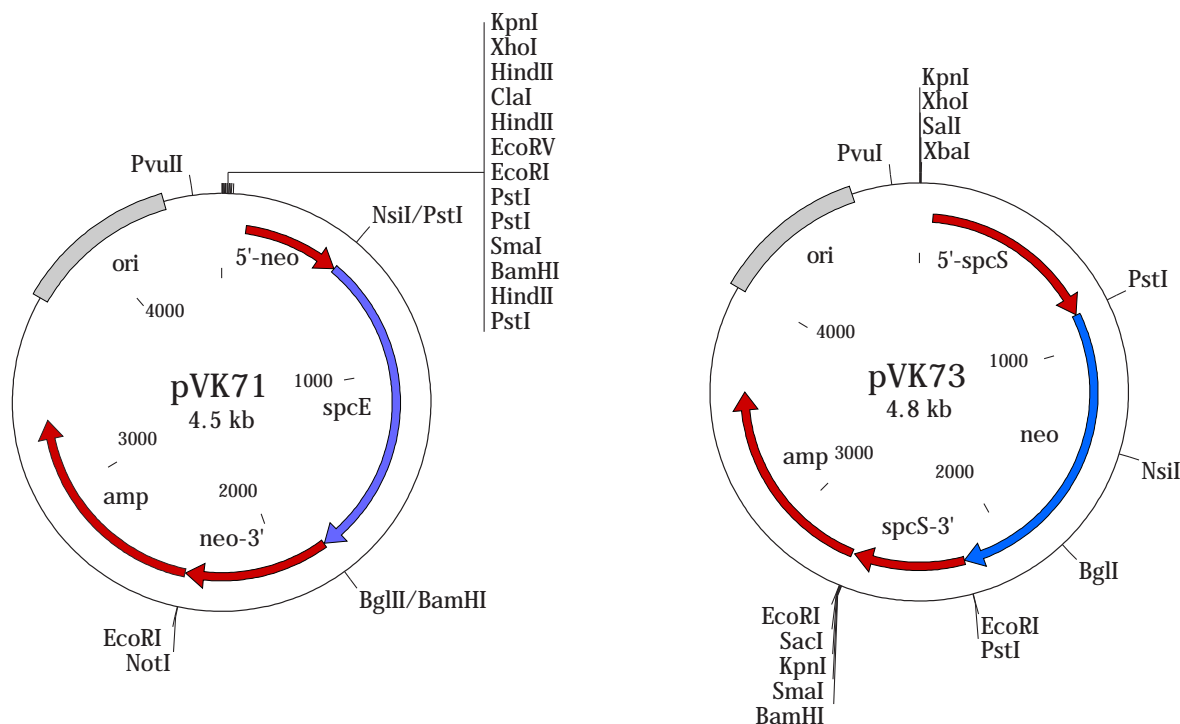


New Antibiotic-Resistance Cassette Switching Vectors



Vasant K. Chary from the Patrick Piggot lab at Temple University School of Medicine has kindly donated a pair of novel antibiotic-cassette switching vectors for use in *Bacillus subtilis* and other Gram-positive organisms. The ability to alter antibiotic cassettes greatly simplifies the construction of mutant strains with multiple gene knockouts. These novel vectors are described in Chary, V. K., E. I. Amaya, and P. J. Piggot. 1997. Neomycin- and spectinomycin-resistance replacement vectors for *Bacillus subtilis*. *FEMS Microbiol. Letts.* 153:135-139. **BGSC No. ECE140** is *Escherichia coli* DH5a containing pVK71. **BGSC No. ECE141** is DH5 α containing pVK73.

One of the most commonly used determinants in *B. subtilis* strains is the neomycin-kanamycin resistance gene originally from plasmid pUB110 (usually modified to contain a strong promoter). Plasmid pVK71 bears the spcE gene, originally from the *Enterococcus faecalis* plasmid pAT21, flanked by the 5' and 3' ends of the neo gene. Transforming a chromosomal kan insertion mutant with pVK71 DNA with selection for spectinomycin resistance, results in an antibiotic-cassette switch. Plasmid pVK73, in contrast, bears the neo gene flanked by the 5' and 3' ends of a second spectinomycin cassette, spcS. This determinant is used in certain mini-Tn10 transposon vectors designed for in Gram-positives. DNA from pVK73 alters transposon mutants to express kanamycin- rather than spectinomycin resistance.

Other Switching Vectors Available from the BGSC

BGSC No.	Original Code	Change from:	Change to:
ECE72	JM103(pCm::Er)	chloramphenicol	erythromycin
ECE73	JM103(pCm::Nm)	chloramphenicol	neomycin
ECE74	JM103(pCm::Sp)	chloramphenicol	spectinomycin
ECE75	JM103(pCm::Tc)	chloramphenicol	tetracycline
ECE76	JM103(pEr::Cm)	erythromycin	chloramphenicol
ECE77	JM103(pEr::Nm)	erythromycin	neomycin
ECE78	JM103(pEr::Pm)	erythromycin	phleomycin
ECE79	JM103(pEr::Sp)	erythromycin	spectinomycin
ECE80	JM103(p917::Sm)	Tn917	mini-Tn917::Sp ^R

Each of these vectors is described in: Steinmetz, M. and R. Richter. 1994. Plasmids designed to alter the antibiotic resistance expressed by insertion mutations in *Bacillus subtilis*, through in vivo recombination. *Gene* 142:79-83.