CONSTRUCTION OF A TRANSFER VECTOR FOR A CLONAL ISOLATE OF LDNPV

Shivanand T. Hiremath, Martha Fikes, and Audrey Ichida
USDA Forest Service, Northeastern Forest Experiment Station
359 Main Road, Delaware, OH 43015

ABSTRACT

Deoxyribonucleic acid from a clonal isolate of LdNPV (CI A2-1), obtained by *in vivo* cloning procedures, was used to construct genomic libraries in phage (lamda Gem 11) and cosmid (pHC79) vectors. Overlapping clones were selected to generate a restriction enzyme map. The restriction enzyme map, covering about 85% of the CI A2-1 genome, was determined. Efforts are underway to clone and characterize the missing regions.

A BglII fragment (10.5 kbp) containing the entire polyhedrin gene was subcloned into pUC18. This new plasmid was characterized for various restriction enzyme sites and the exact location of polyhedrin coding sequences. Several manipulations of the plasmid DNA were carried out which included: 1) deletion of a 6.2 kbp EcoRI fragment; 2) destruction of an existing HindIII site; 3) deletion of a 211 bp fragment containing sequence coding for N-terminal region of polyhedrin; and 4) replacing it with an oligonucleotide containing sites for NotI, HindIII, BamHI, SmaI, and ApaI enzymes.

The final product, pLdS1, was 6.6 kbp in size. It had 1.5 kbp region containing the promoter for polyhedrin gene and upstream sequences. Immediately following this were sites for NotI, BamHI, HindIII, SmaI and ApaI. The multiple cloning site was sequencially followed by 550 bp sequence coding for the C-terminal region of polyhedrin and 1.8 kbp sequences downstream from the polyhedrin coding region. Analysis of pLdS1 DNA suggests that this will be a suitable transfer vector for introducing foreign genes under polyhedrin gene promoter into CI A2-1.

A reporter gene, betagalactosidase, was subcloned into pLdS1 to produce pLdS1-Bgal. Both pLdS1 and pLdS1-Bgal will be tested by co-transfection experiments to determine whether pLdS1 can be used for introducing foreign genes into CI A2-1.

Using polyclonal antibodies, several cDNA clones corresponding to juvenile hormone esterase (JHE) were isolated from a lamda gt11 cDNA library constructed from poly(A) RNA from 5th instar, day 6 larval fat bodies. These are being analyzed to determine whether any contain the entire coding sequence for JHE. Meanwhile, these clones are being used as probes to isolate the JHE gene from a gypsy moth genomic library constructed in EMBL3 and labda Gem11 vectors.