pLP12

Vector ID	V004953
Antibiotic Resistance	Chloramphenicol
Length	3885 bp
Vector Type	Suicide vector

Product Information

Replication Origin	R6K γ ori
Source/Author	Luo P, He X, Liu Q, Hu C.
Promoter	araBAD
Growth Strain(s)	DH5α-λpir
Growth Temperature	30℃
Comment	pLP12 is a novel suicide T-vector developed for rapid and efficient gene deletion in Vibrio species. To use pLP12, first, homologous arms (flanking sequences of the target gene) are amplified by PCR. These arms are directly ligated into AhdI-digested linearized pLP12. The recombinant plasmid is transformed into E. coli β2163 (a donor strain with efficient conjugation machinery, pir+) and transferred into Vibrio (pir-) via conjugation. Transconjugants with plasmid integration (first recombination) are selected on chloramphenicol-containing plates with D-glucose (to repress vmi480). For counterselection, these integrants are plated on medium with L-arabinose to induce vmi480, and surviving colonies (undergoing second recombination) are verified as target gene deletion mutants via PCR and sequencing.

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 ℃.