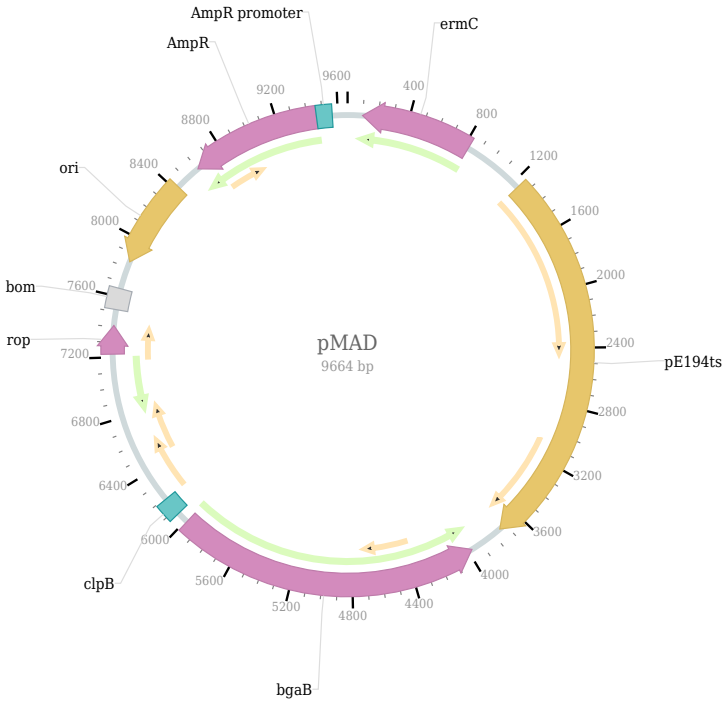
pMAD

Vector ID	V007385
Antibiotic Resistance	Ampicillin
Length	9664 bp
Vector Type	Prokaryotic Expression Vectors

Product Information

Replication Origin	ori
Growth Strain(s)	DH10B
Growth Temperature	37℃
Comment	The pMAD vector is a shuttle vector designed for efficient allelic replacement in naturally nontransformable, low-GC-content Gram-positive bacteria. It features two key components: (i) a temperature-sensitive replication origin (pE194ts) derived from the Staphylococcus aureus plasmid pE194, which enables replication at permissive temperatures (≤32°C) but is fully blocked at nonpermissive temperatures (≥37°C); and (ii) a constitutive clpB promoter (pclpB) from Staphylococcus aureus that drives expression of the thermostable β-galactosidase gene (bgaB) from Bacillus stearothermophilus, facilitating blue-white screening on X-Gal plates without IPTG induction. Below is the step-by-step protocol for gene deletion using this vector. 1. Recombinant vector construction: Tandemly assemble "upstream homology arm of target gene – selection cassette (in the middle) – downstream homology arm", and clone into pMAD's multiple cloning site (e.g., BamHI/NcoI). 2. Transformation & primary screening: Electrotransform recombinant plasmid into Gram-positive bacteria; spread on "erythromycin + X-Gal" solid medium, incubate at 30°C for 2–3 days. Blue colonies indicate successful vector entry and free replication (pE194ts functions at permissive temperature; bgaB expressed via pclpB promoter). 3. Single crossover (vector integration): Pick 3–5 blue colonies, inoculate into erythromycin-containing liquid medium, shake-culture at 39–42°C for 6–12h (nonpermissive temperature blocks pE194ts replication, forcing single crossover-mediated chromosomal integration). Serial dilute and spread on "erythromycin + X-Gal" medium, incubate at 39–42°C for 18–24h. Select uniform blue colonies (successful integrants: erythromycin-resistant, vector retained). 4. Double crossover (gene deletion + vector excision): Pick 1–2 blue integrant colonies, inoculate into antibiotic-free liquid medium, culture at 30°C for 6h (permissive temperature promotes double crossover and vector excision from chromosome). Transfer to 42°C for 3–6h (nonpermissive temperature eliminates non-excised vectors). 5. Double crossover screening: Serial dilute and spread on "antibiotic-free + X-Gal" medium. White colonies = successful double crossover (vector lost, bgaB removed, target gene deleted); blue colonies = unexcised integrants (discard).

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 ℃.