NOTEBOOK
August 2023

August

HUBU-SKY-China

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8.1 Sterilization & purification & glue (F7, N23), transformation (N23)

Tuesday, August 1st, 2023

Overview

- The bacteria containing A2 and M8-related proteins were broken, eluted and collected with different concentrations of imidazole, and then the collected proteins were analyzed for glue
- Because very little N23 protein is eluted with imidazole during purification, it needs to be reconverted

Experimental equipment

- PMSF protease inhibitors, imidazole solutions in different concentrations
- Buffer buffer, F7, N23 bacterial solution
- 2 ml EP tube, 10 ml EP tube, G250 dye
- Maker, loading
- Pipette gun, tip of the pipette

Experimental steps

(1) Sterilization

- 1. Weigh the bacteria on the balance and add 10 ml of the bacteria to resuspend
- 2. Before sterilization, add 1% protease inhibitor PMSF to the resuspended bacterial solution, the volume is ten

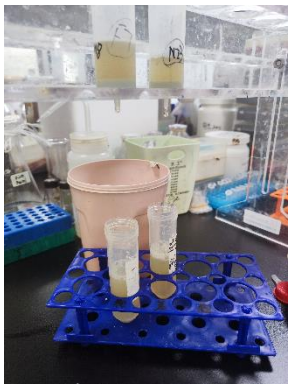
times the grammage

- 3. Wash the high-pressure cell disruptor twice with RO water, and then wash twice with xbuffer, and keep the pressure at 300bar-400bar during cleaning
- 4. Add bacteria liquid to the instrument, and the sterilization pressure is kept at 900bar-1100bar
- 5. Repeat the sterilization 3-4 times so that the bacterial liquid is no longer viscous
- 6. After the sterilization is completed, use 2ml EP tube for aliquoting and centrifuge in a centrifuge at 12000rpm 10° C for 30min (leave a tube without centrifugation for sample preparation).
- 7.After centrifugation, keep one tube of pellet and one tube of supernatant (sample preparation). The rest can be collected in the supernatant

(2) Purification

- 1. Clean the purification column with tap water and RO water
- 2. Soak the purification column with NaOH with a concentration of 0.1M for 15min
- 3. Wash off the NaOH in the purification column and rinse with RO water

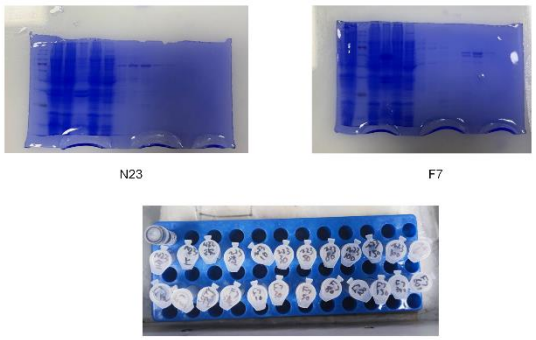
- 4. First add an appropriate amount of nickel beads to the purification column, and then wash twice separately with RO water and buffer.
- 5. After cleaning, add an appropriate amount of 10mM imidazole to the purified beads with nickel beads and soak for 5min to activate the nickel beads.
- 6. After activating the nickel beads, immediately add the collected upper clearing night, and close the tube mouth with parafilm and mix in a silent mixer for 90min (so that the target protein fully binds to the nickel beads).
- 7. After the mixing is completed, take the flow penetration liquid and place it in ice.
- 8. Elution is performed with 10mM imidazole, 30mM imidazole, 50mM imidazole, 80mM imidazole, 100mM imidazole, 150mM imidazole, 300mM imidazole, 600mM imidazole. Each concentration of imidazole is washed until the eluate encounters G250 Coomassie brilliant blue and does not change blue



purification

(3) Running glue

- 1. Prepare samples of collected precipitate, supernatant, flow-through, whole, and various concentrations of imidazole eluate. Take 40ul loading and 10ul samples for mixing, and boil at 100° C for 10min.
- 2. After sample preparation, the protein of interest is tested by SDS-PAGE. During the loading process, take 10ul for each sample and 2ul for protein marker.



SDS-PAGE result

(4) Transformation

- Add 10 ul of competent cells and 1.5 ul of plasmid to the 1.5 ml EP tube in the ultra-clean bench (plasmids can

be added to the table).

- 2. Heat excitation: Bath water in a 42° C water bath for 45s, and then immediately ice bath for 2min
- 3. Add 150 ul of NZY medium to the ultra-clean bench
- 4. Incubate at constant temperature in a shaker at 37° C for 40min-60min
- 5. After incubation, 100 ul of bacterial solution was coated in LA resistant plates andreared overnight at 37° C

8.2 F7, N23 ultrafiltration, measured concentration, N23 clone transformation

Overview

- F7, N23 protein ultrafiltration concentrate
- N23 reconverted

Experimental equipment

- 30000KD ultrafiltration tube, PBS buffer, G250 dye
- Pipette gun, tip of piper, marker
- LB plate, ampicillin
- 23a-N23 plasmid, G-competent cells

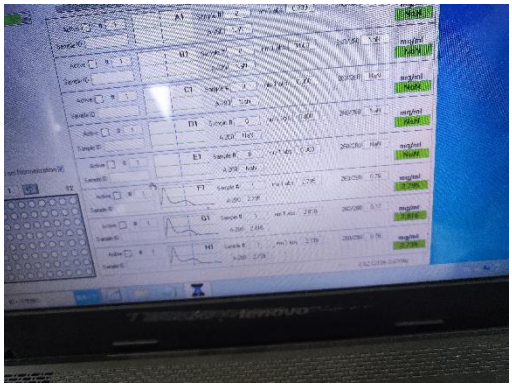
Experimental steps

① Ultrafiltration, concentration measurement

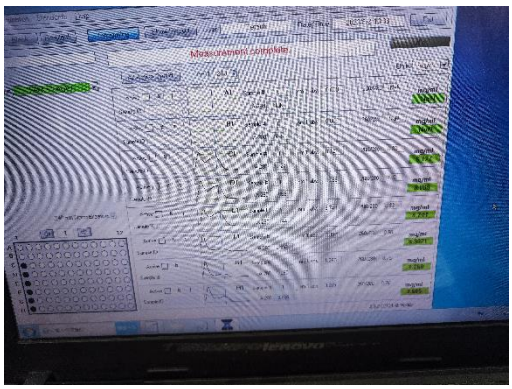
- 1. Select an ultrafiltration tube with a pore size of 30000KD and wash it with RO water
- 2. Use RO water and buffer ultrafiltration for 1-2min (rinsing + leak detection) respectively
- 3. Add the collected eluate to the inner tube of the ultrafiltration tube, and centrifuge it in the centrifuge at 4000rpm 10° C for 15 min after trimming
- 4. Use G250 to detect the inner and outer tubes separately, if the protein does not leak out of the inner tube, pour the outer tube liquid, and continue to add the corresponding eluent to the inner tube layer
- 5. Repeat step 4
- 6. After the eluate is fully concentrated, add buffer to the ultrafiltration tube for centrifugation to remove imidazole.
- 7. Continue centrifugation to the inner tube after repeated 3 times, and the solution volume is less than 1ml
- 8. After the ultrafiltration is completed, the concentrate in the inner tube is divided into 1.5ml EP tubes, each tube is 200ul
- 9. Rinse the injection well of the nucleic acid instrument

with water and PBS solution, debug the equipment, and absorb the water and PBS solution of the injection hole with absorbent paper

