Experimental Protocol

Content

Seamless Cloning

1. General Experiments

Introduction

General Experiments refer to the preparation of materials used in experiments, including the preparation of LB culture medium and competent cells.

Material

1. Peptone
2. NaCl
3. Yeast extract
4. Agar
5. ddH_2O
6. Commercial BL21 competent cells
7. 0.1M $CaCl_2$

Procedure

Preparation of LB Culture Medium

- LB culture medium should be prepared in a flask, sealed, and sterilized. 1 L of LB medium recipe: 10 g peptone, 10 g NaCl, 5 g yeast extract, 15 g agar (if for liquid medium, no agar).

- For LB agar plates, add the appropriate antibiotic after sterilization. Then pour the mixture onto the Petri dishes.

- For liquid LB medium, it should be divided by 5 mL into test tubes before sterilization.

Preparation of Competent Cells for Transformation

- Streak an antibiotic-free agar plate with commercial BL21 competent cells to obtain single colonies. Incubate at 37°C for 12 h.

- Transfer a single colony to 5 mL of liquid LB medium in test tubes. Shake the tubes at 37°C, 220 rpm, for 6 h.

- Inoculate the suspension from the test tube into 300 mL of LB medium. Shake them at 37°C, 220 rpm, for 1-2 h. Continuously monitor the optical density (OD) of the bacterial suspension to ensure the value of OD 600 does not exceed 0.4.

- When the OD value comes up to 0.3, incubate the suspension in an ice bath for 15 min, and pre-chill a 0.1M $CaCl_2$ solution on ice.

- Transfer the suspension into a 50 mL centrifuge tube and centrifuge at 3400 rpm for 10 min.

- Discard the supernatant and resuspend the bacterial pellet in 30 mL of $CaCl_2$ solution. Incubate in an ice bath for 30 min and centrifuge at 3400 rpm for 10 min.

- Discard the supernatant and resuspend the bacterial pellet in 6 mL of $CaCl_2$ solution.

- Seal the resuspended cells in pre-sterilized 1.5 mL EP tubes.

- Rapidly freeze the cells in liquid ammonia and store at -80°C in a freezer.

2. PCR Amplification of Mutants

Introduction

PCR, or Polymerase Chain Reaction, is a fundamental molecular biology technique. It replicates

DNA by repeatedly heating the sample, allowing researchers to amplify specific DNA segments exponentially. This method is crucial for various applications, such as genetic analysis and DNA sequencing, thanks to its ability to create millions of copies of a target DNA region from just a tiny initial sample.

Material

1. Primer
2. DNA template
3. 2× Phanta Max Buffer
4. ddH_2O

Procedure

- Use pET22b (+) (Ceres) (Demetra: pET15b, pET21a, pET22b, pET28a, pET28bs, pET32m, pGEX-6p-1) as carrier. Use NdeI and XhoI for digestion and binding. The final plasmid is supplied by the company.

- Design the primers. Centrifuge them at 12,000 rpm for 2 min before use and add 10 times the required volume of ddH_2O to achieve a primer concentration of 10 μmol/L.

- Prepare the reaction mix in PCR tubes according to Table 1.

Component	*Volume / μL*
ddH_2O	20
2× Phanta Max Buffer	25
Primer-5' (10 μmol/L)	2
Primer-3' (10 μmol/L)	2
DNA template	1
Total	50

Table 1 Components for PCR

- Place the reaction tubes into the PCR machine with the following temperature settings. Set the hot lid temperature to 105℃.

a. Initial Denaturation: 98℃ for 3 min
b. Denaturation: 98℃ for 10 s
c. Annealing: 60℃ for 20 s
d. Extension: 72℃ for 30 s per 1000 bp (30 cycles of b-c-d)
e. Final Extension: 72℃ for 5 min
f. Hold at: 4℃

3. Template DNA Digestion

Introduction

DpnI is a type of restriction endonuclease that specifically cleaves methylated DNA strands. To distinguish between the original DNA and mutant DNA, DpnI is used to enzymatically digest the extension products. Since the template plasmid is derived from regular Escherichia coli and is modified by dam methylation, it is sensitive to DpnI and gets cleaved. In contrast, DNA with mutated sequences synthesized in vitro remains intact because it lacks methylation.

