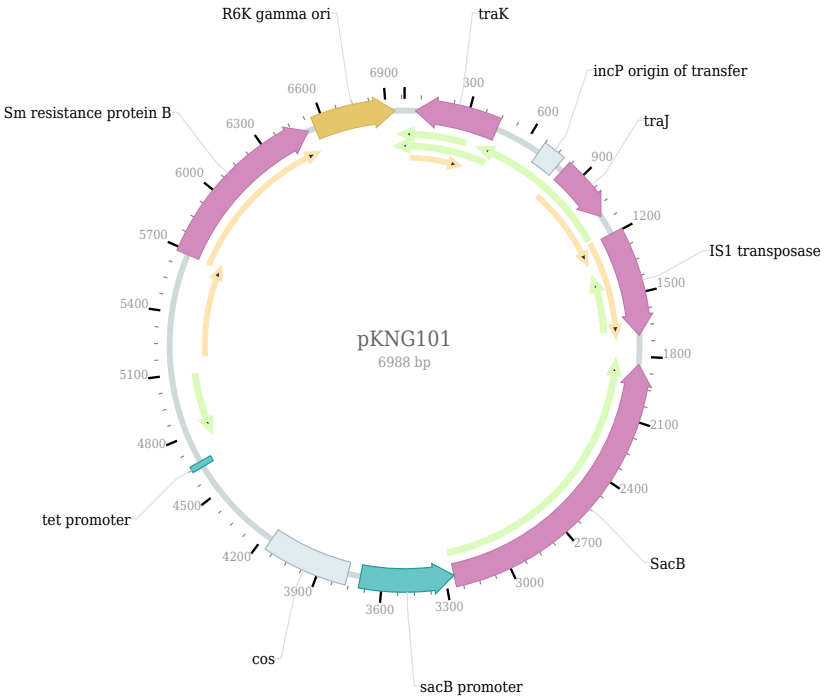
pKNG101

Vector ID	V005203
Antibiotic Resistance	Streptomycin
Length	6988 bp
Vector Type	Suicide vector

Product Information

Replication Origin	R6K γ ori
Source/Author	De Schamphelaire W, Olbrechts A, Meert J, Verhelst K, Roggeman Fonseca M, Vanhoucke M, Beyaert R.
Promoter	sacB
Comment	Throughout the plasmid cultivation process, use only sucrose-free culture medium (animal-derived medium is preferred).The SacB gene, from Bacillus subtilis and related to sugar metabolism, converts sucrose to levan. pKNG101 is a 6.8-kb mobilizable wide-host-range suicide vector designed for reverse-genetics allelic exchange in Gram-negative bacteria. It carries the pir-dependent conditional replication origin oriR6K, conjugative transfer origin mobRK2, streptomycin-resistance strAB, counter-selectable sacB for sucrose sensitivity, and multiple cloning sites for target-gene fragment insertion. After conjugative transfer from λpir-positive E. coli donors, this plasmid cannot replicate in recipient Gram-negative hosts and integrates into the chromosome via single homologous recombination, which is selected by streptomycin resistance. The sacB-mediated sucrose lethality enables positive selection for the second recombination step, facilitating generation of marked or unmarked chromosomal mutations such as the blaA-luxAB insertion in Yersinia enterocolitica described in the original study.

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 ℃.