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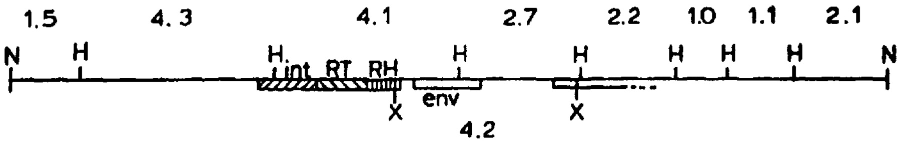

(57) Abstract

Retroviral and retroviral-like polynucleotides, and vectors, proteins and antibodies derived therefrom, that are useful for the introduction of genetic information into soybeans and other plant species are described. Specifically, the retroviral SIRE-1 genomic clone is described.

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# PLANT RETROVIRAL POLYNUCLEOTIDES AND METHODS FOR USE THEREOF

## Cross-reference to Related Applications

This application claims the benefit of U.S. Provisional Application No. 60/025,853, filed 9 September 1996.

## Field of Invention

The present invention relates generally to retroviruses, pro-retroviral polynucleotides including pro-retroviral DNA, pro-retroviral-like DNA and more specifically to recombinant vectors derived therefrom for use in delivering genetic information to susceptible target plant cells.

## Background of Invention

Repetitive DNA sequences are a common feature of the genomes of higher eukaryotes. Repetitive DNA family members in animals and higher plants are tandemly repeated or interspersed with other sequences (Walbot and Goldberg, 1979; Flavell, 1980), and may constitute more than 50% of the genome (Walbot and Goldberg, 1979). Estimates of the proportion of repetitive DNA in the soybean genome range from 36% to 60% (Goldberg, 1978; Gurley *et al.*, 1979).

High copy-number repeats on the order of $10^5$ per haploid genome comprise only 3% of the soybean genome, whereas moderately repetitive sequences with copy-numbers in the $10^3$ range occupy 30-40% of the genome (Goldberg, 1978). Electron micrographic examination of these moderately repetitive sequences demonstrate that they average about 2 kb in length; however, 4% of those observed exceed 11 kb (Pellegrini and Goldberg, 1979).

Most of the highly repetitive sequences in higher eukaryotic genomes are relatively short and are organized in tandem arrays. For example, the chromosomal region adjacent to the centromere in higher eukaryotes is composed of very long blocks of highly repetitive DNA, called satellite DNA, in which simple sequences are repeated thousands of times or more. Tandemly repeated elements found in the soybean genome also include the ribosomal RNA (rRNA)-encoding genes. The approximately 800 rDNA copies are organized as one or more clusters of tandemly repeated 8-kb or 9-kb units (Friedrich *et al.*, 1979; Varsanyi-Breiner *et al.*, 1979).

The genomes of most higher eukaryotes also contain highly repetitive sequences that are distributed evenly throughout the genome, interspersed with longer stretches of unique (or moderately repetitive) DNA. These interspersed repetitive DNA elements are variable in length, are recognizably related but not precisely conserved in sequence, and exhibit relatively small repeat frequencies (Lapitan, 1992).

The dispersal pattern of interspersed repetitive elements in higher eukaryotic genomes has led to the suggestion that they are, or once were, transposable elements known as transposons (Flavell, 1986; Lapitan, 1992). Transposons are genetic elements that can move from one chromosomal location to another, without necessarily altering the general architecture of the chromosomes involved. The existence of transposons has only found general acceptance within the last few decades. Genes were originally believed to have fixed chromosomal locations that only change as a result of chromosomal rearrangements resulting from illegitimate crossing-over between incompletely homologous short sections of DNA. Then, in the late 1940's, McClintock's pioneering experiments with maize showed that certain genetic elements regularly "jump", or transpose, to new locations in the genome (McClintock, 1984).

Transposable elements (TEs) reside in the genomes of virtually all organisms (Berg and Howe, 1989). TEs encode enzymes that bring about the insertion of an identical copy of themselves into a new DNA site. Transposition events involve both recombination and replication processes that frequently generate two daughter copies of the original transposable element; one remains at the parental site, while the other appears at the target site (Shapiro, 1983).

Two major classes of eukaryotic TEs have been identified, which are distinguished by their mode of transposition (Finnegan, 1989). Class I elements transpose via the creation of an RNA intermediate that is then reverse-transcribed to create a DNA copy that integrates at the target site. This class includes several families of retroelements - retrotransposons and retroviruses - including the *copia* elements of *Drosophila melanogaster*, the *gypsy/Ty3* family, the *Ty1* element of yeast, and the mammalian immunodeficiency and Rous sarcoma (RSV) retroviruses. Each of these retroelement families are characterized in part by the presence of long terminal repeats (LTRs) at their borders (Finnegan, 1989); however, this class also includes non-LTR-containing elements like *Cin4* from maize (Schwarz-Sommer and Saedler, 1988) and the mammalian L1 family (Hutchinson *et al.* 1989).

The *copia* elements in *D. melanogaster* possess long terminal direct repeats. There are more than 11 families of *copia*-like elements; the members of each are well-conserved and are located at 5 to 100 different sites in the *Drosophila* genome. These elements are about 5000 base pairs (bp) long, with long terminal repeats (LTRs) several hundred bp in length that vary in both sequence and length between families. At the termini of each element are short imperfect inverted repeats of about 10 bp.

Insertion of *copia* into a new chromosomal site is accompanied by replication of a 3-6 bp stretch of target DNA; the length, but not the sequence, of the direct repeats that consequently appear immediately before and after the element is the same for all members of the same family. *Copia* elements have one long open reading frame (ORF) that encodes proteins homologous to those of RNA tumor viruses: homologies to reverse transcriptase, integrase, and nucleic acid-binding proteins suggest that these proteins function to create an RNA intermediate for *copia* transposition.

Class II elements, like the *Drosophila melanogaster* P element (Engels, 1989; Rio, 1990) and the maize *Ac/Ds* element (Federoff, 1989), transpose directly to new sites without the formation of an RNA intermediate. P elements reside at multiple sites in the *Drosophila* genome and are 0.5 to 1.4 kb in length, bounded by perfect inverted repeats of 31 bp. They represent internally deleted versions of a larger element of about 3 kb called a P factor, which occurs in one or a few copies only in so-called "P strains" of *Drosophila*. Upon insertion into a new site in the genome, P elements create 8 bp duplications of the target sequence.

The Ac/Ds system in maize consists of Ds elements, which, like the P elements of *Drosophila*, are derived from a larger complete element called Ac. Ds elements exist in several different lengths, from 0.4 to 4 kb. Unlike P elements, Ds elements remain stationary within the chromosome unless an Ac element is also present. Ds elements contain perfect inverted repeats of 11 bp at their termini, flanked by 6-8 bp direct repeats of the target DNA. When a Ds (or Ac) element transposes, it leaves behind imperfect but recognizable duplications of the 6-8 bp target sequence.

As stated above, it appears likely that many interspersed repetitive DNA families are, or once were, transposons. In soybean, an interspersed repetitive DNA family whose structural characteristics clearly define it as a transposon family is the *Tgm* family. The *Tgm* family is related to the maize *En/Spm* transposons and consists of fewer than 50 members ranging in size from under 2 kb to greater than 12 kb (Rhodes and Vodkin, 1988).

Retroviruses are type I transposons consisting of an RNA genome that replicates through a DNA intermediate. Although the viral genome is RNA, the intermediate in replication is a double-stranded DNA copy of the viral genome called the provirus (Watson *et al.*, 1987). The provirus resembles a cellular gene and must integrate into host chromosomes in order to serve as a template for transcription of new viral genomes (Varmus, 1982). New genomes are processed in the nucleus by unmodified cellular machinery.

The viral genome RNA looks like a cellular messenger RNA (mRNA), but does not serve as such following infection of a cell. Instead, an enzyme called reverse transcriptase (which is not present in the cell, but is instead carried by the virion) makes a DNA copy of the viral RNA genome, which then undergoes integration into cellular chromosomal DNA as a provirus. Integration of the viral DNA is precise with respect to the viral genome, but is semi-random with respect to the host cell genome, in that some sites are utilized more frequently than others (Shih *et al.*, 1988). The integrated provirus serves as a template for production of new viral RNA genomes, which move to the cell membrane to assemble into virions. These bud from the cell membrane without killing the cell.

Retrovirus virions have icosahedral nucleocapsids surrounded by a proteinaceous envelope. The retroviral genome is diploid, and its general organization is well-known in the art. Typical retroviruses have three protein-encoding genes: *gag* (group-specific antigen) encodes a precursor polypeptide that is cleaved to yield the capsid proteins; *pol* is cleaved to yield reverse transcriptase and an enzyme involved in proviral integration; and *env* encodes the precursor to the envelope glycoprotein. A fourth type of retroviral gene, called *tat*, has been found at the 3′ end of the HTLV-I and -II genomes, which serves as a transcriptional enhancer. A few retroviruses have additional genes, such as *onc*, that give them the ability to rapidly induce certain types of cancer.

Retroviral genomes contain LTR sequences at both their 5′ and 3′ ends (Weiss, 1984). These sequences include signals needed for replication, transcription, and post-transcriptional processing of viral RNA transcripts. The LTRs are perfect direct repeats created by the addition of sequences (called $U_5$ and $U_3$, derived from the opposite ends of the viral genome) to each end of the viral genome during the creation of the double-stranded DNA intermediate. The $U_5$ region appears to be essential for initiation of reverse transcription and in packaging of viral transcripts (Murphy and Goff, 1988). The $U_3$ region contains a number of *cis*-acting signals for viral replication, and sequences responsible for much or all of the transcriptional control over viral genes.

Retroviral genomes also contain a primer binding site (PBS) near the 5′ end (Dahlberg *et al.*, 1974). This sequence is complementary to the 3′ end of a cellular tRNA. The tRNA is stolen from the host cell during replication and serves as a primer for reverse transcription of the RNA genome soon after infection.

Once the provirus is integrated into cellular chromosomal DNA, it is stable and replicates along with the host cell DNA. Proviruses are never excised from the site of integration, although they may be lost as a result of deletions. Retrovirus infections usually do not harm the cell, and infected cells continue to divide, with the integrated provirus serving as a template to direct viral RNA synthesis.

Like all viruses, retroviruses have a specific requirement for interaction with a target cell-surface receptor molecule for infection. In all cases known (and suspected), this molecule is a protein that interacts specifically with a specific virion *env* protein. The best-studied of virion envelope protein-cell surface receptor interaction is that of HIV with the CD4 receptor on human T-cells (Dalgleish *et al.*, 1984). The *env* protein appears to bind to a small region on the receptor not involved in cell-cell recognition or any other known function. Another retrovirus whose cellular receptor has been identified is

Moloney murine leukemia virus (MMLV), which interacts with a cell surface protein that resembles a membrane pore or channel protein. Although the mechanism of interaction of many retroviruses is not yet well understood, it does appear that retroviruses interact with a wide variety of receptor types (Weiss, 1982).

Retroviruses have been studied intensely over the past several decades, mainly because of their ability to cause tumors in animals and to transform cells in culture. The ability of retroviruses to transform cells is based on at least two mechanisms. The first is that certain viruses have incorporated activated proto-oncogenes that upon mutation have acquired the ability to transform cellular growth. The second mechanism of transformation results from insertional mutagenesis upon integration of the viral genome. Because the viral LTRs have promoter and enhancer activities, insertion of an LTR sequence in either orientation adjacent to a cellular gene may lead to inappropriate expression of that gene. If the cellular gene is involved in regulation of cell growth, over- or under-expression or insertional mutagenesis of that gene may lead to uncontrolled growth of the cell.

Retroviral integration is thus potentially mutagenic. Integration of retrotransposons within exonic coding regions may inactivate those genes, while integration within introns or flanking regions may create novel regulatory patterns with significant developmental and evolutionary implications (McDonald, 1990; Robins and Samuelson, 1993; Schwarz-Sommer and Saedler, 1987; Weil and Wessler, 1990; White *et al.*, 1994). Enhancers and *trans*-activating sequences have been found in retroviral and retrotransposon LTRs (Boeke, 1989; Cavarec, *et al*, 1994; Choi and Faller, 1994; Lohning and Ciriacy, 1994; Mellentin-Michelotti *et al.*, 1994; Varmus and Brown, 1989), and retrotransposon insertions between coding regions and enhancers disrupt gene expression (Cal and Levine, 1995;

Georgiev and Corces, 1995; Geyer and Corces, 1992; White *et al.*, 1994).

Element mobilization not only modifies target gene activity, it restructures genomic architecture (King, 1992, Lim and Simmons, 1994; McDonald, 1993; Shapiro, 1992). In fact, one of the major genomic differences between related taxonomic groups appears to be the identity and distribution of repetitive elements, not single-copy coding sequences (McDonald, 1993; Shapiro, 1992). White *et al.* (1994) have demonstrated that the flanking regions of many maize genes are embedded in sequences containing traces of retrotransposon DNA. Moreover, Palmgren (1994) has found that the *BstI* retroelement from maize encodes two conserved domains found in plant membrane $H^+$-ATPases, suggesting that element acquisition of host sequences is not confined to vertebrate retroviruses.

McClintock (1984) has proposed that genetic variation, induced in part by transposable element-mediated insertional mutagenesis, is a directed response to conditions that create "genomic stress." Many TEs and retroviruses preferentially insert in transcriptionally active regions of the genome (Engels, 1989; Sandmeyer *et al.*, 1990; Varmus and Brown, 1989). The *Ty1* retrotransposon in yeast can be activated by growth in sub-optimal temperatures (Paquin and Williamson, 1988) and by exposure to radiation (McEntee and Bradshaw, 1988). Similar observations have been made in *Drosophila* (McDonald *et al.*, 1988; Strand and McDonald, 1985), maize (McClintock, 1984), and soybean (Sheridan and Palmer, 1977).

In plants, TEs are activated during the induction of tissue culture (Hirochika, 1993; Peschke and Phillips, 1991) and may contribute to somaclonal variation observed for a number of higher plant species including soybean (Amberger *et al.*, 1992; Freytag *et al.*, 1989; Graybosch *et al.*, 1987; Roth *et al.*, 1989). In maize, the activation of transposable elements is correlated with changes in the pattern of DNA methylation that occur during induction of cultures (Brettell and Dennis, 1991; Kaeppler and Phillips, 1993; Peschke *et al.*, 1991), providing a well-characterized basis for gene activation.

In plants, most transposon-like sequences appear to be extinct (Grandbastien, 1992). Although a number of plant species harbor these sequences (Flavell *et al.*, 1992; Grandbastien, 1992; Voytas *et al.*, 1992), active transposition has only been demonstrated or directly implicated in tobacco (Grandbastien, *et al.*, 1989; Pouteau *et al.*, 1994) and maize (Johns *et al.*, 1985). RNA transcripts and cDNAs from transposons have been recovered from tobacco (Pouteau, *et al.*, 1994; Hirochika, 1993) and maize (Hu *et al.*, 1995), and transposable element-related proteins have been detected in maize (Hu *et al.*, 1995).

The stable introduction of foreign genes into plants represents one of the most significant developments in a continuum of advances in agricultural technology that includes modern plant breeding, hybrid seed production, farm mechanization, and the use of agrichemicals to provide nutrients and control pests. Genetic engineering has been applied to many species in efforts to improve production efficiency and environmental conservation. Genetic engineering complements plant breeding efforts by increasing the diversity of genes and germplasm available for incorporation into crops and shortening the time required for the production of new varieties and hybrids, while also providing opportunities to develop new agricultural products and manufacturing processes.

The first transgenic plants were tobacco plants transformed with a chimeric neomycin phosphotransferase gene carried on the Ti plasmid of *Agrobacterium tumefaciens* (Horsch *et al.*, 1984). *Agrobacterium*-mediated Ti plasmid transfer has proved to be an efficient, versatile method of plant transformation. The range of plant species amenable to genetic engineering using *Agrobacterium* is fairly large. In those systems where *Agrobacterium*-mediated transformation is efficient, it is the method of choice because of the facile and defined nature of the gene transfer.

Few monocotyledonous plants appear to be natural hosts for *Agrobacterium*, however, although transgenic plants have been produced in asparagus and transformed tumors have been observed in yam. Many commercially valuable crop species, such as cereal grains (*e.g.*, rice, maize, and wheat) are not efficiently transformed by *Agrobacterium*, despite extensive efforts made in this direction. This appears to be due to differences in the wound response; those species recalcitrant to *Agrobacterium*-mediated transformation probably do not express the required appropriate wound response (Potrykus, 1991).

Physical methods of gene delivery have been developed in order to transform plants not susceptible to *Agrobacterium*. These methods include biolistic projection ("particle gun"), microinjection, electroporation, and lipofection (Potrykus, 1991). Most physical transformation experiments have utilized plant protoplasts as the recipient cells; however, other regenerable explants have been utilized, including leaves, stems, and roots. Many plant species have been successfully transformed with physical techniques, but some, notably legumes and cereals, have proved difficult to stably transform by these methods. The applicability of such physical methods to these plants is limited by the difficulties involved in regenerating plants from protoplasts, although some success in this regard has been achieved with some cereals and rice. Little success has been achieved with soybean or maize.

Little experimentation has been reported regarding the use of viral vectors for transformation of plants. Plant viruses exist in a variety of forms; they contain either DNA or RNA as their genetic material, have either rod- or polyhedral-shaped capsids, and can be transmitted either by insects, bacteria, or contact with wounded regions (Robertson, *et al.*, 1983). Most known plant viruses contain single (+) strand RNA as their genetic material. (+) strand plant viruses can further be divided into those which possess a single RNA chain and those which have several RNA chains, each necessary for viral infectivity and which are separately encapsulated into separate virions. Cowpea mosaic virus, for example, contains two RNAs, one encoding several proteins including terminal protein and a protease, with the other chain encoding capsid proteins. There also exist segmented double-strand RNA plant viruses. The best-known of these is wound tumor virus (WTV) which contains 12 different segments and which can replicate in either insect or plant cells.

There are fewer plant DNA viruses. Only two known classes exist, one of which contains double strand DNA and which has a polyhedral capsid. The best understood of this class is cauliflower mosaic virus (CMV). The second class of DNA plant viruses are the geminiviruses that consist of paired capsids held together like twins with each capsid containing a circular single-stranded DNA of about 2500 nucleotides. In some cases, the two paired genomes are identical, while in other cases, the two bear almost no sequence relationship.

Early work with a DNA virus showed that a small bacterial antibiotic resistance gene integrated into such a virus could spread systemically throughout infected plants and confer resistance (Brisson, *et al.*, 1984). It has been suggested that the small size of DNA viral genomes is prohibitory to the wide application of such vectors as useful transforming agents in plants. However, little has been done to follow up on this work.

Even less work has been performed in plants regarding the application of genetic engineering to the far larger group of plant RNA viruses (Ahlquist *et al.*, 1987; Ahlquist and Pacha, 1990). It has been suggested that because the viral RNA does not integrate into the host genome, and is excluded from the meristems and offspring, the usefulness of such RNA viruses in plant transformation is limited at best (Potrykus, 1991).

## Summary of the Invention

In one aspect, the present invention provides retroviral and retroviral-like polynucleotides derived from a plant wherein such polynucleotides are capable of integration into the genome of a plant cell. The invention is also directed to other plant retroviral or retroviral-like polynucleotides obtainable by hybridization under stringent conditions (see, *e.g.*, Sambrook *et al.*) with the retroviral or retroviral-like polynucleotides expressly disclosed herein. Also within the scope of this aspect of the invention are regulatory sequences comprising, for example, plant retroviral long terminal repeat (LTR) sequences that may be operably linked to a gene so as to modulate expression of the linked gene.

In a second aspect, the invention is directed to plant retroviral or retroviral-type elements capable of targeted integration into a specific region in the plant genome and further to methods for accomplishing such integration.

In a third aspect, the present invention is directed to vectors containing all or part of a regulatory sequence derived from a plant retrovirus or retrovirus-like polynucleotide, and to vectors comprising all or part of the retroviral or retroviral-like genome and a heterologous gene.

In a fourth aspect, the invention is directed to vectors containing one or more plant retroviral or retroviral-like regulatory sequences operably linked to a heterologous gene. A heterologous gene in the context of the present application refers to a gene or gene fusion or a part of a gene derived from a source other than the plant pro-retrovirus, or a cDNA, or a plant retroviral gene under the regulatory control of a promoter other than its natural promoter.

In a fifth aspect, the invention is directed to isolated purified proteins encoded by the polynucleotides disclosed herein, and to analogs, homologs, and fragments of such proteins that retain at least one biological property of the proteins.

In a sixth aspect, the invention is directed to isolated purified proteins produced by expression of a heterologous gene using the vectors of the present invention.

In a seventh aspect, the invention is directed to methods for using vectors comprising all or part of a plant proretroviral or retroviral genome and vectors comprising plant retroviral regulatory sequences operably linked to a heterologous gene to introduce a heterologous gene or a regulatory element into a plant genome, wherein the expression product of the gene comprises a polypeptide or an antisense RNA and wherein the regulatory element is a transcriptional regulatory element.

In an eighth aspect, the invention is directed to a plant retrovirus comprising a plant retroviral or retroviral-like polynucleotide, a capsid, and an envelope.

In a ninth aspect, the invention is directed to methods for producing a plant retrovirus, in which the plant retroviral polynucleotide is packaged in a capsid and envelope, preferably through the use of a packaging cell line, but alternatively by use of other vector systems or by *in vitro* constitution of the retroviral capsid and envelope.

In a tenth aspect, the invention is directed to plant cells that have been transformed by transduction of a plant retroviral polynucleotide or transformed by a plant retrovirus comprising a heterologous gene according to the methods of the present invention.

## Brief Description of the Drawings

Figure 1 shows the DNA sequence of the oligonucleotide used as a primer in the polymerase chain reaction that generated the plant pro-retrovirus *SIRE-1* cDNA Gm776 (SEQ ID NO:1). The 5′ and 3′ ends of the oligonucleotide are indicated, and degenerate sites (wherein the oligonucleotide mix contained equal proportions of two nucleotides at a given site) are indicated in parentheses.

Figure 2 presents the nucleotide sequence of the *SIRE-1* cDNA Gm776 (SEQ ID NO:2). The regions corresponding to the oligonucleotide primer used to amplify the cDNA are underlined.

Figure 3 depicts a restriction map of the *SIRE-1* Gm776 cDNA sequence.

Figure 4 shows a statistical analysis of sequence similarities between Gm776 and retrotransposons from *A. thaliana* and *Saccharomyces cerevisiae*.

Figures 5A and 5B set forth the DNA sequences of oligonucleotides (SEQ ID NOS: 12-24) utilized in sequencing Gm776 and the 2.4 kb *SIRE-1* cDNA.

Figure 6 sets out the nucleotide sequence (SEQ ID NO: 3) of the 2.4 kb *SIRE-1* cDNA isolated from a lambda gt11 soybean cDNA library.

Figure 7 depicts a restriction map of the 2.4 kb *SIRE-1* cDNA.

Figure 8 depicts the organization of the 2.4 kb *SIRE-1* cDNA.

Figure 9 shows a comparison of the predicted *SIRE-1* $CX_2CX_4HX_4C$ nucleic acid-binding site sequences (SEQ ID NO: 4) with the amino acid sequences of those in other nucleocapsid proteins.

Figure 10 shows a comparison of the predicted amino acid sequence (SEQ ID NO:5) of the putative *SIRE-1* protease domain with the amino acid sequences of other retroelement proteases.

Figure 11 shows an alignment of the RNA sequence (SEQ ID NO: 6) of the putative *SIRE-1* primer binding site to the 3'-end of soybean tRNA$^{met-1}$. Identity between the sequences is indicated by a vertical line (|).

Figure 12 shows a sequence alignment between the 3'-termini of the putative 5' LTR of *SIRE-1* (SEQ ID NO: 7) and the 5' LTR of the potato retrotransposon *Tst1*. Identity between the sequences is indicated by a vertical line (|).

Figure 13 sets out the DNA sequence (SEQ ID NO: 8) of the 4.2 kb fragment of the *SIRE-1* genomic clone isolated from a lambda bacteriophage FIX II soybean genomic library.

Figure 14 depicts the organization of the 4.2 kb *SIRE-1* genomic fragment.

Figure 15 shows the predicted amino acid sequence (SEQ ID NO: 9) encoded by the *SIRE-1* open reading frames ORF1 (single underline) and ORF2 (double underline) encoded by the 4.2 kb *SIRE-1* genomic fragment.

Figure 16 shows the predicted amino acid sequence (SEQ ID NO: 10) encoded by the *SIRE-1* open reading frame ORF2. The putative signal peptide sequence (residues 22-43) and hydrophobic anchor sequence (residues 511-531) are underlined.

Figure 17 shows a comparison of the predicted amino acid sequence (SEQ ID NO: 11) of the *SIRE-1* ORF1 with the C-terminal region of the *copia* RNase H polypeptide. Vertical lines (|) indicate identity between the sequences, whereas conservative and semi-conservative substitutions are indicated by (:) or (.) respectively.

Figure 18 shows a restriction map of the *SIRE-1* genomic clone isolated from a λ bacteriophage FIX II soybean genomic library. The 5' and 3' ends of the insert are at the left and right, respectively. The numbers above and below the schematic indicate the approximate lengths of the restriction fragments. The restriction endonuclease recognition sites are indicated by single letter codes: H represents a Hind III site; X represents an Xba I site; and N represents a Not I site. The boxed regions of the schematic represent open reading frames encoding SIRE-1 proteins: int represents the integrase domain; RT represents the reverse transcriptase domain; RH represents the Ribonuclease H domain; and env represents the envelope protein domain. The rightmost (open) box represents the 3′ soybean flanking region.

Figure 19 shows the DNA sequences (SEQ ID NOS: 25-38) of oligonucleotide primers used to sequence the 4.2 kb genomic fragment. The numbering in the second column indicates the position of the primer sequence with reference to the predicted sense strand of the genomic fragment.

Figure 20 shows the results of a computer analysis performed on the predicted ORF2 amino acid sequence using the computer program NNpredict (Kneller *et al.* 1990).

Figure 21 shows a nucleotide sequence comparison among the *SIRE-1* 3′ LTR (LTR2) and the *gag* R1 and R2 regions. The numbers following the sequence designations indicate the respective locations of the regions within the *SIRE-1* 4.2 kb genomic fragment.

Figure 22 depicts a nucleotide sequence comparison between Gm776 (SEQ ID NO: 2) and the 2.4 kb *SIRE-1* cDNA (SEQ ID NO: 3). The Gm776 DNA sequence is in reverse orientation (*i.e.*, in the 3′ to 5′ orientation) to the 2.4 kb cDNA sequence.

Figure 23 shows the predicted amino acid sequence (SEQ ID NO: 10) of ORF2. The putative hydrophobic transmembrane regions are indicated by a single underline. The predicted coiled-coil regions are indicated by a double underline. The proline rich region is indicated by a dotted underscore. The predicted α-helical regions are indicated in boldface type. The potential SU/TM cleavage sites are indicated by boxes.

Figure 24 depicts an agarose gel electrophoretic analysis of restriction endonuclease digestion of the *SIRE-1* λFIXII genomic DNA by *Hind* III. Lane 1 contains λ DNA size markers. Lane 2 contains the *SIRE-1* λFIXII genomic DNA digested by *Hind* III. The relative lengths of the *Hind* III fragments are indicated by the numbers (*e.g.*, 2.1 H is a 2.1 kb *Hind* III fragment).

Figure 25 shows a schematic representation of the results of restriction endonuclease digestion and Southern hybridization analyses of the *SIRE-1* genomic clone. The length and nature of each fragment is indicated by the alphanumerical designation at the left (*e.g.*, 1.5H is a 1.5 kb *Hind* III fragment). The fragment(s) recognized by each probe (*i.e.*, *env*, *gag*, LTR) are indicated by the arrows.

Figure 26 presents the result of a restriction endonuclease digestion and Southern hybridization analysis of the *SIRE-1* genomic clone. The *SIRE-1* genomic clone was digested with *Sac* I and *Hind* III. The length of the hybridizable fragments is indicated to the left. The Southern hybridization was performed with a radioactively labeled *env* probe derived from the 4.2 kb *Xba* I fragment.

Figure 27 presents a schematic of the pEG4.1 vector construct. The 4.1 kb *SIRE-1* insert is indicated by the thick bolded clockwise arrow.

Figure 28 depicts the result of restriction endonuclease digestion and Southern hybridization analysis of the pEG4.3 vector construct comprising the 4.3 kb *SIRE-1* *Hind* III fragment. The Southern hybridization was performed using a radioactively labeled *gag* probe derived from the 4.2 kb *SIRE-1* *Xba* I fragment.

Figure 29 presents a schematic of the pEG4.3 vector construct. The 4.3 kb *SIRE-1* insert is indicated by the thick bolded clockwise arrow.

Figure 30 presents the sequences (SEQ ID NOS: 39-49) of oligonucleotide primers utilized in the sequencing of the 4.1 kb and 4.3 kb *SIRE-1* *Hind* III fragments contained in pEG4.1 and pEG4.3, respectively. The lower-case c following a primer designation indicates that the primer was utilized for sequencing the (-) strand of the insert.

Figure 31(a)-(c) presents the nucleotide sequence (SEQ ID NO: 50) of the *SIRE-1* genomic clone derived from the sequences of the 4.1 and 4.3 kb *SIRE-1 Hind* III fragments. The first 321 nucleotides of the sequence are derived from the 3′ terminus of the 4.3 kb *Hind* III fragment, and the remaining sequence is derived from the 4.1 kb *Hind* III fragment. The *Hind* III restriction endonuclease recognition site is indicated in boldface (nt 322-327).

Figure 32 presents the amino acid sequence (SEQ ID NO: 51) of the predicted open reading frame encoded by the combined nucleotide sequences of the 4.3 kb and 4.1 kb *Hind* III fragments of the *SIRE-1* genomic clone.

Figure 33 presents a comparison of the predicted amino acid sequence (SEQ ID NO: 52) of the *SIRE-1 int* domain with the integrase domain of the *Opie-2* retroelement from maize. The amino acid residues constituting the HHCC and D(10)D(35)E conserved motifs are presented in boldface. A (.) represents a gap in the sequence required for optimal alignment. A (|) represents identity between the residues. A (:) represents similarity between the residues.

Figure 34 presents a comparison of the predicted amino acid sequence (SEQ ID NO: 53) of the *SIRE-1* reverse transcriptase (RT) domain and the reverse transcriptase domain of the *Opie-2* retroelement from maize. The regions corresponding to conserved retroelement RT domains are presented in boldface. A (|) represents identity between the residues. A (:) represents similarity between the residues.

Figure 35 presents a comparison of the predicted amino acid sequence (SEQ ID NO: 54) of the *SIRE-1* Ribonuclease H (RH) domain and the Ribonuclease H domain of the *Opie-2* retroelement from maize. The conserved DEDD motif is indicated by boldface. A (|) indicates identity between the residues. A (:) indicates similarity between the residues. A (.) indicates a gap in the sequence required for optimal alignment.

## Detailed Description of the Invention

The present invention provides novel plant retroviruses, proretroviruses, proretroviral polynucleotides, proretroviral DNAs, proretroviral-like polynucleotides and plant retroviral derivatives that are useful for genetic engineering in plants. More particularly, the plant retroviruses, proretroviruses, proretroviral polynucleotides, proretroviral DNAs, proretroviral-like polynucleotides, and plant retroviral derivatives derived therefrom are useful for: introducing a heterologous DNA of interest into plant cells where the peptide or polynucleotide encoded by that sequence will be expressed; for introducing a DNA sequence of interest into plant cells where the RNA encoded by that sequence is complementary (antisense) to an endogenous plant polynucleotide; for introducing a DNA sequence into a plant cell where that sequence becomes integrated into a plant genome; for integrating gene regulatory elements such as transcriptional regulatory sequences into a plant genome; and for identifying the location of such integrations.

The invention provides vector constructs comprising plant proretroviral polynucleotides, proretroviral DNAs, proretroviral-like polynucleotides, fragments thereof, and retroviral derivatives derived therefrom that are useful for: expressing desired proteins in target plant cells, for example, proteins that confer enhanced growth, disease resistance, or herbicide tolerance to plant cells, or to express "antisense" RNA complementary to an endogenous plant polynucleotide.

The invention also provides methods for: producing a plant retroviral vector; using a plant retroviral polynucleotide to identify genetic loci and to characterize the function of a gene within a plant genome;

introducing mutations into a plant genome or disrupting an endogenous plant gene ("knockout"); and inserting genes or gene regulatory elements into genomic loci of plants.

The following examples are illustrative of certain embodiments of the present invention but are not to be construed as limiting thereof.

Example 1 describes the isolation and characterization of the *SIRE-1* cDNA.

Example 2 describes the isolation and characterization of a full-length *SIRE-1* clone from a soybean genomic library.

Example 3 describes the analysis of transcriptional activity from the *SIRE-1* pro-retrovirus in soybean and other plants.

Example 4 describes the detection of *SIRE-1* retrovirally encoded protein expression in plant tissues by Western blot analysis.

Example 5 describes the *in vitro* production of polypeptides from *SIRE-1*-encoded mRNAs.

Example 6 describes the use of *SIRE-1* in non-replicative transduction of plant cells.

Example 7 describes methods and products for production of plant retrovirus packaging cells.

Example 8 describes methods for transduction of plant retroviral polynucleotides into plant cells.

Example 9 describes the use of *SIRE-1* as a gene transfer vector.

Example 10 describes the use of *SIRE-1* to induce and tag mutations in plant genomes.

Example 11 describes the modification of *SIRE-1* to effect directed integration at a specific locus in a plant genome.

Example 12 describes the use of *SIRE-1* and flanking DNA sequences to determine the site of *SIRE-1* insertion in the soybean genome.

## Example 1

### Isolation and Characterization of *SIRE-1* cDNA

The initial characterization of the *SIRE-1* retroviral DNA was based on the fortuitous recovery and analysis of a 776-bp DNA fragment (Gm776) generated by the polymerase chain reaction (PCR) in an attempt to amplify soybean DNA coding for a cytokinin biosynthetic enzyme (Laten and Morris, 1993). Amplification of either total DNA (from etiolated plumules of *Glycine max* cv Williams, isolated by the method of Doyle and Doyle, 1990) or nuclear DNA (from *G. max* cv Wayne, isolated by the method of Hagen and Guilfoyle, 1985) with the single 22-nt oligonucleotide primer (**Figure 1**; SEQ ID NO: 1) generated high levels of Gm776. The amount of Gm776 generated in each PCR amplification suggested that *SIRE-1* is a member of a multi-copy DNA family, and the absence of additional bands suggested that the family is relatively conserved.

