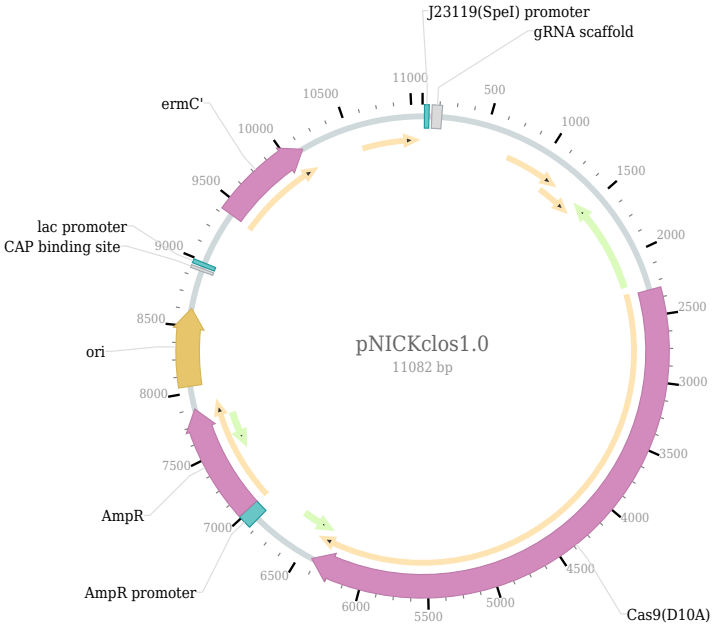
pNICKclos1.0

载体编号 Vector ID	V016659
抗性 Antibiotic Resistance	Ampicillin
长度 Length	11082 bp
载体类型 Vector Type	Gene knockout
宿主 Host	Clostridium

产品信息

复制子 Replication Origin	ori
筛选标记 Selection Marker	Ery
启动子 Promoter	J23119(SpeI)
培养菌株 Growth Strain(s)	DH10B
培养温度 Growth Temperature	37℃
备注 Comment	pNICKclos1.0 is an all-in-one CRISPR-Cas9 nickase shuttle plasmid built on pIMP1-Pptb backbone for Clostridium acetobutylicum ATCC 824 genome editing. It carries D10A-mutated Cas9 nickase, target sgRNA driven by Pj23119, and homologous repair templates flanking target genes. Unlike wild-type Cas9 plasmids, it generates single-strand nicks instead of lethal double-strand breaks to boost transformation and editing efficiency. It enables scarless gene knockout in 3 days, followed by 2-day plasmid curing for successive rounds of mutagenesis in solventogenic clostridia.

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 ℃.