2024.5.15-2024.6.8

1. **Design the verification primers of the plasmid pET32a-L6KD-PT linker-Mtu ΔI-CM-GLP-1**

   GLP-1-F：5’GACTGACGACAGTTTTGACAC

   GLP-1-R：5’TCTTTATCATGCAACTCGTAGG

   size：4665 bp

2. **Transfer pET32a-L6KD-PT linker-Mtu ΔI-CM-GLP-1 plasmid to DH5αcloning strain, and plasmid extraction(the concentration is: 43.05 ng/μL、 59.10 ng/μL).**

3. **The concentration of extracted plasmid is very low, design several experiments of "optimization practice of plasmid extraction steps".**

optimization practice of extraction plasmid steps			
Time	The volume of harvested bacteria	Other optimization actions	the concentration of extracted plasmid （ng/μL）
2024.5.24	2	culture 18 h	43.05 ng/μL
	4	culture 18 h	59.10 ng/μL
2024.5.28	2	culture 12 h	38.00 ng/μL
	2	culture 12 h	43.15 ng/μL
	4	culture 18 h	46.90 ng/μL
	4	culture 18 h	56.85 ng/μL
2024.5.29	10	culture 18 h centrifuge twice	106.30 ng/μL↑
	10	culture 18 h Centrifuge twice，and	111.90 ng/μL↑

		ish twice	

Result of the experiments shows: increase the volume of harvested bacteria, the concentration increases obviously. The use of extraction plasmid kits will be improved.

4. **Transfer pET32a-L6KD-PT linker-Mtu ΔI-CM-GLP-1 plasmid whose concentration is 59.10 ng/μL to BL21(DE3) expression strain, to verify through agarose gel electrophoresis after monoclonal colony PCR**

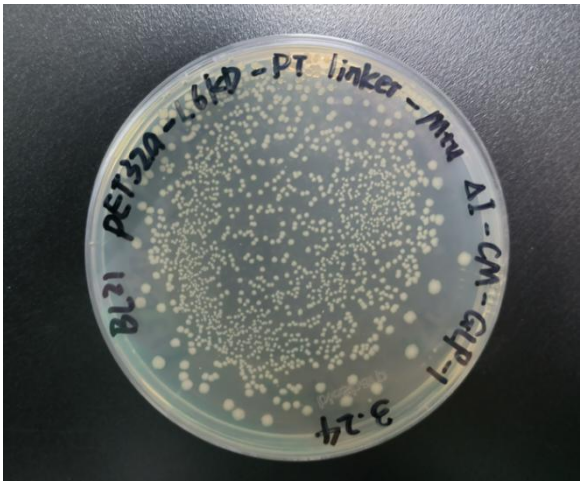

Figure 1 primers:GLP-1-F/GLP-1-R size:4665bp

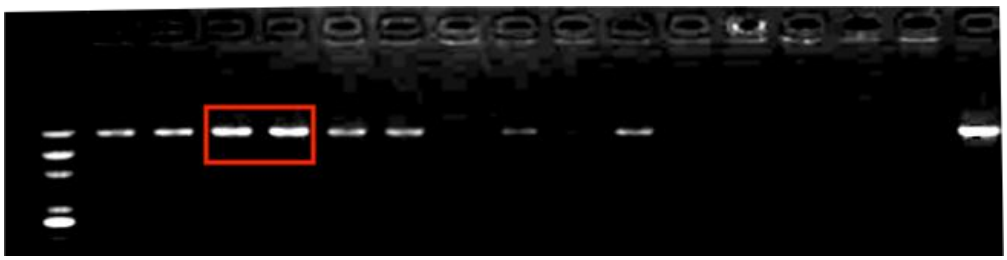

Figure 2 The Result of Agarose Gel Electrophoresis of pET32a-cSAT-GLP-1

5. **Pick two single colonies that have passed PCR verification and add them to 10 mL of LA liquid culture medium for overnight incubation for 18 hours. Preserve the bacteria and prepare for protein expression. Optimize the steps, extract the plasmid (concentration: 106.30 ng/μL, 111.90 ng/μL), and store it in the refrigerator at 4℃.**