Material

1. DpnI

Procedure

- Add 1 μL of DpnI enzyme to each PCR reaction tube and incubate at 37°C in the PCR machine for 30 min. Set the hot lid temperature to 37°C.

4. Agarose Gel Electrophoresis Analysis

Introduction

Agarose gel electrophoresis analysis is a fundamental laboratory technique used to separate and analyze DNA fragments based on their size. In this process, a mixture of DNA molecules is loaded onto an agarose gel and subjected to an electric field. Smaller DNA fragments move through the gel more quickly than larger ones, resulting in distinct bands that can be visualized under ultraviolet light.

Material

1. 1× TAE
2. Agarose
3. ddH_2O
4. Ethidium bromide
5. DNA Marker

Procedure

Preparation of 1% Agarose Gel

- To make a small gel (6 wells, 40 mL), add 40 mL 1× TAE buffer and 0.4 g agarose in a 250 mL conical flask. 1 L of 50× TAE buffer recipe: Dissolve 242g Tris, 37.2g Na_2EDTA in 1 L beaker, add 600 mL of ddH_2O and 57.1 mL of ice acetic acid, mix thoroughly, and then bring up to 1 L.
- Microwave until boiling.
- After slight cooling, add 2 μL of ethidium bromide and mix gently.
- Pour the mixture into the gel casting tray, insert the comb, and wait for the gel to solidify.

Sample Preparation and Loading

- After complete gel solidification, remove the comb and place the gel casting tray into the electrophoresis chamber.
- In the sample wells, load 10 μL of DNA Marker and all PCR products.

Electrophoresis

- Set the voltage to 140 V and run for 20 min, then turn off the power supply.
- Remove the gel and use a gel imaging system for observation and photography of the electrophoresis results.

Attention: Be sure to wear appropriate safety equipment, such as gloves and goggles, when handling ethidium bromide.

5. Gel Extraction

Introduction

Gel extraction is to purify and isolate DNA of interest from the gel.

Material

1. ddH_2O

2. TIANGEN's Gel Purification Kit (Enhanced)

Procedure

- Excise the desired DNA band from the gel, place it in a centrifuge tube, and record its weight.

- Add gel purification solution PE to the gel piece in a 3-fold volume, incubate at room temperature for 20 min, gently invert the centrifuge tube periodically to ensure complete dissolution of the gel piece.

- Transfer the solution obtained from the previous step to column CA5 (place the column in a collection tube). Allow it stand at room temperature for 2 min.

- Centrifuge at 12,000 rpm for 1 min, discard the waste liquid in the collection tube, and return the column CA5 to the collection tube.

- Add 600 μL of wash buffer PW (before use, ensure that anhydrous ethanol has been added) to column CA5.

- Centrifuge at 12,000 rpm for 1 min, discard the waste liquid in the collection tube, and return the column CA5 to the collection tube.

- Repeat the previous two steps, and preheat a metal bath to 55°C.

- Centrifuge the column CA5 at 12,000 rpm for 2 min to remove residual wash buffer as much as possible. Transfer column CA5 into a clean centrifuge tube, place it in the metal bath for 10 min to thoroughly dry.

- Add 30 μL of elution buffer (ddH_2O) to the center of the adsorption membrane in column CA5. Allow it to stand in the metal bath for 2 min.

- Centrifuge the column CA5 at 12,000 rpm for 2 min, collect the DNA solution.

6. Seamless Cloning for Plasmid Acquisition

Introduction

Seamless cloning is a sophisticated molecular biology technique used to precisely insert DNA fragments into vectors without leaving any unwanted scars or sequences behind.

Material

1. ddH_2O
2. 2× HiFi Seamless Cloning Mix
3. Linearized vector
4. Insert fragment

Procedure

- Determine the necessary volumes to add for the linearized vector (X) and the insert fragment (Y) based on rhe experimental requirements.

- Prepare the reaction mix in PCR tubes on ice according to Table 2.

