CHAPTER III

RESULTS

3.1 DNA extraction

Genomic DNA was extracted from a frozen pleopod of each 3 and 5-month-old juveniles using a phenol-chloroform-proteinase K method. The quality of extracted genomic DNA was electrophoretically determined using a 0.8 % agarose gel. High molecular weight DNA at 23.1 kb along with sheared DNA was obtained (Figure 3.1). The ratio of OD_{260}/OD_{280} of extracted DNA ranged from 1.8 - 2.0 indicating that the quality of extracted DNA samples is acceptable for further used. High ratio of OD_{260}/OD_{280} (> 1.8) in some samples indicated RNA contamination and visualized as the smear at the bottom of the gel after electrophoresis.

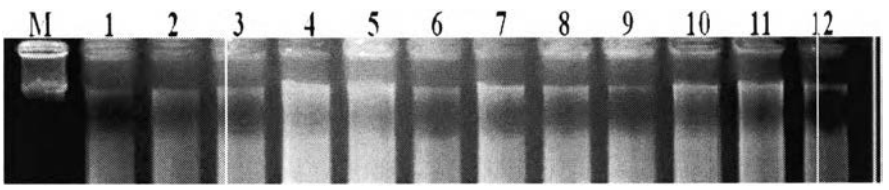


Figure 3.1 A 0.8% ethidium bromide-stained agarose gel showing the quality of genomic DNA extracted from a pleopod of *P. monodon*. Lane M = 200 ng of undigested lambda DNA. Lanes 1 - 12 = genomic DNA from different individuals of *P. monodon*

3.2 Amplification of the genomic gene segments of various growth-related genes by PCR

The genomic sequences of transcripts functionally related with growth including *calponin1* (*PmCnn1*, using two sets of primers; Cnn1-F/R and Cnn1-F3/R3), *cyclin C* (*PmCyC*) and *Cdc25* (*PmCdc25*) were amplified from different samples of juvenile *P. monodon*; 3-month-old (BUM03 and SNP3A) and 5-month-old (PM05) juveniles.

The amplification product of each shrimp was initially analyzed by agarose gel electrophoresis. Polymorphism of the amplified gene segments was further analyzed by SSCP.

3.2.1 *PmCnn1*

The complete genomic sequence of *calponin 1* of *P. monodon* was successfully isolated by genome walking. The *PmCnn1* gene contained 3 exons (185, 206 and 169 bp) and 2 introns (214 and 306 bp) with the open reading frame (ORF) of 561 bp deducing to a polypeptide of 186 amino acids (Buaklin, 2005). Two pairs of primers (primers Cnn1-F/R and Cnn1-F3/R3, Table 2.2) were designed for amplification of its genomic DNA.

The amplified $PmCnn1_{530}$ gene segment covering the partial exon 1, intron 1 and partial exon 2 was generated from primers Cnn1-F/R. The amplification product was 530 bp long containing an intron of 214 bp in size (Figure 3.2).

