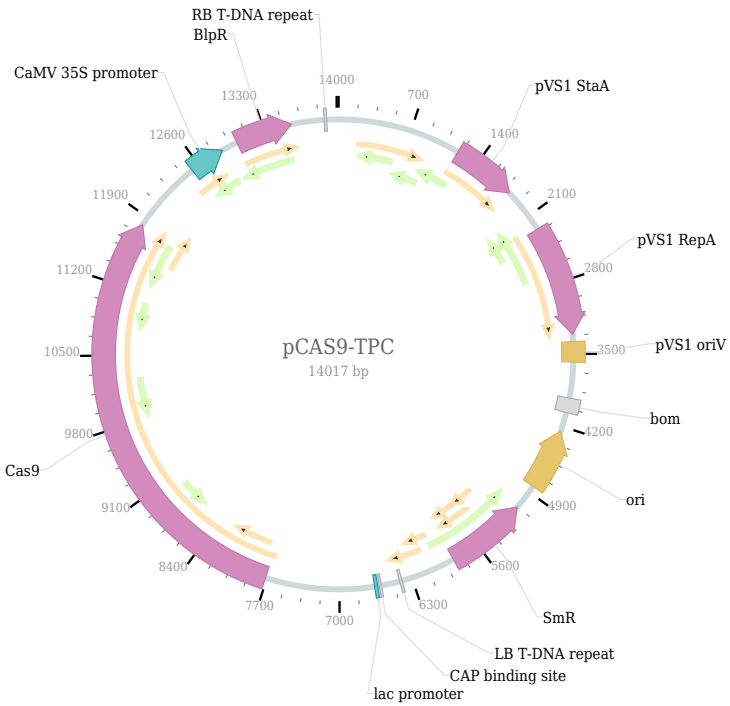
pCAS9-TPC

载体编号 Vector ID	V016618
抗性 Antibiotic Resistance	Streptomycin
长度 Length	14017 bp
载体类型 Vector Type	Gene knockout
宿主 Host	Plants

产品信息

复制子 Replication Origin	ori
筛选标记 Selection Marker	Bar
启动子 Promoter	UBI
培养菌株 Growth Strain(s)	DH5a
培养温度 Growth Temperature	37℃
备注 Comment	pCAS9-TPC (T = pea3A terminator, P = PcUbi4-2 promoter, C = codon-optimized Cas9) is an Agrobacterium binary CRISPR/Cas9 vector developed by Holger Puchta's lab for targeted gene knockout in Arabidopsis thaliana, published in The Plant Journal (2014). Derived from modified pPZP201 backbone, it carries an A. thaliana codon-optimized wild-type SpCas9 nuclease driven by the constitutive PcUbi4-2 promoter, terminated by pea3A polyA signal. The T-DNA harbors a Bar cassette for Basta selection of transgenic plants, with an AvrII site to insert AtU6-26-controlled sgRNA cassettes from intermediate plasmid pChimera via standard restriction-ligation cloning. This vector generates site-specific DNA double-strand breaks (DSBs) to trigger error-prone NHEJ, yielding heritable indel mutations with frequencies up to 70% in T2 progeny. Its counterpart pCAS9-D10A bears a D10A nickase mutation, which barely induces NHEJ but retains robust homologous recombination activity.

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 ℃.