Insertion Element IS102 Resides in Plasmid pSC101

HISAKO OHTSUBO, MICHAEL ZENILMAN, AND EIICHI OHTSUBO*

Department of Microbiology, School of Medicine, State University of New York at Stony Brook, Stony Brook, New York 11794

In vivo recombination was found to occur between plasmid pHs1, a temperature-sensitive replication mutant of pSC101 carrying tetracycline resistance, and plasmid ColE1 after selection for tetracycline resistance at the restrictive temperature, 42°C. Extensive analysis of the physical structures of three of these recombinant plasmids, using restriction endonucleases and the electron microscope heteroduplex method, revealed that the plasmid pHs1 was integrated into different sites on ColE1. The recombinant plasmids contained a duplication of a unique 1-kilobase (kb) sequence of pHs1 in a direct orientation at the junctions between the two parental plasmid sequences. This was confirmed by comparing the nucleotide sequence of the recombinants and their parental plasmids. Nucleotide sequence analysis further revealed that nine nucleotides at the site of recombination of ColE1 were duplicated at the junction of each of the 1-kb sequences. The formation of recombinants was independent of RecA function. Based on our previous finding that a plasmid containing a deoxyribonucleic acid insertion (IS) element can recombine with a second plasmid to generate a duplication of the IS element, we conclude that the 1-kb sequence is an insertion sequence, which we named IS102. For convenience, we have also denoted the IS102 sequence as ρφ to assign the orientation of the sequence. Eighteen nucleotides at one end (η end) were found to be repeated in an inverted orientation at the other end (φ end) of IS102. The nucleotide sequence of the η end of the sequence was found to be identical to the sequence at the ends of the transposon Tn903, which is responsible for transposition of the kanamycin resistance gene.

We have previously described a simple genetic system used to study the cointegration of two different plasmid genomes (18). Studies on in vivo recombination using this system have shown that a plasmid containing the insertion element IS1 can act like a transposon since the whole genome can insert into a second plasmid. Upon insertion, the IS1 and nine base pairs at the insertion site are duplicated in a direct orientation at the junctions of the two plasmids as represented schematically in Fig. 1.

This paper describes the analysis of in vivo recombination between the plasmid pHs1 (6), a temperature-sensitive replication mutant of the plasmid pSC101, and a second plasmid, ColE1, and demonstrates that a particular DNA sequence, 1 kilobase (kb) in length, is originally present in pHs1 and mediates the cointegration of these two plasmids just as IS1 does as represented in Fig. 1. These results enable us to conclude that the 1-kb sequence present in pHs1, and therefore in pSC101 (3), is an insertion sequence which we have named IS102.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacteria used were Escherichia coli K-12 strains JE5507 and JE5519. JE5519 is a RecA- derivative of JE5507 (18). The plasmid pHs1 is a temperature-sensitive replication mutant of pSC101 carrying tetracycline resistance (6). The recombinant plasmids pH01, pH02, and pH03 were isolated by recombination between ColE1 and pHs1 in the RecA+ strain; pH04 and pH05 were isolated in the RecA- strain. These plasmids are described in Results.

Covalently closed circular DNA was isolated from cells harboring each plasmid according to the method described by Ohtsubo et al. (17). The pure DNA was stored in TE buffer containing 0.01 M Tris and 0.001 M EDTA, pH 8.0.

Construction of strains carrying pHs1 and ColE1. Bacterial strains carrying both pHs1 and ColE1 were constructed by successive transformation of ColE1 DNA and pHs1 DNA as described by Kretzschmer et al. (9).

