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Entrapment Vectors – How to Capture a Functional Transposable Element

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Abstract

Transposable elements (*i.e.* insertion sequences and transposons) are components of nearly all bacterial genomes. The majority of these elements have been identified as a result of various sequencing projects. However, in most cases, their activity was not experimentally confirmed. For this reason several strategies have been developed that allow direct cloning and identification of functional transposable elements. Most of the methods are based on the ability of transposable elements to inactivate or activate particular genes by insertion. In this review we describe and critically discuss different cloning strategies that employ various entrapment vectors, carrying (i) conditionally lethal genes, (ii) antibiotic selection cartridges, (iii) promoter-less genes or (iv) suicide replicons. These tools, besides facilitating the identification of new transposable elements, also enable the investigation of various DNA rearrangement mutations, which are related to the transposition process.

Key words: insertion sequence, transposon, entrapment vector, transposition

1. Transposable elements and the plasticity of bacterial genomes

Transposable elements (TE) [insertion sequences (IS) and transposons (Tn)] are the most recombinogenic factors within bacterial genomes. Their transposition promotes structural changes in DNA that lead to the formation of various mutations (insertions, deletions, inversions, translocations or replicon fusion) (Galas and Chandler, 1989). The insertion of TEs can lead to gene inactivation or, on the contrary, to activation (or overexpression) of adjacent genes by the formation of upstream promoters, which may result in various phenotypic changes (*e.g.* Hubner and Hendrickson, 1997; Weightman *et al*., 2002).

Insertion sequences, being the simplest forms of transposable elements, carry genetic information necessary exclusively for their own transposition (Chandler and Mahillon, 2002). In contrast, transposons are larger structures, which in addition to transposition mechanisms, may encode various phenotypic traits (*e.g.* resistance to antibiotics or heavy metals, ability to utilize different carbon sources *etc.*) (*e.g.* Providentii *et al*., 2001; Ajdič *et al*., 2002). Both types of TEs are frequently harboured by other mobile elements (such as plasmids and bacteriophages), which can propagate them by lateral transfer between various bacterial populations.

These elements, thus, play the role of a factor that significantly enhances variability and consequently the adaptative and evolutionary capacities of their hosts. Identification (and analysis of distribution) of functional TEs in various bacterial species, besides assisting in characterization many new elements, provides data from which conclusions may be drawn regarding the frequency and direction of lateral transfer.

2. Methods of identification of transposable elements

The detection of active transposons carrying genes that influence a host's phenotype is relatively simple. However, it is much more difficult to identify insertion sequences or cryptic transposons. Quite often such

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elements are found by chance, during the course of unrelated research (*e.g.* they may reside in close proximity to cloned genes), or by detection of rapid phenotypic changes resulting from their transposition. The first insertion sequence was identified following the latter scenario, when it caused a mutation in the galactose operon of *Escherichia coli* (Shapiro, 1969).

Since the nucleotide sequences of many bacterial genomes have been determined the identification of mobile genetic elements is now much easier. It is thus possible to search for sequences characteristic of transposable elements, *e.g.* for a transposase gene or terminal inverted repeats (IRs). Nonetheless, such analyses do not show whether the element studied is actually capable of transposition. Theoretically all DNA is potentially mobile (Campbell, 2002), which basically means that any DNA fragment, bordered by two, often different, transposable elements, could form a composite transposon. However, whether such DNA segments actually are mobile has to be verified experimentally. Therefore, there is still the need for methods allowing both the identification of transposable elements and the detection of their transposition activity.

2.1. Entrapment vectors carrying conditionally lethal genes

The first attempts at designing a method for the direct detection and isolation of phenotypically "silent" transposable elements were carried out almost twenty years ago. Most strategies were based on insertional gene inactivation, hence the use of vectors carrying genes, whose expression (under certain conditions) was lethal to the studied host. In such circumstances, disruption of a lethal gene caused by insertion of a transposable element enabled growth of the thus mutated clones.

