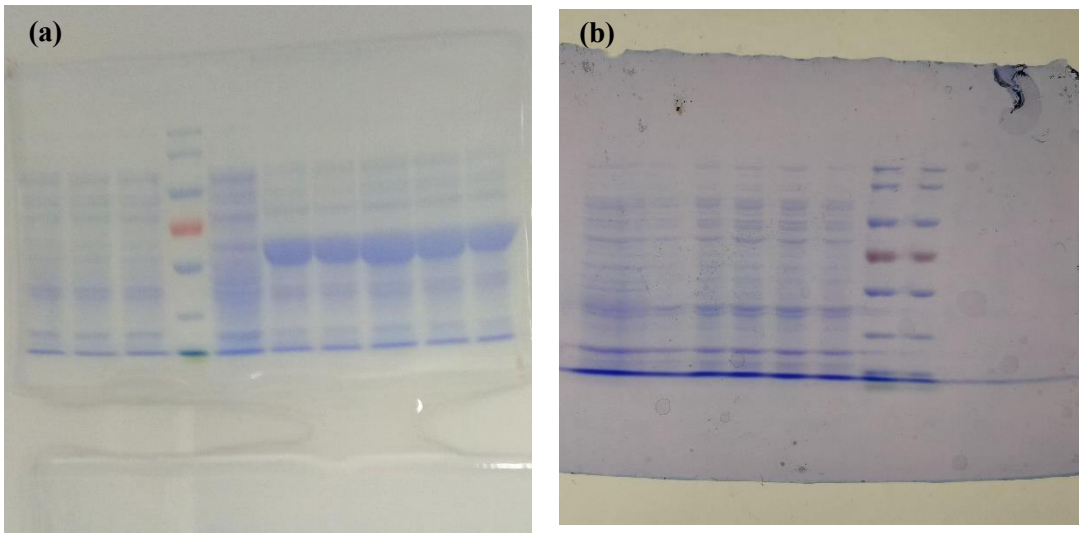


Figure 1 (a) ExoF2 37℃IPTG induced protein electrophoresis; (b) ExoF1 37℃ iptg-induced protein electrophoresis (complement)

Red marker was 70kD (the last marker was 100kD and the next marker was 50kD), and the target protein was about 51.5kd, which could induce the target protein in terms of its size, which needed to be determined by western blot after purification. In addition, the bacteria grew too fast at 37℃, which may have formed inclusion bodies (not properly folded, no function). Try again 25℃, 30℃ induction temperature. (a picture of exo F3 is missing, the hand is washed off by mistake

Experiment Name： Exo F1.5, exoF2.4 IPTG small amount of induction conditions

Date: 2019/07/31

Operators: Liang siwen Bai ningyu Yan xueshan

Detailed Steps	Remarks
⑦ Absorb 50 mu L overnight culture (press 1:100) and transfer it to 5mL fresh culture medium. When OD600 is 0.6-0.8 (about two and a half hours), set an IPGT 0 as uninduced (before induction).Inducible IPTG was added and the inducible gradient was set at 0,0.2, 0.4, 0.6, 0.8, 1.0mm, 37℃, 220rpm for induction for 3h. ⑧ Calculation method of IPTG addition: $V(IPTG) = \frac{IPTG\ \text{final concentration}c \times \text{Bacteria liquid product}v}{IPTG\text{original concentration}}$ IPTG reservoir: 0.8 mol/L So add 0, 1.25, 2.5, 3.75, 5, and 6.25 respectively ⑨ OD_{600}=1.26 (IPTG gradient 0) was measured. 795 micron bacterial solution (1 OD) was taken as the solution for each group. After induction, the solution was centrifuged at 12000rpm for 1min to remove the supernatant. ⑩ Add 1× supernatant buffer 100 mul (50 mul 2X supernatant buffer + ddH2O) to the bacterial precipitate. Boil at 100℃ for 10min, boil and centrifuge at 12,000 RPM for 5min. ⑪ Sds-page electrophoresis detection, sample loading volume calculation: 20 mul (according to the bacteria solution OD600 before and after induction, OD_{600} was converted into 0.2 sample loading). 8% separation glue. ⑫ dyeing for 30min, decolorization	Cook for 2 minutes and then remove the lid

Experiment Results

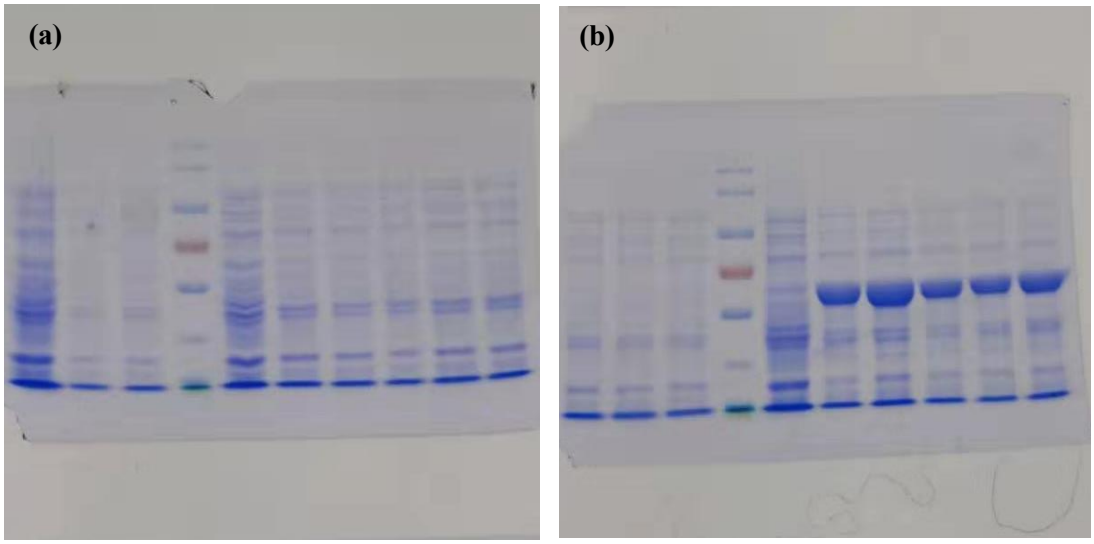


Figure 2 (a) exoF1 30℃IPTG Induced protein electrophoretogram；(b) exoF2 30℃ IPTG Induced protein electrophoretogram

Red marker was 70kD (the last marker was 100kD and the next marker was 50kD), and the target protein was about 51.5kd, which could induce the target protein in terms of its size, which needed to be determined by western blot after purification. Target proteins can also be induced at 30℃. As you can see, the temperature goes down, the induction goes down.