Isolation of cells carrying recombinant plasmids. Purified cells containing both plasmids were grown in 5 ml of Penassay broth (Dirco Laboratories) at 30°C overnight. A 0.1-ml sample of a 1:106 dilution was then inoculated into several tubes containing 5 ml (for RecA+) or 10 ml (for RecA-) of Penassay broth and incubated overnight at 30°C. Then 0.1 ml from each tube was plated on a prewarmed 10-μg/ml tetracycline plate, and the plate was incubated at 42°C for 24 h. The colonies were then purified at 30°C, and the presence of recombinant plasmids was tested by the crude lysis method described below. Only one
Heteroduplex molecules were prepared for electron microscopy as described by Sharp et al. (30). ColE1 DNA was used as the internal length standard for double strands, and φX174 phage DNA was used as a single-stranded internal length standard.

**Enzymes.** EcoRI was purchased from Miles Laboratories, Inc., and PstI, Hhal, Alul, Hinfi, and HaeII were obtained from Bio Labs, Inc. BamHI and SstII were purchased from Bethesda Research Laboratories. These enzymes were assayed as recommended by the laboratory from which they were obtained. HaeII was isolated according to Roberts et al. (25). The reaction mixtures for these endonucleases contained 6 mM MgCl₂, 6 mM 2-mercaptoethanol, 6 mM Tris-hydrochloride (pH 7.9), and bovine serum albumin (500 µg/ml). Polynucleotide kinase (P-L Biochemicals Co.) was used to phosphorylate the 5' ends of the restriction fragments used in sequencing with [γ-³²P]ATP (Amersham Corp.). The reaction mixture contained 50 mM Tris-hydrochloride (pH 7.5), 10 mM MgCl₂, and 5 mM dithiothreitol. Bacterial alkaline phosphatase (36 U/ml), obtained from Worthington Biochemicals Corp., was used in 0.1 M Tris-hydrochloride (pH 8.0)–10 mM MgCl₂.

**Gel electrophoresis.** Native duplex DNA fragments were separated by gel electrophoresis on a 0.7% agarose (13 by 15 by 0.2 cm) or on 4% polyacrylamide slab gels (acrylamide/bisacrylamide = 2:1). DNA bands were visualized by staining with ethidium bromide (0.4 µg/ml) under short-wavelength UV light.

**Determination of nucleotide sequence.** DNA fragments ³²P labeled at the 5' ends were obtained by strand separation or by further cleavage of double-stranded DNA labeled at both 5' ends with another restriction endonuclease (12). The nucleotide sequence of the 5' labeled fragments was determined as described by Maxam and Gilbert (12). To separate the chemically modified and degraded products of the base-specific reactions, thin gels (0.04 by 20 by 40 cm) (28) were used in addition to those described by Maxam and Gilbert (12).

**RESULTS**

**Isolation of cells harboring pHS1 and ColE1 recombinant plasmids.** Figure 2 summarizes the genetic and physical properties of the parental plasmids used for the isolation of recombinants. pHS1, 9.43 kb in length, is a temperature-sensitive replication mutant of pSC101 (6) carrying the tetracycline resistance gene. It can replicate normally at 30°C, but cannot replicate at 42°C within a bacterial cell. ColE1, 6.65 kb in length, is a plasmid responsible for production of the bactericidal protein colicin E1. It replicates at both 30 and 42°C. These two plasmids belong to different incompatibility groups and can therefore replicate independently within the same cell when grown at 30°C.

When cells harboring pHS1 and ColE1 were plated on media containing 10 µg of tetracycline per ml and incubated at 42°C for 24 h, we observed tetracycline-resistant colonies (Tc') at an average frequency of 1 Tc' cell per 2.3 × 10⁸ colony which was identified to carry large plasmids was saved from each culture tube to obtain independent recombinants as described by Luria and Delbrück (11).

**Crude lysis.** A quick and simple way to visualize and compare the plasmid closed circular DNA in bacterial cells is the crude lysis method. We used a modified procedure of Michel and Bauer (13), in which 40 µl of the crude lysate containing mostly plasmid DNA was mixed with 10 µl of 50% glycerol-0.025% bromophenol blue dye solution and run on a 0.7% agarose gel.

