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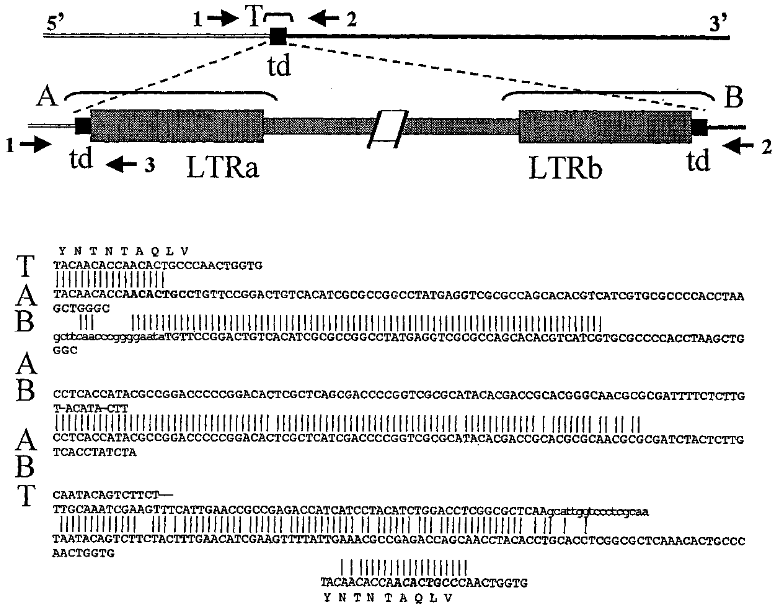

(57) Abstract: Nucleic acid (DNA) probes are provided which will specifically identify a gene for resistance of Bt in insect populations. Sequences are identified associated with the onset of resistance to *Bacillus thuringiensis* toxins. The sequences are used as probes to monitor the presence of acquired insect resistance associated with transgenic crops.

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POLYNUCLEOTIDE ENCODING A GENE CONFERRING RESISTANCE TO BACILLUS THURINGIENSIS TOXINS

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The United States Government may have rights to this invention under the terms of a sponsored research agreement by the National Science Foundation, grant number MCB-9816056.

RELATED APPLICATIONS

This application claims the benefit of US provisional application having serial number 60/276,180 filed on March 15, 2001, and which is incorporated herein by reference.

FIELD OF THE INVENTION

This invention is directed towards the occurrence and identification of pesticide tolerance of certain insects. The invention makes use of specific polynucleotide sequences associated with the onset of resistance to *Bacillus thuringiensis* toxins which are used as probes to monitor the presence of acquired insect resistance associated with transgenic crops. The specific polynucleotide sequences are also used to monitor changes in the frequencies of alleles which confer the resistance to the toxins.

BACKGROUND OF THE INVENTION

The bacterium *Bacillus thuringiensis* (Bt) contains genes encoding insecticidal proteins. Bt proteins are toxic when ingested by susceptible insect larvae. The protein attacks the insect's midgut, causes cessation of feeding, and eventually kills the insect. Bt toxins have been produced as fermentation products of Bt cultures and used in spray formulations for crop protection. Bt genes have also been used commercially to transform crop plants; these transgenic crop plants' cells then produce the insecticidal protein which attacks susceptible insects that attempt to feed on the plant.

The general mode of action of Bt toxins is well known in the art and is described for example by Rajamohan F, Lee MK, Dean DH (1998) *Progress in Nucleic Acid Research and Molecular Biology* 60: 1-27. The protein produced by the bacterium is usually a protoxin, which itself is not toxic until it is proteolytically cleaved by the insect's own proteases. The smaller protein resulting from proteolysis is the active toxin. This toxin diffuses through the peritrophic membrane to the midgut epithelium, where it binds to one or more sites in the membrane. This initial binding step may be reversible, but eventually the toxin becomes irreversibly bound to the membrane. A conformational change occurs in the toxin, whereby membrane-spanning alpha helices are inserted into the membrane, where they aggregate and form pores. These pores disrupt the normal osmotic balance of the epithelial cells. The cells swell and lyse, leading to destruction of the midgut epithelial cell layer and eventual death of the insect.

The initial binding step is believed to be necessary for toxin action; consequently there have been many studies on binding interactions of Bt toxins and components of the midgut, described for example by Pietrantonio PV and Gill SS (1996) in *Biology of the Insect Midgut*, Chapman & Hall, London, pp 345-372. Techniques used to study binding often start with the isolation of brush border membrane vesicles (BBMVs) from the microvillar portion of columnar epithelial cells. Binding to BBMVs in suspension can be measured using labeled toxin. Alternatively, proteins can be isolated from BBMVs, separated by denaturing electrophoresis conditions, transferred to membranes, and probed with toxin. In addition, histological sections of insect midguts can be prepared and binding of labeled toxin can be visualized using microscopy.

Binding of Bt toxins to specific insect proteins can also be measured. Several proteins that interact with Bt toxins are well known in the art. Aminopeptidases exist in many different forms in insect midguts, and many of them have been shown to bind Bt toxins (Knight PJK, Knowles BH, Ellar DJ (1995) *Journal of Biological Chemistry* 270 (30): 17765-17770; Gill SS, Cowles EA, Francis V (1995) *Journal of Biological Chemistry* 270 (45): 27277-27282; Luo K, Sangadala S, Masson L, Mazza A, Brousseau R, Adang MJ (1997) *Insect Biochemistry and Molecular Biology* 27 (8-9): 735-743). Members of the cadherin superfamily have also been shown to bind Bt toxins (Vadlamudi RK, Weber E, Ji IH, Ji TH, and Bulla LA (1995) *Journal of Biological Chemistry* 270: 5490-5494; and Nagamatsu Y, Koike T, Sasaki K, Yoshimoto A, Furukawa Y, (1999) *FEBS Letters* 460: 385-390). Phosphatase enzymes have also been implicated in Bt toxin binding (Sangadala S, Walters FS, English LH, Adang MJ, (1994) *Journal of Biological Chemistry* 269 (13): 10088-10092). TPP-75, an elastase-like serine protease, binds to certain Bt toxins and causes them to precipitate (Milne RE, Pang ASD, Kaplan H (1995) *Insect Biochemistry and Molecular Biology* 25 (10): 1101-1114). BTR-270, a peptidoglycan, binds Cry1A toxins with high affinity (Valaitis AP, Jenkins JL, Lee MK, Dean DH, Garner KJ (2001) *Archives of Insect Biochemistry and Physiology* 46 (4): 186-200). Bt toxins have also been shown to bind to nonprotein components of midgut epithelial membranes. Glycolipids from *Manduca sexta* have been shown to bind Cry1A toxins using an overlay technique (Garczynski SF and Adang MJ (2000) in *Entomopathogenic Bacteria: From Laboratory to Field Application*, Kluwer Academic Publishers, pp 181-197). Neutral lipids are involved in Bt toxin binding to *Manduca sexta* brush border membranes (Sangadala S, Azadi P, Carlson R, Adang MJ (2001) *Insect Biochemistry and Molecular Biology* 32 (1): 97-107). Neutral glycolipids, especially hexa- and tri-saccharylceramides, are implicated in Cry1A toxin binding in diamondback moth (Kumaraswami NS, Maruyama T, Kurabe S, Kishimoto T, Mitsui T, Hori H, (2001) *Comparative Biochemistry and Physiology B- Biochemistry & Molecular Biology* 129 (1): 173-183).

The relationship between binding targets for Bt-toxins and susceptibility or resistance to Bt is very complicated and not completely understood at the present time. Several hundred strains of *Bacillus thuringiensis* exist, with considerable specificity toward various groups of insects. Co-evolution between the insects and Bt has resulted in specificity of the interaction between Bt-toxin and the membranes of insect gut cells. The Bt-toxin of a particular strain of *Bacillus thuringiensis* may bind to the gut of some insect larvae but not to others. Thus, the Bt-toxins may have a high specificity for a small number of insect pest species while having no significant activity against beneficial insects, wildlife, or humans.

Plants transformed to carry Bt genes and express insecticidal proteins are known in the art and include potato, cotton, tomato, corn, tobacco, lettuce, and canola. Transformed plants are known in the art as reflected in US Patent Nos. 5,608,142; 5,495,071; 5,349,124; and 5,254,799, the specifications of which are incorporated in their entirety herein by reference. The use of genetically engineered plants is designed to reduce the use of broad spectrum insecticides.

There is concern that resistance may evolve to Bt toxins, whether they are applied to plants in spray formulations or the plants are genetically engineered to express them. The development of resistance to Bt-toxin expressing crops may also result in resistance to commercial formulations of fermented strains of Bt such as DIPEL® (Abbott Laboratories).

Rapid, reliable methods for broad screening to distinguish and detect the development of Bt resistance in populations of insects are needed. Heretofore, all methods require living or fresh-frozen insect larvae or preparations derived from them. The simplest methods employ bioassays on living insects, in which survivorship or larval metabolic rates are determined over time following a diet containing a specified concentration of a Bt-toxin. One such bioassay based on reduced metabolic rates after exposure to low doses of toxin mixed into artificial diet is discussed in US Patent No. 6,060,039 to Roe *et al.* which is incorporated herein by reference. Other bioassays are based on survival after exposure to a single, high diagnostic dose of toxin (for example, Diaz-Gomez O, Rodriguez JC, Shelton AM, Lagunes-T A, Bujanos-M R, (2000) *Journal of Economic Entomology* 93 (3): 963-970).

In principle, these bioassay methods can detect resistance no matter what its biochemical or physiological mechanism is. However, they require living, healthy larvae for their use, which are not always available. A more severe limitation on these methods is that, depending on the frequency of resistance genes in the populations, millions of individuals may need to be tested to detect a single resistant larva. High-level resistance to Bt is usually recessive, which means that an insect must have two copies of the resistance gene to be resistant. To a very good approximation, the frequency of such homozygous individuals is given by the square of the frequency of the resistance allele. For example, if the resistance allele frequency is one in a thousand, the frequency of homozygous resistant individuals is one in a million. In this example, more than a million larvae would need to be screened to detect resistance.

One solution to this problem is to develop methods for detecting the resistance genes directly. In the example just given, the frequency of heterozygous carriers of one copy of the resistance allele is 2 x 0.001 x 0.999 or approximately 2 in a thousand. When resistance is recessive, these individuals would not be identified by bioassay because the one resistance allele they carry is not enough to make them fully resistant. But a direct, DNA-based method for detecting the resistance allele would identify these individuals, and sample sizes on the order of a thousand, rather than a million, would suffice.

The main limitation to developing DNA-based methods for detecting resistance alleles is that, up to now, the identity of resistance-causing genes has been unknown. In spite of much work on Bt toxin mode of action, prior to the invention described herein there has not been a demonstration of which genes, when mutated, actually cause resistance. Accordingly, there is room for variation and improvement in the art of screening assays useful in detecting the presence of genes conferring Bt resistance in natural populations.

SUMMARY OF THE INVENTION

It is one aspect of one of the present inventions to provide a genetic probe to identify and monitor resistance for the Bt-toxin in target insect populations. One such insect pest is the tobacco budworm (*Heliothis virescens*) which is a major economic pest of cotton.

It is yet another aspect of one of the present inventions to develop a DNA probe and assay protocol which distinguishes between the conditions of homozygotes and heterozygotes with respect to resistance to Bt in populations of *Heliothis virescens* and other insects.

It is yet another aspect of one of the present inventions to provide a process and useful sequences in which nucleotide probes are used to monitor the presence of acquired insect resistance associated with a transgenic crop.

It is yet another aspect of one of the present inventions to provide a process and useful nucleotide sequences which are used to monitor population changes in the frequency of alleles which are associated with the resistance to Bt toxin.

These and other features, aspects, and advantages of the present invention will become better understood with reference to the following description and appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

A full and enabling disclosure of the present invention, including the best mode thereof, to one of ordinary skill in the art, is set forth more particularly in the remainder of the specification, including reference to the accompanying drawings.

Figure 1 is a QTL map of the Cry1Ac resistance trait on linkage group 9 of *Heliothis virescens.*

Figure 2 is a conceptual translation of HevCaLP (s1 allele and r1 allele) in alignment with BmBtR175 of *Bombyx mori* and BtR1 of *Manduca sexta*.

