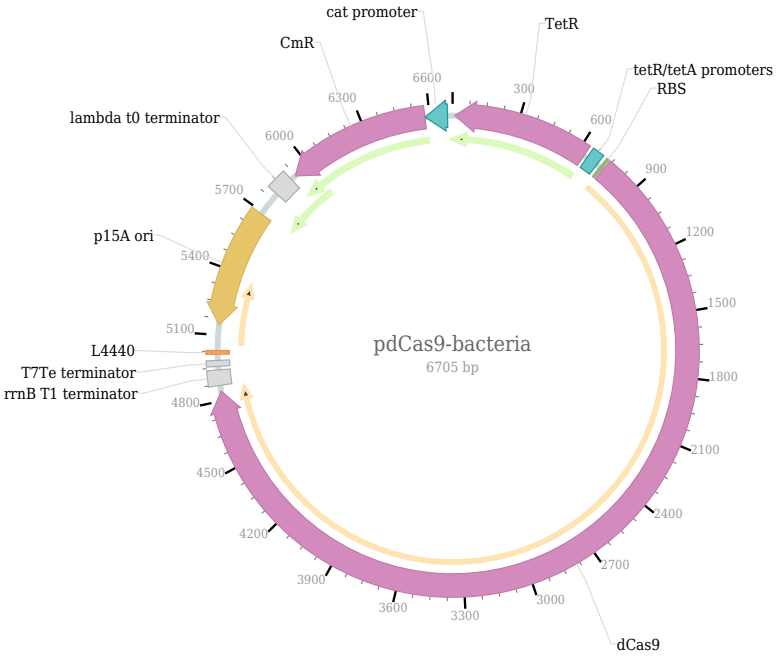
pdCas9-bacteria

Vector ID	V006766
Antibiotic Resistance	Chloramphenicol
Length	6705 bp
Vector Type	Bacterial Expression, CRISPR

Product Information

Replication Origin	p15A ori
Copy Number	Low Copy
Promoter	pLtetO-1
Cloning Method	Restriction Enzyme
5' Primer	ttcaaaaGATCTAAAGAGGAGAAAGGATCTATGGATAAGAAATACTCAATAG
3' Primer	tgcctggagatccttactcgaGTTAGTCACCTCCTAGCTGACTCAAATCAAT
Growth Strain(s)	Stbl3
Comment	pdCas9-bacteria is a low-copy bacterial plasmid from the Qi lab that expresses catalytically dead dCas9 (D10A/H841A mutant S. pyogenes Cas9) under anhydrotetracycline (aTc) induction. It carries chloramphenicol resistance and a p15A replication origin, compatible with pgRNA-bacteria for dual-plasmid CRISPRi in E. coli. The dCas9 protein lacks DNA-cutting activity but forms sgRNA-guided DNA-binding complexes to sterically block bacterial transcription initiation or elongation. Its inducible expression enables reversible, tunable gene knockdown without toxic genomic double-strand breaks from wild-type Cas9.

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 ℃.