Component	*Volume*
2× HiFi Seamless Cloning Mix	5 μL
Linearized vector	X
Inserts	Y
ddH_2O	Bring up to 10 μL

Table 2 Components for Seamless Cloning Reaction

- Gently mix the reaction mixture and incubate at 50°C in the PCR machine for 15 min.

7. Transformation of Recombinant Products into E. coli DH5α

Introduction

The transformation of recombinant products into competent cells is a crucial step in molecular biology. It involves introducing foreign DNA, typically plasmids containing desired genes, into bacterial cells made competent through chemical treatment. This process enables the host cells to take up and express the foreign DNA, allowing the production of recombinant proteins or other biotechnological applications.

Material

1. ddH_2O
2. Competent cell DH5α
3. recombinant product or plasmid
4. LB medium
5. Agar plates

Procedure

- Retrieve competent cells from the -80°C freezer and thaw them on ice for 5 min until melted. Add 10 μL of the recombinant product (1 μL of plasmid) to 100 μL of DH5α competent cells, gently flick the tube to mix, and let it sit on ice for 30 min.

- Heat shock at 42°C for 90 s, then immediately place on ice for 5 min.

- In a bechtop, add 500 μL of LB medium without antibiotics. Shake them at 37°C, 220 rpm, for 45 min.

- Centrifuge at 3400 rpm for 5 min, discard 500 μL of the supernatant in the bechtop, and resuspend the cell pellet in the remaining medium.

- Take up the cell suspension and evenly spread it on agar plates containing the corresponding antibiotic, and incubate them overnight at 37°C.

- Take out the plates from the 37°C incubator.

- Add 2.5 μL of ampicillin (100 mg/mL) to 5 mL of LB medium and mix thoroughly.

- Pick 3 single colonies from each plate and transfer each colony to a test tube containing 5 mL of LB medium with ampicillin. Shake the tubes at 37°C, 220 rpm for 10 h.

- Seal the plates with parafilm and store them in a -4°C refrigerator.

8. Plasmid Extraction

Introduction

Plasmid extraction involves the isolation of plasmid DNA from competent cells. This process allows researchers to obtain pure and concentrated plasmid DNA for various applications, such as gene cloning, sequencing, and genetic engineering. By separating the plasmid DNA from cellular components, plasmid extraction enables the study and manipulation of specific genes or genetic elements within a laboratory setting.

Material

1. ddH_2O

2. TIANGEN's Plasmid Small Extraction Kit

Procedure

- Place the purification column CP3 into a collection tube and add 500 μL of equilibration buffer BL to the column. Centrifuge at 12,000 rpm for 1 min, discard the waste liquid from the collection tube, and return the purification column to the collection tube.

- Take 1.5 mL of overnight culture, transfer it to a centrifuge tube. Centrifuge at 12,000 rpm for 1 min and remove as much supernatant as possible.

- Add 250 μL of solution P1 to the centrifuge tube with the bacterial pellet. Thoroughly resuspend the bacterial pellet by using a pipette or a vortex mixer.

- Add 250 μL of solution P2 to the centrifuge tube, gently invert it 6-8 times.

- Add 350 μL of solution P3 to the centrifuge tube, immediately invert it 6-8 time.

- Centrifuge at 12,000 rpm for 10 min.

- Transfer the collected supernatant to the purification column CP3 (place the column in a collection tube), centrifuge at 12,000 rpm for 1 min, discard the waste liquid from the collection tube, and return the column CP3 to the collection tube.

- Add 600 μL of wash buffer PW to column CA5 (ensure that anhydrous ethanol has been added before use).

- Centrifuge at 12,000 rpm for 1 min, discard the waste liquid in the collection tube, and return the column CA5 to the collection tube.

- Repeat the previous two steps, and preheat a metal bath to 55°C.

- Centrifuge the column CA5 at 12,000 rpm for 2 min to remove residual wash buffer as much as possible. Transfer column CA5 into a clean centrifuge tube, place it in the metal bath for 10 min to thoroughly dry.

- Add 30 μL of elution buffer (ddH_2O) to the center of the adsorption membrane in column CA5. Allow it to stand in the metal bath for 2 min.

