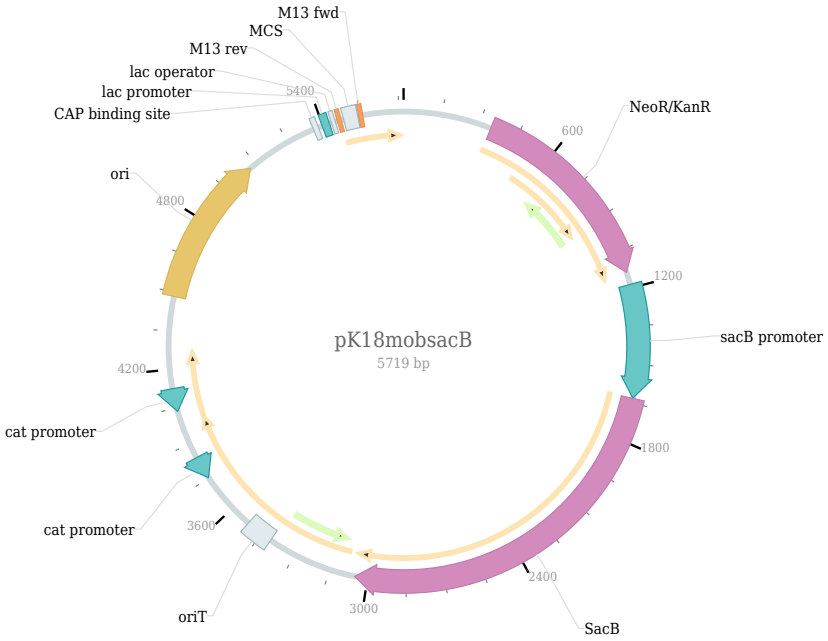
pK18mobsacB

Vector ID	V005269
Antibiotic Resistance	Kanamycin
Length	5719 bp
Vector Type	Corynebacterium glutamicum knockout vectors

Product Information

Replication Origin	ori
Source/Author	Kvitko BH, Collmer A.
Promoter	sacB
Cloning Method	Enzyme digestion and ligation
Growth Strain(s)	DH10B
Growth Temperature	37℃
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.

Vector Map



Plasmid Resuspension Protocol

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 ℃.