One of the first positive selection vectors was pUCD800 (Km^r) which carried the replicator region of the broad host range plasmid pSa together with the *sacB* gene of *Bacillus subtilis* (Gay *et al*., 1985). The *sacB* gene (expressed under the control of its own untranslated leader region *sacR*) encodes levansucrase (sucrose:2,6-β-D-fructan 6-β-D-fructosyltransferase, E.C. 2.4.1.10), an exoenzyme that catalyses the breakdown of sucrose. This reaction results in the formation of levan, which is toxic to bacterial cells (Lepesant *et al*., 1974; Steinmetz *et al*., 1983; Gay *et al*., 1983). The expression of *sacB* gene is induced by the presence of sucrose. Cultivation of bacteria in a medium containing sucrose results, therefore, in cell lysis (Gay *et al*., 1985). This allows direct selection of *sacB* mutants, whose growth, in these conditions, is not affected.

Using pUCD800 it was possible to identify several novel insertion sequences of *Agrobacterium tumefaciens* (Gay *et al*., 1985; de Meirsman *et al*., 1989), *Pseudomonas syringae* (Romantschuk *et al*., 1991), *P. alcaligenes* (Yeo and Poh, 1996), and *Sphingomonas* sp. (Feng *et al*., 1997). Analogous vectors (carrying the *sacRB* cassette) were also employed for the isolation of TEs of gramnegative bacteria, such as *Ralstonia eutropha* (Dong *et al*., 1992), *R. solanacearum* (Lee *et al*., 2001), *Rhizobium leguminosarum* (Ulrich and Puhler, 1994), *Pseudomonas putida* (Lauf *et al*., 1998), *Desulfovibrio vulgaris* (Fu and Voordouw, 1998), and also of grampositive hosts – *Brevibacterium flavum*, *B. lactofermentum*, *Corynebacterium glutamicum*, *C. herculis*, *Rhodococcus fascians* (Vertes *et al*., 1994; Jäger *et al*., 1995), *R. erythropolis* (Lessard *et al.*, 1999) as well as the cyanobacterium *Anabaena* sp. (Cai and Wolk, 1990).

The frequency of appearance of sucrose-resistant mutants varies, depending on the strain studied. In several cases, however, a large number of mutants was obtained, none of which carried a trapped element. This presumably was the result of point mutations, small deletions or insertions within the *sacB* gene. Another possibility is that other mutations in the genome may affect localization of the levansucrase or substrate transport, resulting in sucrose resistance (Jäger *et al*. 1995). In *Zymomonas mobilis*, for example, none of over one thousand sucrose resistant clones tested contained a captured TE. An alternative identification method was used to identify the novel insertion sequence IS*Zm1068* (Galeros *et al.*, 2001).

Several alternative selection methods, that employ genes other than *sacB*, have been developed. One utilizes the *pheS* gene of *E. coli*, encoding the α subunit of phenylalanyl tRNA synthetase. The activity of this enzyme is inhibited by the amino acid analogue p-fluorophenylalanine (PFP). This particular method has serious limitations since it can only be applied to mutated bacterial strains, which lack the wild-type *pheS* gene. Such strains are able to grow in the presence of PFP, but the introduction of a plasmid carrying a functional *pheS* gene results in regained sensitivity to this inhibitor. In such circumstances, only cells carrying inactivated plasmid-borne *pheS* can form colonies (Simon *et al*., 1991).

A similar idea led to the construction of an entrapment vector carrying the *rpsL* (*strA*) gene of *E. coli*, coding for ribosomal protein S12, which is the target site of streptomycin action (its presence is required for

sensitivity to this antibiotic; Sm^s). Interestingly, the *rpsL* gene is transdominant and even naturally streptomycin-resistant bacterial strains become sensitive, when a wild-type copy of *rpsL* is introduced *in trans*. The plasmid pSUP104-rpsL (Tc^r; carries the replicator region of promiscuous plasmid RSF1010) was used to analyse Sm^r derivatives of various strains of *Rhizobium leguminosarum* and *Xanthomonas campestris*, and this led to the entrapment of 12 insertion sequences (Simon *et al*., 1991).

