1.Quorum sensing

1.1 Obtain DNA fragment, AfeR and plasmid DNA

1.1.1 PCR

The DNA fragment was synthesized by Genscript: AfeR-GFP, and the product sent by the company was built on pUC57.

Primer sequence:

FP-afe:5’-ACTTTAAGAAGGAGATATAATGGACCCGTGGAAAGAAGATCTGC-3’

RP-afe:5’-AAGCTTGTCGACGGAGCTCGAATTCTTATTGTATAGTTCATCCATGCCAT-3’

Table1 Preparation of foreign gene PCR system

Total volume	50/μL
DDW	36
10×Buffer	5
dNTP（2.5mM）	5
FP-afe（10μM）	1
RP-afe（10μM）	1
pUC57-AfeR-GFP	1
EasyPfu DNA Polymerase	1

Table2 PCR Settings

Pre-denaturation	94℃	5min	
Transsexual	94℃	30s	30circles
Annealing	60℃	30s	
Extend	72℃	2min	
Extend	72℃	5min	

We run PCR products on a gel and observe the DNA band positions. The PCR product was purified using an AP-PCR-250 kit.

1) Add 3 times volumes of Buffer PCR-A to the PCR reaction solution; mix and transfer to the preparation tube.Place the preparation tube in a 2 ml centrifuge tube, centrifuge at 12000*g for 1 min and discard the filtrate.
2) Place the preparation tube back into a 2 ml centrifuge tube, add 700 μL Buffer W2, centrifuge at 12000*g for 1 min, and discard the filtrate.
3) Place the preparation tube back in a 2 ml centrifuge tube, add 400 μL Buffer W2, centrifuge at 12000*g for 1 min, and discard the filtrate.
4) Reset the preparation tube to the centrifuge and centrifuge at 12000*g for 1 min.
5) The preparation tube is placed at 65℃ to dry to an ethanol-free odor, and the preparation tube is placed in a clean 1.5 ml centrifuge tube, and 25-30 μL of Eluent or sterile water is added to the center of the preparation tube, and allow to stand at room temperature for 1 min. The DNA is eluted by centrifugation at 12000*g for 1 min.

1.1.2 Extraction plasmid pET-28d

We replaced the T7 promoter with J23119, and we handed the company to synthesize this engineered plasmid. We named it pET-28d.

1) 1-4 mL of the bacterial solution was centrifuged at 12,000 rpm for 2 min, and the bacterial solution was discarded.
2) Resuspend by adding 200μLSolutionI, then add 400μL SolutionII to cleave, slowly

invert upside down until the bacterial solution is transparent,

3) Add 300μLSolutionIII and mix.
4) Add 300 μL of chloroform was added and mixed, and centrifuged at 13,000 rpm for 2 minutes.
5) 700 μL of the supernatant were removed into a new 1.5 mL centrifuge tube, and 490 μL of 70% isopropanol was added.
6) Centrifuge at 13,000 rpm for 2 minutes, and the supernatant was discarded.
7) After rinsing the precipitate twice with 500 μL of 75% ethanol, centrifuge at 12,000 rpm for 2 min, dry at 65℃, and dissolve 15-20 mL of sterile water previously heated.

1.2 Construction of recombinant expression vector

1.2.1 Enzyme digestion

Table3.Enzyme digestion system

Component	Volume（μL）
DDW	10
10×Buffer	5
Plasmid DNA	33
NcoI	1
BamHI	1
Total	50

Digestion conditions: 37℃water bath overnight

1.2.2gel extraction

The enzymatically digested product is electrophoresed, and the gel containing the target fragment was cut out under a gel imager. The GK2041 gel recovery kit was used for gel recovery. The specific steps are as follows:

1) Tapping
2) Add 400ul Binding Solution and bath at 55°C for 4min
3) Transfer the above mixture to the adsorption column GC-3u with a 2 ml collection tube, place it at room temperature for 2 min, centrifuge at 6000 rpm for 1 min at room temperature, and drain the waste liquid from the collection tube.
4) Put the adsorption column back into the collection tube, add 500 μL WA Solution, centrifuge at 12000 rpm for 1 min at room temperature, and drain the waste liquid.
5) Resorb the column again, add 500 μL Wash Solution, centrifuge at 12000 rpm for 1 min at room temperature, and drain the waste.
6) Repeat (5)
7) The adsorption column is put back again, centrifuge at 12000 rpm for 1 min, then open the lid at room temperature for 10 min.
8) Place the adsorption column into a clean 1.5ml collection tube, add 25μL Elution

   Buffer to the center of the membrane, cover the lid, place at 37℃ for 2min, and

   centrifuge at 12000rpm for 1min.

