2024.7.2-2024.8.7

1. Bacteria cultured in a 37℃ shaker, plasmid extraction after bacteria preservation Extracted plasmid concentration:

(1) pTZ28a-1913-GLP-1 glycerol bacteria: 118.9 ng/μL
(2) pTZ28a-sfGFP-cSAT-GLP-1-8: 119.0 ng/μL
(3) pTZ28a-sfGFP-cSAT-GLP-1-9: 101.65 ng/μL
(4) pTZ28a-sfGFP-cSAT-GLP-1-12: 128.75 ng/μL
(5) DH5a pET32a-His6 circular self-ligation - 10': 69.25 ng/μL
(6) DH5a pET32a-His6 circular self-ligation - 11': 52.6 ng/μL
(7) DH5a pET32a-His6 circular self-ligation - 12': 57.65 ng/μL
(8) DH5a pET32a-His6 circular self-ligation - 16': 54.75 ng/μL

2. Polymerase chain reaction

(1) Primers:

V-pTZ28a-F new 3C site-R

DNA: Activated glycerol bacteria of pTZ28a-1913-GLP-1 Size: 6,398 bp

Result: The concentration of the recovered plasmid fragment is 18.50 ng/μL.

(2) Primers:

V-pTZ28a-F new 3C site-R

DNA: pTZ28a-sfGFP-cSAT-GLP-1 plasmid Size: 6,398 bp

Result: The concentration of the recovered plasmid fragment is 19.35 ng/μL.

3. Monoclonal colony PCR

(1) Primers:

sfGFP15-F T5-4GLP-1/LV-R-28 Arm

DNA: DH5a pTZ28a-sfGFP-4GLP-1-5LV Size: 1,431 bp

Result: The band size of agarose gel electrophoresis is correct compared with the marker.

(2) Primers:

V-pTZ28a-F new 3C site-R

DNA: DH5a pTZ28a-sfGFP Size: 6,398 bp

Result: There is a band in agarose gel electrophoresis and the size is correct.

(3) Primers:

GLP-1-F GLP-1-R

DNA: DH5a pET32a-4GLP-1-5LV Size: 4,650 bp

Result: The bands of agarose gel electrophoresis are all correct.

(4) Primers:

V-pTZ28a-F new 3C site-R

DNA: Activated glycerol bacteria of pTZ28a-1913-GL-1 Size: 6,398 bp

Result: There is no band in agarose gel electrophoresis.

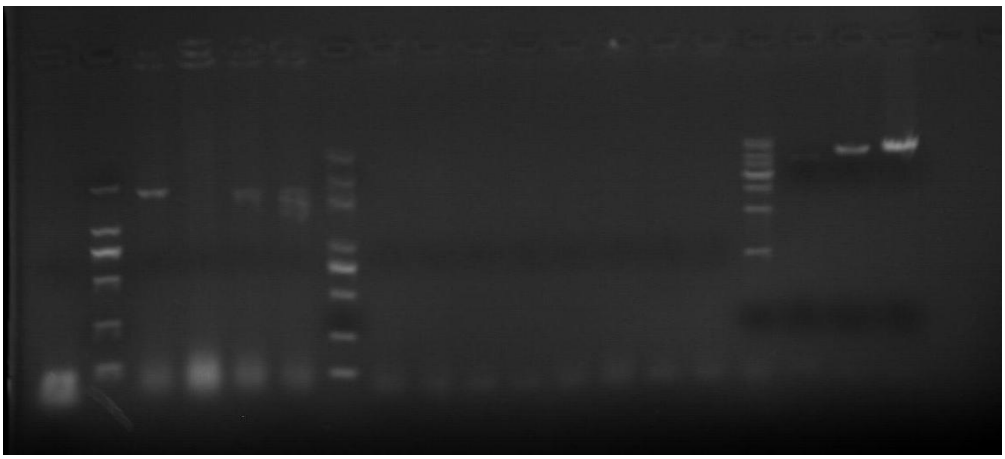

Figure 6

4. Bacterial Cultivation

For the water-soluble bacteria with correct band sizes in the agarose gel electrophoresis of single-clone colonies, 9 μL of each is separately aspirated and added to 10 ml of liquid culture meduim with the corresponding resistance. These cultures are then placed in a 37℃ shaker for overnight incubation. Strains such as DH5α pTZ28a empty strain, DH5α pTZ28a-sfGFP-4GLP-1-5LV strain, and DH5α pET32a-4GLP-1-5LV strain are obtained.

5. Preserve the bacteria and send it for sequencing.

After thorough mixing of each tube of bacteria, 200 μL from each is aspirated into 1.5 ml EP tubes. The submission forms are filled out, placed in the submission bags, and the samples are stored in a 4℃ refrigerator awaiting sampling. The samples are sent to a biological company for sequencing to further verify the correctness of the transformants.

