Preparation of *E. coli* competent cells

Components and Materials:

Escherichia coli culture medium, solution A, solution B

Procedure:

1．Take 1ml of the cultured *E. coli* cell culture solution in a 1.5ml microtube. Centrifuge at 1500 g for 10 min at 4°C, and discard the supernatant.

2．Add 100μl of solution A pre-cooled in water to each microtube, and gently move the microtube to suspend the precipitate.

3．Add 100μl of solution B pre-cooled in ice to each microtube, and gently move the microtube to suspend the precipitate.

4．Competent cells have been made. Freshly prepared competent cells can be used for transformation experiments directly, or the cells can be frozen at -80°C.

DNA transformation of Escherichia coli competent cells

Components and Materials:

Medium (without antibiotics), LB medium (with antibiotics), sterile ddH2O, IPTG, X-gal.

Procedure:

1. Adjust the temperature of the constant temperature water bath to 42°C in advance.

2. Take out a tube (100 μl) of competent bacteria from the -80℃ ultra-low temperature freezer, immediately use your fingers to warm and melt it and insert it on the ice, and ice bath for 5-10 minutes.

3. Add 3-10μl of the ligated plasmid mixture (the DNA content does not exceed 100 ng), shake gently, and place on ice for 30 min.

4. Shake gently and insert it in a 42℃-water bath for 90 seconds for heat shock, then quickly put it back on ice and let it stand for 3~5 minutes.

5. In the ultra-clean workbench, add 890 μl LB medium (without antibiotics) to each of the above tubes and mix gently, then fix it on the spring rack of the shaker and shake at 37°C for 1 h. Take it out and centrifuge them at 10000xg for 1min, discarded 900ul supernatant.

6. Take 100-300 μl of the above conversion mixture in the ultra-clean workbench, drop them into a solid LB plate petri dish containing appropriate antibiotics, and spread them evenly with a glass coating rod burned in an alcohol lamp.

7. If the carrier and host bacteria are suitable for blue-white spot screening, add 40 μl 2% X-gal, 8 μl 20% IPTG to the plate after dripping the bacteria liquid, and coat it with a glass coating rod burned in an alcohol lamp. Evenly.

8. Mark the painted petri dish and place it in a 37°C constant temperature incubator for 30-60 minutes until the liquid on the surface penetrates into the culture medium, then put it upside down and put it in the 37°C constant temperature incubator overnight.

9. Observe the colony clones that grow on the plate, and it is better that the colonies can be separated from each other. Note the white plaque.

Colony PCR

Components and Materials:

Escherichia coli culture medium, solution A, solution B

Procedure:

1. Preparation of PCR mixture

(1) Taq buffer (10x) 75 μl

(2) Primer Forward 6 μl

(3) Primer Reverse 6 μl

(4) ddH2O 60 μl

2. Randomly select the transformants on the transformation plate at room temperature, use a sterilized toothpick or pipette tip to pick a single bacterium, tap on the LB agarose plate, and make a copy.

3. Then put the toothpick or pipette tip stained with bacteria in the corresponding PCR tube.

4. After picking the monoclonal bacteria, add the previously prepared PCR mixture to the system to be 25 μl.

5. Place the PCR mixture mixed with bacteria in a PCR machine, and amplify according to conventional conditions.

6. Add bromophenol blue or other dyes to the amplified reaction solution, and

electrophoresis to detect whether the target fragment is obtained. If there is a positive clone.

7. Place the plate that has been inoculated with colonies in a 37℃ incubator overnight to expand the colonies.

8. On the next day, select positive clones for further screening or culture.

Agarose gel electrophoresis

Components and Materials:

Agarose solution、Running buffer (usually 1X TAE or 0.5X TBE)、Ethidium bromide or SYBR Gold stain、6X gel loading buffer、DNA sample、DNA size standard

Procedure:

1. Seal the open sides of the plastic tray or the edges of the clean and dry glass plate with edge banding to form a mold, and place it on a horizontal support.

2. Prepare enough running buffer (1X TAE or 0.5X TBE) to fill the electrophoresis tank and prepare the gel.

3. Prepare agarose solution of appropriate concentration with electrophoresis buffer according to the size of DNA fragments to be separated: accurately weigh the dry agarose powder and add it to a Erlenmeyer flask or glass bottle containing a predetermined amount of electrophoresis buffer.

