

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
2 September 2010 (02.09.2010)

PCT

(10) International Publication Number
WO 2010/099301 A2

(51) International Patent Classification:

C12N 15/12 (2006.01) C12N 5/10 (2006.01)
C12N 15/63 (2006.01) A61K 48/00 (2006.01)

(21) International Application Number:

PCT/US2010/025386

(22) International Filing Date:

25 February 2010 (25.02.2010)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/155,207 25 February 2009 (25.02.2009) US

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(81) Designated States (unless otherwise indicated, for every

kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every

kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report (Rule 48.2(g))

(54) Title: PIGGYBAC TRANSPOSON VARIANTS AND METHODS OF USE

(57) Abstract: The present invention provides hyperactive piggyBac transposons, in particular hyperactive piggyBac transposons from *Trichoplusia ni* (cabbage looper moth) that transpose at a higher frequency than wildtype. The invention also features integration defective piggyBac transposons. The piggyBac transposons and transposases can be used in gene transfer systems for stably introducing nucleic acids into the DNA of a cell. The gene transfer system can be used in methods, for example, but not limited to, gene therapy, insertional mutagenesis, or gene discovery.



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PIGGYBAC TRANSPOSON VARIANTS AND METHODS OF USE

CROSS-REFERENCE TO RELATED APPLICATION

5 This application claims the benefit of U.S. Provisional Application No. 61/155,207, filed on February 25, 2009, the entire contents of which is incorporated herein by reference.

BACKGROUND OF THE INVENTION

10 Typical methods for introducing DNA into a cell include DNA condensing reagents such as calcium phosphate, polyethylene glycol, lipid-containing reagents, such as liposomes, multi-lamellar vesicles, as well as virus-mediated strategies. However, such methods can have certain limitations. For example, there are size constraints associated with DNA condensing reagents and virus-mediated strategies. Further, the amount of nucleic acid that can be transfected into a cell is limited in virus strategies. Not all methods facilitate insertion of the delivered nucleic acid into cellular nucleic acid, and while DNA condensing methods and lipid-containing reagents are relatively easy to prepare, the insertion of nucleic acid into viral vectors can be labor intensive. Virus-mediated strategies can be cell-type or tissue-type specific, and the use of virus-mediated strategies can create immunologic problems when used *in vivo*.

 One suitable tool to address these issues are transposons. Transposons, or transposable elements, include a (short) nucleic acid sequence, with terminal repeat sequences upstream and downstream. Active transposons encode enzymes that facilitate the excision and insertion of the nucleic acid into target DNA sequences.

 Transposable elements represent a substantial fraction of many eukaryotic genomes. For example, ~50% of the human genome is derived from transposable element sequences, and other genomes, for example plants, may consist of substantially higher proportions of transposable element-derived DNA. Transposable elements are typically divided into two classes, class 1 and class 2. Class 1 is represented by the retrotransposons (LINEs, SINEs, LTRs, and ERVs). Class 2 includes the "cut-and-paste" DNA transposons, which are characterized by terminal inverted repeats (TIRs) and are mobilized by an element-encoded transposase.

Currently, 10 superfamilies of cut-and-paste DNA transposons are recognized in eukaryotes (Feschotte and Pritham, 2007).

While class 2 elements are widespread and active in a variety of eukaryotes, they have been thought to be transpositionally inactive in mammalian genomes. This conclusion was based primarily on the initial analyses of the human and mouse genome sequences. While both species harbor a significant number and a diverse assortment of DNA transposons, they show no signs of recent activity (Lander et al. 2001; Waterston et al. 2002). For example, there are more than 300,000 DNA elements recognizable in the human genome, which are grouped into 120 families and belong to five superfamilies. A large subset of these elements (40 families; ~98,000 copies) were integrated in the last 40-80 million years (Myr), but there remains no evidence for any human DNA transposon families younger than ~37 Myr (Pace and Feschotte, 2007).

The natural process of horizontal gene transfer can be mimicked under laboratory conditions. In plants, transposons of the Ac/Ds and Spm families have been routinely transfected into heterologous species (Osborne and Baker, 1995 Curr. Opin. Cell Biol. 7, 406-413). In animals, however, a considerable obstacle to the transfer of an active transposon system from one species to another has been that of species-specificity of transposition due to the requirement for factors produced by the natural host.

Both invertebrate and vertebrate transposons hold potential for transgenesis and insertional mutagenesis in model organisms. Particularly, the availability of alternative transposon systems in the same species opens up new possibilities for genetic analyses.

There still remains a need for new methods for introducing DNA into a cell, and particularly methods that promote the efficient insertion of transposons of varying sizes into the nucleic acid of a cell or the insertion of DNA into the genome of a cell while allowing more efficient transcription/translation results than constructs as available in the state of the art.

SUMMARY OF THE INVENTION

As described in more detail below, the piggyBac transposon from *Trichoplusia ni* (cabbage looper moth) has been shown to be an active element in a number of insects, mice, swine and mammalian cells, including human. The present

inventors have isolated *Trichoplusia ni* piggyBac variants that transpose at a higher frequency than wildtype. The hyperactive transposons can be used in gene transfer systems for stably introducing nucleic acids into the DNA of a cell. Moreover, the present inventors have identified integration defective piggyBac transposons. The gene transfer systems of the present invention can be used in methods, for example, but not limited to, gene therapy, insertional mutagenesis, or gene discovery.

Accordingly, in a first aspect, the invention features a transposon comprising one or more hyperactive piggyBac nucleic acid sequences and variants, derivatives and fragments thereof that retain transposon activity.

In one embodiment, the hyperactive piggyBac transposon has a higher level of transposon excision compared to a wildtype piggyBac transposon.

In another embodiment, the transposon comprises 2, 3, 4, 5 or more hyperactive piggyBac nucleic acid sequences and variants, derivatives and fragments thereof that retain transposon activity.

In another further embodiment, the hyperactive piggyBac nucleic acid sequence is from the family *Noctuidae*. In a further related embodiment, the hyperactive piggyBac nucleic acid sequence is from the species *Trichoplusia ni*.

In another further embodiment, the nucleic acid sequence is selected from SEQ ID NO: 34 – SEQ ID NO: 63 or SEQ ID NO: 70 SEQ ID NO: 96.

In a further embodiment, the nucleic acid sequence encodes an amino acid sequence selected from SEQ ID NO: 3 – SEQ ID NO: 32.

In another aspect, the present invention features a transposon comprising one or more integration defective piggyBac nucleic acid sequences and variants, derivatives and fragments thereof.

In one embodiment, the integration defective piggyBac transposon has a lower rate of integration as compared to a wildtype piggyBac transposon.

In another embodiment, the integration defective piggyBac nucleic acid sequence is from the family *Noctuidae*. In a further related embodiment, the integration defective piggyBac nucleic acid sequence is from the species *Trichoplusia ni*.

In one embodiment, the nucleic acid sequence is selected from SEQ ID NO: 67 – SEQ ID NO: 69.

In a further embodiment, the nucleic acid sequence encodes an amino acid sequence selected from SEQ ID NO: 64 – SEQ ID NO: 66.

In another further embodiment, the wildtype piggyBac transposon comprises a nucleic acid sequence corresponding to SEQ ID NO: 1.

In certain exemplary embodiments, the hyperactive variants comprise an amino acid change in SEQ ID NO: 2 selected from the group consisting of: G2C,
5 Q40R, S3N, S26P, I30V, G165S, T43A, Q55R, T57A, S61R, I82V, I90V, S103P,
S103T, N113S, M185L, M194V, S230N, R281G, M282V, G316E, P410L, I426V,
Q497L, K501N, K565I, N505D, S573L, S509G, N570S, N538K, K575R, Q591P,
Q591R, F594L.

In one embodiment of the above aspects, the transposon is capable of inserting
10 into the DNA of a cell.

In another embodiment of the above aspects, the transposon further comprises a marker protein.

In still another embodiment of the above aspects, the transposon is inserted in a plasmid.

15 In one embodiment, the transposon further comprises at least a portion of an open reading frame. In another embodiment, the transposon further comprises at least one expression control region. In still another further embodiment, the expression control region is selected from the group consisting of a promoter, an enhancer or a silencer. In another related embodiment, the transposon further comprises a promoter
20 operably linked to at least a portion of an open reading frame.

In one embodiment, the cell is obtained from an animal.

In another embodiment, the cell is from a vertebrate or an invertebrate.

In a further embodiment, the vertebrate is a mammal.

In one embodiment, the invention features a gene transfer system comprising a
25 transposon according to any one of the above aspects; and a piggyBac transposase.

In one embodiment, the piggyBac transposase is from the family *Noctuidae*. In a related embodiment, the piggyBac transposase is from the species *Trichoplusia ni*.

In a further embodiment, the piggyBac transposase comprises an amino acid sequence corresponding to SEQ ID NO: 33.

30 In another embodiment, the piggyBac transposase is a mammalian piggyBac transposase.

In one embodiment, the transposon is inserted into the genome of the cell.

In another embodiment, the cell is obtained from an animal.

In another embodiment, the cell is from a vertebrate or an invertebrate.

In a further embodiment, the vertebrate is a mammal.

The present invention also features in certain embodiments a cell comprising a transposon of any one of the above-described aspects.

5 In other aspects, the present invention features a pharmaceutical composition comprising a transposon comprising a hyperactive piggyBac nucleic acid sequence and a piggyBac transposase, together with a pharmaceutically acceptable carrier, adjuvant or vehicle.

10 The present invention also features a method for introducing exogenous DNA into a cell comprising contacting the cell with the gene transfer system of the above-described aspects, thereby introducing exogenous DNA into a cell.

In one embodiment, the cell is a stem cell.

The present invention also features a kit comprising: a transposon comprising a hyperactive piggyBac nucleic acid sequence and instructions for introducing DNA into a cell.

15 In one embodiment, the hyperactive piggyBac nucleic acid sequence is from the family *Noctuidae*. In a further embodiment, the hyperactive piggyBac nucleic acid sequence is from the species *Trichoplusia ni*.

In another embodiment, the nucleic acid is sequence selected from SEQ ID NO: 34 – SEQ ID NO: 63 or SEQ ID NO: 70 – SEQ ID NO: 96.

20 In another aspect, the present invention also features a kit comprising: a transposon comprising a integration defective piggyBac nucleic acid sequence and instructions for use.

In one embodiment, the nucleic acid sequence is selected from SEQ ID NO: 67 – SEQ ID NO: 69.

25

Other features and advantages of the invention will be apparent from the detailed description, and from the claims.

Definitions

30 The practice of the present invention will employ, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA and immunology, which are within the capabilities of a person of ordinary skill in the art. Such techniques are explained in the literature. See, for example, J. Sambrook, E. F. Fritsch, and T. Maniatis, 1989, *Molecular Cloning: A Laboratory*

Manual, Second Edition, Books 1-3, Cold Spring Harbor Laboratory Press; Ausubel, F. M. et al. (1995 and periodic supplements; Current Protocols in Molecular Biology, ch. 9, 13, and 16, John Wiley & Sons, New York, N.Y.); B. Roe, J. Crabtree, and A. Kahn, 1996, DNA Isolation and Sequencing: Essential Techniques, John Wiley & Sons; J. M. Polak and James O'D. McGee, 1990, In Situ Hybridization: Principles and Practice; Oxford University Press; M. J. Gait (Editor), 1984, Oligonucleotide Synthesis: A Practical Approach, Irl Press; D. M. J. Lilley and J. E. Dahlberg, 1992, Methods of Enzymology: DNA Structure Part A: Synthesis and Physical Analysis of DNA Methods in Enzymology, Academic Press; Using Antibodies: A Laboratory Manual: Portable Protocol NO. I by Edward Harlow, David Lane, Ed Harlow (1999, Cold Spring Harbor Laboratory Press, ISBN 0-87969-544-7); Antibodies: A Laboratory Manual by Ed Harlow (Editor), David Lane (Editor) (1988, Cold Spring Harbor Laboratory Press, ISBN 0-87969-3,4-2), 1855. Handbook of Drug Screening, edited by Ramakrishna Seethala, Prabhavathi B. Fernandes (2001, New York, N.Y., Marcel Dekker, ISBN 0-8247-0562-9); and Lab Ref: A Handbook of Recipes, Reagents, and Other Reference Tools for Use at the Bench, Edited Jane Roskams and Linda Rodgers, 2002, Cold Spring Harbor Laboratory, ISBN 0-87969-630-3. Each of these general texts is herein incorporated by reference.

As used herein, the following terms have the meanings ascribed to them below, unless specified otherwise.

In this disclosure, "comprises," "comprising," "containing" and "having" and the like can have the meaning ascribed to them in U.S. Patent law and can mean "includes," "including," and the like; "consisting essentially of" or "consists essentially" likewise has the meaning ascribed in U.S. Patent law and the term is open-ended, allowing for the presence of more than that which is recited so long as basic or novel characteristics of that which is recited is not changed by the presence of more than that which is recited, but excludes prior art embodiments.

As used herein, the term "integration defective" is meant to refer to a transposon that integrates at a lower frequency into the host genome than a corresponding wild type transposon. In certain exemplary embodiments, the inventive transposons integrate by conventional integration mechanisms.

As used herein, the term "nucleotide" or "polynucleotide" is meant to refer to both double- and single-stranded DNA and RNA, and combinations thereof. A polynucleotide may include nucleotide sequences having different functions,

including for instance coding sequences, and non-coding sequences such as regulatory sequences. A polynucleotide can be obtained directly from a natural source, or can be prepared with the aid of recombinant, enzymatic, or chemical techniques. A polynucleotide can be linear or circular in topology. A polynucleotide can be, for example, a portion of a vector, or a fragment. A "coding sequence" or a "coding region" is a polynucleotide that encodes a polypeptide and, when placed under the control of appropriate regulatory sequences, expresses the encoded polypeptide. The boundaries of a coding region are generally determined by a translational start codon at its 5' end and a translational stop codon at its 3' end. A regulatory sequence is a nucleotide sequence that regulates expression of a coding region to which it is operably linked. Non-limiting examples of regulatory sequences include promoters, transcriptional initiation sites, translational start sites, translational stop sites, transcriptional terminators (including, for instance, poly-adenylation signals), and intervening sequences (introns).