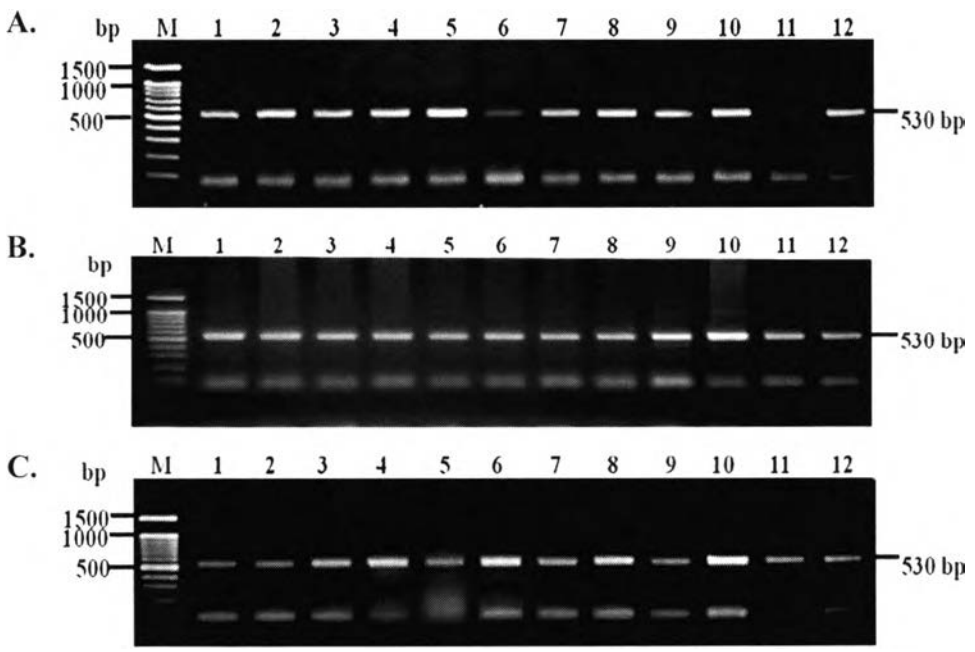


Figure 3.2 A 1.5% ethidium bromide-stained agarose gel showing the amplification result of the *calponin 1* gene segment ($PmCnn1_{530}$) against genomic DNA of *P. monodon* juveniles using primers Cnn1-F/R. Lanes 1-12 (A) = genomic DNA of 3-month-old juveniles (BUM03 sample). Lanes 1–12 (B) = genomic DNA of 3-month-old juveniles (SNP3A sample), and Lanes 1 – 12 (C) = genomic DNA of 5-month-old juveniles (PM05 sample). Lanes M are a 100 bp DNA ladder.

Similarly, the amplified *PmCnn1*$_{425}$ gene segment covering the partial exon 2, complete intron 2 and partial exon 3 was generated from primers Cnn1-F3/R3. The amplified fragment was 425 bp in length containing an intron of 306 bp in size was observed (Figure 3.3). No obvious length polymorphism was observed from the amplification of *PmCnn1* across different sample sets of juvenile shrimp in the present study.

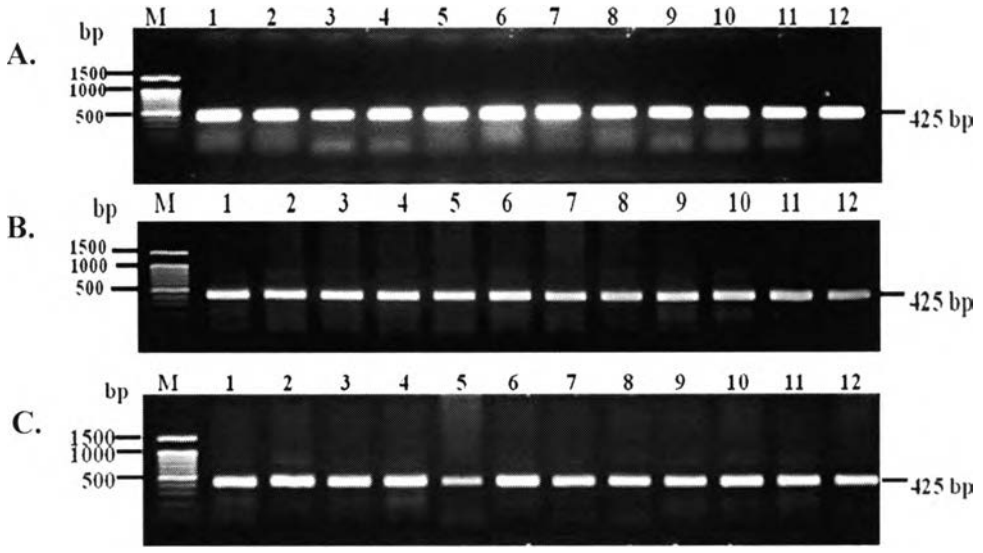
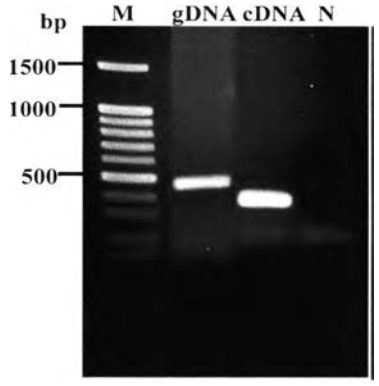


Figure 3.3 A 1.5% ethidium bromide-stained agarose gel showing the amplification result of the *PmCnn1*$_{425}$ gene segment against genomic DNA of juvenile *P. monodon* using Cnn1-F3/R3. Lanes 1-12 (A) = genomic DNA of 3-month-old juveniles (BUM03 sample). Lanes 1–12 (B) = genomic DNA of 3-month-old juveniles (SNP3A sample), and Lanes 1 – 12 (C) = genomic DNA of 5-month-old juveniles (PM05 sample). Lanes M are a 100 bp DNA ladder.

