2024.4.11-2024.5.15

1. Construction and Testing of Combined Strains

Mix 30 µL of the empty editing plasmid pL2R with the BsaI enzyme and incubate at 37℃ for 5-8 hours for enzymatic digestion. Subsequently, recover the digested products to obtain the digested vector. Under the T4 ligase system, ligate the EUP fragment with the digested vector using T4 ligase at 22℃ for 4-5 hours to obtain the editing plasmid pL2R-EUP. Integrate the EUP operon into the ZMO1094 locus of the ZMNPTΔ2 strain, from which ZMO1089 has been knocked out, to obtain the strain ZMNPTΔ3.

2. Preparation of Competent Cells

Prepare the ZMNPTΔ3 strain into competent cells following the competent cells preparation protocol.

3. Electroporation

Electroporate the plasmid pEZ-PtZT1 into the ZMNPTΔ3 competent cells. Verify the electroporation plates through Monoclonal colony PCR. Inoculate the water-soluble bacteria of the correct strain into a 50 mL centrifuge tube containing 8 mL of RS liquid culture medium, and incubate overnight in a 30℃ shaker. Preserve the bacteria to obtain the strain ZMNPTΔ3-PtZT1^{EUP}.

4. Have both NPTΔ1-PtZT1EUP and NPTΔ3-PtZT1EUP undergo fermentation in RMG5 medium with a carbon-to-nitrogen ratio (C/N) of 50/10 and in a medium with a C/N ratio of 50/5, respectively.

5. Analysis by High Performance Liquid Chromatography (HPLC)

Centrifuge the samples at 12,000 rpm for 4 minutes, collect the supernatant, filter it through a 0.22 µm filter, and transfer 400 µL samples to an HPLC injection vial for detection of the contents of glucose, ethanol, and 3-HB in the HPLC (High-Performance Liquid Chromatography).

6. Search the hypoglycetic peptide sequence and choose the sequence GLP-1.

7. **Search the protein purification method and determine the cSAT method.**

8. **Construct the pET32a-L6KD-PT linker-Mtu ΔI-CM-GLP-1 plasmid to express GLP-1 through cSAT method by snapgene, and synthesized it through biotechnology company.**

9. **Improve the codons of L6KD-PT linker-Mtu ΔI-CM-GLP-1, and synthesized it through biotechnology company. Construct pET32a-L6KD-PT linker-Mtu ΔI-CM-GLP-1 plasmid through T5.**