pLP12

载体编号 Vector ID	V004953
抗性 Antibiotic Resistance	Chloramphenicol
长度 Length	3885 bp
载体类型 Vector Type	Suicide vector

产品信息

复制子 Replication Origin	R6K γ ori
来源 Source/Author	Luo P, He X, Liu Q, Hu C.
启动子 Promoter	araBAD
培养菌株 Growth Strain(s)	DH5α-λpir
培养温度 Growth Temperature	30℃
备注 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.

质粒图谱



复溶方法

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 ℃.