The minimum internal and external sequence requirements for transposition of the eukaryotic transformation vector piggyBac

Abstract The piggyBac element from Trichoplusia ni is recognized as a useful vector for transgenesis of a wide variety of species. This transposable element is 2472 bp in length, and has a complex repeat configuration consisting of an internal repeat (IR), spacer, and terminal repeat (TR) at both ends, and a single ORF encoding the transposase. Excision assays performed in microinjected T. ni embryos using plasmids deleted for progressively larger portions of the piggyBac internal sequence reveal that the 5' and 3' IR, spacer, and TR configuration is sufficient for precise excision of piggyBac when transposase is provided in trans. Interplasmid transposition assays using plasmids carrying varying lengths of intervening sequence between the piggyBac termini in T. ni demonstrate that a minimum of 55 bp of intervening sequence is required for optimal transposition, while lengths less than 40 bp result in a dramatic decrease in transposition frequency. These results suggest that the piggyBac transposase may bind both termini simultaneously before cleavage can occur, and/or that the formation of a transposition complex requires DNA bending between the two termini. Based on these results we constructed a 702-bp cartridge with minimal piggyBac 5' and 3' terminal regions separated by an intervening sequence of optimal length. Interplasmid transposition assays demonstrate that the minimal terminal configuration is sufficient to mediate transposition, and also verify that simply inserting this cartridge into an existing plasmid converts that plasmid into a non-autonomous piggyBac transponon. We also constructed a minimal piggyBac vector, pXL-Bac, that contains an internal multiple cloning site sequence between the minimal terminal regions. These vectors should greatly facilitate the utilization of the piggyBac transposon in a wide range of hosts.

Keywords Excision · Microinjection · piggyBac · Transposon · Transposition

Introduction

The piggyBac element was originally isolated from the Trichoplusia ni cell line TN-368 as a gene-disrupting insertion within spontaneously arising baculovirus plaque morphology mutants (Fraser et al. 1983; Cary et al. 1989). The initial sequence analysis indicated that piggyBac is a 2475-bp element with short inverted repeats; it has an asymmetric terminal repeat structure with a 3-bp spacer between the 5' 13-bp TR (terminal repeat) and 19-bp IR (internal repeat), and a 31-bp spacer between the 3' TR and IR (Cary et al. 1989), and the single 2.1-kb ORF encodes a functional transposase (Fraser et al. 1995). Recent resequencing of the piggyBac element has revealed that it is 2472 bp in length (X. Li and M. Fraser, unpublished; P. Shirk, personal communication).

The piggyBac element is capable of precise excision from baculovirus insertion sites, and this process involves site-specific recombination (Fraser et al. 1995, 1996). It is also capable of precise excision and transposition in plasmid-based assays (Elick et al. 1996a; Lobo et al. 1999, 2001; Thibault et al. 1999). Assays for excision and interplasmid transposition confirmed that piggyBac transposes via a strict “cut-and-paste” mechanism (Elick et al. 1996b; Lobo et al. 1999; Thibault et al. 1999), inserting exclusively at 5'-TTAA-3' target sites that are duplicated upon insertion (Cary et al. 1989; Wang and Fraser 1993; Fraser et al. 1995; Elick et al. 1996b), and excising precisely (Elick et al. 1996b; Fraser et al. 1996) leaving no footprint. Excision assays using...
both wild-type and mutagenized piggyBac terminal sequences demonstrated that the element does not discriminate between proximal and distal duplicated ends, and suggest that the transposase does not first recognize an internal binding site and then scan towards the ends (Elick et al. 1997). These results differ from those obtained in similar experiments using the P element, for which binding to the proximal terminal repeat is highly favored (Mullins et al. 1989). Mutagenesis of the terminal trinucleotides or the terminal-proximate 3 bp of the TAA target sequence eliminates excision at the altered terminus (Elick et al. 1997).

The piggyBac element has shown great potential as a germ line transformation vector in insects. Successful transformations have been reported for all species tested, including Ceratitis capitata (Handler et al. 1998), Drosophila melanogaster (Handler and Harrell 1999), Tribolium castaneum (Berghammer et al. 1999), Bombyx mori (Tamura et al. 2000), Pectinophora gossypiella (Peloquin et al. 2000), Bactrocera dorsalis (Handler and McCombs 2000), and Anastrepha suspensa (Handler and Harrell 2001) as well as Aedes aegypti (Lobo and Fraser, in press). In addition to these successful germ line transformations, piggyBac mobility has been demonstrated by interplasmid transposition assays in T. ni, Ae. albopictus, and Ae. triseriatus (Lobo et al. 2001) and in Anopheles gambiae (Grossman et al. 2000).

