Experiment Name: preparation of culture medium, sterilization

Date：2019/05/28 **Operators: Xu yang**

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. (1) Use electronic scale to weigh 2g peptone, 1g yeast extract and 0.2g glucose respectively, and put them into 1000 mL beaker (2) Add 100 mL of deionized water to the beaker and stir with glass rod until the solid is basically dissolved (3) Pour the solution into a measuring cylinder, where the solution is fixed to 200 mL with deionized water (4) Divide the culture solution into 4 150 mL conical cylinders, and each conical flask was roughly filled with 50 mL culture solution (5) Weigh 1g AGAR twice with an electronic scale and add it to two 150 mL conical flasks containing culture liquid (6) Seal conical bottle with rubber band, sealing film and kraft paper (7) Use autoclave for sterilization at 121℃ for 30 min	This column indicates special phenomena or unexpected errors in operation.

Reagent preparation

Date：2019/05/28 **Operator: Xu Yang**

Reagent	Volume
Peptone	2g
Yeast Extract	1g
Glucose	0.2g
Deionized Water	Fixed volume to 200 mL
AGAR (solid medium)	2g

Transfer *Rhodobacter sphaeroides* to PYG Liquid Medium from Solid Medium

Date：2019/05/29 **Operators: Li Zixuan**

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. (1) Sterilize the clean bench by uv for 30min in advance (2) Operate in a sterile environment on the clean bench. Use the inoculating loop to get some *Rhodobacter sphaeroides* colonies cultured at 34℃ for 5 days from the slant solid medium. Place the colonies into the prepared PYG liquid medium and gently shake the inoculation loop to disperse the bacteria into the liquid medium (3) Culture the PYG liquid culture medium with bacteria at a temperature of 34℃ and a rotation speed of 150rpm.	This column indicates special phenomena or unexpected errors in operation.

Experiment Results

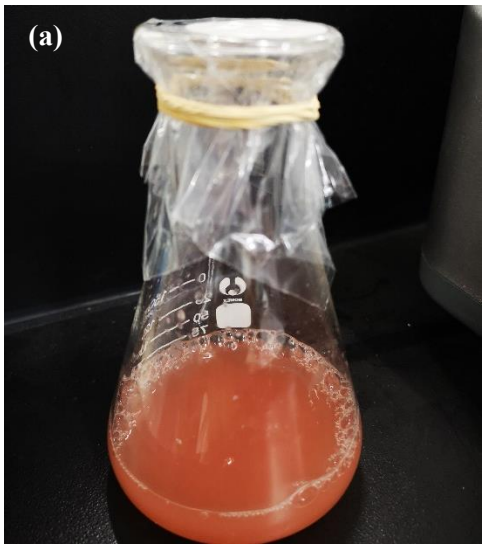


Figure 1 (a) culture of *Rhodobacter sphaeroides.*

Experiment Name: Preparation of Culture Medium for I *Rhodospirillum rubrum* & *Pantothenic y Agglomeration*

Date：2019/05/22 **Operators: Liang Siwen & Lu Jinwang**

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. (4) According to the formula，prepare 600 mL of each of that meat juice agar medium and the PYG medium, wherein the agar component was prepared separately, and the glucose in the PYG medium was prepared separately (different sterilization temperature); (5) Four bottles of the two prepared culture mediums are respectively packed into 100mL * 2 and 200mL * 2, and one bottle of 100mL	This column indicates special phenomena or unexpected errors in operation.

Date：2019/05/22 **Operators: Liang Siwen & Lu Jinwang**

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.
culture mediums is added with agar powder in equal proportion, which is heated and dissolved in a microwave oven, and the label is made; rest 500mL was used as liquid medium without AGAR. (6) The 100 mL culture solution added with agar was used to make the test tube slope, 5 mL/tube, so 40 empty tubes were prepared in advance, plugged, marked and 5 bundles were made; (7) Eight bottles of culture solution and eight bundles of empty test tubes were sterilized at 121 degrees for 20 minutes, and the glucose mother solution was sterilized at 115 degrees for 20 minutes;	
(8) The sterilized agar medium is quickly packed into test tubes under the super-clean stage to make the slope.	The gravy ramp is small in number and uneven (may need to be redone)

Reagent preparation

Reagent name: Nutrient Gravy Agar (*Y Pantothenic*)

Date：2019/05/22 **Operators: Liang Siwen Lu Jinwang**

Reagent	Volume
Peptone	6.0g
Beef extract	1.8g
NaCl	3.0g
Distilled Water	600mL
pH	7.0
Agar	1.5g

Reagent name: PYG medium (*I Rhodospirillum rubrum*)

Date：2019/05/22 **Operators: Lu Jinwang & Liang Siwen**

Reagent	Volume
Peptone	6.0g
Yeast Extract	3.0g
Distilled Water	600mL
pH	6.8-7.0
Glucose	0.6g / 3mL
Agar	1.5g

Experiment Name：Extraction of DNA from *Rhodobacter sphaeroides*

Date：2019/06/03 **Operators: Liao Shujie & Li Zixuan**

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.
(1) 2 mL culture medium of *Rhodobacter sphaeroides* was taken from the ultra-clean table. The supernatant of *Rhodobacter sphaeroides* was aspirated as much as possible by centrifugation at 10,000 rpm (~ 11,500xg) for 1 min.	
(2) A 200 μL buffer GA was added to the bacterial precipitate with a 1000 mL pipette gun, and the bacterial precipitate was immediately dispersed in the solution with the pipette gun. Vortex oscillated until the bacterial precipitate was completely suspended.	It is difficult to suspend the bacteria completely without immediate inhalation.

Date：2019/06/03 **Operators: Liao Shujie & Li Zixuan**

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.
(3) 20 μL Proteinase K solution is added to that tube and the Vortex is oscillate and mixed uniformly. (4) 220 μL of buffer solution GB was added, oscillated for 15 sec, placed at 70 ℃ for 10 min in a metal bath, that solution was strain clear, and centrifuged briefly to remove water droplets on the inner wall of the tube cover.	
(5) Add 220 μL anhydrous ethanol, mix with full oscillation for 15 sec, at which time flocculent precipitation may occur, and centrifuge briefly to remove water droplets from the inner wall of the cap.	This step allows centrifugation at 12,000 rpm for 30 sec to precipitate the cell fragments. Do not add flocculent precipitation to the adsorption column, otherwise the concentration of DNA extracted is very low.
(6) The solution obtained in the previous step is added to an adsorption column CB3 (the adsorption column is placed in a collection tube), centrifuged at 12,000 rpm (~ 13,400 × G) for 30 sec, the waste liquid is poured out, and the adsorption column CB3 is placed in the collection tube.	
(7) 500 μL of buffer GD was added to that adsorption column CB3, and the adsorption column CB3 was centrifuged for 30 sec at 12,000 rpm (~ 13,400 X G), the waste liquid was pour, and the adsorption column CB3 was placed in a collection tube. (8) 600 μL of rinse solution PW was added to adsorption column CB3, and centrifuge at 12,000 rpm (~ 13,400 X G) for 30 sec. Waste solution was poured out and adsorption column CB3 was put into a collection tube. (9) Repeat Step 8.	

Date：2019/06/03 **Operators: Liao Shujie & Li Zixuan**

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.
(10) The adsorption column CB3 was put back into the collecting tube, centrifuged at 12,000 rpm (~ 13,400 × G) for 2 min, and the waste liquid was poured out.	
(11) The adsorption column CB3 was transferred into a clean centrifuge tube, and 50 μL of elution buffer TE was dropped into the middle part of the adsorption membrane, and the solution was collected into the centrifuge tube after 5 min at room temperature and 2 min centrifugation at 12,000 rpm (~ 13,400 × G).	
(12) The solution obtained by centrifugation is added to the adsorption column CB3 and centrifuged at 12,000 rpm (~ 13,400 × G) for 2 min, and the solution is collected in the centrifuge tube.	It was supposed to stay at room temperature for another 2 minutes.
(13) DNA concentration was determined by DNA/RNA concentration detector.	

Experiment Name: Measurement of DNA Concentration

Date：2019/06/03 **Operators: Fan Xuqian & Xu Yang**

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.
(1) Take the lifted DNA to Mr. Yang Jinshui's laboratory (a sister teacher on the stage is responsible for the DNA microanalyzer).	
(2) Turn on the DNA microanalyzer.	

Date：2019/06/03 **Operators: Fan Xuqian & Xu Yang**

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. (3) Take out the colorimetric cup and put it in the hole of the colorimetric cup. (4) Cup lid selection lid10 (5) Wash the lid and colorimetric cup with pure water three times before use. (6) When sampling, the gun head should not touch the colorimetric cup, and the sample should be dropped into the upper sample hole, and then the cup lid should be closed. (7) Note: The colorimetric cup is stored on the smooth side of the mirror paper, and at the same time, the spot hole and cup cover of the colorimetric cup should be dipped in and cleaned with the smooth side of the mirror paper, and should not be wiped back and forth. (8) Select nucleic acid, nucleic acid, dsDNA. (9) Zero with sample solution (buffer TE this time) (10) Hang the sample drop in the upper sample hole and cover the cup lid. (11) Click sample. (12) There is no need to clean the sample hole and lid of the colorimetric cup between the sample and the sample, but the remaining liquid needs to be dipped in the smooth surface of the mirror paper. (13) After the test, wash the cup and lid with pure water three times, wrap the cup in the glossy surface of mirror paper, and put the lid on. (14) Return the instrument to the main page, turn off the power, plug. Do the registration	This column indicates special phenomena or unexpected errors in operation.

Experiment Results

Figure 1 (a) (b) in step 6, the concentration of DNA of only the supernatant was transferred to the adsorption column; (c) (d) in step 6, the concentration of DNA of transfer the supernatant and precipitation to the adsorption column

It can be seen from the figure that only the supernatant transferred into the adsorption of the higher concentration of DNA, may be due to the sediment into the adsorption column after blocking the adsorption column packing, resulting in DNA cannot be successfully adsorbed or eluted.

A260/A280 is high in (a) (b), indicating possible RNA impurities, but should not affect subsequent experiments.

Experiment Name: *Escherichia coli* transformation

Date：2019/06/04 **Operators: Pang Zheng**

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. (1) Four tubes of competent DH5α cells thaw in an ice-water bath, and the plasmids pACYC184-M (CAT resistant) and ptrc99A-M (AMP resistant) were added to the competent DH5α cells (2 tubes of competent DH5α cells per plasmid, 5 μL per tube of plasmid) by pipette. Dial the bottom of the tube with finger and mix it gently (2) Keep in ice bath for 30 minutes without shaking. (3) 42 ℃ heat shock 60 s, do not shake. (4) Ice water bath for 2 minutes, do not shake. (5) 500μL of sterile LB liquid culture medium was added. (6) The cells were incubated in a shaker at 37 ℃ for 60 min at 150-200 rpm. (7) Centrifuge at 6000 rpm for 30 s, and suck the supernatant until about 100 μL of liquid is left. Suck and blow the bacteria at the bottom of the tube with a pipette to mix evenly, and coat the resistant LB plate with a coating rod. (8) After the liquid was dried, the plates were inverted and cultured at 37 ℃ for 12-16 h.	This column indicates special phenomena or unexpected errors in operation.

Experiment Results

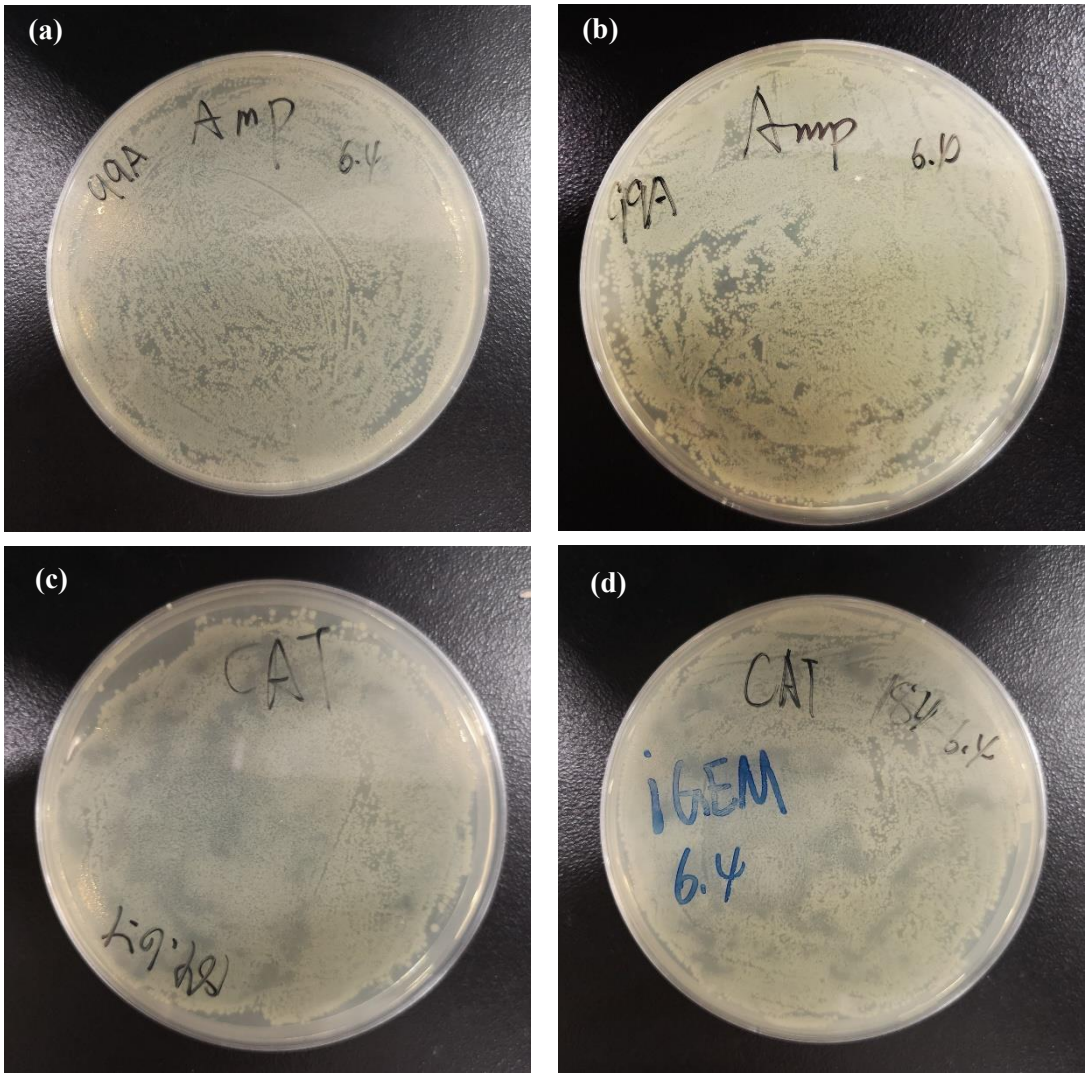


Figure 1 (a) (b) 99A plasmid was transformed into *E.coli*; (C) (d) 184M plasmid was transformed into *E.coli.*

E. coli grew on all resistant plates, indicating that the plasmid transformation was successful. *E. coli* colonies were too dense, probably because the concentration of bacterial solution was too high when coating, so it was necessary to draw a line on the plate to obtain single colonies.

Experiment Name：Liquid medium preparation and strain incubation

Date：2019/06/05 **Operators: Yan Xueshan & Liao Shujie**

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. (1) Amp and Chl were added to two bottles of LB liquid medium (200 ml), respectively. Add Amp (100 μl) and Chl (200 μl) to 2 bottles of LB liquid medium respectively. The LB Amp + liquid medium was poured into 2 test tubes, each tube was 3-4 ml, and a gun was added to the test tube to facilitate the mixing when shaking the bed for incubation. (2) Decant the Amp+ medium into test tubes (x2) for 3-4 ml each. Add one tip into each tube. (3) 100 μl of DH5α UESTC bacterial solution was added to that test tube. Add bacteria liquid DH5α UESTC 100μl into each tube. (4) The plasmid was incubated overnight on a shaker at 37 ℃. Incubate in 37°C orbital incubator shaker overnight for later plasmid extraction. (5) For strain 99A (3 tubes) in Amp + medium, cat (2 tubes) in Chl + medium, repeat the above steps. Steps repeated for strain: 99A (x3) in Amp+ medium, cat (x2) in chl+medium.	This column indicates special phenomena or unexpected errors in operation. Conduct in clean bench. Amp storing concentration should be 50 mg/ml, using in 1000x dilution. (50 μg/ml) Chl storing concentration is 10 mg/ml, using in 1000x dilution for tight plasmids.

Reagent preparation

Reagent name：

Date：2019/06/05 **Operators: Yan Xueshan & Liao Shujie**

Reagent	Volume
LB liquid medium	200 ml x2
Ampicillin (100 mg/ml)	100μl

Strain name：

E．coli: DH5α UESTC

99A

cat

Experiment Results

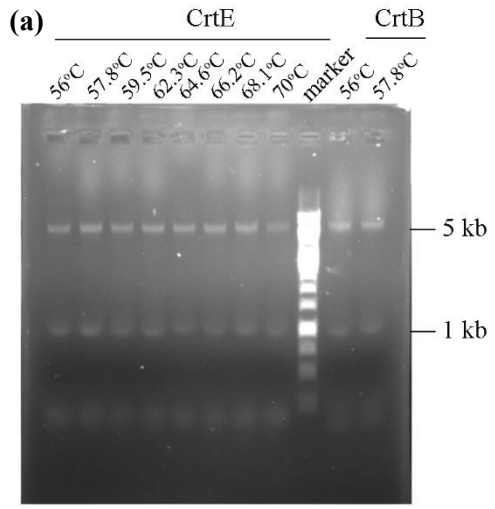


Figure SEQ Figure \ * Arabic 1 (a) crte, crtb PCR results.

1、The amount of marker (10 μL) is too much.

2、Although there are bands about 1 kb, the size of CrtE and CrtB bands is the same, and the brightness of 1 kb band is very low, so it is not the target band.

3、The temperature gradient of CrtE has little effect on the PCR results, so we can consider setting the temperature gradient to 60-72 next time.

4、The size of the band is about 5 kb. It is possible that CrtE and CrtB were amplified together with the middle fragment.

Panspermia aGGlomerans preservation, reculture, DNA extraction

Operators: Fan Xuqian
Lu Jinwang

Date：2019/06/30

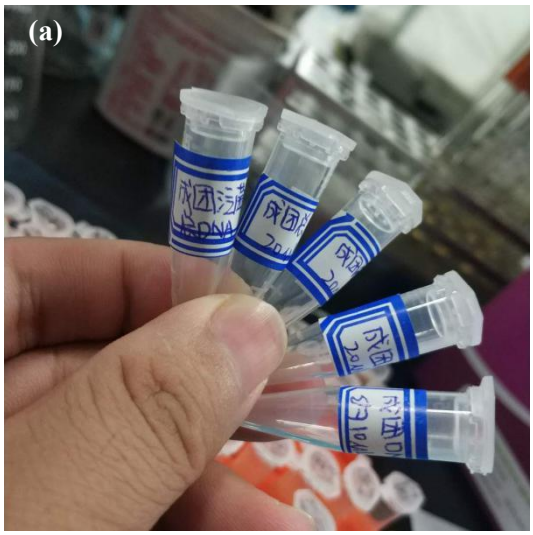
Detailed Steps	Remarks
Please specify the operation name, reagent name, reagent dosage, temperature, reaction time and instrument setting parameters in detail; when adding steps, please stop the cursor at the top of the lower left corner of each line, and click after the plus sign appears.	This column indicates special phenomena or unexpected errors in operation.
(1) Take the mass culture liquid of Pantothenic in the refrigerator at 4 ℃ on June 8, pack 9 tubes separately with 800 μl, add 800 μl glycerol into each tube, seal the bag and store at -80 ℃.	
(2) 4 ml of that remain bacterial st° Ck solution was adde to 150 ml of a meat quality medium (May 31 configuration), shaking the bacteria at 37° C, and culturing two bottles.	Preservation of remaining bacterial st° Ck solution at 4 ℃
(3) The culture medium was centrifuged at 10,000 rpm (~ 11,500xg) for 1 min, and the supernatant was aspirated as much as possible.	
(4) 200 μl of buffer GA was adde to that bacterial precipitate, which was sucked by a gun and vibrated by Vortex until the bacterial suspension was complete.	
(5) 20 μl Proteinase K solution is adde to that tube and the Vortex is oscillate and mixed uniformly.	
(6) 220 μl of buffer solution gB was adde, that buffer solution was oscillated for 15 sec, the metal bath was kept at 70° C for 10 min, the solution strain was clear, and the water droplets on the inner wall of the tube cover were removed by brief centrifuGAtion.	Waited for about 4 minutes after shaking because didn't open the metal bath ahead of time.
(7) Add 220 μl anhydrous ethanol, fully oscillate and mix for 15 sec, at which time fl° Cculent precipitation may ° Ccur, and centrifuge briefly to remove water droplets from the inner wall of the tube cover.	
(8) The solution and the fl° Cculent precipitate obtained in the previous step are added to an adsorption column CB3 (the adsorption column is placed in a collection tube), centrifuged at 12,000 rpm (~ 13,400 ×g) for 30 sec, the waste liquid is poured out, and the adsorption column CB3 is placed in the collection tube.	

Operators: Fan Xuqian
Lu Jinwang

Date：2019/06/30

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.
(9) 500 μl of buffer gD (first check for absolute ethanol) was adde to adsorption column CB3, centrifuged for 30 sec at 12,000 rpm (~ 13,400 ×g), and that waste liquid was pour away and the adsorption column CB3 was placed in a collection tube.	
(10) 600 μl of rinse solution PW (check for anhydrous ethanol first) was adde to adsorption column CB3, centrifuged for 30 sec at 12, 000 rpm (~ 13, 400 ×g), and that waste solution was poured out and the adsorption column CB3 was put into a collection tube.	
(11) The adsorption column CB3 was put back into the collection tube, centrifuged at 12,000 rpm (~ 13,400 ×g) for 2 min, and the waste liquid was poured out.	
(12) The adsorption column CB3 was transferred into a clean centrifuge tube, 50 μl of elution buffer TE was dropped into the middle part of the adsorption membrane, and the solution was collected into the centrifuge tube at room temperature for 2-5 min, 12 000 rpm (~ 13 400 ×g) centrifuGAtion for 2 min.	
(13) The solution obtained by centrifuGAtion was added to the adsorption column CB3, placed at room temperature for 2 min, and centrifuged at 12,000 rpm (~ 13,400 ×g) for 2 min.	
(14) Four tubes of 20 μl and one tube of 10 μl were packed separately, labeled and stored at -20 ℃.	**There's no concentration!**

Experiment Results



(Cut the picture into a square and paste it here)

The DNA labels extracted from Figure SEQ Figure 1 (a) are stored in the lowest layer of the refrigerator at -20 ℃ after baGGing.