- 10. Pipette 2 ul of protein solution sequentially and place it into the well
- 11. Close the instrument lid and click test
- 11. Record the concentration of different plasmids and analyze, take pictures, write the date and concentration on the EP tube
- 12. Flash freeze the aliquoted egg whites with liquid nitrogen and store in a -80° C freezer for use



F7 Concentration results



N23 Concentration results

②invert

- 1. Add competent cells 15 ul and plasmid 5 ul to the 1.5 ml EP tube in the ultra-clean workbench (plasmids can be added to the bench), and ice bath for 30min
- 2. Heat excitation: Bath water in a 42° C water bath for 45s, and then immediately ice bath for 2min
- 3. Add 150 ul of NZY medium to the ultra-clean bench
- 4. Incubate at constant temperature in a shaker at 37° C for 40min-60min
- 8. Take 120ul after incubation and apply the bacterial solution to LA resistant plate, make markings, and culture overnight at 37 ° C

N23 clone transformation

8.3 N23 provoked bacteria, A2, F7 combined to measure activity

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Overview

- N23 picked bacteria and sent N23, A2, 8F, F7, M8 plasmids

for testing

- After measuring the concentration of A2, the activity of F7 and A2 was explored, the activity of A2 was low, and F7 was almost inactive

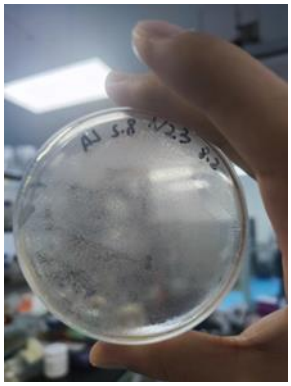
Experimental equipment

- LA medium, 1.5 ml EP tube, marker, pipette gun, tip of the piper
- Different pH buffers
- pipette gun, tip of pipette,
- Nad+, 10mM methanol, 10mM formaldehyde

Experimental steps

(1) Pick bacteria

- 1. Pour LA medium into 1.5 ml EP tubes separately on the bench
- 2. Carefully pick a single colony with the tip of the gun, transfer it into the corresponding 1.5 ml EP tube, shake well and mark it accordingly



(2) Test activity

- 1. Open the software and set the measurement parameters and the address where the data is saved
- 2. First, add 15ul 1M NAD+, 15ul 10mMmethanol/formaldehyde, and an appropriate amount of pH buffer to a 1.5ml EP tube (negative control only add enzyme and pH buffer).
- 3. Transfer the above solution to the cuvette by pipette and zero it (find the AUTO-ZERO button on your computer)
- 4. After zeroing, add 30ug protein solution to the cuvette. Quickly press the "measure" button on your computer to start measuring
- 5. The reaction is 60-120S to stop the measurement and observe the curve

Experimental result

1. A2, F7, ph6.0, 15ulnad, 15ul 1mM methanol 150ul system

Time/Conditions	1 mM methanol, 100ugA2	Methanol and NAD+ are free, with negative controls for A2	1 mM methanol, 100ugA2, F7	1mM methanol, 100ugA2, F7(2)	Methanol and NAD+ are free, with negative controls for A2
0S	0.488	0.462	1.098	0.322	0.497
60S	0.779	0.648	1.380	0.616	0.772
Difference	0.291	0.186	0.282	0.294	0.275
Relative value	0.105		0.007	0.019	

Analysis: A2 activity is low

2. Ph6.5 100ugF7 15ulnad, 1mM formaldehyde 150ul system

Time/Conditions	Negative control	1 mM formaldehyde
0s	0.185	0.267
60s	0.212	0.364
Difference	0.027	0.097
Relative value		0.070

Analysis: F7 is almost inactive

8.4 Transbacterial bottles (N23)

Friday, August 28th, 2023

Overview

- Transfer the N23-containing E. coli cultured in a 1.5 ml EP tube to a bacterial vial and expand the culture

Experimental equipment

- Bacterial bottles
- LA medium

Experimental steps

- Pour 1/3 of LA medium into the bacterial bottle
- Aspirate the bacterial solution in the 10ulEP tube and add to the bacterial bottle
- Place in a 37 ° C shaker and incubate

N23 Go to the bacterial bottle

8.5 N23 plasmid, N23, A2, F7 transformation, F7 clonal transformation

Overview

- N23 plasmid is purified and concentrated, prepared for future expression transformation, measured concentration, and run nucleic acid glue to determine whether the plasmid is mutated
- N23, A2, F7 plasmids are transferred into expressed transformed cells
- F7 clones are transformed to obtain more plasmids

Experimental equipment

- Plasmid extraction kit, 1.5 ml 2 ml EP tubes
- Pipette gun, tip of the pipette
- Kanamycin, LB medium
- LK Tablet

Experimental steps

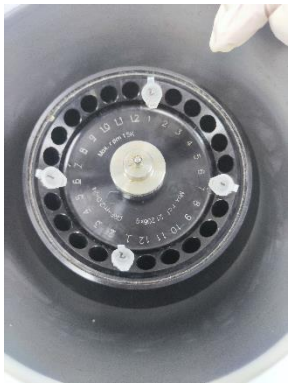
①N23 Plasmid, concentration measurement, run nucleic acid glue

1.Draw plasmids

- 1. Take 4 2ml centrifuge tubes to make markings, pour different bacterial liquids into centrifuge tubes and centrifuge at 12000rpm for 1min, pour the supernatant, collect the bacteria, and repeat this operation until the bacterial liquid is centrifuged
- 2. Add 250ul solution P1 (be sure to add RNase A) to the centrifuge tube with the bacterial pellet, and use pipette to suspend
- 3. Add 250ulP2 to the centrifuge tube and gently flip up and down 6-8 times to fully lyse the bacteria
- 4. Add 350ulP3 to the centrifuge tube, immediately gently flip up and down 6-7 times, mix thoroughly, white flocculent precipitate appears, centrifuge at 12000rpm for 10min
- 5. Transfer the supernatant collected in the previous part to the adsorption column CP3 with a pipette (the adsorption column is placed in the collection tube), pay attention not to take out the pellet as much as possible, centrifuge at 12000rpm for 1min, drain the waste liquid

in the collection tube, and put the adsorption column CP3 into the collection tube

- 6. Add 600ul rinse solution PW to the adsorption column CP3, centrifuge at 12000rpm for 1min, drain the waste liquid in the collection tube, and repeat this step once
- 7. Put the adsorption column CP3 into the collection tube, centrifuge at 12000rpm for 2min, and remove the residual rinse solution in the adsorption column
- 8. Place the adsorption column CP3 in a clean centrifuge tube, add 50ul elution buffer EB dropwise to the middle of the adsorption membrane, place it at room temperature for 2min, centrifuge at 12000rpm for 2min to collect the plasmid solution into the centrifuge tube, and make corresponding marks on the centrifuge tube



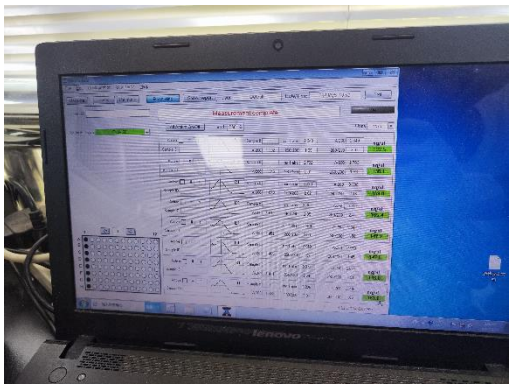
collect N23 plasmid

2.Measure concentration

- 1. Rinse the injection well of the nucleic acid instrument with water and EB solution, and absorb the water and EB

solution of the injection well with absorbent paper

- 2. Pipette 2-4ul plasmid solution sequentially with a pipette gun and place it into the wells
- 3. Close the instrument cover, debug the equipment, and click test
- 4. Record the concentration of different plasmids and analyze, take pictures



