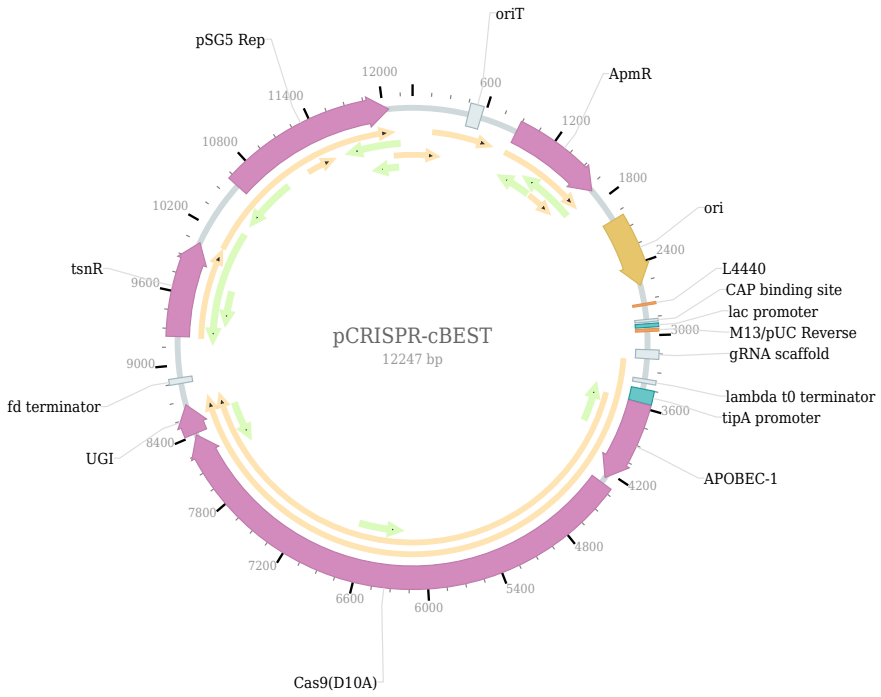
pCRISPR-cBEST

Vector ID	V001084
Antibiotic Resistance	Apramycin
Length	12247 bp
Vector Type	CRISPR, Synthetic Biology

Product Information

Replication Origin	ori
Copy Number	High Copy
Promoter	ermE*/tipA
Cloning Method	Gibson Cloning
Growth Strain(s)	DH10B
Growth Temperature	37℃
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.

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 ℃.