2.2. Entrapment vectors with antibiotic selection cartridges

Another method for *in vivo* "cloning" of TEs employs a two-gene selection cartridge containing, in a divergent orientation: (i) a "silent" antibiotic resistance gene controlled by the P_R promoter of phage λ and (ii) the gene *cI* coding for λ repressor. The *cI* gene product represses the P_R promoter, which completely blocks expression of the resistance gene. Any mutation that inactivates the *cI* gene leads to derepression of the previously "silenced" gene. It is, thus, another strategy for detecting transposition events by positive selection, since only bacteria with a damaged *cI* gene (caused by TE insertion) can grow in the presence of antibiotic. Occasionally, the activation of a "silent" resistance gene may occur when a transposable element integrates outside the *cI* gene. This is due to the presence of IS-encoded outreading promoters, which can drive expression of adjacent genes.

The first selection cartridge of this type, containing a "silent" neomycin/kanamycin resistance gene (*neo*), was constructed by Raabe *et al*. (1988), and cloned within plasmids pRAB1 (Cm^r; carries replicator region of pACYC184) and pRAB2 (Ap^r; *ori* of pUC7). Both plasmids were used to analyze several enterobacterial strains and enabled the identification of TEs from *E. coli* and *Enterobacter cloacae* (Raabe *et al*., 1988). The same selection cassette, linked to different replicons, was later employed to examine other species, *e.g. Pseudomonas glumae* (Hasebe *et al*., 1998).

In order to detect transposition in soil bacteria, in which neomycin/kanamycin selection failed, another vector (pGBG1; Cm^r), containing an analogous cartridge with a "silenced" tetracycline resistance gene *tetA* (cI-Tc), was constructed. Plasmid pGBG1 (carrying the replication system of the small broad-host-range plasmid pBBR1) was used to analyze *Azospirillum* sp., *Burkholderia cepacia, Agrobacterium tumefaciens*, *Ralstonia eutropha* and two strains of *E. coli*, which led to the identification of five ISs and a new Tn*21*-like transposon (Schneider *et al.*, 2000; Miché *et al*., 2001).

The selection cartridge from pGBG1 was also used in the construction of a mobilizable shuttle vector pMEC1 (Km^r), which served as a tool for the identification of TEs in strains of *Paracoccus pantotrophus* and *P. solventivorans* (Bartosik *et al*., 2003a; 2003b). The analysis of several hundred of pMEC1 insertional mutants (which appeared at a frequency of 10^{-3} to 10^{-6}, depending on the host) led to the discovery of 8 novel ISs, as well as two transposons of the Tn*3* family. In the course of these studies a set of cartridge-specific nested PCR primers was designed (complementary to the sequence of the *cI* gene), which make it possible to (i) determine the precise location of the target site for a given element, (ii) roughly estimate the size of the element and (iii) choose the most appropriate primers for DNA sequencing of the terminal regions of individual TEs.

The size of transposable elements captured by pMEC1 did not exceed 6 kb. However, modification of the vector, by replacing its replication system [originating from the small (2.7 kb) multicopy plasmid pWKS1 of *P. pantotrophus* DSM 11072 (Bartosik *et al*., 2002)] with a *repABC* replicon [derived from the large (107 kb) low-copy-number plasmid pTAV1 of *P. versutus* UW1 (Bartosik *et al*., 1998)], permitted the identification of a composite transposon (designated Tn*Ppa*1) in *P. pantotrophus*, which exceeded 40 kb in size (Bartosik, unpublished results). The mobilizable plasmid derivative pMMB2 (Km^r) ensures stable maintenance of large plasmid genomes and, therefore, appears to be a convenient tool for "cloning" of large transposons or even transposable genomic islands.

Before using resistance cartridges in a positive selection strategy, it is important to determine the appropriate selective dose of the antibiotic. During analyses performed with vector pMEC1 (carries cI-Tc cartridge), different levels of tetracycline resistance were observed for individual strains of *P. pantotrophus*. It was therefore essential to identify the selective Tc concentration (in the range 0.5–3 μg/ml) by application of a mutated pMEC1 derivative (deletion within *cI* gene) constitutively expressing the *tetA* gene (Bartosik *et al*., 2003a). On the other hand, it was observed that the repressor gene *cI* is not expressed in some paracoccal hosts (*e.g. P. aminophilus* JCM 7686, *P. zeaxanthinifaciens* ATTC 21588), which results in constitutive tetracycline resistance (Bartosik, unpublished results). Such a block in gene expression is a strong limiting factor restricting the use of this type of the selection cartridge in some bacterial hosts.