Experiment Name： exo F1.5、exoF2.4 IPTG small amount of induction conditions

Date： 2019/08/02

Operators： Liang siwen Bai ningyu Yan xueshan

Detailed Steps	Remarks
IPTG 诱导 1.Absorb 50 mu L overnight culture (press 1:100) and transfer it to 5mL fresh culture medium. When OD600 is 0.6-0.8 (about two and a half hours), set an IPGT 0 as uninduced (before induction).Inducible IPTG was added and the inducible gradient was set at 0,0.2, 0.4, 0.6, 0.8, 1.0mm, 37℃, 220rpm for induction for 3h. Calculation method of IPTG addition: $V(\mathrm{IPTG}) = \frac{\mathrm{IPTG\ final\ concentration} c \times \mathrm{Bacteria\ liquid\ product} v}{\mathrm{IPTG original\ concentration}}$ IPTG reservoir： 0.8 mol/L So add 0, 1.25, 2.5, 3.75, 5, and 6.25 respectively 2.OD600 (IPTG gradient 0) was measured. 330 micron solution (1 OD) was taken as the solution for each group. After induction, the solution was centrifuged at 12000rpm for 1min to remove the supernatant. 3. Add 1× supernatant buffer 100 mul (50 mul 2X supernatant buffer + ddH2O) to the bacterial precipitate. Boil at 100℃ for 10min, boil and centrifuge at 12,000 RPM for 5min. 4. Sds-page electrophoresis detection, sample loading volume calculation: 20 mul (according to the bacteria solution OD600 before and after induction, OD600 was converted into 0.2 sample loading). 8%separation gel。 ⑤ Dye for 30min and decolorize Genome acquisition of Streptomyces cerulean ① A toothpick was used to pick a spot from a medium full of Streptomyces cerulostreptomyces and disperse it into 100 microns of ddH2O. ② in boiling water for 10 minutes.	**If it's over the range, it's diluted 10 times** **I forgot to centrifuge it for 5 minutes... But it doesn't seem to affect the glue** **There is a piece of rubber running very strange, uneven front, and ran alone for 50 minutes**

Experiment Results

(a)

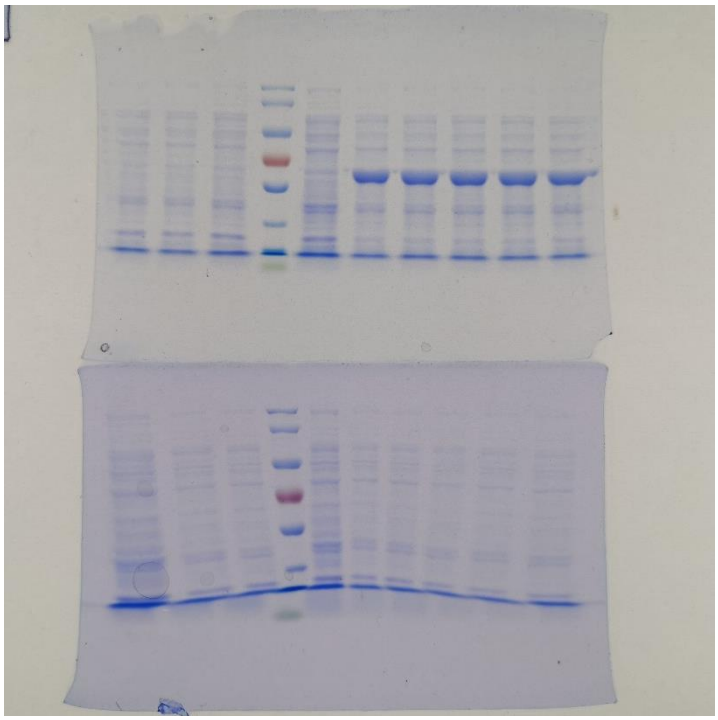


Figure 3 (a) exoF2 25℃IPTG Electrophoretogram of induced protein (top); exoF1 30℃IPTG Electrophoretogram of induced protein (bottom)

Red marker was 70kD (the last marker was 100kD and the next marker was 50kD), and the target protein was about 51.5kd, which could induce the target protein in terms of its size, which needed to be determined by western blot after purification. Target proteins can also be induced at 25℃. As you can see, the temperature goes down, the induction goes down.

Experiment Name：PCR for β-glucosidase gene

Date： 2019/08/03 **Operators：Siwen Liang**

Preparation Protocol	Notes
(1)Add the mycelium of *Streptomyces* into a 1.5ml tube with the 100 μl sterile water by a sterilized toothpick. (2) Mix *Streptomyces* with water by vortex (3) Boil the tube for 10 minutes	Culture incubation requires 48h

Date： 2019/08/03	**Operators：Siwen Liang**
Preparation Protocol	**Notes**
(1)Add the mycelium of *Streptomyces* into a 1.5ml tube with the 100 μl sterile water by a sterilized toothpick. (4) Add the mixture into PCR mixture as the template	Culture incubation requires 48h

PCR Mixture

Target DNA Product Name： β－glucosidase

Reagent	Volume
Buffer	4 μL
10 mM dNTPs	0.4 μL
Primers	1 + 1 μL
Template	2 μL
Phusion DNA Polymerase	0.25 μL
DMSO (Optional)	0.6 μL
$MgCl_2$ (Optional)	0.2 μL
ddH_2O	Up to 20 μL
Total Volume	20 μL

PCR Program

Initial Denaturation	Denaturation	Annealing	Extension	Cycles	Final Extension	Hold
98ºC	98ºC	56ºC	72ºC		72ºC	4ºC
30 s	10 s	30 s	1min	30×	5 min	∞