**Electron microscopy.** Molecular lengths of open circular and linear duplex DNA were determined by electron microscopic measurement of molecular spreads by the standard aqueous basic protein film technique (4). For each calculation, about 40 individual molecules, as well as internal length standards, were measured and averaged. The length of our internal standard, colicin E1 plasmid DNA, was determined to be 6.65 kb from aqueous spreading with double-stranded RFII φX174, which is known from sequence data to be 5.38 kb long (18, 27).
cells in a RecA+ background and 1 per 5.2 × 10⁶ cells in a RecA− background. We isolated plasmid DNA from clones which were independently isolated from three different colonies from the RecA+ strain and two from the RecA− strain. We found that the plasmid DNA was larger than either parental plasmid. We named these pH plasmids. Three of them, pH01, pH02, and pH03, which were isolated from RecA+, were subjected to further physical analysis.

Table 1 summarizes the molecular lengths of the plasmids pH01, pH02, and pH03 as determined by electron microscopy. The pH0 plasmids were approximately identical in size, but their lengths were slightly larger than the sum of the parental plasmids, pH51 and ColE1.

When each pH0 plasmid DNA was digested with BamHI, which cleaves pH51 once but not ColE1 (see Fig. 2), single bands were generated which migrated identically in all recombinants tested (Fig. 3). When pH0 plasmid DNA was digested with EcoRI, which cleaves once in both pH51 and ColE1, two fragments, which differed

Table 1. Molecular lengths of the plasmids and their EcoRI fragments

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Length in kilobases (ColE1 unit)</th>
<th>Length of EcoRI fragment in kilobases (ColE1 unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH51</td>
<td>5.38 (0.810 ± 0.028)*</td>
<td>8.30 (1.249 ± 0.147)*</td>
</tr>
<tr>
<td>pH01</td>
<td>16.61 (2.497 ± 0.147)</td>
<td>5.86 (0.881 ± 0.051)</td>
</tr>
<tr>
<td>pH02</td>
<td>16.36 (2.459 ± 0.120)</td>
<td>10.68 (1.906 ± 0.479)</td>
</tr>
<tr>
<td>pH03</td>
<td>16.91 (2.543 ± 0.203)</td>
<td>3.05 (0.459 ± 0.031)</td>
</tr>
</tbody>
</table>

* Data taken from Sanger et al. (27) and Ohtsubo et al. (18). Uncertainties are one standard deviation.

† Based on the observation that two EcoRI fragments of pH01 comigrate in an agarose gel (see Fig. 3).

Fig. 2. Physical structures of the plasmids pH51 and ColE1. Cleavage sites on the plasmids for the restriction endonucleases EcoRI and BamHI are shown by the arrows. The distance between the EcoRI and the BamHI site on pH51 is 0.39 kb (32). The filled box represents the IS102 (or γβ) sequence, 1 kb in length, which is described in the text. Note that ColE1 is not cut by BamHI. The approximate location of the origins of replication of the two plasmids and the direction of replication of ColE1 (2, 7, 10, 34) are shown.

Fig. 3. (A) 0.7% agarose gel showing purified parental and recombinant pH plasmids. In each column, closed circular DNA molecules (the lower band) and open circular DNA molecules (the upper band) are visualized. (B) 0.75% agarose gel showing EcoRI digests (a), BamHI digests (c), and EcoRI/BamHI double digests (b) of the parental and recombinant plasmids. EcoRI/BamHI double digests generated a small fragment of 0.38 kb which was not visualized on the gel. The size marker is EcoRI digested λ DNA (I).
in size for each pHO plasmid, were generated (see Fig. 3 and Table 1). This suggests that the structures of the pHO plasmids differ. BamHI cleaved one of the EcoRI fragments generated from the pHO plasmids, removing a common 380-base pair fragment identical to that seen in the parental pHS1 plasmid (see Fig. 2 and 3).