Experiment Name: PCR condition groping, CrtB glue recycling

Date：2019/07/02

Operators: Fan Xuqian, Liu Ruogu, Lu Jinwang

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. (1) The PCR system was premixed by adding polymerase GC Buffer 3 μl, primer 0.75 μl, 10 mM dNTPs 0.3 μl and DNA high fidelity polymerase 0.15 μl to the PCR system. (2) Adjust the amount of CrtB total DNA (dissolved in TE or double distilled water), Mg2 and DMSO in the system, and fill each tube to 10.05 μl with ddH2O; add premix 4.95 μl/tube in (1). (3) A total of 24 tubes were used for PCR, and the annealing temperature was set at 60.0 ° C, 30s on the PCR apparatus.	This column indicates special phenomena or unexpected errors in operation. A reset restart was interrupted because an

Date：2019/07/02

Operators: Fan Xuqian, Liu Ruogu, Lu Jinwang

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. error ° Ccurred during initial setup.
(4) AGAr gel preparation was carried out in the course of waiting for the amplified fragments, and the preparation was made with 0.8% aGArose concentration.	
(5) After PCR, add 2 μl loading dye to each tube sample (15 μl), and mix well.	
(6) All the samples were taken, from left to right, 100 BP DNA marker/1-24 tubes, 2 μL marker.	
(7) Turn on the electrophoresis apparatus, keep the voltage at 170V, press "RUN" to start electrophoresis; wait until the front reaches glue 3/5, press "STOP" to stop electrophoresis, and turn off the power supply.	
(8) Place the gel in the center of the gel imager, open the software and select "UV-AE" to get the gel image, save the file and output it as a picture.	
(9) AGArose gel containing the desired DNA (20, 24 well samples of about 1000 BP) was cut under ultraviolet light and put into a 1.5 mL centrifuge tube to obtain gelatin 0.97-0.86 = 0.11 g.	The gel was unscrewed.
(10) 400 μl of DE-A solution was adde and heated at 75° C until that gel was completely melted and evenly mix every 2-3 minutes.	According to DEA 400 μl, DEB 200 μl adding sample; the metal bath is not opened in advance, and is completely dissolved by heating only once.

Date：2019/07/02

Operators: Fan Xuqian, Liu Ruogu, Lu Jinwang

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.
(11) Add 200 μL DE-B solution and mix evenly until the solution is completely yellow.	After oscillation, the liquid is hung on the wall and centrifuged briefly.
(12) The DNA recovery preparation tube is placed in a 2 ml centrifuGAl tube, the solution obtained in the previous step is transferred to the DNA recovery preparation tube, the 12000 g is centrifuged for 1 min, the filtrate is poured back into the preparation tube, the 12000 g is centrifuged for 1 min aGAin, and the filtrate is discarded.	Weighing with 1.41 g ddH2O + centrifuge tube
(13) 500 μl of that buff W1 washing preparation tube, 12000 g centrifuGAtion for 30 s, and discard the filtrate.	
(14) 700 μl of buffer W2 washing preparation tube, centrifuge 12000 g for 30 s, discard filtrate, and washing aGAin in that same way for 2 minutes.	
(15) Put the preparation pipe back into the centrifuge pipe, 12000 g, centrifuge for 1 min.	Centrifuge a small amount of liquid
(16) The preparation tube was placed in a clean 1.5 ml centrifuge tube, dried at 65 ℃ for 3-5 min, preheated at 65 ℃ with 25 μl ddh2o in the center of the preparation film, and then placed at room temperature for 1 min, 12000 g and centrifuged for 1 min to elute DNA.	Use ddH2O (not Eluent)
(17) Label,-20 ℃ refrigerator storage.	

PCR Special Form

gene Name：CrtB

Primer sequence：R:GGAGTTTCATCTAGATCGGGTTGGCCCGGTTCCC

F: GCCCGCGTCTGAATTGCCTCTGCCGATCTCGAT

Date：2019/07/02 **Operators: Fan Xuqian Liu Ruogu Lu Jinwang**

Reagent	Volume	Total Volume
PCR buffer (GC)	3 μ L	15 μ L
10 mM dNTPs	0.3 μ L	
5 '-primer/3' -primer	0.75+0.75=1.5 μ L	
DNA polymerase	0.15 μ L	
DD H_2O	Up to 15 μ L	
Template DNA	0.15/0.3/0.6 μ L	
DMSO	0/0.45 μ L	
Mg2+	0/3.75 μ L	

PCR System Composition：

Date：2019/07/02 **Operators: Fan Xuqian Liu Ruogu Lu Jinwang** **Total volume: 15 μ l/tube**

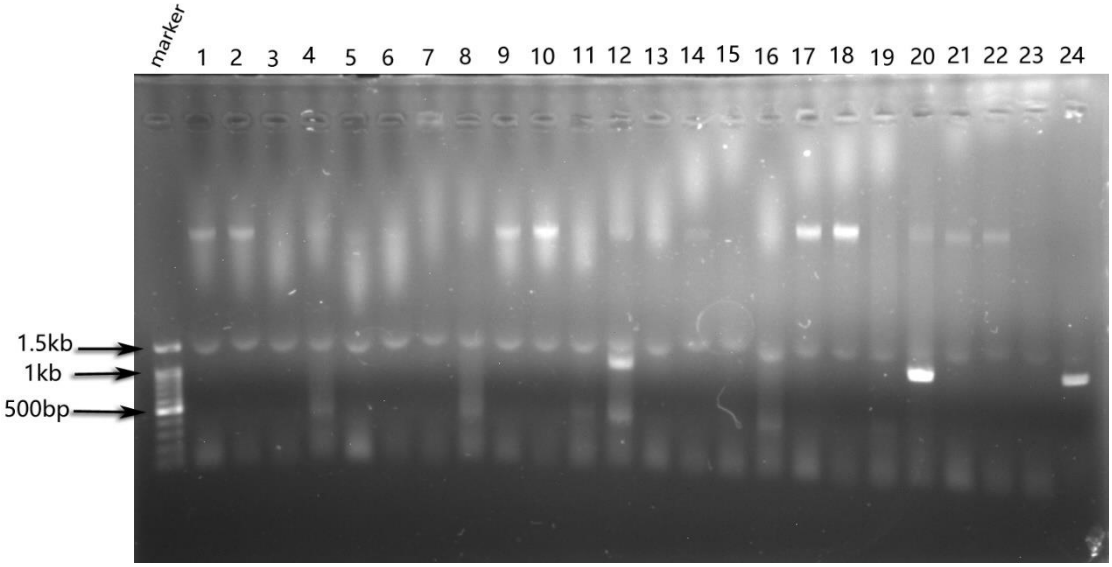
No.	Template (TE)/μl	Template (ddH2O)/μl	Mg2+/μl	DMSO/μl	ddH2O/μl
1	0.15	-	-	-	9.9
2	0.15	-	3.75	-	6.15
3	0.15	-	3.75	0.45	5.7
4	0.15	-	-	0.45	9.45
5	-	0.15	-	-	9.9
6	-	0.15	3.75	-	6.15
7	-	0.15	3.75	0.45	5.7
8	-	0.15	-	0.45	9.45
9	0.3	-	-	-	9.75
10	0.3	-	3.75	-	6.0
11	0.3	-	3.75	0.45	5.55
12	0.3	-	-	0.45	9.3

13	-	0.3	-	-	9.75
14	-	0.3	3.75	-	6.0
15	-	0.3	3.75	0.45	5.55
16	-	0.3	-	0.45	9.3
17	0.6	-	-	-	9.45
18	0.6	-	3.75	-	5.7
19	0.6	-	3.75	0.45	5.25
20	0.6	-	-	0.45	9.0
21	-	0.6	-	-	9.45
22	-	0.6	3.75	-	5.7
23	-	0.6	3.75	0.45	5.25
24	-	0.6	-	0.45	9.0

PCR Program

FIRST UNSPIN	UNSPIN	Annealing	Extension	Cycle	Deactivation	
95° C	95° C	60°C	72°C	cycle	72°C	4°C
3 min	30 s	30 s	30s	20~30×	5 min	∞

Experiment Results



(Description and analysis of results)

1、The results showed that the 12th tube (weak and more heterozygous), the 20th tube (the brightest, but with heterozygous), the 24th tube (bright and no heterozygous, weaker than the 20th tube) may be due to the concentration of total DNA template dissolved by ddH2O was lower than that dissolved by TE.

2、Among them: 1, 2, 9, 10, 17, 18 possession of a very clear large heterozygous band, these conditions of treatment conditions: dissolved template DNA (red ball bacteria) for TE solution, and no DMSO.

3、The size of 1.5 kb heterozygous band is common.

4、It is concluded that the condition of Crt B gene PCR (15 μl system) is adding 0.9 μl template DNA, 0.45 μl DMSO and no Mg ion.

5、At the same time, we can use ddH2O to dissolve the template DNA of Rhodobacteria (because of the low concentration, we can consider to do a grope to continue to increase the amount of template).

Experiment Name: to verify the setting of CrtB and CrtE PCR PCR, CrtE, CrtB gel recovery

Date：2019/07/03 **Operators: Fan Xuqian, Liu Ruogu, Lu Jinwang**

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.
(1) On July 2, the condition of CrtB (15 μl system) was expanded to 50 μl system, and a PCR of CrtE with the same condition was made, but the effect was not good, then the condition of 24 tubes on July 2 was repeated for 5 tubes, and 3 tubes of PCR with the fragment recovered from July 2 as template was made, and CrtE was groped again for PCR condition.	The effect is very bad, there are many miscellaneous bands and the target band is not obvious (CrtB & CrtE).
(2) CrtB fragment treatment: adding polymerase GC Buffer 3 μl, primer 0.75 μl, 10 mM dNTPs 0.3 μl, DNA high fidelity polymerase 0.15 μl, template 0.6 μl, DMSO 0.45 μl, deionized water 15 μl into each PCR system according to the	The PCR condition of large system is not as good as that

Date：2019/07/03

Operators: Fan Xuqian, Liu Ruogu, Lu Jinwang

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.
ratio given by PCR self-contained prot° Col, 5 tubes premixed and packed separately.	of small system, and it is suGGested that the maximum PCR system should not exceed 25 μl.
(3) CrtB fragment treatment: polymerase GC Buffer 3 μl, primer before and after 0.75 μl, 10 mM dNTPs 0.3 μl, DNA high fidelity polymerase 0.15 μl, with 7.2-recovered CrtB as template to add 8 μl, DMSO to 0.45 μl, deionized water to fill 15 μl system, 3 tubes premixing, packaging.	
(4) The conditions of CrtE PCR were as follows: adding polymerase GC Buffer 3 μl, primer 0.75 μl, 10mM dNTPs 0.3 μl, DNA high fidelity polymerase 0.15 μl to the PCR system respectively.	
(5) The conditions of CrtE PCR were as follows: adjusting the total amount of CrtE DNA (dissolved in double distilled water) 0.3 μl/0.6 μl/0.9 μl, the amount of DMSO 0.45 μl/0.75 μl, MGCl2 free/3.75 μl, and adding 15 μl deionized water.	In the future, the total DNA of Rhodobacter sphaeroides dissolved in double-distilled water will be used as a template for PCR.
(6) A total of 8 + 12 = 20 tubes were used for PCR, and the annealing temperature was set at 60.0 ° C, 30s.	
(7) AGAr gel preparation was carried out in the course of waiting for the amplified fragments, and the preparation was made with 0.8% aGArose concentration.	
(8) After PCR, add 2 μl loading dye to each tube sample (15 μl), and mix well.	
(9) 10 μl of sample are taken and divided into two piece of glue, one is CrtB, one is CrtE, that sequence of sample is shown in the following figure; the marker is 3 μl.	After that, in order to recover the target fragments as much as possible, the remaining samples

Date：2019/07/03

Operators: Fan Xuqian, Liu Ruogu, Lu Jinwang

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.
	were mixed with rubber, and the target fragments were collected into the rubber recovery system of 50 μl.
(10) Turn on the electrophoresis apparatus, keep the voltage at 170V, press "RUN" to start electrophoresis; wait until the front reaches glue 3/5, press "STOP" to stop electrophoresis, and turn off the power supply.	
(11) Place the gel in the center of the gel imager, open the software and select "UV-AE" to get the gel image, save the file and output it as a picture.	
	When the glue needs to be recovered for a long time, the glue is divided into two or more centrifuge tubes to avoid insufficient space for subsequent reagent adding centrifuge tubes.
(12) The aGArose gel containing the target DNA was cut under the ultraviolet light and put into a 1.5 mL centrifuge tube (weighed), and then the total weight was weighed and the quality of the recovered gel was calculated	
(13) According to the gel recovery kit: add 3 times the volume of DE-A (1g = 1ml) and heat at 75 ℃ until the gel completely melts, mixing intermittently every 2-3 minutes.	
(14) Add DE-A general volume of DE-B solution, mix evenly until the solution is completely yellow.	
(15) The DNA recovery preparation tube is placed in a 2 ml centrifuge tube, the solution obtained in the previous step is transferred to the DNA recovery preparation tube, the 12000 g is centrifuged for 1 min, the filtrate is inverted back to the preparation tube (the DNA in all the obtained solutions is filtered in a column for enrichment), and the 12000 g is centrifuged aGAin for 1 min to discard the filtrate.	This step restores all the cut glue together, allowing a higher concentration of PCR products to be recovered.

Date：2019/07/03 **Operators: Fan Xuqian, Liu Ruogu, Lu Jinwang**

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.
(16) 500 μl of that buff W1 washing preparation tube, 12000 g centrifuGAtion for 30 s, and discard the filtrate.	
(17) 700 μl of buffer W2 washing preparation tube, centrifuge 12000 g for 30 s, discard filtrate, and washing aGAin in that same way for 2 minutes.	
(18) Put the preparation pipe back into the centrifuge pipe, 12000 g, centrifuge for 1 min.	
(19) The preparation tube was placed in a clean 1.5 ml centrifuge tube, dried at 65 ℃ for 3-5 min, preheated at 65 ℃ with 25 μl ddh2o in the center of the preparation film, and then placed at room temperature for 1 min, 12000 g and centrifuged for 1 min to elute DNA.	Use ddH2O (not Eluent)
(20) Label,-20 ℃ refrigerator storage.	

PCR Special Form

gene Name：CrtB

Primer sequence：R:GGAGTTTCATCTAGATCGGGTTGGCCCGGTTCCC

F: GCCCGCGTCTGAATTGCCTCTGCCGATCTCGAT

Date：2019/07/03 **Operators: Fan Xuqian, Liu Ruogu**

Reagent	Volume	Total Volume
PCR buffer (GC)	3 μL	
10 mM dNTPs	0.3 μL	
5 '-primer/3' -primer	0.75+0.75=1.5 μL	
DNA polymerase	0.15 μL	15 μL
dd H_2O	Up to 15 μL	
Template DNA	0.6 μL/8 μL (recovered)	

DMSO	0.45 μ L

gene Name：CrtE

Primer sequence：R: GGCAATCATTCAGACGCGGGCCGCGACCTGCg

F:
GAGTCGACCTGCAGGCATGCAATGAGGCACAAGATGGCGTTTGAACAGCGG

Date：2019/07/03 **Operators: Fan Xuqian, Liu Ruogu**

Reagent	Volume	Total Volume
PCR buffer (GC)	3 μ L	15 μ L
10 mM dNTPs	0.3 μ L	
5 '-primer/3' -primer	0.75+0.75=1.5 μ L	
DNA polymerase	0.15 μ L	
dd H_2O	Up to 15 μ L	
Template DNA	0.3/0.6/0.9 μ L	
DMSO	0.45 μ L/0.75 μ L	
Mg2+	0/3.75 μ L	

PCR System Composition: CRTE CONDITIONS

Total volume: 15 μl/tube

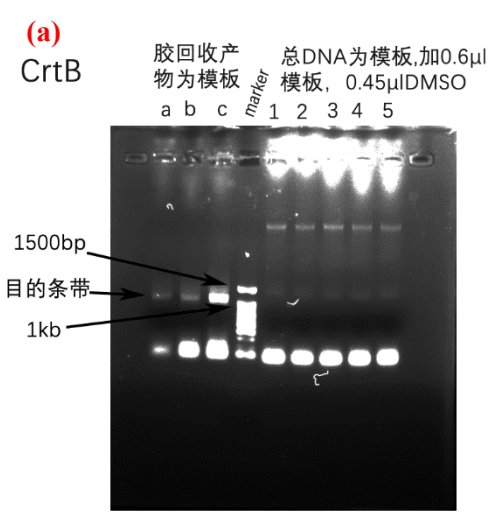
No.	Template (ddH2O)/μl	Mg2+/μl	DMSO/μl	ddH2O/μl
1	0.3	-	0.45	9.3
2	0.3	-	0.75	9.0
3	0.3	3.75	0.45	5.55
4	0.3	3.75	0.75	5.25
5	0.6	-	0.45	9.
6	0.6	-	0.75	8.7

7	0.6	3.75	0.45	5.25
8	0.6	3.75	0.75	4.95
9	0.9	-	0.45	8.7
10	0.9	-	0.75	8.4
11	0.9	3.75	0.45	4.95
12	0.9	3.75	0.75	4.65

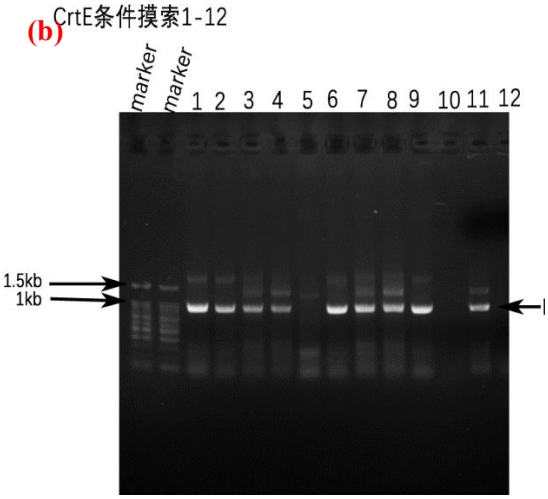
PCR Program

FIRST UNSPIN	UNSPIN	Annealing	Extension	Cycle	Deactivation	
95° C	95° C	60°C	72°C	cycle	72°C	4°C
3 min	30 s	30 s	30s	20~30×	5 min	∞

Experiment Results



(Cut the picture into a square and paste it here)



(Cut the picture into a square and paste it here)

Figure SEQ Figure \ * ARABIC 1 (a) crtb PCR; (B) crte condition groping.

(Description and analysis of results)

1、Using the condition obtained on July 2 to PCR CrtB aGAin (see figure a 1-5 lane), there is no purpose band, it is suGGested to continue to do a CrtB condition exploration tomorrow.

2、Figure a a-C lane using July 2 rubber recycling products as a template, to get the target strip, the rubber recovery.

3、In the condition of CrtE PCR, all the bands had the size of target gene except the bands in lane 5, 10 and 12, and the bands in lane 1, 6 and 9 were the brightest.

Study on the Culture Medium of Experiment Name: PYg and the Activation of Rhodospirillum rubrum

Date：2019/07/05 **Operators: Liang Siwen**

Detailed Steps	Remarks
Rhodospirillum rubrum activation	This column indicates special phenomena or unexpected errors in operation.
(1) Clap the strain label;	
(2) Wipe the tip of the glass tube with alcohol, hold it in the back of the glass tube with your hand, and burn the tip with an alcohol lamp (do not burn your hand, because the bacteria on your hand is also burned);	
(3) After a while, drop sterile water around the sharp mouth, and the glass tube explodes;	They were all black and had not been broken, so they burned a tweezer and knocked it open.
(4) Suck 800 μL from the flask into the glass tube, shake gently and mix evenly to form a uniform bacterial liquid;	
(5) 400 μl of bacterial solution were sucked into test tube and cultured at 30° C (one shake and one non-shake).	

Experiment Results

(a)

Figure SEQ Figure * ARABIC 1 BACTERIA LABEL

Reagent preparation

Reagent name: PYg medium (500 mL)

Date：2019/07/05 **Operators: Liangsiwen**

Reagent	Volume
Peptone	5.0g
Yeast Extract	2.5g
glucose	0.15g+0.35g→3.5mL
AGAr	2.25g (solid medium only)
dd H_2O	500mL
pH6.8~7.0	

Liquid: (test tube) 3mL × 5

(Flask) 50mL × 2 + 200mL

(Sterilize first, match the glucose mother liquor (C = 0.0001g/ μ L), sterilize and then add it in the super-clean table)

Sterilization: 121 ℃, 20 min

Solids: (test tube) 5mL × 10

100 mL (then inverted plate)

Add the glucose and sterilize it. The glucose will go out with the pile.

Sterilization: 108 ℃, 20 min

Experiment Name：Amplication of CrtY and Speculation

Date：2019/07/13 **Operators: Wang Ran, Liu Ruogu**

Detailed Steps	Remarks
PCR amplification of CrtY, DNA gel electrophoresis test, detection and sent to the company for sequencing.	This column indicates special phenomena or unexpected errors in operation.
(1) dd H_2O, polymerase buffer, primer, dNTPs, total DNA and DNA high fidelity polymerase were added according to the prot° Col of PCR. The single tube system was 15 μ l, total 12 tubes.	
(2) According to the operation pr° Cess on the machine, the annealing temperature is set at 60.0° C and the chain extension time is set at 45s on the PCR instrument.	Since the phusion enzyme was used up yesterday, the enzyme used in today's PCR is pfu enzyme borrowed from B081.
(3) The aGArose gel was prepared by 1% aGArose concentration and 40 ml of aGArose gel was prepared.	
(4) After PCR was completed, Marker and amplified fragments were added, and electrophoresis was carried out for 30 minutes, and then the results were observed under ultraviolet light.	
(5) The desired DNA band was cut and the DNA was extracted by the kit method. The desired DNA (CrtB gene fragment) was obtained.	

PCR Special Form

gene Name：CrtY

Primer sequence：R:TCATTGCATCGCCTGTTGAC

F:CCTGGTCTAAATGCCGCGGTATGATCTGAT

Date：2019/07/13	**Operators: Wang Ran, Liu Ruogu**	
Reagent	**Volume**	**Total Volume**
PCR buffer	3 μ L	
2.5 mM dNTPs	0.3 μ L	
5 '-primer/3' -primer	0.75 μ L	
pfu enzyme	0.15 μ L	15 μ L
DMSO	0.45 μ L	
dd H_2O	9 μ L	
Template DNA	0.6 μ L	

PCR Program

FIRST UNSPIN	UNSPIN	Annealing	Extension	Cycle	Deactivation	
95° C	95° C	60°C	72.0° C	cycle	68° C	4° C
3 min	30 s	30 s	45s	30×	5 min	∞

Experiment Results

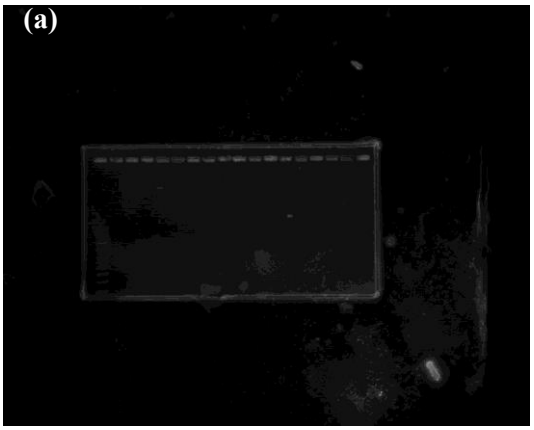


Figure SEQ Figure ARABIC 1 (a) CRTY ELECTROPHORESIS

In this experiment, we used phusion enzyme to amplify CrtY gene for company sequencing. But after electrophoresis, we found no obvious results in CrtY lane. The preliminary judgment may be: the use of phusion enzyme and add buffer does not match. Now the problems are: 1, the lack of phusion enzyme; 2, the original CrtY gene amplification fragment quantity is too small.

