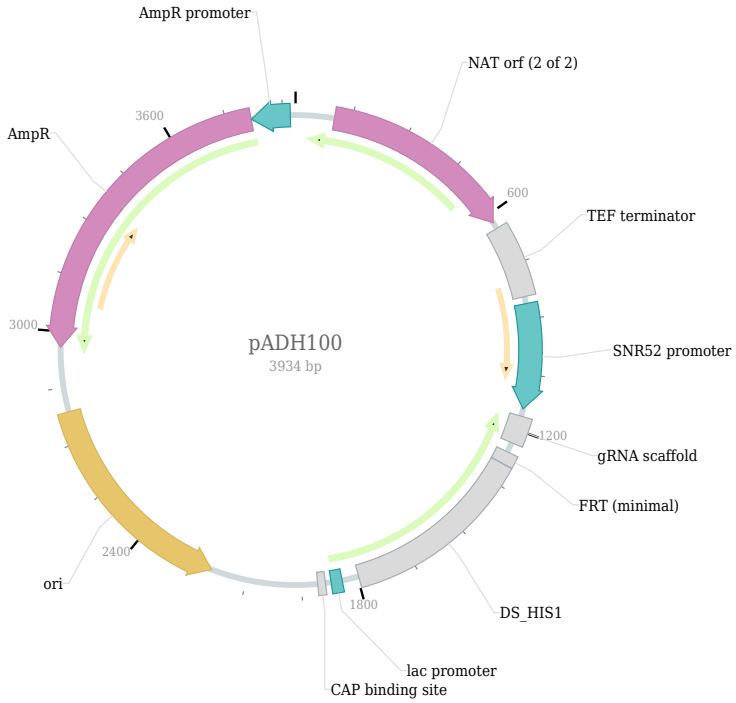
pADH100

Vector ID	V014668
Antibiotic Resistance	Ampicillin
Length	3934 bp
Vector Type	Genome editing
Host	Yeast

Product Information

Replication Origin	ori
Selection Marker	NAT
Growth Strain(s)	DH10B
Growth Temperature	37℃
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. pADH100 is the blank sgRNA backbone vector with the second half of split NAT marker (NAT 2 of 2). Core elements: DS_HIS1 downstream homology arm, another FRT site, pSNR52 sgRNA expression scaffold, partial NAT fragment, and universal sgRNA cloning/stitching template. pADH100 functions: Provides 3′ homologous sequence for HIS1 locus integration; accepts target-specific 20 nt guide sequence via cloning-free stitching PCR or soCPEC; completes functional NAT marker when recombined with pADH99's NAT segment. 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.

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 ℃.