As used herein, the term "operably linked" is meant to refer a nucleotide sequence that is placed in a functional relationship with another nucleotide sequence. For example, if a coding sequence is operably linked to a promoter sequence, this generally means that the promoter may promote transcription of the coding sequence. Operably linked means that the DNA sequences being linked are typically contiguous and, where necessary join two protein coding regions, contiguous and in reading frame. Since enhancers may function when separated from the promoter by several kilobases and intron sequences may be of variable lengths, some nucleotide sequences may be operably linked but not contiguous..

As used herein, the term "polypeptide" is meant to refer to a polymer of amino acids of any length. Thus, for example, the terms peptide, oligopeptide, protein, antibody, and enzyme are included within the definition of polypeptide. This term also includes post-expression modifications of the polypeptide, for example, glycosylations (e.g., the addition of a saccharide), acetylations, phosphorylations and the like.

As used herein, the term "transposon" or "transposable element" is meant to refer to a polynucleotide that is able to excise from a donor polynucleotide, for instance, a vector, and integrate into a target site, for instance, a cell's genomic or extrachromosomal DNA. A transposon includes a polynucleotide that includes a nucleic acid sequence flanked by cis-acting nucleotide sequences on the termini of the

transposon. A nucleic acid sequence is "flanked by" cis-acting nucleotide sequences if at least one cis-acting nucleotide sequence is positioned 5' to the nucleic acid sequence, and at least one cis-acting nucleotide sequence is positioned 3' to the nucleic acid sequence. Cis-acting nucleotide sequences include at least one inverted repeat (also referred to herein as an inverted terminal repeat, or ITR) at each end of the transposon, to which a transposase, preferably a member of the mammalian piggyBac family of transposases, binds. In certain preferred embodiments, the transposon is from the family Noctuidae. In further preferred embodiments, the transposon is a *Trichoplusia ni* (Cabbage looper moth) piggyBac transposon.

10 As used herein "*Trichoplusia ni*" is meant to refer to a member of the moth family *Noctuidae*.

An "isolated" polypeptide or polynucleotide means a polypeptide or polynucleotide that has been either removed from its natural environment, produced using recombinant techniques, or chemically or enzymatically synthesized.

15 Preferably, a polypeptide or polynucleotide of this invention is purified, i.e., essentially free from any other polypeptide or polynucleotide and associated cellular products or other impurities.

As used herein, the term "transposase" is meant to refer to a polypeptide that catalyzes the excision of a transposon from a donor polynucleotide (e.g., a vector) and the subsequent integration of the transposon into the genomic or extrachromosomal DNA of a target cell. Preferably, the transposase binds an inverted sequence or a direct repeat. The transposase may be present as a polypeptide. Alternatively, the transposase is present as a polynucleotide that includes a coding sequence encoding a transposase. The polynucleotide can be RNA, for instance an mRNA encoding the transposase, or DNA, for instance a coding sequence encoding the transposase. When the transposase is present as a coding sequence encoding the transposase, in some aspects of the invention the coding sequence may be present on the same vector that includes the transposon, i.e., in cis. In other aspects of the invention, the transposase coding sequence may be present on a second vector, i.e., in trans. In certain preferred
25
30
embodiments, the transposase is a mammalian piggyBac transposase.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a Table (Table 2) that shows the amino acid changes and fold increase in transposition from that of wildtype (normalized to 1) in inventive hyperactive variants.

5 Figure 2 (A and B). Panel (A) shows the nucleic acid sequence of the wild type *Trichoplusia ni* transposon, corresponding to SEQ ID NO: 1 and the corresponding amino acid sequence (SEQ ID NO: 2). Panel (B) shows the amino acid sequence corresponding to the wild type *Trichoplusia ni* transposase, corresponding to SEQ ID NO: 33.

10 Figure 3 shows identification of excision hyperactive piggyBac mutants.

Figure 4 shows the amino acid sequences of the integration defective piggyBac transposons, corresponding to SEQ ID NOs 64 – 66.

DETAILED DESCRIPTION

15 The ability to use a transposon for genome engineering is highly dependent upon the frequency with which it can move. For example if 1 progeny in 10 has a transposon event, it will be much easier to isolate derivatives of the desired type than if the transposition event occurs in 1 in 1000 progeny. The present inventors have isolated hyperactive piggyBac transposons from *Trichoplusia ni* (cabbage looper
20 moth) that transpose at a higher frequency than wildtype. Transposons such as piggyBac are widely used for genome engineering by insertional mutagenesis and transgenesis in a wide variety of organisms. The piggyBac transposon from *Trichoplusia ni* has been shown to be an active element in a number of insect, mice, swine and mammalian cells including human.

25 Accordingly, the present invention features hyperactive piggyBac transposons. A hyperactive piggyBac transposon is meant to refer to a transposon that has a transposon event at a higher frequency than wild type piggyBac transposon. For example, in certain exemplary embodiments, in a hyperactive piggyBac transposon transposition occurs 0.5 fold, 1 fold, 2 fold, 3 fold, 4 fold, 5 fold, 6 fold, 7 fold, 8 fold,
30 9 fold, 10 fold, 15 fold, 20 fold, 25 fold, 30 fold, 40 fold, 45 fold, 50 fold or more.

According to certain preferred embodiments of the present invention, a hyperactive piggyBac transposon is bound by a transposase, contains a pair of repeat sequences. In certain preferred embodiments, the first repeat is typically located upstream to the nucleic acid sequence and the second repeat is typically located

downstream of the nucleic acid sequence. Accordingly, the second repeat represents the same sequence as the first repeat, but shows an opposite reading direction as compared with the first repeat (5' and 3' ends of the complementary double strand sequences are exchanged). These repeats are then termed "inverted repeats" (IRs), due to the fact that both repeats are just inversely repeated sequences. In certain embodiments, repeats may occur in a multiple number upstream and downstream of the above mentioned nucleic acid sequence. Preferably, the number of repeats located upstream and downstream of the above mentioned nucleic acid sequence is identical. In certain embodiments, the repeats are short, between 10 – 20 base pairs, and preferably 15 base pairs.

The repeats (IRs) as described herein preferably flank a nucleic acid sequence which is inserted into the DNA of a cell. The nucleic acid sequence can include all or part of an open reading frame of a gene (i.e., that part of a protein encoding gene), one or more expression control sequences (i.e., regulatory regions in nucleic acid) alone or together with all or part of an open reading frame. Preferred expression control sequences include, but are not limited to promoters, enhancers, border control elements, locus-control regions or silencers. In a preferred embodiment, the nucleic acid sequence comprises a promoter operably linked to at least a portion of an open reading frame. According to certain preferred embodiments, hyperactive transposons of the present invention can preferably occur as a linear transposons (extending from the 5' end to the 3' end, by convention) that can be used as a linear fragment or circularized, for example in a plasmid.

The present invention features hyperactive piggyBac nucleic acid sequence and variants, derivatives and fragments thereof that retain transposon activity. In preferred embodiments of the invention, the hyperactive piggyBac transposon has a higher level of transposon excision compared to a wildtype piggyBac transposon

In certain preferred embodiments, the hyperactive piggyBac transposon nucleic acid sequence is from the family *Noctuidae*. In further exemplary embodiments, the hyperactive piggyBac transposon nucleic acid sequence is from *Trichoplusia ni*.

Preferred embodiments of the present invention refer to nucleic acids encoding a hyperactive piggyBac transposon as defined herein.

It will be understood by a skilled person that numerous different polynucleotides can encode the same polypeptide as a result of the degeneracy of the genetic code. In addition, it is to be understood that skilled persons may, using routine techniques, make nucleotide substitutions that do not affect the polypeptide sequence
5 encoded by the polynucleotides used in the invention to reflect the codon usage of any particular host organism in which the polypeptides are to be expressed. The polynucleotides may be modified by any method available in the art. Such modifications may be carried out in order to enhance the in vivo activity or life span of the polynucleotides of the invention.

10 Polynucleotides such as DNA polynucleotides may be produced recombinantly, synthetically, or by any means available to those of skill in the art. They may also be cloned by standard techniques.

Longer polynucleotides will generally be produced using recombinant means, for example using PCR (polymerase chain reaction) cloning techniques. This will
15 involve making a pair of primers (e.g. of about 15 to 30 nucleotides) flanking a region of the lipid targeting sequence which it is desired to clone, bringing the primers into contact with mRNA or cDNA obtained from an animal or human cell, performing a polymerase chain reaction under conditions which bring about amplification of the desired region, isolating the amplified fragment (e.g. by purifying the reaction mixture
20 on an agarose gel) and recovering the amplified DNA. The primers may be designed to contain suitable restriction enzyme recognition sites so that the amplified DNA can be cloned into a suitable cloning vector.

It will be appreciated that the polynucleotide of the invention may contain only coding regions. However, it is preferred if the polynucleotide further comprises,
25 in operable linkage, a portion of nucleic acid that allows for efficient translation of the coding sequence. It is further preferred if the polynucleotide (when in a DNA form) further comprises a promoter in operable linkage which allows for the transcription of the coding region and the portion of nucleic acid that allows for efficient translation of the coding region in a target cell.

30 Nucleic acids according to the present invention typically comprise ribonucleic acids, including mRNA, DNA, cDNA, chromosomal DNA, extrachromosomal DNA, plasmid DNA, viral DNA or RNA. In certain preferred

embodiments, a nucleic acid is preferably selected from any nucleic sequence encoding the same amino acid sequence of a hyperactive piggyBac transposon due to degeneration of its genetic code. These alternative nucleic acid sequences may lead to an improved expression of the encoded fusion protein in a selected host organism.

- 5 Tables for appropriately adjusting a nucleic acid sequence are known to a skilled person. Preparation and purification of such nucleic acids and/or derivatives are usually carried out by standard procedures (see Sambrook et al. 2001, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, N.Y.). Other variants of these native nucleic acids may have one or more codon(s) inserted, deleted and/or
- 10 substituted as compared to native nucleic acid sequences. These sequence variants preferably lead to integration defective and/or hyperactive piggyBac transposon proteins having at least one amino acid substituted, deleted and/or inserted as compared to the native nucleic acid sequences of transposons. Therefore, nucleic acid sequences of the present invention may code for modified (non-natural) transposon
- 15 sequences. Further, promoters or other expression control regions can be operably linked with the nucleic acids encoding the proteins described herein to regulate expression of the protein in a quantitative or in a tissue-specific manner.

In a particular embodiment, the *Tichoplusia ni* wildtype piggyBac transposon comprises a nucleic acid sequence corresponding to SEQ ID NO: 1.

- 20 SEQ ID NO: 1

a transposon that integrates at a lower frequency into the host genome than a corresponding wild type transposon. In certain exemplary embodiments, the inventive transposons integrate by conventional integration mechanisms.

Integration defective piggyBac transposons, in certain exemplary
 5 embodiments, are derived from the wildtype piggyBac sequence, SEQ ID NO: 2. In exemplary embodiments, the integration defective piggyBac transposon comprises a change in SEQ ID NO: 2 selected from R372A or K375A.

In certain preferred embodiments, the integration defective piggyBac
 transposon comprises an amino acid sequence selected from SEQ ID NO: 64, SEQ
 10 ID NO: 65 or SEQ ID NO: 66.

In certain embodiments, the amino acid change in SEQ ID NO: 2 comprises R372A and corresponds to SEQ ID NO: 64.

SEQ ID NO: 64

MGSSLDDEHILSALLQSDDELVGEDSDSEISDHVSEDDVQSDTEEAFIDEVHE
 15 VQPTSSGSEILDEQNVIEQPGSSLASNRILTLPQRTIRGKNKHCWSTSKSTRRSR
 VSALNIVRSQRGPTRMCRNIYDPLLCFKLFFTDEIISEIVKWTNAEISLKRRESM
 TGATFRDTNEDEIYAFFGILVMTAVRKDNHMSTDDLFDRLSMVYVSVMMSRD
 RFDFLIRCLRMDDKSIRPTLRENDVFTVPRKIWDLFIHQCIQNYTPGAHLTIDE
 QLLGFRGRCPFRMYIPNKPSKYGIKILMMCDSGTKYMINGMPYLGRGTQTNG
 20 VPLGEYYVKELSKPVHGSCRNITCDNWFTSIPLAKNLLQEPYKLTIVGTVASN
 KREIPEVLKNSRSRPVGTSMFCFDGPLTLVSYKPKPAKMVYLLSSCEDEDASIN
 ESTGKPMVMYYNQTKGGVDTLQMCVMTCSRKTNRWPMALLYGMINIA
 CINSFIYSHNVSSKGEKVQSRKKFMRNLYMSLTSSFMRKRLEAPTLKRYLRD
 NISNILPNEVPGTSDDSTEPPVMKKRTYCTYCPSKIRRKANASCKKCKKVICRE
 25 HNIDMCQSCF

The integration defective variant encoded by SEQ ID NO: 64 corresponds to a nucleotide change of CGA to GCA in SEQ ID NO: 1, and corresponds to SEQ ID NO: 67.

In other certain embodiments, the amino acid change in SEQ ID NO: 2
 30 comprises K375A and corresponds to SEQ ID NO: 65.