Hybridization and restriction digest analyses were performed to characterize the element size of the *SIRE-1* family. Soybean genomic DNA was cleaved with *Bam*HI, *Eco*RI, *Hae*III, *Hind*III, *Hpa*I, and *Mbo*I, respectively, electrophoresed through 0.7% agarose, and blotted to a nylon membrane. The blot was hybridized with radiolabeled Gm776 cDNA in 0.05 M Tris, 1 M NaCl pH 7.5 in 50% formamide at 42°C, washed, and exposed to autoradiography (Southern, 1975). These analyses indicated that the *SIRE-1* family is composed of several hundred, non-tandem, highly homogeneous copies, each in excess of 10.6 kb in length.

*Xba*I linkers were ligated to agarose gel electrophoresis (AGE)-purified Gm776 (modified Gm776) (Sambrook *et al.*, 1989; Titus, 1991). The modified Gm776 DNA was extracted with phenol/chloroform and chloroform, ethanol- precipitated, and redissolved in 10 mM Tris-HCl, 1 mM EDTA, pH 7.6. pUC19 was linearized with *Xba*I and dephosphorylated (Sambrook *et al.*, 1989). Linearized pUC19 DNA and the modified Gm776 DNA insert with the ligated *Xba*I linkers were ligated, and DH5-$\alpha$ cells were transformed with the ligation products. Transformants were identified by resistance to the antibiotic ampicillin (amp$^r$), and the presence of plasmids containing the insert in the amp$^r$lac$^-$ colonies was determined by hybridization with $^{32}$P-labeled probe synthesized from PCR-amplified, PAGE-purified Gm776 DNA. Plasmid DNA from colonies giving positive hybridization signals was isolated by alkaline lysis (Sambrook *et al.*, 1989).

The recovered pGm776 plasmid DNA was sequenced by dideoxynucleotide chain termination using Sequenase 2.0 (U.S. Biochemical, Cleveland, OH) and plasmid-specific and insert-specific primers according to the manufacturer's instructions (Figure 2, SEQ ID NO: 2; Figure 5A and B, SEQ ID NOS: 12-24). Sequence analysis suggested that *SIRE-1* is a member of the *copia/Ty1* retrotransposon family. *SIRE-1* sequences were subsequently detected by hybridization studies using the Gm776 cDNA probe in the genome of *G. max* cv Williams, in several different cultivars, and in the ancestral species, *Glycine soja.* The copy number of the element among these sources varies from a few hundred to over a thousand. The variation in copy number, especially among domestic cultivars, suggested that the family remains active, e.g., capable of replication and transposition. The homogeneity of the sizes of the *SIRE-1* family members also suggested that most are relatively young and have not had time to accumulate a large number of mutations.

The nucleotide and all six possible peptide translations of the Gm776 sequence were compared to sequences in the GenBank and EMBL databases (Devereux *et al.* 1984). No closely related sequences were revealed in these searches. However, statistical analyses of sequence similarities between Gm776 and retrotransposons from *A. thaliana* and *Saccharomyces cerevisiae* were performed using the Gap computer program (Devereux *et al.* 1984), and revealed lengthy, albeit weak, sequence similarities. The results of the analyses are set forth in Figure 4. Column (a) in Figure 4 denotes the nucleotide ranges within Gm776 that exhibit sequence similarities to other retrotransposon elements, and column (b) denotes the retrotransposon elements that exhibit nucleotide sequence homology to the sequences in column (a). Column (c) shows the percentage identity between the sequence ranges in columns (a) and (b), with gap weights of 3.0 for *Ta1* and 2.0 for *Ty1* and a gap length weight of 0.3. Two overlapping 300-plus bp regions between nt 150 and 670 of Gm776 exhibit over 50% identity to adjacent regions overlapping the *Ta*1 RNA binding domain. The alignments include seven gaps in each sequence, averaging 2.5 bp per gap.

When the six potential Gm776 translation sequences were compared to the sequence of the *Ta*1 polyprotein in the region of DNA similarity, no similarities were observed. However, 51% of the nucleotides between bp 390 and 630 of Gm776 are identical to a sequence within the reverse transcriptase gene of the *Saccharomyces cerevisiae* retrotransposon *Ty*1. The alignment requires five gaps averaging 2 bp per gap. There is no significant similarity between any of the six potential Gm776 translation sequences and the corresponding region of the *S. cerevisiae* reverse transcriptase. Sequence comparisons with several other plant transposons, including the *copia*-like elements *Tnt*1 from tobacco (Grandbastien *et al.* 1989), *Tst*1 from potato (Camirand et al. 1990), and PDR1 from pea did not reveal significant similarities.

Column (d) in Figure 4 denotes the "qualities" of sequence matches denoted in column (c), and column (e) denotes the qualities and standard deviations of randomized sequence alignments of the same lengths and base compositions. Column (h) represents the probabilities (P) for normal distribution calculated using the equation $P=0.3989e^{-(x2/2)}$ where x=(Q-meanQ)/S.D. The results indicate that the derived similarities are quite significant, especially as approximately 150,000 nucleotides in 30 transposons were analyzed.

A soybean cDNA lambda gt11 bacteriophage library (Clontech) was screened for the presence of *SIRE-1* cDNAs by hybridization methods well-known in the art (Sambrook *et al.* 1989). The radiolabeled probe was generated from the pGm776 plasmid using the Multiprime DNA Labeling kit (Amersham, Arlington Heights, IL). Three phage plaques (out of 6,000 screened) showed positive hybridization signals and were isolated by limiting dilution and rescreening. Recombinant phage DNA from one of the clones was isolated from plate lysates (Sambrook *et al.*, 1989) and purified on a Qiagen-100 column as recommended by the manufacturer (Qiagen, Chatsworth, CA). The clone contained a 4.0 kilobasepair (kb) insert that was transferred from the phage vector to pUC18 as follows. The purified phage DNA was digested with *Eco*RI, extracted with phenol/chloroform and chloroform, ethanol precipitated, and redissolved in 10 mM Tris-HCl, 1 mM EDTA, pH 7.6. pUC18 was linearized with *Eco*RI and dephosphorylated (Sambrook *et al.*, 1989). Linearized pUC18 DNA and the 4.0 kb *Eco*RI DNA insert were ligated, and DH5-$\alpha$ cells were transformed with the ligation product. Transformants were identified by resistance to the antibiotic ampicillin (amp$^r$), and the presence of plasmids containing the insert in the amp$^r$lac$^-$ colonies was determined by hybridization with $^{32}$P-labeled probe synthesized from PCR-amplified, gel-purified Gm776 DNA.

Plasmid DNA from colonies giving positive hybridization signals was purified over a Qiagen-100 column as described above. Initially, digestion of plasmid DNAs with *Eco*RI generated insert fragments of 2.4 and 1.6 kb. Only the former hybridized to the Gm776 probe. However, the recombinant plasmid isolated for sequencing contained only the 2.4 kb *SIRE-1* fragment, and re-isolation of the original construct proved difficult. The 2.4 kb cDNA insert was sequenced by dideoxynucleotide chain termination using Sequenase 2.0 (U.S. Biochemical, Cleveland, OH) and plasmid-specific and insert-specific primers according to the manufacturer's instructions, and was found to be 2389 bp in length **(Figure 6**; SEQ ID NO: 3; GenBank Accession No. U22103).

The cDNA was found to contain an uninterrupted 617-codon open reading frame (ORF) beginning at nucleotide (nt) 236 **(Figures 6 and 8**; SEQ ID NOS: 8,9). A second 87-codon ORF begins at nt 2155 and continues through the end of the truncated fragment **(Figures 6 and 8)**. The ATG codon at nt 236 is the fourth ATG in the sequence. Extended leader regions with ATGs upstream of the actual translational start site are not unknown among retroelement mRNAs (Varmus and Brown, 1989). In the *SIRE-1* cDNA (SEQ ID NO: 8), the first ATG at nt 28 is followed immediately by a stop codon, and initiations at the two other upstream ATGs each may produce only a dipeptide. It has been suggested that 40S ribosomal subunits can reinitiate and resume scanning beyond very short, upstream ORFs (Kozak, 1991). The ATG at nt 236 is closely followed by another in-frame ATG at nt 242. The latter is actually in a more representative context for translational initiation than is the former (Heidecker *et al*., 1986).

The ORF1 of *SIRE-1* (**Figures 6, 8, and 9**; SEQ ID NO: 9) contains three regions that are characteristically highly conserved among retroviral and retrotransposon polyproteins (Katz and Jentoft, 1989; Varmus and Brown, 1989). The first two are $CX_2CX_4HX_4C$ (where C represents cysteine, H represents histidine, and X denotes any amino acid) nucleic acid-binding motifs (i.e., CCHC boxes) found in retroviral and retrotransposon nucleocapsid (NC) proteins encoded by *gag*, and the third is a catalytic domain (LDSG: lysine-aspartic acid-serine-glycine) characteristic of *prot*-encoded aspartic proteases that cleave retroelement polyproteins.

In a few characterized retroelements, the CCHC boxes in the *gag* region are repeated. The repetition of the CCHC boxes in *SIRE-1* is unique in that the boxes are separated by 189 codons, rather than by just a few codons as in other retroelements (**Figure 8**). As NC proteins are generally less than 100 amino acids in length, it is possible that the *SIRE-1* boxes are expressed in two distinct proteins.

Both *SIRE-1* CCHC boxes are flanked by highly basic regions, especially the region between the boxes: seven of nine amino acids that precede the downstream box are lysine or arginine. This is characteristic of retroelement NC proteins, which are highly basic and are dominated by polar amino acids. Although the boundaries of the *SIRE-1* NC proteins are not yet defined, CCHC boxes are generally found near the carboxy-terminus. The putative NC protein encompasses roughly amino acids 260 to 525. This region is highly basic (23%) and very polar (62%). Sequence comparisons between the *SIRE-1* protease peptide sequence and those of other retroelements firmly places *SIRE-1* in the *copia/Ty1* family (**Figures 9 and 10**).

Retroelement (-) strand replication is usually primed by a host tRNA, often the initiator tRNA. A 22-nt primer binding site (PBS) complementary to the 3' end of soybean tRNA$^{met-1}$ lies upstream of the *SIRE-1* ORFs, between nucleotides 180 and 201 (**Figure 11**; SEQ ID NO: 6). Retroelement PBSs are generally located adjacent to the 5'-LTR (Boeke, 1989). Two bases separate the 5' end of the *SIRE-1* PBS from the dinucleotide CA, found at the 3' end of nearly every LTR. The sequence of the downstream LTR from a genomic clone **(see Example 2)** confirms that this dinucleotide marks the end of the LTR. The putative *SIRE-1* LTR shows significant homology to the terminal 17 nt of the 5' LTR of the potato retrotransposon *Tst1* (**Figure 12**; SEQ ID NO: 7).

An unusual feature of *SIRE-1* is the presence of a 95-bp, nearly tandem, direct repeat between nt 2096 and 2299 (**Figure 6**; SEQ ID NO: 3). The repeats are separated by 3 bp. The upstream member has an 11-bp insertion that is absent in the downstream member. Otherwise, the sequences are 95% identical. The 5% divergence makes it very unlikely that the duplication was created during the cloning process.

The 2.4 kb cDNA sequence was aligned to the corresponding region of Gm776, and it was found that the amplified fragment lies completely within the *gag* region of the 2.4 kb fragment, and that the two sequences differ by only 2% (**Figure 22**). Of the 13 bp differences, seven retain the same amino acid. Of the remaining six, three result in the substitution of one non-polar amino acid for another -- isoleucine for phenylalanine, isoleucine for valine, and leucine for methionine -- and two are substitutions of threonine by isoleucine. The last substitution generates a stop codon in Gm776. Among the amino acid changes, only the threonine to isoleucine substitution is not considered to be a conservative replacement. The predominance of silent and conserved substitutions strongly suggests that the differences reflect the slightly diverged, evolutionary relationship between two *SIRE-1* family members.

## Example 2

## Isolation and Characterization of the *SIRE-1* Genomic Clone

Oligonucleotide primers (**Figure 5B**; SEQ ID NOS: 15-24) were utilized in PCR to amplify fragments from the *gag* and *pol* regions and from part of the adjacent LTR of the 2.4 kb cDNA clone. These amplified fragments and synthetic oligonucleotides (**Figure 5**) were used to generate *gag*- and LTR-specific radiolabeled probes. A λFIXII soybean genomic library (Stratagene, La Jolla CA) was probed with radiolabeled *SIRE-1 gag* probes and positively-hybridizing plaques were purified by limiting dilution screening (Sambrook *et al.*, 1989). DNA was prepared from phage recovered from liquid culture (Burmeister and Lehrach, 1996).

The phage DNAs containing the putative *SIRE-1* genomic clones were digested with the restriction endonuclease *Not* I to release the DNA inserts from the phage. The largest DNA inserts obtained thereby were digested with *Xba* I, and Southern blots of the digested DNAs were probed with an end-labeled, LTR-specific oligonucleotide to identify clones carrying two LTRs. Analyses of one clone yielded two hybridizing bands, indicating that this clone contained two LTRs and was a probable source of a full-sized, intact copy of *SIRE-1*. The purified phage DNA containing the full-length *SIRE*-1 genomic clone was deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville MD 20852 on 12 August 1997 (ATCC accession number 209200) in accordance with the Budapest Treaty requirements.

Restriction endonuclease digestion of the phage DNA with *Xba* I yielded three fragments of 8.5, 6.5 and 4.2 kb. Southern hybridization of the electrophoretically separated fragments with a radioactively labeled 2.4 kb *SIRE*-1 cDNA probe revealed that the *SIRE*-1 2.4 kb cDNA sequence extends across the 12.5 kb and 4.2 kb *Xba* I fragments.

The fragments were each subcloned into a pSPORT-1 plasmid (Life Technologies, Gaithersburg MD) for automated DNA sequencing. Some of these subclones were unstable, but the one carrying the 4.2 kb *Xba* I fragment that hybridized to the LTR probe, but not to the *gag* probe, displayed no evidence of rearrangement. Both strands of this 4.2 kb clone were sequenced on ABI Prism 377 DNA sequencers using pUC universal primers and the oligonucleotide primers listed in **Figure 19** (SEQ ID NOS: 25-38). This sequence (**Figure 13**; SEQ ID NO: 8) is made available as GenBank Accession number U96295.

The 4.2 kb *Xba*I fragment encompasses the 3′ end of the genomic clone and contains the distal 3.7 kb of *SIRE*-1 along with 538 bp of presumably single-copy flanking DNA (**Figure 14**). Analysis and predicted translation of the *SIRE*-1 genomic sequence revealed the presence of two ORFs (**Figure 14**). The first, ORF1 (**Figure 15**; SEQ ID NO: 11), extends from nucleotide (nt) 1 to nt 191, and is clearly the 3′ end of a retroelement ribonuclease H (RH)-encoding sequence. The 3′ terminus of the *SIRE-1* RH coding region exhibits significant amino acid sequence homology (*i.e.*, 53% identity and 87% similarity) with the carboxy-terminus of RNase H from *copia* **(Figure 17)**. In all *copia/Ty1*-like retrotransposons, the RH coding sequence is at the 3' end of the *pol* gene and is closely followed by a polypurine tract (PPT) and the 3' LTR. However, the RH coding region of *pol* in *SIRE-1* is followed by a long ORF in the region corresponding to retroviral *env* (see below).

The second ORF within this fragment, *i.e.*, ORF2, extends from nt 219 to nt 1958. The predicted translation product suggests that ORF2 encodes a full-length, envelope (*env*)-like glycoprotein characteristic of animal retroviruses (**Figures 15 and 16**; SEQ ID NO: 10). Retroviral envelope proteins are synthesized from a spliced transcript in which the initiation codon is supplied by the *gag* region, which for *SIRE-1* was found in the 2.4 kb cDNA clone (**Example 1**; SEQ ID NO: 3). The amino-terminal one-third of the *SIRE-1 env* sequence is rich in proline, serine, and threonine codons, with the latter two possibly serving as O-glycosylation sites. There are also a small number of asparagines in this region that might serve as N-glycosylation sites.

Although the predicted amino acid sequence of ORF2 does not exhibit significant amino acid homology with the known *env* proteins, its predicted secondary structure is typical of animal retrovirus *env* proteins. Failure to find high amino acid homology with other retroviral proteins is not surprising, as it is likely that *SIRE-1* and the animal retroviruses diverged before either had acquired an *env* encoding region.

A typical retroviral *env* protein has a signal peptide near the amino-terminus. There is a likely hydrophobic signal peptide at codons 22-43 of the *SIRE-1 env* sequence (**Figure 16**; SEQ ID NO: 10). Near the carboxy-terminus of retroviral envelope proteins, a hydrophobic domain serves to anchor the molecules in the membrane such that the protein is oriented with the N-terminus outside the cell and the C-terminus within the cytoplasm. Codons 511 to 531 of the *SIRE-1 env* sequence (SEQ ID NO: 10) constitute a hydrophobic region that may provide this function (**Figure 16**). These assignments and the appropriate membrane orientations are strongly supported by analysis with the transmembrane prediction computer program TMpredict (Hofman and Stofel, 1993) (see below).

ORF2 is 647 codons in length (SEQ ID NO: 10), and the derived, unmodified theoretical protein has a molecular weight of 70 kD. Despite its location immediately downstream of *pol*, the translated *env* amino acid sequence does not exhibit significant sequence identity to any reported retroviral *env* protein. This result is not entirely unexpected because known *env* sequences constitute a very heterogeneous population, and pair-wise comparisons often fail to demonstrate significant sequence congruence (Doolittle, *et al.*, 1989; McClure, 1991). Alternatively, ORF2 could be a transduced cellular sequence. For example, *Bst1* from maize, a low copy-number LTR retrotransposon that lacks its own RT (Johns, *et al.*, 1989; Jin and Bennetzen, 1989), encodes domains derived from a maize plasma membrane H-ATPase (Bureau, *et al.*, 1994; Palmgren, 1994).

Retroviral *env* genes encode polypeptides that are cleaved by host proteases into surface (SU) and transmembrane (TM) peptides, respectively, which are subsequently rejoined through disulfide linkages (Hunter and Swanstrom, 1990). While the primary sequences of these proteins may be diverse, all retroviral *env* proteins are glycosylated and share three functionally conserved hydrophobic domains: a signal peptide near the amino terminus of SU, a membrane fusion peptide near the amino terminus of TM, and a distal anchor peptide (Hunter and Swanstrom, 1990).

Retroviral *env* glycoproteins contain between four and thirty N-glycosylated asparagines at Asn-Xaa-Ser/Thr motifs (Hunter and Swanstrom, 1990), with SU generally more heavily glycosylated than TM. The conceptual translation product of ORF2 from *SIRE-1* has only two Asn in this context. However, retroelement *env* proteins are also known to be O-glycosylated at Ser and Thr residues (Pinter and Honnen, 1988). O-glycosylation is correlated with clusters of hydroxy amino acids with elevated frequencies of Pro (Wilson *et al.*, 1991). The amino half of the theoretical *SIRE-1* protein (corresponding to SU) conforms to this pattern, and many of the hydroxy amino acids in the carboxyl half of the protein are adjacent to Pro. The amino acid composition of one extended proline-rich region encompassing amino acids 60 through 127 (SEQ ID NO: 10) is similar to the 60-amino acid proline-rich neutralization (PRN) domain of SU from feline leukemia virus (FeLV) (Fontenot *et al.*, 1994). Pro makes up 18% in both and hydroxy amino acids are 20% in the FeLV PRN and 22% in *SIRE-1*. Gln is 9% in FeLV and 10% in *SIRE-1*, and while the PRN of FeLV contains no aromatic amino acids, the comparable *SIRE-1* region contains only one. In *SIRE-1*, the spacing of many of the Pro residues in this region and beyond (Xaa-Pro-Yaa)$_n$ or (Xaa-Pro)$_n$ is characteristic of many structural membrane proteins from both eukaryotes and prokaryotes (Williamson, 1994).

The putative *env* protein sequence was evaluated for the presence of hydrophobic, membrane-spanning helices using TMpredict (Hofmann and Stoffel, 1993). The program returned two possible transmembrane regions with high confidence values and a third somewhat below the margin of significance (**Figure 23**). The first predicted helix encompasses amino acids 22 to 43 (SEQ ID NO: 10), a typical signal peptide location. The second predicted transmembrane helix extends from amino acid 510 to amino acid 530 (SEQ ID NO: 10), and corresponds to the general location of retroviral anchor peptides. Although of questionable statistical significance, the third predicted transmembrane helix, from amino acids 465 to 485, is in a location that could correspond to that of viral membrane fusion peptides.

Only two retroviral *env* peptides have been structurally characterized by X-ray crystallography (Chan *et al.*, 1997; Fass *et al.*, 1996), but several *env* SU and TM sequences have been analyzed by structural prediction computational programs (Hunter and Swanstrom, 1990; Gallaher *et al.*, 1995; Gallaher *et al.*, 1989). Analysis of the ORF2 sequence using the computer program NNpredict (Kneller *et al.*, 1990) suggests the presence of long α-helices and regions of β-sheets (**Figure 20**) typically found in *env* proteins. The evaluation of ORF2 using several other programs (Deleage and Roux, 1987; Georjon and Deleage, 1995; Georjon and Deleage, 1994; Gibrat *et al.*, 1987; Levin *et al.*, 1986), yielded predictions of multiple α-helices similar to those of corresponding regions of other retroviral *env* proteins (Hunter and Swanstrom, 1990; Gallaher *et al.*, 1995; Gallaher *et al.*, 1989).

ORF2 (SEQ ID NO: 10) was also evaluated for the possible presence of coiled-coils (Lupas *et al.*, 1991). Amino acids 580 to 611 were predicted to form a coiled-coil with very high confidence (**Figure 23**). The sequence adheres well to the heptad repeat sequence identified in several virus fusion peptides (Chambers *et al.*, 1990). The predicted coiled-coil in the TM domains of HIV and Moloney murine leukemia virus have recently been confirmed by X-ray crystallography (Chan *et al.*, 1997; Fass *et al.*, 1996).

Retroviral *env* proteins are generated from spliced transcripts (Varmus and Brown, 1989; Hunter and Swanstrom, 1990). In the case of some avian retroviruses, splicing leads to an in-frame fusion of the *gag* start codon with the 5' end of the *env* coding region (Hunter and Swanstrom, 1990), obviating the need for an initiating AUG in *env*. An analogous splice in a *SIRE-1* transcript would serve the same purpose, although no splice donor or acceptor consensus sequences are present in the expected regions. Cleavage of *env* proteins into SU and TM generally occurs at a conserved site containing the consensus sequence Arg-Xaa-Lys-Arg (Hunter and Swanstrom, 1990). This sequence does not appear in the putative *SIRE-1 env*, but there are several similarly basic tetrapeptide candidates for such a cleavage site (**Figure 23**). The Lys-Lys-Gly-Lys at residues 439-442 would generate a TM protein of 22.3 kD with the fusion peptide near the amino terminus. The corresponding SU would be 48.7 kD.

To confirm that the putative *env* gene was not a library or cloning artifact, and that most, if not all, genomic copies of *SIRE-1* were organized in the same way as the clone, *SIRE-1* genomic DNA was digested with several restriction enzymes and a Southern blot was probed with sequences from the *env* and *gag* subclone regions. The intensity of hybridization of an *env* probe to genomic DNA (data not shown) was similar to that for the *gag* probe that had previously been used to establish the moderately high copy number of *SIRE-1* (Laten and Morris, 1993). In addition, *gag* and *env* probes hybridized to the same 10.5 kb *Hpa*I fragment (data not shown). Although the possibility cannot be ruled out, this *env*-like ORF is probably not a transduced host gene. The presence of this ORF in most if not all of the several hundred copies of *SIRE-1* suggests that this gene is an integral part of the retroelement genome.

Alternate splicing could result in an additional ORF extending from nt 1834 to 2166, thereby encoding a 110-amino acid peptide. Such alternate splicing of retroviral transcripts at similar sites has been shown to lead to the production of *trans*-acting factors, which may be useful in modulating gene expression in accordance with the present invention.

To identify the LTR, the DNA sequence (SEQ ID NO: 8) from the 4.2 kb *Xba*I fragment was aligned with that from the *SIRE-1* cDNA clone (SEQ ID NO: 3) which contained the last 178 bp of the 5' LTR. Sequence alignments were made using the Genetics Computer Group package (Devereux *et al.*, 1984). The GCG analysis confirmed that the genomic subclone contained a 3' LTR and fixed the location of the 3' end of the LTR at nt 3686 in the sequence AATTTCA (**Figure 3**; SEQ ID NO: 8), beyond which the two sequences diverged. Although the region of LTR overlap was virtually identical (98% sequence identity), the moderately high copy number of *SIRE-1* makes it unlikely that the cDNA and genomic clones represent copies of the same element.

Upstream of the genomic LTR there are several polypurine regions ranging in length from 11 to 16 nucleotides (**Figures 13 and 14**). Such sites are known to serve as origins for initiation of retroelement plus-strand synthesis. In addition, the *SIRE-1* LTR contains appropriately located sequences that strongly resemble consensus sequences for retroviral promoter elements and polyadenylation signals.

The 538 nucleotides of flanking DNA adjacent to the 3'-end of the *SIRE-1* sequence (SEQ ID NO: 8) comprises an uninterrupted open reading frame (**Figure 14**). This strongly suggests that the *SIRE-1* insertion disrupted a functional gene. As the *G. max* cultivar is essentially a tetraploid, its genome can accommodate some gene disruptions without major phenotypic consequences. The predicted translation product of the flanking DNA is relatively hydrophilic and is rich in asparagine and glutamine codons. No significant homology was found with known plant proteins, however.

To obtain other subclones of *SIRE-1*, the genomic *SIRE-1* λFIXII bacteriophage DNA was double-digested with *Hind* III (which does not digest λFIXII DNA) and *Sac* I (which does digest λFIXII DNA in the multicloning region). This digest generated 10 fragments (**Figure 24**). The two largest fragments, 20 kb and 9 kb, respectively, are known to constitute the lambda phage arms. The other eight fragments collectively constituted 19 kb of *SIRE-1* genomic sequence. Individual digests of the genomic clone with *Hind* III and *Sac* I, respectively, revealed that the 2.1 kb and 1.5 kb fragments produced in the double digest were adjacent to the lambda phage arms (data not shown). Therefore, these two fragments each have *Hind* III and *Sac* I termini, while the other 6 fragments have only *Hind* III termini.

Southern blot hybridizations were conducted with the *Hind* III/*Sac* I double-digested *SIRE-1* DNA using probes derived from the LTR, *gag*, and *env* regions of the 4.2 kb *Xba* I fragment, respectively (**Figure 25**). These experiments revealed that the *env* sequence lies within the 4.1 kb fragment (**Figure 26**); the LTR regions are contained within the 4.3 kb and 2.7 kb fragments; and the *gag* region is also contained within the 4.3 kb fragment (**Figure 27**).

The 4.1 kb fragment (containing at least a portion of the *env* region) and the 4.3 kb fragment (containing at least a portion of the *gag* region) were each subcloned into pSPORT-1 vectors and the constructs were separately transformed into DH10B *E. coli* cells. Recombinant plasmids were detected by restriction digestion and Southern hybridization. The vector construct comprising the 4.1 kb fragment was named pEG4.1 (**Figure 28**), and the vector construct comprising the 4.3 kb fragment was named pEG4.3 (**Figure 29**).

The pEG4.1 construct was sequenced using M13/pUC universal primers (pUC-forward and -reverse; SEQ ID NOS: 12, 14) and *SIRE-1* specific primers (**Figure 30**; SEQ ID NOS: 39-49) as described above. Translation of the nucleotide sequence obtained thereby (**Figure 31a-c**; SEQ ID NO: 50) revealed a long uninterrupted open reading frame encoding 942 amino acids (**Figure 32**; SEQ ID NO: 51). The 3′ terminus of the 4.1 kb *Hind* III fragment overlapped the 5′ terminus of the 4.2 kb *Xba* I fragment (described above, containing the *env* region) by approximately 1.5 kb. Translation of the remaining 2.6 kb sequence revealed regions exhibiting strong homologies to the integrase, reverse transcriptase, and RNase H regions of known retrotransposons.

The 4.3 kb *Hind* III fragment contained in pEG4.3 was partially sequenced using pUC universal primers (**REF**; SEQ ID NOS: 12,14). The 5′ terminal region of the 4.3 kb fragment was found to contain sequence identical to that of the putative 3′ LTR contained within the 3′ terminal region of the 4.2 kb *Xba* I (*env*-containing) fragment (SEQ ID NO: 8). The 3′ terminal region of the 4.3 kb *Xba* I fragment contained sequences exhibiting strong homology to the amino-terminal region of the integrase (*int*) domain of known retrotransposons.

A region encompassing 400 amino acid residues predicted from the contiguous nucleotide sequences of the 3′-terminal region of the 4.3 kb fragment and the 5′-terminal region of the 4.1 kb fragment, respectively, appears to constitute an integrase (*int*) domain (SEQ ID NO: 52). The predicted amino acid sequence of this putative *int* domain was compared against the BLAST-P peptide database. Significant homology was found with *copia*-like retrotransposons, with the strongest homology being to the *Opie-2* element from maize, which exhibited 39.8% identity and 58.5% similarity at the amino acid level, with three sequence gaps (**Figure 33**). The putative *SIRE-1* and *Opie-2* elements each contain a conserved HHCC (H-X4-H, C-X2-C) motif, which is usually found at the amino-terminus of retrotransposon integrase domains (**Figure 33**). The *SIRE-1* and *Opie-2* elements also each contain a D(10)D(35)E motif (*i.e.*, two aspartate residues within 10 residues of each other, and a glutamate residue within 35 residues of the pair in the carboxy-terminal direction) (**Figure 33**).

The break point between the integrase (*int*) and the reverse transcriptase (RT) domains of *SIRE-1* was determined by comparison of the 4.1 kb fragment sequence with the sequences of retroelements where the break point has been determined experimentally (Doolittle *et al.*, 1989; McClure, 1991; Springer and Britten, 1993; Taylor *et al.*, 1994; Rogers *et al.*, 1995). The predicted amino acid sequence (SEQ ID NO: 53) of the reverse transcriptase domain extends from residue 401 to residue 781. This predicted sequence was compared against the BLAST-P peptide sequence database. Significant homology was found between the putative *SIRE-1* RT region and the RT regions of *copia*-like retrotransposons (**Figure 34**). Again, the most significant match was to *Opie-2* from maize, which exhibited 56% identity and 71% similarity at the amino acid level, with one sequence gap (**Figure 34**). Several regions in which the *SIRE-1* RT exhibits near identity to that of *Opie-2* encompass sequences that have proved useful in studying the phylogenetic relationships of retroelements (Xiong and Eickbush, 1990).

The break point between the reverse transcriptase (RT) and Ribonuclease H (RH) regions of the *SIRE-1* 4.1 kb fragment sequence was also predicted by comparison against those of known retroelements. The RH domain of *SIRE-1* appears to encompass the predicted amino acids 782 to 942. This predicted sequence (SEQ ID NO: 54) was compared against the BLAST-P peptide sequence database. Not surprisingly, the strongest homology was found with the RH element of maize *Opie-2*, which exhibited 53.1% identity and 71.0% similarity to the predicted *SIRE-1* RH region (**Figure 35**). The *SIRE-1* RH domain also contains the DEDD motif found in the RH elements of most known retrotransposons (**Figure 35**).

These data confirm that *SIRE-1* is a retroviral family whose genomic structure is based on a *copia/Ty1*-like organization. The genomic organization of all animal retroviruses (from vertebrates and *Drosophila*) is patterned after *gypsy/Ty3*-like retrotransposons. Neither retroviral genomes nor virions have been reported in plants, although both classes of retrotransposons are widespread. In plants, virus spread is mediated by intercellular movement (Mushegian and Koonin, 1993). However, very few plant virus genomes encode an *env* gene. Those that do -- rhabdoviruses and bunyaviruses (Matthews, 1991) -- also infect animal hosts where *env* proteins mediate viral-host cell membrane fusion. Plant cell walls may preclude this mode of virus transfer, and whether the *env* proteins of these viruses serve any function in their plant hosts is not known. Thus, the presence of an *env* gene in *SIRE-1* suggests that *SIRE-1* may have originally been an infectious invertebrate retrovirus.

The overall restriction site homogeneity, the presence of long, uninterrupted ORFs within and adjacent to *SIRE-1*, and the near identity of the 5′ and 3′ *SIRE-1* LTRs suggest that *SIRE-1* is not an evolutionary relic, and may be modified to function as an infectious retrovirus and/or intracellular retrotransposon.

The genomic clone may be used as a *SIRE-1* genomic probe. The probe may be hybridized to Southern blots of complete and partial digests of soybean DNA to generate a consensus restriction map (Sambrook *et al.*, 1989). Additionally, restriction maps of additional clones and the genomic DNA consensus may be compared to more fully assess *SIRE-1* heterogeneity. The polymorphic sequences of clone populations may then be used to determine expression-related features and phylogenetic relationships to other plant and animal elements.

The *env*, *gag*, and *pol* nucleotide sequences may be used to generate oligonucleotide or cDNA probes to detect transcription of these regions (Navot *et al.*, 1989), and antibodies generated against *SIRE-1* proteins may be used to detect the presence of retroviral protein expression in various plant tissues (Hsu and Lawson, 1991). Moreover, reverse transcriptase (RT) and integrase (*int*) probes may be created by restriction digestion or PCR and used to assess the functional significance of the unprecedented length of *SIRE-1*.

