High frequency of double crossover recombination facilitates genome engineering in Pseudomonas aeruginosa PA14 and clone C strains

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Abstract

Pseudomonas aeruginosa is a key opportunistic human pathogen. An established procedure to replace a target gene is two-step allelic exchange, i.e. selection of single crossover at homologous sequences and subsequent counter selection to induce double crossover for excision of the suicide vector. In this study, we found that certain strains of P. aeruginosa display a high rate of instant double crossover upon introduction of a suicide vector containing an antibiotic resistance cassette flanked by adjacent sequences for gene replacement, making the counter selection step to achieve the second crossover superfluous. Assessment of a limited panel of target genes commonly showed negligible double crossover with a frequency <20 % in the genetic reference strain PAO1, whereas a high double crossover frequency of >70 % was observed for PA14 and clone C strains. Consequently, for certain P. aeruginosa strains replacement of an ORF by a antibiotic resistance cassette can be shortened by directly selecting for double crossover recombination.

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