Heteroduplex analysis of the pHO plasmids. In this section, we will first describe the final structures of the pHO plasmids and then the heteroduplex analysis results used to determine them. Figure 4 shows a linear representation of the physical structures of the pHO plasmids. The critical features of these structures are as follows. (i) Both of the parental plasmids, pHS1 and ColEl, were present in the pHO plasmids in their entirety. A 1-kb DNA sequence, originally present in pHS1 in one copy, occurred at the two junctions between the parental plasmids. This sequence, which we call IS102 or, in the heteroduplex analysis, \( y \), appeared in a directly repeated orientation; thus each pHO plasmid had an extra IS102 sequence. (ii) The site of recombination on ColEl differed in each pHO, but it was always at IS102 in pHS1. (iii) The relative sequence orientation of the parental plasmids was always the same.

These conclusions were obtained by heteroduplexing each pHO plasmid DNA molecule with a parental pHS1 and a ColEl derivative, pNT1. In the actual experiments, each pHO plasmid and pHS1 were cleaved with BamHI, generating linear molecules and thereby allowing us to define reference points on the pHO plasmid as well as on the pHS1 plasmid. pNT1, which was used to determine the location of the ColEl sequence in each pHO plasmid, lacks about 50% of ColEl (33) but contains a foreign DNA sequence of 0.41 kb at the EcoRI site. The sequence homology between ColEl and pNT1 is shown in Fig. 5. pNT1 plasmid DNA cleaved with EcoRI was used for these heteroduplex experiments.

Figure 6 shows a schematic representation of the heteroduplex molecules. The heteroduplex molecule pHO1/pHS1/pNT1 (Fig. 6A-a) had the following features. (i) pHO1 contained sequences which were hybridizable to the entire sequence of pHS1 and ColEl. (ii) A sequence 1 kb in length was present in addition to the entire pHS1 and ColEl sequences of pHO1. This is clearly shown by the two single strands connecting the parental plasmid sequences in the heteroduplex molecules. (iii) The junction of the ColEl sequence in the pHO1 was fixed in a unique position which could be determined by the location of pNT1. (iv) The heteroduplex junction \( x \) between the pHO1 and pHS1 sequence could not be fixed to a single point, but mapped at an area on the pHS1 sequence.

**Fig. 4.** Linear representation of the circular structures of the pHO plasmids. The DNA sequences of the parental plasmids pHS1 and ColEl in each pHO plasmid are denoted by thick lines and sawtooth lines, respectively. Restriction endonuclease cutting sites for EcoRI and BamHI and the origins of replication of the parental plasmids are assigned to indicate the sequence orientation of the parental plasmid sequences. Note that IS102 (also called \( y \)) is located at each junction in the same orientation. Note also that the relative orientation of ColEl is the same with respect to pHS1 in each pHO plasmid.

**Fig. 5.** (A) Schematic representation of circular heteroduplex molecules showing the sequence relation between ColEl and its derivative pNT1. pNT1 was digested with EcoRI before heteroduplexing. (B) Heteroduplex molecules of ColEl and pNT1, both of which were digested with EcoRI. The duplex region was 3.27 ± 0.15 kb, and the two single strands were 0.41 ± 0.13 kb and 3.07 ± 0.38 kb.
A NEW INSERTION ELEMENT, IS102

Fig. 6. Heteroduplex structure of the pHO plasmids with pHSI and pNT1 plasmids. The pHSI sequence is shown by solid lines; ColEI and pNT1 sequences are denoted by sawtooth lines. The thick solid lines in the pHSI and pHO plasmids designate the single-stranded IS102 sequence, 1 kb in length. The IS102 sequence appears as a duplication at the junctions of the pHSI and ColEI sequences in each pHO plasmid. (A) a, Structure of the pHO1/pHSI/pNT1 heteroduplex; b and c, heteroduplex molecules of pHSI with broken pHO1 strands. (B) Structure of the pHO2/pHSI/pNT1 heteroduplex molecules. (C) Structure of the pHO3/pHSI/pNT1 heteroduplex molecule. In the heteroduplex solution of pHO2/pHSI/pNT1 and of pHO3/pHSI/pNT1, we observed heteroduplex structures similar to those shown in A-b and -c.

