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**Complete sequence of the suicide vector pJP5603
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SHORT COMMUNICATION

Complete sequence of the suicide vector pJP5603

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Abstract

We have sequenced the complete R6K-based and mobilizable suicide vector pJP5603. For the replication of the vector a *trans* supply of the *pir*-encoded π protein of plasmid R6K is essential. The 3.126 kb plasmid encodes a kanamycin resistance cassette for selection and contains a lacZ α -system that allows a blue-white selection of cloned fragments.

Keywords: pJP5603, suicide vector, recombinant DNA, site-directed mutagenesis

Abstract

We have sequenced the complete R6K-based and mobilizable suicide vector pJP5603. For the replication of the vector a *trans* supply of the *pir*-encoded π protein of plasmid R6K is essential. The 3.126 kb plasmid encodes a kanamycin resistance cassette for selection and contains a lacZ α -system that allows a blue-white selection of cloned fragments.

Introduction

Plasmid suicide vectors are widely used and efficient genetic tools for site-directed mutagenesis in bacterial hosts (Kaniga et al., 1991). These vectors are conditional for their replication and are commonly used for the integration of specific DNA fragments into a recipient strain in which the plasmid cannot replicate.

Integration of DNA into the host chromosome is mediated via homology between the cloned fragment and the corresponding region of the recipient chromosome. The specific DNA sequence can be integrated via transposon-steered genetic manipulation as well as via single or double homologues recombination using matching sequences between the plasmid and the chromosome.

For transposon-steered genetic manipulation, conservative (“cut-out”, non-replicative) as well as replicative transposition (“copy and paste”) are well-known (Griffiths et al., 2000). In the conservative mechanism, the element of interest is excised from the containing vector and is integrated into the site of interest. The replicative mechanism results in a duplication of the transposable element, one copy present at the new site of interest as well as one copy remaining at the original site. Single homologous recombination between a targeting suicide vector and the chromosome of the host cell results in an insertion of the complete vector into the bacterial genome.

Generation of reporter strains based on luciferase and β -galactosidase fusions is often accomplished by single homologous recombination (Podbielski et al., 1996).

Double homologous recombination results in a sequence exchange between the bacterial host genome and the suicide vector.

A prototypical application for double homologous recombination are gene deletion strategies replacing the gene of interest with an antibiotic resistance marker.

Here we report the sequencing of the suicide vector pJP5603 that was constructed by Penfold and Pemberton in 1992. The vector contains a R6K origin of replication (*oriR6K*), which is different to most ColE-like replicons. ColE-like replicons do not suicide in all bacteria (Penfold and Pemberton, 1992). In contrast utilization of an *oriR6K* strictly requires in *trans* supplementation of the π protein, encoded by the *pir* gene, for plasmid replication (Kolter et al., 1978; Wu et al., 1994). *E. coli* strain JM109 λ *pir* containing one chromosomal copy of the *pir* gene is a suitable cloning host (Miller and Mekalanos, 1988).

Constructed 20 years ago the suicide vector pJP5603 was widely used for the genetic manipulation of different strains (Bi et al., 2007; Chae and Zylstra, 2006; Ingham and Pemberton, 1995; Koerdt et al., 2009; Nishi et al., 2003; Paulick et al., 2009; Ridge et al., 2007; Sheikh et al., 2002; Steiner et al., 2000; Weir et al., 2006; Woods et al., 2001; Wu et al., 1994). However the complete vector sequence was so far elusive and is presented here.

Materials and Methods

The plasmid pJP5603 was maintained in an *E. coli* JM109 λ *pir* strain, which was deposited at the DSMZ (= DSM 26091). For sequencing, DNA was isolated from the culture using the NucleoSpin® Plasmid Kit (Macherey & Nagel, Düren, Germany) according to the manufacturer's protocol. The isolated suicide vector pJP5603 was directly sequenced on an ABI sequencer 3730XL using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, USA) and the primers shown

in table 1. Sequences were manually assembled using BLAST (Altschul et al., 1990) resulting in the circular plasmid sequence of pJP5603.

Results and Discussion

The complete pJP5603 vector has a sequence size of 3.126 kb with a GC content of 56.2% (GenBank Accession No. JQ769377). The various components described by Penfold and Pemberton could be fully confirmed: It encodes a kanamycin resistance cassette of 807 bp as selection marker (Figure 1). Furthermore it contains the *lacZ* α -system and the associated multiple cloning site (MCS) from the pUC19 vector (Yanisch-Perron et al., 1985). To use transfer genes of the IncP-type plasmid RP4 for plasmid mobilization, the vector bears the RP4 *mob* site from the pSUP202 (Simon et al., 1983). The *oriR6K* is derived from the pUTKm vector (Herrero et al., 1990) (Figure 1). Additionally we identified a truncated transposase encoding gene of 258 bp (Figure 1). The complete and manually assembled sequence of the suicide vector pJP5603 was analyzed for restriction sites of commonly used restriction enzymes to simplify genetic manipulation (Figure 2).

Conclusion

We have determined the complete sequence of suicide vector pJP5603 (GenBank Accession No. JQ769377). Our sequencing approach of pJP5603 could be very helpful for further genetic manipulations of strains.

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pJP5603 suicide vector (also in consultation with Penfold and Pemberton). We sincerely thank our colleagues from the Genome Analytics Group of the Helmholtz-Centre for Infection Research for sequencing our vector samples as well as Christine Rohde for deposition of the strain at the Leibniz Institute DSMZ-German Collection for Microorganisms and Cell Cultures. TR and IWD gratefully acknowledge funding by the DFG Transregio-SFB 51 Roseobacter.

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Figures and Tables:

Table 1. Primers. Primers used for sequencing.

Figure 1. pJP5603-Map. The suicide vector pJP5603 consists of a kanamycin resistance cassette (Mazodier et al., 1985), followed by the lacZ α -peptide encoding gene from pUC19 (Yanisch-Perron et al., 1985) with an adjacent MCS. Furthermore the plasmid contains the RP4 *mob* site from pSUP202 (Simon et al., 1983) and the *ori*R6K from pUTKm (Herrero et al., 1990) as originally described by Penfold and Pemberton in 1992. The map was generated with Geneious v.5.5.6 (Drummond A.J. et al., 2011) and includes additionally a truncated transposase gene and sequencing primer positions.

Figure 2. Virtual Gel. The generated sequence of pJP5603 was analyzed for commonly used restriction sites with Geneious v.5.5.6 (Drummond A.J. et al., 2011). The vector was single digested with 37 different commonly used restriction enzymes.

Table 1.

name	sequence (5'-3')
mobF	CTC TTC GTT OGT CTG GAA GG
lacZF	TTC ACT GGC CGT OGT TTT AC
lacZR	GCG TAA TAG OGA AGA GGC C
KanR	GGC CAT TTT CCA CCA TGA TA

Figure 1.

Figure 2.