

[0161] In general, plasmid vectors containing replicon and control sequences that are derived from species compatible with the host cell are used in connection with bacterial hosts. The vector ordinarily carries a replication site, as well as marking sequences that are capable of providing phenotypic selection in transformed cells. For example, *E. coli* is typically transformed using pBR322, a plasmid derived from an *E. coli* species (see, e.g., Bolivar et al., *Gene* 2:95 (1977)). pBR322 contains genes for ampicillin and tetracycline resistance and thus provides easy means for identifying transformed cells. The pBR322 plasmid, or other microbial plasmid or phage, also generally contains, or is modified to contain, promoters that can be used by the microbial organism for expression of the selectable marker genes.

[0162] Nucleic acid molecules encoding *D. v. virgifera* proteins or fragments thereof may be expressed not only directly, but also as a fusion with another polypeptide, preferably a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the polypeptide encoding DNA that is inserted into the vector. The heterologous signal sequence selected should be one that is recognized and processed (i.e., cleaved by a signal peptidase) by the host cell. For bacterial host cells that do not recognize and process the native polypeptide signal sequence, the signal sequence is substituted by a bacterial signal sequence selected, for example, from the group consisting of the alkaline phosphatase, β -lactamase, or heat-stable enterotoxin II leaders and the like.

[0163] Both expression and cloning vectors contain a nucleotide sequence that enables the vector to replicate in one or more selected host cells. Generally, in cloning vectors this sequence is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria.

[0164] Expression and cloning vectors also generally contain a selection gene, also termed a selectable marker. This gene encodes a protein necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for Bacilli. One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous protein or fragment thereof produce a protein conferring drug resistance and thus survive the selection regimen.

[0165] The expression vector for producing a polypeptide 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 encoding the *D. v. virgifera* protein or fragment thereof of interest. Inducible promoters suitable for use with bacterial hosts include the β -lactamase, *E. coli* λ phage P_L and P_R , and *E. coli* galactose, arabinose, alkaline phosphatase, tryptophan (*trp*), and lactose operon promoter systems and variations thereof (Chang et al., *Nature* 275:615 (1978); Goeddel et al., *Nature* 281:544 (1979); Guzman et al., *J. Bacteriol.* 174: 7716-7728 (1992); Goeddel, *Nucleic Acids Res.* 8:4057

(1980); EP 36,776) and hybrid promoters such as the tac promoter (deBoer et al., *Proc. Natl. Acad. Sci. (USA)* 80:21-25 (1983)). However, other known bacterial inducible promoters are suitable (Siebenlist et al., *Cell* 20:269 (1980)).

[0166] Promoters for use in bacterial systems also generally contain a Shine-Dalgarno (S.D.) sequence or a consensus sequence thereof operably linked to the DNA encoding the polypeptide of interest. The promoter can be removed from the bacterial source DNA by restriction enzyme digestion and inserted into the vector containing the desired DNA coding sequence, or vice versa.

[0167] Alternatively, the expression constructs can be integrated into the bacterial genome with an integrating vector. Integrating vectors typically contain at least one sequence homologous to the bacterial chromosome that allows the vector to integrate. Integrations appear to result from recombinations between homologous DNA in the vector and the bacterial chromosome. For example, integrating vectors constructed with DNA from various *Bacillus* strains integrate into the *Bacillus* chromosome (E.P.O. Pub. No. 127,328). Integrating vectors may also be comprised of bacteriophage or transposon sequences.

[0168] Construction of suitable vectors containing one or more of the above-listed components employs standard recombinant DNA techniques. Isolated plasmids or DNA fragments are cleaved, tailored, and re-ligated in the form desired to generate the plasmids required. Examples of available bacterial expression vectors include, but are not limited to, the multifunctional *E. coli* cloning and expression vectors such as Bluescript™ (Stratagene, La Jolla, Calif.), in which, for example, a *D. v. virgifera* protein or fragment thereof, may be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β -galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke and Schuster *J. Biol. Chem.* 264:5503-5509 (1989)); and the like. pGEX vectors (Promega, Madison Wis.) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such-fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems are designed to include heparin, thrombin or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

[0169] It is, of course, necessary to select the appropriate bacteria taking into consideration replicability of the replicon in the cells of a bacterium. For example, *E. coli*, *Serratia*, or *Salmonella* species can be suitably used as the host when well known plasmids such as pBR322, pBR325, pACYC177, or pKN410 are used to supply the replicon. *E. coli* strain W3110 is a preferred host or parent host because it is a common host strain for recombinant DNA product fermentations. Preferably, the host cell should secrete minimal amounts of proteolytic enzymes.

[0170] Host cells are transfected and preferably transformed with the above-described vectors and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

[0171] Numerous methods of transfection are known to the ordinarily skilled artisan, for example, calcium phosphate and electroporation. Depending on the host cell used, transformation is done using standard techniques appropriate to