In this report, we use both excision (Elick et al. 1996a) and interplasmid transposition assays (Sarkar et al. 1997; Lobo et al. 1999) in microinjected T. ni embryos to determine the relative importance for movement of the piggyBac element of sequences internal to, or external to, the TR and IR sequence configurations. Progressive deletions within the internal sequence of the element have no noticeable effect on either excision or transposition. In contrast, deletion of the 3′ IR eliminates excision of the element. We also verify that the length of the intervening sequence between piggyBac termini in the donor plasmid also affects the piggyBac transposition frequency. A minimum of 55 bp is required for optimal transposition of the element. Based on these results, a small cartridge having only intact 5′ and 3′ repeat domains was constructed. Interplasmid transposition assays indicate that this cartridge is capable of converting any existing plasmid into a non-autonomous piggyBac transposon that is capable of transposition when provided with a helper plasmid expressing the transposase. This novel system facilitates the utilization of piggyBac as a transformation vector, and the strategy described here might also be useful for transgenic analyses using other transposable elements.

### Materials and methods

#### Construction of the p3E1.2 deletion series

The plasmid p3E1.2 (Cary et al. 1989; Fraser et al. 1995) was first linearized using BamHI and EcoRI, blunt-ended with the Klenow fragment, and then religated to construct the p3E1.2(ΔMCS), eliminating the MCS from the pUC18 sequence. Internal deletions were made using the Erase-A-Base system (Promega). p3E1.2(ΔMCS) was cut at the unique SacI site within the piggyBac element, generating an ExoIII-resistant end, and then cut at the BglII site to generate an ExoIII-sensitive end. Aliquots of the ExoIII deletion reaction from the BglII site toward the 3′ terminus were taken every 30 s and the reaction was immediately stopped; 240-s reactions were used in the cloning. The deletions were recircularized and transformed into DH5α cells. The sizes of the recovered plasmids were analyzed using a rapid screening method (Sekar 1987). The presence of intact 3′ termini was confirmed by digestion with BsrWI followed by sequencing. Nine consecutive 100–200 bp deletion plasmids were recovered and named p3E1.2-d-1 to p3E1.2-d-9, with p3E1.2-d-9 having the largest deletion (Fig. 1).

#### The pIAO-P/L series

The plasmid p3E1.2 B/X was constructed as a pCRII TA clone (Invitrogen) carrying the entire piggyBac transposon and flanking TTA target sites, which was amplified by PCR from the plasmid p3E1.2 using the BamHI/Xhol-tailed primer MF34 (5′-GGATCCCCCTAGATACGCTAATTCTGTTAG-3′) and the other with a terminal kpnI site (5′-AATCGTACCCAGCCTGGGAGAAGCGTTCGCCG-3′), were used to generate a linear Apl-I KpnI-tailed fragment. This fragment was ligated to a PCR fragment containing the β-lactamase gene and E. coli replication origin amplified from pUC18 using an Apl-tailed (5′-GAAAGGGCCGCTGATACGCTAATTCTGTTAG-3′) and the other with a terminal kpnI site (5′-AATCGTACCCAGCCTGGGAGAAGCGTTCGCCG-3′) primer pair. The resulting plasmid, pIAO, contains the circularized piggyBac transposon with ends separated by an 18-bp fragment of DNA having the restriction site configuration XbaI/BamHI/XbaI, with a β-lactamase gene and the E. coli origin of replication. The lacZ gene under the control of

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<th>p3E1.2 deletion series plasmids</th>
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<td>p3E1.2</td>
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<td>p3E1.2-d-9</td>
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![Fig 1](image)  
**Fig 1** p3E1.2 deletion series plasmids. Exonuclease III was used to make progressively larger deletions in the plasmid p3E1.2. The three plasmids with the largest deletions, p3E1.2-d-7, p3E1.2-d-8 and p3E1.2-d-9, were used, together with the helper plasmid, to perform excision assays in T. ni embryos. Sequencing analysis showed that all three deletion plasmids retain 125 bp of the 5′ terminal sequence which includes the complete 5′ terminal repeat, spacer and internal repeat. The plasmids p3E1.2-d-7 and p3E1.2-d-8 retain the complete 3′ terminal repeat configuration (TR, spacer and IR), while p3E1.2-d-9 only contains 3′ TR and 21 bp of spacer sequence