7A shows the actual map position of the junction x seen in various heteroduplex molecules. The position of the junction was, however, limited to a region of about 1 kb. As demonstrated in Fig. 8, this result could be explained by branch migration (15) due to the presence of the 1-kb IS102 sequence which appeared twice in a direct repeat at the junction between the pHSI and ColEI sequences of the pHO1 plasmid.

We also observed two additional types of heteroduplex molecules with broken strands of pHO1 DNA (Fig. 6A-b and -c). The positions of the heteroduplex junctions γ and θ in these molecules are the ends of the IS102 sequence. Thus these molecules were available to fix the positions of the IS102 sequence as presented in Fig. 7.

The heteroduplex molecules of pHO2 or pHO3 with pHSI and pNT1 are represented in Fig. 6B and 6C. The position of pNT1 in the ColEI loops varied between pHO1, pHO2, and pHO3, indicating that the sites of integration of pHS1 into ColEI were different in each pHO plasmid. Aside from this, the heteroduplex features of pHO2 and -3 with pHS1 were very similar to those found in pHO1/pHS1/pNT1 molecules. The heteroduplex junction x between pHO and pHS1 showed branch migration as demonstrated by plotting the junctions as shown in Fig. 7B and 7C. The additional heteroduplex molecules of pHS1 with broken pHO2 or pHO3 were also observed to be similar to those shown in Fig. 6A-b and -c. The heteroduplex junctions γ and θ in these molecules fixed the area of branch migration as discussed above.

The relative orientation of the two sequences
Fig. 7. Position of the junctions seen in the heteroduplex molecule of the pH0 plasmid and the pHSI plasmid. (A) Position of the junctions x, y, and θ, seen in the pH01/pHS1 heteroduplexes shown in Fig. 6A-a, -b, and -c, respectively. Their positions were plotted on the pHSI sequence from the BamHI site. (B) Position of the junction x seen in the pH02/pHS1 heteroduplex molecules shown in Fig. 6B. The heteroduplex molecules with junctions η and θ are not shown in Fig. 6 but are similar to those shown in Fig. 6A-b and -c. (C) Position of the junction x seen in the pH03/pHS1 heteroduplex region shown in Fig. 6C. The heteroduplex molecules with junctions η and θ are not shown in Fig. 6 but are similar to those represented in Fig. 6 A-b and -c.

Fig. 8. Interpretation of the branch migration phenomenon occurring at the IS102 sequence in the heteroduplex molecules of pH0 and pHSI plasmids. In this figure, a b c d denotes a strand of IS102. a'b'c'd' denotes the complementary strand of the IS102 sequence a b c d. Since the sequence a b c d appears as a duplication in the pH0 plasmids and competes in the hybridization with pHSI for the a'b'c'd' strand, the position of junction x on the left side of the molecule can migrate to the x' position of the right side of molecule or vice versa by a branch migration mechanism.

of parental plasmids present in each pH0 plasmid was always the same, although the ColE1 was connected at a unique site by the IS102 sequence of the pHSI plasmid in each pH0 plasmid.

Cleavage analysis with SstII and HaeII. Cleavage analysis with the restriction enzymes SstII and HaeII showed interesting results. Digestion of the pH01-3 plasmids with SstII, which cleaves both pHSI and ColE1 only once, generated three bands, rather than two bands as expected (Fig. 9A). The largest band in the pH0 digest was constant and identical to pHSI in size, although the other two fragments were unique to each pH0. Digestion of the pH0 plasmids with HaeII generated all of the HaeII fragments of the pHSI parent (see Fig. 9B). All of the ColE1 bands except one, unique for each pH0, were also generated, whereas two new bands, different in each pH0 plasmid and not found in either parental plasmid, appeared. Furthermore, we observed that two of the pHSI bands at 0.28 and 0.48 kb had a higher relative brightness than the same band generated by pHSI DNA alone.