1.2.3 Seamless cloning

The Ezmax one-step rapid cloning kit was used to recombine the purified PCR product and the linearized and purified vector.

Table4.Ezmax system

Component	Volume（μL）
5×Buffer for Ezmax One-Step Cloning	4
Linearization vector	8
Exogenous DNA fragment	2
Ezmax recombinase	2
ddH_2O	4
Total volume	20

After 37 min of water bath at 37℃, the reaction product was placed on ice for 5 min to stop the reaction, and the reaction product was directly used for conversion or frozen at -20℃.

1.3 Preparation of competent cells

1.3.1 Preparation of competent cells by DH5ɑ

1) DH5ɑ is activated on LB plates and is divided into single colonies
2) Pick a single colony and inoculate it in 2-3 mL of LB liquid medium overnight.
3) Inoculate the seed solution in fresh LB medium at 37℃for 1.5-3 h at a ratio of 1%. When the OD600 reaches about 0.4-0.6, the culture is taken out and placed on ice for 15-30 min, then transferred to pre-cooled 50 mL. Centrifuge the tube at 5000 rpm for 5 min at4℃, discard the supernatant, and wash the cells once with sterile water.
4) Add 5mL of pre-cooled 0.1M calcium chloride solution to each centrifuge tube, and place it on ice for 15-30min, then gently shake the centrifuge tube until the bacteria at the bottom of the centrifuge tube are scattered (the whole process should be kept low temperature, gently Mix)
5) Then centrifuge at 5000 rpm for 5 min at 4℃ discard the supernatant, and then add an appropriate amount of pre-cooled 0.1 M calcium chloride solution containing 10% glycerol. Place on ice for 5 min, then shake the bacteria gently, then follow each 90 μL of the tube was dispensed into a pre-cooled 1.5 mL EP tube and stored at -80℃for later use.

1.4 transformation

1) A tube of prepared E. coli competent cells was placed in an ice water bath.
2) Add 10 ul of DNA ligation solution to 90 μL competent cell suspension, mix gently, and place in an ice water bath for 30 min.
3) Mix well, pulse for 90s in a 42-degree water bath, transfer quickly to an ice water bath, add 800ul LB medium this tube.Then incubate for 45min on a 37-degree shaker.

1.5 Identification

1.5.1 Colony PCR

Pick 5-10 monoclonal clones from the culture dish and incubate them in a 10 mL centrifuge tube and carry out colony PCR.

Table5. Colony PCR System

Component	Volume（μL）
dH_2O	3

2×PCR Mix	5
FP-afe（10μM）	0.5
RP-afe（10μM）	0.5
DNA	1
Total	10

The colony PCR program was the same as the colony of the above PCR program, and then ran electrophoresis.

1.5.2 Sequencing

Send bacteria or plasmid to Thermo Fisher Scientific (China) Co, Ltd.

1.6 HSL characterization

Bacteria is cultured in LB culture medium adding with 0.1% ampicillin. After bacteria grows to logarithmic phase, adding HSL until the final concentration is 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}M. Then light absorption is measured.