Figure 3 is a northern analysis of mRNA isolated from susceptible and resistant strains following probing with the gene sequences set forth herein.

Figure 4 sets forth the insertion point of the Hel-1 element in the r1 allele of HevCaLP.

Figure 5 shows the multi-copy occurrence of Hel-1 in genomic DNA of resistant and susceptible strains of *Heliothis virescens*.

BRIEF DESCRIPTION OF THE SEQUENCE LISTINGS

The accompanying sequence ID listings are identified below. The sequence listings appear following the claims and are incorporated herein by reference.

The first sequence 1 identifies SEQ ID NO: 1 which is the DNA sequence of the susceptible allele s1of HevCaLP.

Sequence 2 is the protein sequence SEQ ID NO: 2 of a conceptual translation of allele s1 as used in the protein alignment to *Bombyx* and *Manduca*.

Sequence 3 is the DNA sequence of SEQ ID NO: 3 which is the resistant allele r1 of HevCaLP, including the Hel-1 insert and the duplicated target sequences.

Sequence 4 is the DNA insert identified as SEQ ID NO: 4 for the Hel-1 insert which does not include duplicated target sequences.

Sequence 5, having SEQ ID NO: 5, is a DNA sequence corresponding to the left LTR of the Hel-1 insert.

Sequence 6, having SEQ ID NO: 6, is a DNA sequence corresponding to the right LTR of the Hel-1 insert.

Sequence 7, having SEQ ID NO: 7, is a DNA sequence of primer F1 corresponding to bases 1982 to 2001 of SEQ ID NO: 3.

Sequence 8, having SEQ ID NO: 8, is a DNA sequence corresponding to primer R2 consisting of the reverse complement of bases 4322 to 4351 of SEQ ID NO: 3.

Sequence 9, having SEQ ID NO: 9, is a DNA sequence corresponding to primer R3 consisting of the reverse complement of bases 2029 to 2052 of SEQ ID NO: 3.

DESCRIPTION OF THE PREFERRED EMBODIMENT

Reference now will be made in detail to the embodiments of the invention, one or more examples of which are set forth below. Each example is provided by way of explanation of the invention, not limitation of the invention. In fact, it will be apparent to those skilled in the art that various modifications and variations can be made in the present invention without departing from the scope or spirit of the invention. For instance, features illustrated or described as part of one embodiment, can be used on another embodiment to yield a still further embodiment. Thus, it is intended that the present invention cover such modifications and variations as come within the scope of the appended claims and their equivalents. Other objects, features, and aspects of the present invention are disclosed in the following detailed description. It is to be understood by one of ordinary skill in the art that the present discussion is a description of exemplary embodiments only and is not intended as limiting the broader aspects of the present invention, which broader aspects are embodied in the exemplary constructions.

In describing the various figures herein, the same reference numbers are used throughout to describe the same material, apparatus or process pathway. To avoid redundancy, detailed descriptions of much of the apparatus once described in relation to a figure is not repeated in the descriptions of subsequent figures, although such apparatus or process is labeled with the same reference numbers.

Applicants' protocols and procedures may be found in reference to *"Identification of a Gene Associated with Bt resistance in Heliothis virescens"* which was published in Science, volume 293, pp 857-860, on August 3, 2001; and which is incorporated herein by reference.

A resistant strain of *Heliothis virescens* was previously developed in the laboratory by selection using artificial diet containing various concentrations of Bt toxin (Gould F, Anderson A, Reynolds A, Bumgarner L, Moar W (1995) *Journal of Economic Entomology* 88 (6): 1545-1559). The strain, named YHD2, is 10,000 fold more resistant to the toxin Cry1Ac and is conditioned in a large part by a single recessive gene named BtR-4 which is located in linkage group 9 of *H. virescens.* The initial localization of the resistance gene BtR-4 has been reported in the Applicants' prior publication (Heckel DG, Gahan LC, Gould F, Anderson A (1997) *Journal of Economic Entomology* 90: 75-86) and which is incorporated herein by reference.

Further localization of BtR-4 to a particular region of linkage group 9 was carried out using a total of 11 polymorphic markers spanning a length of 105 cM. The markers were scored on a segregating backcross family derived from YHD2 females crossed with susceptible males. The linkage group was scanned for quantitative trait loci (QTLs) conferring Bt resistance following the methods of Lander, ES and Botstein D (1989) *Genetics* 121: 185-193. A single, highly significant peak of the log-likelihood function indicated that the BtR-4 resistance gene is located between A14 and MPI as set forth in Fig. 1.

The cadherin superfamily was chosen as a candidate for BtR-4. Partially degenerate oligonucleotide primers Bmtp5 and Bmtp8 as shown in Table 1 were designed based on published sequence of the BtR175 gene from *Bombyx mori* (GenBank Accession No AB026260, described by

Nagamatsu Y, Toda S, Koike T, Miyoshi Y, Shigematsu S, Kogure M (1998) *Bioscience, Biotechnology and Biochemistry* 62 (4): 727-734). These primers were used in the polymerase chain reaction (PCR) with cDNA prepared from midgut mRNA of larval *Heliothis virescens*. A PCR product of 334 basepairs designated Hvcad58 was amplified, cloned and sequenced using conventional methodology well-known to those skilled in the art. The sequence of Hvcad58 corresponds to bases 4279 to 4612 of SEQ ID NO: 1.

Radiolabeled Hvcad58 was used to probe Southern filters made from additional segregating backcross families for further mapping on linkage group 9. Finer scale QTL mapping in this region using 268 backcross progeny yielded a single peak of the log-likelihood function directly above the map location of Hvcad58 (Fig. 1). The data clearly indicates that the gene containing Hvcad58 is a strong candidate for the BtR-4 resistance gene.

The Hvcad58 probe was used to screen midgut cDNA libraries made from resistant (YHD2) and susceptible strains of *Heliothis virescens*. Clones recovered from these libraries were sequenced and used to design additional primers to amplify the full-length coding sequence from susceptible cDNA. In addition to the cDNA methods, a five-prime RACE (rapid amplification of cDNA ends) technique was used to complete the full sequence.

The sequencing yielded one transcript (s1) cloned from a susceptible strain as given in SEQ ID NO: 1. Conceptual translation of this transcript produced a protein product (that we have named HevCaLP, *Heliothis virescens* cadherin-like protein) of 1732 amino acids as given in SEQ ID NO: 2. HevCaLP is 70% identical to the BtR175 protein, sharing a signal sequence at the amino terminus, 11 extra-cellular cadherin-type repeats, a non-cadherin proximal membrane region, a transmembrane region, and a highly conserved cytoplasmic domain at the carboxy terminus as shown in Fig. 2. It shows somewhat less similarity to the BT-R1 protein from *Manduca sexta*, as given in GenBank Accession No. AAB33758 and reported by Vadlamudi RK, Weber E, Ji IH, Ji TH, and Bulla LA (1995) *Journal of Biological Chemistry* 270: 5490-5494. The transmembrane and cytoplasmic domains are absent from that sequence of BT-R1.

Expression of the mRNA encoding HevCaLP in susceptible and resistant larval midguts was studied using northern analysis and sequencing of clones from the resistant library. As shown in Fig. 3, susceptible larvae show a single transcript of 5.5 kb. YHD2 larvae show three transcripts. The sequence of the rarest (7.9 kb) is denoted as the r1 allele, and given as set forth in SEQ ID NO: 3. It is similar to the susceptible transcript except for a 2.3 kb insert denoted as Hel-1 as given in the accompanying SEQ ID NO: 4. Hel-1 shows several hallmarks of the LTR-type retrotransposons. Hel-1 has an approximately 255 nucleotide long terminal repeat (LTR) sequence at both ends and an unrelated sequence in the middle. The left LTR sequence, LTRa, is given in SEQ ID NO: 5 and the right LTR sequence, LTRb, is given in SEQ ID NO: 6. Hel-1 is flanked by an 8-nt duplication of the host sequence ACACTGCC, as shown in Fig. 4. The transcript of intermediate abundance (4.4 kb) is an abbreviated form, truncated at the second LTR of Hel-1 by a poly-A tail. The third, highly abundant transcript (2.1 kb), is truncated at the first LTR of Hel-1 by a poly-A tail.

Because of an in-frame stop codon 30 bases into the first LTR of Hel-1, conceptual translation of the three different YHD2 transcripts produces the same truncated 622-aa protein (as shown in the translation of the r1 allele in Fig. 2). Multiple stop codons in all three reading frames of the LTR follow the initial stop codon, preventing translation of a larger protein containing the carboxy-terminus of HevCaLP. Thus, the predicted protein product of the YHD2 r1 allele (if one is produced) would possess the same signal sequence as HevCaLP (possibly directing its secretion into the midgut lumen) but no predicted transmembrane domain or toxin-binding region.

Genomic Southern blots probed with the LTR region of Hel-1 show that it occurs with a copy number of 10-15 in both YHD2 and susceptible insects (Fig. 5). Insertion of this Hel-1 element into the gene encoding HevCaLP has created the novel, knockout r1 allele which confers resistance when homozygous (present in two copies in an individual insect). This insertion event could have occurred in the laboratory during the Bt-resistance selection protocol that produced YHD2, or may already have been present in the field-collected founders of the selection line. Thus it is now evident that a DNA-based method for detecting Bt resistance in *Heliothis virescens* may be devised, based on detection of the specific insertion of the Hel-1 element into the gene encoding HevCaLP, producing the r1 allele.

To illustrate detection of the r1 allele, a PCR assay was designed using two primers flanking the insertion point (F1 and R2) and a third (R3) internal to the left LTR (Fig. 4). Primer F1 consists of bases 1982 to 2001 of SEQ ID NO: 3, 5' ATA CGA GCT GAC GAC ACG CTG GGA GA 3', primer R2 consists of the reverse complement of bases 4322 to 4351 of SEQ ID NO: 3, 5' TCT GAG CGT AGG AGG TGT GTT GTT GAT GTC 3', and primer R3 consists of the reverse complement of bases 2029 to 2052 of SEQ ID NO: 3, 5' GCG CGA TGT GAC AGT CCG GAA CAG 3'. Primers F1 and R3 produce a 71-bp band from the r1 allele. Primers F1 and R2 amplify a 99-bp band from s1 or other susceptible alleles lacking the Hel-1 insert. Heterozygotes produce both bands. This is a marked improvement on a conventional bioassay, which would not distinguish heterozygotes from homozygous susceptibles because the resistant allele is recessive. It also confirms that the resistant strain is fixed for the r1 allele, as all YHD2 individuals examined to date have the 71-bp band only. It will be evident to those skilled in the art that the detection method for the r1 alelle is not limited to PCR with these specific primers, and that there are many other molecular methods of detecting the specific insertion of the Hel-1 element into the HevCaLP gene, based on the sequence information disclosed herein.

It is believed that the gene encoding HevCaLP is identical to BtR-4, the major resistance gene in YHD2. Recessivity of the resistant allele at BtR-4 is explained by Hel-1 inactivation of HevCaLP. HevCaLP functions as a "lethal target" of Bt-toxin, since two copies of the disrupted allele are required for 10,000-fold resistance. Heterozygotes still present a "lethal target" since they have one copy of the susceptible allele.

The normal physiological function of HevCaLP is unknown, although other members of the cadherin superfamily are involved in cell adhesion and signalling (T. Uemura (1998) *Cell* 93 (7): 1095-1098). Whatever its function, it is not essential for life, as YHD2 is viable and fertile under laboratory conditions, despite being a "natural knockout" strain for HevCaLP. Whether its absence confers a fitness disadvantage in the field has important implications for resistance management, and this question can now be addressed with the information developed here. Target-site resistance to other insecticides usually involves modification but not knockout of the target, which is generally essential for life (e.g., acetylcholinesterase for organophosphates, sodium channel for pyrethroids, GABA receptor for cyclodienes) (French-Constant RH, Pittendrigh B, Vaughan A, Anthony N (1998) *Philosophical Transactions of the Royal Society of London Series B--Biological Sciences* 353 (1376): 1685-1693,). However, methoprene resistance in *Drosophila melanogaster* provides another example of resistance by gene inactivation (Wilson TG & Ashok M, (1998) *Proceedings of the National Academy of Sciences of the USA* 95 (24): 14040-14044).