Experiment Name：Measuring concentration of CrtB,CrtE,CrtI,CrtY gene

Date：2019/07/14 **Operators: Wang Ran**

Detailed Steps	Remarks
The concentrations of CrtB, CrtE, CrtI and CrtY genes were determined and re-labeled. (1) The DNA concentration of CrtB, CrtE, CrtI and CrtY genes were measured in B081 laboratory by DNA concentration detector, and the data were recorded.	This column indicates special phenomena or unexpected errors in operation.

Experiment Results

CrtE	CrtB	CrtI	CrtY
9.1ng/ μ L	28.0 ng/ μ L	24.5 ng/ μ L	9.4 ng/ μ L
3.2 ng/ μ L	4.1 ng/ μ L	22.0 ng/ μ L	7.3 ng/ μ L
4.0 ng/ μ L	9.4 ng/ μ L	7.8 ng/ μ L	3.0 ng/ μ L
11.4 ng/ μ L		5.9 ng/ μ L	
63.5 ng/ μ L		11.2 ng/ μ L	
		8.1 ng/ μ L	
		3.3 ng/ μ L	
		0.5 ng/ μ L	
		1.5 ng/ μ L	
		9.3 ng/ μ L	
		10.4 ng/ μ L	
		11.0 ng/ μ L	

This morning, the concentration of the first four genes was detected, and found that the concentration of DNA in most EP tubes is low, especially the concentration of CrtI is low, the collection of tubes is obvious, at present, it is suGGested that after running glue, the recovery stage will be carried out, the corresponding target bands of each lane, if the collected genes are the same, it is suGGested that each gel be collected in a tube first, and then collected through the kit, in order to achieve the effect of enrichment and concentration of target DNA.Only in this way can we lay a good foundation for the next sequencing, seamless connection and other experiments, and the success rate of the next experiment can be improved.

Experiment Name: linearization of plasmid by enzyme cutting, running gel, recycling gel, adding LB liquid culture medium, activating the preserved strain with 184M plasmid, and disposing mother liquid of chloramphenicol (CAT)

Date：2019/07/16

Operators: Fan Xuqian, Liu Ruogu, Wang Ran

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.
(1) Linearization of plasmids by restriction endonuclease: The concentration of plasmids (184M and 99A) was measured.	
(2) The concentrations of 99A: 163.2ng/μl; 99A1: 34.0ng/μl; 184M1: 21.5ng/μl; 184M2: 20.5ng/μl were measured.	
(3) 99A tube and 184M1 enzyme digestion were selected.	
(4) The two plasmids were digested with two 25μl restriction endonuclease systems, adding Hind III 0.5μl, cut smart 2.5μl, plasmid 0.5μg (in which the concentration of 184M plasmid was low, the concentration of 99A was high except for enzyme and buffer), and ddH2O was added to complete the 25μl system.	In this step, due to my personal calculation error, 1μg plasmid was added to the 99A enzyme digestion system.
(5) Water bath at 37 ℃ for 2 hr	
(6) Run gel: configure 0.8% gel, put all the samples on, 5μl 1kb marker.	
(7) Turn on the electrophoresis apparatus, keep the voltage at 170V, press "RUN" to start electrophoresis; wait until the front reaches gel 3/5, press "STOP" to stop electrophoresis, and turn off the power supply.	

Date：2019/07/16

Operators: Fan Xuqian, Liu Ruogu, Wang Ran

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.
(8) Place the gel in the center of the gel imager, open the software and select "UV-AE" to get the gel image, save the file and output it as a picture.	
(9) Gel recovery: cut the gel according to the protocol, combine the two holes to increase the concentration, and finally wash the particles with ddH2O.	
(10) Activated strain: the antibiotic of 184 M plasmid is chloramphenicol, the working concentration of chloramphenicol is 34 μg/ml, the strain preserved now (-80 ℃) is not the strain of single colony, the teacher suggested that we continue to draw a line when cultivating the strain (because the transformation of each strain is different), and then shake the strain in liquid culture medium.	Strain preservation: 800 μl bacterial solution + 800 μl glycerol, marked as cat, one tube is used this time, one tube is left after -80, and remember to preserve the strain after activation of shaking bacteria this time.
(11) Take out a tube of bacteria stored at-80 ℃ and dissolve at room temperature.	
(12) In the super-clean worktable, 500 μl of bacterial solution was taken into the LB plate containing chloramphenicol, uniformly coated, and 3 plates were coated.	
(13) 37 ℃, overnight culture	
(14) Disposition of chloramphenicol (CAT) mother liquid: weigh 0.5 g of chloramphenicol powder, dissolve in absolute ethanol, fix volume to 10 ml, and dispose of 50 mg/ml of chloramphenicol mother liquid.	
(15) The mother liquor was packed into 1.5 μl centrifugal tubes, 10 tubes were divided, 2 tubes were returned to the upstairs laboratory, and 8 tubes were left, which should be enough for later use.	

Date：2019/07/16	Operators: Fan Xuqian, Liu Ruogu, Wang Ran

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. (16) LB liquid culture medium configuration: 200 ml liquid LB culture medium configuration is reserved. (17) So far, there are 400 μl liquid culture medium, solid LB has been used up, follow up, please extinguish the point culture medium.	This column indicates special phenomena or unexpected errors in operation.

Experiment Results

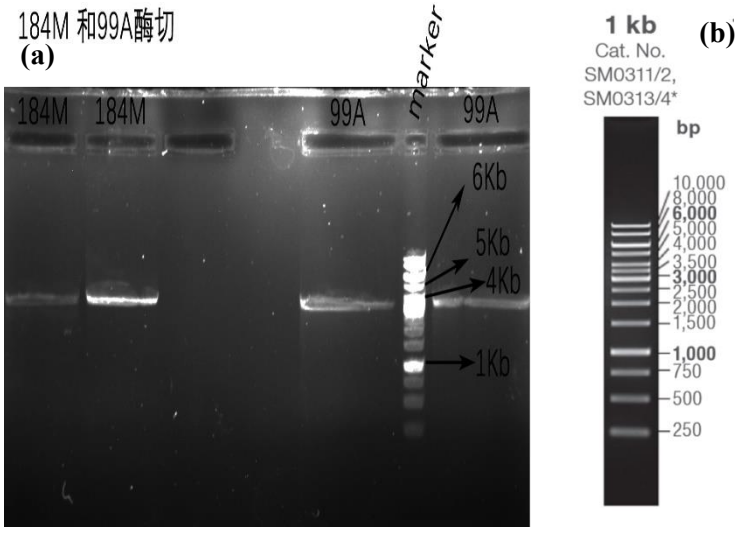


Figure SEQ Figure 1 (a) the bands of 184M and 99A after enzyme digestion; (B) The bands corresponding to marker.

(Description and analysis of results)

1、No circular plasmid was added as control, it was not clear whether the digestion was successful or not, but the bands were single and all appeared near the target size of the plasmid, so the gel was recovered and used as a raw material for exploring the conditions of seamless connection.

2、This time on the 5 μl marker, marker did not run away, the next proposal or on the 3 μl.

3、The concentration of 184M plasmid is low, and less than 50 μl of undigested plasmid is left to culture *Escherichia coli* with 184M plasmid for subsequent experiments.

Reagent preparation

Reagent Name Prepares Linear 184M

Date：2019/07/16 **Operators: Fan Xuqian**

Reagent	Volume	Total Volume
Plasmid	22 μl	25 μl
Buffer（10x cut smart）	2.5 μl	
HindⅢ	0.5 μl	

Reagent Name Preparing Linear 99A

Date：2019/07/16 **Operators: Fan Xuqian**

Reagent	Volume	Total Volume
Plasmid	6 μl	25 μl
Buffer（10x cut smart）	2.5 μl	
HindⅢ	0.5 μl	
DDH2O	13 μl	

Reagent name: LB medium

Date：2019/07/16 **Operators: Liu Ruogu**

Reagent	Volume	Total Volume
Peptone	2g	200ml
Yeast Extract	1g	
NaCl	1g	
ddH2O	Fixed volume to 200ml	

Reagent name: Chloramphenicol (CAT) mother solution (50mg/ml)

Date：2019/07/16 **Operators: Fan Xuqian**

Reagent	Volume	Total Volume
Chloromycetin	0.5g	10ml
Anhydrous ethanol	Fixed volume to 10ml	

Experiment Name：CrtY gene amplication and Extraction

Date：2019/07/17 **Operators: Wang Ran Liu Ruogu**

Detailed Steps	Remarks
The CrtY gene was amplified by PCR and analyzed by DNA gel electrophoresis to obtain high concentration of CrtY DNA. (1) DdH _ 2O, polymerase Buffer, primer, dNTPs, total DNA and DNA high fidelity polymerase were added according to the protocol of PCR. The single tube system was 15 μl, 10 tubes in total, 5 tubes were CrtY gene and 5 tubes were total DNA of P. agglomerans. (2) According to the operation process on the machine, the annealing temperature is set at 60.0° C and the chain extension time is set at 45s on the PCR instrument. (3) The agarose gel was prepared by 1% agarose concentration and 40 ml of agarose gel was prepared. (4) After PCR was completed, Marker and amplified fragments were added, and electrophoresis was carried out for 30 minutes, and then the results were observed under ultraviolet light. (5) The desired DNA band was cut and the DNA was extracted by the kit method. The desired DNA (CrtY gene fragment) was obtained.	This column indicates special phenomena or unexpected errors in operation.

PCR Special Form

Gene Name：CrtY

Primer sequence：R:

F:

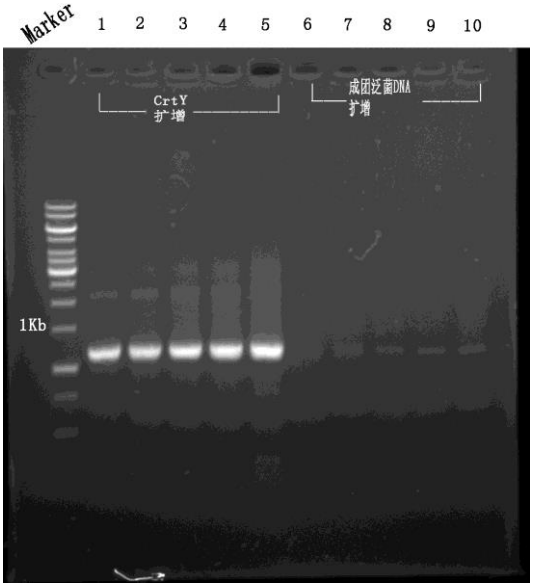
Date：2019/07/17 **Operators: Wang Ran Liu Ruogu**

Reagent	Volume	Total Volume
PCR buffer	3 μ L	
2.5 mM dNTPs	0.3 μ L	
5 '-primer/3' -primer	0.75 μ L	
Phusion enzyme	0.15 μ L	15 μ L
DMSO	0.45 μ L	
DEIONIZED WATER	9 μ L	
Template DNA	0.6 μ L	

PCR Program

FIRST UNSPIN	UNSPIN	Annealing	Extension	Cycle	Deactivation	
95° C	95° C	60°C	72°C	cycle	72° C	4° C
3 min	30 s	20 s	45s	29×	5 min	∞

Experiment Results



PCR amplification of ARABIC 1 in Figure SEQ Figure.

From the final electrophoretic map, we can find that the CrtY gene can effectively amplify the required bands after PCR, and we can still get the product we want when the concentration is 9.1 ng/μL, so we should be able to get the higher concentration CrtY DNA fragments we want after the gel recovery after enrichment, in order to prepare the raw materials for the next seamless connection.

Experiment Name：Combination of CrtB,CrtE and CrtI with plasmid p184M(For appropriate conditions of combination)

Date：2019/07/18 **Operators: Wang Ran**

Detailed Steps	Remarks
The CrtB, CrtE, CrtI genes and 184M plasmids were connected seamlessly for the first time, and then the transformation experiments were carried out to try to obtain the target plasmids and explore the conditions in order to obtain the best connection conditions and ratio and the target plasmids.	This column indicates special phenomena or unexpected errors in operation.
(6) By measuring the concentration of CrtB, CrtE, CrtI and 184M plasmid to calculate, according to the final vector and gene ratio of 1:1 to design, get the amount of each addition.	There was an error setting the Seamless connection time from 30 minutes to 30 seconds.
(7) Premix, gene fragment, vector and nuclease free water were adde to prepare a 20 μl system	The water bath pot was not opened in time, which led to the prolongation of the conversion and adsorption process time.

Date：2019/07/18 **Operators: Wang Ran**

Detailed Steps	Remarks
The CrtB, CrtE, CrtI genes and 184M plasmids were connected seamlessly for the first time, and then the transformation experiments were carried out to try to obtain the target plasmids and explore the conditions in order to obtain the best connection conditions and ratio and the target plasmids.	This column indicates special phenomena or unexpected errors in operation.
(8) The final product was obtained by incubating at 50 ° C for 30 min.	The plate is not matched immediately, resulting in a longer follow-up conversion time.
(9) 5 μ L of that product was mix with commercial competent state (50 to 100 μ L), and the ice bath was used for 30 min	No resistance screening was added to the plate, resulting in crazy growth.
(10) Heat shock for 90 s in a 42 ° C water bath, then cool in an ice bath for 3-5 min	
(11) The product was added to 500 μ L LB culture medium and cultured in 37 ° C shaker for 1hr.	
(12) After centrifugation, 10 ~ 50 μ L LB is left, mixed uniformly, and then coated on a solid LB plate overnight.	
(13) The next day, the colonies were cultured and the plasmids were extracted and tested.	

Seamless Combination experiment:

Gene Name: CrtE, CrtB, CrtI, p184M (experimental group)

Date：2019/07/17 **Operators: Wang Ran**

Reagent	Volume	Total Volume
2X Seamless Premix	10 μ L	20 μ L
CrtE	1 μ L	

CrtB	2.5 μ L	
CrtI	2.5 μ L	
Nuclease free water	1 μ L	
184M plasmid	3 μ L	

Positive Control Group

Date：2019/07/17 **Operators: Wang Ran**

Reagent	Volume	Total Volume
2X Seamless Premix	10 μ L	20 μ L
DNA fragment	1 μ L	
Nuclease free water	8 μ L	
VECTOR PLASMID	1 μ L	

Negative Control Group

Date：2019/07/17 **Operators: Wang Ran**

Reagent	Volume	Total Volume
2X Seamless Premix	10 μ L	20 μ L
Nuclease free water	10 μ L	

PCR Program

Incubation	Connection
50° C	4° C
30 min	∞

Experiment Results

1 SEQ Chart \ * ARABIC \ s 11
Negative Positive Control

1 SEQ Chart \ * ARABIC \ s 12
Experiment Group Results

This experiment is the first experiment of seamless connection, mainly to explore the experiment process, try the first seamless cloning and proportion calculation, the first experiment because there are too many operational errors and material preparation is not timely, resulting in the final result is not satisfactory, but also for the next large number of seamless connection experiments lay a good foundation, give lessons, at the same time in the follow-up experiments have had sufficient experimental design ideas, hope that in the next experiment do not have such low-level errors.

Experiment Name：E.coli cultivate and plasmid extraction

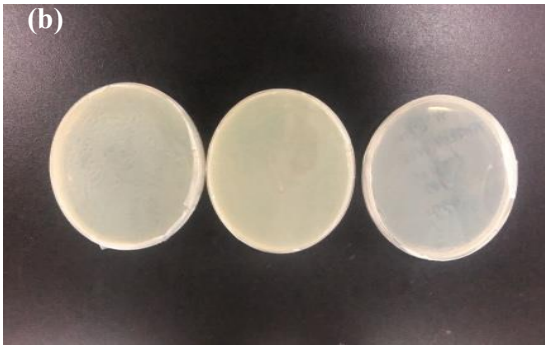
Date：2019/07/19 **Operators: Wang Ran**

Detailed Steps	Remarks
E. coli was separated by streaking to obtain single colony, then cultured in liquid culture medium, and finally collected, the plasmid was extracted and detected by kit.	This column indicates special phenomena or unexpected errors in operation.
(8) 7.17 At 8 o'clock in the morning of the same day, the bacteria were picked out and separated in LB culture medium, and single colonies were isolated overnight.	
(9) 7.18 obtain a single colony, taking a test tube, adde LB culture liquid containing 34 μ g/ml chloramphenicol into that test tube, culturing in a 37 ° C shaking table, and shaking the bacteria overnight;	

Date：2019/07/19 **Operators: Wang Ran**

Detailed Steps	Remarks
E. coli was separated by streaking to obtain single colony, then cultured in liquid culture medium, and finally collected, the plasmid was extracted and detected by kit. (10) After centrifugation, the plasmid was extracted by the kit. (11) The extracted plasmids were identified by electrophoresis.	This column indicates special phenomena or unexpected errors in operation.

Experiment Results



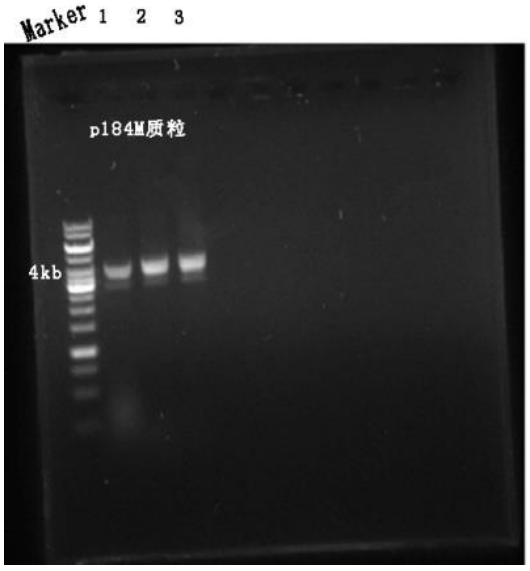


Figure SEQ Figure * Arabic 1 (a) flat line; (B) Flat Coating; (C) P184m electrophoresis results.

The single colony was separated after streaking, and then the plasmid was extracted by amplification, finally the plasmid was verified to be p184M, and the concentration was very high, and the concentration was determined to be 115.5ng/μL

Experiment Name：Combination of CrtB,CrtE and CrtI with plasmid p184M(The third time)

Date：2019/07/20 **Operators: Wang Ran, Pang Zheng**

Detailed Steps	Remarks
The CrtB and CrtE genes were amplified by PCR with temperature gradient, and DNA gel electrophoresis was used to determine the optimal annealing temperature of primers.	This column indicates special phenomena or unexpected errors in operation.
(12) The concentrations of CrtB, CrtE, CrtI, 184M and 99A plasmids were measured and calculated. According to the final vector and gene ratio of 1:1, the quantity of each plasmid was obtained.	Because the bacteria did not grow on the plate in the previous two experiments, so today the empty plasmid coated plate is seamlessly connected in different proportions, and the positive and negative blank control is carried out on the previous plate.
(13) Premix, gene fragment, vector and nuclease free water were adde to prepare a 20 μl system	
(14) The final product was obtained by incubating at 50 ° C for 30 min.	
(15) 5 μL of that product was mix with commercial competent state (50 to 100 μL), and the ice bath was used for 30 min	

Date：2019/07/20 **Operators: Wang Ran, Pang Zheng**

Detailed Steps	Remarks
The CrtB and CrtE genes were amplified by PCR with temperature gradient, and DNA gel electrophoresis was used to determine the optimal annealing temperature of primers.	This column indicates special phenomena or unexpected errors in operation.
(16) Heat shock for 90 s in a 42 ° C water bath, then cool in an ice bath for 3-5 min.	
(17) The product was added to 500 μl LB culture medium and cultured in 37 ° C shaker for 1 hr. (18) After centrifugation, 10 ~ 50 μl LB was left, mixed, and then coated on a solid LB antibiotic plate overnight.	In this experiment, we used the plasmid p184M and 99A, the resistance were chloramphenicol and ampicillin, the concentration were 34 μg/ml and 100 μg/ml.
(19) The next day, the colonies were cultured and the plasmids were extracted and tested.	

Seamless Combination experiment:

Gene Name: CrtE, CrtB, CrtI, p184M (experimental group 1-1)

Date：2019/07/20 **Operators: Pang Zheng, Wang Ran**

Reagent	Volume	Total Volume
2X Seamless Premix	10 μl	20 μl
CrtE	1 μl	
CrtB	2.5 μl	
CrtI	2.5 μl	
Nuclease free water	1 μl	
184M plasmid	3 μl	

Gene Name: CrtE, CrtB, CrtI, p184M (experimental group 1-2)

Date：2019/07/20 **Operators: Pang Zheng, Wang Ran**

Reagent	Volume	Total Volume
2X Seamless Premix	10 μl	20 μl
CrtE	1 μl	
CrtB	3 μl	
CrtI	3 μl	
Nuclease free water	1 μl	
184M plasmid	2 μl	

Gene Name: CrtE, CrtB, CrtI, p99A (experimental group 2-1)

Date：2019/07/20 **Operators: Pang Zheng, Wang Ran**

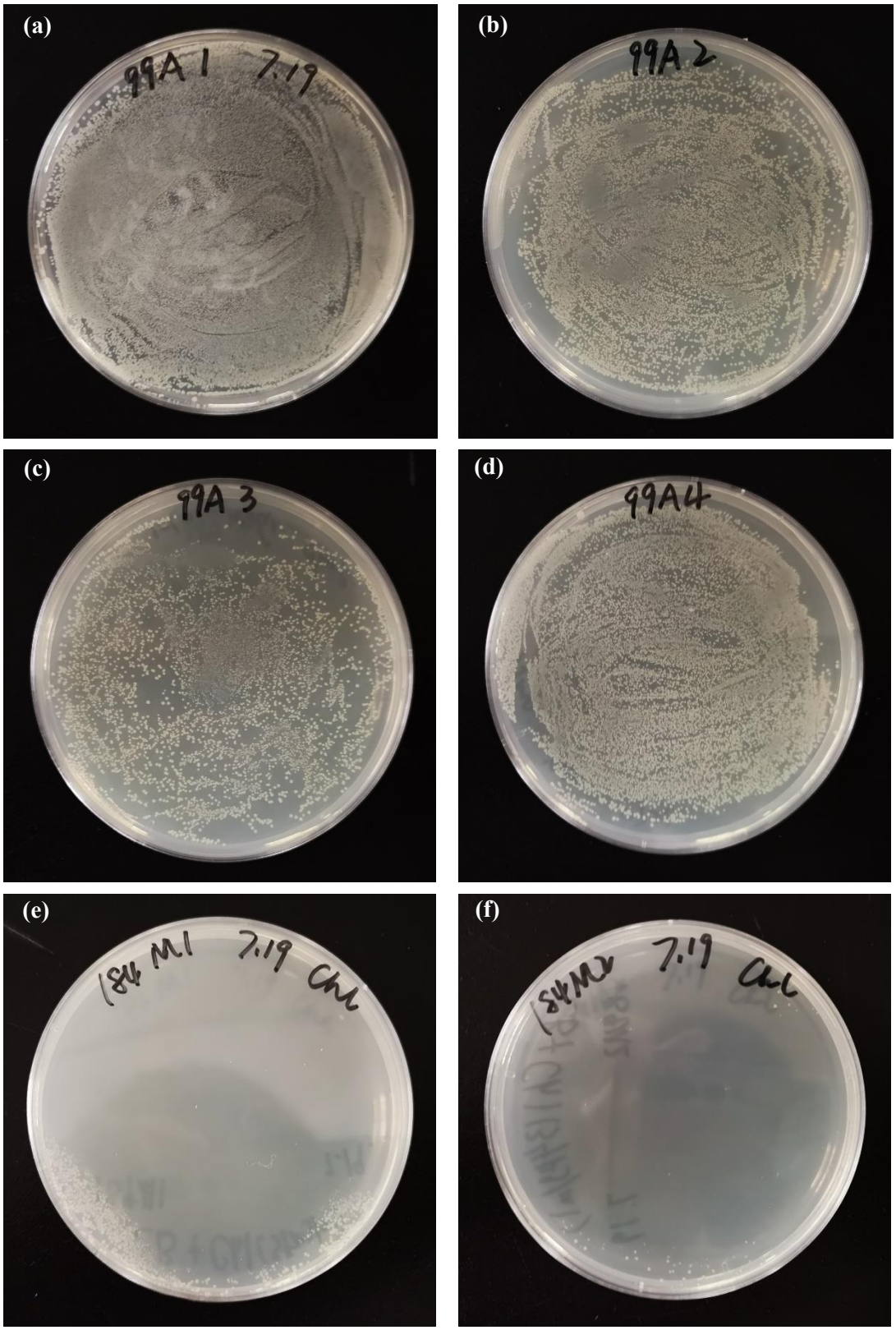
Reagent	Volume	Total Volume
2X Seamless Premix	10 μl	20 μl
CrtE	1 μl	
CrtB	2.5 μl	
CrtI	2.5 μl	
Nuclease free water	1 μl	
184M plasmid	3 μl	