2 rust remover

2.1 Siderophore gene cluster amplification

Primer sequence:

EntCE-BamHⅠ-XbaⅠ:5’-CTAGTCTAGACGCGGATCCATTTTGTGGAGGATGATATGGATACGTCACTG-3’

EntBA-PstⅠ-HindⅢ:5’-CCCAAGCTTAACTGCAGTTATGCCCCCAGCGTTGAGCC-3’

EntF-XbaⅠ:5’-CTACTAGTCTAGATTGCAGGAGGCACAATGAGC-3’

EntF-XmaⅠ:5’-TCCCCCGGGTCATTACTTTTAGCGTGCGCGATTTC-3’

EntD-XmaⅠ: 5’-TCCCCCCGGGGCAGGAGC-ACATGAAACCACGCATACCTCCCTCC-3’

EntD-SacⅠ:5-‘CGAGCTCGTTAATCGTGTTGGCACAGCGTTATGAC-3’

EntFD-P1:5’-GGTAGATTCGCTTTCATAGACAAAGGATCCTTGCAGGAGGCACAATGGCC4’

EntFD-P2:5‘-GACACCTTGCCCTTTTTGCCGGACTGCAGGCTCGAGTTAATCGTGTTGGCACAGCGTTATGAC-3’

Ent-Afe:5’-TGCGCTCAACGCTGGGGCATAACTGCAGCTCACCTTCGGGGG-3’

1) PCR was carried out using the EntCE-BamHI/EntBA-PstⅠ-HindⅢ primer to obtain the product entceba. BamHI/PstI double-cut entceba and pUC57-Afe-GFP, and S1 was linked to Afe-entCEBA.

Thermocycling

The PCR machine should be set to run the following steps.

Table6. PCR Settings

Pre-denaturation	94℃	5min	
Transsexual	94℃	30s	35circles
Annealing	60℃	30s	
Extend	72℃	3min	
Extend	72℃	5min	

2） Using EntF-XbaI/EntF-XmaI and EntD-XmaI/EntD-SacI, respectively, PCR was performed to obtain EntF and EntD, and then EntF was digested by XbaI/XmaI, entD was digested with XmaI/SacI, and double digestion with XbaI/SacI was performed. pUC19, S1 is connected to pUC19-EntFD

Thermocycling

The PCR machine should be set to run the following steps

Table7. PCR Settings

Pre-denaturation	94℃	5min	
Transsexual	94℃	30s	35circles
Annealing	61℃	30s	
Extend	72℃	4min	
Extend	72℃	5min	

Thermocycling

The PCR machine should be set to run the following steps

Table8. PCR Settings

Pre-denaturation	94℃	5min	
Transsexual	94℃	30s	35circles
Annealing	61℃	30s	
Extend	72℃	1min	
Extend	72℃	5min	

2) Using pUC19-EntFD in (2) as a template and EntFD-P1/EntFD-P2 as primers for PCR, EntFD.BamHI/PstI double-digested pUC57-Afe-GFP, and the enzyme digestion product and EntFD were cloned seamlessly. Afe-EntFD.

Thermocycling

The PCR machine should be set to run the following steps

Table9. PCR Settings

Pre-denaturation	94℃	5min	
Transsexual	94℃	30s	30circles
Annealing	60℃	30s	
Extend	72℃	2min	
Extend	72℃	5min	

3) PCR was performed using afe-EntFD in (3) as a template and Ent-Afe/EntFD-P2 as a primer. Afe-EntBACE was digested with PstI, and the digested product and PCR product were cloned seamlessly to obtain Afe-EntBACEFD.

Thermocycling

The PCR machine should be set to run the following steps

Table10. PCR Settings

Pre-denaturation	94℃	5min	
transsexual	94℃	30s	30circles
annealing	60℃	30s	
extend	72℃	5min	
extend	72℃	5min	

2.2 Preparation of competent cells and transformation

2.3 Identification

2.3 1enzyme digestion identification

HindⅢ/EcoRⅠ was double-digested to identify Afe-EntFD, and BamHI was digested to identify Afe-EntBACEFD.

2.3.2 Sequencing

The bacteria solution was sent to Thermo Fisher Scientific (China) Co., Ltd. for sequencing.

2.4 Enterobactin characterization

2.4.1 Enterobactin purification

1) The culture solution was centrifuged at 10,000 rpm for 20 min to take the supernatant.
2) Add 10N HCl to pH=2
3) Equal volume of ethyl acetate extraction 3 times
4) 45℃rotary drying
5) The precipitate was dissolved in 200 ul of methanol, and the supernatant was centrifuged at 13,000rpm for 5 min at 21℃.

