Office de la Propriété Intellectuelle du Canada
Un organisme d'Industrie Canada

Canadian Intellectual Property Office
An agency of Industry Canada

CA 2690903 A1 2008/12/18

(21) 2 690 903

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2008/06/09
(87) Date publication PCT/PCT Publication Date: 2008/12/18
(85) Entrée phase nationale/National Entry: 2009/12/08
(86) N° demande PCT/PCT Application No.: EP 2008/057132
(87) N° publication PCT/PCT Publication No.: 2008/152008
(30) Priorité/Priority: 2007/06/15 (US60/944,229)

(51) Cl.Int./Int.Cl. *C12N 15/82* (2006.01), *A01H 5/00* (2006.01), *C12N 15/11* (2006.01)

(71) Demandeur/Applicant:
BASF PLANT SCIENCE GMBH, DE

(72) Inventeurs/Inventors:
REN, PEIFENG, US;
CHAUDHURI, SUMITA, US;
TALTON, LAWRENCE WINFIELD, US;
HUANG, XIANG, US;
MCMILLAN, JOHN, US

(74) Agent: ROBIC

(54) Titre : COMPOSITIONS ET METHODES D'UTILISATION DE L'INTERFERENCE ARN POUR LUTTER CONTRE LES NEMATODES

(54) Title: COMPOSITIONS AND METHODS OF USING RNA INTERFERENCE FOR CONTROL OF NEMATODES

(57) Abrégé/Abstract:
The present invention relates to the use of RNA interference to inhibit expression of plant parasitic nematode target let-70 genes, and relates to the generation of plants that have increased resistance to parasitic nematodes.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 December 2008 (18.12.2008)

PCT

(10) International Publication Number
WO 2008/152008 A3

(51) International Patent Classification:
C12N 15/82 (2006.01) *A01H 5/00* (2006.01)
C12N 15/11 (2006.01)

(21) International Application Number:
PCT/EP2008/057132

(22) International Filing Date: 9 June 2008 (09.06.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/944,229 15 June 2007 (15.06.2007) US

(71) Applicant *(for all designated States except US)*: **BASF PLANT SCIENCE GMBH** [DE/DE]; 67056 Ludwigshafen (DE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **REN, Peifeng** [US/US]; 206 Wedgemere Street, Cary, NC 27519 (US). **CHAUDHURI, Sumita** [US/US]; 102 Barnes Spring Court, Cary, NC 27519 (US). **TALTON, Lawrence Winfield** [US/US]; 108 Cricket Hearth, Sanford, NC 27330 (US). **HUANG, Xiang** [CN/US]; 2007 Sassacus Lane, Apex, NC 27523 (US). **MCMILLAN, John** [US/US]; 8345 Nantahala Drive, Raleigh, NC 27612 (US).

(74) Agent: **POPP, Andreas**; BASF SE, Gvx/b - C 6, 67056 Ludwigshafen (DE).

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

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

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*
— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

(88) Date of publication of the international search report:
12 March 2009

WO 2008/152008 A3

(54) Title: COMPOSITIONS AND METHODS OF USING RNA INTERFERENCE FOR CONTROL OF NEMATODES

(57) Abstract: The present invention relates to the use of RNA interference to inhibit expression of plant parasitic nematode target let-70 genes, and relates to the generation of plants that have increased resistance to parasitic nematodes.

COMPOSITIONS AND METHODS OF USING RNA INTERFERENCE FOR CONTROL OF NEMATODES

[0001] This application claims priority benefit of U.S. provisional patent application Serial Number 60/944,229, filed June 15, 2007.

[0002] The field of this invention is the control of nematodes, in particular the control of soybean cyst nematodes. The invention also relates to the introduction of genetic material into plants that are susceptible to nematodes in order to increase resistance to nematodes.

BACKGROUND OF THE INVENTION

[0003] Nematodes are microscopic roundworms that feed on the roots, leaves and stems of more than 2,000 row crops, vegetables, fruits, and ornamental plants, causing an estimated $100 billion crop loss worldwide. A variety of parasitic nematode species infect crop plants, including root-knot nematodes (RKN), cyst- and lesion-forming nematodes. Root-knot nematodes, which are characterized by causing root gall formation at feeding sites, have a relatively broad host range and are therefore pathogenic on a large number of crop species. The cyst- and lesion-forming nematode species have a more limited host range, but still cause considerable losses in susceptible crops.

[0004] Pathogenic nematodes are present throughout the United States, with the greatest concentrations occurring in the warm, humid regions of the South and West and in sandy soils. Soybean cyst nematode (Heterodera glycines), the most serious pest of soybean plants, was first discovered in the United States in North Carolina in 1954. Some areas are so heavily infested by soybean cyst nematode (SCN) that soybean production is no longer economically possible without control measures. Although soybean is the major economic crop attacked by SCN, SCN parasitizes some fifty hosts in total, including field crops, vegetables, ornamentals, and weeds.

[0005] Signs of nematode damage include stunting and yellowing of leaves, and wilting of the plants during hot periods. However, nematode infestation can cause significant yield losses without any obvious above-ground disease symptoms. The primary causes of yield reduction are due to root damage underground. Roots infected by SCN are dwarfed or stunted. Nematode infestation also can decrease the number of nitrogen-fixing nodules on the roots, and may make the roots more susceptible to attacks by other soil-borne plant pathogens.

[0006] The nematode life cycle has three major stages: egg, juvenile, and adult. The life cycle varies between species of nematodes. For example, the SCN life cycle can usually be completed in 24 to 30 days under optimum conditions whereas other species can take as long as a year, or longer, to complete the life cycle. When temperature and moisture levels become favorable in the spring, worm-shaped juveniles hatch from eggs in the soil. Only nematodes in the juvenile developmental stage are capable of infecting soybean roots.

[0007] The life cycle of SCN has been the subject of many studies, and as such are a useful example for understanding the nematode life cycle. After penetrating soybean roots, SCN juveniles move through the root until they contact vascular tissue, at which time they stop migrating and begin to feed. With a stylet, the nematode injects secretions that modify certain root cells and transform them into specialized feeding sites. The root cells are morphologically trans-

formed into large multinucleate syncytia (or giant cells in the case of RKN), which are used as a source of nutrients for the nematodes. The actively feeding nematodes thus steal essential nutrients from the plant resulting in yield loss. As female nematodes feed, they swell and eventually become so large that their bodies break through the root tissue and are exposed on the surface of the root.

[0008] After a period of feeding, male SCN nematodes, which are not swollen as adults, migrate out of the root into the soil and fertilize the enlarged adult females. The males then die, while the females remain attached to the root system and continue to feed. The eggs in the swollen females begin developing, initially in a mass or egg sac outside the body, and then later within the nematode body cavity. Eventually the entire adult female body cavity is filled with eggs, and the nematode dies. It is the egg-filled body of the dead female that is referred to as the cyst. Cysts eventually dislodge and are found free in the soil. The walls of the cyst become very tough, providing excellent protection for the approximately 200 to 400 eggs contained within. SCN eggs survive within the cyst until proper hatching conditions occur. Although many of the eggs may hatch within the first year, many also will survive within the protective cysts for several years.

[0009] A nematode can move through the soil only a few inches per year on its own power. However, nematode infestation can be spread substantial distances in a variety of ways. Anything that can move infested soil is capable of spreading the infestation, including farm machinery, vehicles and tools, wind, water, animals, and farm workers. Seed sized particles of soil often contaminate harvested seed. Consequently, nematode infestation can be spread when contaminated seed from infested fields is planted in non-infested fields. There is even evidence that certain nematode species can be spread by birds. Only some of these causes can be prevented.

[0010] Traditional practices for managing nematode infestation include: maintaining proper soil nutrients and soil pH levels in nematode-infested land; controlling other plant diseases, as well as insect and weed pests; using sanitation practices such as plowing, planting, and cultivating of nematode-infested fields only after working non-infested fields; cleaning equipment thoroughly with high pressure water or steam after working in infested fields; not using seed grown on infested land for planting non-infested fields unless the seed has been properly cleaned; rotating infested fields and alternating host crops with non-host crops; using nematicides; and planting resistant plant varieties.

[0011] Methods have been proposed for the genetic transformation of plants in order to confer increased resistance to plant parasitic nematodes. U.S. Patent Nos. 5,589,622 and 5,824,876 are directed to the identification of plant genes expressed specifically in or adjacent to the feeding site of the plant after attachment by the nematode. The promoters of these plant target genes can then be used to direct the specific expression of detrimental proteins or enzymes, or the expression of antisense RNA to the target gene or to general cellular genes. The plant promoters may also be used to confer nematode resistance specifically at the feeding site by transforming the plant with a construct comprising the promoter of the plant target gene linked to a gene whose product induces lethality in the nematode after ingestion.

[0012] Recently, RNA interference (RNAi), also referred to as gene silencing, has been proposed as a method for controlling nematodes. When double-stranded RNA (dsRNA) corresponding essentially to the sequence of a target gene or mRNA is introduced into a cell, expression from the target gene is inhibited (See e.g., U.S. Patent No. 6,506,559). U.S. Patent No. 6,506,559 demonstrates the effectiveness of RNAi against known genes in Caenorhabditis elegans, but does not demonstrate the usefulness of RNAi for controlling plant parasitic nematodes.

[0013] Use of RNAi to target essential nematode genes has been proposed, for example, in PCT Publication WO 01/96584, WO 01/17654, US 2004/0098761, US 2005/0091713, US 2005/0188438, US 2006/0037101, US 2006/0080749, US 2007/0199100, and US 2007/0250947. US 2007/0271630, US 2007/0250947, and WO 2007/095469 each disclose a large number of SCN genes, including a gene encoding the class I E2 ubiquitin conjugating enzyme designated LET-70. The C. elegans let-70 gene was identified in screens for essential genes and phenotypic analyses indicate that loss of let-70 activity results in embryonic and larval lethality with defects in sarcomere assembly. Purified LET-70 can stimulate the self-ubiquitylation activities of CHN-1 and UFD-2, E4 ubiquitin conjugation factors and, in addition, can stimulate the CHN-1- and UFD-2-dependent multiubiquitylation of UNC-45, a myosin-directed chaperone. In this manner, LET-70 may regulate UNC-45 degradation and myosin assembly. RNA blot analyses and a let-70-lacZ reporter fusion reveal that let-70 is abundantly expressed at all stages of development, including the dauer larval stage; strong staining is observed in most somatic tissues until adult stages, when expression generally becomes restricted to the nervous system (embryonic lethal, adult lethal, protruding vulva).

[0014] A number of models have been proposed for the action of RNAi. In mammalian systems, dsRNAs larger than 30 nucleotides trigger induction of interferon synthesis and a global shut-down of protein syntheses, in a non-sequence-specific manner. However, U.S. Patent No. 6,506,559 discloses that in nematodes, the length of the dsRNA corresponding to the target gene sequence may be at least 25, 50, 100, 200, 300, or 400 bases, and that even larger dsRNAs were also effective at inducing RNAi in C. elegans. It is known that when hairpin RNA constructs comprising double stranded regions ranging from 98 to 854 nucleotides were transformed into a number of plant species, the target plant genes were efficiently silenced. There is general agreement that in many organisms, including nematodes and plants, large pieces of dsRNA are cleaved into about 19-24 nucleotide fragments (siRNA) within cells, and that these siRNAs are the actual mediators of the RNAi phenomenon.

[0015] Although there have been numerous efforts to use RNAi to control plant parasitic nematodes, to date no transgenic nematode-resistant plant has been deregulated in any country. Accordingly, there continues to be a need to identify safe and effective compositions and methods for the controlling plant parasitic nematodes using RNAi, and for the production of plants having increased resistance to plant parasitic nematodes.

SUMMARY OF THE INVENTION

[0016] The present invention provides nucleic acids, transgenic plants, and methods to over-

come or alleviate nematode infestation of valuable agricultural crops such as soybeans. The nucleic acids of the invention are capable of decreasing expression of parasitic nematode target genes by RNAi. In accordance with the invention, the parasitic nematode target gene is the let-70 gene.

[0017] The nucleic acids of the invention encode double stranded RNA comprising (a) a first strand having a sequence substantially identical to from about 19 to about 400 or 500 consecutive nucleotides of a let-70 target gene having a sequence selected from the group consisting of nucleotides 75-574 of SEQ ID NO:1, nucleotides 1 to 548 of SEQ ID NO: 2, nucleotides 1 to 715 of SEQ ID NO: 3, and nucleotides 1 to 668 of SEQ ID NO:4; and (b) a second strand having a sequence substantially complementary to the first strand.

[0018] The invention is further embodied as a pool of double stranded RNA molecules comprising a multiplicity of short interfering RNA molecules each comprising a double stranded region having a length of about 19 to 24 nucleotides, wherein said RNA molecules are derived from a polynucleotide selected from the group consisting of a polynucleotide having a sequence consisting of nucleotides 75-574 of SEQ ID NO:1; a polynucleotide comprising nucleotides 1 to 548 of SEQ ID NO: 2, a polynucleotide comprising nucleotides 1 to 715 of SEQ ID NO: 3, and a polynucleotide comprising nucleotides 1 to 668 of SEQ ID NO:4

[0019] In another embodiment, the invention provides a double stranded RNA molecule comprising a first strand having a sequence which is substantially identical to an oligonucleotide selected from the group consisting of SEQ ID NO:9 (motif A); SEQ ID NO:10 (motif B); SEQ ID NO: 11 (motif C); SEQ ID NO:12 (motif D); SEQ ID NO:13 (motif E); SEQ ID NO:14 (motif F); and SEQ ID NO:15 (motif G); and a second strand having a sequence substantially complementary to the first strand.

[0020] In another embodiment, the invention provides a transgenic plant comprising a nucleic acid construct that encodes a dsRNA capable of specifically decreasing expression of a parasitic nematode let-70 target gene, wherein the plant is resistant to parasitic nematode infection. In this embodiment, the transgenic plant may further comprise a second nucleic acid construct capable of specifically decreasing expression of a second parasitic nematode target gene, or alternatively, capable of overexpressing a gene that encodes a protein that reduces parasitic nematode infestation.

[0021] In another embodiment, the invention provides a transgenic plant capable of expressing a pool of dsRNA molecules, wherein each dsRNA molecule comprises a double stranded region having a length of about 19-24 nucleotides and wherein the RNA molecules are derived from polynucleotides substantially identical to a portion of a let-70 parasitic nematode target gene.

[0022] The invention further encompasses a method of making a transgenic plant capable of expressing a dsRNA that is substantially identical to a portion of a let-70 target gene of a parasitic nematode, said method comprising the steps of: (a) preparing a nucleic acid fragment comprising a region that is substantially identical to at least 19 contiguous nucleotides of nucleotides 75-574 of SEQ ID NO:1, nucleotides 1 to 548 of SEQ ID NO: 2, nucleotides 1 to 715 of SEQ ID NO: 3, or nucleotides 1 to 668 of SEQ ID NO:4, wherein the nucleic acid fragment is able to form a double-stranded transcript once expressed in the plant; (b) transforming a recipi-

ent plant with said nucleic acid fragment; (c) producing one or more transgenic offspring of said recipient plant; and (d) selecting the offspring for nematode resistance.

[0023] The invention further provides a method of conferring nematode resistance to a plant, said method comprising the steps of: (a) preparing a nucleic acid fragment comprising a region that is substantially identical to at least 19 contiguous nucleotides of nucleotides 75-574 of SEQ ID NO:1, nucleotides 1 to 548 of SEQ ID NO: 2, nucleotides 1 to 715 of SEQ ID NO: 3, or nucleotides 1 to 668 of SEQ ID NO:4, wherein the nucleic acid fragment is able to form a double-stranded transcript once expressed in the plant; (b) transforming a recipient plant with said nucleic acid fragment; (c) producing one or more transgenic offspring of said recipient plant; and (d) selecting the offspring for nematode resistance.

[0024] The invention further provides an expression cassette and an expression vector comprising a nucleic acid fragment which is substantially identical to at least 19 contiguous nucleotides of nucleotides 75-574 of SEQ ID NO:1, nucleotides 1 to 548 of SEQ ID NO: 2, nucleotides 1 to 715 of SEQ ID NO: 3, or nucleotides 1 to 668 of SEQ ID NO:4.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] Figure 1 shows the table of SEQ ID NOs assigned to corresponding nucleotide and amino acid sequences from H. glycines and other species.

[0026] Figure 2 shows the amino acid alignment of let-70 like sequences: H. glycines let-70 (SEQ ID NO:5); a Globodera rostochiensis let-70 like sequence from Genbank accession number BM344005 (SEQ ID NO:6), a Globodera pallida let-70 like sequence from Genbank accession number CV578667 (SEQ ID NO:7), and a Meloidogyne incognita let-70 like sequence from Genbank accession number CK984217 (SEQ ID NO:8) using the Vector NTI software suite v10.3.0 (gap opening penalty = 10, gap extension penalty = 0.05, gap separation penalty = 8).