Experiment Results

Experiment Name：Bacteria Culture Preparation for

Plasmid Extraction

Date： 2019/08/20 **Operator Name：Xueshan Yan**

Protocol	**Notes**
(1) Prepare 5 ml sterile LB liquid medium (2) For INP-pUC57 Kan^+: Pick a single colony from the plate by a tip then inoculate to the prepared culture. (3) For endo-pET30a Kan+, exo-pET Kan+: Add 50μl volume from saturated liquid bacterial germ to the new medium (100x) (4) Add Kana to the new culture for 5μL each (1000x) (5) Incubate at 37℃ shaker overnight	x2 tubes each

Plasmids` Name：

INP-pUC57 Kan+

endo-pET30a Kan+

exo-pET Kan+

Experiment Name：Plasmid Purification by Magen Plasmid Purification Mini Kit

Date： 2019/08/21 **Operator Name：Xueshan Yan**

Protocol	**Notes**
(4) Harvest the bacterial cells by centrifugation at 8000 rpm for 30 seconds. (5) Discard the supernatant (6) Add 250μl of Buffer P1 (RNase A added) and resuspend the bacterial pellet. (7) Add 250μl of Buffer P2 and mix thoroughly by inverting the sealed tube 8 - 10 times (8) Add 350μl of Buffer NP3 and immediately invert the sealed tube 8 - 10 times (9) Centrifuge at 13000rpm for 2 mins (10) Transfer the supernatant (not more than 700μl at a time) into HiPure DNA Mini Column II loaded in the collection tube. Centrifuge at 13000rpm for 1 min. (11) Discard the centrifuged supernatant. (12) Wash the column with 500μl Buffer PW1, centrifuge at 13000rpm for 30 s (13) Discard the centrifuged supernatant. And wash the column with 600μl Buffer PW2. (14) Repeat the step 10. Centrifuge at 13000rpm for 30 s.	x2 tubes each

(15) Discard the centrifuged supernatant. Centrifuge at 13000rpm for 2 mins to remove ethanol. (16) Elute the purified plasmids with 30μl ddH_2O.	

Plasmids` Name：

INP-pUC57 Kan+

endo-pET30a Kan+

exo-pET30a Kan+

Experiment Name：INP-N-endo & INP-N-exo vector construction

Date： 2019/08/26 **Operators：Liang Siwen Yan Xueshan**

Detailed Steps	Remarks
INP-N & pET-endo/exo digestion (10) EcoRV & NcoI double **digestion** Plasmid /PCR fragment 30 μ L Buffer 3 μ L EcoRV 1 μ L NcoI 1 μ L Enzyme 1 h The enzyme digestion product was recovered from the gel of the beta PCR product β PCR TD PCR and ordinary PCR were performed once (25 microns), and the target band of ordinary PCR was found to be brighter. Then normal PCR was used for 4 groups (25 microns).Plastic recycling	

PCR Special Form

Gene Name： β

Primer sequence：R:β-7558-R1

F: β -7558-F2

Date：2019/08/26 **Operators: Liangsiwen Yan Xueshan**

Reagent	Volume	Total Volume
PCR buffer	25μL	50
10 mM dNTPs	1μL	
5 '-primer/3' -primer	2+2μL	
Phanta enzyme	1μL	
DEIONIZED WATER	16μL	
Template DNA	1μL	

Mixed 50 μL system, divided into two parts (25 × 2)

PCR Program

First unspin	unspin	Annealing	Extension	Cycle	Deactivation	
95℃	95 ℃	62 ℃	72 ℃	cycle	72 ℃	4 ℃
00:30 min	30 s	15 s	90s	30×	5 min	∞

Touch-Down PCR Program

① 95℃，15:00
② 95℃，00:45
③ 75℃，00:30，Inc -2℃/Cyc
④ 72℃，01:30
⑤ Goto2,5 More times
⑥ 95℃，00:30
⑦ 62℃，00:30
⑧ 72℃，01:30
⑨ 30x
⑩ 72℃，05:00

Experiment Results

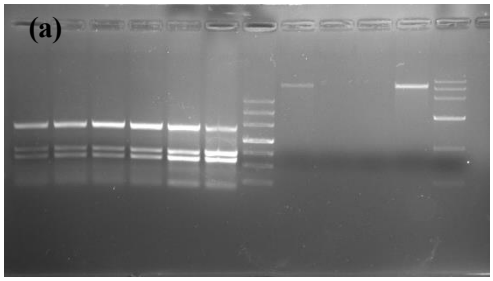


Figure 1 (a) β PCR products & ENDO, EXO recovery of enzyme cut products

The result of TD PCR is not as good as that of common PCR.
Only the endo is cut out, but the amount is very small, and the recycling effect will not be too good.P again tomorrow.

Experiment Name: INP-N-endo & Construction of INP-N-exo Vector

Date：2019/08/27 **Operators: Liangsiwen Yan Xueshan**

Detailed Steps	Remarks
INP-N & pET-endo/exo digestion (11) PET-endo/exo: EcoRV-NcoI double enzyme digestion Plasmid 30 μL Buffer 3μL EcoRV 1 μL NcoI 1 μL (12) INP: EcoRV-EcoRI double enzyme digestion Plasmid 30 μL Buffer 3μL EcoRV 1 μL EcoRI 1 μL (13) β: HindIII-EcoRI double enzyme digestion PCR fragment 30 μL Buffer 3μL EcoRV 1 μL EcoRI 1 μL (14) PET30a: HindIII & EcoRV double enzyme digestion PCR fragment 30 μL	

Date：2019/08/27 **Operators: Liangsiwen Yan Xueshan**

Detailed Steps	Remarks
INP-N & pET-endo/exo digestion Buffer 3μL EcoRV 1 μ L EcoRI 1 μ L Enzyme digestion time: 1h30 **Fragment linking** （1） pET-INP-exo/endo Plasmid 6 μ L INP 2 μ L Buffer 1μL T4 enzyme 1 μ L （2） pET-INP- β Plasmid 6 μ L INP 2 μ L Buffer 1μL T4 enzyme 1 μ L 25 DEG C, connect 2h30 **DH5α conversion (see previous experimental report for steps)**	

