CHAPTER 3

RESULTS

3.1 DNA Isolation

Total DNA was isolated from frozen pleopods and hemolymph samples as described in method 2.6.1 and 2.6.2 respectively. From 0.7 % agarose gel as compared with standard DNA markers (λ DNA/*Hin*d III), the extracted DNA consisted of high molecular weight DNA greater than 23.1 kb (Fig. 3.1). The concentration of DNA was determined by measuring the absorbance at 260 nm and assuming that 1.0 O.D. was equivalent to 50 mg DNA per millilitre. From the absorption spectrum of the extracted DNA, the ratio of $O.D._{260}/O.D._{280}$ was higher than 1.8 reflecting good quality of the DNA obtained.

3.2 Primer Screening and Selection

The arbitrary decanucleotide primers (10-base long) were purchased from the University of British Columbia (UBC) and Operon Technologies, Inc. Seven primers from UBC were selected according to Tassanakajon et al. (1997). These primers were UBC 101, 174, 268, 428, 456, 457 and 459 (Table 3.1). Forty-two primers from Operon Technologies were tested for amplification of *P. monodon* DNA. These primers were OPA 1 - 20, OPB 1-20, OPM-09 and OPZ-09 (Table 3.1). All primers, except OPB-08 and OPB-09, could amplified the genomic DNA. From the amplification patterns, primers which yielded intense or consistent bands or both were selected for further analysis. Of the primer screened, 26 primers were selected for their abilities to differentiate the RAPD patterns between normal and viral tolerance *P. monodon* (Table 3.2). Examples of primer screening and selection were shown by Fig. 3.2 and 3.3.

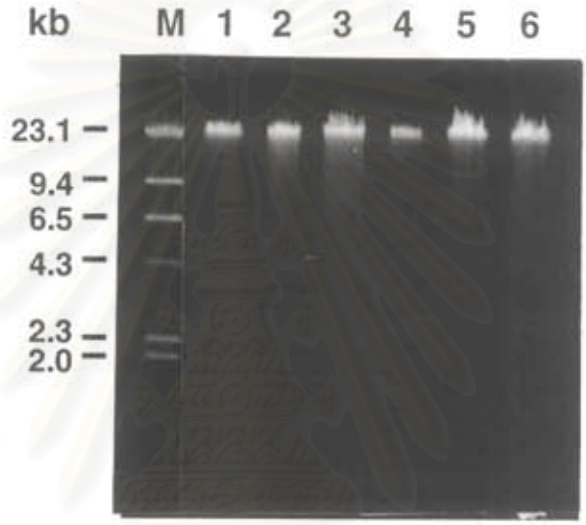


Fig. 3.1 Ethidium bromide stained gel showing high molecular weigth genomic DNA of *P. monodon*.

lane M : λ DNA/*Hind* III, standard marker

lanes 1-3 : genomic DNA from pleopod of 3 different *P. monodon* individuals using proteinase K-phenol-chloroform method

lanes 4-6 : genomic DNA from hemolymph of 3 different *P. monodon* individuals using a modification of Cook method

Table 3.1 Sequences of arbitrary primers used for primer screening

Primers	Sequences (5' to 3')
University of British Columbia	
101	GCG CCT GGA G
174	AAC GGG CAG C
268	AGG CCG CTT A
428	GGC TGC GGT A
456	GCG GAG GTC C
457	CGA CGC CCT G
459	GCG TCG AGG G
Operon Technologies	
OPA-01	CAG GCC CTT C
OPA-02	TGC CGA GCT G
OPA-03	AGT CAG CCA C
OPA-04	AAT CGG GCT G
OPA-05	AGG GGT CTT G
OPA-06	GGT CCC TGA C
OPA-07	GAA ACG GGT G
OPA-08	GTG ACG TAG G
OPA-09	GGG TAA CGC C
OPA-10	GTG ATC GCA G
OPA-11	CAA TCG CCG T
OPA-12	TCG GCG ATA G
OPA-13	CAG CAC CCA C
OPA-14	TCT GTG CTG G
OPA-15	TTC CGA ACC C

Table 3.1 (continued)

Primers	Sequence (5' to 3')
OPA-16	AGC CAG CGA A
OPA-17	GAC CGC TTG T
OPA-18	AGG TGA CCG T
OPA-19	CAA ACG TCG G
OPA-20	GTT GCG ATC C
OPB-01	GTT TCG CTC C
OPB-02	TGA TCC CTG G
OPB-03	CAT CCC CCT G
OPB-04	GGA CTG GAG T
OPB-05	TGC GCC CTT C
OPB-06	TGC TCT GCC C
OPB-07	GGT GAC GCA G
OPB-08	GTC CAC ACG G
OPB-09	TGG GGG ACT C
OPB-10	CTG CTG GGA C
OPB-11	GTA GAC CCG T
OPB-12	CCT TGA CGC A
OPB-13	TTC CCC CGC T
OPB-14	TCC GCT CTG G
OPB-15	GGA GGG TGT T
OPB-16	TTT GCC CGG A
OPB-17	AGG GAA CGA G
OPB-18	CCA CAG CAG T
OPB-19	ACC CCC GAA G
OPB-20	GGA CCC TTA C

Table 3.1 (continued)

Primers	Sequence (5' to 3')
OPM-09	GTC TTG CGG A
OPZ-09	CAC CCC AGT C

Table 3.2 Sequences of selected primers used to differentiate RAPD patterns between normal and viral tolerance *P. monodon*

Primers	Sequence (5' to 3')
University of Birtish Columbia	
101	GCG CCT GGA G
174	AAC GGG CAG C
268	AGG CCG CTT A
428	GGC TGC GGT A
456	GCG GAG GTC C
457	CGA CGC CCT G
459	GCG TCG AGG G
Operon Technologies	
OPA-01	CAG GCC CTT C
OPA-02	TGC CGA GCT G
OPA-04	AAT CGG GCT G
OPA-05	AGG GGT CTT G
OPA-08	GTG ACG TAG G
OPA-09	GGG TAA CGC C
OPA-10	GTG ATC GCA G
OPA-16	AGC CAG CGA A
OPA-18	AGG TGA CCG T
OPB-04	GGA CTG GAG T
OPB-06	TGC TCT GCC C
OPB-07	GGT GAC GCA G
OPB-08	GTC CAC ACG G
OPB-12	CCT TGA CGC A
OPB-05	TGC GCC CTT C

Table 3.2 (continued)

Primers	Sequence (5' to 3')
OPB-14	TCC GCT CTG G
OPB-15	GGA GGG TGT T
OPB-18	CCA CAG CAG T
OPB-20	GGA CCC TTA C

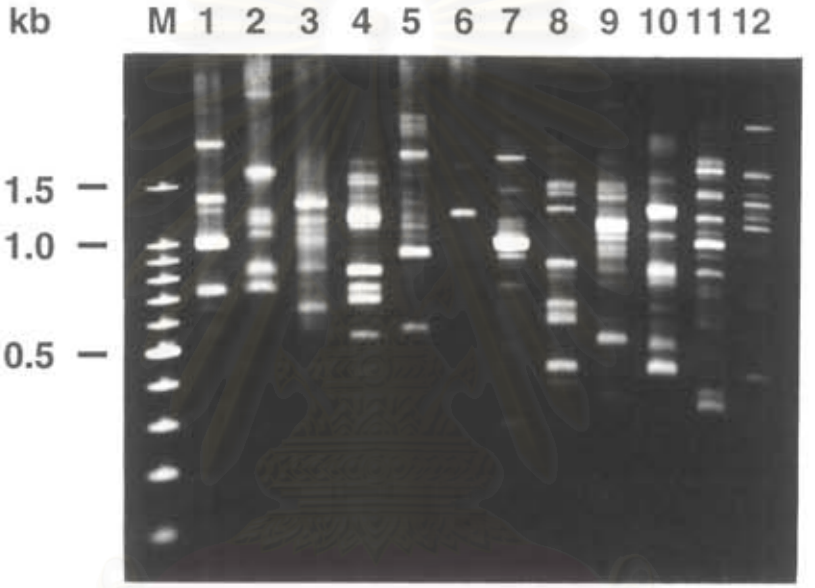


Fig. 3.2 Amplification patterns of *P. monodon* genomic DNA using various RAPD primers.