[0027] Figure 3 shows the nucleotide alignment of the full length H. glycines let-70 coding region (SEQ ID NO:1), the G. rostochiensis let-70 fragment (SEQ ID NO:2) and G. pallida let-70 fragment (SEQ ID NO:3). Conserved motifs are indicated by bold text and are listed in Figure 4. The alignment was done using the Vector NTI software suite v10.3.0 (gap opening penalty = 15, gap extension penalty = 6.66, gap separation penalty = 8).

[0028] Figure 4 shows a table of conserved nucleotide motifs identified from let-70 genes as described in Figure 3.

[0029] Figures 5a and 5b show global percent identity of exemplary let-70 like sequences (Figure 5a is amino acid identity and Figure 5b is nucleotide identity). Percent identity was calculated from multiple alignments using the Vector NTI software suite v10.3.0.

[0030] Figures 6a-6r show various 21mers possible in SEQ ID NO:1, 2, 3, 4, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20,21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35 by nucleotide position.

DETAILED DESCRIPTION OF THE INVENTION

[0031] The present invention may be understood more readily by reference to the following detailed description of the preferred embodiments of the invention and the examples included

herein. Unless otherwise noted, the terms used herein are to be understood according to conventional usage by those of ordinary skill in the relevant art. In addition to the definitions of terms provided below, definitions of common terms in molecular biology may also be found in Rieger et al., 1991 Glossary of genetics: classical and molecular, 5th Ed., Berlin: Springer-Verlag; and in Current Protocols in Molecular Biology, F.M. Ausubel et al., Eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1998 Supplement). It is to be understood that as used in the specification and in the claims, "a" or "an" can mean one or more, depending upon the context in which it is used. Thus, for example, reference to "a cell" can mean that at least one cell can be utilized It is to be understood that the terminology used herein is for the purpose of describing specific embodiments only and is not intended to be limiting.

[0032] Throughout this application, various publications are referenced. The disclosures of all of these publications and those references cited within those publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains. Standard techniques for cloning, DNA isolation, amplification and purification, for enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like, and various separation techniques are those known and commonly employed by those skilled in the art. A number of standard techniques are described in Sambrook et al., 1989 Molecular Cloning, Second Edition, Cold Spring Harbor Laboratory, Plainview, N.Y.; Maniatis et al., 1982 Molecular Cloning, Cold Spring Harbor Laboratory, Plainview, N.Y.; Wu (Ed.) 1993 Meth. Enzymol. 218, Part I; Wu (Ed.) 1979 Meth Enzymol. 68; Wu et al., (Eds.) 1983 Meth. Enzymol. 100 and 101; Grossman and Moldave (Eds.) 1980 Meth. Enzymol. 65; Miller (Ed.) 1972 Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.; Old and Primrose, 1981 Principles of Gene Manipulation, University of California Press, Berkeley; Schleif and Wensink, 1982 Practical Methods in Molecular Biology; Glover (Ed.) 1985 DNA Cloning Vol. I and II, IRL Press, Oxford, UK; Hames and Higgins (Eds.) 1985 Nucleic Acid Hybridization, IRL Press, Oxford, UK; and Setlow and Hollaender 1979 Genetic Engineering: Principles and Methods, Vols. 1-4, Plenum Press, New York. Abbreviations and nomenclature, where employed, are deemed standard in the field and commonly used in professional journals such as those cited herein.

[0033] As used herein, "RNAi" or "RNA interference" refers to the process of sequence-specific post-transcriptional gene silencing in nematodes, mediated by double-stranded RNA (dsRNA). As used herein, "dsRNA" refers to RNA that is partially or completely double stranded. Double stranded RNA is also referred to as short interfering RNA (siRNA), short interfering nucleic acid (siNA), micro-RNA (mRNA), and the like. In the RNAi process, dsRNA comprising a first strand that is substantially identical to a portion of a target gene and a second strand that is complementary to the first strand is introduced into a nematode, preferably by soaking and more preferably by feeding. After introduction into the nematode, the target gene-specific dsRNA is processed into relatively small fragments (siRNAs) and can subsequently become distributed throughout the nematode, leading to a loss-of-function mutation having a phenotype that, over the period of a generation, may come to closely resemble the phenotype arising from a com-

plete or partial deletion of the target gene. Alternatively, the target gene-specific dsRNA is processed into relatively small fragments by a plant cell containing the RNAi processing machinery; and when the plant-processed small dsRNA is ingested by a parasitic nematode, the loss-of-function phenotype is obtained.

[0034] As used herein, taking into consideration the substitution of uracil for thymine when comparing RNA and DNA sequences, the term “substantially identical” as applied to dsRNA means that the nucleotide sequence of one strand of the dsRNA is at least about 80%-90% identical to 20 or more contiguous nucleotides of the target gene, more preferably, at least about 90-95% identical to 20 or more contiguous nucleotides of the target gene, and most preferably at least about 95%, 96%, 97%, 98% or 99% identical or absolutely identical to 20 or more contiguous nucleotides of the target gene. 20 or more nucleotides means a portion, being at least about 20, 21, 22, 23, 24, 25, 50, 100, 200, 300, 400, 500, 1000, 1500, consecutive bases or up to the full length of the target gene.

[0035] As used herein, “complementary” polynucleotides are those that are capable of base pairing according to the standard Watson-Crick complementarity rules. Specifically, purines will base pair with pyrimidines to form a combination of guanine paired with cytosine (G:C) and adenine paired with either thymine (A:T) in the case of DNA, or adenine paired with uracil (A:U) in the case of RNA. It is understood that two polynucleotides may hybridize to each other even if they are not completely complementary to each other, provided that each has at least one region that is substantially complementary to the other. As used herein, the term “substantially complementary” means that two nucleic acid sequences are complementary over at least at 80% of their nucleotides. Preferably, the two nucleic acid sequences are complementary over at least at 85%, 90%, 95%, 96%, 97%, 98%, 99% or more or all of their nucleotides. Alternatively, “substantially complementary” means that two nucleic acid sequences can hybridize under high stringency conditions. As used herein, the term “substantially identical” or “corresponding to” means that two nucleic acid sequences have at least 80% sequence identity. Preferably, the two nucleic acid sequences have at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% of sequence identity.

[0036] Also as used herein, the terms “nucleic acid” and “polynucleotide” refer to RNA or DNA that is linear or branched, single or double stranded, or a hybrid thereof. The term also encompasses RNA/DNA hybrids. When dsRNA is produced synthetically, less common bases, such as inosine, 5-methylcytosine, 6-methyladenine, hypoxanthine and others can also be used for antisense, dsRNA, and ribozyme pairing. For example, polynucleotides that contain C-5 propyne analogues of uridine and cytidine have been shown to bind RNA with high affinity and to be potent antisense inhibitors of gene expression. Other modifications, such as modification to the phosphodiester backbone, or the 2’-hydroxy in the ribose sugar group of the RNA can also be made.

[0037] As used herein, the terms “contacting” and “administering” are used interchangeably, and refer to a process by which dsRNA of the present invention is delivered to a cell of a parasitic nematode, in order to inhibit expression of an essential target gene in the nematode. The dsRNA may be administered in a number of ways, including, but not limited to, direct introduc-

tion into a cell (i.e., intracellularly); or extracellular introduction into a cavity, interstitial space, or into the circulation of the nematode, oral introduction, the dsRNA may be introduced by bathing the nematode in a solution containing dsRNA, or the dsRNA may be present in food source. Methods for oral introduction include direct mixing of dsRNA with food of the nematode, as well as engineered approaches in which a species that is used as food is engineered to express a dsRNA, then fed to the organism to be affected. For example, the dsRNA may be sprayed onto a plant, or the dsRNA may be applied to soil in the vicinity of roots, taken up by the plant and/or the parasitic nematode, or a plant may be genetically engineered to express the dsRNA in an amount sufficient to kill or adversely affect some or all of the parasitic nematode to which the plant is exposed.

[0038] As used herein, the term “control,” when used in the context of an infection, refers to the reduction or prevention of an infection. Reducing or preventing an infection by a nematode will cause a plant to have increased resistance to the nematode; however, such increased resistance does not imply that the plant necessarily has 100% resistance to infection. In preferred embodiments, the resistance to infection by a nematode in a resistant plant is greater than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% in comparison to a wild type plant that is not resistant to nematodes. Preferably the wild type plant is a plant of a similar, more preferably identical genotype as the plant having increased resistance to the nematode, but does not comprise a dsRNA directed to the target gene. The plant’s resistance to infection by the nematode may be due to the death, sterility, arrest in development, or impaired mobility of the nematode upon exposure to the dsRNA specific to an essential gene. The term “resistant to nematode infection” or “a plant having nematode resistance” as used herein refers to the ability of a plant, as compared to a wild type plant, to avoid infection by nematodes, to kill nematodes or to hamper, reduce or stop the development, growth or multiplication of nematodes. This might be achieved by an active process, e.g. by producing a substance detrimental to the nematode, or by a passive process, like having a reduced nutritional value for the nematode or not developing structures induced by the nematode feeding site like syncytia or giant cells. The level of nematode resistance of a plant can be determined in various ways, e.g. by counting the nematodes being able to establish parasitism on that plant, or measuring development times of nematodes, proportion of male and female nematodes or, for cyst nematodes, counting the number of cysts or nematode eggs produced on roots of an infected plant or plant assay system.

[0039] The term “plant” is intended to encompass plants at any stage of maturity or development, as well as any tissues or organs (plant parts) taken or derived from any such plant unless otherwise clearly indicated by context. Plant parts include, but are not limited to, stems, roots, flowers, ovules, stamens, seeds, leaves, embryos, meristematic regions, callus tissue, anther cultures, gametophytes, sporophytes, pollen, microspores, protoplasts, hairy root cultures, and the like. The present invention also includes seeds produced by the plants of the present invention. In one embodiment, the seeds are true breeding for an increased resistance to nematode infection as compared to a wild-type variety of the plant seed. As used herein, a “plant cell” includes, but is not limited to, a protoplast, gamete producing cell, and a cell that regenerates into a whole plant. Tissue culture of various tissues of plants and regeneration of plants therefrom is

well known in the art and is widely published.

[0040] As used herein, the term “transgenic” refers to any plant, plant cell, callus, plant tissue, or plant part that contains all or part of at least one recombinant polynucleotide. In many cases, all or part of the recombinant polynucleotide is stably integrated into a chromosome or stable extra-chromosomal element, so that it is passed on to successive generations. For the purposes of the invention, the term “recombinant polynucleotide” refers to a polynucleotide that has been altered, rearranged, or modified by genetic engineering. Examples include any cloned polynucleotide, or polynucleotides, that are linked or joined to heterologous sequences. The term “recombinant” does not refer to alterations of polynucleotides that result from naturally occurring events, such as spontaneous mutations, or from non-spontaneous mutagenesis followed by selective breeding.

[0041] As used herein, the term “amount sufficient to inhibit expression” refers to a concentration or amount of the dsRNA that is sufficient to reduce levels or stability of mRNA or protein produced from a target gene in a parasitic nematode. As used herein, “inhibiting expression” refers to the absence or observable decrease in the level of protein and/or mRNA product from a target gene. Inhibition of target gene expression may be lethal to the parasitic nematode, or such inhibition may delay or prevent entry into a particular developmental step (e.g., metamorphosis), if plant disease is associated with a particular stage of the parasitic nematode’s life cycle. The consequences of inhibition can be confirmed by examination of the outward properties of the nematode (as presented below in the examples).

[0042] In accordance with the invention, a parasitic nematode is contacted with a dsRNA, which specifically inhibits expression of a target gene that is essential for survival, metamorphosis, or reproduction of the nematode. Preferably, the parasitic nematode comes into contact with the dsRNA after entering a plant that expresses the dsRNA. In one embodiment, the dsRNA is encoded by a vector that has been transformed into an ancestor of the infected plant. Preferably, the nucleic acid sequence expressing said dsRNA is under the transcriptional control of a root specific promoter, a parasitic nematode induced feeding cell-specific promoter or a constitutive promoter.

[0043] In accordance with the invention, the parasitic nematode target gene is a let-70 gene comprising a sequence selected from the group consisting of: nucleotides 75-574 of SEQ ID NO:1, nucleotides 1 to 548 of SEQ ID NO: 2 (BM344005), and nucleotides 1 to 715 of SEQ ID NO: 3 (CV578667); and nucleotides 1 to 668 of SEQ ID NO: 4 (CK984217). In one embodiment, the parasitic nematode let-70 gene comprises an oligonucleotide selected from the group consisting of SEQ ID NO:9 (motif A); SEQ ID NO:10 (motif B); SEQ ID NO: 11 (motif C); SEQ ID NO:12 (motif D); SEQ ID NO:13 (motif E); SEQ ID NO:14 (motif F); and SEQ ID NO:15 (motif G)

[0044] Complete cDNAs corresponding to the parasitic nematode target of the invention may be isolated from parasitic nematodes other than H. glycines, G. rostochiensis, G. pallida, and M. incognita using the information provided herein and techniques known to those of skill in the art of biotechnology. For example, a nucleic acid molecule from a parasitic nematode that hybridizes under stringent conditions to a nucleotide sequence of SEQ ID NO:1, 2, 3, or 4 can be iso-

lated from parasitic nematode cDNA libraries. As used herein with regard to hybridization for DNA to a DNA blot, the term “stringent conditions” refers to hybridization overnight at 60°C in 10X Denhart’s solution, 6X SSC, 0.5% SDS, and 100 μg/ml denatured salmon sperm DNA. Blots are washed sequentially at 62°C for 30 minutes each time in 3X SSC/0.1% SDS, followed by 1X SSC/0.1% SDS, and finally 0.1X SSC/0.1% SDS. As also used herein, in a preferred embodiment, the phrase “stringent conditions” refers to hybridization in a 6X SSC solution at 65°C. In another embodiment, “highly stringent conditions” refers to hybridization overnight at 65°C in 10X Denhart’s solution, 6X SSC, 0.5% SDS and 100 μg/ml denatured salmon sperm DNA. Blots are washed sequentially at 65°C for 30 minutes each time in 3X SSC/0.1% SDS, followed by 1X SSC/0.1% SDS, and finally 0.1X SSC/0.1% SDS. Methods for nucleic acid hybridizations are described in Meinkoth and Wahl, 1984, Anal. Biochem. 138:267-284; well known in the art. Alternatively, mRNA can be isolated from parasitic nematode cells, and cDNA can be prepared using reverse transcriptase. Synthetic oligonucleotide primers for polymerase chain reaction amplification can be designed based upon the nucleotide sequence shown in SEQ ID NOs:1, 2, 3, or 4. Nucleic acid molecules corresponding to the parasitic nematode target genes of the invention can be amplified using cDNA or, alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid molecules so amplified can be cloned into appropriate vectors and characterized by DNA sequence analysis.

[0045] Accordingly, in one embodiment the dsRNA of the invention comprises a first strand that is substantially identical to at least 19 contiguous nucleotides of a let-70 target gene from a plant parasitic nematode and a second strand that is substantially complementary to the first strand. In preferred embodiments, the target gene is selected from the group consisting of a polynucleotide consisting of nucleotides 75-574 of SEQ ID NO:1, a polynucleotide comprising nucleotides 1 to 548 of SEQ ID NO: 2, a polynucleotide comprising nucleotides 1 to 715 of SEQ ID NO: 3, and a polynucleotide comprising nucleotides 1 to 668 of SEQ ID NO:4.

[0046] As discussed above, fragments of dsRNA larger than about 19-24 nucleotides in length are cleaved intracellularly by nematodes and plants to siRNAs of about 19-24 nucleotides in length, and these siRNAs are the actual mediators of the RNAi phenomenon. The table in Figures 6a - 6l sets forth exemplary 21-mers of the SCN let-70 gene (SEQ ID NO:1), the G. rostochiensis let-70 gene (SEQ ID NO:2), the G. pallida let-70 gene (SEQ ID NO:3) , and the M. incognita let-70 gene (SEQ ID NO:4), and the respective fragments and homologs thereof, as indicated by SEQ ID NOs set forth in the table. This table can also be used to calculate the 19, 20, 22, 23, or 24-mers by adding or subtracting the appropriate number of nucleotides from each 21mer. Thus the dsRNA of the present invention may range in length from about 19 nucleotides to about 500 consecutive nucleotides or up to the whole length of the target gene. The dsRNA of the invention may be embodied as a miRNA which targets a single site within a parasitc nematode target gene. Alternatively, the dsRNA of the invention has a length from about 21 nucleotides to about 600 consecutive nucleotides. Further, the dsRNA of the invention has a length from about 21 nucleotides to about 400 consecutive nucleotides, or from about 21 nucleotides to about 300 consecutive nucleotides.