2.3. Entrapment vectors carrying promoter-less genes

Another method for the identification of TEs exploits the fact that many elements carry outward directed promoters, which may induce expression of genes located adjacent to the integration site. Additionally, it has been shown that transposition of various ISs can fortuitously lead to the formation of fusion promoters composed of a –35 hexamer present in the terminal part of the mobile element and a –10 box located close to the size of insertion (*e.g.* Hubner and Hendrickson, 1997; Nagy and Chandler, 2004). The entrapment of such TEs in vectors carrying promoter-less (or poorly expressed) genes may activate their expression, which can result in a selectable phenotype.

Such a strategy was employed for the analysis of TEs of *Pseudomonas cepacia*. In this case a broad-host-range conjugative plasmid pRP1, carrying a β-lactamase gene (weakly expressed in the studied strain) was used as an entrapment vector. Several selected clones (with increased ampicillin resistance) carried four new insertion sequences and one transposon, whose presence activated expression of the β-lactamase gene (Scordilis *et al*., 1987).

A more universal entrapment vector (pAW1326), carrying two promoter-less genes coding for kanamycin and chloramphenicol resistance, was constructed by Szeverenyi *et al*. (1996). This vector (Ap^r, Sm^r; based on the pBR322 replicon) allowed "cloning" of seven elements from *E. coli*, and thus proved to be useful for the isolation of TEs.

One major advantage of the above method is that in most cases, acquisition of antibiotic resistance is related to transposition events. For example, it is less likely that the expression of a marker gene could be induced by point mutations. On the other hand only those mobile genetic elements which possess (complete or partial) outward directed promoters may be identified. For this reason, the frequency of appearance of clones expressing the marker genes is lower than for methods based on insertional gene inactivation.

2.4. Suicide plasmids as entrapment vectors

In several cases suicide vectors (unable to replicate in the studied host) have been used for the identification of TEs. The introduction of such a vector into bacterial cells may result in the formation of a transient cointegrate between the donor and target replicon. In the absence of DNA homology between the vector and genomic sequences, such integration is possible due to the activity of transposable elements. Formation of cointegrates (which are intermediates in the intermolecular transposition pathway) involves the combined action of transposase and the host cell replication machinery. As a result, the integrated suicide vector is flanked by two identical copies of a transposable element, which can facilitate its direct cloning. It is obvious that this method may only be applied to the identification of elements that transpose *via* a replicative pathway.

The donor-target cointegrates formed by insertion sequences are relatively stable, which has for example permitted the isolation of a novel insertion sequence IS*1248* from *Paracoccus denitrificans* (Van Spanning *et al*., 1995). In contrast, transposons usually form transient structures, due to the presence of their own recombinase systems for cointegrate resolution (*e.g.* Grindley, 2002). Nevertheless, many transposons [*e.g.* Tn*1000* residing within the F plasmid (Guyer, 1978) or Tn*2501* from plasmid pGC1 from *Yersinia enterocolitica* (Michiels and Cornelis, 1984)] were discovered due to their ability to form cointegrates.

3. Conclusions

The methods described above have been very effective in the isolation and characterization of transposable elements in many bacterial species. In this mini-review we have described both the advantages and drawbacks of different types of entrapment vectors. The choice of an appropriate method as well as the selection factor depends mostly on which system works best in a given strain or species.

Such analyses, carried out *in vivo*, also allow determination of the frequency of transposition of a particular type of TE in a studied strain and the singling out of elements that are significantly expansive. A good example is the streptomycin-resistant Tn*5393*, which transposed into the entrapment vector pMEC1 in *Paracoccus pantotrophus* LMD 82.5, with a very high frequency (10^{-3}) (Bartosik *et al*., 2003a). These observations explain the wide spread of this transposon, which has been found in many environmental isolates (*e.g.* Tauch *et al*., 2000; 2003).

