Protocol

Plasmid Extraction

Performed according to the TIANprep Mini Plasmid Kit

PCR

1. For a 20 μL reaction：
2. In a PCR tube on ice, combine 0.5 μL of template DNA, 1.0 μL of 10 μM forward primer,1.0 μL of 10 μM reverse primer to a PCR tube on ice, 10 μL of PrimeSTAR Max Premix(2X) and sterile water up to 20 μL.
3. Gently mix the reaction
4. Collect the liquid to the bottom of the PCR tube by centrifuging briefly
5. Transfer the PCR tube from ice to a PCR machine preheated to 95°C to begin thermocycling
6. Thermocycling:
7. The PCR machine should be set to run the following steps:

step	Temperature(℃)	Time
Initial denaturation	95	3 minutes
30-40 cycles	95(denaturation) Tm-5(annealing) 72(extension)	30seconds 30seconds 20seconds per kb
Final extension	72	10 minutes
Hold	12	indefinitely

Colony PCR

1.Selection of 8 Monoclonal colonies from Agar Plate.

2.Cultured in LB medium containing required antibiotics in 1.5ml centrifuge tube for 2 to 3 hours (constant temperature shaking bed 37 ℃, 200rpm).

3.Verification of PCR positive Recombinant plasmid using bacterial liquid as cDNA template.

4.According to the synthetic target gene sequence.

5.Specific primers containing the required restriction sites were designed.

6. The conditions of colony PCR amplification system were the same as those of common pcr.

Agarose-gel electrophoresis

1. Prepare a 1% agarose-gel: Dissolve the agarose in 1X TAE by boiling and add Nucleic acid

dye(10 µl per 100 ml agarose solution).
For short DNA fragments, gels with higher agarose percentage should be used.

2. Pour the gel and let it curdle
3. Add 1ul 6X blue loading buffer to sample(5ul)
4. Place the gel into the tank and add 1X TAE buffer so that the gel is fully covered.
5. Pipet sample and marker into the pockets.
6. Run the gel at 110 V for 30 min.
7. Image the gel using ultraviolet light. If necessary, cut out bands.

DNA recovery from agarose gel

Performed according to the TIANgel Midi Purification Kit

Restriction Digest

1.Add 1ug of DNA to be digested, and adjust with dH20 for a total volume of 16ul.
2.Add 5.0ul of NEBuffer 2.
3.Add 1.0ul of BSA.
4.Add 1.0ul of EcoRI.
5.Add 1.0ul of PstI.
6.adjust with dH20 for a total volume of 50ul.
7.Mix well and spin down briefly.
8.Incubate the restriction digest at 37C° for 30min, and then 80C° for 20min to heat kill the enzymes. We incubate in a thermal cycler with a heated lid
9.Run a portion of the digest on a gel (6ul), to check that both plasmid backbone and part length are accurate.

Ligation

1.Add 2.5ul of digested plasmid backbone (31.2 ng)
2.Add 3 ul of EcoRI-HF SpeI digested fragment
3.Add 3 ul of XbaI PstI digested fragment
4.Add 1 ul T4 DNA ligase buffer. Note: Do not use quick ligase
5.Add 0.5 ul T4 DNA ligase
6.Add water to 10 ul
7.Ligate 16C/30 min, heat kill 80C/20 min

Chemical Transformation of E.Coli

1. Thaw 100 µL DH5α competent cells on ice
2. Pipet plasmid into competent cells and mix them thoroughly
3. Incubate on ice for half an hour
4. Heat shock at 42 ℃ for 60 seconds
5. Incubate on ice for 2 minutes
6. Add 1 ml SOC solution and incubate for 1h at 37℃ on a shaker shaking at 200-300rpm.
7. Pipette 100µL of each transformation onto petri plates Spread with sterilized spreader or glass beads immediately
8. Spin down cells at 6800g for 3mins and discard 800µL of the supernatant. Resuspend the cells in the remaining 100µL, and pipette each transformation onto petri plates Spread with sterilized spreader or glass beads immediately
9. Incubate transformations overnight (14-18hr) at 37°C: Incubate the plates upside down (agar side up)
10. Pick single colonies: Pick single colonies from transformations: do a colony PCR to verify part size, make glycerol stocks, grow up cell cultures and miniprep

Bacterial cell culture

1. Streak an agar plate from glycerol stock. Incubate plates until colonies grow.
2. Adding 20ml liquid medium to aseptic dried conical flask.
3. Pick up one colony by tapping a small (0.1 µl) pipette tip (held on a pipette) on the surface of the plate.
4. use a sterile metal loop (sterile by flaming) to place the colony in the bottle.
5. Pipette 20µl of antibiotic into each bottle （per 20 ml)
6. Vortex each Conical bottle for 1-2 seconds to mix well.
7. Place the conical bottle evenly mixed in a 37 degree constant temperature shaker.
8. Wait overnight or until your cells have reached the desired concentration.

