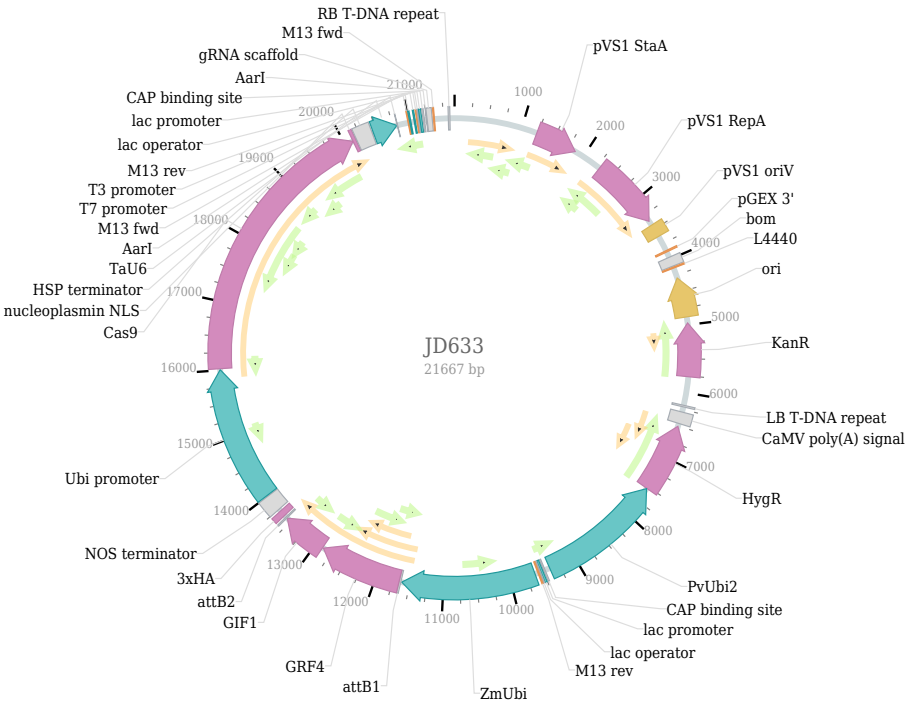
JD633

Vector ID	V012098
Antibiotic Resistance	Kanamycin
Length	21667 bp
Vector Type	Binary vector

Product Information

Replication Origin	ori
Selection Marker	Hygromycin
Promoter	Ubi
Growth Strain(s)	stbl3
Comment	JD633 is an Agrobacterium binary vector optimized for high-efficiency CRISPR-Cas9 genome editing in wheat and other monocot crops. The chimeric GRF4-GIF1 fusion protein and wheat codon-optimized TaCas9 are both driven by the maize Zea mays ZmUbiquitin promoter. This fusion drastically boosts somatic regeneration efficiency, expands the range of transformable wheat genotypes, and enables cytokinin-free plant regeneration. Target gRNA is transcribed by the endogenous Triticum aestivum TaU6 Pol III promoter. Two flanking AarI type IIS restriction sites are integrated downstream of TaU6, enabling one-step Golden Gate assembly to rapidly insert custom gRNA spacer sequences via simultaneous AarI digestion and ligation. The hygromycin resistance gene (hptII/hygr) for transgenic cell selection is controlled by the Panicum virgatum PvUbi2 promoter with a CAT1 intron to enhance expression in monocot callus. All functional expression cassettes are assembled within a single T-DNA, combining regeneration enhancement and targeted gene editing in one construct. After genome editing, the GRF4-GIF1 and CRISPR-Cas9 components can be genetically segregated in T_1 progeny to generate transgene-free edited wheat plants.

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 ℃.