pNZ5319

Vector ID	V004242
Antibiotic Resistance	Erythromycin
Length	3781 bp
Vector Type	Mutagenesis vector

Product Information

Replication Origin	p15A ori
Source/Author	Lambert JM, Bongers RS, Kleerebezem M.
Copy Number	Medium copy number
Growth Strain(s)	DH5alpha
Growth Temperature	37℃
Comment	For L. plantarum, 10 μg/ml chloramphenicol and 10 μg/ml or (for replica plating) 30 μg/ml erythromycin were used. For E. coli, 10 μg/ml chloramphenicol (coud be as high as 37.5 μg/ml) and 250 μg/ml erythromycin were used. pNZ5319 is a mutagenesis vector designed for targeted gene deletion in Gram-positive bacteria like Lactobacillusand Lactococcusspecies. It functions as a suicide vector in these hosts, carrying a lox66-P32-cat-lox71 cassette to replace the target gene via double-crossover homologous recombination. The vector contains origins of replication and selectable markers (chloramphenicol and erythromycin resistance) for propagation in E. coli, facilitating cloning. Its key feature is compatibility with the Cre-loxP system, allowing subsequent excision of the antibiotic resistance marker to generate marker-free mutants.

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 ℃.