



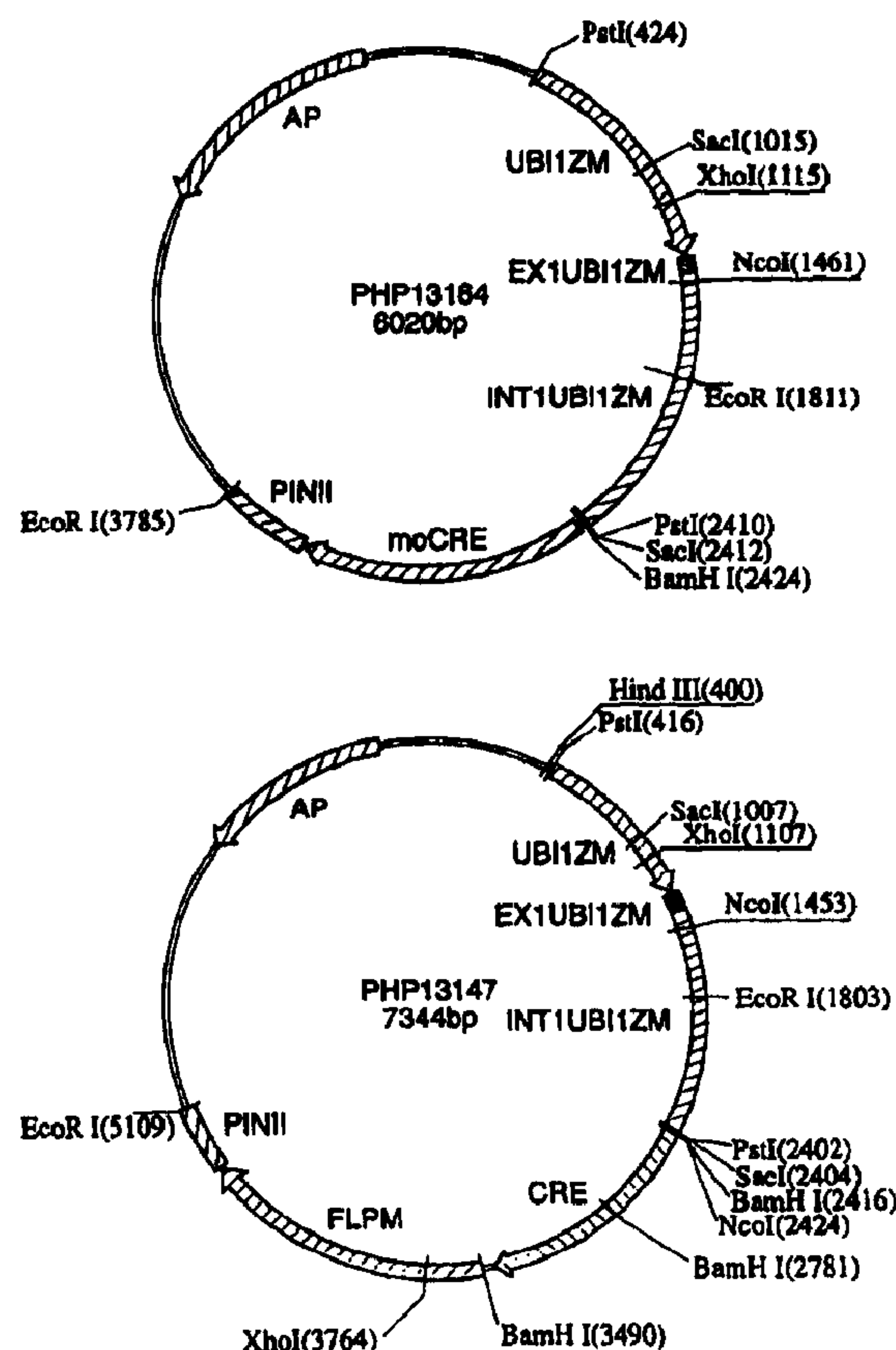
(86) Date de dépôt PCT/PCT Filing Date: 1998/11/17
(87) Date publication PCT/PCT Publication Date: 1999/05/27
(45) Date de délivrance/Issue Date: 2008/08/05
(85) Entrée phase nationale/National Entry: 2000/05/18
(86) N° demande PCT/PCT Application No.: US 1998/024608
(87) N° publication PCT/PCT Publication No.: 1999/025840
(30) Priorités/Priorities: 1997/11/18 (US60/065,627);
1997/11/18 (US60/065,613); 1998/09/08 (US60/099,435)

(51) Cl.Int./Int.Cl. *C12N 15/52* (2006.01),
A01H 1/00 (2006.01), *A01H 5/00* (2006.01),
C07K 19/00 (2006.01), *C12N 15/62* (2006.01),
C12N 15/82 (2006.01), *C12N 15/90* (2006.01),
C12N 5/10 (2006.01), *C12N 9/00* (2006.01)

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(54) Titre : NOUVEAU PROCEDE D'INTEGRATION D'ADN ETRANGER DANS DES GENOMES EUKARYOTYQUES
(54) Title: A NOVEL METHOD FOR THE INTEGRATION OF FOREIGN DNA INTO EUKARYOTIC GENOMES



(57) Abrégé/Abstract:

Compositions and methods for introducing a DNA of interest into a genomic target site are provided. In particular, the methods and compositions involve the use of a combination of target sites for two site specific recombinases and expression of a chimeric



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(57) **Abrégé(suite)/Abstract(continued):**

recombinase with dual target site specificity. Thus, the compositions comprise novel site-specific recombinases with specificities to multiple target sites, and nucleotide sequences and expression cassettes encoding these recombinases or target sites. The methods involve transforming a eukaryotic cell having target sites for the novel recombinase with a DNA of interest that is flanked by corresponding target sites. Expression of the recombinase results in integration of the DNA of interest into the genome of the cell. The compositions and methods of the invention have use in the construction of stably transformed eukaryotic cells, and in particular, plant cells. The methods result in the efficient targeted genomic intergration of DNA by site-specific recombination.



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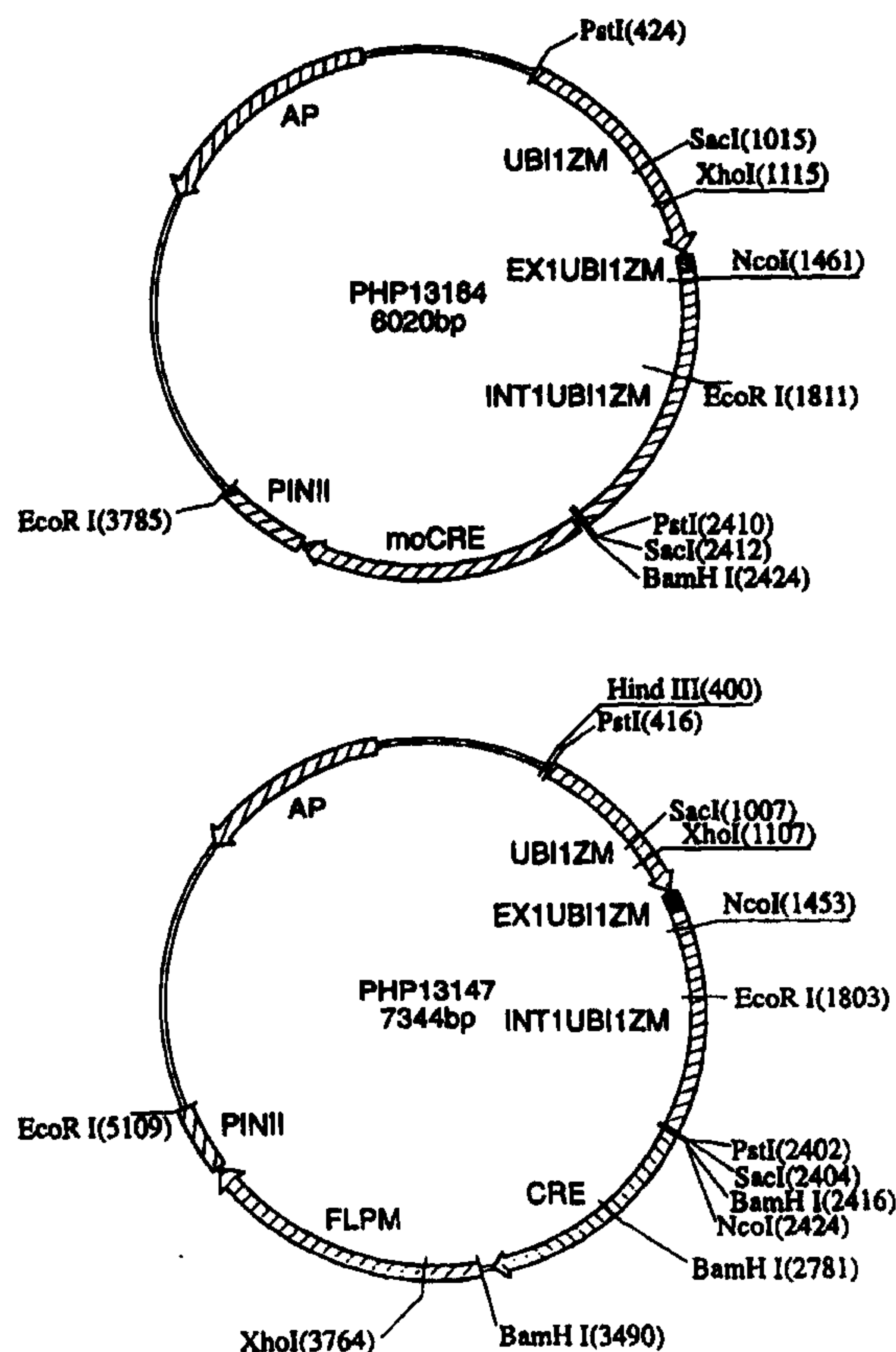
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/52, 15/62, 15/82, 15/90, 5/10, A01H 5/00, 5/10	A1	(11) International Publication Number: WO 99/25840 (43) International Publication Date: 27 May 1999 (27.05.99)
(21) International Application Number: PCT/US98/24608 (22) International Filing Date: 17 November 1998 (17.11.98) (30) Priority Data: 60/065,613 18 November 1997 (18.11.97) US 60/065,627 18 November 1997 (18.11.97) US 60/099,435 8 September 1998 (08.09.98) US (71) Applicant (for all designated States except US): PIONEER HI-BRED INTERNATIONAL, INC. [US/US]; 800 Capital Square, 400 Locust Street, Des Moines, IA 50319 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): BASZCZYNSKI, Christo- pher, L. [CA/US]; 7305 Benton Drive, Urbandale, IA 50322 (US). LYZNIK, Leszek, Alexander [PL/US]; 5960 Greendale Place #105, Johnston, IA 50131 (US). GOR- DON-KAMM, William J. [US/US]; 3916 67th Street, Ur- bandale, IA 50322 (US). RAO, Aragula, Gururaj [US/US]; 4734 74th Street, Urbandale, IA 50322 (US). TAGLIANI, Laura, A. [US/US]; 7552 N.W. 28th Street, Ankeny, IA 50021 (US). GUAN, Xueni [CN/US]; 3668 Ruelle de Ville, San Diego, CA 82139 (US).		(74) Agents: SPRUILL, W., Murray et al.; Bell Seltzer Intellectual Property Law Group, Alston & Bird LLP, P.O. Drawer 34009, Charlotte, NC 28234 (US). (81) Designated States: AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>

(54) Title: A NOVEL METHOD FOR THE INTEGRATION OF FOREIGN DNA INTO EUKARYOTIC GENOMES

(57) Abstract

Compositions and methods for introducing a DNA of interest into a genomic target site are provided. In particular, the methods and compositions involve the use of a combination of target sites for two site specific recombinases and expression of a chimeric recombinase with dual target site specificity. Thus, the compositions comprise novel site-specific recombinases with specificities to multiple target sites, and nucleotide sequences and expression cassettes encoding these recombinases or target sites. The methods involve transforming a eukaryotic cell having target sites for the novel recombinase with a DNA of interest that is flanked by corresponding target sites. Expression of the recombinase results in integration of the DNA of interest into the genome of the cell. The compositions and methods of the invention have use in the construction of stably transformed eukaryotic cells, and in particular, plant cells. The methods result in the efficient targeted genomic intergration of DNA by site-specific recombination.



**A NOVEL METHOD FOR THE INTEGRATION OF FOREIGN
DNA INTO EUKARYOTIC GENOMES
FIELD OF THE INVENTION**

The invention relates to the genetic modification of chromosomes. In particular, methods and compositions for the integration of DNA into a eukaryotic genome are provided.

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BACKGROUND OF THE INVENTION

Several approaches have been used to integrate a DNA of interest into the genome of a plant. In the simplest method, DNA is introduced into a cell and randomly integrates into the genome through illegitimate recombination. One drawback to this method is that positional effects due to random integration make gene expression difficult to analyze.

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As an alternative to illegitimate recombination, integration may be targeted to a particular site on the genome through the use of homologous recombination or site-specific recombination. In plants, where homologous recombination technology has not been developed, site-specific recombination is used to integrate a sequence of interest into an integration site that has been previously inserted into the plant host genome. If site-specific integration occurs by a single cross-over event between a chromosome and a circular extrachromosomal replicon, the entire replicon will be inserted into the chromosome. When insertion of the entire replicon is undesirable, a fragment of the replicon comprising the DNA of interest, flanked by target sites for a site-specific recombinase, may be introduced by a double reciprocal cross-over event, into a chromosome having an integration site corresponding to the target sites which flank the DNA of interest. In either case, integration is inefficient because it is reversible, that is, the integrated DNA may be excised by subsequent site-specific recombination between the target sites flanking the integrated DNA.

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Several approaches have been taken to avoid excision of an integrated DNA. In one approach, expression of a site-specific recombinase, such as Cre or FLP, is temporally regulated. See O'Gorman *et al.* (1991) *Science* 251:1351-1355; Logie and Stewart (1995) *Proc Natl Acad Sci* 92:5940-5944; Zhang *et al.* (1996) *Nuc Acid Res* 24:543-548; Nichols *et al.* (1997) *Mol Endocrinol* 11:950-961; and Feil *et al.* (1997) *Biochem Biophys Res Comm* 237:752-757.

In these methods, the recombinase is briefly expressed, either transiently or inducibly, in order to allow integration. However, excision of the integrated DNA may occur before active recombinase disappears from the cell. Furthermore, intramolecular excision is kinetically favored over bi-molecular integration. Therefore, integrated DNA is inherently unstable in the presence of recombinase.

A second approach reduces excision of integrated DNA by using pairs of singly mutated target sites on both the chromosome and flanking the DNA of interest. See Albert *et al.* (1995) *Plant J* 7:649-659; Schlake and Bode (1994) *Biochemistry* 33:12746-12751; O'Gorman *et al.* (1997) *Proc Natl Acad Sci* 94:14602-14607; and Araki *et al.* (1997) *Nuc Acid Res* 25:868-872.

Recombination between singly mutated target sites results in doubly mutated target sites flanking the DNA inserted into the chromosome. The doubly mutated target sites are not well recognized by the recombinase. Thus, the inserted DNA is excised from the chromosome by a reverse reaction only at low levels. This system, however, has the disadvantage that the singly mutated target sites often do not act as efficient recombination substrates and thus the frequency of integration is reduced. In addition, transformants are unstable because excision may still occur, although at reduced frequency.

Accordingly, it is an object of the invention to provide efficient methods for site-specific integration of DNA into eukaryotic genomes which avoid subsequent excision reactions and other non-productive recombination reactions.

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SUMMARY OF THE INVENTION

Compositions and methods for introducing a DNA of interest into a genomic integration site are provided. In particular, the methods and compositions involve the use of a combination of target sites for two distinctive site-specific recombinases, such as Cre and FLP, and expression of a chimeric recombinase with dual target site specificity. Thus, the compositions comprise novel site-specific recombinases with specificities to multiple target sites, and nucleotides sequences and expression cassettes encoding these recombinases or target sites. The methods involve transforming a eukaryotic cell having target sites for the novel recombinase with a DNA of interest that is flanked by corresponding target sites. Expression of either the novel chimeric recombinase or two site-specific recombinases in the eukaryotic cell results in integration of the DNA of interest into the genome. The compositions and methods of the invention have use in the construction of stably transformed eukaryotic cells, and in particular, plant cells. The methods result in the efficient targeted genomic integration of DNA by site-specific recombination.

One aspect of the invention provides a recombinant protein comprising a first site-specific recombinase fused in frame with a distinct second site-specific recombinase. The first and second site-specific recombinases may be members of the integrase family of recombinases, an active derivative of a member of the integrase family of recombinases, or an active fragment of a member of the integrase family of recombinases, wherein said recombinase, said active derivative, or said active fragment catalyze site-specific recombination. The first and said second recombinases may be selected from the group consisting of

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Cre, FLP, an active derivative of Cre, an active fragment of Cre, an active fragment of FLP, and an active derivative of FLP, wherein said recombinase, said active derivative, or said active fragment catalyze site-specific recombination.

5 Another aspect of the invention provides a recombinant protein comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 3, 6, 9, and 11.

 Another aspect of the invention provides a nucleic
10 acid molecule comprising a nucleotide sequence encoding a first site-specific recombinase fused in frame with a distinct second site-specific recombinase. The first and said second site-specific recombinases may be members of the integrase family of recombinases, an active derivative of a
15 member of the integrase family of recombinases, or an active fragment of a member of the integrase family of recombinases, wherein said recombinase, said active derivative, or said active fragment catalyze site-specific recombination. The first and said second recombinases may
20 be selected from the group consisting of Cre, FLP, an active derivative of Cre, an active fragment of Cre, an active fragment of FLP, and an active derivative of FLP, wherein said recombinase, said active derivative, or said active fragment catalyze site-specific recombination. The first
25 and said second recombinase may be encoded by a nucleotide sequence comprising moCre and FLPm.

 Another aspect of the invention provides a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of:

30 a) a nucleotide sequence comprising the sequence set forth in any one of SEQ ID NOS: 4, 5, 7, and 8; and,

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b) a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set forth in any one of SEQ ID NOS: 3, 6, 9, and 11.

Another aspect of the invention provides a
5 eukaryotic cell, a transformed plant, or cell within a plant seed having stably incorporated into its genome the nucleic acid molecule of the invention.