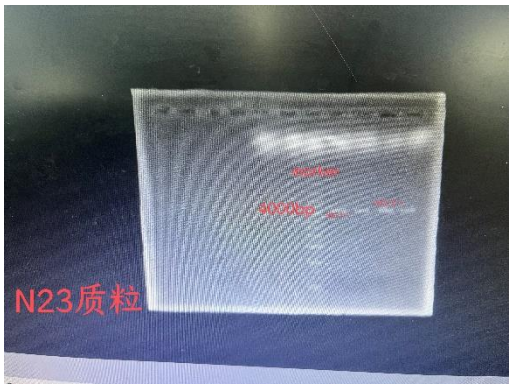
N23 Plasmid concentration

3.Run nucleic acid glue

- 1. Glue: 0.24g agarose, 30ml ETB buffer mixed well, microwave oven heated and put into a splint to fix for 30min to make nucleic acid glue
- 2. Mix the loading of 2ul with different plasmid solutions of 1ul and evenly add them into the void, and then add 2ul of 2000 and 1kd makers to the void respectively
- 3. Put it into an electrophoresis tank for 20-30min
- 4. Take out the glue board and carefully clean it with water, then put it into the dyeing solution and dye for

20min

- 5. Put the dyed rubber plate into the glue illuminator for observation



N23 RUN PAGE result

②A2, F7, N23 transformation, F7 clone transformation

- Add 10ul of competent cells and 1.5 ul of plasmid to the 1.5 ml EP tube in the ultra-clean bench (plasmids can be added to the bench)
- 2. Heat excitation: Bath water in a 42° C water bath for 45s, and then immediately ice bath for 2min
- 3. Add 150 ul of NZY medium to the ultra-clean bench
- 4. Incubate at constant temperature in a shaker at 37° C for 40min-60min
- 5. After incubation, 120 ul of bacterial solution was coated in LA/LK resistant plates and reared overnight at 37° C (N23 for LB, A2, F7 for LK).
- 6.10ulF7 cloned transformed competent cells were placed

in a bacterial bottle containing LK medium and cultured overnight in a shaker at 37 ° C

Conversion preparation

8.7 Plasmid (F7), N23 to large bottle and induction and sterilization (A2, F7)

Monday, August 7th, 2023

Overview

- N23 plasmid is purified and concentrated, prepared for future expression transformation, measured concentration, and run nucleic acid glue to determine whether the plasmid is mutated
- E. coli containing the N23 protein gene in the bacterial bottle was transferred to the vial medium to expand the culture, and then induce it to produce the protein of interest
- Collect E. coli bacteria containing A2 and F7 protein genes, respectively, after expanded culture

Experimental equipment

- Plasmid extraction kit, 1.5 ml, 2 ml EP tubes
- Ampicillin, TB medium, 4×phosphate buffer buffer, IPTG
- Harvest bottles, centrifuge tubes, centrifuges, PBSbuffers

Experimental steps

③F7 takes plasmids, measures concentration, and runs nucleic acid glue

1.Draw plasmids

- 1. Take 4 2ml centrifuge tubes to make markings, pour different bacterial liquids into centrifuge tubes and centrifuge at 12000rpm for 1min, pour the supernatant, collect the bacteria, and repeat this operation until the bacterial liquid is centrifuged
- 2. Add 250ul solution P1 (be sure to add RNase A) to the centrifuge tube with the bacterial pellet, and use pipette to suspend
- 3. Add 250ulP2 to the centrifuge tube and gently flip up and down 6-8 times to fully lyse the bacteria
- 4. Add 350ulP3 to the centrifuge tube, immediately gently flip up and down 6-7 times, mix thoroughly, white flocculent precipitate appears, centrifuge at 12000rpm for 10min

- 5. Transfer the supernatant collected in the previous section to the adsorption column CP3 with a pipette (the adsorption column is placed in the collection tube), pay attention not to take out the pellet as much as possible, centrifuge at 12000rpm for 1min, drain the waste liquid in the collection tube, and put the adsorption column CP3 into the collection tube
- 6. Add 600ul rinse solution PW to the adsorption column CP3, centrifuge at 12000rpm for 1min, drain the waste liquid in the collection tube, and repeat this step once
- 7. Put the adsorption column CP3 into the collection tube, centrifuge at 12000rpm for 2min, and remove the residual rinse solution in the adsorption column
- 8. Place the adsorption column CP3 in a clean centrifuge tube, add 50ul elution buffer EB dropwise to the middle of the adsorption membrane, place it at room temperature for 2min, centrifuge at 12000rpm for 2min, collect the plasmid solution into the centrifuge tube, and make corresponding marks on the centrifuge tube

F7 draw plasmid

2.Measure concentration

- 1. Rinse the injection well of the nucleic acid instrument with water and EB solution, and absorb the water and EB solution of the injection well with absorbent paper
- 2. Pipette 2-4ul plasmid solution sequentially with a pipette gun and place it into the wells
- 3. Close the instrument cover, debug the equipment, and click test
- 4. Record the concentration of different plasmids and analyze, take pictures

3.Run nucleic acid glue

- 1. Glue: 0.24g agarose, 30ml ETB buffer mixed well, microwave oven heated and put into a splint to fix for 30min to make nucleic acid glue
- 2. Mix the loading of 2ul with different plasmid solutions of 1ul and evenly add them into the void, and then add 2ul of 2000 and 1kd makers to the void respectively

- 3. Put it into an electrophoresis tank for 20-30min
- 4. Take out the glue board and carefully clean it with water, and then put it into the dyeing solution to dye for 20min
- 5. Put the dyed rubber plate into the glue illuminator for observation

F7 Sample loading

④N23 turn the vial and induce

- Place the required items (sterilization solution) in the ultra-clean workbench and irradiate ultraviolet for 10min
- Add 25ml of 4x phosphate buffer, 500ul ampicillin, and 1/2 of the corresponding bacterial liquid bottle to the TB medium, and mark the bottle body
- The sterilized medium was incubated in a constant temperature shaker at 37° C for about 8h
- IPTG 750ul was added to the cultured bacterial solution and placed at 18 ° C to induce for 16-18 h

⑤A2, F7 harvesting

- Divide the bacterial liquid in the large bottle into the collection bottle, and the two pairs are flat
- After 6,000 rpm in a centrifuge for 10 min, pour the supernatant
- Resuspend the centrifuged bacteria with PBSbuffer and transfer to 50 ml centrifuge tubes with pair-leveling
- Centrifuge in a centrifuge at 4000rmp 10° C for 15 min and then take out
- Pour the supernatant and store at -20 ° C in the refrigerator

8.8 N23 harvesting, A2, F7 sterilization, purification, running nucleic acid glue

Overview

- Collect N23 bacteria and store in a -20 ° C freezer for later use
- A2, F7 sterilization, purified protein, run glue to identify the target protein position

Experimental equipment

- N23 bacterial solution, centrifuge bottle, PBS buffer, 50 ml centrifuge tube

- PMSF protease inhibitors, 10 ml, 2 ml, 1.5 ml EP tubes
- G250 dye, pipette gun, tip of the gun,
- Imidazole solution in different concentrations, nickel column
- loading, maker