Gene Name: CrtE, CrtB, CrtI, p99A (experimental group 2-2)

Date：2019/07/20 **Operators: Pang Zheng, Wang Ran**

Reagent	Volume	Total Volume
2X Seamless Premix	10 μl	20 μl
CrtE	1 μl	
CrtB	3 μl	
CrtI	3 μl	
Nuclease free water	1 μl	
184M plasmid	2 μl	

Experiment Results


(a)
99A 1 7.19
(b)
99A 2
(c)
99A 3
(d)
99A4
(e)
(f)

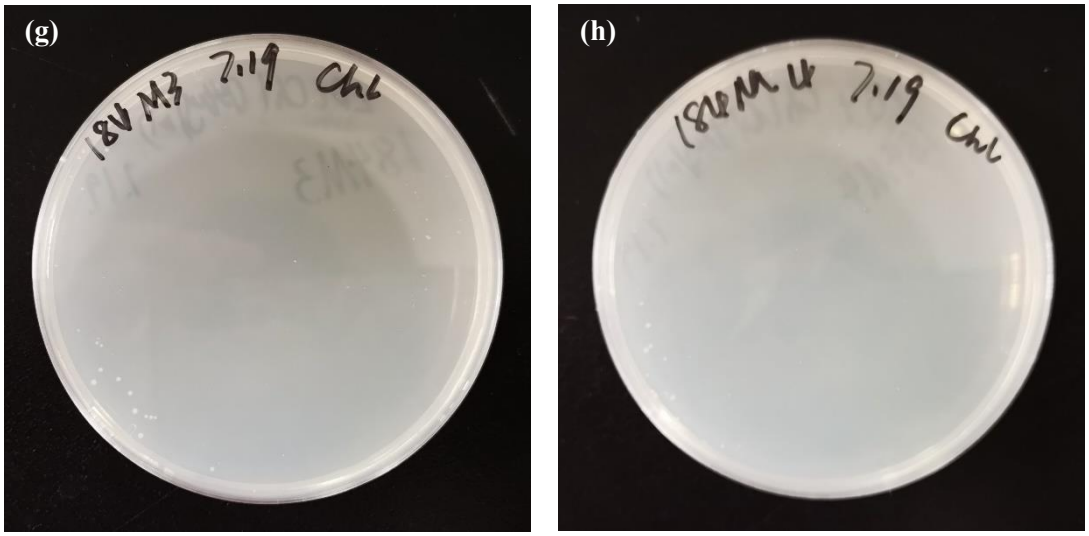


Figure SEQ Figure * Arabic 1 (a) 99a blank plasmid coating; (B) 99a Seamless Connection Experiment Group 1; (C) 99a Seamless Connection Experiment Group 2; (d) 99a Seamless Connection Experiment Group 3; (e) 184m blank plasmid plate condition; (f) 184m Seamless Connection Experiment Group 1; (G) 184m Seamless Connection Experiment Group 2; (H) 184m Seamless Connection Experiment Group 3.

The results showed that there was no plasmid inactivation in 184M and 99A plasmids. At the same time, transformation *Escherichia coli* appeared in the experimental group, but from the results of bacterial p electrophoresis, it could be concluded that there was no successful result of ligation.

Experiment Name: Colony PCR Identification of Vector Linkage

Date：2019/07/21 **Operators: Liao Shujie, Li Zixuan**

Detailed Steps	Remarks
(20) Preparation of colony PCR reaction system (premixed and then subpacked into PCR tube)	
(21) 24 single colonies were selected from the plate with transformants with white spear heads, transferred to a new resistant plate, numbered 1-24, and the spear heads were inserted into the PCR tube.	The number of isolated colonies varies from case to case.
(22) Take out the gun head, put the PCR tube into the PCR machine to amplify.	

Date：2019/07/21 **Operators: Liao Shujie, Li Zixuan**

Detailed Steps	Remarks
(23) Results of agarose gel electrophoresis	

PCR Special Form

Gene Name：99A：CrtE-CrtB-CrtI、184M：CrtE-CrtB-CrtI

Primer sequence：F: CrtE F

R: CrtI R

Date：2019/05/22 **Operators: Please write down the names of all operators.**

Reagent	Volume	Total Volume
High GC buffer	3 μl	15 μl
10 mM dNTPs	0.3 μl	
5 '-primer/3' -primer	0.75 μl	
Phusion enzyme	0.15 μl	
DEIONIZED WATER	9.3 μl	
DMSO	0.75 μl	

PCR Program

FIRST UNSPIN	UNSPIN	Annealing	Extension	Cycle	Deactivation	
95° C	95° C	58-61°C (0.1 °C increase per cycle)	72° C	cycle	72° C	4° C
5 min	30 s	30 s	3 min	30×	5 min	∞

Experiment Results

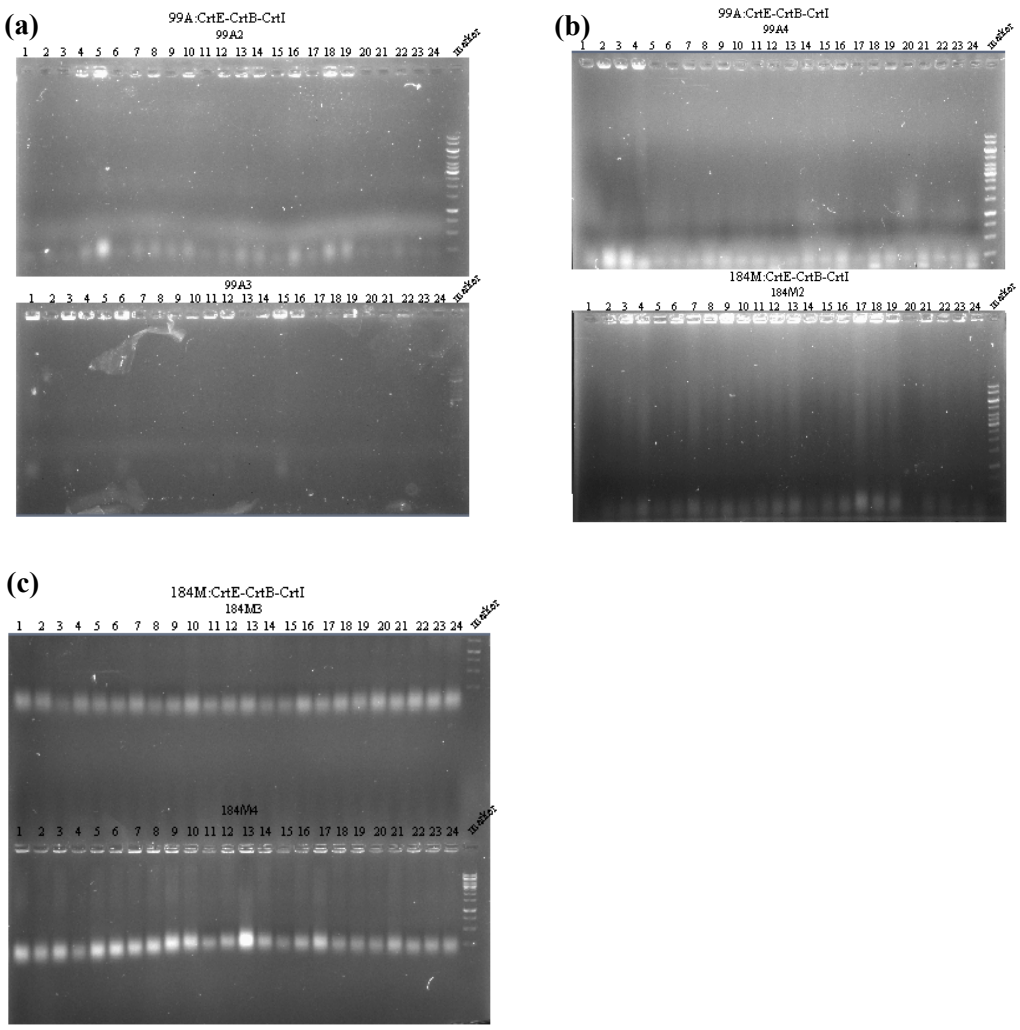


Figure SEQ Figure 1 (a) 99A: crte-crtb-crti plate 2, 3 bacteria p results; (B) 99A: crte-crtb-crti plate 4, 184m: crte-crtb-crti plate 2 bacteria p results; (C) 184m: crte-crtb-crti plate 3, 4 bacteria p results.

The possible reasons were as follows: (1) the plasmid was not linearized completely, and there were many false-positive clones; (2) the fragment between the P primers was too long; (1) the circular plasmid was used as control, and the restriction enzyme digestion was determined by electrophoresis; if the restriction enzyme digestion effect was not ideal, the restriction enzyme digestion time should be prolonged; (2) the false positive rate was too high when the plasmid was transformed into *Escherichia coli* and not transformed into *Escherichia coli*; (3) the P primers were redesigned, and only the fragment of several hundred BP at the interface was amplified.

Reagent preparation

Reagent name: Amp $^{+}$ resistant plate

Date：2019/07/21 **Operators: Liao Shujie**

Reagent	Volume
LB solid medium	200 mL
100 mg/mL Amp	200 μl

Experiment Name：Overlapping PCR of CrtE and CrtB

Date： 2019/07/22 **Operators：** Ran Wang, Shujie Liao

Detailed Steps	Notes
Fusoin PCR gel electrophoresis for CrtB and CrtE, explore the condition, and. (24) ddH2O, polymerase Buffer, primer, dNTPs, total DNA and DNA high-fidelity polymerase were added respectively according to the proportion given by the protocol of PCR. The single tube system was 15 μl, with a total of tubes, and the DNA was mixed with CrtE and CrtB. (25) According to the operation process on the machine, the annealing temperature is 60.0℃ and the chain extension time is 2 minutes on the PCR machine. (26) Agrose gel of 30ml with 3 μl nucleic acid dye was prepared at 1% agarose concentration when waiting for fragments amplication. (27) After the PCR, the fragments were added to electrophoresis at 150V for 20min, then the results were observed under ultraviolet light.	

PCR Special Form

Gene Name：CrtE CrtB

Primer sequence： R: GGAGTTTCATCTAGATCGGGTTGGCCCGGTTCCC

F: GAGTCGACCTGCAGGCATGCAATGAGGCACAAGATGGCGTTTGAACAGCGG

Date： 2019/07/22 **Operators：** Ran Wang,Shujie Liao

Reagent	Volume	Total Volume
PCR Buffer	3μL	
2.5 mM dNTPs	0.3μL	
5’-primer/3’-primer	0.75μL	
Phusion DNA Polymerase	0.15μL	15μL
ddH2O	μL	
CrtB	μL	
CrtE	μL	

PCR Program

Initial Denaturation	Denaturation	Annealing	Extension	Cycles	Final Extension	Hold
95ºC	95ºC	60ºC	72ºC	cycle	72ºC	4ºC
3 min	30 s	30 s	2min	20~30×	5 min	∞

Experiment Results

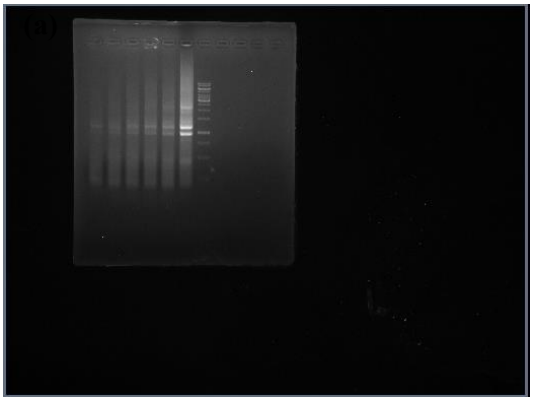


Figure 2 (a)CrtE,CrtB fusion PCR.

The result was not ideal for there were too many miscellaneous bands in the background, affecting the observation of experiment result. Further exploration on experiment condition is necessary

Experiment Name：

Date： 2019/07/24 **Operators：** Ran Wang,Zixuan Li

Detailed Steps	Notes
The CrtE gene was amplified by PCR temperature gradient and detected by DNA gel electrophoresis. The 184M plasmid was digested by HindIII and examined.	
(28) Add ddH2O, polymerase Buffer, forward and reverse primers, dNTPs, total DNA and DNA high-fidelity polymerase according to the proportions given by protocol. The single tube system is 15 μl, with a total of 6 tubes, which are CrtE genes.	DNA samples ran out of the gel and can not be detected
(29) Digestion of p184M plasmid was performed before PCR. Cut smart	because of the

Date： 2019/07/24 **Operators：** Ran Wang, Zixuan Li

Detailed Steps	Notes
The CrtE gene was amplified by PCR temperature gradient and detected by DNA gel electrophoresis. The 184M plasmid was digested by HindIII and examined.	
buffer, p184M plasmid, ddH 2, Hind III endonuclease were added according to the calculation.	long running electrophoresis time.
(30) Water bath for 2h at 37℃.	
(31) Run PCR according to the established program on PCR machine.	
(32) Agar gel was prepared during when waiting for amplification, and the agarose gel of 30ml with 3 μl nucleic acid dye was prepared at 1% agarose concentration.	The plasmid was added too little to retrieve
(33) After PCR, Marker and amplified fragments were added to electrophoresis for 20 minutes, and then the results were observed under ultraviolet light.	

PCR Special Form

Gene Name：CrtE

Primer sequence： R:

F:
GAGTCGACCTGCAGGCATGCAATGAGGCACAAGATGGCGTTTGAACAGCGG

Date： 2019/07/24 **Operators：** Ran Wang, Zixuan Li

Reagent	Volume	Total Volume
PCR Buffer	3μL	
2.5 mM dNTPs	0.3μL	
5’-primer/3’-primer	0.75μL	
Phusion DNA Polymerase	0.15μL	15μL
ddH2O	9μL	
DMSO	0.45μL	
TemplateDNA	0.6μL	

PCR Program

Initial Denaturation	Denaturation	Annealing	Extension	Cycles	Final Extension	Hold
95ºC	95ºC	60ºC	72ºC	cycle	60ºC	4ºC
3 min	30 s	20 s	45s	30×	5 min	∞

Experiment Results

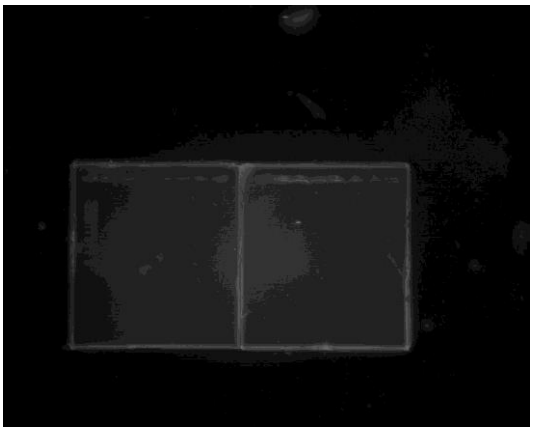


Figure 3 (a) CrtE amplication (left) (b)p184M enzyme digest(right)

Because of the long running time and the small amount of p184M plasmid, the experimental results can not be shown, which leads to the failure of the experiment.

Experiment Name：CrtE amplication

Date： 2019/07/25 **Operators：** Ran Wang

Detailed Steps	Notes
The CrtE gene was amplified by PCR from the total DNA, and DNA gelw was retrieved by electrophoresis and was used as a reserve resource.。 (34) Add ddH2O, polymerase Buffer, forward and reverse primers, dNTPs, total DNA and DNA high-fidelity polymerase according to the proportions given by protocol. The single tube system is 25 μl, with a total of 6 tubes. (35) Run PCR according to established programe on PCR machine	

Date： 2019/07/25 **Operators：** Ran Wang

Detailed Steps	Notes
The CrtE gene was amplified by PCR from the total DNA, and DNA gelw was retrieved by electrophoresis and was used as a reserve resource.。 (36) Agar gel was prepared during when waiting for amplification, and the agarose gel of 30ml with 3 μl nucleic acid dye was prepared at 1% agarose concentration. (37) After PCR, Marker and amplified fragments were added to electrophoresis for 20 minutes, and then the results were observed under ultraviolet light. (38) Cut the interested DNA band and retrieve with kit.	

PCR Special Form

Gene Name：CrtE

Primer sequence： R:

F:

Date： 2019/07/25 **Operators：** Ran Wang

Reagent	Volume	Total Volume
PCR Buffer	5μL	25μL
2.5 mM dNTPs	0.5μL	
5’-primer/3’-primer	1.25μL	
KOD 酶	0.25μL	
ddH2O	5.75μL	
TemplateDNA	1μL	

PCR Program

Initial Denaturation	Denaturation	Annealing	Extension	Cycles	Final Extension	Hold
95ºC	95ºC	60ºC	72ºC	cycle	72ºC	4ºC
3 min	30 s	30 s	45s	29×	5 min	∞

Experiment Results

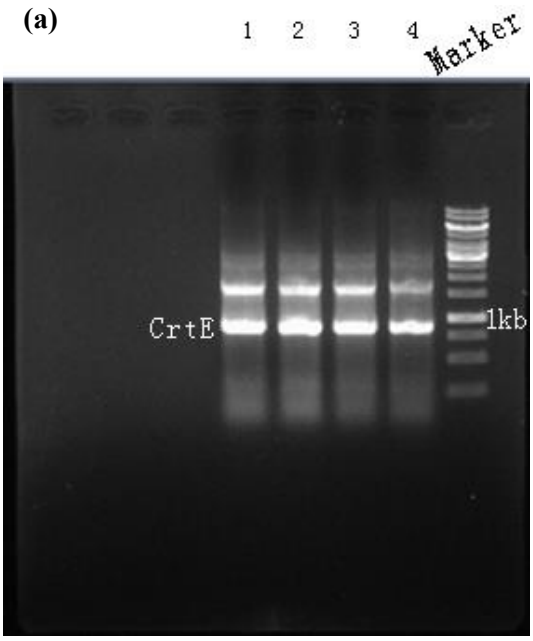


Figure 4 (a) CrtE PCR amplication

The results showed that non-specific amplification appeared in the PCR, ignoring the fragment and cutting the band below about 1 kb, which was the band of CrtE. However, it is still uncertain that it is the CrtE gene, which needs to be sent to the company for sequencing before accurate results can be obtained.

Experiment Name：pET Plasmid Extraction

Date：2019/08/02 **Operators：**Ran Wang

Detailed Steps	Notes
(39) Pour the bacterial liquid into 2 ml centrifugal tube, centrifuge 10,000 ×g for 1 minute, and collect the bacteria.	5 mL bacterial liquid was collected in a tube.
(40) Disposal the medium, add 250 mu L Buffer P1/RNase A mixture, and shake with vortex.；	
(41) Add 250 mu L Buffer P2 to the suspension and mix it upside down (6-8 times);	
(42) Add 350 mu L Buffer NP3 and immediately reverse 8-10 times to completely neutralize the solution.	
(43) Centrifugation at 13000×g for 1 min;	
(44) put HiPure DNA Mini Column II in collecting pipe.Pour the supernatant into the column. 13,000 ×g centrifugation for 1 min	
(45) Discard the filtrate and recycle the column sleeve into the header. Add 500 mL Buffer PW1 to the column. 13,000 x g centrifugation for 1 min;	
(46) Discard the filtrate and recycle the column sleeve into the header. 13,000 × g centrifugal drying column for 2 min. Open the lid and dry for a few minutes.	
(47) Put the column in a sterilized 1.5mL centrifugal tube. Add 50 ml Elution Buffer to the center of the membrane of the column. DNA was eluted by centrifugation for 1 minute and 13,000 x g for 1 minute.	
(48) The column was removed and the quality of plasmid was detected by agarose gel. The plasmid concentration was detected by DNA concentration detector in B081 laboratory.	Electrophoresis with the cut plasmid on August 3.

Experiment Results

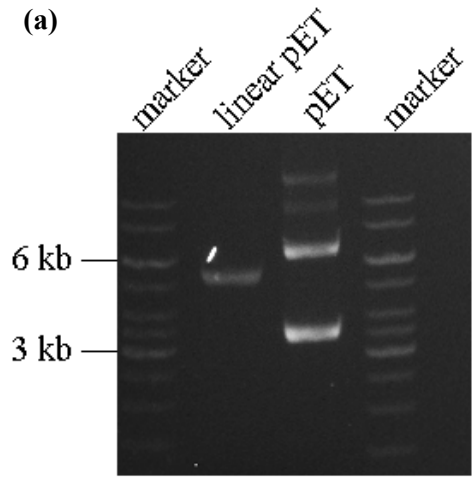


Figure 5 (a) The results of electrophoresis before and after plasmid pET digestion.

The size of pET plasmid is 5422 bp. There are two bright bands and two dark bands in the picture. The bands smaller than 6 KB should be superhelical pET plasmid. The bands larger than 6 KB may be circular pET with different conformations. The superhelix band of the extracted pET was bright in the electrophoresis test, and no linear band was found. The concentration of the extracted pET was 94.0 ng/μL by DNA concentration detector, which indicated that the quality of the extracted plasmid was good.

Experiment Name：CrtE、CrtB fusion PCR condition exploration

Date： 2019/08/02 **Operators：** Ran Wang ,Shujie Liao

Detailed Steps	Notes
(1) Preparing fusion PCR system according to PCR special form and setting up the program of PCR. (2) Electrophoresis is carried out after PCR.	

PCR Special Form

Gene Name：CrtB、CrtE fusion

Primer sequence： F:CrtE F（no RBS，conect to 184M homologous arm）

R:CrtB R（no RBS，conect to CrtI homologous arm）

Date：2019/08/02 **Operators：**Ran Wang, Shujie Liao

Reagent	Volume	Total Volume
GC buffer	3 μL	15 μL
10 mM dNTPs	0.3 μL	
5’-primer/3’-primer	0.75 μL	
DMSO	0.75 μL	
Tag DNA polymerase	0.15 μL	
ddH2O	6.3 μL	
CrtB	1.5 μL	
CrtE	1.5 μL	

PCR Program

Initial Denaturation	Denaturation	Annealing	Extension	Cycles	Final Extension	Hold
95ºC	95ºC	60ºC 65ºC 70ºC	72ºC	cycle	72ºC	4ºC
3 min	30 s	20 s	1 min	30×	5 min	∞

Experiment Name：CrtE、CrtB、CrtI、CrtY PCR

Date：2019/08/02 **Operators：**Ran Wang, Shujie Liao

Detailed Steps	Notes
(3) Preparing fusion PCR system according to PCR special form and setting up the program of PCR. (4) Electrophoresis is carried out after PCR.	