- Centrifuge the column CA5 at 12,000 rpm for 2 min, collect the plasmid solution.

- Measure plasmid concentration on Nanodrop and record on the tube. Transfer 10 μL of recombinant plasmid to a new EP tube, label appropriately, and send for DNA sequencing.

- Store the rest of plasmid at -20°C freezer.

9. Agarose Gel Electrophoresis Analysis

Introduction

The obtained plasmids were verified by agarose gel electrophoresis again. If you can observe a single and correctly sized band, subsequent experiments will be ready to be performed. Otherwise, homologous recombination was not successful.

Procedure

- Agarose Gel Electrophoresis Analysis, following the same procedure as Step 4.

10. Sequencing

- 10μL plasmid were sent to Jinweizhi for sequencing.

Expression and Purification of the Protein

11. Transformation of Plasmid into E. coli BL21

- Transform plasmid into BL21 competent cells, following the same procedure as Step 7.

12. Protein Expression

Introduction

Protein expression involves the production of specific proteins within a host organism. This procedure allows researchers to generate large quantities of a desired protein for various applications, including structural studies, functional assays, and therapeutic developments. By inducing protein expression in bacterial cultures and carefully controlling the conditions, scientists can obtain the target protein efficiently and effectively.

Material

1. LB medium
2. Ampicillin
3. ddH_2O
4. 5× Loading Buffer
5. IPTG (Isopropyl β-D-1-thiogalactopyranoside)

Procedure

- Select 3-4 single colonies and inoculate them into 5 mL of LB medium containing 2.5 μL of ampicillin. Incubate at 37°C with shaking at 220 rpm for 6 h.
- Place 2 L conical flasks with 900 mL of LB medium in a UV hood for 10-20 min.
- Add 300 μL of ampicillin each flask and transfer the entire 5 mL LB culture into the 900 mL medium. Incubate at 37°C with shaking at 220 rpm for 6 h.
- Cool the shaker to the desired temperature (16°C) half an hour before induction.
- Once the large bottles' shaker reaches 16°C, add 450 μL of IPTG to induce protein expression.
- Continue shaking at 16°C for 16-18 hours.
- Pour the 900 mL medium into collection buckets, balance them, centrifuge at 4000 rpm for 20 min, discard the supernatant, and transfer the pellet to a 50 mL centrifuge tube. Store at -20°C.

13. Cell Disruption and Protein Separation

Introduction

Cell disruption and protein separation are critical steps in the process of isolating proteins from microbial cultures. This procedure aims to break down the cell walls or membranes to release the cellular contents, including the target proteins. Once the cells are disrupted, the mixture is separated into supernatant and pellet fractions, allowing for the subsequent purification and analysis of the proteins of interest. This process is crucial for obtaining purified and active proteins for various research and industrial applications.

Material

1. Cell disruption buffer
2. ddH_2O

3. 5× Loading Buffer
4. 20% ethanol

Procedure

- Place the tubes containing cells on ice for ten min. Pre-chill the high-pressure cell disruptor, high-speed centrifuge, and 4°C centrifuge in advance.
- Resuspend the cells in 30 mL of cell disruption buffer.
- Set the pressure to 1200 for E. coli and maintain the temperature at 4°C.
- Empty the ethanol from the cell disruptor, wash it twice with ddH_2O, and then rinse it with cell disruption buffer.
- Pour the resuspended cells into the cell disruptor and apply high pressure.
- Disrupt the cells 3-5 times until the liquid flows out smoothly without viscosity and appears watery.
- After use, rinse the cell disruptor with ddH_2O at least twice, ensuring the sample port is thoroughly cleaned, free from foam, and rinse once with 20% ethanol. Finally, store it in 20% ethanol.
- Turn off the cell disruptor.
- Pour the disrupted bacterial culture into high-speed centrifuge tubes, ensuring an even balance.
- Centrifuge at 18,000 rpm, 4°C for 50 min.
- Place the supernatant obtained after centrifugation in a 50 mL centrifuge tube and start subsequent protein purification steps immediately.