The present invention now makes possible the application of molecular methods to Bt-resistance monitoring. We previously estimated the frequency of YHD2-type resistant alleles in field populations of *Heliothis virescens* prior to widespread planting of transgenic Bt-cotton to be 0.002 (Gould F, Anderson A, Jones A, Sumerford D, Heckel DG, Lopez J, Micinski S, Leonard R, Laster M (1997) *Proceedings of the National Academy of Sciences of the USA* 94 (8): 3519-3523). This labor-intensive, bioassay-based estimate was derived by testing progeny of more than 1,000 field-caught males mated to YHD2 females, for alleles which would confer resistance when heterozygous with r1. Our results now suggest that this estimate covers the entire class of HevCaLP knockouts regardless of the nature of the molecular lesion, as well as other mutants preventing any expressed HevCaLP from functioning as a toxic target. Development of efficient DNA-based methods to detect these other types of mutants at BtR4 should be a high priority and is now possible with the methods described herein.

Only by monitoring allele frequencies at resistance genes like BtR-4 will it be possible to verify that the high-dose/refuge resistance management strategy for Bt-cotton mandated by the US Environmental Protection Agency (EPA) is actually working to keep resistance allele levels low. The present invention affords a new method of complying with EPA regulations which require monitoring resistance levels in *Heliothis virescens*. The present invention provides a nucleic acid probe that will specifically identify genes for resistance in field populations. Further, the probes and protocols set forth herein provide for a method of monitoring the population of homozygous and heterozygous resistant individuals in field populations.

Bt resistance in *Heliothis virescens* caused by other types of mutations that inactivate the HevCaLP gene product may also be screened for using the information provided herein. Such methods may include obtaining portions of the gene or its homologues by cDNA cloning or the polymerase chain reaction, determining the DNA sequence by standard methods, and examining the sequence for the occurrence mutations that may include nucleotide substitution, insertions, or deletions. Such mutations may affect protein sequences encoded by the gene by causing amino acid substitutions, insertions, or deletions as well as incorrect intron splicing, premature chain termination due to nonsense mutations, or errors in the normal initiation or termination of the transcription or translation.

By way of example, DNA or RNA isolated from individual *Heliothis virescens* is used as the template for PCR using primers specifically designed from SEQ ID NO: 1. The PCR products are directly sequenced, or cloned and sequenced, using standard methods. The sequences are examined using commercially available computer programs well known in the art, such as the Wisconsin Genetics Computer Group package. Mutations, such as individual nucleotide substitutions, insertions, or deletions; or insertions or deletions of several nucleotides, are detected by comparison to SEQ ID NO: 1. Such mutations may alter the amino acid in the protein sequence, leading to reduced binding of Bt toxins to the HevCaLP gene product and thereby conferring resistance. Or such mutations may cause frameshifts or premature occurrence of stop codons, resulting in a truncated or absent protein that fails to bind to Bt toxins and thereby confers resistance.

In the course of this invention, an isolated nucleic acid molecule of the present invention includes a nucleic acid that is at least about 85%, preferably at least about 90%, and still more preferably at least about 95%, and even more preferably at least about 99% identical to the sequence of the susceptible allele s1 of HevCaLP. Additionally, any isolated polynucleotide or naturally occurring polynucleotide that hybridizes to the sequence set forth in SEQ ID NO: 1 at 60°C in 1X SSC will have properties useful in carrying out the present invention.

Other embodiments of the present invention include isolated nucleic acid molecules that are at least about 85%, preferably at least about 90%, still more preferably at least about 95%, and even more preferably at least about

99%, identical to the sequences set forth in SEQ ID NO: 3 and SEQ ID NO: 4.

Bt resistance in other insect species may also be screened for using the same approach. These species may contain one or more genes homologous to the *Heliothis virescens* HevCaLP gene, whose products interact with Bt toxins. Resistance in these other species can be detected by obtaining the sequence of those genes, designing PCR primers, and amplifying and sequencing DNA from individual insects collected from the field or reared in the laboratory. Examination of the sequence for inactivating mutations as described herein will detect Bt resistance in those species. Representative sequences of HevCaLP homologues in other species and which may be used in the screening process described herein include the following:

1) *Manduca sexta* BT-R1, GenBank Accession No. I77078, US Patent No. 5693491 (SEQ ID NO: 1) and US Patent No. 6,007,981 (SEQ ID NO: 1);

2) *Bombyx mori* BtR175, GenBank Accession No. AB026260, described by Nagamatsu Y, Toda S, Koike T, Miyoshi Y, Shigematsu S, Kogure M (1998) Bioscience, Biotechnology and Biochemistry 62 (4): 727-734;

3) *Pectinophora gossypiella* BT-R2, GenBank Accession No. AX150183, Patent Application, International Publication No. WO01/34807 (SEQ ID NO: 1);

4) *Ostrinia nubilalis*, GenBank Accession No. AX147201, Patent application, International Publication No. WO 01/36639 (SEQ ID NO: 1);

5) *Helicoverpa zea*, GenBank Accession No. AX147203, Patent application, International Publication No. WO01/36639 (SEQ ID NO: 3);

6) *Spodoptera frugiperda*, GenBank Accession No. AX147205, Patent application, International Publication No. WO0/136639 (SEQ ID NO: 5); and

7) *Lymantria dispar* BTR-CAD, GenBank Accession No. AF317621.

The above identified sequences and the referenced publications are all incorporated herein by reference as is set forth in their entirety.

The current methodology includes detecting resistance to *Bacillus thuringiensis* endotoxin in insect populations by screening for mutations that alter the structure or function of a protein as set forth in SEQ ID NO: 2. For the purposes of screening protocols, it is believed that using the sequence set forth in SEQ ID NO: 2 may include homologues and other species which would display at least 60% similarity to the sequence set forth in SEQ ID NO: 2. More preferably, the sequence similarity is at least about 75%, preferably at least about 80%, more preferably at least about 85%, even more preferably at least about 90%, still more preferably at least about 95%, and even more preferably at least about 99% identical to the amino acid sequence set forth in SEQ. ID. NO: 2.

Several of the mutations in other species detected by this approach may not have an obvious effect of activating the HevCaLP homologue. In that case, evidence that the mutation confers resistance may be obtained by conducting a linkage analysis and mapping the gene as described herein for *Heliothis virescens*. For that purpose, a strain of the species of interest with the mutation is crossed with a wild-type strain, and the F1 hybrids are intercrossed or backcrossed to one of the parental strains. The F2 or backcross progeny are tested for resistance by any of the bioassay methods described previously and well known in the art, and DNA is isolated from each individual progeny. The DNA is analyzed for the presence of the mutation, using restriction fragment polymorphism analysis, allele-specific PCR, denaturing gradient gel electrophoresis, single-stranded conformation polymorphism, denaturing high-performance liquid chromatography, or any other mutation detection system well known in the art. Evidence that the mutation confers resistance is obtained from the correlation across progeny between presence of the mutation and presence of resistance.

A straightforward extension of this method of detecting Bt- resistance is to examine the DNA sequence of genes encoding other proteins that interact with Bt toxins, including but not limited to aminopeptidases, alkaline phosphatases, elastin-like serine proteases, and peptidoglycans.

All cited references, publications, and sequence listings set forth herein are incorporated by reference in their entirety.

These and other modifications and variations to the present invention may be practiced by those of ordinary skill in the art, without departing from the spirit and scope of the present invention. In addition, it should be understood that aspects of the various embodiments may be interchanged both in whole or in part. Furthermore, those of ordinary skill in the art will appreciate that the foregoing description is by way of example only, and is not intended to limit the invention.

Primers Used in Determining the Structure of BtR4, the Cadherin-like Polynucleotide in Heliothis virescens

Bmtp 5	5′- GTR CTG ACK CTT AAY ATC CAG CCC ACK GC –3′
Bmtp 8	5′- TAC GGG YAC RTT RTC SCG KAT GAA GTG KCC –3′
Hvtp05	5′- AGC CCA CTG CAT CTA TGC ACG GCA TGT TTG A –3′
Hvtp08	5′- CCT GAC TTC GGT CTG GTC GTC CCT GGC –3′
CGp1	5′- TGT GGA GTC AGC TTC CAT AGA GTC TTG TAT CAG CGT GTA –3′
CGnotp2	5′- GAT ACG CGG CCG CAG GTC AGC AGA GCT CTG TTG ATG GTG TCG AGG GTG GAG A –3′
T7p1	5′- TAA GTT GGG TAA CGC CAG GGT TTT CCC AGT CAC –3′
T7p2	5′- GGC CAG TGA ATT GTA ATA CGA CTC ACT ATA GGG CG –3′
T3p1	5′- GAT AAC AAT TTC ACA CAG GAA ACA GCT ATG ACC ATG –3′
T3p2	5′- GAA ATT AAC CAC CCT TAA AGG GAA CAA AAG CTG GAG –3′
CGp3	5′- GGC ACG TTT TTT TCC ACT GAC GGG GTC GTG CG –3′
Cgnotp4	5′- GAT ACG CGG CCG CGG GCA GTC TGA GCG TAG GAG GTG TGT TGT TGA T –3′
RC36T4	5′- GAC GTG TGT TCG CCT GAT CCT AAC TAC T –3′
RC36cg5	5′- AGC CTC TTA AAT CCA TAG CGG TCT CCA G –3′
RC36cg5+	5′- CTG GAG ACC GCT ATG GAT TTA AGA –3′
SC3T6	5′- ATG TTC GAG GTG CTG TAC CTC ACC G –3′
SC3cg7	5′- ACA CGA ACA CAG GAT CGT GGA AGT T –3′
CGp5	5′- TGT ATC TTC TGG AAC TCC GGC ACT TCG AAG TC –3′
CGnotp6	5′- GAT ACG CGG CCG CAT GTG ATG GTT CTG CGT GCC GAC GAT GAA GGA CTG –3′
Sint1	5′- GCT AAG GAC CGG GAT ATT GAT GAT AGA GT –3′
Sint2	5′- CGT GCG GGG CAG TCT GAG AGT AG –3′
RUNI1	5′- CAT ACA CGA CCG CAC GCG CAA CG –3′
RUNI2	5′- TGA GCG CCG AGG TGC AGG TGT AGG –3′
Hvtp13	5′- CTG TAC ACA GCC GGC ATC TCC AC –3′
Hvtp14	5′- CTG GAA GTT GAG GGT CAG CAC TCC AGT –3′
Hvtp15	5′- AAC CGT CGT CTG GAA GCT CT –3′
Hvtp16	5′- TCT TCG ATG CCG ATC AGA TCC GAG TC –3′
Hvtp17	5′- GCG GCG CCG GGC ACC AAC AAG CA –3′
HvA11-RT	5′- AAT AGA TGC TCT TAC ATA ATA CGA GTA TCT TAC –3′
5′R5A4/8	5′- GAT ACG CGG CCG CGA GAA CTA TGA GAT GGC AGT CGA CGT GAG AAT A –3′
HvA11F1	5′- GAA CTA TGA GAT GGC AGT CGA CGT GAG AAT –3′
HvA11F3	5′- TTA ACT TTC GCG CAA GAT TGT TCC TAT ATG –3′
HvA11R2	5′- GAA CTC TGG GCT GAA GGG GGT AGC –3′
HvA11R4	5′- CCC GAA GTT RTT GTT ATG GTT TGC TAC TGA –3′
USTP01	5′- ATG GGC AAC GCA GTT AAC TAC CTG –3′
USTP02	5′- CAT CCT CGT GAC AAT CGA CGA TGC 3′
USTP03	5′- CAG ACA GAA CGA GCT CTT TGT GCA –3′
F771-5Ksp1	5′- GCC GTG CAG CAG TTC GAT GAG AAG –3′
F771-5Ksp2	5′- CTC CCA CTG TAT CAG TAG CCA TCA –3′
738-3.4Ksp1	5′- ACA ATC CTT CAG GGT TCG AGC CAT C –3′
738-3.4Ksp2	5′- GTA CAA GAG AAA ATC GCG CGT TGC GT –3′
738-3.4Ksp3	5′- CCT GAT CAA CTG GAA CGA TGA GCT G –3′
738-3.4Ksp4	5′- CCA AAG TCC ACG GGC GGT TGC GCA C –3′