Experimental steps

①N23 harvesting

- 1. Divide the bacterial liquid in the large bottle into the collection bottle, and match the two pairs
- 2. After 6000 rpm in the centrifuge for 10 min, pour the supernatant
- 3. Resuspend the centrifuged bacteria with an appropriate buffer and transfer to a 50 ml centrifuge tube with pairing
- 4. Centrifuge at 6000rmp 10° C in a centrifuge for 20min and take out
- 5. Pour the supernatant, store it in the refrigerator at -20 ° C, and write the date of the strain

N23 harvesting

②A2, F7 sterilization, purification, running glue

1.Sterilization

- 1. Weigh the bacteria on the balance, add 10ml of the bacteria to 10ml buffer for resuspending
- 2. Before sterilization, add 1% protease inhibitor PMSF to the resuspended bacterial solution and add 100ul per 10ml of bacterial solution
- 3. Clean the high-pressure cell disruptor 2 times with RO water, and then wash twice with buffer, and keep the pressure at 300bar-400bar during cleaning
- 4. Add bacteria liquid to the instrument, and the sterilization pressure is kept at 900bar-1100bar
- 5. Repeat the sterilization 3-4 times so that the bacterial liquid is no longer viscous
- 6. After the sterilization is completed, use 2ml EP tube for aliquoting and centrifuge in a centrifuge at 12000rpm 10° C for 30min (leave one tube without centrifugation

for sample preparation)

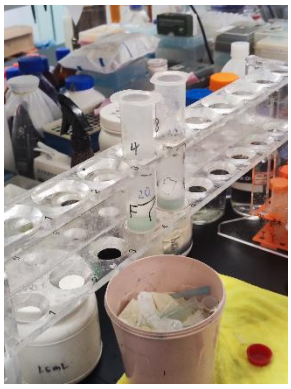
- 7. After centrifugation, keep one tube of pellet and one tube of supernatant (sample preparation). The rest can be collected in the supernatant.

2.Purification

- 1. Clean the purification column with tap water and RO water
- 2. Soak the purification column with NaOH with a concentration of 0.1M for 15min
- 3. Wash off the NaOH in the purification column and rinse with RO water
- 4. First add an appropriate amount of nickel beads to the purification column, and then wash twice separately with RO water and buffer.
- 5. After cleaning, add an appropriate amount of 10mM imidazole to the purified beads with nickel beads and soak for 5min to activate the nickel beads.
- 6. After activating the nickel beads, immediately add the collected upper clearing night, and close the tube mouth with parafilm and mix in a silent mixer for 90min (so that the target protein fully binds to the nickel beads).
- 7. After the mixing is completed, take the flow

penetration liquid and place it in ice.

- 8. Elution is performed with 10mM imidazole, 30mM imidazole, 50mM imidazole, 80mM imidazole, 100mM imidazole, 150mM imidazole, 300mM imidazole, 600mM imidazole. Each concentration of imidazole is washed until the eluate encounters G250 Coomassie brilliant blue and does not change blue.

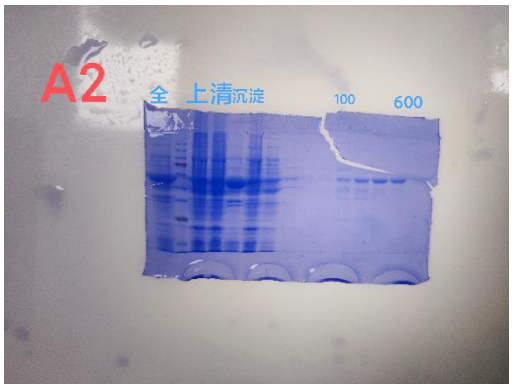


A2,F7 Sterilization

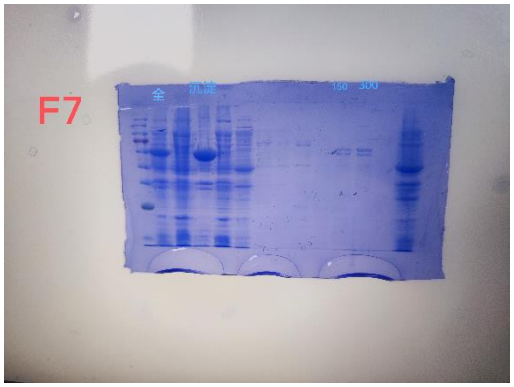
3. Run glue

- 1. Prepare samples of collected precipitate, supernatant, flow-through, whole, and various concentrations of imidazole eluate. Take 40ul loading and 10ul samples for mixing (precipitation, appropriate dilution as a whole), and boil at 100 ° C for 10min.
- 2. After sample preparation, the protein of interest is tested by SDS-PAGE. During the loading process, take 10ul for each sample and 2ul for protein marker
- 3.After 40-60min of electrophoresis, take the glue and

stain for 2-3h, decolorize for 2h, and then analyze and take pictures



A2 SDS-PAGE result



F7 SDS-PAGE result

8.10-11 A2, F7, 8F expression transformation, pronging, induction, 8F cloning transformation

Overview

- In order to explore the effect of different competent states on the expression of the protein of interest, we designed this experiment, changing the competent state to C43, re-converting it, and seeing which protein would have a higher expression compared with the previous experiment using B21

- Expand the culture of E. coli containing the A2, F7, and 8F gene of interest, respectively, and induce it to produce the protein of interest
- The cloning and transformation of 8F increases the number of gene of interest containing 8F

Experimental equipment

- 1.5ml EP 管
- Pipette gun, tip of the pipette
- Kanamycin, LB medium
- LK Tablet
- IPTJ

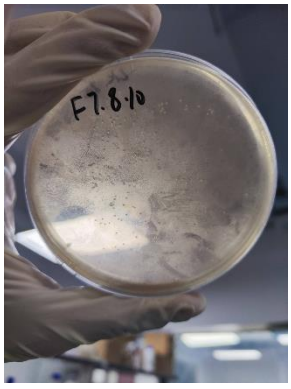
Experimental steps

⑥A2, F7, 8F expression transformation, 8F cloning transformation

- Add 10ul of competent cells and 1.5 ul of plasmid to the 1.5 ml EP tube in the ultra-clean bench (plasmids can be added to the bench)
- 2. Heat excitation: Bath water in a 42° C water bath for 45s, and then immediately ice bath for 2min
- 3. Add 150 ul of NZY medium to the ultra-clean bench
- 4. Incubate at constant temperature in a shaker at 37° C for 40min-60min

- 5. After incubation, 120 ul of bacterial solution was coated in LK resistant plates and reared overnight at 37° C

A2 Conversion results



F7 Conversion results

8F Conversion results

⑦ Pick fungi

- Add 12.5 ul of kanamycin to a small Erlenmeyer flask containing 25 ml of LB medium

- Carefully pick 3-4 single colonies with the tip of the gun, transfer them into the corresponding small Erlenmeyer flask, shake well and mark accordingly
- Place in a 37 ° C shaker and incubate for 6-8 h

⑧induce

- Add 12.5ulIPTG to the cultured bacterial solution and induce it at 18° C for 16-18 h

8.12 8F pick bacteria, take plasmids to measure the concentration and run nucleic acid glue; A2, F7 sterilization, crushing, running glue, activity measurement