14. Protein Purification

Affinity Chromatography

Introduction

Affinity chromatography is a powerful technique used in protein purification to selectively capture and separate proteins based on their specific interactions with immobilized ligands. In this method, a nickel sulfate-containing matrix is utilized to capture proteins containing a histidine tag. This process allows for the isolation of target proteins with a high degree of purity and specificity.

Material

1. G250
2. Gravity column
3. Nickel sulfate
4. ddH_2O
5. Cell disruption buffer
6. 5× Loading Buffer
7. Wash buffer
8. 8M urea
9. 0.1M EDTA

Procedure

Prepare EP tubes in advance for sampling and PCR tubes with 50 mL of G250 for protein detection.

- Column Equilibration: Drain the nickel sulfate from the gravity column, rinse with ddH_2O, and then rinse again with cell disruption buffer.

- Incubation: Add the supernatant after cell disruption into the gravity column, resuspend the medium in the gravity column, and pour it back into the centrifuge tube. Incubate at 4°C on a roller for 120 min.

- Column Loading: Pour the incubated sample back into the gravity column and drain it from the bottom.

- Protein Detection Method: Take 50 mL of G250 into PCR tubes, add 6 μL of wash buffer, mix by repeated aspiration, and check for blue color in G250. If it turns blue, it contains protein.

- Column Washing: Add wash buffer into the gravity column, resuspend the medium, let it sit for 2 min, and then drain (add 15 mL at a time, repeat multiple times until the liquid drained no longer contains protein, i.e., it does not turn blue).

- Elution: Add 10 mL of 8M urea into the gravity column, resuspend the medium, let it sit for 2 min, and drain. Rinse the column with ddH_2O twice. Add 10 mL of 0.1M EDTA into the gravity column, resuspend the medium, let it sit for 2 min, and drain. Rinse the column with ddH_2O twice. Add 10 mL of 0.1M nickel sulfate into the gravity column, resuspend the medium, and store at 4°C.

Concentration

Introduction

Concentration of the eluted protein solution is an essential step in protein purification. This process involves removing excess liquid to achieve a higher concentration of the target protein. Concentrating the protein solution is crucial for downstream applications and analyses.

Procedure

- Pre-chill the centrifuge, add 15 mL of the protein solution obtained from elution to the ultrafiltration tube, and centrifuge at 3400 rpm for 30 min (or longer until the final volume in the ultrafiltration tube is less than 500 μL, ensuring precise balance).

- Transfer the concentrated protein solution into a 1.5 mL centrifuge tube.

- Centrifuge the concentrated protein solution at 4°C, 12,000 rpm, for 10 min.

AKTA with Desalting

Introduction

The AKTA system with desalting is employed to further purify and desalt the concentrated protein solution. It separates the target protein from salts and other impurities, ensuring that the final protein sample is of high purity and suitable for various applications.

Material

1. Molecular-exclusion chromatography buffer
2. ddH_2O

Procedure

- Two desalting columns are connected in series and mounted on the instrument. Wash the system successively with ddH_2O and molecular-exclusion chromatography buffer. The columns are balanced for 5 column volumes.

- Concentrate the protein to less than 1mL by centrifuging at 4'C, 3400rpm for 10 minutes in a highspeed centrifuge to remove insoluble substances and bubbles. Then concentrated protein solution is loaded into the system.

- Protein is eluted at a flow rate of 5mL/min. The eluate is collected according to the peak position.

- Concentrate protein to approximately 2mL and stored in -80°C freezer through frozen in liquid nitrogen.

SDS-PAGE Protein Gel

Introduction

SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis) is a widely used technique for analyzing and characterizing proteins based on their size. It separates proteins in a gel matrix according to their molecular weight, allowing for the assessment of protein purity and molecular weight determination.