[0047] As disclosed herein, 100% sequence identity between the dsRNA and the target gene is not required to practice the present invention. Preferably, the dsRNA of the invention comprises a 19-nucleotide portion which is substantially identical to at least 19 contiguous nucleotides of the target gene. While a dsRNA comprising a nucleotide sequence identical to a portion of the parasitic nematode target genes of the invention is preferred for inhibition, the invention can tolerate sequence variations that might be expected due to gene manipulation or synthesis, genetic mutation, strain polymorphism, or evolutionary divergence. Thus the dsRNAs of the invention also encompass dsRNAs comprising a mismatch with the target gene of at least 1, 2, or more nucleotides. For example, it is contemplated in the present invention that the 21mer dsRNA sequences exemplified in Figures 6a - 6l may contain an addition, deletion or substitution of 1, 2, or more nucleotides, so long as the resulting sequence still interferes with the parasitic nematode target gene function.

[0048] Sequence identity between the dsRNAs of the invention and the parasitic nematode target genes may be optimized by sequence comparison and alignment algorithms known in the art (see Gribskov and Devereux, Sequence Analysis Primer, Stockton Press, 1991, and references cited therein) and calculating the percent difference between the nucleotide sequences by, for example, the Smith-Waterman algorithm as implemented in the BESTFIT software program using default parameters (e.g., University of Wisconsin Genetic Computing Group). Greater than 80 % sequence identity, 90% sequence identity, or even 100% sequence identity, between the inhibitory RNA and at least 19 contiguous nucleotides of the target gene is preferred.

[0049] When dsRNA of the invention has a length longer than about 21 nucleotides, for example from about 50 nucleotides to about 1000 nucleotides, it will be cleaved randomly to dsRNAs of about 21 nucleotides within the plant or parasitic nematode cell, the siRNAs. The cleavage of a longer dsRNA of the invention will yield a pool of 21mer dsRNAs, derived from the longer dsRNA. This pool of 21mer dsRNAs is also encompassed within the scope of the present invention, whether generated intracellularly within the plant or nematode or synthetically using known methods of oligonucleotide synthesis.

[0050] The siRNAs of the invention have sequences corresponding to fragments of about 19-24 contiguous nucleotides across the entire sequence of the let-70 target gene. For example, a pool of siRNA of the invention derived from the H. glycines target gene as set forth in SEQ ID NO:1 may comprise a multiplicity of RNA molecules which are selected from the group consisting of oligonucleotides substantially identical to the 21mer nucleotides of SEQ ID NO:1 described in Figures 6a - 6l. A pool of siRNA of the invention derived from the H. glycines target gene of SEQ ID NO:1 may also comprise any combination of the specific RNA molecules having any of the 21 contiguous nucleotide sequences derived from SEQ ID NO:1 set forth in Figures 6a - 6l. The pool of siRNAs of the invention is also embodied in pools of 21mers of fragments and homologs of the G. Rostochiensis, G. pallida, and M. incognita let-70 target genes as set forth in the table of Figures 6a-6l. Further, as multiple specialized Dicers in plants generate siRNAs typically ranging in size from 19nt to 24nt (See Henderson et al., 2006. Nature Genetics 38:721-725.), the siRNAs of the present invention can may range from about 19 contigu-

ous nucleotide sequences to about 24 contiguous nucleotide sequences. Accordingly, the pool of siRNA of the invention may comprise a multiplicity of RNA molecules having any 19, 20, 21, 22, 23, or 24 contiguous nucleotide sequences derived from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4. Alternatively, the pool of siRNA of the invention may comprise a multiplicity of RNA molecules having a combination of any 19, 20, 21, 22, 23,and/or 24 contiguous nucleotide sequences derived from SEQ ID NO: 1; SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4.

[0051] The dsRNA of the invention may optionally comprise a single stranded overhang at either or both ends. Preferably, the single stranded overhang comprises at least two nucleotides at the 3´ end of each strand of the dsRNA molecule. The double-stranded structure may be formed by a single self-complementary RNA strand (i.e. forming a hairpin loop) or two complementary RNA strands. RNA duplex formation may be initiated either inside or outside the cell. When the dsRNA of the invention forms a hairpin loop, it may optionally comprise an intron, as set forth in US 2003/0180945A1 or a nucleotide spacer, which is a stretch of sequence between the complementary RNA strands to stabilize the hairpin transgene in cells. Methods for making various dsRNA molecules are set forth, for example, in WO 99/53050 and in U.S.Pat.No. 6,506,559. The RNA may be introduced in an amount that allows delivery of at least one copy per cell. Higher doses of double-stranded material may yield more effective inhibition.

[0052] In another embodiment, the invention provides an isolated recombinant expression vector comprising a nucleic acid encoding a dsRNA molecule as described above, wherein expression of the vector in a host plant cell results in increased resistance to a parasitic nematode as compared to a wild-type variety of the host plant cell. As used herein, the term “vector” refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a “plasmid,” which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host plant cell into which they are introduced. Other vectors are integrated into the genome of a host plant cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as “expression vectors.” In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, “plasmid” and “vector” can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., potato virus X, tobacco rattle virus, and Gemini virus), which serve equivalent functions.

[0053] The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host plant cell, which means that the recombinant expression vector includes one or more regulatory sequences, e.g. promoters, selected on the basis of the host plant cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. With respect to a recombinant expression vector, the terms “operatively linked” and “in operative association” are interchange-

able and are intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in a host plant cell when the vector is introduced into the host plant cell). The term "regulatory sequence" is intended to include promoters, enhancers, and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990) and Gruber and Crosby, in: Methods in Plant Molecular Biology and Biotechnology, Eds. Glick and Thompson, Chapter 7, 89-108, CRC Press: Boca Raton, Florida, including the references therein. Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cells and those that direct expression of the nucleotide sequence only in certain host cells or under certain conditions. It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of dsRNA desired, and the like. The expression vectors of the invention can be introduced into plant host cells to thereby produce dsRNA molecules of the invention encoded by nucleic acids as described herein.

[0054] In accordance with the invention, the recombinant expression vector comprises a regulatory sequence operatively linked to a nucleotide sequence that is a template for one or both strands of the dsRNA molecules of the invention. In one embodiment, the nucleic acid molecule further comprises a promoter flanking either end of the nucleic acid molecule, wherein the promoters drive expression of each individual DNA strand, thereby generating two complementary RNAs that hybridize and form the dsRNA. In another embodiment, the nucleic acid molecule comprises a nucleotide sequence that is transcribed into both strands of the dsRNA on one transcription unit, wherein the sense strand is transcribed from the 5' end of the transcription unit and the antisense strand is transcribed from the 3' end, wherein the two strands are separated by 3 to 500 base or more pairs, and wherein after transcription, the RNA transcript folds on itself to form a hairpin. In accordance with the invention, the spacer region in the hairpin transcript may be any DNA fragment.

[0055] According to the present invention, the introduced polynucleotide may be maintained in the plant cell stably if it is incorporated into a non-chromosomal autonomous replicon or integrated into the plant chromosomes. Alternatively, the introduced polynucleotide may be present on an extra-chromosomal non-replicating vector and be transiently expressed or transiently active. Whether present in an extra-chromosomal non-replicating vector or a vector that is integrated into a chromosome, the polynucleotide preferably resides in a plant expression cassette. A plant expression cassette preferably contains regulatory sequences capable of driving gene expression in plant cells that are operatively linked so that each sequence can fulfill its function, for example, termination of transcription by polyadenylation signals. Preferred polyadenylation signals are those originating from Agrobacterium tumefaciens t-DNA such as the gene 3 known as octopine synthase of the Ti-plasmid pTiACH5 (Gielen et al., 1984, EMBO J. 3:835) or functional equivalents thereof, but also all other terminators functionally active in plants are suitable. As plant gene expression is very often not limited on transcriptional levels, a plant expression

cassette preferably contains other operatively linked sequences like translational enhancers such as the overdrive-sequence containing the 5'-untranslated leader sequence from tobacco mosaic virus enhancing the polypeptide per RNA ratio (Gallie et al., 1987, Nucl. Acids Research 15:8693-8711). Examples of plant expression vectors include those detailed in: Becker, D. et al., 1992, New plant binary vectors with selectable markers located proximal to the left border, Plant Mol. Biol. 20:1195-1197; Bevan, M.W., 1984, Binary Agrobacterium vectors for plant transformation, Nucl. Acid. Res. 12:8711-8721; and Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, Vol. 1, Engineering and Utilization, eds.: Kung and R. Wu, Academic Press, 1993, S. 15-38.

[0056] Plant gene expression should be operatively linked to an appropriate promoter conferring gene expression in a temporal-preferred, spatial-preferred, cell type-preferred, and/or tissue-preferred manner. Promoters useful in the expression cassettes of the invention include any promoter that is capable of initiating transcription in a plant cell present in the plant's roots. Such promoters include, but are not limited to those that can be obtained from plants, plant viruses and bacteria that contain genes that are expressed in plants, such as Agrobacterium and Rhizobium. Preferably, the expression cassette of the invention comprises a root-specific promoter, a pathogen inducible promoter, or a nematode inducible promoter. More preferably the nematode inducible promoter is or a parasitic nematode feeding site-specific promoter. A parasitic nematode feeding site-specific promoter may be specific for syncytial cells or giant cells or specific for both kinds of cells. A promoter is inducible, if its activity, measured on the amount of RNA produced under control of the promoter, is at least 30%, 40%, 50% preferably at least 60%, 70%, 80%, 90% more preferred at least 100%, 200%, 300% higher in its induced state, than in its un-induced state. A promoter is cell-, tissue- or organ-specific, if its activity , measured on the amount of RNA produced under control of the promoter, is at least 30%, 40%, 50% preferably at least 60%, 70%, 80%, 90% more preferred at least 100%, 200%, 300% higher in a particular cell-type, tissue or organ, then in other cell-types or tissues of the same plant, preferably the other cell-types or tissues are cell types or tissues of the same plant organ, e.g. a root. In the case of organ specific promoters, the promoter activity has to be compared to the promoter activity in other plant organs, e.g. leaves, stems, flowers or seeds.

[0057] The promoter may be constitutive, inducible, developmental stage-preferred, cell type-preferred, tissue-preferred or organ-preferred. Constitutive promoters are active under most conditions. Non-limiting examples of constitutive promoters include the CaMV 19S and 35S promoters (Odell et al., 1985, Nature 313:810-812), the sX CaMV 35S promoter (Kay et al., 1987, Science 236:1299-1302), the Sep1 promoter, the rice actin promoter (McElroy et al., 1990, Plant Cell 2:163-171), the Arabidopsis actin promoter, the ubiquitin promoter (Christensen et al., 1989, Plant Molec. Biol. 18:675-689); pEmu (Last et al., 1991, Theor. Appl. Genet. 81:581-588), the figwort mosaic virus 35S promoter, the Smas promoter (Velten et al., 1984, EMBO J. 3:2723-2730), the GRP1-8 promoter, the cinnamyl alcohol dehydrogenase promoter (U.S. Patent No. 5,683,439), promoters from the T-DNA of Agrobacterium, such as mannopine synthase, nopaline synthase, and octopine synthase, the small subunit of ribulose biphosphate carboxylase (ssuRUBISCO) promoter, and the like. Promoters that express the dsRNA in a cell

that is contacted by parasitic nematodes are preferred. Alternatively, the promoter may drive expression of the dsRNA in a plant tissue remote from the site of contact with the nematode, and the dsRNA may then be transported by the plant to a cell that is contacted by the parasitic nematode, in particular cells of, or close by nematode feeding sites, e.g. syncytial cells or giant cells.

[0058] Inducible promoters are active under certain environmental conditions, such as the presence or absence of a nutrient or metabolite, heat or cold, light, pathogen attack, anaerobic conditions, and the like. For example, the promoters TobRB7, AtRPE, AtPyk10, Gemini19, and AtHMG1 have been shown to be induced by nematodes (for a review of nematode-inducible promoters, see Ann. Rev. Phytopathol. (2002) 40:191-219; see also U.S. Pat. No. 6,593,513). Method for isolating additional promoters, which are inducible by nematodes are set forth in U.S. Pat. Nos. 5,589,622 and 5,824,876. Other inducible promoters include the hsp80 promoter from Brassica, being inducible by heat shock; the PPDK promoter is induced by light; the PR-1 promoter from tobacco, Arabidopsis, and maize are inducible by infection with a pathogen; and the Adh1 promoter is induced by hypoxia and cold stress. Plant gene expression can also be facilitated via an inducible promoter (For review, see Gatz, 1997, Annu. Rev. Plant Physiol. Plant Mol. Biol. 48:89-108). Chemically inducible promoters are especially suitable if time-specific gene expression is desired. Non-limiting examples of such promoters are a salicylic acid inducible promoter (PCT Application No. WO 95/19443), a tetracycline inducible promoter (Gatz et al., 1992, Plant J. 2:397-404) and an ethanol inducible promoter (PCT Application No. WO 93/21334).

[0059] Developmental stage-preferred promoters are preferentially expressed at certain stages of development. Tissue and organ preferred promoters include those that are preferentially expressed in certain tissues or organs, such as leaves, roots, seeds, or xylem. Examples of tissue preferred and organ preferred promoters include, but are not limited to fruit-preferred, ovule-preferred, male tissue-preferred, seed-preferred, integument-preferred, tuber-preferred, stalk-preferred, pericarp-preferred, and leaf-preferred, stigma-preferred, pollen-preferred, anther-preferred, a petal-preferred, sepal-preferred, pedicel-preferred, silique-preferred, stem-preferred, root-preferred promoters and the like. Seed preferred promoters are preferentially expressed during seed development and/or germination. For example, seed preferred promoters can be embryo-preferred, endosperm preferred and seed coat-preferred. See Thompson et al., 1989, BioEssays 10:108. Examples of seed preferred promoters include, but are not limited to cellulose synthase (celA), Cim1, gamma-zein, globulin-1, maize 19 kD zein (cZ19B1) and the like.

[0060] Other suitable tissue-preferred or organ-preferred promoters include, but are not limited to, the napin-gene promoter from rapeseed (U.S. Patent No. 5,608,152), the USP-promoter from Vicia faba (Baeumlein et al., 1991, Mol Gen Genet. 225(3):459-67), the oleosin-promoter from Arabidopsis (PCT Application No. WO 98/45461), the phaseolin-promoter from Phaseolus vulgaris (U.S. Patent No. 5,504,200), the Bce4-promoter from Brassica (PCT Application No. WO 91/13980), or the legumin B4 promoter (LeB4; Baeumlein et al., 1992, Plant Journal, 2(2):233-9), as well as promoters conferring seed specific expression in monocot plants like

maize, barley, wheat, rye, rice, etc. Suitable promoters to note are the lpt2 or lpt1-gene promoter from barley (PCT Application No. WO 95/15389 and PCT Application No. WO 95/23230) or those described in PCT Application No. WO 99/16890 (promoters from the barley hordein-gene, rice glutelin gene, rice oryzin gene, rice prolamin gene, wheat gliadin gene, wheat glutelin gene, oat glutelin gene, Sorghum kasirin-gene, and rye secalin gene).

[0061] Other promoters useful in the expression cassettes of the invention include, but are not limited to, the major chlorophyll a/b binding protein promoter, histone promoters, the Ap3 promoter, the β-conglycin promoter, the napin promoter, the soybean lectin promoter, the maize 15kD zein promoter, the 22kD zein promoter, the 27kD zein promoter, the g-zein promoter, the waxy, shrunken 1, shrunken 2, and bronze promoters, the Zm13 promoter (U.S. Patent No. 5,086,169), the maize polygalacturonase promoters (PG) (U.S. Patent Nos. 5,412,085 and 5,545,546), and the SGB6 promoter (U.S. Patent No. 5,470,359), as well as synthetic or other natural promoters.

[0062] In accordance with the present invention, the expression cassette comprises an expression control sequence operatively linked to a nucleotide sequence that is a template for one or both strands of the dsRNA. The dsRNA template comprises (a) a first stand having a sequence substantially identical to from about 19 to about 400–500, or up to the full length, consecutive nucleotides derived from nucleotides 75-574 of SEQ ID NO:1, nucleotides 1 to 548 of SEQ ID NO: 2, nucleotides 1 to 715 of SEQ ID NO: 3, or nucleotides 1 to 668 of SEQ ID NO:4; and (b) a second strand having a sequence substantially complementary to the first strand. In further embodiments, a promoter flanks either end of the template nucleotide sequence, wherein the promoters drive expression of each individual DNA strand, thereby generating two complementary RNAs that hybridize and form the dsRNA. In alternative embodiments, the nucleotide sequence is transcribed into both strands of the dsRNA on one transcription unit, wherein the sense strand is transcribed from the 5' end of the transcription unit and the antisense strand is transcribed from the 3' end, wherein the two strands are separated by about 3 to about 500 base pairs, and wherein after transcription, the RNA transcript folds on itself to form a hairpin.