2.4.2 Enterobactin remove rust

1) .Arnow quantitative method and make the DHB(2,3-dihydroxybenzoic acid) standard curve. DHB is diluted to 10μM, 50μM, 100μM, 200μM, 300μM, 400μM, 500μM, 600μM, 700μM, respectively. Take 1ml each in a test tube, add 1 ml of 0.5M HCl, 1 ml of molybdate solution (1 g of sodium nitrite + 1 g of sodium molybdate plus water to 10 ml), 1 ml of 1M NaOH, mix, and measure the absorbance at 510 nm. The siderophore has three DHB precursors, and the content is equivalent to 1/3 of the calibration
2) Making a ferrous ion standard curve.
   Add 40 μL of 1,10-phenanthroline (0.3%, w/v), 40 μL of hydroxylamine hydrochloride (1%, w/v), 40 μL of ammonium acetate to each well of a 96-well plate. (0.1M). Then, add 0, 10, 20, 30, 40, 50, 90 μL of ferrous ion working solution to the well plate containing the above mixed solution, and adjust the final volume to 250 μl with high purity water. The concentration of ferrous ions is respectively 0, 1, 2, 3, 4, 5 and 9 mg/L. This step was repeated twice and three sets of parallel experiments were performed. Next, blow three times with a pipette to thoroughly mix. The solution was allowed to stand for 20 min to completely develop color. The absorbance was measured at 510 nm. Finally, record the data, place it and fit it.
3) Sample descaling experiment
   Take 1ml of sample solution into the rust sample, shake at 22℃, 220 rpm, collect the supernatant after 2h. Add sample 20μl, 0.3% 1,10-phenanthroline (w/v) 40μl, 1% hydroxylamine hydrochloride(w/v) 40μl, 0.1M ammonium acetate 40μl and 110μl water respectively. Then mix the solution and react for 20 min. Light absorption is measured under 510nm.Light absorption is plugged into ferrous iron standard curve then concentration of ferric iron is got. After centrifugation, the rust sample was taken, washed, and then added with 10% oxalic acid solution, 22℃, shaking at 220 rpm for 30 min, and the iron ion concentration was measured in the same manner.
4) Prepare 10^{-1} and 10^{-6}M oxalic acid solution as the derusting reaction sample,and the specific steps are the same as 3)

3.Biofilm remover

3.1 Obtain the target gene,DspB and plasmid DNA

3.1.1 PCR

Primer sequence

FP-DspB1:5'-GGCAGCCATATGAAGAAAGCAATTACACT-3'

RP-DspB1:5'-GTGGTGCTCGAGTTAATGAGATTTCGGATCATTTG-3'

Thermocycling

The PCR machine should be set to run the following steps

Table11. PCR Settings

<table>
<tr><td>Pre-denaturation</td><td>94℃</td><td colspan="2">5min</td></tr>
<tr><td>Transsexual</td><td>94℃</td><td>30s</td><td rowspan="3">30circles</td></tr>
<tr><td>Annealing</td><td>55℃</td><td>30s</td></tr>
<tr><td>Extend</td><td>72℃</td><td>3min</td></tr>
<tr><td>Extend</td><td>72℃</td><td colspan="2">5min</td></tr>
</table>

The PCR product was purified using an AP-PCR-250 kit.

3.1.2. Extracting pET-28a(+)

3.2 Recombinant expression vector construction

1) BamHⅠ/XhoⅠ double-cleavage of the purified products of the PCR products DspB and pET-28a(+);

2) Measure the concentration, calculate and formulate the S1 connection system.

3.3 BL21 competent cell preparation and transformation

3.4 Identification

3.4.1 Colony PCR

If the bacteria P is positive, keep the 10mL centrifuge tube and continue the shaker culture, extract the plasmid to the OD of about 0.6, and perform the PCR again using the plasmid as a template.

