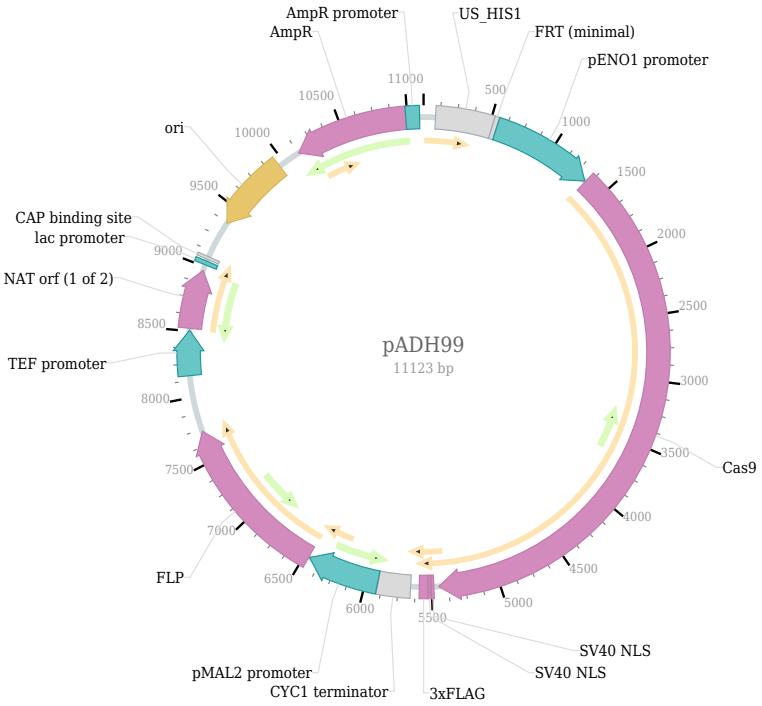
pADH99

载体编号 Vector ID	V014678
抗性 Antibiotic Resistance	Ampicillin
长度 Length	11123 bp
载体类型 Vector Type	Genome editing
宿主 Host	Yeast

产品信息

复制子 Replication Origin	ori
筛选标记 Selection Marker	NAT
启动子 Promoter	TEF
培养菌株 Growth Strain(s)	DH10B
培养温度 Growth Temperature	37℃
备注 Comment	pADH99 and pADH100 are the two-component plasmids of the HIS-FLP recyclable CRISPR-Cas9 genome editing toolkit for Candida albicans. They must be co-transformed as linearized DNA fragments to achieve markerless, iterative gene manipulation in nourseothricin-sensitive strains. pADH99 is the Cas9 expression vector carrying the first half of split NAT marker (NAT 1 of 2). Core elements: US_HIS1 upstream homology arm, FRT recombination site, pENO1-driven Cas9 cassette, maltose-inducible FLP recombinase, and partial NAT resistance fragment. pADH99 functions: Delivers Cas9 nuclease and FLP recombinase; provides 5′ homologous sequence for integration at the HIS1 genomic locus; supplies half of NAT fragment to reconstitute intact nourseothricin resistance after recombination with pADH100. After maltose induction, FLP excises all CRISPR and NAT components flanked by dual FRT sites. Linearized pADH99 and pADH100 co-transform C. albicans. Split NAT fragments recombine to form a full NAT cassette for nourseothricin selection. US_HIS1 and DS_HIS1 direct the whole CRISPR cassette to integrate at the endogenous HIS1 locus. Cas9-sgRNA complex creates DNA double-strand breaks at target genes, stimulating HDR-mediated homozygous knockout, point mutation or gene addback without permanent markers at edited loci. Maltose-induced FLP recombinase removes all integrated Cas9, sgRNA and NAT sequences, leaving only a single FRT scar at HIS1. The recovered nourseothricin-sensitive strain can undergo multiple rounds of CRISPR editing.

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 ℃.