PCR Special Form

Gene Name：CrtB、CrtE、CrtY

Primer sequence：F: pET-CrtE F、pET-CrtB F、pET-CrtY F

R: pET-CrtE R、pET-CrtB R、pET-CrtY R

Date：2019/08/02 Operators：Ran Wang, Shujie Liao

Reagent	Volume	**Total Volume**
GC buffer	3 μL	15 μL
10 mM dNTPs	0.3 μL	
5'-primer/3'-primer	0.75 μL	
DMSO	0.45 μL	
Phusion DNA Polymerase	0.15 μL	
ddH2O	9 μL	
TemplateDNA	0.6 μL	

Gene Name：**CrtI**

Primer sequence：F: pET-CrtI F

R: pET-CrtI R

Date：2019/08/02 Operators：Ran Wang, Shujie Liao

Reagent	Volume	**Total Volume**
GC buffer	3 μL	15 μL
10 mM dNTPs	0.3 μL	
5'-primer/3'-primer	0.75 μL	
DMSO	0.75 μL	
Phusion DNA Polymerase	0.15 μL	
ddH2O	8.4 μL	
Template DNA	0.9 μL	

PCR Program

Initial Denaturation	Denaturation	Annealing	Extension	Cycles	Final Extension	Hold
95ºC	95ºC	60ºC	72ºC	cycle	72ºC	4ºC
3 min	30 s	20 s	1 min	30×	5 min	∞

Experiment Results

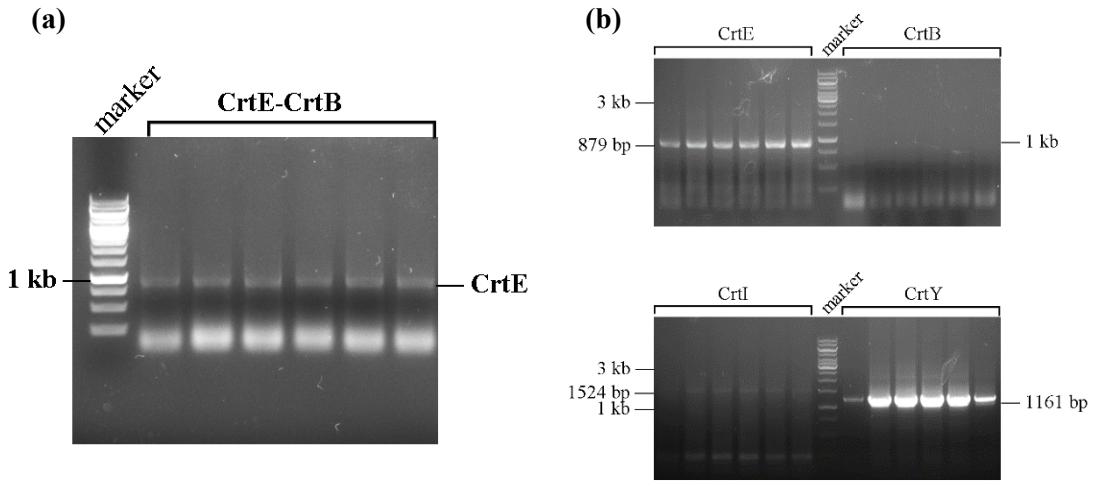


Figure 6 (a) CrtE-CrtB fusion PCRresult; (b) CrtE,CrtB,CrtI,CrtY PCR result.

CrtE-CrtB fusion PCR was the target band, but only the band which might be CrtE (879 bp) appeared. It may be that the CrtB template degraded due to the storage time was too long. The four genes of Crt E, CrtB, CrtI and CrtY were amplified by PCR with newly synthesized primers. The target bands of CrtE and Crt Y appeared bright, the target bands of CrtI were dark, and the target bands of CrtB did not appear. Because the template used by CrtB was the same as that used by fusion PCR, it was considered that template degradation resulted in no target bands. It is suggested that the CrtB gene should be re-amplificated with the total DNA of *Rhodobacter sphaeroides*.

Experiment Name：DNA Gel Extraction

Date： 2019/08/02 **Operators：** Ran Wang, Shujie Liao

Detailed Steps	Notes
(1) cut the agarose gel containing the target DNA into the 1.5 mL centrifuge tube under ultraviolet lamp and weigh the quality of the block.	
(2) add 3 times the DE-A volume of the gel volume and heat it to 75 degrees until the gel melts completely.	The size of the gel is too large, so it is melted into one tube

Date： 2019/08/02 **Operators：** Ran Wang, Shujie Liao

Detailed Steps	Notes
	and then divided into two tubes.
(3) Add DE-B solution of 0.5 times DE-A volume and mix evenly until the solution is completely yellow.	
(4) Place the DNA retrieve preparation tube in a 2 mL centrifugal tube, transfer the solution obtained from the previous step to the DNA retrieve preparation tube, centrifuge 12 000×g for 1 minute, pour the filtrate back into the preparation tube, centrifuge 12 000 g for 1 minute, and discard the filtrate.	CrtE and CrtY are recycled into two tubes, and CrtI is merged into one tube.
(5) 500 UL buffer W1 scrubbing preparation tube, 12 000 g centrifugal 30 s, discarded filtrate	
(6) 700 mL buffer W2 washing preparation tube, 12 000 g centrifuge for 30 seconds, discarded filtrate, washed again in the same way, centrifuge for 1 minute	
(7) Place the prepared tube back into centrifugal tube, 12000 g, centrifugal for 1 minute.	
(8) Place the preparation tube in a clean 1.5 mL centrifugal tube, add 25-30 μL ddH2O (heated at 65 C) in the center of the preparation membrane, and leave it at room temperature for 1 minute, 12 000 g, centrifuge for 1 minute, and elute DNA.	
(9) Measure the concentration of the DNA with B081	

Experiment Name：CrtE、CrtB、CrtI、CrtY amplication

Date： 2019/08/05 **Operators：** Ran Wang, Shujie Liao

Detailed Steps	Remarks
Amplify CrtB,CrtE,CrtI and CrtY by PCR, examine by DNA gel electrophoresis and retrieve	
(49) According to the proportions given by protocol, ddH2O, polymerase buffer, forward and reverse primers, dNTPs, total DNA	

Date： 2019/08/05 **Operators：** Ran Wang, Shujie Liao

Detailed Steps	Remarks
Amplify CrtB,CrtE,CrtI and CrtY by PCR, examine by DNA gel electrophoresis and retrieve and DNA high-fidelity polymerase were added respectively. The single tube system was 15 μl, and each gene contained 7 tubes, totally 28 tubes. (50) Adjusting annealing temperature and elongation time to run according to the established program on the machine. (51) Agar gel was prepared during the process of waiting for amplification, and the agarose gel of 60ml was prepared by 1% agarose concentration. In addition, 6μ L nucleic acid dye should be noticed. (52) After the completion of the PCR, Marker and amplified fragments were electrophoresised at 150 V for 20 minutes, then the results were observed under ultraviolet light. (53) The DNA fragments were cut and extracted by kit. Get the interested DNA fragments and detect the concentration.	

PCR Special Form

Gene Name： CrtE

Primer sequence： R:PET-CrtE R

F: PET-CrtE F

Date： 2019/08/05 **Operators：** Ran Wang

Reagent	Volume	Total Volume
PCR buffer	3μL	
2.5 mM dNTPs	0.3μL	
5’-primer/3’-primer	0.75μL	15μL
Phusion DNA Polymerase	0.15μL	
DMSO	0.45μL	

ddH2O	9.0μL	
Template DNA	0.6μL	

Gene Name：CrtB

Primer sequence：R: PET-CrtB R

F: PET-CrtB F

Date：2019/08/05 **Operators：**Ran Wang

Reagent	Volume	Total Volume
PCR buffer	3μL	
2.5 mM dNTPs	0.3μL	
5’-primer/3’-primer	0.75μL	
Phusion DNA Polymerase	0.15μL	15μL
DMSO	0.75μL	
ddH2O	8.7μL	
Template DNA	0.6μL	

Gene Name：CrtI

Primer sequence：R: PET-CrtI R

F: PET-CrtI F

Date：2019/08/05 **Operators：**Ran Wang

Reagent	Volume	Total Volume

PCR buffer	3μL	
2.5 mM dNTPs	0.3μL	
5’-primer/3’-primer	0.75μL	
Phusion DNA Polymerase	0.15μL	15μL
DMSO	0.75μL	
ddH2O	8.4μL	
Template DNA	0.9μL	

Gene Name： CrtY

Primer sequence： R: PET-CrtY R

F: PET-CrtY F

Date： 2019/08/05 **Operators：** Ran Wang

Reagent	Volume	Total Volume
PCR Buffer	3μL	
2.5 mM dNTPs	0.3μL	
5’-primer/3’-primer	0.75μL	
Phusion DNA Polymerase	0.15μL	15μL
DMSO	0.45μL	
ddH2O	9.0μL	
Template DNA	0.6μL	

PCR Program

Initial Denaturation	Denaturation	Annealing	Extension	Cycles	Final Extension	Hold
95ºC	95ºC	60ºC	72ºC	cycle	72ºC	4ºC
3 min	30 s	20s	1min	29×	5 min	∞

Experiment Results

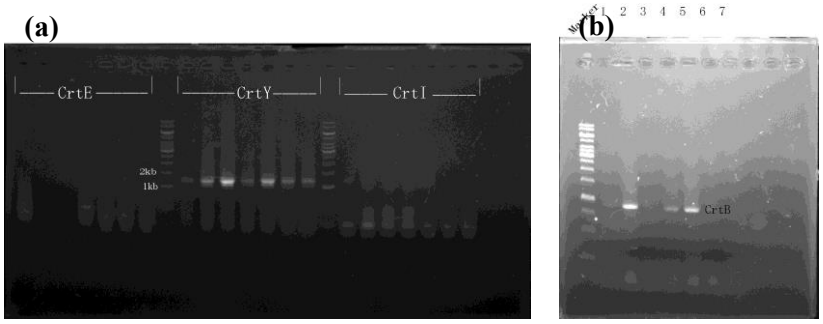


Figure 7 (a) CrtE、CrtY、CrtI PCR amplification result;(b) CrtB PCR amplification result.

From gel images, we can see that the genes of CrtE and CrtI have not been successfully obtained, which may be related to the unmatching of raw materials, genes and primers, and the amount of gene added. The CrtY gene is successfully amplified, and the next step of the enzyme digestion experiment can be carried out. CrtB is partly amplified. In the subsequent PCR, some conditions can be changed, and the influencing factors can be observed and foun

Experiment Name：CrtE、CrtB、CrtI、CrtY amplication

Date： 2019/08/06 **Operators：** Zheng Pang

Detailed Steps	Notes
(1) Adding ddH_2O, polymerase Buffer, pre-and post-primers, dNTPs, total DNA and DNA high-fidelity polymerase according to the protocol. The single tube system is 15 ml, 7 tubes each gene. There are 28 tubes. (2) Adjusting annealing temperature and prolonging time to run according to the established program on the machine (3) Agarose gel was prepared with the concentration of 1% agarose. 6 μ l μ nucleic acid dye was added into 60 ml agarose gel before it's been cooled down. (4) Marker and PCR products were added into the gel. Setting Electrophoresis to 150V for 20 min. The results were then observed under ultraviolet light. (5) The DNA bands were cut and extracted by kit method. Getting the DNA fragments we want and measuring the concentration.	

Gene Name： CrtI

Primer sequence： R: PET-CrtI R

F: PET-CrtI F

Date： 2019/08/06 **Operators：** Zheng Pang

Reagent	Volume	Total Volume
GC buffer	3μL	15μL
10 mM dNTPs	0.3μL	
5'-primer/3'-primer	Each 0.75μL	
Phusion DNA Polymerase	0.15μL	
DMSO	0.75μL	
ddH2O	8.4μL	

TemplateDNA	0.9μL	

PCR Program

Initial Denaturation	Denaturation	Annealing	Extension	Cycles	Final Extension	
95ºC	95ºC	60ºC	72ºC	Cycle	72ºC	4ºC
3 min	30 s	20s	1min	29×	5 min	∞

Date： 2019/08/06 **Operators：** Shujie Liao

Detailed Steps	Notes
(6) Adding ddH_2O, polymerase Buffer, pre-and post-primers, dNTPs, total DNA and DNA high-fidelity polymerase according to the protocol. The single tube system is 15 ml, 7 tubes each gene. There are 28 tubes. (7) Adjusting annealing temperature and prolonging time to run according to the established program on the machine (8) Agarose gel was prepared at the concentration of 1% agarose and 60 ml agarose gel was prepared. Attention should be paid to the addition of 6 mu L nucleic acid dye. (9) Marker and amplified fragments were added after the PCR. Electrophoresis at 150V for 20 min. The results were then observed under ultraviolet light. (10) The DNA bands were cut and extracted by kit method. Get the DNA fragments we want and measure the concentration.	

Gene Name： CrtI

Primer sequence： R: PET-CrtI R

F: PET-CrtI F

Date： 2019/08/06 **Operators：** Shujie Liao

Reagent	Volume	Total Volume
GC buffer	3μL	15μL
10 mM dNTPs	0.3μL	

5’-primer/3’-primer	Each 0.75μL
Phusion DNA Polymerase	0.15μL
DMSO	0.75μL
ddH2O	8.4μL
TemplateDNA	0.9μL

PCR Program

Initial Denaturation	Denaturation	Annealing	Extension	Cycles	Final Extension	
95ºC	95ºC	58ºC/62 ºC/64 ºC/66 ºC/68 ºC/70 ºC	72ºC	cycle	72ºC	4ºC
3 min	30 s	20s	1min	29×	5 min	∞

Experiment Results

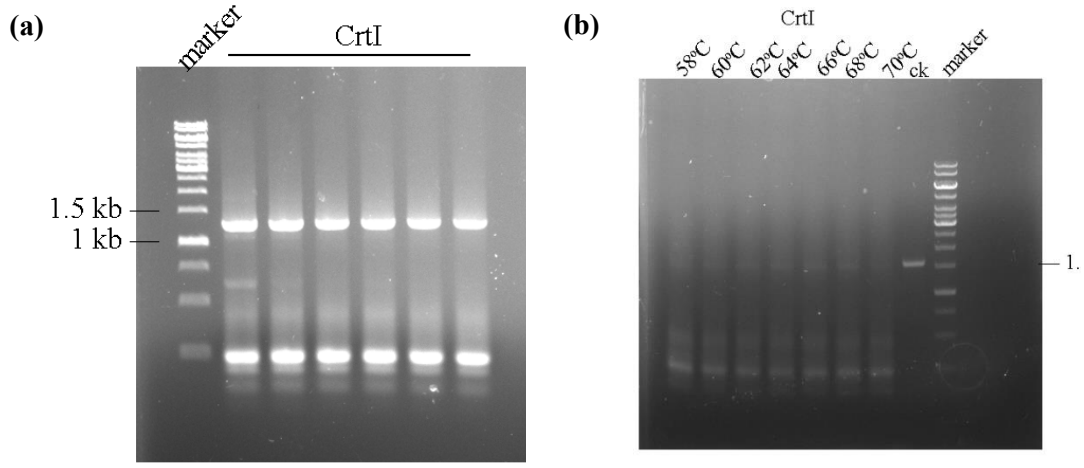


Figure 8 (a) CrtI PCR amplification; (b) CrtI temperatuer gradient PCR amplification.

The size of CrtI is 1500 bp. Bright bands appear in figure a, but the size is slightly less than 1500 bp. It may be that the dye or loading buffer causes the band to be pre-positioned. After gel retrieve, re-electrophoresis detection (ck in figure b)

showed correct band size. It shows that the band of about 1300 BP in figure a is the product of CrtI amplification.

Only weak bands appeared in CrtI temperature gradient PCR, which may be due to the long storage time of template and hight rate of degradation.

Experiment Name：Linking pET-CrtY

Date： 2019/08/06 **Operators：** Zheng Pang

Detailed Steps	Notes
(11) Prepare system according to the table below (12) Mental bath at 25℃ for 2h. (13) Transform E.coli DH5α (14) Overnight at 37℃	

Date： 2019/08/05 **Operators：** Shujie Liao

Reagent	Volume	Total Volume
pET	5 μL	10 μL
CrtY	3 μL	
T4 ligase buffer	1 μL	
T4 ligase	1μL	

Experiment Results

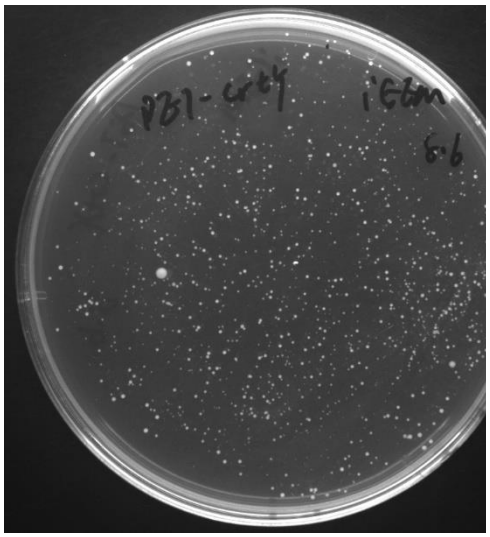


Figure 9 (a) pET-CrtB transforming result.

A transformant appeared 24 hours after culture, and more transformants appeared 48 hours after culture, but only the transformant which appeared the first day was positive according to the following colony PCR.

Experiment Name：pET-CrtE、pET-CrtB strain PCR

Date： 2019/08/07 **Operators：** Shujie Liao

Detailed Steps	Notes
(54) Prepare the PCR system according to the tabel below. (55) Select single colony with small gun head, mark on new resistance plate and insert the gun head to coresponding PCR tube. (56) After all the colonies have been picked out, gently shake the PCR tube, and then remove the small gunhead from the PCR tube. (57) Run PCR program (58) Agarose gel electrophoresis	

PCR Special Form

Gene Name：CrtE，CrtB

Primer： pET-CrtE F/R、pET-CrtB F/R

Date： 2019/08/07 **Operators：** Shujie Liao

Reagent	Volume	Total Volume
One Taq Quick-Load 2x Mix	7.5 μL	
5'-primer/3'-primer	0.75 μL	15 μL
ddH2O	6 μL	

PCR Program

Initial Denaturation	Denaturation	Annealing	Extension	Cycles	Final Extension	
95ºC	95ºC	60ºC/65ºC	68ºC	cycle	68ºC	4ºC
3 min	30 s	20s	1min	30×	5 min	∞

Experiment Results

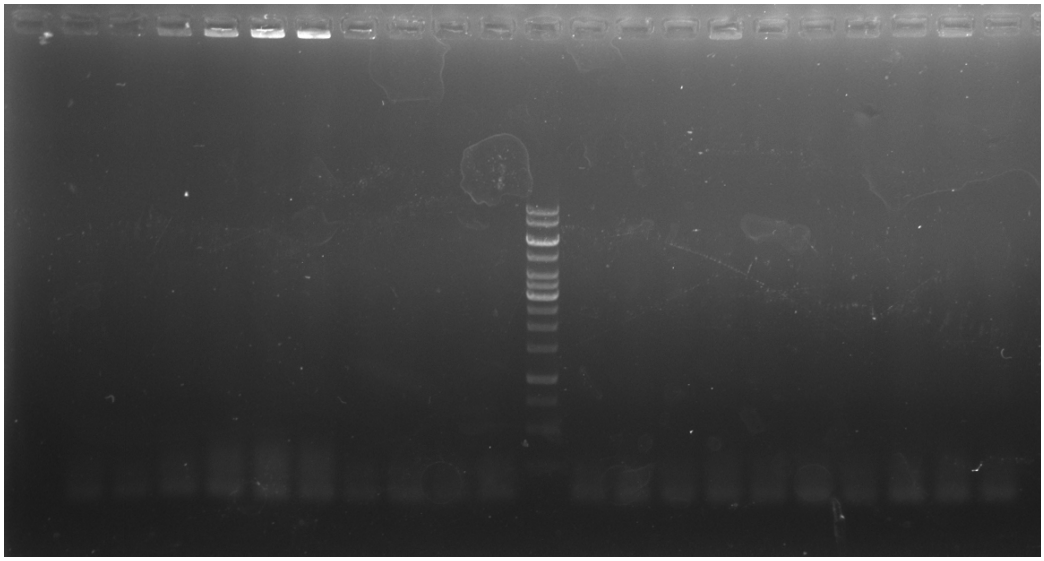


Figure 10 (a)CrtE, Crt colony PCR.

No P bands were found, which may be the inappropriate ratio of carrier and fragment in the ligation system, or the high GC content of target fragment and the inappropriate buffer of Taq enzyme mix.

Experiment Name：pET，CrtY seamless connection

Date： 2019/08/07 **Operators：Ran Wang**

Detailed Steps	Notes
(1) Prepare the system according to the table below. (2) Mental bath at 50℃ for 15 min. (3) Transform the DH5α with the product (4) Overnight at 37℃	

	CK (-)	CK (+)	pET-CrtY
CrtY	-	-	2 μL
linear pET	5 μL	-	6 μL
pUC	-	1 μL	-
2×Mix	10 μL	10 μL	10 μL
standard DNA	-	1 μL	-
ddH2O	5 μL	8 μL	2 μL
total volume		20 μL	

Experiment Name：Amplication CrtE、CrtB、CrtI、CrtY

Date： 2019/08/08 **Operators：** Shujie Liao

Detailed Steps	Notes
(59) Prepare amplification system according to protocol. (60) Run PCR program (61) agarose gel electrophoresis	

PCR Special Form

Gene Name：CrtE、CrtB、CrtI、CrtY

Primer： pET-CrtE F/R、pET-CrtB F/R、pET-CrtI F/R、pET-CrtY F/R

Date： 2019/08/08 **Operators：** Shujie Liao

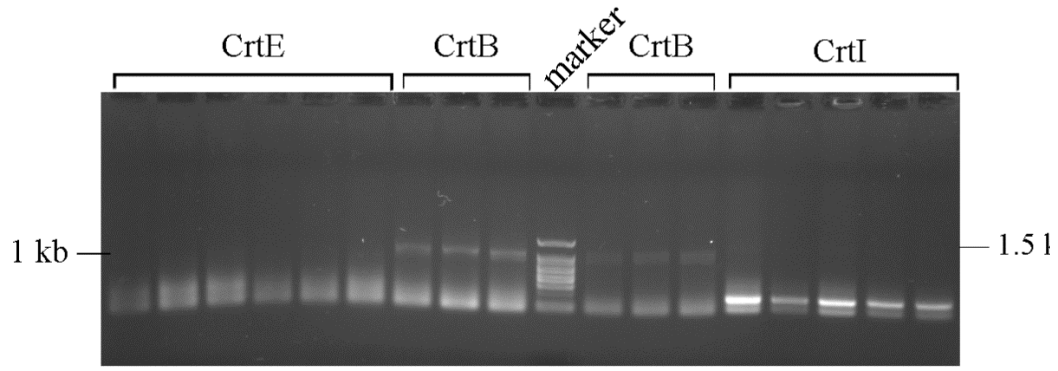
Reagent	Volume	Total Volume
One Taq Quick-Load 2x Mix	7.5 μL	15 μL
5’-primer/3’-primer	0.75 μL	
Template DNA	0.6 μL	
ddH2O	5.4 μL	

PCR Program

Initial Denaturation	Denaturation	Annealing	Extension	Cycles	Final Extension	
95ºC	95ºC	60ºC/65ºC	68ºC	cycle	68ºC	4ºC
3 min	30 s	20s	1min	30×	5 min	∞

Experiment Results

(a)



(b)

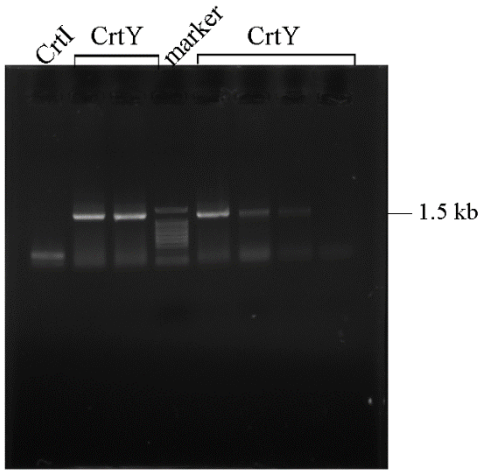


Figure 11 amplification of CrtE、CrtB、CrtI、CrtY with enzyme taq

It can be seen from the figure that only CrtB and CrtY of four genes can be amplified by using Taq polymerase. This indicates that the absence of target bands in colony PCR may not be the target fragment which means the gene didn't connect with the vector successfully. Therefore, we suggested that high GC buffer Taq mix or high fidelity enzyme + high GC buffer be used in colony PCR of CrtE and CRTI in the future.