[0063] In another embodiment, the vector contains a bidirectional promoter, driving expression of two nucleic acid molecules, whereby one nucleic acid molecule codes for the sequence substantially identical to a portion of a parasitic nematode let-70 target gene and the other nucleic acid molecule codes for a second sequence being substantially complementary to the first strand and capable of forming a dsRNA, when both sequences are transcribed. A bidirectional promoter is a promoter capable of mediating expression in two directions.

[0064] In another embodiment, the vector contains two promoters, one mediating transcription of the sequence substantially identical to a portion of a parasitic nematode let-70 target gene and another promoter mediating transcription of a second sequence being substantially complementary to the first strand and capable of forming a dsRNA, when both sequences are transcribed. The second promoter might be a different promoter.

[0065] A different promoter means a promoter having a different activity in regard to cell or tissue specificity, or showing expression on different inducers for example, pathogens, abiotic stress or chemicals. For example, one promoter might by constitutive or tissue specific and an-

other might be tissue specific or inducible by pathogens. In one embodiment one promoter mediates the transcription of one nucleic acid molecule suitable for over expression of a first strand of a parasitic nematode let-70 gene, while another promoter mediates tissue- or cell-specific transcription or pathogen inducible expression of the complementary nucleic acid.

[0066] The invention is also embodied in a transgenic plant capable of expressing the dsRNA of the invention and thereby inhibiting the let-70 genes in parasitic nematodes. The plant or transgenic plant may be any plant, such like, but not limited to trees, cut flowers, ornamentals, vegetables or crop plants. The plant may be from a genus selected from the group consisting of Medicago, Lycopersicon, Brassica, Cucumis, Solanum, Juglans, Gossypium, Malus, Vitis, Antirrhinum, Populus, Fragaria, Arabidopsis, Picea, Capsicum, Chenopodium, Dendranthema, Pharbitis, Pinus, Pisum, Oryza, Zea, Triticum, Triticale, Secale, Lolium, Hordeum, Glycine, Pseudotsuga, Kalanchoe, Beta, Helianthus, Nicotiana, Cucurbita, Rosa, Fragaria, Lotus, Medicago, Onobrychis, trifolium, Trigonella, Vigna, Citrus, Linum, Geranium, Manihot, Daucus, Raphanus, Sinapis, Atropa, Datura, Hyoscyamus, Nicotiana, Petunia, Digitalis, Majorana, Ciahorium, Lactuca, Bromus, Asparagus, Antirrhinum, Heterocallis, Nemesis, Pelargonium, Panieum, Pennisetum, Ranunculus, Senecio, Salpiglossis, Browaalia, Phaseolus, Avena, and Allium, or the plant may be selected from a genus selected from the group consisting of Arabidopsis, Medicago, Lycopersicon, Brassica, Cucumis, Solanum, Juglans, Gossypium, Malus, Vitis, Antirrhinum, Brachipodium, Populus, Fragaria, Arabidopsis, Picea, Capsicum, Chenopodium, Dendranthema, Pharbitis, Pinus, Pisum, Oryza, Zea, Triticum, Triticale, Secale, Lolium, Hordeum, Glycine, Pseudotsuga, Kalanchoe, Beta, Helianthus, Nicotiana, Cucurbita, Rosa, Fragaria, Lotus, Medicago, Onobrychis, trifolium, Trigonella, Vigna, Citrus, Linum, Geranium, Manihot, Daucus, Raphanus, Sinapis, Atropa, Datura, Hyoscyamus, Nicotiana, Petunia, Digitalis, Majorana, Ciahorium, Lactuca, Bromus, Asparagus, Antirrhinum, Heterocallis, Nemesis, Pelargonium, Panicum, Pennisetum, Ranunculus, Senecio, Salpiglossis, Browaalia, Phaseolus, Avena, and Allium. In one embodiment the plant is a monocotyledonous plant or a dicotyledonous plant.

[0067] Preferably the plant is a crop plant. Crop plants are all plants, used in agriculture. Accordingly in one embodiment the plant is a monocotyledonous plant, preferably a plant of the family Poaceae, Musaceae, Liliaceae or Bromeliaceae, preferably of the family Poaceae. Accordingly, in yet another embodiment the plant is a Poaceae plant of the genus Zea, Triticum, Oryza, Hordeum, Secale, Avena, Saccharum, Sorghum, Pennisetum, Setaria, Panicum, Eleusine, Miscanthus, Brachypodium, Festuca or Lolium. When the plant is of the genus Zea, the preferred species is Z. mays. When the plant is of the genus Triticum, the preferred species is T. aestivum, T. speltae or T. durum. When the plant is of the genus Oryza, the preferred species is O. sativa. When the plant is of the genus Hordeum, the preferred species is H. vulgare. When the plant is of the genus Secale, the preferred species S. cereale. When the plant is of the genus Avena, the preferred species is A. sativa. When the plant is of the genus Saccarum, the preferred species is S. officinarum. When the plant is of the genus Sorghum, the preferred species is S. vulgare, S. bicolor or S. sudanense. When the plant is of the genus Pennisetum, the preferred species is P. glaucum. When the plant is of the genus Setaria, the preferred spe-

cies is S. italica. When the plant is of the genus Panicum, the preferred species is P. miliaceum or P. virgatum. When the plant is of the genus Eleusine, the preferred species is E. coracana. When the plant is of the genus Miscanthus, the preferred species is M. sinensis. When the plant is a plant of the genus Festuca, the preferred species is F. arundinaria, F. rubra or F. pratensis. When the plant is of the genus Lolium, the preferred species is L. perenne or L. multiflorum. Alternatively, the plant may be Triticosecale.

[0068] Alternatively, in one embodiment the plant is a dicotyledonous plant, preferably a plant of the family Fabaceae, Solanaceae, Brassicaceae, Chenopodiaceae, Asteraceae, Malvaceae, Linacea, Euphorbiaceae, Convolvulaceae Rosaceae, Cucurbitaceae, Theaceae, Rubiaceae, Sterculiaceae or Citrus. In one embodiment the plant is a plant of the family Fabaceae, Solanaceae or Brassicaceae. Accordingly, in one embodiment the plant is of the family Fabaceae, preferably of the genus Glycine, Pisum, Arachis, Cicer, Vicia, Phaseolus, Lupinus, Medicago or Lens. Preferred species of the family Fabaceae are M. truncatula, M, sativa, G. max, P. sativum, A. hypogea, C. arietinum, V. faba, P. vulgaris, Lupinus albus, Lupinus luteus, Lupinus angustifolius or Lens culinaris. More preferred are the species G. max A. hypogea and M. sativa. Most preferred is the species G. max. When the plant is of the family Solanaceae, the preferred genus is Solanum, Lycopersicon, Nicotiana or Capsicum. Preferred species of the family Solanaceae are S. tuberosum, L. esculentum (also known as Solanum lycopersicon), N. tabaccum or C. chinense. More preferred is S. tuberosum. Accordingly, in one embodiment the plant is of the family Brassicaceae, preferably of the genus Brassica or Raphanus. Preferred species of the family Brassicaceae are the species B. napus, B. oleracea, B. juncea or B. rapa. More preferred is the species B. napus. When the plant is of the family Chenopodiaceae, the preferred genus is Beta and the preferred species is the B. vulgaris. When the plant is of the family Asteraceae, the preferred genus is Helianthus and the preferred species is H. annuus. When the plant is of the family Malvaceae, the preferred genus is Gossypium or Abelmoschus. When the genus is Gossypium, the preferred species is G. hirsutum or G. barbadense and the most preferred species is G. hirsutum. A preferred species of the genus Abelmoschus is the species A. esculentus. When the plant is of the family Linacea, the preferred genus is Linum and the preferred species is L. usitatissimum. When the plant is of the family Euphorbiaceae, the preferred genus is Manihot, Jatropa or Rhizinus and the preferred species are M. esculenta, J. curcas or R. comunis. When the plant is of the family Convolvulaceae, the preferred genus is Ipomea and the preferred species is I. batatas. When the plant is of the family Rosaceae, the preferred genus is Rosa, Malus, Pyrus, Prunus, Rubus, Ribes, Vaccinium or Fragaria and the preferred species is the hybrid Fragaria x ananassa. When the plant is of the family Cucurbitaceae, the preferred genus is Cucumis, Citrullus or Cucurbita and the preferred species is Cucumis sativus, Citrullus lanatus or Cucurbita pepo. When the plant is of the family Rubiaceae, the preferred genus is Coffea and the preferred species is C. arabica or C. canephora. When the plant is of the family Sterculiaceae, the preferred genus is Theobroma and the preferred species is T. cacao. When the plant is of the genus Citrus, the preferred species is C. sinensis, C. limon, C. reticulata, C. maxima and hybrids of Citrus species, or the like. In a preferred embodiment of the invention, the plant is a soybean, a potato or a corn plant

[0069] Suitable methods for transforming or transfecting host cells including plant cells are well known in the art of plant biotechnology. Any method may be used to transform the recombinant expression vector into plant cells to yield the transgenic plants of the invention. General methods for transforming dicotyledenous plants are disclosed, for example, in U.S. Pat. Nos. 4,940,838; 5,464,763, and the like. Methods for transforming specific dicotyledenous plants, for example, cotton, are set forth in U.S. Pat. Nos. 5,004,863; 5,159,135; and 5,846,797. Soybean transformation methods are set forth in U.S. Pat. Nos. 4,992,375; 5,416,011; 5,569,834; 5,824,877; 6,384,301 and in EP 0301749B1 may be used. Transformation methods may include direct and indirect methods of transformation. Suitable direct methods include polyethylene glycol induced DNA uptake, liposome-mediated transformation (US 4,536,475), biolistic methods using the gene gun (Fromm ME et al., Bio/Technology. 8(9):833-9, 1990; Gordon-Kamm et al. Plant Cell 2:603, 1990), electroporation, incubation of dry embryos in DNA-comprising solution, and microinjection. In the case of these direct transformation methods, the plasmids used need not meet any particular requirements. Simple plasmids, such as those of the pUC series, pBR322, M13mp series, pACYC184 and the like can be used. If intact plants are to be regenerated from the transformed cells, an additional selectable marker gene is preferably located on the plasmid. The direct transformation techniques are equally suitable for dicotyledonous and monocotyledonous plants.

[0070] Transformation can also be carried out by bacterial infection by means of Agrobacterium (for example EP 0 116 718), viral infection by means of viral vectors (EP 0 067 553; US 4,407,956; WO 95/34668; WO 93/03161) or by means of pollen (EP 0 270 356; WO 85/01856; US 4,684,611). Agrobacterium based transformation techniques (especially for dicotyledonous plants) are well known in the art. The Agrobacterium strain (e.g., Agrobacterium tumefaciens or Agrobacterium rhizogenes) comprises a plasmid (Ti or Ri plasmid) and a T-DNA element which is transferred to the plant following infection with Agrobacterium. The T-DNA (transferred DNA) is integrated into the genome of the plant cell. The T-DNA may be localized on the Ri- or Ti-plasmid or is separately comprised in a so-called binary vector. Methods for the Agrobacterium-mediated transformation are described, for example, in Horsch RB et al. (1985) Science 225:1229. The Agrobacterium-mediated transformation is best suited to dicotyledonous plants but has also been adapted to monocotyledonous plants. The transformation of plants by Agrobacteria is described in, for example, White FF, Vectors for Gene Transfer in Higher Plants, Transgenic Plants, Vol. 1, Engineering and Utilization, edited by S.D. Kung and R. Wu, Academic Press, 1993, pp. 15 - 38; Jenes B et al. Techniques for Gene Transfer, Transgenic Plants, Vol. 1, Engineering and Utilization, edited by S.D. Kung and R. Wu, Academic Press, 1993, pp. 128-143; Potrykus (1991) Annu Rev Plant Physiol Plant Molec Biol 42:205- 225. Transformation may result in transient or stable transformation and expression. Although a nucleotide sequence of the present invention can be inserted into any plant and plant cell falling within these broad classes, it is particularly useful in crop plant cells.

[0071] In certain embodiments the nucleic acid sequences of the present invention can be stacked with any combination of polynucleotide sequences of interest to create desired phenotypes, thus creating a “stack” of transgenes in the plant and/or its progeny. These stacked

combinations can alternatively be created by cross breeding plants using conventional methods or by genetic transformation. The combinations can produce plants with a variety of trait combinations including but not limited to disease resistance, herbicide tolerance, yield enhancement, cold and drought tolerance.

[0072] In accordance with the invention, "gene stacking" is preferably accomplished by transferring two or more genes, either sequentially or in unison, into a plant cell nucleus by transformation. For example, multiple parasitic nematode resistance genes (plant genes or nematode target genes) can be down-regulated by gene silencing mechanisms, specifically RNAi, by using a single transgene targeting multiple linked dsRNA constructs that target different parasitic nematode genes. Alternatively, dsRNA constructs that target nematode genes may be stacked with dsRNA constructs that target a plant gene that is required for maintenance of parasitic nematode resistance. As another alternative, a dsRNA construct may be combined with one or more constructs that over-express genes that confer resistance to parasitic nematodes. For example if two genes are to be introduced, the two sequences can be contained in separate transformation cassettes or on the same transformation cassette. The expression of the sequences can be driven by the same or different promoters.

[0073] In accordance with the invention, in addition to comprising a transgene encoding the let-70 specific dsRNA, the transgenic plant may further comprise one or additional more nucleic acids that enhance nematode resistance. In one embodiment, dsRNAs constructs that target other parasitic nematode genes may be stacked with the let-70 specific dsRNA described herein. For example, in addition to containing the let-70 specific dsRNA, the transgenic plant of the invention may comprise a dsRNA that targets a second plant parasitic nematode gene. Any second plant parasitic nematode gene may be targeted in accordance with the invention. For example, plant parasitic nematode genes such as those disclosed in US 2005/0188438, US 2006/0037101, US 2004/098761, US 2007/0271630, US 2007/0250947, WO 2007/095469, and the like, may be targeted by a second dsRNA construct in accordance with this embodiment of the invention. Preferably, the second plant parasitic nematode target gene is selected from the group consisting of a pat-10 gene (SEQ ID NO:16; see, US2005/0188438); a pas-5 gene (SEQ ID NO:17; see, US 2006/0037101); a sca1 gene (SEQ ID NO:18); a tcp-1 gene (SEQ ID NO:19); an innexin-like gene (SEQ ID NO:20); a polymerase delta s gene (SEQ ID NO:21); a pas-1 gene (SEQ ID NO:22); a snurportin-1-like gene (SEQ ID NO:23); a prs-4 gene (SEQ ID NO:24); an rtp-1 gene (SEQ ID NO:25); and an rpn-5 gene (SEQ ID NO:26). Alternatively, the second dsRNA construct may target a plant gene which is required for maintenance of parasitic nematode infection, such as the G. max CAD-like gene (SEQ ID NO:27), or the CDPK gene (SEQ ID NO:32).

[0074] In another embodiment, the let-70 specific dsRNA construct may be stacked with a construct comprising a polynucleotide which encodes a protein that confers or increases resistance to plant parasitic nematodes when transformed into susceptible plants. Examples of such polynucleotides include a sucrose isomerase encoding polynucleotide (SEQ ID NO:28); a chitinase encoding polynucleotide (SEQ ID NO:29); an OPR3 encoding polynucleotide (SEQ ID NO:30); a trehalase encoding polynucleotide (SEQ ID NO:31); an alanine racemase encoding

polynucleotide (SEQ ID NO:33); a pEARLI1 encoding polynucleotide (SEQ ID NO:34); an MTHFR encoding polynucleotide (SEQ ID NO:35); and the like.

[0075] Stacking of other constructs that confer parasitic nematode resistance may also occur through breeding. The transgenic plants of the invention may be crossed with similar transgenic plants or with transgenic plants lacking the nucleic acids of the invention or with non-transgenic plants, using known methods of plant breeding, to prepare seeds. The seed is then planted to obtain a crossed fertile transgenic plant comprising the nucleic acid of the invention. The crossed fertile transgenic plant may have the particular expression cassette inherited through a female parent or through a male parent. The second plant may be an inbred plant. The crossed fertile transgenic may be a hybrid. Also included within the present invention are seeds of any of these crossed fertile transgenic plants. The seeds of this invention can be harvested from fertile transgenic plants and be used to grow progeny generations of transformed plants of this invention including hybrid plant lines comprising the DNA construct.

[0076] In accordance with this embodiment, the transgenic plant of the invention is produced by a method comprising the steps of preparing an expression cassette having a first region that is substantially identical to at least 19 contiguous nucleotides of a parasitic nematode let-70 gene such as nucleotides 75-574 of SEQ ID NO:1, nucleotides 1 to 548 of SEQ ID NO: 2, nucleotides 1 to 715 of SEQ ID NO: 3, or nucleotides 1 to 668 of SEQ ID NO:4, and a second region which is complementary to the first region, transforming the expression cassette into a plant, and selecting progeny of the transformed plant which express the dsRNA construct of the invention.