Studies made using entrapment vectors, besides identifying transposable elements, also enable the observation of DNA rearrangement mutations, which, in other circumstances, are impossible to link to a given transposition event, or are based on speculation alone. Recent reports indicating the presence of transposable elements of atypical structure (Kholodii *et al*., 2004), that cannot be distinguished simply by nucleotide sequence analysis should encourage wider use of entrapment vectors and more detailed analysis of the large pool of insertional mutants obtained. Even when carried out in bacteria whose genomes have been fully sequenced, such analyses may produce many interesting and even surprising findings.

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Literature

Ajdič D., W.M. McShan, R.E. McLaughlin, G. Savic, J. Chang, M.B. Carson, C. Primeaux, R. Tian, S. Kenton, H. Jia, S. Lin, Y. Qian, S. Li, H. Zhu, F. Najar, H. Lai, J. White, B.A. Roe and J.J. Ferretti. 2002. Genome sequence of *Streptococcus mutans* UA159, a cariogenic dental pathogen. *Proc. Natl. Acad. Sci. USA*. **99**: 14434–14439.

Bartosik D., J. Baj and M. Wlodarczyk. 1998. Molecular and functional analysis of pTAV320, a *repABC*-type replicon of the *Paracoccus versutus* composite plasmid pTAV1. *Microbiology* **144**: 3149–3157.

Bartosik D., J. Baj, M. Sochacka, E. Piechucka and M. Wlodarczyk. 2002. Molecular characterization of functional modules of plasmid pWKS1 of *Paracoccus pantotrophus* DSM 11072. *Microbiology* **148**: 2847–2856.

Bartosik D., M. Sochacka and J. Baj. 2003a. Identification and characterisation of transposable elements of *Paracoccus pantotrophus*. *J. Bacteriol*. **185**: 3753–3763.

Bartosik D., M. Szymanik and J. Baj. 2003b. Identification and distribution of insertion sequences of *Paracoccus solventivorans*. *Appl. Environ. Microbiol*. **69**: 7002–7008.

Campbell A. 2002. Eubacterial genomes, pp. 1024–1039. In: N.L. Craig, R. Craigie, M. Gellert and A.M. Lambowitz (eds). Mobile DNA II, American Society for Microbiology, Washington D.C.

Cai Y. and P. Wolk. 1990. Use of conditionally lethal gene in *Anabaena* sp. strain PCC 7120 to select for double recombinants and to entrap insertion sequences. *J. Bacteriol*. **172**: 3138–3145.

Chandler M. and J. Mahillon. 2002. Insertion sequences revised, pp. 305–366. In: N.L. Craig, R. Craigie, M. Gellert and A.M. Lambowitz (eds). Mobile DNA II, American Society for Microbiology, Washington D.C.

Dong Q., A. Sadouk, D. van der Lelie, S. Taghavi, A. Ferhat, J.M. Nuyten, B. Borremans, M. Mergeay and A. Toussaint. 1992. Cloning and sequencing of IS*1086*, an *Alcaligenes eutrophus* insertion element related to IS*30* and IS*4351*. *J. Bacteriol.* **174**: 8133–8138.

Feng X., L.T. Ou and A. Ogram. 1997. Cloning and sequence analysis of a novel insertion element from plasmids harbored by the carbofuran-degrading bacterium, *Sphingomonas* sp. CFO6. *Plasmid* **37**: 169–79.

Fu R. and G. Voordouw. 1998. IS*D1*, an insertion element from the sulfate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough: structure, transposition and distribution. *Appl. Environ. Microbiol.* **64**: 53–61.

Galas D.J. and M. Chandler. 1989. Bacterial insertion sequences, pp. 109–162. In: D.E. Berg and M.M. Howe (eds). Mobile DNA. American Society for Microbiology, Washington D.C.

Galeros M., K.M. Pappas, E. Beletsiotis and M.A. Typas. 2001. IS*Zm1068*: an IS*5*-like insertion element from *Zymomonas mobilis*. *Arch. Microbiol*. **175**: 323–333.

Gay P., D. LeCoq, M. Steinmetz, E. Ferrari and J.A. Hoch. 1983. Cloning structural gene *sacB***,** which codes for exoenzyme levansucrase of *Bacillus subtilis*: expression of the gene in *Escherichia coli*. *J. Bacteriol*. **153**: 1424–1431.

