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Piggybac transposon-based genetic transformation system for insects

**Abstract**

The present invention is directed to nucleic acid and amino acid sequences for transformation constructs containing piggyBac or tagalong transposable elements. These constructs allow for the precise excision and insertion of heterologous DNA into a host cell.

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Appl. No.: **844274**

Filed: **April 18, 1997**

**Current U.S. Class:** **435/455; 435/320.1; 435/462; 435/465; 435/466; 536/23.1**

**Intern'l Class:** **C12N 005/10; C12N 015/09; C12N 015/85**

**Field of Search:** **800/2 435/320.1,348,455,462,465,466 536/23.1**

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**References Cited** [\[Referenced By\]](#)

**Other References**

O'Brochta et al., "Transposable elements and gene transformation in non-drosophilid insects", *Insect Biochem. Molec. Biol.* 26(8-9): 739-753, 1996.\*

Cary, L. C., "Analyses of *Trichoplusia ni* transposon IFP2 insertions within the FP-locus of nuclear polyhedrosis viruses", Ph.D. dissertation, Univ. of Notre Dame (172 pages), 1989.\*

Fraser et al., "Assay for movement of Lepidopteran transposon IFP2 in insect cells using a baculovirus genome as the target", *Virology* 211 (2): 397-407, Aug. 1995.\*

Lidholm et al., "The transposable element mariner mediates germline transformation in *Drosophila melanogaster*", *Genetics* 134: 859-868, 1993.\*

Ashburner M. (*Drosophila*, a Laboratory Handbook, CSH, 1989, pp. 1024, 1035, 1051).\*

Crampton J. M. (Symp. R. Entomol. Soc. London, 1992, vol. Date 1991, 16th, 3-20).\*

Unsal et al. (*Journal of Molecular Biology*, (1995) 248, 812-823).\*

Cary, L. (*Dissertation Abstracts International*, (1989) vol. 50, No. 12B, p. 5451), Abstract only.\*

Wang, et al. (*Insect Molecular Biology*, (1993) vol. 1, No. 3, pp. 109-116).\*

Karess et al. (*Cell*, vol. 38, 135-146, Aug. 1984).

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**Parent Case Text**

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This application is a non-provisional application claiming benefit of provisional application No. 60/016,234, filed Apr. 19, 1996.

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**Claims**

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We claim:

1. A transformation system comprising:

(a) a first DNA comprising a non-transposon heterologous DNA sequence inserted between a pair of inverted repeats of a piggyBac transposon, and

(b) a second DNA encoding a transposase active on the pair of inverted repeats,

wherein the second DNA is incapable of transposition caused by said transposon.

2. The transformation system of claim 1 wherein the pair of inverted repeats is a pair of 13 base-pair inverted repeats.

3. The transformation system of claim 2 wherein the pair of 13 base-pair inverted repeats consists of SEQ ID NO 2 and SEQ ID NO 3.

4. The transformation system of claim 1 wherein the pair of inverted repeats is a pair of 17 base-pair inverted repeats.

5. The transformation system of claim 4 wherein the pair of 17 base-pair inverted repeats consists of SEQ ID NO 4 and SEQ ID NO 5.

6. The transformation system of claim 1 wherein the non-transposon heterologous DNA sequence inserted between the pair of inverted repeats is inserted into an endonuclease restriction site within a polylinker sequence.

7. The transformation system of claim 1 wherein the second DNA is contained within the genome of a cell targeted for transformation.

8. The transformation system of claim 1 wherein the second DNA is a plasmid contained within a cell targeted for transformation.

9. The transformation system of claim 1 wherein said second DNA is physically linked to said first DNA.

10. The transformation system of claim 1 wherein the second DNA is a separate DNA molecule from the first DNA.

11. A method of introducing a heterologous DNA sequence into the genome of a cultured cell of interest comprising:

(a) introducing into the cell a first nucleic acid comprising a non-transposon heterologous DNA sequence inserted between a pair of inverted repeats of a piggyBac transposon; and

(b) providing the cell with a second nucleic acid that encodes a transposase active on the pair of inverted repeats such that the heterologous DNA sequence is inserted into the genome of the cell, said second nucleic acid characterized in that it is incapable of transposition by the transposase.

12. The method of claim 11 wherein the providing step comprises transfecting with a nucleic acid encoding the transposase.

13. The method of claim 11 wherein the providing step comprises inducing expression from a genomic sequence encoding the transposase.

14. The method of claim 11 wherein the first and second nucleic acid are introduced by microinjection, high velocity propulsion, permeabilization, fusion with DNA-containing entities, or electroporation.
15. The method of claim 11 wherein the pair of inverted repeats is a pair of 13 base-pair inverted repeats.
16. The method of claim 15 wherein the pair of 13 base-pair inverted repeats consists of SEQ ID NO 2 and SEQ ID NO 3.
17. The method of claim 11 wherein the pair of inverted repeats is a pair of 17 base-pair inverted repeats.
18. The method of claim 17 wherein the pair of 17 base-pair inverted repeats consists of SEQ ID NO 4 and SEQ ID NO 5.
19. The method of claim 11 wherein the non-transposon heterologous DNA sequence inserted between the pair of inverted repeats is inserted into an endonuclease restriction site within a polylinker sequence.
20. A cell comprising:
- (a) a first DNA comprising a non-transposon heterologous DNA sequence inserted between a pair of inverted repeats of a piggyBac transposon; and
  - (b) a second DNA encoding a transposase active on a pair of inverted repeats, which second DNA is incapable of transposition caused by the transposase.
21. The cell of claim 20 wherein the second DNA sequence is operably linked to an inducible promoter.
22. The cell of claim 21 wherein the inducible promoter comprises a heat shock promoter, a metallothionein promoter, or a glucocorticoid response element.
23. The cell of claim 20 wherein the non-transposon heterologous DNA sequence comprises a selectable marker.
24. The cell of claim 23 wherein the selectable marker is antibiotic resistance, pesticide resistance, insecticide resistance, herbicide resistance, green fluorescent protein, amber mutation, or lacZ.
25. The cell of claim 20 wherein the second DNA is physically linked to the first DNA.
26. The cell of claim 20 wherein the second DNA is a separate DNA molecule from the first DNA.
27. A cell comprising:
- (a) a first DNA comprising a non-transposon heterologous DNA sequence operably linked to an inducible promoter and inserted between a pair on inverted repeats of a piggyBac transposon; and
  - (b) a second DNA encoding a transposase active on a pair of inverted repeats.
28. The cell of claim 27 wherein the second DNA is a separate DNA molecule from the first DNA.

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*Description*

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## BACKGROUND OF THE INVENTION

### 1. Field of the Invention

The present invention is directed to DNA transformation constructs encoding mobile elements and their use for transforming eukaryotic cells. In particular transposons are used as a mechanism for inserting DNA sequences into the cell's genome after introduction of the transformation construct into the cell.

## 2. Description of the Related Art

Certain natural DNA sequences in eukaryotic and prokaryotic cells have the ability to move from one genomic locus to a second locus. These genetic elements are referred to generally as transposable elements or transposons. Advantageously, these transposable elements can be used as tools for genetically manipulating cells. In particular, transposable elements isolated from eukaryotes are anticipated as having the greatest potential for use in producing transgenic organisms.

Transposable elements can be divided into two classes. Class I are the retro-transposons that replicate through an RNA intermediate and utilize reverse transcriptase to produce a DNA molecule that is inserted into the host cell's genome. The Class II transposons include all other mobile elements and include P, hobo, mariner, Tc1, and Ac elements (Berg & Howe, *Mobile DNA*, American Society for Microbiology, Washington, D.C. 1989). Members of this transposon class have short inverted repeats at their termini and generate direct duplications of a host target sequence upon insertion. Many of these elements are currently being developed as general transformation vectors in insects and plants (Rubin & Spradling, *Science*, Volume 218, 348-353 1982; Lidholm, Lohe & Hartl, *Genetics*, Volume 134, 859-868 1993; O'Brochta & Handler, *Prospects and possibilities for gene transfer techniques in insects*, 451-488; in *Molecular Approaches to Fundamental and Applied Entomology*, ed. Oakeshott et al, Springer-Verlag, New York, 1993).

The P element has been used effectively for *Drosophila* transformation but has limited use as a general transformation vector because it is not active in species other than *Drosophila* (O'Brochta & Handler, 1993 supra; Rubin & Spradling, 1982 supra). The mariner element is phylogenetically dispersed (Robertson, H. *Insect Physiol.*, Volume 41, 99-105, 1995), and therefore apparently has the capability of movement in a number of diverse species. In addition, the hobo element has demonstrated mobility in diverse genetic backgrounds and is a promising candidate for development as a genetic engineering tool (Atkinson, Warren & O'Brochta, *PNAS USA*, Volume 90, 9693-9697 1993; O'Brochta & Handler, 1993 supra; O'Brochta et al., *Mol. Gen. Genet.*, Volume 244, 9-14, 1994).

PiggyBac (previously described as IFP2) and tagalong elements are unique Lepidopteran transposons structurally related to the Class II DNA transposable elements (Finnegan, *Curr. Opin. Cell Bio.*, Volume 2, 471-477 1990). These transposons were isolated from the cabbage looper moth, *Trichoplusia ni* Hubner (Lepidoptera: Noctuidae). The piggyBac element was first identified as an insertion within *Galleria mellonella* or *Autographa californica* nuclear polyhedrosis virus genomes following passage of the viruses in the *Trichoplusia ni* insect cell line, TN-368 (Fraser et al., *Virology*, Volume 145, 356-361, 1985; Fraser et al., *J. Virology*, Volume 47, 287-300, 1983).

The piggyBac and tagalong elements are unusual among Class II transposons in that those elements always target and duplicate the tetranucleotide, TTAA, upon insertion in Baculovirus-infected cells (Cary et al., *Virology*, Volume 172, 156-169, 1989). The specificity for TTAA target sites is exhibited by other Lepidopteran transposon-like insertions as well (Beames & Summers, *Virology*, Volume 162, 206-220 1988; Beames & Summers, *Virology*, Volume 174, 354-363 1990; Carstens, *Virology*, Volume 161, 8-17, 1987; Oellig et al., *J. Virology*, Volume 61, 3048-3057, 1987; Schetter, Oellig & Doerfler, *J. Virology*, Volume 64, 1844-1850, 1990). Thus the piggyBac and tagalong elements are part of a subclass of the Class II transposons.