Another aspect of the invention provides a method for integrating a DNA of interest into the genome of an
10 eukaryotic cell such as a plant cell wherein the genome comprises at least one stably integrated integration site, said integration site comprising a first target site for a first site-specific recombinase and a second target site for a distinct second site-specific recombinase, said method
15 comprising:

a) introducing into said eukaryotic cell a nucleic acid comprising said DNA of interest, wherein said DNA of interest is flanked by said first and said second target sites; and,

20 b) providing in said eukaryotic cell a recombinant protein comprising said first recombinase, an active derivative of said first recombinase, or an active fragment of said first recombinase fused in frame with said distinct second recombinase, an active derivative of said distinct
25 second recombinase, or an active fragment of said distinct second recombinase, wherein said first and said distinct second recombinase, said active derivative, or said active fragment catalyze a site-specific recombination event,

wherein the DNA of interest is integrated into the
30 genome at the integration site.

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Another aspect of the invention provides a plant cell, or cell within a plant seed, having stably incorporated into a chromosome, at least one integration site comprising a target site for a first site-specific recombina-
5 recombina- and a target site for a distinct second site-specific recombina- , wherein said target sites are contiguous.

Another aspect of the invention provides a method for integrating a DNA of interest into the genome of an
10 eukaryotic cell wherein the genome comprises at least one stably integrated integration site, said integration site comprising a first target site for a first site-specific recombina- and a second target site for a distinct second site-specific recombina- , comprising:

15 a) introducing into said eukaryotic cell a nucleic acid comprising said DNA of interest, wherein said DNA of interest is flanked by said first and said second target sites; and,

b) providing in said cell said first recombina- ,
20 an active derivative of said first recombina- , or an active fragment of said first recombina- and said distinct second recombina- , an active derivative of said distinct second recombina- , or an active fragment of said distinct second recombina- , wherein said first and said second
25 recombina- , said active derivative, or said active fragment thereof catalyze a site-specific recombination event.

Another aspect of the invention provides a system for integrating a DNA of interest into the genome of an
30 eukaryotic cell, comprising:

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a) an eukaryotic cell containing at least one stably integrated integration site, said integration site comprising a first target site for a first site-specific recombinase and a second target site for a distinct second
5 site-specific recombinase;

b) a nucleic acid comprising said DNA of interest, wherein said DNA is flanked by said first and said second target sites;

c) a recombinant protein comprising said first
10 recombinase, an active derivative of said first recombinase, or an active fragment of said first recombinase fused in frame with said distinct second recombinase, an active derivative of said distinct second recombinase, or an active fragment of said distinct second recombinase, wherein said
15 first and said second recombinase, said active derivative, or said active fragment catalyze a site-specific recombination event;

d) means for introducing the nucleic acid of (b) into the cell; and,

20 e) means for introducing the recombinant protein of (c) into the cell,

wherein the DNA of interest is integrated into the genome at the integration site.

Another aspect of the invention provides a system
25 for integrating a DNA of interest into the genome of an eukaryotic cell, comprising:

a) an eukaryotic cell containing at least one stably integrated integration site, said integration site comprising a first target site for a first site-specific

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recombinase and a second target site for a distinct second site-specific recombinase;

b) a nucleic acid comprising said DNA of interest, wherein said DNA of interest is flanked by said first and
5 said second target sites;

c) the first recombinase, an active derivative of said first recombinase, or an active fragment of said first recombinase and, the distinct second recombinase, an active derivative of said distinct second recombinase, or an active
10 fragment of said distinct second recombinase, wherein said first and said second recombinase, said active derivative, or said active fragment catalyze a site-specific recombination event;

d) means for introducing the nucleic acid of (b)
15 into the cell; and,

e) means for introducing the first and the second recombinase, or said active derivative thereof, or said active fragment thereof of (c) into the cell;

wherein the DNA of interest is integrated into the
20 genome at the integration site.

Another aspect of the invention provides use of a genetically modified plant as a crop, wherein the genetically modified plant has stably incorporated in its genome the nucleic acid molecule of the invention.

25 Another aspect of the invention provides a process for generating a genetically modified plant, the process comprising crossing a genetically modified first parent plant with a second plant, wherein the genetically modified first parent plant comprises a nucleic acid molecule of the
30 invention.

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Another aspect of the invention provides use of a genetically modified plant as a crop, wherein the genetically modified plant has stably integrated into a chromosome, at least one integration site comprising a first
5 target site for a first site-specific recombinase and a second target site for a distinct second site-specific recombinase, wherein said target sites are contiguous.

Another aspect of the invention provides a process for generating a genetically modified plant, the process
10 comprising crossing a genetically modified first parent plant with a second plant, wherein the genetically modified first parent plant has stably integrated into a chromosome, at least one integration site comprising a first target site for a first site-specific recombinase and a second target
15 site for a distinct second site-specific recombinase, wherein said target sites are contiguous.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 schematically represents plant transformation vectors, PHP13164 and PHP13147, for
20 expression of moCRE recombinase and Cre:FLPm recombinase, respectively.

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Figure 2 graphically represents activation of GUS expression by FLPm or CRE:FLPm mediated excision of a sequence flanked by FRT sites that separates the ubiquitin promoter and the GUS open reading frame.

- 5 Figure 3 graphically represents activation of GUS expression by CRE:FLPm mediated excision of a sequence flanked by loxP sites that separates the ubiquitin promoter and the GUS open reading frame.

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DETAILED DESCRIPTION OF THE INVENTION

Compositions and methods for site-specific integration of DNA into predetermined genomic integration sites in a host genome are provided. The invention provides for the use of chimeric recombinases that catalyze site-specific recombination between target sites that originate from different site-specific recombination systems. Such a dual function chimeric recombinase ensures that the two ends of foreign DNA do not ligate with each other, but instead, recombine with their cognate partner target sites residing in the genomic DNA. The methods facilitate the directional targeting of desired genes and nucleotide sequences into corresponding integration sites previously introduced into the genome.

In the methods of the invention, a combination of target sites for two site-specific recombinases are introduced into the genome of an organism of interest, establishing an integration site for insertion of nucleotide sequences of interest. For the purposes of the invention, an integration site will comprise flanking target sites where the target sites correspond to the recombination sites for two distinctive site-specific recombinases. These recombination or target sites may flank other nucleotide sequences or may be contiguous. Methods for the production of transgenic plants containing specific recombination sites integrated in the plant genome are described in U.S. Patent No. 6,187,994, entitled Compositions and Methods for Genetic Modification of Plants, filed 18 November 1997. Once a stable plant or cultured tissue is established, a transfer cassette comprising a DNA of interest, flanked by target sites corresponding to those of the genomic integration site, is introduced into the stably transformed plant or tissues in the presence of a chimeric recombinase with

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specificities to each of the target sites. Alternatively, two distinct recombinases corresponding to the target sites may be present in the cell in lieu of a chimeric recombinase. This process results in exchange of the nucleotide sequences between
5 the two identical target sites of the genomic integration site and the transfer cassette.

Thus, the invention provides a method for integrating a DNA of interest into the genome of a eukaryotic cell, comprising: a) transforming said cell with a transfer cassette
10 comprising said DNA, wherein said DNA is flanked by a target site for a first site-specific recombinase and a target site for a second site-specific recombinase, and said genome contains an integration site comprising target sites corresponding to said target sites flanking said DNA; and b)
15 providing in said cell a recombinant protein comprising said first recombinase fused in frame with said second recombinase.

The invention further provides a method for integrating a DNA of interest into the genome of a eukaryotic cell, comprising: a) transforming said cell with a transfer
20 cassette comprising said DNA, wherein said DNA is flanked by a target site for a first site-specific recombinase and a target site for a second site-specific recombinase, and said genome contains an integration site comprising target sites corresponding to said target sites flanking said DNA; and b)
25 providing in said cell said first recombinase and said second recombinase.

By "site-specific recombinase" is meant any enzyme that catalyzes conservative site-specific recombination between its corresponding recombination sites. For reviews of site-
30 specific recombinases, see Sauer (1994) *Current Opinion in Biotechnology* 5:521-527; and Sadowski (1993) *FASEB* 7:760-767.

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The first and second site-specific recombinases may be full length recombinases and/or active fragments or derivatives thereof. Site-specific recombinases useful for creating the chimeric recombinases of the invention, include
5 recombinases from the integrase family, derivatives thereof, and any other naturally occurring or recombinantly produced enzyme or derivative thereof, that catalyzes conservative site-specific recombination between specified DNA sites. The

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integrase family of recombinases has over thirty members and includes FLP, Cre, Int and R. Preferably, the recombinases do not require cofactors or a supercoiled substrate. Most preferably the recombinases are Cre and FLP. The bacteriophage P1 *loxP*-Cre and the *Saccharomyces* 2 μ plasmid FRT/FLP site-specific

5 recombinations systems have been extensively studied and their uses are well known to those skilled in the art. Cre and FLP are known to function in a variety of organisms, including bacteria, yeast, *Drosophila*, mammals and monocotyledonous and dicotyledonous plants. In addition these recombinases do not require auxiliary factors to function.

10 The site-specific recombinases and sequences encoding them that are used in the methods and compositions of the invention may be variants of naturally occurring recombinases and the genes encoding them. The term "conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers
15 to those nucleic acids which encode identical or conservatively modified variants of the amino acid sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be
20 altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations" and represent one species of conservatively modified variation. One of ordinary skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine) can be modified to yield a functionally identical molecule.

25 As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid

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with a chemically similar amino acid. Thus, any number of amino acid residues selected from the group of integers consisting of from 1 to 15 can be so altered. Thus, for example, 1, 2, 3, 4, 5, 7, or 10 alterations can be made. Conservatively modified variants typically provide similar biological activity as the unmodified polypeptide sequence from which they are derived. For example, substrate specificity, enzyme activity, or ligand/receptor binding is generally at least 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the native protein for its native substrate. Conservative substitution tables providing functionally similar amino acids are well known in the art.

10 The following six groups each contain amino acids that are conservative substitutions for one another:

- 1) Alanine (A), Serine (S), Threonine (T);
- 2) Aspartic acid (D), Glutamic acid (E);
- 3) Asparagine (N), Glutamine (Q);
- 15 4) Arginine (R), Lysine (K);
- 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and
- 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

See Creighton (1984) Proteins, W.H. Freeman and Company.

20 Rather than use full length recombinases, functional fragments of site-specific recombinases may be used in the methods and compositions of the invention. Functional fragments of site-specific recombinases can be identified using a variety of techniques. For example, functional fragments of the FLP protein may be identified by their ability, upon introduction to cells containing appropriate FRT substrates, to catalyze site-specific recombination and result in the excision of an assayable marker gene.

25 A general approach of such functional analysis involves subcloning DNA fragments of a genomic clone, cDNA clone or synthesized gene sequence into an expression vector, introducing the expression vector into a heterologous host, and screening to detect the product of recombination (*i.e.* using restriction analysis

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to verify the product of recombination at the nucleic acid level, or relying on an assay system for recombination as described above). Methods for generating fragments of a cDNA or genomic clone are well known. Variants of an isolated DNA encoding a site-specific recombinase can be produced by deleting, adding
5 and/or substituting nucleotides. Such variants can be obtained, for example, by oligonucleotide-directed mutagenesis, linker-scanning mutagenesis, mutagenesis using the polymerase chain reaction, and the like. See, for example, Ausubel, Current Protocols In Molecular Biology, Wiley Interscience (1990) pages 8.0.3 - 8.5.9, and McPherson (ed.), *Directed Mutagenesis: A Practical Approach*, (IRL Press, 1991).
10

The dual function recombinant proteins of the invention comprise a first site-specific recombinase fused in frame with a second site-specific recombinase. It will be recognized that in the methods of invention, the recombinases comprising the chimeric recombinase must correspond to the target
15 sites of the transformed organism and the targeting cassette. That is, if *FRT* and *loxP* sites are utilized, a chimeric FLP:Cre recombinase will be needed.

The open reading frames encoding the first and second recombinases may be directly fused to each other or may be joined by a linker that maintains the correct reading frame of the chimeric recombinase. It is understood that the
20 recombinases may be fused amino to carboxy terminus, amino to amino terminus, or carboxy to amino terminus.

Genes encoding chimeric site-specific recombinases and recombination sites can be made using standard recombinant methods, synthetic techniques, or combinations thereof. Use of cloning vectors, expression vectors,
25 adapters, and linkers is well known in the art and can be found in such references as Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd ed. (Cold Spring Harbor, New York, 1989). A variety of strategies are available for ligating fragments of DNA, the choice of which depends on the nature of the termini of the DNA fragments and which choices can be readily made by those of skill in the art.

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The FLP recombinase gene from yeast (*Saccharomyces cerevisiae*) is commercially available in plasmid pOG44 from Stratagene Cloning Systems (11011 North Torrey Pines Road, La Jolla, CA 92037). For a description of the FLP gene and various nucleic acids see, for example, Stratagene Cloning Systems, Catalogs 1995, 1996, 1997 (La Jolla, CA); and, Amersham Life Sciences, Inc, Catalog 1997 (Arlington Heights, IL). Similarly, the sequences of many other site-specific recombinases and their cognate recombination sites are publicly or commercially available. Genes encoding FLP and Cre can also be obtained, for example, by synthesizing the genes with mutually priming long oligonucleotides see, for example, Ausubel et al. (eds.), Current Protocols In Molecular Biology, pages 8.2.8 to 8.2.13, Wiley Interscience (1990). Also, see Wosniak et al. (1987) Gene 60:115. Moreover, current techniques using the polymerase chain reaction provide the ability to synthesize genes as large as 1.8 kilobases in length (Adang et al. (1993) *Plant Mol. Biol.* 21:1131; Bombat et al. (1993) *PCR Methods and Applications* 2:266).

When the nucleic acid is prepared or altered synthetically, advantage can be taken of known codon preferences of the intended host where the nucleic acid is to be expressed. For example, although nucleic acid sequences of the present invention may be expressed in both monocotyledonous and dicotyledonous plant species, sequences can be modified to account for the specific codon preferences and GC content preferences of monocotyledons and dicotyledons as these preferences have been shown to differ (Murray et al. (1989) *Nucl. Acids Res.* 17:477-498; and Campbell et al. (1990) *Plant Physiol.* 92:1). Thus, the maize preferred codon for a particular amino acid may be derived from known gene sequences from maize. Maize codon usage for 28 genes from maize plants are listed in Table 4 of Murray et al., *supra*.

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Examples of genes encoding recombinases, using maize preferred codons include, FLPm, described in U.S. Patent No. 5,929,301, and moCre, shown in SEQ. ID NOS. 1 and 2. FLPm is derived from the *Saccharomyces* 2 μ plasmid FLP

recombinase, but is encoded by a nucleic acid sequence utilizing maize-preferred codons. While the FLPm nucleic acid sequence includes preferred codons for expression of amino acids in maize, it is understood that a useful sequence may contain codons occurring in maize with less than the highest reported maize codon frequencies. Examples of nucleic acids encoding chimeric recombinases include
5 Cre:FLPm (SEQ. ID NO. 4), moCre:FLPm (SEQ. ID NO. 5), Cre:FLP (SEQ. ID NO. 7) and FLPm:Cre (SEQ. ID NO. 8).

The invention also provides expression cassettes containing a nucleic acid sequence encoding a chimeric site-specific recombinase, operably linked to a
10 promoter that drives expression in a eukaryotic cell. Preferably the promoter is a plant promoter. For example, the plant expression vector PHP13147, shown in Figure 1, contains an expression cassette for Cre:FLPm, wherein the gene encoding the chimeric recombinase is operably linked to a ubiquitin promoter. As used herein "operably linked" includes reference to a functional linkage between a
15 promoter and a second sequence, wherein the promoter sequence initiates and mediates transcription of the DNA sequence corresponding to the second sequence. Generally, operably linked means that the nucleic acid sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in the same reading frame.

20 As used herein "promoter" includes reference to a region of DNA upstream from the start of transcription and involved in recognition and binding of RNA polymerase and other proteins to initiate transcription. A "plant promoter" is a promoter capable of initiating transcription in plant cells. Exemplary plant promoters include, but are not limited to, those that are obtained from plants, plant
25 viruses, and bacteria genes that are expressed in plant cells such as those of *Agrobacterium* or *Rhizobium*. Both heterologous and non-heterologous (i.e., endogenous) promoters can be employed to direct expression of a sequence encoding a site-specific recombinase. The promoter may be constitutive, inducible or tissue specific.

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Many different constitutive promoters can be utilized in the instant invention. Exemplary constitutive promoters include the promoters from plant viruses such as the 35S promoter from CaMV (Odell *et al.* (1985) *Nature* 313:810-812) and the promoters from such gene as rice actin (McElroy *et al.* (1990) *Plant Cell* 2:163-171); ubiquitin (Christensen *et al.* (1989) *Plant Mol. Biol.* 12:619-632 and Christensen *et al.* (1992) *Plant Mol. Biol.* 18:675-689); pEMU (Last *et al.* (1991) *Theor. Appl. Genet.* 81:581-588); MAS (Velten *et al.* (1984) *EMBO J.* 3:2723-2730); maize H3 histone (Lepetit *et al.* (1992) *Mol. Gen. Genet.* 231: 276-285 and Atanassova *et al.* (1992) *Plant Journal* 2(3):291-300); the 1'- or 2'- promoter derived from T-DNA of *Agrobacterium tumefaciens*, the Smas promoter, the cinnamyl alcohol dehydrogenase promoter (U.S. Patent No. 5,683,439), the *Nos* promoter, the Pemu promoter, the rubisco promoter, the GRP1-8 promoter, and other transcription initiation regions from various plant genes known to those of skill. The ALS promoter, a XbaI/NcoI fragment 5-prime to the *Brassica napus* ALS3 structural gene (or a nucleotide sequence that has substantial sequence similarity to said XbaI/NcoI fragment), represents a particularly useful constitutive promoter. (See co-pending Pioneer Hi-Bred International US Patent Application 08/409,297.)

A variety of inducible promoters can be used in the instant invention. See Ward *et al.* (1993) *Plant Mol. Biol.* 22:361-366. Exemplary inducible promoters include that from the ACE1 system which responds to copper (Mett *et al.* (1993) *PNAS* 90:4567-4571); In2 gene from maize which responds to benzenesulfonamide herbicide safeners (Hershey *et al.* (1991) *Mol. Gen. Genetics* 227:229-237 and Gatz *et al.* (1994) *Mol. Gen. Genetics* 243:32-38); the Adh1 promoter which is inducible by hypoxia or cold stress, the Hsp70 promoter which is inducible by heat stress, and the PPDK promoter which is inducible by light; or Tet repressor from Tn10 (Gatz *et al.* (1991) *Mol. Gen. Genet.* 227:229-237. A particularly preferred inducible promoter is a promoter that responds to an inducing agent to which plants do not normally respond. An exemplary inducible promoter is

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the inducible promoter from a steroid hormone gene the transcriptional activity of which is induced by a glucocorticosteroid hormone (Schena *et al.* (1991) *Proc. Natl. Acad. Sci. U.S.A.* 88:10421).