SEQ ID NO: 65

MGSSLDDEHILSALLQSDDELVGEDSDSEISDHVSEDDVQSDTEEAFIDEVHE
 VQPTSSGSEILDEQNVEIQPGSSLASNRILTLPQRTIRGKNKHCWSTSKSTRRSR
 VSALNIVRSQRGPTRMCRNIYDPLLCFKLFFTDEIISEIVKWTNAEISLKRRESM
 5 TGATFRDTNEDEIYAFFGILVMTAVRKDNHMSTDDLDFDRSLSMVYVSVMSRD
 RFDFLIRCLRMDDKSIRPTLRENDVFTPVRKIWDLFIHQCIQNYTPGAHLTIDE
 QLLGFRGRCPFRMYIPNKPSKYGIKILMMCDSGTKYMINGMPYLGRTQTNG
 VPLGEYYVKELSKPVHGSCRNITCDNWFTSIPLAKNLLQEPYKLTIVGTVASN
 KREIPEVLKNSRSPVGTSMFCFDGPLTLVSYKPKPAKMVYLLSSCEDEDASIN
 10 ESTGKPMVMYYNQTKGGVDTLQMCVMTCRKTNRWPMALLYGMINIA
 CINSFIYSHNVSSKGEKVQSRKKFMRNLYMSLTSSFMRKRLEAPTLKRYLRD
 NISNILPNEVPGTSDDSTEELVMKKRTYCTYCPSKIRKANASCKKCKKVICRE
 HNIDMCQSCF

The integration defective variant encoded by SEQ ID NO: 65 corresponds to a
 15 nucleotide change of AAA to GCA in SEQ ID NO: 1, and corresponds to SEQ ID
 NO: 68.

In other certain embodiments, the amino acid change in SEQ ID NO: 2
 comprises R372A, K375A and corresponds to SEQ ID NO: 66.

20 SEQ ID NO: 66

MGSSLDDEHILSALLQSDDELVGEDSDSEISDHVSEDDVQSDTEEAFIDEVHE
 VQPTSSGSEILDEQNVEIQPGSSLASNRILTLPQRTIRGKNKHCWSTSKSTRRSR
 VSALNIVRSQRGPTRMCRNIYDPLLCFKLFFTDEIISEIVKWTNAEISLKRRESM
 TGATFRDTNEDEIYAFFGILVMTAVRKDNHMSTDDLDFDRSLSMVYVSVMSRD
 25 RFDFLIRCLRMDDKSIRPTLRENDVFTPVRKIWDLFIHQCIQNYTPGAHLTIDE
 QLLGFRGRCPFRMYIPNKPSKYGIKILMMCDSGTKYMINGMPYLGRTQTNG
 VPLGEYYVKELSKPVHGSCRNITCDNWFTSIPLAKNLLQEPYKLTIVGTVASN
 KREIPEVLKNSRSPVGTSMFCFDGPLTLVSYKPKPAKMVYLLSSCEDEDASIN
 ESTGKPMVMYYNQTKGGVDTLQMCVMTCRKTNRWPMALLYGMINIA
 30 CINSFIYSHNVSSKGEKVQSRKKFMRNLYMSLTSSFMRKRLEAPTLKRYLRD
 NISNILPNEVPGTSDDSTEELVMKKRTYCTYCPSKIRKANASCKKCKKVICRE
 HNIDMCQSCF

The integration defective variant encoded by SEQ ID NO: 66 corresponds to a nucleotide change of CGA to GCA/AAA to GCA in SEQ ID NO: 1, and corresponds to SEQ ID NO: 69.

As described herein, the present invention also features hyperactive piggyBac
5 transposons.

In certain preferred embodiments, the hyperactive piggyBac transposons are generated from the integration defective piggyBac variants. That is, alterations, preferably one or more mutations, are made in the integration defective piggyBac transposon sequence. In other embodiments, the hyperactive piggyBac transposons
10 are generated from the wildtype sequences. That is, alterations, preferably one or more mutations, are made in the wild type piggyBac transposon sequence.

In exemplary embodiments, the hyperactive piggyBac comprises an amino acid sequence selected from the group consisting of: SEQ ID NO: 3 – SEQ ID NO: 32.

15 In other exemplary embodiments, the hyperactive piggyBac comprises a nucleic acid sequence selected from the group consisting of: SEQ ID NO: 34 – SEQ ID NO: 63.

The hyperactive piggyBac can preferably comprise one or more nucleic acid sequences selected from the group consisting of: SEQ ID NO: 34 – SEQ ID NO: 63.
20 For example, the hyperactive piggyBac can preferably comprise 1, 2, 3, 4, 5 or more nucleic acid sequences selected from the group consisting of: SEQ ID NO: 34 – SEQ ID NO: 63.

In certain exemplary embodiments, the hyperactive variants comprise an amino acid change in SEQ ID NO: 2 selected from the group consisting of: L15P,
25 D19N/F395L, S31P/T164A, H33Y, E44K/K334R, E45G, C97R/T242I, S103P, R189K/G120G, R189R/D450N/R526R, M194T, M194V, S213S/V436I, I221T, S373P, N384T, 453S/N571S, T560A, N571S, S573A, S584P, M589V, M589V/D170D, S592G, F594L, Stop/WLESCN, Stop595ELESCN/H33H.

In certain exemplary embodiments, the hyperactive variants comprise an
30 amino acid change in SEQ ID NO: 64 or 65 selected from the group consisting of: L15P, D19N/F395L, S31P/T164A, H33Y, E44K/K334R, E45G, C97R/T242I, S103P, R189K/G120G, R189R/D450N/R526R, M194T, M194V, S213S/V436I, I221T,

S373P, N384T, 453S/N571S, T560A, N571S, S573A, S584P, M589V,
M589V/D170D, S592G, F594L, Stop/WLESCN, Stop595ELESCN/H33H.

In certain exemplary embodiments, the hyperactive variants comprise an amino acid change in SEQ ID NO: 2 selected from the group consisting of: G2C,
5 Q40R, S3N, S26P, I30V, G165S, T43A, Q55R, T57A, S61R, I82V, I90V, S103P, S103T, N113S, M185L, M194V, S230N, R281G, M282V, G316E, P410L, I426V, Q497L, K501N, K565I, N505D, S573L, S509G, N570S, N538K, K575R, Q591P, Q591R, F594L.

In certain embodiments, the amino acid change in SEQ ID NO: 64 or 65
10 comprises L15P and corresponds to SEQ ID NO: 3. The hyperactive variant encoded by SEQ ID NO: 3 corresponds to a nucleotide change of CUG to CCG in SEQ ID NO: 64 or 65, and corresponds to SEQ ID NO: 34 or 70.

In certain embodiments, the amino acid change in SEQ ID NO: 64 or 65
15 comprises D19N/F395L and corresponds to SEQ ID NO: 4. The hyperactive variant encoded by SEQ ID NO: 4 corresponds to a nucleotide change of GAC to AAC/UUU to CUU in SEQ ID NO: 1, and corresponds to SEQ ID NO: 35 or 71.

In certain embodiments, the amino acid change in SEQ ID NO: 64 or 65
20 comprises S31P/T164A and corresponds to SEQ ID NO: 5. The hyperactive variant encoded by SEQ ID NO: 5 corresponds to a nucleotide change of UCA to CCA/ACA to GCA in SEQ ID NO: 64 or 65 and corresponds to SEQ ID NO: 36 or 72.

In certain embodiments, the amino acid change in SEQ ID NO: 64 or 65
comprises H33Y and corresponds to SEQ ID NO: 6. The hyperactive variant encoded by SEQ ID NO: 6 corresponds to a nucleotide change of CAC to UAC in SEQ ID NO: 64 or 65, and corresponds to SEQ ID NO: 37 or 73.

25 In certain embodiments, the amino acid change in SEQ ID NO: 64 or 65 comprises E44K/K334R and corresponds to SEQ ID NO: 7. The hyperactive variant encoded by SEQ ID NO: 7 corresponds to a nucleotide change of GAA to AAA/AAG to AGG in SEQ ID NO: 1, and corresponds to SEQ ID NO: 38 or 74.

In certain embodiments, the amino acid change in SEQ ID NO: 64 or 65
30 comprises E45G and corresponds to SEQ ID NO: 8. The hyperactive variant encoded by SEQ ID NO: 8 corresponds to a nucleotide change of GAA to GGA in SEQ ID NO: 1, and corresponds to SEQ ID NO: 39 or 75.

In certain embodiments, the amino acid change in SEQ ID NO: 64 or 65 comprises C97R/T242I and corresponds to SEQ ID NO: 9. The hyperactive variant encoded by SEQ ID NO: 9 corresponds to a nucleotide change of UGU to CGU/ACU to AUU in SEQ ID NO: 1, and corresponds to SEQ ID NO: 40 or 76.

5 In certain embodiments, the amino acid change in SEQ ID NO: 64 or 65 comprises S103P and corresponds to SEQ ID NO: 10. The hyperactive variant encoded by SEQ ID NO: 10 corresponds to a nucleotide change of UCC to CCC in SEQ ID NO: 1, and corresponds to SEQ ID NO: 41 or 77.

10 In certain embodiments, the amino acid change in SEQ ID NO: 64 or 65 comprises R189K/G120G and corresponds to SEQ ID NO: 11. The hyperactive variant encoded by SEQ ID NO: 11 corresponds to a nucleotide change of AGA to AAA/GGU to GGC in SEQ ID NO: 1, and corresponds to SEQ ID NO: 42 or 78.

15 In certain embodiments, the amino acid change in SEQ ID NO: 64 or 65 comprises R189R/D450N/R526R and corresponds to SEQ ID NO: 12. The hyperactive variant encoded by SEQ ID NO: 12 corresponds to a nucleotide change of AGA to AGG/GAC to AAC in SEQ ID NO: 1, and corresponds to SEQ ID NO: 43 or 79.

20 In certain embodiments, the amino acid change in SEQ ID NO: 64 or 65 comprises M194T and corresponds to SEQ ID NO: 13. The hyperactive variant encoded by SEQ ID NO: 13 corresponds to a nucleotide change of AUG to ACG in SEQ ID NO: 1, and corresponds to SEQ ID NO: 44 or 80.

25 In certain embodiments, the amino acid change in SEQ ID NO: 64 or 65 comprises M194V and corresponds to SEQ ID NO: 14. The hyperactive variant encoded by SEQ ID NO: 14 corresponds to a nucleotide change of AUG to GUG in SEQ ID NO: 1, and corresponds to SEQ ID NO: 45 or 81.

In certain embodiments, the amino acid change in SEQ ID NO: 64 or 65 comprises S213S/V436I and corresponds to SEQ ID NO: 15. The hyperactive variant encoded by SEQ ID NO: 15 corresponds to a nucleotide change of AGU to AGC in SEQ ID NO: 1, and corresponds to SEQ ID NO: 46 or 82.

30 In certain embodiments, the amino acid change in SEQ ID NO: 64 or 65 comprises I221T and corresponds to SEQ ID NO: 16. The hyperactive variant

encoded by SEQ ID NO: 16 corresponds to a nucleotide change of AUA to ACA in SEQ ID NO: 1, and corresponds to SEQ ID NO: 47 or 83.

In certain embodiments, the amino acid change in SEQ ID NO: 64 or 65 comprises S373P between M6+ and corresponds to SEQ ID NO: 17. The hyperactive
5 variant encoded by SEQ ID NO: 17 corresponds to a nucleotide change of UCA to CCA in SEQ ID NO: 1, and corresponds to SEQ ID NO: 48 or 84.

In certain embodiments, the amino acid change in SEQ ID NO: 64 or 65 comprises N384T and corresponds to SEQ ID NO: 18. The hyperactive variant
10 encoded by SEQ ID NO: 18 corresponds to a nucleotide change of AAC to ACC in SEQ ID NO: 1, and corresponds to SEQ ID NO: 49 or 85.

In certain embodiments, the amino acid change in SEQ ID NO: 64 or 65 comprises C453S/N571S and corresponds to SEQ ID NO: 19. The hyperactive variant
15 encoded by SEQ ID NO: 19 corresponds to a nucleotide change of UGU to AGU/AAU to AGU in SEQ ID NO: 1, and corresponds to SEQ ID NO: 50 or 86.

In certain embodiments, the amino acid change in SEQ ID NO: 64 or 65 comprises T560A and corresponds to SEQ ID NO: 20. The hyperactive variant
20 encoded by SEQ ID NO: 20 corresponds to a nucleotide change of ACU to GCU in SEQ ID NO: 1, and corresponds to SEQ ID NO: 51 or 87.

In certain embodiments, the amino acid change in SEQ ID NO: 64 or 65
25 comprises N571S and corresponds to SEQ ID NO: 21. The hyperactive variant encoded by SEQ ID NO: 21 corresponds to a nucleotide change of AAU to AAG in SEQ ID NO: 1, and corresponds to SEQ ID NO: 52 or 88.

In certain embodiments, the amino acid change in SEQ ID NO: 64 or 65
30 comprises S573A and corresponds to SEQ ID NO: 22. The hyperactive variant encoded by SEQ ID NO: 22 corresponds to a nucleotide change of UCG to GCG in SEQ ID NO: 1, and corresponds to SEQ ID NO: 53 or 89.

In certain embodiments, the amino acid change in SEQ ID NO: 64 or 65
35 comprises S584P and corresponds to SEQ ID NO: 23. The hyperactive variant encoded by SEQ ID NO: 23 corresponds to a nucleotide change of UCU to CCU in SEQ ID NO: 1, and corresponds to SEQ ID NO: 54 or 90.

In certain embodiments, the amino acid change in SEQ ID NO: 64 or 65 comprises M598V and corresponds to SEQ ID NO: 24. The hyperactive variant encoded by SEQ ID NO: 24 corresponds to a nucleotide change of AUG to GUG in SEQ ID NO: 1, and corresponds to SEQ ID NO: 55 or 91.

5 In certain embodiments, the amino acid change in SEQ ID NO: 64 or 65 comprises M589V/D170D and corresponds to SEQ ID NO: 25. The hyperactive variant encoded by SEQ ID NO: 25 corresponds to a nucleotide change of ATG to GUG/GAC to GAU in SEQ ID NO: 1, and corresponds to SEQ ID NO: 56 or 92.

10 In certain embodiments, the amino acid change in SEQ ID NO: 64 or 65 comprises S592G and corresponds to SEQ ID NO: 26. The hyperactive variant encoded by SEQ ID NO: 26 corresponds to a nucleotide change of AGU to GGU in SEQ ID NO: 1, and corresponds to SEQ ID NO: 57 or 93.