lane M : a 100 bp DNA ladder

lanes 1-12 : RAPD patterns using OPA-01 to OPA-12, respectively

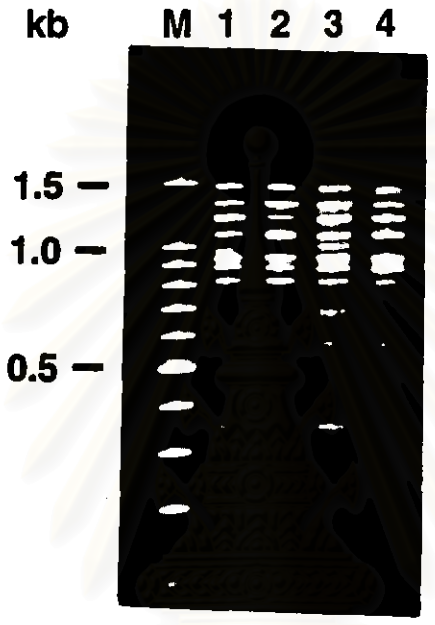


Fig. 3.3 Amplification patterns of *P. monodon* genomic DNA using the selected primer, OPA-18.

lane M : a 100 bp DNA ladder

lanes 1-4 : genomic DNA from 4 normal *P. monodon* individuals

3.3 Detection of Differentiated RAPD Patterns between Normal and Viral Tolerance *P. monodon*

Two separated groups of *P. monodon*, normal and viral tolerance shrimps were detected. The RAPD analysis of the two groups (3 individuals each) using selected positive primers produced scorable bands ranging in size from 200 to more than 1,500 bp. Only 2 selected primers, OPA-04 and OPB-20, showed DNA bands which consistently appeared only in normal shrimps. Using OPA-04, a DNA band with size about 800 bp existed only in the normal *P. monodon* samples (Fig. 3.4). Using primer OPB-20, DNA band with size about 1,250 bp appeared only in the normal *P. monodon* samples (Fig. 3.5). To confirmed whether these bands can be used as DNA markers to differentiate between normal and viral tolerance shrimps, more shrimp samples were tested. The two DNA bands still existed in all normal shrimps but not in the viral tolerance shrimps (Figs 3.6 and 3.7).

However, amplification of normal *P. monodon* from other geographical samples in Thailand using primer OPA-04 did not showed the fragment of 800 bp. The samples from Satun-Trang collected later in 1997 showed only fainted band of 800 bp (Fig. 3.8)

By increasing the number of samples in each group to the final of twenty individuals using primer OPA-04 and OPB-20, the 800 bp existed in all normal shrimp but the 1,250 band existed only in 12 out of 19 shrimps (63.2 %) (see Figs 3.5, 3.7 and Appendix 6).

3.4 Dot Blot Hybridization of Genomic DNA of *P. monodon*

To clarify whether the 800 bp fragment existed in the genome of normal and viral tolerance shrimp, the samples of normal and viral tolerance *P. monodon* from Satun, Trang, Trat, Angsila, Chumphon and Phangnga, were examined by dot blot

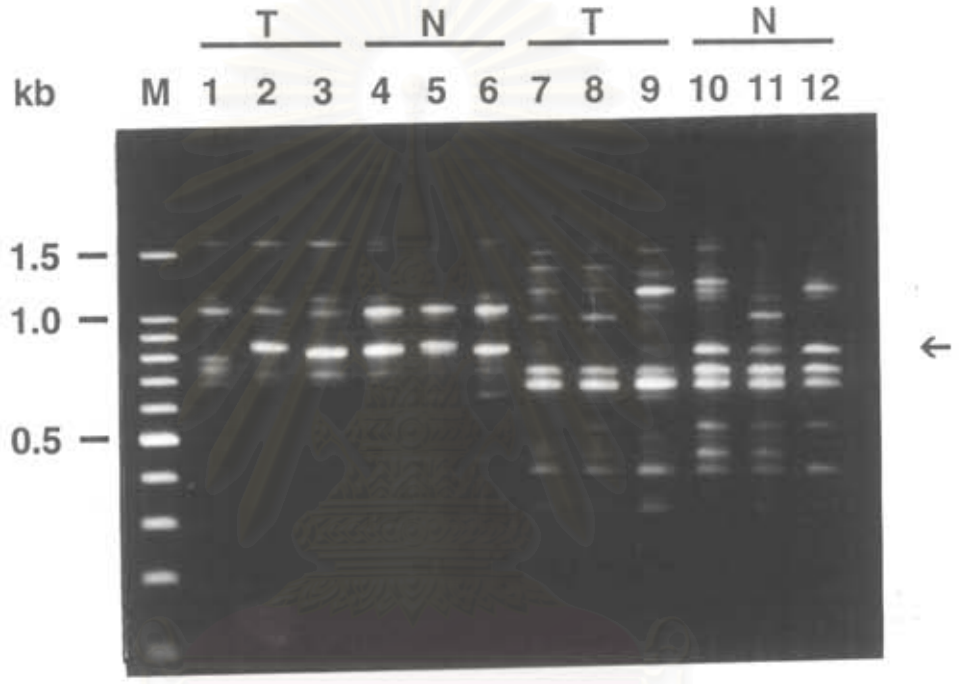


Fig. 3.4 RAPD patterns of normal and viral tolerance *P. monodon* using primers OPA-02 and OPA-04.

lane M : a 100 bp DNA ladder

lanes 1-6 : results from the using of primer OPA-02

lanes 7-12 : results from the using of primer OPA-04

← : DNA band with size about 800 bp

T : viral disease tolerance *P. monodon*

N : normal *P. monodon*

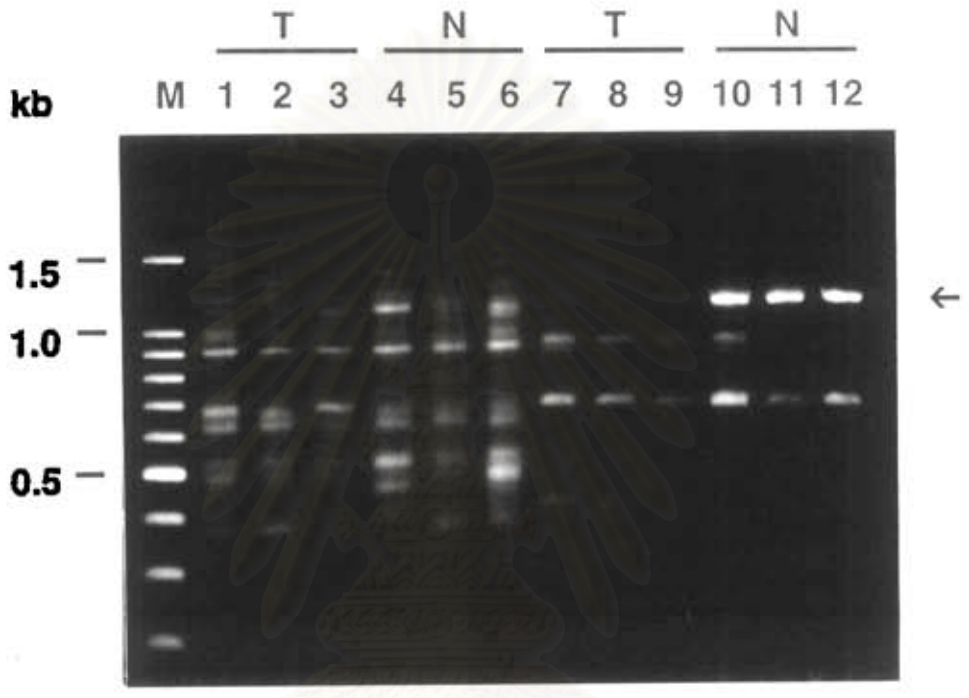


Fig. 3.5 RAPD patterns of normal and viral tolerance *P. monodon* using primers OPB-18 and OPB-20.

