The construction and characterization of an effective transpositional system based on IS30

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Abstract **We constructed an in vivo system to detect transpositional rearrangements induced by the insertion sequence IS30. The transposase protein expressed from the transposase producer plasmids catalyzed rearrangements on different target sequences presented in *trans*. High yields, up to 83%, of transpositional frequencies were observed. The frequency of rearrangements correlated with the amount of transposase protein produced and the attractivity of the target sequences. Alteration in the frequency of transposition was observed in the *recA*$^-$ *E. coli* strains JM109 and TG2. Remarkable structural and functional analogy was found with site-specific recombination systems.**

Key words: IS30; Overproduction of IS30 transposase; In vivo assay; Transpositional frequency; *Escherichia coli*

1. Introduction

The insertion sequence IS30 (Fig. 1A) is a low copy number mobile genetic element residential in the K-12 and C strains of *Escherichia coli* [1]. The transposition of IS30 proceeds in a conservative pathway, via an intermediate that consists of two elements joining to each other with their left and right terminal inverted repeats (IR) [2]. The highly unstable $(IS30)_2$ intermediate is formed by means of site-specific dimerization and mediates further DNA rearrangements characteristic of the element. IS30 appears to be bifunctional as it catalyzes site-specific reactions (dimerization) and 'classical' transpositional events: simple insertion, deletion, inversion and transpositional fusion (cointegration). IS30 has a defined target specificity: natural hot spot sequences were described from lambda and P1 bacteriophages [2], but its own inverted repeats are also preferred targets. The 1221 bp long element contains one large open reading frame (ORF-A, bp 63–1211) coding for the 44.3 kDa transposase protein (Fig. 1A) [3]. The promoter of the transposase gene, *P30A*, overlaps with the left IR of the element [4,5]. The N-terminal part of the IS30 transposase (ORF-A-N) containing the DNA-binding domain, placed under the control of the inducible *tac* promoter, was overproduced and purified [6].

It was previously demonstrated that the genomic copies of IS30 were unable to complement the mutations in the transposase gene (Olasz et al., manuscript submitted) [2]. The lack of detectable complementation can be explained in two ways: (1) the amount of available transposase protein (synthesized by the genomic copies of the element) is too low to carry out transpositional reactions effectively; (2) the transposase protein can act only in *cis*, i.e. on the same replicon from which it was produced – as was shown in the case of Tn*5* [7–9].

To clarify this question we studied the in *trans* activity of the IS30 transposase in reactions characteristic of the element, and investigated the correlation between the expression level of IS30 transposase and the transpositional frequency. In our experiments the IS30 transposase was able to perform site-specific and 'classical' transpositional reactions in *trans* (dimerization and deletion, respectively), at very high frequencies, up to 82.7%. The system described here represents a simple and useful tool in the isolation and characterization of mutants and in the development of an in vitro system.

2. Materials and methods

2.1. Bacterial strains, media, enzymes and chemicals

The experiments were carried out in the *recA*$^-$ *E. coli* strains JM109 [10] and TG2 [11]. LB medium was prepared as described by Miller [12]. The primary antibody used with an Amersham immunoblotting detection kit (RPN23) was developed against IS30 transposase. Antibiotics were used in the following final concentrations: kanamycin (Km) 20 μg/ml; ampicillin (Ap) 150 μg/ml; chloramphenicol (Cm) 20 μg/ml. All other DNA techniques were carried out according to Sambrook et al. [11]. The structure of the plasmids used in the experiments is shown in Fig. 1.

2.2. Induction of the IS30 transposase

TG2 colonies harboring the different transposase producer plasmids were grown for 18 h in 2 ml LB Ap at 37°C in a waterbath, with or without 0.01 mM IPTG induction. Cells were harvested, protein samples were analyzed by SDS-PAGE (according to Laemmli [15]) and immunoblotting. The transposase fraction was determined using a UVP image analyzer and GelBase software.