5 Examples of promoters under developmental control include promoters that initiate transcription only, or preferentially, in certain tissues, such as leaves, roots, fruit, seeds, or flowers. The operation of a promoter may also vary depending on its location in the genome. Thus, an inducible promoter may become fully or partially constitutive in certain locations.

10 The chimeric recombinase must be expressed in the plant cell in order for integration of the DNA of interest into the host chromosome. Accordingly, the expression cassette encoding the site-specific recombinase may be supplied *in cis* to the DNA of interest; *in trans* on a host chromosome or extrachromosomal replicon; or may be transferred to the host and transiently expressed near to the time that recombination is desired.

15 The compositions of the invention include transfer cassettes comprising nucleotide sequences encoding the chimeric recombinases of the invention. By transfer cassette is meant any nucleotide sequence that may be used to transform a cell of interest. For example, the transfer cassette may be an independent replicon such as a plasmid, shuttle vector, Ti plasmid, viral vector or
20 the like. Alternatively, the transfer cassette could be a nucleic acid that is not capable of independent replication, yet could be transferred into an organism of interest by a variety of transformation protocols, such as particle bombardment, electroporation, and the like. Thus, the invention provides a transfer cassette comprising a nucleotide sequence encoding a recombinant protein comprising a first
25 site-specific recombinase fused in frame with a second site-specific recombinase, wherein said nucleotide sequence is operably linked to a promoter that drives expression in a eukaryotic cell.

In the compositions and methods of the invention, the DNA of interest is flanked by target sites for two distinct site-specific recombinases. By

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"flanked by" is meant that the recombination or target sites may be directly contiguous with the DNA of interest, or there may be one or more intervening sequences present between one or both ends of the DNA of interest and the site-specific

5 recombination sites. Intervening sequences of particular interest would include linkers, adapters, selectable markers and/or other sites which aid in vector construction or analysis and expression cassette for a gene of interest. Target sites for site-specific recombinases are known to those skilled in

10 the art and are discussed in WO 99/25854. Examples of target sites include, but are not limited to FRT, FRT1, FRT5, FRT6, FRT7, other FRT mutants, loxP, loxP mutants, and the like. See, for example, Schlake and Bode (1994) *Biochemistry* 33:12746-12751; Huang et al. (1991) *Nucleic Acids Research*

15 19:443-448; Sadowski (1995) *In Progress in Nucleic Acid Research and Molecular Biology* vol. 51, pp. 53-91; Cox (1989) *In Mobile DNA*, Berg and Howe (eds) American Society of Microbiology, Washington D.C., pp. 116-670; Dixon et al. (1995) 18:449-458; Umlauf and Cox (1988) *The EMBO Journal* 7:1845-1852;

20 Buchholz et al. (1996) *Nucleic Acids Research* 24:3118-3119; Kilby et al. (1993) *Trends Genet.* 9:413-421; Rossant and Geagy (1995) *Nat. Med.* 1:592-594; Lox Albert et al. (1995) *The Plant J.* 7:649-659; Bayley et al. (1992) *Plant Mol. Biol.* 18:353-361; Odell et al. (1990) *Mol. Gen. Genet.* 223:369-378; and Dale and

25 Ow (1991) *Proc. Natl. Acad. Sci. USA* 88:10558-105620; Qui et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:1706-1710; Stuurman et al. (1996) *Plant Mol. Biol.* 32:901-913; and Dale et al. (1990) *Gene* 91:79-85.

By "target site for a site-specific recombinase" is

30 meant a DNA sequence that is recognized by a particular site-specific recombinase. A variety of recombination sites are known to those skilled in the art and may be used in the methods and compositions of the invention. The site may have

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the sequence of the cognate site for a given recombinase, or
may be modified, so long as it is capable of acting as a
recombination site. The site may contain the minimal sequences
necessary for recombination, or it may contain additional
5 sequences that enhance

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recombination. Examples of recombination sites for use in the invention are known in the art and include FRT and *loxP* sites (See, for example, Schlake and Bode (1994) *Biochemistry* 33:12746-12751; Huang et al. (1991) *Nucleic Acids Research* 19:443-448; Paul D. Sadowski (1995) *In progress in Nucleic Acid Research and Molecular Biology* vol. 51, pp. 53-91; Michael M. Cox (1989) *In Mobile DNA*, Berg and Howe (eds) American Society of Microbiology, Washington D.C., pp. 116-670; Dixon et al. (1995) 18:449-458; Umlauf and Cox (1988) *The EMBO Journal* 7:1845-1852; Buchholz et al. (1996) *Nucleic Acids Research* 24:3118-3119; Kilby et al (1993) *Trends Genet.* 9:413-421; Rossant and Geagy (1995) *Nat. Med.* 1:592-594; Lox Albert et al. (1995) *The Plant J.* 7:649-659; Bayley et al. (1992) *Plant Mol. Biol.* 18:353-361; Odell et al. (1990) *Mol. Gen. Genet.* 223:369-378; and Dale and Ow (1991) *Proc. Natl. Acad. Sci. USA* 88:10558-105620; Qui et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:1706-1710; Stuurman et al. (1996) *Plant Mol. Biol.* 32:901-913; Hartley et al. (1980) *Nature* 286:860-864; Sauer (1994) *Current Opinion in Biotechnology* 5:521-527; and Dale et al. (1990) *Gene* 91:79-85.)

Each *loxP* and FRT site contains two 13 base pair inverted repeats which flank an 8 base pair spacer. The FRT site contains an additional non-essential 13 base pair repeats. The sequences of the *loxP* and FRT sites have been described in many publications. A minimal FRT site comprising two 13 base pair repeats, separated by an 8 base spacer, is:

5'-GAAGTTCCTATTC [TCTAGAAA] GTATAGGAACTTC3'

wherein the nucleotides within the brackets indicate the spacer region (SEQ ID NO:10). The nucleotides in the spacer region

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can be replaced with a combination of nucleotides, so long as the two 13-base repeats are separated by eight nucleotides. FLP is a conservative, site-specific recombinase, capable of catalyzing inversion of a nucleic acid sequence positioned
5 between two inversely oriented FRTs; recombination between two molecules each containing a FRT site; and excision between FRT sites. The core region is not symmetrical, and its asymmetry dictates the directionality of

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the reaction. Recombination between inverted FRT sites causes inversion of a DNA sequence between them, whereas recombination between directly oriented sites leads to excision of the DNA between them.

5 Nucleotide sequences containing a DNA of interest flanked by target sites, transfer cassettes for two distinct site-specific recombinases and vectors carrying these sequences may be constructed using standard molecular biology techniques. See, for example, Sambrook *et al.* (eds.) *Molecular Cloning: A Laboratory Manual*, Second Edition, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY 1989).

10 Techniques for transforming a wide variety of eukaryotic cells, including higher plant species are well known and described in the technical, scientific, and patent literature. See, for example, Weising *et al.*, *Ann. Rev. Genet.* 22: 421-477 (1988). These methods are useful for transforming a plant cell with the chimeric recombinase expression cassettes of the invention and DNAs of interest
15 flanked by target sites for the chimeric recombinase. The expression cassette encoding the site-specific recombinase may be present in the plant genome prior to transformation of the DNA of interest, or may be transformed into the plant around the time of transformation with the T-DNA to the plant cell so that it will be transiently expressed. For example, the DNA construct may be introduced directly
20 into the genomic DNA of the plant cell using techniques such as electroporation, PEG poration, particle bombardment, silicon fiber delivery, or microinjection of plant cell protoplasts or embryogenic callus.

Agrobacterium tumefaciens-mediated transformation techniques are well described in the scientific literature. See, for example Horsch *et al.*, *Science*
25 233: 496-498 (1984), Fraley *et al.*, *Proc. Natl. Acad. Sci.* 80: 4803 (1983) and Kado, (1991), *Crit. Rev. Plant Sci.* 10:1. Descriptions of the *Agrobacterium* vector systems and methods for *Agrobacterium*-mediated gene transfer are provide in Gruber *et al.*, *supra*; Miki, *et al.*, *supra*; and Moloney *et al.* (1989), *Plant Cell Reports* 8:238. Although *Agrobacterium* is useful primarily in dicots, certain

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monocots can be transformed by *Agrobacterium*. For instance, *Agrobacterium* transformation of maize is described in U.S. Patent No. 5,550,318. Other methods of agroinfection include *Agrobacterium rhizogenes*-mediated transformation (see, e.g., Lichtenstein and Fuller In: Genetic Engineering, vol. 6, PWJ Rigby, Ed., London, Academic Press, 1987; and Lichtenstein, C. P., and Draper, J., In: DNA Cloning, Vol. II, D. M. Glover, Ed., Oxford, IRI Press, 1985), Application PCT/US87/02512 (WO 88/02405 published Apr. 7, 1988) describes the use of *A. rhizogenes* strain A4 and its Ri plasmid along with *A. tumefaciens* vectors pARC8 or pARC16.

Optimized methods and vectors for *Agrobacterium*-mediated transformation of plants in the family Graminae, such as rice and maize have been described by Heath *et al.* (1997) *Mol. Plant-Microbe Interact.* 10:221-227; Hiei *et al.* (1994) *Plant J.* 6:271-282 and Ishida *et al.* (1996) *Nat. Biotech.* 14:745-750.

The efficiency of maize transformation is affected by a variety of factors including the types and stages of tissue infected, the concentration of *Agrobacterium*, the tissue culture media, the Ti vectors and the maize genotype. Super binary vectors carrying the vir genes of *Agrobacterium* strains A281 and A348 are useful for high efficiency transformation of monocots.

The introduction of DNA constructs using polyethylene glycol precipitation is described in Paszkowski *et al.*, *Embo J.* 3: 2717-2722 (1984). Electroporation techniques are described in Fromm *et al.*, *Proc. Natl. Acad. Sci.* 82: 5824 (1985). Ballistic transformation techniques are described in Klein *et al.*, *Nature* 327: 70-73 (1987).

Viral means of introducing DNA into mammalian cells are known in the art. In particular, a number of vector systems are known for the introduction of foreign or native genes into mammalian cells. These include SV40 virus (See, e.g., Okayama *et al.* (1985) *Molec. Cell Biol.* 5:1136-1142); Bovine papilloma virus (See, e.g., DiMaio *et al.* (1982) *Proc. Natl. Acad. Sci. USA* 79:4030-4034);

adenovirus (See, e.g., Morin *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:4626; Yifan *et al.* (1995) *Proc. Natl. Acad. Sci. USA* 92:1401-1405; Yang *et al.* (1996) *Gene Ther.* 3:137-144; Tripathy *et al.* (1996) *Nat. Med.* 2:545-550; Quantin *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:2581-2584; Rosenfeld *et al.* (1991) *Science* 252:431-434; Wagner (1992) *Proc. Natl. Acad. Sci. USA* 89:6099-6103; Curiel *et al.* (1992) *Human Gene Therapy* 3:147-154; Curiel (1991) *Proc. Natl. Acad. Sci. USA* 88:8850-8854; LeGal LaSalle *et al.* (1993) *Science* 259:590-599); Kass-Eisler *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:11498-11502); adeno-associated virus (See, e.g., Muzyczka *et al.* (1994) *J. Clin. Invest.* 94:1351; Xiao *et al.* (1996) *J. Virol.* 70:8098-8108); herpes simplex virus (See, e.g., Geller *et al.* (1988) *Science* 241:1667; Huard *et al.* (1995) *Gene Therapy* 2:385-392; U.S. Patent No. 5,501,979); retrovirus-based vectors (See, for example, Curran *et al.* (1982) *J. Virol.* 44:674-682; Gazit *et al.* (1986) *J. Virol.* 60:19-28; Miller, A.D. (1992) *Curr. Top. Microbiol. Immunol.* 158:1-24; Cavanaugh *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:7071-7075; Smith *et al.* (1990) *Molecular and Cellular Biology* 10:3268-3271). See also, Wu *et al.* (1991) *J. Biol. Chem.* 266:14338-14342; Wu and Wu (*J. Biol. Chem.* (1988)) 263:14621-14624; Wu *et al.* (1989) *J. Biol. Chem.* 264:16985-16987; Zenke *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:3655-3659; Wagner *et al.* (1990) 87:3410-3414.

DNA can also be introduced into plants by direct DNA transfer into pollen as described by Zhou *et al.*, *Methods in Enzymology*, 101:433 (1983); D. Hess, *Intern Rev. Cytol.*, 107:367 (1987); Luo *et al.*, *Plant Mol. Biol. Reporter*, 6:165 (1988). Expression of polypeptide coding genes can be obtained by injection of the DNA into reproductive organs of a plant as described by Pena *et al.*, *Nature*, 325:274 (1987). DNA can also be injected directly into the cells of immature embryos and the rehydration of desiccated embryos as described by Neuhaus *et al.*, *Theor. Appl. Genet.*, 75:30 (1987); and Benbrook *et al.*, in *Proceedings Bio Expo* 1986, Butterworth, Stoneham, Mass., pp. 27-54 (1986). A variety of plant viruses

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that can be employed as vectors are known in the art and include cauliflower mosaic virus (CaMV), geminivirus, brome mosaic virus, and tobacco mosaic virus.

Plants cells stably transformed with a chimeric recombinase expression cassette can be regenerated, e.g., from single cells, callus tissue or leaf discs according to standard plant tissue culture techniques. It is well known in the art that various cells, tissues, and organs from almost any plant can be successfully cultured to regenerate an entire plant. Plant regeneration from cultured protoplasts is described in Evans *et al.*, *Protoplasts Isolation and Culture, Handbook of Plant Cell Culture*, Macmillan Publishing Company, New York, pp. 124-176 (1983); and Binding, *Regeneration of Plants, Plant Protoplasts*, CRC Press, Boca Raton, pp. 21-73 (1985).

The regeneration of plants containing the recombinant genes can be achieved as described by Horsch *et al.*, *Science*, 227:1229-1231 (1985). In this procedure, transformants are grown in the presence of a selection agent and in a medium that induces the regeneration of shoots in the plant species being transformed as described by Fraley *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 80:4803 (1983). This procedure typically produces shoots within two to four weeks and these transformant shoots are then transferred to an appropriate root-inducing medium containing the selective agent and an antibiotic to prevent bacterial growth. Transgenic plants of the present invention may be fertile or sterile.

Regeneration can also be obtained from plant callus, explants, organs, or parts thereof. Such regeneration techniques are described generally in Klee *et al.*, *Ann. Rev. of Plant Phys.* 38: 467-486 (1987). The regeneration of plants from either single plant protoplasts or various explants is well known in the art. See, for example, *Methods for Plant Molecular Biology*, A. Weissbach and H. Weissbach, eds., Academic Press, Inc., San Diego, Calif. (1988). This regeneration and growth process includes the steps of selection of transformant cells and shoots, rooting the transformant shoots and growth of the plantlets in soil. For maize cell culture and regeneration see generally, *The Maize Handbook*, Freeling and Walbot,

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Eds., Springer, New York (1994); *Corn and Corn Improvement*, 3rd edition, Sprague and Dudley Eds., American Society of Agronomy, Madison, Wisconsin (1988).

5 One of skill will recognize that after a DNA, such as a chimeric recombinase expression cassette or target site for a chimeric recombinase is stably incorporated in transgenic plants and confirmed to be operable, it can be introduced into other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

10 The methods and compositions of the invention are useful to integrate a DNA of interest into the genome of any host cell, including any plant host. As used herein, the term "plant" includes reference to whole plants, plant organs (e.g., leaves, stems, roots, etc.), seeds and plant cells and progeny of same. Plant cell, as used herein includes, without limitation, seeds suspension cultures, embryos, meristematic regions, callus tissue, leaves, roots, shoots, gametophytes, sporophytes, pollen, and microspores. The class of plants which can be used in the methods of the invention is generally as broad as the class of higher plants amenable to transformation techniques, including both monocotyledonous and dicotyledonous plants. A particularly preferred monocot is maize. Other monocots of particular interest include wheat, rice, barley, sorghum and rye. Dicots of particular interest include soybean, Brassica, sunflower, alfalfa, and safflower.

20 Because of the use of the chimeric site-specific recombinases and target sites provided herein, the cells transformed by the methods of the invention may be distinguishable from other transformation methods as the modified cells of the invention will contain nucleotide sequences of interest inserted into the genome flanked by target sites for distinct recombinases.

25 The following examples are offered by way of illustration not by way of limitation.

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EXPERIMENTAL

Example 1

Construction of Vectors Containing a DNA of Interest Flanked By Target Sites For
a Chimeric Site-Specific Recombinase

5

DNA fragments containing a DNA of interest flanked by *loxP* and
FRT target sites are constructed either by synthesizing, annealing and ligating
complementary oligonucleotides or by creating primers for PCR amplification of a
DNA of interest with containing the *loxP* and FRT sites in addition to restriction
sites useful for cloning into a vector of choice.

10

For example, long PCR primers may be designed wherein the 3' end
of the primer hybridizes to the 5' end of the DNA of interest and the 5' end of the
primers further contain *loxP* or *FRT* sites and useful cloning sites. The resulting
PCR product is digested with the appropriate restriction enzyme and inserted into an
appropriate vector.

15

Example 2

Excision of FRT Site by FLPm and the Cre:FLPm Chimeric Recombinase

5 A transfer cassette encoding a Cre-FLPm chimeric recombinase was transformed into plant cells having an expression cassette encoding GUS driven by the ubiquitin promoter, wherein a sequence flanked by either identical FRT or loxP sites interrupted the GUS open reading frame. Figures 2 and 3 show that the Cre-FLPm chimeric recombinase is functional independently at either the FRT site or the loxP site, as measured by the ability to activate GUS activity following excision of sequences between two identical target sites, thereby bringing GUS activity under
10 the control of the ubiquitin promoter.

All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains.

15

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the
20 scope of the appended claims.

