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Cloning of the *npt*II gene of *Escherichia coli* and construction of a recombinant strain harboring functional *rec*A and *npt*II antibiotic resistance

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ABSTRACT. In an attempt to clone the ORF of the *npt*II gene of *Escherichia coli* K12 (ATCC 10798), two degenerate primers were designed based on the *npt*II sequence of its Tn*5* transposon. The *npt*II ORF was placed under the control of the *E. coli* hybrid *trc* promoter, in the pKK388-1 vector, transformed into *E. coli* DH5α Δ*rec*A (recombinant, deficient strain). Transferred cells were tested for ampicillin, tetracycline, kanamycin, neomycin, geneticin, paromomycin, penicillin, and UV resistance. The neomycin phosphotransferase gene of *E. coli* was cloned successfully and conferred kanamycin, neomycin, geneticin, and paromomycin resistance to recombinant DH5α; this did not inhibit insertion of additional antibiotic resistance against ampicillin and tetracycline, meaning the *trc* promoter can express two different genes carried by two different plasmids harbored in the same cell. This resistance conferral process could be considered as an emulation of horizontal gene transfer occurring in nature and would be a useful tool for understanding mechanisms of evolution of multidrug-resistant strains.

Key words: *Escherichia coli*; Neomycin phosphotransferase gene (*npt*II); Homologous recombination gene (*rec*A); Aminoglycoside resistance