2.3. The measurement of the in trans activity of IS30 transposase

The transposase producer plasmids pJKI110, pJKI132 and pJKI154 were introduced into JM109 cells. One colony of each transformation was chosen to make competent cells that were subsequently transformed with one of the substrate plasmids pJKI216, pJKI213, pJKI225 or pTFA2. In the control reactions we omitted the first transformation step, thus the bacterial host contained no transposase producer plasmid. After selection for the Cm^R phenotype of the substrate plasmids, three individual colonies from each category (corresponding to a unique transposase producer-substrate plasmid combination) were picked and grown for 18 h in 2 ml LB Km at 37°C in a waterbath, with or without IPTG induction. Cells were harvested and plasmid DNA was purified. To detect the rearrangements in the substrate plasmids, it was necessary to separate the Cm^S segregants from the original Cm^R plasmids existing in the same cell, so the plasmid DNA population was introduced into TG2 cells. The transformants were spread on solid LB medium containing Km, incubated overnight at 37°C and replica-plated on LB Km and LB Cm plates. The Cm^R and Cm^S colonies were counted and the frequency of transpositional rearrangement was calculated as the ratio of Cm^S versus all, i.e. Cm^S + Cm^R colonies. Plasmid DNA was purified from at least 10 colonies in each category and analyzed by restriction mapping.

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3. Results

3.1. The construction of the system

To study the in *trans* activity of IS30 transposase, the following criteria had to be fulfilled. (1) The transposase producing ORF-A had to be separated from the possible transpositional target sequences, including the left and right inverted repeat of the element. Therefore, two groups of plasmids were constructed: the transposase producer replicons (Fig. 1B) contained the intact ORF of the IS30 transposase, but carried no target sequences: the left and right inverted repeats of the element were inactivated (see legend to Fig. 1); the compatible substrate replicons (Fig. 1C) carried different transpositional target sequences and produced no functional tranposase. (2) To obtain a higher level of expression and transpositional activity, and to be able to investigate the correlation between them, the original promoter of the ORF-A was replaced with the inducible *tac* promoter in the transposase producer plasmid pJKI110 and pJKI132 (Fig. 1B). The cloned *lacI*^{q} repressor gene provided tight control over the transposase expression in pJKI132. The control plasmid pJKI154 contained no functional promoter region. (3) It was crucial to develop a simple method to detect transpositional rearrangements at a very low frequency. In the substrate plasmids, the transpositional reactions led to the loss of the Cm^R gene by means of transpositional deletion (pJKI225) or site-specific dimerization (pTFA2, pJKI213, pJKI216, see Fig. 3B), so the bacteria carrying the original and rearranged substrate plasmids were easily identified as Cm^R and Cm^S clones, respectively.

3.2. The expression level of IS30 transposase in the transposase producer plasmids

To assure that the transposase producer plasmids were overexpressing the IS30 transposase, and to study the effect of induction, bacterial cells harboring the plasmids pJKI110, pJKI132 and pJKI154 were grown under inducing or non-inducing conditions. Samples were analyzed by SDS-PAGE and immunoblotting (Fig. 2). No transposase was detectable in the bacteria without transposase producer plasmids, although the host cells contained the genomic copies of IS30, or in the bacteria carrying pJKI154, where the *P30A* promoter was truncated. In the cells harboring the transposase producer plasmids pJKI110 and pJKI132, in which the transposase gene was placed under the control of the *tac* promoter, the transposase represented 17–21% of the total protein under inducing conditions. From pJKI110 the same level of transposase was produced without IPTG induction, while in the case of pJKI132 the *lacI*^{q} repressor effectively regulated the expression of the transposase gene.

3.3. The measurement of the in trans activity of IS30

One transposase producer and one substrate plasmid were introduced into the same *recA*$^-$ JM109 bacterial host. In this way, 16 transposase producer-substrate plasmid combination were created (we used 4 substrate and 3 transposase producer plasmids; in the control reaction there was no transposase producer plasmid). The bacteria were incubated under inducing or non-inducing conditions, and the transpositional frequencies were measured (see Section 2.3). The schematic representation of the assay is shown in Fig. 3A, while the experimental data are presented in Table 1A. In the absence of any transposase producer plasmids, the background activ-