SEQUENCE LISTING

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<120> A Novel Method For The Integration Of Foreign DNA Into
Eukaryotic Genomes

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<140> 2,306,188

<141> 1998-11-17

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(moCRE)

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Asp	Ala	Thr	Ser	Asp	Glu	Val	Arg	Lys	Asn	Leu	Met	Asp	Met	Phe	Arg
			20					25					30		

Asp	Arg	Gln	Ala	Phe	Ser	Glu	His	Thr	Trp	Lys	Met	Leu	Leu	Ser	Val
		35					40					45			

Cys	Arg	Ser	Trp	Ala	Ala	Trp	Cys	Lys	Leu	Asn	Asn	Arg	Lys	Trp	Phe
	50					55					60				

Pro	Ala	Glu	Pro	Glu	Asp	Val	Arg	Asp	Tyr	Leu	Leu	Tyr	Leu	Gln	Ala
	65				70					75				80	

Arg	Gly	Leu	Ala	Val	Lys	Thr	Ile	Gln	Gln	His	Leu	Gly	Gln	Leu	Asn
				85					90					95	

Met	Leu	His	Arg	Arg	Ser	Gly	Leu	Pro	Arg	Pro	Ser	Asp	Ser	Asn	Ala
			100					105					110		

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Val Ser Leu Val Met Arg Arg Ile Arg Lys Glu Asn Val Asp Ala Gly
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Glu Arg Ala Lys Gln Ala Leu Ala Phe Glu Arg Thr Asp Phe Asp Gln
    130                                135                        140

Val Arg Ser Leu Met Glu Asn Ser Asp Arg Cys Gln Asp Ile Arg Asn
    145                                150                        155                        160

Leu Ala Phe Leu Gly Ile Ala Tyr Asn Thr Leu Leu Arg Ile Ala Glu
    165                                170                        175

Ile Ala Arg Ile Arg Val Lys Asp Ile Ser Arg Thr Asp Gly Gly Arg
    180                                185                        190

Met Leu Ile His Ile Gly Arg Thr Lys Thr Leu Val Ser Thr Ala Gly
    195                                200                        205

Val Glu Lys Ala Leu Ser Leu Gly Val Thr Lys Leu Val Glu Arg Trp
    210                                215                        220

Ile Ser Val Ser Gly Val Ala Asp Asp Pro Asn Asn Tyr Leu Phe Cys
    225                                230                        235                        240

Arg Val Arg Lys Asn Gly Val Ala Ala Pro Ser Ala Thr Ser Gln Leu
    245                                250                        255

Ser Thr Arg Ala Leu Glu Gly Ile Phe Glu Ala Thr His Arg Leu Ile
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Tyr Gly Ala Lys Asp Asp Ser Gly Gln Arg Tyr Leu Ala Trp Ser Gly
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His Ser Ala Arg Val Gly Ala Ala Arg Asp Met Ala Arg Ala Gly Val
    290                                295                        300

Ser Ile Pro Glu Ile Met Gln Ala Gly Gly Trp Thr Asn Val Asn Ile
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Val Met Asn Tyr Ile Arg Asn Leu Asp Ser Glu Thr Gly Ala Met Val
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      P1, maize preferred codons (moCRE)

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acctggaaga tgctgctctc cgtctgccgc tcctgggctg catggtgcaa gctgaacaac 180

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cgcgcccgca agaacggggg ggctgcccct agcgccacca gccaaactcag cacgaggggc 780
ttggaaggta ttttcgaggc caccaccgc ctgatctac gcgcgaagga tgacagcggt 840
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cgcgccgggt tttccatccc cgaaatcatg caggcgggtg gatggacgaa cgtgaacatt 960
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 <223> Description of Artificial Sequence: Cre:FLPm
 polypeptide, Cre from Bacteriophage P1 and FLP
 from Saccharomyces with maize preferred codons

<400> 3
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 1 5 10 15
 Asp Ala Thr Ser Asp Glu Val Arg Lys Asn Leu Met Asp Met Phe Arg
 20 25 30
 Asp Arg Gln Ala Phe Ser Glu His Thr Trp Lys Met Leu Leu Ser Val
 35 40 45
 Cys Arg Ser Trp Ala Ala Trp Cys Lys Leu Asn Asn Arg Lys Trp Phe
 50 55 60
 Pro Ala Glu Pro Glu Asp Val Arg Asp Tyr Leu Leu Tyr Leu Gln Ala
 65 70 75 80
 Arg Gly Leu Ala Val Lys Thr Ile Gln Gln His Leu Gly Gln Leu Asn
 85 90 95
 Met Leu His Arg Arg Ser Gly Leu Pro Arg Pro Ser Asp Ser Asn Ala
 100 105 110
 Val Ser Leu Val Met Arg Arg Ile Arg Lys Glu Asn Val Asp Ala Gly
 115 120 125
 Glu Arg Ala Lys Gln Ala Leu Ala Phe Glu Arg Thr Asp Phe Asp Gln
 130 135 140
 Val Arg Ser Leu Met Glu Asn Ser Asp Arg Cys Gln Asp Ile Arg Asn
 145 150 155 160
 Leu Ala Phe Leu Gly Ile Ala Tyr Asn Thr Leu Leu Arg Ile Ala Glu
 165 170 175

Ile	Ala	Arg	Ile	Arg	Val	Lys	Asp	Ile	Ser	Arg	Thr	Asp	Gly	Gly	Arg	180	185	190	
Met	Leu	Ile	His	Ile	Gly	Arg	Thr	Lys	Thr	Leu	Val	Ser	Thr	Ala	Gly	195	200	205	
Val	Glu	Lys	Ala	Leu	Ser	Leu	Gly	Val	Thr	Lys	Leu	Val	Glu	Arg	Trp	210	215	220	
Ile	Ser	Val	Ser	Gly	Val	Ala	Asp	Asp	Pro	Asn	Asn	Tyr	Leu	Phe	Cys	225	230	235	240
Arg	Val	Arg	Lys	Asn	Gly	Val	Ala	Ala	Pro	Ser	Ala	Thr	Ser	Gln	Leu	245	250	255	
Ser	Thr	Arg	Ala	Leu	Glu	Gly	Ile	Phe	Glu	Ala	Thr	His	Arg	Leu	Ile	260	265	270	
Tyr	Gly	Ala	Lys	Asp	Asp	Ser	Gly	Gln	Arg	Tyr	Leu	Ala	Trp	Ser	Gly	275	280	285	
His	Ser	Ala	Arg	Val	Gly	Ala	Ala	Arg	Asp	Met	Ala	Arg	Ala	Gly	Val	290	295	300	
Ser	Ile	Pro	Glu	Ile	Met	Gln	Ala	Gly	Gly	Trp	Thr	Asn	Val	Asn	Ile	305	310	315	320
Val	Met	Asn	Tyr	Ile	Arg	Asn	Leu	Asp	Ser	Glu	Thr	Gly	Ala	Met	Val	325	330	335	
Arg	Leu	Leu	Glu	Asp	Gly	Asp	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Gly	340	345	350	
Gly	Gly	Ser	Asp	Pro	Thr	Met	Pro	Gln	Phe	Asp	Ile	Leu	Cys	Lys	Thr	355	360	365	
Pro	Pro	Lys	Val	Leu	Val	Arg	Gln	Phe	Val	Glu	Arg	Phe	Glu	Arg	Pro	370	375	380	
Ser	Gly	Glu	Lys	Ile	Ala	Leu	Cys	Ala	Ala	Glu	Leu	Thr	Tyr	Leu	Cys	385	390	395	400
Trp	Met	Ile	Thr	His	Asn	Gly	Thr	Ala	Ile	Lys	Arg	Ala	Thr	Phe	Met	405	410	415	
Ser	Tyr	Asn	Thr	Ile	Ile	Ser	Asn	Ser	Leu	Ser	Phe	Asp	Ile	Val	Asn	420	425	430	
Lys	Ser	Leu	Gln	Phe	Lys	Tyr	Lys	Thr	Gln	Lys	Ala	Thr	Ile	Leu	Glu	435	440	445	
Ala	Ser	Leu	Lys	Lys	Leu	Ile	Pro	Ala	Trp	Glu	Phe	Thr	Ile	Ile	Pro	450	455	460	
Tyr	Tyr	Gly	Gln	Lys	His	Gln	Ser	Asp	Ile	Thr	Asp	Ile	Val	Ser	Ser	465	470	475	480
Leu	Gln	Leu	Gln	Phe	Glu	Ser	Ser	Glu	Glu	Ala	Asp	Lys	Gly	Asn	Ser	485	490	495	

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His Ser Lys Lys Met Leu Lys Ala Leu Leu Ser Glu Gly Glu Ser Ile
      500                      505                      510

Trp Glu Ile Thr Glu Lys Ile Leu Asn Ser Phe Glu Tyr Thr Ser Arg
      515                      520                      525

Phe Thr Lys Thr Lys Thr Leu Tyr Gln Phe Leu Phe Leu Ala Thr Phe
      530                      535                      540

Ile Asn Cys Gly Arg Phe Ser Asp Ile Lys Asn Val Asp Pro Lys Ser
545                      550                      555                      560

Phe Lys Leu Val Gln Asn Lys Tyr Leu Gly Val Ile Ile Gln Cys Leu
      565                      570                      575

Val Thr Glu Thr Lys Thr Ser Val Ser Arg His Ile Tyr Phe Phe Ser
      580                      585                      590

Ala Arg Gly Arg Ile Asp Pro Leu Val Tyr Leu Asp Glu Phe Leu Arg
      595                      600                      605

Asn Ser Glu Pro Val Leu Lys Arg Val Asn Arg Thr Gly Asn Ser Ser
      610                      615                      620

Ser Asn Lys Gln Glu Tyr Gln Leu Leu Lys Asp Asn Leu Val Arg Ser
625                      630                      635                      640

Tyr Asn Lys Ala Leu Lys Lys Asn Ala Pro Tyr Ser Ile Phe Ala Ile
      645                      650                      655

Lys Asn Gly Pro Lys Ser His Ile Gly Arg His Leu Met Thr Ser Phe
      660                      665                      670

Leu Ser Met Lys Gly Leu Thr Glu Leu Thr Asn Val Val Gly Asn Trp
      675                      680                      685

Ser Asp Lys Arg Ala Ser Ala Val Ala Arg Thr Thr Tyr Thr His Gln
      690                      695                      700

Ile Thr Ala Ile Pro Asp His Tyr Phe Ala Leu Val Ser Arg Tyr Tyr
705                      710                      715                      720

Ala Tyr Asp Pro Ile Ser Lys Glu Met Ile Ala Leu Lys Asp Glu Thr
      725                      730                      735

Asn Pro Ile Glu Glu Trp Gln His Ile Glu Gln Leu Lys Gly Ser Ala
      740                      745                      750

Glu Gly Ser Ile Arg Tyr Pro Ala Trp Asn Gly Ile Ile Ser Gln Glu
      755                      760                      765

Val Leu Asp Tyr Leu Ser Ser Tyr Ile Asn Arg Arg Ile
      770                      775                      780

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<210> 4

<211> 2346

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Nucleotide
sequence encoding a Cre:FLPm polypeptide, Cre
from Bacteriophage P1 and FLP (Maize preferred
codons) from Saccharomyces

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acctggaaaa tgcttctgtc cgtttgccgg tcgtgggagg catggtgcaa gttgaataac 180
cggaaatggg ttcccgcaga acctgaagat gttcgcgatt atcttctata tcttcaggcg 240
cgcggtctgg cagtaaaaac tatccagcaa catttgggccc agctaaacat gcttcatcgt 300
cgggtccgggc tgccacgacc aagtgcagac aatgctgttt cactgggttat gcggcggatc 360
cgaaaagaaa acgttgatgc cgggtgaacgt gcaaaacagg ctctagcgtt cgaacgcact 420
gatttcgacc aggttcgttc actcatggaa aatagcgatc gctgccagga tatacgtaat 480
ctggcatttc tggggattgc ttataacacc ctgttacgta tagccgaaat tgccaggatc 540
agggttaaag atatctcacg tactgacggg gggagaatgt taatccatat tggcagaacg 600
aaaacgctgg ttagcaccgc aggtgtagag aaggcactta gcctgggggt aactaaactg 660
gtcgagcgat ggatttcgtt ctctgggtgta gctgatgatc cgaataacta cctgttttgc 720
cgggtcagaa aaaatgggtg tgccgcgcca tctgccacca gccagctatc aactcgcgcc 780
ctggaaggga tttttgaagc aactcatcga ttgatttacg gcgctaagga tgactctggg 840
cagagatacc tggcctgggtc tggacacagt gcccggtgctg gagccgcgcg agatatggcc 900
cgcgctggag tttcaatacc ggagatcatg caagctgggt gctggaccaa tgtaaataat 960
gtcatgaact atatccgtaa cctgggatagt gaaacagggg caatgggtgcg cctgctggaa 1020
gatggcgatg gtggcggcag cgggtggcggc tccggcgggt gctcggatcc aacaatgcc 1080
cagttcgaca tcctctgcaa gacccccccc aagggtgctc tgaggcagtt cgtggagagg 1140
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tggatgatca cccacaacgg caccgccatt aagaggggcca ccttcatgtc atacaacacc 1260
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accagaagg ccaccatcct cgaggcctcc ctcaagaagc tcatccccgc ctgggagttc 1380
accatcatcc cctactacgg ccagaagcac cagtccgaca tcaccgacat cgtgtcatcc 1440
ctccagcttc agttcgagtc ctccgaggag gctgacaagg gcaactccca ctccaagaag 1500
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gtgtacctcg acgagttcct caggaactca gagcccgtgc tcaagagggt gaacaggacc 1860
ggcaactcct cctccaacaa gcaggagtag cagctcctca aggacaacct cgtgagggtcc 1920
tacaacaagg ccctcaagaa gaacgccccc tactccatct tcgccatcaa gaacggcccc 1980
aagtcccaca tcggtaggca cctcatgacc tccttctctt caatgaaggg cctcaccgag 2040
ctcaccaacg tgggtgggcaa ctggtccgac aagagggcct ccgcccgtggc caggaccacc 2100
tacaccacc agatcaccgc catccccgac cactacttcg ccctcgtgtc aaggactac 2160
gcctacgacc ccatctccaa ggagatgatc gccctcaagg acgagactaa ccccatcgag 2220
gagtggcagc acatcgagca gctcaagggc tccgccgagg gctccatcag gtaccccgcc 2280
tggaacggca tcatctccca ggagggtgctc gactacctct cctcctacat caacaggagg 2340
atctga 2346

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<210> 5

<211> 2346

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: sequence
encoding moCre:FLPm, Cre from Bacteriophage P1 and
FLP from Saccharomyces, both maize preferred
codons

<220>

<221> CDS

<222> (1) .. (2346)

<400> 5

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1 5 10 15	
gac gcg acg tcc gat gaa gtc agg aag aac ctc atg gac atg ttc cgc	96
Asp Ala Thr Ser Asp Glu Val Arg Lys Asn Leu Met Asp Met Phe Arg	
20 25 30	
gac agg caa gcg ttc agc gag cac acc tgg aag atg ctg ctc tcc gtc	144
Asp Arg Gln Ala Phe Ser Glu His Thr Trp Lys Met Leu Leu Ser Val	
35 40 45	
tgc cgc tcc tgg gct gca tgg tgc aag ctg aac aac agg aag tgg ttc	192
Cys Arg Ser Trp Ala Ala Trp Cys Lys Leu Asn Asn Arg Lys Trp Phe	
50 55 60	
ccc gct gag ccc gag gac gtg agg gat tac ctt ctg tac ctg caa gct	240
Pro Ala Glu Pro Glu Asp Val Arg Asp Tyr Leu Leu Tyr Leu Gln Ala	
65 70 75 80	
cgc ggg ctg gca gtg aag acc atc cag caa cac ctt gga caa ctg aac	288
Arg Gly Leu Ala Val Lys Thr Ile Gln Gln His Leu Gly Gln Leu Asn	
85 90 95	
atg ctt cac agg cgc tcc ggc ctc ccg cgc ccc agc gac tcg aac gcc	336
Met Leu His Arg Arg Ser Gly Leu Pro Arg Pro Ser Asp Ser Asn Ala	
100 105 110	
gtg agc ctc gtc atg cgc cgc atc agg aag gaa aac gtc gat gcc ggc	384
Val Ser Leu Val Met Arg Arg Ile Arg Lys Glu Asn Val Asp Ala Gly	
115 120 125	
gaa agg gca aag cag gcc ctc gcg ttc gag agg acc gat ttc gac cag	432
Glu Arg Ala Lys Gln Ala Leu Ala Phe Glu Arg Thr Asp Phe Asp Gln	
130 135 140	
gtc cgc agc ctg atg gag aac agc gac agg tgc cag gac att agg aac	480
Val Arg Ser Leu Met Glu Asn Ser Asp Arg Cys Gln Asp Ile Arg Asn	
145 150 155 160	
ctg gcg ttc ctc gga att gca tac aac acg ctc ctc agg atc gcg gaa	528
Leu Ala Phe Leu Gly Ile Ala Tyr Asn Thr Leu Leu Arg Ile Ala Glu	
165 170 175	
att gcc cgc att cgc gtg aag gac att agc cgc acc gac ggc ggc agg	576
Ile Ala Arg Ile Arg Val Lys Asp Ile Ser Arg Thr Asp Gly Gly Arg	
180 185 190	
atg ctt atc cac att ggc agg acc aag acg ctc gtt tcc acc gca ggc	624
Met Leu Ile His Ile Gly Arg Thr Lys Thr Leu Val Ser Thr Ala Gly	
195 200 205	
gtc gaa aag gcc ctc agc ctc gga gtg acc aag ctc gtc gaa cgc tgg	672
Val Glu Lys Ala Leu Ser Leu Gly Val Thr Lys Leu Val Glu Arg Trp	
210 215 220	