3.4.2 SDS-PAGE

Table12.SDS-PAGE Separation and Concentrate Preparation

Component	Separation gel (μL)	Concentrated gel (μL)
Double distilled water	1650	2700
30% acrylamide	2000	670
Separation gel buffer	1250	--
Concentrated Gum Buffer	--	500
10%SDS	50	40
10% ammonium persulfate	50	40
TEMED	2.5	2.5
Total volume	5000	4000

After the gelatinization is completed, the protein electrophoresis is carried out. After about 45 minutes, the electrophoresis is completed, stained with Coomassie brilliant blue, stained for a period of time, and the gel is immersed in a petri dish and decolorized until the non-stripe portion of the gel is discolored.

3.4.3 Sequencing

Send bacteria or plasmid to Thermo Fisher Scientific (China) Co, Ltd.

3.5. Characterization

3.5.1 preparation

1) Cells cultured condition: LB culture medium+0.1% kanamycin
Cultured under 37℃，220 rpm overnight, then inoculated into fresh medium and cultured until the logarithmic phase.
2) Add IPTG to a final concentration of 20 and culture overnight at 25 ℃ .Final OD_{600}=0.437(diluted 16 fold,SD=0,017).MW of target protein is about 40 kDa.
3) 20 ml bacteria solution are disrupted and supernatant acts as DSPB enzyme liquid.

3.5.2 Antibiofilm experiments

1) E coli DH5a is cultured in LB culture medium under 37℃，220 rpm overnight
2) Transferred to 96-well microtiter plates. After 24 hours' standing at 37℃，supernatant is abandoned.
3) Biofilm is washed by PBS buffer and acted with enzyme agent overnight.
4) Biofilm is dyed by Crystal violet and washed by ethanol.
5) Solution is transferred into new plates and measured the light absorption of 570nm. The lower the OD_{570} is, the better the antibiofilm activity is.

3.5.3 Activity measurement

4-nitrophenyl-N-acetyl-β-D-glucosaminide(NP-GlcNAc) is hydrolysed by DspB and releases 4-nitrophenol having the maximum light absorption under 405nm.

4 Fur inverter

4.1 DNA fragment amplification

Designed in the downstream of the -35 region of the lacI promoter, in the middle of the -35 region and the -10 region, and upstream of the -10 region, respectively, the furbox sequence was replaced, and the modified promoter sequences fur1, fur2, fur3 were obtained and submitted to Genscript.to synthesize.

Primer sequence

RP-fur:5' CCAAACTGCGCGCCGCTACTATTATCACTGCCGCTTTCCAGTCG-3'

FP-fur1:5'-
TGCCGGAATTCGCGGCCGCTTCTAGAGGACACCATCGAATGGCGGAATGATAATCAT
TATC-3'

FP-fur2:5'- TTCGEATTCGCGCCTCTAGAGTATATCATTATCGATGGCGCA-3

FP-fur3:5'-TGCGCTCAACGCTGGGGCATAACTGCGCTCCCTTCGCGTGG-3'

4.1.1fur1 Thermocycling

The PCR machine should be set to run the following steps

Table13. PCR Settings

Pre-denaturation	94℃	5min	
Transsexual	94℃	30s	30circles
Annealing	65℃	30s	
Extend	72℃	3min	
Extend	72℃	5min	

The PCR product was purified using an AP-PCR-250 kit.

4.1.21fur2 Thermocycling

The PCR machine should be set to run the following steps

Table14. PCR Settings

Pre-denaturation	94℃	5min

Transsexual	94℃	30s	30circles
Annealing	65℃	30s	
Extend	72℃	3min	
Extend	72℃	5min	

The PCR product was purified using an AP-PCR-250 kit.

4.1.3 fur3 Thermocycling

The PCR machine should be set to run the following steps

Table15. PCR Settings

Pre-denaturation	94℃	5min	
transsexual	94℃	30s	30circles
annealing	65℃	30s	
extend	72℃	3min	
extend	72℃	5min	

The PCR product was purified using an AP-PCR-250 kit.

4.2 Construction of recombinant expression vector

1) BamH Ⅰ/Xho Ⅰ double-cleavage of pET-28d to obtain linearized plasmid
2) The synthetic sequence was seamlessly cloned with the linearized vector.

