Resource Summary Report

Generated by RRID on Aug 19, 2026

pKOV

RRID:Addgene_25769
Type: Plasmid

Proper Citation

RRID:Addgene_25769

Plasmid Information

URL: http://www.addgene.org/25769

Proper Citation: RRID:Addgene_25769

Bacterial Resistance: Chloramphenicol

Defining Citation: PMID:9335267

Vector Backbone Description: Backbone Marker:N/A; Backbone Size:8673; Vector Backbone:pMAK700, pMAK705, pBS-TS; Vector Types:Bacterial Expression; Bacterial Resistance:Chloramphenicol

Comments: pKOV is identical to the pKO3 vector described in J. Bacteriology 179: 6228-6237, except for the addition of a 3kb stuffer sequence in the multiple cloning site. This stuffer permits (i) directional cloning using NotI and BamHI and (ii) clean separation of doubly cut vector from singly cut contaminants when using this pair of enzymes. The pKOV cloning site is: 5' - SmaI - NotI - SmaI- stuffer - BamHI - SalI - 3'. BamHI and SalI are not used together, nor is SmaI used with NotI. BglII & BclI cut ends are compatible with BamHI. PmeI & SwaI are compatible with SmaI. NOTE: pKOV has a temperature sensitive pSC101 replication origin. To recover the plasmid, strains harboring the plasmid must be grown at 30 deg C under chloramphenicol selection. Gene replacement: Mutant alleles cloned into the pKOV gene replacement vector are electroporated into recombination proficient strains (eg. EMG2) and allowed to recover for 1 h at 30 deg C. The cells are plated on prewarmed chloramphenicol/LB plates and incubated at 42 deg C. To measure the integration frequency, the electroporated cells are also plated on chloramphenicol/LB plates at 30 deg C. From the 42 deg C plate, 1-5 colonies are picked into 1 ml of LB broth, serially diluted, and immediately plated at 30&degree;C on either 5% w/v sucrose or 5% sucrose+antibiotic plates. The 5% sucrose plates are replica plated to chloramphenicol plates at 30 deg C to test for loss of the replacement vector (cms). The gene replacement is confirmed by either PCR using primers flanking the targeted open reading frame or by genomic Southern's. Note from depositor: If sucrose plates do not select correctly, pKOV should be used in liquid selection on a plate reader with a LB + Cm

control growth and a LB + Cm + sucrose growth. pKOV can sometimes still allow survival in the presence of sucrose, but significantly decreases fitness, so tracking the kinetic growth can be helpful. The sacB gene loses its efficacy,, sacB is toxic in E. coli even in the absence of sucrose.

Plasmid Name: pKOV

Record Creation Time: 20220422T222115+0000

Record Last Update: 20260815T081247+0000

Ratings and Alerts

No rating or validation information has been found for pKOV.

No alerts have been found for pKOV.

Data and Source Information

Source: Addgene

Usage and Citation Metrics

We found 10 mentions in open access literature.

Listed below are recent publications. The full list is available at RRID .

Zierke L, et al. (2025) Influence of the polysaccharide capsule on virulence and fitness of Klebsiella pneumoniae. Frontiers in microbiology, 16, 1450984.

Gudeta DD, et al. (2024) Versatile allelic replacement and self-excising integrative vectors for plasmid genome mutation and complementation. Microbiology spectrum, 12(1), e0338723.

Azam AH, et al. (2024) Selective bacteriophages reduce the emergence of resistant bacteria in bacteriophage-antibiotic combination therapy. Microbiology spectrum, 12(6), e0042723.

Venkatesan M, et al. (2023) Molecular mechanism of plasmid-borne resistance to sulfonamide antibiotics. Nature communications, 14(1), 4031.

Kravchuk V, et al. (2022) A universal coupling mechanism of respiratory complex I. Nature, 609(7928), 808.

Ma P, et al. (2021) Genetic determinants facilitating the evolution of resistance to carbapenem antibiotics. eLife, 10.

Ma??achowska A, et al. (2018) TECS: a toxin expression control strategy as a tool for optimization of inducible promoters. Microbial cell factories, 17(1), 40.

Semanjski M, et al. (2018) The kinases HipA and HipA7 phosphorylate different substrate pools in Escherichia coli to promote multidrug tolerance. Science signaling, 11(547).

Wytock TP, et al. (2018) Experimental evolution of diverse Escherichia coli metabolic mutants identifies genetic loci for convergent adaptation of growth rate. PLoS genetics, 14(3), e1007284.

March C, et al. (2013) Role of bacterial surface structures on the interaction of Klebsiella pneumoniae with phagocytes. PloS one, 8(2), e56847.