2024.1.19-2024.1.31

Increase the number of TAB operon copies

1. Polymerase chain reaction

Pgap-T1AB operon is obtained by using pEZ15A-PgT1 plasmid as template.

2. T4 Ligase-mediated

The plasmid PL2R-PGT1 is obtained by reacting the recovered P*gap*-T1AB fragment with pL2R vector at 22℃ for 4-5 hours under T4 enzyme ligation system. pL2R-PgT1 edited plasmid is used to replace the ZMO1650 site of the ZMNP genome with the P*gap*-T1AB operon to obtain strain NPTΔ1, and then the recombinant plasmid pEZ15A-PtT1 is transferred to obtain 3-HB multi-copy strain NPTΔ1-PtT1. Mix 1 mL of bacterial liquid with 1 mL of 60% glycerin in the cryopreservation tube in the super-clean bench, and store it in the refrigerator at -80℃.

3. Fermentation

(1) Firstly, 100 μL of glycerol bacteria are inoculated into a cryotube containing 1 mL of RMG5 (containing 100 μg/mL spectinomycin) medium and statically activated in a 30℃ incubator until turbidity is achieved.

(2) The activated bacterial liquid in the cryotube is poured into a 50 mL centrifuge tube containing an appropriate amount of RMG5 (with corresponding antibiotics) medium as the fermentation seed liquid and statically cultured in a 30℃ incubator until the middle and late logarithmic phase. It is inoculated into the RMG5 (with corresponding antibiotics) medium with 80% filling volume in a 50 mL Erlenmeyer flask, with the initial OD600nm controlled at 0.1, and fermented at 100 rpm and 30℃.

(3) At fixed intervals, 1 mL of the sample is taken out in a super-clean bench for collection. The OD600nm of the bacterial liquid is detected using a UV-1800 UV spectrophotometer and recorded. The remaining samples are frozen at -80℃ for subsequent detection.

(4) The data graphs are plotted using Graphpad 9.0 (Insightful Science, CA, USA) software.

4. Analysis by High Performance Liquid Chromatography (HPLC)

The samples are centrifuged at 12,000 rpm for 4 minutes, and the supernatant is collected and filtered through a 0.22 µm filter. 400 µL samples are taken into an HPLC injection vial for the detection of the contents of glucose, ethanol, and 3-HB in the HPLC (High Performance Liquid Chromatography).