## Example 3

## Northern Hybridization Analysis of *SIRE-1* Transcriptional Activity

The use of the *SIRE-1* polynucleotide as a tool for genetic engineering may require the expression of sequences therefrom. It may therefore be desirable to determine growing conditions under which plants or plant cell cultures that have been infected or transduced with *SIRE-1*-derived DNA exhibit elevated or depressed transcriptional activity. There are many examples in which the transcriptional activity of a virus is enhanced during periods in which its host experiences environmental stress. Therefore, experiments may be conducted to determine growth conditions (or conditions of stress) optimal for the regulation of *SIRE-1* expression.

The presence of *SIRE-1*-specific transcripts in plants such as soybean may be evaluated by Northern hybridization (Sambrook *et al.*, 1989). For example, several *G. max* cultivars, including the Asgrow Mutable line, an unstable soybean isolate (Groose & Palmer, 1987; Groose *et at*, 1983), and *Glycine soja* strains (from a range of origins) may be grown from seed obtained from the U.S. Regional Soybean Laboratory in Urbana, Illinois.

Plants may be grown under optimal and adverse (stress) conditions in growth chambers or in a greenhouse, and the transcriptional activity of *SIRE-1* in plants subjected to adverse conditions may then be compared to that in plants grown in normal conditions.

Many potential adverse growing conditions are well-known in the art. For example, seedlings may be grown in vermiculite and subjected to temperatures ranging from 15°C to 40°C. Plants may also be subjected to salt stress by applying NaCl solutions ranging up to 2%, or to osmotic stress by adding solutions containing PEG 8000. Plants growing under each or several of these conditions may be harvested at various times to assess the temporal relationship of the adverse condition to the transcriptional activity of *SIRE-1*. To assess the impact of viral infection, leaf tissue may be inoculated with a virus such as soybean mosaic virus and harvested at 2, 5, 10 and 20 days after infection (Mansky *et al.*, 1991).

In addition, the transcriptional activity of *SIRE-1* may be assessed in plant tissue cultures. Tissue cultures may be initiated from roots, cotyledons, or leaves from selected cultivars as described (Amberger *et al*, 1992; Roth *et al.*, 1989; Shoemaker *et al.*, 1991). Tissue can then be transferred to Petri plates containing Gamborg's B5 medium supplemented with kinetin, casein hydrolysate and concentrations of 2,4-D ranging from 1 to 20 $\mu$M. After the formation of callus, suspension cultures may be initiated and maintained in liquid medium (Roth *et al.*, 1989). These cultures may then be exposed to adverse growing conditions as described above.

Total RNA may be isolated from seeds, cotyledons, leaves, roots, shoot tips, or cultured cells using commercial kits such as RNeasy$^{TM}$ (Qiagen, Chatsworth, CA). If necessary, polyadenylated RNA may be isolated from total RNA using the PolyATtract$^{TM}$ mRNA isolation system (Promega, Madison, WI). Isolated RNA may then be applied to nylon membranes (Gene Screen Plus$^{TM}$, New England Nuclear, Boston, MA) using a slot-blot apparatus, denatured, and probed with end-labeled oligomers or radiolabeled cDNAs corresponding to the *gag* or *pol* regions of *SIRE-1* (Sambrook *et al.*, 1989). RNA samples that give positive signals may be fractionated on 1% agarose-formaldehyde gels, blotted to nylon membranes, and probed as above. Preliminary studies of *SIRE-1* RNA transcripts in *G. max* (using the slot-blot procedures described above) have revealed the presence of high levels of *gag* transcripts in leaf tissues.

As retro-elements commonly produce polyprotein-encoding transcripts that traverse nearly the entire element, functional *SIRE-1* transcripts could exceed 10 kb in length. This could limit the applicability of agarose-formaldehyde gel separations. Alternatively, isolated RNA can be analyzed for the presence of *SIRE-1* transcripts by ribonuclease (RNase) protection assays well-known in the art. For example, RNA isolated from plants grown in the above-described conditions can be hybridized to *SIRE-1*-

derived radiolabeled RNA probe in solution and then exposed to one or more of several available RNases. The double-stranded hybrid formed by the probe and target RNA is protected from RNase digestion. The protected RNA can be fractionated on a denaturing polyacrylamide gel, blotted to a nylon membrane, and visualized by autoradiography.

### Example 4

### Detection of Retroelement Proteins by Western Hybridization Analysis

Plant tissue samples that contain *SIRE-1*-specific transcripts may be analyzed for the presence of *SIRE-1*-specific proteins or for proteins expressed by heterologous genes inserted into a *SIRE-1* derived vector. Protein recovered from these tissues may be spotted on nylon membranes and assayed for the presence of nucleocapsid, protease, and RT polypeptides by Western hybridization (Sambrook *et al.*, 1989).

Polyclonal antisera against *SIRE-1* proteins (or fusion constructs containing *SIRE-1* and heterologous peptide sequences) to be detected in these hybridizations can be obtained using methods well-known in the art. For example, oligopeptides may be designed and synthesized using sequence information from the cDNA and genomic clones. The synthetic oligopeptides may be coupled to carrier protein using for example gluteraldehyde, and antibodies against these raised in rabbits and affinity-purified as is well-known in the art (Harlow and Lane, 1988).

Alternatively, polyclonal antisera may be raised against fusion proteins produced by inserting the appropriate *SIRE-1* DNA fragments (or DNA encoding the heterologous proteins) in a protein expression vector like pPROEX-1 (Life Technologies, Gaithersburg, MD) and isolating the fusion protein according to the manufacturer's instructions.

Monoclonal antibody preparations against *SIRE-1* proteins or fusion proteins may also be isolated from hybridoma cells derived from splenocytes or thymocytes of mice immunized with such proteins according to methods well-known in the art (Harlow and Lane, 1988).

## Example 5

### *In vitro* Transcription and Translation of *SIRE-1* Transcripts

It may be desirable to produce *SIRE-1* polypeptides *in vitro* for use in producing antibodies or for capsid reconstitution studies and to provide reagents for *in vitro* packaging of retroviral polynucleotides. Production of *SIRE-1* polypeptides in a cell-free environment may be accomplished by creating cDNAs from *SIRE-1* mRNA transcripts, inserting those cDNAs into plasmids, propagating the plasmids, and utilizing such plasmids in *in vitro* transcription/translation reactions as are well-known in the art. cDNAs may be recovered from full-length *SIRE-1* transcripts isolated from soybean total or poly-A-selected RNA. Such cDNAs may be produced using reagents and reactions optimized for long transcripts (Nathan *et al.*, 1995). Total or poly-A-selected soybean RNA may be reverse-transcribed with SuperScript II™ reverse transcriptase (Life Technologies, Gaithersburg, MD) using an oligo(dT) primer. RNase H may be added and the single-stranded cDNA amplified using LA *Taq* DNA polymerase (Oncor) with oligo(dT) and 5′ primers derived from the proximal end of the *SIRE-1 gag* and/or *env* cDNA sequences. The 5′ end of each PCR primer may contain a restriction enzyme recognition sequence for subsequent vector ligation in the appropriate orientation and sequences that would facilitate enhanced transcription and/or translation.

Amplified cDNAs may be initially characterized by agarose gel electrophoresis and Southern hybridization using *gag*-, *pol*- and *env*-specific cDNA or oligonucleotide probes. The amplified DNAs may be ligated into pSPORT-1 (Life Technologies, Gaithersburg, MD), a vector designed to carry large inserts, and the recombinant plasmids used to transform competent *E. coli* DH5$\alpha$ cells (Life Technologies, Gaithersburg, MD). Plasmid DNA may be recovered from transformants and evaluated by restriction mapping and Southern hybridization as described above. Selected regions of several cDNAs may be sequenced with primers based on the sequence obtained from the genomic *SIRE-1* clone. cDNA variability may be assessed and quantitatively compared to that observed with *Tnt1* transcripts in tobacco, which constitute a quasispecies-like collection (Casacuberta *et al.*, 1995). The transcriptional initiation site(s) may be evaluated by primer extension and/or S1 nuclease digestion (Sambrook *et al.*, 1989).

Alternatively, a parallel series of experiments may be run to generate translatable mRNAs. *SIRE-1*-specific cDNAs may be generated as above, except that the 5′ PCR primer may be derived from the beginning of the *gag* and *pol* coding regions. The cDNA sequence suggests that a single *gag-pol* ORF may not be present in *SIRE-1*, and translation of the downstream *pol* region requires readthrough of a stop codon and/or a frameshift. It is probable that the ribosomes in the *in vitro* translation system may not emulate the *in vivo* translation. For expression of the *pol* region, the cDNAs may be amplified using a 5′ primer derived from the proximal end of the *pol* ORF.

Plasmid DNAs containing *SIRE-1* cDNAs may be recovered, and coupled *in vitro* transcription-translation assays may be run (Switzer and Heneine, 1995) using a reticulocyte lysate system (Promega, Madison, WI). Translation products may be analyzed by SDS-PAGE and Western hybridization as described above.

As an alternative to coupled *in vitro* transcription and translation, *SIRE-1* cDNAs may be cloned into the protein expression vector pPROEX-1 (Life Technologies, Gaithersburg, MD), and fusion proteins expressed in *E. coli* and recovered as described by the manufacturer. *SIRE-1* cDNAs utilized in the above-mentioned reactions could include those encoding analogs, homologs, or fragments of the full-length SIRE-1 *gag*, *pol*, or *env* proteins. These proteins, although not identical to proteins encoded by the *SIRE-1* polynucleotides disclosed herein, may nevertheless be useful if they retain at least one biological property of *SIRE-1* proteins. Such proteins may be used for antibody generation as described above, or for subsequent protein conformation studies.

### Example 6
### Modification of *SIRE-1* for Use in Non-Replicative Transduction of Plant Cells

*SIRE-1* may be adopted for use as a retroviral vector in legumes, *e.g.*, soybean, common beans, and alfalfa, cereals, *e.g.*, rice, wheat, and barley, and other agronomically important crops such as fruit trees, conifers, and hardwoods. The use of a plant retrovirus for introduction of DNA sequences into plant cells presents several advantages over previously-known methods. First, unlike other plant viral vectors (Joshi and Joshi, 1991; Potrykus, 1991), the *SIRE-1* pro-retrovirus may integrate into the host genome and generate stable transformants (Crystal, 1995; Miller, 1992; Smith, 1995).

Second, although other vectors have been used to introduce nucleic acid into plant genomes, they have serious limitations. For example, Ti plasmid-based vectors lead to integrative transformation, but their bacterial host, *Agrobacterium tumefaciens*, has a limited host range that does not include many legumes or most cereals (Christou, 1995; Potrykus, 1991).

Finally, physical transformation methods (*i.e.*, biolistic projection or microinjection) are far less efficient than viral infection in introducing DNA constructs into desired cells. These physical methods also generally require regeneration of adult plants by somatic embryogenesis (Christou, 1995; Potrykus, 1991).

A full-length *SIRE-1* pro-retroviral DNA and vectors derived therefrom will be competent to effect transduction into plant host cells and integration into the host genome, using any of the foregoing methods. However, it may be desirable to modify *SIRE-1* vectors so as to limit the region of integration, to restrict subsequent transposition events, to add DNA sequences to promote homologous recombination between a vector and a target region of the genome, and to insure against infectious spread of a potentially pathogenic agent.

*SIRE-1* may be modified in a manner analogous to that used for vertebrate retroviruses to create recombinant viral vectors that may infect host cells but not complete an infection cycle. For vertebrate retroviral vectors, this is accomplished by deleting or disabling the *trans*-acting elements (*i.e.*, *gag*, *pol*, and *env*) from the vector to be transduced into the host cell, while leaving intact the *cis*-acting elements (*i.e.*, LTRs and packaging signals). This is followed by transduction of the modified vector into retrovirus packaging cell lines or tissue cultures (Miller, 1992; Smith, 1995) that may contribute the necessary *trans*-acting elements.

Thus, the present invention contemplates *SIRE-1* constructs in which sequences encoding the *trans*-acting factors (*e.g.*, *gag*, *pol*, and *env*), the LTRs, or the packaging signals have been mutated or deleted, either singly or in combination. Mutations may be easily accomplished using PCR-mediated site-directed or cassette mutagenesis techniques as are well-known in the art.

The *trans*-factor encoding sequences may be deleted by digestion of the *SIRE-1* viral DNA with appropriate restriction enzymes. Those of ordinary skill in the art will be readily able to determine the appropriate restriction enzyme recognition sites in the *SIRE-1* DNA that will allow for removal of the appropriate *trans*-factor DNA segments while leaving intact essential *cis* element sequences. One approach would be to digest the *SIRE-1* DNA with a restriction enzyme that would cleave at sites located at or near the 5′ and 3′ boundaries of the ORF2 region (**Figure 14**) such that all or part of the *env*-encoding region could be removed from the vector.

Restriction digestion may be followed by recovery and purification of the digested vector DNA fragments containing *cis* factor sequences, followed by religation of the digested termini (Sambrook *et al.* 1989). Alternatively, appropriate double-stranded DNA linkers may be ligated to the digested ends of the vector DNA in order to maintain or create a proper reading frame. As another possibility, linker sequences containing one or more endonuclease restriction enzyme recognition sites may be ligated to the ends of the digested vector DNA, and these ends then religated in order to facilitate subsequent insertion of heterologous gene sequences.

Infection of packaging cells or tissue cultures with the modified *SIRE-1* vector may allow for the recovery and use of a non-replicative recombinant vector in a functional virion particle that may be capable of intercellular transport (for example, through plasmodesmata), host cell penetration, nuclear targeting, and chromosomal integration, but incapable of further transposition. Reporter genes like GUS (ß-glucuronidase, Jefferson *et al.*, 1981) or *Npt-II* (Neomycin phosphoryltransferase, Pridmore, 1987) and others (Croy, 1994) may also be incorporated into *SIRE-1* or vectors derived therefrom to allow detection of integration events.

**Example 7**

**Production of Plant Retroviral Packaging Cells**

Modification of pro-retroviruses for use as vectors is fairly straightforward. In essence, retroviral vectors are simple, containing the 5′ and 3′ LTRs, a packaging sequence, and a transcription unit composed of the recombinant gene or genes of interest and appropriate regulatory elements which include LTRs but which may also include heterologous regulatory elements. To grow the vector, however, the missing *trans*-factors must be provided using a so-called packaging cell line. Such a cell is engineered to contain integrated copies of *gag*, *pol*, and *env*, but to lack a packaging signal so that no "helper virus" sequences become encapsidated. Additional features may be added to or removed from the vector and packaging cell line to render the vectors more efficacious or to reduce the possibility of contamination by "helper virus."

A packaging cell line is produced by means of transfection of a helper virus plasmid encoding *gag*, *pol*, and *env* and by selecting for cells that express the proteins and that can support vector production (Miller, 1990). To avoid replication of helper sequences, one may make deletions in, for example, the packaging signal regions. To avoid recombination between the packaging vector and the replicating vector, the 3′ LTR is commonly deleted and replaced with a polyadenylation sequence (Dougherty *et al.*, 1989). Deletions may also be incorporated into the 5′ LTR to reduce its ability to replicate, and a heterologous promoter may be inserted downstream to maintain expression of the *trans*-factors (Miller, 1989). Finally, the viral genome may be split into two transcription units, one encoding *gag* and *pol* and a second encoding *env* (Markowitz, 1988). The *cis*-acting factors may be deleted or modified from these vectors in order to prevent production of replication-competent retrovirus by the packaging cells.

The *trans*-acting factors encoded by the helper virus construct may include the native factors from *SIRE-1*, modified *SIRE-1* factors, or other proretrovirus-derived factors that may result in an increased or alternative host range or higher efficiency of viral production or transduction efficiency (Smith, 1995). Thus, the present invention encompasses vectors containing sequences encoding the *trans*-acting factors from *SIRE-1*, either singly or in various combination, for use in creating packaging cells, and the packaging cells themselves.

To manipulate target cell specificity, the *env* gene of the helper virus/packaging cell line may be varied. A successful approach has been to remove sequences from the *env* gene and replace them with sequences encoding proteins with a different specificity (Russell *et al.*, 1993). For example, erythropoietin sequences have been incorporated into mammalian retroviruses to target the EPO receptor (Kassahara *et al.*, 1994). Another approach has been to incorporate a single-chain antibody into the *env* sequence (Chu *et al.*, 1994). Finally, the ability of retroviruses to incorporate glycoproteins from other viruses into their envelope has been utilized to produce so-called pseudotypes (Dong *et al.*, 1992). The pseudotype retrovirus acquires the infective range of the glycoprotein donor, and usually is more stable as well. Analogous strategies may be used in *SIRE-1* retroviral vectors to manipulate the host range beyond soybean by inserting into the *SIRE-1 env* gene ligand-, receptor-, or single-chain antibody-encoding fragments that could recognize, or be recognized by, proteins from other plant species, such as rice or maize.

## Example 8

## Transduction of the *SIRE-1* Plant Proretrovirus into Plant Cells

If the *SIRE-1* proretrovirus or vectors derived therefrom integrate into the genome of a cell transduced with such DNA, all cells derived from the original cell transfected with the *SIRE-1* vector may contain the retroviral insertion. Infections are commonly targeted to embryonic, meristematic, or germ line cells to enable transmission to progeny plants. Since certain plants (such as *G. max*) are self-fertilizing, transfection of embryos or meristematic tissue may lead to homozygosity of inserted DNA in some $F_1$ offspring, although the proportion of seed homozygous for a particular insertion event may need to be empirically tested. Dominant changes may be manifested in heterozygous progeny. Transfection of various adult tissues, especially meristems and ovaries, or seeds, pollen, protoplasts, or callus, may be performed by standard inoculation and/or co-incubation techniques which are well known (Potrykus, 1991). Viruses may also be inoculated into phloem for transport to distant sites. In some cases, physical methods such as biolistic projection, microinjection, or macroinjection may be necessary or preferred to transduce *SIRE-1* into plant cells or tissues (Draper and Scott, 1991; Potrykus, 1991).

**Example 9**

**Use of *SIRE-1* as a Gene Transfer Vector**

*SIRE-1* may be modified to carry useful gene sequences (e.g., gene sequences encoding useful proteins) or, alternatively, genes to produce antisense transcripts against undesirable endogenous sequences or to introduce into the genome gene regulatory elements which may regulate transcription of an adjacent gene. This may be easily accomplished by restriction enzyme digestion of the vector DNA at sites near the 5′ and 3′ boundaries of the ORFs encoding the *gag*, *pol*, and/or *env* proteins (as described above), isolating the remaining vector DNA, and either ligating a heterologous DNA fragment between the digested vector termini or alternatively by recombinantly inserting a multicloning site (Sambrook, *et al.*, 1989) between the digested vector termini to allow for subsequent facile restriction enzyme digestion and recombination of digested vector and heterologous DNAs. Heterologous gene sequences may be operably linked to (heterologous) host-cell specific promoter sequences (Waugh and Brown 1991), or their transcription may be driven by the *SIRE-1* LTR promotor activity. The heterologous gene sequences may encode any of a variety of polypeptides whose expression may result in useful phenotypic changes of the host cell and plant. By way of example, introduction and expression of these heterologous gene sequences in plants may result in the generation of the following exemplary phenotypic variations:

**A. Disease Resistance**

Many agronomically important crops are susceptible to a variety of diseases, viral infections, and bacterial or fungal infestations. Resistance to these conditions results in higher crop yields and decreased use of bacteriocidal and fungicidal compositions. Transfer of genes conferring resistance to diseases and/or viral or bacterial infection is an object of the present invention.

Many plant genomes, including soybean, are currently being mapped (Keim *et al.* 1996). In addition, genetic loci associated with disease resistance have been identified in many plant lines. For example, resistance markers and quantitative trait loci (QTL) for many soybean diseases have been linked to restriction fragment length polymorphism (RFLP), RAPD (Randomly Amplified Polymorphic DNA), and STS (Sequence Tag Sites) genome markers. These include bacterial blight, downy mildew (Bernard and Cremeens, 1971), phytophthora root rot (Diers *et al.* 1992), powdery mildew (Lohnes and Bernard, 1992), soybean root-knot nematode infection (Luzzi *et al.* 1994), phomopsis seed decay, cyst nematode infection (Baltazar and Mansur 1992; Boutin *et al.* 1992; Rao-Arelli *et al.* 1992; Young 1996), soybean mosaic virus (Chen *et al.* 1993), soybean rust (Hartwig and Bromfield 1983), stem canker (Bowers *et al.* 1993; Kilen and Hartwig 1987), sudden death syndrome (Prabhu *et al.* 1996), purple seed stain and leaf blight, and brown spot disease.

Both YAC (yeast artificial chromosome) and BAC (bacterial artificial chromosome) soybean libraries have been constructed (Funk and Colchinsky, 1994), and resistance markers have been assigned to particular clones in these libraries. The availability of these gene sequences will allow for insertion of DNA fragments encoding such genes into *SIRE-1* proretrovirus-derived vectors of the present invention using standard recombinant techniques as have been described above (Sambrook *et al.*, 1989). The recombinant vector may then be transduced into target plant cells, where the resistance gene may be expressed episomally or following integration of the vector into the host plant genome.

Transfer of resistance to viral infection to target plant cells is an important object of the present invention. The expression of a viral coat protein in a plant has been shown to diminish the ability of the virus to subsequently infect the plant and spread systemically; thus viral resistance may be mediated by vector-sponsored transfer of viral gene sequences into susceptible plant hosts (Beachy, 1990; Fitchen and Beachy, 1993). Many different viral coat protein genes have been introduced into plant genomes, expressed, and found to confer viral tolerance, including tobacco mosaic virus, cucumber mosaic virus, alfalfa mosaic virus, tobacco streak virus, tobacco rattle virus, potato viruses X and Y, and tobacco etch virus (Beachy, 1990; Gasser and Fraley, 1989; Golemboski *et al.*, 1990; Hemenway *et al.*, 1988; Hill *et al.*, 1991). This approach to viral resistance is especially promising, as the introduction of a viral coat protein from one virus using the vectors of the present invention may often confer tolerance to a range of seemingly unrelated viruses (Beachy, 1990). Moreover, transgenic plants expressing viral coat proteins exhibit viral tolerance in the field as well as in a laboratory setting (Nelson *et al.*, 1988).

Plants may also be transformed with a retroviral vector encoding an antisense RNA complementary to a plant virus polynucleotide. Expression of antisense RNA against viral sequences may provide tolerance against the virus by interfering with either the translation of viral mRNAs or the replication of the viral genome. Expression of antisense RNA has been found to confer viral resistance in, among others, potato, tobacco, and cucumber plants (Beachy, 1990; Day *et al.*, 1991; Hemenway *et al.*, 1988; Rezaian *et al.*, 1988).

Using the present invention, DNA fragments encoding viral coat proteins or antisense RNA complementary to viral RNA transcripts may be recombinantly inserted into the *SIRE-1* proretrovirus, transduced into susceptible plants, and expressed to confer resistance to a virus.

### B. Herbicide Tolerance

The use of herbicides is limited in part by their toxicity to crop species and by the development of resistance in "weed" species (Hathaway, 1989). Increasing tolerance to herbicides may increase yield and augment the spectrum of herbicides available for use to curtail weed growth. A wider range of suitable herbicides may also retard the development of resistance in weed species (LeBaron and McFarland, 1990), thereby decreasing the overall need for herbicides. Herbicide classes include, for example, acetanilides (e.g., alachlor), aliphatics (e.g., glyphosphate), dinitroanilines (e.g., trifluralin), diphenyl esters (e.g., acifluorfen), imidazolinones (e.g., imazapyr), sulfonylureas (e.g., chlorsulfuron), and triazines (e.g., atrazine).

Two general approaches may be taken in engineering herbicide tolerance: one may alter the level or sensitivity of the target enzyme for the herbicide (such as by altering the enzyme itself, or by decreasing the level or activity of a herbicide transporter), or incorporate or increase the activity of a gene that will detoxify the herbicide (Hathaway, 1989; Stalker, 1991).

An example of the first approach is the introduction (using the vectors and viruses of the present invention) into various crops of genetic constructs leading to overexpression of the enzyme EPSPS (5-*enol*pyruvylshikimate-3-phosphate synthase), or isoenzymes thereof exhibiting increased tolerance, which confers resistance to the active ingredient in the widely-used herbicide Roundup™, glyphosphate (Shah *et al.*, 1986). The gene for EPSPS was isolated from glyphosphate-resistant *E. coli*, given a plant promoter, and introduced into plants, where it conferred resistance to the herbicide. Transgenic species carrying resistance to glyphosphate have been developed in tobacco, petunia, tomato, potato, cotton, and *Arabidopsis* (della-Cioppa *et al.*, 1987; Gasser and Fraley, 1989; Shah *et al.*, 1986).

Similarly, resistance to sulfonylurea compounds, the active ingredients in Glean™ and Oust™ herbicides, has been produced by the introduction of site-specific mutant forms of the gene encoding acetolactate synthase (ALS) into plants (Haughn *et al.*, 1988). Resistance to sulfonylureas has been transferred using this method to tobacco, *Brassica*, and *Arabidopsis* (Miki *et al.*, 1990).

Bromoxynil is a herbicide that acts by inhibiting photosystem II. Rather than attempting to modify the target plant gene, resistance to bromoxynil has been conferred by the introduction of a gene encoding a bacterial nitrylase, which can inactivate the compound before it contacts the target enzyme. This strategy has been used to confer bromoxynil resistance to tobacco plants (Stalker *et al.*, 1988).

Genes encoding wild-type or mutant forms of endogenous plant enzymes targeted by herbicide compounds, or enzymes that inactivate herbicide compounds, may be recombinantly inserted into *SIRE-1* or vectors derived therefrom and transduced into plant cells. The genes may then be expressed under the control of plant- or tissue-specific promoters (Perlak *et al.*, 1991) to confer herbicide resistance to the transformed plant. The overexpression of normal or mutant forms of enzymes normally present in the wild-type progenitor plant is preferred, as this may decrease the probability of deleterious effects on crop performance or product quality.

### C. Insect Resistance

Transduction of functional genes encoding insecticidal products into plants may lead to crop strains that are intrinsically tolerant of insect predators. Such plants would not have to be treated with expensive and ecologically hazardous chemical pesticides. In addition, such insecticides would be effective at much lower concentrations than exogenously applied synthetic pesticides, and because biological insecticides are very specific, they are generally not hazardous to the food consumers.

Insect resistance in plants is generally provided by toxins or repellents (Gatehouse *et al.*, 1991). Using the present invention, insecticidal protoxin genes derived from, for example, several subspecies of *Bacillus thuringiensis* (Vaeck *et al.*, 1987), may be transduced into plant cells and constitutively expressed therein. This protoxin does not persist in the environment and is non-hazardous to mammals, making it a safe means for protecting plants. The gene for the toxin has been introduced and selectively expressed in a number of plant species including tomato, tobacco, potato, and cotton (Gasser and Fraley, 1989; Brunke and Meussen, 1991).

The trypsin inhibitor protein from cowpea is also an effective insecticide against a variety of insects: its presence restricts the ability of insects to digest food by interfering with hydrolysis of plant proteins (Hilder *et al.*, 1987). As the trypsin inhibitor is a natural plant protein, it may be expressed in plants without adversely affecting the physiology of the host. There are several potential drawbacks to the use of the cowpea trypsin inhibitor, however. Relative to the *B. thuringiensis* toxin, higher concentrations of inhibitor are required for insecticidal effectiveness (Brunke *et al.*, 1991). Thus, production of the inhibitor may require a more powerful transcriptional promoter (Perlak *et al.*, 1991), and may be more energetically costly for the host plant. In addition, the inhibitor is active in mammalian digestive systems unless inactivated prior to consumption. Inactivation may be accomplished by heating, however, so this may not be a significant drawback to the use of the inhibitor in most crop plants. Moreover, in most crops, the expression of the inhibitor may be restricted to those plant tissues such as leaves or roots that are most exposed to insect predators but are not consumed by mammals through the use of tissue-specific promoter sequences operably linked to the inhibitor gene (Perlak *et al.*, 1991).

These exemplary genes conferring insect resistance or repellence may be inserted into *SIRE-1* proretrovirus derived vectors using recombinant methods well-known in the art. These recombinant vectors may then be transduced into soybean and other plants. As more insect resistance and repellence genes are identified, these may be recombinantly inserted into the *SIRE-1*-derived gene transfer vector and expressed in host plants.

### D. Enhanced Nitrogen Fixation and/or Nodulation

Genes whose expression contributes to greater nitrogen fixation and nodulation (Gresshoff and Landau-Ellis, 1994; Qian *et al.* 1996) may be overexpressed in plant cells by transduction of a recombinant *SIRE-1* vector containing DNA fragments from which those genes may be expressed. Alternatively, expression of those genes whose expression leads to reduced nitrogen fixation or nodulation (Wu *et al.* 1995) may be modulated by the *SIRE-1*-mediated expression of recombinantly inserted DNA fragments encoding antisense transcripts. Manipulation of these genes may lessen or obviate the current great need for nitrogen-based fertilizers.

### E. Enhanced Vigor and/or Growth

Genes from wild progenitor species or non-related species whose expression results in economically valuable growth traits often found in wild progenitor species or non-related species have been discovered (Allen, 1994; Takahashi and Asanuma, 1996). Such genes or gene fragments may be placed under the control of heterologous or native promoters to create a gene cassette, and such cassettes may be recombinantly inserted into *SIRE-1* or vectors derived therefrom. These recombinant vectors may then be transduced into plant cells, where expression of the proteins encoded by such genes may lead to the development of plant phenotypes exhibiting economically valuable growth characteristics.

### F. Altered Seed Oil/Carbohydrate/Protein Production

Markers have been identified for several genes associated with soybean seed protein and oil content (Lee *et al.* 1996; Moreira *et al.* 1996). Transduction and expression of these genes within plants may result in greater seed oil production with lowered linolenic acid content, enhanced seed storage protein production, diminished raffinose-derived oligosaccharide levels, decreased lipoxygenase levels, or decreased protease inhibitor content (which may decrease the nutritive value of some plant proteins in animal feed due to decreased hydrolysis in the digestive tracts of animals). Such genes may be recombinantly inserted into *SIRE-1* proretrovirus or vectors derived therefrom, and the recombinant virus or vector may then be used to introduce such genes into plants or plant cells where they may be expressed and may influence the plant phenotype.

The potential food value of certain grains may be improved by altering the amino acid composition of the seed storage proteins. This may be accomplished in at least two ways. First, genes encoding heterologous seed storage proteins composed of a more desirable amino acid mix may be transferred into plants using the vectors and methods of the present invention with an undesirable seed storage protein amino acid composition. This approach has been utilized in several model studies: an oleosin gene from maize was successfully transferred and expressed in *Brassica* (Lee *et al.*, 1991), and a phaseolin gene from a legume was expressed, and the seed storage protein was appropriately compartmentalized, in tobacco plants (Altenbach *et al.*, 1989).

Second, genes encoding endogenous seed storage proteins may be mutated to contain a more desirable amino acid composition and reintroduced into the host plant using the vectors of the present invention (Hoffman *et al.*, 1988). The effect of these amino acid substitutions on protein conformation and compartmentalization may be lessened by targeting the substitutions to the hypervariable regions near the carboxy-terminus of most seed storage proteins (Dickinson *et al.*, 1990). Genes encoding proteins with altered amino acid compositions may be incorporated into the *SIRE-1* retroviral or vectors derived therefrom, and the recombinant virus or vector may then be used to introduce the genes into plant cells in order to introduce changes in protein amino acid composition.

### G. Heterologous Protein Production

The present invention contemplates recombinant *SIRE-1* virus or vectors derived therefrom that may be used to introduce genes encoding technical enzymes, heterologous storage proteins, or novel polymer-producing enzymes, thus allowing crops to become a novel source for these products.

## Example 10
## Use of *SIRE-1* to Induce and Tag Mutations in a Plant Genome

An important object of this invention is the use of the *SIRE-1* proretrovirus to establish new landmarks in plant genomes, and to induce and trace new mutations. *SIRE-1* may be used to link mutagenesis and element expression. Somaclonal variation has been demonstrated for soybean (Amberger *et al.*, 19921- Freytag *et al.*, 1989; Graybosch *et al.*, 1987; Roth *et al.*, 1989), for example, but little is known about the agents that induce the heritable changes. Persons of ordinary skill in the art will be able to identify new *SIRE-1* insertion sites in plant genomes and to correlate these new sites with variant phenotypes. Homozygosity at insertion sites may theoretically be achieved in the $F_1$ progeny, while dominant insertions may be differentiated from pre-existing integration events if the active element possesses a reporter gene like GUS or Npt. Phenotypes may then be correlated with the newly tagged genomic sites, and sequences flanking the sites may be easily cloned and sequenced (Sambrook, *et al.*, 1989).

*SIRE-1* may also be used to investigate the relationship between "genomic stress" and transposable element activity by seeking clues in the LTR regions to the identity of host proteins that might regulate element expression. The presence and expression of these proteins may then be correlated with the adverse conditions known to induce element expression.

The availability of a functional proretrovirus in a major plant group has far-ranging applications to applied genetic manipulations and to basic biological problems concerning gene function, genome organization, and evolution. A better understanding of these issues may be valuable in identifying and mapping important new loci. Understanding the relationships between plant health and element mobilization may provide invaluable insights into short- and long-term consequences of transposition. If retroelements have played a significant role in adaptive mutation in natural populations, then plant geneticists may be able to accelerate and direct the process to generate new resistant alleles. New insertion sites would be "tagged" by the element and it may be possible to distinguish these sites from pre-existing loci by competitive hybridization schemes. It should then be possible to clone and characterize the disrupted loci. In addition, if the element has contributed to genotypic changes that have persisted under the pressure of selection, then important loci may be closely linked to the element, a feature that may make it easier to map and isolate coding regions by element-anchored polymorphisms.

## Example 11

## Modification of *SIRE-1* Vectors to Effect Directed Integration

Retroviral integration systems show little target site specificity, and random insertions into a target cell genome may have undesirable consequences: integration near cellular proto-oncogenes may lead to ectopic gene activation and tumor production (Shiramazu *et al.*, 1994), and random integration may also inactivate essential or desirable genes (Coffin, 1990). Therefore, the ability to direct the integration of a plant proretrovirus to a limited region of a target plant cell genome is very desirable.