lane M : a 100 bp DNA ladder

lanes 1-6 : results from the using of primer OPB-18

lanes 7-12 : results from the using of primer OPB-20

← : DNA band with size about 1,250 bp

T : viral disease tolerance *P. monodon*

N : normal *P. monodon*

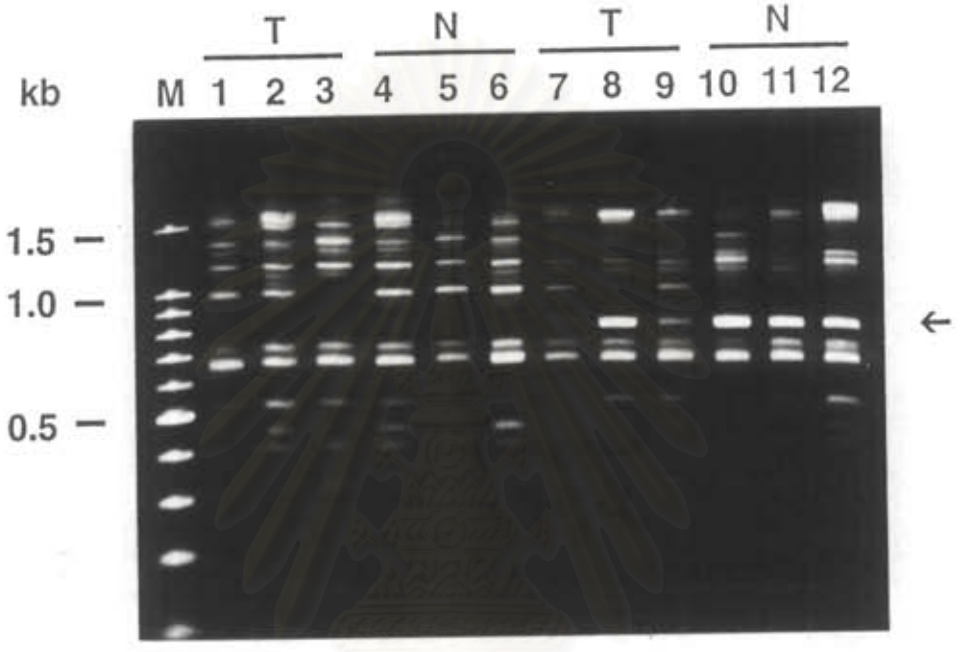


Fig. 3.6 RAPD patterns of normal and viral tolerance *P. monodon* using primer OPA-04.

lane M : a 100 bp DNA ladder

lanes 1-6 : viral tolerance *P. monodon*

lanes 7-12 : normal *P. monodon*

← : DNA band with size about 800 bp

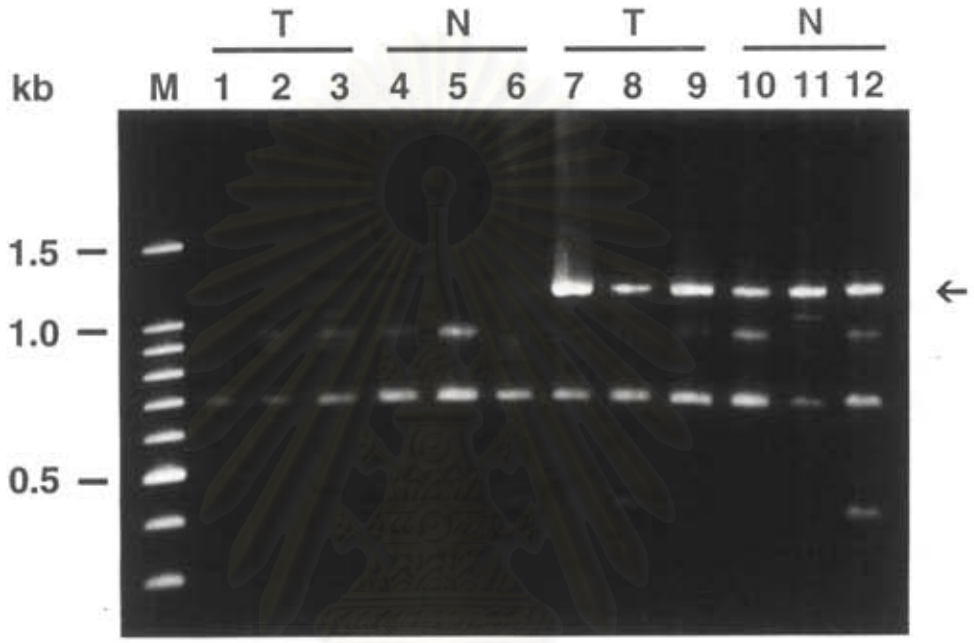


Fig. 3.7 RAPD patterns of normal and viral tolerance *P. monodon* using primer OPB-20.

lane M : a 100 bp DNA ladder

lanes 1-6 : viral tolerance *P. monodon*

lanes 7-12 : normal *P. monodon*

← : DNA band with size about 1,250 bp

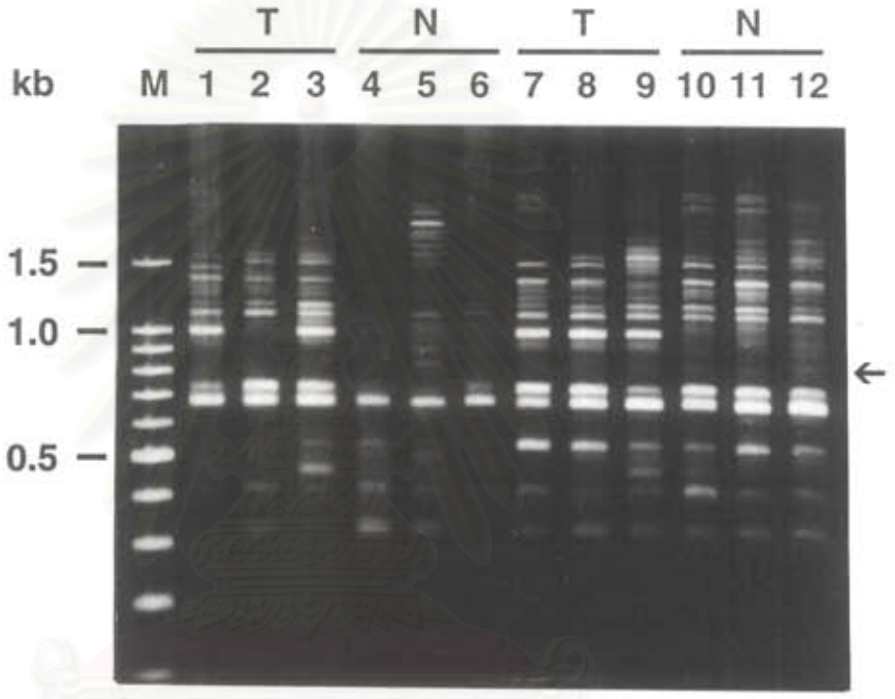


Fig. 3.8 RAPD patterns of geographically different *P. monodon* using OPA-04.

lane M : a 100 bp DNA ladder

lanes 1-3 : individuals from Trat

lanes4-6 : individuals from Angsila

lanes 7-9 : individuals from Satun (collected in 1997)

lanes 10-12 : individuals from Trang (collected in 1997)

← : DNA band with size about 800 bp

hybridization with digoxigenin-labeled 800 bp DNA fragment as a probe. The sample from Satun-Trang (normal shrimp) were collected in 1996. Additional samples were collected from Satun and Trang in 1997. Genomic DNA of normal shrimp from Satun-Trang showed strong hybridization signal while those from other geographically separated shrimps showed weak hybridizaton signal. Viral tolerance shrimp showed very faint signal and probably resulted from the hybridization background (see Fig. 3.9).