4.3DH5α competent cell preparation and transformation

4.4 Identification

1) Pick a single clone for colony PCR
2) If the colony PCR is positive,this monoclonal continue the shaker culture, extract the plasmid to the OD of about 0.6, and perform the PCR again using the plasmid as a template.
3) Submit the bacterial liquid to the sequencing of Thermo Fisher Scientific (China) Co., Ltd.

4.5 characterization

Bacteria is cultured in LB culture medium adding with 0.1% kanamycin. Fluorescence intensity is measured at excitation wavelength of 587nm and emission wavelength of 610nm.

Fe deficit medium is added 2,2'-bipyridine to final concentration of 200uM.Fe excess medium is added $FeCl_3$ to a final concentration of 100uM. Bacteria is inoculated at 1% seed culture medium in Fe deficit medium and Fe excess medium and cultured for 12 hours. Then fluorescence intensity is measured.

5Auto-Lysin

5.1 Obtain and amplify DNA fragment

Primer sequence

FP:5'-AGCTCGAATTCGGATCCTTACTGCGTTTCCAC-3'

RP:5'-AAGGAGATATACCATGGCCCAATGAAAAAAATAACAGGG-3'

Thermocycling

The PCR machine should be set to run the following steps

Table16. PCR Settings

Pre-denaturation	94℃	5min	
Transsexual	94℃	30s	30circles
Annealing	58℃	30s	
Extend	72℃	3min	
Extend	72℃	5min	

5.2 Recombinant expression vector construction

1) BamH Ⅰ/Nco Ⅰ double-cleavage of pET-28d to obtain linearized plasmid
2) The synthetic sequence was seamlessly cloned with the linearized vector

5.3 Preparation of competent cells and transformation

5.4 Identification

1) Pick a single clone for colony PCR
2) If the colony PCR is positive,this monoclonal continue the shaker culture, extract the plasmid to the OD of about 0.6, and perform the PCR again using the plasmid as a template
3) Sequencing

5.5 Characterization

bacteria are cultured by LB medium adding with 0.1% kanamycin till logarithmic phase and induced by IPTG. Then culture solution is transferred into 96-well microtiter plates and measured the light absorption of 600 nm.

6.Anti-bacterial peptide

6.1Obtain the gene of interest and plasmid DNA

6.1.1 PCR

Primer sequence

FP:5’-GAGCTCGAATTCGGATAACTAGCCAAGG-3’

RP:5’-AGAAGGAGATATACCATGGCACTCGAGAAAAGA-3’

Thermocycling

The PCR machine should be set to run the following steps

Table17. PCR Settings

Pre-denaturation	94℃	5min	
Transsexual	94℃	30s	30circles
Annealing	57℃	30s	
Extend	72℃	3min	
Extend	72℃	5min	

6.1.2. Extraction of plasmid

6.2 Construction of recombinant expression vector

1) BamH/Nco double-cleavage of pET-28d to obtain linearized plasmid.
2) The synthetic sequence was seamlessly cloned with the linearized vector.

6.3 Preparation of competent cells and transformation.

6.4 Identification

1) Pick a single clone for colony PCR
2) If the colony PCR is positive,this monoclonal continue the shaker culture, extract the plasmid to the OD of about 0.6, and perform the PCR again using the plasmid as a

template.

3) Sequencing. Send bacteria or plasmid to Thermo Fisher Scientific (China) Co, Ltd.

6.5 Characterization.

1) Iron bacteria are cultured in Winogradsky culture medium with 1% inoculum size from seed medium.
2) Then medium solution is transferred into 96-well microtiter plates with different culture conditions. Then bacteria are cultured for 24 hours and OD_{600} is measured.

7.Light suicide

7.1 Obtaining the gene of interest and plasmid

7.1.1 PCR

Vector is cut by PstⅠ and SpeⅠ, Mnase is from biobrick BBa_K902019.Exogenous gene is amplified by PCR

Primer sequence

FP:5’-AATGGTTGCATGTACTAAGGAGGTACTAGTATGACAGAGTATCTCCTAAGCGCG-3’

RP:5’-CACCTTGTAGATGAACTCGCCGTCCTGCAGTTATTATTATTGACCGCTATCCGCGTTATCTTC-3’

Thermocycling

The PCR machine should be set to run the following steps

Table18. PCR Settings

Pre-denaturation	94℃	5min	
Denaturation	94℃	30s	30 circles
Annealing	63℃	30s	
Extension	72℃	1min	
Extension	72℃	5min	

The PCR product was electrophoresed and the position of the strip was observed and compared with the position of the envisaged strip. The PCR product was purified using the AP-PCR-250 kit.