TABLE 1

Primers Used in Determining the Structure of BtR4, the Cadherin-like Polynucleotide in Heliothis virescens (continued)

738-3.8sp6	5'- GTG TAA CGT AGT GTG CTC GTG TAA TGC -3'
738-C10sp8-	5'- CCG TCT GAA ACA TGT CGA AGT CAT -3'
TBR01	5'- GAG ACT AGC ACC TAC ACG GTC GCT -3'
TBR02	5'- TCC AAC GAG CTG TTC CTG CTG ACG -3'
CR9TBR	5'- CAC TGT TAC TGT CAA TGT TCG AGA -3'
LTR-Pr1	5'- CAC ACG TCA TCG TGC GCC CCA CCT AAG CTG -3'
LTR-Pr2	5'- CTG GCG CGA CCT CAT AGG CCG GCG CGA TGT -3'
LTR-1.9Ksp1	5'- CGA ATC AGC TGA TTC ATT GTC GCT -3'
LTR-1.9Ksp2	5'- GTA GTG TGT GAT GTG ATC CAG -3'
Rint-Fwd1	5'- ATA CGA GCT GAC GAC ACG CTG GGA GAG CC -3'
Rint-Rev2	5'- TCT GAG CGT AGG AGG TGT GTT GTT GAT GTC -3'
C36RESEQ-F	5'- CCC GGC ACC GAC AAC TCC -3'
C36RESEQ-R	5'- CTC CAT GGT CGT ATG CCT TGA CAT GTA -3'
pc11Fa	5'- GAG ATG GCA GTC GAC GTG AGA ATA CTG A -3'
pc12Fa	5'- CCC GTT TCG CCG TGT TCA GGA ATG TC -3'
pc12Ra	5'- TGG TAC CTC GGT AGT TAA GCC TGG CAA T -3'
pc13Fa	5'- GAA CAC GGC GAA ACG GGC ACC ACA GA -3'
pc13Ra	5'- TGC CAG GCT TAA CTA CCG AGG TAC CA -3'
pc14Fa	5'- AAC CCG CTG CAT TTG TTT AGA GTT ACA G -3'
pc14Ra	5'- CGA ACT GCT GCA CGG CGA AGA TCT CCA T -3'
pc15Ra	5'- TTC CTT CCA CGT CAT TGT CGC CAT ATT T -3'
RintS-F1	5'- ATA CGA GCT GAC GAC ACG CTG GGA GA -3'
RintS-R2	5'- TCT GAG CGT AGG AGG TGT GTT GTT GAT GTC -3'
RintR-R3	5'- GCG CGA TGT GAC AGT CCG GAA CAG -3'
RintR-F4	5'- ACG CGC AAC GCG CGA TCT ACT CTT -3'

TABLE 1

THAT WHICH IS CLAIMED IS:

1. An isolated polynucleotide sequence selected from the group consisting of SEQ ID NOs: 1, 3, 4, 7, 8 and 9.

2. A nucleotide sequence having at least an 85% identity of one of the sequences set forth in Claim 1.

3. An isolated nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 2.

4. Any isolated polynucleotide or naturally occurring polynucleotide that hybridizes the sequence in Claim 1 at 60°C in 1X SSC.

5. An isolated polynucleotide encoding an allele of a gene which when homozygous confers resistance to *Bacillus thuringiensis* endotoxin comprising the sequence of SEQ ID NO: 3.

6. A nucleotide sequence having at least an 85% identity to the sequence set forth in Claim 5.

7. An isolated polynucleotide encoding a mobile genetic element capable of being inserted into the genomic DNA of insects, comprising the sequence of SEQ ID NO: 4 .

8. A nucleotide sequence having at least an 85% identity to the sequence set forth in Claim 7.

9. The complement of any of the sequences set forth in Claim 1.

10. A method of detecting resistance to *Bacillus thuringiensis* endotoxin in *Heliothis virescens* populations by screening for the presence of mutations having a sequence selected from the group consisting of SEQ ID NO: 3 or SEQ ID NO: 4.

11. A method of detecting resistance to *Bacillus thuringiensis* endotoxin in insect populations by screening for mutations that alter the structure or function of any protein encoded by the nucleotide sequence set forth in SEQ ID NO: 1.

12. A method of detecting resistance to *Bacillus thuringiensis* endotoxin in insect populations by screening for mutations that alter the structure or function of any protein having at least a 60% similarity to the sequence of SEQ ID NO: 2.

13. A method for detecting mutations in genes from insect populations by screening for the presence of insertions of a DNA sequence set forth in Claim 8.

14. A process for monitoring Bt resistance associated with the presence of an r1 allele in an insect population associated with transgenic crops comprising the steps of:

obtaining DNA from an individual insect;

amplifying said DNA using primers having nucleotide sequences of SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 9.

measuring the molecular size of said amplified DNA, thereby determining whether said individual is a susceptible, a heterozygote, or a homozygote for said r1 allele.

15. A process for determining Bt resistance in an insect species containing one or more genes homologous to the HevCaLP gene comprising the steps of:

identifying a first gene encoding a cadherin-like protein;

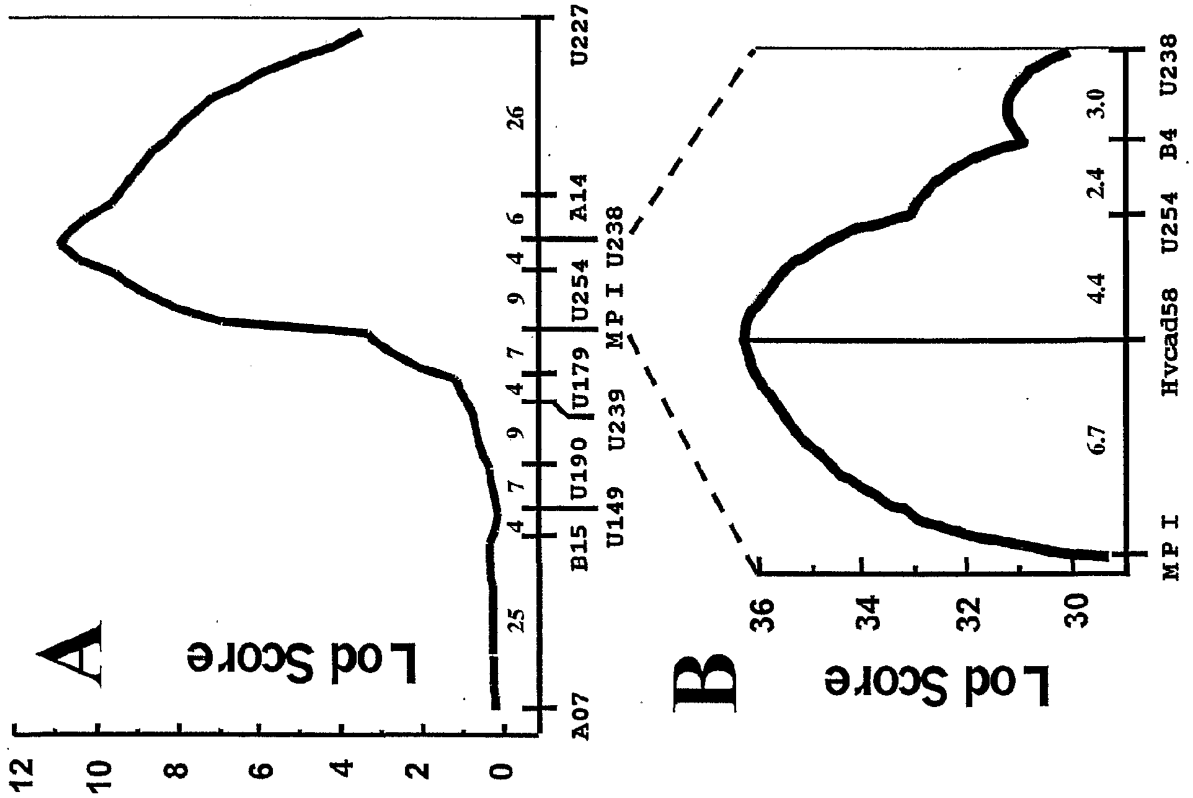
identifying a second gene, said second gene a mutant of said first gene, said mutant gene associated with Bt resistance;

producing a set of primers for PCR amplification of a sample of DNA containing either one of said first gene or said second gene, said set of primers having at least one individual primer unique for a mutated portion of said second gene;

amplifying DNA from an insect using said set of primers;

separating said amplified DNA according to size;

determining from said separated DNA whether said individual insect is a homozygote, a heterozygote, or a susceptible individual with respect to said mutant gene.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Lod Score
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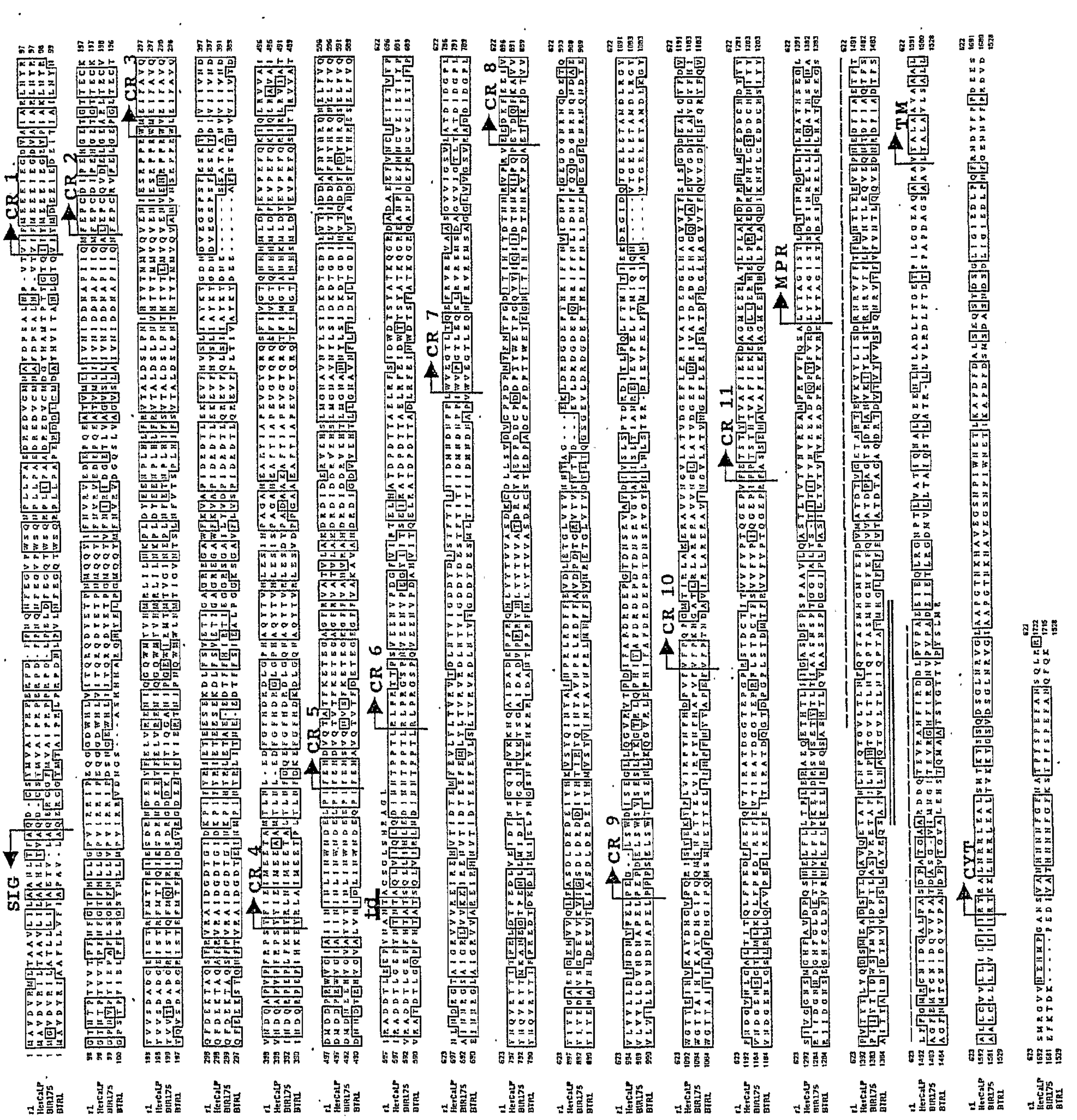
Fig. 1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CR 4
CR 5
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CR 8
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BtR175
BtR1