Experiment Results

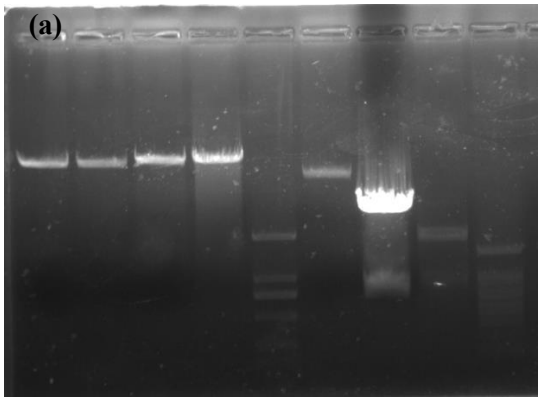


Figure 1 (a) enzymatic digestion product glue

From left to right: endo × 2, exo × 2, marker (D2000), pET, INP, β, marker (our garbage marker)

Experiment Name: INP-N-endo & INP-N-exo carrier bacteria p verification, glucose standard curve determination

Date：2019/08/28 **Operators: Liangsiwen Yan Xueshan**

Detailed Steps	Remarks
Verification of pET-INP-endo/exo Ligation Product Bacteria p (see table below) Exo, endo 16, β 40 (15) pET-INP-endo No banding: 9, 14, 15 Very bright: 5, 6, 7, 8, 10, 11, 12, 13, 16 (16) pET-INP-exo No banding: 5, 8, 11, 12 Bright: 6, 13, 14, 15 (17) pET-INP-β There's no tape. Suspicion is because beta is hard to p, maybe just p doesn't come out (doesn't mean it can't be connected). (18) PET30a: HindIII & EcoRV double enzyme digestion PCR fragment 30 μL Buffer 3μL EcoRV 1 μL EcoRI 1 μL Enzyme digestion time: 1h30 **Fragment digestion: β & pET** Plasmid/fragment 30 μL Buffer 3μL	

Date：2019/08/28	Operators: Liangsiwen Yan Xueshan
Detailed Steps	**Remarks**
Hind III 1 μ L EcoR I 1 μ L **Fragment linking** pET- β Plasmid 3.5 μ L β 4.5 μ L Buffer 1μL T4 enzyme 1 μ L 16 DEG C, connected overnight **Plasmids: pET-endo, pET-exo** **Determination of glucose standard curve** 0, 2, 4, 6, 8 and 10 ml of 0.1% glucose standard solution were drawn in a 50 ml centrifuge tube and diluted with distilled water to prepare dilute standard solution containing 0, 0.4, 0.8, 1.2, 1.6 and 2 mg of glucose per ml respectively. 2 ml of dilute standard solution with different concentrations were taken in a test tube and 2 ml of DNS reagent were boiled in a boiling water bath for 5 min. After cooling, the solution was diluted to 8 mL and the absorbance was measured with a spectrophotometer at 530 nm using a 10 mm colorimetric dish.	It looks, but it's not very linear.

PCR Special Form

Gene Name：pET-INP-exo、pET-INP-endo

Primer sequence：R: INP-EcoRV

F: INP-EcoRI

Date：2019/08/28	Operators: Liangsiwen Yan Xueshan	
Reagent	**Volume**	**Total Volume**
Taq mix	10μL	
H2O	8μL	
5 '-primer/3' -primer	1+1μL	
Template bacteria		

PCR Program: Taq

Gene Name：pET-INP-β

Primer sequence：R:β-7558-R

F:β-7559-F

Date：2019/08/28 **Operators: Liangsiwen Yan Xueshan**

Reagent	Volume	Total Volume
2×buffer	5μL	
Phanta	0.5 μ L	
H2O	1.5μL	
dNTP	1 μ L	
5 '-primer/3' -primer	1+1μL	
Template bacteria		

