

nucleotides, for example, about 21-nucleotide duplexes with about 19 base pairs and 3'-terminal mononucleotide, dinucleotide, or trinucleotide overhangs. In yet another embodiment, siNA molecules of the invention comprise duplex nucleic acid molecules with blunt ends, where both ends are blunt, or alternatively, where one of the ends is blunt.

**[0169]** An siNA molecule of the present invention may comprise modified nucleotides while maintaining the ability to mediate RNAi. The modified nucleotides can be used to improve in vitro or in vivo characteristics such as stability, activity, and/or bioavailability. For example, a siNA molecule of the invention can comprise modified nucleotides as a percentage of the total number of nucleotides present in the siNA molecule. As such, a siNA molecule of the invention can generally comprise about 5% to about 100% modified nucleotides (e.g., about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% modified nucleotides). The actual percentage of modified nucleotides present in a given siNA molecule will depend on the total number of nucleotides present in the siNA. If the siNA molecule is single stranded, the percent modification can be based upon the total number of nucleotides present in the single stranded siNA molecules. Likewise, if the siNA molecule is double stranded, the percent modification can be based upon the total number of nucleotides present in the sense strand, antisense strand, or both the sense and antisense strands.

#### **[0170]** VII. Nucleic Acid Constructs

**[0171]** A recombinant nucleic acid vector may, for example, be a linear or a closed circular plasmid. The vector system may be a single vector or plasmid or two or more vectors or plasmids that together contain the total nucleic acid to be introduced into the genome of the bacterial host. In addition, a bacterial vector may be an expression vector. Nucleic acid molecules as set forth in SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 49-158, 159, 160, 161, 162, 163, 168, 173, 178, 183, 188, 193, 198, 203, 208, 215, 220, 225, 230, 240-246, 247, 249, 251, 253, 255, 257, 259, 275-472, 473, 478, 483, 488, 493, 498, 503, 508-512, 513, 515, 517, 519, 521, 533-575, 576, 581, 586, 591, 596, 601, 603, 605, 607, 609, 621-767, 768, 773, 778, 783, 788, 793, 795, 797, 799, 801, 813-862, 863, 868, 873, 878, 883, 888, 890, 892, 894, 896, 908-1040, 1041, 1046, 1051, 1056, 1061, 1066-1070, 1071, 1073, 1075, 1077, 1079, 1081, 1083, 1085, 1087, 1089, 1091, 1093, 1095, 1097, 1099, 1101, 1103, 1105, 1107, 1109, 1111, 1113, 1161-1571, 1572, 1577, 1582, 1587, 1592, 1597, 1602, 1607, 1612, 1617, 1622, 1627, 1632, 1637, 1642, 1647, 1652, 1657, 1662, 1667, 1672, 1677, 1682, 1684, 1686, 1688, 1690, 1692, 1694, 1696, 1698, 1700, 1702, 1704, 1730-2039, 2040, 2045, 2050, 2055, 2060, 2065, 2070, 2075, 2080, 2085, 2090, 2095, 2100, 2102, 2104, 2106, 2108, 2120-2338, 2339, 2344, 2349, 2354, 2359, 2364, 2366, 2368, 2370, 2372, 2384-2460, 2461, 2466, 2471, 2476 and 2481, or fragments thereof can, for example, be suitably inserted into a vector under the control of a suitable promoter that functions in one or more microbial hosts to drive expression of a linked coding sequence or other DNA sequence. Many vectors are available for this purpose, and selection of the appropriate vector will depend mainly on the size of the nucleic acid to be inserted into the vector and the particular host cell to be transformed with the vector. Each vector contains various components depending on its function (amplification of DNA or expression of DNA) and the particular host cell with which it is compatible. The vector components for bacterial transforma-

tion generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more selectable marker genes, and an inducible promoter allowing the expression of exogenous DNA.

#### **[0172]** Promoters

**[0173]** "Operably linked", as used in reference to a regulatory sequence and a structural nucleotide sequence, means that the regulatory sequence causes regulated expression of the linked structural nucleotide sequence. "Regulatory sequences" or "control elements" refer to nucleotide sequences located upstream (5' noncoding sequences), within, or downstream (3' non-translated sequences) of a structural nucleotide sequence, and which influence the timing and level or amount of transcription, RNA processing or stability, or translation of the associated structural nucleotide sequence. Regulatory sequences may include promoters, translation leader sequences, introns, enhancers, stem-loop structures, repressor binding sequences, and polyadenylation recognition sequences and the like.

**[0174]** An expression vector for producing a mRNA can also contain an inducible promoter that is recognized by the host bacterial organism and is operably linked to the nucleic acid encoding, for example, the nucleic acid molecule coding the *D. v. virgifera* mRNA or fragment thereof of interest. Inducible promoters suitable for use with bacterial hosts include  $\beta$ -lactamase promoter, *E. coli*  $\lambda$  phage PL and PR promoters, and *E. coli* galactose promoter, arabinose promoter, alkaline phosphatase promoter, tryptophan (trp) promoter, and the lactose operon promoter and variations thereof and hybrid promoters such as the tac promoter. However, other known bacterial inducible promoters are suitable.

**[0175]** In certain embodiments, the genes can be derived from different insects in order to broaden the range of insects against which the agent is effective. When multiple genes are targeted for suppression or a combination of expression and suppression, a polycistronic DNA element can be fabricated as illustrated and disclosed in Fillatti, Application Publication No. US 2004-0029283.

#### **[0176]** Selectable Marker Genes

**[0177]** A recombinant DNA vector or construct of the present invention will typically comprise a selectable marker that confers a selectable phenotype on transformed cells. Selectable markers may also be used to select for cells that contain the exogenous nucleic acids encoding polypeptides or proteins of the present invention. The marker may encode biocide resistance, such as antibiotic resistance (e.g., kanamycin, G418 bleomycin, hygromycin, etc.). Examples of selectable markers include, but are not limited to, a neo gene which codes for kanamycin resistance and can be selected for using kanamycin, G418, etc., a bar gene which codes for bialaphos resistance; a nitrilase gene which confers resistance to bromoxynil, and a methotrexate resistant DHFR gene. Examples of such selectable markers are illustrated in U.S. Pat. Nos. 5,550,318; 5,633,435; 5,780,708 and 6,118,047.

**[0178]** A recombinant vector or construct of the present invention may also include a screenable marker. Screenable markers may be used to monitor expression. Exemplary screenable markers include a  $\beta$ -glucuronidase or uidA gene (GUS) which encodes an enzyme for which various chromogenic substrates are known (Jefferson, 1987; Jefferson et al., 1987); a  $\beta$ -lactamase gene (Sutcliffe et al., 1978), a gene which encodes an enzyme for which various chromogenic substrates are known (e.g., PADAC, a chromogenic cephalosporin); a luciferase gene (Ow et al., 1986) a xyleE gene