Fig. 2

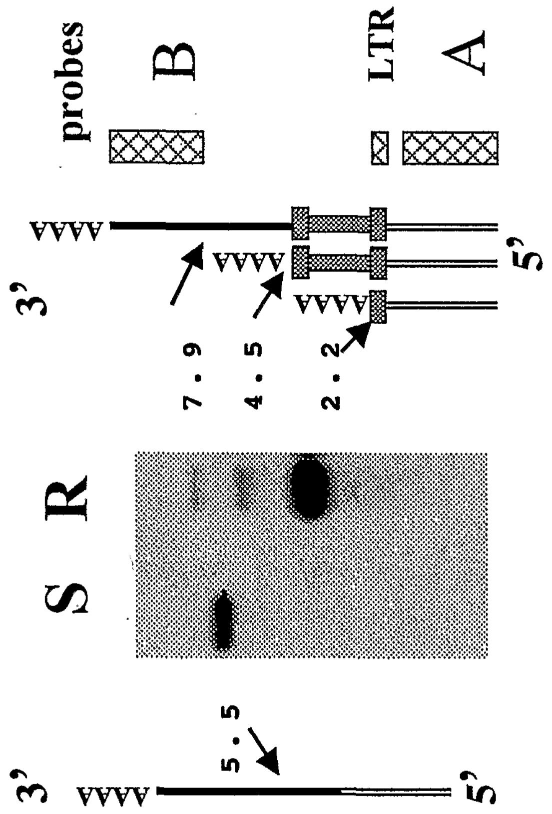
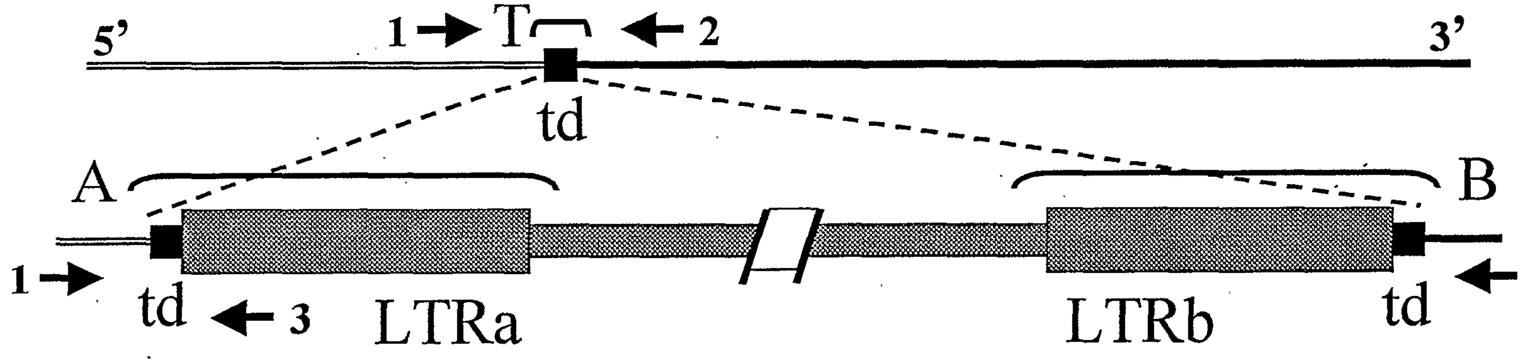


Fig. 3



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    Y N T N T A Q L V
T   TACAACACCAACACTGCCCAACTGGTG
    |||||||||||||||||
A   TACAACACCAACACTGCCTGTTCCGGACTGTCACATCGCGCCGGCCTATGAGGTCGCGCCAGCACACGTCATCGTGCGCCCCACCTAA
    GCTGGGC
B      |||      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
    gcttcaacccggggaataTGTTCCGGACTGTCACATCGCGCCGGCCTATGAGGTCGCGCCAGCACACGTCATCGTGCGCCCCACCTAAGCTG
    GGC
A

B   CCTCACCATACGCCGGACCCCCGGACACTCGCTCAGCGACCCCGGTCGCGCATACACGACCGCACGGGCAACGCGCGATTTTCTCTTG
    T-ACATA-CTT
    ||||||||||||||||||||||||||||||||||| |||||||||||||||||||||||||||||| |||||||||||| | ||||||| || || ||
A   CCTCACCATACGCCGGACCCCCGGACACTCGCTCATCGACCCCGGTCGCGCATACACGACCGCACGCGCAACGCGCGATCTACTCTTG
    TCACCTATCTA
B

T   CAATACAGTCTTCT—
    TTGCAAATCGAAGTTTCATTGAACCGCCGAGACCATCATCCTACATCTGGACCTCGGCGCTCAAgcattggtccctcgcaa
     ||||||||||||   ||| | |||||||||| |||||| |||||||||||| || |||||| ||| |||||||||||||| ||| |  |
    TAATACAGTCTTCTACTTTGAACATCGAAGTTTATTGAAACGCCGAGACCAGCAACCTACACCTGCACCTCGGCGCTCAAACACTGCCC
    AACTGGTG
                                           | | ||||||||||||||||||
Fig. 4                                    TACAACACCAACACTGCCCAACTGGTG
                                          Y N T N  T A Q L V
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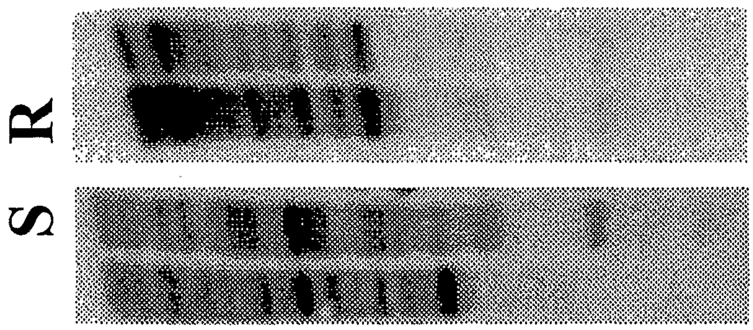

Fig. 5

SEQUENCE LISTING<110> Clemson University<120> Polynucleotide encoding a gene conferring resistance to Bacillus thuringiensis toxins<130> CXU-352-PCT<160> 9 <170> PatentIn version 3.1<210> 1<211> 5355<212> DNA<213> Heliothis virescens<220><221> CDS<222> (10)..(5208)<223>

<300><301> Linda J. Gahan and David G. Heckel<302> Identification of a gene associated with Bt resistance in Heliothis virescens<303> Science<304> 293<305> 5531<306> 857-860<307> 2001-08-03<308> GenBank AF367362<309> 2001-08-12<313> (1)..(5355)<300><308> GenBank AF367362<309> 2001-08-12<313> (1)..(5355)<400> 1

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          Met Ala Val Asp Val Arg Ile Leu Thr Ala Ala Val Leu Ile
          1               5                   10

ctc gct gct cat tta acg gtc gcg caa gat tgt tct tac atg gta gca       99
Leu Ala Ala His Leu Thr Val Ala Gln Asp Cys Ser Tyr Met Val Ala
15                  20                  25                  30

att cct aga cca gag cga cca gat ttt cct aat caa aat ttc gaa gga      147
Ile Pro Arg Pro Glu Arg Pro Asp Phe Pro Asn Gln Asn Phe Glu Gly
                35                  40                  45

gta cca tgg agt cag aac ccc ctg tta cca gcg gag gat agg gaa gat      195
Val Pro Trp Ser Gln Asn Pro Leu Leu Pro Ala Glu Asp Arg Glu Asp
            50                  55                  60

gtg tgc atg aac gcg ttc gac ccg agt gcc ttg aac ccc gtc acc gtc      243
Val Cys Met Asn Ala Phe Asp Pro Ser Ala Leu Asn Pro Val Thr Val
        65                  70                  75

atc ttc atg gag gag gag atc gaa ggg gac gtg gcc att gcc agg ctt      291
Ile Phe Met Glu Glu Glu Ile Glu Gly Asp Val Ala Ile Ala Arg Leu
    80                  85                  90

aac tac cga ggt acc aat act ccg acc gtg gta act cca ttt aac ttt      339
Asn Tyr Arg Gly Thr Asn Thr Pro Thr Val Val Thr Pro Phe Asn Phe
95                  100                 105                 110

ggt acc ttc cac ttg ttg ggg ccg gtc ata cgt agg atc ccc gag caa      387
Gly Thr Phe His Leu Leu Gly Pro Val Ile Arg Arg Ile Pro Glu Gln
                115                 120                 125

ggg ggg gac tgg cat ctt gtt att acg cag agg cag gac tat gag acc      435
Gly Gly Asp Trp His Leu Val Ile Thr Gln Arg Gln Asp Tyr Glu Thr
            130                 135                 140

ccg aac atg cag cag tat atc ttc aac gtg agg gta gaa gac gag ccc      483
Pro Asn Met Gln Gln Tyr Ile Phe Asn Val Arg Val Glu Asp Glu Pro
        145                 150                 155

cag gaa gcc act gtg atg ctc atc att gtc aac att gac gac aac gct      531
Gln Glu Ala Thr Val Met Leu Ile Ile Val Asn Ile Asp Asp Asn Ala
    160                 165                 170

cct atc ata cag atg ttc gag cct tgt gac att cct gaa cac ggc gaa      579
Pro Ile Ile Gln Met Phe Glu Pro Cys Asp Ile Pro Glu His Gly Glu
175                 180                 185                 190

acg ggc acc aca gaa tgc aag tac gtg gtg agc gat gct gac ggc gag      627
Thr Gly Thr Thr Glu Cys Lys Tyr Val Val Ser Asp Ala Asp Gly Glu
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                195                 200                 205

atc agc aca cgt ttc atg acg ttc caa atc gag agc gat cga aac gat        675
Ile Ser Thr Arg Phe Met Thr Phe Gln Ile Glu Ser Asp Arg Asn Asp
            210                 215                 220

gaa gaa tat ttc gaa ctc gtc aga gag aat att cag gga cag tgg atg        723
Glu Glu Tyr Phe Glu Leu Val Arg Glu Asn Ile Gln Gly Gln Trp Met
        225                 230                 235

tac gtc cat atg agg ctt atc ctc aac aaa cct ctg gac tat gag gaa        771
Tyr Val His Met Arg Leu Ile Leu Asn Lys Pro Leu Asp Tyr Glu Glu
    240                 245                 250

aac ccg ctg cat ttg ttt aga gtt aca gct ttg gat tcc cta cca aac        819
Asn Pro Leu His Leu Phe Arg Val Thr Ala Leu Asp Ser Leu Pro Asn
255                 260                 265                 270

gtt cac aca gta acg atg atg gtg caa gta gag aac ata gag agc aga        867
Val His Thr Val Thr Met Met Val Gln Val Glu Asn Ile Glu Ser Arg
                275                 280                 285

cca cca cgg tgg atg gag atc ttc gcc gtg cag cag ttc gat gag aag        915
Pro Pro Arg Trp Met Glu Ile Phe Ala Val Gln Gln Phe Asp Glu Lys
            290                 295                 300

aca gca caa gcc ttc agg gtt cga gcc atc gat gga gac acg gga atc        963
Thr Ala Gln Ala Phe Arg Val Arg Ala Ile Asp Gly Asp Thr Gly Ile
        305                 310                 315

gat aaa cct att ttc tat agg atc gaa act gaa gaa agc gag aaa gat       1011
Asp Lys Pro Ile Phe Tyr Arg Ile Glu Thr Glu Glu Ser Glu Lys Asp
    320                 325                 330