3.5 Cloning of 800 bp RAPD Marker

To facilitate DNA cloning, the 800 bp DNA fragment was reamplified by the RAPD primer which contained *Bam*H I site (Fig. 3.10). After amplification, PCR products were phenol-chloroform extracted, digested with *Bam*H I and further ligated into pUC18/*Bam*H I/BAP vector. The ligation mixture was transformed into DH5α competent cell prepared by $CaCl_2$ method as described in section 2.8.2.4. Recombinant clones were further selected according to the insert size on 0.7 % agarose gel electrophoresis using undigested pUC18 and λ DNA/Hind III as markers. The results in Fig 3.11 presented 6 recombinant clones (clone no. 5, 10, 21, 24, 28 and 35) with sizes greater than undigested pUC18. To determine the insert size, recombinant clones were digested with *Bam*H I and analyzed on 1.6 % agarose gel. From Fig. 3.12, only clone no 28 contained the 800 bp inserted fragment whereas the other clones consisted of the inserted fragment smaller than 800 bp.

To ensure that the insert fragment of clone no. 28 was the 800 bp RAPD marker, the clone no. 28 was digested with *Bam*H I, subjected to agarose gel electrophoresis and then analyzed by Southern blot hybridization techniques as described in section 2.10. Positive signals were detected with both the 800 bp fragment from clone no. 28 and the 800 bp fragment eluted DNA from RAPD

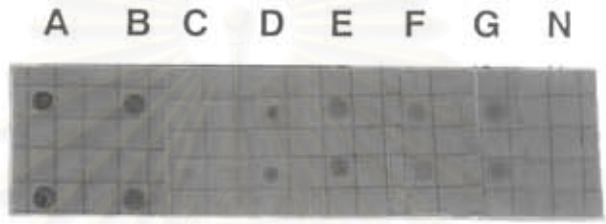


Fig. 3.9 Dot blot hybridization of genomic DNA of *P. monodon* using the Dig-labeled 800 bp fragment as a probe (B -G contained 2.0 µg of genomic DNA).

A : 800 bp DNA fragment (100 ng)

B : genomic DNA of normal *P. monodon* from Satun-Trang (collected in 1996)

C : genomic DNA of viral tolerance *P. monodon*

D : genomic DNA from Satun (collected in 1997)

E : genomic DNA from Phang-nga

F : genomic DNA from Chumphon

G : genomic DNA from Angsila

N : negative control (500 ng of undigested pUC18)

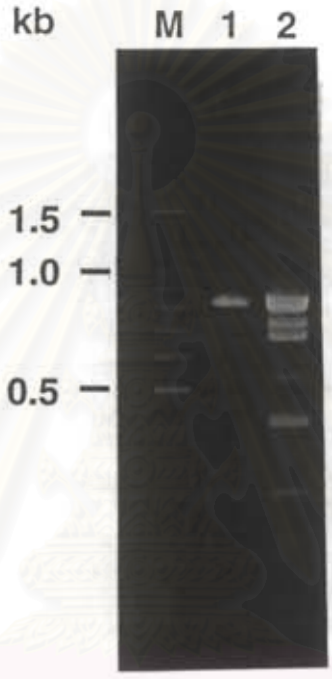


Fig. 3.10 RAPD patterns of reamplification of 800 bp eluted fragment using the oligonucleotide primer containing *Bam*H I site.

(5' CGGGATCCCGAATCGGGCTG 3')

lane M.: a 100 bp DNA ladder

lane 1 : a 800 bp eluted fragment (control)

lane 2 : products of reamplified 800 bp fragment

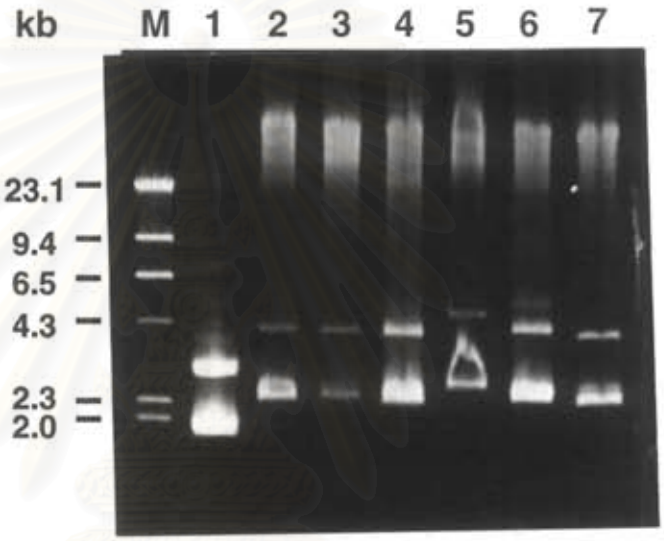


Fig. 3.11 Ethidium bromide staining of recombinant clones on 0.7 % agarose gel.
lane M : λ DNA/*Hin*d III standard marker
lane 1 : undigested pUC18 vector
lanes 2-7 : recombinant clones no. 5, 10, 21, 24, 28 and 35, respectively

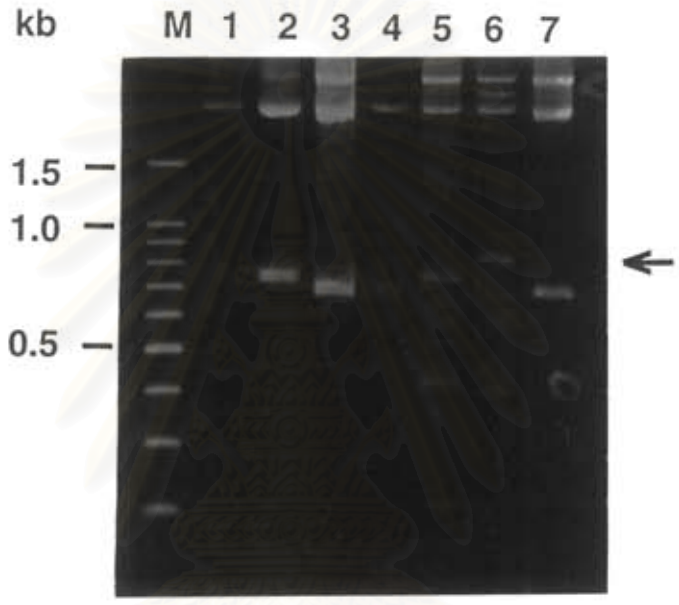


Fig. 3.12 Ethidium bromide staining of recombinant plasmids digested with *Bam*H I on 1.6 % agarose gel.

lane M : a 100 bp DNA ladder

lane 1 : pUC 18 vector

lanes 2-7 : recombinant clones no. 5, 10, 21, 24, 28 and 35, respectively

←: DNA band with size about 800 bp

amplification using primer OPA-04 (Fig. 3.13).

3.6 DNA Sequence Analysis of the 800 bp RAPD Marker

The 800 bp insert in the clone no. 28 was sequenced by the ABI-PRISM automated sequencer (Fig. 3.14). The sequence was compared to the other DNAs in the GenBank using BLAST program as described in section 2.11. The result of sequence comparison showed that it was not significantly similar to any other DNAs in the GenBank (Fig. 3.15). Analysis of the nucleotide sequence did not reveal any open reading frame (ORF) (data not shown). The nucleotide sequence was converted into amino acid sequence. The amino acid sequence without the interrupted stop codons was compared to other proteins in the GenBank deposited protein sequence using BLAST program. The results as shown in Fig. 3.16 did not reveal any similarity to any proteins in the GenBank.

3.7 Specificity Test of the 800 bp Fragment

Specific primers (upper and lower), 20 base in the length, were designed from the nucleotide sequences of the 800 bp fragment using Oligo 4.0s program as shown:

upper primer : 5' ACG GTT ACA AAA TAG GTT CC 3'

lower primer : 5' TCA GCT ATC TCT CTC CAA GC 3'

Amplification of *P. monodon* DNA using these specific primers yielded a PCR product with size about 173 bp.