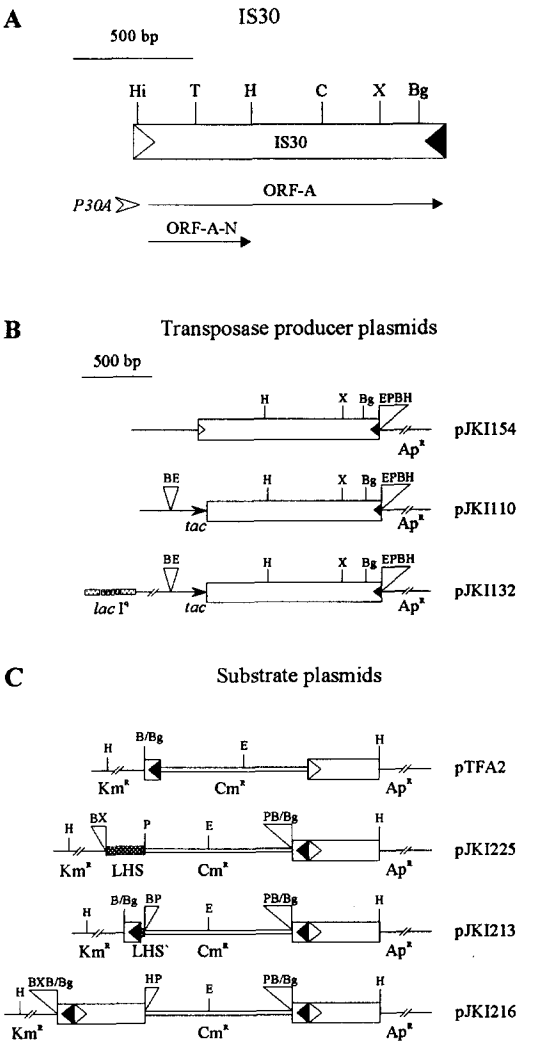

Fig. 1. The structure of IS30 (A), the transposase producer (B) and substrate (C) plasmids. A rectangle represents the IS30 sequence, the white and black triangles indicate the left and right inverted repeats, respectively. The IS30 transposase protein is encoded by the ORF-A and transcribed from the *P30A* promoter (white arrowhead). ORF-A-N is coding for the N-terminal truncated part of the transposase (see Section 1). The transposase producer plasmids were derivatives of pAW380 [6], in which the original *P30A* promoter was deleted and substituted by the *tac* promoter (black arrowhead). The inactivation of the right IR of the element by the introduction of a new *Eco*RI site at 1216 bp (Olasz et al., manuscript submitted) and subsequent cloning steps resulted in pJKI110, in which the right IR was partially deleted (small black triangle). The insertion of the *lacI*^{q} gene (dotted rectangle) from pDMI,1 [13] into pJKI110 yielded pJKI132. In pJKI154 the *tac* promoter was removed and the left IR was truncated (small white triangle). pJKI154 contained the IS30 sequence from the unique *Hin*cII site to the newly introduced *Eco*RI site (bp 17–1216). The substrate plasmids were pACYC177 derivatives. pTFA2 was a deletion product of pAW444 [2], and contained the Cm^R gene flanked by the right (bp 1115–1221) and the left ends (bp 1–459) of IS30. The three other substrate plasmids were pAW441 [2] derivatives and carried an $(IS30)_2$ intermediate structure (bp 1115–459) and the lambda hot spot (LHS, small dark rectangle) from pAW782 [2] in pJKI225, the 120 bp fragment of pFOL553 (unpublished) containing the right IR (bp 1115–1221) joined to the synthetic hot spot (LHS′) in pJKI213, and another intermediate structure from pAW136 [14] in pJKI216. Abbreviations: Ap^R: Ap resistance gene (β-lactamase); Km^R: Km resistance gene (aminoglucoside-3′-phosphotransferase); Cm^R: Cm resistance gene (chloramphenicol acetyltransferase). Restriction sites: B: *Bam*HI; B/Bg: *Bam*HI-*Bgl*II hybrid recognition site; Bg: *Bgl*II; C: *Cla*I; H: *Hin*dIII; Hi: *Hin*cII; E: *Eco*RI; P: *Pst*I; T: *Tth*111I; X: *Xba*I.