15 In certain embodiments, the amino acid change in SEQ ID NO: 64 or 65 comprises F594L and corresponds to SEQ ID NO: 27. The hyperactive variant encoded by SEQ ID NO: 27 corresponds to a nucleotide change of UUC to TTA in SEQ ID NO: 1, and corresponds to SEQ ID NO: 58 or 94.

20 In certain embodiments, the amino acid change in SEQ ID NO: 64 or 65 comprises Stop/WLESCN and corresponds to SEQ ID NO: 28. The hyperactive variant encoded by SEQ ID NO: 28 corresponds to a nucleotide change of TGA to TGG in SEQ ID NO: 1, and corresponds to SEQ ID NO: 59 or 95.

25 In certain embodiments, the amino acid change in SEQ ID NO: 64 or 65 comprises Stop595ELESCN/H33H and corresponds to SEQ ID NO: 29. The hyperactive variant encoded by SEQ ID NO: 29 corresponds to a nucleotide change of TGA to GGA/CAC to CAU in SEQ ID NO: 1, and corresponds to SEQ ID NO: 60 or 96.

30 In another preferred embodiment the nucleic acid encoding the hyperactive piggyBac transposon is selected from a nucleic acid sequence encoding the hyperactive piggyBac transposon as defined above and being capable of hybridizing to a complement of a nucleic acid sequence as defined above under stringent conditions. In another preferred embodiment the nucleic acid encoding the hyperactive piggyBac transposon is selected from a nucleic acid sequence encoding the hyperactive piggyBac transposase as defined above and being capable of

hybridizing to a complement of a nucleic acid sequence as defined above under stringent conditions. Stringent conditions are, for example: 30% (v/v) formamide in 0.5*SSC, 0.1% (w/v) SDS at 42 C for 7 hours.

Assays for measuring the excision of a transposon from a vector, the
5 integration of a transposon into the genomic or extrachromosomal DNA of a cell, and the ability of transposase to bind to an inverted repeat are described herein and are known to the art (see, for instance, (Ivics et al. Cell, 91, 501-510 (1997); WO 98/40510 (Hackett et al.); WO 99/25817 (Hackett et al.), WO 00/68399 (McIvor et al.), incorporated by reference in their entireties herein. For purposes of determining
10 the frequency of transposition of a transposon of the present invention, the activity of the baseline transposon is normalized to 100%, and the relative activity of the transposon of the present invention determined. Preferably, a transposon of the present invention transposes at a frequency that is, in increasing order of preference, at least about 50%, at least about 100%, at least about 200%, most preferably, at least
15 about 300% greater than a baseline transposon. Preferably, both transposons (i.e., the baseline transposon and the transposon being tested) are flanked by the same nucleotide sequence in the vector containing the transposons.

The invention also features protein sequence showing at least 80%, preferably at least 85%, more preferably at least 90%, even more preferably at least 95% and
20 most preferably at least 98% sequence identity with the protein sequence of any one of SEQ ID NOs 3 – 32.

The invention also features protein sequence showing at least 80%, preferably at least 85%, more preferably at least 90%, even more preferably at least 95% and
25 most preferably at least 98% sequence identity with the protein sequence of any one of SEQ ID NOs 3 - 32.

The term "identity" is understood as the degree of identity between two or more proteins, nucleic acids, etc., which may be determined by comparing these sequences using known methods such as computer based sequence alignments (basic local alignment search tool, S. F. Altschul et al., J. Mol. Biol. 215 (1990), 403-410).
30 Such methods include without being limited thereto the GAG programme, including GAP (Devereux, J., et al., Nucleic Acids Research 12 (12): 287 (1984); Genetics Computer Group University of Wisconsin, Madison, (WI)); BLASTP or BLASTN,

and FASTA (Altschul, S., et al., J. Mol. Biol. 215:403-410) (1999)). Additionally, the Smith Waterman-algorithm may be used to determine the degree of identity between two sequences.

Functional derivatives according to the present invention preferably maintain the biological function of the mammalian transposase, i.e. the transposase activity, the excision of the nucleic acid sequence and its insertion activity concerning the excised sequences into specific target sequences. Functional derivatives according to the present invention may comprise one or more amino acid insertion(s), deletion(s) and/or substitution(s) of the hyperactive variants as described herein, for example, as those corresponding to SEQ ID NOs 3 - 32.

Amino acid substitutions as described herein are preferably conservative amino acid substitutions, which do not alter the biological activity of the transposon or transposase protein. Conservative amino acid substitutions are characterized in that an amino acid belonging to a group of amino acids having a particular size or characteristic can be substituted for another amino acid, particularly in regions of the inventive protein that are not associated with catalytic activity or DNA binding activity, for example. Other amino acid sequences may include, for example, amino acid sequences containing conservative changes that do not significantly alter the activity or binding characteristics of the resulting transposase. Substitutions for an amino acid sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and tyrosine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Such alterations are not expected to substantially affect apparent molecular weight as determined by polyacrylamide gel electrophoresis or isoelectric point. Particularly preferred conservative substitutions include, but are not limited to, Lys for Arg and vice versa to maintain a positive charge; Glu for Asp and vice versa to maintain a negative charge; Ser for Thr so that a free --OH is maintained; and Gln for Asn to maintain a free NH₂.

Amino acid insertions and substitutions are preferably carried out at those sequence positions of that do not alter the spatial structure or which relate to the

catalytic center or binding region of the piggyBac transposon or transposase. A change of a spatial structure by insertion(s) or deletion(s) can be detected readily with the aid of, for example, CD spectra (circular dichroism spectra) (Urry, 1985, Absorption, circular Dichroism and ORD of Polypeptides, in: Modern Physical
5 Methods in Biochemistry, Neuberger et al. (Ed.), Elsevier, Amsterdam). Suitable methods for generating proteins with amino acid sequences which contain substitutions in comparison with the native sequence(s) are disclosed for example in the publications U.S. Pat. No. 4,737,462, U.S. Pat. No. 4,588,585, U.S. Pat. No. 4,959,314, U.S. Pat. No. 5,116,943, U.S. Pat. No. 4,879,111 and U.S. Pat. No.
10 5,017,691, incorporated by reference in their entireties herein. Other functional derivatives may be additionally stabilized in order to avoid physiological degradation. Such stabilization may be obtained by stabilizing the protein backbone by a substitution of by stabilizing the protein backbone by substitution of the amide-type bond, for example also by employing [beta]-amino acids.

15 According to certain preferred embodiments of the present invention, the transposon of the present invention may further comprise a marker protein. For example, in certain preferred embodiments, the nucleic acid sequence can be of any variety of recombinant proteins, e.g. any protein known in the art. e.g. the protein encoded by the nucleic acid sequence can be a marker protein such as green
20 fluorescent protein (GFP), the blue fluorescent protein (BFP), the photo activatable-GFP (PA-GFP), the yellow shifted green fluorescent protein (Yellow GFP), the yellow fluorescent protein (YFP), the enhanced yellow fluorescent protein (EYFP), the cyan fluorescent protein (CFP), the enhanced cyan fluorescent protein (ECFP), the monomeric red fluorescent protein (mRFP1), the kindling fluorescent protein (KFP1),
25 aequorin, the autofluorescent proteins (AFPs), or the fluorescent proteins JRed, TurboGFP, PhiYFP and PhiYFP-m, tHc-Red (HcRed-Tandem), PS-CFP2 and KFP-Red (all available commercially available), or other suitable fluorescent proteins chloramphenicol acetyltransferase (CAT). The protein further may be selected from growth hormones, for example to promote growth in a transgenic animal, or from
30 beta-galactosidase (lacZ), luciferase (LUC), and insulin-like growth factors (IGFs), alpha-anti-trypsin, erythropoietin (EPO), factors VIII and XI of the blood clotting system, LDL-receptor, GATA-1, etc. The nucleic acid sequence further may be a suicide gene encoding e.g. apoptotic or apoptose related enzymes and genes including

Alf, Apaf e.g. Apaf-1, Apaf-2, Apaf-3, or APO-2 (L), APO-3 (L), Apopain, Bad, Bak, Bax, Bcl-2, Bcl-x.sub.L, Bcl-x.sub.S, bik, CAD, Calpain, Caspases e.g. Caspase-1, Caspase-2, Caspase-3, Caspase-4, Caspase-5, Caspase-6, Caspase-7, Caspase-8, Caspase-9, Caspase-10, Caspase-11, or Granzyme B, ced-3, ced-9, Ceramide, c-Jun, c-Myc, CPP32, crm A, Cytochrome c, D4-GDP-DI, Daxx, CdR1, DcR1, DD, DED, DISC, DNA-PK.sub.CS, DR3, DR4, DR5, FADD/MORT-1, FAK, Fas, Fas-ligand CD95/fas (receptor), FLICE/MACH, FLIP, Fodrin, fos, G-Actin, Gas-2, Gelsolin, glucocorticoid/glucocorticoid receptor, granzyme A/B, hnRNPs C1/C2, ICAD, ICE, JNK, Lamin A/B, MAP, MCL-1, Mdm-2, MEKK-1, MORT-1, NEDD, NF-.sub.kappa.B, NuMa, p53, PAK-2, PARP, Perforin, PITSLRE, PKC.delta., pRb, Presenilin, prICE, RAIDD, Ras, RIP, Sphingomyelinase, SREBPs, thymidine kinase from Herpes simplex, TNF-.alpha., TNF-alpha receptor, TRADD, TRAF2, TRAIL-R1, TRAIL-R2, TRAIL-R3, Transglutaminase, U1 70 kDa snRNP, YAMA, etc.

The inventive piggyBac transposons, preferably in combination with a piggyBac transposase, has several advantages compared to approaches in the prior art, e.g. with respect to viral and retroviral methods. For example, unlike proviral insertions, transposon insertions can be (re)mobilized by supplying the transposase activity in trans. Thus, for example, instead of performing time-consuming microinjections, it is possible according to the present invention to generate transposon insertions at new loci.

The inventive piggyBac transposons, in combination with transposase proteins as defined above can be transfected into a cell as a protein or as ribonucleic acid, including mRNA, as DNA, e.g. as extrachromosomal DNA including, but not limited to, episomal DNA, as plasmid DNA, or as viral nucleic acid. Furthermore, the nucleic acid encoding the transposase protein can be transfected into a cell as a nucleic acid vector such as a plasmid, or as a gene expression vector, including a viral vector. Therefore, the nucleic acid can be circular or linear. A vector, as used herein, refers to a plasmid, a viral vector or a cosmid that can incorporate nucleic acid encoding the transposase protein or the transposon of this invention. The terms "coding sequence" or "open reading frame" refer to a region of nucleic acid that can be transcribed and/or translated into a polypeptide in vivo when placed under the control of the appropriate regulatory sequences.

DNA encoding the transposase protein can be stably inserted into the genome of the cell or into a vector for constitutive or inducible expression. Where the transposase protein is transfected into the cell or inserted into the vector as nucleic acid, the transposase encoding sequence is preferably operably linked to a promoter.

- 5 There are a variety of promoters that could be used including, but not limited to, constitutive promoters, tissue-specific promoters, inducible promoters, and the like. Promoters are regulatory signals that bind RNA polymerase in a cell to initiate transcription of a downstream (3' direction) coding sequence. A DNA sequence is operably linked to an expression-control sequence, such as a promoter when the
- 10 expression control sequence controls and regulates the transcription and translation of that DNA sequence. The term "operably linked" includes having an appropriate start signal (e.g., ATG) in front of the DNA sequence to be expressed and maintaining the correct reading frame to permit expression of the DNA sequence under the control of the expression control sequence to yield production of the desired protein product.
- 15 Exemplary nucleic acid sequences encoding the hyperactive piggyBac transposon are provided as SEQ ID NO: 3 – SEQ ID NO: 32 or other hyperactive variants as described herein. In addition to the conservative changes discussed above that would necessarily alter the transposon-encoding nucleic acid sequence (all of which are disclosed herein as well), there are other DNA or RNA sequences encoding the
- 20 hyperactive piggyBac transposon protein. These DNA or RNA sequences have the same amino acid sequence as a hyperactive piggyBac transposon protein, but take advantage of the degeneracy of the three letter codons used to specify a particular amino acid. For example, it is well known in the art that various specific RNA codons (corresponding DNA codons, with a T substituted for a U) can be used
- 25 interchangeably to code for specific amino acids.

Methods for manipulating DNA and proteins are known in the art and are explained in detail in the literature such as Sambrook et al, (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press or Ausubel, R. M., ed. (1994). *Current Protocols in Molecular Biology*.

30

Gene Transfer System

The present invention also features a gene transfer system comprising an inventive transposon, an integration defective transposon or a hyperactive piggyBac transposon as described herein, and a piggyBac transposase as described herein.

As mentioned above, the piggyBac transposase protein preferably recognizes repeats (e.g. IRs) on the hyperactive piggyBac transposon. The gene transfer system of this invention, therefore, preferably comprises two components: the transposase as described herein and a hyperactive transposon or integration defective transposon as described herein. Preferably, in certain embodiments, the transposon has at least two repeats (e.g. IRs). When put together these two components provide active transposon activity and allow the transposon to be relocated. In use, the transposase binds to the repeats and promotes insertion of the intervening nucleic acid sequence into DNA of a cell as defined below.

In further exemplary embodiments, the gene transfer system comprises an inventive piggyBac transposon as defined above in combination with a piggyBac transposase protein (or nucleic acid encoding a piggyBac transposase protein to provide its activity in a cell). This combination preferably results in the insertion of the nucleic acid sequence into the DNA of the cell. Alternatively, it is possible to insert the transposon of the present invention into DNA of a cell through non-homologous recombination through a variety of reproducible mechanisms. In either event the inventive transposon can be used for gene transfer by using this gene transfer system.

In certain preferred embodiments, the gene transfer system mediates insertion of the hyperactive piggyBac transposon into the DNA of a variety of cell types and a variety of species by using the piggyBac transposase protein. Preferably, such cells include any cell suitable in the present context, including but not limited to animal cells or cells from bacteria, fungi (e.g., yeast, etc.) or plants. Preferred animal cells can be vertebrate or invertebrate. For example, preferred vertebrate cells include cells from mammals including, but not limited to, rodents, such as rats or mice, ungulates, such as cows or goats, sheep, swine or cells from a human.

