Genomic DNA extraction：

We choose TIANGEN company's TIANamp Bacteria DNA Kit to achieve genomic DNA extraction. Protocol as follow.

1. Pipet 1-5 ml bacterial culture suspension in a centrifuge tube, centrifuging for 1 min at 10,000 rpm (~11,500 × g), discard supernatant as possible.
2. Add 200 µl Buffer GA. Mix thoroughly by vortex.
3. Add 20 µl Proteinase K. Mix thoroughly by vortex.
4. Add 220 µl Buffer GB to the sample, vortex for 15 s, and incubate at 70°C for 10 min to yield a homogeneous solution. Briefly centrifuge the 1.5 ml centrifuge tube to remove drops from the inside of the lid.
5. Add 220 µl ethanol (96-100%) to the sample, and mix thoroughly by vortex for 15 s. A white precipitate may form on addition of ethanol. Briefly centrifuge the 1.5 ml centrifuge tube to remove drops from the inside of the lid.
6. Pipet the mixture from step 5 into the Spin Column CB3 (in a 2ml collection tube) and centrifuge at 12,000 rpm (~13,400 × g) for 30 s. Discard flow-through and place the spin column into the collection tube.
7. Add 500 µl Buffer GD (Ensure ethanol (96-100%) has been added) to Spin Column CB3, and centrifuge at 12,000 rpm (~13,400 × g) for 30 s, then discard the flow-through and place the spin column into the collection tube.
8. Add 600 µl Buffer PW (Ensure ethanol (96-100%) has been added) to Spin Column CB3, and centrifuge at 12,000 rpm (~13,400 × g) for 30 s. Discard the flow-through and place the spin column into the collection tube.
9. Repeat Step 8.
10. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min to dry the membrane completely.
11. Place the Spin Column CB3 in a new clean 1.5 ml centrifuge tube, and pipet 50-200 µl Buffer TE or distilled water directly to the center of the membrane. Incubate at room temperature (15-25°C) for 2-5 min, and then centrifuge for 2 min at 12,000 rpm (~13,400 × g).

Plasmid extraction：

We choose TIANGEN company's HiPure Plasmid Micro Kit to achieve plasmid extraction. Details as follow.

（1） Pellet 1-5 ml of an overnight *E.coli* culture by centrifuging at 10,000 × g for 1 minute. Discard the supernatant.

（2） Completely resuspend the bacterial pellet with 250 µl Buffer P1/RNase A by vortex.

（3） Add 250 µl of the Buffer P2. Immediately mix the contents by gentle inversion (6-8 times) until the mixture becomes clear and viscous.

（4） Add 350 µl of the Buffer NP3. Gently invert the tube 8-10 times. Pellet the cell debris

by centrifuging at 13,000 × g for 1 minute.

（5） Insert a HiPure DNA Mini Column II into a provided microcentrifuge tube. Add supernatant to the Mini Column and centrifuge at 13,000 × g for 1 minute. Discard the flow-through liquid.

（6） Add 500 µl of the Buffer PW1 to the column. Centrifuge at 13,000 × g for 1 minute. Discard the flow-through liquid.

（7） Add 600 µl of the Buffer PW2 to the column. Centrifuge at 13,000 × g for 1 minute. Discard the flow-through liquid. Repeat this step once more.

（8） Centrifuge at 13,000 × g for 2 minutes without any additional Wash Solution to remove excess ethanol.

（9） Transfer the column to a fresh collection tube. Add 50 µl Elution Buffer to the column. After putting the tube at room temperature for 1 minute, centrifuge at 13,000 × g for 1 minute. The DNA is present in the eluate.

Gel Extraction

We choose Axygen company's Axyprep DNA Gel Extraction Kit to achieve gel extraction. Protocol as follow.