Experiment Name：pET-CrtY colony PCR

Date： 2019/08/08 **Operators：** Ran Wang

Detailed Steps	Notes
(62) Prepare the PCR system according to the table below (63) Selecting the single colony, mark it on new resistence plate and insert the gun head to corresponding PCR tube. (64) After all the colonies have been picked out, gently shake the PCR tube, and then remove the small gunhead from the PCR tube. (65) Run PCR program (66) Agarose gel electrophoresis	

PCR Special Form

Gene Name：CrtY

Primer： pET-CrtY F/R

Date：2019/08/08 **Operators：**Ran Wang

Reagent	Volume	Total Volume
One Taq Quick-Load 2x Mix	7.5 μL	
5'-primer/3'-primer	0.75 μL	15 μL
ddH2O	6 μL	

PCR Program

Initial Denaturation	Denaturation	Annealing	Extension	Cycles	Final Extension	
95ºC	95ºC	60ºC	68ºC	cycle	68ºC	4ºC
3 min	30 s	20s	1min	30×	5 min	∞

Experiment Results

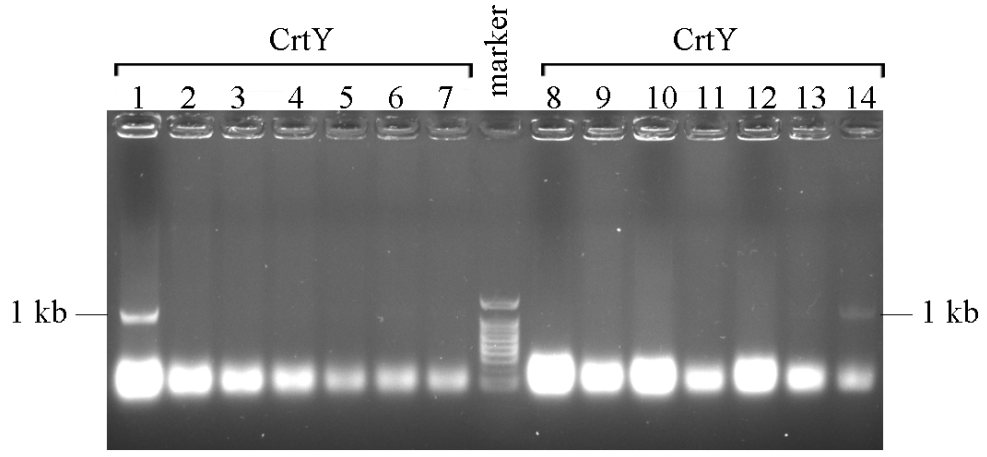


Figure 12 (a) pET-CrtY colony PCR.

Bacteria No. 1 and No. 14 showed amplified bands, and the size was the same as the CrtY, but the bands of No. 14 were weak. Bacteria 1 and 4 were cultured overnight in liquid medium, and plasmids were extracted and send to be sequenced.

Experiment Name：digestion of CrtB、pET

Date：2019/08/08 **Operators：**Yang Xu

Detailed Steps	Notes
(67) Prepare the digestion system according to the protocol. (68) Water bath at 37℃ for 2h. (69) Agarose gel electrophoresis, retrieve	

Date：2019/08/08 **Operators：**Yang Xu

Reagent	Volume	Total Volume
Hind III	1 μL	35.5 μL
BamH I	1 μL	
CrtB（PCR gel retrieve）	30 μL	
HF buffer	3.5 μL	

Date：2019/08/08 **Operators：**Yang Xu

Reagent	Volume	Total Volume
Hind III	1 μL	50 μL
BamH I	1 μL	
pET	25 μL	
cutsmart	5 μL	
ddH2O	18 μL	

Experiment Results

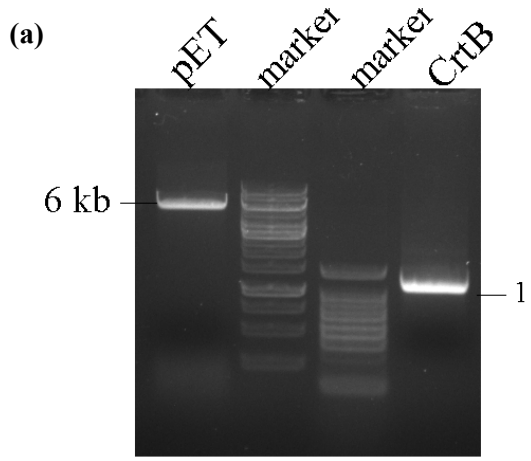


Figure 13 (a) digestion of **CrtB、pET**.

The size of pET band was consistent with that of linear pET, indicating that the plasmid was successfully digested and the concentration of CrtB fragment was higher after digestion.

Experiment Name：lingking pET-CrtB

Date： 2019/08/08 **Operators：Xu Yang**

Detailed Steps	Notes
(15) Prepare the reaction system according to the following tabel. (16) Mental bath at 25℃ for 2h. (17) DH5αTransform E.coli DH5α. (18) Overnight at 37℃.	

Date： 2019/08/05 **Operators：** Shujie Liao

Reagent	Volume	Total Volume
pET	1 μL	
CrtY	7 μL	10 μL
T4 ligase buffer	1 μL	

T4 ligase	1μL

Experiment Name：small induction of CrtZ and BKT

Date： 2019/08/09 **Operators：** Shujie Liao

Detailed Steps	Notes
(70) The seed liquid (pET, pET-CrtE, pET-CrtB) of overnight culture was taken and added into the new 5 mL medium. Kanamycin was added at 1:1000 to make the final concentration of kanamycin 50 μg/mL, 37℃, 200 rpm for 2-2.5 h. (71) Distilled water was the control group and the bacterial OD_{600} was measured. When OD_{600} was between 0.6-0.8, 0.08 M IPTG was added, the final concentration is 0 mM, 0.02 mM, 0.04 mM, 0.06 mM, 0.08 mM, 0.1 mM each, 30℃ 200 rpm for 3 h (72) Preparing 8% SDS-PAGE separation gel and 5% concentrate gel according to protocol	
(73) OD_{600} of induced bacterial solution was measured, 1 OD bacterial solution was collected, centrifuged at 12000 rpm for 30 seconds, and water was discarded	When OD_{600} is higher than 0.9, it should be diluted before measurement.
(74) Re-suspension the bacteria with 100 μL 1×SDS Loading buffer, boiling for 5min	
(75) Centrifuge in 12000 rpm for 5 min.	
(76) The SDS-PAGE protein gel was packed in the electrophoresis tank, added 1 x running buffer, pulled out the comb and checked for leakage.	
(77) Add 20 μL sample and 12 μL marker	
(78) electrophoresis:80 V 20 min，120 V 50 min，55 V 30 min	
(79) Coomassie brilliant blue dyeing for 30 minutes. Then decolorize gel overnight.	

Experiment Results

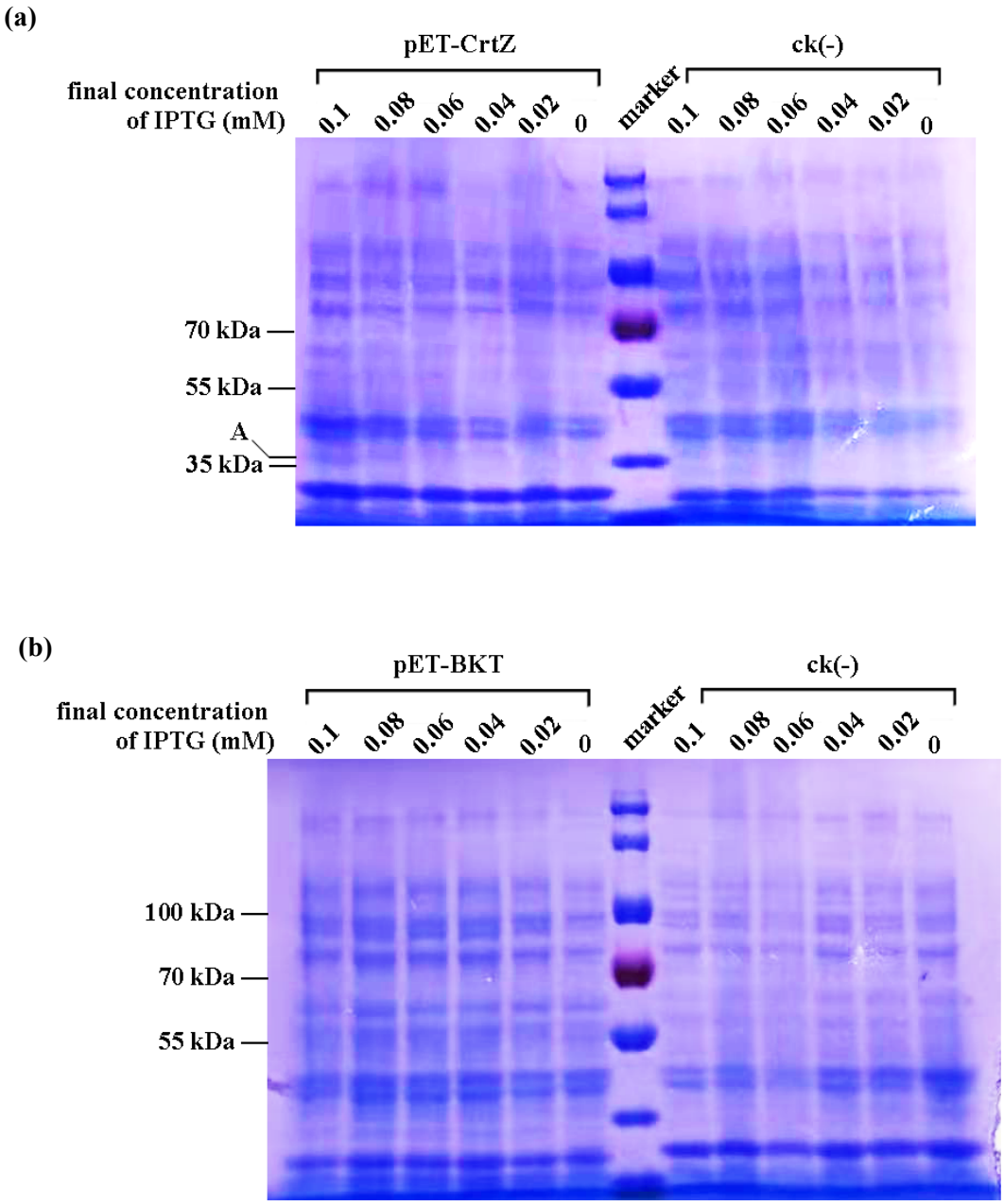


Figure 14 (a) CrtZ small induction(b) BKT small induction.

When the final concentration of IPTG was 0.1 mM and 0.08 mM in Fig. a, the induction band of pET-CrtZ appeared vaguely, but the size of CrtZ protein was about 27 kDa, which was slightly different from the result. No positive results appeared in Fig. B. It was speculated that the reason for this induction was that the strain DH5alpha had low protein expression, and the plasmid should be transferred to BL21 before induction.

Experiment Name：Small induction of CrtZ

Date：2019/08/10 **Operators：**Shujie Liao

Detailed Steps	Remarks
(80) The seed liquid (pET, pET-CrtE, pET-CrtB) of overnight culture was taken and added into the new 5 mL medium. Kanamycin was added at 1:1000 to make the final concentration of kanamycin 50 μg/mL, 37℃, 200 rpm for 2-2.5 h.	
(81) Distilled water was the control group. The bacterial OD_{600} was measured. When OD_{600} was between 0.6-0.8, 0.08 M IPTG was added, the final concentration of medium was 0 mM, 0.02 mM, 0.04 mM, 0.06 mM, 0.08 mM, 0.1 mM each, 30℃ 200 rpm for 3 h	
(82) Preparing 8% SDS-PAGE separation gel and 5% concentrate gel according to protocol.	
(83) OD_{600} of induced bacterial solution was measured, 1 OD bacterial solution was collected, centrifuged at 12000 rpm for 30 seconds, and water was discarded	When OD_{600} is higher than 0.9, it should be diluted before measurement.
(84) Re-suspension the bacteria with 100 μL 1×SDS Loading buffe, boiling for 5min	
(85) Centrifuge at 12000 rpm for 5 min.	
(86) The SDS-PAGE protein gel was packed in the electrophoresis tank, added 1 x running buffer, pulled out the comb and checked for leakage.	
(87) Add 20 μL sample and 12 μL marker	
(88) electrophoresis:80 V 20 min，120 V 50 min，55 V 30 min	
(89) Coomassie brilliant blue dyeing for 30 minutes then decolorizing the gel overnight.	

Experiment Results

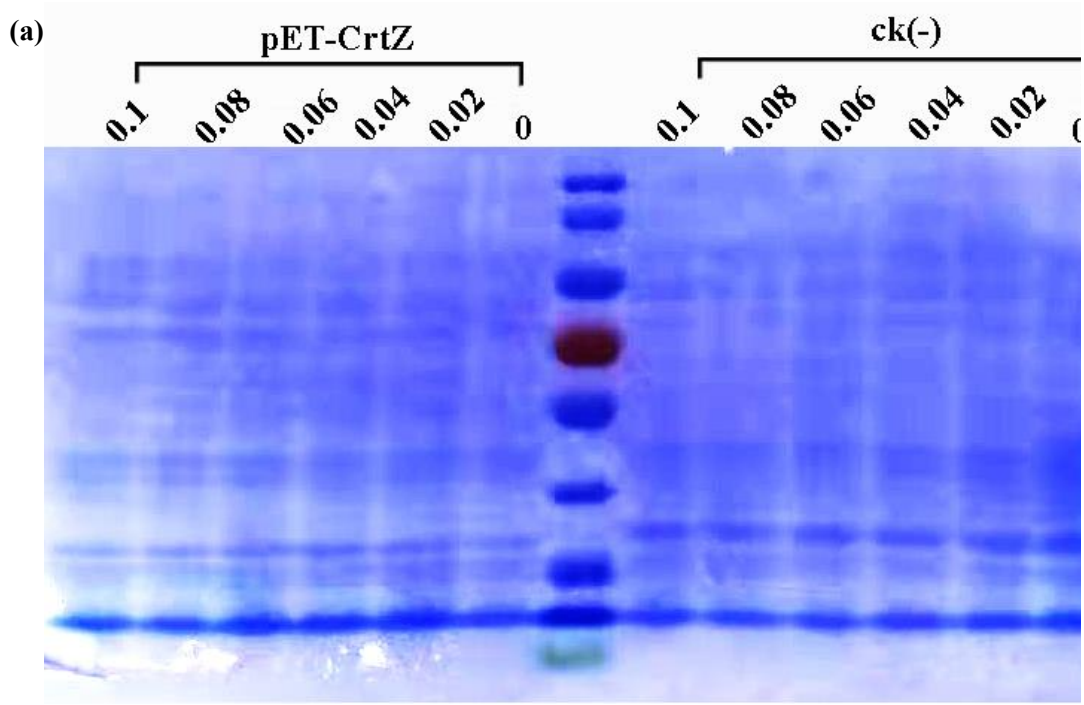


Figure 15 (a) small induction of CrtZ.

The result is still negative because the *E.coli* used in induciton is still DH5α.

Experiment Name：184M E&B&I overlap

Date： 2019/08/11 **Operators：** Xuqian Fan

Detailed Steps	Notes
(90) Step of overlap PCR: a) Take 5 μl of each PCR products for electrophoresis ,leaving the rest of products,to see whether there is a band. If there is a band, do the second step. b) Taking two kinds of PCR products, 1 μL each, without primers, and the rest are the same as the normal PCR system (15 mu l), 7 cycles. c) Take 5 μl product and run gel to see if there is a target band. If there is, add all the remaining solution of 15 ml PCR system to the remaining product. The primers are F of the first sequence and R, 30 cycles of the second sequence. Run gel and cut gel to get the target DNA.	If the overlap after the single PCR is too late, step 2 can add the recovered products of 1 ml of each CR gel. In step 3,the remaining 10 ml of PCR products, plus other solutions, will exceed 15 ml, but that's OK Overlap takes into account the extension time, which is determined by the size of the strip.

Date： 2019/08/11 **Operators：** Xuqian Fan

Detailed Steps	Notes
(91) PCR for CrtE, CrtB and CrtI with vazyme (92) Target strips were observed with 5 μl of the product was used for electrophoresis (93) Take the brighter bands for overlap and retrieve the remaining bands (94) Overlap E&B and B&I for 7 cycles. (95) Take 5 μl for electrophoresis, and observe that the target strip appears vaguely in E & B, but there is no strip in B & I. (96) Overlap E&B and B&I for 30 cycles. (97) Take 5 μl for electrophoresis and check the result, retrive.	

PCR Special Form

Gene Name：Crt E

Primer sequence： 184M- Crt E F

184M- Crt E R

Date： 2019/08/11 **Operators：** Xuqian Fan

Reagent	Volume	Total Volume
Buffer	7.5 μL	15μL
10 mM dNTPs	0.3μL	
5’-primer/3’-primer	0.6+0.6=1.2μL	
vazyme	0.3μL	
ddH2O	Fit to15 μ	
Template	0.6 μl/0.9 μl	

Gene Name：Crt B

Primer sequence： 184M- Crt B F

184M - Crt B R

Date： 2019/08/11 **Operators：** Xuqian Fan

Reagent	Volume	Total Volume
Buffer	7.5 μL	15μL
10 mM dNTPs	0.3μL	
5’-primer/3’-primer	0.6+0.6=1.2μL	
vazyme	0.3μL	
ddH2O	补齐 15 μ	
Template	0.6 μ l/0.9 μ l	

Gene Name：Crt I

Primer sequence： 184M - Crt I F

184M - Crt I R

Date： 2019/08/11 **Operators：** Xuqian Fan

Reagent	Volume	Total Volume
Buffer	7.5 μL	15μL
10 mM dNTPs	0.3μL	
5’-primer/3’-primer	0.6+0.6=1.2μL	
vazyme	0.3μL	
ddH2O	Fit to15 μ	
Template	0.6 μ l/0.9 μ l	

PCR Program

Initial Denaturation	Denaturation	Annealing	Extension	Cycles	Final Extension

on					n	
95ºC	95ºC	60ºC	72ºC	cycle	72ºC	4ºC
3 min	15 s	15 s	60s	2~30×	5 min	∞

Experiment Results

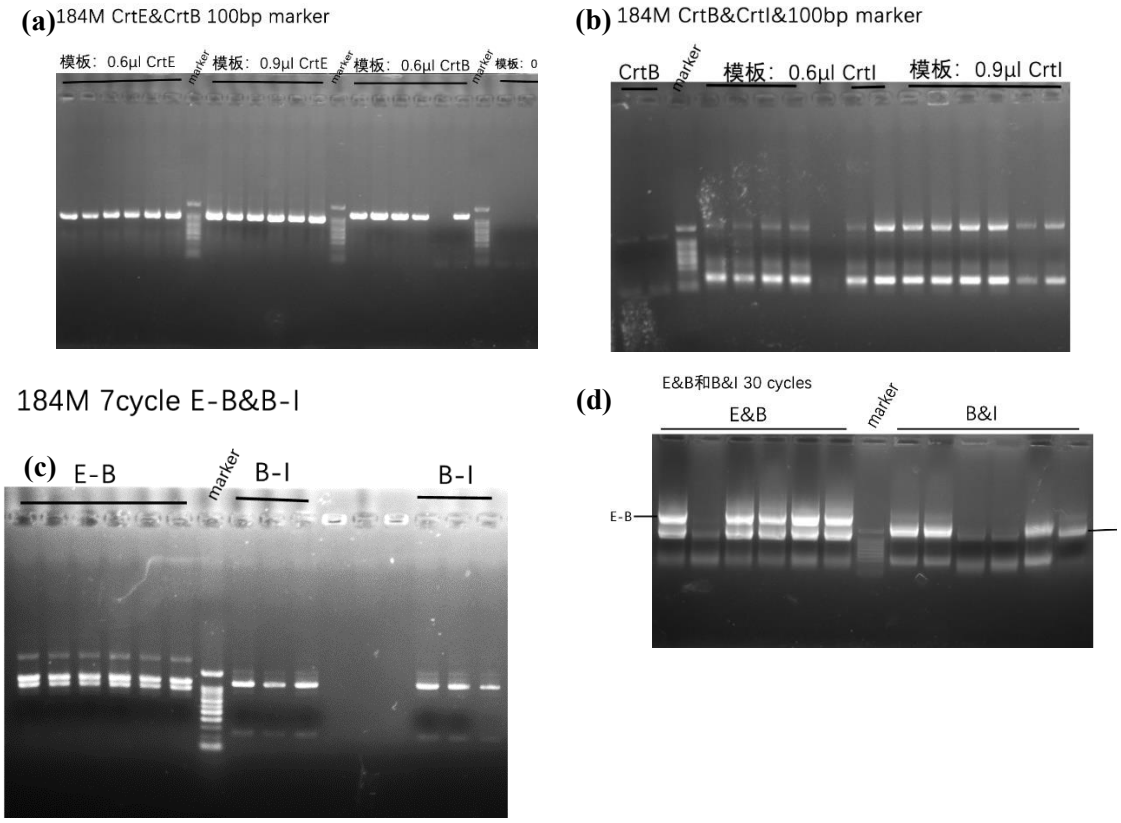


Figure 16 (a)B&B PCR (b)B&I PCR (b) 7 cycles E-B and B-I (b) 30cycles E-B and B-I

According to the PCR, all the objective bands appears.And E-B objective bands was appeared according to the electrophoresis for overlap PCR, but there are some other bright bands.No B-I bands appeared.