In addition to TTAA target specificity, all Lepidopteran transposons having the TTAA target specificity terminate in at least two C residues at the 5' ends of their inverted repeats. Given their similarity in insertion site selection and duplication, all of these TTAA specific elements are likely to excise in a similar manner.

Furthermore piggyBac and tagalong elements excise precisely upon transposition *in vivo*, leaving behind

the single TTAA target sequence upon excision. The excision events of piggyBac and tagalong are dissimilar to the transposase-associated excision events of the hAT family of transposons. This family includes hobo, hermes, Ac and Tam3 (Calvi et al., *Cell*, Volume 66, 465-471, 1991). Elements in the hAT family vary in the length and nucleotide sequence of their inverted terminal repeats (Calvi et al., 1991; supra), but have a conserved A.sub.2 G.sub.5 motif within these repeats, and generate 8 bp target site duplications (Warren et al., *Genet. Research*, Volume 64, 87-97, 1994). These elements excise imprecisely in the presence of an element-encoded transposase and leave behind characteristic footprints that have proven useful in distinguishing transposase-associated excision events (Atkinson et al., 1993 supra; Warren et al., 1994 supra).

Most of the transposase-associated excisions of P-elements are imprecise events, leaving behind part or all of the 31 bp terminal inverted repeat and adding `filler` sequences at the excision breakpoints (O'Brochta et al, *Mol. Gen. Genet.*, Volume 225, 387-394, 1991; Takasu-Ishikawa et al., *Mol. Gen. Genet.*, Volume 232, 17-23, 1992). In the case of the hobo element of *Drosophila melanogaster*, excision from plasmids in microinjected fertile eggs most often involves the complete removal of hobo and some flanking nucleotides with the addition of filler sequences related to flanking host DNA at the excision breakpoints (Atkinson, Warren & O'Brochta, 1993 supra; Handler & Gomez, *Mol. Gen. Genet.*, Volume 247, 399-408 1995; O'Brochta & Handler, 1993 supra).

In contrast with these other Class II elements, precise excision of piggyBac and tagalong is the rule rather than the exception. Precise excision of genetically tagged piggyBac elements was first demonstrated in Baculovirus genomes of infected cells (Fraser et al, *Virology* 211, 397-407 1995). However, the precise excision of the piggyBac element has also been demonstrated in non-virus infected cells indicating the excision of piggyBac is not dependent on Baculovirus protein products. The frequency of precise excision events in transiently transfected IPLB-SF21AE cells is greatly enhanced by the presence of a helper element encoding a full-length transposase. The excision event is believed to be a non-conservative event involving double-strand breaks at or near the transposon termini.

The present invention, discussed below, provides recombinant DNA vectors derived from the piggyBac and tagalong transposons which are different from related art vectors. Furthermore, the present invention provides a method to produce transgenic organisms using the recombinant DNA vectors. The transposon genetic transformation system of the present invention provides vectors and broad spectrum methods for the introduction of foreign genes that do not currently exist.

#### SUMMARY OF THE INVENTION

It is therefore an object of the present invention to provide DNA sequences capable of allowing for almost precise excision of a second DNA sequence inserted into a plasmid and insertion of said second DNA sequence into a host cell after transformation of said host cell with a transformation construct containing said first and second DNAs.

Another object of the present invention is to provide transformation constructs including DNA derived from a piggyBac transposon element which allow for the almost precise excision of a second DNA sequence included in the construct and insertion of said second DNA sequence into a host cell after introduction of a transformation construct containing said first and second DNAs into said host cell.

A further object of the present invention is to provide a transformation construct containing transposing elements combined with a DNA sequence capable of being expressed in a transformed host cell.

A still further object of the present invention is to provide a DNA sequence capable of being expressed in a transformed cell flanked by piggyBac or tagalong terminal inverted repeats.

Another object of the present invention is to provide a method for making a transgenic organism by inserting a transformation construct containing a DNA sequence, capable of being expressed in a transformed cell, flanked by piggyBac or tagalong inverted repeats into a cell; wherein the DNA sequence will excise from the construct and will insert into the host cell at least at the target sequence TTAA in said

host cell genome and using the transformed cell to obtain said transgenic organism.

Further objects and advantages of the present invention will become apparent from the following description.

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A shows the construction of the pIFP2BX-supF excision donor plasmid. The piggyBac element from p3E1.2 (top) was altered to remove flanking viral sequences (crosshatched bars) by PCR amplification using the primer MF34 (see materials and methods). MF34 anneals to both of the piggyBac terminal inverted repeats. The resulting piggyBac element (solid black bar) was flanked by TTAA target sites and BamHI/XbaI restriction sites. The 2.5 kB PCR product was cloned into pCRII (Invitrogen). The pCRII-piggyBac clone was digested with BamHI and the 2.5 kB BamHI-piggyBac fragment was subcloned into BamHI-digested pUC18. This clone was designated pIFP2BX (middle). pIFP2BX was digested with BglIII (piggyBac nucleotide position 675) and genetically tagged by the addition of a 250 bp BglIII fragment containing the supF gene. This plasmid was designated pIFP2BX-supF (bottom) and served as the donor plasmid in excision assays.

FIG. 1B shows that excision of the piggyBac element from either p3E1.2 (top) or pIFP2BX-supF (bottom) produces plasmids with different restriction enzyme profiles at the excision breakpoints. p3E1.2-piggyBac precise excision products are sensitive to digestion with MluI and EcoRV whereas pIFP2BX-supF-piggyBac precise excision products are insensitive to digestion with these same enzymes.

FIG. 2 shows the experimental strategy for analysis of piggyBac excision from the donor plasmid pIFP2BX-supF. *S. frugiperda* cells were transfected with donor DNA in the presence or absence of the helper transposon p3E1.2. Low molecular weight DNA was isolated at 48 hours post transfection and digested with the restriction enzymes EcoRV, MluI, and HpaI to select against non-excised piggyBac plasmids and the p3E1.2 helper plasmid. The digested DNA was used to transform MBL50 *E. coli*. White colonies containing plasmids that were not digested by the enzyme mix were analyzed by restriction digestion with PstI and AseI (FIG. 3) to determine if they resulted from piggyBac excision. Putative excision clones were then sequenced to confirm the sequence at the excision breakpoints (FIGS. 4A and 4B).

FIG. 3 is a photograph of a gel showing restriction enzyme analysis of a representative piggyBac excision clone and a non-excised plasmid clone. Digestion of the pIFP2BX-supF donor plasmid with PstI generates two products of 4641 bp and 803 bp. Digestion of pIFP2BX-supF with AseI generates three products of 2792, 1358, and 1294 bp. If piggyBac excises precisely from this plasmid, a single product of 2714 bp is produced from PstI digestion and a diagnostic 1257 bp product is produced from AseI digestion due to creation of a new ATTAAT AseI site at the point of excision (see sequence, FIG. 4). Another diagnostic AseI band of 163 bp is not resolved on this gel but is resolved on overloaded 2.5% agarose gels (not shown).

FIG. 4A is a photograph of a gel showing dideoxy sequencing analysis of four representative piggyBac excisions from pIFP2BX-supF.

FIG. 4B shows that precise excision of piggyBac from the pIFP2BX-supF donor plasmid generates the characteristic BamHI/XbaI-TTAA-XbaI/BamHI nucleotide sequence SEQ ID NO 21 and SEQ ID NO 22.

FIGS. 5a-5e is the entire nucleic acid, SEQ ID NO 11 and amino acid sequence, SEQ ID NO 12, for the piggyBac transposon element.

FIGS. 6a-6g, SEQ ID NO 13 is the entire nucleic acid sequence for the plasmid p3E1.2, also called the p3E1.2 H/S clone. This represents a clone of the Hind/Sal fragment containing the piggyBac insertion from an *Autographa californica* nuclear polyhedrosis virus FP mutant.

FIGS. 7a-7e is the entire nucleic acid sequence, SEQ ID NO 14 for the piggyBac/opd plasmid.

FIG. 8 is a Southern blot of the PiA-3 and PiA-11 piggyBac transformed *Plodia interpunctella* strains. Genomic DNA was extracted from G.sub.10 larvae of the PiA-3 and PiA-11 strains of *P. interpunctella* strains that were coinjected with piggyBac/opd and p3E1.2.DELTA.TRL. Lanes A-C contain 2.5 micrograms each of PiA-11 genomic DNA; lanes D and J are blank; lanes E-H contain piggyBac/opd DNA; lane I contains 2.5 micrograms of wild type *P. interpunctella* DNA; lanes K-M contain 2.5 micrograms of PiA-3 DNA. Lanes A,E and K are PstI digests; lanes B,F and L are EcoRI digests; lanes C,G and M are ApaI digests. Lane H is uncut DNA. The blot was hybridized with PCR labeled probe to hsp70/opd.

FIGS. 9a-9j is the entire nucleic acid sequence, SEQ ID NO 15 of a pCRII clone of the piggyBac sequence amplified from the p3E1.2 plasmid using the primer MF34.

FIGS. 10a-10h is the entire nucleic acid sequence, SEQ ID NO 16 for plasmid IFP2B/Xpuc18.1.

FIGS. 11a-11h is the entire nucleic acid sequence, SEQ ID NO 17 for plasmid IFP2B/XsupF4H.

FIG. 12a shows the nucleic acid sequence, SEQ ID NO 18 (top) and SEQ ID NO 19 (bottom), of two primers used in the PCR amplification of the hs/opd fragment.

FIGS. 12b-m is the entire nucleic acid sequence, SEQ ID NO 20 of the p3E1.2hs/opd plasmid.