From PCR optimization, the PCR profiles were 35 cycles of 15 sec 94 $^{\circ}$C, 30 sec 56 $^{\circ}$C and 30 sec 72 $^{\circ}$C. The PCR was performed with a total volume of 20 µl containing 50 ng of DNA template, 2.0 m M $MgCl_2$, 200 µM of each dNTPs, 2 µM of each primers and 0.25 unit of *Taq* DNA polymerase.

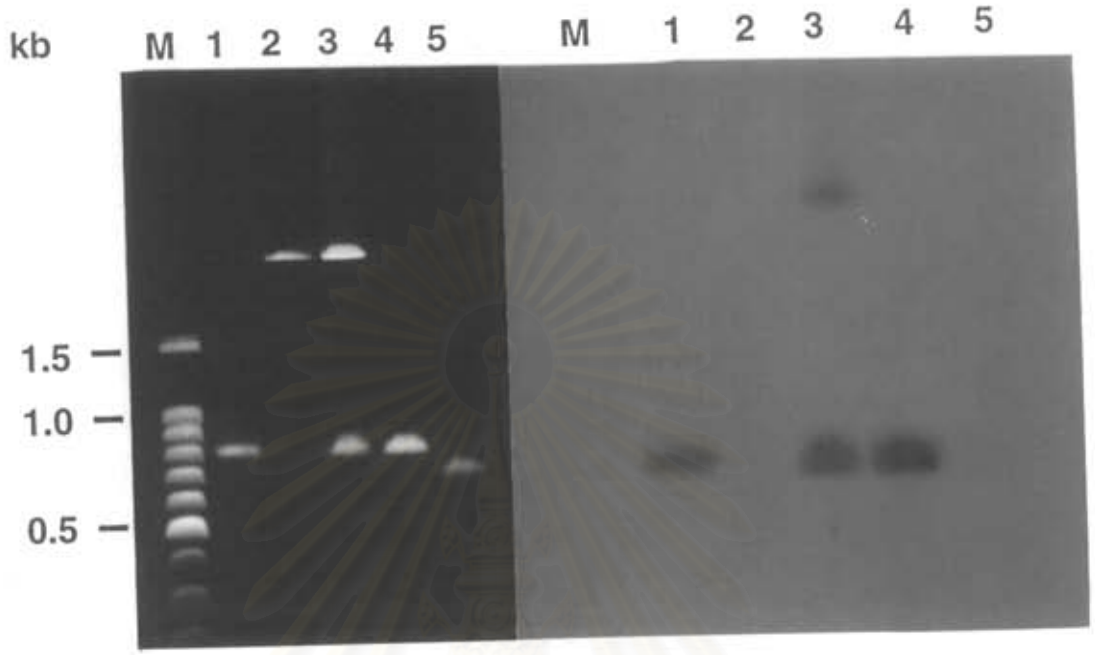


Fig. 3.13 Analysis of the 800 bp DNA fragment by Southern blot hybridization using digoxigenin-labeled probe.

lane M : a 100 bp DNA ladder

lane 1 : a 800 bp eluted fragment

lane 2 : *Bam*H I digested pUC18

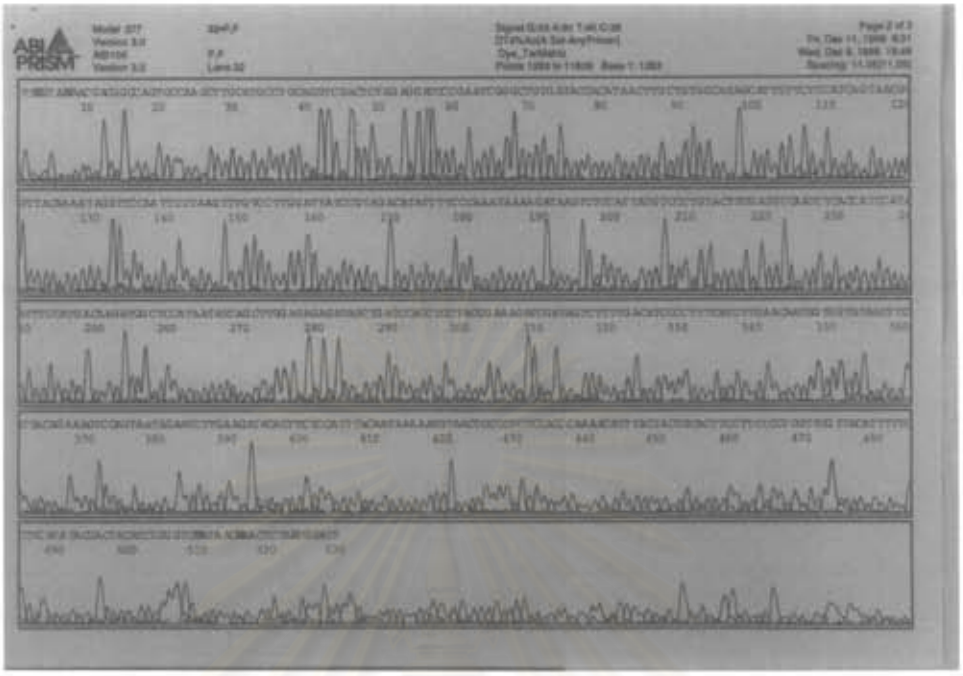
lane 3: *Bam*H I digested recombinant plasmid no. 28

lane 4 : a 800 bp eluted fragment from *Bam*H I digested recombinant plasmid no. 28

lane 5 : negative control (700 bp digested fragment of pBlueScript vector)

(a) Ethidium bromide stained gel

(b) Southern blot hybridization of (a)



A

AATCGGGCTGTGATACCACATAACTTGCTGTGGCAGAGCATTGTTCTTCATCAGTAACGGT
TACAAAATAGGTTCCAATTCCTAAGTTTGTCCTTGGATTATCCGTAGACATATTTTCCCAAAT
AAAAGATAAGTCTCCATTATGTCCCTGTACTTCGATGTCAATCTCACCATCCATATTTCCATG
ACAAGATGGCTCCATAATATCAGCTTGGAGAGAGATAGCTGATCCACCTCCTACGGAAAGA
TCGATAGTCTTTTGACATCCCCTTTCATCTTGAACAATGGTGGTGTAATTTCCTACAGAAAG
TCCAGTAATAGAATCTTGAAGATACACTTCTCCATTTACAATAAAAATGTAACTCCCCTCTCC
ACCCAAAACATTTATTACTGCACTTCCTTCTCCTGGTTGGTTACATTTTTCTTCATATACGAC
TACATCTGGGTCAATAACCAACTCTAGTGGATT

B

Fig. 3.14 Sequencing of the 800 bp fragment using the ABI-PRISM automated sequencer.

A : The sequencing profiles of the 800 bp fragment of clone no. 28

B : Nucleotide sequences of the 800 bp fragment

```
Query= tmpseq_1
     (469 letters)

Database: Non-redundant GenBank+EMBL+DDBJ+PDB sequences
       410,274 sequences; 997,242,718 total letters.
Searching.....done

                                                                  Smallest
                                                                    Sum
                                                                  High  Probability
Sequences producing High-scoring Segment Pairs:                   Score  P(N)     N

gb|AC004962|AC004962 Homo sapiens clone DJ1099N07, comple...  147  0.076    1
gb|U41546|CELT25B6  Caenorhabditis elegans cosmid T25B6       119  0.95     2
gb|AC004095|AC004095 Human Cosmid g1248a143 from 7q31.3, ...  124  0.999    1
gb|AC004021|AC004021 Human PAC clone DJ0186K10 from 5q31,...  123  0.9996   1
gb|M57473|MPMCGA  Murine polyomavirus, complete genome        123  0.9996   1
gb|M55904|MPMCG  Mouse polyomavirus T-antigen (TAg), ...      123  0.9996   1
gb|AC004160|AC004160 Homo sapiens BAC clone GS164B05 from...  123  0.9996   1
gb|AC002464|AC002464 Human BAC clone RG331P03, complete s...  123  0.9996   1


gb|AC004962|AC004962 Homo sapiens clone DJ1099N07, complete sequence
       [Homo sapiens]
       Length = 143,749

 Plus Strand HSPs:

 Score = 147 (40.6 bits), Expect = 0.080, P = 0.076
 Identities = 35/42 (83%), Positives = 35/42 (83%), Strand = Plus / Plus

Query:  384 TTTATTACTGCACTTCCTTCTCCTGGTTGGTTACATTTTTCT 425
           |||||| | |||||| || | ||||||||||||| |||
Sbjct: 1319 TGTATTACTACTCTTCCTTTTCGTTGTTGGTTACATTTATCT 1360
```