PCR Program: Phanta

Experiment Results

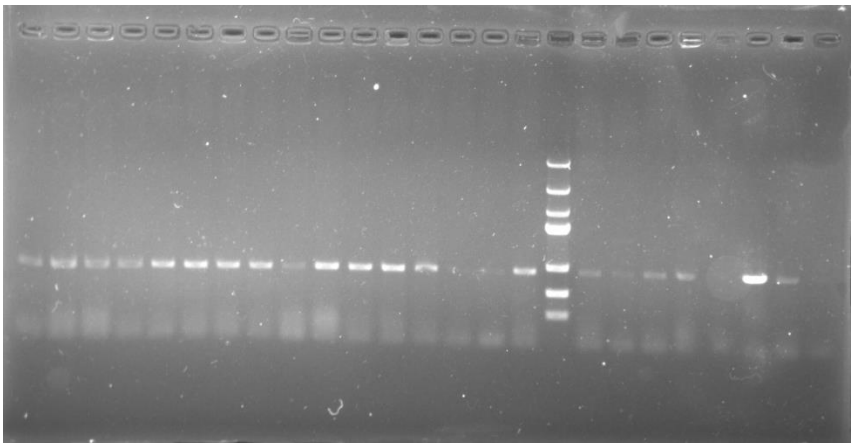


Figure SEQ Figure * ARABIC 1 (a) PET-INP-EXO & ENDOMYCES

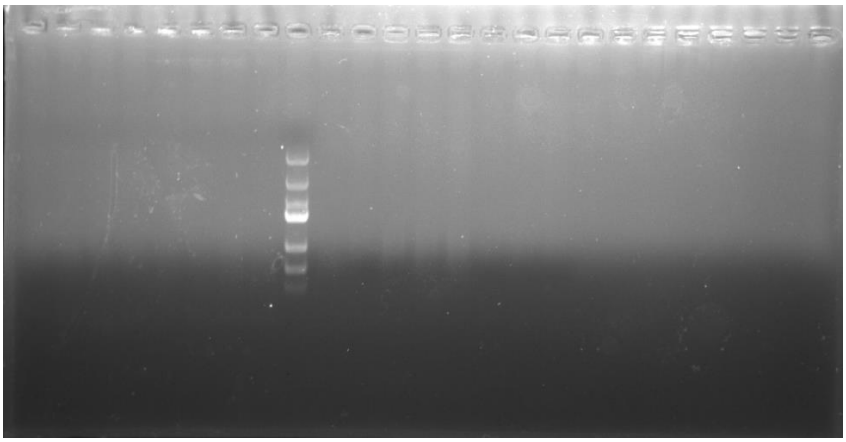


Figure 2 (B) pET-INP-beta p

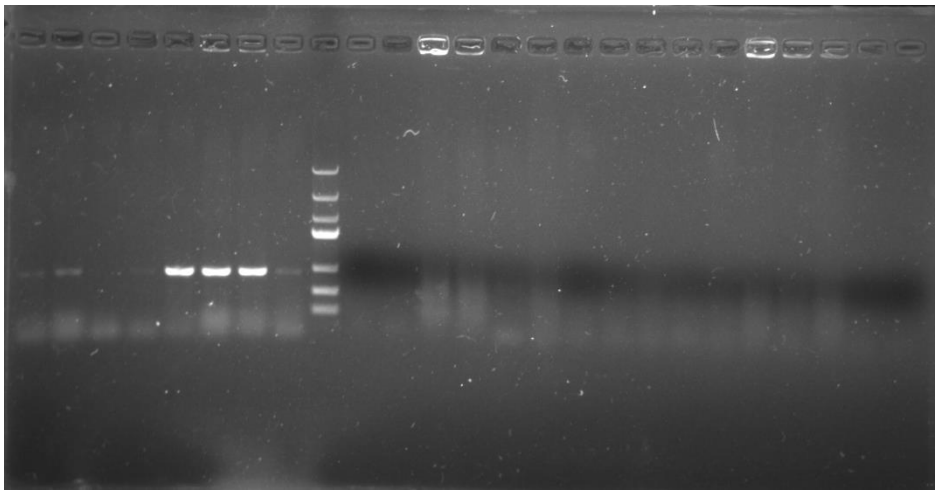


Figure 3 (C) pET-INP-endo & beta.

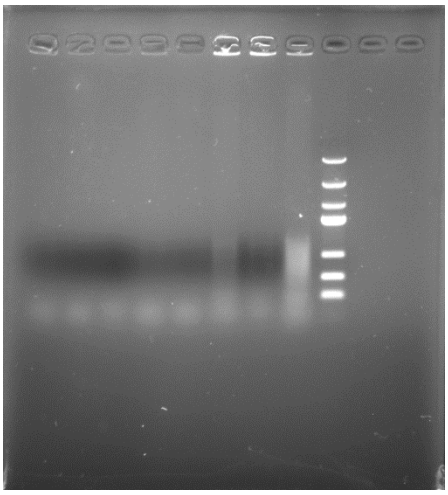


Figure 4 (d) pET-INP-beta p

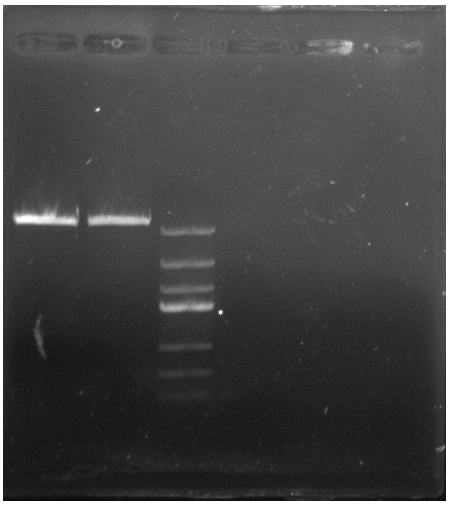


Figure 5 (e) pET HindIII, EcoRI digestion

Both pET-INP-endo and pET-INP-exo had successfully ligated products;

PET-INP-β did not connect successfully, first connect INP-β.

Experiment Name: PET-β Bacteria Verification

Date：2019/08/29 **Operators: Liangsiwen Yan Xueshan**

Detailed Steps	Remarks
Extracted plasmid: exo × 4, endo × 4, β × 2 (See previous experimental records) **CONVERSION OF LINKAGE PRODUCTS: PET-β** (See previous experimental records)	

Experiment Name: PET-β Bacteria Verification

Date：2019/08/30	Operators: Liangsiwen
Detailed Steps	**Remarks**
Verification of pET-β Ligation Products by Bacteria p (8) (19) pET-INP- β Zone 4.	

PCR Special Form

Gene Name：pET-INP-β

Primer sequence：R:β-7558-R

F:β-7559-F

Date：2019/08/28 **Operators: Liangsiwen Yan Xueshan**

Reagent	Volume	Total Volume
2×buffer	5μL	
Phanta	0.5 μ L	
H2O	1.5μL	
dNTP	1 μ L	
5 '-primer/3' -primer	1+1μL	
Template bacteria		

PCR Program: Phanta

Experiment Results

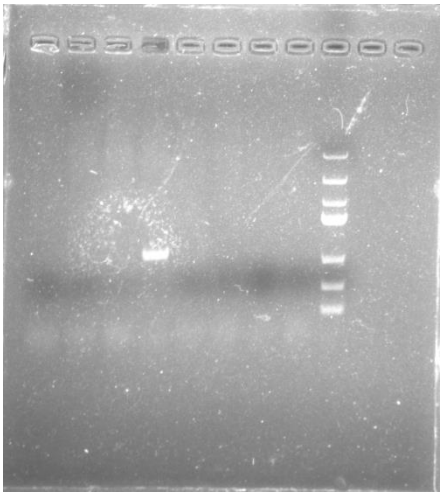


Figure 1 (a) PET-β

No.4. But considering that this gene is difficult to p, it may also be linked to p and can not come out, and then choose one to sequence.