Overview

- 8F Singles bacteria expanded the culture, extracted plasmid, measured plasmid concentration, and ran glue to determine plasmid size
- A2, F7 harvesting, crushing its precipitation and supernatant running glue, simple activity measurement

Experimental equipment

- Pipette gun, tip of pipette, LK plate
- Plasmid extraction cassette, 1.5, 2 ml EP tubes
- 50 ml centrifuge tubes, cell ultrasonic disruptor,
- Loading, maker, 10mM methanol, formaldehyde, 1mMnad

Experimental steps

①8F pick bacteria, take plasmids, measure concentration, run nucleic acid glue

1.Pick fungi

- 1. Pick a single colony on the 8F plate and place it in a bacterial bottle containing LK medium
- 2. Mark the bottle body and put it in a 37 ° C shaker to incubate for 8h

8F pick bacteria

2.Draw plasmids

- 1. Take 4 2ml centrifuge tubes to make markings, pour different bacterial liquids into centrifuge tubes and centrifuge at 12000rpm for 1min, pour the supernatant, collect the bacteria, and repeat this operation until the bacterial liquid is centrifuged
- 2. Add 250ul solution P1 (be sure to add RNase A) to the centrifuge tube with the bacterial pellet, and use pipette to suspend

- 3. Add 250ulP2 to the centrifuge tube and gently flip up and down 6-8 times to fully lyse the bacteria
- 4. Add 350ulP3 to the centrifuge tube, immediately gently flip up and down 6-7 times, mix thoroughly, white flocculent precipitate appears, centrifuge at 12000rpm for 10min
- 5. Transfer the supernatant collected in the previous part to the adsorption column CP3 with a pipette (the adsorption column is placed in the collection tube), pay attention not to take out the pellet as much as possible, centrifuge at 12000rpm for 1min, drain the waste liquid in the collection tube, and put the adsorption column CP3 into the collection tube
- 6. Add 600ul rinse solution PW to the adsorption column CP3, centrifuge at 12000rpm for 1min, drain the waste liquid in the collection tube, and repeat this step once
- 7. Put the adsorption column CP3 into the collection tube, centrifuge at 12000rpm for 2min, and remove the residual rinse solution in the adsorption column
- 8. Place the adsorption column CP3 in a clean centrifuge tube, add 50ul elution buffer EB dropwise to the middle of the adsorption membrane, place it at room temperature

for 2min, centrifuge at 12000rpm for 2min to collect the plasmid solution into the centrifuge tube, and make corresponding marks on the centrifuge tube

3. Measure concentration

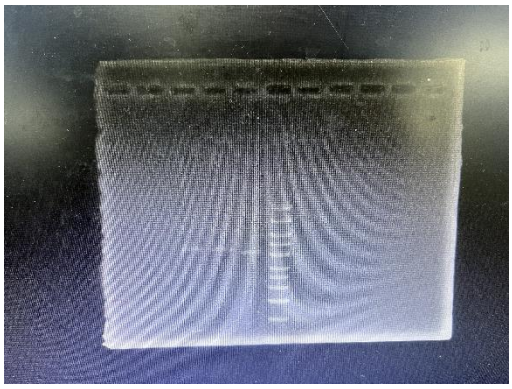
- 1. Rinse the injection well of the nucleic acid instrument with water and EB solution, and absorb the water and EB solution of the injection well with absorbent paper
- 2. Pipette 2-4ul plasmid solution sequentially with a pipette gun and place it into the wells
- 3. Close the instrument cover, debug the equipment, and click test
- 4. Record the concentration of different plasmids and analyze, take pictures

4. Run nucleic acid glue

- 1. Glue: 0.24g agarose, 30ml ETB buffer mixed well, microwave oven heated and put into a splint to fix for 30min to make nucleic acid glue
- 2. Mix the loading of 2ul with different plasmid solutions of 1ul and evenly add them into the void, and then add 2ul of 2000 and 1kd makers to the void respectively
- 3. Put it into an electrophoresis tank for 20-30min
- 4. Take out the glue board and carefully clean it with

water, then put it into the dyeing solution and dye for 20min

- 5. Put the dyed rubber plate into the glue illuminator for observation



8F Plasmid running results

②A2, F7 sterilization, crushing, running glue, activity measurement

1.Harvest bacteria

- 1. Pour the induced bacterial solution into a 50ml centrifuge tube, and put it into a centrifuge at 4000rpm for centrifuge for 10min
- 2.Pour the supernatant and change the PBS buffer to continue away for 10 min
- 3.Keep the separated bacteria on ice for later use

2.Broken

- 1. Weigh the collected bacteria, 01 g of bacteria plus 1 ml of PBS pipette and resuspend and transfer into a 2 ml

centrifuge tube

- 2. Set the parameters of the ultrasonic crusher: engineering 2, 100w, 10min
- 3.To clean the instrument, first wash with water, then spray with alcohol, then wash with water, and then dry with a paper towel
- 4. Put the bacterial liquid away, test it, and then click to work to run
- 5.Put the broken bacteria into a low-temperature centrifuge for 12000rpm and centrifuge for 30min

Cell ultrasonic disruptor

3.Run glue

- 1. Retention precipitate and supernatant preparation: 10ulloading + 40ul protein (precipitation to be diluted: 10ulloading + 30ulpbs + 10ul precipitation).
- 2.The prepared sample metal bath is 100 ° C for 10min
- 3.Loading, maker 2ul, protein 1ul
- 4.After 40-60min of electrophoresis, staining for 30min,

decolorization for 60min (microwave), observe the results and take pictures to record

A2,F7SDS-PAGE result

4. Measure activity

- 1. Open the software and set the measurement parameters and the address where the data is saved
- 2. First, add 15ul 1M NAD+, 15ul 10mM methanol/formaldehyde, and an appropriate amount of ul pH solution to a 1.5ml EP tube, respectively, in which the negative control only adds pH solution and enzyme
- 3. Transfer the above solution to the cuvette by pipette and zero (find the AUTO-ZERO button on the computer)
- 4. After zeroing, add 50ul A2/F7 protein solution to the cuvette. Quickly press the "measure" button on your computer to start measuring
- 5. The reaction can stop the measurement and observe the curve after 1min
- 6. The results are inactive

8.14 A2, F7 harvesting, sterilization, running glue

Overview

- A2, F7 harvest the bacteria, crush it to precipitate and supernatant running glue

Experimental equipment

- 50 ml centrifuge tubes, cell ultrasonic disruptor, 2 ml EP tubes
- Loading, marker,PBSbuffer

Experimental steps

1.Harvest bacteria

- 1. Pour the induced bacterial solution into a 50ml centrifuge tube, and put the equalization into a centrifuge at 4000rpm to centrifuge for 15min
- 2.Pour the supernatant and change the PBS buffer to continue away for 10 min
- 3.Keep the separated bacteria on ice for later use

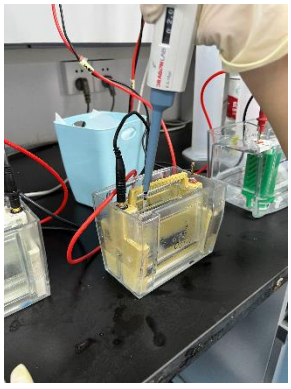
2.Broken

- 1. Weigh the collected bacteria, 01 g of bacteria plus 1 ml of PBS pipette and resuspend and transfer into a 2 ml centrifuge tube
- 2. Set the parameters of the ultrasonic crusher: engineering 2, 100w, 10min