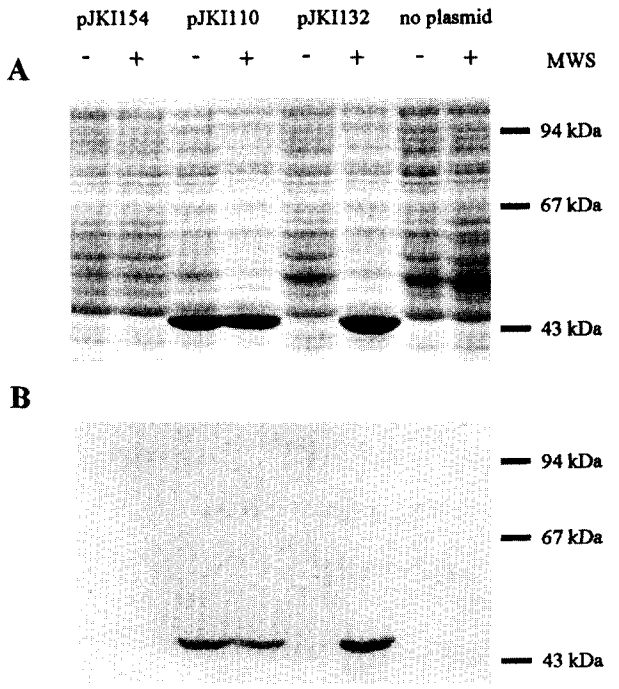

Fig. 2. SDS-PAGE (A) and immunoblot (B) analysis of the IS30 transposase produced by the transposase producer plasmids. Bacterial hosts harboring the plasmids pJKI154, pJKI110, pJKI132 or no transposase producer plasmid were incubated under inducing (+) and non-inducing (−) conditions (see Section 2.2). Protein samples were separated by SDS-PAGE (10% w/v gel) and blotted. Gel was stained with Coomassie blue (A), the immunoblot was developed with anti-IS30 transposase antibody (B) (MWS: molecular weight standard).

ity (due to genomic copies of IS30) was very low: only one transpositional rearrangement was detected out of 23065 cases investigated. The promoterless transposase producer plasmid pJKI154 also had very low activity (< 0.04%), with the exception of pJKI216 as substrate plasmid, where five rearrangements were found in 6803 colonies. pJKI110 (containing the *tac* promoter) yielded transpositional activities in the range of 0.03–82.7%, and reached its maximum value with the substrate plasmid pJKI216 (carrying two intermediate structures). pJKI110 showed no response to induction: with the same substrate plasmid it resulted in 78.7% and 82.7% under inducing and non-inducing conditions, respectively. pJKI132 (containing the *tac* promoter and the $lacI^q$ gene) resulted in similar values (< 0.03–57.6%), but due to the stringent control of the *tac* promoter the effect of induction was evident: the addition of IPTG doubled the transpositional frequency in the case of pJKI216 (from 29.4% to 57.6%). These findings are in agreement with the result of the induction assay: the expression level of the transposase correlates with the transpositional frequency (see Section 3.2 and below). A significant trend was observed in the attractivity of the target sequences, too. pJKI216 (two intermediate structures) was the most active substrate plasmid, pJKI213 (an intermediate structure and a right end joined to a synthetic LHS′ sequence) and pTFA2 (the 'inner' left and right inverted repeats of a transposon) were of the same activity, while pJKI225 (an intermediate structure and a LHS) proved to be the least active. High DNA rearrangement frequencies (9.1–82.7%) were obtained with the combination of the transposase producer plasmids pJKI110 or pJKI132 (in which the transposase was transcribed from the *tac* promoter) and the substrate plasmid pJKI216 (containing two intermediate structures). In the case of the same transposase producer plasmids the induction had no influence on the transpositional frequency with the less active substrate plasmids pTFA2, pJKI225 and pJKI213. This finding suggests that in these reactions it is presumably the target structure and not the amount of the transposase protein that determined the efficiency of the reaction.