1. Excise the agarose gel slice containing the DNA fragment of interest with a clean, sharp scalpel under ultraviolet illumination. Briefly place the excised gel slice on absorbent toweling to remove residual buffer. Transfer the gel slice to a piece or plastic wrap or a weighing boat. Mince the gel into small pieces and weigh. In this application, the weight of gel is regarded as equivalent to the volume.
2. Add a 3x sample volume of Buffer DE-A.
3. Heat at 75°C until the gel is completely dissolved (typically, 6-8 minutes). IMPORTANT: Gel must be completely dissolved or the DNA fragment recovery will be reduced. IMPORTANT: Do not heat the gel for longer than 10 minutes.
4. Add 0.5x Buffer DE-A volume of Buffer DE-B. Please make sure the contents are a uniform yellow color before proceeding.
5. Place a Miniprep column into a 2 ml microfuge tube (provided). Transfer the solubilized agarose from Step 4 into the column. Centrifuge at 12,000xg for 1 minute.
6. Discard the filtrate from the 2 ml microfuge tube. Return the Miniprep column to the 2 ml microfuge tube and add 500 µl of Buffer W1. Centrifuge at 12,000xg for 30 seconds.
7. Discard the filtrate from the 2 ml microfuge tube. Return the Miniprep column to the 2 ml microfuge tube and add 700 µl of Buffer W2. Centrifuge at 12,000xg for 30 seconds.
8. Discard the filtrate from the 2 ml microfuge tube. Place the Miniprep column back into the 2 ml microfuge tube. Add a second 700 µl aliquot of Buffer W2 and centrifuge at 12,000xg for 1 minute.
9. Discard the filtrate from the 2 ml microfuge tube. Place the Miniprep column back into the 2 ml microfuge tube. Centrifuge at 12,000xg for 1 minute.
10. Transfer the Miniprep column into a clean 1.5 ml microfuge tube (provided). To elute the DNA, add 25-30 µl of Eluent or deionized water to the center of the membrane. Let it stand for 1 minute at room temperature. Centrifuge at 12,000xg for 1 minute.

Note: Pre-warming the Eluent at 65°C will generally improve elution efficiency.
Note: Deionized water can also be used to elute the DNA fragments.

Polymerase Chain Reaction

Gene Name： cenA (link with pET-30a).

Primer sequence： R: acg cgt ccc aac gcg cgt tac

F: cgc gga tcc atg agc acc cgt cgt ac

表 1

Reagent	Volume	Total Volume
5×GC buffer	4 μL	20 μL
10 mM dNTPs	0.4 μL	
5'-primer/3'-primer	1+1 μL	
Phusion polymerase	0.2 μL	
ddH_2O	12.4 μL	
Template DNA	1 μL	

PCR Program

Primary Denaturation	Denaturation	Annealling	Extension	Circulation	Deactivation	
98°C	98°C	57.5°C	72°C	cycle	72°C	4°C
30 s	10 s	30 s	60 s	30×	5 min	α

Gene Name：cex（link with pET-30a）

Primer sequence： R：acgcgtcgacACCCACGGTGCACGG

F: cgcggatccATGCCGCGTACCACCCC

Reagent	Volume	Total Volume
5×GC buffer	4 μl	
10 mM dNTPs	0.4 μl	
DMSO	0.4 μl	
5'-primer/3'-primer	1+1 μl	20 μl
Phusion polymerase	0.2 μl	
ddH_2O	12 μl	
Template DNA	1 μl	

PCR Program

Primary Denaturation	Denaturation	Annealling	Extension	Circulation	Deactivation	
98°C	98°C	56°C	72°C	cycle	72°C	4°C
30 s	10 s	30 s	60 s	30×	5 min	α

Gene Name：β-glucosidase（link with pET-30a）

Primer sequence： R: cccaagcttGGCGCCGGGCAGTA
F: ccggaattcGTGGTCACCGCAGCACA

Reagent	Volume	Total Volume
2×Phanta PCR buffer	25 μl	
10 mM dNTPs	1 μl	
5'-primer/3'-primer	2+2 μl	50 μl
Phanta polymerase	1 μl	
ddH_2O	16 μl	
Template DNA	1 μl	

PCR Program

Primary Denaturation	Denaturation	Annealling	Extension	Circulation	Deactivation	
95°C	95°C	62°C	72°C	cycle	72°C	4°C
30 s	10 s	30 s	90 s	30×	5 min	α

Touch-Down PCR Program

① 95℃，15:00

② 95℃，00:45

③ 75℃，00:30，Inc -2℃/Cyc

④ 72℃，01:30

⑤ Goto2,5 More times

⑥ 95℃，00:30

⑦ 62℃，00:30

⑧ 72℃，01:30

⑨ 30x

⑩ 72℃，05:00

Gene Name：INP（link with cenA/cex-pET-30a）

Primer sequence： R: cttgatatctgccagttcaccac

F: catgccatggatGTGATGACCCTGGAT

Reagent	Volume	Total Volume
2×Phanta PCR buffer	25 μl	50 μl
10 mM dNTPs	1 μl	
5'-primer/3'-primer	2+2 μl	
Phanta polymerase	1 μl	

ddH_2O	16 μl
Template DNA	1 μl

PCR Program

Primary Denaturation	Denaturation	Annealling	Extension	Circulation	Deactivation	
95°C	95°C	57°C	72°C	cycle	72°C	°C
5 min	30 s	30 s	50 s	30×	5 min	