Gay P., D. le Coq, M. Steinmetz, T. Berkelman and C.I. Kado. 1985. Positive selection procedure for entrapment of insertion sequence elements in gram-negative bacteria. *J. Bacteriol*. **164**: 918–921.

Grindley N.D.F. 2002. The movement of Tn*3*-like elements: transposition and cointegrate resolution , pp. 272–302. In: N.L. Craig, R. Craigie, M. Gellert and A. M. Lambowitz (eds). Mobile DNA II, American Society for Microbiology, Washington D.C.

Guyer M.S. 1978. The gamma delta sequence of F is an insertion sequence. *J. Mol. Biol*. **126**: 347–365.

Hasebe A., S. Tsushima and S. Iida. 1998. Isolation and characterization of IS*1416* from *Pseudomonas glumae*, a new member of the IS*3* family. *Plasmid* **39**: 196–204.

Hubner A. and Hendrickson W. 1997. A fusion promoter created by a new insertion sequence, IS*1490*, activates transcription of 2,4,5-trichlorophenoxyacetic acid catabolic genes in *Burkholderia cepacia* AC1100. *J. Bacteriol*. **179**: 2717–2723.

Jäger W., A. Schafer, J. Kalinowski and A. Puhler. 1995. Isolation of insertion elements from gram-positive *Brevibacterium*, *Corynebacterium* and *Rhodococcus* strains using the *Bacillus subtilis sacB* gene as a positive selection marker. *FEMS Microbiol. Lett*. **126**: 1–6.

Kholodii G., S. Mindlin, Z. Gorlenko, M. Petrova, J. Hobman and V. Nikiforov. 2004. Translocation of transposition-deficient (Tn^{d}*PKLH2*-like) transposons in the natural environment: mechanistic insights from the study of adjacent DNA sequences. *Microbiology* **150**: 979–992.

Lauf U., C. Muller and H. Herrmann. 1998. The transposable elements resident on the plasmids of *Pseudomonas putida* strain H, Tn*5501* and Tn*5502*, are cryptic transposons of the Tn*3* family. *Mol. Gen. Genet.* **259**: 674–678.

Lee Y.A., S.C. Fan, L.Y. Chiu and K.C. Hsia. 2001. Isolation of an insertion sequence from *Ralstonia solanacearum* race 1 and its potential use for strain characterisation. *Appl. Environ. Microbiol.* **67**: 3943–3950.

Lepesant J.A., J. Lepesant-Kejzlarova, M. Pascal, F. Kunst, A. Billault and R. Dedonder. 1974. Identification of the structural gene of levansucrase in *Bacillus subtilis* Marburg. *Mol. Gen. Genet.* **128**: 213–221.

Lessard P.A., X.M. O'Brien, N.A. Ahlgren, S.A. Ribich and A.J. Sinskey. 1999. Characterization of IS*1676* from *Rhodococcus erythropolis* SQ1. *Appl. Microbiol. Biotechnol.* **52**: 811–819.

de Meirsman C., C. Croes, J. Desair, C. Verreth, A. Van Gool and J. Vanderleyden. 1989. Identification of insertion sequence element IS*427* in pTiT37 plasmid DNA of an *Agrobacterium tumefaciens* T37 isolate. *Plasmid* **21**: 129–137.

Miché L., D. Faure, M. Blot, E. Cabanne-Giuli and J. Balandreau. 2001. Detection and activity of insertion sequences in environmental strains of *Burkholderia*. *Environ. Microbiol*. **3**: 766–773.

Michiels T. and G. Cornelis. 1984. Detection and characterisation of Tn*2501*, a transposon included within the lactose transposon Tn*951*. *J. Bacteriol*. **158**: 866–871.

Nagy Z. and M. Chandler. 2004. Regulation of transposition in bacteria. *Res. Microbiol.* **155**: 387–398.

Providenti M.A. and R.C. Wyndham. 2001. Identification and functional characterization of CbaR, a MarR-like modulator of the *cbaABC*-encoded chlorobenzoate catabolism pathway. *Appl. Environ. Microbiol.* **67**: 3530–3541.

Raabe T., E. Jenny and J. Meyer. 1988. A selection cartridge for rapid detection and analysis of spontaneous mutations including insertions of transposable elements in Enterobacteriaceae. *Mol. Gen. Genet.* **215**: 176–180.

