

(1980); Zoller and Smith, *Methods Enzymol.* 100: 468-500 (1983); Dalbadie-McFarland et al., *Proc. Natl. Acad. Sci. (U.S.A.)* 79: 6409-6413 (1982)) and methods based upon PCR (Scharf et al., *Science* 233: 1076-1078 (1986); Higuchi et al., *Nucleic Acids Res.* 16: 7351-7367 (1988)). Site-directed mutagenesis approaches are also described in EP 0 385 962, EP 0 359 472, and PCT Patent Application WO 93/07278.

**[0237]** Any of the nucleic acid molecules of the present invention may either be modified by site-directed mutagenesis or used as, for example, nucleic acid molecules that are used to target other nucleic acid molecules for modification. It is understood that mutants with more than one altered nucleotide can be constructed using techniques that practitioners skilled in the art are familiar with such as isolating restriction fragments and ligating such fragments into an expression vector (see, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press (1989)). In a preferred embodiment of the present invention, one or more of the nucleic acid molecules or fragments thereof of the present invention may be modified by site-directed mutagenesis.

**[0238]** Receptors identified from the cDNA libraries of the present invention will find great use in the design of straightforward *in vivo* screening assays for toxins which will interact successfully with these receptors resulting in measurable effects on the cells in which the receptors reside. Briefly, suitable host cells, which contain expression vectors appropriate to the hosts for the production of the receptors of the invention displayed on the surfaces of the cells. Preferably, the host cells are eucaryotic cells such as mammalian, insect and yeast. Since the receptors are natively membrane proteins, no particular design of the expression system is required in order to effect their disposition at the cell surface. Expression vectors suitable for any desired host are generally known in the art. Prokaryotic cells such as *E. coli* also may be adapted for expression of the receptor in the assay, for instance by using a reporter gene under the control of cyclic AMP and operably linked to the receptor via protein G such that toxin binding will interrupt adenyl cyclase activity and thereby produce a detectable change in reporter gene activity.

**[0239]** In the *in vivo* assays, the modified cells are contacted with the candidate toxin and the effect on metabolism or morphology is noted in the presence and absence of the candidate. The effect may be cytotoxic—i.e., the cells may themselves exhibit one of the indices of cell death, such as reduced thymidine uptake, slower increase in optical density of the culture, reduced exclusion of vital dyes (e.g., trypan blue), increased release of viability markers such as chromium and rubidium, and the like. The differential response between the toxin-treated cells and the cells absent the toxin is then noted. The strength of the toxin can be assessed by noting the strength of the response.

**[0240]** These assays may be conducted directly as described above or competitively with known toxins. For example, one approach might be to measure the diminution in binding of labeled BT cry toxin in the presence and absence of the toxin candidate.

**[0241]** In addition to simply screening candidates, the screen can be used to devise improved forms of toxins which are more specific or less specific to particular classes of insects as desired. The ability to determine binding affinity (K<sub>sub.a</sub> and K<sub>sub.d</sub>), dissociation and association rates, and cytotoxic effects of a candidate allows quick, accurate and

reproducible screening techniques for a large number of toxins and other ligands under identical conditions which was not possible heretofore. Such information will facilitate the selection of the most effective toxins and ligands for any given receptor obtained from any desired host cell.

**[0242]** Competition assays may also employ RNA aptamers, peptide aptamers and antibodies that have high affinity and specificity for the receptor. The information and the structural characteristics of toxins and ligands tested will permit a rational approach to designing more efficient toxins and ligands. Additionally, such assays will lead to a better understanding of the function and the structure/function relationship of both toxin/ligand and toxin receptors on gut surface. In turn, this will allow the development of highly effective toxins/ligands.

**[0243]** In addition to the above discussed procedures, practitioners are familiar with the standard resource materials which describe specific conditions and procedures for the construction, manipulation and isolation of macromolecules (e.g., DNA molecules, plasmids, etc.), generation of recombinant organisms and the screening and isolating of clones, (see for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press (1989); Mailga et al., *Methods in Plant Molecular Biology*, Cold Spring Harbor Press (1995); Birren et al., *Genome Analysis: Analyzing DNA*, 1, Cold Spring Harbor, New York).

**[0244]** Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

#### Example 1

##### Construction of the cDNA Library LIB149

**[0245]** The cDNA library LIB 149 was generated from midguts of WCR third instar larvae. Disinfected WCR eggs were suspended in a 0.1% (w/v) agar solution and dispensed into petri dishes containing 2% (w/v) agar and filter paper. The WCR eggs were incubated first at 20° C. and 60% relative humidity for 3 days and then at 25° C. and 60% RH for 10 days. After 13 days of incubation, the eggs were washed from the dishes into sweater boxes containing soil (2 parts Metro-Mix 200:1 part Redi-Earth; steam sterilized) and germinated corn mats, which were prepared by germinating corn seeds of Pioneer varieties 3615, 3732, or CD5 in sweater boxes containing germination paper wet with 1.0% (w/v) 3336<sup>F</sup> fungicide. Larvae were reared in sweater boxes in a growth chamber (25° C., 70% relative humidity, irrigated for 10 minutes every 2 days, and fertilized every 4 days). Third instar larvae were dissected to separate midguts from other body (cuticle, head, fore- and hindgut, as well as fat body). The harvested midguts were placed in chilled m.c. tube with 25 mM Tris buffer (pH 7.4), thoroughly saturated with the buffer, and then centrifuged for 5 minutes at 14,000 g at 4° C. Supernatant was discarded and midgut pellets were immediately frozen in liquid nitrogen and stored at -80° C. until total RNA preparation. RNA was purified and the cDNA library was constructed as described in Example 2.

##### Construction of the cDNA Library LIB150

**[0246]** The cDNA library LIB 150 was generated from WCR pupae. Disinfected WCR eggs were suspended in a 0.1% (w/v) agar solution and dispensed into petri dishes containing 2% (w/v) agar and filter paper. The WCR eggs