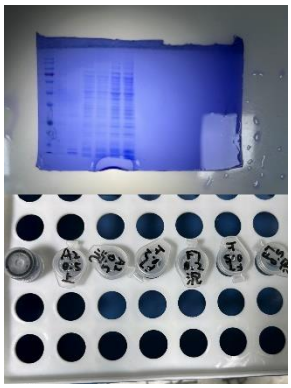
- 3. To clean the instrument, first wash with water, then spray with alcohol, then wash with water, and then dry with a paper towel
- 4. Put the bacterial liquid away, test it, and then click the work to run
- 5. Put the broken bacteria into a low-temperature centrifuge for 12000rpm and centrifuge for 30min

3.Run glue

- 1. Retention precipitate and supernatant preparation: 10ulloading + 20ulpbs + 20ul precipitation or supernatant
- 2. The prepared sample metal bath is 100 ° C for 10min
- 3. Loading, maker 2ul, protein 1ul
- 4. After 40-60min of electrophoresis, staining for 30min, decolorization for 60min (microwave), observe the results and take pictures to record



sample loading



A2,F7 SDS-PAGE result

Experiment result:

- Running glue display, no target protein

8.19 A2, F7, 8F pick, induce

Sunday, July 2, 2023

Overview

- Pick the three bacteria into 150 ml of LK medium and culture
- Add an appropriate amount of inducers to induce it to express the protein

Experimental equipment

- Pipette gun, tip of the pipette
- LK medium, markers
- IPTG inducers

Experimental steps

①Pick bacteria and induce

- 1. Pick single colonies on A2, F7, 8F plates and place in

an Erlenmeyer flask containing LK medium

- 2. Mark the bottle body and put it in a 37° C shaker to incubate for 8h
- 3. Add 75ul of inducer to the bacterial solution and put it into an 18° C shaker to induce for 16-18h

8.20 A2, F7 sterilization, sterilization, glue, activity measurement, A2, F7 picking

Overview

- A2, F7 harvesting, crushing its precipitation and supernatant running glue, supernatant activity measured
- E. coli containing A2 and F7 protein genes were inoculated into bacterial vials containing LB medium for culture

Experimental equipment

- Pipette gun, tip of pipette, LK plate
- Centrifuge bottles, 50 ml centrifuge tubes, 2 ml EP tubes
- Loading, marker,PBSbuffer
- 10 mM methanol, formaldehyde, 1 mMnad

Experimental steps

(1) A2, F7 sterilization, crushing, running glue, testing activity

1.Harvest bacteria

- 1. Pour the induced bacterial liquid into the collection bottle and balance the two pairs
-
- 2. After 6000 rpm in a centrifuge, after 7 min, pour the supernatant
- 3. Resuspend the centrifuged bacteria with xxxx buffer and transfer to a 50 ml centrifuge tube with pair-by-pair leveling
- 4. Centrifuge in a centrifuge at 3000RMP for 15min and then take out

2.Broken

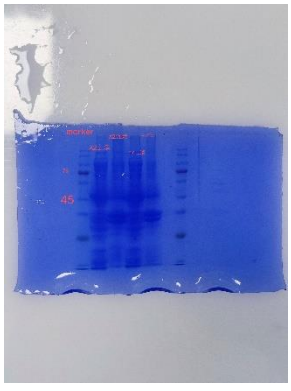
- 1. Weigh the collected bacteria, 1g of bacteria plus 10ml PBS blowing and resuspending
- 2. Clean the high-pressure cell disruptor twice with RO water, and then wash twice with xx buffer, and keep the pressure at 300bar-400bar during cleaning
- 3. Add bacteria liquid to the instrument, and the sterilization pressure is kept at 900bar-1100bar
- 4. Repeat the sterilization 3-6 times to make the bacterial liquid no longer viscous
- 5. After the sterilization is completed, use 2ml EP tube

for aliquoting and centrifuge in a centrifuge at 14000rpm 10° C for 30min (leave a tube without centrifugation for sample preparation)

- 6. After centrifugation, keep one tube of pellet and one tube of supernatant (sample preparation). The rest can be collected in the supernatant.

3.Run glue

- 1. Retention precipitate and supernatant preparation: 10ulloading + 20ulpbs + 20ul precipitation or supernatant
- 2.The prepared sample metal bath is 100 ° C for 10min
- 3.Loading, maker 2ul, protein 1ul
- 4.After 40-60min of electrophoresis, staining for 30min, decolorization for 60min (microwave), observe the results and take pictures to record



SDS-PAGE result

4. Measure activity

- 1. Open the software and set the measurement parameters and the address where the data is saved

- 2. First, add 15ul 1M NAD+, 15ul 10mMmethanol/formaldehyde, and an appropriate amount of ul pH solution to a 1.5ml EP tube, in which the negative control only adds pH solution and enzyme-containing supernatant
- 3. Transfer the above solution to the cuvette by pipette and zero it (find the AUTO-ZERO button on your computer)
- 4. After zeroing, add 50/100ul supernatant containing A2/F7 to the cuvette. Quickly press the "measure" button on your computer to start measuring
- 5. The reaction can stop the measurement and observe the curve after 1min

(2) A2, F7 pick

- 1. Pick a single colony on the A2 and F7 plates and put them into the bacterial bottles containing LK medium
- 2. Mark the bottle body and put it in a 37 ° C shaker to incubate for 8h

8.22 A2, F7 harvesting, sterilization, running glue

Overview

- A2, F7 harvesting, in which the vial-induced (0.2) bacteria are taken about 1g with the other two gradients

(0.1) ultrasonic sterilization, precipitation and supernatant sample running for analysis

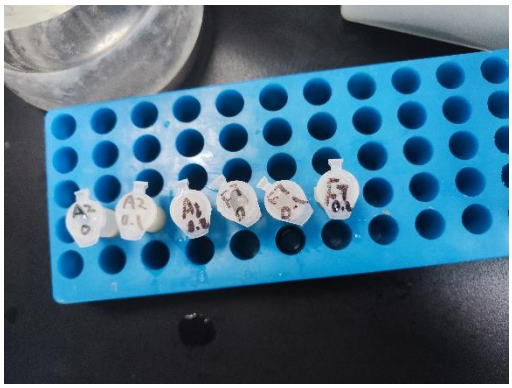
Experimental equipment

- Harvesting bottle, PBS buffer
- 1.5mlEP 管, marker, loading
- Pipette gun, tip of the pipette

Experimental steps

①Harvest bacteria

- 1. Divide the bacterial liquid in the large bottle into the collection bottle, and match the two pairs
- 2. After 6000 rpm in the centrifuge for 10 min, pour the supernatant
- Resuspend the centrifuged bacteria with 20-30 ml buffer and transfer to a 50 ml centrifuge tube with pair-equalization
- 4. Centrifuge at 4000rmp 10° C for 15min in a centrifuge and take out (50ml of bacterial liquid centrifugation and then add 10ml PBS to clean centrifugation).