These results, combined with the heteroduplex data, indicate that an additional sequence of IS102 is present in pH0 and that IS102 contained one SstII cutting site and at least three HaeII cutting sites. This IS102 sequence is from pHSI and is duplicated at the junctions of the two parental plasmid sequences in a direct orientation as shown in Fig. 4.

pH04 and pH05 were recombinant plasmids isolated from a RecA+ strain harboring pHSI and ColE1. Results of cleavage with SstII showed that pH04 and pH05 generated three bands (Fig. 9A); the largest band corresponded to linear pHSI DNA; others were unique for each respective recombinant. Digestion of pH04 and pH05 with HaeII generated two characteristic pHSI bands with a higher relative brightness as was observed after HaeII digestion of pH01-3 (Fig. 9B). These results indicated that the formation of pH04 and pH05 was mediated by the IS102 and that again this process led to
a duplication of IS102 at the junctions of the pHS1 and ColE1 sequences in a direct orientation.

Nucleotide sequencing analysis. We have examined the nucleotide sequence of the DNA fragments in ColE1 containing the target site for recombination with pHS1 and the two fragments of the recombinants pH01 or pH02 containing the junctions of ColE1 and pHS1. These fragments were readily identified by comparison of the cleavage patterns of the pH0 plasmid with those of the parental plasmids. For example, upon digestion with the restriction endonuclease HaeIII, the F fragment (approximately 410 base pairs long) of ColE1 (see Tomizawa et al. [33]) was not seen in the HaeIII digest of pH01, indicating that this HaeIII fragment contained the site of recombination with pHS1. The HaeIII digest of pH01, however, generated two new fragments (approximately 440 and 270 base pairs) which were not seen in the HaeIII digests of the parental plasmids, indicating that those fragments were due to recombination between ColE1 and pHS1 and must therefore contain the junction regions of the pH01 plasmid.

After purification of these three HaeIII fragments by electrophoretic elution from polyacrylamide gels, the DNA fragments were subjected to further digestion with other restriction endonucleases, as listed in Materials and Methods, to determine secondary cleavage sites or to prepare shortened fragments for sequence analysis by the Maxam and Gilbert method (12). Figure 10 shows the regions A through F which were sequenced. It also shows the DNA strands used for sequencing. Figure 11 summarizes the nucleotide sequences determined. The results of nucleotide sequences show that parts of the sequence in the region A of ColE1 appeared in the sequences in the regions B and C of pH01 and were connected with different sequences, which are probably the two ends of IS102, defined as η end and θ end (Fig. 11). Similar results were obtained by sequencing the region D of ColE1 and the regions E and F of pH02 (see Fig. 10 and 11). Note that the nucleotide sequences connected with the ColE1 sequences were identical to those defined as η end and θ end of IS102 (see bottom of Fig. 11) and that 18 nucleotides at η and θ ends appear as inverted repeats. It is interesting that the sequence of η end of IS102 is identical to the sequence at the end of Tn903 described by Oka et al. (23). The sequence results also showed that nine base pairs originally present in ColE1 appeared as a direct duplication at the junction with the IS102 sequence.

DISCUSSION

We have shown that two different plasmids, pH01 and ColE1, can recombine to form co-integrated large plasmids by the transposition of pHS1 into ColE1. Systematic analysis of the recombinations with restriction endonucleases, electron microscopy, and DNA sequencing has shown that the parental pH01 plasmid is always
integrated at a particular 1-kb sequence into the other parent, ColEl, at any number of sites. This 1-kb sequence is present at both junctions of recombination as a direct repeat. The sequencing analysis demonstrated that nine bases at the target site on ColEl had been duplicated in a direct orientation of pHS1 into ColEl. This observation is completely analogous to the case of the plasmid pSM1, which has one copy of the element IS1, which, after recombining with the plasmid pHS1, gives rise to an IS1 duplication at the junction of the two plasmids (18). The 1-kb sequence, present in the plasmid pHS1, can therefore be classified as an insertion element; we have named it IS102.

