

FROM CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, 5th ed., vol. 1-2, ed. Ausubel et al., John Wiley & Sons, Inc., 2002; GENOME ANALYSIS: A LABORATORY MANUAL, vol. 1-2, ed. Green et al., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1997.

[0041] Various techniques using PCR are described, for example, in Innis et al., PCR PROTOCOLS: A GUIDE TO METHODS AND APPLICATIONS, Academic Press, San Diego, 1990 and in Dieffenbach and Dveksler, PCR PRIMER: A LABORATORY MANUAL, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2003. PCR-primer pairs can be derived from known sequences by known techniques such as using computer programs intended for that purpose, e.g., Primer, Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge, Mass. Methods for chemical synthesis of nucleic acids are discussed, for example, in Beaucage & Caruthers, *Tetra. Letts.* 22: 1859-62 (1981), and Matteucci & Caruthers, *J. Am. Chem. Soc.* 103: 3185 (1981).

[0042] Restriction enzyme digestions, phosphorylations, ligations, and transformations were done as described in Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, 2nd ed. (1989), Cold Spring Harbor Laboratory Press. All reagents and materials used for the growth and maintenance of bacterial cells were obtained from Aldrich Chemicals (Milwaukee, Wis.), DIFCO Laboratories (Detroit, Mich.), Invitrogen (Gaithersburg, Md.), or Sigma Chemical Company (St. Louis, Mo.) unless otherwise specified.

[0043] Biological activity refers to the biological behavior and effects of a protein or peptide and its manifestations on a pest. For example, an inventive RNAi may prevent translation of a particular mRNA, thereby inhibiting the biological activity of the protein encoded by the mRNA or other biological activity of the pest.

[0044] In the present description, an RNAi molecule may inhibit a biological activity in a pest, resulting in one or more of the following attributes: reduction in feeding by the pest, reduction in viability of the pest, death of the pest, inhibition of differentiation and development of the pest, absence of or reduced capacity for sexual reproduction by the pest, muscle formation, juvenile hormone formation, juvenile hormone regulation, ion regulation and transport, maintenance of cell membrane potential, amino acid biosynthesis, amino acid degradation, sperm formation, pheromone synthesis, pheromone sensing, antennae formation, wing formation, leg formation, development and differentiation, egg formation, larval maturation, digestive enzyme formation, haemolymph synthesis, haemolymph maintenance, neurotransmission, cell division, energy metabolism, respiration, apoptosis, and any component of a eukaryotic cells' cytoskeletal structure, such as, for example, actins and tubulins.

[0045] Complementary DNA (cDNA) refers to single-stranded DNA synthesized from a mature mRNA template. Though there are several methods, cDNA is most often synthesized from mature (fully spliced) mRNA using the enzyme reverse transcriptase. This enzyme operates on a single strand of mRNA, generating its complementary DNA based on the pairing of RNA base pairs (A, U, G, C) to their DNA complements (T, A, C, G). Two nucleic acid strands are substantially complementary when at least 85% of their bases pair.

[0046] Desired Polynucleotide: a desired polynucleotide of the present invention is a genetic element, such as a promoter, enhancer, or terminator, or gene or polynucleotide that is to be transcribed and/or translated in a transformed cell that comprises the desired polynucleotide in its genome. If the desired

polynucleotide comprises a sequence encoding a protein product, the coding region may be operably linked to regulatory elements, such as to a promoter and a terminator, that bring about expression of an associated messenger RNA transcript and/or a protein product encoded by the desired polynucleotide. Thus, a "desired polynucleotide" may comprise a gene that is operably linked in the 5'- to 3'-orientation, a promoter, a gene that encodes a protein, and a terminator. Alternatively, the desired polynucleotide may comprise a gene or fragment thereof, in a "sense" or "antisense" orientation, the transcription of which produces nucleic acids that may affect expression of an endogenous gene in the host cell. A desired polynucleotide may also yield upon transcription a double-stranded RNA product upon that initiates RNA interference of a gene to which the desired polynucleotide is associated. A desired polynucleotide of the present invention may be positioned within a vector, such that the left and right border sequences flank or are on either side of the desired polynucleotide. The present invention envisions the stable integration of one or more desired polynucleotides into the genome of at least one host cell. A desired polynucleotide may be mutated or a variant of its wild-type sequence. It is understood that all or part of the desired polynucleotide can be integrated into the genome of a host. It also is understood that the term "desired polynucleotide" encompasses one or more of such polynucleotides. Thus, a vector of the present invention may comprise one, two, three, four, five, six, seven, eight, nine, ten, or more desired polynucleotides.

[0047] "Exposing" encompasses any method by which a pest may come into contact with a dsRNA, wherein the dsRNA comprises annealed complementary strands, one of which has a nucleotide sequence which is complementary to at least part of the nucleotide sequence of a pest target gene to be down-regulated. A pest may be exposed to the dsRNA by direct uptake (e.g. by feeding), which does not require expression of dsRNA within the pest. Alternatively, a pest may come into direct contact with a composition comprising the dsRNA. For example, a pest may come into contact with a surface or material treated with a composition comprising a dsRNA. A dsRNA may be expressed by a prokaryotic (for instance, but not limited to, a bacterial) or eukaryotic (for instance, but not limited to, a yeast) host cell or host organism.

[0048] Foreign: "foreign," with respect to a nucleic acid, means that that nucleic acid is derived from non-host organisms. According to the present invention, foreign DNA or RNA represents nucleic acids that are naturally occurring in the genetic makeup of viruses, mammals, fish or birds, but are not naturally occurring in the host that is to be transformed. Thus, a foreign nucleic acid is one that encodes, for instance, a polypeptide that is not naturally produced by the transformed host. A foreign nucleic acid does not have to encode a protein product.

[0049] Gene: refers to a polynucleotide sequence that comprises control and coding sequences necessary for the production of a polypeptide or precursor. The polypeptide can be encoded by a full length coding sequence or by any portion of the coding sequence. A gene may constitute an uninterrupted coding sequence or it may include one or more introns, bound by the appropriate splice junctions. Moreover, a gene may contain one or more modifications in either the coding or the untranslated regions that could affect the biological activity or the chemical structure of the expression product, the rate of expression, or the manner of expression control. Such modifications include, but are not limited to, mutations, insertions,