Protocols

1. PCR

1) Takara rTaq®

Components	Volume
TaKaRa Taq（5 U/μl）	0.25 μl
10×PCR Buffer（Mg^{2+} Plus）	5 μl
dNTP Mixture（2.5 mM each）	4 μl
Template DNA（*E. Coli* genomic DNA）	10 ng～100 ng
primer 1（20 μM）	1 μl
primer 2（20 μM）	1 μl
Autoclaved, distilled water	up to 50 μl

Program:

1	94℃	5’	
2	94℃	30’’	30 Cycles
3	Based on the temperature of primers	30’’	
4	72℃	Based on the length (1Kb/min)	
5	72℃	5’	
6	4℃	-/-	
7	END		

2) Toyobo KOD plus®

Components	Volume	Final Concentration
Autoclaved, distilled water	(33 - X) μl	
10× PCR Buffer for KOD -Plus- Neo	5 μl	1×
2 mM dNTPs	5 μl	0.2 mM each
25 mM MgSO4	3 μl	1.5 mM
primers (10 μM each)	1.5 μl	0.3 μM each
template	X μl	Genomic DNA ~200 ng/50μl Plasmid DNA ~50 ng/50μl cDNA ~200 ng/50μl
KOD -Plus- Neo (1 U/μl)	1 μl	1 U/50μl
Total	50 μl	

Program:

1	94℃	5’	
2	94℃	30’’	30~35 Cycles
3	Based on the	30’’	

	temperature of primers		
4	68℃	Based on the length (1Kb/min)	
5	68℃	5’	
6	4℃	-/-	
7	END		

3) Takara PrimeStar®

Components	Volume
5×PrimeSTAR® Buffer（Mg^{2+} plus）	10 μl
dNTP Mixture（2.5 mM, each）	4 μl
Primer 1（10 μM）	1 μl
Primer 2（10 μM）	1 μl
Template DNA（*E. Coli* genomic DNA）	1 ng～10 ng
PrimeSTAR® HS	
DNA Polymerase（2.5 U/μl）	0.5 μl
Autoclaved, distilled water	up to 50 μl

Program:

1	94℃	5’	
2	94℃	30’’	30~35 Cycles
3	Based on the temperature of primers	30’’	
4	72℃	Based on the length (1Kb/min)	
5	72℃	5’	
6	4℃	-/-	
7	END		

4) Go Taq®

Components	Volume
2×Go Taq®	5 μl
Autoclaved, distilled water	4 μl
Primer 1	0.5 μl
Primer 2	0.5 μl
Template (DNA or clony)	25 ng
Total	10 μl

Program:

1	94℃	5’	
2	94℃	30’’	25 Cycles
3	Based on the temperature of primers	30’’	
4	72℃	Based on the length (1Kb/min)	
5	72℃	5’	

6	4℃	-/-	
7	END		

5) Recombinant PCR

One-step program:

Usual system			
1	94℃	5’	
2	94℃	30’’	30 Cycles
3	Based on the temperature of primers	30’’	
4	72℃	Based on the length (1Kb/min)	
5	72℃	5’	
6	4℃	-/-	
7	END		

Two-step program:

Usual system without primers			
1	94℃	5’	
2	94℃	30’’	10 Cycles
3	Based on the temperature of complementary primers	30’’	
4	72℃	Based on the length (1Kb/min)	
5	72℃	5’	
Add end-side-primers primers			
6	94℃	5’	
7	94℃	30’’	30 Cycles
8	Based on the temperature of end-side-primers	30’’	
9	72℃	Based on the length (1Kb/min)	
10	72℃	5’	
11	4℃	-/-	
12	END		

2. Digestion (NEB Restrictive Enzymes)

Components	Volume
DNA or plasmids	20~30 μl
Autoclaved, distilled water	10~20 μl
NEB Buffer	5 μl

NEB Restrictive Enzymes	1.5~2.0 μl
Total	50 μl

Condition: DNA, 37℃ for 2~2.5h; Plasmids, 37℃ for 4.5~5h.

3. Ligation (NEB T4 Ligase)

Components	Volume
T4 Ligase	1 μl
T4 Ligase Buffer	2 μl
Autoclaved, distilled water + gene + plasmid	17 μl
Total	20 μl

Note: The units of gene and plasmid ligated in 20 μl system are based on their relative concentration in the water, or the relative intensity of brightness on the gel, which has formula below:

$$3 = \frac{\mathrm{ax/m}}{\mathrm{by/n}}$$

Where a and b are the relative intensity of brightness, m and n are lengths of gene and plasmid, x and y are gene and plasmid, respectively.

