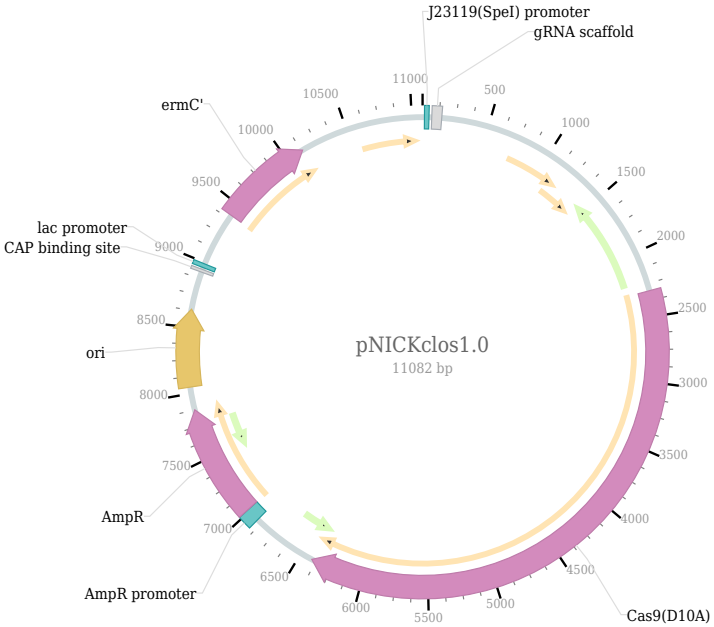
pNICKclos1.0

Vector ID	V016659
Antibiotic Resistance	Ampicillin
Length	11082 bp
Vector Type	Gene knockout
Host	Clostridium

Product Information

Replication Origin	ori
Selection Marker	Ery
Promoter	J23119(SpeI)
Growth Strain(s)	DH10B
Growth Temperature	37℃
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.

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 ℃.