Experiment Name: β-INP-pET plasmid digestion verification

Date：2019/08/31 **Operators: Liangsiwen**

Detailed Steps	Remarks
β-INP-pET plasmid (10 μL system) EcoRI 1 μL EcoRV 1 μL HindIII 1 μL Buffer 1μL Plasmid 6 μL 37℃，2h	

Experiment Results

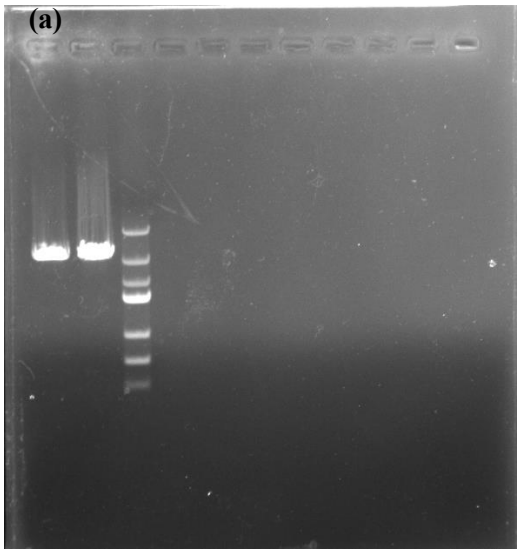


Figure 1 (a) B-INP-PET plasmid restriction endonuclease;Error! No sequence specified.

There's only one band, no plasmid cut. Probably not attached, both false positives.

Experiment Name: pET-exo, pET-endo

Date：2019/09/01

Operators: Liangsiwen Dong Xinran Shi Jing

Detailed Steps	Remarks
Moderate inducement 1.Single colonies were picked up with a gun and cultured in 5mL fresh medium (including antibiotics) at 37℃ and 220rpm overnight. 2. Overnight culture was transferred to 50mL fresh medium at 1:100 (i.e., 500 μL bacterial solution was absorbed) and incubated until OD600 was 0.6-0.8. The induction substance IPTG was added into the bacterial liquid to the final concentration of 0.02mm (exo) and 0.2mm (endo, the mother liquid was used wrongly and was not diluted 10 times), and the induction was conducted at 25℃ and 220rpm for 3h. 3. Exo centrifugation: 1, 0000 rpm for 5 min	

Date：2019/09/01	Operators: Liangsiwen Dong Xinran Shi Jing

Detailed Steps	Remarks
4. Divide 50 mL of the collected bacteria into two centrifuge tubes and dissolve them with 1 mL lysis buffer respectively.	

Experiment Name: exo Ultrasonic Crushing & Enzyme Activity Determination

Date：2019/08/31	Operators: Liangsiwen

Detailed Steps

Remarks

Ultrasonic breakage

Using the ultrasonic crusher of Innovation Laboratory, 30% power, breaking 1 s, stopping 3 s, 5 min × 2

But the feeling did not change the special clarification.

CMC enzyme activity assay

	matched group	Determination of group
	0.8mLCMC+0.2mL Enzyme solution (original solution, different dilution)	
	1ml 0.4M NaOH	
	50℃Water bath (60 min)	
		1mL 0.4M NaOH
	2mL 3, 5-dinitrosalicylic acid, water bath (5min), cooling	
	Dilute with buffer to 8mL	

Date：2019/08/31	Operators: Liangsiwen
Detailed Steps	**Remarks**
In the experimental group, 0.2ml of the enzyme solution was absorbed accurately, preheated at 50℃ for 5 min, 0.8ml of CMC solution preheated at 50 ℃ was added (three parallel test tubes were made for each sample at the same time), then the reaction was precisely carried out in water bath at 50℃ for 60 min, 1mL of NaOH (0.4M) was added to stop the reaction, 2.0ml of DNS reagent was added, 4mL buffer was added to dilute to 8mL, and the absorbance of the sample was determined according to the standard curve making step. Control group: take CMC solution 0.8ml, at 50 ℃ water bath precise reaction for 60 minutes, then add 1 mL NaOH (0.4M) to stop the reaction, add diluted enzyme solution 0.2ml, then add DNS reagent 2.0ml, the other steps are the same as the sample determination. Calculation: X = CN/ (0.2 * 60)	

Experiment Results

Control group: OD: 0.014;
OD: 0.041;
Dilute 2 times enzyme solution OD: 0.025.
Glucose standard: OD = 0.1734c (C is glucose concentration, mg/mL)
The enzyme activity was 0.013 U/mg.
Diluted double enzyme activity was 0.011 U/mg.

EXPERIMENTAL NAME: extracted plasmid

Date：2019/09/08 **Operators: Shi Jing**

Detailed Steps	Remarks
Plasmid UESTC × 2	
(20) 12 000xg centrifugation for 1 min;	
(21) Discard the culture medium, add 250 μL Buffer P1/RNase A mixture, and shake the suspension with vortex; (Ensure that RNase A has been added to ensure that the bacteria has been thoroughly re-suspended.)	
(22) Adding 250 μL Buffer P2 into the heavy suspension, and reversing the mixing (6-8 times); (Thick and clear solution indicates full lysis of bacteria; if necessary, place at room temperature for 2 min, inverting the mixture several times; when treating multiple samples, the operation time of this step should not exceed 4 min)	
(23) Adding 350 μL Buffer NP3 and immediately reversing 8 to 10 times to completely neutralize the solution; (Immediately invert that mix to prevent precipitation and agglomeration from affect the neutralization effect)	
(24) 13,000 × G centrifugation for 1 min;	
(25) Put the HiPure DNA Mini Column II in the collecting pipe and pour the supernatant into the Column. centrifuge for 1 min at 13,000 × G; (Do not add flocculent precipitate)	
(26) Discard the filtrate and loop the column back into the collection tube. Add 500 μL Buffer PW1 to the column. 13, 000 × G centrifuge for 1 min;	
(27) Discard the filtrate and loop the column back into the collection tube. Add 600 μL Buffer PW2 to the column. Centrifuge 13,000 × G for 1 min; repeat once.	
(28) Discard the filtrate and loop the column back into the collection tube. 13, 000 X G centrifuge for 2 min to dry the column. If you can still smell ethanol in the column, remove the lid and dry for a few	

minutes; (29) The column was placed in a sterilized 1.5 mL centrifuge tube, and 50 μL ddH2O was added to the center of the column membrane; (The lowest elution volume of the column is 30 μL. Less than 30 μL will lead to a decrease in elution efficiency. 30 μL can elute 60-70% of plasmid DNA. 50 μL can elute 80-85% of plasmid DNA. If maximum yield is required, elute twice without poking the membrane when ddH2O or Elute Buffer is added.) The column was discarded and the plasmid was stored in 1.5 ml EP tube.	