[0077] As increased resistance to nematode infection is a general trait wished to be inherited into a wide variety of plants, the present invention may be used to reduce crop destruction by any plant parasitic nematode. Preferably, the parasitic nematodes belong to nematode families inducing giant or syncytial cells. Nematodes inducing giant or syncytial cells are found in the families Longidoridae, Trichodoridae, Heterodidae, Meloidogynidae, Pratylenchidae or Tylenchulidae. In particular in the families Heterodidae and Meloidogynidae.

[0078] Accordingly, parasitic nematodes targeted by the present invention belong to one or more genus selected from the group of Naccobus, Cactodera, Dolichodera, Globodera, Heterodera, Punctodera, Longidorus or Meloidogyne. In a preferred embodiment the parasitic nematodes belong to one or more genus selected from the group of Naccobus, Cactodera, Dolichodera, Globodera, Heterodera, Punctodera or Meloidogyne. In a more preferred embodiment the parasitic nematodes belong to one or more genus selected from the group of Globodera, Heterodera, or Meloidogyne. In an even more preferred embodiment the parasitic nematodes belong to one or both genera selected from the group of Globodera or Heterodera. In another embodiment the parasitic nematodes belong to the genus Meloidogyne.

[0079] When the parasitic nematodes are of the genus Globodera, the species are preferably from the group consisting of G. achilleae, G. artemisiae, G. hypolysi, G. mexicana, G. millefolii, G. mali, G. pallida, G. rostochiensis, G. tabacum, and G. virginiae. In another preferred embodiment the parasitic Globodera nematodes includes at least one of the species G. pallida, G. tabacum, or G. rostochiensis. When the parasitic nematodes are of the genus Heterodera, the

species may be preferably from the group consisting of H. avenae, H. carotae, H. ciceri, H. cruciferae, H. delvii, H. elachista, H. filipjevi, H. gambiensis, H. glycines, H. goettingiana, H. graduni, H. humuli, H. hordecalis, H. latipons, H. major, H. medicaginis, H. oryzicola, H. pakistanensis, H. rosii, H. sacchari, H. schachtii, H. sorghi, H. trifolii, H. urticae, H. vigni and H. zeae. In another preferred embodiment the parasitic Heterodera nematodes include at least one of the species H. glycines, H. avenae, H. cajani, H. gottingiana, H. trifolii, H. zeae or H. schachtii. In a more preferred embodiment the parasitic nematodes includes at least one of the species H. glycines or H. schachtii. In a most preferred embodiment the parasitic nematode is the species H. glycines. When the parasitic nematodes are of the genus Meloidogyne, the parasitic nematode may be selected from the group consisting of M. acronea, M. arabica, M. arenaria, M. artiellia, M. brevicauda, M. camelliae, M. chitwoodi, M. cofeicola, M. esigua, M. graminicola, M. hapla, M. incognita, M. indica, M. inornata, M. javanica, M. lini, M. mali, M. microcephala, M. microtyla, M. naasi, M. salasi and M. thamesi. In a preferred embodiment the parasitic nematodes includes at least one of the species M. javanica, M. incognita, M. hapla, M. arenaria or M. chitwoodi.

[0080] The following examples are not intended to limit the scope of the claims to the invention, but are rather intended to be exemplary of certain embodiments. Any variations in the exemplified methods that occur to the skilled artisan are intended to fall within the scope of the present invention.

EXAMPLE 1: IDENTIFICATION AND ISOLATION OF H. GLYCINES RNAi TARGET GENES.

[0081] Using total RNA isolated from SCN J2 stage, RT-PCR was used to isolate an H. glycines let-70 cDNA fragment approximately 400-500 bp in length that was used to construct the binary vector described in Example 2. The PCR products were cloned into TOPO pCR2.1 vector (Invitrogen, Carlsbad, CA) and inserts were confirmed by sequencing. The full-length H. glycines let-70 gene (SEQ ID NO:1) was obtained using a commercially available RT-PCR method, based on highly conserved spliced leader sequence (SL1) present in many nematode species.

EXAMPLE 2: BINARY VECTOR CONSTRUCTION FOR SOYBEAN TRANSFORMATION.

[0082] The H. glycines let-70 cDNA fragment isolated in Example 1 (nucleotides 75-574 of SEQ ID NO:1) was used to make the binary vector RDM103, which consisted of an antisense fragment of the H. glycines let-70 gene, a spacer fragment, the sense fragment of the H. glycines let-70target and a vector backbone. In this vector, dsRNA for the target gene was expressed under a constitutive Super Promoter (see US 5955,646, incorporated herein by reference). The selection marker for transformation was a mutated acetohydroxyacid synthase (AHAS) gene from Arabidopsis thaliana that confers resistance to the herbicide ARSENAL (Imazapyr, BASF Corporation, Florham Park, NJ). The expression of the mutated AHAS was driven by a ubiquitin promoter.

Example 3 Bioassay of dsRNA targeted to H. glycines target genes

[0083] . The binary vector RDM103 described in Example 2 was transfected into the disarmed

A. rhizogenes strain K599, and soybean cotyledons containing the proximal end from its connection with the seedlings were used as the explant for transformation. Two to three weeks after inoculation and root induction in accordance with the method of commonly assigned copending USSN 12/001,234, incorporated herein by reference, transformed roots were formed on the cut ends of the explants. Soybean roots were excised from the rooted explants, subcultured, and one to five days after subculturing, the roots were inoculated with surface sterilized SCN J2 juveniles in multi-well plates for the gene of interest construct assay. As controls, soybean cultivar Williams 82 control vector and Jack control vector roots were used. Four weeks after nematode inoculation, the cysts in each well were counted. Bioassay results for the construct RDM103 resulted in multiple lines with reduced cyst count, showing a general trend of reduced cyst count over many of the lines tested.

Example 4 Description of homologs and DNA sequence motifs

[0084] As disclosed in Example 3, the construct RDM103 results in the expression of a double stranded RNA molecule that targets SEQ ID NO:1 and results in reduced cyst count when operably linked to a constitutive promoter and expressed in soybean roots. As disclosed in Example 1, the putative full length transcript sequence of the gene described by SEQ ID NO:1 contains an open reading frame with the amino acid sequence disclosed as SEQ ID NO:5. Plant parasitic nematode genes with DNA and LET-70 amino acid sequences homologous to SEQ ID NO:1 and SEQ ID NO:5, respectively, were identified and are set forth in SEQ ID NOs 2, 3, 4, 6, 7, and 8. The amino acid alignment of these LET-70 homologs to SEQ ID NO:5 is shown in Figure 2. A matrix table showing the amino acid percent identity among the various LET-70 homologs identified herein is shown in Figure 5a. The DNA sequence alignment of the various let-70 homologs identified herein shown in Figure 3. Regions of high homology alignment over 21 nucleotides or more are marked as Motif A through Motif G in Figure 3. The motif sequences corresponding to Motif A through Motif F are described by SEQ ID NOs 9-15 found in Figure 4. A matrix table showing the DNA sequence percent identity of SEQ ID NO:1 and the identified homologs to each other is shown in Figure 5b.

[0085] Those skilled in the art will recognize, or will be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

1. A double stranded RNA molecule comprising (a) a first strand having a sequence substantially identical to from about 19 to about 400 or 500 consecutive nucleotides of a target gene having a sequence selected from the group consisting of nucleotides 75-574 of SEQ ID NO:1, nucleotides 1 to 548 of SEQ ID NO: 2, nucleotides 1 to 715 of SEQ ID NO: 3, and nucleotides 1 to 668 of SEQ ID NO:4; and (b) a second strand having a sequence substantially complementary to the first strand.

2. A pool of double stranded RNA molecules comprising a multiplicity of short interfering RNA molecules each comprising a double stranded region having a length of about 19 to 24 nucleotides, wherein said RNA molecules are derived from a polynucleotide selected from the group consisting of a polynucleotide having a sequence consisting of nucleotides 75-574 of SEQ ID NO:1; a polynucleotide comprising nucleotides 1 to 548 of SEQ ID NO: 2, a polynucleotide comprising nucleotides 1 to 715 of SEQ ID NO: 3, and a polynucleotide comprising nucleotides 1 to 668 of SEQ ID NO:4.

3. A double stranded RNA molecule comprising a first strand having a sequence which is substantially identical to an oligonucleotide selected from the group consisting of SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO: 11; SEQ ID NO:12; SEQ ID NO:13; SEQ ID NO:14; and SEQ ID NO:15, and a second strand having a sequence substantially complementary to the first strand.

4. A transgenic plant comprising a nucleic acid construct that encodes a dsRNA capable of specifically decreasing expression of a parasitic nematode let-70 target gene, wherein the plant is resistant to parasitic nematode infection.

5. The transgenic plant of claim 4, wherein the let-70 target gene is selected from the group consisting of nucleotides 75-574 of SEQ ID NO:1; nucleotides 1 to 548 of SEQ ID NO: 2, nucleotides 1 to 715 of SEQ ID NO: 3, and nucleotides 1 to 668 of SEQ ID NO:4.

6. The transgenic plant of claim 4, wherein the dsRNA comprises an oligonucleotide selected from the group consisting of SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO: 11; SEQ ID NO:12; SEQ ID NO:13; SEQ ID NO:14; and SEQ ID NO:15.

7. The transgenic plant of claim 4, further comprising a second nucleic acid construct capable of specifically decreasing expression of a second parasitic nematode target gene.

8. The transgenic plant of claim 4, further comprising a second nucleic acid construct capable of overexpressing a gene that encodes a protein that reduces parasitic nematode infestation.

9. A transgenic plant capable of expressing a pool of dsRNA molecules, wherein each dsRNA molecule comprises a double stranded region having a length of about 19-24 nucleotides and wherein the RNA molecules are derived from polynucleotides substantially identical to a portion of a let-70 parasitic nematode target gene.

10. The transgenic plant of claim 9, wherein the let-70 target gene is selected from the group consisting of nucleotides 75-574 of SEQ ID NO:1; nucleotides 1 to 548 of SEQ ID NO: 2, nucleotides 1 to 715 of SEQ ID NO: 3, and nucleotides 1 to 668 of SEQ ID NO:4

11. The transgenic plant of claim 9, wherein the dsRNA comprises an oligonucleotide selected from the group consisting of SEQ ID NO:9; SEQ ID NO:10; SEQ ID NO: 11; SEQ ID NO:12; SEQ ID NO:13; SEQ ID NO:14; and SEQ ID NO:15.

12. A method of making a transgenic plant capable of expressing a dsRNA that is substantially identical to a portion of a let-70 target gene of a parasitic nematode, said method comprising the steps of:
(a) preparing a nucleic acid fragment comprising a region that is substantially identical to at least 19 contiguous nucleotides of nucleotides 75-574 of SEQ ID NO:1, nucleotides 1 to 548 of SEQ ID NO: 2, nucleotides 1 to 715 of SEQ ID NO: 3, or nucleotides 1 to 668 of SEQ ID NO:4, wherein the nucleic acid fragment is able to form a double-stranded transcript once expressed in the plant;
(b) transforming a recipient plant with said nucleic acid fragment;
(c) producing one or more transgenic offspring of said recipient plant; and
(d) selecting the offspring for nematode resistance.

13. A method of conferring nematode resistance to a plant, said method comprising the steps of:
(a) preparing a nucleic acid fragment comprising a region that is substantially identical to at least 19 contiguous nucleotides of nucleotides 75-574 of SEQ ID NO:1, nucleotides 1 to 548 of SEQ ID NO: 2, nucleotides 1 to 715 of SEQ ID NO: 3, or nucleotides 1 to 668 of SEQ ID NO:4, wherein the nucleic acid fragment is able to form a double-stranded transcript once expressed in the plant;
(b) transforming a recipient plant with said nucleic acid fragment;
(c) producing one or more transgenic offspring of said recipient plant; and
(d) selecting the offspring for nematode resistance.

14. An expression cassette and an expression vector comprising a nucleic acid fragment which is substantially identical to at least 19 contiguous nucleotides of nucleotides 75-574 of SEQ ID NO:1, nucleotides 1 to 548 of SEQ ID NO: 2, nucleotides 1 to 715 of SEQ ID NO: 3, or nucleotides 1 to 668 of SEQ ID NO:4.

Figure 1

Gene, promoter or motif name	Species	SEQ ID NO:
Hg_let-70 DNA	*Heterodera glycines*	1
BM344005 DNA	*Globodera rostochiensis*	2
CV578667 DNA	*Globodera pallida*	3
CK984217 DNA	*Meloidogyne incognita*	4
Hg_let-70 Protein	*Heterodera glycines*	5
BM344005 Protein	*Globodera rostochiensis*	6
CV578667 Protein	*Globodera pallida*	7
CK984217 Protein	*Meloidogyne incognita*	8
let70_motif_A	*Synthetic*	9
let70_motif_B	*Synthetic*	10
let70_motif_C	*Synthetic*	11
let70_motif_D	*Synthetic*	12
let70_motif_E	*Synthetic*	13
let70_motif_F	*Synthetic*	14
let70_motif_G	*Synthetic*	15
Hg_pat-10 DNA	*Heterodera glycines*	16
Hg_pas-5 DNA	*Heterodera glycines*	17
Hg_sca-1 DNA	*Heterodera glycines*	18
Hg_tcp-1 DNA	*Heterodera glycines*	19
Hg_innexin like DNA	*Heterodera glycines*	20
Hg_polymerase delta sub unit DNA	*Heterodera glycines*	21
Hg_pas-1 DNA	*Heterodera glycines*	22
Hg_snurportin-1 like DNA	*Heterodera glycines*	23
Hg_prs-4 DNA	*Heterodera glycines*	24
Hg_rpt-1 DNA	*Heterodera glycines*	25
Hg_rpn-5 DNA	*Heterodera glycines*	26
CAD like DNA	*Glycine max*	27
Sucrose isomerase DNA	*Erwinia rhapontici*	28
Chitinase like DNA	*Heterodera glycines*	29
OPR3 like DNA	*Glycine max*	30
Trehalase like DNA	*Glycine max*	31
CDPK like DNA	*Glycine max*	32
Alanine racemase DNA	*Escherichia coli*	33
pEARLI1 like DNA	*Arabidopsis thaliana*	34
MTHFR like DNA	*Glycine max*	35

Figure 2

```
SEQ ID NO:7     (1) MALKRIQKELQDLGRDPPAQCSAGPVGDDLFHWQATIMGPPESPYQGGVF
SEQ ID NO:6     (1) MALKRIQKELQDLGRDPPAQCSAGPVGDDLFHWQATIMGPPESPYQGGVF
SEQ ID NO:5     (1) MALKRIQKELQDLGRDPPAQCSAGPVGDDLFHWQATIMGPPESPYQGGVF
SEQ ID NO:8     (1) MALKRIQKELQDLGRDPPAQCSAGPVGDDLFHWQATIMGPPESPYQGGVF

SEQ ID NO:7    (51) FLTIHFPTDYPFKPPKVAFTTRIYHPNINSNGSICLDILRSQWSPALTIS
SEQ ID NO:6    (51) FLTIHFPTDYPFKPPKVAFTTRIYHPNINSNGSICLDILRSQWSPALTIS
SEQ ID NO:5    (51) FLTIHFPTDYPFKPPKVAFTTRIYHPNINSNGSICLDILRSQWSPALTIS
SEQ ID NO:8    (51) FLTIHFPTDYPFKPPKVAFTTRIYHPNINSNGSICLDILRSQWSPALTIS

SEQ ID NO:7   (101) KVLLSICSLLCDPNPDDPLVPEIARIYKTDRDRYNTLAREWTQKYAM
SEQ ID NO:6   (101) KVLLSICSLLCDPNPDDPLVPEIARIYKTDRDRYNTLAREWTQKYAM
SEQ ID NO:5   (101) KVLLSICSLLCDPNPDDPLVPEIARIYKTDRERYNTLAREWTQKYAM
SEQ ID NO:8   (101) KVLLSICSLLCDPNPDDPLVPEIARIYKTDRERYNTLAREWTQKYAM
```