3.4. Alteration in the transpositional activity in different bacterial hosts

To test the applicability of the system in other bacterial strains, the experimental procedure described in the previous section was repeated in the $recA^-$ strain TG2. The results are shown in Table 1B. The general tendencies were similar to the ones observed in JM109, with the same ranking being set among the transposase producer and substrate plasmids. However, the transpositional frequencies were lower in TG2 compared to JM109, e.g. the values dropped to one-third and one-half with transposase producer plasmid pJKI132 (*tac* and $lacI^q$) and substrate plasmid pJKI216 (two intermediate structures), and to one-eighth and one-tenth with transposase producer plasmid pJKI110 (*tac*) and substrate plasmid pTFA2 (the left and right IR). There was only one case where the difference in the transpositional activities was insignificant: the transposase producer plasmid pJKI110 (*tac*) and the substrate plasmid pJKI216 (two intermediate structures) resulted in similar values in both strains under IPTG induction (TG2: 76.5%, JM109: 78.7%). The phenomenon can be explained if we presume that we reached the 'saturation level' of the transpositional reactions in the system. The fact that the same transposase producer-substrate plasmid combination (pJKI110 and pJKI216) showed no response to induction in JM109, but did so in strain TG2, also supports this hypothesis: as the frequency of transposition was 82.7% in JM109 under non-inducing conditions, it reached the 'saturation level' and could not be increased further by induction, while in TG2 the transpositional frequency was able to grow from 23.3% to 76.5% upon induction.

3.5. The effect of transcription level on the transpositional frequency

To study the effect of induction on the frequency of transposition, three TG2 colonies harboring the transposase producer plasmid pJKI132 and the substrate plasmid pJKI216 were incubated in liquid media containing 0; 0.1; 1; 10; 50 and 100 μM IPTG, under the same conditions used in the measurement of the in *trans* activity (see Section 3.3 and Fig. 4). The addition of the inducer had no significant effect on the transpositional frequency until 1 μM. In the range of 10–50 μM IPTG concentration, the frequency of rearrangement increased dramatically, until it had its maximum at 50 μM, then slightly declined, probably due to the lethal effects of the induction and the transpositional rearrangements. Relatively high transpositional activity (14%) was observed without induction of the transposase gene in pJKI132, despite the very small amount of transposase produced (Fig. 2), indicating that the repression by the $lacI^q$ was not efficient in this respect. The results suggest that little amount of IS30 transposase can effectively catalyze the transposition.

