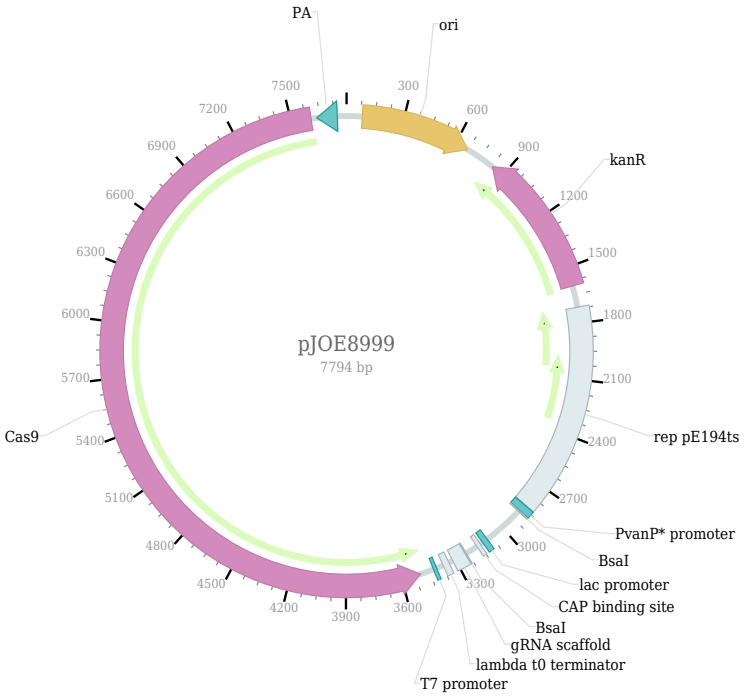
pJOE8999

Vector ID	V047698
Antibiotic Resistance	Kanamycin
Length	7794 bp
Vector Type	CRISPR; shuttle vector
Host	Bacillus subtilis

Product Information

Replication Origin	ori
Copy Number	Low copy number
Promoter	manP
Growth Strain(s)	DH10B
Growth Temperature	30℃
Expression Method	mannose induced
Comment	pJOE8999 is a single-plasmid CRISPR-Cas9 shuttle vector designed for scar-free genome editing in Bacillus subtilis. It carries the pUC origin for propagation in Escherichia coli, the temperature-sensitive pE194ts replicon for B. subtilis, and a kanamycin-resistance marker for selection in both hosts. Its cas9 gene is controlled by the mannose-inducible manP promoter, while sgRNA is constitutively transcribed; BsaI sites enable easy replacement of sgRNA spacers and SfiI sites accept homologous-repair templates for recombination-mediated DNA repair of Cas9-triggered double-strand breaks. This vector supports large-fragment deletions, point-mutation repair, and can be cured at high temperature, offering an alternative to traditional selection-counterselection editing methods and showing potential for adaptation to other firmicutes.

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 ℃.