

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^F fungicide. The sweater boxes containing eggs were placed in a growth chamber (25° C., 70% relative humidity, irrigated for 10 minutes every 2 days, and fertilized every 4 days) for 10 days. 10-day old larval mats were removed from the sweater boxes and transferred into an emergence box containing soil (2 parts Metro-Mix 200:1 part Redi-Earth; steam sterilized) and germinated corn lining the inside of the box. The emergence boxes were placed in a growth chamber (25° C., 70% RH, irrigated for 10 minutes every 2 days, and fertilized every 4 days) for 7 days. WCR pupae were collected, 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 LIB3026

[0247] The cDNA library LIB3026 was generated from midguts of WCR first 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^F 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). First instar larva were dissected to separate midguts from other body (cuticle, head, fore- and hindgut, as well as fat body). The harvested midguts are 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 LIB3373

[0248] The cDNA library LIB3373 was generated from whole guts of WCR third instar larvae. Western corn root-worm eggs were prepared weekly. Eggs were separated from soil by floating in a separatory funnel containing a 1.25 specific gravity solution of magnesium sulfate. Once separated, the eggs were surface disinfected in 0.25% peroxyacetic acid, rinsed, treated with Nystatin (2 mg/ml), and plated onto sterile filter disks on 2% agar. Egg plates were incubated at 25° C. for two weeks then transferred to corn seedling boxes (described below).

[0249] Corn seedlings for rearing the hatching larvae were prepared weekly by treating approximately 1 kg of corn seeds with 25% bleach for five minutes, followed by rinsing. Corn seed was divided into twelve sterile tissue culture boxes, each containing germination paper and 200 ml of 1% Domain fungicide solution. Germination temperature was 29° C. and

germinating seedlings were ready for infestation one week after preparation. Following infestation, rearing boxes were incubated at 25° C.

[0250] Third instar larvae were harvested from the seedling boxes eight to twelve days following infestation. Insects were harvested by placing infested corn mats onto a screen within a large funnel (60 cm). As insects crawled out of the root mass, they would fall through the funnel into a 50 ml screw-cap tube (on ice).

[0251] Third instar larva were dissected to separate guts from other body (cuticle, head, and fat body). The harvested guts 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 gut pellets were immediately frozen in liquid nitrogen and stored at -80° C. until total RNA preparation.

[0252] Total RNA was isolated using the Pharmacia Quick-Prep Total RNA Extraction Kit, following the protocol recommended by the manufacturer. Poly A+ RNA (mRNA) was purified using QuickPrep kit from Amersham Pharmacia Biotech, Inc. (Piscataway, N.J.), essentially as recommended by the manufacturer.

[0253] Construction of cDNA libraries is well-known in the art and a number of cloning strategies exist. A number of cDNA library construction kits are commercially available. The Stratagene cDNA Synthesis Kit (Stratagene, La Jolla, Calif.) was used, following the conditions suggested by the manufacturer. The cDNA was ligated into a Lambda Zap vector.

Example 2

[0254] Total RNA was purified using Trizol reagent from Life Technologies (Gibco BRL, Life

[0255] Technologies, Gaithersburg, Md. U.S.A.), essentially as recommended by the manufacturer. Poly A+ RNA (mRNA) was purified using magnetic oligo dT beads essentially as recommended by the manufacturer (Dynabeads, Dynal Corporation, Lake Success, New York U.S.A.).

[0256] Construction of cDNA libraries is well-known in the art and a number of cloning strategies exist. A number of cDNA library construction kits are commercially available. The Superscript™ Plasmid System for cDNA synthesis and Plasmid Cloning (Gibco BRL, Life Technologies, Gaithersburg, Md. U.S.A.) was used, following the conditions suggested by the manufacturer. The cDNA was ligated into pSPORT cloning vector.

Example 3

[0257] The cDNA libraries were plated on LB agar containing the appropriate antibiotics for selection and incubated at 37° for a sufficient time to allow the growth of individual colonies. Single colonies were individually transferred to each well of 96-well microtiter plates containing LB liquid including selective antibiotics. The plates were incubated overnight at approximately 37° C. with gentle shaking to promote growth of the cultures. The plasmid DNA was isolated from each clone using Qiaprep plasmid isolation kits, using the conditions recommended by the manufacturer (Qiagen Inc., Santa Clara, Calif. U.S.A.).

[0258] The template plasmid DNA clones were used for subsequent sequencing. For sequencing the cDNA libraries LIB 149, LIB 150, and LIB3026, a commercially available sequencing kit, such as the ABI PRISM dRhodamine Termi-