atc tcc gtg tcc ggc gtc gcg gac gac cca aac aac tac ctc ttc tgc	720
Ile Ser Val Ser Gly Val Ala Asp Asp Pro Asn Asn Tyr Leu Phe Cys	
225 230 235 240	
cgc gtc cgc aag aac ggg gtg gct gcc cct agc gcc acc agc caa ctc	768
Arg Val Arg Lys Asn Gly Val Ala Ala Pro Ser Ala Thr Ser Gln Leu	
245 250 255	
agc acg agg gcc ttg gaa ggt att ttc gag gcc acc cac cgc ctg atc	816
Ser Thr Arg Ala Leu Glu Gly Ile Phe Glu Ala Thr His Arg Leu Ile	
260 265 270	
tac ggc gcg aag gat gac agc ggt caa cgc tac ctc gca tgg tcc ggg	864
Tyr Gly Ala Lys Asp Asp Ser Gly Gln Arg Tyr Leu Ala Trp Ser Gly	
275 280 285	
cac tcc gcc cgc gtt gga gct gct agg gac atg gcc cgc gcc ggt gtt	912
His Ser Ala Arg Val Gly Ala Ala Arg Asp Met Ala Arg Ala Gly Val	
290 295 300	
tcc atc ccc gaa atc atg cag gcg ggt gga tgg acg aac gtg aac att	960
Ser Ile Pro Glu Ile Met Gln Ala Gly Gly Trp Thr Asn Val Asn Ile	
305 310 315 320	
gtc atg aac tac att cgc aac ctt gac agc gag acg ggc gca atg gtt	1008
Val Met Asn Tyr Ile Arg Asn Leu Asp Ser Glu Thr Gly Ala Met Val	
325 330 335	
cgc ctc ctg gaa gat ggc gat ggt ggc ggc agc ggt ggc ggc tcc ggc	1056
Arg Leu Leu Glu Asp Gly Asp Gly Gly Ser Gly Gly Gly Ser Gly	
340 345 350	
ggt ggc tcg gat cca aca atg ccc cag ttc gac atc ctc tgc aag acc	1104
Gly Gly Ser Asp Pro Thr Met Pro Gln Phe Asp Ile Leu Cys Lys Thr	
355 360 365	
ccc ccc aag gtg ctc gtg agg cag ttc gtg gag agg ttc gag agg ccc	1152
Pro Pro Lys Val Leu Val Arg Gln Phe Val Glu Arg Phe Glu Arg Pro	
370 375 380	
tcc ggc gag aag atc gcc ctc tgc gcc gcc gag ctc acc tac ctc tgc	1200
Ser Gly Glu Lys Ile Ala Leu Cys Ala Ala Glu Leu Thr Tyr Leu Cys	
385 390 395 400	
tgg atg atc acc cac aac ggc acc gcc att aag agg gcc acc ttc atg	1248
Trp Met Ile Thr His Asn Gly Thr Ala Ile Lys Arg Ala Thr Phe Met	
405 410 415	
tca tac aac acc atc atc tcc aac tcc ctc tcc ttc gac atc gtg aac	1296
Ser Tyr Asn Thr Ile Ile Ser Asn Ser Leu Ser Phe Asp Ile Val Asn	
420 425 430	
aag tcc ctc cag ttc aaa tac aag acc cag aag gcc acc atc ctc gag	1344
Lys Ser Leu Gln Phe Lys Tyr Lys Thr Gln Lys Ala Thr Ile Leu Glu	
435 440 445	
gcc tcc ctc aag aag ctc atc ccc gcc tgg gag ttc acc atc atc ccc	1392
Ala Ser Leu Lys Lys Leu Ile Pro Ala Trp Glu Phe Thr Ile Ile Pro	
450 455 460	

tac tac ggc cag aag cac cag tcc gac atc acc gac atc gtg tca tcc	1440
Tyr Tyr Gly Gln Lys His Gln Ser Asp Ile Thr Asp Ile Val Ser Ser	
465 470 475 480	
ctc cag ctt cag ttc gag tcc tcc gag gag gct gac aag ggc aac tcc	1488
Leu Gln Leu Gln Phe Glu Ser Ser Glu Glu Ala Asp Lys Gly Asn Ser	
485 490 495	
cac tcc aag aag atg ctg aag gcc ctc ctc tcc gag ggc gag tcc atc	1536
His Ser Lys Lys Met Leu Lys Ala Leu Leu Ser Glu Gly Glu Ser Ile	
500 505 510	
tgg gag atc acc gag aag atc ctc aac tcc ttc gag tac acc tcc agg	1584
Trp Glu Ile Thr Glu Lys Ile Leu Asn Ser Phe Glu Tyr Thr Ser Arg	
515 520 525	
ttc act aag acc aag acc ctc tac cag ttc ctc ttc ctc gcc acc ttc	1632
Phe Thr Lys Thr Lys Thr Leu Tyr Gln Phe Leu Phe Leu Ala Thr Phe	
530 535 540	
atc aac tgc ggc agg ttc tca gac atc aag aac gtg gac ccc aag tcc	1680
Ile Asn Cys Gly Arg Phe Ser Asp Ile Lys Asn Val Asp Pro Lys Ser	
545 550 555 560	
ttc aag ctc gtg cag aac aag tac ctc ggc gtg atc atc cag tgc ctc	1728
Phe Lys Leu Val Gln Asn Lys Tyr Leu Gly Val Ile Ile Gln Cys Leu	
565 570 575	
gtg acc gag acc aag acc tcc gtg tcc agg cac atc tac ttc ttc tcc	1776
Val Thr Glu Thr Lys Thr Ser Val Ser Arg His Ile Tyr Phe Phe Ser	
580 585 590	
gct cgc ggc agg atc gac ccc ctc gtg tac ctc gac gag ttc ctc agg	1824
Ala Arg Gly Arg Ile Asp Pro Leu Val Tyr Leu Asp Glu Phe Leu Arg	
595 600 605	
aac tca gag ccc gtg ctc aag agg gtg aac agg acc ggc aac tcc tcc	1872
Asn Ser Glu Pro Val Leu Lys Arg Val Asn Arg Thr Gly Asn Ser Ser	
610 615 620	
tcc aac aag cag gag tac cag ctc ctc aag gac aac ctc gtg agg tcc	1920
Ser Asn Lys Gln Glu Tyr Gln Leu Leu Lys Asp Asn Leu Val Arg Ser	
625 630 635 640	
tac aac aag gcc ctc aag aag aac gcc ccc tac tcc atc ttc gcc atc	1968
Tyr Asn Lys Ala Leu Lys Lys Asn Ala Pro Tyr Ser Ile Phe Ala Ile	
645 650 655	
aag aac ggc ccc aag tcc cac atc ggt agg cac ctc atg acc tcc ttc	2016
Lys Asn Gly Pro Lys Ser His Ile Gly Arg His Leu Met Thr Ser Phe	
660 665 670	
ctc tca atg aag ggc ctc acc gag ctc acc aac gtg gtg ggc aac tgg	2064
Leu Ser Met Lys Gly Leu Thr Glu Leu Thr Asn Val Val Gly Asn Trp	
675 680 685	
tcc gac aag agg gcc tcc gcc gtg gcc agg acc acc tac acc cac cag	2112
Ser Asp Lys Arg Ala Ser Ala Val Ala Arg Thr Thr Tyr Thr His Gln	
690 695 700	

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atc acc gcc atc ccc gac cac tac ttc gcc ctc gtg tca agg tac tac 2160
Ile Thr Ala Ile Pro Asp His Tyr Phe Ala Leu Val Ser Arg Tyr Tyr
705                      710                      715                      720

gcc tac gac ccc atc tcc aag gag atg atc gcc ctc aag gac gag act 2208
Ala Tyr Asp Pro Ile Ser Lys Glu Met Ile Ala Leu Lys Asp Glu Thr
                      725                      730                      735

aac ccc atc gag gag tgg cag cac atc gag cag ctc aag ggc tcc gcc 2256
Asn Pro Ile Glu Glu Trp Gln His Ile Glu Gln Leu Lys Gly Ser Ala
                      740                      745                      750

gag ggc tcc atc agg tac ccc gcc tgg aac ggc atc atc tcc cag gag 2304
Glu Gly Ser Ile Arg Tyr Pro Ala Trp Asn Gly Ile Ile Ser Gln Glu
                      755                      760                      765

gtg ctc gac tac ctc tcc tcc tac atc aac agg agg atc tga 2346
Val Leu Asp Tyr Leu Ser Ser Tyr Ile Asn Arg Arg Ile
                      770                      775                      780

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<210> 6
<211> 781
<212> PRT
<213> Artificial Sequence

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<220>
<223> Description of Artificial Sequence: sequence
      encoding moCre:FLPm, Cre from Bacteriophage P1 and
      FLP from Saccharomyces, both maize preferred
      codons

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<400> 6
Met Ser Asn Leu Leu Thr Val His Gln Asn Leu Pro Ala Leu Pro Val
  1                      5                      10                      15

Asp Ala Thr Ser Asp Glu Val Arg Lys Asn Leu Met Asp Met Phe Arg
      20                      25                      30

Asp Arg Gln Ala Phe Ser Glu His Thr Trp Lys Met Leu Leu Ser Val
      35                      40                      45

Cys Arg Ser Trp Ala Ala Trp Cys Lys Leu Asn Asn Arg Lys Trp Phe
      50                      55                      60

Pro Ala Glu Pro Glu Asp Val Arg Asp Tyr Leu Leu Tyr Leu Gln Ala
      65                      70                      75                      80

Arg Gly Leu Ala Val Lys Thr Ile Gln Gln His Leu Gly Gln Leu Asn
      85                      90                      95

Met Leu His Arg Arg Ser Gly Leu Pro Arg Pro Ser Asp Ser Asn Ala
      100                     105                     110

Val Ser Leu Val Met Arg Arg Ile Arg Lys Glu Asn Val Asp Ala Gly
      115                     120                     125