Figure 3

```
SEQ ID NO:2   (1) ------TATATTGATATTTAACGAATCGCCGTTGACTGTTATCTAACAGA
SEQ ID NO:3   (1) TTTGAGTATATTGATATTTAACGAATCGCCGTTGACTGTTATCCGACAGA
SEQ ID NO:1   (1) ----------------------------------------------

SEQ ID NO:2  (45) CGCTTCCATGGCTTTGAAACGCATTCAGAAGGAGCTCCAGGACCTTGGTC
SEQ ID NO:3  (51) CGCTTCCATGGCTTTGAAACGCATTCAGAAGGAGCTCCAGGACCTTGGTC
SEQ ID NO:1   (1) -------ATGGCACTGAAACGTATACAAAAGGAGCTGCAGGACCTCGGCC

                        Motif A
SEQ ID NO:2  (95) GTGATCCACCAGCACAGTGCAGTGCTGGTCCAGTTGGCGACGATCTTTTT
SEQ ID NO:3 (101) GTGATCCACCAGCACAGTGCAGTGCTGGTCCAGTTGGCGACGATCTTTTT
SEQ ID NO:1  (44) GTGACCCACCCGCCCAGTGCAGTGCTGGCCCTGTCGGCGATGACCTTTTC

                                          Motif B
SEQ ID NO:2 (145) CATTGGCAGGCCACTATTATGGGCCCACCCGAATCGCCTTATCAGGGCGG
SEQ ID NO:3 (151) CATTGGCAGGCCACCATTATGGGCCCACCCGAATCGCCTTATCAGGGCGG
SEQ ID NO:1  (94) CATTGGCAAGCCACCATTATGGGGCCACCAGAATCGCCTTACCAGGGCGG

                        Motif B                                 Motif C
SEQ ID NO:2 (195) CGTCTTCTTTCTGACCATCCACTTTCCGACAGACTACCCGTTCAAACCGC
SEQ ID NO:3 (201) CGTCTTCTTTCTGACCATCCACTTTCCGACAGACTATCCGTTCAAACCGC
SEQ ID NO:1 (144) CGTCTTCTTTCTGACCATCCACTTCCCGACAGACTATCCGTTTAAGCCAC

                        Motif C                 Motif D
SEQ ID NO:2 (245) CAAAGGTGGCGTTCACCACACGCATTTATCATCCGAACATCAACAGCAAC
SEQ ID NO:3 (251) CAAAGGTGGCGTTCACCACACGCATTTATCACCCGAACATCAACAGCAAC
SEQ ID NO:1 (194) CGAAGGTGGCGTTCACCACTCGTATTTATCATCCGAACATCAACAGCAAC

                        Motif D                                 Motif E
SEQ ID NO:2 (295) GGGAGCATTTGTCTTGATATTCTGAGATCTCAGTGGTCTCCTGCGCTGAC
SEQ ID NO:3 (301) GGGAGCATTTGTCTTGATATTCTGAGATCTCAATGGTCTCCGGCGCTGAC
SEQ ID NO:1 (244) GGGAGCATTTGCCTTGATATTCTGAGATCTCAATGGTCTCCTGCACTGAC

                        Motif E
SEQ ID NO:2 (345) TATCTCAAAAGTCTTGCTCTCGATTTGCTCTCTTCTTTGTGACCCGAATC
SEQ ID NO:3 (351) TATCTCAAAAGTCTTGCTCTCGATTTGCTCTCTTCTTTGTGACCCGAATC
SEQ ID NO:1 (294) TATCTCCAAAGTCTTGCTTTCGATTTGCTCTCTTCTCTGTGATCCGAATC

                                  Motif F
SEQ ID NO:2 (395) CGGATGATCCGCTGGTTCCTGAGATAGCGCGTATCTACAAGACTGACCGT
SEQ ID NO:3 (401) CGGATGATCCGTTGGTTCCGGAGATAGCGCGTATCTACAAGACTGACCGT
SEQ ID NO:1 (344) CCGATGATCCATTGGTTCCGGAGATAGCACGCATCTACAAGACGGATCGC

                        Motif G
SEQ ID NO:2 (445) GACAGATACAATACCTTGGCGCGGGAATGGACTCAGAAGTATGCGATGTG
SEQ ID NO:3 (451) GACAGATACAATACCTTGGCACGGGAATGGACTCAGAAGTATGCGATGTG
SEQ ID NO:1 394) GAAAGATACAATACGTTGGCGCGGGAATGGACTCAGAAATATGCGATGTG
```

```
SEQ ID NO:2(495) ATCGACGGACACTGGCACCCGGGAAGACGACCTTCACGGACATCTTTTTT
SEQ ID NO:3(501) ATCGACGGACACTGGCATCCGGGAAGACGACCTTCACGAACATCTTTTTT
SEQ ID NO:1(444) A--------------------------------------------

SEQ ID NO:2(545) AAGC------------------------------------------
SEQ ID NO:3(551) AAGCCACCCGAGTTTTCTTTTGGATACTTTTTTGTGTGAAGGCAAAATTC
SEQ ID NO:1(445) ---------------------------------------------

SEQ ID NO:2(549) ---------------------------------------------
SEQ ID NO:3(601) TGGAAAATCGGACTTTCTTTCGCTATGTCAACAAATGCACCTGTTTTTCA
SEQ ID NO:1(445) ---------------------------------------------

SEQ ID NO:2(549) ----------------------------------------
SEQ ID NO:3(651) ACACCACGTAAATAAATTTGGTTGAAGCACAAAAAAAAA
SEQ ID NO:1(445) ----------------------------------------
```

Motif A consensus sequence is represented by SEQ ID NO:9
Motif B consensus sequence is represented by SEQ ID NO:10
Motif C consensus sequence is represented by SEQ ID NO:11
Motif D consensus sequence is represented by SEQ ID NO:12
Motif E consensus sequence is represented by SEQ ID NO:13
Motif F consensus sequence is represented by SEQ ID NO:14
Motif G consensus sequence is represented by SEQ ID NO:15

Figure 4

Nucleotide motif name	SEQ ID NO:	Sequence
let70_motif_A	9	CCACCNGCNCAGTGCAGTGCTGG
let70_motif_B	10	CCACCNGAATCGCCTTANCAGGGCGGCGTCTTCTTTCTG ACCATCCACTTNCCGACAGACTANCCGTT
let70_motif_C	11	CCNCCNAAGGTGGCGTTCACCACNCGNATTTATCA
let70_motif_D	12	CCGAACATCAACAGCAACGGGAGCATTTGNCTTGATATT CTGAGATCTCANTGGTCTCCNGC
let70_motif_E	13	CTGACTATCTCNAAAGTCTTGCTNTCGATTTGCTCTCTT CTNTGTGANCCGAATCC
let70_motif_F	14	GAGATAGCNCGNATCTACAAGAC
let70_motif_G	15	AGATACAATACNTTGGCNCGGGAATGGACTCAGAANTAT GCGATGTGA

Figure 5a.

Percent Identity (let-70 Amino acid)

	CV578667 (SEQ ID NO:7)	BM344005 (SEQ ID NO:6)	Hg_let-70 (SEQ ID NO:5)	CK984217 (SEQ ID NO:8)
CV578667 (SEQ ID NO:7)		100	99	99
BM344005 (SEQ ID NO:6)			99	99
Hg_let-70 (SEQ ID NO:5)				100
CK984217 (SEQ ID NO:8)				

Figure 5b.

Percent Identity (let-70 Nucleotide)

	BM344005 (SEQ ID NO:2)	CV578667 (SEQ ID NO:3)	CK984217 (SEQ ID NO:4)	HG_let-70 (SEQ ID NO:1)
BM344005 (SEQ ID NO:2)		98	82	89
CV578667 (SEQ ID NO:3)			82	89
CK984217 (SEQ ID NO:4)				82
HG_let-70 (SEQ ID NO:1)				

Figure 6a

nucleotide positions of possible 21mers selected from a group consisting of SEQ ID NO:1, 2, 3, 4, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
1	21	37	57	73	93	109	129	145	165
2	22	38	58	74	94	110	130	146	166
3	23	39	59	75	95	111	131	147	167
4	24	40	60	76	96	112	132	148	168
5	25	41	61	77	97	113	133	149	169
6	26	42	62	78	98	114	134	150	170
7	27	43	63	79	99	115	135	151	171
8	28	44	64	80	100	116	136	152	172
9	29	45	65	81	101	117	137	153	173
10	30	46	66	82	102	118	138	154	174
11	31	47	67	83	103	119	139	155	175
12	32	48	68	84	104	120	140	156	176
13	33	49	69	85	105	121	141	157	177
14	34	50	70	86	106	122	142	158	178
15	35	51	71	87	107	123	143	159	179
16	36	52	72	88	108	124	144	160	180
17	37	53	73	89	109	125	145	161	181
18	38	54	74	90	110	126	146	162	182
19	39	55	75	91	111	127	147	163	183
20	40	56	76	92	112	128	148	164	184
21	41	57	77	93	113	129	149	165	185
22	42	58	78	94	114	130	150	166	186
23	43	59	79	95	115	131	151	167	187
24	44	60	80	96	116	132	152	168	188
25	45	61	81	97	117	133	153	169	189
26	46	62	82	98	118	134	154	170	190
27	47	63	83	99	119	135	155	171	191
28	48	64	84	100	120	136	156	172	192
29	49	65	85	101	121	137	157	173	193
30	50	66	86	102	122	138	158	174	194
31	51	67	87	103	123	139	159	175	195
32	52	68	88	104	124	140	160	176	196
33	53	69	89	105	125	141	161	177	197
34	54	70	90	106	126	142	162	178	198
35	55	71	91	107	127	143	163	179	199
36	56	72	92	108	128	144	164	180	200

Figure 6b

nucleotide positions of possible 21mers selected from a group consisting of SEQ ID NO:1, 2, 3, 4, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
181	201	217	237	253	273	289	309	325	345
182	202	218	238	254	274	290	310	326	346
183	203	219	239	255	275	291	311	327	347
184	204	220	240	256	276	292	312	328	348
185	205	221	241	257	277	293	313	329	349
186	206	222	242	258	278	294	314	330	350
187	207	223	243	259	279	295	315	331	351
188	208	224	244	260	280	296	316	332	352
189	209	225	245	261	281	297	317	333	353
190	210	226	246	262	282	298	318	334	354
191	211	227	247	263	283	299	319	335	355
192	212	228	248	264	284	300	320	336	356
193	213	229	249	265	285	301	321	337	357
194	214	230	250	266	286	302	322	338	358
195	215	231	251	267	287	303	323	339	359
196	216	232	252	268	288	304	324	340	360
197	217	233	253	269	289	305	325	341	361
198	218	234	254	270	290	306	326	342	362
199	219	235	255	271	291	307	327	343	363
200	220	236	256	272	292	308	328	344	364
201	221	237	257	273	293	309	329	345	365
202	222	238	258	274	294	310	330	346	366
203	223	239	259	275	295	311	331	347	367
204	224	240	260	276	296	312	332	348	368
205	225	241	261	277	297	313	333	349	369
206	226	242	262	278	298	314	334	350	370
207	227	243	263	279	299	315	335	351	371
208	228	244	264	280	300	316	336	352	372
209	229	245	265	281	301	317	337	353	373
210	230	246	266	282	302	318	338	354	374
211	231	247	267	283	303	319	339	355	375
212	232	248	268	284	304	320	340	356	376
213	233	249	269	285	305	321	341	357	377
214	234	250	270	286	306	322	342	358	378
215	235	251	271	287	307	323	343	359	379
216	236	252	272	288	308	324	344	360	380

Figure 6c

nucleotide positions of possible 21mers selected from a group consisting of SEQ ID NO:1, 2, 3, 4, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
361	381	397	417	433	453	469	489	505	525
362	382	398	418	434	454	470	490	506	526
363	383	399	419	435	455	471	491	507	527
364	384	400	420	436	456	472	492	508	528
365	385	401	421	437	457	473	493	509	529
366	386	402	422	438	458	474	494	510	530
367	387	403	423	439	459	475	495	511	531
368	388	404	424	440	460	476	496	512	532
369	389	405	425	441	461	477	497	513	533
370	390	406	426	442	462	478	498	514	534
371	391	407	427	443	463	479	499	515	535
372	392	408	428	444	464	480	500	516	536
373	393	409	429	445	465	481	501	517	537
374	394	410	430	446	466	482	502	518	538
375	395	411	431	447	467	483	503	519	539
376	396	412	432	448	468	484	504	520	540
377	397	413	433	449	469	485	505	521	541
378	398	414	434	450	470	486	506	522	542
379	399	415	435	451	471	487	507	523	543
380	400	416	436	452	472	488	508	524	544
381	401	417	437	453	473	489	509	525	545
382	402	418	438	454	474	490	510	526	546
383	403	419	439	455	475	491	511	527	547
384	404	420	440	456	476	492	512	528	548
385	405	421	441	457	477	493	513	529	549
386	406	422	442	458	478	494	514	530	550
387	407	423	443	459	479	495	515	531	551
388	408	424	444	460	480	496	516	532	552
389	409	425	445	461	481	497	517	533	553
390	410	426	446	462	482	498	518	534	554
391	411	427	447	463	483	499	519	535	555
392	412	428	448	464	484	500	520	536	556
393	413	429	449	465	485	501	521	537	557
394	414	430	450	466	486	502	522	538	558
395	415	431	451	467	487	503	523	539	559
396	416	432	452	468	488	504	524	540	560

Figure 6d

nucleotide positions of possible 21mers selected from a group consisting of SEQ ID NO:1, 2, 3, 4, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
541	561	577	597	613	633	649	669	685	705
542	562	578	598	614	634	650	670	686	706
543	563	579	599	615	635	651	671	687	707
544	564	580	600	616	636	652	672	688	708
545	565	581	601	617	637	653	673	689	709
546	566	582	602	618	638	654	674	690	710
547	567	583	603	619	639	655	675	691	711
548	568	584	604	620	640	656	676	692	712
549	569	585	605	621	641	657	677	693	713
550	570	586	606	622	642	658	678	694	714
551	571	587	607	623	643	659	679	695	715
552	572	588	608	624	644	660	680	696	716
553	573	589	609	625	645	661	681	697	717
554	574	590	610	626	646	662	682	698	718
555	575	591	611	627	647	663	683	699	719
556	576	592	612	628	648	664	684	700	720
557	577	593	613	629	649	665	685	701	721
558	578	594	614	630	650	666	686	702	722
559	579	595	615	631	651	667	687	703	723
560	580	596	616	632	652	668	688	704	724
561	581	597	617	633	653	669	689	705	725
562	582	598	618	634	654	670	690	706	726
563	583	599	619	635	655	671	691	707	727
564	584	600	620	636	656	672	692	708	728
565	585	601	621	637	657	673	693	709	729
566	586	602	622	638	658	674	694	710	730
567	587	603	623	639	659	675	695	711	731
568	588	604	624	640	660	676	696	712	732
569	589	605	625	641	661	677	697	713	733
570	590	606	626	642	662	678	698	714	734
571	591	607	627	643	663	679	699	715	735
572	592	608	628	644	664	680	700	716	736
573	593	609	629	645	665	681	701	717	737
574	594	610	630	646	666	682	702	718	738
575	595	611	631	647	667	683	703	719	739
576	596	612	632	648	668	684	704	720	740

Figure 6e

nucleotide positions of possible 21mers selected from a group consisting of SEQ ID NO:1, 2, 3, 4, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
721	741	757	777	793	813	829	849	865	885
722	742	758	778	794	814	830	850	866	886
723	743	759	779	795	815	831	851	867	887
724	744	760	780	796	816	832	852	868	888
725	745	761	781	797	817	833	853	869	889
726	746	762	782	798	818	834	854	870	890
727	747	763	783	799	819	835	855	871	891
728	748	764	784	800	820	836	856	872	892
729	749	765	785	801	821	837	857	873	893
730	750	766	786	802	822	838	858	874	894
731	751	767	787	803	823	839	859	875	895
732	752	768	788	804	824	840	860	876	896
733	753	769	789	805	825	841	861	877	897
734	754	770	790	806	826	842	862	878	898
735	755	771	791	807	827	843	863	879	899
736	756	772	792	808	828	844	864	880	900
737	757	773	793	809	829	845	865	881	901
738	758	774	794	810	830	846	866	882	902
739	759	775	795	811	831	847	867	883	903
740	760	776	796	812	832	848	868	884	904
741	761	777	797	813	833	849	869	885	905
742	762	778	798	814	834	850	870	886	906
743	763	779	799	815	835	851	871	887	907
744	764	780	800	816	836	852	872	888	908
745	765	781	801	817	837	853	873	889	909
746	766	782	802	818	838	854	874	890	910
747	767	783	803	819	839	855	875	891	911
748	768	784	804	820	840	856	876	892	912
749	769	785	805	821	841	857	877	893	913
750	770	786	806	822	842	858	878	894	914
751	771	787	807	823	843	859	879	895	915
752	772	788	808	824	844	860	880	896	916
753	773	789	809	825	845	861	881	897	917
754	774	790	810	826	846	862	882	898	918
755	775	791	811	827	847	863	883	899	919
756	776	792	812	828	848	864	884	900	920