Making a long term stock of bacteria

1. Pick a single colony of the clone off a plate and grow an overnight in the appropriate selectable liquid medium (3-5ml).
2. Add 400 ul of 60% glycerol in H2O to a Aseptic centrifuge tube.
3. Add 800 ul sample from the culture of bacteria to be stored.
4. Gently vortex the Centrifugal tube to ensure the culture and glycerol is well-mixed.
5. Alternatively, pipet to mix.
6. On the side of the Centrifugal tube list all relevant information - part, vector, strain, date, researcher, etc.
7. Freeze glycerol stock in liquid nitrogen and store in a -80C° freezer.

LB Culturing medium recipe

LB Broth Formulation per one liter
10g Tryptone
5g Yeast Extract
5g Sodium Chloride
If you need to prepare a solid medium, you need to add 1.5-2.0 g Agar powder (per 100ml liquid medium)

M9 Culturing medium recipe

M9 mother liquor per one liter
64g Na2HPO4
15g KH2PO4
2.5gNACL
5.0g NH4Cl
Sterilization(Note that magnesium sulfate and M9 mother liquor should be sterilized separately)
Take M9 mother liquor 200ml and water 700 ml, add 2ml 1mol/L sterilized magnesium sulfate, add 100 uL sterilized 1mol/L calcium chloride (can be added but not added)

Data measurement 1 (phenylalanine induction test)

1. Escherichia coli was cultured in LB medium until the OD600 value reached 0.6-0.8, Diluted to M9 medium (final concentration of 0.4% glucose, 0.24 mg / ml magnesium sulfate, 111 μ g / ml calcium chloride, 0.3 μ mol thiamine hydrochloride and 50 μ g / ml kanamycin).
2. 100 uL M9 medium was added to sterile 96-well plate, and M9 medium containing phenylalanine / tyrosine was diluted to 0,12.5,25,50,100,200uM according to concentration gradient.
3. The 96-well plate was put into the automatic enzyme labeling instrument. Incubate overnight at 37 ℃, and record the fluorescence value and OD600 of each hole every 30 minutes.
4. Each group should be repeated at least 3 times

Data Measurement II (Comparative experiment)

1. Escherichia coli was cultured in LB medium until the OD600 value reached 0.6-0.8, Diluted to M9 medium (final concentration of 0.4% glucose, 0.24 mg / ml magnesium sulfate, 111 μ g / ml calcium chloride, 0.3 μ mol thiamine hydrochloride and 50 μ g / ml kanamycin).
2. 100 μ L M9 medium and 100 microphenylalanine, 1.8% urea and 0.05% uric acid were added to two aseptic cone bottles, respectively. Fresh M9 medium was used as blank control.

3. Incubated with 200rpm at 37 ℃ in a shaking bed. The fluorescence and OD values are measured every eight hours.
4. Each group should be repeated at least 3 times

Colorimetric card

1. Purple, blue, yellow and pink pigment proteins were overexpressed in E. coli, centrifuged with 8000rpm for 3 min, resuscitated with pbs buffer and repeated three times.
2. Ultrasonic crushing of Escherichia coli.
3. Four kinds of broken Escherichia coli were added to four aseptic eight tubes, and the yellow and pink pigment proteins were diluted to 0, 20, 40, 60, 80, 100, 120, 140 according to the concentration gradient. Purple and blue diluted to 140, 120, 100, 80, 60, 40, 20, 0 according to concentration gradient.
4. The diluted yellow pigment protein was mixed with purple pigment protein and blue pigment protein, and the pink pigment protein was mixed with purple pigment protein and blue pigment protein respectively.

Data measurement

1. Spectral scanning of BBa_K1033910、BBa_K1033932、BBa_K1033919、BBa_K592010
2. Looking for their maximum absorption peaks.And find out if they have peaks of excited and emitted light.