Experiment Name：Transformation of E.coli

Date： 2019/08/11 **Operators：** Ran Wang, Yang Xu

Detailed Steps	Notes
The recombinant E. coli was obtained by conjugating pET plasmid with CTB gene and transforming E. coli.	The pET plasmid was not retrieved after rubbing,
(98) Enzyme digestion of pET plasmid and CrtB（HindII and BamHI）.	
(99) pET and CrtB were ligated overnight after enzymatic digestion (T4 ligase).	This may lead to the problem of low connection effect on following step.
(100) Transform receptive cell DH5 α with ligation product.	
(101) Heat shock for 90s, then add in 500μL LB liguid medium.	
(102) Coating the plate and observing the result the next day.	

Cutting program

Date： 2019/08/11 **Operators：** Ran Wang

Reagent	Volume	Total Volume
BamHI	1μL	10μL
HindIII		
Cutsmart buffer	1μL	
pET Plasmid	5μL	

Linking Program

37ºC
2h

Date： 2019/08/11 **Operators：** Ran Wang

Reagent	Volume	Total Volume
BamHI	1μL	50μL
HindIII	1μL	
DdH_2O	18μL	
Cutsmart buffer	5μL	
CrtB Gene	25μL	

Linking Program

37°C
2h

linking program

Date： 2019/08/11 **Operators：** Ran Wang

Reagent	Volume	Total Volume
T4 ligase	1μL	10μL
ligase buffer	1μL	
pET Plasmid	5μL	
CrtB gene	3μL	

Linking Program

16°C
16h

Experiment Results

Experiment Name：CrtE、CrtB、CrtI、CrtY amplication

Date： 2019/08/05 **Operators：** Ran Wang, Shujie Liao

Detailed Steps	Notes
CrtB, CrtE, CrtI and CrtY genes were amplified by PCR and check and retrieve by DNA gel electrophoresis. (103) Add ddH_2O, polymerase Buffer, F and R primers, dNTPs, total DNA and DNA high-fidelity polymerase according to protocol. The single tube system is 15 μl, 7 tubes each gene, the total is 28 tubes. (104) Setting annealing temperature and elonging time to run according to the PCR program on the machine (105) Agar gel was prepared during the process of waiting for amplification, and the agar gel of 60 μl was prepared by 1% agar concentration. Adding 6 μL nucleic acid dye. (106) Electrophoresis at 150V for 20 min for marker and amplified fragments after PCR. The results were then observed under ultraviolet light. (107) The DNA bands were cut and extracted. Get the DNA fragments we want and measure the concentration.	

PCR Special Form

Gene Name： CrtE

Primer sequence： R:PET-CrtE R

F: PET-CrtE F

Date： 2019/08/05 **Operators：** Ran Wang

Reagent	Volume	Total Volume
PCRBuffer	3μL	15μL
2.5 mM dNTPs	0.3μL	

5’-primer/3’-primer	0.75μL	
Phusion DNA Polymerase	0.15μL	
DMSO	0.45μL	
ddH2O	9.0μL	
TemplateDNA	0.6μL	

Gene Name：CrtB

Primer sequence：R: PET-CrtB R

F: PET-CrtB F

Date：2019/08/05 **Operators：**Ran Wang

Reagent	Volume	Total Volume
PCRBuffer	3μL	
2.5 mM dNTPs	0.3μL	
5’-primer/3’-primer	0.75μL	
Phusion DNA Polymerase	0.15μL	15μL
DMSO	0.75μL	
ddH2O	8.7μL	
TemplateDNA	0.6μL	

Gene Name：CrtI

Primer sequence：R: PET-CrtI R

F: PET-CrtI F

Date：2019/08/05 **Operators：**Ran Wang

Reagent	Volume	Total Volume
PCRBuffer	3μL	15μL
2.5 mM dNTPs	0.3μL	

5’-primer/3’-primer	0.75μL	
Phusion DNA Polymerase	0.15μL	
DMSO	0.75μL	
ddH2O	8.4μL	
TemplateDNA	0.9μL	

Gene Name： CrtY

Primer sequence： R: PET-CrtY R

F: PET-CrtY F

Date： 2019/08/05 **Operators：** Ran Wang

Reagent	Volume	Total Volume
PCRBuffer	3μL	
2.5 mM dNTPs	0.3μL	
5’-primer/3’-primer	0.75μL	
Phusion DNA Polymerase	0.15μL	15μL
DMSO	0.45μL	
ddH2O	9.0μL	
TemplateDNA	0.6μL	

PCR Program

Initial Denaturation	Denaturation	Annealing	Extension	Cycles	Final Extension	
95ºC	95ºC	60ºC	72ºC	cycle	72ºC	4ºC
3 min	30 s	20s	1min	29×	5 min	∞

Experiment Results

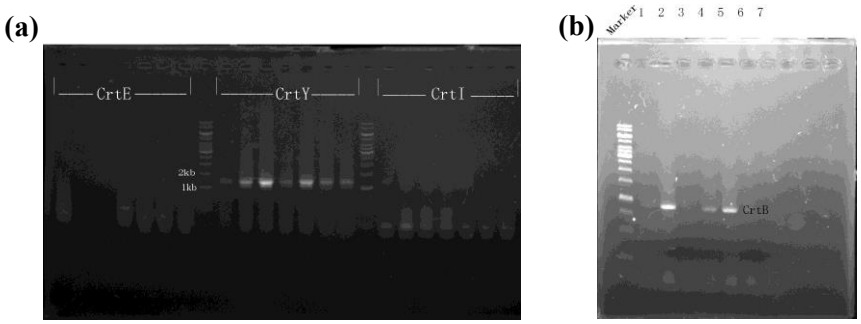


Figure 17 (a) CrtE、CrtY、CrtI PCR amplified result; (b) CrtB PCRamplified result.

From the image, we can see that the gene of CrtE and CrtI has not been successfully amplified, which may be related to the inability to pair with the original material genes, genes and primers, and the amount of gene added. The successful amplification of CrtY gene can be carried out in the next step of the practical enzymatic test. The phenomenon of partial amplification of CrtB occurs. In the next step of PCR, the conditions can be changed partially, and the influencing factors can be observed and found.

Experiment Name：Splicing pET-CrtI

Date： 2019/08/22 **Operators：** Shujie Liao

Detailed Steps	Notes
(19) Preparing the connection system according to the following table	
(20) Metal bath at 25℃ for 2 hours	
(21) Transforming E.coli DH5α	Contrast with pET no-load
(22) Overnight incubation at 37℃	

Date： 2019/08/05 **Operators：** Shujie Liao

Reagent	Volume	Total Volume
pET	1 μL	10 μL
CrtI	7 μL	
T4 ligase buffer	1 μL	
T4 ligase	1μL	

Experiment Results

No transformant was found and the ligation failed. Probably because the restriction sites at both ends of the PCR products were difficult to identify and the restriction efficiency was low. It is suggested that CrtI should be linked to T vector by T-A cloning and then digested. On the one hand, it can improve the recognition efficiency of restriction endonuclease sites, on the other hand, it is convenient to test the success of digestion.

Experiment Name：Enzyme digestion of pET-CrtE and pET-CrtB to verify the correctness of the linked plasmids

Date： 2019/08/22 **Operators：** Shujie Liao

Detailed Steps	Notes
(108) Preparing the connection system according to the following table (109) Water bath for 2h at 37℃ (110) agar gel electrophoresis, get the target DNA	

Date： 2019/08/22 **Operators：** Shujie Liao

Reagent	Volume	Total Volume
Hind III	1 μL	
BamH I	1 μL	
pET-CrtE / pET-CrtB	15 μL	30 μL
cutsmart	3 μL	
ddH2O	10 μL	

Experiment Results

No obvious CrtE, CrtB bands were found and plasmids were extracted for sequencing.

Experiment Name：CrtI T-A cloning

Date： 2019/08/22 **Operators：** Shujie Liao

Detailed Steps	Notes
(1) tailing of PCR products: tailing system was prepared according to table 1 below and incubated at 72 ℃ for 10 minutes. (2) T-A connection: The connection system was prepared according to Table 2 below and incubated at room temperature for 5 minutes. (3) Transforming: 50 μL DH5α was added into the 5 μL conjugated product, and placed at room temperature for 5 min. 500 μL was added into the super-clean table without antibiotics. The culture was resumed at 37 ℃ for 10 min. 200 μL bacterial solution was taken to coat the plate.	The standard DNA fragments equipped with the kit were used as control.

Table 1

Reagent	Volume	Total Volume
One Taq Quick Mix	7.5 μL	15 μL

CrtI	1 μL	
ddH2O	6.5μL	

Table 2

Reagent	Volume	Total Volume
vector	0.5 μL	
CrtI with A	4 μL	5 μL
10X enhancer	0.5 μL	

Experiment Results

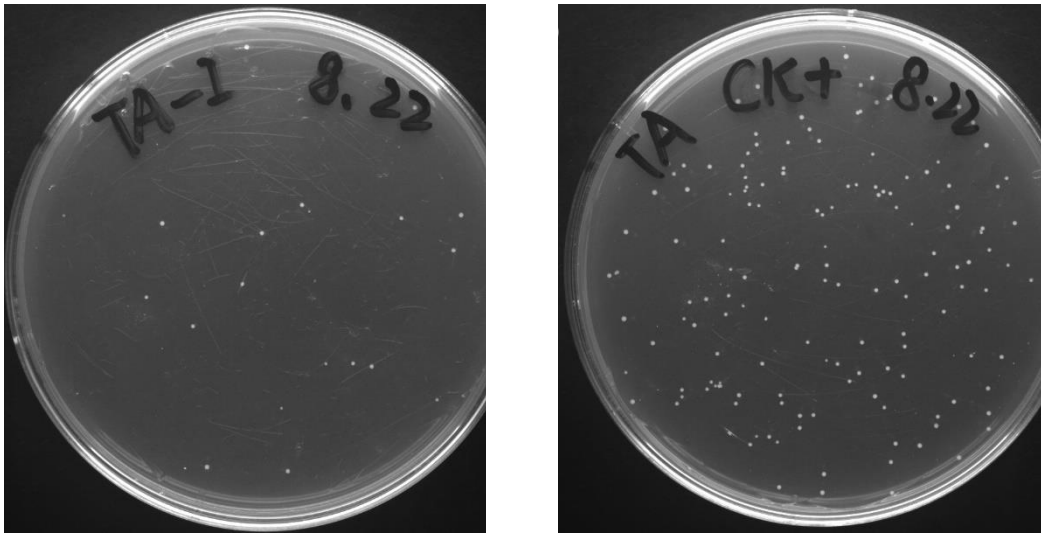


Figure 18 (a) CrtI T-A cloning; (b) T-A cloning positive control.

Transformers were grown on both CrtI T-A clones and positive control plates. There were more transformers on the positive control plate. It may be that the standard DNA fragment 3' A rate provided by the kit was higher than that of the experimental group, so the conjugation efficiency was higher. But the number of T-A clone transformants of CrtI is enough. The next step is to verify by colony PCR.

Experiment Name：small induction CrtE、CrtB

Detailed Steps	Notes
(111) The seed liquid (pET no-load, pET-CrtE, pET-CrtB) of overnight culture was taken and added into the new 5 mL medium. Kanamycin was added at 1:1000 to make the final concentration of kanamycin 50 μg/mL, 37℃, 200 rpm for 2.5 h. (112) Distilled water was the control. The bacterial OD_{600} was measured. When OD_{600} was between 0.6-0.8, 0.08 M IPTG was added, resulting in the final concentration of 0 mM, 0.02 mM, 0.04 mM, 0.06 mM, 0.08 mM, 0.1 mM, 30℃ 200 rpm for 3 h. (113) Preparing 10% SDS-PAGE separation gum and 5% concentrate gum according to formula (114) After three times dilution, OD_{600} of induced bacterial solution was measured, 1 OD bacterial solution was collected, centrifuged at 12000 rpm for 30 seconds, and supernatant was discarded (115) Re-suspension the bacteria with 100 μL 1×SDS Loading buffe,boil for 5min (116) Centrifugal at 12000 rpm for 5 min. (117) The SDS-PAGE protein glue was packed in the electrophoresis tank, added 1 x running buffer, pulled out the comb and checked for leakage. (118) Add 20 μL sample and 12 μL marker (119) electrophoresis:80 V 20 min，120 V 50 min，55 V 30 min (120) Coomassie brilliant blue dyeing for 30 minutes, decolorize gel overnight	

Experiment Results

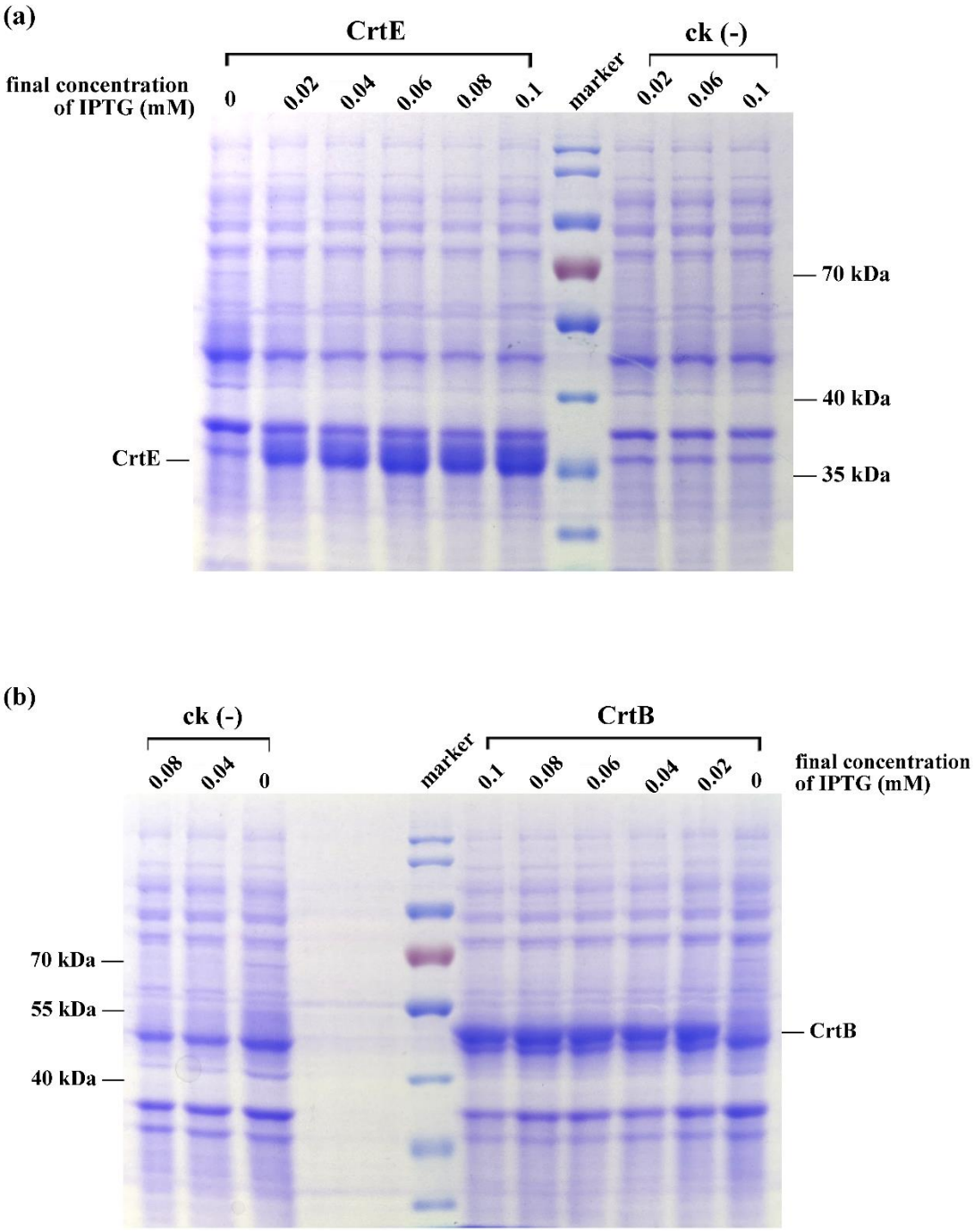


Figure 19 (a) CrtE small induction; (b) CrtBsmall induction.

Picture (a):When the final concentration of IPTG is 0.02 mM to 0.1 mM, the induction band of pET-CrtE appears obviously and pET empty plasmid and pET-CrtE with no IPTG appears no bands. The band size is between 35 kDa and 40 kDa, which coincides with the size of CRTE-His-tag (37.5 kDa). Therefore, it is considered that CrtE can be induced by IPTG at 30℃.The band width of CrtE increased with the increase of IPTG concentration, especially between 0.04 mM and 0.06 mM, but after 0.06 mM, the band width of CrtE protein increased slightly. Therefore, 0.06 mM IPTG was considered as the best inducing concentration.

Picture(b): The inducing band of CrtB also appears in figure b, but the band width does not change significantly with the concentration of IPTG. Therefore, 0.02 mM IPTG is considered as the best inducing condition of CrtB.

Experiment Name：T vector-CrtI colony PCR

Date： 2019/08/23 **Operators：** Shujie Liao

Detailed Steps	Notes
(121) Prepare PCR system according to the table below. (122) Selecting single colony with small gunhead, marking it on a new resistant medium and inserting the gunhead into the corresponding PCR tube. (123) After all the colonies have been picked out, gently shake the PCR tube, and then remove the small gunhead from the PCR tube. (124) Run PCR program. (125) Agar gel electrophoresis.	

PCR Special Form

Gene Name：CrtI

Primer： pET-CrtI F/R

Date： 2019/08/23 **Operators：** Shujie Liao

Reagent	Volume	Total Volume
One Taq Quick-Load 2x Mix	7.5 μL	
5’-primer/3’-primer	0.75 μL	15 μL
ddH2O	6 μL	

PCR Program

Initial Denaturation	Denaturation	Annealing	Extension	Cycles	Final Extension

95ºC	95ºC	60ºC/65ºC	68ºC	cycle	68ºC	4ºC
3 min	30 s	20s	1min	30×	5 min	∞

Experiment Results

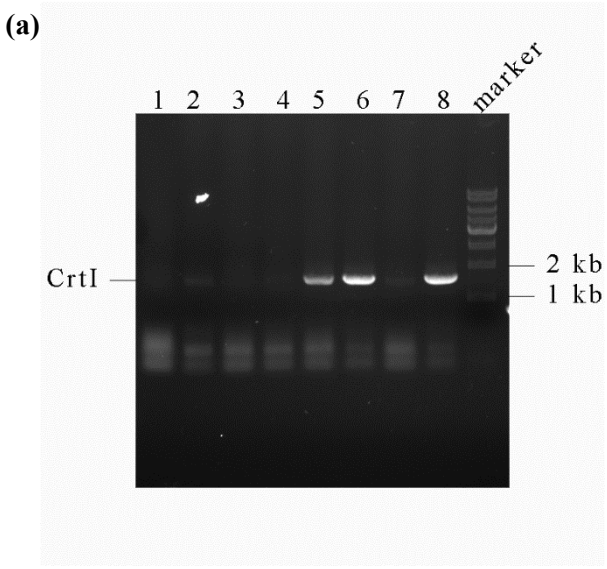


Figure 20 (a) T vector-CrtI colony PCR.

Bright bands appeared in lanes 5, 6 and 8, about 1.5 kb in size. It was considered that bacteria 5, 6 and 8 were connected successfully colonies and could be used in following experiments.

Experiment Name：Enzyme digestion 184M

Date： 2019/08/25 **Operators：** Shujie Liao

Detailed Steps	Notes
(126) Prepare the enzyme digestion system according to the table below.	
(127) Water bath at 37℃ for 2h.	
(128) Agarose gel electrophoresis and retrieve target DNA.	

Date： 2019/08/25 **Operators：** Shujie Liao

Reagent	Volume	Total Volume
Kpn I	1 μL	
BamH I	1 μL	
184M	25 μL	50 μL
cutsmart	5 μL	
ddH2O	18 μL	

Experiment Results

The measured concentration after rubber recovery is 1.8 ng/μL。

Experiment Name：seamless splicing 184M-EB-I

Date： 2019/08/25 **Operators：** Shujie Liao

Detailed Steps	Notes
(23) Prepare the system according to the table below. (24) Mental bath at 50℃ for 15min. (25) Transform *E.coli* DH5α (26) Culture *E.coli* overnight at 37℃	

Date： 2019/08/05 **Operators：** Shujie Liao

Reagent	Volume	Total Volume
linear 184M	7 μL	20 μL
CrtI	1 μL	
overlap CrtE-CrtB	2 μL	
cloning mix	10 μL	

Experiment Results

8 transformants appears.

Experiment Name： Extract plasmid pET-CrtI

Experiment Name： pET-CrtI、pET-CrtY transform BL21

Experiment Name： Prepare plate with Chl antibiotic

Experiment Name： overlap CrtE-CrtB 和 CrtI

Date： 2019/08/25 **Operators：Shujie Liao**

Detailed Steps	Notes
(27) Add ddH_2O, polymerase Buffer, dNTPs, total DNA and DNA high-fidelity polymerase according to protocol. 7 cycles without primers. The single tube system is 15 μl, with 4 tubes in total. (28) Adjusting annealing temperature and prolonging time to run according to the established program on the machine. (29)]Agar gel was prepared before PCR finished, and the agarose gel of 60ml was prepared by 1% agarose concentration with 6μL nucleic acid dye. (30) After the PCR, 5 μl of the product was taken for electrophoresis at a voltage of 150 V and for 20 minutes. The results were then observed under ultraviolet light.。 (31) If there are weak splicing bands, primers (CrtE F, CrtI R) and enzymes are added to the 7-cycle PCR system for 30-cycle PCR.	Enzyme was forgot to be added in this step

Gene Name：CrtE-B overlap CrtI

Primer sequence： R: 184M-CrtI R

F: 184M-CrtE F

Date： 2019/08/25 **Operators：** Shujie Liao

Reagent	Volume	Total Volume
GC buffer	3 μL	15μL
10 mM dNTPs	0.3 μL	

Phusion DNA Polymerase	0.15 μL
DMSO	0.75 μL
ddH2O	9.8 μL
CrtEB/CrtI	0.5 μL
Adding primers at 30 circle	0.75 μL
Adding enzyme at 30 circle	0.15 μL

PCR Program

Initial Denaturation	Denaturation	Annealing	Extension	Cycles	Final Extension	
95ºC	95ºC	60ºC	72ºC	cycle	72ºC	4ºC
3 min	30 s	20 s	1 min	7×	5 min	∞

Initial Denaturation	Denaturation	Annealing	Extension	Cycles	Final Extension	
95ºC	95ºC	60ºC	72ºC	cycle	72ºC	4ºC
3 min	30 s	20 s	2 min	30×	5 min	∞

Experiment Results

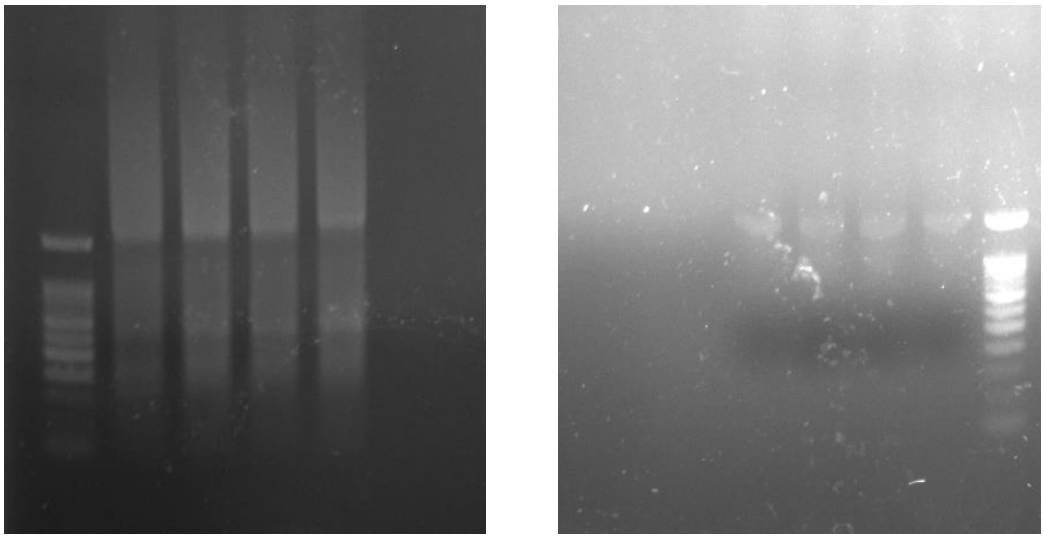


Figure 21 (a)7 circle overlap PCR; (b)3 circle overlap PCR.