ttg ttc agt gtt gaa aca ata gga gct ggt cgg gaa ggt gct tgg ttt       1059
Leu Phe Ser Val Glu Thr Ile Gly Ala Gly Arg Glu Gly Ala Trp Phe
335                 340                 345                 350

aaa gtc gct cca ata gac agg gac act cta gaa aag gaa gtt ttc cac       1107
Lys Val Ala Pro Ile Asp Arg Asp Thr Leu Glu Lys Glu Val Phe His
                355                 360                 365

gtg tct cta ata gcg tac aaa tat ggc gac aat gac gtg gaa gga agt       1155
Val Ser Leu Ile Ala Tyr Lys Tyr Gly Asp Asn Asp Val Glu Gly Ser
            370                 375                 380

ccg tca ttc gag tcg aag acc gat atc gtc att att gtg aac gac gtg       1203
Pro Ser Phe Glu Ser Lys Thr Asp Ile Val Ile Ile Val Asn Asp Val
        385                 390                 395

aat gat cag gcg ccg gtg cca ttc cgt cct tca tac tac att gaa att       1251
Asn Asp Gln Ala Pro Val Pro Phe Arg Pro Ser Tyr Tyr Ile Glu Ile
    400                 405                 410

atg gag gaa gct gcg atg aca ttg aat tta gag gac ttt ggt ttc cac       1299
Met Glu Glu Ala Ala Met Thr Leu Asn Leu Glu Asp Phe Gly Phe His
415                 420                 425                 430

gat aga ggt ctt ggt ccg cac gca cag tac aca gtg cac ctg gag agc       1347
Asp Arg Gly Leu Gly Pro His Ala Gln Tyr Thr Val His Leu Glu Ser
                435                 440                 445
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atc tcc ccg gcg gga gcg cac gag gcg ttc tac atc gcg ccg gag gtg       1395
Ile Ser Pro Ala Gly Ala His Glu Ala Phe Tyr Ile Ala Pro Glu Val
            450                 455                 460

ggc tac cag cga cag tcc ttc atc gtc ggc acg cag aac cat cac atg       1443
Gly Tyr Gln Arg Gln Ser Phe Ile Val Gly Thr Gln Asn His His Met
        465                 470                 475

ctc gac ttt gaa gtg cca gag ttc caa aag ata caa ctt agg gcg gta       1491
Leu Asp Phe Glu Val Pro Glu Phe Gln Lys Ile Gln Leu Arg Ala Val
    480                 485                 490

gcc ata gac atg gac gat ccc agg tgg gtt ggt atc gcg att ata aac       1539
Ala Ile Asp Met Asp Asp Pro Arg Trp Val Gly Ile Ala Ile Ile Asn
495                 500                 505                 510

att aac ctg atc aac tgg aac gat gag ctg ccg atc ttc gag cac gac       1587
Ile Asn Leu Ile Asn Trp Asn Asp Glu Leu Pro Ile Phe Glu His Asp
                515                 520                 525

gtg cag act gtc acc ttc aag gag acg gag ggc gct ggc ttc cgg gtc       1635
Val Gln Thr Val Thr Phe Lys Glu Thr Glu Gly Ala Gly Phe Arg Val
            530                 535                 540

gcc act gtt ctg gca aag gac agg gat att gat gat aga gtc gaa cat       1683
Ala Thr Val Leu Ala Lys Asp Arg Asp Ile Asp Asp Arg Val Glu His
        545                 550                 555

tct cta atg ggc aac gca gtg aac tac ctg agt atc gac aaa gac acc       1731
Ser Leu Met Gly Asn Ala Val Asn Tyr Leu Ser Ile Asp Lys Asp Thr
    560                 565                 570

ggc gac atc ctc gtg aca atc gac gat gca ttt aac tat cac aga cag       1779
Gly Asp Ile Leu Val Thr Ile Asp Asp Ala Phe Asn Tyr His Arg Gln
575                 580                 585                 590

aac gag ctc ttt gtg cag ata cga gct gat gac acg ttg gga gag ccg       1827
Asn Glu Leu Phe Val Gln Ile Arg Ala Asp Asp Thr Leu Gly Glu Pro
                595                 600                 605

tat aat acc aac acc gcc caa ctg gtg ata cag ctg caa gac atc aac       1875
Tyr Asn Thr Asn Thr Ala Gln Leu Val Ile Gln Leu Gln Asp Ile Asn
            610                 615                 620

aac aca cct cct acg ctc aga ctg cct cgc acg act ccg tca gtg gaa       1923
Asn Thr Pro Pro Thr Leu Arg Leu Pro Arg Thr Thr Pro Ser Val Glu
        625                 630                 635

gag aac gtg ccg gac ggg ttc gtg atc ccc acc gag ctg cac gcc acc       1971
Glu Asn Val Pro Asp Gly Phe Val Ile Pro Thr Glu Leu His Ala Thr
    640                 645                 650

gac ccc gac acc acc gcc gag ctg cgc ttc agc atc gac tgg gac act       2019
Asp Pro Asp Thr Thr Ala Glu Leu Arg Phe Ser Ile Asp Trp Asp Thr
655                 660                 665                 670

tcc tat gcc acc aag cag gga cga gat gct gat gct gag gag ttt gtt       2067
Ser Tyr Ala Thr Lys Gln Gly Arg Asp Ala Asp Ala Glu Glu Phe Val
                675                 680                 685
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aat tgt ata gaa atc gag acg gta tac ccg aac ttg aac gac cga ggc      2115
Asn Cys Ile Glu Ile Glu Thr Val Tyr Pro Asn Leu Asn Asp Arg Gly
            690                 695                 700

acc gcc atc ggc cgc gtg gtg gtt cgc gag atc cgg gaa cac gtc acc      2163
Thr Ala Ile Gly Arg Val Val Val Arg Glu Ile Arg Glu His Val Thr
        705                 710                 715

atc gac tac gag atg ttc gag gtg ctg tac ctc acc gtc agg gtc acg      2211
Ile Asp Tyr Glu Met Phe Glu Val Leu Tyr Leu Thr Val Arg Val Thr
    720                 725                 730

gat ctc aac acg gtc att gga gac gac tat gat ata tca aca ttc acg      2259
Asp Leu Asn Thr Val Ile Gly Asp Asp Tyr Asp Ile Ser Thr Phe Thr
735                 740                 745                 750

atc att ata ata gac atg aac gac aac cct ccg ctg tgg gtg gaa ggc      2307
Ile Ile Ile Ile Asp Met Asn Asp Asn Pro Pro Leu Trp Val Glu Gly
                755                 760                 765

acg ctg acg cag gag ttc cgc gtg cga gag gtc gcc gcc tcg gga gtt      2355
Thr Leu Thr Gln Glu Phe Arg Val Arg Glu Val Ala Ala Ser Gly Val
            770                 775                 780

gtt ata gga tcc gta ctc gcc act gat atc gac gga cct ctt tat aat      2403
Val Ile Gly Ser Val Leu Ala Thr Asp Ile Asp Gly Pro Leu Tyr Asn
        785                 790                 795

caa gtg cgg tat acc atc act cct aga tta gat act cca gaa gac cta      2451
Gln Val Arg Tyr Thr Ile Thr Pro Arg Leu Asp Thr Pro Glu Asp Leu
    800                 805                 810

gtg gag atc gac ttc aat tcg ggt cag atc tca gtg aag aag cac cag      2499
Val Glu Ile Asp Phe Asn Ser Gly Gln Ile Ser Val Lys Lys His Gln
815                 820                 825                 830

gcc atc gac gcg gac gag ccg ccg cgc cag cac ctc tac tac acc gtg      2547
Ala Ile Asp Ala Asp Glu Pro Pro Arg Gln His Leu Tyr Tyr Thr Val
                835                 840                 845

gtc gcc agc gac aag tgc gac ctg ctc tct gtc gac gtg tgt ccg cct      2595
Val Ala Ser Asp Lys Cys Asp Leu Leu Ser Val Asp Val Cys Pro Pro
            850                 855                 860

gat cct aac tac ttc aac aca ccg gga gac ata acg atc cac ata aca      2643
Asp Pro Asn Tyr Phe Asn Thr Pro Gly Asp Ile Thr Ile His Ile Thr
        865                 870                 875

gac acg aac aac agg gtg cct cga gtg gaa gag gac aag ttc gag gaa      2691
Asp Thr Asn Asn Arg Val Pro Arg Val Glu Glu Asp Lys Phe Glu Glu
    880                 885                 890

att gtc tat atc tac gag ggc gcg gag gac gga gaa cac gtc gtg cag      2739
Ile Val Tyr Ile Tyr Glu Gly Ala Glu Asp Gly Glu His Val Val Gln
895                 900                 905                 910

ctc ttc gcc agc gat ctg gat aga gat gaa atc tac cac aaa gtg agc      2787
Leu Phe Ala Ser Asp Leu Asp Arg Asp Glu Ile Tyr His Lys Val Ser
                915                 920                 925

tac cag atc aac tac gcg atc aac cct cgt ctc cgc gac ttc ttc gag      2835
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Tyr Gln Ile Asn Tyr Ala Ile Asn Pro Arg Leu Arg Asp Phe Phe Glu
            930                 935                 940

gta gac ctg gag acc ggc ctg gtg tac gtc aac aac acg gcc ggg gag      2883
Val Asp Leu Glu Thr Gly Leu Val Tyr Val Asn Asn Thr Ala Gly Glu
        945                 950                 955

aag ctc gac cgg gac ggc gat gaa ccc acg cat cgg atc ttc ttc aac      2931
Lys Leu Asp Arg Asp Gly Asp Glu Pro Thr His Arg Ile Phe Phe Asn
    960                 965                 970

gtt atc gat aac ttc tat ggg gaa gga gat ggc aac cgg aac cag gac      2979
Val Ile Asp Asn Phe Tyr Gly Glu Gly Asp Gly Asn Arg Asn Gln Asp
975                 980                 985                 990

gag aca caa gtg tta gtg gtg ctg ttg gac  atc aac gac aac tat  ccg    3027
Glu Thr Gln Val Leu Val Val Leu Leu Asp  Ile Asn Asp Asn Tyr  Pro
                995                 1000                1005

gag ctg cct gag  ggt ctc tca tgg gat  atc tct gag gga ttg  cta       3072
Glu Leu Pro Glu  Gly Leu Ser Trp Asp  Ile Ser Glu Gly Leu  Leu
            1010                 1015                 1020

cag ggt gtc cgt  gta acc cca gat atc  ttc gcc ccg gac cgc  gac       3117
Gln Gly Val Arg  Val Thr Pro Asp Ile  Phe Ala Pro Asp Arg  Asp
            1025                 1030                 1035

gag ccc ggc acc  gac aac tcc cgc gtg  gcg tac gac atc gtc  agc       3162
Glu Pro Gly Thr  Asp Asn Ser Arg Val  Ala Tyr Asp Ile Val  Ser
            1040                 1045                 1050

ctc tcg ccc acc  gac agg gac atc aca  ctt cct caa ctc ttc  acc       3207
Leu Ser Pro Thr  Asp Arg Asp Ile Thr  Leu Pro Gln Leu Phe  Thr
            1055                 1060                 1065

atg atc acc ata  gag aag gac agg ggc  atc gac cag act gga  gag       3252
Met Ile Thr Ile  Glu Lys Asp Arg Gly  Ile Asp Gln Thr Gly  Glu
            1070                 1075                 1080

ctg gag acc gct  atg gat tta aga ggc  tat tgg ggc act tat  gaa       3297
Leu Glu Thr Ala  Met Asp Leu Arg Gly  Tyr Trp Gly Thr Tyr  Glu
            1085                 1090                 1095

ata cat gta aag  gca tac gac cat gga  gta cct caa agg att  tcc       3342
Ile His Val Lys  Ala Tyr Asp His Gly  Val Pro Gln Arg Ile  Ser
            1100                 1105                 1110

tac gag aag tac  ccg cta gtt att aga  cct tac aac ttc cac  gac       3387
Tyr Glu Lys Tyr  Pro Leu Val Ile Arg  Pro Tyr Asn Phe His  Asp
            1115                 1120                 1125