In other further exemplary embodiments, such cells, particularly cells derived from a mammals as defined above, can be pluripotent (i.e., a cell whose descendants can differentiate into several restricted cell types, such as hematopoietic stem cells or

other stem cells) and totipotent cells (i.e., a cell whose descendants can become any cell type in an organism, e.g., embryonic stem cells). These cells are advantageously used in order to affirm stable expression of the transposase or to obtain a multiple number of cells already transfected with the components of the inventive gene transfer system. Additionally, cells such as oocytes, eggs, and one or more cells of an embryo may also be considered as targets for stable transfection with the present gene transfer system.

In certain preferred embodiments of the invention, the cells are stem cells.

Cells receiving the inventive piggyBac transposon and/or the piggyBac transposase protein and capable of inserting the transposon into the DNA of that cell also include without being limited thereto, lymphocytes, hepatocytes, neural cells, muscle cells, a variety of blood cells, and a variety of cells of an organism, embryonic stem cells, somatic stem cells e.g. hematopoietic cells, embryos, zygotes, sperm cells (some of which are open to be manipulated by an in vitro setting).

In other certain exemplary embodiments, the cell DNA that acts as a recipient of the transposon of described herein includes any DNA present in a cell (as mentioned above) to be transfected, if the inventive piggyBac transposon, e.g. the hyperactive piggyBac transposon, is in contact with an piggyBac transposase protein within the cell. For example, the DNA can be part of the cell genome or it can be extrachromosomal, such as an episome, a plasmid, a circular or linear DNA fragment. Typical targets for insertion are e.g. double-stranded DNA.

The components of the gene transfer system described herein, i.e. the piggyBac transposase protein (either as a protein or encoded by a nucleic acid as described herein) and an inventive piggyBac transposon can be transfected into a cell, preferably into a cell as defined above, and more preferably into the same cell. Transfection of these components may furthermore occur in subsequent order or in parallel. E.g. the piggyBac transposase protein or its encoding nucleic acid may be transfected into a cell as defined above prior to, simultaneously with or subsequent to transfection of the mammalian piggyBac transposon. Alternatively, the transposon may be transfected into a cell as defined above prior to, simultaneously with or subsequent to transfection of the piggyBac transposase protein or its encoding nucleic acid. If transfected parallel, preferably both components are provided in a separated

formulation and/or mixed with each other directly prior to administration in order to avoid transposition prior to transfection. Additionally, administration of at least one component of the gene transfer system may occur repeatedly, e.g. by administering at least one, two or multiple doses of this component.

5 For any of the above transfection reactions, the gene transfer system may be formulated in a suitable manner as known in the art, or as a pharmaceutical composition or kit as described herein.

In further preferred embodiments, the components of the gene transfer system may preferably be transfected into one or more cells by techniques such as particle
10 bombardment, electroporation, microinjection, combining the components with lipid-containing vesicles, such as cationic lipid vesicles, DNA condensing reagents (e.g., calcium phosphate, polylysine or polyethyleneimine), and inserting the components (i.e. the nucleic acids thereof into a viral vector and contacting the viral vector with the cell. Where a viral vector is used, the viral vector can include any of a variety of
15 viral vectors known in the art including viral vectors selected from the group consisting of a retroviral vector, an adenovirus vector or an adeno-associated viral vector.

As already mentioned above the nucleic acid encoding the piggyBac transposase protein may be RNA or DNA. Similarly, either the nucleic acid encoding
20 the piggyBac transposase protein or the transposon of this invention can be transfected into the cell as a linear fragment or as a circularized fragment, preferably as a plasmid or as recombinant viral DNA.

Furthermore, the nucleic acid encoding the piggyBac transposase protein is thereby preferably stably or transiently inserted into the genome of the cell to
25 facilitate temporary or prolonged expression of the piggyBac transposase protein in the cell.

The gene transfer system as disclosed above represents a considerable refinement of non-viral DNA-mediated gene transfer. For example, adapting viruses as agents for gene therapy restricts genetic design to the constraints of that virus
30 genome in terms of size, structure and regulation of expression. Non-viral vectors, as described herein, are generated largely from synthetic starting materials and are therefore more easily manufactured than viral vectors. Non-viral reagents are less

likely to be immunogenic than viral agents making repeat administration possible. Non-viral vectors are more stable than viral vectors and therefore better suited for pharmaceutical formulation and application than are viral vectors. Additionally, the inventive gene transfer system is a non-viral gene transfer system that facilitates
5 insertion into DNA and markedly improves the frequency of stable gene transfer.

The present invention further provides an efficient method for producing transgenic animals, including the step of applying the inventive gene transfer system to an animal. Transgenic DNA has not been efficiently inserted into chromosomes. Only about one in a million of the foreign DNA molecules is inserted into the cellular
10 genome, generally several cleavage cycles into development. Consequently, most transgenic animals are mosaic (Hackett et al. (1993). The molecular biology of transgenic fish. In *Biochemistry and Molecular Biology of Fishes* (Hochachka & Mommsen, eds) Vol. 2, pp. 207-240). As a result, animals raised from embryos into which transgenic DNA has been delivered must be cultured until gametes can be
15 assayed for the presence of inserted foreign DNA. Many transgenic animals fail to express the transgene due to position effects. A simple, reliable procedure that directs early insertion of exogenous DNA into the chromosomes of animals at the one-cell stage is needed. The present system helps to fill this need.

In certain preferred embodiments, the gene transfer system of this invention
20 can readily be used to produce transgenic animals that carry a particular marker or express a particular protein in one or more cells of the animal. Generally, methods for producing transgenic animals are known in the art and incorporation of the inventive gene transfer system into these techniques does not require undue experimentation, e.g. there are a variety of methods for producing transgenic animals for research or for
25 protein production including, but not limited to Hackett et al. (1993, supra). Other methods for producing transgenic animals are described in the art (e.g. M. Markkula et al. *Rev. Reprod.*, 1, 97-106 (1996); R. T. Wall et al., *J. Dairy Sci.*, 80, 2213-2224 (1997)), J. C. Dalton, et al. (*Adv. Exp. Med. Biol.*, 411, 419-428 (1997)) and H. Lubon et al. (*Transfus. Med. Rev.*, 10, 131-143 (1996)).

30 In another embodiment, the present invention features a transgenic animal produced by the methods described herein, preferably by using the gene transfer system presently described. For example, transgenic animals may preferably contain a nucleic acid sequence inserted into the genome of the animal by the gene transfer

system, thereby enabling the transgenic animal to produce its gene product, e.g. a protein. In transgenic animals this protein is preferably a product for isolation from a cell, for example the inventive protein can be produced in quantity in milk, urine, blood or eggs. Promoters can be used that promote expression in milk, urine, blood or eggs and these promoters include, but are not limited to, casein promoter, the mouse urinary protein promoter, beta-globin promoter and the ovalbumin promoter respectively. Recombinant growth hormone, recombinant insulin, and a variety of other recombinant proteins have been produced using other methods for producing protein in a cell. Nucleic acids encoding these or other proteins can be inserted into the transposon of this invention and transfected into a cell. Efficient transfection of the inventive transposon as defined above into the DNA of a cell occurs when mammalian piggyBac transposase protein is present. Where the cell is part of a tissue or part of a transgenic animal, large amounts of recombinant protein can be obtained.

Transgenic animals may be selected from vertebrates and invertebrates, e.g. fish, birds, mammals including, but not limited to, rodents, such as rats or mice, ungulates, such as cows or goats, sheep, swine or humans.

The present invention furthermore provides a method for gene therapy comprising the step of introducing the gene transfer system into cells as described herein. Therefore, the inventive piggyBac transposons as described herein preferably comprises a gene to provide a gene therapy to a cell or an organism. Preferably, the gene is placed under the control of a tissue specific promoter or of a ubiquitous promoter or one or more other expression control regions for the expression of a gene in a cell in need of that gene. Presently, a variety of genes are being tested for a variety of gene therapies including, but not limited to, the CFTR gene for cystic fibrosis, adenosine deaminase (ADA) for immune system disorders, factor IX and interleukin-2 (IL-2) for blood cell diseases, alpha-1-antitrypsin for lung disease, and tumor necrosis factors (TNFs) and multiple drug resistance (MDR) proteins for cancer therapies. These and a variety of human or animal specific gene sequences including gene sequences to encode marker proteins and a variety of recombinant proteins are available in the known gene databases such as GenBank.

An advantage of the inventive gene transfer system for gene therapy purposes is that it is not limited to a great extent by the size of the intervening nucleic acid sequence positioned between the repeats. There is no known limit on the size of the

nucleic acid sequence that can be inserted into DNA of a cell using the mammalian piggyBac transposase protein.

In particular preferred embodiments, for gene therapy purposes, but also for other inventive purposes the gene transfer system may be transfected into cells by a variety of methods, e.g. by microinjection, lipid-mediated strategies or by viral-mediated strategies. For example, where microinjection is used, there is very little restraint on the size of the intervening sequence of the transposon of this invention. Similarly, lipid-mediated strategies do not have substantial size limitations. However, other strategies for introducing the gene transfer system into a cell, such as viral-mediated strategies could limit the length of the nucleic acid sequence positioned between the repeats.

Accordingly, in certain exemplary embodiments, the gene transfer system as described herein can be delivered to cells via viruses, including retroviruses (such as lentiviruses, etc.), adenoviruses, adeno-associated viruses, herpes viruses, and others. There are several potential combinations of delivery mechanisms that are possible for the hyperactive piggyBac transposon portion containing the transgene of interest flanked by the terminal repeats and the gene encoding the transposase. For example, both the transposon and the transposase gene can be contained together on the same recombinant viral genome; a single infection delivers both parts of the gene transfer system such that expression of the transposase then directs cleavage of the transposon from the recombinant viral genome for subsequent insertion into a cellular chromosome. In another example, the transposase and the transposon can be delivered separately by a combination of viruses and/or non-viral systems such as lipid-containing reagents. In these cases either the transposon and/or the transposase gene can be delivered by a recombinant virus. In every case, the expressed transposase gene directs liberation of the transposon from its carrier DNA (viral genome) for insertion into chromosomal DNA.

In certain preferred embodiments of the present invention, inventive piggyBac transposons may be utilized for insertional mutagenesis, preferably followed by identification of the mutated gene. DNA transposons, particularly the transposons, have several advantages compared to approaches in the prior art, e.g. with respect to viral and retroviral methods. For example, unlike proviral insertions, transposon insertions can be remobilized by supplying the transposase activity in trans. Thus,

instead of performing time-consuming microinjections, it is possible according to the present invention to generate transposon insertions at new loci by crossing stocks transgenic for the above mentioned two components of the transposon system, the inventive transposon and the inventive transposase. In a preferred embodiment the gene transfer system is directed to the germline of the experimental animals in order to mutagenize germ cells. Alternatively, transposase expression can be directed to particular tissues or organs by using a variety of specific promoters. In addition, remobilization of a mutagenic transposon out of its insertion site can be used to isolate revertants and, if transposon excision is associated with a deletion of flanking DNA, the inventive gene transfer system may be used to generate deletion mutations. Furthermore, since transposons are composed of DNA, and can be maintained in simple plasmids, inventive transposons and particularly the use of the inventive gene transfer system is much safer and easier to work with than highly infectious retroviruses. The transposase activity can be supplied in the form of DNA, mRNA or protein as defined above in the desired experimental phase.

In another embodiment, the present invention also provides an efficient system for gene discovery, e.g. genome mapping, by introducing an inventive piggyBac transposon, as defined above into a gene using a gene transfer system as described in the present invention. In one example, the hyperactive piggyBac transposon in combination with the piggyBac transposase protein or a nucleic acid encoding the piggyBac transposase protein is transfected into a cell. In certain preferred embodiments, the transposon preferably comprises a nucleic acid sequence positioned between at least two repeats, wherein the repeats bind to the piggyBac transposase protein and wherein the transposon is inserted into the DNA of the cell in the presence of the piggyBac transposase protein. In certain preferred embodiments, the nucleic acid sequence includes a marker protein, such as GFP and a restriction endonuclease recognition site. Following insertion, the cell DNA is isolated and digested with the restriction endonuclease. For example, if the endonuclease recognition site is a 6-base recognition site and a restriction endonuclease is used that employs a 6-base recognition sequence, the cell DNA is cut into about 4000-bp fragments on average. These fragments can be either cloned or linkers can be added to the ends of the digested fragments to provide complementary sequence for PCR primers. Where linkers are added, PCR reactions are used to amplify fragments using primers from

the linkers and primers binding to the direct repeats of the repeats in the transposon. The amplified fragments are then sequenced and the DNA flanking the direct repeats is used to search computer databases such as GenBank.

5 Using the gene transfer system for methods as disclosed above such as gene discovery and/or gene tagging, permits, for example, identification, isolation, and characterization of genes involved with growth and development through the use of transposons as insertional mutagens or identification, isolation and characterization of transcriptional regulatory sequences controlling growth and development.

10 In another exemplary embodiment of the present invention, the invention provides a method for mobilizing a nucleic acid sequence in a cell. According to this method the hyperactive piggyBac transposon is inserted into DNA of a cell, as described herein. Hyperactive piggyBac protein or nucleic acid encoding the piggyBac transposase protein is transfected into the cell and the protein is able to mobilize (i.e. move) the transposon from a first position within the DNA of the cell to
15 a second position within the DNA of the cell. The DNA of the cell is preferably genomic DNA or extrachromosomal DNA. The inventive method allows movement of the transposon from one location in the genome to another location in the genome, or for example, from a plasmid in a cell to the genome of that cell.