No splicing bands were found during the 7 cycle, and the bands of CrtI and CrtEB were not clear. Diffusion bands appeared, which may be the partial degradation of template DNA. There was no overlap band in the 30 cycle because there was no enzyme added.

Experiment Name：

Date： 2019/08/28 **Operators：Xuqian Fan, Shujie Liao**

Detailed Steps	Remarks
(129) Transfer DH5α *E.coli* containing 184M-EBI to new LB. There are and 4 (No.2, 3, 4, 8) of 6 bacteria (No.2, 3, 4, 7, 8, 9) turned into red. Among those 4 bacteria No.8 was the reddest. (130) Extract the plasmid from the rest bacteria. (131) Bacterial 2, 3, 4, 8 result is positive while bacterial 7, 9 showed negative results according to the electrophoresis. (132) Send bacterial 8 for Sequencing. (133) Plasmid of bacteria No.3, pET-CrtY, CrtY(pET serial primer) were digested(HindIII、BamHI) and linked. The product was used to transform DH5α. Coating overnight at 37℃. (134) The plasmids of BacterialNo.2 and Bacteria No.4 were transformed into receptive cell BL21 and cultured at 37 ℃ for overnight.。 (135) Configuration of Kanamycin antibiotics (50mg/ml): Take 0.5g Kana and dissolve it in 100ml ddH_2O. Then filter the solution by sterilizing filter.	When linking, it is better to use 1.5ml or 2ml centrifugal tube for mental bath or use PCR tube for PCR machine.

Experiment Results

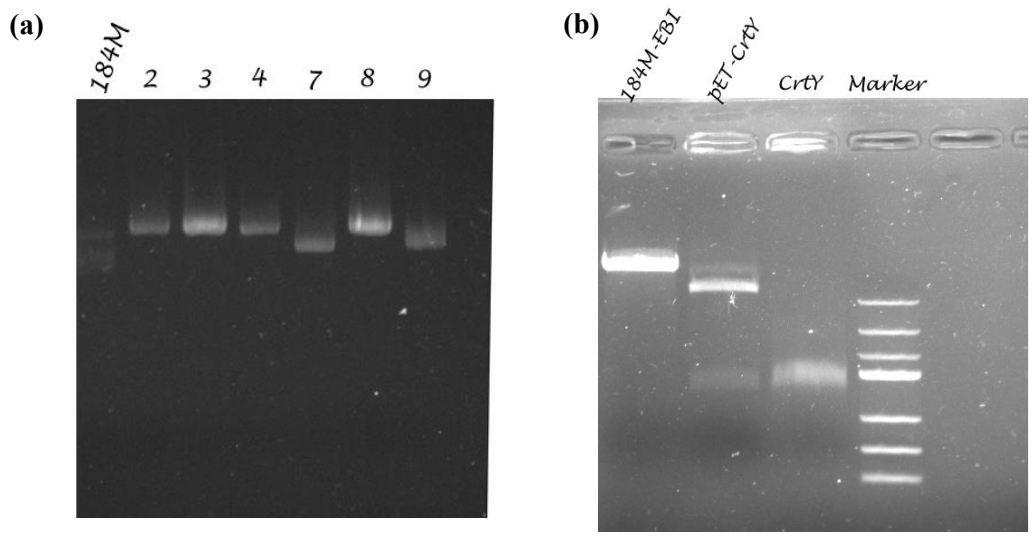


Figure 22 (a) The results of Plasmid Extraction from 184M-EBI strain (from left to right were 184M empty plasmid, bactera 2, bacteria 3, bacteria 4, bacteria 7, bacteria 8, bacteria 9, respectively); (b) The results of enzymatic digestion were used to connect the 184M-EBI plasmid with CrtY.

Figure (a): The result of bacteria 7 and 9 was negative. The sizes of their plasmids were smaller than others. The size of plasmids of bacteria 2, 3, 4 and 8 was close to the target band. So we thought the result was positive.

Figure (b) is the Gel electrophoresis image after enzymatic digestion. The concentration of CrtY is relatively low, and more products were added in the later stage of connection.

Reagent preparation

Reagent name：Kanamycin（50mg/ml）

Date：2019/08/28 **Operators**：Shujie Liao

Reagent	Volume
Kanamycin powder	0.5g
ddH_2O	100ml
Filtration sterilization	

Experiment Name：Bacterial PCR of CrtY

Date： 2019/08/29 **Operators：** Xuqian Fan

Detailed Steps	Remarks
(136) The strains DH5α of containing 184M-EBI (2, 3, 4, 8) were preserved, and the remaining bacterial liquid was used to extract plasmids. (137) Bacteria grew in the plate of 184M-EBI-Y (DH5α), which was linked to CrtY, and were cultured at 37℃.	

Experiment Results

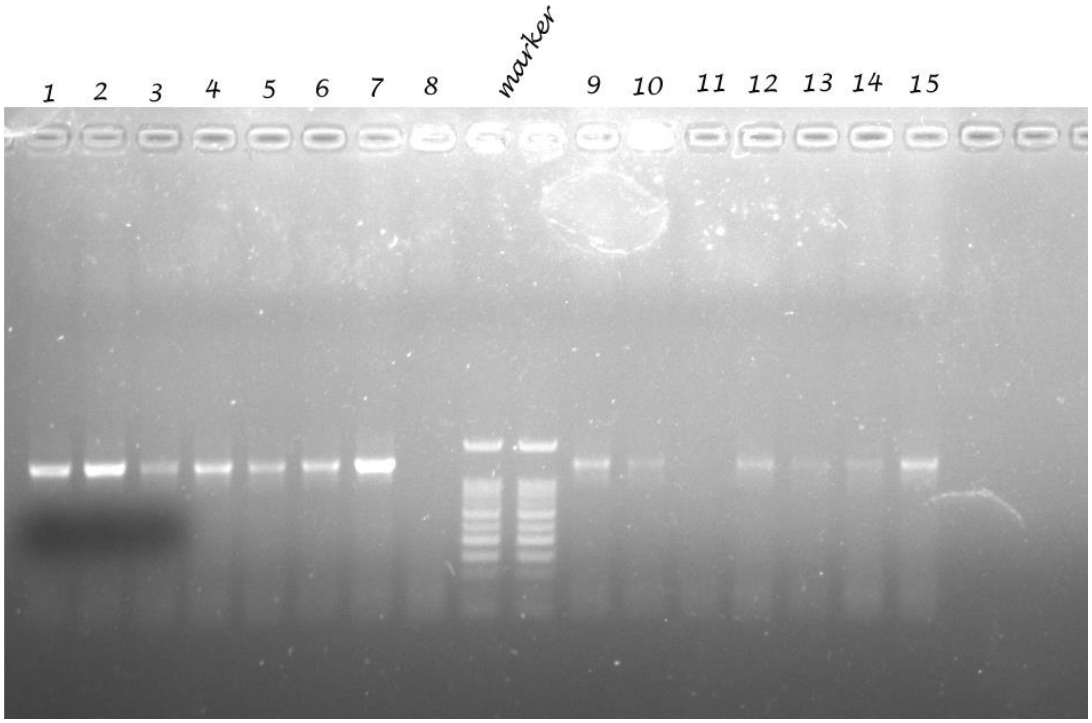


Figure 23 (a) CrtY in 184M-EBI-Y colony PCR

It can be observed that bacterial 1, 2, 3, 4, 5, 6, 7, 9, 10, 12, 13, 14, 15 showed positive result, of which bacterial 1, 2, 7 showed the brightest bands, which can be used for the following experiment

Experiment Name：PCR BKT and CrtZ

Operators ： Xuqian Fan, Ran wang, Shujie Liao

Date： 2019/08/30

Detailed Steps	Remarks
(138) The bacteria transferred to 184M-EBI-Y turned yellow. Yesterday's electrophoresis showed that the bands of strains 1, 2 and 7 were relatively bright, so the three were cultured in liquid medium. (139) PCR for CrtZ and BKT with newly designed 184M primers. (140) The PCR results of the first CrtZ and BKT are not ideal, and then further conditions are explored by temperature gradient PCR, BKT (58-65 C, extension time 1 min 45s), CrtZ (60-65 C, extension time 30 s), but no target bands appeared due to the template. (141) Coating BL21 and 184M with pET-BKT and pET-CrtZ respectively for further IPTG induction, overnight culture at 37℃. (142) Transform pET-BKT and pET-CrtZ to BL21, coating , overnight culture at 37℃.	

PCR Special Form

Gene Name：PCR CrtZ & BKT

Primer sequence： R:184M-CrtZ-R &184M-BKT-R

F: 184M-CrtZ-F &184M-BKT-F

Date： 2019/08/30 **Operators：** 樊旭倩、Ran Wang

Reagent	Volume	Total Volume
buffer	7.5 μL	15μL
dNTP mix	0.3 μL	
5’-primer/3’-primer	0.6 μL	
Vazyme	0.3 μL	
ddH_2O	5.4 μL	
Template DNA	0.3 μL	

PCR Program

Unwind	Unwind	Anneal	Prolong		Denature	
95ºC	95ºC	60ºC	68ºC	cycle	72ºC	4ºC
3 min	15 s	15 s	90 s	30×	5 min	∞

Experiment Results

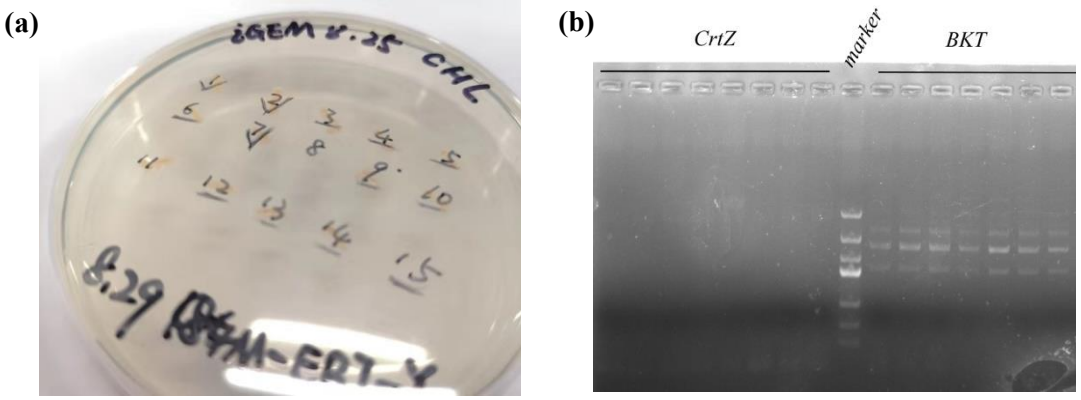


Figure 24 (a) colony with 184M-EBI-Y turned yellow; (b) PCR result of CrtZ and BKT.

Figure (a) showed that CrtY was successfully inserted into the 184M-EBI plasmid and produced yellow β -carotene.

Figure(b) showed the result of the first PCR of CrtZ and BKT. No CrtZ bands appeared and bands of BKT appeared slightly.

Possible reason: the size of CrtZ is too small for the setting time to prolong, 30s is recommended. Plus the annealing time of CrtZ and BKT is not appropriate. A temperature gradient PCR is recommended.

Experiment Name: 99A, 184M-EBIY Co-transformation BL21

Date：2019/09/28 **Operators: Liao Shujie**

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.
(1) Configure the connection system according to the following table.	
(2) Metal bath at 50 ℃ for 15 min	
(3) Transforming Escherichia coli DH5 α	
(4) 37 ℃ overnight culture	

Date：2019/09/28 **Operators: Liao Shujie**

Reagent（7x）	Volume	Total Volume
99A	2.5 μL	
Z-BKT	7.5 μL	20 μL
Cloning mix	10 μL	

Date：2019/09/28 **Operators: Liao Shujie**

Reagent（7x）	Volume	Total Volume
184M-EBIY	8 μL	
Z-BKT	2 μL	20 μL
Cloning mix	10 μL	

Experiment Results：

Experiment Name: CrtE

Date：2019/09/29 **Operators: Liao Shujie, Li Zixuan**

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. (143) Calculate the PCR system and add the Pre-mix package. (144) Put the separately packed PCR tube into the PCR instrument, set the condition to grope for the file to run, wait for the amplification to be completed, and select the most suitable condition. (145) Glue making. (146) Take out 5 μl from the amplified PCR tube and add into loading buffer, mix evenly and sample. (147) Point the right amount of marker and start running the glue. (148) The results were observed in the gel scanner, and the tubes with the brightest and clearest strips were taken for follow-up experiments. (149) 20 μl of PCR system was prepared by adding GC buffer, dNTP and other reagents in the amplification results screened in the previous step, and the ligation results were observed by running glue.	This column indicates special phenomena or unexpected errors in operation.

PCR Special Form

Date：2019/09/29 **Operators: Liao Shujie**

Reagent（7x）	Volume	Total Volume
PCR buffer	3 μL	15 μL
2.5 mM dNTPs	0.3 μL	

5 '-primer/3' -primer	1 μ L
Enzyme	0.15 μ L
DEIONIZED WATER	9.1 μ L
Template DNA	-
DMSO	0.45 μ L

Date：2019/09/29 **Operators: Liao Shujie**

Reagent (30x add-on)	Volume	Total Volume
PCR buffer	2 μ L	
2.5 mM dNTPs	0.2 μ L	
5 '-primer/3' -primer	0.75 μ L	
Enzyme	0.1 μ L	20 μ L
DEIONIZED WATER	6 μ L	
Template DNA	10 μ L	
DMSO	0.3 μ L	

PCR Program

First unspin	unspin	Annealing	Extension	Cycle	Deactivation	
95oC	95oC	62oC	72oC	cycle	72oC	4oC
3 min	30 s	30 s	1min	7×	5 min	∞

First unspin	unspin	Annealing	Extension	Cycle	Deactivation	
95oC	95oC	62oC	72oC	cycle	72oC	4oC
3 min	30 s	30 s	2min	30×	5 min	∞

Experiment Results: The results of electrophoresis at 60 ℃, 61 ℃ and 62 ℃ for 7 cycles of fragment amplification were ideal, and 30 cycles of ligation were successful (the bands were very shallow).

iment Results

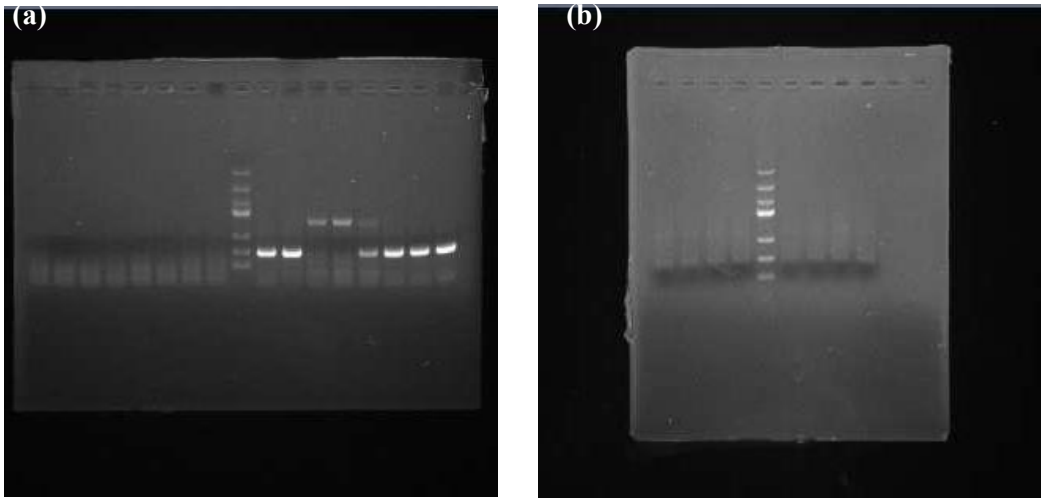


Figure 1 (a) result of crte gene fragment amplification (B) result of crte gene fragment ligation

Experiment Name: Inducing BKT, CrtZ Gene Expression, Measuring Enzyme Activity

Date：2019/10/01 **Operators: Fan Xuqian**

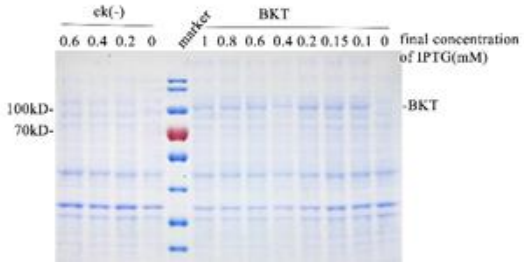
Detailed Steps	Remarks
Please specify the operation name, reagent name, reagent dosage, temperature, reaction time and instrument setting parameters in detail; when adding steps, please stop the cursor at the top of the lower left corner of each line, and click after the plus sign appears. (150) 16 ℃ overnight induction (151) The expression was induced at 30 ℃ for 4 HR. (152) Press the table to add the reagent to destroy the enzyme reaction product released by the cell and measure the enzyme activity.	This column indicates special phenomena or unexpected errors in operation.

Reagent：

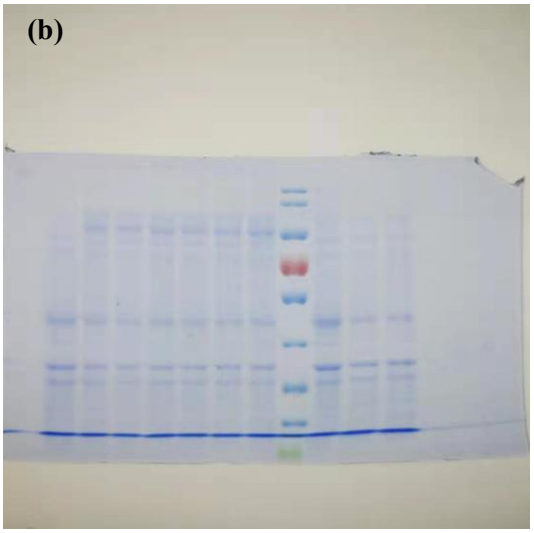
Date：2019/10/01	Operators: Fan Xuqian	
Reagent	**Volume**	**Total Volume**
1M Tris/HCl	400 μ L	4ml
DTT	20 μ L	
Cock tail	40 μ L	
5% GLYCERIN	200 μ L	

Experiment Results：

(a)



(b)



(Cut the picture into a square and paste it here)

Figure 1 (a) overnight expression at 16 ℃; (B) 4hr expression at 30 ℃.

Experiment Name: Amplified CrtE and Recovery

Date：2019/10/02 **Operators: Fan Xuqian**

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. (153) The PCR system was calculated and pre-mix was prepared for packaging. (154) Put the repackaged PCR tube into the PCR instrument, run the applicable condition file before selecting, and wait for the amplification to complete. (155) Glue making. (156) Loading buffer was added into the PCR tube, and the mixture was uniform. (157) A suitable amount of marker was applied and the electrophoresis was started (170v for 15min). (158) Observe the results in the gel scanner, and cut the brightest and clearest strips for gel recovery.	This column indicates special phenomena or unexpected errors in operation.

PCR Special Form

Date：2019/10/02 **Operators: Liao Shujie**

Reagent（7x）	Volume	Total Volume
PCR buffer	3 μ L	
2.5 mM dNTPs	0.3 μ L	
5 '-primer/3' -primer	1 μ L	
Enzyme	0.15 μ L	15 μ L
DEIONIZED WATER	9.1 μ L	
Template DNA	-	
DMSO	0.45 μ L	

Date：2019/10/02	Operators: Liao Shujie	
Reagent (30x add-on)	**Volume**	**Total Volume**
PCR buffer	2 μ L	
2.5 mM dNTPs	0.2 μ L	
5 '-primer/3' -primer	0.75 μ L	
Enzyme	0.1 μ L	20 μ L
DEIONIZED WATER	6 μ L	
Template DNA	10 μ L	
DMSO	0.3 μ L	

PCR Program

First unspin	unspin	Annealing	Extension	Cycle	Deactivation	
95oC	95oC	65oC	72oC	cycle	72oC	4oC
3 min	30 s	30 s	1min	7×	5 min	∞

First unspin	unspin	Annealing	Extension	Cycle	Deactivation	
95oC	95oC	65oC	72oC	cycle	72oC	4oC
3 min	30 s	30 s	2min	30×	5 min	∞

Experiment Results: Amplification of the 7 loop fragment yielded results, but ligation of the 30 loop was not successful.

Experiment Name： expression after BKT gene transformation (enzyme activity)

Date： 2019/10/03 **Operators：** Liao Shu jie

Detailed Steps	Remarks
Please specify the operation name, reagent name, reagent dosage, temperature, reaction time and instrument setting parameters. When you need to add steps, please stop the cursor at the lower left corner of each line and click after the plus sign appears. (159) 1M Tris/HCl (pH8.0) was added to re-suspend the bacteria and destroy the cell release protein (160) 0.1g carotene was dissolved in 10ml 0.4M Tris/HCl as the substrate for the enzymatic reaction (161) Press the table to add reagents as reference solution (162) The enzyme solution was added to the reaction substrate (and the reference solution) to perform the enzymatic reaction step by step (163) After the reaction, the sample solution was placed in an ultraviolet spectrophotometer to measure OD475 and OD600 and recorded	This column indicates special phenomena or unexpected errors in operation

Reagent：

Date： 2019/10/03 **Operators：** Liao Shu jie

Reagent	Volume	Total Volume
1M Tris/HCl	400μL	4ml
DTT	20μL	
Cock tail	40μL	
5% glycerinum	200μL	

Experiment Results：

iment Results

Experiment Name： The expression of BKT gene was induced and enzyme activity was measured

Date： 2019/10/05 **Operators：** Xu yang

Detailed Steps	Remarks
Please specify the operation name, reagent name, reagent dosage, temperature, reaction time and instrument setting parameters. When you need to add steps, please stop the cursor at the lower left corner of each line and click after the plus sign appears. (164) The inducer was added and the expression was induced at 25℃ and 3h (165) The result of induced expression was tested (enzyme activity was measured).	This column indicates special phenomena or unexpected errors in operation

Reagent：

Date： 2019/10/05 **Operators：Xu Yang**

Reagent	Volume	Total Volume
1M Tris/HCl	400μL	4ml
DTT	20μL	
Cock tail	40μL	
5% glycerinum	200μL	

Experiment Results： **No dilution was carried out when the light absorption value was measured, and the data was negative.**