#### DETAILED DESCRIPTION OF THE INVENTION

The identification and isolation of autonomous mobile elements from the piggyBac transposon according to the present invention enables the transformation of cells and the production of transgenic organisms wherein DNA capable of being expressed in the transformed cell or transgenic organism is excised from a transformation construct and inserted into the genome of a cell used to produce a transgenic organism. The term cell for the purposes of this invention includes any cell capable of being transformed by the transformation construct of the present invention and preferably includes any eukaryotic cell. More preferably, the cell is any arthropod cell and most preferably the cell is an insect cell. Furthermore, cells are transformed with DNA sequences that are introduced into the cell and targeted for insertion at a TTAA sequence of the cell's DNA. Typically the introduced DNA sequences include functional genes that are flanked by the piggyBac transposon inverted repeats to form a transformation construct. For the purposes of this invention the introduced transformation construct comprises a targeted functional DNA sequence flanked by a pair of transposon terminal inverted repeats from TTAA piggyBac or tagalong transposons. Targeted functional DNA sequence for the purposes of this invention is any heterologous sequence capable of being expressed in a host cell and/or a transgenic organism. In one embodiment of the present invention, the inverted repeats comprise at least 13 bps of the inverted repeats of the piggyBac transposon which include the sequence: left end CCCTAGAAAGATA, SEQ ID NO 2; right end TATCTTTCTAGGG, SEQ ID NO 3. The sequence for a 17 bp inverted repeat is: left end TTAACCCTAGAAAGATA, SEQ ID NO 4; right end TATCTTTCTAGGGTTAA, SEQ ID NO 5. In another embodiment the transformation construct also encodes a transposase gene whose product interacts with the transposon inverted repeats to induce transposition of the targeted sequence. The targeted functional DNA sequence typically will encode a gene that is capable of being expressed in the host cell. This gene can be expressed under the control of an inducible promoter. The targeted DNA can also include a selectable marker gene if the targeted gene to be inserted into the host cell's genome does not itself provide a selectable marker functionality. In one embodiment the transformation construct also can comprise a polylinker flanked by a pair of at least 13 bps of the inverted repeats of the piggyBac transposon. For the purposes of this application, a polylinker is a short length of DNA that contains numerous different endonuclease restrictions sites located in close proximity. The presence of the polylinker is advantageous because it allows various exogenous sequences, such as expression cassettes, to be easily inserted and removed, thus simplifying the process of making a transformation construct containing a particular targeted DNA fragment. When this transformation construct is introduced into a host cell, in the presence of transposase activity specific for the flanking inverted repeats, the targeted DNA sequence will be excised from the introduced construct and will be inserted into a new location. Transposition of the targeted DNA located within the transformation construct

is enhanced in the presence of transposase activity. The gene encoding the transposase can either be physically linked to the transformation construct, already present in the host cell's genome, or introduced into the cell as part of a separate DNA molecule. Inducible promoters can be used as a means of triggering the production or transposase activity.

The present invention utilizes the transposon machinery of the TTAA specific transposons to excise and insert the targeted DNA sequence into the genome of the host cell. The resulting transformed cell or group of cells are stable transformants which are then used to make a transgenic organism, using techniques known to the skilled artisan, which will pass the introduced gene to all subsequent progeny.

The above described transformation construct can also be part of a larger construct. The additional sequences of the larger construct comprising DNA sequences capable of replicating the entire DNA molecule in a bacterial host and DNA sequences encoding a bacterial selectable marker such as for example genes encoding for ampicillin or tetracycline resistance. This larger construct, which can be a plasmid, can be used to transform bacterial cells. These transformed bacterial cells can then be cultured to produce large quantities of the plasmid DNA. The plasmid DNA can then be purified and the specific transformation construct can optionally be removed from the DNA sequences utilized to replicate the plasmid in the bacterial cell using techniques well known to those familiar with the art.

In one embodiment of the invention, the target functional DNA sequence encodes a gene operably linked to an inducible promoter. Inducible promoters include any promoter capable of increasing the amount of gene product produced, by a given gene, in response to exposure to an inducer. Thus the use of this construct allows for control of the expression of the target functional gene introduced into the transgenic organism. Inducible promoters are known to those familiar with the art and a variety exists that could be used to drive expression of the transposase gene. Inducible systems include, for example, the heat shock promoter system, the metallothionein system, the glucocorticoid system, tissue specific promoters, etc. Promoters regulated by heat shock, such as the promoter normally associated with the gene encoding the 70-kDa heat shock protein, can increase expression several-fold after exposure to elevated temperatures. The glucocorticoid system also functions well in triggering the expression of genes. The system consists of a gene encoding glucocorticoid receptor protein (GR) which in the presence of a steroid hormone (i.e. glucocorticoid or one of its synthetic equivalents such as dexamethasone) forms a complex with the hormone. This complex then binds to a short nucleotide sequence (26 bp) named the glucocorticoid response element (GRE), and this binding activates the expression of linked genes. Thus inducible promoters can be used as an environmentally inducible promoter for controlling the expression of the introduced gene. Other means besides inducible promoters for controlling the functional activity of a gene product are known to those familiar with the art.

Specifically, the transformation construct of the present invention includes DNA derived from a TTAA specific transposon of the Lepidopteran transposons, piggyBac and tagalong. The piggyBac and tagalong transposons were isolated as insertions in the nuclear polyhedrosis virus, *Galleria mellonella* (GmMNPV), following maintenance of that virus in the TN-368 cell line, a *T. ni* derived cell line (Fraser et al., *J. Virology*, Volume 47, 287-300, 1983 herein incorporated by reference). Both elements have also been associated with repeated insertion events within the *Autographa californica* NPV (AcMNPV) genome (Cary et al, *Virology*, Volume 172, 156-169, 1989; Kumar & Miller, *Virus Res.*, Volume 7, 335-349, 1987; Wang & Fraser, *J. Insect Mol. Bio.*, Volume 1, 1-7 1992).

The piggyBac (IFP2) element is 2.5 kb in length and is bounded by 13 bp inverted terminal repeats, with additional internal 19 bp inverted repeats located asymmetrically with respect to the ends. The entire nucleic acid, SEQ ID NO 11, and amino acid, SEQ ID NO 12, sequence of the piggyBac element is shown in FIGS. 5a-5e. The internal sequence contains a consensus RNA polymerase II promoter region and a poly-adenylation signal (Cary et al., 1989 supra) flanking a single major open reading frame. The open reading frame encodes a single transcript of approximately 2.1 kb in length with a 5' end that maps to a consensus cap recognition sequence (Cary et al., 1989 supra). This open reading frame encodes transposase activity that enhances the transposition of the piggyBac element.

The tagalong (TFP3) element is considerably smaller (780 bp) with no apparent coding potential (Fraser et

al., 1983, 1985 supra; Wang et al., *Gene*, Volume 81, 97-108, 1989; Wang & Fraser, 1992 supra). The element is bounded by 13/15 bp imperfect inverted repeats, and is repeated and dispersed within the genome of all *T. ni* derived cell lines tested, as well as laboratory colonies of *T. ni* (Fraser et al., 1983 supra; Wang et al., 1989 supra; Wang & Fraser, 1992 supra). Comparative sequence analyses of tagalong elements and their insertion sites within baculovirus genomes and host cell genomes (Wang & Fraser, 1992 supra) have demonstrated that these elements transpose in an identical fashion whether they are moving in baculovirus-infected cells or in uninfected cells.

Both piggyBac and tagalong elements excise from their insertion sites entirely and in a precise fashion, regenerating a single copy of the TTAA target site at the point of excision. Precise excision of both elements is not restricted to the cell line of origin, TN368, but can also occur in other eukaryotic cells as well.

The creation of a transformed cell requires that the DNA first be physically placed within the host cell. Current transformation procedures utilize a variety of techniques to introduce DNA into a cell. In one form of transformation, the DNA is microinjected directly into cells through the use of micropipettes. Alternatively, high velocity ballistics can be used to propel small DNA associated particles into the cell. In another form, the cell is permeabilized by the presence of polyethylene glycol, thus allowing DNA to enter the cell through diffusion. DNA can also be introduced into a cell by fusing protoplasts with other entities which contain DNA. These entities include minicells, cells, lysosomes or other fusible lipid-surfaced bodies. Electroporation is also an accepted method for introducing DNA into a cell. In this technique, cells are subject to electrical impulses of high field strength which reversibly permeabilizes biomembranes, allowing the entry of exogenous DNA sequences. One preferred method of introducing the transformation construct into cells in accordance with the present invention is to microinject fertilized eggs with the construct. The DNA sequence flanked by the transposon inverted repeats will be inserted into the genome of the fertilized egg during development of the organism, this DNA will be passed on to all of the progeny cells to produce a transgenic organism. The microinjection of eggs to produce transgenic animals has been previously described and utilized to produce transformed mammals and insects (Rubin et al., *Science*, Volume 218,384-393, 1982; Hogan et al., *Manipulating The Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Plainview, N.Y., 1986; Shirk et al., *In Biotechnology For Crop Protection*, Hedin et al (eds.), ACS Books, Washington D.C., 135-146, 1988; Morgan et al., *Annu. Rev., Biochem.*, Volume 62, 191-217, 1993; all herein incorporated by reference). Accordingly transgenic organisms can be produced that have an exogenous DNA sequence that is flanked by the sequence 5' TTAACCC . . . GGGTTAA 3', SEQ ID NO 1 and SEQ ID NO 6 respectively. Accordingly a method of producing stably transformed insects includes the step of microinjecting a transformation construct comprising the inverted repeats of a TTAA specific transposon into a cell, preferably a fertile insect egg. The resulting transgenic insect has an exogenous DNA sequence inserted into its genomic DNA at the sequence TTAA, wherein the inserted exogenous DNA is located between the sequence 5' TTAACCC . . . GGGTTAA 3', SEQ ID NO 1 and SEQ ID NO 6 respectively.