Material

1. Glass plates for gel casting
2. Sealed bags
3. Running buffer
4. Protein marker
5. Coomassie Brilliant Blue R250 staining solution
6. 20% ethanol

Procedure

- Sampling and sample preparation: There are seven samples, namely supernatant, precipitation, Column Loading, first wash, final wash, first elution and final elution. For supernatant and precipitation, take 100 μL of the disrupted solution, centrifuge at 12,000 rpm, 4°C for 10 min. Transfer the supernatant to a new 1.5 mL EP tube, add 24 μL and 6 μL of 5× Loading Buffer, and heat at 100°C for 3 min. Slowly wash the pellet twice with a small amount of ddH_2O, resuspend in 100 μL ddH_2O, and add 24 μL and 6 μL of 5× Loading Buffer, then heat at 100°C for 3 min. For the others, take 24 μL of the liquid that flowed down, transfer it to a 1.5 mL EP tube, add 6 μL of 5× Loading Buffer, and heat at 100°C for 3 min.

- Prepare the gel plate: Clean the glass plates, rinse with ddH_2O, and dry with lint-free paper. Place the long glass plate and short glass plate together on the stand, secure them with clips.

- Gel Casting: Prepare the separating gel according to the recipe, pour it between the glass plates until it's slightly below the green plane of the stand, then add water to the top of the short glass plate to level with it, and press the separating gel flat. Once the separating gel solidifies, pour off the water from the top and blot any remaining with a paper towel. Prepare the stacking gel according to the recipe, pour it on top of the separating gel until it reaches just above the short glass plate, insert the comb, and let the stacking gel solidify.

- Storage: If not using the protein gel immediately, place it in a sealed bag, add 1× running buffer to the sequencing bag, and store it at 4°C.

- Load the gel plate: Secure the short glass plate against the ridge of the sealing rubber, ensuring a tight seal. Insert the comb, creating wells, and add 1× running buffer to the gel chamber until it reaches the top of the long glass plate. Wait a few min to check if the liquid level drops. If it does, the gel plate needs to be reloaded.

- Sample Loading: Fill the gel chamber with 1× running buffer, remove the comb, and add 3 μL of protein marker to the first well and 5 μL of sample to the remaining wells.

- Electrophoresis: Place the gel plate in the electrophoresis apparatus, add the quantified 1× running buffer as indicated on the electrophoresis device, apply a voltage of 180 V, and run the gel

for 40 min.

- Staining and Destaining: After electrophoresis, add half of the Coomassie Brilliant Blue R250 staining solution to a staining box. Use plastic tweezers to gently separate the protein gel, and place it upside down in the staining box. Microwave for 2-3 min, remove the staining box and collect the Coomassie Brilliant Blue R250 staining solution. Add tap water or 20% ethanol to the box, and microwave for 7-8 min. Repeat this process until complete destaining is achieved.

Characterization of Protein Properties

15. Measuring the Enzyme Tm

Introduction

Tm is an important indicator of protein thermostability, called transition temperature. This value represents the temperature required for transition from native 3D conformation to unfolded state under certain conditions. We measured Tm of proteins by DSF. With the increase of temperature, the quaternary structure of protein was destroyed. Fluorescent dye binds to the exposed hydrophobic region and its fluorescence intensity changes. By measuring this change, Tm can be calculated.

Material

1. ddH_2O
2. Sypro orange staining solution
3. Purified protein
4. Molecular sieve buffer

Procedure

- Launch the Nanodrop software and select the "Protein A280" function. After zeroing, add 1.5 μL of the sample and measure the protein concentration.

- Prepare the reaction mix in PCR tubes according to Table 3. Set up three parallel sets for each protein sample, adjusting the protein volume based on its concentration.

Component	*Volume*
Sypro orange staining solution	3 μL
1 mg/ml protein	3 μL
Molecular sieve buffer	24 μL
Total Volume	30 μL

Table 3 Components for qPCR

- Gently mix the reaction mixture and place into the qPCR machine to initiate the reaction.

- Process the data using Graphpad to obtain the Tm of the samples.

16. Measuring Activity of Enzyme

Introduction

By reading literatures, we determined the protocol for PEase's reaction with PE. First, it is necessary to pre-treat PE film to remove possible surface stains and microbial biofilms. Next, protein is continuously supplemented to ensure the continuity of reaction. Enzyme activity of the mutants

could be qualitatively analyzed by comparing mass of films before and after the reaction.