7.1.2. Extraction of plasmid

7.2 Construction of recombinant expression vector

7.3 Preparation of competent cells and transformation

1) PstⅠ/SpeⅠ double-cleavage of pET-28d(+) to obtain linearized plasmid
2) The synthetic sequence was seamlessly cloned with the linearized vector

7.4 Identification

1) Pick a single clone for colony PCR
2) If the colony PCR is positive, this monoclonal continue the shaker culture, extract the plasmid to the OD of about 0.6, and perform the PCR again using the plasmid as a template
3) Sequencing. Send bacteria or plasmid to Thermo Fisher Scientific (China) Co, Ltd.

Send bacteria or plasmid to Thermo Fisher Scientific (China) Co, Ltd.

7.5 Characterization

7.5.1 Light-on mcherry characterization

Bacteria is cultured in LB culture medium adding with 0.1% streptomycin overnight, then 1% inoculum of culture solution is operated. Cells with light illumination all the time, with light illumination at the logarithmic phase, with light illumination at late period of logarithmic phase and cells in dark are measured fluorescence intensity. Wavelength of exciting light is 587nm, and wavelength of emitted light is 610nm

7.5.2 Light-on Mnase characterization

Bacteria are cultured in LB culture medium adding with 0.1% streptomycin. We divided these bacteria into two groups. One is cultured in the dark. The other is under the light. We measured OD600 to determine cell viability

Medium Formulation

1. MKB medium:

Casein acid hydrolysate	5g/L
Glycerol	15ml/L
$MgSO_4 \cdot 7H_2O$	2.5g/L
K_2HPO_4	2.5g/L
pH	7.2

$MgSO_4 \cdot 7H_2O$, K_2HPO_4 and casein acid hydrolysate need to sterilize separately. The glycerol can mix with casein acid hydrolysate before sterilization.

2. LB medium and LB iron limited medium:

Tryptone	10g/L
Yeast extract	5g/L
NaCl	10g/L
pH	7.2-7.4

When we need to limit the concentration of iron to characterize iron responsive element. We add 2,2-bipyridine. The final concentration of 2,2-bipyridine is 200mM.

3. M9 medium:

a. 5×M9 saline solution:

$Na_2HPO_4 \cdot 7H_2O$	12.8g
KH_2PO_4	3.0g
NaCl	0.5g
NH_4Cl	1.0g
ddH_2O	200ml

b. Aseptic operation to compound M9 medium:

5×M9 saline solution	200ml/L
1mol/L $MgSO_4$	2ml/L
20% glucose	20ml/L
1mol/L $CaCl_2$	0.1ml/L
Add ddH_2O	778ml /L

5×M9 saline solution, $MgSO_4$ and $CaCl_2$ need to sterilize separately. We can use 0.22μm filter head to filter bacteria for glucose. And when we use M9 saline solution to culture E.coli, adding1ml 100mM thiamine hydrochloride is required.

4. CAS plat:

2×Chromeazurol S	47 ml/100ml
2×Hexadecyl trimethyl ammonium bromide (HDTMA)	47ml/100ml
PBS(pH=6.8)	5ml/100ml
$FeCl_3$	1ml/100ml
Agar	0.9%

CAS and HDTMA need to sterilize separately. And we should use 0.22μm filter head to filter bacteria for $FeCl_3$.

5. IB medium:

$MgSO_4$	0.5g/L
$(NH_4)_2SO_4$	0.5g/L
KH_2PO_4	0.5g/L
$CaCl_2$	0.2g/L
$NaNO_3$	0.5g/L
Ammonium ferric citrate brown	10.0g/L
pH	6.8-7.2