# pJOE8999

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| **载体编号 Vector ID** | V047698 |
| **抗性 Antibiotic Resistance** | Kanamycin |
| **长度 Length** | 7794 bp |
| **载体类型 Vector Type** | CRISPR; shuttle vector |
| **宿主 Host** | Bacillus subtilis |

## 产品信息

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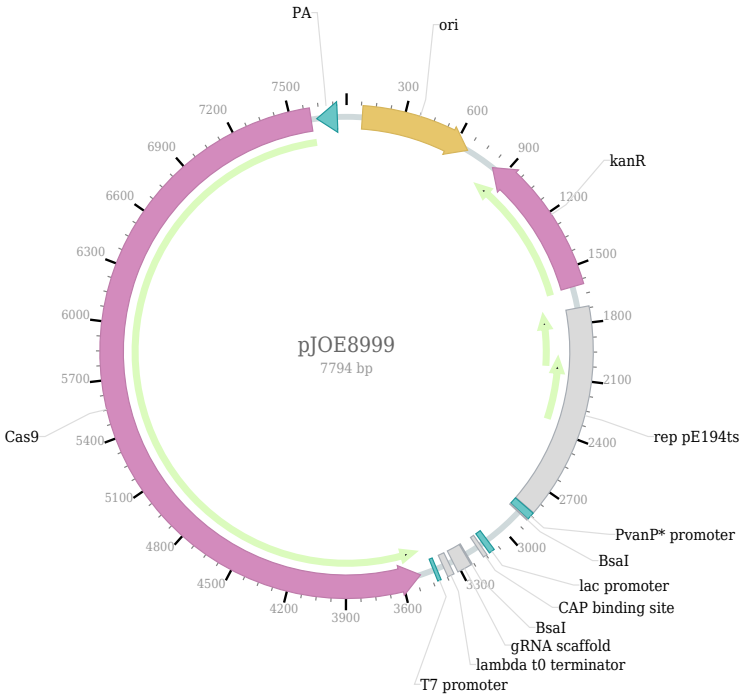
| **复制子 Replication Origin** | ori |
| **拷贝数 Copy Number** | Low copy number |
| **启动子 Promoter** | manP |
| **培养菌株 Growth Strain(s)** | DH10B |
| **培养温度 Growth Temperature** | 30℃ |
| **表达方式 Expression Method** | mannose induced |
| **备注 Comment** | pJOE8999 is a single-plasmid CRISPR-Cas9 shuttle vector 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. |

## 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 ℃.