Gene Name：CrtE

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

PCR Program

Primary Denaturation	Denaturation	Annealing	Extension	Cycle	Deactivation	
95° C	95° C	70°C	72°C	cycle	72° C	4° C
3 min	30 s	15 s	45 s	29×	5 min	∞

Gene Name：CrtB

Reagent	Volume	Total Volume
PCR buffer	3 μl	
2.5 mM dNTPs	0.3 μl	
5 '-primer/3' -primer	0.75 μl	
Phusion enzyme	0.15 μl	15 μl
DMSO	0.75 μl	
DEIONIZED WATER	8.7 μl	
Template DNA	0.6 μl	

PCR Program

Primary Denaturation	Denaturation	Annealing	Extension	Cycle	Deactivation	
95° C	95° C	65°C	72°C	cycle	72° C	4° C
3 min	30 s	20 s	45 s	29×	5 min	∞

Gene Name：CrtI

Reagent	Volume	Total Volume
PCR buffer	3 μl	
2.5 mM dNTPs	0.3 μl	
5 '-primer/3' -primer	0.75 μl	
Phusion enzyme	0.15 μl	15 μl
DMSO	0.75 μl	
DEIONIZED WATER	8.4 μl	
Template DNA	0.9 μl	

PCR Program

Primary	Denaturation	Annealing	Extension	Cycle	Deactivation

Denaturation						
95° C	95° C	60°C	72°C	cycle	72° C	4° C
3 min	30 s	20 s	45 s	29×	5 min	∞

Gene Name：CrtY

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

PCR Program

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

Seamless splicing-E. coli transformation：

(1) By measuring the concentration of fragment and plasmid to calculate, according to the final vector and gene ratio of 3:1 (1-2 fragments) or to design, get the amount of each addition.

(2) Premix, gene fragments, vector and nuclease free water are added to prepare a 20 μl system

(3) The final product is obtained by incubating at 50 °C for 15 min.

(4) 5μl of that product is mix with commercial competent state (50 to 100 μl), and the ice bath is used for 30 min

(5) Heat shock for 90 s in a 42 °C water bath, then cool in an ice bath for 3-5 min

(6) The product is added to 500 μl LB culture medium and cultured in 37 °C shaker for 1hr.

(7) After centrifugation, 10 ~ 50 μl LB is left, mixed uniformly, and then coated on a solid LB plate overnight.

(8) The next day, the colonies will be cultured and the plasmids will be extracted and tested.

Experiment Group

Reagent	Volume	Rate (mol)	Total Volume
2X Seamless Premix	10 μl		
Fragment 1	10-100 ng	3	
Fragment 2 (if have)	10-100 ng	3	20 μl
plasmid	50-100 ng	1	
Nuclease free water	Up to 20 μl		

Positive Control Group

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

Negative Control Group

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

LB medium

Reagent	Volume	Total Volume
Peptone	10 g	1 L
Yeast Extract	5 g	
NaCl	10 g	
ddH_2O	Fixed volume to 1 L	

Autoclave：120℃ 20min

Chloramphenicol (Chl) mother solution (50mg/ml)

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

Though 0.2 μm filter to remove bacteria; Store at -20℃

Colonies PCR

Reagent	Volume	Total Volume
2 x Taq Mix with standard buffer	5 μl	10 μl
Primers	0.75 μl+0.75 μl	
ddH_2O	3.5 μl	

The PCR conditions are determined by the fragment we needed.