Harvest bacteria

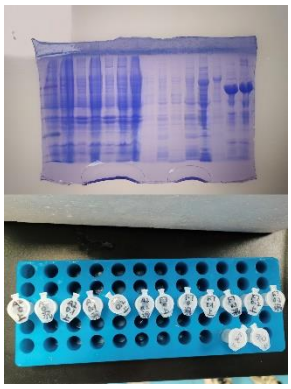
②Sterilization

- 1. Aliquot into 6 EP tubes according to the gradient, add 1ml pbs to puff and resuspend
- 2. Clean the ultrasonic sterilizer, wash each with water and alcohol
- 3. Set parameters: time 10min, power 110w, working 2s, stop 4s
- 4. Put the EP tube containing the bacterial solution into the sterilizer and break the bacteria in turn

③Run glue

- 1.Put the broken bacteria into a 12000 rpm centrifuge and centrifuge at 10 ° C for 30min
- 2. The obtained precipitate and supernatant were prepared separately: 10ulloading + 20ulpbs + 20ul sample
- 3.The prepared sample is 100 ° C metal bath for 10min
- 4.Sample: marker2ul, sample 10ul
- 5.After 40-50min of electrophoresis, staining for 2h, and

then decolorization for 1h, the glue pattern was analyzed



SDS-PAGE result

8.25A2, F7, 8F conversion (BL, BZ) A2 clone transformation

Overview

- A2, F7, 8F transformed, with different competent states (BL, BZ).
- A2 clonal transformation

Experimental equipment

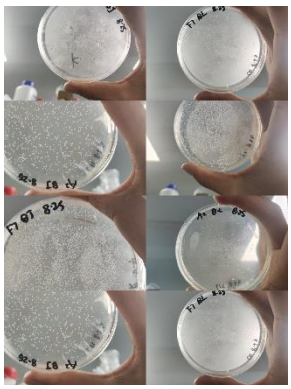
- Competent cells BL, BZ
- Different plasmids (A2, F7, 8F).
- NZY, LK Tablet
- Pipette gun, tip of the pipette

Experimental steps

①invert

- 1.Add 30ul of competent cells and 1.5ul of plasmid to a 1.5 ml EP tube in the ultra-clean bench (plasmids can be added to the bench).

- 2. Ice bath 30min
- 3. Heat excitation: Bath water in a 42° C water bath for 45s, and then immediately ice bath for 2min
- Add NZY medium 150 ul to the ultra-clean bench
- 5. Incubate at constant temperature in a shaker at 37 ° C for 40min-60min
- 8. Take 150ul of the bacteria solution after incubation and apply it to LK resistant plates and rearing overnight at 37° C



conversion result

8.26 A2, F7, 8F Picking, inducing

Overview

- E. coli containing A2, F7, and 8F protein genes were inoculated into TB medium for culture and induced with different concentrations of IPTG (0, 0.2, 0.3).

Experimental equipment

- Pipette gun, tip

- Kanamycin, TB medium, 4×phosphate buffer buffer, IPTG
- Markers, ultra-clean workbenches, alcohol bottles, alcohol lamps

Experimental step

③A2, F7, 8F pick and induce

- Add 4x phosphate buffer 625ul of corresponding amount of kanamycin to TB medium
- Single colonies on A2, F7, and 8F plates were picked and inoculated into TB medium, and the bottles were labeled
- The sterilized medium was incubated in a constant temperature shaker at 37° C for about 8h
- IPTG 0ul, 5ul and 7.5ul were added to the cultured bacterial solution, and placed at 18 ° C to induce for 16-18h

8.29 A2, F7pcr run nucleic acid glue, A2 clone transformation

Overview

- A2, F7 re-PCR, PAO nucleic acid glue detection, not p
- A2 clonal transformation

Experimental equipment

- A2, F7 plasmid, sterile water, positive and negative primers, primer star enzyme

- D competent cells, NZY medium
- LK plate, pipette gun, tip of the piper
- Nucleic acids Marker, loading

Experimental steps

①PCR, run nucleic acid glue

- 1. PCR system (10ul): add 3ul sterile water, 1ul template, 0.5ul positive primer, 0.5ul reverse primer, 5ul primer star enzyme (10s/kb) to the PCR tube
- 2. Set the parameters of PCR instruments: predenaturation 95° C for 3min, denaturation 95° C for 20s, renaturation 61° C for 20s, extension 72° C for 75s, compound extension 72° C for 5min, protection instrument 12° C for 2min
- 3. Nucleic acid glue configuration (0.8%): Add 30 ml of 1xTAE, 0.24 g agarose to the Erlenmeyer flask and dissolve in the microwave
- 4. Nucleic acid glue dosing: 2ul loading + 1ul sample mixed, transferred to the pores, add marker 2ul
- 5. Nucleic acid electrophoresis: after 150v constant pressure electrophoresis about XXmin, staining in the dark for 20min, gently rinsing with clean water and observing under the glue machine

②Clone transformation

- 1. (Ordinary) Add 10ul of competent cells and 0.5ul of plasmid to the 1.5ml EP tube in the ultra-clean workbench (plasmids can be added to the bench), and ice bath for 30min
- 2. Heat excitation: Bath water in a 42° C water bath for 45s, and then immediately ice bath for 2min
- 3. Add 150 ul of NZY medium to the ultra-clean bench
- 4. Incubate at constant temperature in a shaker at 37° C for 40min-60min
- 8. After incubation, 150 ul of bacterial solution was coated in LK resistant plates and reared overnight at 37° C

A2 Clone transformation result

8.30 A2 pick bacteria, plasmid run nucleic acid glue concentration, F7, 8F clonal transformation

Overview

- A2 plasmid extraction, running glue concentration,

sending test, A2concentration 150ng/ul

- F7,8F cloning transformation,

Experimental equipment

- Bacterial bottles, pipette guns, tips
- LK medium, LK plates
- Plasmid extraction kit, nucleic acid glue
- D competent state, NZY medium, F7, 8F plasmids

Experimental steps

①A2 plasmid extraction

1. Pick fungi

- 1. Pick a single colony on the A2 plate and place it in a bacterial vial containing LK medium
- 2. Mark the bottle body and put it in a 37° C shaker to incubate for 7h

2. Draw plasmids

- 1. Take two 2ml centrifuge tubes to make markings, pour different bacterial liquids into centrifuge tubes and centrifuge at 12000rpm for 1min, pour the supernatant, collect the bacteria, and repeat this operation until the bacterial liquid is centrifuged
- 2. Add 250ul solution P1 (be sure to add RNase A) to the centrifuge tube with the bacterial pellet, and use pipette

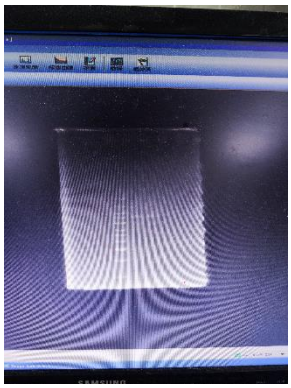
to suspend

- 3. Add 250ulP2 to the centrifuge tube and gently flip up and down 6-8 times to fully lyse the bacteria
- 4. Add 350ulP3 to the centrifuge tube, immediately gently flip up and down 6-7 times, mix thoroughly, white flocculent precipitate appears, centrifuge at 12000rpm for 10min
- 5. Transfer the supernatant collected in the previous section to the adsorption column CP3 with a pipette (the adsorption column is placed in the collection tube), pay attention not to take out the pellet as much as possible, centrifuge at 12000rpm for 1min, drain the waste liquid in the collection tube, and put the adsorption column CP3 into the collection tube
- 6. Add 600ul rinse solution PW to the adsorption column CP3, centrifuge at 12000rpm for 1min, drain the waste liquid in the collection tube, and repeat this step once
- 7. Put the adsorption column CP3 into the collection tube, centrifuge at 12000rpm for 2min, remove the residual rinse solution in the adsorption column, and bake in the oven for 5min to remove alcohol
- 8. Place the adsorption column CP3 in a clean centrifuge

tube, add 50ul elution buffer EB dropwise to the middle of the adsorption membrane, place it at room temperature for 2min, centrifuge at 12000rpm for 2min, collect the plasmid solution into the centrifuge tube, and make corresponding marks on the centrifuge tube