Transformed cells and/or transgenic organisms (those containing the DNA inserted into the host cell's DNA) can be selected from untransformed cells and/or transformed organisms if a selectable marker was included as part of the introduced DNA sequences. Selectable markers include, for example, genes that provide antibiotic, pesticide, insecticide, herbicide resistance; genes that modify the physiology of the host, such as for example eye color or green fluorescent protein, to produce an altered visible phenotype; etc. Cells and/or organisms containing these genes are capable of surviving in the presence of antibiotic, insecticides or herbicide concentrations that kill untransformed cells/organisms or producing an altered visible phenotype. Using standard techniques known to those familiar with the field, techniques such as, for example, Southern blotting and polymerase chain reaction, DNA can be isolated from transgenic cells and/or organisms to confirm that the introduced DNA has been inserted.

Specifically, the TTAA specific transposon based constructs of the present invention are utilized in a method to genetically transform insects. The method comprises the steps of introducing the construct into the egg of the organism wherein the transposon excises from the plasmid and is inserted into the genome of the host. A piggyBac derived construct has been used to transform the cabbage looper moth. The construct was microinjected into eggs at a pre-blastula stage and the piggyBac DNA was induced to move from the

plasmid DNA to stably integrate into the chromosomal DNA of germ cells of the cabbage looper moth. Thus, the piggyBac transposon is useful as a vector to move foreign genes into cabbage looper moth chromosomes and, as a consequence, produce genetically transformed insects. The piggyBac transposon genetic transformation system provides a broad spectrum method that does not currently exist for the introduction of foreign genes into insects.

Genetic modification of insects with new genetic elements provides a means to control populations of agriculturally pestiferous or beneficial insects. The ability to control pest insects through genetically based sterile insect programs or genetically introduced targeted conditional susceptibilities will result in significant cost savings to agribusiness. In addition, introduction of genes that impart resistance to chemicals (including herbicides, pesticides and insecticides) can improve the efficacy of beneficial insects. This technology can also be used for detection and monitoring of insect populations and infestations where the piggyBac transposon is present in the population. Each of these applications will result in more efficient pest control programs.

Enhancing the resistance of beneficial insects to pesticides will enhance the efficacy of the beneficial insects and may allow for the simultaneous use of chemical control and biological control of pests. Some of the beneficial insects that would make good candidates for such transformations include Hymenopteran parasitoids of *Heliothis* spp: *Microplitis croceipes* and *Cardiochiles nigriceps*; Hymenopteran parasitoid of Diamondback moth, *Plutella xylostella*: *Diadegma insolare*; Hymenopteran parasitoid of the Indianmeal moth, *Plodia interpunctella*: *Bracon hebetor*; and Hemipteran predators: *Xylocoris flavipes* and *Podisus maculatus*.

The following examples are intended only to further the invention and are not intended to limit the scope of the invention as described by the claims.

#### EXAMPLE 1

The piggyBac-deficient *Spodoptera frugiperda* cell line, IPLB-SF21AE (Vaughn et al., *In Vitro*, Volume 13, 213-217, 1977 herein incorporated by reference) was maintained as described in Fraser, Smith & Summers, *J. Virol.* 47: p. 287-300, 1983; herein incorporated by reference. Twenty-four hours prior to use, cells were seeded to early-log phase to insure optimum growth at transfection.

#### EXAMPLE 2

In order to clone plasmid excision constructs, plasmid p3E1.2 (FIGS. 6a-6g), containing an active piggyBac element, was used as a template for PCR reactions with the inverted terminal repeat-specific BamHI/XbaI-ended primer MF34 to remove flanking viral sequences from p3E1.2 (FIGS. 2 and 6, SEQ ID NO 13). A primer oligonucleotide, MF34, having the sequence 5' GGATCCTCTAGATTAACCCTAGAAAGATA 3', SEQ ID NO 7, annealed to both piggyBac terminal repeats and generated a full-length piggyBac transposon product. The MF34 primer is tailed with BamHI and XbaI sites immediately adjacent to the TTAA target site and terminal inverted repeat sequences. This single primer amplified the entire piggyBac element and target site duplication because of its homology to both ends of the element through the terminal repeat sequences. The PCR reaction contained approximately 5 Units Taq polymerase (Promega), about 2 mM MgCl<sub>2</sub>, about 1 mM dNTPs, about 50 mM KCl, about 10 mM Tris-Cl pH 9, 0.1% Triton X, and about 100 pmols MF34 primer. The 2.5 kB amplified BamHI/XbaI-ended piggyBac PCR product is tailed with BamHI and XbaI sites flanking the TTAA target sites on both sides, and was cloned into pCRII (Invitrogen) TA cloning vector (FIGS. 9a-9j, SEQ ID NO 15) to generate a piggyBac element flanked by TTAA target sites and BamHI/XbaI restriction sites (Elick et al, *Genetica*, Volume 98, 33-41, 1996; herein incorporated by reference in its entirety). This is analyzed extensively by restriction digestion to insure the PCR product did not contain significant mutations due to infidelity of the Taq polymerase. An approximately 2.5 kB BamHI fragment was then subcloned into pUC18 and designated pIFP2BX (FIGS. 1A and 10a-10h, SEQ ID NO 16). Both orientations of the BamHI insert are cloned. About a 250 bp BamHI fragment containing the *E. coli* tRNA suppresser gene, supF (Ariza et al., 1993), was band-isolated from pKFSupF (kindly supplied by Dr. D. O'Brochta) and cloned into the unique BglIII site of pIFP2BX by interrupting the open reading frame at the unique BglIII site (nucleotide position 673 and adding a BglIII-

compatible 250 bp BamHI cartridge containing the supF gene (FIGS. 1a and 11a-11h, SEQ ID NO 17) (Elick et al, *Genetica*, Volume 98, 33-41, 1996; herein incorporated by reference). This plasmid was designated piFp2BX-supF (FIGS. 1A and 11a-11h, SEQ ID NO 17) and was the donor plasmid in excision assays.

The supF gene encodes a tRNA (Ariza. et al., *Carcinogenesis*, Volume 14, 303-305, 1993; herein incorporated by reference) that suppressed an amber mutation in the 9-galactosidase gene of the E. coli strain MBL50 to produce blue colonies in the presence of X-gal. If the piggyBac element tagged with the supF gene is excised from the plasmid piFp2BX-supF, the amber mutation in the MBL50 B-galactosidase gene was not suppressed and the resulting colonies were white in the presence of Xgal.

### EXAMPLE 3

The CaPO.sub.4 co-precipitation protocol was used to cotransfect plasmid excision vectors into piggyBac-deficient IPLB-SF21AE cells (Corsaro & Fraser, *J. Tiss. Cul. Meth.*, Volume 12, 7-12, 1989; Graham & Van der Eb, *Virology*, Volume 52, 456-467, 1973; Summers & Smith, *A Manual of methods for baculovirus vectors and insect cell culture procedures*, Texas Agricultural Experiment Station Bulletin, 1987; all herein incorporated by reference). The IPLB-SF21AE cell line is used because this cell line lacks piggyBac-homologous sequences (Cary et al., 1989 supra; Elick et al., *Genetica*, Volume 97, 127-139, 1996; herein incorporated by reference). It was expected that co-transfection of the piFp2BX-supF plasmid with the helper plasmid p3E1.2 would increase the number of white excision products if excision of piggyBac was enhanced by the presence of the presumptive transposase. About 5 .mu.g of supercoiled piFp2BX-supF donor plasmid DNA was combined with about 5 .mu.g of supercoiled p3E1.2 helper plasmid in about 1 ml 1.times. HEPES, pH about 7.1. Transfections done in the absence of the helper plasmid contained twice as much (approximately 10 .mu.g) donor plasmid DNA. After incubation the co-transfection mixtures were placed onto monolayers of IPLB-SF21AE cells and incubated for one hour with gentle agitation approximately every 15 minutes. The monolayers were then fed with about 1 ml TNM-FH +approximately 8% FBS and incubated for about another 4 hours at 27.degree. C. Transfected cells were harvested approximately 48 hours post transfection for extraction of plasmid DNA.

### EXAMPLE 4

Low molecular weight DNA, i.e., extrachromosomal DNA, was isolated from transfected cells of Example 3 according to the method of Hirt (*Hirt, J. Mol. Bio.*, Volume 26, 365-369, 1967; herein incorporated by reference). The media was removed from the cells and approximately 800 .mu.l of Hirt extraction buffer (about 25 mM Tris-Cl, about 10 mM EDTA, about 0.6% SDS, pH about 7.5) was added. Cell lysates were scraped into microcentrifuge tubes after about 5 minutes and NaCl was added to a final concentration of about 1M. The lysates were incubated on ice for about 2 hours and centrifuged for about 15 minutes at about 15K and about 4.degree. C. Supernatants containing low molecular weight DNA were placed into fresh tubes, extracted with phenol/chloroform and precipitated with ethanol according to standard protocols (Sambrook, Fritsch & Maniatis, *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Press, 1989; herein incorporated by reference). The extracted DNA was resuspended in water and used in restriction digestions and electrotransformations of MBL50 E. coli.

### EXAMPLE 5

The Hirt isolated DNAs from Example 4 were digested with approximately 5' U each of MluI, EcoRV, and HpaI (Promega) in approximately 100 .mu.l reactions containing approximately 1 .mu.g DNA. Each of these enzymes act within the piggyBac element. These reactions were carried out as recommended by the manufacturer. The loss of piggyBac from piFp2BX-supF prevented digestion with this enzyme mix (see FIG. 1B). The loss of piggyBac from the helper plasmid p3E1.2 did not prevent digestion with EcoRV or MluI (see FIG. 1B). The p3E1.2 plasmids as well as any plasmids resulting from excision of piggyBac from p3E1.2 were selectively degraded.

The MBL50 E. coli strain was transformed with approximately 10 .mu.g of Hirt isolated, digested DNA using a Bio-Rad Gene Pulser. Briefly, approximately 40 .mu.l of electrocompetent MBL50 E. coli were

combined with the digested DNA on ice, placed into an approximately 0.2 cm gap electroporation cuvette and pulsed at settings of about 25 .mu.Fd, about 25 kV, about 200 .OMEGA.A, about 1.5 ml aliquot of SOC (about 2% w/v bacto-tryptone, about 0.5% w/v bacto-yeast extract, about 8.5 mM NaCl, about 2.5 mM KCl, about 10 MM MgCl.sub.2, about 20 mM glucose) was added after electroporation. The electroporated bacteria were collected immediately by centrifugation at about 2000.times.g for about 5 minutes at room temperature, resuspended in about 100 .mu.ls SOC and spread on LB plates (about 150.times.15 mm) containing approximately 100 .mu.ls of about 2% X-gal and approximately 50 .mu.g/ml ampicillin.