Glu Arg Ala Lys Gln Ala Leu Ala Phe Glu Arg Thr Asp Phe Asp Gln
      130                     135                     140

```

Val	Arg	Ser	Leu	Met	Glu	Asn	Ser	Asp	Arg	Cys	Gln	Asp	Ile	Arg	Asn	145	150	155	160
Leu	Ala	Phe	Leu	Gly	Ile	Ala	Tyr	Asn	Thr	Leu	Leu	Arg	Ile	Ala	Glu	165	170	175	
Ile	Ala	Arg	Ile	Arg	Val	Lys	Asp	Ile	Ser	Arg	Thr	Asp	Gly	Gly	Arg	180	185	190	
Met	Leu	Ile	His	Ile	Gly	Arg	Thr	Lys	Thr	Leu	Val	Ser	Thr	Ala	Gly	195	200	205	
Val	Glu	Lys	Ala	Leu	Ser	Leu	Gly	Val	Thr	Lys	Leu	Val	Glu	Arg	Trp	210	215	220	
Ile	Ser	Val	Ser	Gly	Val	Ala	Asp	Asp	Pro	Asn	Asn	Tyr	Leu	Phe	Cys	225	230	235	240
Arg	Val	Arg	Lys	Asn	Gly	Val	Ala	Ala	Pro	Ser	Ala	Thr	Ser	Gln	Leu	245	250	255	
Ser	Thr	Arg	Ala	Leu	Glu	Gly	Ile	Phe	Glu	Ala	Thr	His	Arg	Leu	Ile	260	265	270	
Tyr	Gly	Ala	Lys	Asp	Asp	Ser	Gly	Gln	Arg	Tyr	Leu	Ala	Trp	Ser	Gly	275	280	285	
His	Ser	Ala	Arg	Val	Gly	Ala	Ala	Arg	Asp	Met	Ala	Arg	Ala	Gly	Val	290	295	300	
Ser	Ile	Pro	Glu	Ile	Met	Gln	Ala	Gly	Gly	Trp	Thr	Asn	Val	Asn	Ile	305	310	315	320
Val	Met	Asn	Tyr	Ile	Arg	Asn	Leu	Asp	Ser	Glu	Thr	Gly	Ala	Met	Val	325	330	335	
Arg	Leu	Leu	Glu	Asp	Gly	Asp	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Gly	340	345	350	
Gly	Gly	Ser	Asp	Pro	Thr	Met	Pro	Gln	Phe	Asp	Ile	Leu	Cys	Lys	Thr	355	360	365	
Pro	Pro	Lys	Val	Leu	Val	Arg	Gln	Phe	Val	Glu	Arg	Phe	Glu	Arg	Pro	370	375	380	
Ser	Gly	Glu	Lys	Ile	Ala	Leu	Cys	Ala	Ala	Glu	Leu	Thr	Tyr	Leu	Cys	385	390	395	400
Trp	Met	Ile	Thr	His	Asn	Gly	Thr	Ala	Ile	Lys	Arg	Ala	Thr	Phe	Met	405	410	415	
Ser	Tyr	Asn	Thr	Ile	Ile	Ser	Asn	Ser	Leu	Ser	Phe	Asp	Ile	Val	Asn	420	425	430	
Lys	Ser	Leu	Gln	Phe	Lys	Tyr	Lys	Thr	Gln	Lys	Ala	Thr	Ile	Leu	Glu	435	440	445	
Ala	Ser	Leu	Lys	Lys	Leu	Ile	Pro	Ala	Trp	Glu	Phe	Thr	Ile	Ile	Pro	450	455	460	

Tyr	Tyr	Gly	Gln	Lys	His	Gln	Ser	Asp	Ile	Thr	Asp	Ile	Val	Ser	Ser	465	470	475	480
Leu	Gln	Leu	Gln	Phe	Glu	Ser	Ser	Glu	Glu	Ala	Asp	Lys	Gly	Asn	Ser		485	490	495
His	Ser	Lys	Lys	Met	Leu	Lys	Ala	Leu	Leu	Ser	Glu	Gly	Glu	Ser	Ile		500	505	510
Trp	Glu	Ile	Thr	Glu	Lys	Ile	Leu	Asn	Ser	Phe	Glu	Tyr	Thr	Ser	Arg		515	520	525
Phe	Thr	Lys	Thr	Lys	Thr	Leu	Tyr	Gln	Phe	Leu	Phe	Leu	Ala	Thr	Phe		530	535	540
Ile	Asn	Cys	Gly	Arg	Phe	Ser	Asp	Ile	Lys	Asn	Val	Asp	Pro	Lys	Ser	545	550	555	560
Phe	Lys	Leu	Val	Gln	Asn	Lys	Tyr	Leu	Gly	Val	Ile	Ile	Gln	Cys	Leu		565	570	575
Val	Thr	Glu	Thr	Lys	Thr	Ser	Val	Ser	Arg	His	Ile	Tyr	Phe	Phe	Ser		580	585	590
Ala	Arg	Gly	Arg	Ile	Asp	Pro	Leu	Val	Tyr	Leu	Asp	Glu	Phe	Leu	Arg		595	600	605
Asn	Ser	Glu	Pro	Val	Leu	Lys	Arg	Val	Asn	Arg	Thr	Gly	Asn	Ser	Ser	610	615	620	
Ser	Asn	Lys	Gln	Glu	Tyr	Gln	Leu	Leu	Lys	Asp	Asn	Leu	Val	Arg	Ser	625	630	635	640
Tyr	Asn	Lys	Ala	Leu	Lys	Lys	Asn	Ala	Pro	Tyr	Ser	Ile	Phe	Ala	Ile		645	650	655
Lys	Asn	Gly	Pro	Lys	Ser	His	Ile	Gly	Arg	His	Leu	Met	Thr	Ser	Phe		660	665	670
Leu	Ser	Met	Lys	Gly	Leu	Thr	Glu	Leu	Thr	Asn	Val	Val	Gly	Asn	Trp		675	680	685
Ser	Asp	Lys	Arg	Ala	Ser	Ala	Val	Ala	Arg	Thr	Thr	Tyr	Thr	His	Gln	690	695	700	
Ile	Thr	Ala	Ile	Pro	Asp	His	Tyr	Phe	Ala	Leu	Val	Ser	Arg	Tyr	Tyr	705	710	715	720
Ala	Tyr	Asp	Pro	Ile	Ser	Lys	Glu	Met	Ile	Ala	Leu	Lys	Asp	Glu	Thr		725	730	735
Asn	Pro	Ile	Glu	Glu	Trp	Gln	His	Ile	Glu	Gln	Leu	Lys	Gly	Ser	Ala		740	745	750
Glu	Gly	Ser	Ile	Arg	Tyr	Pro	Ala	Trp	Asn	Gly	Ile	Ile	Ser	Gln	Glu		755	760	765
Val	Leu	Asp	Tyr	Leu	Ser	Ser	Tyr	Ile	Asn	Arg	Arg	Ile				770	775	780	

<210> 7
 <211> 2346
 <212> DNA
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: sequence
 encoding a Cre:FLP polypeptide , Cre from
 Bacteriophage P1 and FLP from Saccharomyces

<400> 7

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gatgaggttc gcaagaacct gatggacatg ttcagggatc gccaggcggt ttctgagcat 120
acctggaaaa tgcttctgtc cgtttgccgg tcgtgggagg catggtgcaa gttgaataac 180
cggaaatggg ttcccgcaga acctgaagat gttcgcgatt atcttctata tcttcaggcg 240
cgcggtctgg cagtaaaaac tatccagcaa catttgggccc agctaaacat gcttcatcgt 300
cgggtccgggc tgccacgacc aagtgcagac aatgctgttt cactgggttat gcggcggatc 360
cgaaaagaaa acgttgatgc cgggtgaacgt gcaaaacagg ctctagcggt cgaacgcact 420
gatttcgacc aggttcgttc actcatggaa aatagcgatc gctgccagga tatacgtaat 480
ctggcatttc tggggattgc ttataacacc ctgttacgta tagccgaaat tgccaggatc 540
agggttaaag atatctcacg tactgacggt gggagaatgt taatccatat tggcagaacg 600
aaaacgctgg ttagcacccg aggtgtagag aaggcactta gcctgggggt aactaaactg 660
gtcgagcgat ggatttcctg ctctggtgta gctgatgatc cgaataacta cctgttttgc 720
cgggtcagaa aaaatgggtg tgccgcgcca tctgccacca gccagctatc aactcgcgcc 780
ctggaaggga tttttgaagc aactcatcga ttgatttacg gcgctaagga tgactctggt 840
cagagatacc tggcctggtc tggacacagt gcccgtgtcg gagccgcgcg agatatggcc 900
cgcgctggag tttcaatacc ggagatcatg caagctggtg gctggaccaa tgtaaataat 960
gtcatgaact atatccgtaa cctggatagt gaaacagggg caatggtgcg cctgctggaa 1020
gatggcgatg gtggcggcag cgggtggcgg tccggcgggt gctcggatcc aacaatgcca 1080
caatttgata tattatgtaa aacaccacct aagggtgctt ttcgtcagtt tgtggaaagg 1140
tttgagagac cttccggaga gaaaatagca ttatgtgctg ctgaactaac ctatttatgt 1200
tggatgatta cacataacgg aacagcaatc aagagagcca cattcatgag ctataatact 1260
atcataagca attcgctgag tttggatatc gtcaacaagt cactgcagtt taaatacaag 1320
acgcaaaaag caacaattct ggaagcctca ttaaagaaat tgattcctgc ttgggaattt 1380
acaattattc cttactatgg acaaaaacat caatctgata tcactgatat tgtaagtagt 1440
ttgcaattac agttcgaatc atcgggaaga gcagataagg gaaatagcca cagtaaaaaa 1500
atgcttaaag cacttctaag tgagggtgaa agcatctggg agatcactga gaaaatacta 1560
aattcgtttg agtatacttc gagatttaca aaaacaaaaa ctttatacca attcctcttc 1620
ctagctactt tcatcaattg tgggaagatt agcgatatta agaacggtga tccgaaatca 1680
tttaaattag tccaaaataa gtatctggga gtaataatcc agtgtttagt gacagagaca 1740
aagacaagcg ttagtaggca catatacttc tttagcgcaa ggggtaggat cgatccactt 1800
gtatattttg atgaattttt gaggaattct gaaccagtcc taaaacgagt aaataggacc 1860
ggcaattctt caagcaacaa gcaggaatac caattattaa aagataactt agtcagatcg 1920
tacaacaaag ctttgaagaa aaatgcgcct tattcaatct ttgctataaa aaatggccca 1980
aaatctcaca ttggaagaca tttgatgacc tcatttcttt caatgaaggg cctaacggag 2040
ttgactaatg ttgtgggaaa ttggagcgat aagcgtgctt ctgccgtggc caggacaacg 2100
tatactcatc agataacagc aatacctgat cactacttcg cactagtctt tcggtactat 2160
gcatatgatc caatatcaaa ggaaatgata gcattgaagg atgagactaa tccaattgag 2220
gagtggcagc atatagaaca gctaaagggt agtgctgaag gaagcatacg ataccccgca 2280
tggaatggga taatatcaca ggagggtacta gactaccttt catcctacat aaatagacgc 2340
atataa                                     2346

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<210> 8
 <211> 2346
 <212> DNA
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Sequence
encoding a FLPm:Cre polypeptide, FLP from
Saccharomyces (maize preferred codons), and Cre
from Bacteriophage P1

<220>

<221> CDS

<222> (1)..(2346)

<400> 8

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Met Pro Gln Phe Asp Ile Leu Cys Lys Thr Pro Pro Lys Val Leu Val	
1 5 10 15	
agg cag ttc gtg gag agg ttc gag agg ccc tcc ggc gag aag atc gcc	96
Arg Gln Phe Val Glu Arg Phe Glu Arg Pro Ser Gly Glu Lys Ile Ala	
20 25 30	
ctc tgc gcc gcc gag ctc acc tac ctc tgc tgg atg atc acc cac aac	144
Leu Cys Ala Ala Glu Leu Thr Tyr Leu Cys Trp Met Ile Thr His Asn	
35 40 45	
ggc acc gcc att aag agg gcc acc ttc atg tca tac aac acc atc atc	192
Gly Thr Ala Ile Lys Arg Ala Thr Phe Met Ser Tyr Asn Thr Ile Ile	
50 55 60	
tcc aac tcc ctc tcc ttc gac atc gtg aac aag tcc ctc cag ttc aaa	240
Ser Asn Ser Leu Ser Phe Asp Ile Val Asn Lys Ser Leu Gln Phe Lys	
65 70 75 80	
tac aag acc cag aag gcc acc atc ctc gag gcc tcc ctc aag aag ctc	288
Tyr Lys Thr Gln Lys Ala Thr Ile Leu Glu Ala Ser Leu Lys Lys Leu	
85 90 95	
atc ccc gcc tgg gag ttc acc atc atc ccc tac tac ggc cag aag cac	336
Ile Pro Ala Trp Glu Phe Thr Ile Ile Pro Tyr Tyr Gly Gln Lys His	
100 105 110	
cag tcc gac atc acc gac atc gtg tca tcc ctc cag ctt cag ttc gag	384
Gln Ser Asp Ile Thr Asp Ile Val Ser Ser Leu Gln Leu Gln Phe Glu	
115 120 125	
tcc tcc gag gag gct gac aag ggc aac tcc cac tcc aag aag atg ctg	432
Ser Ser Glu Glu Ala Asp Lys Gly Asn Ser His Ser Lys Lys Met Leu	
130 135 140	
aag gcc ctc ctc tcc gag ggc gag tcc atc tgg gag atc acc gag aag	480
Lys Ala Leu Leu Ser Glu Gly Glu Ser Ile Trp Glu Ile Thr Glu Lys	
145 150 155 160	
atc ctc aac tcc ttc gag tac acc tcc agg ttc act aag acc aag acc	528
Ile Leu Asn Ser Phe Glu Tyr Thr Ser Arg Phe Thr Lys Thr Lys Thr	
165 170 175	
ctc tac cag ttc ctc ttc ctc gcc acc ttc atc aac tgc ggc agg ttc	576
Leu Tyr Gln Phe Leu Phe Leu Ala Thr Phe Ile Asn Cys Gly Arg Phe	
180 185 190	

tca gac atc aag aac gtg gac ccc aag tcc ttc aag ctc gtg cag aac	624
Ser Asp Ile Lys Asn Val Asp Pro Lys Ser Phe Lys Leu Val Gln Asn	
195 200 205	
aag tac ctc ggc gtg atc atc cag tgc ctc gtg acc gag acc aag acc	672
Lys Tyr Leu Gly Val Ile Ile Gln Cys Leu Val Thr Glu Thr Lys Thr	
210 215 220	
tcc gtg tcc agg cac atc tac ttc ttc tcc gct cgc ggc agg atc gac	720
Ser Val Ser Arg His Ile Tyr Phe Phe Ser Ala Arg Gly Arg Ile Asp	
225 230 235 240	
ccc ctc gtg tac ctc gac gag ttc ctc agg aac tca gag ccc gtg ctc	768
Pro Leu Val Tyr Leu Asp Glu Phe Leu Arg Asn Ser Glu Pro Val Leu	
245 250 255	
aag agg gtg aac agg acc ggc aac tcc tcc tcc aac aag cag gag tac	816
Lys Arg Val Asn Arg Thr Gly Asn Ser Ser Ser Asn Lys Gln Glu Tyr	
260 265 270	
cag ctc ctc aag gac aac ctc gtg agg tcc tac aac aag gcc ctc aag	864
Gln Leu Leu Lys Asp Asn Leu Val Arg Ser Tyr Asn Lys Ala Leu Lys	
275 280 285	
aag aac gcc ccc tac tcc atc ttc gcc atc aag aac ggc ccc aag tcc	912
Lys Asn Ala Pro Tyr Ser Ile Phe Ala Ile Lys Asn Gly Pro Lys Ser	
290 295 300	
cac atc ggt agg cac ctc atg acc tcc ttc ctc tca atg aag ggc ctc	960
His Ile Gly Arg His Leu Met Thr Ser Phe Leu Ser Met Lys Gly Leu	
305 310 315 320	
acc gag ctc acc aac gtg gtg ggc aac tgg tcc gac aag agg gcc tcc	1008
Thr Glu Leu Thr Asn Val Val Gly Asn Trp Ser Asp Lys Arg Ala Ser	
325 330 335	
gcc gtg gcc agg acc acc tac acc cac cag atc acc gcc atc ccc gac	1056
Ala Val Ala Arg Thr Thr Tyr Thr His Gln Ile Thr Ala Ile Pro Asp	
340 345 350	
cac tac ttc gcc ctc gtg tca agg tac tac gcc tac gac ccc atc tcc	1104
His Tyr Phe Ala Leu Val Ser Arg Tyr Tyr Ala Tyr Asp Pro Ile Ser	
355 360 365	
aag gag atg atc gcc ctc aag gac gag act aac ccc atc gag gag tgg	1152
Lys Glu Met Ile Ala Leu Lys Asp Glu Thr Asn Pro Ile Glu Glu Trp	
370 375 380	
cag cac atc gag cag ctc aag ggc tcc gcc gag ggc tcc atc agg tac	1200
Gln His Ile Glu Gln Leu Lys Gly Ser Ala Glu Gly Ser Ile Arg Tyr	
385 390 395 400	
ccc gcc tgg aac ggc atc atc tcc cag gag gtg ctc gac tac ctc tcc	1248
Pro Ala Trp Asn Gly Ile Ile Ser Gln Glu Val Leu Asp Tyr Leu Ser	
405 410 415	
tcc tac atc aac agg agg atc ggt ggc ggc agc ggt ggc ggc tcc ggc	1296
Ser Tyr Ile Asn Arg Arg Ile Gly Gly Gly Ser Gly Gly Gly Ser Gly	
420 425 430	

ggt ggc tcg gat cca acc atg gcc aat tta ctg acc gta cac caa aat	1344
Gly Gly Ser Asp Pro Thr Met Ala Asn Leu Leu Thr Val His Gln Asn	
435 440 445	
ttg cct gca tta ccg gtc gat gca acg agt gat gag gtt cgc aag aac	1392
Leu Pro Ala Leu Pro Val Asp Ala Thr Ser Asp Glu Val Arg Lys Asn	
450 455 460	
ctg atg gac atg ttc agg gat cgc cag gcg ttt tct gag cat acc tgg	1440
Leu Met Asp Met Phe Arg Asp Arg Gln Ala Phe Ser Glu His Thr Trp	
465 470 475 480	
aaa atg ctt ctg tcc gtt tgc cgg tcg tgg gcg gca tgg tgc aag ttg	1488
Lys Met Leu Leu Ser Val Cys Arg Ser Trp Ala Ala Trp Cys Lys Leu	
485 490 495	
aat aac cgg aaa tgg ttt ccc gca gaa cct gaa gat gtt cgc gat tat	1536
Asn Asn Arg Lys Trp Phe Pro Ala Glu Pro Glu Asp Val Arg Asp Tyr	
500 505 510	
ctt cta tat ctt cag gcg cgc ggt ctg gca gta aaa act atc cag caa	1584
Leu Leu Tyr Leu Gln Ala Arg Gly Leu Ala Val Lys Thr Ile Gln Gln	
515 520 525	
cat ttg ggc cag cta aac atg ctt cat cgt cgg tcc ggg ctg cca cga	1632
His Leu Gly Gln Leu Asn Met Leu His Arg Arg Ser Gly Leu Pro Arg	
530 535 540	
cca agt gac agc aat gct gtt tca ctg gtt atg cgg cgg atc cga aaa	1680
Pro Ser Asp Ser Asn Ala Val Ser Leu Val Met Arg Arg Ile Arg Lys	
545 550 555 560	
gaa aac gtt gat gcc ggt gaa cgt gca aaa cag gct cta gcg ttc gaa	1728
Glu Asn Val Asp Ala Gly Glu Arg Ala Lys Gln Ala Leu Ala Phe Glu	
565 570 575	
cgc act gat ttc gac cag gtt cgt tca ctc atg gaa aat agc gat cgc	1776
Arg Thr Asp Phe Asp Gln Val Arg Ser Leu Met Glu Asn Ser Asp Arg	
580 585 590	
tgc cag gat ata cgt aat ctg gca ttt ctg ggg att gct tat aac acc	1824
Cys Gln Asp Ile Arg Asn Leu Ala Phe Leu Gly Ile Ala Tyr Asn Thr	
595 600 605	
ctg tta cgt ata gcc gaa att gcc agg atc agg gtt aaa gat atc tca	1872
Leu Leu Arg Ile Ala Glu Ile Ala Arg Ile Arg Val Lys Asp Ile Ser	
610 615 620	
cgt act gac ggt ggg aga atg tta atc cat att ggc aga acg aaa acg	1920
Arg Thr Asp Gly Gly Arg Met Leu Ile His Ile Gly Arg Thr Lys Thr	
625 630 635 640	
ctg gtt agc acc gca ggt gta gag aag gca ctt agc ctg ggg gta act	1968
Leu Val Ser Thr Ala Gly Val Glu Lys Ala Leu Ser Leu Gly Val Thr	
645 650 655	
aaa ctg gtc gag cga tgg att tcc gtc tct ggt gta gct gat gat ccg	2016
Lys Leu Val Glu Arg Trp Ile Ser Val Ser Gly Val Ala Asp Asp Pro	
660 665 670	

```

aat aac tac ctg ttt tgc cgg gtc aga aaa aat ggt gtt gcc gcg cca 2064
Asn Asn Tyr Leu Phe Cys Arg Val Arg Lys Asn Gly Val Ala Ala Pro
      675                      680                      685

tct gcc acc agc cag cta tca act cgc gcc ctg gaa ggg att ttt gaa 2112
Ser Ala Thr Ser Gln Leu Ser Thr Arg Ala Leu Glu Gly Ile Phe Glu
      690                      695                      700

gca act cat cga ttg att tac ggc gct aag gat gac tct ggt cag aga 2160
Ala Thr His Arg Leu Ile Tyr Gly Ala Lys Asp Asp Ser Gly Gln Arg
705                      710                      715                      720

tac ctg gcc tgg tct gga cac agt gcc cgt gtc gga gcc gcg cga gat 2208
Tyr Leu Ala Trp Ser Gly His Ser Ala Arg Val Gly Ala Ala Arg Asp
      725                      730                      735

atg gcc cgc gct gga gtt tca ata ccg gag atc atg caa gct ggt ggc 2256
Met Ala Arg Ala Gly Val Ser Ile Pro Glu Ile Met Gln Ala Gly Gly
      740                      745                      750

tgg acc aat gta aat att gtc atg aac tat atc cgt aac ctg gat agt 2304
Trp Thr Asn Val Asn Ile Val Met Asn Tyr Ile Arg Asn Leu Asp Ser
      755                      760                      765

gaa aca ggg gca atg gtg cgc ctg ctg gaa gat ggc gat tag 2346
Glu Thr Gly Ala Met Val Arg Leu Leu Glu Asp Gly Asp
      770                      775                      780

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<210> 9
 <211> 781
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Sequence
 encoding a FLPm:Cre polypeptide, FLP from
 Saccharomyces (maize preferred codons), and Cre
 from Bacteriophage P1

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<400> 9
Met Pro Gln Phe Asp Ile Leu Cys Lys Thr Pro Pro Lys Val Leu Val
  1                      5                      10                      15

Arg Gln Phe Val Glu Arg Phe Glu Arg Pro Ser Gly Glu Lys Ile Ala
      20                      25                      30

Leu Cys Ala Ala Glu Leu Thr Tyr Leu Cys Trp Met Ile Thr His Asn
      35                      40                      45

Gly Thr Ala Ile Lys Arg Ala Thr Phe Met Ser Tyr Asn Thr Ile Ile
      50                      55                      60

Ser Asn Ser Leu Ser Phe Asp Ile Val Asn Lys Ser Leu Gln Phe Lys
      65                      70                      75                      80