3.Run nucleic acid glue

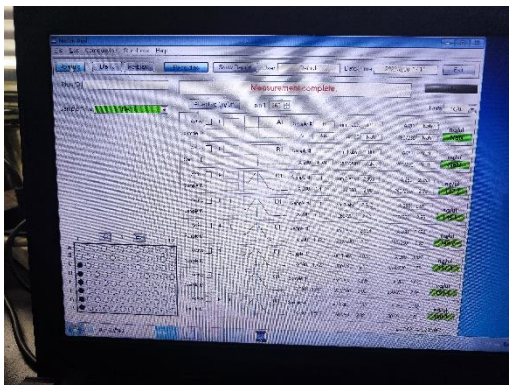
- 1. Glue: 0.24g agarose, 30ml ETB buffer mixed well, microwave oven heated and put into a splint to fix for 30min to make nucleic acid glue
- 2. Mix the loading of 2ul with different plasmid solutions of 1ul and evenly add them into the void, and then add 2ul 1kd maker to the void
- 3. Put it into an electrophoresis tank for 20-30min
- 4. Take out the glue board and carefully clean it with water, and then put it into the dyeing solution to dye for 20min
- 5. Put the dyed rubber plate into the glue illuminator for observation



A2 Run nucleic acid glue

4. Measure concentration

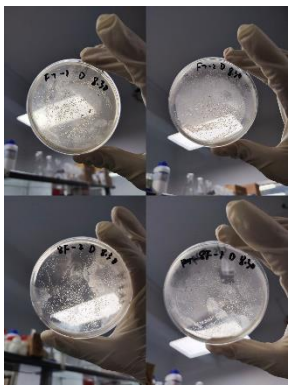
- 1. Rinse the injection well of the nucleic acid instrument with water and EB solution, and absorb the water and EB solution of the injection well with absorbent paper
- 2. Pipette 2-4ul plasmid solution sequentially with a pipette gun and place it into the wells
- 3. Close the instrument cover, debug the equipment, and click test
- 4. Record the concentration of different plasmids and analyze, take pictures



A2 concentration result

② F7,8F clone transformation

- 1. (Ordinary) Add 15ul of competent cells and 1.5ul of plasmid to the 1.5ml EP tube in the ultra-clean workbench (plasmids can be added to the bench) ice bath for 30min
- 2. Heat excitation: Bath water in a 42° C water bath for 45s, and then immediately ice bath for 2min
- 3. Add 150 ul of NZY medium to the ultra-clean bench
- 4. Incubate at constant temperature in a shaker at 37° C for 40min-60min
- 5. Take 100ul of bacteria after incubation and apply it to a 100ul resistant plate, and rearing overnight at 37° C



F7,8F clone transformation result

8.31 F7, 8F to pick bacteria, plasmid run nucleic acid glue concentration, A2PCR

Overview

- F7, 8F plasmid extraction, run glue to measure concentration, send for testing
- A2PCR, not p

Experimental equipment

- Bacterial bottles, pipette guns, tips
- Plasmid extraction kit, nucleic acid glue
- A2, F7 plasmids, sterile water, positive and negative primers, primerstar enzyme
- Nucleic acids Marker, loading

Experimental steps

First, F7, 8F plasmid extraction

1. Pick fungi

- 1. Pick a single colony on the A2 plate and place it in a bacterial vial containing LK medium
- 2. Mark the bottle body and put it in a 37° C shaker to incubate for 7h

Pick fungi

2. Draw plasmids

- 1. Take two 2ml centrifuge tubes to make markings, pour different bacterial liquids into centrifuge tubes and centrifuge at 12000rpm for 1min, pour the supernatant,

collect the bacteria, and repeat this operation until the bacterial liquid is centrifuged

- 2. Add 250ul solution P1 (be sure to add RNase A) to the centrifuge tube with the bacterial pellet, and use pipette to suspend
- 3. Add 250ulP2 to the centrifuge tube and gently flip up and down 6-8 times to fully lyse the bacteria
- 4. Add 350ulP3 to the centrifuge tube, immediately gently flip up and down 6-7 times, mix thoroughly, white flocculent precipitate appears, centrifuge at 12000rpm for 10min
- 5. Transfer the supernatant collected in the previous section to the adsorption column CP3 with a pipette (the adsorption column is placed in the collection tube), pay attention not to take out the pellet as much as possible, centrifuge at 12000rpm for 1min, drain the waste liquid in the collection tube, and put the adsorption column CP3 into the collection tube
- 6. Add 600ul rinse solution PW to the adsorption column CP3, centrifuge at 12000rpm for 1min, drain the waste liquid in the collection tube, and repeat this step once
- 7. Put the adsorption column CP3 into the collection tube,

centrifuge at 12000rpm for 2min, remove the residual rinse solution in the adsorption column, and bake in the oven for 5min to remove alcohol

- 8. Place the adsorption column CP3 in a clean centrifuge tube, add 50ul elution buffer EB dropwise to the middle of the adsorption membrane, place it at room temperature for 2min, centrifuge at 12000rpm for 2min, collect the plasmid solution into the centrifuge tube, and make corresponding marks on the centrifuge tube

3.Run nucleic acid glue

- 1. Glue: 0.24g agarose, 30ml ETB buffer mixed well, microwave oven heated and put into a splint to fix for 30min to make nucleic acid glue
- 2. Mix the loading of 2ul with different plasmid solutions of 1ul and evenly add them into the void, and then add 2ul 1kd maker to the void
- 3. Put it into an electrophoresis tank for 20-30min
- 4. Take out the glue board and carefully clean it with water, and then put it into the dyeing solution to dye for 20min
- 5. Put the dyed rubber plate into the glue illuminator for observation

nucleic acid glue

4. Measure concentration

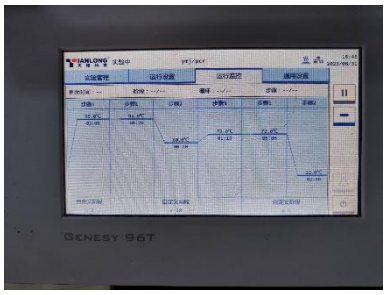
- 1. Rinse the injection well of the nucleic acid instrument with water and EB solution, and absorb the water and EB solution of the injection well with absorbent paper
- 2. Pipette 2-4ul plasmid solution sequentially with a pipette gun and place it into the wells
- 3. Close the instrument cover, debug the equipment, and click test
- 4. Record the concentration of different plasmids and analyze, take pictures

Second, PCR, run nucleic acid glue

- 1. PCR system (10ul): add 3ul sterile water, 1ul template, 0.5ul positive primer, 0.5ul reverse primer, 5ulprimerstar enzyme (10s/kb) to the PCR tube
- 2. Set the parameters of PCR instruments: predenaturation 95° C3min, denaturation 95° C20s, renaturation 61° C20s, extension 72 ° C75s, compound extension 72 ° C5min,

protection instrument 12° C2min

- 3. Nucleic acid glue sampling: 2ulloading + 1ul sample mixed, transferred to the pores, add marker2ul
- 4. Nucleic acid electrophoresis: 150V constant pressure electrophoresis for about 25min, stained in the dark for 20min, gently rinsed with clean water and observed under the glue machine



PCR setup