SDS-PAGE

Separating gel	8%	10%
ddH_2O (ml)	7.25	6.25

30% Acr-Bis (ml)	4.0	5.0
4x Tris-HCl (ml)	3.75	3.75
10% APS (ml)	0.15	0.15
TEMED (ml)	0.009	0.006
Total volume (ml)	15	15

Stacking gel	**6%**
ddH_2O (ml)	3.42
30% Acr-Bis (ml)	1.02
4x Tris-HCl (ml)	1.5
10% APS (ml)	0.06
TEMED (ml)	0.006
Total volume (ml)	6

Loading the gel: samples + marker

Run Gel:

(1) 60 V 20 min

(2) 110 V 60 min

(3) 55 V 20 min

Overlap PCR

Two fragments overlap PCR

1. Get the PCR product (15 µl system) of each fragment then take 5 µl to run DNA gel electrophoresis. If the band with predicted molecular weight is obtained, go to the next step
2. Taking two kinds of PCR products, 1µl each, without primers, and the rest are the same as the normal PCR system (15 µl), 7 cycles.
3. Take 5 µ l product and run gel electrophoresis to see if there is a target band. If there is, add all the remaining solution as Template to normal PCR system (15 µl). The primers are the Forward of the first fragment and the Reverse of the second fragment, 30 cycles. Run gel electrophoresis. if we get the target band, do gel extraction.

Lycopene extraction

1. Take 1 ml bacteria in EP tube, Centrifugate at 12000 rpm/s, 5 min.
2. Discard the supernatant and drain the residual medium.
3. Add 1 ml ddH_2O and mix, Centrifugate at 12000 rpm/s, 5 min.
4. Discard the supernatant and drain the residual medium.
5. Add 1 ml of acetone and mix
6. In the dark, 55 ° C metal bath for 15 min (inverted every 5 min)
7. Centrifugate at 12000 rpm/s, 5 min.
8. Measure the supernatant of the supernatant at 473 nm spectrum (use acetone as CK).

β-carotene extraction

9. Take 1 ml bacteria in EP tube, Centrifugate at 12000 rpm/s, 5 min.
10. Discard the supernatant and drain the residual medium.
11. Add 1 ml ddH_2O and mix, Centrifugate at 12000 rpm/s, 5 min.
12. Discard the supernatant and drain the residual medium.
13. Add 1 ml of acetone and mix
14. In the dark, 55 ° C metal bath for 15 min (inverted every 5 min)
15. Centrifugate at 12000 rpm/s, 5 min
16. Measure the supernatant of the supernatant at 453 nm spectrum (use acetone as CK).

Transformation

1. Mix Plasmid (1~3 μl) or the product of ligation(10 μl) with commercial competent state cells (50 μl)，then put them in ice bath for 30 min;
2. Heat shock the mixture for 90 s in a 42℃ water bath, then cool it in the ice bath for 3-5 min;
3. Add 500 μl LB culture medium to the product and culture them in 37℃ shaker for 1 hr;
4. Take 200 μl LB culture medium and then coat on a solid LB antibiotic plate overnight.

Restriction Enzyme Digestion

Reagent	Volume	Total Volume
ddH_2O	15 μl	
CutSmart buffer	5 μl	
PCR production（vector）	28 μl	50 μl
Restriction Enzyme 1	1 μl	
Restriction Enzyme 2	1 μl	

Enzyme Ligation

Reagent	Volume	Rate (mol)	Total Volume
Fragment	10-100 ng	3~7	
Vector	50-100 ng	1	10 μl
T4 DNA Ligase	1 μl		
T4 DNA Ligation Buffer	1 μl		

Imosone solid medium

Reagent	Volume	Total Volume
Glucose	10 g	
Tryptone	4 g	
Yeast Extract	10 g	
Beef extract	4 g	1L
NaCl	2.5 g	
ddH_2O	Fixed volume to 1 L	
Agar	18 g	

Adjust pH to 7.0

Kohlberg's no. 1 medium

Reagent	Volume	Total Volume
Soluble starch	20.0 g	1 L
KNO_3	1.0 g	
$K_2HPO_4 \cdot 3H_2O$	0.5 g	
$MgSO_4 \cdot 7H_2O$	0.5 g	
$FeSO_4 \cdot 7H_2O$	0.5 g	
NaCl	0.5 g	
ddH_2O	Fixed volume to 1 L	
Agar	20.0 g	

Adjust pH to 7.2~7.4

Extracting Genome of Streptomyces coelicolor

1. Streak the fresh Streptomyces spores onto a Imosone solid medium, then invert culture for 48 h (30℃);
2. Select the young mycelium in a 1.5 mL centrifuge tube containing 100 μl of Milli-Q water;
3. Use a sterile toothpick to disperse the mycelium fully in the Milli-Q water, use the Vortex to mix it evenly;
4. Boil it in boiling water for 10 min;
5. Take the supernatant as template in PCR.