4. Stopper the neck of the Erlenmeyer flask loosely with Kimwipes, such as a

glass bottle, be sure to cover it loosely. Heat in a boiling water bath or microwave until the agarose melts.

5. Use isolation gloves or clamps to transfer the Erlenmeyer flask or glass bottle to a 55°C water bath. After the melted gel is cooled slightly, add ethidium bromide to a final concentration of 0.5 μg/ml. Swirl gently to mix the gel solution thoroughly.

6. While the agarose solution is cooling, use a suitable comb to form a sample hole. The position of the comb teeth should be 0.5~1.0 mm on the bottom of the tray, so that when the agarose is poured onto the tray, it will form a sample hole that meets the requirements.

7. Pour the warm agarose solution into the mold.

8. Let the gel solution completely congeal, at room temperature for 30 to 45 minutes. Add a small amount of running buffer to the top of the gel and carefully pull out the comb. Pour out the running buffer. Gently tear off the edge banding tape and place the gel in the electrophoresis tank.

9. Add the electrophoresis buffer to the electrophoresis tank, just about 1 mm below the gel.

10. Mix the DNA sample with 0.2 volumes of 10X loading buffer.

11. Use a micropipette and a disposable tip, or an elongated Pasteur pipette, or a glass capillary tube to slowly add the sample mixture to the sample hole of the immersion gel. The molecular mass standards should be added to the two holes on the left and right sides of the sample well.

12. Close the cover of the electrophoresis tank and connect the electrode plug. The DNA should swim towards the anode (red plug) side. Give a voltage of 1~5 V/cm, where the distance is measured from the anode to the cathode. If the electrode plug is connected correctly, the anode and cathode will generate bubbles due to electrolysis, and the bromophenol blue will migrate from the sample hole into the colloid within a few minutes. Stop the electrophoresis after bromophenol blue and xylene cyanide FF have moved to an appropriate distance.

13. When the DNA sample or dye has migrated a sufficient distance in the gel, turn off the power, pull out the electrode plug, and open the electrophoresis tank cover. If the gel and buffer contain ethidium bromide, observe the gel and take pictures with an ultraviolet light. Otherwise, the gel is immersed in water containing ethidium bromide (0.5 μg/ml) or electrophoresis buffer at room temperature for 30~45 min, or with electrophoresis buffer 1:10000 diluted SYBR Gold stock solution.

Extraction of Escherichia coli Plasmid DNA

Components and Materials:

Solution I (1% glucose, 50mM/L EDTA pH8.0, 25mM/L Tris-HCl pH8.0)

Solution II (0.2 mM/L NaOH, 1% SDS)

Solution III (5 mol/L KAc, pH4.8)

TE buffer

HBC buffer

DNA wash buffer

Elution buffer

Procedure:

1. Connect 1% *E. coli* cells containing plasmid to 2ml LB medium.

2. Incubate with shaking at 37°C overnight.

3. Take 1.5ml of bacteria in Ep tube, centrifuge at 1000xg for 1minute, discard the supernatant.

4. Add 250ulsolution I and mix well.

5. Add 250ul solution II, gently invert to mix, and place in an ice bath for 5 min.

6. Add 350ul of pre-cooled solution III, gently invert to mix, and place in an ice bath for 5 min.

7. Centrifuge at 13000xg for 10 minutes, and transfer the supernatant to another new Ep tube

8.Insert a HiBind DNA Mini Column into a 2mL Collection Tube. Centrifuge at maximum speed for 1 minute. Discard the fltrate and reuse the collection tube.

9. Add 500uL HBC Buffer.Centrifuge at maximum speed for 1 minute.Discard the filtrate and reuse collection tube.

10. Add 700uL DNA Wash Buffer.

11. Centrifuge the empty HiBind DNA Mini Column for 2 minutes at maximum speed to dry the column matrix.

12. Transfer the HiBind DNA Mini Column to a clean 1.5 mL microcentrifuge tube.Add 30-100uL Elution Buffer or sterile deionized water directly to the center of the column membrane. Let sit at temperature for 1 minute.