One manner by which directed integration may be effected is via "tethering" of the integration machinery to a specific target sequence. This may be accomplished by fusion of a sequence-specific DNA-binding domain to the integrase sequence of the *SIRE-1* proretrovirus (Kirchner *et al.*, 1995). The nucleotide sequence encoding the DNA-binding domain from a protein known to bind to a specific locus in the genome of a plant (i.e., a transcriptional enhancer for a gene whose expression is commercially disadvantageous) may be recombinantly inserted in-frame and just downstream from the 3′ end of the *SIRE-1* nucleotide sequence encoding the carboxy-terminus of the *pol* region (i.e., at the carboxy-terminus of the integrase protein, which is a product of *pol* cleavage). The DNA-binding domain may then act to "guide" the integrase protein and the *SIRE-1* polynucleotide to the genetic locus to be insertionally mutated by *SIRE-1*.

## Example 12
## Determination of the *SIRE-1* Insertion Site in the Soybean Genome

The sequence of the flanking genomic DNA from the *SIRE-1* genomic clone may be used to generate probes for determination of the genomic insertion site. Restriction enzyme digests of genomic DNA from a variety of *G. max* cultivars, *G. soja*, and other plant species (for example, *G. tabacina*, *G. canescens*, and *G. tormentella*) will be electrophoretically fractionated on agarose gels, transferred to nylon membranes, and hybridized with the flanking DNA probe(s). If a band to which the probe(s) hybridize is polymorphic, the relation of the polymorphism to the presence of a *SIRE-1* insert may be determined by hybridization with a *SIRE-1* LTR-specific probe. A *SIRE-1*-

related polymorphism among cultivars would strongly support functional transposition of the *SIRE-1* family in the recent past.

The above examples support that conclusion that *SIRE-1* is an endogenous family of proretroviruses whose genomic structure is based on a *copia*-like organization. In contrast, the genomic organization of all animal retroviruses (from vertebrates and *Drosophila*) is patterned after *gypsy*-like retrotransposons. Thus, *SIRE-1* is clearly a plant retroviral element that is evolutionarily far diverged from animal retroviruses.

Neither retroviral genomes nor virions have been reported in plants, although both classes of retrotransposons are otherwise widespread in nature. Therefore, *SIRE-1* is the first known plant proretrovirus. Few plant virus genomes encode an envelope protein. Those that do -- rhabdoviruses and bunyaviruses -- also infect animal hosts where envelope proteins sponsor viral-host cell membrane fusion. It is not known whether plant cell walls would preclude this mode of transfer.

*SIRE-1* may originally have been an invertebrate retrovirus. Its ability to integrate into plant genomes and the presence of envelope protein-encoding regions suggests the possibility that at one time it may have served as a "shuttle vector" between and among animal and plant hosts. Judging by its copy number it has clearly been successful in *G. max*.

The overall restriction site homogeneity of family members, the presence of long, uninterrupted ORFs within and adjacent to the retroviral insert, the strong homologies of the *env, gag, int*, RT and RH domains to those from known retrotransposons, and the near-identity of the LTRs indicate that *SIRE-1* is not an evolutionary relic, but an active proretrovirus. As such, it may be utilized to influence the organization and expression of soybean and possibly other plant genomes.

From the foregoing it may be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention (as set out in the appended claims).

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Laten, Howard M.

(ii) TITLE OF INVENTION: PLANT RETROVIRAL POLYNUCLEOTIDES AND
METHODS OF USE THEREOF

(iii) NUMBER OF SEQUENCES: 58

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Marshall, O'Toole, Gerstein, Murray and Borun
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(C) CITY: Chicago
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(E) COUNTRY: United States of America
(F) ZIP: 60606-6402

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Clough, David W.
(B) REGISTRATION NUMBER: 36,107
(C) REFERENCE/DOCKET NUMBER: 27013/33214 US

(ix) TELECOMMUNICATION INFORMATION:
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(B) TELEFAX: (312) 474-0448

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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(C) STRANDEDNESS: single
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CTTCTCACTT TTGATGCATA GTTCTCTATA GGATATAGCA AGCTCATCAA AAGTGATTTC     360

ACTATCTGTA TCACTTGAAT CTTCAGCAGA TTCAAATCTC CCAGTGAGTG CATTCACATC     420

TCTGTCAGAA TCACTTCTTG TTCACTCTCT GTATCATCAG ACCGACATAC AGAAAGTCCT     480

TTCCTCTGCT TCTTGAGATG AGTGGGACAT TCAGCTTTGA TGTGTCCATA GCCTTCACAC     540

CCATGGCATT GAATTCCTTT GCTGTGACTG GGCTTTTCAT CTGACCTTTT CTGGTATTCA     600

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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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(2) INFORMATION FOR SEQ ID NO:4:

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          (C) STRANDEDNESS: not relevant
          (D) TOPOLOGY: not relevant

    (ii) MOLECULE TYPE: peptide



    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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(2) INFORMATION FOR SEQ ID NO:5:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 10 amino acids
          (B) TYPE: amino acid
          (C) STRANDEDNESS: not relevant
          (D) TOPOLOGY: not relevant

    (ii) MOLECULE TYPE: peptide



    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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(2) INFORMATION FOR SEQ ID NO:6:

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          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "PBS"



    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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(2) INFORMATION FOR SEQ ID NO:7:

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          (A) LENGTH: 17 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "SIRE-1"



    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
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TTGGTATCAG AATTTCA 17

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4224 base pairs
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(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
GCTCGCGGCC GCGAGCTCTA ATACGACTCA CTATAGGGCG TCGACTCGAT CTTGTTGATG      60
ATAAAGTTAT CACACTGGAG CATGTTGACA CTGAGGAACA AATAGCAGAT ATTTTCACAA     120
AGGCATTGGA TGCAAATCAG TTTGAAAAAC TGAGGGGCAA GCTGGGCATT TGTCTGCTAG     180
AGGATTTATA GCAATTACTT TTATCTGAAC GTGCTTAAAC GTTAATAGCG CGTTCTCTAC     240
TGGGCCAAAA CAAATTCGAC CGTTGCTTCA CACGTCCCTC TACATTCCTC ATTCAAACTC     300
ATATTTTCGT GGTAATCTCG TTTTCAGCAT TCCCCAACAG CTCTCAGAGA TTTACGAAAC     360
CATTCCAAAG GCTCTGCTTC TCCATGGCTA CCTCACCAAA AGATACTTCA TCTCCTGGTT     420
CACCCTCTGT ACCATCATCT CCATCATCCA CCAAAGCACC ATCAAACCAG GAACAACCTG     480
AATTCCATAT CCAACCCATA CAAATGATTC CTGGTCTAGC CCCTGTTCCT GAGAAACTGG     540
TCCCCATAAG ACAACAGGGA GTGAAGATTT CTGAAAACCC TAGCATTGCA ACAAGTCCTA     600
GGGAATTGAC ACGGGAGATG GATAAGAAGA TCCGCAGTAT TGTGAGTAGT ATTCTGAAAA     660
ATGCTTCTGT CCCTGATGCT GATAAAGATG TTCCAACATC TTCCACCCCA AATGCTGAAG     720
TCCTCTCTTC ATCCAGTAAA GAGGAATCAA CAGAGGAAGA GGAACAAGCC ACAGAGGAGA     780
CCCCTGCACC AAGGGCACCA GAACCTGCTC CAGGTGACCT CATTGACCTA GAAGAAGTAG     840
AATCTGATGA GGAACCCATT GCCAACAAGT TGGCACCTGG CATTGCAGAA AGATTACAAA     900
GCAGAAAGGG AAAAACCCCC ATTACTAGGT CTGGACGAAT CAAAACTATG GCACAGAAGA     960
AGAGCACACC AATCACTCCT ACCACATCCA GATGGAGCAA AGTTGCAATC CCTTCCAAGA    1020
AGAGGAAAGA ATTTTCCTCA TCTGATTCTG ATGATGATGT CGAACTAGAT GTTCCCGACA    1080
TCAAGAGGGC CAAGAAATCT GGGAAAAAGG TGCCTGGAAA TGTCCCTGAT GCACCATTGG    1140
ACAACATTTC ATTCCACTCC ATTGGCAATG TTGAAAGGTG GAAATTTGTA TATCAACGCA    1200
GACTTGCCTT AGAAAGAGAA CTGGGAAGAG ATGCCTTGGA TTGCAAGGAG ATCATGGACC    1260
TCATCAAGGG CTGCTGGACT GCTGAAAACA GTCACCAAGT TGGGAGATGT TATGAAAGCC    1320
TAGTCAGGGA ATTCATTGTC AACATTCCCT CTGACATAAC AAACAGAAAG AGTGATGAGT    1380
ATCAGAAAGT GTTTGTCAGA GGAAAATGTG TTAGATTCTC CCCTGCTGTA ATCAACAAAT    1440
ACCTGGGCAG ACCTACTGAA GGAGTGGTGG ATATTGCTGT TTCTGAGCAT CAAATTGCCA    1500
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AGGAAATCAC TGCCAAACAA GTCCAGCATT GGCCAAAGAA AGGGAAGCTT TCTGCAGGGA    1560
AGCTAAGTGT GAAGTATGCA ATCCTGCACA GGATTGGCGC TGCAAACTGG GTACCCACCA    1620
ATCATACTTC CACAGTTGCC ACAGGTTTGG GTAAATTTCT GTATGCTGTT GGAACCAAGT    1680
CCAAATTTAA TTTTGGAAAG TATATTTTTG ATCAAACTGT TAAGCATTCA GAATCATTTG    1740
CTGTCAAATT ACCCATTGCC TTCCCAACTG TATTGTGTGG CATTATGTTG AGTCAACATC    1800
CCAATATTTT AAACAACATT GACTCTGTGA TGAAGAAAGA ATCGGCTCTG TCCCTGCATT    1860
ACAAACTGTT TGAGGGGACA CATGTCCCAG ACATTGTCTC GACATCAGGG AAAGCTGCTG    1920
CTTCAGGTGC TGTATCCAAG GGATGCTTTG ATTGCTGAAC TCAAGGACAC ATGCAAGGTG    1980
CTGGAAGCAA CCATCAAAGC CACCACAGAG AAGAAAATGG AGCTGGAACG CCTGATCAAA    2040
AGACTCTCAG ACAGTGGCAT TGATGATGGT GAAGCAGCTG AGGAAGAAGA AGAAGCCGCT    2100
GAGGAAGAGA AAGATGCAGC AGAAGATACA GAATCAGATG ATGATGATTC TGATGCCACC    2160
CCATGACCAT CAGACCTTTA TTTTTGCTTT TTACTCTTAC TAGCTATAGG GCATGTCCCT    2220
TTGAACAATT GATTGCTATT GGTCTGTAAT ATTTGCATGC ATTCTACTTT TGTCAAATTC    2280
TGTCTAAAAA GGGGATATAT ATTATGCATG ATTTTGAGTA GTAGATACTA TGTTGCAATA    2340
GTATATTATG CATAATTTAT GATTTTGAGT AGTAGGATAC GATGTATGCA TGATTCATGA    2400
TTTTGAGGGG GAGTTGTAAG TATATGATTT TGAGGGGGAG TAGTATCTGA TGATGCTGAT    2460
AGAAGATGGC ATGGAGACAG GGGGAGCAGA AAGCTGATGT CACGTGAGAT GTCTTGACAT    2520
CCTGGAAACG ACTTGCAACT TGCAGAATTT TGCTGTCGCC CCTACAGATA CCGCTGTGCT    2580
TGATTACTCT GATAATGAAA GTTGCTGATC CCACTTGCAT AACTGCTCGT ACCTGCTCAG    2640
GAAGTGTCTA AGTATGTTTT AGACAAAATT TGCCAAAGGG GGAGATTGTT AGTGCTTAGC    2700
TTTACTGAGT TTTAAAAGAT TGGCTAAAAT TTTGTTAAAA CATAAGCACT TAGACAATGA    2760
AGGAAAGCTG GAGTTGCTGC ACAGGATGTC CAACGTTATG TCAAGGAATC AGATTGGGCT    2820
CCACAATGCA CAAGGCAAGA TAAAAGGTCA AATGAAGAAT TGAAGCTGCA GGATCCACGA    2880
TGTCGGATAC AATGTCCAGG ACATCCTGCC CGAAAATACT GGACACATAA ATCTGTTATA    2940
TCTTTAACAG ATTAATGTGC AGTTAGCAAC AGATTTGGCG ATCTATCTTT AGGAACGAAT    3000
TAAAAGATAA TTAAAGTTCG AATTACAAAC TTGAATAGTT CGTTCAGGGA TTAAAGATTA    3060
AAGATAAAAA CTAAAAGATC AAACTGTATC TTTTAGATCT TTAAGTGCAG ATTTTTCAGG    3120
AGAATGATAG ATCTTATCCA GCGCAAGATG TTGCAGCCCA GATACGCACA CTGCTATATA    3180
AACATGAAGG CTGCACGAGT TTTCTACCAA GTCCGGGATT GAAGAGTTAT TTTGTGAGTT    3240
TTGGGACTTG AGTGTTTTGT GAGCCACCTT GATGTTACCC TAACATCAAG TGTTGGACCT    3300
GAGTGTGTAG AGTTGATCTC TATTGTTCAG AGAGCAATCT CTGGTGTGTC TTTGATTTAT    3360
TTGTAAACAC GGGAGAGTGA TTGAGAGGGA GTGAGAGGGG TTCTCATATC TAAGAGTGGC    3420
TCTTAGGTAG AGGTTGCACG GGTAGTGGTT AGGTGAGAAG GTTGTAAACA GTGGCTGTTA    3480
GATCTTCGAA CTAACACTAT TTTAGTGGAT TTCCTCCCTG GCTTGGTAGC CCCCAGATGT    3540
```

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AGGTGAGGTT GCACCGAACT GGGTTAACAA TTCTCTTGTG TTATTTACTT GTTTAATCTG    3600

TTCATACTGT CAAATATAAT CTGCATGTTC TGAAGCGTGA TGTCGTGACA TCCGGTACGA    3660

CATCTGTCAT TGGTATCAGA ATTTCATGCT GCAAATATTT ACAATAGACC TCCTCAACCT    3720

CAACAGCAAA ATCAACCACA GCAGAACAAT TATGACCTCT CCAGCAACAG ATACAACCCT    3780

GGATGGAGGA ATCACCCTAA CCTCAGATGG TCCAGCCCTC AGCAACAACA ACAGCAGCCT    3840

GCTCCTTCCT TCCAAAATGC TGTTGGCCCA AGCAGACCAT ACATTCCTCC ACCAATCCAA    3900

CAACAGCAAC AACCCCAGAA ACAGCCAACA GTTGAGGCCC TCCACAACTT CCTTCGAAGA    3960

ACTTGTGAGG CAAATGACTA TGCAGAACAT GCAGTTTCAG CAAGAGACTA GAGCCTCCAT    4020

TCAGAGCTTA ACCAATCAGA TGGGACAATT GGCTACCCAA TTGAATCAAC AACAGTCCCA    4080

GAATTCTGAC AAGTTGCCTT CTCAAGCTGT CCAAAATCCC AAAAATGTCA GTGCCATTTC    4140

ATTGAGGTCG GGAAAGCAGT GTCAAGGACC TCAACCCGTA GCACCTTCCT CATCTGCAAA    4200

TGAACCTGCC AAACTTCACT CTAC                                           4224
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 695 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein
Xaa=stop codon

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Ser Arg Pro Arg Ala Leu Ile Arg Leu Thr Ile Gly Arg Arg Leu Asp
1               5                   10                  15

Leu Val Asp Asp Lys Val Ile Thr Leu Glu His Val Asp Thr Glu Glu
            20                  25                  30

Gln Ile Ala Asp Ile Phe Thr Lys Ala Leu Asp Ala Asn Gln Phe Glu
        35                  40                  45

Lys Leu Arg Gly Lys Leu Gly Ile Cys Leu Leu Glu Asp Leu Xaa Gln
    50                  55                  60

Leu Leu Leu Ser Glu Arg Ala Xaa Thr Leu Ile Ala Arg Ser Leu Leu
65                  70                  75                  80

Gly Gln Asn Lys Phe Asp Arg Cys Phe Thr Arg Pro Ser Thr Phe Leu
                85                  90                  95

Ile Gln Thr His Ile Phe Val Val Ile Ser Phe Ser Ala Phe Pro Asn
            100                 105                 110

Ser Ser Gln Arg Phe Thr Lys Pro Phe Gln Arg Leu Cys Phe Ser Met
        115                 120                 125

Ala Thr Ser Pro Lys Asp Thr Ser Ser Pro Gly Ser Pro Ser Val Pro
    130                 135                 140

Ser Ser Pro Ser Ser Thr Lys Ala Pro Ser Asn Gln Glu Gln Pro Glu
```

```
145                 150                 155                 160

Phe His Ile Gln Pro Ile Gln Met Ile Pro Gly Leu Ala Pro Val Pro
                165                 170                 175

Glu Lys Leu Val Pro Ile Arg Gln Gln Gly Val Lys Ile Ser Glu Asn
            180                 185                 190

Pro Ser Ile Ala Thr Ser Pro Arg Glu Leu Thr Arg Glu Met Asp Lys
        195                 200                 205

Lys Ile Arg Ser Ile Val Ser Ser Ile Leu Lys Asn Ala Ser Val Pro
    210                 215                 220

Asp Ala Asp Lys Asp Val Pro Thr Ser Ser Thr Pro Asn Ala Glu Val
225                 230                 235                 240

Leu Ser Ser Ser Ser Lys Glu Glu Ser Thr Glu Glu Glu Glu Gln Ala
                245                 250                 255

Thr Glu Glu Thr Pro Ala Pro Arg Ala Pro Glu Pro Ala Pro Gly Asp
            260                 265                 270

Leu Ile Asp Leu Glu Glu Val Glu Ser Asp Glu Glu Pro Ile Ala Asn
        275                 280                 285

Lys Leu Ala Pro Gly Ile Ala Glu Arg Leu Gln Ser Arg Lys Gly Lys
    290                 295                 300

Thr Pro Ile Thr Arg Ser Gly Arg Ile Lys Thr Met Ala Gln Lys Lys
305                 310                 315                 320

Ser Thr Pro Ile Thr Pro Thr Thr Ser Arg Trp Ser Lys Val Ala Ile
                325                 330                 335

Pro Ser Lys Lys Arg Lys Glu Phe Ser Ser Ser Asp Ser Asp Asp Asp
            340                 345                 350

Val Glu Leu Asp Val Pro Asp Ile Lys Arg Ala Lys Lys Ser Gly Lys
        355                 360                 365

Lys Val Pro Gly Asn Val Pro Asp Ala Pro Leu Asp Asn Ile Ser Phe
    370                 375                 380

His Ser Ile Gly Asn Val Glu Arg Trp Lys Phe Val Tyr Gln Arg Arg
385                 390                 395                 400

Leu Ala Leu Glu Arg Glu Leu Gly Arg Asp Ala Leu Asp Cys Lys Glu
                405                 410                 415

Ile Met Asp Leu Ile Lys Gly Cys Trp Thr Ala Glu Asn Ser His Gln
            420                 425                 430

Val Gly Arg Cys Tyr Glu Ser Leu Val Arg Glu Phe Ile Val Asn Ile
        435                 440                 445

Pro Ser Asp Ile Thr Asn Arg Lys Ser Asp Glu Tyr Gln Lys Val Phe
    450                 455                 460

Val Arg Gly Lys Cys Val Arg Phe Ser Pro Ala Val Ile Asn Lys Tyr
465                 470                 475                 480

Leu Gly Arg Pro Thr Glu Gly Val Val Asp Ile Ala Val Ser Glu His
                485                 490                 495

Gln Ile Ala Lys Glu Ile Thr Ala Lys Gln Val Gln His Trp Pro Lys
            500                 505                 510
```

```
Lys Gly Lys Leu Ser Ala Gly Lys Leu Ser Val Lys Tyr Ala Ile Leu
        515                 520                 525

His Arg Ile Gly Ala Ala Asn Trp Val Pro Thr Asn His Thr Ser Thr
    530                 535                 540

Val Ala Thr Gly Leu Gly Lys Phe Leu Tyr Ala Val Gly Thr Lys Ser
545                 550                 555                 560

Lys Phe Asn Phe Gly Lys Tyr Ile Phe Asp Gln Thr Val Lys His Ser
                565                 570                 575

Glu Ser Phe Ala Val Lys Leu Pro Ile Ala Phe Pro Thr Val Leu Cys
            580                 585                 590

Gly Ile Met Leu Ser Gln His Pro Asn Ile Leu Asn Asn Ile Asp Ser
        595                 600                 605

Val Met Lys Lys Glu Ser Ala Leu Ser Leu His Tyr Lys Leu Phe Glu
    610                 615                 620

Gly Thr His Val Pro Asp Ile Val Ser Thr Ser Gly Lys Ala Ala Ala
625                 630                 635                 640

Ser Gly Ala Val Ser Lys Gly Cys Phe Asp Cys Xaa Thr Gln Gly His
                645                 650                 655

Met Gln Gly Ala Gly Ser Asn His Gln Ser His His Arg Lys Lys Asn
            660                 665                 670

Gly Ala Gly Thr Pro Asp Gln Lys Thr Leu Arg Gln Trp His Xaa Xaa
        675                 680                 685

Trp Xaa Ser Ser Xaa Gly Arg
    690                 695
```

(2) INFORMATION FOR SEQ ID NO:10:

```
    (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 578 amino acids
         (B) TYPE: amino acid
         (C) STRANDEDNESS: not relevant
         (D) TOPOLOGY: not relevant

   (ii) MOLECULE TYPE: protein



   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Thr Leu Ile Ala Arg Ser Leu Leu Gly Gln Asn Lys Phe Asp Arg Cys
1               5                   10                  15

Phe Thr Arg Pro Ser Thr Phe Leu Ile Gln Thr His Ile Phe Val Val
            20                  25                  30

Ile Ser Phe Ser Ala Phe Pro Asn Ser Ser Gln Arg Phe Thr Lys Pro
        35                  40                  45

Phe Gln Arg Leu Cys Phe Ser Met Ala Thr Ser Pro Lys Asp Thr Ser
    50                  55                  60

Ser Pro Gly Ser Pro Ser Val Pro Ser Ser Pro Ser Ser Thr Lys Ala
65                  70                  75                  80

Pro Ser Asn Gln Glu Gln Pro Glu Phe His Ile Gln Pro Ile Gln Met
```

```
                85                  90                  95

Ile Pro Gly Leu Ala Pro Val Pro Glu Lys Leu Val Pro Ile Arg Gln
            100                 105                 110

Gln Gly Val Lys Ile Ser Glu Asn Pro Ser Ile Ala Thr Ser Pro Arg
        115                 120                 125

Glu Leu Thr Arg Glu Met Asp Lys Lys Ile Arg Ser Ile Val Ser Ser
    130                 135                 140

Ile Leu Lys Asn Ala Ser Val Pro Asp Ala Asp Lys Asp Val Pro Thr
145                 150                 155                 160

Ser Ser Thr Pro Asn Ala Glu Val Leu Ser Ser Ser Ser Lys Glu Glu
                165                 170                 175

Ser Thr Glu Glu Glu Glu Gln Ala Thr Glu Glu Thr Pro Ala Pro Arg
            180                 185                 190

Ala Pro Glu Pro Ala Pro Gly Asp Leu Ile Asp Leu Glu Glu Val Glu
        195                 200                 205

Ser Asp Glu Glu Pro Ile Ala Asn Lys Leu Ala Pro Gly Ile Ala Glu
    210                 215                 220

Arg Leu Gln Ser Arg Lys Gly Lys Thr Pro Ile Thr Arg Ser Gly Arg
225                 230                 235                 240

Ile Lys Thr Met Ala Gln Lys Lys Ser Thr Pro Ile Thr Pro Thr Thr
                245                 250                 255

Ser Arg Trp Ser Lys Val Ala Ile Pro Ser Lys Lys Arg Lys Glu Phe
            260                 265                 270

Ser Ser Ser Asp Ser Asp Asp Asp Val Glu Leu Asp Val Pro Asp Ile
        275                 280                 285

Lys Arg Ala Lys Lys Ser Gly Lys Lys Val Pro Gly Asn Val Pro Asp
    290                 295                 300

Ala Pro Leu Asp Asn Ile Ser Phe His Ser Ile Gly Asn Val Glu Arg
305                 310                 315                 320

Trp Lys Phe Val Tyr Gln Arg Arg Leu Ala Leu Glu Arg Glu Leu Gly
                325                 330                 335

Arg Asp Ala Leu Asp Cys Lys Glu Ile Met Asp Leu Ile Lys Gly Cys
            340                 345                 350

Trp Thr Ala Glu Asn Ser His Gln Val Gly Arg Cys Tyr Glu Ser Leu
        355                 360                 365

Val Arg Glu Phe Ile Val Asn Ile Pro Ser Asp Ile Thr Asn Arg Lys
    370                 375                 380

Ser Asp Glu Tyr Gln Lys Val Phe Val Arg Gly Lys Cys Val Arg Phe
385                 390                 395                 400

Ser Pro Ala Val Ile Asn Lys Tyr Leu Gly Arg Pro Thr Glu Gly Val
                405                 410                 415

Val Asp Ile Ala Val Ser Glu His Gln Ile Ala Lys Glu Ile Thr Ala
            420                 425                 430

Gln Val Gln His Trp Pro Lys Lys Gly Lys Leu Ser Ala Gly Lys Leu
        435                 440                 445
```

```
Ser Val Lys Tyr Ala Ile Leu His Arg Ile Gly Ala Ala Asn Trp Val
    450                 455                 460

Pro Thr Asn His Thr Ser Thr Val Ala Thr Gly Leu Gly Lys Phe Leu
465                 470                 475                 480

Tyr Ala Val Gly Thr Lys Ser Lys Phe Asn Phe Gly Lys Tyr Ile Phe
                485                 490                 495

Asp Gln Thr Val Lys His Ser Glu Ser Phe Ala Val Lys Leu Pro Ile
            500                 505                 510

Ala Phe Pro Pro Val Leu Cys Gly Ile Met Leu Thr Gln His Pro Asn
        515                 520                 525

Ile Leu Asn Asn Ile Asp Ser Val Met Lys Lys Glu Ser Ala Leu Ser
    530                 535                 540

Leu His Tyr Lys Leu Phe Glu Gly Thr His Val Pro Asp Ile Val Ser
545                 550                 555                 560

Thr Ser Gly Lys Ala Ala Ala Ser Gly Ala Val Ser Lys Gly Cys Phe
                565                 570                 575

Asp Cys
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 62 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
Ser Arg Pro Arg Ala Leu Ile Arg Leu Thr Ile Gly Arg Arg Leu Asp
1               5                   10                  15

Leu Val Asp Asp Lys Val Ile Thr Leu Glu His Val Asp Thr Glu Glu
            20                  25                  30

Gln Ile Ala Asp Ile Phe Thr Lys Ala Leu Asp Ala Asn Gln Phe Glu
        35                  40                  45

Lys Leu Arg Gly Lys Leu Gly Ile Cys Leu Leu Glu Asp Leu
    50                  55                  60
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCCAGTCACG ACGTTGTAAA ACG 23

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TCCTTTAAGT TCAGAGATT 19

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

AGCGGATAAC AATTTCACAC AGG 23

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GTAATGGTCA ACCAGACCAC AGTT 24

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

```
        (A) DESCRIPTION: /desc = "oligonucleotide"


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GACGAATTGG CACTTGG                                                   17

(2) INFORMATION FOR SEQ ID NO:17:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 18 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "oligonucleotide"


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TTTGCACTGC CTTGGGAG                                                  18

(2) INFORMATION FOR SEQ ID NO:18:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 17 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "oligonucleotide"


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CCAAGGAGCA CAACTGC                                                   17

(2) INFORMATION FOR SEQ ID NO:19:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 20 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "oligonucleotide"


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GCTGAACAGA ATGGACAGGA                                                20

(2) INFORMATION FOR SEQ ID NO:20:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 19 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "oligonucleotide"


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

AAAGATATAA CAAGATTTA                                                  19

(2) INFORMATION FOR SEQ ID NO:21:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 20 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "oligonucleotide"


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CCCGATCTTA TTCCTTGACA                                                 20

(2) INFORMATION FOR SEQ ID NO:22:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 18 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "oligonucleotide"


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CTTGCCACAG TAGTGACA                                                   18

(2) INFORMATION FOR SEQ ID NO:23:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 18 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "oligonucleotide"


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TCTTCCCAAG CTGTAGCA                                                   18
```

```
(2) INFORMATION FOR SEQ ID NO:24:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 19 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "oligonucleotide"



    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TCCTTTAAGT TCAGAGATT                                                    19

(2) INFORMATION FOR SEQ ID NO:25:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 20 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "oligonucleotide"



    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

AGCGCGTTCT CTACTGGGCC                                                   20

(2) INFORMATION FOR SEQ ID NO:26:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 20 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "oligonucleotide"



    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CCACCAAAGC ACCATCAAAC                                                   20

(2) INFORMATION FOR SEQ ID NO:27:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 20 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "oligonucleotide"
```

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GGCACAGAAG AAGAGCACAC 20

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

TGCAAGGAGA TCATGGACCT 20

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CACAGGATTG GCGCTGCAAA 20

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

TCCCTGGCTT GGTAGCCCCC AGATGTAGG 29

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

```
          (A) DESCRIPTION: /desc = "oligonucleotide"


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GGCCCTCCAC AACTTCCTTC G                                                  21

(2) INFORMATION FOR SEQ ID NO:32:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 20 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "oligonucleotide"


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CAGATGAGGA AGGTGCTACG                                                    20

(2) INFORMATION FOR SEQ ID NO:33:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 30 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "oligonucleotide"


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CCCAGTTCGG TGCAACCTCA CCTACATCTG                                         30

(2) INFORMATION FOR SEQ ID NO:34:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 20 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "oligonucleotide"


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GGTGGCTCAC AAAACACTCA                                                    20

(2) INFORMATION FOR SEQ ID NO:35:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 20 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "oligonucleotide"


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

TGTGTCCAGT ATTTTCGGGC                                                  20

(2) INFORMATION FOR SEQ ID NO:36:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 20 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "oligonucleotide"


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

TCATCAGATA CTACTCCCCC                                                  20

(2) INFORMATION FOR SEQ ID NO:37:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 22 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "oligonucleotide"


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

CCTAGGACTT GTTGCAATGC TA                                               22

(2) INFORMATION FOR SEQ ID NO:38:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 20 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "oligonucleotide"


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

ATGAGGAATG TAGAGGGACG                                                  20
```

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

CTCATGAGTT CTCTGCAGCC 20

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GACAATGTTG CAGATACAGC TAAAAGTGC 29

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

CCAGATGGAT GTGAAGAGCG 20

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

```
    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

TGGGATGGAA AATGCCAGC                                                     19

(2) INFORMATION FOR SEQ ID NO:43:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 20 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "oligonucleotide"


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

AGAACTGTGT GTCCCTATCC                                                    20

(2) INFORMATION FOR SEQ ID NO:44:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 20 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "oligonucleotide"


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CCTCAGTGTC AACATGCTCC                                                    20

(2) INFORMATION FOR SEQ ID NO:45:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 20 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "oligonucleotide"


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

ATCCCATAGT CACTGGTGCC                                                    20

(2) INFORMATION FOR SEQ ID NO:46:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 20 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
```

```
        (A) DESCRIPTION: /desc = "oligonucleotide"



    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

CTCTGTTAGC CTTTCATACC                                                20

(2) INFORMATION FOR SEQ ID NO:47:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 20 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "oligonucleotide"



    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

CTTGATCTTG TAGTGACTCC                                                20

(2) INFORMATION FOR SEQ ID NO:48:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 20 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "oligonucleotide"



    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

ATACAGTGTG GTTGGAGTCC                                                20

(2) INFORMATION FOR SEQ ID NO:49:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 20 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "oligonucleotide"



    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

GAAGTCTTAG ACTCAACTCC                                                20

(2) INFORMATION FOR SEQ ID NO:50:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 2826 amino acids
```

(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

```
Gly Ala Thr Gly Ala Ala Gly Gly Ala Thr Thr Cys Ala Ala Thr Gly
1               5                   10                  15

Thr Ala Gly Ala Cys Thr Thr Cys Ala Cys Ala Gly Ala Gly Thr Cys
            20                  25                  30

Ala Gly Ala Ala Thr Gly Cys Thr Thr Gly Ala Thr Gly Ala Cys Ala
        35                  40                  45

Ala Ala Ala Gly Ala Gly Ala Ala Gly Ala Gly Ala Gly Ala Ala Gly
    50                  55                  60

Thr Cys Cys Thr Ala Ala Thr Gly Ala Ala Gly Gly Gly Cys Gly Gly
65                  70                  75                  80

Cys Ala Gly Ala Thr Cys Ala Ala Ala Gly Gly Ala Cys Ala Ala Cys
                85                  90                  95

Thr Gly Thr Thr Ala Cys Cys Thr Gly Thr Gly Gly Ala Cys Ala Cys
            100                 105                 110

Cys Thr Cys Ala Ala Gly Ala Ala Ala Cys Cys Ala Gly Thr Thr Ala
        115                 120                 125

Cys Thr Cys Cys Thr Cys Cys Ala Cys Ala Thr Gly Thr Cys Thr Ala
    130                 135                 140

Thr Thr Cys Thr Cys Cys Ala Ala Ala Gly Ala Ala Gly Ala Thr Gly
145                 150                 155                 160

Ala Ala Gly Thr Cys Ala Ala Ala Ala Thr Ala Thr Gly Gly Cys Ala
                165                 170                 175

Thr Cys Ala Ala Ala Gly Ala Thr Thr Thr Gly Gly Ala Cys Ala Thr
            180                 185                 190

Cys Thr Gly Cys Ala Cys Thr Thr Ala Gly Gly Ala Gly Gly Cys Ala
        195                 200                 205

Thr Gly Ala Ala Gly Ala Ala Ala Ala Thr Cys Ala Thr Thr Gly Ala
    210                 215                 220

Cys Ala Ala Ala Gly Gly Thr Gly Cys Thr Gly Thr Thr Ala Gly Ala
225                 230                 235                 240

Gly Gly Cys Ala Thr Thr Cys Cys Cys Ala Ala Thr Cys Thr Gly Ala
                245                 250                 255

