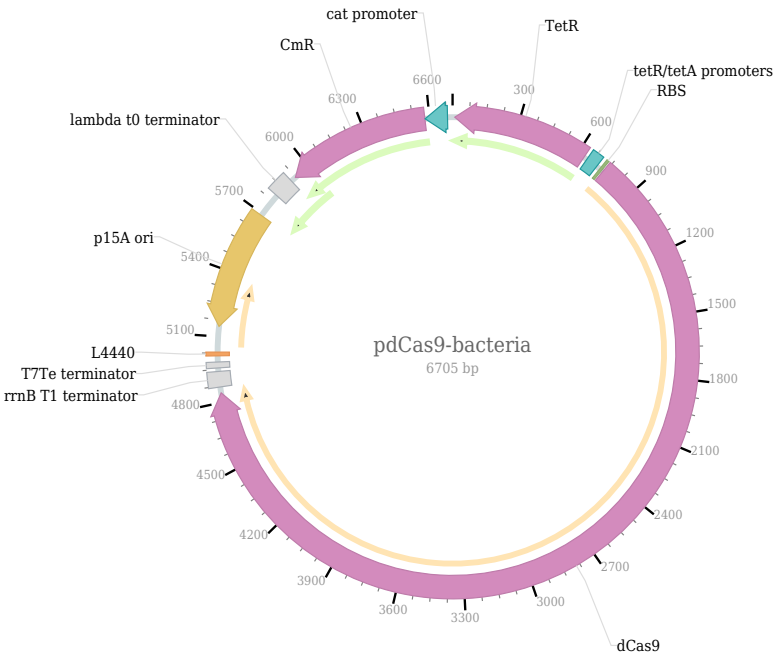
pdCas9-bacteria

载体编号 Vector ID	V006766
抗性 Antibiotic Resistance	Chloramphenicol
长度 Length	6705 bp
载体类型 Vector Type	Bacterial Expression, CRISPR

产品信息

复制子 Replication Origin	p15A ori
拷贝数 Copy Number	Low Copy
启动子 Promoter	pLtetO-1
克隆方法 Cloning Method	Restriction Enzyme
5' 引物 5' Primer	ttcaaaaGATCTAAAGAGGAGAAAGGATCTATGGATAAGAAATACTCAATAG
3' 引物 3' Primer	tgcctggagatccttactcgaGTTAGTCACCTCCTAGCTGACTCAAATCAAT
培养菌株 Growth Strain(s)	Stbl3
备注 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.

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 ℃.