In another exemplary embodiments, the inventive gene transfer system can
20 also be used as part of a method involving RNA-interference techniques. RNA interference (RNAi), is a technique in which exogenous, double-stranded RNAs (dsRNAs), being complementary to mRNA's or genes/gene fragments of the cell, are introduced into this cell to specifically bind to a particular mRNA and/or a gene and thereby diminishing or abolishing gene expression. The technique has proven
25 effective in *Drosophila*, *Caenorhabditis elegans*, plants, and recently, in mammalian cell cultures. In order to apply this technique in context with the present invention, the inventive transposon preferably contains short hairpin expression cassettes encoding small interfering RNAs (siRNAs), which are complementary to mRNA's and/or genes/gene fragments of the cell. These siRNAs have preferably a length of 20 to 30
30 nucleic acids, more preferably a length of 20 to 25 nucleic acids and most preferably a length of 21 to 23 nucleic acids. The siRNA may be directed to any mRNA and/or a gene, that encodes any protein as defined above, e.g. an oncogene. This use, particularly the use of mammalian piggyBac transposons for integration of siRNA

vectors into the host genome provides a long-term expression of siRNA in vitro or in vivo and thus enables a long-term silencing of specific gene products.

Induced Pluripotent Stem cells (iPS)

5 In certain preferred embodiments, the present invention may include a reprogramming vector that includes a polycistronic expression cassette comprising a transcriptional regulatory element, one or more reprogramming factors, and one or more hyperactive piggyBac transposons as described herein. Preferably, the reprogramming factor encoded is Sox, Oct, Nanog, Klf4, or c-Myc

10 In general, stem cells are undifferentiated cells which can give rise to a succession of mature functional cells. For example, a hematopoietic stem cell may give rise to any of the different types of terminally differentiated blood cells. Embryonic stem (ES) cells are derived from the embryo and are pluripotent, thus possessing the capability of developing into any organ or tissue type or, at least
15 potentially, into a complete embryo.

 Induced pluripotent stem cells, commonly abbreviated as iPS cells or iPSCs, are a type of pluripotent stem cells artificially derived from non-pluripotent cells, typically adult somatic cells, by inserting certain genes. Induced pluripotent stem cells are believed to be identical to natural pluripotent stem cells, such as embryonic stem
20 cells in many respects, for example, in the expression of certain stem cell genes and proteins, chromatin methylation patterns, doubling time, embryoid body formation, teratoma formation, viable chimera formation, and potency and differentiability, but the full extent of their relation to natural pluripotent stem cells is still being assessed.

 iPS cells were first produced in 2006 (Takahashi et al., 2006, incorporated by
25 reference in its entirety herein) from mouse cells and in 2007 from human cells (Takahashi et al., 2007, incorporated by reference in its entirety herein). This has been cited as an important advancement in stem cell research, as it may allow researchers to obtain pluripotent stem cells, which are important in research and potentially have therapeutic uses, without the controversial use of embryos.

30 "Reprogramming" is a process that confers on a cell a measurably increased capacity to form progeny of at least one new cell type, either in culture or in vivo, than it would have under the same conditions without reprogramming. More specifically, reprogramming is a process that confers on a somatic cell a pluripotent

potential. This means that after sufficient proliferation, a measurable proportion of progeny having phenotypic characteristics of the new cell type if essentially no such progeny could form before reprogramming; otherwise, the proportion having characteristics of the new cell type is measurably more than before reprogramming.

5 Under certain conditions, the proportion of progeny with characteristics of the new cell type may be at least about 1%, 5%, 25% or more in the in order of increasing preference.

Embryonic stem (ES) cells" are pluripotent stem cells derived from early embryos. An ES cell was first established in 1981, which has also been applied to
10 production of knockout mice since 1989. In 1998, a human ES cell was established, which is currently becoming available for regenerative medicine.

Unlike ES cells, tissue stem cells have a limited differentiation potential. Tissue stem cells are present at particular locations in tissues and have an undifferentiated intracellular structure. Therefore, the pluripotency of tissue stem cells
15 is typically low. Tissue stem cells have a higher nucleus/cytoplasm ratio and have few intracellular organelles. Most tissue stem cells have low pluripotency, a long cell cycle, and proliferative ability beyond the life of the individual. Tissue stem cells are separated into categories, based on the sites from which the cells are derived, such as the dermal system, the digestive system, the bone marrow system, the nervous system,
20 and the like. Tissue stem cells in the dermal system include epidermal stem cells, hair follicle stem cells, and the like. Tissue stem cells in the digestive system include pancreatic (common) stem cells, liver stem cells, and the like. Tissue stem cells in the bone marrow system include hematopoietic stem cells, mesenchymal stem cells, and the like. Tissue stem cells in the nervous system include neural stem cells, retinal stem
25 cells, and the like.

"Induced pluripotent stem cells," commonly abbreviated as iPS cells or iPSCs, refer to a type of pluripotent stem cell artificially prepared from a non-pluripotent cell, typically an adult somatic cell, or terminally differentiated cell, such as fibroblast, a hematopoietic cell, a myocyte, a neuron, an epidermal cell, or the like, by inserting
30 certain genes, referred to as reprogramming factors.

The generation of iPS cells is crucial on the genes used for the induction. The following factors or combination thereof could be used in the present invention. In certain aspects, nucleic acids encoding Sox and Oct (preferably Oct3/4) will be included into the reprogramming vector. For example, a reprogramming vector may

comprise expression cassettes encoding Sox2, Oct4, Nanog and optionally Lin-28, or expression cassettes encoding Sox2, Oct4, Klf4 and optionally c-myc. Nucleic acids encoding these reprogramming factors may be comprised in the same expression cassette, different expression cassettes, the same reprogramming vector, or different
5 reprogramming vectors.

Oct-3/4 and certain members of the Sox gene family (Sox1, Sox2, Sox3, and Sox15) have been identified as crucial transcriptional regulators involved in the induction process whose absence makes induction impossible. Additional genes, however, including certain members of the Klf family (Klf1, Klf2, Klf4, and Klf5),
10 the Myc family (C-myc, L-myc, and N-myc), Nanog, and LIN28, have been identified to increase the induction efficiency. Oct-3/4 (Pou5fl) is one of the family of octamer ("Oct") transcription factors, and plays a crucial role in maintaining pluripotency. The absence of Oct-3/4 in Oct-3/4+ cells, such as blastomeres and embryonic stem cells, leads to spontaneous trophoblast differentiation, and presence of Oct-3/4 thus gives
15 rise to the pluripotency and differentiation potential of embryonic stem cells. Various other genes in the "Oct" family, including Oct-3/4's close relatives, Oct1 and Oct6, fail to elicit induction, thus demonstrating the exclusiveness of Oct-3/4 to the induction process.

The Sox family of genes is associated with maintaining pluripotency similar to
20 Oct-3/4, although it is associated with multipotent and unipotent stem cells in contrast with Oct-3/4, which is exclusively expressed in pluripotent stem cells. While Sox2 was the initial gene used for induction by Yamanaka et al. (2007), Jaenisch et al. (1988) and Yu et al. (2007), other genes in the Sox family have been found to work as well in the induction process. Sox1 yields iPS cells with a similar efficiency as Sox2,
25 and genes Sox3, Sox15, and Sox18 also generate iPS cells, although with decreased efficiency.

In embryonic stem cells, at least an Oct member such as Oct-3/4 and at least a Sox member such as Sox2, are necessary in promoting pluripotency. Yamanaka et al. (2007) reported that Nanog was unnecessary for induction although Yu et al. (2007)
30 has reported it is possible to generate iPS cells with Nanog as one of the factors and Nanog certainly enhances reprogramming efficiency dose-dependently.

Klf4 of the Klf family of genes was initially identified by Yamanaka et al. and confirmed by Jaenisch et al. (1988) as a factor for the generation of mouse iPS cells and was demonstrated by Yamanaka et al. (2007) as a factor for generation of human

iPS cells. However, Thompson et al. reported that Klf4 was unnecessary for generation of human iPS cells and in fact failed to generate human iPS cells. Klf2 and Klf4 were found to be factors capable of generating iPS cells, and related genes Klf1 and Klf5 did as well, although with reduced efficiency.

5 The Myc family of genes are proto-oncogenes implicated in cancer. Yamanaka et al. and Jaenisch et al. (1988) demonstrated that c-myc is a factor implicated in the generation of mouse iPS cells and Yamanaka et al. demonstrated it was a factor implicated in the generation of human iPS cells. However, Thomson et al. and Yamanaka et al. (2007) reported that c-myc was unnecessary for generation of
10 human iPS cells. Usage of the "myc" family of genes in induction of iPS cells is troubling for the eventuality of iPS cells as clinical therapies, as 25% of mice transplanted with c-myc-induced iPS cells developed lethal teratomas. N-myc and L-myc have been identified to induce in the stead of c-myc with similar efficiency.

15 **Pharmaceutical Compositions**

The present invention further refers to pharmaceutical compositions containing either a piggyBac transposase as a protein or encoded by a nucleic acid, and/or a hyperactive piggyBac transposon, or a gene transfer system as described herein comprising a piggyBac transposase as a protein or encoded by a nucleic acid,
20 in combination with a hyperactive piggyBac transposon.

The pharmaceutical composition may optionally be provided together with a pharmaceutically acceptable carrier, adjuvant or vehicle. In this context, a pharmaceutically acceptable carrier, adjuvant, or vehicle according to the invention refers to a non-toxic carrier, adjuvant or vehicle that does not destroy the
25 pharmacological activity of the component(s) with which it is formulated. Pharmaceutically acceptable carriers, adjuvants or vehicles that may be used in the compositions of this invention include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial
30 glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium

carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

The pharmaceutical compositions of the present invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally,
5 buccally, vaginally or via an implanted reservoir.

The term parenteral as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques. Preferably, the pharmaceutical compositions are administered orally, intraperitoneally or
10 intravenously. Sterile injectable forms of the pharmaceutical compositions of this invention may be aqueous or oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent
15 or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium.

For this purpose, any bland fixed oil may be employed including synthetic
20 mono- or di-glycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as carboxymethyl cellulose or similar dispersing agents that are
25 commonly used in the formulation of pharmaceutically acceptable dosage forms including emulsions and suspensions. Other commonly used surfactants, such as Tweens, Spans and other emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation.

30 The pharmaceutically acceptable compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use,

carriers commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried cornstarch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and
5 suspending agents. If desired, certain sweetening, flavoring or coloring agents may also be added.

Alternatively, the pharmaceutically acceptable compositions of this invention may be administered in the form of suppositories for rectal administration. These can be prepared by mixing the inventive gene transfer system or components thereof with
10 a suitable non-irritating excipient that is solid at room temperature but liquid at rectal temperature and Therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

The pharmaceutically acceptable compositions of this invention may also be administered topically, especially when the target of treatment includes areas or
15 organs readily accessible by topical application, including diseases of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas or organs.

For topical applications, the pharmaceutically acceptable compositions may be formulated in a suitable ointment containing the inventive gene transfer system or
20 components thereof suspended or dissolved in one or more carriers. Carriers for topical administration of the components of this invention include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene component, emulsifying wax and water. Alternatively, the pharmaceutically acceptable compositions can be formulated in a
25 suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetaryl alcohol, 2-octyldodecanol, benzyl alcohol and water.

For ophthalmic use, the pharmaceutically acceptable compositions may be
30 formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions in isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively, for ophthalmic uses, the

pharmaceutically acceptable compositions may be formulated in an ointment such as petrolatum.

The pharmaceutically acceptable compositions of this invention may also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents.

The amount of the components of the present invention that may be combined with the carrier materials to produce a composition in a single dosage form will vary depending upon the host treated, the particular mode of administration. It has to be noted that a specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the activity of the specific component employed, the age, body weight, general health, sex, diet, time of administration, rate of excretion, drug combination, and the judgment of the treating physician and the severity of the particular disease being treated. The amount of a component of the present invention in the composition will also depend upon the particular component(s) in the composition.

The pharmaceutical composition is preferably suitable for the treatment of diseases, particular diseases caused by gene defects such as cystic fibrosis, hypercholesterolemia, hemophilia, immune deficiencies including HIV, Huntington disease, .alpha.-anti-Trypsin deficiency, as well as cancer selected from colon cancer, melanomas, kidney cancer, lymphoma, acute myeloid leukemia (AML), acute lymphoid leukemia (ALL), chronic myeloid leukemia (CML), chronic lymphocytic leukemia (CLL), gastrointestinal tumors, lung cancer, gliomas, thyroid cancer, mamma carcinomas, prostate tumors, hepatomas, diverse virus-induced tumors such as e.g. papilloma virus induced carcinomas (e.g. cervix carcinoma), adeno carcinomas, herpes virus induced tumors (e.g. Burkitt's lymphoma, EBV induced B cell lymphoma), Hepatitis B induced tumors (Hepato cell carcinomas), HTLV-1 und HTLV-2 induced lymphoma, lung cancer, pharyngeal cancer, anal carcinoma, glioblastoma, lymphoma, rectum carcinoma, astrocytoma, brain tumors, stomach cancer, retinoblastoma, basalioma, brain metastases, medullo blastoma, vaginal cancer, pancreatic cancer, testis cancer, melanoma, bladder cancer, Hodgkin

syndrome, meningioma, Schneeberger's disease, bronchial carcinoma, pituitary cancer, mycosis fungoides, gullet cancer, breast cancer, neurinoma, spinalioma, Burkitt's lymphoma, laryngeal cancer, thymoma, corpus carcinoma, bone cancer, non-Hodgkin lymphoma, urethra cancer, CUP-syndrome, oligodendroglioma, vulva
5 cancer, intestinal cancer, oesphagus carcinoma, small intestine tumors, craniopharyngeoma, ovarian carcinoma, ovarian cancer, liver cancer, leukemia, or cancers of the skin or the eye; etc.

Kits

10 The present invention also features kits comprising a piggyBac transposase as a protein or encoded by a nucleic acid, and/or a hyperactive piggyBac transposon; or a gene transfer system as described herein comprising a piggyBac transposase as a protein or encoded by a nucleic acid as described herein, in combination with a hyperactive piggyBac transposon; optionally together with a pharmaceutically
15 acceptable carrier, adjuvant or vehicle, and optionally with instructions for use.