4. Transformation

Components	Volume
Product of ligation or plasmids	2~3 μl
Competent cell	100~200 μl

Process	Condition
electro-transformation	2500V, 4.5~6.0 ms
culture	37℃ for 40min
smear on the medium	100 μl liquid containing *E. Coli*

5. Preparation of competent cells for electro-transformation

1) Inoculate the DH5α of *E. Coli* in 5mL LB medium at 37℃ for 14~16h.
2) Amplifying culture the 5mL bacterial liquid into 55mL LB medium with 2% sucrose for 4~5 h at 18℃.
3) Inoculate the bacterial liquid for 30 min in ice (about 0℃).
4) Use two 50mL BD tubes to centrifuge the bacterial liquid 10 min at 5,000×g at 4℃ to collect

the bacterial and discard the liquid.
5) Use 40mL autoclaved, distilled water to suspend the bacterial, then centrifuge the liquid 15 min at 4,000×g at 4℃ to collect the bacterial and discard the liquid.
6) Repeat step (5).
7) Use 40mL 10% glycerol to suspend the bacterial, then centrifuge the liquid 15 min at 4,000×g at 4℃ to collect the bacterial and discard 38mL liquid.
8) Resuspend the bacterial and then transfer 200 μl to each microcentrifuge tube, then reserve at -80℃.

6. Agarose gel electrophoresis

1) Test gel: 120V, 25~30 min.
2) Collect gel: 80V, 55~60 min.

7. Prescription of usual reagents and mediums

1) Agarose of DNA electrophoresis: 1% agarose.
2) Buffer of agarose gel electrophoresis (50×TAE): 2M Tris-acetic acid, 100mM EDTA.
3) LB liquid medium: 10g NaCl, 10g tryptone and 5g yeast extract in 1L distilled water.
4) LB solid medium: 10g NaCl, 10g tryptone, 5g yeast extract and 18g agar in 1L distilled water.
5) IPTG: 24mg/mL IPTG.
6) Antibiotics: 100mg/mL Ampicilin, 50mg/mL kanamycini, 50 mg/mL chloramphenicol, 50μg/mL Nalidixic acid.

8. Cs^+ absorbance

1. Culture the pET32a-BL21 E.coli and the trkD-pET32a-BL21 E.coli with 5ml LB medium overnitht.
2. Put 3ml culture of the pET32a-BL21 E.coli and the trkD-pET32a-BL21 E.coli into 100ml CsCl-LB solution respectively. Two hours later, measure their respective OD values. Then induce them with IPTG (concentration: 0.1mol/L). Four to six hours later, measure their respective OD values. Then keep them still in the 37℃ incubator for 4 to 8 hours. Centrifugate the culture to collect the E.coli, then wash them with PBS for two times. In the end, suspend the E.coli with about 20ml ddH_2O respectively. Then send the two samples of the pET32a-BL21 E.coli and the trkD-pET32a-BL21 E.coli to China National Analytical Center, Guangzhou for the analysis of Cs element.

9. Fluorescence Detection

1. Inoculate two strains with GFP, cheZ-GFP-pUC18-BL21 and trkD-GFP-pUC18-BL21
2. Mix 1ml incula with 100 LB to intermediately culture the two strains(place the conical flask containing inocula in 37° shaker at 220 rpm) for 90 to 120mins. Measure the OD value of the incula from 90min. Stop shaking when the OD of the inocula reaches 0.4. Then start induction with IPTG.
3. Induction with IPTG at three final concentrations: 0.1ug/ml，0.5ug/ml and 1ug/ml. Shake at 37° and 220 rpm with the control group for 4h.
4. Mix 5ul inocula and 5ul fluorescence anti-fade reagent on a slide and cover with a coverslip. Exam the bacterial fluorescence with the Laser Scanning Confocal Microscope.

10. Western Blot

Before Western Blot

1. Culture E.coli in 5mL LB at 37℃ for 16h
2. Amplication culture at 37℃ until OD600 reaches 0.4~0.8
3. Induction with IPTG at 0.1mg/mL at 18℃ for18h
4. Total protein extraction after cell discruption by sonic oscillator
5. Acrylamide gel preparation
6. SDS-PAGE
7. Electrophoretic transfer to PVDF membranes in a mixture of ice and water for 2h

Western Blot

8. Blocking the membrance with skimmed milk powder in TBST buffer overnight at 4℃
9. Wash 3 times with TBST, each for 5 min
10. Incubate with primary antibody (mouse anti Histidine) at RT for 2h
11. Remove antibody solution and wash membrane 3 times with TBST, each for 5 min
12. Incubate with second antibody (HRP-Goat anti mouse lgG(H+L)) at RT for 2h
13. Remove antibody solution and wash membrane 3 times with TBST, each for 5 min
14. Add substrate and chemiluminescence analysis