Figure 6f

nucleotide positions of possible 21mers selected from a group consisting of SEQ ID NO:1, 2, 3, 4, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
901	921	937	957	973	993	1009	1029	1045	1065
902	922	938	958	974	994	1010	1030	1046	1066
903	923	939	959	975	995	1011	1031	1047	1067
904	924	940	960	976	996	1012	1032	1048	1068
905	925	941	961	977	997	1013	1033	1049	1069
906	926	942	962	978	998	1014	1034	1050	1070
907	927	943	963	979	999	1015	1035	1051	1071
908	928	944	964	980	1000	1016	1036	1052	1072
909	929	945	965	981	1001	1017	1037	1053	1073
910	930	946	966	982	1002	1018	1038	1054	1074
911	931	947	967	983	1003	1019	1039	1055	1075
912	932	948	968	984	1004	1020	1040	1056	1076
913	933	949	969	985	1005	1021	1041	1057	1077
914	934	950	970	986	1006	1022	1042	1058	1078
915	935	951	971	987	1007	1023	1043	1059	1079
916	936	952	972	988	1008	1024	1044	1060	1080
917	937	953	973	989	1009	1025	1045	1061	1081
918	938	954	974	990	1010	1026	1046	1062	1082
919	939	955	975	991	1011	1027	1047	1063	1083
920	940	956	976	992	1012	1028	1048	1064	1084
921	941	957	977	993	1013	1029	1049	1065	1085
922	942	958	978	994	1014	1030	1050	1066	1086
923	943	959	979	995	1015	1031	1051	1067	1087
924	944	960	980	996	1016	1032	1052	1068	1088
925	945	961	981	997	1017	1033	1053	1069	1089
926	946	962	982	998	1018	1034	1054	1070	1090
927	947	963	983	999	1019	1035	1055	1071	1091
928	948	964	984	1000	1020	1036	1056	1072	1092
929	949	965	985	1001	1021	1037	1057	1073	1093
930	950	966	986	1002	1022	1038	1058	1074	1094
931	951	967	987	1003	1023	1039	1059	1075	1095
932	952	968	988	1004	1024	1040	1060	1076	1096
933	953	969	989	1005	1025	1041	1061	1077	1097
934	954	970	990	1006	1026	1042	1062	1078	1098
935	955	971	991	1007	1027	1043	1063	1079	1099
936	956	972	992	1008	1028	1044	1064	1080	1100

FIGURE 6g

nucleotide positions of possible 21mers selected from a group consisting of SEQ ID NO:1, 2, 3, 4, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
1081	1101	1117	1137	1153	1173	1189	1209	1225	1245
1082	1102	1118	1138	1154	1174	1190	1210	1226	1246
1083	1103	1119	1139	1155	1175	1191	1211	1227	1247
1084	1104	1120	1140	1156	1176	1192	1212	1228	1248
1085	1105	1121	1141	1157	1177	1193	1213	1229	1249
1086	1106	1122	1142	1158	1178	1194	1214	1230	1250
1087	1107	1123	1143	1159	1179	1195	1215	1231	1251
1088	1108	1124	1144	1160	1180	1196	1216	1232	1252
1089	1109	1125	1145	1161	1181	1197	1217	1233	1253
1090	1110	1126	1146	1162	1182	1198	1218	1234	1254
1091	1111	1127	1147	1163	1183	1199	1219	1235	1255
1092	1112	1128	1148	1164	1184	1200	1220	1236	1256
1093	1113	1129	1149	1165	1185	1201	1221	1237	1257
1094	1114	1130	1150	1166	1186	1202	1222	1238	1258
1095	1115	1131	1151	1167	1187	1203	1223	1239	1259
1096	1116	1132	1152	1168	1188	1204	1224	1240	1260
1097	1117	1133	1153	1169	1189	1205	1225	1241	1261
1098	1118	1134	1154	1170	1190	1206	1226	1242	1262
1099	1119	1135	1155	1171	1191	1207	1227	1243	1263
1100	1120	1136	1156	1172	1192	1208	1228	1244	1264
1101	1121	1137	1157	1173	1193	1209	1229	1245	1265
1102	1122	1138	1158	1174	1194	1210	1230	1246	1266
1103	1123	1139	1159	1175	1195	1211	1231	1247	1267
1104	1124	1140	1160	1176	1196	1212	1232	1248	1268
1105	1125	1141	1161	1177	1197	1213	1233	1249	1269
1106	1126	1142	1162	1178	1198	1214	1234	1250	1270
1107	1127	1143	1163	1179	1199	1215	1235	1251	1271
1108	1128	1144	1164	1180	1200	1216	1236	1252	1272
1109	1129	1145	1165	1181	1201	1217	1237	1253	1273
1110	1130	1146	1166	1182	1202	1218	1238	1254	1274
1111	1131	1147	1167	1183	1203	1219	1239	1255	1275
1112	1132	1148	1168	1184	1204	1220	1240	1256	1276
1113	1133	1149	1169	1185	1205	1221	1241	1257	1277
1114	1134	1150	1170	1186	1206	1222	1242	1258	1278
1115	1135	1151	1171	1187	1207	1223	1243	1259	1279
1116	1136	1152	1172	1188	1208	1224	1244	1260	1280

FIGURE 6h

nucleotide positions of possible 21mers selected from a group consisting of SEQ ID NO:1, 2, 3, 4, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
1261	1281	1297	1317	1333	1353	1369	1389	1405	1425
1262	1282	1298	1318	1334	1354	1370	1390	1406	1426
1263	1283	1299	1319	1335	1355	1371	1391	1407	1427
1264	1284	1300	1320	1336	1356	1372	1392	1408	1428
1265	1285	1301	1321	1337	1357	1373	1393	1409	1429
1266	1286	1302	1322	1338	1358	1374	1394	1410	1430
1267	1287	1303	1323	1339	1359	1375	1395	1411	1431
1268	1288	1304	1324	1340	1360	1376	1396	1412	1432
1269	1289	1305	1325	1341	1361	1377	1397	1413	1433
1270	1290	1306	1326	1342	1362	1378	1398	1414	1434
1271	1291	1307	1327	1343	1363	1379	1399	1415	1435
1272	1292	1308	1328	1344	1364	1380	1400	1416	1436
1273	1293	1309	1329	1345	1365	1381	1401	1417	1437
1274	1294	1310	1330	1346	1366	1382	1402	1418	1438
1275	1295	1311	1331	1347	1367	1383	1403	1419	1439
1276	1296	1312	1332	1348	1368	1384	1404	1420	1440
1277	1297	1313	1333	1349	1369	1385	1405	1421	1441
1278	1298	1314	1334	1350	1370	1386	1406	1422	1442
1279	1299	1315	1335	1351	1371	1387	1407	1423	1443
1280	1300	1316	1336	1352	1372	1388	1408	1424	1444
1281	1301	1317	1337	1353	1373	1389	1409	1425	1445
1282	1302	1318	1338	1354	1374	1390	1410	1426	1446
1283	1303	1319	1339	1355	1375	1391	1411	1427	1447
1284	1304	1320	1340	1356	1376	1392	1412	1428	1448
1285	1305	1321	1341	1357	1377	1393	1413	1429	1449
1286	1306	1322	1342	1358	1378	1394	1414	1430	1450
1287	1307	1323	1343	1359	1379	1395	1415	1431	1451
1288	1308	1324	1344	1360	1380	1396	1416	1432	1452
1289	1309	1325	1345	1361	1381	1397	1417	1433	1453
1290	1310	1326	1346	1362	1382	1398	1418	1434	1454
1291	1311	1327	1347	1363	1383	1399	1419	1435	1455
1292	1312	1328	1348	1364	1384	1400	1420	1436	1456
1293	1313	1329	1349	1365	1385	1401	1421	1437	1457
1294	1314	1330	1350	1366	1386	1402	1422	1438	1458
1295	1315	1331	1351	1367	1387	1403	1423	1439	1459
1296	1316	1332	1352	1368	1388	1404	1424	1440	1460

FIGURE 6i

nucleotide positions of possible 21mers selected from a group consisting of SEQ ID NO:1, 2, 3, 4, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
1441	1461	1477	1497	1513	1533	1549	1569	1585	1605
1442	1462	1478	1498	1514	1534	1550	1570	1586	1606
1443	1463	1479	1499	1515	1535	1551	1571	1587	1607
1444	1464	1480	1500	1516	1536	1552	1572	1588	1608
1445	1465	1481	1501	1517	1537	1553	1573	1589	1609
1446	1466	1482	1502	1518	1538	1554	1574	1590	1610
1447	1467	1483	1503	1519	1539	1555	1575	1591	1611
1448	1468	1484	1504	1520	1540	1556	1576	1592	1612
1449	1469	1485	1505	1521	1541	1557	1577	1593	1613
1450	1470	1486	1506	1522	1542	1558	1578	1594	1614
1451	1471	1487	1507	1523	1543	1559	1579	1595	1615
1452	1472	1488	1508	1524	1544	1560	1580	1596	1616
1453	1473	1489	1509	1525	1545	1561	1581	1597	1617
1454	1474	1490	1510	1526	1546	1562	1582	1598	1618
1455	1475	1491	1511	1527	1547	1563	1583	1599	1619
1456	1476	1492	1512	1528	1548	1564	1584	1600	1620
1457	1477	1493	1513	1529	1549	1565	1585	1601	1621
1458	1478	1494	1514	1530	1550	1566	1586	1602	1622
1459	1479	1495	1515	1531	1551	1567	1587	1603	1623
1460	1480	1496	1516	1532	1552	1568	1588	1604	1624
1461	1481	1497	1517	1533	1553	1569	1589	1605	1625
1462	1482	1498	1518	1534	1554	1570	1590	1606	1626
1463	1483	1499	1519	1535	1555	1571	1591	1607	1627
1464	1484	1500	1520	1536	1556	1572	1592	1608	1628
1465	1485	1501	1521	1537	1557	1573	1593	1609	1629
1466	1486	1502	1522	1538	1558	1574	1594	1610	1630
1467	1487	1503	1523	1539	1559	1575	1595	1611	1631
1468	1488	1504	1524	1540	1560	1576	1596	1612	1632
1469	1489	1505	1525	1541	1561	1577	1597	1613	1633
1470	1490	1506	1526	1542	1562	1578	1598	1614	1634
1471	1491	1507	1527	1543	1563	1579	1599	1615	1635
1472	1492	1508	1528	1544	1564	1580	1600	1616	1636
1473	1493	1509	1529	1545	1565	1581	1601	1617	1637
1474	1494	1510	1530	1546	1566	1582	1602	1618	1638
1475	1495	1511	1531	1547	1567	1583	1603	1619	1639
1476	1496	1512	1532	1548	1568	1584	1604	1620	1640

FIGURE 6j

nucleotide positions of possible 21mers selected from a group consisting of SEQ ID NO:1, 2, 3, 4, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
1621	1641	1657	1677	1693	1713	1729	1749	1765	1785
1622	1642	1658	1678	1694	1714	1730	1750	1766	1786
1623	1643	1659	1679	1695	1715	1731	1751	1767	1787
1624	1644	1660	1680	1696	1716	1732	1752	1768	1788
1625	1645	1661	1681	1697	1717	1733	1753	1769	1789
1626	1646	1662	1682	1698	1718	1734	1754	1770	1790
1627	1647	1663	1683	1699	1719	1735	1755	1771	1791
1628	1648	1664	1684	1700	1720	1736	1756	1772	1792
1629	1649	1665	1685	1701	1721	1737	1757	1773	1793
1630	1650	1666	1686	1702	1722	1738	1758	1774	1794
1631	1651	1667	1687	1703	1723	1739	1759	1775	1795
1632	1652	1668	1688	1704	1724	1740	1760	1776	1796
1633	1653	1669	1689	1705	1725	1741	1761	1777	1797
1634	1654	1670	1690	1706	1726	1742	1762	1778	1798
1635	1655	1671	1691	1707	1727	1743	1763	1779	1799
1636	1656	1672	1692	1708	1728	1744	1764	1780	1800
1637	1657	1673	1693	1709	1729	1745	1765	1781	1801
1638	1658	1674	1694	1710	1730	1746	1766	1782	1802
1639	1659	1675	1695	1711	1731	1747	1767	1783	1803
1640	1660	1676	1696	1712	1732	1748	1768	1784	1804
1641	1661	1677	1697	1713	1733	1749	1769	1785	1805
1642	1662	1678	1698	1714	1734	1750	1770	1786	1806
1643	1663	1679	1699	1715	1735	1751	1771	1787	1807
1644	1664	1680	1700	1716	1736	1752	1772	1788	1808
1645	1665	1681	1701	1717	1737	1753	1773	1789	1809
1646	1666	1682	1702	1718	1738	1754	1774	1790	1810
1647	1667	1683	1703	1719	1739	1755	1775	1791	1811
1648	1668	1684	1704	1720	1740	1756	1776	1792	1812
1649	1669	1685	1705	1721	1741	1757	1777	1793	1813
1650	1670	1686	1706	1722	1742	1758	1778	1794	1814
1651	1671	1687	1707	1723	1743	1759	1779	1795	1815
1652	1672	1688	1708	1724	1744	1760	1780	1796	1816
1653	1673	1689	1709	1725	1745	1761	1781	1797	1817
1654	1674	1690	1710	1726	1746	1762	1782	1798	1818
1655	1675	1691	1711	1727	1747	1763	1783	1799	1819
1656	1676	1692	1712	1728	1748	1764	1784	1800	1820

FIGURE 6k

nucleotide positions of possible 21mers selected from a group consisting of SEQ ID NO:1, 2, 3, 4, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
1801	1821	1837	1857	1873	1893	1909	1929	1945	1965
1802	1822	1838	1858	1874	1894	1910	1930	1946	1966
1803	1823	1839	1859	1875	1895	1911	1931	1947	1967
1804	1824	1840	1860	1876	1896	1912	1932	1948	1968
1805	1825	1841	1861	1877	1897	1913	1933	1949	1969
1806	1826	1842	1862	1878	1898	1914	1934	1950	1970
1807	1827	1843	1863	1879	1899	1915	1935	1951	1971
1808	1828	1844	1864	1880	1900	1916	1936	1952	1972
1809	1829	1845	1865	1881	1901	1917	1937	1953	1973
1810	1830	1846	1866	1882	1902	1918	1938	1954	1974
1811	1831	1847	1867	1883	1903	1919	1939	1955	1975
1812	1832	1848	1868	1884	1904	1920	1940	1956	1976
1813	1833	1849	1869	1885	1905	1921	1941	1957	1977
1814	1834	1850	1870	1886	1906	1922	1942	1958	1978
1815	1835	1851	1871	1887	1907	1923	1943	1959	1979
1816	1836	1852	1872	1888	1908	1924	1944	1960	1980
1817	1837	1853	1873	1889	1909	1925	1945	1961	1981
1818	1838	1854	1874	1890	1910	1926	1946	1962	1982
1819	1839	1855	1875	1891	1911	1927	1947	1963	1983
1820	1840	1856	1876	1892	1912	1928	1948	1964	1984
1821	1841	1857	1877	1893	1913	1929	1949	1965	1985
1822	1842	1858	1878	1894	1914	1930	1950	1966	1986
1823	1843	1859	1879	1895	1915	1931	1951	1967	1987
1824	1844	1860	1880	1896	1916	1932	1952	1968	1988
1825	1845	1861	1881	1897	1917	1933	1953	1969	1989
1826	1846	1862	1882	1898	1918	1934	1954	1970	1990
1827	1847	1863	1883	1899	1919	1935	1955	1971	1991
1828	1848	1864	1884	1900	1920	1936	1956	1972	1992
1829	1849	1865	1885	1901	1921	1937	1957	1973	1993
1830	1850	1866	1886	1902	1922	1938	1958	1974	1994
1831	1851	1867	1887	1903	1923	1939	1959	1975	1995
1832	1852	1868	1888	1904	1924	1940	1960	1976	1996
1833	1853	1869	1889	1905	1925	1941	1961	1977	1997
1834	1854	1870	1890	1906	1926	1942	1962	1978	1998
1835	1855	1871	1891	1907	1927	1943	1963	1979	1999
1836	1856	1872	1892	1908	1928	1944	1964	1980	2000