Any of the components of the inventive kit may be administered and/or transfected into cells in a subsequent order or in parallel. e.g. the piggyBac transposase protein or its encoding nucleic acid may be administered and/or transfected into a cell as defined above prior to, simultaneously with or subsequent to
20 administration and/or transfection of the inventive hyperactive transposon. Alternatively, the hyperactive piggyBac transposon may be transfected into a cell as defined above prior to, simultaneously with or subsequent to transfection of the piggyBac transposase protein or its encoding nucleic acid. If transfected parallel, preferably both components are provided in a separated formulation and/or mixed
25 with each other directly prior to administration in order to avoid transposition prior to transfection. Additionally, administration and/or transfection of at least one component of the kit may occur in a time staggered mode, e.g. by administering multiple doses of this component.

30 The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the assay, screening, and therapeutic methods of the invention, and are not intended to limit the scope of what the inventors regard as their invention.

EXAMPLES

Example 1. Identification of integration-defective piggyBac variants

The present experiments describe screening and identification of excision-
5 hyperactive piggyBac transposons using a version of the Cherry gene which produces
a red fluorescent protein. A copy of the piggyBac transposon was put into the gene,
inactivating it such as the cells are NOT red. However, piggyBac excision restores
the gene, leading to the production of the red fluorescent protein. Accordingly,
increased red colony color identifies mutants that excise better.

10 A large collection of mutant transposase genes was made using mutagenic
PCR which was cloned into an expression vector in yeast. Colonies containing
individual mutants were grow up on an agar plate and were then examined with red
fluorescent light. Figure 4 shows excision hyperactives that have been isolated to
date.

15 In certain preferred embodiments, the integration defective piggyBac
comprises an amino acid change in the wild type piggyBac sequence corresponding to
SEQ ID NO: 2. Preferably, the amino acid change is R371A, R373A or R371A,
R373A.

In preferred exemplary embodiments, the integration defective piggyBac
20 corresponds to the amino acid sequence set forth as SEQ ID NO: 64, SEQ ID NO: 65
or SEQ ID NO: 66.

Example 2. Identification of hyperactive variants

Using the integration defective piggyBac mutants as a starting point, the
25 present inventors have identified hyperactive piggyBac transposon mutants. The
yeast excision assay that was developed as described in Mitra R. et al. (piggyBac can
bypass DNA synthesis during cut and paste transposition. EMBO J. Apr
9;27(7):1097-109. Epub 2008 Mar 20) was used to identify the hyperactive mutants.
The piggyBac ORF was mutagenized by mutagenic PCR using primers flanking the
30 ORF as the expression construct and then recovered transformants by co-
transformation of the PCR product with a gapped piggyBac plasmid into the yeast
assay strain containing a ura- to ura+ cassette in which transposon excision results
in formation of ura+ colonies. Following recovery of transformants on SC-Trp-His
plates, colonies were resuspended in water and spotted onto plates lacking uracil to

identify excisions. By comparison to the number of ura+ colonies from the mutagenized transformants to wildtype in these spotting tests, potential hyperactive variants were identified. Each hyperactive candidate strain was then purified and quantitatively reassayed excision. Plasmid DNA containing the piggyBac gene from confirmed hyperactives was then sequenced to identify the piggyBac gene mutation and resulting amino acid change. Amino acid changes and corresponding nucleic acid changes are shown in Table 1, below:

Table 1

L15P	CUG to CCG
D19N/F395L	GAC to AAC/UUU to CUU
S31P/T164A	UCA to CCA/ACA to GCA
H33Y	CAC to UAC
E44K/K334R	GAA to AAA/AAG to AGG
E45G	GAA to GGA
C97R/T242I	UGU to CGU/ACU to AUU
S103P	UCC to CCC
R189K/G120G	AGA to AAA/GGU to GGC
R189R/D450N/R526R	AGA to AGG/GAC to AAC
M194T	AUG to ACG
M194V	AUG to GUG
S213S/V436I	AGU to AGC
I221T	AUA to ACA
S373P between M6+	UCA to CCA
N384T	AAC to ACC
C453S/N571S	UGU to AGU/AAU to AGU
T560A	ACU to GCU
N571S	AAU to AAG
S573A	UCG to GCG
S584P	UCU to CCU
M589V	AUG to GUG
M589V/D170D	ATG to GUG/GAC to GAU
S592G	AGU to GGU
F594L	UUC to TTA
Stop/WLESCN	TGA to TGG
Stop595ELESCN/H33H	TGA to GGA/CAC to CAU

10

The amino acid changes and fold increase in transposition from that of wildtype (normalized to 1) for certain exemplary hyperactive mutants is shown in Table 2 in Figure 1.

15

Hyperactive mutations can occur at many positions within the transposase and it is expected that many more hyperactive piggyBac variants will be found. These variants may be altered in a single amino acid or multiple amino acids. Variants can

be identified using the yeast assay as a screen. PCR mutagenesis of the entire gene as well as targeted mutagenesis using smaller piggyBac fragments or oligonucleotide-directed mutagenesis to regions that have been identified as giving hyperactive mutations will be used.

5

Example 3. Induced pluripotent stem cell generation using the hyperactive transposon

In certain exemplary embodiments, the hyperactive piggyBac transposons can be used to create induced pluripotent stem cells using a minimal set of genes. In particular, Oct ³/₄, Sox2, Klf4 and c-myc are used as a minimal set of genes. Takahashi et al. (Cell, 131, 861 – 872, November 30, 2007), incorporated by reference in its entirety herein, teach methods of generating induced pluripotent stem cells (iPS) from human dermal fibroblasts using Oct 3/4 , Sox2, klf4, and c-Myc.

10

15 Other Embodiments

From the foregoing description, it will be apparent that variations and modifications may be made to the invention described herein to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

20

The recitation of a listing of elements in any definition of a variable herein includes definitions of that variable as any single element or combination (or subcombination) of listed elements. The recitation of an embodiment herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

25

All patents and publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent patent and publication was specifically and individually indicated to be incorporated by reference.

What is claimed is:

1. A transposon comprising: one or more hyperactive piggyBac nucleic acid sequences and variants, derivatives and fragments thereof that retain transposon activity.
5
2. The transposon of claim 1, wherein the hyperactive piggyBac transposon has a higher level of transposon excision compared to a wildtype piggyBac transposon.
- 10 3. The transposon of claim 1, comprising 2, 3, 4, 5 or more hyperactive piggyBac nucleic acid sequences and variants, derivatives and fragments thereof that retain transposon activity.
4. The transposon of claim 1, wherein the hyperactive piggyBac nucleic acid
15 sequence is from the family *Noctuidae*.
5. The transposon of claim 1, wherein the hyperactive piggyBac nucleic acid sequence is from the species *Trichoplusia ni*.
- 20 6. The transposon of claim 1, comprising a nucleic acid sequence selected from the group consisting of: SEQ ID NO: 34 – SEQ ID NO: 63 or SEQ ID NO: 70 SEQ ID NO: 96.
7. The transposon of claim 6, wherein the nucleic acid sequence encodes an
25 amino acid sequence selected from the group consisting of: SEQ ID NO: 3 – SEQ ID NO: 32.
8. A transposon comprising: one or more integration defective piggyBac nucleic acid sequences and variants, derivatives and fragments thereof.
- 30 9. The transposon of claim 8, wherein the integration defective piggyBac transposon has a lower rate of integration as compared to a wildtype piggyBac transposon.

10. The transposon of claim 8, wherein the integration defective piggyBac nucleic acid sequence is from the family *Noctuidae*.
11. The transposon of claim 8, wherein the integration defective piggyBac nucleic acid sequence is from the species *Trichoplusia ni*.
12. The transposon of claim 8, comprising a nucleic acid sequence selected from the group consisting of: SEQ ID NO: 67 – SEQ ID NO: 69.
13. The transposon of claim 12, wherein the nucleic acid sequence encodes an amino acid sequence selected from the group consisting of: SEQ ID NO: 64 – SEQ ID NO: 66.
14. The transposon of claim 2 or claim 9, wherein the wildtype piggyBac transposon comprises a nucleic acid sequence corresponding to SEQ ID NO: 1.
15. The transposon of claim 1 or claim 8, wherein the transposon is capable of inserting into the DNA of a cell.
16. The transposon of claim 1 or claim 8, wherein the transposon further comprises a marker protein.
17. The transposon of claim 1 or claim 8, wherein the transposon is inserted in a plasmid.
18. The transposon of claim 17 wherein the transposon further comprises at least a portion of an open reading frame.
19. The transposon of claim 17 wherein the transposon further comprises at least one expression control region.
20. The transposon of claim 17 wherein the expression control region is selected from the group consisting of a promoter, an enhancer or a silencer.

21. The transposon of claim 17 wherein the transposon further comprises a promoter operably linked to at least a portion of an open reading frame.
22. The transposon of claim 15 wherein the cell is obtained from an animal.
- 5 23. The transposon of claim 22 wherein the cell is from a vertebrate or an invertebrate.
24. The transposon of claim 17 wherein the vertebrate is a mammal.
- 10 25. A gene transfer system comprising: a transposon according to claim 1 or claim 8; and a piggyBac transposase.
26. The gene transfer system of claim 25, wherein the piggyBac transposase is from the family *Noctuidae*.
- 15 27. The gene transfer system of claim 26, wherein the piggyBac transposase is from the species *Trichoplusia ni*.
- 20 28. The gene transfer system of claim 27, wherein the piggyBac transposase comprises an amino acid sequence corresponding to SEQ ID NO: 33.
29. The gene transfer system of claim 25, wherein the piggyBac transposase is a mammalian piggyBac transposase.
- 25 30. The gene transfer system of claim 25 wherein the transposon is inserted into the genome of the cell.
31. The gene transfer system of claim 30, wherein the cell is obtained from an animal.
- 30 32. The gene transfer system of claim 30, wherein the cell is from a vertebrate or an invertebrate.

33. The gene transfer system of claim 32, wherein the vertebrate is a mammal.
34. A cell comprising the transposon of claim 1.
- 5 35. A cell comprising the transposon of claim 8.
36. A pharmaceutical composition comprising: a transposon comprising a hyperactive piggyBac nucleic acid sequence and a piggyBac transposase, together with a pharmaceutically acceptable carrier, adjuvant or vehicle.
- 10 37. A method for introducing exogenous DNA into a cell comprising:
contacting the cell with the gene transfer system of claim 25, thereby introducing exogenous DNA into a cell.
- 15 38. The method of claim 37, wherein the cell is a stem cell.
39. A kit comprising: a transposon comprising a hyperactive piggyBac nucleic acid sequence and instructions for introducing DNA into a cell.
- 20 40. The kit of claim 39, wherein the hyperactive piggyBac nucleic acid sequence is from the family *Noctuidae*.
41. The kit of claim 39, wherein the hyperactive piggyBac nucleic acid sequence is from the species *Trichoplusia ni*.
- 25 42. The kit of claim 39, comprising a nucleic acid sequence selected from the group consisting of: SEQ ID NO: 34 – SEQ ID NO: 63 or SEQ ID NO: 70 – SEQ ID NO: 96.
- 30 43. A kit comprising: a transposon comprising a integration defective piggyBac nucleic acid sequence and instructions for use.
44. The kit of claim 43, comprising a nucleic acid sequence selected from the group consisting of: SEQ ID NO: 67 – SEQ ID NO: 69.

FIG. 1

Table 2

PiggyBac hyperactives
- fold increase

wildtype	1
G2C, Q40R	8.7
S3N	23.7
S26P	12.3
I30V, G165S	16.4
T43A	15.9
Q55R	8.1
T57A	5.3
S61R	23.9
I82V	6.8
I90V	13.7
S103P	12.3
S103T	5.0
N113S	8.87
M185L	7.3
M194V	9.1
S230N	11.7
R281G	12.6
M282V	15.7
G316E	8.4
P410L	13.8
I426V	10.7
Q497L	8.1
K501N, K565I	59
N505D, S573L	10.9
S509G, N570S	11.1
N538K	16.4
K575R	16.9
Q591P	156
Q591R	31.8
F594L	42.2

