A wide-host-range suicide vector for improving reverse genetics in gram-negative bacteria: inactivation of the blaA gene of Yersinia enterocolitica

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A new suicide vector (pKNG101) that facilitates the positive selection of double recombination events in Gram-bacteria has been developed. It contains a conditional origin of replication (oriR6K), the strAB genes encoding the streptomycin phosphotransferase (SmR), an origin of transfer (mobRK2), the sacB gene mediating sucrose sensitivity, and multiple cloning sites. It was used to mutate the blaA gene of Yersinia enterocolitica, by marker-exchange mutagenesis. To do this, we have first cloned into the suicide vector pKNG101, a 2.5-kb fragment of Y. enterocolitica chromosomal DNA encoding the 20-kDa beta-lactamase A. Gene blaA was then mutated in vitro by insertion of luxAB, which resulted in pKNG105. The disrupted blaA gene was then reintroduced into Y. enterocolitica chromosome by homologous recombinations in two steps. First, E. coli SM10 lambda pir (pKNG105) was mated with strains of Y. enterocolitica. This led to the integration of pKNG105 into the chromosome, by a single homologous recombination event. The transconjugants, selected for SmR, were sensitive to sucrose due to the synthesis of levans (toxic compounds), catalysed by levansucrase, the product of sacB. For the second step, a single colony from the first step was grown in rich medium deprived of antibiotic, allowing the occurrence of a second crossing-over that replaced the wild-type allele blaA with the mutant one, and then excised the plasmid-borne sacB from the chromosome. Such blaA mutants were selected on their ability to grow on TSA medium containing 5% sucrose.(ABSTRACT TRUNCATED AT 250 WORDS)

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