Experiment Results

Experiment Name: INP PCR (New Primer) & Restriction Enzyme

Date：2019/09/09 **Operators: Liangsiwen**

Detailed Steps	Remarks
INP new primer PCR System: 50 μL × 4 **INP digestion** 35 μL: PCR product 30 μL Buffer 3.5 μL NcoI 1 μL EcoRV 1μL	

PCR Special Form

Gene Name：INP

Primer sequence：R: INP-EcoRV-new

F: INP-NcoI-new

Date：2019/09/09	Operators: Liangsiwen	
Reagent	**Volume**	**Total Volume**
Buffer	25μL	50 μ L
5 '-primer/3' -primer	2+2μL	
DEIONIZED WATER	16μL	
dNTP	1 μ L	
Phanta	1 μ L	
Template DNA	1 μ L	

PCR Program

FIRST UNSPIN	UNSPIN	Annealing	Extension	Cycle	Deactivation	
95oC	95oC	57oC	72oC	cycle	72oC	12oC
5 min	30 s	30 s	50s	20~30×	5 min	∞

Experiment Results

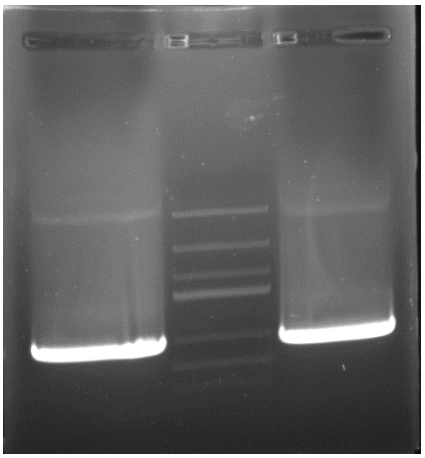
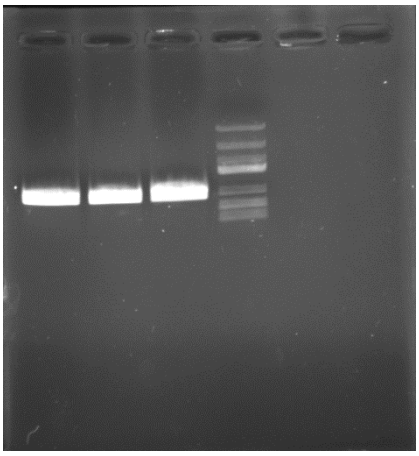


Figure SEQ Figure \ * Arabic 1 (a) Inp PCR (new primers);



(B) digestion of the INP PCR product;

P came out and cut out, the concentration looks good ~

Experiment Name: Recovery of INP Enzymatic Digestion Products & Linking

Date：2019/09/10 **Operators: Liangsiwen**

Detailed Steps	Remarks
Recovery of INP ENZYME CUT RUBBER **INP and endo-pET/exo-pET Connection** 35 μ L： INP 1 μ L Carrier 7 μ L T4 buffer 1 μ L T4 enzyme 1 μL 25 DEG C, connected for 4 H **CONVERSION OF LINKAGE PRODUCTS** Overnight culture at 37 ℃	

The concentration of INP digestion product is very high.

Experiment Name: INP Conjugate Bacteria P Verification

Date：2019/09/11	Operators: Liangsiwen
Detailed Steps	**Remarks**
β -pET extracted plasmid Number 4 & 6, each with two tubes. **INP-endo-pET & Verification of INP-exo-Pet P** Pick 24 colonies each, phanta enzyme (because Taq is gone) **Endo-pET & exo-pET overnight induction at 16 ℃** IPTG concentration: 0.2	

PCR Special Form

Gene Name：INP

Primer sequence：R: INP-EcoRV-new

F: INP-NcoI-new

Date：2019/09/12 **Operators: Liangsiwen**

Reagent	Volume	Total Volume
2×taq mix	10μL	
5 '-primer/3' -primer	1+1μL	
DEIONIZED WATER	8μL	

PCR Program

FIRST UNSPIN	UNSPIN	Annealing	Extension	Cycle	Deactivation	
95oC	95oC	57oC	72oC	cycle	72oC	12oC
5 min	30 s	30 s	50s	20~30×	5 min	∞

Experiment Results

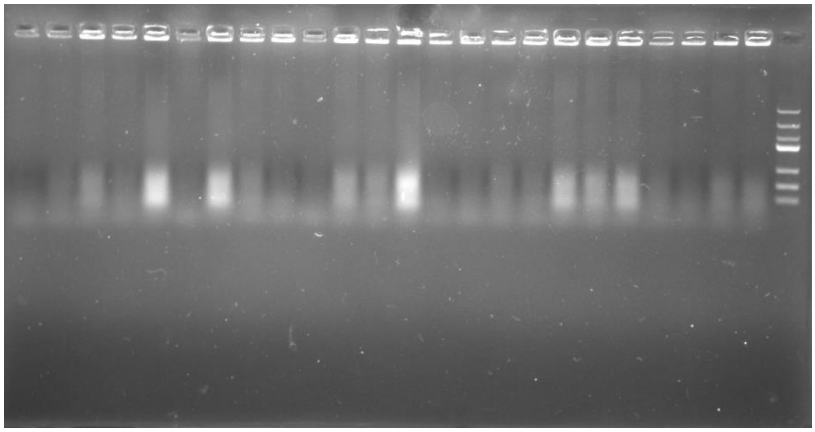
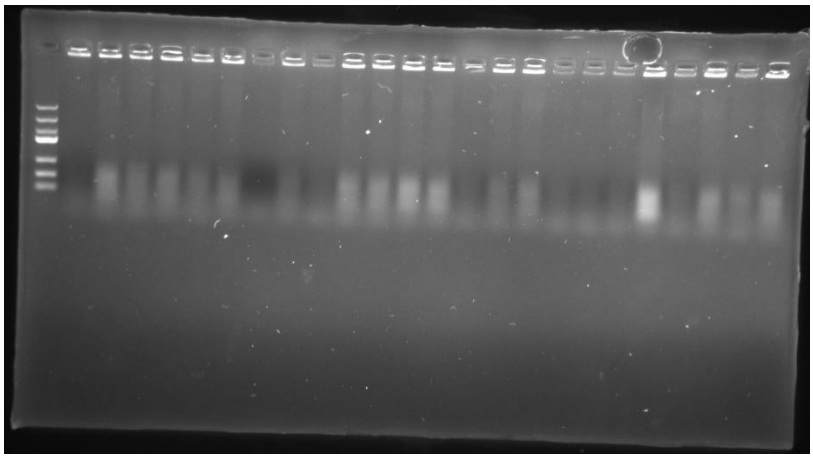
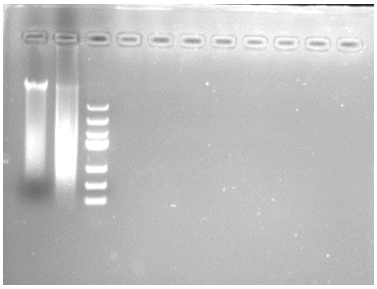