FIG. 2A-1

SEQ ID NO: 1

>Trichoplusia ni piggyBac transposon

CCTAGAAAGATAGTCTGCGTAAAATTGACGCATGCATTCTTGAAAATATTGCTCTCTCTTCTAAAATAGCGGAAATCCGTCGCTGTGCGT
 TTAGGACATCTCAGTCGCCCTGGAGCTCCCGTGAGCGTCTTGTCAATCGGTAAGTGTCACTGATTTTGAACATATAACGACCGCGGT
 GAGTCAAAATGACGCATGATTATCTTTTACGTGACTTTAAGATTTAACTCATACGATAATTATATTGTTATTTTCATGTTCTACTTACGT
 GATAACTTATATATATATATTTTCTGTTATAGATATCGTGACTAATAATAATAAAATGGTAGTCTTTAGACGATGAGCATAATCCT
 CTCTGCTCTTCTGAAAAGCGATGACGAGCTTGTGGTGAGGATCTGACAGTGAATAATCAGATCACGTAAGTGAAGATGACGTCCAGAG
 CGATACAGAAAGCGTTTATAGATGAGGTACATGAAGTGCAGCCAAACGTCAAAGCGTAGTGAATAATTAGACGAAACAAAATGTTATTGA
 ACAACGAGGTTCTTCATTGGCTTTAAACAAAATCTTGACCTTGCCACAGAGGACTATTAGAGGTAAGAAATAAACATTTGTTGGTCAACTTC
 AAAGTCCACGAGCGTAGCCGAGTCTCTGCACCTCAATGATGAGATAATTTCCGGAATTTGTAATAATGGACAAAATGCTGAGATATCATTGAAAACGTCGGGA
 ACTTTTATGCTTCAAACTATTTTTTACTGATGAGATAATTTCCGGAATTTGTAATAATGGACAAAATGCTGAGATAATCATTGAAAACGTCGGGA
 ATCTATGACAGGTGCTACATTTCTGTGACACGAAATGAAGATGAAATCTATGCTTCTTGTGATTTGTAATGACAGCAGTGAAGAAAAGA
 TAACCACATGTCCACAGATGACCTCTTTGATCGATCTTTGTCAATGGTGTACGCTCTGTAAATGAGTCGTGATCGTTTGTGATTTTGTGAT
 ACGATGCTTTAGAATGGATGACAAAAGTATACGCCCCACACTTCGAGAAAACGATGTATTTACTCCTGTAGAAAATAATGGGATCTCTT
 TATCCATCAGTGCAATAAAAATTACACTCCAGGGCTCATTTGACCCATAGATGAACAGTTACTTGGTTTLAGAGGACCGTGTCCGTTTAG
 GATGTATATCCAAAACAGCCAAAGTAAAGTATGGAATAAAAATCCTCATGATGTGACAGTGTACAAAAGTATATGATAAATGGAATGCC
 TTATTTGGGAAAGAGAAACACAGACCACCGGAGTACCCTCGGTGAATACCTGAAAGGAGTTATCAAAGCCTGTGCACGGTAGTTGTCTG
 TAATATTACGTGTGACAAATGGTTCACTCAATCCCTTTGGCAAAAACCTTACTACAAGAACCGTATAAAGTTAACCATTTGTGGGAACCCGT
 GCGATCAAAACAACCGGAGATACCGGAAGTACTGAAAACAGTCCGCTCCAGCCAGTGGAAACATCGATGTTTGTGTTTGACGGACCCCT
 TACTCTGCTCATATAAACCGAAGCCAGCTAAGATGGTATACCTTATATCATCTTGTGATGAGGATGCTTCTATCAACGAAAGTACCCGG
 TAAACCGCAAATGGTTATGTATTAATCAAACATAAAGCGGAGTGGACACCGTAGACCCAAAATGTGTTCTGTGATGACCTGCAGTAGGAA
 GACGAAATAGGTGCCCTATGGCATTATTTGACGGAATGATAAACATTTGCCATGAAATCTTTTATATATACAGCCATAAATGTCAGTAG
 CAAGGGAGAAAAGGTTCAAAGTCGCAAAAATTTATGAGAAAACCTTTACATGAGCCTGACGTGATCGTTTATGCGTAAGCGTTTAGAAGC
 TCCCTACTTTGAAGAGATATTGCGCGATAATACTCTAATATTTGCCAAATGAAGTGCCTGTACATCAGATGACAGTACTGAAGAGCC
 AGTAAACGAAAACGTACTTACTGTACTTACTGCCCTCTAAAATAAGCGGAAAGGCAAATGCATCGTGCAAAATAATGCAAAAAGTTAT
 TTGTCGAGAGCATAAATTTGATATGTGCCAAAAGTTGTTTCTGACTGACTAATAAGTATAATTTGTTCTTATATGATAAAGTTAAGCTAA
 TTACTTATTTATAAATACAACATGACTGTTTTTAAAAGTACAAAATAAGTTTTATTTTGTAAAAGAGAGAATGTTTAAAAGTTTTGTTACT
 TTTATAGAAGAAATTTGAGTTTTTGTGTTTTTTTAAATAAATAAATAAACATAAATAAATAGTTTGTGATTTATATAGTATGTAAGT
 GTAATAATAAATAAATTAATGATTTCAAATTAATAAATAAACCCTCGATAATACAGACCGGATAAAAACACATGCGTCAATTTTACGCATA
 TTATCTTTAACGTACGTCACAAATATGATTAATCTTTCTAGGG

FIG. 2A-2

SEQ ID NO: 2
 MGSSLDDEHILSALLQSDDELVGEDSDSEISDHVSEDDVQSDTEEAFIDEVHEVQPTSSGSEILDEQNVIEQPGSSLASNRILTLPQRTI
 RGKNKHCWSTSKSTRRSRVSALNIVRSQRGPTRMCRNIYDPLLCFKLFFTDEIIEIVKWTNAEISLKRRESMTGATFRDTNEDEIYAFF
 GILVMTAVRKDNHMSTDDLFDRLSLMVYVSVMSRDRFDLIRCLRMDDKSI RPTLRENDVFTPVRKIWDLFIHQCIQNYTPGAHLTIDEQ
 LLGFRGRCPFRMYIPNKPSKYGIKILMCDSTGTYKYMINGMPYLGRTQNGVPLGEYYVKELSKPVHGSCRNITCDNWFTSIPLAKNLLQ
 EPYKLTIVGTVRSNKREIPEVLKNSRSPVGTSMFCFDGPLTLVSYKPKPAKMVYLLSSCDEEDASINESTGKPMVMYINQTKGGVDTLQ
 QMCSVMTCSRKTNRWPMALLYGMINIACINSFIYSHNVSSKGEKVQSRKKFMRNLYMSLTSSFMRKRLEAPTLKRYLRDNI SNILPNEV
 PGTSDDSTEPEVMKKRTYCTYCPKIRRKANASCKKCKVICREHNIDMCQSCF

FIG. 2B

SEQ ID NO: 33
 >Trichoplasia ni piggyBac transposase
 MGSSLDDEHILSALLQSDDELVGEDSDSEISDHVSEDDVQSDTEEAFIDEVHEVQPTSSGSEILDEQNVIEQPGSSLASNKILTLPQRTI
 RGKNKHCWSTSKSTRRSRVSALNHVRSQRGPTRMCRNIYDPLLCFKLFFTDEIIEIVKWTNAEISLKRRESMTGATFRDTNEDEIYAFF
 GILVMTAVRKDNHMSTDDLFDRLSLMVYVSVMSRDRFDLIRCLRMDDKSI RPTLRENDVFTPVRKIWDLFIHQCIQNYTPGAHLTIDEQ
 LLGFRGRCPFRMYIPNKPSKYGIKILMCDSTGTYKYMINGMPYLGRTQNGVPLGEYYVKELSKPVHGSCRNITCDNWFTSIPLAKNLLQ
 EPYKLTIVGTVRSNKREIPEVLKNSRSPVGTSMFCFDGPLTLVSYKPKPAKMVYLLSSCDEEDASINESTGKPMVMYINQTKGGVDTLQ
 QMCSVMTCSRKTNRWPMALLYGMINIACINSFIYSHNVSSKGEKVQSRKKFMRNLYMSLTSSFMRKRLEAPTLKRYLRDNI SNILPNEV
 PGTSDDSTEPEVMKKRTYCTYCPKIRRKANASCKKCKVICREHNIDMCQSCF

FIG. 3

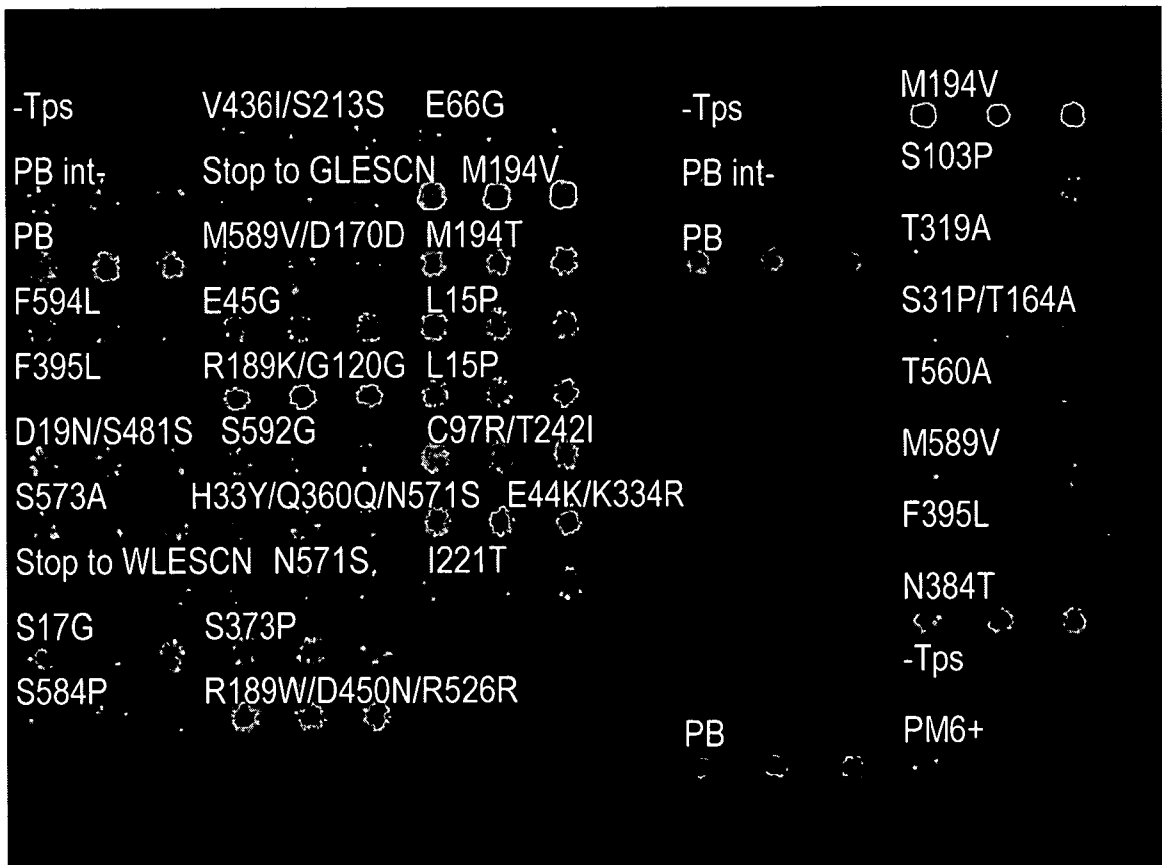


FIG. 4

SEQ ID NO: 64
 MGSSLDDEHILSALLQSDDELVGEDSDSEISDHVSEDDVQSDTEEEAFIDEVHEVQPTSSGSEILDEQNVIEQPGSSLASNRILTLPQRTI
 RGKNKHCWSTSKTRRSRVSALNIVRSQRGPTMCRNIYDPLLCFKLFFTDEI ISEIVKWTNAEISLKRRESMTGATFRDTNEDEIYAFF
 GILVMTAVRKDNHMSTDDLFRSLSMVYVSMRDRDFLIRCLRMDDKSI RPTLRENDVFTPVRKIWDLFIHQCIQNYTPGAHLTIDEQ
 LLGFRGRCFFRMYIPNKPSKYGIKILMCDSGTKYMINGPYLGRGTQNGVPLGEYVVKELSKPVHGSCRNITCDNWFTSIPLAKNLLQ
 EPYKLTIVGTV**AS**NKREIPEVLKNSRSPVGTSMFCFDGPLTLVSYKPKPAKMVYLLSSCDEEDASINESTGKPKQMVVYNNQTKGGVDTLQ
 QMCSVMTCSRKTNRWPMALLYGMINIAINSFIIYSHNVSSKGEKVQSRKKFMRNLVMSLTSSFMRKRLEAPT LKRYLRDNI SNILPNEV
 PGTSDSDSTE EPVMKKRTYCTYCP SKIRRKANASCKKCKKVICREHNIDMCQSCF

SEQ ID NO: 65
 MGSSLDDEHILSALLQSDDELVGEDSDSEISDHVSEDDVQSDTEEEAFIDEVHEVQPTSSGSEILDEQNVIEQPGSSLASNRILTLPQRTI
 RGKNKHCWSTSKTRRSRVSALNIVRSQRGPTMCRNIYDPLLCFKLFFTDEI ISEIVKWTNAEISLKRRESMTGATFRDTNEDEIYAFF
 GILVMTAVRKDNHMSTDDLFRSLSMVYVSMRDRDFLIRCLRMDDKSI RPTLRENDVFTPVRKIWDLFIHQCIQNYTPGAHLTIDEQ
 LLGFRGRCFFRMYIPNKPSKYGIKILMCDSGTKYMINGPYLGRGTQNGVPLGEYVVKELSKPVHGSCRNITCDNWFTSIPLAKNLLQ
 EPYKLTIVGTV**AS**NKREIPEVLKNSRSPVGTSMFCFDGPLTLVSYKPKPAKMVYLLSSCDEEDASINESTGKPKQMVVYNNQTKGGVDTLQ
 QMCSVMTCSRKTNRWPMALLYGMINIAINSFIIYSHNVSSKGEKVQSRKKFMRNLVMSLTSSFMRKRLEAPT LKRYLRDNI SNILPNEV
 PGTSDSDSTE EPVMKKRTYCTYCP SKIRRKANASCKKCKKVICREHNIDMCQSCF

SEQ ID NO: 66
 MGSSLDDEHILSALLQSDDELVGEDSDSEISDHVSEDDVQSDTEEEAFIDEVHEVQPTSSGSEILDEQNVIEQPGSSLASNRILTLPQRTI
 RGKNKHCWSTSKTRRSRVSALNIVRSQRGPTMCRNIYDPLLCFKLFFTDEI ISEIVKWTNAEISLKRRESMTGATFRDTNEDEIYAFF
 GILVMTAVRKDNHMSTDDLFRSLSMVYVSMRDRDFLIRCLRMDDKSI RPTLRENDVFTPVRKIWDLFIHQCIQNYTPGAHLTIDEQ
 LLGFRGRCFFRMYIPNKPSKYGIKILMCDSGTKYMINGPYLGRGTQNGVPLGEYVVKELSKPVHGSCRNITCDNWFTSIPLAKNLLQ
 EPYKLTIVGTV**AS**NKREIPEVLKNSRSPVGTSMFCFDGPLTLVSYKPKPAKMVYLLSSCDEEDASINESTGKPKQMVVYNNQTKGGVDTLQ
 QMCSVMTCSRKTNRWPMALLYGMINIAINSFIIYSHNVSSKGEKVQSRKKFMRNLVMSLTSSFMRKRLEAPT LKRYLRDNI SNILPNEV
 PGTSDSDSTE EPVMKKRTYCTYCP SKIRRKANASCKKCKKVICREHNIDMCQSCF