Nucleotide sequence analysis of the junction region between pHS1 and ColEl in the pHO plasmids has shown that a nine-nucleotide sequence at the recombination site was duplicated in the recombinant. The molecular mechanism for the formation of a recombination of this type has been discussed, and we believe it to be related to the phenomenon by which the insertion element IS1 generates a duplication of the nine-nucleotide sequence at the target site (18).

Although we also used pHS1 in our previous study of recombination between pSM1 and pHS1, we never observed the involvement of the IS102 present in pHS1; only the IS1 present on pSM1 was involved in the formation of recombinant plasmids. This is probably due to the frequency of transposition, which is much higher when IS1 is used than when IS102 is used. Ohsumi et al. (15) reported a case of in vivo recombination between the filamentous bacteriophage fl and the plasmid pSC101. Restriction endonucleases and sequence analysis of two derivatives suggest that the recombinant contained the two genomes flanked by a 200-nucleotide-long sequence in a direct orientation (5). This sequence, thought to be present in pSC101, generates a five-base direct repeat at the fl target site. Using pH1, a temperature-sensitive mutant of pSC101, we never found this sequence in pHS1 to be specifically involved in the formation of pHO plasmids; IS102, present originally in pHS1, was the only one involved.

Nomura et al. (14) reported that a transposable element, Tn903, contained inverted repeats of about 1 kb flanking the gene responsible for resistance to kanamycin. It is interesting that the nucleotide sequence of one end (q end) of IS102 was found to be the same as that of both ends of Tn903. From this observation, it is reasonable to assume that IS102 is the same as the inverted repeat sequence in Tn903. The transposon Tn903 was initially present in the resistance plasmid R6 and has been seen by electron microscopy as an inversion loop structure with a duplex stem after denaturation and renaturation of the plasmid DNA (29). This sequence has been named the θ0 sequence (20, 21). There-
fore we assume that the notation $\eta^f$ used for IS102 and the orientation of the sequence used in this paper are the same as those used previously.

Most of the transposable elements are known to be flanked by two copies of insertion sequences. The inverted stem sequence in transposon Tn1891 was shown to be the insertion sequence IS1 (31). The inverted stem sequence of 1.3 kb in Tn10 is said to be the insertion sequence IS10 (26, 29). It has also been shown that DNA segments flanked by insertion sequences in a direct orientation act as transposons, as demonstrated by the structure of the transposon Tn9, in which two IS1 sequences flank the DNA segment containing the chloramphenicol resistance genes (8). It is therefore possible that when IS102 flanks a segment in a direct orientation, the two IS102 sequences as well as the sequence between them are transposed.

As described earlier, IS102 was found to contain inverted repeat sequences of 18 nucleotides in length which appear at the exact ends of IS102. It has previously been thought that presence of about a 35-nucleotide sequence at the ends of an insertion element in an inverted order is probably vital for transposition of the element. This is based on the observation that all the known IS and Tn elements contained inverted repeats of about 35-nucleotide sequences or more (19, 22, 24). Our finding, however, suggests that only the first 18 bases from the end of the element may be important for translocation of the element. It is also possible that although an inverted repeat is necessary, its length depends upon the particular insertion element and is probably dependent upon the proteins involved in catalyzing the translocation reaction.

We have shown in this paper that this recombination system, originally described by Ohtsubo et al. (18), is useful in detecting new insertion...
sequences and to assay their transposition activity. Since the molecules are small, they can be easily subjected to analysis on the molecular level as demonstrated in this paper.

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LITERATURE CITED