Fig. 3.15 Comparison of nucleotides of the 800 bp fragment using the ABI-PRISM automated sequencer with those deposited in the GenBank.

gb|U41546|CELT25B6 Caenorhabditis elegans cosmid T25B6
Length = 33,086

Minus Strand HSPs:

Score = 95 (26.3 bits), Expect = 3.0, Sum P(2) = 0.95

Identities = 27/37 (72%), Positives = 27/37 (72%), Strand = Minus / Plus

Query: 433 GTATATGAAGAAAAATGTAACCAACCAGGAGAAGGAA 397
Sbjct: 8394 GTTTGTGAGCAAAAATATATTTCACAAGGAGAAGGAA 8430

Score = 119 (32.9 bits), Expect = 3.0, Sum P(2) = 0.95
Identities = 39/58 (67%), Positives = 39/58 (67%), Strand = Minus / Plus

Query: 405 GAGAAGGAAGTGCAGTAATAAATGTTTTGGGTGGAGAGGGGAGTTACATTTTTATTGT 348
Sbjct: 18311 GGGAAGGAAGTGCACTAAACAGTGTTTTTGTGGCAAAATGGGAATACATATCTAAGGT 18368

gb|AC004095|AC004095 Human Cosmid g1248a143 from 7q31.3, complete sequence [Homo sapiens]
Length = 38,162

Minus Strand HSPs:

Score = 124 (34.3 bits), Expect = 6.5, P = 1.0
Identities = 32/41 (78%), Positives = 32/41 (78%), Strand = Minus / Plus

Query: 431 ATATGAAGAAAAATGTAACCAACCAGGAGAAGGAAGTGCAG 391
Sbjct: 29847 ATGTGAAGAAAGATGTCAACATACAGGTACAGGAAGTGCAG 29887

Fig. 3.15 (continued)

gb|AC004021|AC004021 Human PAC clone DJ0186K10 from 5q31, complete sequence [Homo sapiens]
Length = 171,370

Minus Strand HSPs:

Score = 123 (34.0 bits), Expect = 7.9, P = 1.0
Identities = 31/39 (79%), Positives = 31/39 (79%), Strand = Minus / Plus

Query: 402 AAGGAAGTGCAGTAATAAATGTTTTGGGTGGAGAGGGGA 364
Sbjct: 151197 AAGGAAGTGCAGTAATATACATTCTAGGTGATGTGGGGA 151235

gb|M57473|MPMCGA Murine polyomavirus, complete genome
Length = 4754

Plus Strand HSPs:

Score = 123 (34.0 bits), Expect = 7.9, P = 1.0
Identities = 39/57 (68%), Positives = 39/57 (68%), Strand = Plus / Plus

Query: 301 CTACAGAAAGTCCAGTAATAGAATCTTGAAGATACACTTCTCCATTTACAATAAAAA 357
Sbjct: 4562 CTACAGAAAGTCCGGTAATAGAGGCAAGGTCGAATACTTCAGCAAGTACAGCAAGAA 4618

gb|M55904|MPMCG Mouse polyomavirus T-antigen (TAg), t-antigen (tAg), capsid proteins VP1, VP2 and VP3 genes, complete genome.
Length = 4754

Plus Strand HSPs:

Score = 123 (34.0 bits), Expect = 7.9, P = 1.0
Identities = 39/57 (68%), Positives = 39/57 (68%), Strand = Plus / Plus

Query: 301 CTACAGAAAGTCCAGTAATAGAATCTTGAAGATACACTTCTCCATTTACAATAAAAA 357
Sbjct: 4562 CTACAGAAAGTCCGGTAATAGAGGCAAGGTCGAATACTTCAGCAAGTACAGCAAGAA 4618

Fig. 3.15 (continued)

gb|AC004160|AC004160 Homo sapiens BAC clone GS164B05 from 7p21-p22, complete sequence [Homo sapiens]
Length = 143,751

Plus Strand HSPs:

Score = 123 (34.0 bits), Expect = 7.9, P = 1.0
Identities = 35/48 (72%), Positives = 35/48 (72%), Strand = Plus / Plus

Query: 94 TTGGATTATCCGTAGACATATTTTCCCAAATAAAAGATAAGTCTCCAT 141
Sbjct: 34645 TTAGATTATGTTGAGATATTACTTCCAAATTAAAAGATAAGTTTCTAT 34692

gb|AC002464|AC002464 Human BAC clone RG331P03, complete sequence [Homo sapiens]
Length = 123,805

Plus Strand HSPs:

Score = 123 (34.0 bits), Expect = 7.9, P = 1.0
Identities = 27/30 (90%), Positives = 27/30 (90%), Strand = Plus / Plus

Query: 340 CTCCATTTACAATAAAAATGTAACTCCCCT 369
Sbjct: 18145 CACCATTTAAAATAAAAATGTAACTCCCTT 18174

Parameters:

V=100
B=50
H=0

Lambda K H
0.192 0.173 0.357

E S T X
10.0 123 0 73

Database: Non-redundant GenBank+EMBL+DDBJ+PDB sequences
Posted date: Mar 11, 1999 9:01 PM
# of letters in database: 997,242,718
# of sequences in database: 410,274

Fig. 3.15 (continued)

NRAVIPHNLLWQSIVLHQZRLQNRFQFLSLSLDYPZTYFPKZKISLHYVPVLRCQSHHPYFHDKM
APZYQLGERZLIHLLRKDRZSFDIPFHLEQWWCNFLQKVQZZNLEDTLLHLQZKCNSPLHPKHL
LLHFLLLVGYIFLHIRLHLGQZPTLVD

A

Query= tmpseq_1
(156 letters)

Database: Non-redundant SwissProt sequences
77,419 sequences; 27,864,727 total letters.
Searching.....done

Sequences producing High-scoring Segment Pairs:	Smallest Sum High Probability Score	P(N)	N
sp|P20010|LIG_PHLRA LIGNINASE III PRECURSOR (LIGNIN PERO...	60	0.88	1
sp|P29622|KAIN_HUMAN KALLISTATIN PRECURSOR (KALLIKREIN IN...	58	0.98	1
sp|P27738|RRPO_ACLSP RNA-DIRECTED RNA POLYMERASE (RNA REP...	50	0.995	2
sp|P04919|B3AT_MOUSE BAND 3 ANION EXCHANGE PROTEIN (MEB3)	57	0.997	1
sp|P55067|PGCN_RAT NEUROCAN CORE PROTEIN PRECURSOR (245...	47	0.9991	2
sp|P06181|LIG8_PHACH LIGNINASE H8 PRECURSOR (LIGNIN PEROX...	56	0.9997	1
sp|P31838|LIGB_PHACH LIGNINASE B PRECURSOR (LIGNIN PEROXI...	56	0.9997	1
sp|P31837|LIGA_PHACH LIGNINASE A PRECURSOR (LIGNIN PEROXI...	56	0.9997	1
sp|P11542|LIG4_PHACH LIGNINASE H2 PRECURSOR (LIGNIN PEROX...	56	0.9997	1
sp|P21764|LIG3_PHACH LIGNINASE LG3 PRECURSOR (LIGNIN PERO...	56	0.9997	1
sp|P50622|LIG6_PHACH LIGNINASE LG5 PRECURSOR (LIGNIN PERO...	56	0.9997	1
sp|P11543|LIG5_PHACH LIGNINASE LG5 PRECURSOR (LIGNIN PERO...	56	0.9997	1

B

Fig. 3.16 Sequence and alignment of amino acids deduced from the 800 bp fragment.