Tyr Lys Thr Gln Lys Ala Thr Ile Leu Glu Ala Ser Leu Lys Lys Leu
      85                      90                      95

```

Ile	Pro	Ala	Trp	Glu	Phe	Thr	Ile	Ile	Pro	Tyr	Tyr	Gly	Gln	Lys	His			
			100					105					110					
Gln	Ser	Asp	Ile	Thr	Asp	Ile	Val	Ser	Ser	Leu	Gln	Leu	Gln	Phe	Glu			
		115					120					125						
Ser	Ser	Glu	Glu	Ala	Asp	Lys	Gly	Asn	Ser	His	Ser	Lys	Lys	Met	Leu			
	130					135					140							
Lys	Ala	Leu	Leu	Ser	Glu	Gly	Glu	Ser	Ile	Trp	Glu	Ile	Thr	Glu	Lys			
145					150					155					160			
Ile	Leu	Asn	Ser	Phe	Glu	Tyr	Thr	Ser	Arg	Phe	Thr	Lys	Thr	Lys	Thr			
				165					170					175				
Leu	Tyr	Gln	Phe	Leu	Phe	Leu	Ala	Thr	Phe	Ile	Asn	Cys	Gly	Arg	Phe			
			180					185					190					
Ser	Asp	Ile	Lys	Asn	Val	Asp	Pro	Lys	Ser	Phe	Lys	Leu	Val	Gln	Asn			
	195						200					205						
Lys	Tyr	Leu	Gly	Val	Ile	Ile	Gln	Cys	Leu	Val	Thr	Glu	Thr	Lys	Thr			
	210					215					220							
Ser	Val	Ser	Arg	His	Ile	Tyr	Phe	Phe	Ser	Ala	Arg	Gly	Arg	Ile	Asp			
225					230					235					240			
Pro	Leu	Val	Tyr	Leu	Asp	Glu	Phe	Leu	Arg	Asn	Ser	Glu	Pro	Val	Leu			
				245					250					255				
Lys	Arg	Val	Asn	Arg	Thr	Gly	Asn	Ser	Ser	Ser	Asn	Lys	Gln	Glu	Tyr			
			260				265						270					
Gln	Leu	Leu	Lys	Asp	Asn	Leu	Val	Arg	Ser	Tyr	Asn	Lys	Ala	Leu	Lys			
		275					280					285						
Lys	Asn	Ala	Pro	Tyr	Ser	Ile	Phe	Ala	Ile	Lys	Asn	Gly	Pro	Lys	Ser			
	290					295					300							
His	Ile	Gly	Arg	His	Leu	Met	Thr	Ser	Phe	Leu	Ser	Met	Lys	Gly	Leu			
305					310					315					320			
Thr	Glu	Leu	Thr	Asn	Val	Val	Gly	Asn	Trp	Ser	Asp	Lys	Arg	Ala	Ser			
				325					330					335				
Ala	Val	Ala	Arg	Thr	Thr	Tyr	Thr	His	Gln	Ile	Thr	Ala	Ile	Pro	Asp			
			340					345					350					
His	Tyr	Phe	Ala	Leu	Val	Ser	Arg	Tyr	Tyr	Ala	Tyr	Asp	Pro	Ile	Ser			
		355					360					365						
Lys	Glu	Met	Ile	Ala	Leu	Lys	Asp	Glu	Thr	Asn	Pro	Ile	Glu	Glu	Trp			
	370					375					380							
Gln	His	Ile	Glu	Gln	Leu	Lys	Gly	Ser	Ala	Glu	Gly	Ser	Ile	Arg	Tyr			
385					390					395					400			
Pro	Ala	Trp	Asn	Gly	Ile	Ile	Ser	Gln	Glu	Val	Leu	Asp	Tyr	Leu	Ser			
				405					410					415				

Ser Tyr Ile Asn Arg Arg Ile Gly Gly Gly Ser Gly Gly Gly Ser Gly
 420 425 430
 Gly Gly Ser Asp Pro Thr Met Ala Asn Leu Leu Thr Val His Gln Asn
 435 440 445
 Leu Pro Ala Leu Pro Val Asp Ala Thr Ser Asp Glu Val Arg Lys Asn
 450 455 460
 Leu Met Asp Met Phe Arg Asp Arg Gln Ala Phe Ser Glu His Thr Trp
 465 470 475 480
 Lys Met Leu Leu Ser Val Cys Arg Ser Trp Ala Ala Trp Cys Lys Leu
 485 490 495
 Asn Asn Arg Lys Trp Phe Pro Ala Glu Pro Glu Asp Val Arg Asp Tyr
 500 505 510
 Leu Leu Tyr Leu Gln Ala Arg Gly Leu Ala Val Lys Thr Ile Gln Gln
 515 520 525
 His Leu Gly Gln Leu Asn Met Leu His Arg Arg Ser Gly Leu Pro Arg
 530 535 540
 Pro Ser Asp Ser Asn Ala Val Ser Leu Val Met Arg Arg Ile Arg Lys
 545 550 555 560
 Glu Asn Val Asp Ala Gly Glu Arg Ala Lys Gln Ala Leu Ala Phe Glu
 565 570 575
 Arg Thr Asp Phe Asp Gln Val Arg Ser Leu Met Glu Asn Ser Asp Arg
 580 585 590
 Cys Gln Asp Ile Arg Asn Leu Ala Phe Leu Gly Ile Ala Tyr Asn Thr
 595 600 605
 Leu Leu Arg Ile Ala Glu Ile Ala Arg Ile Arg Val Lys Asp Ile Ser
 610 615 620
 Arg Thr Asp Gly Gly Arg Met Leu Ile His Ile Gly Arg Thr Lys Thr
 625 630 635 640
 Leu Val Ser Thr Ala Gly Val Glu Lys Ala Leu Ser Leu Gly Val Thr
 645 650 655
 Lys Leu Val Glu Arg Trp Ile Ser Val Ser Gly Val Ala Asp Asp Pro
 660 665 670
 Asn Asn Tyr Leu Phe Cys Arg Val Arg Lys Asn Gly Val Ala Ala Pro
 675 680 685
 Ser Ala Thr Ser Gln Leu Ser Thr Arg Ala Leu Glu Gly Ile Phe Glu
 690 695 700
 Ala Thr His Arg Leu Ile Tyr Gly Ala Lys Asp Asp Ser Gly Gln Arg
 705 710 715 720
 Tyr Leu Ala Trp Ser Gly His Ser Ala Arg Val Gly Ala Ala Arg Asp
 725 730 735

41

Met Ala Arg Ala Gly Val Ser Ile Pro Glu Ile Met Gln Ala Gly Gly
 740 745 750

Trp Thr Asn Val Asn Ile Val Met Asn Tyr Ile Arg Asn Leu Asp Ser
 755 760 765

Glu Thr Gly Ala Met Val Arg Leu Leu Glu Asp Gly Asp
 770 775 780

<210> 10
 <211> 34
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: minimal
 wild-type FRT recombination site

<400> 10
 gaagttccta ttctctagaa agtataggaa cttc

34

<210> 11
 <211> 781
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: Cre:FLP
 polypeptide, Cre from Bacteriophage P1 and FLP
 from Saccharomyces

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 1 5 10 15

Asp Ala Thr Ser Asp Glu Val Arg Lys Asn Leu Met Asp Met Phe Arg
 20 25 30

Asp Arg Gln Ala Phe Ser Glu His Thr Trp Lys Met Leu Leu Ser Val
 35 40 45

Cys Arg Ser Trp Ala Ala Trp Cys Lys Leu Asn Asn Arg Lys Trp Phe
 50 55 60

Pro Ala Glu Pro Glu Asp Val Arg Asp Tyr Leu Leu Tyr Leu Gln Ala
 65 70 75 80

Arg Gly Leu Ala Val Lys Thr Ile Gln Gln His Leu Gly Gln Leu Asn
 85 90 95

Met Leu His Arg Arg Ser Gly Leu Pro Arg Pro Ser Asp Ser Asn Ala
 100 105 110

Val Ser Leu Val Met Arg Arg Ile Arg Lys Glu Asn Val Asp Ala Gly
 115 120 125

Glu Arg Ala Lys Gln Ala Leu Ala Phe Glu Arg Thr Asp Phe Asp Gln
 130 135 140

Val	Arg	Ser	Leu	Met	Glu	Asn	Ser	Asp	Arg	Cys	Gln	Asp	Ile	Arg	Asn	145	150	155	160
Leu	Ala	Phe	Leu	Gly	Ile	Ala	Tyr	Asn	Thr	Leu	Leu	Arg	Ile	Ala	Glu	165	170	175	
Ile	Ala	Arg	Ile	Arg	Val	Lys	Asp	Ile	Ser	Arg	Thr	Asp	Gly	Gly	Arg	180	185	190	
Met	Leu	Ile	His	Ile	Gly	Arg	Thr	Lys	Thr	Leu	Val	Ser	Thr	Ala	Gly	195	200	205	
Val	Glu	Lys	Ala	Leu	Ser	Leu	Gly	Val	Thr	Lys	Leu	Val	Glu	Arg	Trp	210	215	220	
Ile	Ser	Val	Ser	Gly	Val	Ala	Asp	Asp	Pro	Asn	Asn	Tyr	Leu	Phe	Cys	225	230	235	240
Arg	Val	Arg	Lys	Asn	Gly	Val	Ala	Ala	Pro	Ser	Ala	Thr	Ser	Gln	Leu	245	250	255	
Ser	Thr	Arg	Ala	Leu	Glu	Gly	Ile	Phe	Glu	Ala	Thr	His	Arg	Leu	Ile	260	265	270	
Tyr	Gly	Ala	Lys	Asp	Asp	Ser	Gly	Gln	Arg	Tyr	Leu	Ala	Trp	Ser	Gly	275	280	285	
His	Ser	Ala	Arg	Val	Gly	Ala	Ala	Arg	Asp	Met	Ala	Arg	Ala	Gly	Val	290	295	300	
Ser	Ile	Pro	Glu	Ile	Met	Gln	Ala	Gly	Gly	Trp	Thr	Asn	Val	Asn	Ile	305	310	315	320
Val	Met	Asn	Tyr	Ile	Arg	Asn	Leu	Asp	Ser	Glu	Thr	Gly	Ala	Met	Val	325	330	335	
Arg	Leu	Leu	Glu	Asp	Gly	Asp	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Ser	Gly	340	345	350	
Gly	Gly	Ser	Asp	Pro	Thr	Met	Pro	Gln	Phe	Asp	Ile	Leu	Cys	Lys	Thr	355	360	365	
Pro	Pro	Lys	Val	Leu	Val	Arg	Gln	Phe	Val	Glu	Arg	Phe	Glu	Arg	Pro	370	375	380	
Ser	Gly	Glu	Lys	Ile	Ala	Leu	Cys	Ala	Ala	Glu	Leu	Thr	Tyr	Leu	Cys	385	390	395	400
Trp	Met	Ile	Thr	His	Asn	Gly	Thr	Ala	Ile	Lys	Arg	Ala	Thr	Phe	Met	405	410	415	
Ser	Tyr	Asn	Thr	Ile	Ile	Ser	Asn	Ser	Leu	Ser	Leu	Asp	Ile	Val	Asn	420	425	430	
Lys	Ser	Leu	Gln	Phe	Lys	Tyr	Lys	Thr	Gln	Lys	Ala	Thr	Ile	Leu	Glu	435	440	445	
Ala	Ser	Leu	Lys	Lys	Leu	Ile	Pro	Ala	Trp	Glu	Phe	Thr	Ile	Ile	Pro	450	455	460	

Tyr	Tyr	Gly	Gln	Lys	His	Gln	Ser	Asp	Ile	Thr	Asp	Ile	Val	Ser	Ser	465	470	475	480
Leu	Gln	Leu	Gln	Phe	Glu	Ser	Ser	Glu	Glu	Ala	Asp	Lys	Gly	Asn	Ser	485	490		495
His	Ser	Lys	Lys	Met	Leu	Lys	Ala	Leu	Leu	Ser	Glu	Gly	Glu	Ser	Ile	500	505		510
Trp	Glu	Ile	Thr	Glu	Lys	Ile	Leu	Asn	Ser	Phe	Glu	Tyr	Thr	Ser	Arg	515	520		525
Phe	Thr	Lys	Thr	Lys	Thr	Leu	Tyr	Gln	Phe	Leu	Phe	Leu	Ala	Thr	Phe	530	535	540	
Ile	Asn	Cys	Gly	Arg	Phe	Ser	Asp	Ile	Lys	Asn	Val	Asp	Pro	Lys	Ser	545	550	555	560
Phe	Lys	Leu	Val	Gln	Asn	Lys	Tyr	Leu	Gly	Val	Ile	Ile	Gln	Cys	Leu	565	570		575
Val	Thr	Glu	Thr	Lys	Thr	Ser	Val	Ser	Arg	His	Ile	Tyr	Phe	Phe	Ser	580	585		590
Ala	Arg	Gly	Arg	Ile	Asp	Pro	Leu	Val	Tyr	Leu	Asp	Glu	Phe	Leu	Arg	595	600	605	
Asn	Ser	Glu	Pro	Val	Leu	Lys	Arg	Val	Asn	Arg	Thr	Gly	Asn	Ser	Ser	610	615	620	
Ser	Asn	Lys	Gln	Glu	Tyr	Gln	Leu	Leu	Lys	Asp	Asn	Leu	Val	Arg	Ser	625	630	635	640
Tyr	Asn	Lys	Ala	Leu	Lys	Lys	Asn	Ala	Pro	Tyr	Ser	Ile	Phe	Ala	Ile	645	650		655
Lys	Asn	Gly	Pro	Lys	Ser	His	Ile	Gly	Arg	His	Leu	Met	Thr	Ser	Phe	660	665		670
Leu	Ser	Met	Lys	Gly	Leu	Thr	Glu	Leu	Thr	Asn	Val	Val	Gly	Asn	Trp	675	680	685	
Ser	Asp	Lys	Arg	Ala	Ser	Ala	Val	Ala	Arg	Thr	Thr	Tyr	Thr	His	Gln	690	695	700	
Ile	Thr	Ala	Ile	Pro	Asp	His	Tyr	Phe	Ala	Leu	Val	Ser	Arg	Tyr	Tyr	705	710	715	720
Ala	Tyr	Asp	Pro	Ile	Ser	Lys	Glu	Met	Ile	Ala	Leu	Lys	Asp	Glu	Thr	725	730		735
Asn	Pro	Ile	Glu	Glu	Trp	Gln	His	Ile	Glu	Gln	Leu	Lys	Gly	Ser	Ala	740	745		750
Glu	Gly	Ser	Ile	Arg	Tyr	Pro	Ala	Trp	Asn	Gly	Ile	Ile	Ser	Gln	Glu	755	760	765	
Val	Leu	Asp	Tyr	Leu	Ser	Ser	Tyr	Ile	Asn	Arg	Arg	Ile				770	775	780	

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CLAIMS:

1. A recombinant protein comprising a first site-specific recombinase fused in frame with a distinct second site-specific recombinase.

5 2. The recombinant protein of claim 1, wherein said first and said distinct second site-specific recombinases are members of the integrase family of recombinases, an active derivative of a member of the integrase family of recombinases, or an active fragment of a member of the
10 integrase family of recombinases, wherein said recombinase, said active derivative, or said active fragment catalyze site-specific recombination.

3. The recombinant protein of claim 2, wherein said first and said second recombinases are selected from the
15 group consisting of Cre, FLP, an active derivative of Cre, an active fragment of Cre, an active fragment of FLP, and an active derivative of FLP, wherein said recombinase, said active derivative, or said active fragment catalyze site-specific recombination.

20 4. A recombinant protein comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 3, 6, 9, and 11.

5. A nucleic acid molecule comprising a nucleotide sequence encoding a first site-specific recombinase fused in
25 frame with a distinct second site-specific recombinase.

6. The nucleic acid molecule of claim 5, wherein said first and said second site-specific recombinases are members of the integrase family of recombinases, an active derivative of a member of the integrase family of recombinases, or an
30 active fragment of a member of the integrase family of

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recombinases, wherein said recombinase, said active derivative, or said active fragment catalyze site-specific recombination.

7. The nucleic acid molecule of claim 6, wherein said
5 first and said second recombinases are selected from the group consisting of Cre, FLP, an active derivative of Cre, an active fragment of Cre, an active fragment of FLP, and an active derivative of FLP, wherein said recombinase, said active derivative, or said active fragment catalyze site-
10 specific recombination.

8. The nucleic acid molecule of claim 7, wherein said first and said second recombinase are encoded by a nucleotide sequence comprising moCre and FLPm.

9. A nucleic acid molecule comprising a nucleotide
15 sequence selected from the group consisting of:

a) a nucleotide sequence comprising the sequence set forth in any one of SEQ ID NOS: 4, 5, 7, and 8; and,

b) a nucleotide sequence encoding a polypeptide comprising an amino acid sequence set forth in any one of
20 SEQ ID NOS: 3, 6, 9, and 11.

10. A nucleic acid molecule comprising the nucleotide sequence of any one of claims 5 to 9, wherein said nucleotide sequence is operably linked to a promoter that drives expression in an eukaryotic cell.

25 11. An eukaryotic cell having stably incorporated into its genome the nucleic acid molecule of any one of claims 5 to 10.

