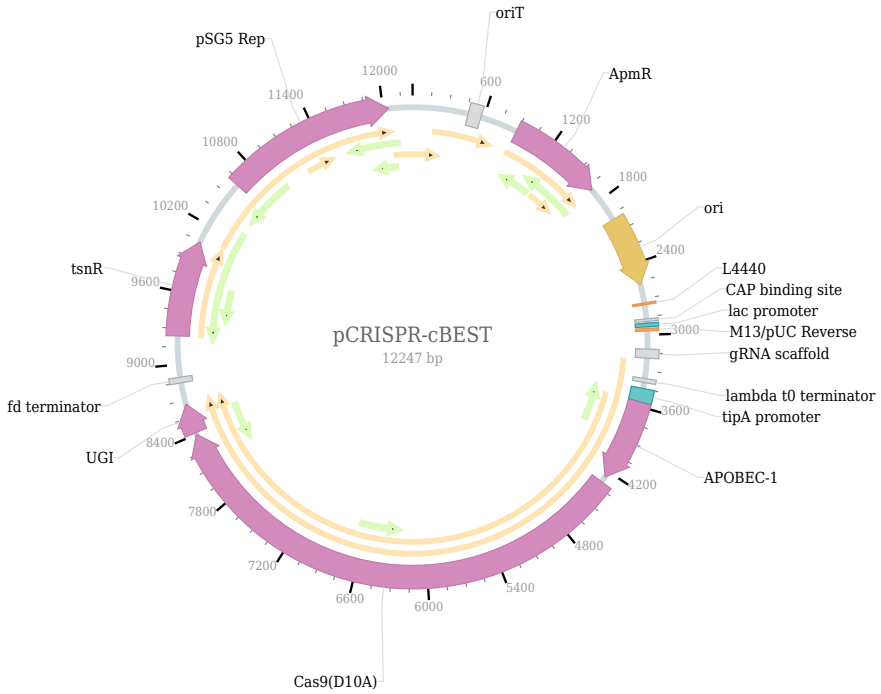
pCRISPR-cBEST

载体编号 Vector ID	V001084
抗性 Antibiotic Resistance	Apramycin
长度 Length	12247 bp
载体类型 Vector Type	CRISPR, Synthetic Biology

产品信息

复制子 Replication Origin	ori
拷贝数 Copy Number	High Copy
启动子 Promoter	ermE*/tipA
克隆方法 Cloning Method	Gibson Cloning
培养菌株 Growth Strain(s)	DH10B
培养温度 Growth Temperature	37℃
备注 Comment	pCRISPR-cBEST is a temperature-sensitive pSG5 shuttle plasmid for DSB-free cytosine base editing across Streptomyces strains, carrying a fusion of codon-optimized rAPOBEC1 deaminase, Cas9n(D10A) and UGI driven by the thiostrepton-inducible PtipA promoter. Guided by sgRNA, it precisely converts C:G to T:A within a 7-nucleotide editing window, with substrate preference of TC > CC > AC > GC and editing efficiency up to nearly 100% to introduce stop codons for gene knockout. It outperforms conventional CRISPR-Cas9 by avoiding lethal large chromosomal deletions, capable of simultaneously mutating two identical duplicated gene copies in non-model Streptomyces species like S. collinus Tü365. Integrated with a Csy4-mediated sgRNA processing module, this all-in-one vector also enables single-plasmid multiplex editing of multiple biosynthetic pathway genes with high fidelity and mild genome-wide off-target effects.

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 ℃.