**Lysogeny broth Medium(LB Medium)：**

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| --- | --- | --- |
| Ingredients | 200ml | 100ml |
| Tryptone | 4g | 2g |
| Yeast extract | 2g | 1g |
| Sodium Chloride (NaCl) | 2g | 1g |
| 5mol/L Sodium Hydroxide (NaOH) Solution | 40**μl** | 20**μl** |

**SDS Polyacrylamide Gel Electrophoresis (SDS PAGE)：**

1.Gel Formula：

Separating Gel：(10ml)

|  |  |  |  |
| --- | --- | --- | --- |
| Ingredients | 8% | 10% | 12% |
| Distilled water | 4.65ml | 4ml | 3.3ml |
| 30%Acr-Bis | 2.65ml | 3.3ml | 4ml |
| 1.5M Tris-HCl pH8.8 | 2.5ml | 2.5ml | 2.5ml |
| 10%SDS | 0.1ml | 0.1ml | 0.1ml |
| 10%APS | 0.1ml | 0.1ml | 0.1ml |
| TEMED | 0.006ml | 0.004ml | 0.004ml |

Acrylamide（5%）：

|  |  |
| --- | --- |
| Ingredients | 4ml |
| Distilled Water | 2.7ml |
| 30%Acr-Bis | 0.67ml |
| 1.5M Tris-HCl pH6.8 | 0.5ml |
| 10%SDS | 0.04ml |
| 10%APS | 0.04ml |
| TEMED | 0.004ml |

2.Configuration of SDS PAGE Gel：

（1）Wash the glass plate (1.5 mm) , comb and trim, and let dry. After the glass plates are aligned, place them in a clamp and tighten them vertically onto the shelf.

（2）Configure 10ml separating gel, according to the configuration formula. Quickly add the gel in the gap between the two glass plates until the remaining plate is 1cm wider than the length of the comb. Add 1ml anhydrous ethanol in the gel.

（3）20 min later, pour out the anhydrous ethanol on the glue, and let the anhydrous ethanol evaporate completely at room temperature for 5 min.

1. Configure 4 ml of 5% concentrated gel according to the formula. After adding the concentrated gel, insert a clean comb (1.5 mm, with 10 teeth) at once. When the concentrated gel has solidified, pull out the comb vertically and gently.

3.Electrophoresis：

（1）Clamp the configured gel plate on the power rack, and add new 1 × SDS into the power rack. Add the recycled 1 × SDS the electrophoresis tank.

1. Load the samples in the order of 1×SDS, marker (2μl), 1×SDS protein sample (30μl).

（3）First, perform electrophoresis at a voltage of 80v. 15-20 minutes later, increase the voltage to 120v for electrophoresis. When the sample moves to a suitable position, stop the electrophoresis.

**Protein detection：**

1. Take 1ml of bacterial solution in a 1.5ml centrifuge tube, 10000rpm for 10min, and discard the supernatant.
2. Add 150μl cell lysate and 37.5μl 5xSDS.
3. Put the centrifuge tube in a 95℃ water bath and heat it for 3 minutes, take it out and shake it evenly, repeat the operation once, and then heat it for another 4 minutes.
4. Load 2μl of marker and 30μl of protein sample.
5. SDS PAGE
6. Cut the polyacrylamide gel and add Coomassie Brilliant Blue to stain for 20 minutes.
7. Observe the position of the strips.

**Plasmid transformation TB1 :**

1. Add 1μl plasmid to competent TB1, flick and mix, and place it on ice for 30min.

2.Warm compress in a 42℃ water bath for 90s.

1. Ice bath For 3min
2. Add 200μl of LB medium.
3. Incubate in a shaker at 37°C and 220 rpm for 40 min.
4. Spread the bacterial solution evenly on the Kana resistant plate and incubate it in a biochemical incubator at 37°C for 12-16 hours.

**Pick monoclones：**

1. Take 10ml of LB medium in a 50ml EP tube, add 10μl Kana. Use a small pipette tip to pick a single colony and place it in the EP tube.

2.Incubate on a shaker overnight at 37°C for 12-16h.

**Cell expansion and induction：**

1. Prepare a bottle of 200ml LB medium and a tube of 10ml LB medium
2. Take 200μl of Kana into 200ml of LB medium, then add 2ml of bacterial solution; take another 10μl of Kana into 10ml of LB medium, add 100μl of bacterial solution as a control group, and expand the incubation for 4 hours.

3.Add 1ml of 100mM IPTG to 200ml medium (IPTG final concentration is 0.5mM), without adding IPTG to the control group, and continue to incubate for 4 hours.

**Protein purification：**

1. Assemble the peristaltic pump, judge the direction of water flow, and wash at 20rpm.

2.Wash at 18rpm PBS.

1. Balance the nickel column: insert the hose into the 5x imidazole and slowly flow through the hose and the nickel column, leaving a section of air column.
2. Insert the hose into the protein sample, start the peristaltic pump at 1 rpm, and render the protein solution flow slowly through the nickel column.
3. Insert the 50x imidazole into the hose and render it flow through the hose slowly and the nickel column. Detect with Coomassie Brilliant Blue every 2 minutes. If the solution changes color, start to elute. Once the color become dormant, the elution is completed.
4. Insert the hose into the 250x imidazole, slowly control the flow rate, detect with Coomassie Brilliant Blue, if the color starts to change, collect the eluent immediately (pre-prepare two sterilized 50ml EP tubes, one for 30ml protein sample, the other for 20ml).
5. Add an appropriate amount of desalting solution to the centrifuge tube, centrifuge at 10000rpm for 40 minutes. Take out the centrifuge tube and discard all the desalting solution in the tube. Add an appropriate amount of protein sample 10000 and centrifuge for 1h, Take out the centrifuge tube, discard the 250× imidazole in the tube and continue to add protein sample. Centrifuge. Repeat until the protein sample in the tube is less than 2ml.
6. Add 2ml of desalting solution to the collected concentrated solution, centrifuge at 10000rpm. Take out the centrifuge tube every 1h and discard the filtrate. Refill the protein sample. After the protein sample is added, store at -20°C.

8.23

Learn the configuration of LB medium and operations of cell expansion and SDS PAGE;

8.24

8.25

8.26：

1. Expands the target gene by polymerase chain reaction(PCR)
2. Obtain the target gene by gel recycle.

8.27：

1. Verify the purity of the gel recycle on 8.26, and perform Agarose Gel Electrophoresis(AGE). Results show that it is highly pure, indicating that there is no problem with the gel recycle operation;
2. Carry out cell expansion and expression;
3. Carry out SDS PAGE.
4. Transfer the empty plasmid pET-28a into TB1;

8.28：

1. Pick out monoclonal antibodies;

2.Configure 100ml and 200ml LB Mediums;

1. Configure 12% separating gel

8.29：

1. AGE(check the plasmid extraction);
2. Pick out monoclonal antibodies
3. Enzyme digestion (vector and target gene)

8.30：

1. AGE(enzyme digestion plasmid, gene and no-load comparison)
2. Plasmid connection

8.31：

1. Ultrasonic break the bacteria added with IPTG, pipette the supernatant into a centrifuge tube (labeled as "supernatant"), add 2μl cell lysate to the sediment, vortex, and pipette 1μl into another centrifuge tube (label As "sediment");
2. Transform the connected plasmid;
3. Configure 12% gel

9.1：

1. Protein purification.
2. Protein concentration.

9.2：

1. Protein concentration detection;
2. Configure 200ml LB medium;
3. Configure 12% gel plate;