Romantschuk M., G.Y. Richter, P. Mukhopadhyay and D. Mills. 1991. IS*801*, an insertion sequence element isolated from *Pseudomonas syringae* pathovar phaseolicola. *Mol. Microbiol*. **5**: 617–622.

Schneider D., D. Faure, M. Noirclerc-Savoye, A.C. Barriere, E. Coursange and M. Blot. 2000. A broad-host-range plasmid for isolating mobile genetic elements in gram-negative bacteria. *Plasmid* **44**: 201–207.

Scordilis G.E., H. Ree and T.G. Lessie. 1987. Identification of transposable elements which activate gene expression in *Pseudomonas cepacia*. *J. Bacteriol*. **169**: 8–13.

Shapiro J.A. 1969. Mutations caused by the insertion of genetic material into the galactose operon of *E. coli*. *J. Mol. Biol*. **40**: 93–105.

Simon R., B. Hotte, B. Klauke and B. Kosier. 1991. Isolation and characterization of insertion sequence elements from gram-negative bacteria by using new broad-host-range, positive selection vectors. *J. Bacteriol*. **173**: 1502–1508.

Steinmetz M., D. LeCoq, H.B. Djemia and P. Gay. 1983. Genetic analysis of *sacB*, the structural gene of a secreted enzyme, levansucrase of *Bacillus subtilis* Marburg. *Mol. Gen. Genet.* **191**: 138–144.

Szeverenyi I., A. Hodel, W. Arber and F. Olasz. 1996. Vector for IS element entrapment and functional characterization based on turning on expression of distal promoterless genes. *Gene* **174**: 103–10.

Tauch A., S. Krieft, J. Kalinowski and A. Puhler. 2000. The 51,409-bp R-plasmid pTP10 from the multiresistant clinical isolate *Corynebacterium striatum* M82B is composed of DNA segments initially identified in soil bacteria and in plant, animal, and human pathogens. *Mol. Gen. Genet.* **263**: 1–11.

Tauch A., A. Schluter, N. Bischoff, A. Goesmann, F. Meyer and A. Puhler. 2003. The 79,370-bp conjugative plasmid pB4 consists of an IncP-1beta backbone loaded with a chromate resistance transposon, the *strA-strB* streptomycin resistance gene pair, the oxacillinase gene bla(NPS-1), and a tripartite antibiotic efflux system of the resistance-nodulation-division family. *Mol. Genet. Genomics* **268**: 570–584.

Ulrich A. and A. Puhler. 1994. The new class II transposon Tn*163* is plasmid-borne in two unrelated *Rhizobium leguminosarum biovar viciae* strains. *Mol. Gen. Genet.* **242**: 505–516.

Van Spanning R.J.M., A.P. De Boer, D.J. Slotboom, W.N. Reijnders and A.H. Stouthamer. 1995. Isolation and characterization of a novel insertion sequence element, IS*1248*, in *Paracoccus denitrificans*. *Plasmid* **34**: 11–21.

Vertes A.A., Y. Asai, M. Inui, M. Kobayashi, Y. Kurusu and H. Yukawa. 1994. Transposon mutagenesis of coryneform bacteria. *Mol. Gen. Genet*. **245**: 397–405.

Weightman A.J., A.W. Topping, K.E. Hill, L.L. Lee, K. Sakai, J.H. Slater and A.W. Thomas. 2002. Transposition of DEH, a broad-host-range transposon flanked by IS*Ppu*12, in *Pseudomonas putida* is associated with genomic rearrangements and dehalogenase gene silencing. *J. Bacteriol*. **184**: 6581–6591.

Yeo C.C. and C.L. Poh. 1996. IS*1394* from *Pseudomonas alcaligenes* N.C.I.B. 9867: identification and characterisation of a member of the IS*30* family of insertion elements. *Gene* **175**: 109–113.

Van Spanning R.J.M., A.P. De Boer, D.J. Slotboom, W.N. Reijnders and A.H. Stouthamer. 1995. Isolation and characterization of a novel insertion sequence element, IS*1248*, in *Paracoccus denitrificans. Plasmid* **34**: 11–21.