Ala Ala Ala Thr Ala Gly Ala Ala Gly Ala Ala Gly Gly Cys Ala Gly
            260                 265                 270

Ala Ala Thr Cys Thr Gly Thr Gly Gly Thr Gly Ala Ala Thr Gly Thr
        275                 280                 285

Cys Ala Gly Ala Thr Thr Gly Gly Ala Ala Ala Gly Cys Ala Ala Gly
    290                 295                 300
```

```
Thr Cys Ala Ala Gly Ala Thr Gly Thr Cys Cys Ala Ala Cys Cys Ala
305                 310                 315                 320

Gly Ala Ala Gly Cys Thr Thr Cys Ala Ala Cys Ala Thr Cys Ala Gly
                325                 330                 335

Ala Cys Cys Ala Cys Thr Thr Cys Cys Ala Gly Gly Gly Thr Gly Cys
            340                 345                 350

Thr Gly Gly Ala Ala Cys Thr Ala Cys Thr Thr Cys Ala Cys Ala Thr
        355                 360                 365

Gly Gly Ala Cys Thr Thr Gly Ala Thr Gly Gly Gly Gly Cys Cys Thr
    370                 375                 380

Ala Thr Gly Cys Ala Ala Gly Thr Thr Gly Ala Ala Ala Gly Cys Cys
385                 390                 395                 400

Thr Thr Gly Gly Ala Ala Gly Ala Ala Ala Ala Ala Gly Gly Thr Ala
                405                 410                 415

Thr Gly Cys Cys Thr Ala Thr Gly Thr Thr Gly Thr Thr Gly Thr Gly
            420                 425                 430

Gly Ala Thr Gly Ala Thr Thr Thr Cys Thr Cys Cys Ala Gly Ala Thr
        435                 440                 445

Thr Thr Ala Cys Cys Thr Gly Gly Gly Thr Cys Ala Ala Cys Thr Thr
    450                 455                 460

Thr Ala Thr Cys Ala Gly Ala Gly Ala Gly Ala Ala Ala Thr Cys Ala
465                 470                 475                 480

Gly Ala Cys Ala Cys Cys Thr Thr Thr Gly Ala Ala Gly Thr Ala Thr
                485                 490                 495

Thr Cys Ala Ala Gly Gly Ala Gly Thr Thr Gly Ala Gly Thr Cys Thr
            500                 505                 510

Ala Ala Gly Ala Cys Thr Thr Cys Ala Ala Ala Gly Ala Gly Ala Ala
        515                 520                 525

Ala Ala Ala Gly Ala Cys Thr Gly Thr Gly Thr Cys Ala Thr Cys Ala
    530                 535                 540

Ala Gly Ala Gly Ala Ala Thr Cys Ala Gly Gly Ala Gly Thr Gly Ala
545                 550                 555                 560

Cys Cys Ala Thr Gly Gly Cys Ala Gly Ala Gly Ala Gly Thr Thr Thr
                565                 570                 575

Gly Ala Ala Ala Ala Cys Ala Gly Cys Ala Ala Gly Thr Thr Thr Ala
            580                 585                 590

Cys Thr Gly Ala Ala Thr Thr Cys Thr Gly Cys Ala Cys Ala Thr Cys
        595                 600                 605

Thr Gly Ala Ala Gly Gly Cys Ala Thr Cys Ala Cys Thr Cys Ala Thr
    610                 615                 620

Gly Ala Gly Thr Thr Cys Thr Cys Thr Gly Cys Ala Gly Cys Cys Ala
625                 630                 635                 640

Thr Thr Ala Cys Ala Cys Cys Ala Cys Ala Ala Cys Ala Ala Ala Ala
                645                 650                 655

Thr Gly Gly Cys Ala Thr Ala Gly Thr Thr Gly Ala Ala Ala Gly Gly
```

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            660                 665                 670

Ala Ala Ala Ala Ala Cys Ala Gly Gly Ala Cys Cys Thr Thr Gly Cys
        675                 680                 685

Cys Ala Gly Ala Ala Gly Cys Thr Gly Cys Thr Ala Gly Gly Gly Thr
    690                 695                 700

Cys Ala Thr Gly Cys Thr Thr Cys Ala Thr Gly Cys Cys Ala Ala Ala
705                 710                 715                 720

Gly Ala Ala Cys Thr Thr Cys Cys Cys Thr Ala Thr Ala Ala Thr Cys
                725                 730                 735

Thr Cys Thr Gly Gly Gly Cys Thr Gly Ala Ala Gly Cys Cys Ala Thr
            740                 745                 750

Gly Ala Ala Cys Ala Cys Ala Gly Cys Ala Thr Gly Cys Thr Ala Cys
        755                 760                 765

Ala Thr Cys Cys Ala Cys Ala Ala Cys Ala Gly Ala Gly Thr Cys Ala
    770                 775                 780

Cys Ala Cys Thr Thr Ala Gly Ala Ala Gly Ala Gly Gly Gly Ala Cys
785                 790                 795                 800

Thr Cys Cys Ala Ala Cys Cys Ala Cys Ala Cys Thr Gly Thr Ala Thr
                805                 810                 815

Gly Ala Ala Ala Thr Cys Thr Gly Gly Ala Ala Ala Gly Gly Gly Ala
            820                 825                 830

Gly Gly Ala Ala Gly Cys Cys Ala Ala Cys Thr Gly Thr Cys Ala Ala
        835                 840                 845

Gly Cys Ala Cys Thr Thr Cys Cys Ala Cys Ala Thr Cys Thr Gly Thr
    850                 855                 860

Gly Gly Ala Ala Gly Thr Cys Cys Ala Thr Gly Thr Thr Ala Cys Ala
865                 870                 875                 880

Thr Thr Thr Thr Gly Gly Cys Ala Gly Ala Thr Ala Gly Ala Gly Ala
                885                 890                 895

Gly Cys Ala Ala Ala Gly Gly Ala Gly Ala Ala Ala Gly Ala Thr Gly
            900                 905                 910

Gly Ala Thr Cys Cys Cys Ala Ala Gly Ala Gly Thr Gly Ala Thr Gly
        915                 920                 925

Cys Ala Gly Gly Gly Ala Thr Ala Thr Thr Cys Thr Thr Gly Gly Gly
    930                 935                 940

Ala Thr Ala Cys Thr Cys Thr Ala Cys Ala Ala Ala Cys Ala Gly Cys
945                 950                 955                 960

Ala Gly Ala Gly Cys Ala Thr Ala Thr Ala Gly Ala Gly Thr Ala Thr
                965                 970                 975

Thr Cys Ala Ala Thr Thr Cys Cys Ala Gly Ala Ala Cys Cys Ala Gly
            980                 985                 990

Ala Ala Cys Thr Gly Thr Gly Ala Thr Gly Gly Ala Ala Thr Cys Cys
        995                 1000                1005

Ala Thr Cys Ala Ala Thr Gly Thr Gly Gly Thr Thr Gly Thr Thr Gly
    1010                1015                1020
```

```
Ala Thr Gly Ala Thr Cys Thr Ala Ala Cys Thr Cys Cys Ala Gly Cys
1025                1030                1035                1040

Ala Ala Gly Ala Ala Ala Gly Ala Ala Gly Gly Ala Thr Gly Thr Cys
                1045                1050                1055

Gly Ala Ala Gly Ala Ala Gly Ala Thr Gly Thr Cys Ala Gly Ala Ala
            1060                1065                1070

Cys Ala Thr Cys Gly Gly Gly Ala Gly Ala Cys Ala Ala Thr Gly Thr
        1075                1080                1085

Thr Gly Cys Ala Gly Ala Thr Ala Cys Ala Gly Cys Thr Ala Ala Ala
    1090                1095                1100

Ala Gly Thr Gly Cys Ala Gly Ala Ala Ala Ala Thr Gly Cys Ala Gly
1105                1110                1115                1120

Ala Ala Ala Ala Cys Thr Cys Thr Gly Ala Thr Thr Cys Thr Gly Cys
                1125                1130                1135

Thr Ala Cys Ala Gly Ala Thr Gly Ala Ala Cys Cys Ala Ala Ala Cys
            1140                1145                1150

Ala Thr Cys Ala Ala Thr Cys Ala Ala Cys Cys Thr Gly Ala Cys Ala
        1155                1160                1165

Ala Gly Ala Gly Ala Cys Cys Cys Thr Cys Cys Ala Thr Thr Ala Gly
    1170                1175                1180

Ala Ala Thr Cys Cys Ala Gly Ala Ala Gly Ala Thr Gly Cys Ala Cys
1185                1190                1195                1200

Cys Cys Cys Ala Ala Gly Gly Ala Gly Cys Thr Gly Ala Thr Thr Ala
                1205                1210                1215

Thr Ala Gly Gly Ala Gly Ala Thr Cys Cys Ala Ala Ala Cys Ala Gly
            1220                1225                1230

Ala Gly Gly Ala Gly Thr Cys Ala Cys Thr Ala Cys Ala Ala Gly Ala
        1235                1240                1245

Thr Cys Ala Ala Gly Gly Gly Ala Gly Ala Thr Thr Gly Ala Gly Ala
    1250                1255                1260

Thr Thr Ala Thr Cys Thr Cys Cys Ala Ala Thr Thr Cys Ala Thr Gly
1265                1270                1275                1280

Thr Thr Thr Thr Gly Thr Cys Thr Cys Cys Ala Ala Ala Ala Thr Thr
                1285                1290                1295

Gly Ala Gly Cys Cys Cys Ala Ala Gly Ala Ala Thr Gly Thr Gly Ala
            1300                1305                1310

Ala Ala Gly Ala Gly Gly Cys Ala Cys Thr Gly Ala Cys Thr Gly Ala
        1315                1320                1325

Thr Gly Ala Gly Thr Thr Cys Thr Gly Gly Ala Thr Cys Ala Ala Thr
    1330                1335                1340

Gly Cys Thr Ala Thr Gly Cys Ala Ala Gly Ala Ala Gly Ala Ala Thr
1345                1350                1355                1360

Thr Gly Gly Ala Gly Cys Ala Ala Thr Thr Cys Ala Ala Ala Ala Gly
                1365                1370                1375

Gly Ala Ala Thr Gly Ala Ala Gly Thr Thr Thr Gly Gly Gly Ala Gly
```

```
            1380                1385                1390

Cys Thr Ala Gly Thr Thr Cys Cys Thr Ala Gly Gly Cys Cys Cys Gly
        1395                1400                1405

Ala Gly Gly Gly Ala Ala Cys Thr Ala Ala Thr Gly Thr Gly Ala Thr
    1410                1415                1420

Thr Gly Gly Cys Ala Cys Cys Ala Ala Gly Thr Gly Gly Ala Thr Cys
1425                1430                1435                1440

Thr Thr Cys Ala Ala Gly Ala Ala Cys Ala Ala Ala Ala Cys Cys Ala
                1445                1450                1455

Ala Thr Gly Ala Ala Gly Ala Ala Gly Gly Thr Gly Thr Thr Ala Thr
            1460                1465                1470

Ala Ala Cys Cys Ala Gly Ala Ala Ala Cys Ala Ala Gly Gly Cys Cys
        1475                1480                1485

Ala Gly Ala Cys Thr Thr Gly Thr Thr Gly Cys Thr Cys Ala Ala Gly
    1490                1495                1500

Gly Cys Thr Ala Cys Ala Cys Thr Cys Ala Gly Ala Thr Thr Gly Ala
1505                1510                1515                1520

Ala Gly Gly Thr Gly Thr Ala Gly Ala Cys Thr Thr Thr Gly Ala Thr
                1525                1530                1535

Gly Ala Ala Ala Cys Thr Thr Thr Thr Gly Cys Cys Cys Cys Thr Gly
            1540                1545                1550

Gly Thr Gly Cys Thr Ala Ala Ala Cys Thr Thr Gly Ala Gly Thr Cys
        1555                1560                1565

Cys Ala Thr Cys Ala Gly Ala Cys Thr Gly Thr Thr Ala Cys Thr Thr
    1570                1575                1580

Gly Gly Thr Gly Thr Ala Gly Cys Thr Thr Gly Cys Ala Thr Cys Cys
1585                1590                1595                1600

Thr Cys Ala Ala Ala Thr Thr Cys Ala Ala Gly Cys Thr Gly Thr Ala
                1605                1610                1615

Cys Cys Ala Gly Ala Thr Gly Gly Ala Thr Gly Thr Gly Ala Ala Gly
            1620                1625                1630

Ala Gly Cys Gly Cys Ala Thr Thr Thr Cys Thr Gly Ala Ala Thr Gly
        1635                1640                1645

Gly Ala Thr Ala Cys Cys Thr Gly Ala Ala Thr Gly Ala Ala Gly Ala
    1650                1655                1660

Ala Gly Cys Cys Thr Ala Thr Gly Thr Gly Gly Ala Gly Cys Ala Gly
1665                1670                1675                1680

Cys Cys Ala Ala Ala Gly Gly Gly Ala Thr Thr Thr Gly Thr Ala Gly
                1685                1690                1695

Ala Thr Cys Cys Ala Ala Cys Thr Cys Ala Thr Cys Cys Ala Gly Ala
            1700                1705                1710

Thr Cys Ala Thr Gly Thr Ala Thr Ala Cys Ala Gly Gly Cys Thr Cys
        1715                1720                1725

Ala Ala Gly Ala Ala Gly Cys Thr Cys Thr Gly Cys Thr Ala Thr Gly
    1730                1735                1740
```

```
Gly Ala Thr Thr Gly Ala Ala Gly Cys Ala Ala Gly Cys Thr Thr Cys
1745                1750                1755                1760

Ala Ala Gly Ala Gly Cys Thr Thr Gly Gly Thr Ala Thr Gly Ala Ala
                1765                1770                1775

Ala Gly Gly Cys Thr Ala Ala Cys Ala Gly Ala Gly Thr Thr Cys Cys
            1780                1785                1790

Thr Thr Ala Cys Thr Cys Ala Gly Cys Ala Ala Gly Gly Gly Thr Ala
        1795                1800                1805

Thr Ala Gly Gly Ala Ala Gly Gly Gly Gly Gly Gly Gly Ala Thr Thr
    1810                1815                1820

Gly Ala Cys Ala Ala Gly Ala Cys Cys Cys Thr Thr Thr Thr Thr Gly
1825                1830                1835                1840

Thr Thr Ala Ala Ala Cys Ala Ala Gly Ala Thr Gly Cys Thr Gly Gly
                1845                1850                1855

Ala Ala Ala Ala Thr Thr Gly Ala Thr Gly Ala Thr Ala Gly Cys Ala
            1860                1865                1870

Cys Ala Gly Ala Thr Ala Thr Ala Thr Gly Thr Thr Gly Ala Thr Gly
        1875                1880                1885

Ala Cys Ala Thr Thr Gly Thr Gly Thr Thr Thr Gly Gly Ala Gly Gly
    1890                1895                1900

Gly Ala Thr Gly Thr Thr Gly Ala Ala Thr Gly Ala Gly Ala Thr Gly
1905                1910                1915                1920

Cys Thr Thr Cys Gly Ala Cys Ala Thr Thr Thr Thr Gly Thr Cys Cys
                1925                1930                1935

Ala Ala Cys Ala Gly Ala Thr Gly Cys Ala Ala Thr Thr Thr Gly Ala
            1940                1945                1950

Ala Thr Thr Thr Gly Ala Gly Ala Thr Gly Ala Gly Thr Thr Thr Thr
        1955                1960                1965

Gly Thr Thr Gly Gly Ala Gly Ala Gly Cys Thr Gly Ala Ala Thr Thr
    1970                1975                1980

Ala Thr Thr Thr Thr Thr Thr Gly Gly Gly Ala Ala Thr Cys Cys Ala
1985                1990                1995                2000

Ala Gly Thr Gly Ala Ala Gly Cys Ala Gly Ala Thr Gly Gly Ala Ala
                2005                2010                2015

Gly Ala Ala Thr Cys Cys Ala Thr Ala Thr Thr Cys Cys Thr Thr Thr
            2020                2025                2030

Cys Ala Cys Ala Ala Ala Gly Cys Ala Ala Gly Thr Ala Thr Gly Cys
        2035                2040                2045

Ala Ala Ala Gly Ala Ala Cys Ala Thr Thr Gly Thr Cys Ala Ala Gly
    2050                2055                2060

Ala Ala Gly Thr Thr Thr Gly Gly Gly Ala Thr Gly Gly Ala Ala Ala
2065                2070                2075                2080

Ala Thr Gly Cys Cys Ala Gly Cys Cys Ala Thr Ala Ala Ala Ala Gly
                2085                2090                2095

Ala Ala Cys Ala Cys Cys Thr Gly Cys Ala Cys Cys Thr Ala Ala Thr
```

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            2100                2105                2110

Cys Ala Ala Thr Thr Gly Ala Ala Gly Cys Thr Gly Thr Cys Ala Ala
        2115                2120                2125

Ala Ala Gly Ala Thr Gly Ala Ala Gly Cys Thr Gly Gly Cys Ala Cys
    2130                2135                2140

Cys Ala Gly Thr Gly Thr Thr Gly Ala Thr Cys Ala Ala Ala Gly Thr
2145                2150                2155                2160

Thr Thr Gly Thr Ala Cys Ala Gly Ala Ala Gly Cys Ala Thr Gly Ala
                2165                2170                2175

Thr Thr Gly Gly Gly Ala Gly Cys Thr Thr Ala Ala Thr Ala Thr Ala
            2180                2185                2190

Thr Thr Thr Ala Ala Cys Ala Gly Cys Thr Ala Gly Cys Ala Gly Ala
        2195                2200                2205

Cys Cys Thr Gly Ala Cys Ala Thr Cys Ala Cys Cys Thr Ala Thr Gly
    2210                2215                2220

Cys Ala Gly Thr Ala Gly Gly Thr Gly Gly Thr Thr Gly Thr Gly Cys
2225                2230                2235                2240

Ala Ala Gly Ala Thr Ala Thr Cys Ala Ala Gly Cys Cys Ala Ala Thr
                2245                2250                2255

Cys Cys Thr Ala Ala Gly Ala Thr Ala Ala Gly Thr Cys Ala Cys Thr
            2260                2265                2270

Thr Gly Ala Ala Thr Cys Ala Ala Gly Thr Ala Ala Ala Gly Ala Gly
        2275                2280                2285

Ala Ala Thr Thr Thr Thr Gly Ala Ala Ala Thr Ala Thr Gly Thr Ala
    2290                2295                2300

Ala Ala Thr Gly Gly Cys Ala Cys Cys Ala Gly Thr Gly Ala Cys Thr
2305                2310                2315                2320

Ala Thr Gly Gly Gly Ala Thr Thr Ala Thr Gly Thr Ala Cys Thr Gly
                2325                2330                2335

Thr Cys Ala Thr Thr Gly Thr Thr Cys Ala Gly Ala Thr Thr Cys Ala
            2340                2345                2350

Ala Thr Gly Cys Thr Gly Gly Thr Thr Gly Gly Gly Thr Ala Thr Thr
        2355                2360                2365

Gly Thr Gly Ala Thr Gly Cys Thr Gly Ala Thr Thr Gly Gly Gly Cys
    2370                2375                2380

Thr Gly Gly Ala Ala Gly Thr Gly Thr Ala Gly Ala Thr Gly Ala Cys
2385                2390                2395                2400

Ala Gly Ala Ala Ala Ala Ala Gly Cys Ala Cys Thr Thr Thr Thr Gly
                2405                2410                2415

Gly Thr Gly Gly Ala Thr Gly Thr Thr Thr Thr Thr Ala Thr Thr Thr
            2420                2425                2430

Gly Gly Gly Ala Ala Cys Cys Ala Ala Thr Thr Thr Thr Ala Thr Thr
        2435                2440                2445

Thr Cys Ala Thr Gly Gly Thr Thr Cys Ala Gly Cys Ala Ala Gly Ala
    2450                2455                2460
```

```
Ala Gly Cys Ala Gly Ala Ala Cys Thr Gly Thr Gly Thr Gly Thr Cys
2465                2470                2475                2480

Cys Cys Thr Ala Thr Cys Cys Ala Cys Thr Gly Cys Ala Gly Ala Ala
                2485                2490                2495

Gly Cys Ala Gly Ala Gly Thr Ala Thr Ala Thr Thr Gly Cys Ala Gly
            2500                2505                2510

Cys Ala Gly Gly Ala Ala Gly Cys Ala Gly Cys Thr Gly Thr Thr Cys
        2515                2520                2525

Ala Cys Ala Ala Cys Thr Ala Gly Thr Thr Thr Gly Gly Ala Thr Gly
    2530                2535                2540

Ala Ala Gly Cys Ala Gly Ala Thr Gly Cys Thr Cys Ala Ala Gly Gly
2545                2550                2555                2560

Ala Gly Thr Ala Cys Ala Ala Thr Gly Thr Cys Gly Ala Ala Cys Ala
                2565                2570                2575

Ala Gly Ala Thr Gly Thr Cys Ala Thr Gly Ala Cys Ala Thr Thr Gly
            2580                2585                2590

Thr Ala Cys Thr Gly Thr Gly Ala Cys Ala Ala Cys Thr Thr Gly Ala
        2595                2600                2605

Gly Thr Gly Cys Thr Ala Thr Thr Ala Ala Thr Ala Thr Thr Thr Cys
    2610                2615                2620

Thr Ala Ala Ala Ala Ala Thr Cys Cys Thr Gly Thr Thr Cys Ala Ala
2625                2630                2635                2640

Cys Ala Cys Ala Gly Cys Ala Gly Ala Ala Cys Cys Ala Ala Gly Cys
                2645                2650                2655

Ala Cys Ala Thr Thr Gly Ala Cys Ala Thr Thr Ala Gly Ala Cys Ala
            2660                2665                2670

Thr Cys Ala Cys Thr Ala Thr Ala Thr Thr Ala Gly Ala Gly Ala Thr
        2675                2680                2685

Cys Thr Thr Gly Thr Thr Gly Ala Thr Gly Ala Thr Ala Ala Ala Gly
    2690                2695                2700

Thr Thr Ala Thr Cys Ala Cys Ala Cys Thr Gly Gly Ala Gly Cys Ala
2705                2710                2715                2720

Thr Gly Thr Thr Gly Ala Cys Ala Cys Thr Gly Ala Gly Gly Ala Ala
                2725                2730                2735

Cys Ala Ala Ala Thr Ala Gly Cys Ala Gly Ala Thr Ala Thr Thr Thr
            2740                2745                2750

Thr Cys Ala Cys Ala Ala Ala Gly Gly Cys Ala Thr Thr Gly Gly Ala
        2755                2760                2765

Thr Gly Cys Ala Ala Ala Thr Cys Ala Gly Thr Thr Thr Gly Ala Ala
    2770                2775                2780

Ala Ala Ala Cys Thr Gly Ala Gly Gly Gly Gly Cys Ala Ala Gly Cys
2785                2790                2795                2800

Thr Gly Gly Gly Cys Ala Thr Thr Thr Gly Thr Cys Thr Gly Cys Thr
                2805                2810                2815

Ala Gly Ala Gly Gly Ala Thr Thr Thr Ala
```

```
                2820                2825
```

(2) INFORMATION FOR SEQ ID NO:51:

```
    (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 942 amino acids
         (B) TYPE: amino acid
         (C) STRANDEDNESS: not relevant
         (D) TOPOLOGY: not relevant

   (ii) MOLECULE TYPE: protein



   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

    Asp Glu Gly Phe Asn Val Asp Phe Thr Glu Ser Glu Cys Leu Met Thr
    1               5                   10                  15

    Lys Glu Lys Arg Glu Val Leu Met Lys Gly Gly Arg Ser Lys Asp Asn
                20                  25                  30

    Cys Tyr Leu Trp Thr Pro Gln Glu Thr Ser Tyr Ser Ser Thr Cys Leu
            35                  40                  45

    Phe Ser Lys Glu Asp Glu Val Lys Ile Trp His Gln Arg Phe Gly His
        50                  55                  60

    Leu His Leu Gly Gly Met Lys Lys Ile Ile Asp Lys Gly Ala Val Arg
    65                  70                  75                  80

    Gly Ile Pro Asn Leu Lys Ile Glu Glu Gly Arg Ile Cys Gly Glu Cys
                    85                  90                  95

    Gln Ile Gly Lys Gln Val Lys Met Ser Asn Gln Lys Leu Gln His Gln
                100                 105                 110

    Thr Thr Ser Arg Val Leu Glu Leu Leu His Met Asp Leu Met Gly Pro
            115                 120                 125

    Met Gln Val Glu Ser Leu Gly Arg Lys Arg Tyr Ala Tyr Val Val Val
        130                 135                 140

    Asp Asp Phe Ser Arg Phe Thr Trp Val Asn Phe Ile Arg Glu Lys Ser
    145                 150                 155                 160

    Asp Thr Phe Glu Val Phe Lys Glu Leu Ser Leu Arg Leu Gln Arg Glu
                    165                 170                 175

    Lys Asp Cys Val Ile Lys Arg Ile Arg Ser Asp His Gly Arg Glu Phe
                180                 185                 190

    Glu Asn Ser Lys Phe Thr Glu Phe Cys Thr Ser Glu Gly Ile Thr His
            195                 200                 205

    Glu Phe Ser Ala Ala Ile Thr Pro Gln Gln Asn Gly Ile Val Glu Arg
        210                 215                 220

    Lys Asn Arg Thr Leu Pro Glu Ala Ala Arg Val Met Leu His Ala Lys
    225                 230                 235                 240

    Glu Leu Pro Tyr Asn Leu Trp Ala Glu Ala Met Asn Thr Ala Cys Tyr
                    245                 250                 255

    Ile His Asn Arg Val Thr Leu Arg Arg Gly Thr Pro Thr Thr Leu Tyr
                260                 265                 270
```

```
Glu Ile Trp Lys Gly Arg Lys Pro Thr Val Lys His Phe His Ile Cys
        275                 280                 285

Gly Ser Pro Cys Tyr Ile Leu Ala Asp Arg Glu Gln Arg Arg Lys Met
    290                 295                 300

Asp Pro Lys Ser Asp Ala Gly Ile Phe Leu Gly Tyr Ser Thr Asn Ser
305                 310                 315                 320

Arg Ala Tyr Arg Val Phe Asn Ser Arg Thr Arg Thr Val Met Glu Ser
                325                 330                 335

Ile Asn Val Val Val Asp Asp Leu Thr Pro Ala Arg Lys Lys Asp Val
            340                 345                 350

Glu Glu Asp Val Arg Thr Ser Gly Asp Asn Val Ala Asp Thr Ala Lys
        355                 360                 365

Ser Ala Glu Asn Ala Glu Asn Ser Asp Ser Ala Thr Asp Glu Pro Asn
    370                 375                 380

Ile Asn Gln Pro Asp Lys Arg Pro Ser Ile Arg Ile Gln Lys Met His
385                 390                 395                 400

Pro Lys Glu Leu Ile Ile Gly Asp Pro Asn Arg Gly Val Thr Thr Arg
                405                 410                 415

Ser Arg Glu Ile Glu Ile Ile Ser Asn Ser Cys Phe Val Ser Lys Ile
            420                 425                 430

Glu Pro Lys Asn Val Lys Glu Ala Leu Thr Asp Glu Phe Trp Ile Asn
        435                 440                 445

Ala Met Gln Glu Glu Leu Glu Gln Phe Lys Arg Asn Glu Val Trp Glu
    450                 455                 460

Leu Val Pro Arg Pro Glu Gly Thr Asn Val Ile Gly Thr Lys Trp Ile
465                 470                 475                 480

Phe Lys Asn Lys Thr Asn Glu Glu Gly Val Ile Thr Arg Asn Lys Ala
                485                 490                 495

Arg Leu Val Ala Gln Gly Tyr Thr Gln Ile Glu Gly Val Asp Phe Asp
            500                 505                 510

Glu Thr Phe Ala Pro Gly Ala Lys Leu Glu Ser Ile Arg Leu Leu Leu
        515                 520                 525

Gly Val Ala Cys Ile Leu Lys Phe Lys Leu Tyr Gln Met Asp Val Lys
    530                 535                 540

Ser Ala Phe Leu Asn Gly Tyr Leu Asn Glu Glu Ala Tyr Val Glu Gln
545                 550                 555                 560

Pro Lys Gly Phe Val Asp Pro Thr His Pro Asp His Val Tyr Arg Leu
                565                 570                 575

Lys Lys Leu Cys Tyr Gly Leu Lys Gln Ala Ser Arg Ala Trp Tyr Glu
            580                 585                 590

Arg Leu Thr Glu Phe Leu Thr Gln Gln Gly Tyr Arg Lys Gly Gly Ile
        595                 600                 605

Asp Lys Thr Leu Phe Val Lys Gln Asp Ala Gly Lys Leu Met Ile Ala
    610                 615                 620

Gln Ile Tyr Val Asp Asp Ile Val Phe Gly Gly Met Leu Asn Glu Met
```

```
625                 630                 635                 640

Leu Arg His Phe Val Gln Gln Met Gln Phe Glu Phe Glu Met Ser Phe
                645                 650                 655

Val Gly Glu Leu Asn Tyr Phe Leu Gly Ile Gln Val Lys Gln Met Glu
            660                 665                 670

Glu Ser Ile Phe Leu Ser Gln Ser Lys Tyr Ala Lys Asn Ile Val Lys
        675                 680                 685

Lys Phe Gly Met Glu Asn Ala Ser His Lys Arg Thr Pro Ala Pro Asn
    690                 695                 700

Gln Leu Lys Leu Ser Lys Asp Glu Ala Gly Thr Ser Val Asp Gln Ser
705                 710                 715                 720

Leu Tyr Arg Ser Met Ile Gly Ser Leu Ile Tyr Leu Thr Ala Ser Arg
                725                 730                 735

Pro Asp Ile Thr Tyr Ala Val Gly Gly Cys Ala Arg Tyr Gln Ala Asn
            740                 745                 750

Pro Lys Ile Ser His Leu Asn Gln Val Lys Arg Ile Leu Lys Tyr Val
        755                 760                 765

Asn Gly Thr Ser Asp Tyr Gly Ile Met Tyr Cys His Cys Ser Asp Ser
    770                 775                 780

Met Leu Val Gly Tyr Cys Asp Ala Asp Trp Ala Gly Ser Val Asp Asp
785                 790                 795                 800

Arg Lys Ser Thr Phe Gly Gly Cys Phe Tyr Leu Gly Thr Asn Phe Ile
                805                 810                 815

Ser Trp Phe Ser Lys Lys Gln Asn Cys Val Ser Leu Ser Thr Ala Glu
            820                 825                 830

Ala Glu Tyr Ile Ala Ala Gly Ser Ser Cys Ser Gln Leu Val Trp Met
        835                 840                 845

Lys Gln Met Leu Lys Glu Tyr Asn Val Glu Gln Asp Val Met Thr Leu
    850                 855                 860

Tyr Cys Asp Asn Leu Ser Ala Ile Asn Ile Ser Lys Asn Pro Val Gln
865                 870                 875                 880

His Ser Arg Thr Lys His Ile Asp Ile Arg His His Tyr Ile Arg Asp
                885                 890                 895

Leu Val Asp Asp Lys Val Ile Thr Leu Glu His Val Asp Thr Glu Glu
            900                 905                 910

Gln Ile Ala Asp Ile Phe Thr Lys Ala Leu Asp Ala Asn Gln Phe Glu
        915                 920                 925

Lys Leu Arg Gly Lys Leu Gly Ile Cys Leu Leu Glu Asp Leu
    930                 935                 940
```

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 400 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

```
Asp Glu Gly Phe Asn Val Asp Phe Thr Glu Ser Glu Cys Leu Met Thr
1               5                   10                  15

Lys Glu Lys Arg Glu Val Leu Met Lys Gly Gly Arg Ser Lys Asp Asn
            20                  25                  30

Cys Tyr Leu Trp Thr Pro Gln Glu Thr Ser Tyr Ser Ser Thr Cys Leu
        35                  40                  45

Phe Ser Lys Glu Asp Glu Val Lys Ile Trp His Gln Arg Phe Gly His
    50                  55                  60

Leu His Leu Gly Gly Met Lys Lys Ile Ile Asp Lys Gly Ala Val Arg
65                  70                  75                  80

Gly Ile Pro Asn Leu Lys Ile Glu Glu Gly Arg Ile Cys Gly Glu Cys
                85                  90                  95

Gln Ile Gly Lys Gln Val Lys Met Ser Asn Gln Lys Leu Gln His Gln
            100                 105                 110

Thr Thr Ser Arg Val Leu Glu Leu Leu His Met Asp Leu Met Gly Pro
        115                 120                 125

Met Gln Val Glu Ser Leu Gly Arg Lys Arg Tyr Ala Tyr Val Val Val
    130                 135                 140

Asp Asp Phe Ser Arg Phe Thr Trp Val Asn Phe Ile Arg Glu Lys Ser
145                 150                 155                 160

Asp Thr Phe Glu Val Phe Lys Glu Leu Ser Leu Arg Leu Gln Arg Glu
                165                 170                 175

Lys Asp Cys Val Ile Lys Arg Ile Arg Ser Asp His Gly Arg Glu Phe
            180                 185                 190

Glu Asn Ser Lys Phe Thr Glu Phe Cys Thr Ser Glu Gly Ile Thr His
        195                 200                 205

Glu Phe Ser Ala Ala Ile Thr Pro Gln Gln Asn Gly Ile Val Glu Arg
    210                 215                 220

Lys Asn Arg Thr Leu Pro Glu Ala Ala Arg Val Met Leu His Ala Lys
225                 230                 235                 240

Glu Leu Pro Tyr Asn Leu Trp Ala Glu Ala Met Asn Thr Ala Cys Tyr
                245                 250                 255

Ile His Asn Arg Val Thr Leu Arg Arg Gly Thr Pro Thr Thr Leu Tyr
            260                 265                 270

Glu Ile Trp Lys Gly Arg Lys Pro Thr Val Lys His Phe His Ile Cys
        275                 280                 285

Gly Ser Pro Cys Tyr Ile Leu Ala Asp Arg Glu Gln Arg Arg Lys Met
    290                 295                 300

Asp Pro Lys Ser Asp Ala Gly Ile Phe Leu Gly Tyr Ser Thr Asn Ser
305                 310                 315                 320
```