6. Plasmid Extraction

(1) DH5α pTZ28a-His6-sfGFP-14: 163.65 ng/μL

(2) DH5α pTZ28a-His6-sfGFP-32: 85.75 ng/μL

(3) DH5α pTZ28a-sfGFP-4GLP-1-5LV-2: 102.8 ng/μL

(4) DH5α pTZ28a-sfGFP-4GLP-1-5LV-4: 186.55 ng/μL

(5) DH5α pTZ28a-sfGFP-4GLP-1-5LV-5: 180.5 ng/μL

(6) DH5α pET32a-4GLP-1-5LV-1: 62 ng/μL

(7) DH5α pET32a-4GLP-1-5LV-4: 68.75 ng/μL

(8) DH5α pET32a-4GLP-1-5LV-5: 61.3 ng/μL

(9) DH5α pET32a-4GLP-1-5LV-6: 76.25 ng/μL

7. Transformation of Escherichia coli

The plasmids pTZ28a-His6-sfGFP-14 and pTZ28a-sfGFP-4GLP-1-5LV-4 are transformed into the trans110 competent cells for demethylation. The transformed bacteria are placed in a 37℃ shaker for 45 minutes to 1 hour. Then, they are spread onto LK medium plates in a super-clean bench and placed in a 37℃ incubator for overnight incubation.

The plasmids pET32a-4GLP-1-5LV-4, pET32a-4GLP-1-5LV-6, pET32a empty vector circular self-ligation - 10', and pET32a-His6 circular self-ligation - 12' are separately transformed into BL21 (DE3) competent cells. The transformed bacteria are placed in a 37℃ shaker for 45 minutes to 1 hour. Then, they are spread onto LA medium plates in a super-clean bench, labeled, and placed in a 37℃ constant temperature incubator upside down for overnight incubation.

8. Electroporation

The plasmids pET32a-4GLP-1-5LV-4, pET32a-4GLP-1-5LV-6, pET32a-His6 circular self-ligation-10', and pET32a-His6 circular self-ligation-12' are respectively electroporated into Nissle1917 competent cells. The electroporated bacteria are placed in a 37℃ shaker for 4-6 hours. Then, they are spread onto LA medium plates in a super-clean bench. Four tubes of bacterial suspension are prepared, each containing 100 μL and 200 μL. Labels are marked, such as Nissle1917 pET32a-4GLP-1-5LV-47.12 CLY 100 μL, Nissle1917 pET32a-4GLP-1-5LV-47.12 CLY 200 μL, and placed in a 37℃ constant temperature incubator upside down for overnight incubation.

9. Monoclonal colony PCR

Perform Monoclonal colony PCR on the plates of pTZ28a-His6-sfGFP-14 and pTZ28a-sfGFP-4GLP-1-5LV-4 plasmid intestinal-transformed trans110 competent cells, using three rows of eight-tube strips. Twenty-three colonies are picked, with tube 24 serving as the plasmid control.

(1) Primers:

V-pTZ28a-F new 3Csite-R

DNA: trans110 pTZ28a-His6-sfGFP Size: 6,398 bp

Result: Agarose gel electrophoresis showed bands of the correct size.

(2) Primers:

GLP-1-F GLP-1-R

DNA: trans110 pTZ28a-sfGFP-4GLP-1-5LV Size: 4,650 bp

Result: Agarose gel electrophoresis showed that except for two bacterial samples, the bands of the remaining 21 bacterial samples are all correct compared to the marker size.

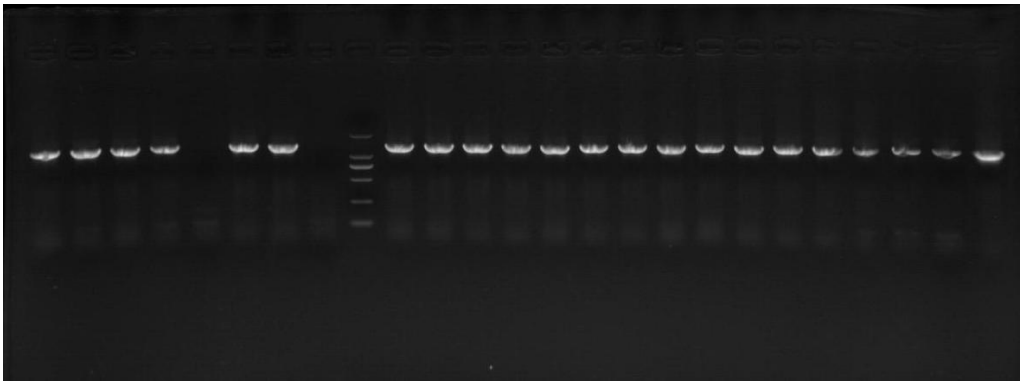

Figure 7

10. Monoclonal colony PCR is conducted on the four plates of intestinal transformation to BL21 and the eight plates of electrotransformation to Nissle1917.

(1) Primer:

GLP-1-F GLP-1-R

DNA: BL21 pET32a-4GLP-1-5LV Size: 4650 bp

Result: A band is observed in agarose gel electrophoresis with the correct size.

(2) Primer:

GLP-1-F GLP-1-R

DNA: BL21 pET32a-His6 circular self-ligation Size: 3978 bp

Result: A band is present and its size is correct.