The pIFP2BX-supF plasmid contains two PstI sites, one in the piggyBac element and the other in the multiple cloning site of pUC18. Digestion of pIFP2BX-supF with PstI generates two fragments of 4641 bp and 803 bp in length (FIG. 3). Excision of the--2750 bp piggyBac element from this plasmid removes one of the PstI sites to generate a single PstI digestion product of 2714 bp. The supF(-) clones exhibiting this single 2714 bp PstI product were then analyzed with AseI. This enzyme recognizes the sequence ATTAAT. Precise excision of piggyBac from pIFP2BX-supF regenerates a single TTAA and a new AseI site is generated at the excision breakpoint. A diagnostic AseI band of 1257 bp is resolved on a 2.5% agarose gel (FIG. 3). The other diagnostic AseI fragment of 163 bp is also resolved on a 2.5% gel when the gel is overloaded (data not shown). In addition, the donor pIFP2BX-supF AseI fragments of 2792 bp and 1358 bp are absent in clones where piggyBac has excised (FIG. 3). After screening by restriction digestion, positive clones were sequenced to confirm the precise excision events that had generated the sequence GGATCCTCTAG(ATTAAT)CTAGAGGATCC, SEQ ID NO 8 at the excision breakpoints (FIGS. 4a and b).

Digested Hirt extracts harvested from cells transfected with 10 .mu.g of pIFP2BX-supF alone (control) generated a total of 51 white colonies in three separate experiments upon transformation of MBL50 cells. No precise excisions of the SupF-tagged piggyBac element were recovered in the absence of the piggyBac transposase (Table 1). Table 1 shows assay results for supF(-) plasmids obtained from transformation of E. coli MBL50 cells either prior to or following transfection of the IPLB-SF21AE insect cell line. The IPLB-SF21AE (SF21AE) cell line was transfected with the pIFP2BX-supF plasmid (psupF) in the absence or presence of the p3E1.2 helper plasmid. At 48 hours post transfection a Hirt extraction was performed to isolate plasmid DNAs. Equivalent amounts of Hirt extracted DNAs were transformed either directly (Total Number of Plasmids) or following treatment with the restriction enzyme mix EcoRV, MluI, and EcoRV (Number supF(-)) into E. coli MBL50 cells and the number of colonies produced was counted. In the control experiment (MBL50) equivalent aliquots of either undigested or pre-digested pIFP2BX-supF plasmid DNA were transformed directly into the bacteria (Total Number of Plasmids and Number supF(-), respectively). The number of and percentage of precise excision events among the white supF(-) plasmids recovered was determined (Number Precise and Percent Precise, respectively) and the frequency of precise excisions was calculated relative to the total number of supF(-) plasmids recovered. In these instances the loss of supF activity apparently resulted from random deletions of supF, piggybac, and/or portions of the pUC18 plasmid DNA.

Transfections of IPLB-SF21AE cells with pIFP2BX-supF in the presence of the p3E1.2 helper transposon also produced plasmids that resisted digestion with the enzyme mix and generated white colonies upon transformation of MBL50 E. coli. A total of 19 supF negative clones isolated from 3 independent experiments were analyzed by restriction digestion.

TABLE 1

	Total Number of Plasmids	Number of Plasmids supF(-)	Number Precise	Percent Precise	Number Precise Frequency
21AE pIFP2BX-supF	1 .times. 10(7)	51	0	0	0
21AE pIFP2BX-supF + 3E1.2	1 .times. 10(5)	19	11	58	1 .times. 10(-4)
L50 pIFP2BX-supF	3 .times. 10(5)	25	0	0	0

## EXAMPLE 6

White colonies resulting from the supF deletions in Example 5, representing putative excision events, were mini-prepped by boiling according to standard protocols (Sambrook et al., 1989 supra) and analyzed by restriction digestion and sequencing. The plasmid DNAs were digested with PstI to identify possible excision events (FIG. 3). PstI digests positive for excision were further characterized by digestion with the enzyme AseI (FIG. 3) since precise excision of piggyBac from pIFP2BX-supF generates a new AseI site (ATTAAT, SEQ ID NO 9) at the excision breakpoint. Double-stranded DNAs from clones representing putative excision events were sequenced by the dideoxy method (Sanger, Nicklen & Coulson, PNAS USA, Volume 74, 5463-5467, 1977 herein incorporated by reference) using the Sequenase version 2.0 kit (Amersham).

Eleven of these clones appeared to be precise piggyBac excision events. The remaining 8 white clones were not characterized further since they reflected extreme deletions or rearrangements that appeared to be unrelated to piggyBac excision. The eleven putative excision clones were sequenced and all were confirmed as precise excision events (Table 1), leaving a single TTAA at the excision breakpoint (FIG. 4).

Overall, 58% percent of the white colonies screened from co-transfections with pIFP2BX-supF and p3E1.2 were derived from precise piggyBac excision events. In contrast, 0% of the recovered plasmids exhibited precise excisions when the helper plasmid was not supplied (Table 1). These results demonstrated that precise excision of piggyBac from plasmids in IPLB-SF21AE cells was significantly enhanced by the addition of the p3E1.2 helper transposon plasmid.

#### EXAMPLE 7

To estimate the frequencies of supF(-) plasmids resulting from precise excision of piggyBac versus those resulting from alternative deletions, equal amounts of Hirt extracted DNAs from transfected IPLB-SF21AE cells were either mock digested or digested with MluI, EcoRV, and HpaI in 100 .mu.l reactions. Equal amounts of these DNAs were then transformed into MBL50 E. coli. The supF deletion frequency was calculated as the number of white colonies produced from the digested preparation divided by the total number of colonies produced from the undigested control.

The frequency of supF deletions that were unrelated to precise piggyBac excision in control transfections with pIFP2BX-supF alone was determined. In the absence of the p3E1.2 helper transposon, a white colony was generated in every 2.times.10.sup.4 pIFP2BX-supF plasmids used to transform MBL50 E. coli. This equals a background supF deletion frequency of 5.times.10.sup.-5 (Table 1).

In the presence of the p3E1.2 helper transposon, approximately one white colony was generated in every 5.8.times.10.sup.3 input plasmids (both pIFP2BX-supF and p3E1.2) and one precise excision was confirmed in every 1.2.times.10.sup.4 input plasmids. This corresponds to a supF deletion frequency of 1.8.times.10.sup.-4 and a piggyBac precise excision frequency of 1.0.times.10.sup.-4 (Table 1).

The possibility existed that that the piggyBac-supF excision events could have occurred in MBL50 E. coli after transformation with the Hirt extracted DNAs rather than in the transfected IPLB-SF21AE cells. As a control, we performed direct transformations of MBL50 E. coli with pIFP2BX-SupF DNA pre-digested with MluI, EcoRV, and HpaI. The transformation mixtures were spread on LB-amp+X-gal plates and plasmids from white colonies were screened by restriction digestion with several diagnostic enzymes. A total of 25 white colonies were generated in three separate experiments from these direct transformations. None of these white colonies resulted from plasmids with precise excisions of the supF-tagged piggyBac element. These results confirmed that the precise excisions of piggyBac must have occurred exclusively in IPLBSF21AE cells and not in transformed bacteria.

The frequency of the imprecise spontaneous supF deletions from plasmids directly transformed into the MBL50 E. coli cells was compared to the previously calculated frequency from Hirt extracted plasmids recovered from transfected IPLB-SF21AE cells to determine if the observed background supF deletion events occurred predominantly in the SF21AE cells or in the bacteria. In three separate transformations, the MBL50 E. coli directly transformed with 3.times.10.sup.5 digested pIFP2BX-supF plasmids generated a

total of 25 white colonies (Table 1). This corresponded to a supF deletion frequency of 8.times.10.sup.-5. This frequency was similar to the supF deletion frequency previously observed for pIFP2BX-supF plasmids that had been introduced into IPLBSF21AE cells in the absence of the helper p3E1.2 (5.times.10.sup.-5). This apparent similarity in supF deletion frequencies suggested the majority of background supF deletion events (those not involving a precise piggyBac excision) had occurred in the transformed bacteria and not in the transfected IPLB-SF21AE cells. Further evidence for this conclusion was apparent from the similarities of restriction fragment patterns among those clones isolated following direct bacterial transformations and those isolated following transfections of IPLB-SF21AE cells (data not shown).

The above establishes that precise excision of piggyBac is enhanced by the addition of the helper p3E1.2 in transfected IPLB-SF21AE cells. This helper plasmid presumably provides a source of the piggyBac transposase. Precise excisions of genetically tagged piggyBac from mutant Baculovirus genomes have been observed in infected IPLB-SF21AE cells in the absence of a helper transposon (data not shown). Precise excisions in transfected IPLB-SF21AE cells in the absence of the helper transposase was not detected. However, precise excisions probably do occur at some baseline frequency.

The inability to find precise excisions in this plasmid assay in the absence of added transposase plasmid is probably the result of an excision frequency that is slightly below the detection limit. Precise excisions from the Baculovirus recombinants results in viruses that are then amplified in the infected cells, and the ability to detect these relatively infrequent excision events is therefore enhanced. The results of the plasmid-based excision assay also confirm that viral-encoded gene products are not necessary for precise excision of piggyBac in these Lepidopteran cells.