```
    Arg Ala Tyr Arg Val Phe Asn Ser Arg Thr Arg Thr Val Met Glu Ser
                    325                 330                 335

    Ile Asn Val Val Val Asp Asp Leu Thr Pro Ala Arg Lys Lys Asp Val
                340                 345                 350

    Glu Glu Asp Val Arg Thr Ser Gly Asp Asn Val Ala Asp Thr Ala Lys
            355                 360                 365

    Ser Ala Glu Asn Ala Glu Asn Ser Asp Ser Ala Thr Asp Glu Pro Asn
        370                 375                 380

    Ile Asn Gln Pro Asp Lys Arg Pro Ser Ile Arg Ile Gln Lys Met His
    385                 390                 395                 400

(2) INFORMATION FOR SEQ ID NO:53:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 381 amino acids
          (B) TYPE: amino acid
          (C) STRANDEDNESS: not relevant
          (D) TOPOLOGY: not relevant

    (ii) MOLECULE TYPE: protein



    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

    Pro Lys Glu Leu Ile Ile Gly Asp Pro Asn Arg Gly Val Thr Thr Arg
    1               5                   10                  15

    Ser Arg Glu Ile Glu Ile Ile Ser Asn Ser Cys Phe Val Ser Lys Ile
                20                  25                  30

    Glu Pro Lys Asn Val Lys Glu Ala Leu Thr Asp Glu Phe Trp Ile Asn
            35                  40                  45

    Ala Met Gln Glu Glu Leu Glu Gln Phe Lys Arg Asn Glu Val Trp Glu
        50                  55                  60

    Leu Val Pro Arg Pro Glu Gly Thr Asn Val Ile Gly Thr Lys Trp Ile
    65                  70                  75                  80

    Phe Lys Asn Lys Thr Asn Glu Glu Gly Val Ile Thr Arg Asn Lys Ala
                    85                  90                  95

    Arg Leu Val Ala Gln Gly Tyr Thr Gln Ile Glu Gly Val Asp Phe Asp
                100                 105                 110

    Glu Thr Phe Ala Pro Gly Ala Lys Leu Glu Ser Ile Arg Leu Leu Leu
            115                 120                 125

    Gly Val Ala Cys Ile Leu Lys Phe Lys Leu Tyr Gln Met Asp Val Lys
        130                 135                 140

    Ser Ala Phe Leu Asn Gly Tyr Leu Asn Glu Glu Ala Tyr Val Glu Gln
    145                 150                 155                 160

    Pro Lys Gly Phe Val Asp Pro Thr His Pro Asp His Val Tyr Arg Leu
                    165                 170                 175

    Lys Lys Leu Cys Tyr Gly Leu Lys Gln Ala Ser Arg Ala Trp Tyr Glu
                180                 185                 190
```

```
Arg Leu Thr Glu Phe Leu Thr Gln Gln Gly Tyr Arg Lys Gly Gly Ile
        195                 200                 205

Asp Lys Thr Leu Phe Val Lys Gln Asp Ala Gly Lys Leu Met Ile Ala
    210                 215                 220

Gln Ile Tyr Val Asp Asp Ile Val Phe Gly Gly Met Leu Asn Glu Met
225                 230                 235                 240

Leu Arg His Phe Val Gln Gln Met Gln Phe Glu Phe Glu Met Ser Phe
                245                 250                 255

Val Gly Glu Leu Asn Tyr Phe Leu Gly Ile Gln Val Lys Gln Met Glu
            260                 265                 270

Glu Ser Ile Phe Leu Ser Gln Ser Lys Tyr Ala Lys Asn Ile Val Lys
        275                 280                 285

Lys Phe Gly Met Glu Asn Ala Ser His Lys Arg Thr Pro Ala Pro Asn
    290                 295                 300

Gln Leu Lys Leu Ser Lys Asp Glu Ala Gly Thr Ser Val Asp Gln Ser
305                 310                 315                 320

Leu Tyr Arg Ser Met Ile Gly Ser Leu Ile Tyr Leu Thr Ala Ser Arg
                325                 330                 335

Pro Asp Ile Thr Tyr Ala Val Gly Gly Cys Ala Arg Tyr Gln Ala Asn
            340                 345                 350

Pro Lys Ile Ser His Leu Asn Gln Val Lys Arg Ile Leu Lys Tyr Val
        355                 360                 365

Asn Gly Thr Ser Asp Tyr Gly Ile Met Tyr Cys His Cys
    370                 375                 380
```

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 166 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

```
Ser Asp Ser Met Leu Val Gly Tyr Cys Asp Ala Asp Trp Ala Gly Ser
1               5                   10                  15

Val Asp Asp Arg Lys Ser Thr Phe Gly Gly Cys Phe Tyr Leu Gly Thr
            20                  25                  30

Asn Phe Ile Ser Trp Phe Ser Lys Lys Gln Asn Cys Val Ser Leu Ser
        35                  40                  45

Thr Ala Glu Ala Glu Tyr Ile Ala Ala Gly Ser Ser Cys Ser Gln Leu
    50                  55                  60

Val Trp Met Lys Gln Met Leu Lys Glu Tyr Asn Val Glu Gln Asp Val
65                  70                  75                  80

Met Thr Leu Tyr Cys Asp Asn Leu Ser Ala Ile Asn Ile Ser Lys Asn
```

```
                85                  90                  95

Pro Val Gln His Ser Arg Thr Lys His Ile Asp Ile Arg His His Tyr
            100                 105                 110

Ile Arg Asp Leu Val Asp Asp Lys Val Ile Thr Leu Glu His Val Asp
        115                 120                 125

Thr Glu Glu Gln Ile Ala Asp Ile Phe Thr Lys Ala Leu Asp Ala Asn
    130                 135                 140

Gln Phe Glu Lys Leu Arg Gly Lys Leu Gly Ile Cys Leu Leu Glu Asp
145                 150                 155                 160

Leu Xaa Asn Pro Xaa Pro
                165
```

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 613 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

```
Thr Leu Ile Ala Arg Ser Leu Leu Gly Gln Asn Lys Phe Asp Arg Cys
1               5                   10                  15

Phe Thr Arg Pro Ser Thr Phe Leu Ile Gln Thr His Ile Phe Val Val
            20                  25                  30

Ile Ser Phe Ser Ala Phe Pro Asn Ser Ser Gln Arg Phe Thr Lys Pro
        35                  40                  45

Phe Gln Arg Leu Cys Phe Ser Met Ala Thr Ser Pro Lys Asp Thr Ser
    50                  55                  60

Ser Pro Gly Ser Pro Ser Val Pro Ser Ser Pro Ser Ser Thr Lys Ala
65                  70                  75                  80

Pro Ser Asn Gln Glu Gln Pro Glu Phe His Ile Gln Pro Ile Gln Met
                85                  90                  95

Ile Pro Gly Leu Ala Pro Val Pro Glu Lys Leu Val Pro Ile Arg Gln
            100                 105                 110

Gln Gly Val Lys Ile Ser Glu Asn Pro Ser Ile Ala Thr Ser Pro Arg
        115                 120                 125

Glu Leu Thr Arg Glu Met Asp Lys Lys Ile Arg Ser Ile Val Ser Ser
    130                 135                 140

Ile Leu Lys Asn Ala Ser Val Pro Asp Ala Asp Lys Asp Val Pro Thr
145                 150                 155                 160

Ser Ser Thr Pro Asn Ala Glu Val Leu Ser Ser Ser Ser Lys Glu Glu
                165                 170                 175

Ser Thr Glu Glu Glu Glu Gln Ala Thr Glu Glu Thr Pro Ala Pro Arg
            180                 185                 190
```

```
Ala Pro Glu Pro Ala Pro Gly Asp Leu Ile Asp Leu Glu Glu Val Glu
        195                 200                 205

Ser Asp Glu Glu Pro Ile Ala Asn Lys Leu Ala Pro Gly Ile Ala Glu
    210                 215                 220

Arg Leu Gln Ser Arg Lys Gly Lys Thr Pro Ile Thr Arg Ser Gly Arg
225                 230                 235                 240

Ile Lys Thr Met Ala Gln Lys Lys Ser Thr Pro Ile Thr Pro Thr Thr
                245                 250                 255

Ser Arg Trp Ser Lys Val Ala Ile Pro Ser Lys Lys Arg Lys Glu Phe
            260                 265                 270

Ser Ser Ser Asp Ser Asp Asp Asp Val Glu Leu Asp Val Pro Asp Ile
        275                 280                 285

Lys Arg Ala Lys Lys Ser Gly Lys Lys Val Pro Gly Asn Val Pro Asp
    290                 295                 300

Ala Pro Leu Asp Asn Ile Ser Phe His Ser Ile Gly Asn Val Glu Arg
305                 310                 315                 320

Trp Lys Phe Val Tyr Gln Arg Arg Leu Ala Leu Glu Arg Glu Leu Gly
                325                 330                 335

Arg Asp Ala Leu Asp Cys Lys Glu Ile Met Asp Leu Ile Lys Gly Cys
            340                 345                 350

Trp Thr Ala Glu Asn Ser His Gln Val Gly Arg Cys Tyr Glu Ser Leu
        355                 360                 365

Val Arg Glu Phe Ile Val Asn Ile Pro Ser Asp Ile Thr Asn Arg Lys
    370                 375                 380

Ser Asp Glu Tyr Gln Lys Val Phe Val Arg Gly Lys Cys Val Arg Phe
385                 390                 395                 400

Ser Pro Ala Val Ile Asn Lys Tyr Leu Gly Arg Pro Thr Glu Gly Val
                405                 410                 415

Val Asp Ile Ala Val Ser Glu His Gln Ile Ala Lys Glu Ile Thr Ala
            420                 425                 430

Lys Gln Val Gln His Trp Pro Lys Lys Gly Lys Leu Ser Ala Gly Lys
        435                 440                 445

Leu Ser Val Lys Tyr Ala Ile Leu His Arg Ile Gly Ala Ala Asn Trp
    450                 455                 460

Val Pro Thr Asn His Thr Ser Thr Val Ala Thr Gly Leu Gly Lys Phe
465                 470                 475                 480

Leu Tyr Ala Val Gly Thr Lys Ser Lys Phe Asn Phe Gly Lys Tyr Ile
                485                 490                 495

Phe Asp Gln Thr Val Lys His Ser Glu Ser Phe Ala Val Lys Leu Pro
            500                 505                 510

Ile Ala Phe Pro Thr Val Leu Cys Gly Ile Met Leu Ser Gln His Pro
        515                 520                 525

Asn Ile Leu Asn Asn Ile Asp Ser Val Met Lys Lys Glu Ser Ala Leu
    530                 535                 540

Ser Leu His Tyr Lys Leu Phe Glu Gly Thr His Val Pro Asp Ile Val
```

```
    545                 550                 555                 560

    Ser Thr Ser Gly Lys Ala Ala Ala Ser Gly Ala Val Ser Lys Gly Cys
                    565                 570                 575

    Phe Asp Cys Thr Gln Gly His Met Gln Gly Ala Gly Ser Asn His Gln
                580                 585                 590

    Ser His His Arg Lys Lys Asn Gly Ala Gly Thr Pro Asp Gln Lys Thr
            595                 600                 605

    Leu Arg Gln Trp His
        610

(2) INFORMATION FOR SEQ ID NO:56:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 183 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "GagR2"


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

GTTGCTGCAC AATGCACAAG GCAAGATAAA AGAAGTGAAG CTGCAGGATC CACGATGTCG      60

GATACGATGT CCAAGACATC TGGCCCGAAA ATACTGGACA CATAAATCTG TTATATCTTT     120

AACAGATTAT TGTGCAGTTA GCAACAGGTT AGACGATCTA TCTTTAGGAA CGAACTCTTC     180

TAG                                                                   183

(2) INFORMATION FOR SEQ ID NO:57:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 138 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: other nucleic acid
          (A) DESCRIPTION: /desc = "GagR1"


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

GACTTCGTTA TGTCAAGGAA TAAGATCGGG CTGCACAATG CACAAGGCAA GATAAAATGT      60

CAAATGAAGA ATTGAAGCTG CAGGATCCAT GATGTCGGAT ACAATGTCCA GGACATCCTG     120

CCCGAAAATA CTGGAGTT                                                   138

(2) INFORMATION FOR SEQ ID NO:58:

     (i) SEQUENCE CHARACTERISTICS:
          (A) LENGTH: 220 base pairs
          (B) TYPE: nucleic acid
          (C) STRANDEDNESS: single
          (D) TOPOLOGY: linear
```

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "LTR2"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

```
TCCAACGTTA TGTCAAGGAA TCAGATTGGG CTCCACAATG CACAAGGCAA GATAAAAGGT        60

CAAATGAAGA ATTGAAGCTG CAGGATCCAC GATGTCGGAT ACAATGTCCA GGACATCCTG       120

CCCGAAAATA CTGGACACAT AAATCTGTTA TATCTTTAAC AGATTAATGT GCAGTTAGCA       180

ACAGATTTGG CGATCTATCT TTAGGAACGA ATTAAAAGAT                             220
```

**I Claim:**

1. An isolated, purified polynucleotide comprising a polynucleotide selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 8, SEQ ID NO: 50, polynucleotides that hybridize under stringent conditions to any one of the foregoing polynucleotides, and fragments thereof.

2. The polynucleotide of claim 1 wherein said fragments comprise all or part of one or more *SIRE-1* long terminal repeats.

3. The polynucleotide of claim 1 further comprising a heterologous DNA.

4. The polynucleotide of claim 3 wherein said heterologous DNA comprises a transcriptional regulatory element.

5. A vector comprising the polynucleotide according to claim 1.

6. The vector of claim 5 further comprising a heterologous DNA.

7. The vector of claim 6 wherein said heterologous DNA comprises a transcriptional regulatory element.

8. The vector of claim 6 wherein said heterologous DNA is operably linked to a transcriptional regulatory element.

9. The vector of claim 8 wherein the heterologous DNA comprises a DNA encoding a protein conferring resistance to a plant disease.

10. The vector of claim 8 wherein said heterologous DNA comprises a DNA encoding a protein conferring resistance to insect infestation.

11. The vector of claim 8 wherein said heterologous DNA comprises a DNA encoding a protein conferring tolerance to a herbicide.

12. The vector of claim 8 wherein said heterologous DNA comprises a DNA encoding a protein conferring tolerance enhanced nitrogen fixation or nodulation.

13. The vector of claim 8 wherein said heterologous DNA comprises a DNA encoding a protein conferring enhanced vigor or growth.

14. The vector of claim 8 wherein said heterologous DNA comprises a DNA encoding a *SIRE-1*-encoded protein.

15. The vector of claim 8 wherein said heterologous DNA comprises a gene or a fragment thereof.

16. The vector of claim 8 wherein said heterologous DNA comprises a DNA encoding an antisense transcript.

17. A method for transforming a host cell comprising the step of introducing a vector according to any of claims 5 to 16 into said host cell.

18. A host cell transformed by the method of claim 17.

19. The host cell according to claim 18 wherein said host cell is a plant cell.

20. The host cell according to claim 19 wherein said plant cell is a soybean cell.

21. An isolated, purified *SIRE-1*-encoded protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, and analogs, homologs, and fragments thereof.

22. The protein of claim 21 wherein said protein is a recombinant protein.

23. A method for making a heterologous protein comprising the steps of:

(a) culturing a host cell according to claim 18 under suitable medium and environmental conditions; and

(b) isolating said protein from said cultured cell or from said medium.

24. A packaging cell comprising a polynucleotide encoding a *SIRE-1* protein having an amino acid sequence selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, and analogs, homologs, and fragments thereof, and wherein said polynucleotide lacks a functional packaging signal sequence.

25. An isolated, purified antibody that specifically recognizes an epitope on a protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, and analogs, homologs, and fragments thereof.

26. A plant retrovirus comprising a polynucleotide according to any one of claims 1 to 4 and a capsid protein.

27. The plant retrovirus of claim 26 further comprising one or more proteins comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, and analogs, homologs, and fragments thereof.

28. A method of producing a plant retrovirus, said method comprising the step of introducing the polynucleotide according to claim 1 into a packaging cell.

29. A method for transforming a plant cell, said method comprising the steps of:

(a) introducing a polynucleotide according to claim 1 into a plant cell; and

(b) culturing said plant cell under suitable nutrient and environmental conditions; and

(c) detecting said polynucleotide in said plant cell.

30. A method for transforming a plant cell, said method comprising the steps of:

(a) introducing a vector according to any one of claims 5 to 8 into a plant cell;

(b) culturing said plant cell under suitable nutrient and environmental conditions for the expression of an expression product of said polynucleotide; and

(c) detecting said expression product.

31. A transformed plant cell produced by the method of claim 29 or claim 30.

32. The transformed plant cell of claim 31 wherein said plant cell is a soybean cell.

33. A transgenic plant comprising a vector according to any of claims 5 to 8.

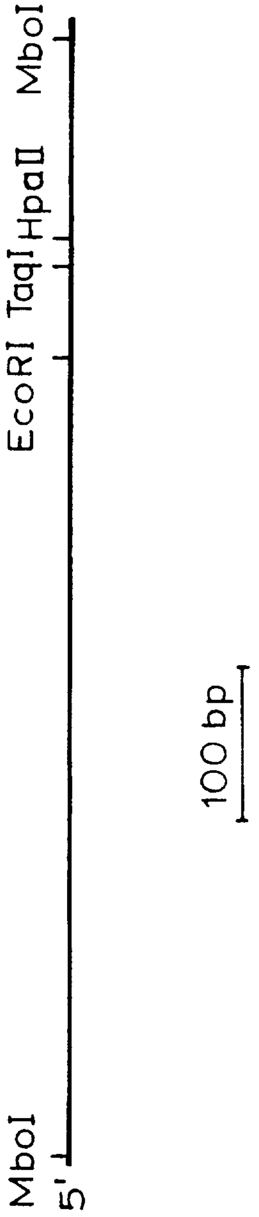
$^{5'}$ TNTTNGATCG(G/T)GTNCA(A/G)TGCTG $^{3'}$

FIGURE 1

```
TATTGGATCG GGTGCAGTGC TGTTTTTGGC AGGAACAAAT   40
TATGTCATGG TTGTTCTGCC AGCAGATTTA TGATTAAATC   80
CAAGTCCTCT CTGGTTTCCA ACATTCTTCC CAAGCTGTAG  120
CACCTCATCA AGCAAATTTG AGCCTTTATT CAGCATCTTT  160
ATTGATTTTG TCATGTTTTC CAGTTTAGAG TTCAGAAAAC  200
CAATTTCTCC TTTAAGTTCA GAGATTTCCT CTTCATGTGC  240
CTCCTTCTCA GCCTCCAGAT TTGCAATGAC CTTCTTTAGT  280
TGTGCTTCTT GCTGAAGAAT CTTCTCACTT TTGATGCATA  320
GTTCTCTATA GGATATAGCA AGCTCATCAA AAGTGATTTC  360
ACTATCTGTA TCACTTGAAT CTTCAGCAGA TTCAAATCTC  400
CCAGTGAGTG CATTCACATC TCTGTCAGAA TCACTTCTTG  440
TTCACTCTCT GTATCATCAG ACCGACATAC AGAAAGTCCT  480
TTCCTCTGCT TCTTGAGATG AGTGGGACAT TCAGCTTTGA  520
TGTGTCCATA GCCTTCACAC CCATGGCATT GAATTCCTTT  560
GCTGTGACTG GGCTTTTCAT CTGACCTTTT CTGGTATTCA  600
CTACCTTTCC TGATGTCGAA AGGGATGTTC CGGACATGTG  640
GTTTCTGCCT CCTGTCCATT CTGTTCAGCA CTTTGTTGAA  680
CTGTTTTCCA AGGAGCACAA CTGCGTTAGT CAGACCTTCA  720
TCAGTATCCA GGTCATACTC ATCTTCTTCT CCTTCAGCAC  760
TGCACCCGAT CCAATA                            776
```

FIGURE 2

FIGURE 3



| Gm776 (nt) | Element (nt) | % Identity | Q | Randomized Mean Q | Randomized S.D. | P |
|---|---|---|---|---|---|---|
| 150-465 | Tal (735-1045) | 50.3 | 127.2 | 111.8 | 2.9 | 0.0000003 |
| 360-670 | Tal (1210-1510) | 51.0 | 122.3 | 109.2 | 3.2 | 0.00009 |
| 144-382 | Tyl (4404-4640) | 51.2 | 106.3 | 93.1 | 3.5 | 0.0003 |

FIGURE 4

# FIGURE 5

## A

| | |
|---|---|
| F1 (M13/pUC reverse) | 5'-CCCAGTCACGACGTTGTAAAACG-3' |
| F2 (208-226) | 5'-TCCTTTAAGTTCAGAGATT-3' |
| R1 (M13/pUC reverse) | 5'-AGCGGATAACAATTTCACACAGG-3' |

## B

| | |
|---|---|
| Sense strand | |
| R1 (M13/pUC reverse) | 5'-CCCAGTCACGACGTTGTAAAACG-3' |
| R2 (320-349) | 5'-GTAATGGTCAACCAGACCACAGTT-3' |
| R3 (455-471) | 5'-GACGAATTGGCACTTGG-3' |
| R4 (708-725) | 5'-TTTGCACTGCCTTGGGAG-3' |
| R5 (983-999) | 5'-CCAAGGAGCACAACTGC-3' |
| R6 (1018-1037) | 5'-GCTGAACAGAATGGACAGGA-3' |
| R7 missing | |

| | |
|---|---|
| Complementary strand | |
| F1 (M13/pUC reverse) | 5'-AGCGGATAACAATTTCACACAGG-3' |
| F2 (2304-2321) | 5'-AAAGATATAACAAGATTTA-3' |
| F3 (2077-2097) | 5'-CCCGATCTTATTCCTTGACA-3' |
| F4 (1747-1766) | 5'-CTTGCCACAGTAGTGACA-3' |
| F5 (1566-1583) | 5'-TCTTCCCAAGCTGTAGCA-3' |
| F6 (1462-1481) | 5'-TCCTTTAAGTTCAGAGATT-3' |

```
TCCGGTCCCT GGCTTGGTAG CCCCCAGATG TAGGTGAGGT    40
TGCACCGAAC TGGGTTAACA ATTCTCTTGT GTTAGTTACT    80
TGTTTAATCT GTTCATACAG TCAAACATAA TCTGCATGTT   120
CTGAAGCGTG ATGTCGTGAC ATCCGGTACG ACATCTGTCA   160
TTGGTATCAG AATTTCAATT GGTATCAGAG CAGGCACTCG   200
AATTCACTGA GTGAGATCTA GGGAGATAAA TTCTGATGAA   240
CATGGAGAAA GAAGGAGGAC CAGTGAACAG ACCACCAATT   280
CTGGATGGAA CCAACTATGA ATACTGGAAA GCAAGGATGG   320
TGGCCTTCCT CAAATCACTG GATAGCAGAA CCTGGAAAGC   360
TGTCATCAAA GACTGGGAAC ATCCCAAGAT GCTGGACACA   400
GAAGGAAAGC CCACTGATGG ATTGAAGCCA GAAGAAGACT   440
GGACTAAAGA AGAAGACGAA TTGGCACTTG GAAACTCCAA   480
AGCTTTGAAT GCTCTATTCA ATGGAGTTGA CAAGAATATC   520
TTCAGACTGA TCAACACATG CACAGTGGCC AAGGATGCAT   560
GGGAGATCCT GAAAACCACT CATGAAGGAA CCTCCAAAGT   600
GAAGATGTCC AGATTGCAAC TATTGGCCAC AAAATTCGAA   640
AATCTGAAGA TGAAGGAGGA AGAGTGTATT CATGACTTTC   680
ACATGAACAT TCTTGAAATT GCCAATGCTT GCACTGCCTT   720
GGGAGAAAGA ATGACTGATG AAAAGCTGGT GAGAAAGATC   760
CTCAGATCCT TGCCTAAGAG ATTTGACATG AAAGTCACTG   800
CAATAGAGGA GGCCCAAGAC ATTTGCAACC TGAGAGTAGA   840
```

FIGURE 6 A

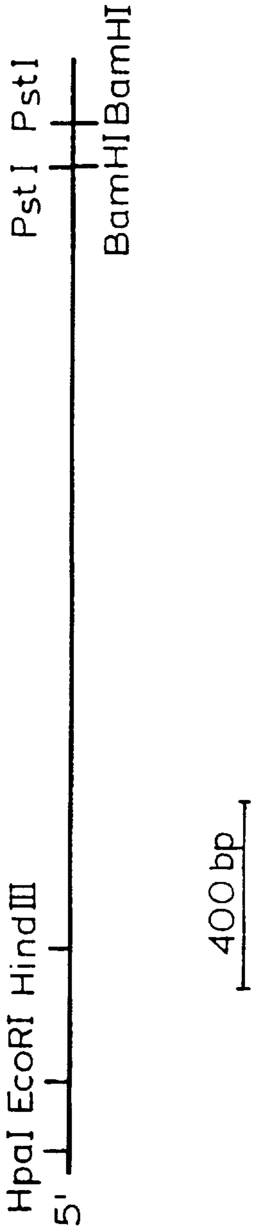
```
TGAACTCATT GGTTCCCTTC AAACCTTTGA GCTAGGACTC    880
TCGGATAGGA CTGAAAAGAA GAGCAAGAAT CTGGCGTTCG    920
TGTCCAATGA TGAAGGAGAA GAAGATGAGT ATGACCTGGA    960
TACAGATGAA GGTCTGACTA ATGCAGTTGT GCTCCTTGGA   1000
AAACAGTTCA ACAAAGTGCT GAACAGAATG GACAGGAGGC   1040
AGAAACCACA TGTCCGGAAC ATCCCTTTCG ACATCAGGAA   1080
AGGTAGTGAA TACCAGAAAA GGTCAGATGA AAAGCCCAGT   1120
CACAGCAAAG GATTTCAATG CCATGGGTGT GAAGGCTATG   1160
GACACATCAA AGCTGAATGT CCCACTCATC TCAAGAAGCA   1200
GAGGAAAGGA CTTTCTGTAT GTCGGTCTGA TGATACAGAG   1240
AGTGAACAAG AAAGTGATTC TGACAGAGAT GTGAATGCAC   1280
TCACTGGGAG ATTTGAATCT GCTGAAGATT CAAGTGATAC   1320
AGACAGTGAA ATCACTTTTG ATGAGCTTGC TACATCCTAT   1360
AGAGAACTAT GCATCAAAAG TGAGAAGATT CTTCAGCAAG   1400
AAGCACAACT GAAGAAGGTC ATTGCAAATC TGGAGGCTGA   1440
GAAGGAGGCA CATGAAGAGG AGATCTCTGA GCTTAAAGGA   1480
GAAGTTGGTT TTCTGAACTC TAAACTGGAA AACATGACAA   1520
AATCAATAAA GATGCTGAAT AAAGGCTCAG ATATGCTTGA   1560
TGAGGTGCTA CAGCTTGGGA AGAATGTTGG AAACCAGAGA   1600
GGACTTGGGT TTAATCATAA ATCTGCTGGC AGAATAACCA   1640
TGACAGAATT TGTTCCTGCC AAAATCAGCA CTGGAGCCAC   1680
```

FIGURE 6 B

```
GATGTCACAA CATCGGTCTC GACATCATGG AACGCAGCAG   1720
AAAAAGAGTA AAAGAAAGAA GTGGAGGTGT CACTACTGTG   1760
GCAAGTATGG TCACATAAAG CCCTTTTGCT ATCATCTACA   1800
TGGCCATCCA CATCATGGAA CTCAAAGTAG CAGCAGCAGA   1840
AGGAAGATGA TGTGGGTTCC AAAACACAAG ATTGTCAGTC   1880
TTGTTGTTCA TACTTCACTT AGAGCATCAG CTAAGGAAGA   1920
TTGGTACCTA GATAGCGGCT GTTCCAGACA CATGACAGGA   1960
GTCAAAGAAT TTCTGGTGAA CATTGAACCC TGCTCCACTA   2000
GCTATGTGAC ATTTGGAGAT GGCTCTAAAG GAAAGATCAC   2040
TGGAATGGGA AAGCTAGTCC ATGATGGACT TCGTTATGTC   2080
AAGGAATAAG ATCGGGCTGC ACAATGCACA AGGCAAGATA   2120
AAATGTCAAA TGAAGAATTG AAGCTGCAGG ATCCATGATG   2160
TCGGATACAA TGTCCAGGAC ATCCTGCCCG AAAATACTGG   2200
AGTTGCTGCA CAATGCACAA GGCAAGATAA AAGAAGTGAA   2240
GCTGCAGGAT CCACGATGTC GGATACGATG TCCAGGACAT   2280
CTGGCCCGAA AATACTGGAC ACATAAATCT GTTATATCTT   2320
TAACAGATTA TTGTGCAGTT AGCAACAGGT TAGACGATCT   2360
ATCTTTAGGA ACGAACTCTT CTAGTTCCGG AATTCGAGCT   2400
CGGTACCCGG GGATCCT                            2417
```

FIGURE 6 C

FIGURE 7



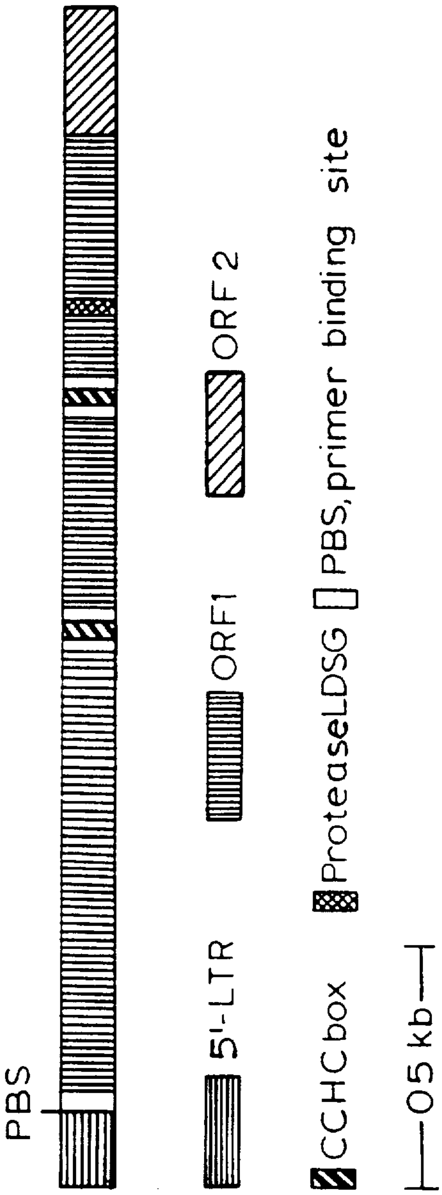

PBS
5'-LTR
ORF1
ORF 2
CCHC box
ProteaseLDSG
PBS, primer binding site
05 kb

FIGURE 8

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SIRE-1a | C | H | G | C | E | G | Y | G | H | I | K | A | E | C |
| SIRE-1b | C | H | Y | C | G | K | Y | G | H | I | K | P | F | C |
| del | C | Y | S | C | G | Q | P | G | H | F | K | A | N | C |
| copia | C | H | H | C | G | R | E | G | H | I | K | K | D | C |
| Tal-2 | C | W | Y | C | K | K | E | G | H | V | K | K | D | C |
| Tnt1 | C | Y | N | C | V | K | P | G | H | F | K | R | D | C |
| HIV-1b | C | W | K | C | G | K | P | G | H | I | M | T | N | C |
| Tst1 | C | D | H | C | K | K | Y | W | H | T | R | E | T | C |
| CaMV | C | W | I | C | N | I | E | G | H | Y | A | N | E | C |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SIRE-1b | C | H | Y | C | G | K | Y | G | H | I | K | P | F | C |
| SIRE-1a | C | H | G | C | E | G | Y | G | H | I | K | A | E | C |
| del | C | Y | S | C | G | Q | P | G | H | F | K | A | N | C |
| copia | C | H | H | C | G | R | E | G | H | I | K | K | D | C |
| Tal-2 | C | W | Y | C | K | K | E | G | H | V | K | K | D | C |
| Tnt1 | C | Y | N | C | V | K | P | G | H | F | K | R | D | C |
| Tst1 | C | D | H | C | K | K | Y | W | H | T | R | E | T | C |
| HIV-1b | C | W | K | C | G | K | P | G | H | I | M | T | N | C |

FIGURE 9

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SIRE-1 | L | D | S | G | C | S | R | H | M | T |
| Tal-2 | L | D | S | G | C | T | S | H | M | S |
| Tnt1 | V | D | T | A | A | S | H | H | A | T |
| copia | L | D | S | G | A | S | D | H | L | T |
| Tst1 | I | D | S | R | A | S | D | H | M | T |
| del | I | D | T | G | S | T | H | S | F | I |
| CaMV | V | D | T | G | A | S | L | C | I | A |
| HIV-1 | L | D | T | G | R | D | D | T | V | L |

FIGURE 10

```
tRNA1-met    3'  -ACCAUAGUCUCGGUCCAAAGCU..
                  ||||||||||||  |   ||||
PBS          180  TGGTATCAGAGCAGGCACTCGA   201
```

FIGURE 11

```
SIRE-1      161 TTG..GTATC.AGAATTTCA   177
                |||  ||||| | | |||||
Tst1        266 TTGCAGTATCTAAACTTTCA   285
```

FIGURE 12