13. Centrifuge at maximum speed for 1 minute.

concentration.Store DNA at-20℃

8. Add equal volume of isoamyl alcohol, mix well and let stand at 0°C for 10 min.

9. Centrifuge again at 10,000 rpm for 20 minutes, and discard the supernatant.

10. Wash once with 0.5ml of 70% ethanol and drain all the liquid.

11. After the precipitate is dried, dissolve it in 0.05ml TE buffer.

PCR

Components and Materials:

Mineral oil MgCl2 Primer dNTP water Bromophenol blue Sepharose EB

Procedure:

1. Adjust the template concentration to 10 ng/ml

2. Prepare the reaction mixture according to the following system, mix well, add a drop of mineral oil, and centrifuge for 5 seconds

Template DNA (20 ng) 2

buffer 2.0 10 μl

MgCl2 (25 mM) 1.5 μl

lmPrimer F (10 mM) 0.2 μl

Primer R (10 mM) 0.2 μl

lmdNTPs (2 mM) 2.0 μl

Taq (5 U/μl) 2.0 μl

Add ddH2O to 20 μl

3. PCR reaction cycle condition setting:

95 ℃ 1 cycle

94 ℃ 55 ℃ 1'72 ℃ 1'30" 35 cycles

72 ℃ 1 cycle

4 ℃ forever

Bromophenol blue, mix well, centrifuge briefly, take 15 ml

4. Detection: add 2 ml reaction product to spot electrophoresis;

5. Spot electrophoresis on a 1% agarose gel; EB staining and UV observation.

Gibson

Procedure:

1. Obtain the vector with homologous ends (overlap) and insert (wherein the vector can also be obtained by restriction digestion) and purify it by PCR.

2. Take out a tube of Master Mix (15 μl), melt it on ice, and add 5 μl of DNA mixture of vector and insert.

3. After adding the DNA mixture, immediately use a pipette to gently pipette 5

to 10 times, and then put the reaction solution in a 50 ℃ water bath for 30 to 60 minutes.

4. When the time is up, take out the reaction solution in time and place it on ice to transform competent cells; it can also be temporarily stored in a refrigerator at -20℃.

5. Take 5~10 μl of the reaction solution to transform 50~100 μl of competent cells, and the rest can be kept in the refrigerator at -20℃ for later use.

Strain activation and inoculation

Components and Materials:

Paul Bacteria tube, Saccharomyces cerevisiae strains, Yeast Liquid Medium

Procedure:

1.Inoculate the Saccharomyces cerevisiae strains stored at -20 ℃ on the liquid culture medium.

2.Place the culture medium at 30 ℃ and activate culture at 180 rpm.

3.When the optical density of the strain in the culture medium to be activated reaches about 1, draw an appropriate amount of bacterial liquid and transfer it to the fermentation medium.

4.Regularly measure the yeast optical density and Residual Sugar in the culture medium and record the data

Measurement of growth curve

Components and Materials:

Uv-vis spectrophotometer, Colorimetric dish, Fermented liquid ,1.5 ml EP tube, YPD medium

Procedure:

1. Turn on the Uv-vis spectrophotometer and preheat for 20 minutes. Adjust the wavelength to 600nm and prepare a clean colorimetric dish.

2. Remove 1000 μl liquid from the fermentation broth every 4 hours into a 1.5 ml EP tube.

3. Dilute the liquid in the EP tube with YPD medium to a factor (2-40 times) , until the OD value is between 0.2 and 1.0

4. Transfer the diluted liquid to a colorimetric dish, and then measured in a spectrophotometer, recorded readings.

5. With time as the abscissa, OD value of the corresponding time as the ordinate. Make yeast cell growth curve.

Measurement of residual sugar

Components and Materials:

Biosensor, Fermented liquid ,1.5 ml EP tube

Procedure:

1. Turn on the biosensor.

2. Remove 1000 μl liquid from the fermentation broth every 4 hours into a 1.5 ml EP tube.

3. Dilute the liquid in the EP tube with deionized water to a factor (2-40 times), until the residual sugar value is no greater than 1.0

4. Confect glucose standard solution, and calibration on the Biosensor.

5.Put the measured liquid into the instrument, click "Test", wait for the measurement data display, and record the data.