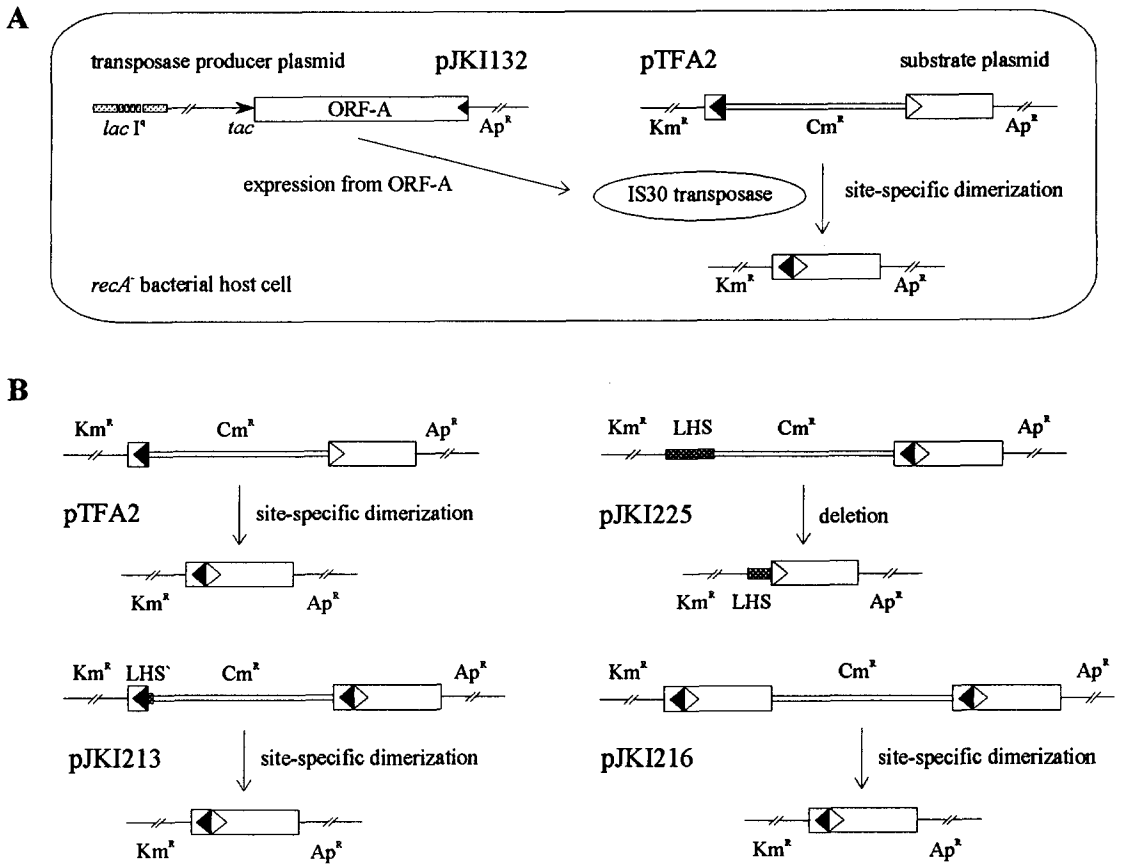

Fig. 3. The schematic presentation of the in vivo transpositional reaction between a transposase producer and substrate plasmid (A). The characteristic reactions of the different substrate plasmids (B). For abbreviations see Fig. 1. Site-specific dimerization designates the reaction which leads to the formation of an $(IS30)_2$ intermediate structure [2]. The structure of the transpositional products was verified by restriction analysis.

4. Discussion

To study the in *trans* activity of the IS30 transposase two sets of plasmids were constructed: the transposase producer replicons expressed the intact protein, but carried no transpositional target sequences. The substrate replicons contained different hot spot regions for the transposition of IS30, but produced no functional transposase. Each substrate plasmid was designed to assay one type of reaction (Fig. 3B) and to measure the different activities of site-specific dimerization (intermediate-formation) and deletion separately. As various target sequences were offered for the same transposase producer plasmid, a significant order was observed in the attractivity of the targets (Fig. 1C, Table 1). High transpositional frequencies, 76.5–82.7%, were observed between the transposase producer replicons, which had the ORF-A under the control of the *tac* promoter (pJKI110 and pJKI132) and the substrate replicon carrying two intermediate structures (pJKI216). In the control reactions (in the absence of the transposase producer plasmid or with the promoterless transposase producer plasmid pJKI154) only a few rearrangements were detected. The phenomenon that there was no complementation with the chromosomal copies of IS30 is in good agreement with the data of previous experiments [2], and is presumably due to the low level of available transposase.

Some differences in the transpositional frequency were observed in the two *E. coli* strains investigated. The comparison of the genetic background revealed no obvious explanation for this finding. The system, however, could be applied for the study of transpositional host factors.

Other possible applications of the system:

1. The verification of the in vivo complementation of IS30 transposase was a step towards the construction of an in vitro system. Our substrate plasmids proved to be efficient for detecting different transpositional rearrangements, and enabled us to study the various reactions separately.
2. The transposase producer plasmid pJKI132 can be used for the overexpression and purification of the IS30 transposase protein, and therefore, also contributes to the development of the in vitro system.
3. The high activity and reproducibility of the *trans* system provides a convenient assay to identify and characterize mutations in the transposase or in the hot spot sequences.

