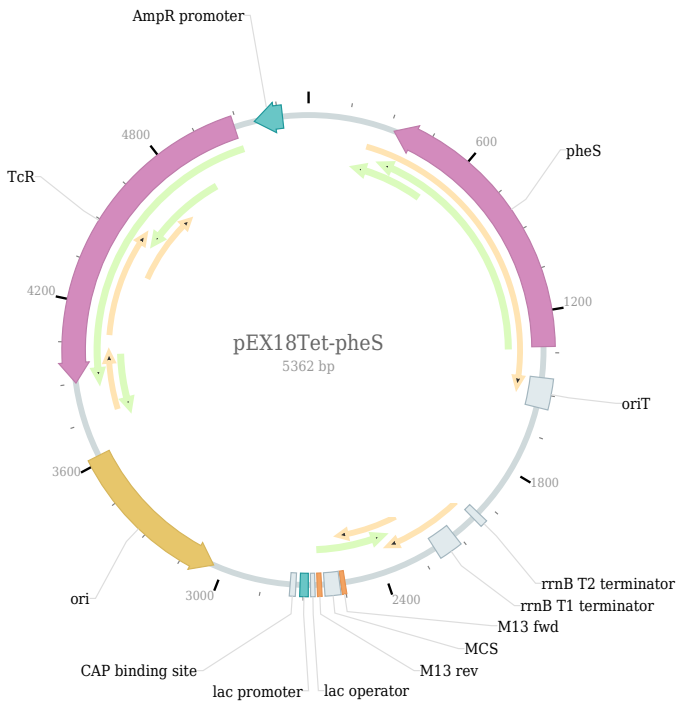
pEX18Tet-pheS

Vector ID	V013759
Antibiotic Resistance	Tetracycline
Length	5362 bp
Vector Type	Allelic exchange in Burkholderia sp.
Host	E. coli

Product Information

Replication Origin	ori
Source/Author	Ashley R. Barrett, Tung T. Hoang (tongh@hawaii.edu)
Selection Marker	pheS Counter-selectable Marker
Copy Number	High Copy
Promoter	lac
5' Primer	M13 fwd
3' Primer	M13 rev
Growth Strain(s)	DH5a
Growth Temperature	37℃
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.

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 ℃.