A : Amino acid sequences of the 800 bp fragment

B : Comparison of the amino acid sequences of the 800 bp fragment to other deposited in the GenBank using BLAST program

sp|P20010|LIG_PHLRA LIGNINASE III PRECURSOR (LIGNIN PEROXIDASE)
Length = 361

Score = 60 (28.5 bits), Expect = 2.1, P = 0.88
Identities = 11/18 (61%), Positives = 13/18 (72%)

Query: 11 WQSIVLHQZRLQNRFQFL 28
WQS Q +LQNRFQF+
Sbjct: 274 WQSFGTDQAKLQNRFQFI 291

sp|P29622|KAIN_HUMAN KALLISTATIN PRECURSOR (KALLIKREIN INHIBITOR)
(PROTEASE INHIBITOR 4)
Length = 427

Score = 58 (27.5 bits), Expect = 4.1, P = 0.98
Identities = 9/33 (27%), Positives = 18/33 (54%)

Query: 35 PZTYFPKZKISLHYVPVLRCQSHHPYFHDKMAP 67
P+ ++ + ++ +L+ Q HH Y HD+ P
Sbjct: 230 PKDFYVDENTTVRVPMMLQDQEHHWYLHDRYLP 262

sp|P27738|RRPO_ACLSP RNA-DIRECTED RNA POLYMERASE (RNA REPLICASE) (216.5
KD PROTEIN) (ORF1)
Length = 1884

Score = 50 (23.7 bits), Expect = 5.4, Sum P(2) = 1.0
Identities = 10/33 (30%), Positives = 18/33 (54%)

Query: 60 YFHDKMAPZYQLGERZLIHLLRKDRZSFDIPFH 92
YFHDK++ ++ H++ K R + I F+
Sbjct: 717 YFHDKVSYPTFEATGEIRHVMMKARSKWGIDFN 749

Score = 46 (21.8 bits), Expect = 5.4, Sum P(2) = 1.0
Identities = 9/23 (39%), Positives = 11/23 (47%)

Query: 97 WCNFLQKVQZZNLEDTLLHLQZK 119
WC + +KV NL D Q K
Sbjct: 1595 WCRYTEKVLTANLPDNYYIHQRK 1617

Fig. 3.16 B (continued)

sp|P04919|B3AT_MOUSE BAND 3 ANION EXCHANGE PROTEIN (MEB3)
Length = 929

Score = 57 (27.1 bits), Expect = 5.7, P = 1.0
Identities = 16/54 (29%), Positives = 24/54 (44%)

Query: 18 QZRLQNRFQFLSLSLDYPZTYFPKZKISLHYVPVLRCQSHHPYFHDKMAPZYQL 71
++ L++ FL SL P T P +K L+ VPV + Y P+ L
Sbjct: 318 EELLRSLESFLDCSLVLPPTDAPSEKALLNLVPVQKELLRRRYLPSPAKPDPNL 371

sp|P55067|PGCN_RAT NEUROCAN CORE PROTEIN PRECURSOR (245 KD EARLY POSTNATAL CORE GLYCOPROTEIN) (CONTAINS: 150 KD ADULT CORE GLYCOPROTEIN)
Length = 1257

Score = 39 (18.5 bits), Expect = 7.0, Sum P(2) = 1.0
Identities = 6/16 (37%), Positives = 11/16 (68%)

Query: 42 ZKISLHYVPVLRCQSH 57
+ S H+V +RC+S+
Sbjct: 1187 EGFSQHHVATIRCRSN 1202

Score = 47 (22.3 bits), Expect = 7.0, Sum P(2) = 1.0
Identities = 10/36 (27%), Positives = 16/36 (44%)

Query: 52 LRCQSHHPYFHDKMAPZYQLGERZLIHLLRKDRZSF 87
+R HHP+ H K +++ +R KD F
Sbjct: 1221 MRRHHHHPHRHHKPRKEHRKHKRHPAEDWEKDEGDF 1256

sp|P06181|LIG8_PHACH LIGNINASE H8 PRECURSOR (LIGNIN PEROXIDASE)
Length = 372

Score = 56 (26.6 bits), Expect = 8.0, P = 1.0
Identities = 11/22 (50%), Positives = 16/22 (72%)

Query: 11 WQSIVLHQZRLQNRFQFLSLSL 32
WQS V +Q +L + FQF+ L+L
Sbjct: 279 WQSFVNNQSKLVDDFQFIFLAL 300

Fig. 3.16 B (continued)

sp|P31838|LIGB_PHACH LIGNINASE B PRECURSOR (LIGNIN PEROXIDASE)
Length = 372

Score = 56 (26.6 bits), Expect = 8.0, P = 1.0
Identities = 11/22 (50%), Positives = 16/22 (72%)

Query: 11 WQSIVLHQZRLQNRFQFLSLSL 32
WQS V +Q +L + FQF+ L+L
Sbjct: 279 WQSFVNNQSKLVDDFQFIFLAL 300

sp|P31837|LIGA_PHACH LIGNINASE A PRECURSOR (LIGNIN PEROXIDASE)
Length = 372

Score = 56 (26.6 bits), Expect = 8.0, P = 1.0
Identities = 11/22 (50%), Positives = 16/22 (72%)

Query: 11 WQSIVLHQZRLQNRFQFLSLSL 32
WQS V +Q +L + FQF+ L+L
Sbjct: 279 WQSFVNNQSKLVSDFQFIFLAL 300

sp|P11542|LIG4_PHACH LIGNINASE H2 PRECURSOR (LIGNIN PEROXIDASE) (LG4)
Length = 372

Score = 56 (26.6 bits), Expect = 8.0, P = 1.0
Identities = 11/22 (50%), Positives = 15/22 (68%)

Query: 11 WQSIVLHQZRLQNRFQFLSLSL 32
WQS V +Q +LQ FQF+ +L
Sbjct: 280 WQSFVNNQTKLQEDFQFIFTAL 301

sp|P21764|LIG3_PHACH LIGNINASE LG3 PRECURSOR (LIGNIN PEROXIDASE)
Length = 372

Score = 56 (26.6 bits), Expect = 8.0, P = 1.0
Identities = 11/22 (50%), Positives = 16/22 (72%)

Query: 11 WQSIVLHQZRLQNRFQFLSLSL 32
WQS V +Q +L + FQF+ L+L
Sbjct: 279 WQSFVNNQSKLVDDFQFIFLAL 300

Fig. 3.16 B (continued)

sp|P50622|LIG6_PHACH LIGNINASE LG5 PRECURSOR (LIGNIN PEROXIDASE)
Length = 372

Score = 56 (26.6 bits), Expect = 8.0, P = 1.0
Identities = 11/22 (50%), Positives = 16/22 (72%)

Query: 11 WQSIVLHQZRLQNRFQFLSLSL 32
WQS V +Q +L + FQF+ L+L
Sbjct: 279 WQSFVNNQSKLVSDFQFIFLAL 300

sp|P11543|LIG5_PHACH LIGNINASE LG5 PRECURSOR (LIGNIN PEROXIDASE)
Length = 371

Score = 56 (26.6 bits), Expect = 8.0, P = 1.0
Identities = 11/22 (50%), Positives = 16/22 (72%)

Query: 11 WQSIVLHQZRLQNRFQFLSLSL 32
WQS V +Q +L + FQF+ L+L
Sbjct: 277 WQSFVNNQSKLVSDFQFIFLAL 298

Parameters:

V=100
B=50
H=0
-filter
SEG

Lambda K H
0.329 0.143 0.464

Cutoff to enter 2nd pass: >= 40 (0.0 bits)

E S T1 T2 X1 X2 W Gap
10.0 57 11 11 -15 -22 40 50

Database: Non-redundant SwissProt sequences
Posted date: Mar 11, 1999 10:30 PM
# of letters in database: 27,864,727
# of sequences in database: 77,419

Fig. 3.16 B (continued)

To determine whether a 173 bp DNA fragment was specific to normal shrimps, 20 individuals in each group of geographically separated *P. monodon* were tested. In normal shrimps, 16 out of 20 individuals showed a 173 bp DNA fragment which was approximately 80 % specificity (Fig. 3.17). For viral tolerance shrimp, this fragment did not appeared (Fig. 3.18). To examine the other normal shrimps in Thailand, samples from Satun (collected in 1997), Trang (collected in 1997), Trat and Chumphon were tested. Only three samples from Satun showed a 173 bp fragment (15 %) as showed in Fig. 3.19 but the other groups did not give the expecting 173 bp fragment (see Appendices 7.1, 7.2 and 7.3).