cct gtg ttc gtg  ttc cct caa cct gga  atg act atc aga ctc  gcg       3432
Pro Val Phe Val  Phe Pro Gln Pro Gly  Met Thr Ile Arg Leu  Ala
            1130                 1135                 1140

aag gag cga gca  gta gtg aac gga gtg  ctg gcg aca gtg gac  ggc       3477
Lys Glu Arg Ala  Val Val Asn Gly Val  Leu Ala Thr Val Asp  Gly
            1145                 1150                 1155

gag ttc ctc gag  cga ata gtc gcc acc  gac gag gat ggc tta  cac       3522
Glu Phe Leu Glu  Arg Ile Val Ala Thr  Asp Glu Asp Gly Leu  His
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            1160                1165                1170

gct gga gtt gtt  acc ttc tct atc tcg  gga gat gat gag gcg  ttg       3567
Ala Gly Val Val  Thr Phe Ser Ile Ser  Gly Asp Asp Glu Ala  Leu
            1175                1180                1185

cag tac ttc gac  gtg ttt aac gac gga  gtg aac ttg ggt gcg  ctg       3612
Gln Tyr Phe Asp  Val Phe Asn Asp Gly  Val Asn Leu Gly Ala  Leu
            1190                1195                1200

acc atc acg cag  ctc ttc cct gaa gac  ttc cga gag ttt cag  gtg       3657
Thr Ile Thr Gln  Leu Phe Pro Glu Asp  Phe Arg Glu Phe Gln  Val
            1205                1210                1215

acg att cgt gct  acg gat ggt ggt acg  gag cct ggt cca agg  agt       3702
Thr Ile Arg Ala  Thr Asp Gly Gly Thr  Glu Pro Gly Pro Arg  Ser
            1220                1225                1230

acc gac tgc acg  atc acc gta gtg ttt  gtg cct acg cag gga  gag       3747
Thr Asp Cys Thr  Ile Thr Val Val Phe  Val Pro Thr Gln Gly  Glu
            1235                1240                1245

cct gtg ttc gaa  act agc acc tac acg  gtc gct ttt atc gag  aaa       3792
Pro Val Phe Glu  Thr Ser Thr Tyr Thr  Val Ala Phe Ile Glu  Lys
            1250                1255                1260

gat gct ggt atg  gaa gag cgg gcc acg  ctg cct ctc gcc aag  gac       3837
Asp Ala Gly Met  Glu Glu Arg Ala Thr  Leu Pro Leu Ala Lys  Asp
            1265                1270                1275

ccg cgt aac ata  atg tgt gaa gat gat  tgt cac gac acc tat  tac       3882
Pro Arg Asn Ile  Met Cys Glu Asp Asp  Cys His Asp Thr Tyr  Tyr
            1280                1285                1290

agc att gtt gga  ggc aac tcg atg ggc  cac ttt gcg gtg gac  cct       3927
Ser Ile Val Gly  Gly Asn Ser Met Gly  His Phe Ala Val Asp  Pro
            1295                1300                1305

cag tcc aac gag  ctg ttc ctg ctg acg  cca ctg gag cgc gcg  gag       3972
Gln Ser Asn Glu  Leu Phe Leu Leu Thr  Pro Leu Glu Arg Ala  Glu
            1310                1315                1320

cag gag acg cac  acc ctc atc atc ggc  gcc agc gac tcg ccc  agc       4017
Gln Glu Thr His  Thr Leu Ile Ile Gly  Ala Ser Asp Ser Pro  Ser
            1325                1330                1335

cca gcc gcc gtg  ctg cag gct tct acc  ctc act gtt act gtc  aat       4062
Pro Ala Ala Val  Leu Gln Ala Ser Thr  Leu Thr Val Thr Val  Asn
            1340                1345                1350

gtt cga gaa gca  aac ccg cgg cca gtg  ttc cag agc gct ctg  tac       4107
Val Arg Glu Ala  Asn Pro Arg Pro Val  Phe Gln Ser Ala Leu  Tyr
            1355                1360                1365

aca gcc ggc atc  tcc acc ctc gac acc  atc aac aga ggt ctg  cta       4152
Thr Ala Gly Ile  Ser Thr Leu Asp Thr  Ile Asn Arg Gly Leu  Leu
            1370                1375                1380

acg cta cac gcg  act cat tca gaa ggc  ttg cct gtg acc tac  acg       4197
Thr Leu His Ala  Thr His Ser Glu Gly  Leu Pro Val Thr Tyr  Thr
            1385                1390                1395
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ctg gta caa gac  tcc atg gaa gct gac  tcc aca ctg caa gct  gtg        4242
Leu Val Gln Asp  Ser Met Glu Ala Asp  Ser Thr Leu Gln Ala  Val
            1400                 1405                 1410

cag gag aca gcc  ttc aac ttg aac cct  cag act gga gtg ctg  acc        4287
Gln Glu Thr Ala  Phe Asn Leu Asn Pro  Gln Thr Gly Val Leu  Thr
            1415                 1420                 1425

ctc aac ttc cag  ccc aca gca tct atg  cac ggc atg ttt gag  ttc        4332
Leu Asn Phe Gln  Pro Thr Ala Ser Met  His Gly Met Phe Glu  Phe
            1430                 1435                 1440

gat gtg atg gct  act gat aca gtg gga  gaa acc gcg cgc acc  gaa        4377
Asp Val Met Ala  Thr Asp Thr Val Gly  Glu Thr Ala Arg Thr  Glu
            1445                 1450                 1455

gtg aag gtg tac  ctg ata tcc gac cgc  aac aga gtg ttc ttc  acg        4422
Val Lys Val Tyr  Leu Ile Ser Asp Arg  Asn Arg Val Phe Phe  Thr
            1460                 1465                 1470

ttc atg aac acg  ctt gaa gaa gtc gaa  ccg aat gaa gat ttc  ata        4467
Phe Met Asn Thr  Leu Glu Glu Val Glu  Pro Asn Glu Asp Phe  Ile
            1475                 1480                 1485

gcg gag aca ttt  acc ctg ttc ttc ggc  atg cgg tgc aac atc  gac        4512
Ala Glu Thr Phe  Thr Leu Phe Phe Gly  Met Arg Cys Asn Ile  Asp
            1490                 1495                 1500

cag gcg ctg ccc  gcc agc gac ccc gcc  acc ggc gcc gcc agg  gac        4557
Gln Ala Leu Pro  Ala Ser Asp Pro Ala  Thr Gly Ala Ala Arg  Asp
            1505                 1510                 1515

gac cag acc gaa  gtc agg gca cac ttc  ata cgc gac gac ctg  cct        4602
Asp Gln Thr Glu  Val Arg Ala His Phe  Ile Arg Asp Asp Leu  Pro
            1520                 1525                 1530

gta cct gct gag  gag atc gaa caa tta  cgc ggc aac ccg acc  cta        4647
Val Pro Ala Glu  Glu Ile Glu Gln Leu  Arg Gly Asn Pro Thr  Leu
            1535                 1540                 1545

gtg gcg acc atc  cag aac gcc ctg cag  gag gag aac ctg aac  ctg        4692
Val Ala Thr Ile  Gln Asn Ala Leu Gln  Glu Glu Asn Leu Asn  Leu
            1550                 1555                 1560

gcc gac ctg ttc  acg ggc gag act ccc  atc ctg ggc ggc gag  gcg        4737
Ala Asp Leu Phe  Thr Gly Glu Thr Pro  Ile Leu Gly Gly Glu  Ala
            1565                 1570                 1575

cag gcg cgg gcg  gtc tat gct ctc gcg  gcg gtg gcg gct gcg  ctc        4782
Gln Ala Arg Ala  Val Tyr Ala Leu Ala  Ala Val Ala Ala Ala  Leu
            1580                 1585                 1590

gcg ctg ctc tgc  gtc gtg ctg ctt ata  ctc ttc ttc atc agg  act        4827
Ala Leu Leu Cys  Val Val Leu Leu Ile  Leu Phe Phe Ile Arg  Thr
            1595                 1600                 1605

agg gcc ctc aac  cgt cgc ctg gaa gcc  cta tcc atg acc aag  tac        4872
Arg Ala Leu Asn  Arg Arg Leu Glu Ala  Leu Ser Met Thr Lys  Tyr
            1610                 1615                 1620
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agt tcc caa gac  tca gga ctc aac cgc  gtg ggt ctg gcg gcg  ccg       4917
Ser Ser Gln Asp  Ser Gly Leu Asn Arg  Val Gly Leu Ala Ala  Pro
            1625                 1630                 1635

ggc acc aac aag  cac gcg gtg gag ggc  tcc aac ccc atc tgg  aac       4962
Gly Thr Asn Lys  His Ala Val Glu Gly  Ser Asn Pro Ile Trp  Asn
            1640                 1645                 1650

gaa act ctt aag  gca ccg gac ttt gat  gct ctt agc gag cag  tcg       5007
Glu Thr Leu Lys  Ala Pro Asp Phe Asp  Ala Leu Ser Glu Gln  Ser
            1655                 1660                 1665

tac gac tcg ggt  ctg atc ggc atc gaa  gac ttg ccg cag ttc  agg       5052
Tyr Asp Ser Gly  Leu Ile Gly Ile Glu  Asp Leu Pro Gln Phe  Arg
            1670                 1675                 1680

aac gac tac ttc  ccg cct gac gag gag  agc tcc atg cgg gga  gtc       5097
Asn Asp Tyr Phe  Pro Pro Asp Glu Glu  Ser Ser Met Arg Gly  Val
            1685                 1690                 1695

gtc aat gaa cac  atg cct gga gct aat  tca gta gca aac cat  aac       5142
Val Asn Glu His  Met Pro Gly Ala Asn  Ser Val Ala Asn His  Asn
            1700                 1705                 1710

aat aac ttc ggg  ttc aac gct acc ccc  ttc agc cca gag ttc  gcg       5187
Asn Asn Phe Gly  Phe Asn Ala Thr Pro  Phe Ser Pro Glu Phe  Ala
            1715                 1720                 1725

aac tcg cag ctc  agg aga taa aacattatag tattttttat ataatattat        5238
Asn Ser Gln Leu  Arg Arg
            1730

aaagaagtga tataacgcac taaaatttac ctataagtat atattgaagt gtaagatact   5298

cgtattatgt aagagcatct attttttac caccagacaa taaaaacttt ataaaag       5355


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Met Ala Val Asp Val Arg Ile Leu Thr Ala Ala Val Leu Ile Leu Ala
1               5                   10                  15


Ala His Leu Thr Val Ala Gln Asp Cys Ser Tyr Met Val Ala Ile Pro
            20                  25                  30


Arg Pro Glu Arg Pro Asp Phe Pro Asn Gln Asn Phe Glu Gly Val Pro
        35                  40                  45


Trp Ser Gln Asn Pro Leu Leu Pro Ala Glu Asp Arg Glu Asp Val Cys
    50                  55                  60


Met Asn Ala Phe Asp Pro Ser Ala Leu Asn Pro Val Thr Val Ile Phe
65                  70                  75                  80


Met Glu Glu Glu Ile Glu Gly Asp Val Ala Ile Ala Arg Leu Asn Tyr
                85                  90                  95
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Arg Gly Thr Asn Thr Pro Thr Val Val Thr Pro Phe Asn Phe Gly Thr
            100                 105                 110

Phe His Leu Leu Gly Pro Val Ile Arg Arg Ile Pro Glu Gln Gly Gly
        115                 120                 125

Asp Trp His Leu Val Ile Thr Gln Arg Gln Asp Tyr Glu Thr Pro Asn
    130                 135                 140

Met Gln Gln Tyr Ile Phe Asn Val Arg Val Glu Asp Glu Pro Gln Glu
145                 150                 155                 160

Ala Thr Val Met Leu Ile Ile Val Asn Ile Asp Asp Asn Ala Pro Ile
                165                 170                 175

Ile Gln Met Phe Glu Pro Cys Asp Ile Pro Glu His Gly Glu Thr Gly
            180                 185                 190

Thr Thr Glu Cys Lys Tyr Val Val Ser Asp Ala Asp Gly Glu Ile Ser
        195                 200                 205

Thr Arg Phe Met Thr Phe Gln Ile Glu Ser Asp Arg Asn Asp Glu Glu
    210                 215                 220

