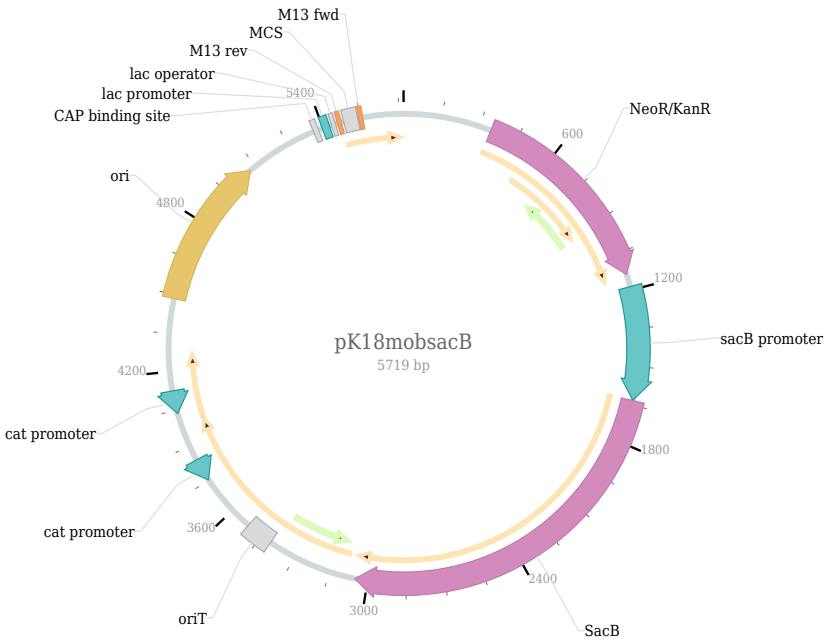
pK18mobsacB

载体编号 Vector ID	V005269
抗性 Antibiotic Resistance	Kanamycin
长度 Length	5719 bp
载体类型 Vector Type	Corynebacterium glutamicum knockout vectors

产品信息

复制子 Replication Origin	ori
来源 Source/Author	Kvitko BH, Collmer A.
启动子 Promoter	sacB
克隆方法 Cloning Method	Enzyme digestion and ligation
培养菌株 Growth Strain(s)	stbl3
培养温度 Growth Temperature	37℃
备注 Comment	The pK18mobsacB vector contains the sacB element. The SacB gene, from Bacillus subtilis and related to sugar metabolism, converts sucrose to levan. In Escherichia coli lacking genes to process levan, expressing SacB leads to the accumulation of levan and causes bacterial death. Thus, by simply adding 10% sucrose to the medium, E. coli can be screened. In the CRISPR Cas9 system for gene knockout and targeted insertion in microorganisms, including E. coli, the sacB gene in the Cas9 plasmid helps eliminate the plasmid post-recombination. When sucrose is present, the protein encoded by SacB converts sucrose to fructose, and fructose accumulation is toxic to E. coli. Strains that can lose the plasmid survive under this pressure, while those unable to do so die.

质粒图谱



复溶方法

1. Centrifuge at 5,000×g for 5 min.
2. Carefully open the tube and add sterile water to dissolve the DNA: add 20 µl for 5 µg plasmid, and 100 µl for 100 µg plasmid.
3. Close the tube and incubate for 10 minutes at room temperature.
4. Briefly vortex the tube and then do a quick spin to concentrate the liquid at the bottom. Speed is less than 5000×g.
5. Store the plasmid at -20 ℃.