The frequent and favored event of piggyBac precise excision is unique among Class II transposons. In the case of the hobo element of *Drosophila melanogaster*, excision from plasmids in microinjected fertile eggs most often involves the complete removal of hobo and some flanking nucleotides with the addition of filler sequences related to flanking host DNA at the excision breakpoints (Atkinson, Warren & O'Brochta, 1993 supra; Handler & Gomez, Mol. Gen. Genet., Volume 247, 399-408, 1995; O'Brochta & Handler, 1993 supra; all herein incorporated by reference). This addition of filler sequence could involve either a polymerase-dependent template-switching process during repair of the excision breakpoint (Saedler & Nevers, J. Eur. Mol. Bio. Org., Volume 4, 585-590, 1985; herein incorporated by reference) or the formation of hairpins at the excision breakpoint that are subsequently nicked, filled in, and religated (Takasu Ishikawa, Ishihara & Hotta, Mol. Gen. Genet., Volume 232, 17-23, 1992; Coen & Carpenter, J. Eur. Mol. Bio. Org., Volume 7, 877-883, 1988). The hobo excision process requires the hobo transposase or may involve cross mobilization by a similar transposase with hobo-like activity (Atkinson, Warren & O'Brochta, 1993 supra; Handler & Gomez, 1995 supra; O'Brochta et al., 1994 supra; Warren, Atkinson & O'Brochta, Genet. Res., Volume 64, 87-97, 1994). The piggyBac element may also be cross mobilized in the absence of added piggyBac transposase by similar elements resident in the IPLB-SF21AE cell line (Fraser et al., Virology 211, p397-407 1995; herein incorporated by reference). The piggyBac element most often excises precisely from plasmids in IPLB-SF21AE cells when supplied with a piggyBac transposase source. piggyBac also preferentially excises precisely from Baculoviruses in infected IPLB-SF21AE cells (data not shown). No extra nucleotides are removed and no filler sequences are inserted at the piggyBac excision breakpoints.

Like piggyBac, the P element can excise precisely from plasmids in vitro in the presence of transposase (Rio, Laski & Rubin, Cell, Volume 44, 21-32, 1986). However, the P element most often excises imprecisely in vivo, leaving behind residual terminal repeat sequences at the excision breakpoints (O'Brochta, Gomez & Handler, 1991 supra; Takasu-Ishikawa, Ishihara & Hotta, 1992 supra). The apparent precise excision events of genomic P elements in vivo are due to homolog dependent gap repair rather than precise excision (Engels et al., Cell, Volume 62, 15-525, 1990). Since there are no ectopic repair templates representing piggyBac empty sites in either plasmid-based assays or in Baculovirus infected cells, piggyBac precise excision is most likely coupled to the cleavage process itself rather than being a consequence of a subsequent repair event.

The IPLB-SF21AE cell line, derived from *S. frugiperda*, was established in the mid 1970's (Vaughn et al., In Vitro, Volume 13, 17-23, 1977; herein incorporated by reference) and is devoid of piggyBac

homologous sequences, yet piggyBac is capable of excising in IPLB-SF21AE cells when supplied with piggyBac transposase. A previous report (Fraser et al., 1995 supra) had established that piggyBac is also capable of transposing in these cells. Since the excision process in IPLB-SF21AE cells apparently reflects the ability of piggyBac to transpose in these cells (Fraser et al., 1995 supra), an excision assay for piggyBac is useful in determining the ability of piggyBac to mobilize in other species as well. tagalong (formerly TFP3), another TTAA specific transposable element, is also capable of precise excision in IPLB-SF21AE cells (Fraser et al., 1995 supra). tagalong has a smaller DNA sequence than piggyBac and has no apparent coding potential (Fraser, Smith & Summers, J. Virology, Volume 47, 287-300, 1983; Wang & Fraser, 1992 supra; Wang, Fraser & Cary, 1989 supra). Like piggyBac, tagalong was originally isolated as an insertion into the Baculovirus genome after passage of the virus in TN-368 cells (Wang & Fraser, 1992 supra).

The extreme specificity for TTAA target sites upon insertion of piggyBac also occurs in uninfected TN-368 cells (Elick et al., 1995 supra) eliminating any possible involvement of virus-specified proteins in the target selection and insertion process. piggyBac contains a single ORF that, when interrupted, abolishes the ability of the element to transpose (Fraser et al., 1995 supra).

The specificity for TTAA target sites is exhibited by other Lepidopteran transposon-like insertions as well (Beames & Summers, Virology 162, 206-220, 1988; Beames & Summers, Virology, Volume 174, 354-363, 1990; Carstens, Virology, Volume 161, 8-17, 1987; Oellig et al., J. Virology, Volume 61, 3048-3057, 1987; Schetter, Oellig & Doerfler, J. Virology, Volume 64, 1844-1850, 1990). In addition to TTAA target specificity, all of these Lepidopteran insertions terminate in at least two C residues at the 5' ends of their inverted repeats. Given their similarity in insertion site selection and duplication, these TTAA specific elements are likely to excise in a similar manner.

The ability of a piggyBac derived construct carrying an exogenous gene as the targeted sequence to transpose in vivo has been demonstrated using a Baculovirus genome as the DNA targeted for insertion (Fraser et al., 1995 supra). This transposition assay demonstrates that a Lepidopteran transposon is capable of transposing while carrying a marker gene in insect cells.

Following the transposition assay the ability of piggyBac or tagalong inserted elements to excise from the Baculovirus genome was examined. Recovery of excision events relied on the blue/white screening of polh/lacZ tagged tagalong or piggyBac insertions. In contrast with tagalong, none of the white revertants we analyzed from piggyBac excision events resulted from mutation of the polh/lacZ gene. The complete lack of alternative mutations leading to the white revertant plaque phenotype demonstrates that the rate of precise excision for piggyBac exceeds the baseline mutation rate in these infected cells. tagalong and piggyBac elements do not necessarily require their own transposon-encoded functions for precise excision in baculovirus-infected cells. tagalong excision was effected in both TN-368 cells having resident copies of this element and in SF21AE cells lacking tagalong homologues. Similarly, the piggyBac element excised repeatedly and precisely in SF21AE cells lacking piggyBac homologues. Since there are transpositionally active TTAA-specific elements resident in the SF21AE cell line (Carstens, 1987 supra; Beames & Summers, 1988, 1990 supra) excision of piggyBac in these cells could reflect the cross-mobilizing activity of some resident TTAA-specific element.

True precise excision is often a site-specific recombination event involving enzymes that recognize specific sequences or structures at or near the termini of the element (for reviews see Sadowski, J. Biol. Chem., Volume 267, 21273-21276, 1993; Plasterk, FASEB, Volume 7, 760-767, 1993. Mizuuchi, Cell, Volume 74, 781-786, 1992). Comparisons between the terminal inverted repeat domains of tagalong and piggyBac, or between these elements and other TTAA specific elements reveal few similarities aside from the target site and three terminal bases, 5' TTAACCC . . . GGGTTAA 3', SEQ ID NO 7 and SEQ ID NO 6, respectively.

## EXAMPLE 8

The feasibility of the microinjection procedure has been established for *T. ni*, *S. frugiperda*, *S. exigua*, *H. zea*, and *P. interpunctella* embryos, yielding survival rates of 70% or better. A major advantage of the lepidopteran egg development is the relatively extended time from egg laying to blastoderm development

(Nagy et al., Dev. Biol., Volume 165, 137-151, 1994). For *T. ni* this period seems to be about 6-8 hours. The amount of DNA injected and the lengthy period before blastoderm formation allows germ line nuclei to become transformed in at least some of the fertile eggs.

The microinjection protocol utilizes approximately two to six hr old *T. ni* eggs. The eggs are attached to a microscope cover slip with double-stick tape without dechorionation. Approximately 2 ml of PBS (about 5 mM KCl, about 100 mM NaH<sub>2</sub>PO<sub>4</sub>, pH about 6.8) containing approximately 100 ug/ml of plasmid DNA is injected directly into each egg. The perforation in the egg resulting from the needle is sealed with a coating of Krazy Glue. The eggs are then maintained at about 22.degree. C. and about 80% relative humidity for approximately twelve hours with a normal photocycle of about 16hr:8hr (light:dark) before being placed on diet.

#### EXAMPLE 9

Evidence indicates that the piggyBac element transposes through a cut-and-paste mechanism. Thus excision of the element is necessary for transposition. Therefore, excision assays with piggyBac in lepidopteran embryos should be an effective predictor of its ability to transpose in that species. This assay has been used with other transposons and is accepted as an effective predictor of the ability of an element to transpose in a given species (Handler, A. M. and Gomez, S. P. (1995), The hobo transposable element has transposase dependent and -independent excision activity in drosophilid species, Mol. Gen. Genet. 247, 399-408; O'Brochta, D. A., Handler, A. M. (1988), Mobility of P-elements in drosophilids and nondrosophilids. Proc. Natl. Acad. Sci. (USA) 85, 6052-6056; all herein incorporated by reference.) Using a supF-tagged piggyBac element (pIFP2BX-supF, FIG. 2), microinjections were performed on *T. ni*, *S. frugiperda*, *S. exigua*, and *H. zea* embryos. Precise excision events characteristic of piggyBac mobilization were observed in all species examined. Surprisingly, these events occurred even in the absence of added helper p3E1.2 plasmid, suggesting the presence of transmobilizing elements in these species. Note there is no possibility for homolog dependent gap repair or homologous exchange with these plasmid constructs, since there are no wild type copies of piggyBac in the cell line used in these studies. The background precise excision suggests there are active cross-mobilizing elements already present in these species. Because excision is a prerequisite for transposition in a cut-and-paste mechanism (see above), the fact that excision occurs is predictive that transposition in these species is possible.

#### EXAMPLE 10

Precise excisions of the tagged piggyBac transposon from the IFP2BX-supF 4H plasmid were recovered following microinjection of fertile insect eggs by Hirt extraction and transformation of MBL50 *E. coli*. *S. exigua*, *H. virescens*, *P. interpunctella*, *T. ni*, *S. frugiperda*, *A. aegypti* and *D. melanogaster* fertile insect eggs were injected as described above. Some injections were done with added helper p3E1.2 plasmid while others were done without the helper plasmid. The results are shown below in Table 2. The characteristic precise excision event associated with mobilization of the piggyBac element was recovered from most of the microinjected insects whether or not helper was added. The inability to recover precise excision events in a couple of species is likely due to a low number of total number of supF(-) plasmids available for analysis. These experiments establish that the characteristic precise excision of piggyBac associated with the transposition event is possible in a wide range of insect species spanning the orders Lepidoptera and Diptera. These results verify that piggyBac may be used for transformation of a wide range of insects.