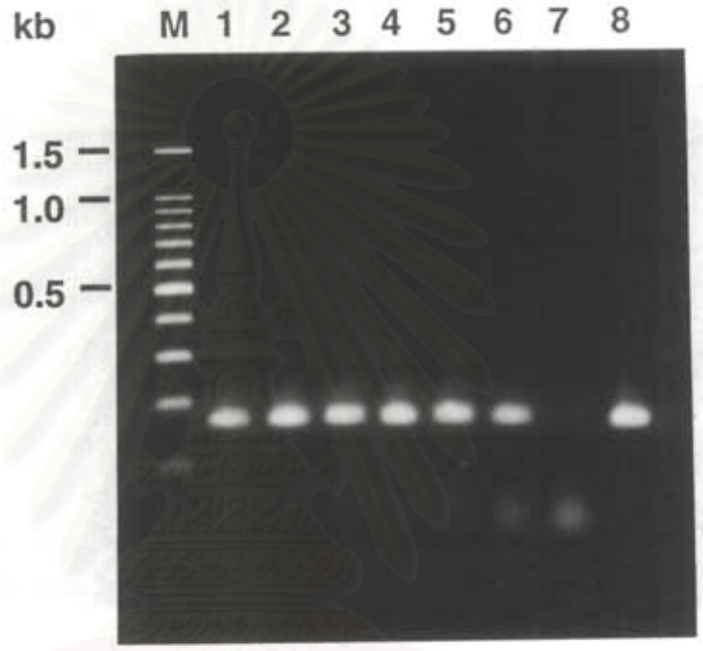


Fig. 3.17 Ethidium bromide stained gel representing a 173 bp PCR product in normal *P. monodon*.

lane M : a 100 bp DNA ladder

lanes 1-6 : normal *P. monodon* individuals (collected in 1996)

lane 7 : negative control

lane 8 : positive control

Fig. 3.17 (continued)

lane M : a 100 bp DNA ladder

lanes 1-14 : normal *P. monodon* individuals (collected in 1996)

lane 15 : negative control

lane 16 : positive control

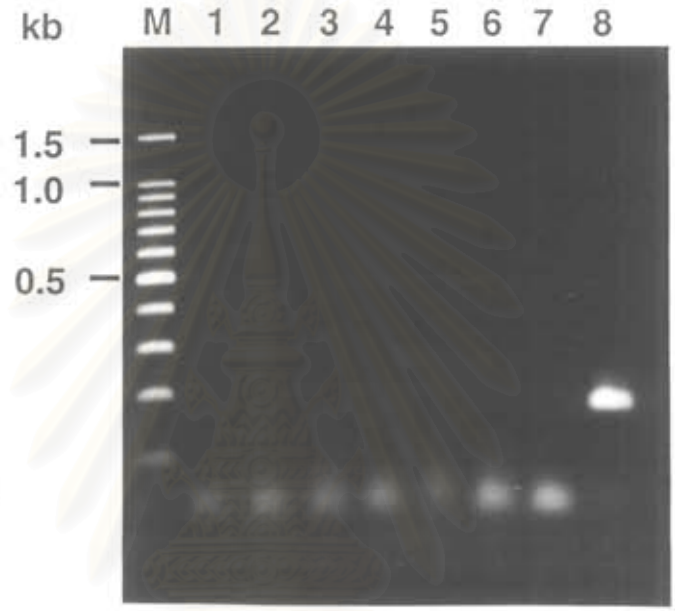


Fig. 3.18 Ethidium bromide stained gel showing the absence of a 173 bp PCR product in viral tolerance *P. monodon*.

lane M : a 100 bp DNA ladder

lanes 1-6 : viral tolerance *P. monodon* individuals

lane 7 : negative control

lane 8 : positive control

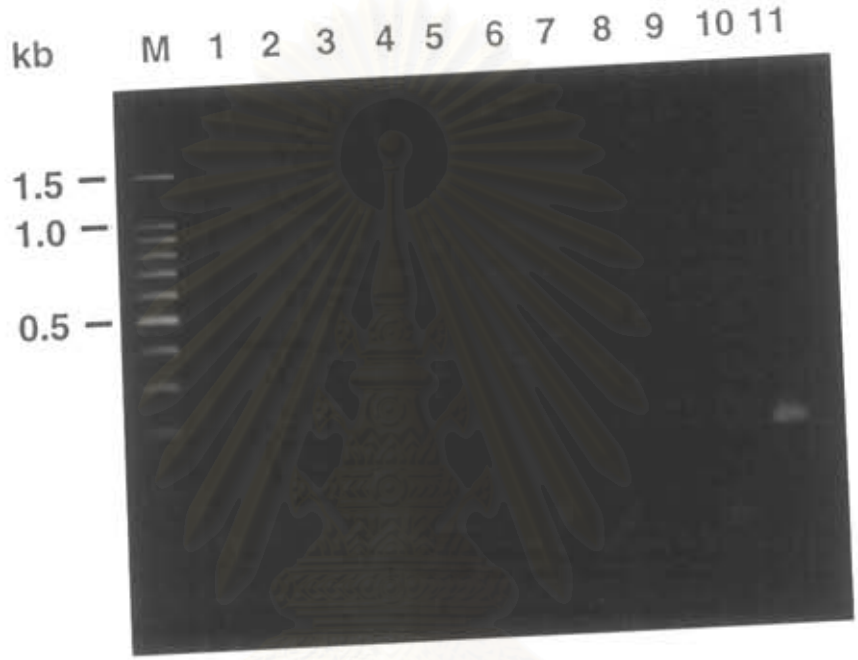


Fig. 3.18 (continued)

lane M : a 100 bp DNA ladder

lanes 1-9 : viral tolerance *P. monodon* individuals

lane 10 : negative control

lane 11 : positive control

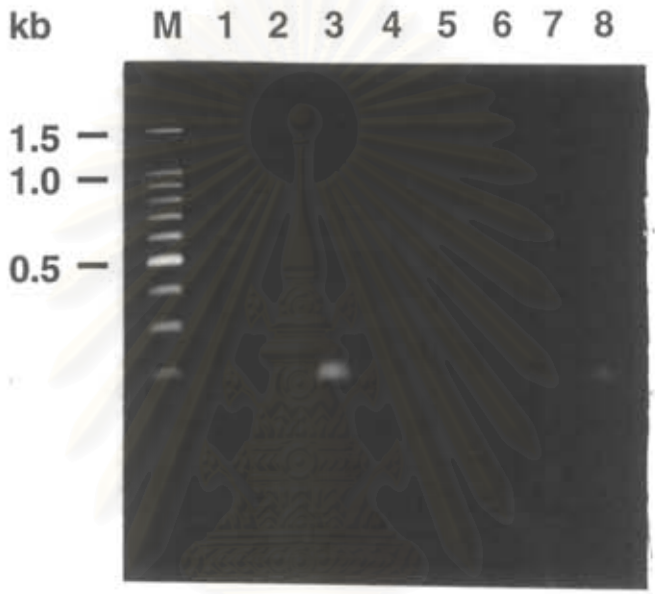


Fig. 3.19 Ethidium bromide staining of a 173 bp PCR product of shrimps from Satun.

lane M : a 100 bp DNA ladder

lanes 1-6 : individuals collected from Satun (collected in 1997)

lane 7 : negative control

lane 8 : positive control

Fig. 3.19 (continued)

lane M : a 100 bp DNA ladder

lanes 1-14 : individuals collected from Satun (collected in 1997)

lane 15 : negative control

lane 16 : positive control