FIGURE 6I

nucleotide positions of possible 21mers selected from a group consisting of SEQ ID NO:1, 2, 3, 4, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
1981	2001	2017	2037	2053	2073	2089	2109	2125	2145
1982	2002	2018	2038	2054	2074	2090	2110	2126	2146
1983	2003	2019	2039	2055	2075	2091	2111	2127	2147
1984	2004	2020	2040	2056	2076	2092	2112	2128	2148
1985	2005	2021	2041	2057	2077	2093	2113	2129	2149
1986	2006	2022	2042	2058	2078	2094	2114	2130	2150
1987	2007	2023	2043	2059	2079	2095	2115	2131	2151
1988	2008	2024	2044	2060	2080	2096	2116	2132	2152
1989	2009	2025	2045	2061	2081	2097	2117	2133	2153
1990	2010	2026	2046	2062	2082	2098	2118	2134	2154
1991	2011	2027	2047	2063	2083	2099	2119	2135	2155
1992	2012	2028	2048	2064	2084	2100	2120	2136	2156
1993	2013	2029	2049	2065	2085	2101	2121	2137	2157
1994	2014	2030	2050	2066	2086	2102	2122	2138	2158
1995	2015	2031	2051	2067	2087	2103	2123	2139	2159
1996	2016	2032	2052	2068	2088	2104	2124	2140	2160
1997	2017	2033	2053	2069	2089	2105	2125	2141	2161
1998	2018	2034	2054	2070	2090	2106	2126	2142	2162
1999	2019	2035	2055	2071	2091	2107	2127	2143	2163
2000	2020	2036	2056	2072	2092	2108	2128	2144	2164
2001	2021	2037	2057	2073	2093	2109	2129	2145	2165
2002	2022	2038	2058	2074	2094	2110	2130	2146	2166
2003	2023	2039	2059	2075	2095	2111	2131	2147	2167
2004	2024	2040	2060	2076	2096	2112	2132	2148	2168
2005	2025	2041	2061	2077	2097	2113	2133	2149	2169
2006	2026	2042	2062	2078	2098	2114	2134	2150	2170
2007	2027	2043	2063	2079	2099	2115	2135	2151	2171
2008	2028	2044	2064	2080	2100	2116	2136	2152	2172
2009	2029	2045	2065	2081	2101	2117	2137	2153	2173
2010	2030	2046	2066	2082	2102	2118	2138	2154	2174
2011	2031	2047	2067	2083	2103	2119	2139	2155	2175
2012	2032	2048	2068	2084	2104	2120	2140	2156	2176
2013	2033	2049	2069	2085	2105	2121	2141	2157	2177
2014	2034	2050	2070	2086	2106	2122	2142	2158	2178
2015	2035	2051	2071	2087	2107	2123	2143	2159	2179
2016	2036	2052	2072	2088	2108	2124	2144	2160	2180

FIGURE 6m

nucleotide positions of possible 21mers selected from a group consisting of SEQ ID NO:1, 2, 3, 4, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
2161	2181	2197	2217	2233	2253	2269	2289	2305	2325
2162	2182	2198	2218	2234	2254	2270	2290	2306	2326
2163	2183	2199	2219	2235	2255	2271	2291	2307	2327
2164	2184	2200	2220	2236	2256	2272	2292	2308	2328
2165	2185	2201	2221	2237	2257	2273	2293	2309	2329
2166	2186	2202	2222	2238	2258	2274	2294	2310	2330
2167	2187	2203	2223	2239	2259	2275	2295	2311	2331
2168	2188	2204	2224	2240	2260	2276	2296	2312	2332
2169	2189	2205	2225	2241	2261	2277	2297	2313	2333
2170	2190	2206	2226	2242	2262	2278	2298	2314	2334
2171	2191	2207	2227	2243	2263	2279	2299	2315	2335
2172	2192	2208	2228	2244	2264	2280	2300	2316	2336
2173	2193	2209	2229	2245	2265	2281	2301	2317	2337
2174	2194	2210	2230	2246	2266	2282	2302	2318	2338
2175	2195	2211	2231	2247	2267	2283	2303	2319	2339
2176	2196	2212	2232	2248	2268	2284	2304	2320	2340
2177	2197	2213	2233	2249	2269	2285	2305	2321	2341
2178	2198	2214	2234	2250	2270	2286	2306	2322	2342
2179	2199	2215	2235	2251	2271	2287	2307	2323	2343
2180	2200	2216	2236	2252	2272	2288	2308	2324	2344
2181	2201	2217	2237	2253	2273	2289	2309	2325	2345
2182	2202	2218	2238	2254	2274	2290	2310	2326	2346
2183	2203	2219	2239	2255	2275	2291	2311	2327	2347
2184	2204	2220	2240	2256	2276	2292	2312	2328	2348
2185	2205	2221	2241	2257	2277	2293	2313	2329	2349
2186	2206	2222	2242	2258	2278	2294	2314	2330	2350
2187	2207	2223	2243	2259	2279	2295	2315	2331	2351
2188	2208	2224	2244	2260	2280	2296	2316	2332	2352
2189	2209	2225	2245	2261	2281	2297	2317	2333	2353
2190	2210	2226	2246	2262	2282	2298	2318	2334	2354
2191	2211	2227	2247	2263	2283	2299	2319	2335	2355
2192	2212	2228	2248	2264	2284	2300	2320	2336	2356
2193	2213	2229	2249	2265	2285	2301	2321	2337	2357
2194	2214	2230	2250	2266	2286	2302	2322	2338	2358
2195	2215	2231	2251	2267	2287	2303	2323	2339	2359
2196	2216	2232	2252	2268	2288	2304	2324	2340	2360

FIGURE 6n

nucleotide positions of possible 21mers selected from a group consisting of SEQ ID NO:1, 2, 3, 4, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
2341	2361	2377	2397	2413	2433	2449	2469	2485	2505
2342	2362	2378	2398	2414	2434	2450	2470	2486	2506
2343	2363	2379	2399	2415	2435	2451	2471	2487	2507
2344	2364	2380	2400	2416	2436	2452	2472	2488	2508
2345	2365	2381	2401	2417	2437	2453	2473	2489	2509
2346	2366	2382	2402	2418	2438	2454	2474	2490	2510
2347	2367	2383	2403	2419	2439	2455	2475	2491	2511
2348	2368	2384	2404	2420	2440	2456	2476	2492	2512
2349	2369	2385	2405	2421	2441	2457	2477	2493	2513
2350	2370	2386	2406	2422	2442	2458	2478	2494	2514
2351	2371	2387	2407	2423	2443	2459	2479	2495	2515
2352	2372	2388	2408	2424	2444	2460	2480	2496	2516
2353	2373	2389	2409	2425	2445	2461	2481	2497	2517
2354	2374	2390	2410	2426	2446	2462	2482	2498	2518
2355	2375	2391	2411	2427	2447	2463	2483	2499	2519
2356	2376	2392	2412	2428	2448	2464	2484	2500	2520
2357	2377	2393	2413	2429	2449	2465	2485	2501	2521
2358	2378	2394	2414	2430	2450	2466	2486	2502	2522
2359	2379	2395	2415	2431	2451	2467	2487	2503	2523
2360	2380	2396	2416	2432	2452	2468	2488	2504	2524
2361	2381	2397	2417	2433	2453	2469	2489	2505	2525
2362	2382	2398	2418	2434	2454	2470	2490	2506	2526
2363	2383	2399	2419	2435	2455	2471	2491	2507	2527
2364	2384	2400	2420	2436	2456	2472	2492	2508	2528
2365	2385	2401	2421	2437	2457	2473	2493	2509	2529
2366	2386	2402	2422	2438	2458	2474	2494	2510	2530
2367	2387	2403	2423	2439	2459	2475	2495	2511	2531
2368	2388	2404	2424	2440	2460	2476	2496	2512	2532
2369	2389	2405	2425	2441	2461	2477	2497	2513	2533
2370	2390	2406	2426	2442	2462	2478	2498	2514	2534
2371	2391	2407	2427	2443	2463	2479	2499	2515	2535
2372	2392	2408	2428	2444	2464	2480	2500	2516	2536
2373	2393	2409	2429	2445	2465	2481	2501	2517	2537
2374	2394	2410	2430	2446	2466	2482	2502	2518	2538
2375	2395	2411	2431	2447	2467	2483	2503	2519	2539
2376	2396	2412	2432	2448	2468	2484	2504	2520	2540

FIGURE 6o

nucleotide positions of possible 21mers selected from a group consisting of SEQ ID NO:1, 2, 3, 4, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
2521	2541	2557	2577	2593	2613	2629	2649	2665	2685
2522	2542	2558	2578	2594	2614	2630	2650	2666	2686
2523	2543	2559	2579	2595	2615	2631	2651	2667	2687
2524	2544	2560	2580	2596	2616	2632	2652	2668	2688
2525	2545	2561	2581	2597	2617	2633	2653	2669	2689
2526	2546	2562	2582	2598	2618	2634	2654	2670	2690
2527	2547	2563	2583	2599	2619	2635	2655	2671	2691
2528	2548	2564	2584	2600	2620	2636	2656	2672	2692
2529	2549	2565	2585	2601	2621	2637	2657	2673	2693
2530	2550	2566	2586	2602	2622	2638	2658	2674	2694
2531	2551	2567	2587	2603	2623	2639	2659	2675	2695
2532	2552	2568	2588	2604	2624	2640	2660	2676	2696
2533	2553	2569	2589	2605	2625	2641	2661	2677	2697
2534	2554	2570	2590	2606	2626	2642	2662	2678	2698
2535	2555	2571	2591	2607	2627	2643	2663	2679	2699
2536	2556	2572	2592	2608	2628	2644	2664	2680	2700
2537	2557	2573	2593	2609	2629	2645	2665	2681	2701
2538	2558	2574	2594	2610	2630	2646	2666	2682	2702
2539	2559	2575	2595	2611	2631	2647	2667	2683	2703
2540	2560	2576	2596	2612	2632	2648	2668	2684	2704
2541	2561	2577	2597	2613	2633	2649	2669	2685	2705
2542	2562	2578	2598	2614	2634	2650	2670	2686	2706
2543	2563	2579	2599	2615	2635	2651	2671	2687	2707
2544	2564	2580	2600	2616	2636	2652	2672	2688	2708
2545	2565	2581	2601	2617	2637	2653	2673	2689	2709
2546	2566	2582	2602	2618	2638	2654	2674	2690	2710
2547	2567	2583	2603	2619	2639	2655	2675	2691	2711
2548	2568	2584	2604	2620	2640	2656	2676	2692	2712
2549	2569	2585	2605	2621	2641	2657	2677	2693	2713
2550	2570	2586	2606	2622	2642	2658	2678	2694	2714
2551	2571	2587	2607	2623	2643	2659	2679	2695	2715
2552	2572	2588	2608	2624	2644	2660	2680	2696	2716
2553	2573	2589	2609	2625	2645	2661	2681	2697	2717
2554	2574	2590	2610	2626	2646	2662	2682	2698	2718
2555	2575	2591	2611	2627	2647	2663	2683	2699	2719
2556	2576	2592	2612	2628	2648	2664	2684	2700	2720

FIGURE 6p

nucleotide positions of possible 21mers selected from a group consisting of SEQ ID NO:1, 2, 3, 4, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
2701	2721	2737	2757	2773	2793	2809	2829	2845	2865
2702	2722	2738	2758	2774	2794	2810	2830	2846	2866
2703	2723	2739	2759	2775	2795	2811	2831	2847	2867
2704	2724	2740	2760	2776	2796	2812	2832	2848	2868
2705	2725	2741	2761	2777	2797	2813	2833	2849	2869
2706	2726	2742	2762	2778	2798	2814	2834	2850	2870
2707	2727	2743	2763	2779	2799	2815	2835	2851	2871
2708	2728	2744	2764	2780	2800	2816	2836	2852	2872
2709	2729	2745	2765	2781	2801	2817	2837	2853	2873
2710	2730	2746	2766	2782	2802	2818	2838	2854	2874
2711	2731	2747	2767	2783	2803	2819	2839	2855	2875
2712	2732	2748	2768	2784	2804	2820	2840	2856	2876
2713	2733	2749	2769	2785	2805	2821	2841	2857	2877
2714	2734	2750	2770	2786	2806	2822	2842	2858	2878
2715	2735	2751	2771	2787	2807	2823	2843	2859	2879
2716	2736	2752	2772	2788	2808	2824	2844	2860	2880
2717	2737	2753	2773	2789	2809	2825	2845	2861	2881
2718	2738	2754	2774	2790	2810	2826	2846	2862	2882
2719	2739	2755	2775	2791	2811	2827	2847	2863	2883
2720	2740	2756	2776	2792	2812	2828	2848	2864	2884
2721	2741	2757	2777	2793	2813	2829	2849	2865	2885
2722	2742	2758	2778	2794	2814	2830	2850	2866	2886
2723	2743	2759	2779	2795	2815	2831	2851	2867	2887
2724	2744	2760	2780	2796	2816	2832	2852	2868	2888
2725	2745	2761	2781	2797	2817	2833	2853	2869	2889
2726	2746	2762	2782	2798	2818	2834	2854	2870	2890
2727	2747	2763	2783	2799	2819	2835	2855	2871	2891
2728	2748	2764	2784	2800	2820	2836	2856	2872	2892
2729	2749	2765	2785	2801	2821	2837	2857	2873	2893
2730	2750	2766	2786	2802	2822	2838	2858	2874	2894
2731	2751	2767	2787	2803	2823	2839	2859	2875	2895
2732	2752	2768	2788	2804	2824	2840	2860	2876	2896
2733	2753	2769	2789	2805	2825	2841	2861	2877	2897
2734	2754	2770	2790	2806	2826	2842	2862	2878	2898
2735	2755	2771	2791	2807	2827	2843	2863	2879	2899
2736	2756	2772	2792	2808	2828	2844	2864	2880	2900

FIGURE 6q

nucleotide positions of possible 21mers selected from a group consisting of SEQ ID NO:1, 2, 3, 4, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
2881	2901	2917	2937	2953	2973	2989	3009		
2882	2902	2918	2938	2954	2974	2990	3010		
2883	2903	2919	2939	2955	2975	2991	3011		
2884	2904	2920	2940	2956	2976	2992	3012		
2885	2905	2921	2941	2957	2977	2993	3013		
2886	2906	2922	2942	2958	2978	2994	3014		
2887	2907	2923	2943	2959	2979	2995	3015		
2888	2908	2924	2944	2960	2980	2996	3016		
2889	2909	2925	2945	2961	2981	2997	3017		
2890	2910	2926	2946	2962	2982	2998	3018		
2891	2911	2927	2947	2963	2983	2999	3019		
2892	2912	2928	2948	2964	2984	3000	3020		
2893	2913	2929	2949	2965	2985	3001	3021		
2894	2914	2930	2950	2966	2986	3002	3022		
2895	2915	2931	2951	2967	2987	3003	3023		
2896	2916	2932	2952	2968	2988	3004	3024		
2897	2917	2933	2953	2969	2989	3005	3025		
2898	2918	2934	2954	2970	2990	3006	3026		
2899	2919	2935	2955	2971	2991	3007	3027		
2900	2920	2936	2956	2972	2992	3008	3028		
2901	2921	2937	2957	2973	2993	3009	3029		
2902	2922	2938	2958	2974	2994	3010	3030		
2903	2923	2939	2959	2975	2995	3011	3031		
2904	2924	2940	2960	2976	2996	3012	3032		
2905	2925	2941	2961	2977	2997	3013	3033		
2906	2926	2942	2962	2978	2998				
2907	2927	2943	2963	2979	2999				
2908	2928	2944	2964	2980	3000	.....	.....		
2909	2929	2945	2965	2981	3001	.....	.....		
2910	2930	2946	2966	2982	3002	n-5	n+15		
2911	2931	2947	2967	2983	3003	n-4	n+16		
2912	2932	2948	2968	2984	3004	n-3	n+17		
2913	2933	2949	2969	2985	3005	n-2	n+18		
2914	2934	2950	2970	2986	3006	n-1	n+19		
2915	2935	2951	2971	2987	3007	n	n+20		
2916	2936	2952	2972	2988	3008				

FIGURE 6r

n = total number of nucleotides of the entire length of a parasitic nematode target gene encoding polynucleotide - 20.

For example:

n = 677 (697-20) for SEQ ID NO:1;
n = 528 (548-20) for SEQ ID NO:2;
n = 669 (689-20) for SEQ ID NO:3;
n = 647 (667-20) for SEQ ID NO:4;
n = 3 (23-20) for SEQ ID NO:9;
n = 48 (68-20) for SEQ ID NO:10;
n = 15 (35-20) for SEQ ID NO:11;
n = 42 (62-20) for SEQ ID NO:12;
n = 36 (56-20) for SEQ ID NO:13;
n = 3 (23-20) for SEQ ID NO:14;
n = 28 (48-20) for SEQ ID NO:15;
n = 750 (770-20) for SEQ ID NO:16;
n = 959 (979-20) for SEQ ID NO:17;
n = 3013 (3033-20) for SEQ ID NO:18;
n = 1771 (1791-20) for SEQ ID NO:19;
n = 1824 (1844-20) for SEQ ID NO:20;
n = 1666 (1686-20) for SEQ ID NO:21;
n = 844 (864-20) for SEQ ID NO:22
n = 1488 (1508-20) for SEQ ID NO:23;
n = 555 (575-20) for SEQ ID NO:24;
n = 1424 (1444-20) for SEQ ID NO:25;
n = 1632 (1652-20) for SEQ ID NO:26;
n = 1096 (1116-20) for SEQ ID NO:27;
n = 444 (1464-20) for SEQ ID NO:28;
n = 1033 (1053-20) for SEQ ID NO:29;
n = 1376 (1396-20) for SEQ ID NO:30;
n = 1654 (1674-20) for SEQ ID NO:31;
n = 300 (320-20) for SEQ ID NO:32;
n = 1051 (1071-20) for SEQ ID NO:33;
n = 707 (727-20) for SEQ ID NO:34;
n = 1249 (1269-20) for SEQ ID NO:35;