(3) Primer:

GLP-1-F GLP-1-R

DNA: Nissle1917 pET32a-4GLP-1-5LV Size: 4650 bp

Result: A band is observed in agarose gel electrophoresis with the correct size, but

the band is rather faint.

(4) Primer

GLP-1-F GLP-R

DNA: Nissle1917 pET32a-His6 circular self-ligation Size: 3978 bp

Result: A band is present and its size is correct, but the band is rather faint.

11. After the verification of the transformed plates by Monoclonal colony PCR, the correct bacteria are aspirated into the corresponding liquid culture meduim and placed in a 37℃ shaker for cultivation, obtaining the trans110 pTZ28a-sfGFP-4GLP-1-5LV strain, BL21(DE3) pET32a-4GLP-1-5LV strain, BL21 (DE3) pET32a empty vector circular self-ligation strain, Nissle1917 pET32a-4GLP-1-5LV strain, and Nissle1917 pET32a empty vector circular self-ligation strain.

In a super-clean bench, 1 mL of liquid-cultured bacterial broth and 1 mL of 60% glycerol are pipetted into a cryotube, which is subsequently stored at -80℃. Concurrently, 1 mL of the bacterial broth is transferred into a 1.5 mL EP tube and dispatched to a biological company for sequencing to further confirm the integrity of the transformant.

12. Plasmids are extracted from trans110 pTZ28a-sfGFP-4GLP-1-5LV and trans110 pTZ28a-His6-sfGFP strains.

(1) pTZ28a-sfGFP-4GLP-1-5LV-1: 193.8 ng/μL

(2) pTZ28a-sfGFP-4GLP-1-5LV-7: 146.2 ng/μL

(3) pTZ28a-sfGFP-4GLP-1-5LV-8: 152.3 ng/μL

(4) pTZ28a-sfGFP-4GLP-1-5LV-19: 186.95 ng/μL

13. Electroporation

The demethylated plasmids pTZ28a-His6-sfGFP-14 and pTZ28a-sfGFP-4GLP-1-5LV are respectively electroporated into ZM4-T7 and ZMNP-T7 competent cells. After electroporation, the bacteria are placed in a 30℃ shaker for 4-6 hours, and then spread onto RK solid medium in a super-clean bench, labeled, and placed in a 30℃ constant temperature incubator upside down for cultivation.

14. After verification by Monoclonal colony PCR of the electroporation plates, the correct bacteria are aspirated into RK liquid medium and placed in a 30℃ shaker for cultivation, obtaining the ZM4-T7 pTZ28a-His6-sfGFP strain, ZMNP-T7 pTZ28a-His6-sfGFP strain, ZM4-T7 pTZ28a-sfGFP-4GLP-1-5LV strain,

and ZMNP-T7 pTZ28a-sfGFP-4GLP-1-5LV strain.

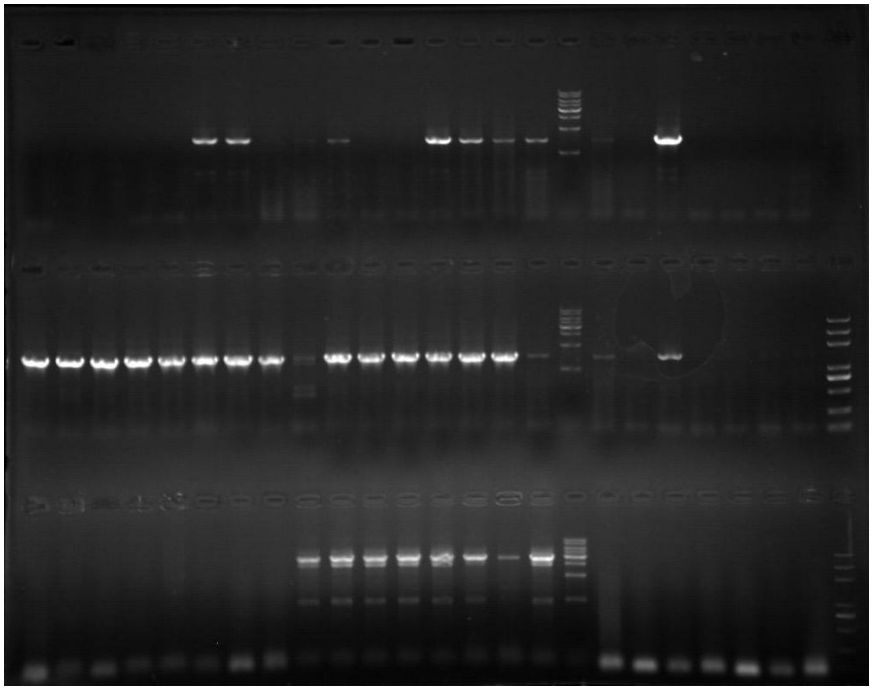

Figure 8

15. Cryopreserve strain and send for sequencing

ZM4-T7 pTZ28a-His6-sfGFP strain, ZMNP-T7 pTZ28a-His6-sfGFP strain, ZM4-T7 pTZ28a-sfGFP-4GLP-1-5LV strain and ZMNP-T7 pTZ28a-sfGFP-4GLP-1-5LV strain, and send them for sequencing.