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Short communication

A novel plasmid vector that uses the glucose kinase gene (*glkA*) for the positive selection of stable gene disruptants in *Streptomyces*[1]

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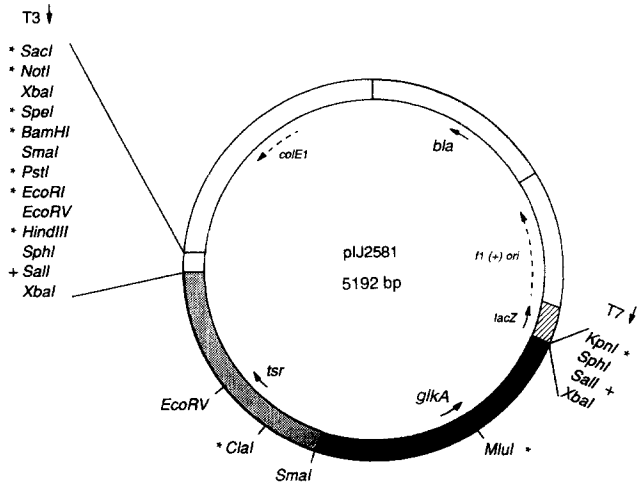
Abstract

We describe an *Escherichia coli* plasmid, pIJ2581, that can be used for the efficient construction of stable gene disruptants and of gene deletions in *Streptomyces*. Integration of pIJ2581 derivatives carrying chromosomal sequences is achieved by selecting for plasmid-encoded thiostrepton resistance, while plasmid excision is secured by counter-selection of the pIJ2581 *glkA* gene, which confers sensitivity to 2-deoxyglucose.

Keywords: 2-Deoxyglucose; Gene replacement; Insertional inactivation; *Streptomyces coelicolor* A3(2); Thiostrepton resistance

The glucose kinase gene (*glkA*; Ikeda et al., 1984; Angell et al., 1992) of *Streptomyces coelicolor* A3(2) has been used for the positive selection of recombinants in gene disruption and gene replacement experiments (Buttner and Lewis, 1992; van Wezel et al., 1995). The presence of an active glucose kinase (Glk) results in sensitivity to the non-utilisable sugar 2-deoxyglucose (Dog) and therefore *glkA* mutants, which are not able to utilise glucose as sole carbon source, are Dog^R (Hodgson, 1982). Conversely, introduction of a plasmid or phage carrying *glkA* into a Glk$^-$ strain results in Dog^S. This principle has been exploited to develop a suicide vector (pIJ2581; Fig. 1) for efficient gene inactivation in *Streptomyces*. The vector is based on the *Escherichia coli* vector pBluescript SK+ (purchased from Stratagene), which has both ColE1 and f1 (+) origins of replication, the latter allowing the production of single-stranded DNA in the presence of a helper phage [Sambrook et al. (1989); single-stranded DNA gives transformation and/or integration frequencies in



+ Not all sites shown

* Unique restriction site

Fig. 1. Restriction map of pIJ2581. Solid arrows indicate the direction of transcription of each gene/promoter; dotted arrows indicate the direction of DNA synthesis from each origin of replication. The sequence of pIJ2581 was deposited in the EMBL database, accession No. X98363.

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[1] On request, the authors will supply detailed experimental evidence for the conclusions reached in this Short communication.

Abbreviations: Ap, ampicillin; bp, base pairs; *bla*, gene encoding Ap^R from *Escherichia coli*; *colE1*, origin of replication of plasmid ColE1; Dog, 2-deoxyglucose; *f1 (+) ori*, origin of replication for single-stranded DNA synthesis from phage f1; Glc$^{+/-}$, able/unable to grow on glucose as sole carbon source; Glk, glucose kinase from *S. coelicolor* A3(2); *glkA*, glucose kinase gene of *S. coelicolor* A3(2); kb, kilobase(s) or 1000 bp; *lacZ*, DNA encoding the promoter and α fragment of the β-galactosidase gene of *E. coli*; *malEFG*, part of the maltose operon of *S. coelicolor* A3(2); MCS, multiple cloning site; ^{R}, resistance/resistant; ^{S}, sensitivity/sensitive; *S.*, *Streptomyces*; T3, phage T3 promoter; T7, phage T7 promoter; Th, thiostrepton; *tsr*, gene encoding Th^R from *S. azureus*.

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Streptomyces that are 10–100 fold higher than the corresponding double-stranded DNA (Hillemann et al., 1991)]. The lack of a *Streptomyces* origin of replication ensures that the plasmid can only be maintained in *Streptomyces* by integration into the chromosome through cloned homologous sequences. *tsr* (conferring Th^R in *Streptomyces*) and *glkA* were both obtained from pIJ2559 (van Wezel et al., 1995).

To test the utility of pIJ2581, a 1.5-kb segment of the *S. coelicolor* A3(2) maltose operon *malEFG* (G.P. van Wezel, unpublished data) was cloned in the MCS, and single- and double-stranded DNAs derived from the resulting construct were isolated from the *E. coli* methylation-deficient strain ET12567 (MacNeil et al., 1992) (*S. coelicolor* possesses a methyl-specific restriction system that drastically reduces transformation frequencies; MacNeil, 1988) and used to transform M480 (Angell et al., 1994), a *glkA* deletion derivative of *S. coelicolor* M145. In this particular experiment, transformation with single-stranded DNA resulted in approximately ten times more Th^R colonies than transformation with a comparable amount of double-stranded DNA. All Th^R colonies obtained after transformation were Dog^S Glc^+ (able to grow on glucose as sole carbon source). Colonies were then plated on minimal medium plates (Hopwood et al., 1985) containing 100 mM Dog (Sigma) with mannitol (1% w/v) as carbon source. All of the several hundred Dog^R colonies tested were Th^S Glc^- (unable to grow on glucose as sole carbon source), indicative of excision of the plasmid from the chromosome by a second cross-over event, which was confirmed by Southern analysis of DNA from four independent Th^S Glc^- isolates.

Although the use of pIJ2581 in *S. coelicolor* A3(2) requires a *glkA* deletion strain, the ability to select for both primary (Th^R) and secondary (Dog^R) cross-over events simplifies the time-consuming screening procedure generally required for gene inactivation in streptomycetes. Should the *glkA* mutation interfere with further analysis, the gene disruption can always be crossed into a $glkA^+$ genetic background (e.g. Buttner and Lewis, 1992). In species that are not closely related to *S. coelicolor* A3(2), and in which the *glkA* DNA sequence has diverged by more than a few percent, it would probably be unnecessary to use a *glkA* deletion mutant, and a point mutant would probably suffice, since the frequency of undesirable recombination events between the plasmid-borne and chromosomal *glkA* genes would be greatly reduced (Chater and Bruton, 1983). pIJ2581 may also be used for the identification of essential genes. Assuming that a primary cross-over event results in the retention of complete or partial gene function, the subsequent failure to obtain Dog^R Th^S colonies containing the required disruption after selecting for Dog^R would be indicative of an essential gene.

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