12. The eukaryotic cell of claim 11, wherein said cell is a plant cell.

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13. A transformed plant cell having stably incorporated into its genome the nucleic acid molecule of any one of claims 5 to 10.

14. The transformed plant cell of claim 13, wherein
5 said plant cell is within a plant seed.

15. A method for integrating a DNA of interest into the genome of an eukaryotic cell wherein the genome comprises at least one stably integrated integration site, said integration site comprising a first target site for a first
10 site-specific recombinase and a second target site for a distinct second site-specific recombinase, said method comprising:

a) introducing into said eukaryotic cell a nucleic acid comprising said DNA of interest, wherein said DNA of
15 interest is flanked by said first and said second target sites; and,

b) providing in said eukaryotic cell a recombinant protein comprising said first recombinase, an active derivative of said first recombinase, or an active fragment
20 of said first recombinase fused in frame with said distinct second recombinase, an active derivative of said distinct second recombinase, or an active fragment of said distinct second recombinase, wherein said first and said distinct second recombinase, said active derivative, or said active
25 fragment catalyze a site-specific recombination event,

wherein the DNA of interest is integrated into the genome at the integration site.

16. The method of claim 15, wherein said eukaryotic cell comprises a plant cell.

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17. The method of claim 15 or 16, wherein said first recombinase is Cre, an active derivative of Cre, or an active fragment of Cre and said second recombinase is FLP, an active derivative of FLP, or an active fragment of FLP, wherein said
5 recombinase, said active derivative, or said active fragment catalyze site-specific recombination.

18. A plant cell having stably incorporated into a chromosome, at least one integration site comprising a target site for a first site-specific recombinase and a target site
10 for a distinct second site-specific recombinase, wherein said target sites are contiguous.

19. The plant cell of claim 18, wherein said plant cell is from a monocot.

20. The plant cell of claim 19, wherein said monocot is
15 maize, wheat, rice, barley, sorghum, or rye.

21. The plant cell of claim 18, wherein said plant cell is from a dicot.

22. The plant cell of claim 21, wherein said dicot is soybean, Brassica, sunflower, alfalfa, or safflower.

20 23. The plant cell of any one of claims 18 to 22, wherein said plant cell is in a seed.

24. A method for integrating a DNA of interest into the genome of an eukaryotic cell wherein the genome comprises at least one stably integrated integration site, said
25 integration site comprising a first target site for a first site-specific recombinase and a second target site for a distinct second site-specific recombinase, comprising:

a) introducing into said eukaryotic cell a nucleic acid comprising said DNA of interest, wherein said DNA of

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interest is flanked by said first and said second target sites; and,

b) providing in said cell said first recombinase, an active derivative of said first recombinase, or an active
5 fragment of said first recombinase and said distinct second recombinase, an active derivative of said distinct second recombinase, or an active fragment of said distinct second recombinase, wherein said first and said second recombinases, said active derivative, or said active fragment thereof
10 catalyze a site-specific recombination event.

25. The method of claim 15 or 16, wherein said first recombinase is FLP, an active derivative of FLP, or active fragment of FLP, and said distinct second recombinase is Cre, an active derivative of Cre, or active fragment of Cre,
15 wherein said first and second recombinases, said active derivative, or said active fragment catalyze a site-specific recombination event.

26. The method of claim 17 or 25, wherein the Cre recombinase is encoded by a nucleotide sequence comprising
20 moCre.

27. The method of any one of claims 17, 25, and 26, wherein the FLP recombinase is encoded by a nucleotide sequence comprising FLPm.

28. The method of claim 24, wherein said eukaryotic
25 cell comprises a plant cell.

29. The method of claim 24 or 28, wherein said first recombinase is Cre, an active derivative of Cre, or an active fragment of Cre, and said second recombinase is FLP, an active derivative of FLP, or an active fragment of FLP, and
30 said first and said second recombinase, said active

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derivative, or said active fragment catalyze a site-specific recombination event.

30. The method of claim 24 or 28, wherein said first recombinase is FLP, an active derivative of FLP, or an active
5 fragment of FLP, and said second recombinase is Cre, an active derivative of Cre, or an active fragment of Cre, and wherein said first and said second recombinase, said active derivative, or said active fragment catalyze a site-specific recombination event.

10 31. The method of claim 29 or 30, wherein the Cre recombinase is encoded by a nucleotide sequence comprising moCre.

32. The method of claim 29, 30, or 31, wherein the FLP recombinase is encoded by a nucleotide sequence comprising
15 FLPM.

33. The method of claim 16 or 28, wherein said plant cell is monocotyledonous.

34. The method of claim 33, wherein said plant cell is from maize, wheat, rice, barley, sorghum, or rye.

20 35. The method of claim 16 or 28, wherein said plant cell is dicotyledonous.

36. The method of claim 35, wherein said plant cell is from soybean, Brassica, sunflower, alfalfa, or safflower.

25 37. A system for integrating a DNA of interest into the genome of an eukaryotic cell, comprising:

a) an eukaryotic cell containing at least one stably integrated integration site, said integration site comprising a first target site for a first site-specific

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recombinase and a second target site for a distinct second site-specific recombinase;

b) a nucleic acid comprising said DNA of interest, wherein said DNA is flanked by said first and said second
5 target sites;

c) a recombinant protein comprising said first recombinase, an active derivative of said first recombinase, or an active fragment of said first recombinase fused in frame with said distinct second recombinase, an active
10 derivative of said distinct second recombinase, or an active fragment of said distinct second recombinase, wherein said first and said second recombinase, said active derivative, or said active fragment catalyze a site-specific recombination event;

15 d) means for introducing the nucleic acid of (b) into the cell; and,

e) means for introducing the recombinant protein of (c) into the cell,

wherein the DNA of interest is integrated into the
20 genome at the integration site.

38. The system of claim 37, wherein the cell is a plant cell.

39. A system for integrating a DNA of interest into the genome of an eukaryotic cell, comprising:

25 a) an eukaryotic cell containing at least one stably integrated integration site, said integration site comprising a first target site for a first site-specific recombinase and a second target site for a distinct second site-specific recombinase;

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b) a nucleic acid comprising said DNA of interest, wherein said DNA of interest is flanked by said first and said second target sites;

c) the first recombinase, an active derivative of said first recombinase, or an active fragment of said first recombinase; and, the distinct second recombinase, an active derivative of said distinct second recombinase, or an active fragment of said distinct second recombinase, wherein said first and said second recombinase, said active derivative, or said active fragment catalyze a site-specific recombination event;

d) means for introducing the nucleic acid of (b) into the cell; and,

e) means for introducing the first and the second recombinase, or said active derivative thereof, or said active fragment thereof of (c) into the cell;

wherein the DNA of interest is integrated into the genome at the integration site.

40. The system of claim 39, wherein the cell is a plant cell.

41. The recombinant protein of claim 3, wherein said Cre recombinase is encoded by a nucleotide sequence comprising moCre and said FLP recombinase is encoded by a nucleotide sequence comprising FLPM.

25 42. Use of a genetically modified plant as a crop, wherein the genetically modified plant has stably incorporated in its genome the nucleic acid molecule of any one of claims 5 to 10.

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43. A process for generating a genetically modified plant, the process comprising crossing a genetically modified first parent plant with a second plant, wherein the genetically modified first parent plant comprises a nucleic acid molecule as defined in any one of claims 5 to 10.

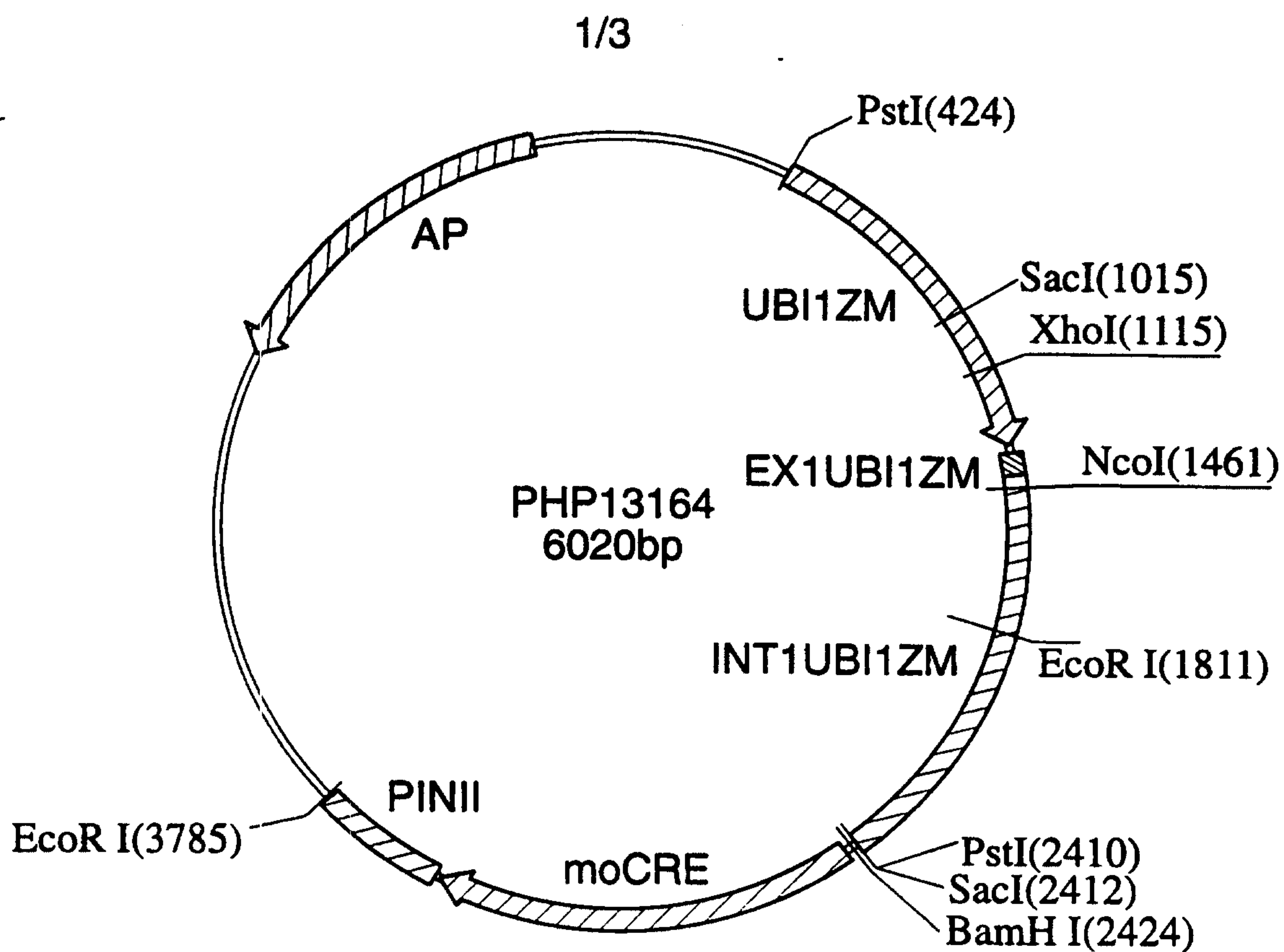
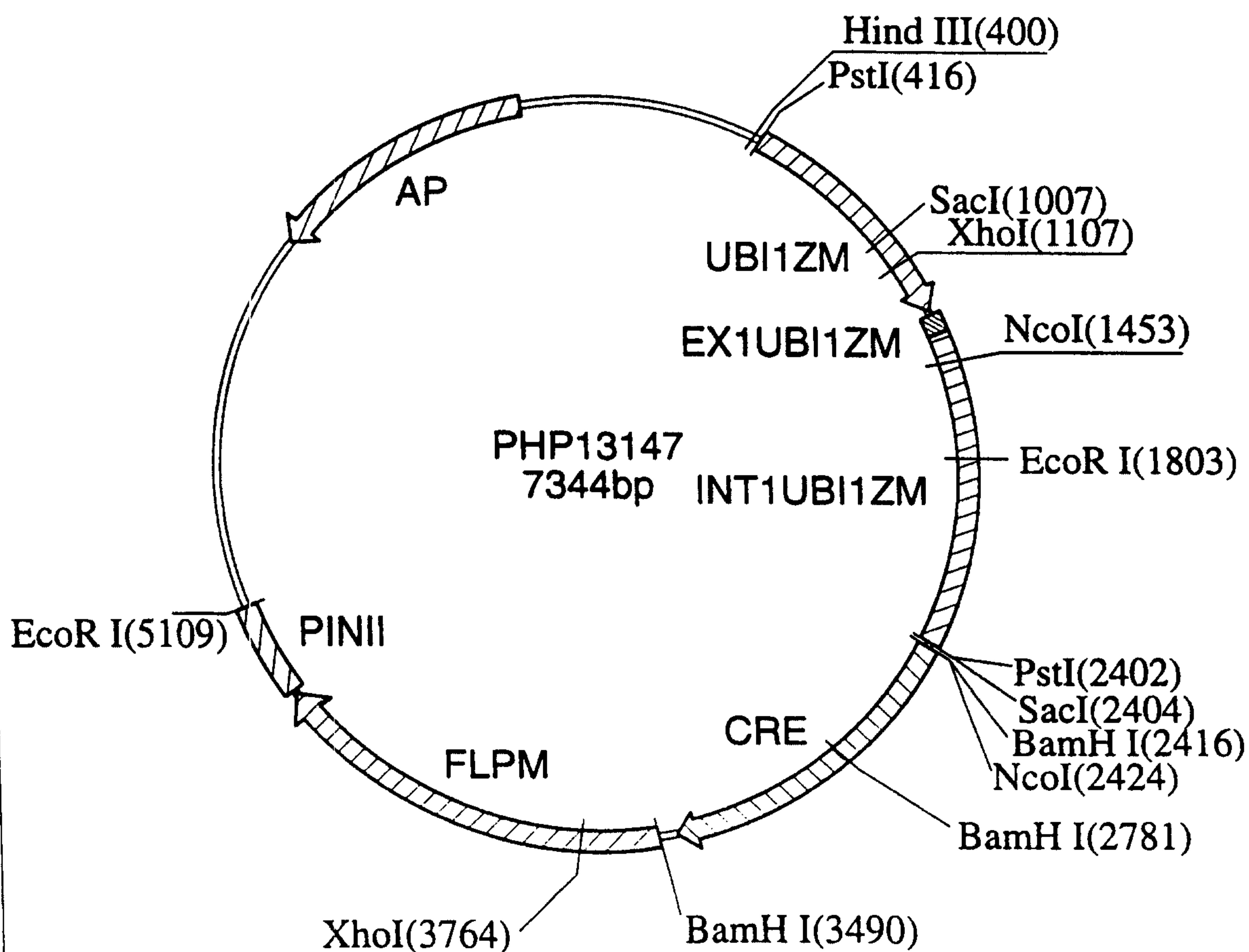
44. Use of a genetically modified plant as a crop, wherein the genetically modified plant has stably integrated into a chromosome, at least one integration site comprising a first target site for a first site-specific recombinase and a second target site for a distinct second site-specific recombinase, wherein said target sites are contiguous.

45. A process for generating a genetically modified plant, the process comprising crossing a genetically modified first parent plant with a second plant, wherein the genetically modified first parent plant has stably integrated into a chromosome, at least one integration site comprising a first target site for a first site-specific recombinase and a second target site for a distinct second site-specific recombinase, wherein said target sites are contiguous.

46. The method of any one of claims 16, 17, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, and 36 wherein introducing comprises transformation.

47. The method of any one of claims 16, 17, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, and 36 wherein introducing comprises sexual breeding.

48. The method of claim 15 or 16, wherein said recombinant protein comprises an amino acid sequence set forth in SEQ ID NO: 3, 6, 9, or 11.

**FIG. 1.**

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EXCISION OF FRT SITE BY FLP_m AND Cre-FLP_m FUSION

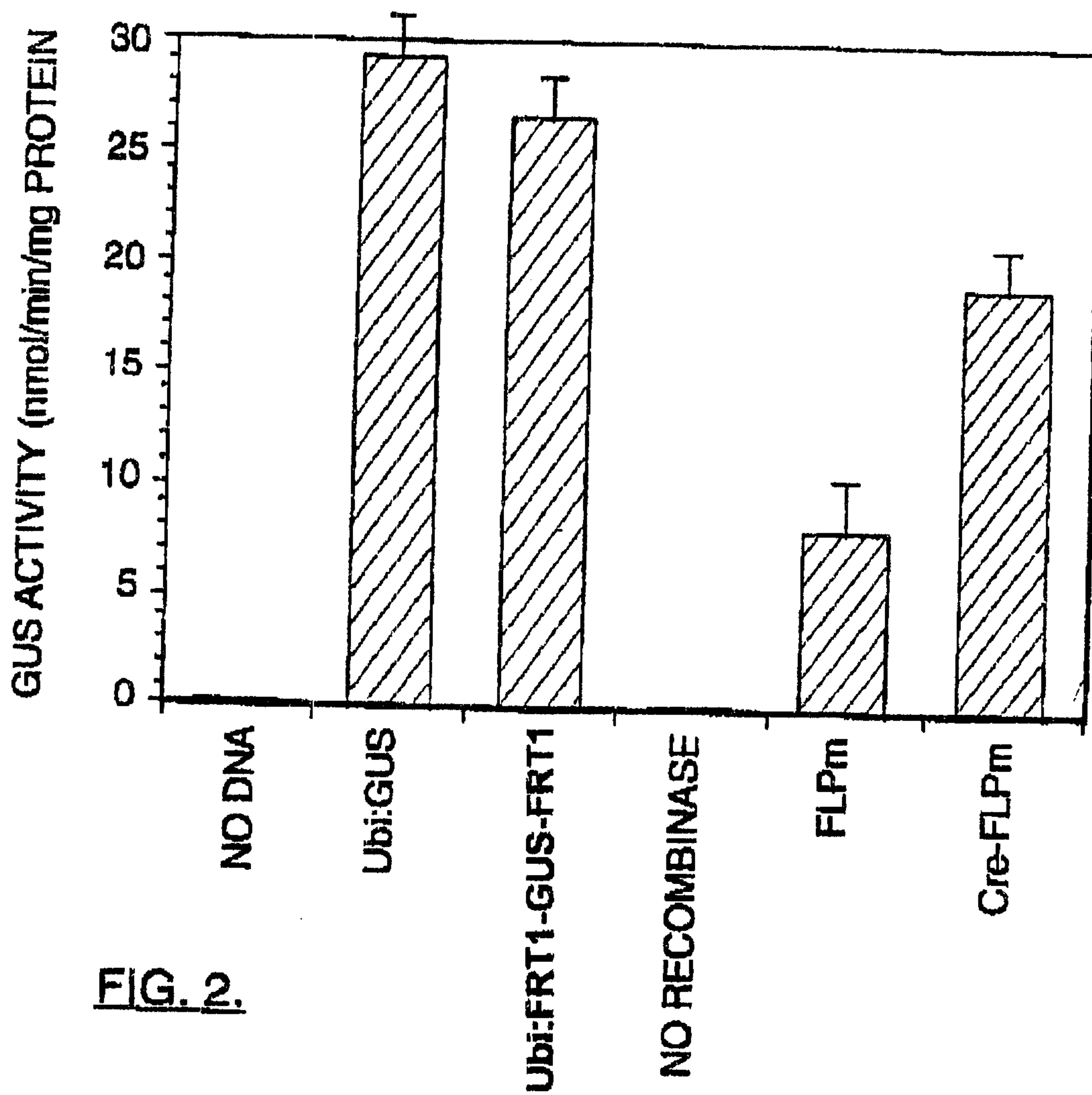


FIG. 2.

FRT1/FRT1
EXCISION VECTOR

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EXCISION OF LoxP SITE BY Cre-FLPm FUSION PROTEIN

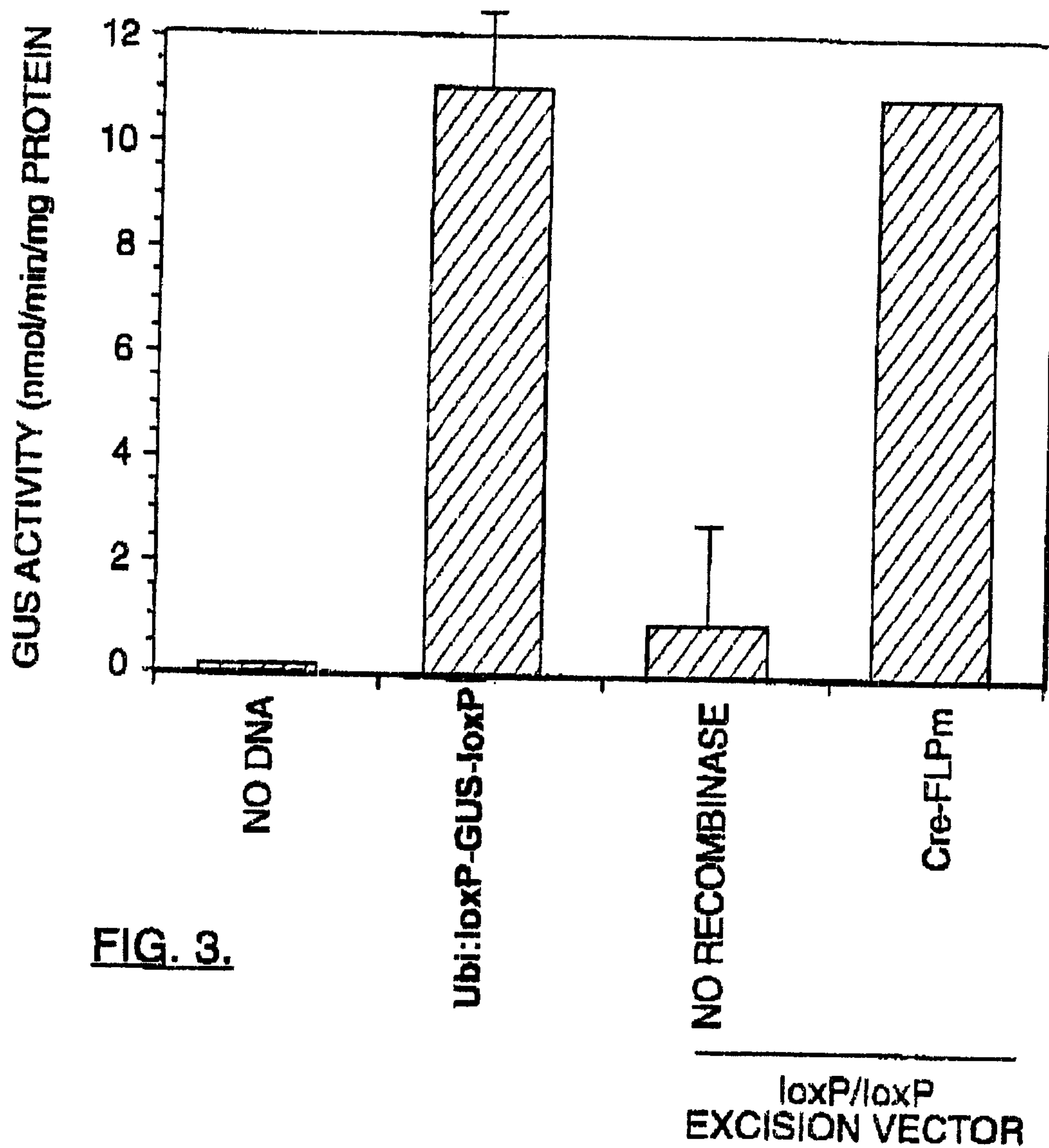


FIG. 3.