Figure 1 (a) inp-endo-pet;



(B) INP-exo-pET bacteria P;



(C) β-pET extract plasmid

Experiment Name: exo/endo Ultrasonic Crushing & Enzyme Activity Determination & Bacterium P

Date：2019/09/12 **Operators: Liangsiwen**

Detailed Steps	**Remarks**

Induction condition: 16 ℃ 0.2 IPTG overnight

Ultrasonic breakage

Use ultrasonic crusher of innovation laboratory, 30% power, break 2S, stop 4s, 20min

CMC enzyme activity assay

	对照组	测定组
	0.8mLCMC+0.2mL 酶液（原液、不同稀释度）	
	1ml 0.4M NaOH	
	50℃水浴(60min)	
		1mL 0.4M NaOH
	2mL 3,5-二硝基水杨酸，水浴(5min)，冷却	
	用 buffer 稀释至 8mL	

In the experimental group, 0.2ml of the enzyme solution was absorbed accurately, preheated at 50 ℃ for 5 min, 0.8ml of CMC solution preheated at 50 ℃ was added (three parallel test tubes were made for each sample at the same time), then the reaction was precisely carried out in water bath at 50 ℃ for 60 min, 1mL of NaOH (0.4M) was added to stop the reaction, 2.0ml of DNS reagent was added, 4mL buffer was added to dilute to 8mL, and the absorbance of the sample was determined according to the standard curve making step.

Control group: take CMC solution 0.8ml, at 50 ℃ water bath precise reaction for 60 minutes, then add 1 mL NaOH (0.4M) to stop the reaction, add diluted enzyme solution 0.2ml, then add DNS reagent 2.0ml, the other steps are the same as the sample determination.

Calculation: X = CN/ (0.2 * 60)

INP-endo-pET & INP-exo-pET colony PCR

Endo：3、5、7、8、13、18、19、20

Exo：2、10、11、12、13、20、22、24

PCR Special Form

Gene Name：INP

Primer sequence：R: INP-EcoRV-new

F: INP-NcoI-new

Date：2019/09/12 **Operators: Liangsiwen**

Reagent	Volume	Total Volume
2×taq mix	10μL	
5 '-primer/3' -primer	1+1μL	
DEIONIZED WATER	8μL	

PCR Program

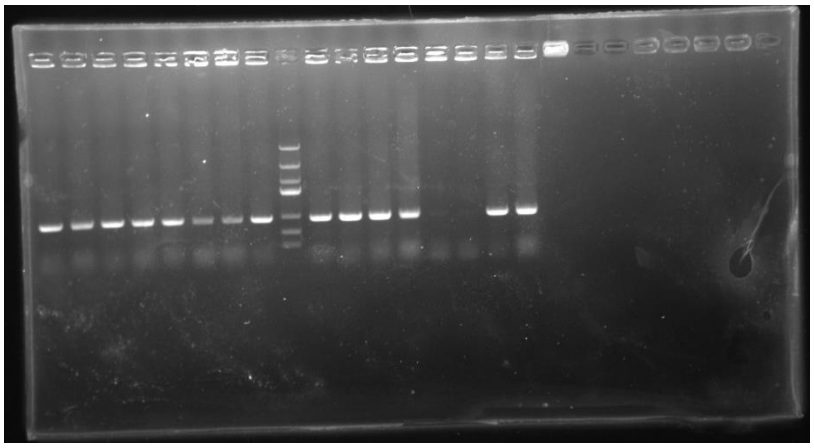
FIRST UNSPIN	UNSPIN	Annealing	Extension	Cycle	Deactivation	
95oC	95oC	57oC	72oC	cycle	72oC	12oC
5 min	30 s	30 s	50s	20~30×	5 min	∞

Experiment Results

Control group: OD: exo: 0.066; endo: 0.022
OD: exo: 0.005; endo: 0.025;
Dilute 2 times enzyme solution: OD: exo: 0.000; endo: -0.040.

REFLECTION: The wrong buffer should be citric acid buffer, not lysis buffer.
It is also possible that the enzyme activity of the enzyme in the precipitation, should also be used to break up the suspension of centrifugation is also measured.

INP-exo-pET & INP-endo-pET conjugate bacteria P



Marker, left: endo
Marker, right: exo
Changed the Taq enzyme, P came out.
Shake the bacteria, improve the plasmid.

Experiment Name: INP-exo-pET & INP-endo-pET plasmid

Date：2019/09/10 **Operators: Liangsiwen**

Detailed Steps	Remarks
INP-endo-pET & INP-exo-pET extract plasmid Endo：3,7,19 Exo：2,11，24 **LB liquid** 50mL×10	

Experiment Name: exo-pET & endo-pET 16 ℃ Overnight Induction

Date：2019/09/10	Operators: Liangsiwen
Detailed Steps	**Remarks**
Induction concentration: 0.2 mM & 0.08 mM Mother liquor: 0.8M IPTG (12.5 μL, 5 μL added respectively) 16 ℃ overnight induction (8:00 p. M. to 11:00 a. M.)	