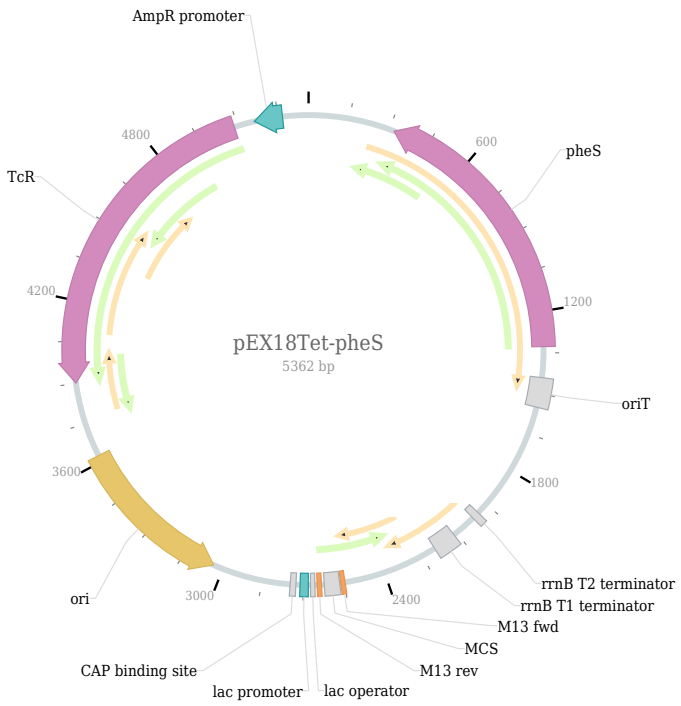
pEX18Tet-pheS

载体编号 Vector ID	V013759
抗性 Antibiotic Resistance	Tetracycline
长度 Length	5362 bp
载体类型 Vector Type	Allelic exchange in Burkholderia sp.
宿主 Host	E. coli

产品信息

复制子 Replication Origin	ori
来源 Source/Author	Ashley R. Barrett, Tung T. Hoang (tongh@hawaii.edu)
筛选标记 Selection Marker	pheS Counter-selectable Marker
拷贝数 Copy Number	High Copy
启动子 Promoter	lac
5' 引物 5' Primer	M13 fwd
3' 引物 3' Primer	M13 rev
培养菌株 Growth Strain(s)	DH5a
培养温度 Growth Temperature	37℃
备注 Comment	The pEX18Tet-pheS is a conjugative plasmid designed for allelic replacement studies in Burkholderia species. One of the most notable features of pEX18Tet-pheS is its dual - selectable marker system. It harbors the Tet (tetracycline) resistance gene, which allows for the selection of transformed cells on media containing tetracycline. Simultaneously, the A304G mutated pheS gene serves as a counter - selectable marker. In the presence of 0.1% p-chlorophenylalanine (cPhe), cells carrying the wild-type pheS gene will be killed, while cells with successful conjugation and allelic replacement events can be effectively selected. To use pEX18Tet-pheS, one should first introduce the plasmid into the target Burkholderia cells, commonly via conjugation with a suitable donor strain (such as E.coli). After conjugation, the cells are plated on tetracycline - containing media to select for plasmid - bearing bacteria. Subsequently, counter-selection on media with 0.1% cPhe is performed to identify the bacteria that have undergone the correct allelic replacement.

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 ℃.