Preparation of Yeast Competent Cells

Components and Materials:

1M Sorbitol solution, Sterile water, Saccharomyces cerevisiae culture medium, Tris-EDTA buffer solution, Lithium acetate solution

Procedure:

1. Place the yeast liquid with an optical density of 1.3-1.5 on ice and incubate for 15 minutes.

2. Transfer the bacteria to a 50ml centrifuge tube, 4℃, 3000rpm, centrifuge for 5min.

3. Discard the supernatant, add 50ml sterile water to resuspend the bacteria, centrifuge at 4℃, 3000rpm for 3min.

4. Discard the supernatant, add 8ml sterile water to resuspend the bacteria, add 1ml 10xTris-EDTA buffer solution and shake well, add 1ml 10x LiAc and shake well, 30℃, 180rpm, shaking and incubate for 45min.

5. Centrifuge the bacterial solution at 4℃ and 3000 rpm, and discard the supernatant.

6. Add 25ml of pre-cooled sterile water, centrifuge at 4°C, 3000rpm, and discard the supernatant.

7. Add 2-5ml of ice-cold 1M sorbitol solution, centrifuge at 4°C, 3000rpm, and discard the supernatant.

8. Add 1 ml of ice-cold 1M sorbitol solution, centrifuge at 4°C and 3000 rpm. Divide the bacterial solution into 1.5 ml eppendorf tubes and store at -80°C.

Preparation of E-coli Competent Cells

Components and Materials:

1.5 ml EP tube, solution A, solution B, centrifugal machine

Procedure:

1. Take 1ml culture medium into the 1.5ml EP tube.

2. 1500 rpm , 4 °C ， centrifuge for 5 mins, abandon the supernatant.

3. Add 100μl solution A pre-cooled in ice to every EP tube , flick it and suspend the sediment.

4. 1500 rpm , 4 °C ， centrifuge for 5 mins, abandon the supernatant.

5. Add 100μl solution B pre-cooled in ice to every EP tube , flick it and suspend the sediment.

6. Divide the solution into 1.5 ml EP tubes and store at -80°C.

Electroconversion of yeast Competent Cells

Components and Materials:

Plasmid, Yeast Competent Cell, YPD medium, Electrotransmitter

Procedure:

1. Put the electroporation cup on ice, add 10 μl plasmid, 50 μl yeast competent cells, and ice bath for 3-5 min.

2. Electrical transfer. +1.5kv, 25μF, 200Ω for electrical transfer.

3. Add 1mL YPD immediately after the electric shock, aspirate and incubate at 30°C for 2-3 h.

4. After centrifugation, discard the 500μl supernatant, blow and suck and mix well, and take a certain amount to coat the plate.

Identification of Gadusol production in *S. cerevisiae*

Components and Materials:

Ultraviolet Spectrophotometer

Centrifuge Tubes

Centrifuge Machine

5 mM Phosphate Buffer

MeOH

Ultrasonic Cell Crusher

Rotary Evaporator

Freeze Drier

Electronic Balance

High Performance Liquid Chromatography

Procedure:

1.The optical density of fermentation liquid was measured with ultraviolet spectrophotometer.

2.The fermentation liquid was collected in 50 ml centrifuge tubes.

3.Centrifuge at 6000 x g for 4 minutes at room temperature.

4.Decant or aspirate and discard the culture media.

5.*S. cerevisiae* cell pellets was suspended in 5 mM phosphate buffer (15 ml).

6.Centrifuge at 6000 x g for 4 minutes at room temperature.

7.Decant or aspirate and discard the supernatant.

8.*S. cerevisiae* cell pellets were suspended in MeOH (20 ml).

9.*S. cerevisiae* cells were broken up by ultrasonication (Pulse on for 2 seconds followed by pulsing off for 4 seconds, the whole process time is 10 minutes).

10.Centrifuge at 10,000 x g for 4 minutes at room temperature.

11.Discard *S. cerevisiae* cell pellets.

12.The supernatant was evaporated, and the concentrated extract was collected in a pre-weighed 50 ml centrifuge tube after being dissolved in 5 mM phosphate buffer (10 ml).

13.The extract was frozen at −80°C overnight.

14.The extract was lyophilized overnight.

15.The extract was weighed.

16.The extract (10 mg) was dissolved in 5 mM phosphate buffer (1 ml) for identification by HPLC, MeOH-5 mM phosphate buffer (1% MeOH for 20 min followed by a gradient from 1 to 95% MeOH in 20 min), flow rate 0.3 ml/min, 296 nm.