```
GCTCGCGGCC GCGAGCTCTA ATACGACTCA CTATAGGGCG    40
TCGACTCGAT CTTGTTGATG ATAAAGTTAT CACACTGGAG    80
CATGTTGACA CTGAGGAACA AATAGCAGAT ATTTTCACAA   120
AGGCATTGGA TGCAAATCAG TTTGAAAAAC TGAGGGGCAA   160
GCTGGGCATT TGTCTGCTAG AGGATTTATA GCAATTACTT   200
TTATCTGAAC GTGCTTAAAC GTTAATAGCG CGTTCTCTAC   240
TGGGCCAAAA CAAATTCGAC CGTTGCTTCA CACGTCCCTC   280
TACATTCCTC ATTCAAACTC ATATTTTCGT GGTAATCTCG   320
TTTTCAGCAT TCCCCAACAG CTCTCAGAGA TTTACGAAAC   360
CATTCCAAAG GCTCTGCTTC TCCATGGCTA CCTCACCAAA   400
AGATACTTCA TCTCCTGGTT CACCCTCTGT ACCATCATCT   440
CCATCATCCA CCAAAGCACC ATCAAACCAG GAACAACCTG   480
AATTCCATAT CCAACCCATA CAAATGATTC CTGGTCTAGC   520
CCCTGTTCCT GAGAAACTGG TCCCCATAAG ACAACAGGGA   560
GTGAAGATTT CTGAAAACCC TAGCATTGCA ACAAGTCCTA   600
GGGAATTGAC ACGGGAGATG GATAAGAAGA TCCGCAGTAT   640
TGTGAGTAGT ATTCTGAAAA ATGCTTCTGT CCCTGATGCT   680
GATAAAGATG TTCCAACATC TTCCACCCCA AATGCTGAAG   720
TCCTCTCTTC ATCCAGTAAA GAGGAATCAA CAGAGGAAGA   760
GGAACAAGCC ACAGAGGAGA CCCCTGCACC AAGGGCACCA   800
GAACCTGCTC CAGGTGACCT CATTGACCTA GAAGAAGTAG   840
```

FIGURE 13 A

```
AATCTGATGA GGAACCCATT GCCAACAAGT TGGCACCTGG   880
CATTGCAGAA AGATTACAAA GCAGAAAGGG AAAAACCCCC   920
ATTACTAGGT CTGGACGAAT CAAAACTATG GCACAGAAGA   960
AGAGCACACC AATCACTCCT ACCACATCCA GATGGAGCAA  1000
AGTTGCAATC CCTTCCAAGA AGAGGAAAGA ATTTTCCTCA  1040
TCTGATTCTG ATGATGATGT CGAACTAGAT GTTCCCGACA  1080
TCAAGAGGGC CAAGAAATCT GGGAAAAAGG TGCCTGGAAA  1120
TGTCCCTGAT GCACCATTGG ACAACATTTC ATTCCACTCC  1160
ATTGGCAATG TTGAAAGGTG GAAATTTGTA TATCAACGCA  1200
GACTTGCCTT AGAAAGAGAA CTGGGAAGAG ATGCCTTGGA  1240
TTGCAAGGAG ATCATGGACC TCATCAAGGG CTGCTGGACT  1280
GCTGAAAACA GTCACCAAGT TGGGAGATGT TATGAAAGCC  1320
TAGTCAGGGA ATTCATTGTC AACATTCCCT CTGACATAAC  1360
AAACAGAAAG AGTGATGAGT ATCAGAAAGT GTTTGTCAGA  1400
GGAAAATGTG TTAGATTCTC CCCTGCTGTA ATCAACAAAT  1440
ACCTGGGCAG ACCTACTGAA GGAGTGGTGG ATATTGCTGT  1480
TTCTGAGCAT CAAATTGCCA AGGAAATCAC TGCCAAACAA  1520
GTCCAGCATT GGCCAAAGAA AGGGAAGCTT TCTGCAGGGA  1560
AGCTAAGTGT GAAGTATGCA ATCCTGCACA GGATTGGCGC  1600
TGCAAACTGG GTACCCACCA ATCATACTTC CACAGTTGCC  1640
ACAGGTTTGG GTAAATTTCT GTATGCTGTT GGAACCAAGT  1680
```

FIGURE 13 B

```
CCAAATTTAA TTTTGGAAAG TATATTTTTG ATCAAACTGT   1720
TAAGCATTCA GAATCATTTG CTGTCAAATT ACCCATTGCC   1760
TTCCCAACTG TATTGTGTGG CATTATGTTG AGTCAACATC   1800
CCAATATTTT AAACAACATT GACTCTGTGA TGAAGAAAGA   1840
ATCGGCTCTG TCCCTGCATT ACAAACTGTT TGAGGGGACA   1880
CATGTCCCAG ACATTGTCTC GACATCAGGG AAAGCTGCTG   1920
CTTCAGGTGC TGTATCCAAG GGATGCTTTG ATTGCTGAAC   1960
TCAAGGACAC ATGCAAGGTG CTGGAAGCAA CCATCAAAGC   2000
CACCACAGAG AAGAAAATGG AGCTGGAACG CCTGATCAAA   2040
AGACTCTCAG ACAGTGGCAT TGATGATGGT GAAGCAGCTG   2080
AGGAAGAAGA AGAAGCCGCT GAGGAAGAGA AAGATGCAGC   2120
AGAAGATACA GAATCAGATG ATGATGATTC TGATGCCACC   2160
CCATGACCAT CAGACCTTTA TTTTTGCTTT TTACTCTTAC   2200
TAGCTATAGG GCATGTCCCT TTGAACAATT GATTGCTATT   2240
GGTCTGTAAT ATTTGCATGC ATTCTACTTT TGTCAAATTC   2280
TGTCTAAAAA GGGGATATAT ATTATGCATG ATTTTGAGTA   2320
GTAGATACTA TGTTGCAATA GTATATTATG CATAATTTAT   2360
GATTTTGAGT AGTAGGATAC GATGTATGCA TGATTCATGA   2400
TTTTGAGGGG GAGTTGTAAG TATATGATTT TGAGGGGGAG   2240
TAGTATCTGA TGATGCTGAT AGAAGATGGC ATGGAGACAG   2280
GGGGAGCAGA AAGCTGATGT CACGTGAGAT GTCTTGACAT   2520
```

FIGURE 13 C

```
CCTGGAAACG ACTTGCAACT TGCAGAATTT TGCTGTCGCC   2560
CCTACAGATA CCGCTGTGCT TGATTACTCT GATAATGAAA   2600
GTTGCTGATC CCACTTGCAT AACTGCTCGT ACCTGCTCAG   2640
GAAGTGTCTA AGTATGTTTT AGACAAAATT TGCCAAAGGG   2680
GGAGATTGTT AGTGCTTAGC TTTACTGAGT TTTAAAAGAT   2720
TGGCTAAAAT TTTGTTAAAA CATAAGCACT TAGACAATGA   2760
AGGAAAGCTG GAGTTGCTGC ACAGGATGTC CAACGTTATG   2800
TCAAGGAATC AGATTGGGCT CCACAATGCA CAAGGCAAGA   2840
TAAAAGGTCA AATGAAGAAT TGAAGCTGCA GGATCCACGA   2880
TGTCGGATAC AATGTCCAGG ACATCCTGCC CGAAAATACT   2920
GGACACATAA ATCTGTTATA TCTTTAACAG ATTAATGTGC   2960
AGTTAGCAAC AGATTTGGCG ATCTATCTTT AGGAACGAAT   3000
TAAAAGATAA TTAAAGTTCG AATTACAAAC TTGAATAGTT   3040
CGTTCAGGGA TTAAAGATTA AAGATAAAAA CTAAAAGATC   3080
AAACTGTATC TTTTAGATCT TTAAGTGCAG ATTTTTCAGG   3120
AGAATGATAG ATCTTATCCA GCGCAAGATG TTGCAGCCCA   3160
GATACGCACA CTGCTATATA AACATGAAGG CTGCACGAGT   3200
TTTCTACCAA GTCCGGGATT GAAGAGTTAT TTTGTGAGTT   3240
TTGGGACTTG AGTGTTTTGT GAGCCACCTT GATGTTACCC   3260
TAACATCAAG TGTTGGACCT GAGTGTGTAG AGTTGATCTC   3300
TATTGTTCAG AGAGCAATCT CTGGTGTGTC TTTGATTTAT   3360
```

FIGURE 13 D

```
TTGTAAACAC GGGAGAGTGA TTGAGAGGGA GTGAGAGGGG   3400
TTCTCATATC TAAGAGTGGC TCTTAGGTAG AGGTTGCACG   3440
GGTAGTGGTT AGGTGAGAAG GTTGTAAACA GTGGCTGTTA   3480
GATCTTCGAA CTAACACTAT TTTAGTGGAT TTCCTCCCTG   3520
GCTTGGTAGC CCCCAGATGT AGGTGAGGTT GCACCGAACT   3560
GGGTTAACAA TTCTCTTGTG TTATTTACTT GTTTAATCTG   3600
TTCATACTGT CAAATATAAT CTGCATGTTC TGAAGCGTGA   3640
TGTCGTGACA TCCGGTACGA CATCTGTCAT TGGTATCAGA   3680
ATTTCATGCT GCAAATATTT ACAATAGACC TCCTCAACCT   3720
CAACAGCAAA ATCAACCACA GCAGAACAAT TATGACCTCT   3760
CCAGCAACAG ATACAACCCT GGATGGAGGA ATCACCCTAA   3800
CCTCAGATGG TCCAGCCCTC AGCAACAACA ACAGCAGCCT   3840
GCTCCTTCCT TCCAAAATGC TGTTGGCCCA AGCAGACCAT   3880
ACATTCCTCC ACCAATCCAA CAACAGCAAC AACCCCAGAA   3920
ACAGCCAACA GTTGAGGCCC TCCACAACTT CCTTCGAAGA   3960
ACTTGTGAGG CAAATGACTA TGCAGAACAT GCAGTTTCAG   4000
CAAGAGACTA GAGCCTCCAT TCAGAGCTTA ACCAATCAGA   4040
TGGGACAATT GGCTACCCAA TTGAATCAAC AACAGTCCCA   4080
GAATTCTGAC AAGTTGCCTT CTCAAGCTGT CCAAAATCCC   4120
AAAAATGTCA GTGCCATTTC ATTGAGGTCG GGAAAGCAGT   4160
GTCAAGGACC TCAACCCGTA GCACCTTCCT CATCTGCAAA   4200
TGAACCTGCC AAACTTCACT CTAC                    4224
```

FIGURE 13 E

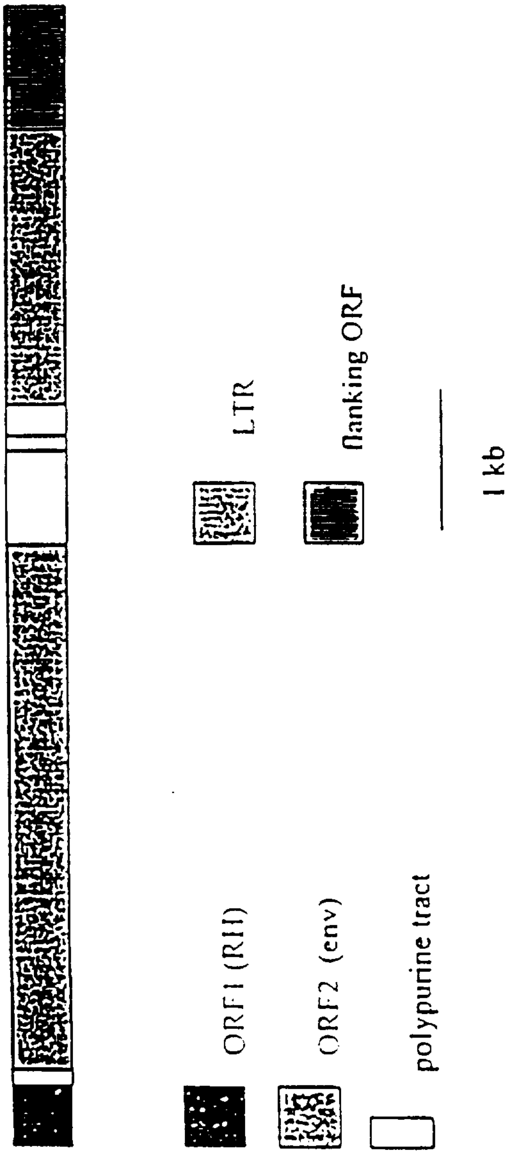


FIGURE 14

S R P R A L I R L T I G R R L D L V D D K V I T L 25

E H V D T E E Q I A D I F T K A L D A N Q F E K L 50

R G K L G I C L L E D L * Q L L L S E R A * T L I 75

A R S L L G Q N K F D R C F T R P S T F L I Q T H 100

I F V V I S F S A F P N S S Q R F T K P F Q R L C 125

F S M A T S P K D T S S P G S P S V P S S P S S T 150

K A P S N Q E Q P E F H I Q P I Q M I P G L A P V 175

P E K L V P I R Q Q G V K I S E N P S I A T S P R 200

E L T R E M D K K I R S I V S S I L K N A S V P D 225

A D K D V P T S S T P N A E V L S S S S K E E S T 250

E E E E Q A T E E T P A P R A P E P A P G D L I D 275

L E E V E S D E E P I A N K L A P G I A E R L Q S 300

R K G K T P I T R S G R I K T M A Q K K S T P I T 325

P T T S R W S K V A I P S K K R K E F S S S D S D 350

FIGURE 15 A

D D V E L D V P D I K R A K K S G K K V P G N V P 375

D A P L D N I S F H S I G N V E R W K F V Y Q R R 400

L A L E R E L G R D A L D C K E I M D L I K G C W 425

T A E N S H Q V G R C Y E S L V R E F I V N I P S 450

D I T N R K S D E Y Q K V F V R G K C V R F S P A 475

V I N K Y L G R P T E G V V D I A V S E H Q I A K 500

E I T A K Q V Q H W P K K G K L S A G K L S V K Y 525

A I L H R I G A A N W V P T N H T S T V A T G L G 550

K F L Y A V G T K S K F N F G K Y I F D Q T V K H 575

S E S F A V K L P I A F P T V L C G I M L S Q H P 600

N I L N N I D S V M K K E S A L S L H Y K L F E G 625

T H V P D I V S T S G K A A A S G A V S K G C F D 650

C * T Q G H M Q G A G S N H Q S H H R K K N G A G 675

T P D Q K T L R Q W H * * W * S S * G R 695

FIGURE 15 B

```
  1 TLIARSLLGQ NKFDRCFTRP STELIQTHIF VVISFSAFPN SSQRFTKPFQ
 51 RLCFSMATSP KDTSSPGSPS VPSSPSSTKA PSNQEQPEFH IQPIQMIPGL
101 APVPEKLVPI RQQGVKISEN PSIATSPREL TREMDKKIRS IVSSILKNAS
151 VPDADKDVPT SSTPNAEVLS SSSKEESTEE EEQATEETPA PRAPEPAPGD
201 LIDLEEVESD EEPIANKLAP GIAERLQSRK GKTPITRSGR IKTMAQKKST
251 PITPTTSRWS KVAIPSKKRK EFSSSDSDDD VELDVPDIKR AKKSGKKVPG
301 NVPDAPLDNI SFHSIGNVER WKFVYQRRLA LERELGRDAL DCKEIMDLIK
351 GCWTAENSHQ VGRCYESLVR EFIVNIPSDI TNRKSDEYQK VFVRGKCVRF
401 SPAVINKYLG RPTEGVVDIA VSEHQIAKEI TAQVQHWPKK GKLSAGKLSV
451 KYAILHRIGA ANWVPTNHTS TVATGLGKFL YAVGTKSKFN FGKYIFDQTV
501 KHSESFAVKL PIAFPPVLCG IMLTQHPNIL NNIDSVMKKE SALSLHYKLF
551 EGTHVPDIVS TSGKAAASGA VSKGCFDC*
```

FIGURE 16

```
ORF1      1 .SRPRAL.IRLTIGRRLDLVDDKVITLEHVDTEEQIADIFTKALDANQFE 48
             .|:: : |:. ::|  : |::.||.||.:.||:|:||||||:|.|..|
copia  1345 HKRAKHIDIKYHFAR..EQVQNNVICLEYIPTENQLADIFTKPLPAARFV 1392

         49 KLRGKLGICLLEDL*
            .||:|||  ||:|
       1393 ELRDKLG..LLQDDQSNAE*
```

FIGURE 17

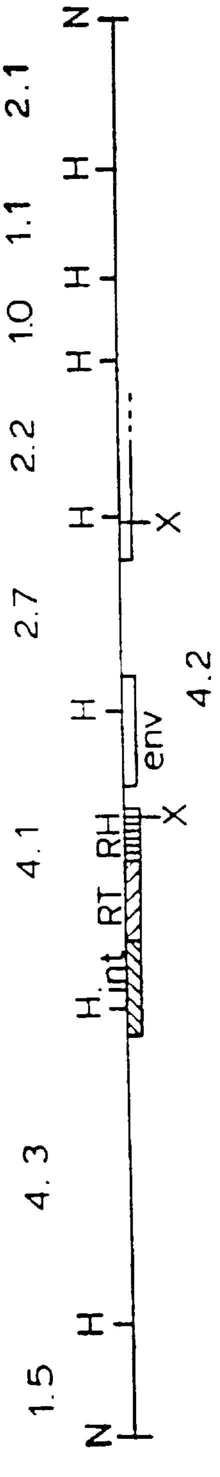


FIGURE 18

# FIGURE 19

| | | |
|---|---|---|
| Sense strand | | |
| F1 (M13/pUC forward) | | 5'-CCCAGTCACGACGTTGTAAAACG-3' |
| F2 (aka pucf2) | 227-246 | 5'-AGCGCGTTCTCTACTGGGCC-3' |
| F3 (aka pucf3a) | 448-467 | 5'-CCACCAAAGCACCATCAAAC-3' |
| F4 (aka pucf4) | 950-969 | 5'-GGCACAGAAGAAGAGCACAC-3' |
| F5 (aka pucf5) | 1242-1261 | 5'-TGCAAGGAGATCATGGACCT-3' |
| F6 (aka pucf6) | 1586-1605 | 5'-CACAGGATTGGCGCTGCAAA-3' |
| F7 (aka AM01) | 3515-3543 | 5'-TCCCTGGCTTGGTAGCCCCCAGATGTAGG-3' |
| F8 (aka 3flankA) | 3936-3956 | 5'-GGCCCTCCACAACTTCCTTCG-3' |
| Complementary strand | | |
| R1 (M13/pUC reverse) | | 5'-AGCGGATAACAATTTCACACAGG-3' |
| R2 (aka 3flankB) | 4196-4177 | 5'-CAGATGAGGAAGGTGCTACG-3' |
| R3 (aka AM02) | 3563-3534 | 5'-CCCAGTTCGGTGCAACCTCACCTACATCTG-3' |
| R4 (aka LTR2a) | 3268-3249 | 5'-GGTGGCTCACAAAACACTCA-3' |
| R5 (aka LTR3) | 2927-2908 | 5'-TGTGTCCAGTATTTTCGGGC-3' |
| R6 (aka LTR4) | 2453-2434 | 5'-TCATCAGATACTACTCCCCC-3' |
| R7 (aka pucfcom3) | 602-581 | 5'-CCTAGGACTTGTTGCAATGCTA-3' |
| R8 (aka pucfcom2) | 292-273 | 5'-ATGAGGAATGTAGAGGGACG-3' |

# FIGURE 20

**Tertiary structure class:** none

**Sequence** ***orf2*****:**

```
TLIARSLLGQNKFDRCFTRPSTFLIQTHIFVVISFSAFPNSEQRFTKPFQRLCFSMATSPKDTSSPGSPSVP
SSPSSTKAPSNQEQPEFHIQPIQMIPGLAPVPEKLVPIRQQGVKISENPSIATSPRELTREMDKKIRSIVES
ILKNAEVPDADKDVPTSSTPNAEVLSSSSKEESTEEEEQATEETPAPRAPEPAPGDLIDLEEVESDEEPIAN
KLAPGIAERLQSRKGKTPITRSGRIKTMAQKKSTPITPTTSRWSKVAIPSKKRKEFSSSDSDDDVELDVPDI
KRAKKSGKKVPGNVPDAPLDNISFHSIGNVERWKFVYQRRLALERELGRDALDCKEIMDLIKGCWTAENSHQ
VGRCYESLVREFIVNIPSDITNRKSDEYQKVFVRGKCVRFSPAVINKYLGRPTEGVVDIAVSEHQIAKEITA
KQVQHWPKKGKLSAGKLSVKYAILHRIGAANWVPTNHTSTVATGLGKFLYAVGTKSKFNFGKYIFDQTVKHS
ESFAVKLPIAFPTVLCGIMLSQHPNILNNIDSVMKKESALSLHYKLFEGTHVPDIVSTSGKAAASGAVSKGC
FDCTQGHMQGAGSNHQSHHRKKNGAGTPDQKTLRQWH
```

**Secondary structure prediction** ***(H = helix, E = strand, - = no prediction):***

```
--HHHHH----------------EEEEEEEEEEFE---------------HHHHE-------------------
----------------------------------------------------------HHHHHHHHHHHHEEHHH
HH---------------------EEE---------HHHH-------------------------------HHH-
-----HHHHHH-------------HHHH-----------------E---------E---------------HH
HHHH--------------------E--------HHHHHHHH-HHHHHH-------HHHHHHH------H----
---HHHHHHHHHEEE-------------HHHEEE-----------H---------EEEEHHHHHHHHHHHHH
HHH-H--------H--HHEHHHEEEHH------------EEHHHHHHHHHEEHH----------EEE-HH----
HHHHHE--------EE--E-------E--HHHHHHHHHHHHHHHHH-------EEE-----HEH--------
-------------------------------HHHH---
```

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```
GagR2  2202/2205                  ....GTTGCTGCACAATGCACAAGGCAAGATAA
                                          |||||||||||||||||||||||||
GagR1  2067 GACTTCGTTATGTCAAGGAATAAGATCGGGCTGCACAATGCACAAGGCAAGATAA
              |  |||||||||||||||| |||| ||||| ||||||||||||||||||||||
LTR2   2789 TCCAACGTTATGTCAAGGAATCAGATTGGGCTCCACAATGCACAAGGCAAGATAA

GagR2  2231 ...........AAGAAGTGAAGCTGCAGGATCCACGATGTCGGATACGATGTCCA
                       ||||| |||||||||||||||||||||||||||||| |||||||
GagR1  2122 AATGTCAAATGAAGAATTGAAGCTGCAGGATCCATGATGTCGGATACAATGTCCA
            || ||||||||||||||||||||||||||||||||||||||||||||||||||||
LTR2   2844 AAGGTCAAATGAAGAATTGAAGCTGCAGGATCCACGATGTCGGATACAATGTCCA

GagR2  2275 AGACATCTGGCCCGAAAATACTGGA  GTT....2204   GagR1
             |||||| ||||||||||||||||| X
GagR1  2177 GGACATCCTGCCCGAAAATACTGGA  CACATAAATCTGTTATATCTTTAACAGAT
            |||||||||||||||||||||||||  |||||||||||||||||||||||||||||
LTR2   2899 GGACATCCTGCCCGAAAATACTGGA--CACATAAATCTGTTATATCTTTAACAGAT

GagR2  2329 TATTGTGCAGTTAGCAACAGGTTAGACGATCTATCTTTAGGAACGAACTCTTCTAG
            || ||||||||||||||||| || | ||||||||||||||||||||| |     |
LTR2   2953 TAATGTGCAGTTAGCAACAGATTTGGCGATCTATCTTTAGGAACGAATTAAAAGAT
```

FIGURE 21

```
 776 ...........TATTGGATCGGGTGCAGTGCTGAAGGAGAAGAAGATGAG 738
                 | ||| || || || |||||||||||||||||||
 900 AGAGCAAGAATCTGGCGTTCGTGTCCAATGATGAAGGAGAAGAAGATGAG 949
              .         .         .         .         .
 737 TATGACCTGGATACTGATGAAGGTCTGACTAACGCAGTTGTGCTCCTTGG 688
     |||||||||||||| ||||||||||||||||| |||||||||||||||||
 950 TATGACCTGGATACAGATGAAGGTCTGACTAATGCAGTTGTGCTCCTTGG 999
              .         .         .         .         .
 687 AAAACAGTTCAACAAAGTGCTGAACAGAATGGACAGGAGGCAGAAACCAC 638
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1000 AAAACAGTTCAACAAAGTGCTGAACAGAATGGACAGGAGGCAGAAACCAC 104
              .         .         .         .         .
 637 ATGTCCGGAACATCCCTTTCGACATCAGGAAAGGTAGTGAATACCAGAAA 588
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1050 ATGTCCGGAACATCCCTTTCGACATCAGGAAAGGTAGTGAATACCAGAAA 109
              .         .         .         .         .
 587 AGGTCAGATGAAAAGCCCAGTCACAGCAAAGGAATTCAATGCCATGGGTG 538
     ||||||||||||||||||||||||||||||||| ||||||||||||||||
1100 AGGTCAGATGAAAAGCCCAGTCACAGCAAAGGATTTCAATGCCATGGGTG 114
              .         .         .         .         .
 537 TGAAGGCTATGGACACATCAAAGCTGAATGTCCCACTCATCTCAAGAAGC 488
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1150 TGAAGGCTATGGACACATCAAAGCTGAATGTCCCACTCATCTCAAGAAGC 119
```

FIGURE 22 A

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```
              .         .         .         .         .
 487 AGAGGAAAGGACTTTCTGTATGTCGGTCTGATGATACAGAGAGTGAACAA 438
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1200 AGAGGAAAGGACTTTCTGTATGTCGGTCTGATGATACAGAGAGTGAACAA 124
              .         .         .         .         .
 437 G.AAGTGATTCTGACAGAGATGTGAATGCACTCACTGGGAGATTTGAATC 389
     | ||||||||||||||||||||||||||||||||||||||||||||||||
1250 GAAAGTGATTCTGACAGAGATGTGAATGCACTCACTGGGAGATTTGAATC 129
              .         .         .         .         .
 388 TGCTGAAGATTCAAGTGATACAGATAGTGAAATCACTTTTGATGAGCTTG 339
     |||||||||||||||||||||||| |||||||||||||||||||||||||
1300 TGCTGAAGATTCAAGTGATACAGACAGTGAAATCACTTTTGATGAGCTTG 134
              .         .         .         .         .
 338 CTATATCCTATAGAGAACTATGCATCAAAAGTGAGAAGATTCTTCAGCAA 289
     ||| ||||||||||||||||||||||||||||||||||||||||||||||
1350 CTACATCCTATAGAGAACTATGCATCAAAAGTGAGAAGATTCTTCAGCAA 139
              .         .         .         .         .
 288 GAAGCACAACTAAAGAAGGTCATTGCAAATCTGGAGGCTGAGAAGGAGGC 239
     ||||||||||| ||||||||||||||||||||||||||||||||||||||
1400 GAAGCACAACTGAAGAAGGTCATTGCAAATCTGGAGGCTGAGAAGGAGGC 144
```

FIGURE 22 B

```
                   .         .         .         .         .
 238 ACATGAAGAGGAAATCTCTGAACTTAAAGGAGAAATTGGTTTTCTGAACT 189
     |||||||||||| |||||||| |||||||||||| |||||||||||||||
1450 ACATGAAGAGGAGATCTCTGAGCTTAAAGGAGAAGTTGGTTTTCTGAACT 1499
                   .         .         .         .         .
 188 CTAAACTGGAAAACATGACAAAATCAATAAAGATGCTGAATAAAGGCTCA 139
     ||||||||||||||||||||||||||||||||||||||||||||||||||
1500 CTAAACTGGAAAACATGACAAAATCAATAAAGATGCTGAATAAAGGCTCA 1549
                   .         .         .         .         .
 138 GATTTGCTTGATGAGGTGCTACAGCTTGGGAAGAATGTTGGAAACCAGAG 89
     ||| ||||||||||||||||||||||||||||||||||||||||||||||
1550 GATATGCTTGATGAGGTGCTACAGCTTGGGAAGAATGTTGGAAACCAGAG 1599
                   .         .         .         .         .
  88 AGGACTTGGATTTAATCATAAATCTGCTGGCAGAACAACCATGACATAAT 39
     ||||||||| ||||||||||||||||||||||||| |||||||||| |||
1600 AGGACTTGGGTTTAATCATAAATCTGCTGGCAGAATAACCATGACAGAAT 1649
                   .         .         .         .         .
  38 TTGTTCCTGCCAAAAACAGCACTGCACCCGATCCAATA............ 1
     ||||||||||||||| |||||||| | ||        |
1650 TTGTTCCTGCCAAAATCAGCACTGGAGCCACGATGTCACAACATCGGTCT 1699
```

FIGURE 22 C

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```
  1  TLIARSLLGQNKFDRCFTRPSTFLIQTHIFVVISFSAFPNSSQRFTKPFQ
 51  RLCFSMATSPKDTSSPGSPSVPSSPSSTKAPSNQEQPEFHIQPIQMIPGQ
101  APVPEKLVPKRQQGVKISENPSIATSPRVDTEMDKKIRSIVSSILKNASV
151  PDADKDVPTSSTPNAEVLSSSSKEESTEEEEQATEETPAPRAPEPAPGDL
201  IDLEEVESDEEPIANKLAPGIAERLQSRKGKTPITRSGRIKTMAQKKSTP
251  ITPTTSRWSKVAIPSKKRKEFSSSDSDDDVELDVPDIKRAKKSGKKVPGN
301  VPDAPLDNISFHSIGNVERWKFVYQRRLALERELGRDALDCKEIMDLIKA
351  AGLLKTVTKLGDCYESLVREFIVNIPSDITNRKSDEYQKVFVRGKCVRFS
401  PAVINKYLGRPTEGVVDIAVSEHQIAKEITAKQVQHWPKKGKLSAGKLSV
451  KYAILHRIGAANWVPTNHTSTVATGLGKFLYAVGTKSKFNFGKYIFDQTV
501  KHSESFAVKLPIAFPTVLCGIMLSQHPNILNNIDSVMKRESALSLHYKLF
551  EGTHVPDIVSTSGKAAASGAVSKDALIAELKDTCKVLEATIKATTEKKME
601  LERLIKRLSDSGIDDGEAAEEEEEAAEEEKDAAEDTESDDDDSDATP*
```

FIGURE 23

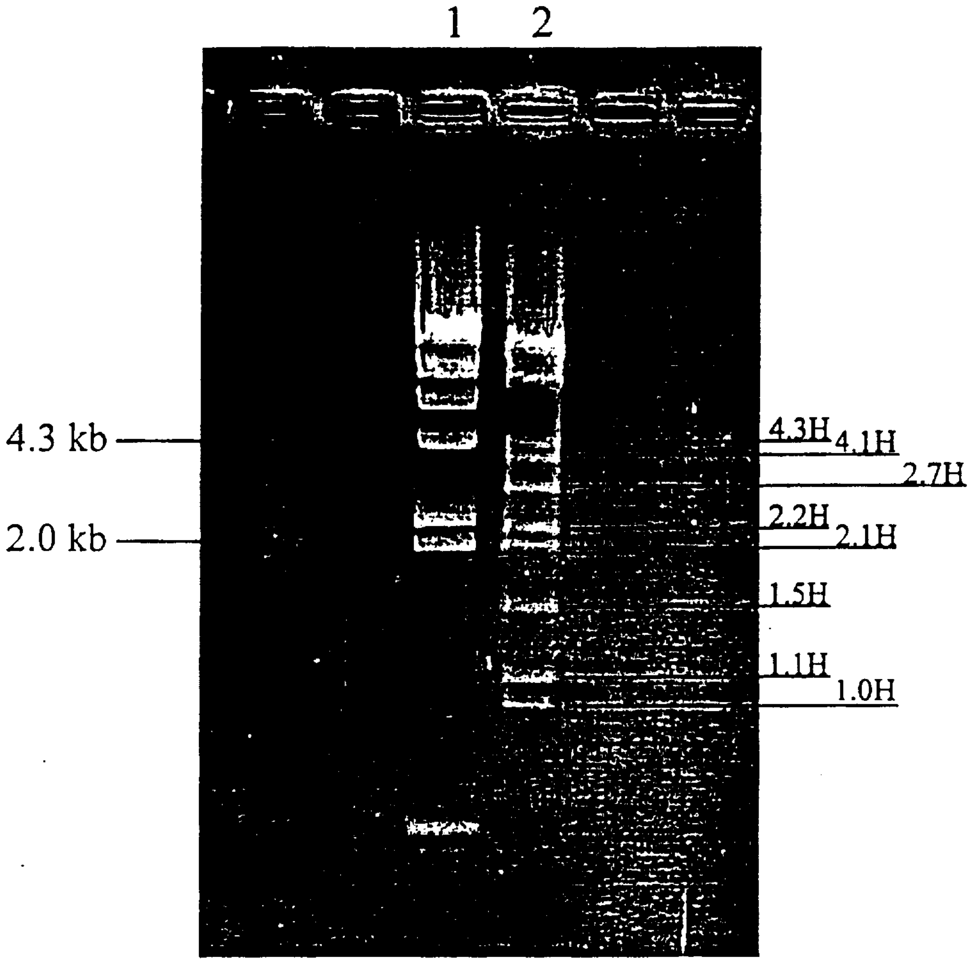

FIGURE 24

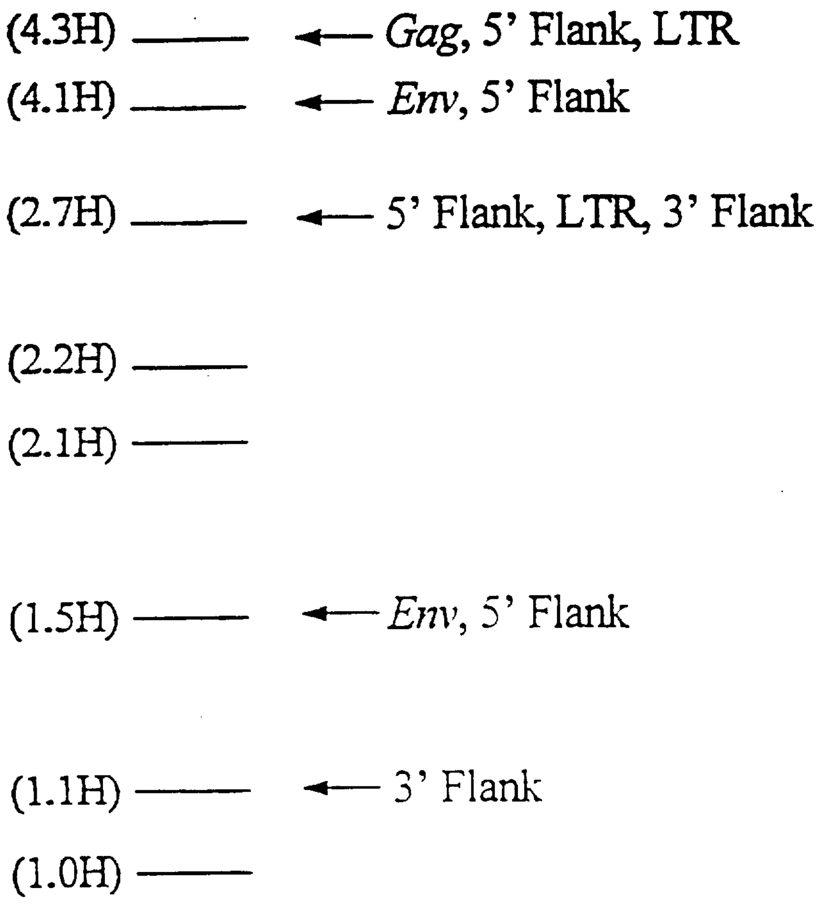
(4.3H)
← *Gag*, 5' Flank, LTR
(4.1H)
← *Env*, 5' Flank
(2.7H)
← 5' Flank, LTR, 3' Flank
(2.2H)
(2.1H)
(1.5H)
← *Env*, 5' Flank
(1.1H)
← 3' Flank
(1.0H)