TABLE 2

Organism	Number supF (-)	Precise Excisions	Near Terminal Excisions
<i>S. exigua</i> - helper	14	1	2
<i>H. virescens</i> - helper	4	1	0
<i>P. interpunctella</i> - helper	3	0	1
<i>T. ni</i> - helper	45	7	2
<i>S. frugiperda</i> + helper	38	6	1
<i>S. frugiperda</i> - helper	21	14	0
<i>A. aegypti</i> + helper	7	2	0

A. aegypti - helper 13	1	0
D. melanogaster	1	0

#### EXAMPLE 11

A phsp 70/opd plasmid and helper p3E1.2 were coinjected into T. ni eggs as described above in example 8. The hsp70/opd gene fusion construct (Benedict et al, Insect Mol. Biol., Volume 3, 247-252, 1194; herein incorporated by reference) was used as the targeted DNA in the transformation construct used for obtaining transgenic T. ni. The opd gene product confers resistance to the insecticide paraoxon. opd is an abbreviation for the parathion hydrolase gene, in this case isolated from Pseudomonas diminuta (Benedict et al; supra). The product of this gene metabolizes numerous organophosphorous nerve agents including the insecticides parathion and paraoxon. The particular gene used in this construct encodes a native, cytoplasmic form of the hydrolase protein, and is therefore referred to as copd. The hsp70 heat shock promoter is an inducible promoter that provides high-level expression of the bacterial opd gene when induced. The hsp70/opd gene construct was inserted into pIFP2BX to form phsp/opd plasmid. The p3E1.2hs/opd plasmid was constructed by inserting a PCR amplified hs/opd fragment using primers tailed with BglII sites directly into the unique BglII site within p3E1.2 plasmid, effectively positioning the hs/opd gene within the piggyBac sequence (FIGS. 12b-12m, SEQ ID NO 20, and SEQ ID NO 19). The primers SEQ ID NO 18 used for the amplification are shown in FIG. 12a.

The eggs were hatched and subsequently mass-nated. The G.sub.1 progeny of the mass-mated microinjected insects were permitted to feed for about 24 ours, heat-shocked for about 60 minutes at about 42.degree. C., rested at about 26.degree. C. and allowed to feed for about an additional 18 hours before being subjected to selection. These heat stressed caterpillars were then allowed to crawl for about 30 minutes on approximately 50 ug/cm.sub.2 paraoxon-treated filter paper disks. Nearly about 30% of all the G2 larvae survived the initial approximately 50 ug/cm.sup.2 dose at a 30 min exposure, while all of the control larvae perished. Cloning of piggyBac sequences seemed to confirm transposition into the genome. Southern blot analysis confirmed the presence of multiple, dispersed copies of piggyBac in the genome of transformed insect progeny (G2) at levels above the two or three copies that serve as background in this insect. All but one of these surviving G1 insects died over the next three days, probably from residual paraoxon. The one putative transformed insect that was obtained was paraoxon resistant and had white eyes. This transformant did not generate fertile eggs and a lineage could not be established.

#### EXAMPLE 12

A helper plasmid construct was prepared that would supply the transposase activity but would not be capable of transposing. The use of this construct allows the production of transgenic insects having only the desired exogenous DNA inserted into the genome. The construct, 3E1.2 delta TRL was prepared by digesting plasmid p3E1.2 with SstI to remove a DNA fragment from nucleotide 3441 to 3724 of p3E1.2. Removal of this fragment deletes the right terminal. repeat of the piggyBac transposon preventing the element from transposing. The sequence of 3E1.2 delta TRL, SEQ ID NO 10, is as follows:

1 TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG GAGACGGTCA

61 CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG  
TCAGCGGGTG

121 TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA GCAGATTGTA  
CTGAGAGTGC

181 ACCATATGCG GTGTGAAATA CCGCACAGAT GCGTAAGGAG AAAATACCGC  
ATCAGGCGCC

241 ATTCGCCATT CAGGCTGCGC AACTGTTGGG AAGGGCGATC GGTGCGGGCC  
TCTTCGCTAT

301 TACGCCAGCT GGC GAAAGGG GGATGTGCTG CAAGGCGATT AAGTTGGGTA  
ACGCCAGGGT

361 TTTCCCAGTC ACGACGTTGT AAAACGACGG CCAGTGCCAA GCTTTGTTTA  
AAATATAACA

421 AAATTGTGAT CCCACAAAAT GAAGTGGGGC AAAATCAAAT AATTAAGTAG  
TGTCCGTAAA

481 CTTGTTGGTC TTCAACTTTT TGAGGAACAC GTTGGACGGC AAATCGTGAC  
TATAACACAA

541 GTTGATTAA TAATTTTAGC CAACACGTCG GGCTGCGTGT TTTTTCGCT CTGTGTACAC

601 GTTGATTAAC TGGTCGATTA AATAATTTAA TTTTGGTTC TTCTTTAAAT CTGTGATGAA

661 ATTTTTTAAA ATAACTTTAA ATTCTTCATT GGTA AAAAAT GCCACGTTTT GCAACTTGTG

721 AGGGTCTAAT ATGAGGTCAA ACTCAGTAGG AGTTTTATCC AAAAAAGAAA  
ACATGATTAC

781 GTCTGTACAC GAACGCGTAT TAACGCAGAG TGCAAAGTAT AAGAGGGTTA  
AAAAATATAT

841 TTTACGCACC ATATACGCAT CGGGTTGATA TCGTTAATAT GGATCAATTT  
GAACAGTTGA

901 TTAACGTGTC TCTGCTCAAG TCTTTGATCA AAACGCAAAT CGACGAAAAT  
GTGTCGGACA

961 ATATCAAGTC GATGAGCGAA AAATAAAAA GGCTAGAATA CGACAATCTC  
ACAGACAGCG

1021 TTGAGATATA CGGTATTCAC GACAGCAGGC TGAATAATAA AAAAATTAGA  
AACTATTATT

1081 TAACCCTAGA AAGATAATCA TATTGTGACG TACGTAAAG ATAATCATGC  
GTAAAATTGA

1141 CGCATGTGTT TTTATCGGTC TGTATATCGA GGTTTATTTA TTAATTTGAA  
TAGATATTAA

1201 GTTTTATTAT ATTTACTT ACATACTAAT AATAAATTCA ACAAACAATT  
TATTTATGTT

1261 TATTTATTTA TTAAAAAAA ACAAAAACCTC AAAATTTCTT CTAAAGTAAC  
AAAACTTTTA

1321 AACATTCTCT CTTTACAAA AATAAACTTA TTTTGTACTT TAAAAACAGT  
CATGTTGTAT

1381 TATAAAATAA GTAATTAGCT TAACTTATAC ATAATAGAAA CAAATTATAC  
TTATTAGTCA

1441 GTCCAGAAAC AACTTTGGCA CATATCAATA TTATGCTCTC GACAAATAAC

TTTTTTGCAT

1501 TTTTTGCACG ATGCATTTGC CTTTCGCCTT ATTTTAGAGG GGCAGTAAGT  
ACAGTAAGTA

1561 CGTTTTTTTCA TTAGTGGCTC TTCAGTACTG TCATCTGATG TACCAGGCAC  
TTCATTTGGC

1621 AAAATATTAG AGATATTATC GCGCAAATAT CTCTTCAAAG TAGGAGCTTC  
TAAACGGTTA

1681 CGCATAAACG ATGACGTCAG GCTCATGTAA AGGTTTCTCA TAAATTTTTT  
GCGACTTTGA

1741 ACCTTTTCTC CTTGCTACT GACATTATGG CTGTATATAA TAAAAGAATT  
TATGCAGGCA

1801 ATGTTTATCA TTCCGTACAA TAATGCCATA GGCCACCTAT TCGTCTTCCT  
ACTGCAGGTC

1861 ATCACAGAAC ACATTTGGTC TAGCGTGTCC ACTCCGCCTT TAGTTTGATT  
ATAATACATA

1921 ACCATTTGCG GTTTACCGGT ACTTTCGTTG ATAGAAGCAT CCTCATCACA  
AGATGATAAT

1981 AAGTATACCA TCTTAGCTGG CTTTCGGTTTA TATGAGACGA GAGTAAGGGG  
TCCGTCAAAA

2041 CAAAACATCG ATGTTCCAC TGGCCTGGAG CGACTGTTTT TCAGTACTTC  
CGGTATCTCG

2101 CGTTTGTTTTG ATCGCACGGT TCCCACAATG GTTAACTTAT ACGGTTCTTG  
TAGTAAGTTT

2161 TTTGCCAAAG GGATTGAGGT GAACCAATTG TCACACGTAA TATTACGACA  
ACTACCGTGC

2221 ACAGGCTTTG ATAACCTT CACGTAGTAT TCACCGAGTG GACTCCGTT  
GGTCTGTGTT

2281 CCTTTCCCA AATAAGGCAT TCCATTTATC ATATACTTCG TACCACTGTC  
ACACATCATG

2341 AGGATTTTTTA TTCCATACTT ACTTGGCTTG TTTGGGATAT ACATCCTAAA  
CGGACACCGT

2401 CCTCTAAAAC CAAGTAACTG TTCATCTATG GTCAAATGAG CCCCTGGAGT  
GTAATTTTGT

2461 ATGCACTGAT GGATAAAGAG ATCCCATATT TTTCTAACAG GAGTAAATAC  
ATCGTTTTCT

2521 CGAAGTGTGG GCCGTATACT TTTGTCATCC ATTCTAAGAC ATCGTATCAA  
AAAATCCAAA

2581 ACGATCCACA GACTCATTAC AGAGACGTAC ACATTGACAA AGATCGATCC  
AAAGAGGTCA

2641 TCTGTGGACA TGTGGTTATC TTTTCTCACT GCTGTCATTA CCAGAATACC  
AAAGAAAGCA

2701 TAGATTTTCAT CTTCATTTCGT GTCACGAAAT GTAGCACCTG TCATAGATTC  
CCGACGTTTC

2761 AATGATATCT CAGCATTGT CCATTTTACA ATTTGCGAAA TTATCTCATC  
AGTAAAAAAT

2821 AGTTTGAAGC ATAAAAGTGG GTCATATATA TTGCGGCACA TACGCGTCGG  
ACCTCTTTGA

2881 GATCTGACAA TGTTCAAGTGC AGAGACTCGG CTACCGCTCG TGGACTTTGA  
AGTTGACCAA

2941 CAATGTTTAT TCTTACCTCT AATAGTCCTC TGTGGCAAGG TCAAGATTCT  
GTTAGAAGCC

3001 AATGAAGAAC CTGGTTGTTT AATAACATTT TGTTTCGTCTA ATATTTCACT  
ACGCTTGACG

3061 TTGGCTGACA CTTCATGTAC CTCATCTATA AACGCTTCTT CTGTATCGCT  
CTGGACGTCT

3121 TCACTTACGT GATCTGATAT TTCACTGTCA GAATCCTCAC CAACAAGCTC  
GTCATCGCCT

3181 TGCAGAAGAG CAGAGAGGAT ATGCTCATCG TCTAAAGAAC ATCCCATTTT  
ATTATATATT

3241 AGTCACGATA TCTATAACAA GAAAATATAT ATATAATAAG TTATCACGTA  
AGTAGAACAT

3301 GAAATAACAA TATTAATTAT CGTATGAGTT AAATCTTAAA AGTCACGTAA  
AAGATAATCA

3361 TGCCTCATTG TGAATCACGC GGTCGTTATA GTTCAAATC AGTGACACTT  
ACCGCATTGA

3421 CAAGCACGCC TCAGCCGAGC TCGAATTCGT AATCATGGTC ATAGCTGTTT  
CCTGTGTGAA

3481 ATTGTTATCC GCTCACAATT CCACACAACA TACGAGCCGG AAGCATAAAG  
TGTAAGCCT

3541 GGGGTGCCTA ATGAGTGAGC TAACTCACAT TAATTGCGTT GCGCTCACTG  
CCCGCTTTCC

3601 AGTCGGGAAA CCTGTCGTGC CAGCTGCATT AATGAATCGG CCAACGCGCG  
GGGAGAGCGG

3661 GTTTGCATAT TGGGCGCTCT TCCGCTTCTT CGCTCACTGA CTCGCTGCGC  
TCGGTCGTTT

3721 GGCTGCGGCG AGCGGTATCA GCTCACTCAA AGGCGGTAAT ACGGTTATCC  
ACAGAATCAG

3781 GGGATAACGC AGGAAAGAAC ATGTGAGCAA AAGGCCAGCA AAAGGCCAGG  
AACCGTAAAA

3841 AGGCCGCGTT GCTGGCGTTT TTCCATAGGC TCCGCCCCC TGACGAGCAT  
CACAAAAATC

3901 GACGCTCAAG TCAGAGGTGG CGAAACCCGA CAGGACTATA AAGATACCAG  
GCGTTTCCCC

3961 CTGGAAGCTC CCTCGTGCGC TCTCCTGTTC CGACCCTGCC GCTTACCGGA  
TACCTGTCCG

4021 CCTTTCTCCC TTCGGGAAGC GTGGCGCTTT CTCAATGCTC ACGCTGTAGG  
TATCTCAGTT

4081 CGGTGTAGGT CGTTCGCTCC AAGCTGGGCT GTGTGCACGA ACCCCCCGTT  
CAGCCCCGACC

4141 GCTGCGCCTT ATCCGGTAAC TATCGTCTTG AGTCCAACCC GGTAAGACAC  
GACTTATCGC

4201 CACTGGCAGC AGCCACTGGT AACAGGATTA GCAGAGCGAG GTATGTAGGC  
GGTGCTACAG

4261 AGTTCTTGAA GTGGTGGCCT AACTACGGCT AACTAGAAG GACAGTATTT  
GGTATCTGCG

4321 CTCTGCTGAA GCCAGTTACC TTCGGAAAAA GAGTTGGTAG CTCTTGATCC  
GGCAAACAAA

4381 CCACCGCTGG TAGCGGTGGT TTTTTTGTTT GCAAGCAGCA GATTACGCGC  
AGAAAAAAG

4441 GATCTCAAGA AGATCCTTTG ATCTTTTCTA CGGGGTCTGA CGCTCAGTGG  
AACGAAAAC

4501 CACGTTAAGG GATTTTGGTC ATGAGATTAT CAAAAAGGAT CTTCACCTAG  
ATCCTTTTAA

4561 ATTAATAATG AAGTTTTAAA TCAATCTAAA GTATATATGA GTAAACTTGG  
TCTGACAGTT

4621 ACCAATGCTT AATCAGTGAG GCACCTATCT CAGCGATCTG TCTATTTTCTG  
TCATCCATAG

4681 TTGCCTGACT CCCCGTCGTG TAGATAACTA CGATACGGGA GGGCTTACCA  
TCTGGCCCCA

4741 GTGCTGCAAT GATACCGCGA GACCCACGCT CACCGGCTCC AGATTTATCA  
GCAATAAACC

4801 AGCCAGCCGG AAGGGCCGAG CGCAGAAGTG GTCCTGCAAC TTTATCCGCC

TCCATCCAGT

4861 CTATTAATTG TTGCCGGGAA GCTAGAGTAA GTAGTTCGCC AGTTAATAGT  
TTGCGCAACG

4921 TTGTTGCCAT TGCTACAGGC ATCGTGGTGT CACGCTCGTC GTTTGGTATG  
GCTTCATTCA

4981 GCTCCGGTTC CCAACGATCA AGGCGAGTTA CATGATCCCC CATGTTGTGC  
AAAAAAGCGG

5041 TTAGCTCCTT CGGTCCTCCG ATCGTTGTCA GAAGTAAGTT GGCCGCAGTG  
TTATCACTCA

5101 TGGTTATGGC AGCACTGCAT AATTCTCTTA CTGTCATGCC ATCCGTAAGA  
TGCTTTTCTG

5161 TGACTGGTGA GTACTCAACC AAGTCATTCT GAGAATAGTG TATGCGGCGA  
CCGAGTTGCT

5221 CTTGCCCGGC GTCAATACGG GATAATACCG CGCCACATAG CAGAACTTTA  
AAAGTGCTCA

5281 TCATTGAAA ACGTTCTTCG GGGCGAAAAC TCTCAAGGAT CTTACCGCTG  
TTGAGATCCA

5341 GTTCGATGTA ACCCACTCGT GCACCCAAC TATCTTCAGC ATCTTTTACT  
TTCACCAGCG

5401 TTTCTGGGTG AGCAAAAACA GGAAGGCAA ATGCCGCAA AAAGGGAATA  
AGGGCGACAC

5461 GGAAATGTTG AATACTCATA CTCTTCCTTT TTCAATATTA TTGAAGCATT  
TATCAGGGTT

5521 ATTGCTCAT GAGCGGATAC ATATTTGAAT GTATTTAGAA AAATAAACAA  
ATAGGGGTTT

5581 CGCGCACATT TCCCCGAAAA GTGCCACCTG ACGTCTAAGA AACCATTATT  
ATCATGACAT

5641 TAACCTATAA AAATAGGCGT ATCACGAGGC CCTTTCGTC

### EXAMPLE 13

The phsp70/opd plasmid (Example 11 supra) was inserted into p3E1.2 by cutting p3E1.2 with Cla I and then ligating with an adaptor containing a Cla I half-site and Apa I site. The construct was then cut with Apa I and Bam HI. The fragment was gel purified and ligated with gel purified Apa I/Bgl II phsp70/opd fragment. This plasmid is designated piggyBac/opd (FIGS. 7a-7e, SEQ ID NO 14). The phsp/opd marker gene was coinjected with the piggyBac element of p3E1.2 plasmid into *Plodia interpunctella* genome to test the marker. This confirmed that the hsp/opd gene is an effective selectable marker gene for detecting transformations in insects (Data not shown).

Transformations were attempted by injecting fertile eggs of the Indianmeal moth, *Plodia interpunctella* with the piggyBac/opd plasmid with the p3E1.2.DELTA.TRL helper (described above in Example 11) as described above in Example 8. The insects were hatched and mass-mated. The G.sub.1 progeny of the

mass-mated microinjected insects were permitted to feed for about 24 hours, heat-shocked for about 60 minutes at about 42.degree. C., rested for about 60 minutes at about 26.degree. C. and allowed to feed before being subjected to selection on paraoxon. The optimum time interval between heat shock and paraoxon treatment for *P. interpunctella* is about 4 to about 8 hours for a maximum period for resistance. The heated stressed caterpillars and then allowed to crawl for about 30 minutes on approximately 50 .mu.gram/cm<sup>2</sup> paraoxon-treated filter paper disks.

Four independent transformed lines were recovered which are now in G.sub.16. Three of these lines are white-eyed mutants. These transformation induced white-eyed mutants of *P. interpunctella* are genetically similar to those recovered from the laboratory strain as spontaneous white-eyed mutations, because matings between the transformation induced white-eyed insects and the spontaneous white-eyed mutants showed no complementation between the strains. Southern blots of genomic DNA from larvae that were in the tenth generation of these lines show positive hybridization profiles for both piggyBac and hsp/opd sequences that are unique for each strain (FIG. 8, compare lane B with L and C with M). Genomic DNA was extracted from G10 larvae of the PiA-3 and PiA-11 strains of *P. interpunctella* that were coinjected with piggyBac/opd and p3E1.2.DELTA.TRL. These results indicate that the Indian meal moth, *P. interpunctella* has been genetically transformed using the piggyBac/opd plasmid as the transforming vector.

The foregoing detailed description is for the purpose of illustration. Such detail is solely for that purpose and those skilled in the art can make variations without departing from the spirit and scope of the invention.

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