6. **Send the plasmid samples to the company for sequencing verification:**

Sequencing primer (T7P-R): 5' tctcaaaaaacccctcaagacccg Size: 1039bp

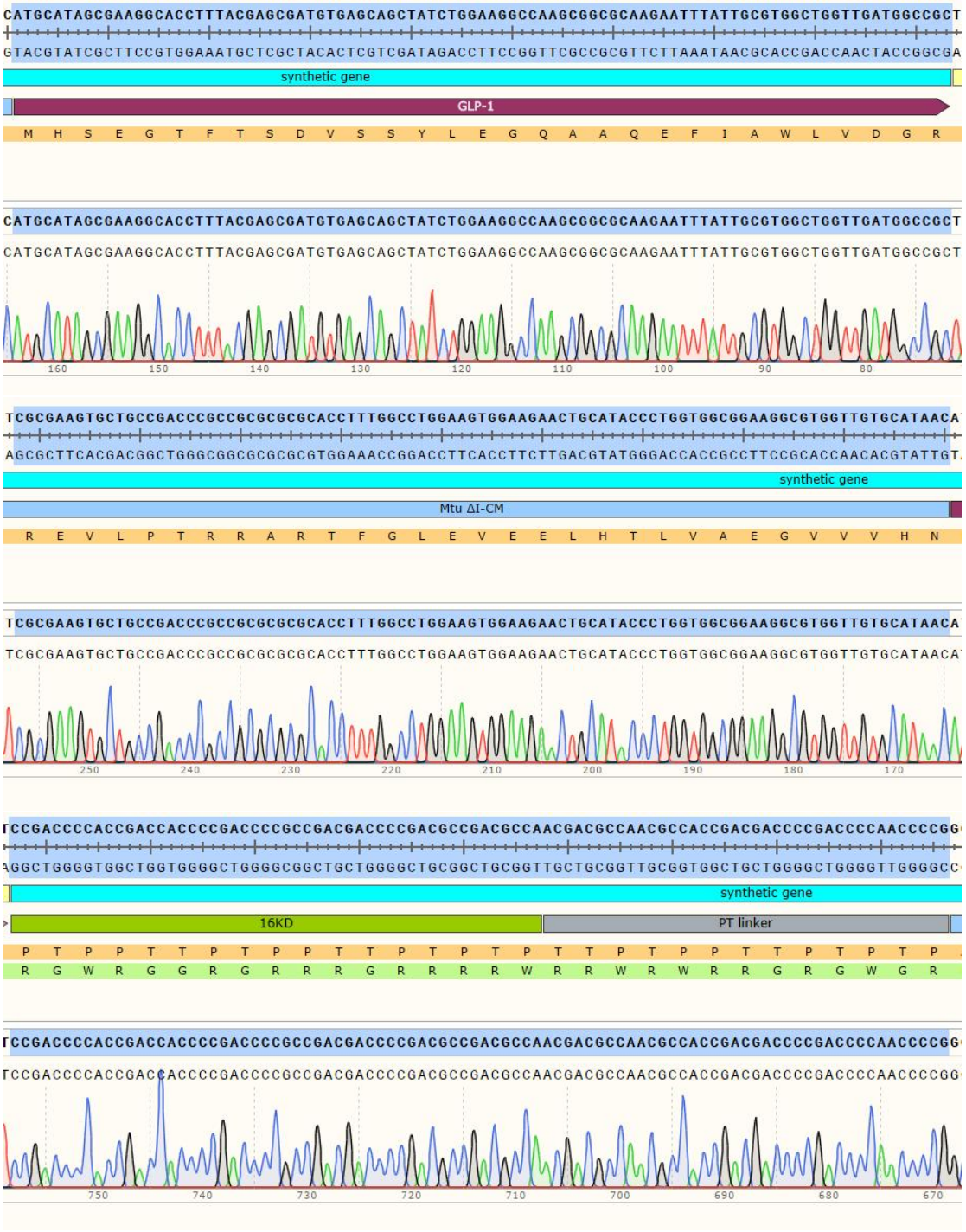

Figure 3 Sequencing Results of the L6KD-PT Linker-Mtu ΔI-CM-GLP-1

7. **Preserve the strain of BL21 pET32a-L6KD-PT linker-Mtu ΔI-CM-GLP-1 (Serial Number： 7, 16, 21, 22) . Prepare for protein expression and purification.**