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

His Met Arg Leu Ile Leu Asn Lys Pro Leu Asp Tyr Glu Glu Asn Pro
                245                 250                 255

Leu His Leu Phe Arg Val Thr Ala Leu Asp Ser Leu Pro Asn Val His
            260                 265                 270

Thr Val Thr Met Met Val Gln Val Glu Asn Ile Glu Ser Arg Pro Pro
        275                 280                 285

Arg Trp Met Glu Ile Phe Ala Val Gln Gln Phe Asp Glu Lys Thr Ala
    290                 295                 300

Gln Ala Phe Arg Val Arg Ala Ile Asp Gly Asp Thr Gly Ile Asp Lys
305                 310                 315                 320

Pro Ile Phe Tyr Arg Ile Glu Thr Glu Glu Ser Glu Lys Asp Leu Phe
                325                 330                 335
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Ser Val Glu Thr Ile Gly Ala Gly Arg Glu Gly Ala Trp Phe Lys Val
340 345 350

Ala Pro Ile Asp Arg Asp Thr Leu Glu Lys Glu Val Phe His Val Ser
355 360 365

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

Phe Glu Ser Lys Thr Asp Ile Val Ile Ile Val Asn Asp Val Asn Asp
385 390 395 400

Gln Ala Pro Val Pro Phe Arg Pro Ser Tyr Tyr Ile Glu Ile Met Glu
405 410 415

Glu Ala Ala Met Thr Leu Asn Leu Glu Asp Phe Gly Phe His Asp Arg
420 425 430

Gly Leu Gly Pro His Ala Gln Tyr Thr Val His Leu Glu Ser Ile Ser
435 440 445

Pro Ala Gly Ala His Glu Ala Phe Tyr Ile Ala Pro Glu Val Gly Tyr
450 455 460

Gln Arg Gln Ser Phe Ile Val Gly Thr Gln Asn His His Met Leu Asp
465 470 475 480

Phe Glu Val Pro Glu Phe Gln Lys Ile Gln Leu Arg Ala Val Ala Ile
485 490 495

Asp Met Asp Asp Pro Arg Trp Val Gly Ile Ala Ile Ile Asn Ile Asn
500 505 510

Leu Ile Asn Trp Asn Asp Glu Leu Pro Ile Phe Glu His Asp Val Gln
515 520 525

Thr Val Thr Phe Lys Glu Thr Glu Gly Ala Gly Phe Arg Val Ala Thr
530 535 540

Val Leu Ala Lys Asp Arg Asp Ile Asp Asp Arg Val Glu His Ser Leu
545 550 555 560

Met Gly Asn Ala Val Asn Tyr Leu Ser Ile Asp Lys Asp Thr Gly Asp
565 570 575

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Ile Leu Val Thr Ile Asp Asp Ala Phe Asn Tyr His Arg Gln Asn Glu
            580                 585                 590

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

Thr Asn Thr Ala Gln Leu Val Ile Gln Leu Gln Asp Ile Asn Asn Thr
    610                 615                 620

Pro Pro Thr Leu Arg Leu Pro Arg Thr Thr Pro Ser Val Glu Glu Asn
625                 630                 635                 640

Val Pro Asp Gly Phe Val Ile Pro Thr Glu Leu His Ala Thr Asp Pro
                645                 650                 655

Asp Thr Thr Ala Glu Leu Arg Phe Ser Ile Asp Trp Asp Thr Ser Tyr
            660                 665                 670

Ala Thr Lys Gln Gly Arg Asp Ala Asp Ala Glu Glu Phe Val Asn Cys
        675                 680                 685

Ile Glu Ile Glu Thr Val Tyr Pro Asn Leu Asn Asp Arg Gly Thr Ala
    690                 695                 700

Ile Gly Arg Val Val Val Arg Glu Ile Arg Glu His Val Thr Ile Asp
705                 710                 715                 720

Tyr Glu Met Phe Glu Val Leu Tyr Leu Thr Val Arg Val Thr Asp Leu
                725                 730                 735

Asn Thr Val Ile Gly Asp Asp Tyr Asp Ile Ser Thr Phe Thr Ile Ile
            740                 745                 750

Ile Ile Asp Met Asn Asp Asn Pro Pro Leu Trp Val Glu Gly Thr Leu
        755                 760                 765

Thr Gln Glu Phe Arg Val Arg Glu Val Ala Ala Ser Gly Val Val Ile
    770                 775                 780

Gly Ser Val Leu Ala Thr Asp Ile Asp Gly Pro Leu Tyr Asn Gln Val
785                 790                 795                 800

Arg Tyr Thr Ile Thr Pro Arg Leu Asp Thr Pro Glu Asp Leu Val Glu
                805                 810                 815

Ile Asp Phe Asn Ser Gly Gln Ile Ser Val Lys Lys His Gln Ala Ile
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            820                 825                 830

Asp Ala Asp Glu Pro Pro Arg Gln His Leu Tyr Tyr Thr Val Val Ala
        835                 840                 845

Ser Asp Lys Cys Asp Leu Leu Ser Val Asp Val Cys Pro Pro Asp Pro
    850                 855                 860

Asn Tyr Phe Asn Thr Pro Gly Asp Ile Thr Ile His Ile Thr Asp Thr
865                 870                 875                 880

Asn Asn Arg Val Pro Arg Val Glu Glu Asp Lys Phe Glu Glu Ile Val
                885                 890                 895

Tyr Ile Tyr Glu Gly Ala Glu Asp Gly Glu His Val Val Gln Leu Phe
            900                 905                 910

Ala Ser Asp Leu Asp Arg Asp Glu Ile Tyr His Lys Val Ser Tyr Gln
        915                 920                 925

Ile Asn Tyr Ala Ile Asn Pro Arg Leu Arg Asp Phe Phe Glu Val Asp
    930                 935                 940

Leu Glu Thr Gly Leu Val Tyr Val Asn Asn Thr Ala Gly Glu Lys Leu
945                 950                 955                 960

Asp Arg Asp Gly Asp Glu Pro Thr His Arg Ile Phe Phe Asn Val Ile
                965                 970                 975

Asp Asn Phe Tyr Gly Glu Gly Asp Gly Asn Arg Asn Gln Asp Glu Thr
            980                 985                 990

Gln Val Leu Val Val Leu Leu Asp  Ile Asn Asp Asn Tyr  Pro Glu Leu
        995                 1000                 1005

Pro Glu  Gly Leu Ser Trp Asp  Ile Ser Glu Gly Leu  Leu Gln Gly
    1010                 1015                 1020

Val Arg  Val Thr Pro Asp Ile  Phe Ala Pro Asp Arg  Asp Glu Pro
    1025                 1030                 1035

Gly Thr  Asp Asn Ser Arg Val  Ala Tyr Asp Ile Val  Ser Leu Ser
    1040                 1045                 1050

Pro Thr  Asp Arg Asp Ile Thr  Leu Pro Gln Leu Phe  Thr Met Ile
    1055                 1060                 1065
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Thr Ile  Glu Lys Asp Arg Gly  Ile Asp Gln Thr Gly  Glu Leu Glu
    1070                 1075                 1080

Thr Ala  Met Asp Leu Arg Gly  Tyr Trp Gly Thr Tyr  Glu Ile His
    1085                 1090                 1095

Val Lys  Ala Tyr Asp His Gly  Val Pro Gln Arg Ile  Ser Tyr Glu
    1100                 1105                 1110

Lys Tyr  Pro Leu Val Ile Arg  Pro Tyr Asn Phe His  Asp Pro Val
    1115                 1120                 1125

Phe Val  Phe Pro Gln Pro Gly  Met Thr Ile Arg Leu  Ala Lys Glu
    1130                 1135                 1140

Arg Ala  Val Val Asn Gly Val  Leu Ala Thr Val Asp  Gly Glu Phe
    1145                 1150                 1155

Leu Glu  Arg Ile Val Ala Thr  Asp Glu Asp Gly Leu  His Ala Gly
    1160                 1165                 1170

Val Val  Thr Phe Ser Ile Ser  Gly Asp Asp Glu Ala  Leu Gln Tyr
    1175                 1180                 1185

Phe Asp  Val Phe Asn Asp Gly  Val Asn Leu Gly Ala  Leu Thr Ile
    1190                 1195                 1200

Thr Gln  Leu Phe Pro Glu Asp  Phe Arg Glu Phe Gln  Val Thr Ile
    1205                 1210                 1215

Arg Ala  Thr Asp Gly Gly Thr  Glu Pro Gly Pro Arg  Ser Thr Asp
    1220                 1225                 1230

Cys Thr  Ile Thr Val Val Phe  Val Pro Thr Gln Gly  Glu Pro Val
    1235                 1240                 1245

Phe Glu  Thr Ser Thr Tyr Thr  Val Ala Phe Ile Glu  Lys Asp Ala
    1250                 1255                 1260

Gly Met  Glu Glu Arg Ala Thr  Leu Pro Leu Ala Lys  Asp Pro Arg
    1265                 1270                 1275

Asn Ile  Met Cys Glu Asp Asp  Cys His Asp Thr Tyr  Tyr Ser Ile
    1280                 1285                 1290
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Val Gly  Gly Asn Ser Met Gly  His Phe Ala Val Asp  Pro Gln Ser
    1295                 1300                 1305

Asn Glu  Leu Phe Leu Leu Thr  Pro Leu Glu Arg Ala  Glu Gln Glu
    1310                 1315                 1320

Thr His  Thr Leu Ile Ile Gly  Ala Ser Asp Ser Pro  Ser Pro Ala
    1325                 1330                 1335

Ala Val  Leu Gln Ala Ser Thr  Leu Thr Val Thr Val  Asn Val Arg
    1340                 1345                 1350

Glu Ala  Asn Pro Arg Pro Val  Phe Gln Ser Ala Leu  Tyr Thr Ala
    1355                 1360                 1365

Gly Ile  Ser Thr Leu Asp Thr  Ile Asn Arg Gly Leu  Leu Thr Leu
    1370                 1375                 1380

His Ala  Thr His Ser Glu Gly  Leu Pro Val Thr Tyr  Thr Leu Val
    1385                 1390                 1395

Gln Asp  Ser Met Glu Ala Asp  Ser Thr Leu Gln Ala  Val Gln Glu
    1400                 1405                 1410

Thr Ala  Phe Asn Leu Asn Pro  Gln Thr Gly Val Leu  Thr Leu Asn
    1415                 1420                 1425

Phe Gln  Pro Thr Ala Ser Met  His Gly Met Phe Glu  Phe Asp Val
    1430                 1435                 1440

Met Ala  Thr Asp Thr Val Gly  Glu Thr Ala Arg Thr  Glu Val Lys
    1445                 1450                 1455

Val Tyr  Leu Ile Ser Asp Arg  Asn Arg Val Phe Phe  Thr Phe Met
    1460                 1465                 1470

Asn Thr  Leu Glu Glu Val Glu  Pro Asn Glu Asp Phe  Ile Ala Glu
    1475                 1480                 1485

Thr Phe  Thr Leu Phe Phe Gly  Met Arg Cys Asn Ile  Asp Gln Ala
    1490                 1495                 1500

Leu Pro  Ala Ser Asp Pro Ala  Thr Gly Ala Ala Arg  Asp Asp Gln
    1505                 1510                 1515
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Thr Glu  Val Arg Ala His Phe  Ile Arg Asp Asp Leu  Pro Val Pro
    1520                 1525                 1530

Ala Glu  Glu Ile Glu Gln Leu  Arg Gly Asn Pro Thr  Leu Val Ala
    1535                 1540                 1545

Thr Ile  Gln Asn Ala Leu Gln  Glu Glu Asn Leu Asn  Leu Ala Asp
    1550                 1555                 1560

Leu Phe  Thr Gly Glu Thr Pro  Ile Leu Gly Gly Glu  Ala Gln Ala
    1565                 1570                 1575

Arg Ala  Val Tyr Ala Leu Ala  Ala Val Ala Ala Ala  Leu Ala Leu
    1580                 1585                 1590

Leu Cys  Val Val Leu Leu Ile  Leu Phe Phe Ile Arg  Thr Arg Ala
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