It is worth mentioning that our IS30 system shows remarkable analogies to the site-specific recombination systems, especially in the case of pJKI216 (Fig. 3B). The joined IS30 ends have the characteristic features of a 'recombinogenic site': the $(IS30)_2$ intermediate structure contains inverted DNA sequences separated by a spacing region, and the rearrangement between the two sites shows analogies to the excision of bacteriophage lambda and to the resolution of Tn3-type transpo-

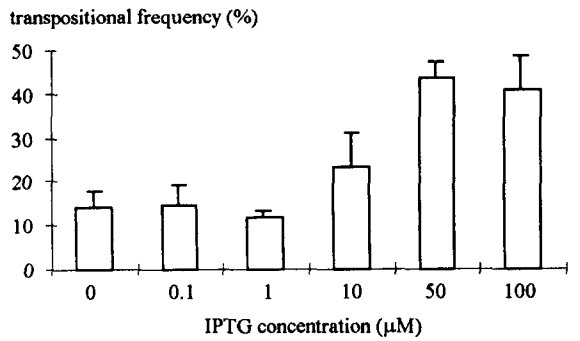

Fig. 4. The correlation between the transpositional frequency and the transcription level of the IS30 transposase gene. The transpositional frequency was measured as the ratio of Cm^S versus all colonies (see Section 2.3 and Table 1) in the presence of different IPTG concentrations.

sons, while the IS30 transposase protein plays the role of a 'site-specific recombinase' (for review see [16,17]). The high frequency of rearrangements detected (above 80%) is also comparable to the efficiency of the site-specific recombination systems. These considerations raise the possibility of an evolutionary relationship between the $(IS)_2$ forming insertion sequences (IS2 [18], IS21 [19,20], IS30 [2]) and certain site-specific recombination systems.

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Table 1
The transpositional frequencies with different transposase producer-substrate plasmid combinations in JM109 (A) and TG2 (B) (see Sections 3.3 and 3.4)

Transposase producer plasmid	Substrate plasmid	No induction			IPTG induction		
		Number of colonies		Transpositional frequency (%)	Number of colonies		Transpositional frequency (%)
		total	Cm^s		total	Cm^s	
(A) Transpositional frequencies in JM109							
none	pTFA2	1943	0	< 0.05	2408	0	< 0.04
	pJKI225	3423	0	< 0.03	4773	0	< 0.02
	pJKI213	3330	0	< 0.03	1855	0	< 0.05
	pJKI216	3945	1	0.02	1388	0	< 0.07
pJKI154	pTFA2	3011	0	< 0.03	3324	0	< 0.03
	pJKI225	3273	0	< 0.03	3633	0	< 0.03
	pJKI213	3136	0	< 0.03	2317	0	< 0.04
	pJKI216	3139	2	0.06	3664	3	0.08
pJKI110	pTFA2	3806	31	0.81	3461	27	0.78
	pJKI225	2496	3	0.12	3436	1	0.03
	pJKI213	1560	15	0.96	3138	36	1.13
	pJKI216	1923	1590	82.68	2588	2037	78.71
pJKI132	pTFA2	3457	30	0.87	2491	17	0.68
	pJKI225	3612	0	< 0.03	3225	2	0.06
	pJKI213	2492	16	0.64	3259	28	0.86
	pJKI216	1464	431	29.44	2441	1405	57.56
(B) Transpositional frequencies in TG							
none	pTFA2	3498	0	< 0.02	1911	0	< 0.05
	pJKI225	1332	0	< 0.07	1143	0	< 0.08
	pJKI213	3903	0	< 0.02	3766	2	0.05
	pJKI216	818	1	0.12	1798	0	< 0.05
pJKI154	pTFA2	271	0	< 0.36	323	0	< 0.31
	pJKI225	570	0	< 0.17	527	0	< 0.19
	pJKI213	2226	0	< 0.04	2198	0	< 0.04
	pJKI216	450	0	< 0.22	1564	0	< 0.06
pJKI110	pTFA2	932	0	< 0.11	1191	0	< 0.08
	pJKI225	824	0	< 0.12	2088	0	< 0.04
	pJKI213	1568	9	0.57	1534	4	0.26
	pJKI216	1034	241	23.30	612	468	76.47
pJKI132	pTFA2	1504	3	0.20	3587	11	0.31
	pJKI225	572	0	< 0.17	1376	1	0.07
	pJKI213	2283	5	0.22	3448	8	0.23
	pJKI216	1246	114	9.15	1956	524	26.79

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