Material

1. PE films
2. 70% Ethanol
3. Ceres
4. Reaction buffer

Procedure

- Pre-treat: Round PE films with a hole punch. 70% ethanol is stirred and soaked for 30min. Wash 2-3 times with ddH_2O. Dry in 37 degrees incubator over night.

- Weigh PE films before reaction with an analytical balance(0.0001 g).

- Reaction: Put PE discs into each EP tube and add 600μL reaction buffer. 5μL of purified enzyme at a concentration of 1-5μg/μL is applied separately 8 sequential times(90min each) on PE films.

- Fish out PE films. Wash 2-3 times with ddH_2O. Wash 2-3 times with 70% ethanol. Finally, wash 2-3 times with ddH_2O. Dry in 37 degrees incubator over night.

- Weigh PE films after reaction with an analytical balance(0.0001 g).

Protein D Test

Introduction

Protein D test(pre-expression test) refers to a series of controlled experiments, aimed to find the best conditions to express D protein, with a set of standard and efficient operating procedures.

Material

Following the same material with steps that have same experiments.

Procedure

- D protein plasmids were constructed according to the procedure described above, and transformed into E.coli.

- Select single colonies into 5ml LB medium containing the corresponding resistance, shaking to OD 0.4-0.6 at 37℃, 220rpm.

- Draw 24μL bacterial solution in clean bench, reserve in EP tube as ample. Then add 6μL 6× SDS-Sample Buffer and heat the metal bath at 100℃ to prepare sample (pre-induction sample);

- Calculate the amount of IPTG working at 1M 0.7M 0.5M 0.3M 0.1M and add it in clean bench. Then shake at 16℃ for 16h.

- Draw 24μL bacterial solution in clean bench, reserve in EP tube as ample. Then add 6μL 6× SDS -Sample Buffer.

- Treat it with 40kHz ultrasonic for 10min, keeping the temperature below 30℃.

- After installing the explosion-proof clip, put the samples on metal bath at 100℃ for 2min for thermal cracking.

- Treat it with 40kHz ultrasonic for another 10min, keeping the temperature below 30℃.

- Centrifuge at 12000rpm for 20min.

- SDS-PAGE Protein Gel, following the same procedure as Step 14.

Supplementary Recipe Sheet

Reagent Formulations

Name	Recipe for 1L
Liquid LB Medium*	10g Peptone, 10g NaCl, 5g Yeast Extract
Solid LB Medium*	10g Peptone, 10g NaCl, 5g Yeast Extract, 15g Agar
IPTG (1M)**	[50mL] 11.92g IPTG Powder, 50mL ddH2O
Kanamycin (100mg/mL)**	[50mL] 5g Kanamycin Powder, 50mL ddH2O
Ampicillin (100mg/mL)**	[50mL] 5g Ampicillin Powder, 50mL ddH2O
EDTA (0.1M)	29.2g EDTA, 1L ddH2O
Nickel Sulfate (0.1M)	15.5g Nickel Sulfate, 1L ddH2O
50% Glycerol*	500mL Glycerol, 500mL ddH2O
Urea (8M)	480g Urea, 1L ddH2O
10× Running Buffer	30.2g Tris, 188g Glycine, 10g SDS
50× TAE	242g Tris-base, 57.1mL Acetic Acid, 100mL 0.5M EDTA , pH=8(RT)
20% Ethanol**	[100mL] 32mL 75% Ethanol, 88mL ddH2O
Wash Buffer**	1.57g Tris-HCl, 2.92g NaCl, 200mL Glycerol, 1.36g Imidazole, pH=8(4°C)
Elution Buffer**	1.57g Tris-HCl, 2.92g NaCl, 200mL Glycerol, 13.6g Imidazole, pH=8(4°C)
Molecular-exclusion chromatography buffer**	1.57g Tris-HCl, 2.92g NaCl, 50mL Glycerol, 0.3085g DTT, pH=8(4°C)
Suspension Buffer**	1.57g Tris-HCl, 2.92g NaCl, 200mL Glycerol, pH=8(4°C)
Reaction Buffer**	4.77g HEPES, 8.76g NaCl, 50mL Glycerol, pH=7.2(RT)

PS: *= Sterilization, **= Vaccum Filtration.