FIGURE 25

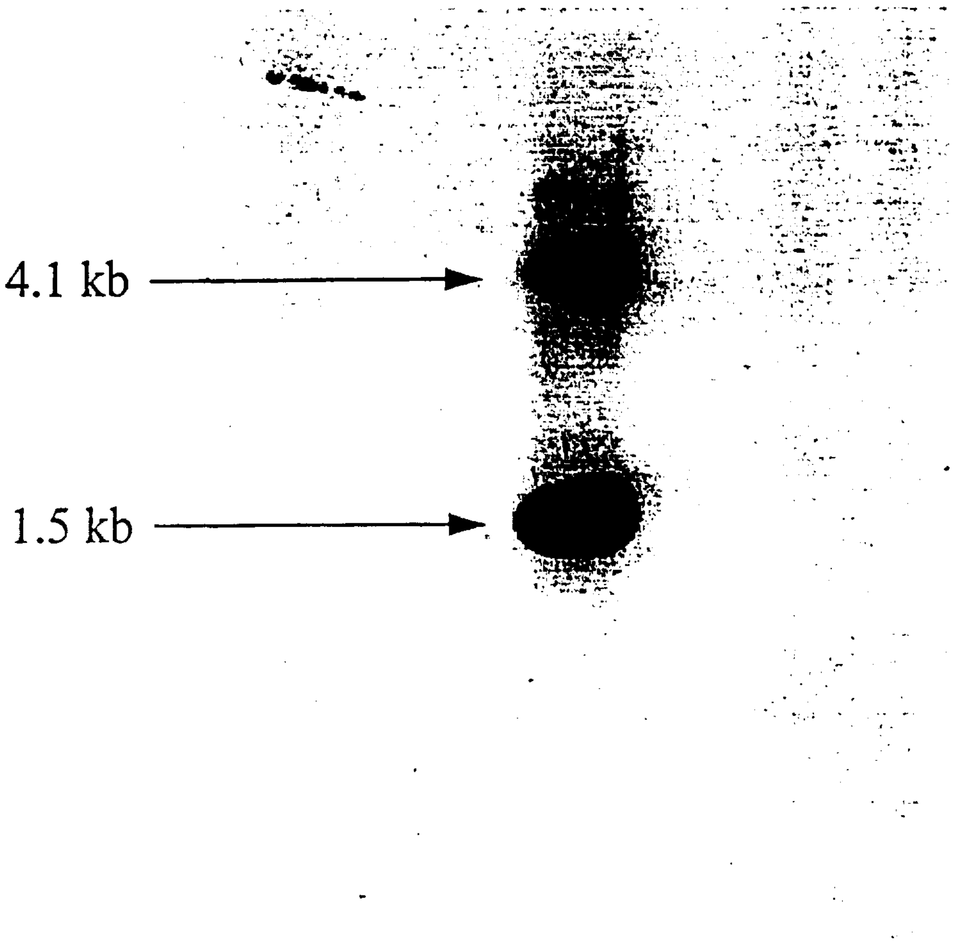

FIGURE 26

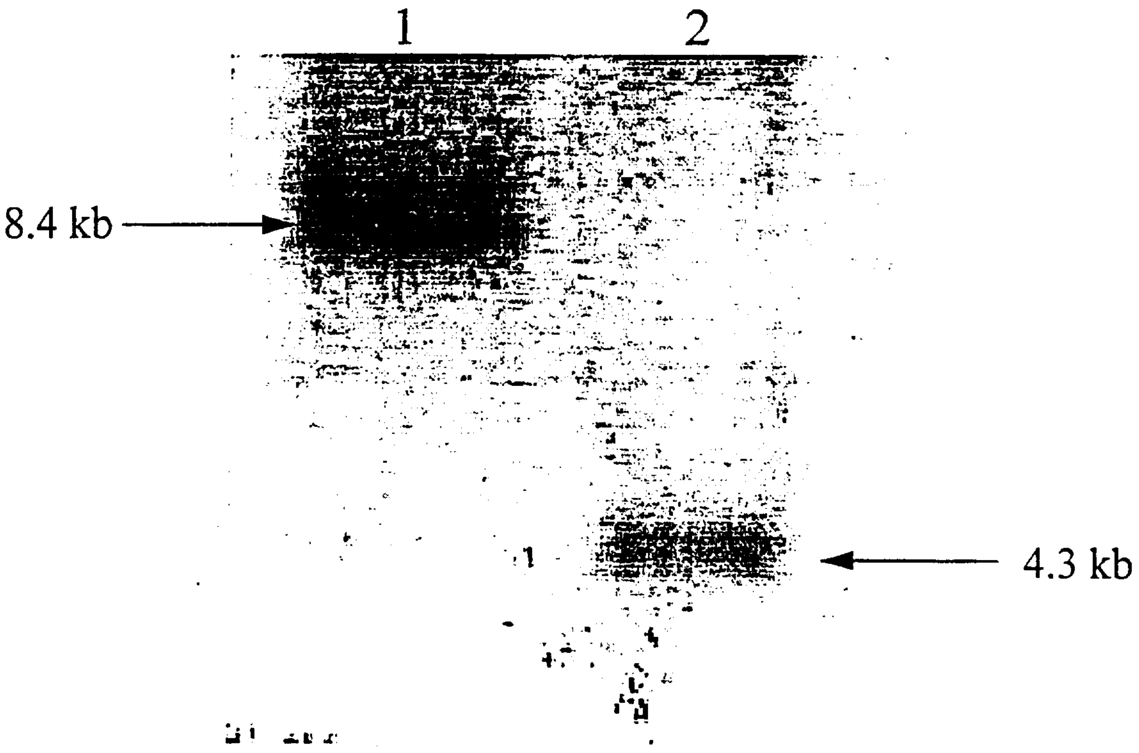

FIGURE 27

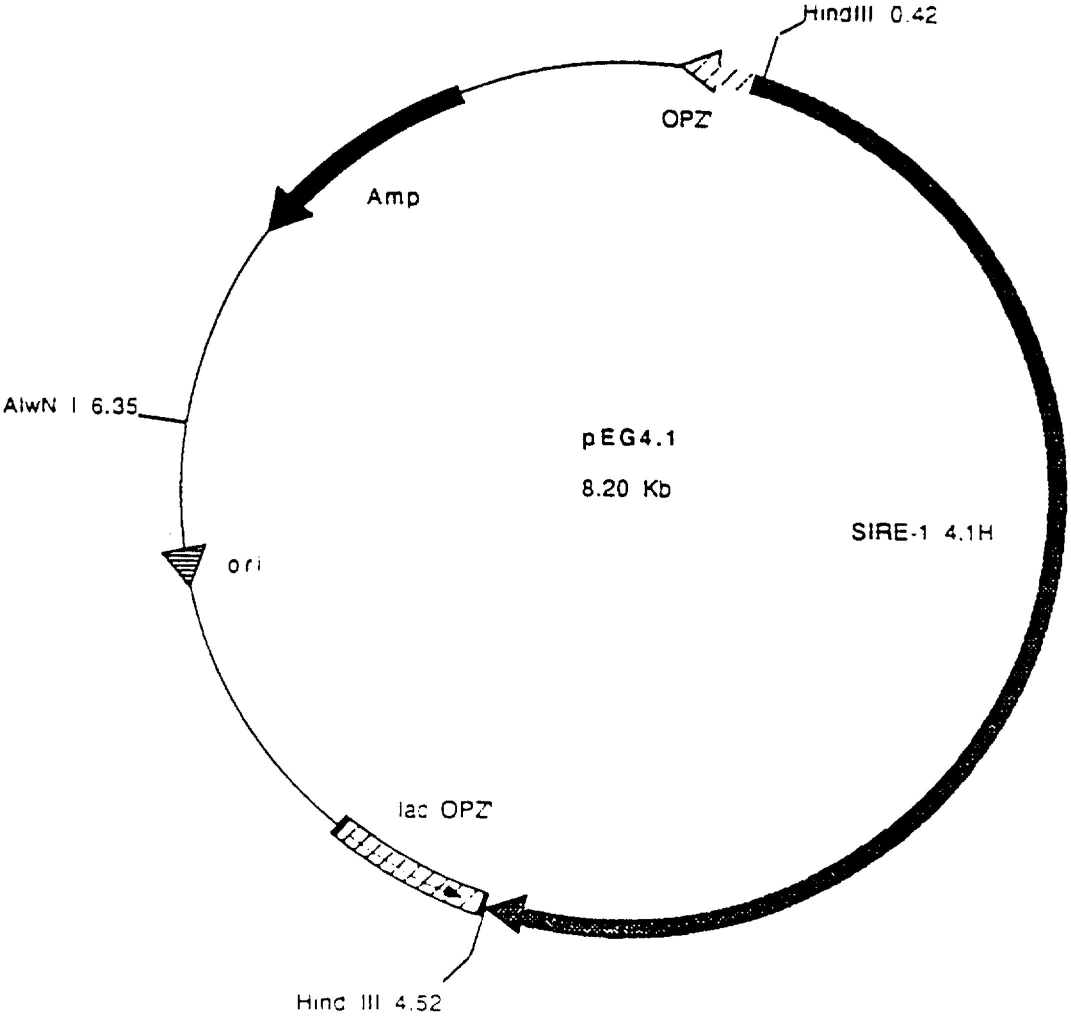
HindIII 0.42
OPZ'
Amp
AlwN I 6.35
pEG4.1
8.20 Kb
SIRE-1 4.1H
ori
lac OPZ'
Hinc III 4.52

FIGURE 28

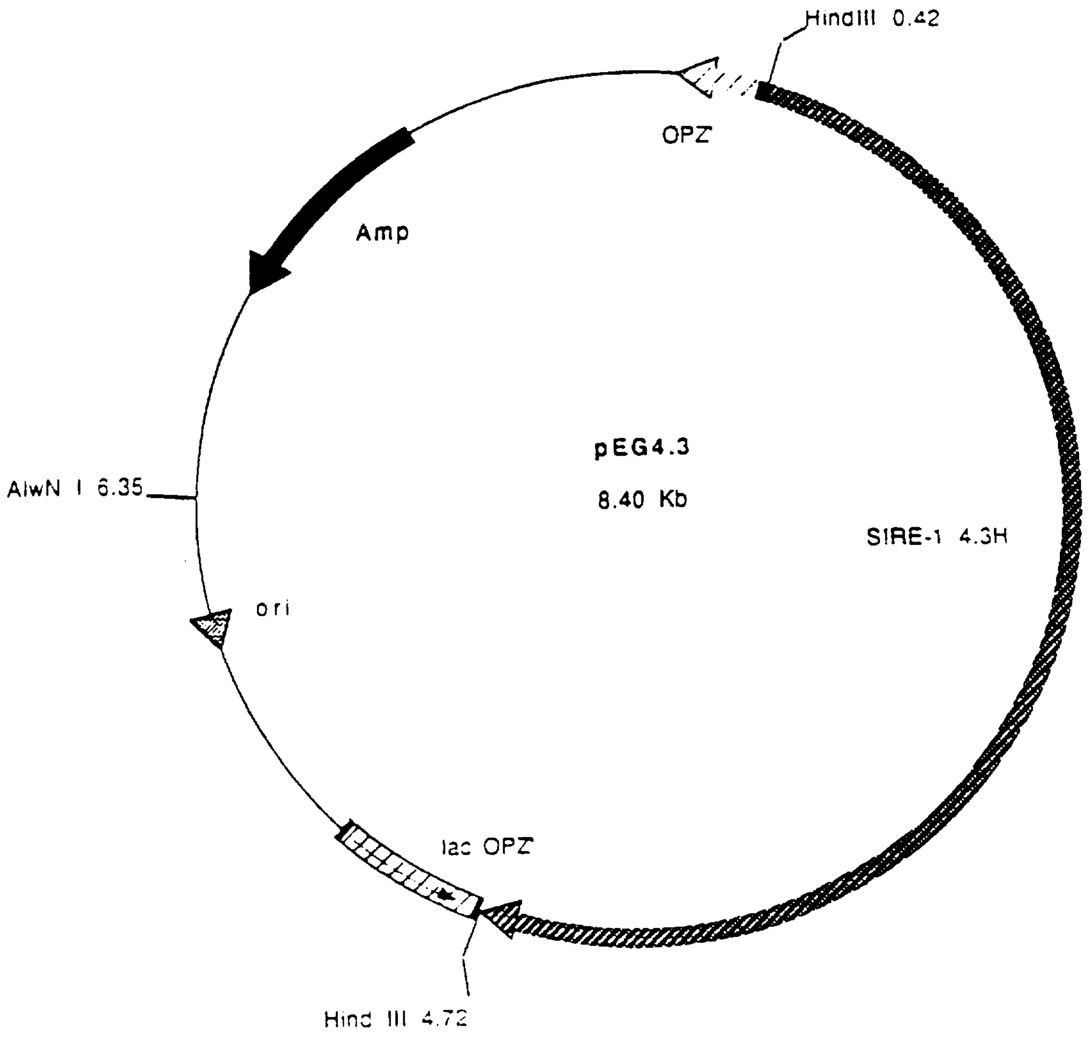
HindIII 0.42
OPZ
Amp
pEG4.3
8.40 Kb
AlwN I 6.35
SIRE-1 4.3H
ori
lac OPZ
Hind III 4.72

FIGURE 29

| | | |
|---|---|---|
| A. | PUC Forward | 5'-CCC AGT CAC GAC GTT GTA AAA CG-3' |
| B. | PUC Reverse | 5'-AGC GGA TAA CAA TTT CAC ACA GG-3' |
| C. | EG4.1H-620 | 5'-CTC ATG AGT TCT CTG CAG CC-3' |
| D. | EG4.1H-1081 | 5'-GAC AAT GTT GCA GAT ACA GCT AAA AGT GC-3' |
| E. | EG4.1H-1617 | 5'-CCA GAT GGA TGT GAA GAG CG-3' |
| F. | EG4.1H-2070 | 5'-TGG GAT GGA AAA TGC CAG C-3' |
| G. | EG4.1H-2468 | 5'-AGA ACT GTG TGT CCC TAT CC-3' |
| H. | EG4.1H-2734c | 5'-CCT CAG TGT CAA CAT GCT CC-3' |
| I. | EG4.1H-2327c | 5'-ATC CCA TAG TCA CTG GTG CC-3' |
| J. | EG4.1H-1788c | 5'-CTC TGT TAG CCT TTC ATA CC-3' |
| K. | EG4.1H-1253c | 5'-CTT GAT CTT GTA GTG ACT CC-3' |
| L. | EG4.1H-816c | 5'-ATA CAG TGT GGT TGG AGT CC-3' |
| M. | EG4.1H-520c | 5'-GAA GTC TTA GAC TCA ACT CC-3' |

FIGURE 30

```
  1  GATGAAGGAT TCAATGTAGA CTTCACAGAG TCAGAATGCT TGATGACAAA
 51  AGAGAAGAGA GAAGTCCTAA TGAAGGGCGG CAGATCAAAG GACAACTGTT
101  ACCTGTGGAC ACCTCAAGAA ACCAGTTACT CCTCCACATG TCTATTCTCC
151  AAAGAAGATG AAGTCAAAAT ATGGCATCAA AGATTTGGAC ATCTGCACTT
201  AGGAGGCATG AAGAAAATCA TTGACAAAGG TGCTGTTAGA GGCATTCCCA
251  ATCTGAAAAT AGAAGAAGGC AGAATCTGTG GTGAATGTCA GATTGGAAAG
301  CAAGTCAAGA TGTCCAACCA GAAGCTTCAA CATCAGACCA CTTCCAGGGT
351  GCTGGAACTA CTTCACATGG ACTTGATGGG GCCTATGCAA GTTGAAAGCC
401  TTGGAAGAAA AAGGTATGCC TATGTTGTTG TGGATGATTT CTCCAGATTT
451  ACCTGGGTCA ACTTTATCAG AGAGAAATCA GACACCTTTG AAGTATTCAA
501  GGAGTTGAGT CTAAGACTTC AAAGAGAAAA AGACTGTGTC ATCAAGAGAA
551  TCAGGAGTGA CCATGGCAGA GAGTTTGAAA ACAGCAAGTT TACTGAATTC
601  TGCACATCTG AAGGCATCAC TCATGAGTTC TCTGCAGCCA TTACACCACA
651  ACAAAATGGC ATAGTTGAAA GGAAAAACAG GACCTTGCCA GAAGCTGCTA
701  GGGTCATGCT TCATGCCAAA GAACTTCCCT ATAATCTCTG GGCTGAAGCC
751  ATGAACACAG CATGCTACAT CCACAACAGA GTCACACTTA GAAGAGGGAC
801  TCCAACCACA CTGTATGAAA TCTGGAAAGG GAGGAAGCCA ACTGTCAAGC
851  ACTTCCACAT CTGTGGAAGT CCATGTTACA TTTTGGCAGA TAGAGAGCAA
901  AGGAGAAAGA TGGATCCCAA GAGTGATGCA GGGATATTCT TGGGATACTC
```

FIGURE 31 A

```
 951  TACAAACAGC AGAGCATATA GAGTATTCAA TTCCAGAACC AGAACTGTGA
1001  TGGAATCCAT CAATGTGGTT GTTGATGATC TAACTCCAGC AAGAAAGAAG
1051  GATGTCGAAG AAGATGTCAG AACATCGGGA GACAATGTTG CAGATACAGC
1101  TAAAAGTGCA GAAAATGCAG AAAACTCTGA TTCTGCTACA GATGAACCAA
1151  ACATCAATCA ACCTGACAAG AGACCCTCCA TTAGAATCCA GAAGATGCAC
1201  CCCAAGGAGC TGATTATAGG AGATCCAAAC AGAGGAGTCA CTACAAGATC
1251  AAGGGAGATT GAGATTATCT CCAATTCATG TTTTGTCTCC AAAATTGAGC
1301  CCAAGAATGT GAAAGAGGCA CTGACTGATG AGTTCTGGAT CAATGCTATG
1351  CAAGAAGAAT TGGAGCAATT CAAAAGGAAT GAAGTTTGGG AGCTAGTTCC
1401  TAGGCCCGAG GGAACTAATG TGATTGGCAC CAAGTGGATC TTCAAGAACA
1451  AAACCAATGA AGAAGGTGTT ATAACCAGAA ACAAGGCCAG ACTTGTTGCT
1501  CAAGGCTACA CTCAGATTGA AGGTGTAGAC TTTGATGAAA CTTTTGCCCC
1551  TGGTGCTAAA CTTGAGTCCA TCAGACTGTT ACTTGGTGTA GCTTGCATCC
1601  TCAAATTCAA GCTGTACCAG ATGGATGTGA AGAGCGCATT TCTGAATGGA
1651  TACCTGAATG AAGAAGCCTA TGTGGAGCAG CCAAAGGGAT TTGTAGATCC
1701  AACTCATCCA GATCATGTAT ACAGGCTCAA GAAGCTCTGC TATGGATTGA
1751  AGCAAGCTTC AAGAGCTTGG TATGAAAGGC TAACAGAGTT CCTTACTCAG
1801  CAAGGGTATA GGAAGGGGGG GATTGACAAG ACCCTTTTTG TTAAACAAGA
1851  TGCTGGAAAA TTGATGATAG CACAGATATA TGTTGATGAC ATTGTGTTTG
1901  GAGGGATGTT GAATGAGATG CTTCGACATT TTGTCCAACA GATGCAATTT
1951  GAATTTGAGA TGAGTTTTGT TGGAGAGCTG AATTATTTTT TGGGAATCCA
2001  AGTGAAGCAG ATGGAAGAAT CCATATTCCT TTCACAAAGC AAGTATGCAA
2051  AGAACATTGT CAAGAAGTTT GGGATGGAAA ATGCCAGCCA TAAAAGAACA
2101  CCTGCACCTA ATCAATTGAA GCTGTCAAAA GATGAAGCTG GCACCAGTGT
2151  TGATCAAAGT TTGTACAGAA GCATGATTGG GAGCTTAATA TATTTAACAG
2201  CTAGCAGACC TGACATCACC TATGCAGTAG GTGGTTGTGC AAGATATCAA
2251  GCCAATCCTA AGATAAGTCA CTTGAATCAA GTAAAGAGAA TTTTGAAATA
```

FIGURE 31 B

```
2301  TGTAAATGGC ACCAGTGACT ATGGGATTAT GTACTGTCAT TGTTCAGATT
2351  CAATGCTGGT TGGGTATTGT GATGCTGATT GGGCTGGAAG TGTAGATGAC
2401  AGAAAAAGCA CTTTTGGTGG ATGTTTTTAT TTGGGAACCA ATTTTATTTC
2451  ATGGTTCAGC AAGAAGCAGA ACTGTGTGTC CCTATCCACT GCAGAAGCAG
2501  AGTATATTGC AGCAGGAAGC AGCTGTTCAC AACTAGTTTG GATGAAGCAG
2551  ATGCTCAAGG AGTACAATGT CGAACAAGAT GTCATGACAT TGTACTGTGA
2601  CAACTTGAGT GCTATTAATA TTTCTAAAAA TCCTGTTCAA CACAGCAGAA
2651  CCAAGCACAT TGACATTAGA CATCACTATA TTAGAGATCT TGTTGATGAT
2701  AAAGTTATCA CACTGGAGCA TGTTGACACT GAGGAACAAA TAGCAGATAT
2751  TTTCACAAAG GCATTGGATG CAAATCAGTT TGAAAAACTG AGGGGCAAGC
2801  TGGGCATTTG TCTGCTAGAG GATTTA
```

FIGURE 31 C

```
  1  DEGFNVDFTE SECLMTKEKR EVLMKGGRSK DNCYLWTPQE TSYSSTCLFS
 51  KEDEVKIWHQ RFGHLHLGGM KKIIDKGAVR GIPNLKIEEG RICGECQIGK
101  QVKMSNQKLQ HQTTSRVLEL LHMDLMGPMQ VESLGRKRYA YVVVDDFSRF
151  TWVNFIREKS DTFEVFKELS LRLQREKDCV IKRIRSDHGR EFENSKFTEF
201  CTSEGITHEF SAAITPQQNG IVERKNRTLP EAARVMLHAK ELPYNLWAEA
251  MNTACYIHNR VTLRRGTPTT LYEIWKGRKP TVKHFHICGS PCYILADREQ
301  RRKMDPKSDA GIFLGYSTNS RAYRVFNSRT RTVMESINVV VDDLTPARKK
351  DVEEDVRTSG DNVADTAKSA ENAENSDSAT DEPNINQPDK RPSIRIQKMH
401  PKELIIGDPN RGVTTRSREI EIISNSCFVS KIEPKNVKEA LTDEFWINAM
451  QEELEQFKRN EVWELVPRPE GTNVIGTKWI FKNKTNEEGV ITRNKARLVA
501  QGYTQIEGVD FDETFAPGAK LESIRLLLGV ACILKFKLYQ MDVKSAFLNG
551  YLNEEAYVEQ PKGFVDPTHP DHVYRLKKLC YGLKQASRAW YERLTEFLTQ
601  QGYRKGGIDK TLFVKQDAGK LMIAQIYVDD IVFGGMLNEM LRHFVQQMQF
651  EFEMSFVGEL NYFLGIQVKQ MEESIFLSQS KYAKNIVKKF GMENASHKRT
701  PAPNQLKLSK DEAGTSVDQS LYRSMIGSLI YLTASRPDIT YAVGGCARYQ
751  ANPKISHLNQ VKRILKYVNG TSDYGIMYCH CSDSMLVGYC DADWAGSVDD
801  RKSTFGGCFY LGTNFISWFS KKQNCVSLST AEAEYIAAGS SCSQLVWMKQ
851  MLKEYNVEQD VMTLYCDNLS AINISKNPVQ HSRTKHIDIR HHYIRDLVDD
901  KVITLEHVDT EEQIADIFTK ALDANQFEKL RGKLGICLLE DL
```

FIGURE 32

```
SIRE-1   1 ...................................DEGFNVDFTESE.CL 14
                                              : |:|  ||: : ::
Opie-2  86 ...................................NMGYNCLFTNIDVSV 100

SIRE-1  15 MTKEKREVLMKGGRSKDNCYLWTPQETSYSSTCLFSKEDEVKIWHQRFGH 64
           : :    : :||      : :  : |      ||: |     :|| |::|
Opie-2 101 FRRCDGSLAFKGVLDGKLYLVDFAKEEAGLDACLIAKTSMGWLWHRRLAH 150

SIRE-1  65 LHLGGMKKIIDKGAVRGIPNLKIEEGRICGECQIGKQVKMSNQKLQHQTT 114
           : :  : |::  : | |: |: :| :| |: || ||||  |::     ||
Opie-2 151 VGMKNLHKLLKGEHVIGLTNVQFEKDRPCAACQAGKQVGGSHHTKNVMTT 200

SIRE-1 115 SRVLELLHMDLMGPMQVESLGRKRYAYVVVDDFSRFTWVNFIREKSDTFE 164
           || ||:|||||:||:   |:|  :|: |:|||||||||| |: |||:| :
Opie-2 201 SRPLEMLHMDLFGPVAYLSIGGSKYGLVIVDDFSRFTWVFFLQEKSETQG 250

SIRE-1 165 VFKELSLRLQREKDCVIKRIRSDHGREFENSKFTEFCTSEGITHEFSAAI 214
            :| :  | | | :  :|:||||:| || |    ||   ||| |||||:
Opie-2 251 TLKRFLRRAQNEFELKVKKIRSDNGSEFKNLQVEEFLEEEGIKHEFSAPY 300

SIRE-1 215 TPQQNGIVERKNRTLPEAARVMLHAKELPYNLWAEAMNTACYIHNRVTLR 264
           ||||||:|||||||| : || ||     |  :| ||:||||   ||| |:
Opie-2 301 TPQQNGVVERKNRTLIDMARTMLGEFKTPECFWTEAVNTACHAINRVYLH 350

SIRE-1 265 RGTPTTLYEIWKGRKPTVKHFHICGSPCYILADREQRRKMDPKSDAGIFL 314
           |    | ||:: | || |  |:: || ||||  ::   |: ||   |::|
Opie-2 351 RILKNTSYELLTGNKPNVSYFRVFGSKCYILVKKGRNSKFAPKAVEGFLL 400

SIRE-1 315 GYSTNSRAYRVFNSRTRTVMESINVVVDDLTPARKKDV.......EEDVR 357
           ||  | :||||||     |  | :|| |:     : :|       |||:
Opie-2 401 GYDSNTKAYRVFNKSSGLVEVSGDVVFDETNGSPREQVVDCDDVDEEDIP 450

SIRE-1 358 TS.................................GDNVADTAKSAENAE 374
           |                                  :: | :   : : :
Opie-2 451 TAAIRTMAIGEVRPQEQDEREQPSPSTMVHPPTQDDEQVHQQEVCDQGGA 500

SIRE-1 375 NSDSATDEPNINQPDKRPSIRIQKMH........................400
             |   :|     |       ||: |
Opie-2 501 QDDHVLEEEAQPAPPTQVRAMIQRDH........................ 526
```

FIGURE 33

```
SIRE-1    1 ..........................PKELIIGDPNRGVTTRSREIEIIS 24
            | : |:||  :||||||| :::
Opie-2  527 ..........................PVDQILGDISKGVTTRSRLVNFCE 550

SIRE-1   25 NSCFVSKIEPKNVKEALTDEFWINAMQEELEQFKRNEVWELVPRPEGTNV 74
            : :||| |||  | ||| |  |: ||||||: ||||||| |||||   ||
Opie-2  551 HNSFVSSIEPFRVEEALLDPDWVLAMQEELNNFKRNEVWTLVPRPKQ.NV 599

SIRE-1   75 IGTKWIFKNKTNEEGVITRNKARLVAQGYTQIEGVDFDETFAPGAKLESI 124
            :||||:|:|| :| ||:||||||||| || |: |:||:||||| |:||||
Opie-2  600 VGTKWVFRNKQDERGVVTRNKARLVAKGYAQVAGLDFEETFAPVARLESI 649

SIRE-1  125 RLLLGVACILKFKLYQMDVKSAFLNGYLNEEAYVEQPKGFVDPTHPDHVY 174
            |:||: |    |:||||||||||||| : || ||||| || |   ||||:
Opie-2  650 RILLAYAAHHSFRLYQMDVKSAFLNGPIKEEVYVEQPPGFEDERYPDHVC 699

SIRE-1  175 RLKKLCYGLKQASRAWYERLTEFLTQQGYRKGGIDKTLFVKQDAGKLMIA 224
            :| |  |||||| ||||| | :||   ::: |  | ||| |   | |::
Opie-2  700 KLSKALYGLKQAPRAWYECLRDFLIANAFKVGKADPTLFTKTCDGDLFVC 749

SIRE-1  225 QIYVDDIVFGGMLNEMLRHFVQQMQFEFEMSFVGELNYFLGIQVKQMEES 274
            |||||||:||:        |   |   ||||::||||||||:||||: ::
Opie-2  750 QIYVDDIIFGSTNQKSCEEFSRVMTQKFEMSMMGELNYFLGFQVKQLKDG 799

SIRE-1  275 IFLSQSKYAKNIVHKFGMENASHKRTPAPNQLKLSKDEAGTSVDQSLYRS 324
             |:|| ||  :::|:||| :|   :||   :     : :| ||||  |||
Opie-2  800 TFISQTKYTQDLLKRFGMKDAKPAKTPMGTDGHTDLNKGGKSVDQKAYRS 849

SIRE-1  325 MIGSLIYLTASRPDITYAVGGCARYQANPKISHLNQVKRILKYVNGTSDY 374
            |||||:|| ||||||   |  |||:| :|| :||  |||||:|: :|  :
Opie-2  850 MIGSLLYLCASRPDIMLSVCMCARFQSDPKECHLVAVKRILRYLVATPCF 899

SIRE-1  375 GIMYCHC........................................... 381
            |: |
Opie-2  900 GLWYPKG........................................... 906
```

FIGURE 34

```
SIRE-1      1 ......SDSMLVGYCDADWAGSVDDRKSTFGGCFYLGTNFISWFSKKQNC 44
                    |   ||||:| |:||:  ||||| | | :||  ::|| |||| :
Opie-2    901 LWYPKGSTFDLVGYSDSDYAGCKVDRKSTSGTCQFLGRSLVSWNSKKQTS 950

SIRE-1     45 VSLSTAEAEYIAAGSSCSQLVWMKQMLKEYNVEQDVMTLYCDNLSAINIS 94
              | ||||||||:||| :| ||:||:| |:::  :   : | ||| ||| :
Opie-2    951 VALSTAEAEYVAAGQCCAQLLWMRQTLRDFGYNLSKVPLLCDNESAIRMA 1000

SIRE-1     95 KNPVQHSRTKHIDIRHHYIRDLVDDKVITLEHVDTEEQIADIFTKALDAN 144
               |||:||||||||||||::||  :   | : || ||:|:||||||:||
Opie-2   1001 ENPVEHSRTKHIDIRHHFLRDHQQKGDIEVFHVSTENQLADIFTKPLDEK 1050

SIRE-1    145 QFEKLRGKLGICLLEDLXNPXP 166
               | :||: | :    :|
Opie-2   1051 TFCRLRSELNVLDSRNLD.... 1068
```

FIGURE 35

## INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14802

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1, 23.72, 24.1; 435/69.1, 172.3, 320.1, 419, 415; 530/350; 800/205

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog, Medline, Biotech, Biosci
Search terms: SIRE-1, retrovirus, plant, vector, transformation, transgenic

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | FLAVELL et al. Extreme Heterogeneity of *Ty1-Copia* Group Retrotransposons in Plants. Molecular General Genetics. 1992, Vol. 231, pages 233-242, especially Figures 1, 2 and 4. | 1-33 |
| A | MARIE-ANGELE, G. Retroelements in Higher Plants. Trends in Genetics. March 1992, Vol. 8, No. 3, pages 103-108, especially page 104. | 1-33 |
| A | FINNEGAN, D. J. Eucaryotic Transposable Elements and Genome Evolution. Trends in Genetics. April 1989, Vol. 5, No. 4, pages 103-107, especially page 103. | 1-33 |

☒ Further documents are listed in the continuation of Box C. □ See patent family annex.

| | | | |
|---|---|---|---|
| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" | document defining the general state of the art which is not considered to be of particular relevance | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" | earlier document published on or after the international filing date | | |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | "&" | document member of the same patent family |

| | |
|---|---|
| Date of the actual completion of the international search<br>06 NOVEMBER 1997 | Date of mailing of the international search report<br>28 NOV 1997 |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No. (703) 305-3230 | Authorized officer<br>DAVID GUZO<br>Telephone No. (703) 308-0196 |

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | DI et al. Production of Transgenic Soybean Lines Expressing the Bean Pod Mottle Virus Coat Protein Precursor Gene. Plant Cell Reports. 1996, Vol. 15, pages 746-750, especially page 747. | 29-33 |

A CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A01H 1/06; C07H 21/02, 21/04; C12N 5/04, 5/10, 7/01, 15/48, 15/63, 15/83; C07K 14/00, 14/15

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

536/23.1, 23.72, 24.1; 435/69.1, 172.3, 320.1, 419, 415; 530/350; 800/205