3.2.2 *PmCyC*

The amplified *PmCyC* gene segment was approximately 400 bp in length which is larger than that (280 bp) expected from the cDNA sequence (Figure 3.4A). The amplified fragment was cloned and sequenced (Figure 3.4B). Pairwise alignment of nucleotide sequences from genomic DNA (403 bp) and EST (280 bp) revealed an intron of 123 bp within the amplified region (Figure 3.4C).

A.



B.

----------TACATGAAGATTATGACCTTCTTTGCTAACT*GTAAGAGACAG*
CAACATCCATAGATATTTACTGTTTTTCCAATAGATGGTTTCATCAGATAGAATTAGTTTAT
*TTTGTATTACTCTATAAAAAACAGTTTTTCATAGGTAACAATAATTTCAG***TTATTCAGCAAC**
TTGGTGAATCACTCAAGCTTAAACAACAGGTCATCGCAACTGCCACATGCTTCCTTAAAAGA
TTCTACGCAAGAAATTCTCTCAAGTGCATTGACCCTCTTCTCCTCGCCCCCACCAGTGTCTT
CCTCTCATCCA----------

C.

```
PmCyC-gDNA    ----------TCGACAGTCAGGACTTGATACAAGAGCGCCAGGCTGACCT
PmCyC-cDNA    ----------TCGACAGTCAGGACTTGATACAAGAGCGCCAGGCTGACCT
              ************************************************************
PmCyC-gDNA    AGAGGTGCTGTCTGAAGAAGAGTACATGAAGATTATGACCTTCTTTGCTAACTGTAAGAG
PmCyC-cDNA    AGAGGTGCTGTCTGAAGAAGAGTACATGAAGATTATGACCTTCTTTGCTAACT-------
              ************************************************
PmCyC-gDNA    ACAGCAACATCCATAGATATTTACTGTTTTTCCAATAGATGGTTTCATCAGATAGAATTA
PmCyC-cDNA    ------------------------------------------------------------

PmCyC-gDNA    GTTTATTTTGTATTACTCTATAAAAAACAGTTTTTCATAGGTAACAATAATTTCAGTTAT
PmCyC-cDNA    ------------------------------------------------------------TTAT
                                                                          ****
PmCyC-gDNA    TCAGCAACTTGGTGAATCACTCAAGCTTAAACAACAGGTCATCGCAACTGCCACATGCTT
PmCyC-cDNA    TCAGCAACTTGGTGAATCACTCAAGCTTAAACAACAGGTCATCGCAACTGCCACATGCTT
              ************************************************************
PmCyC-gDNA    CCTTAAAAGATTCTACGCAAGAAATTCTCTCAAGTGCATTGACCCTCTTCTCCTCGCCCC
PmCyC-cDNA    CCTTAAAAGATTCTACGCAAGAAATTCTCTCAAGTGCATTGACCCTCTTCTCCTCGCCCC
              ************************************************************
PmCyC-gDNA    CACCAGTGTCTTCCTCTCATCTA----------
PmCyC-cDNA    CACCAGTGTCTTCCTCTCATCTA----------
              **********************************
```

Figure 3.4 (A). A 1.5% ethidium bromide-stained agarose gel showing the amplification result of the *cyclin C* gene segment (*PmCyC*) against genomic DNA (gDNA) and cDNA of juvenile *P. monodon* using primers cyclinC-F/R. Lane M = a 100 bp DNA ladder. (B). Nucleotide sequence of the amplified genomic segment of *PmCyC*. Primer sequences are dashed and boldfaced. An intron is italicized. (C). Pairwise alignment between nucleotide sequences from coding sequence (CDS) and genomic DNA of *cyclin C*.

No obvious length polymorphism was observed from the amplification of *PmCyC* across different sample sets of juvenile shrimp (BUM03and SNP3A and PM05) (Figure 3.5).

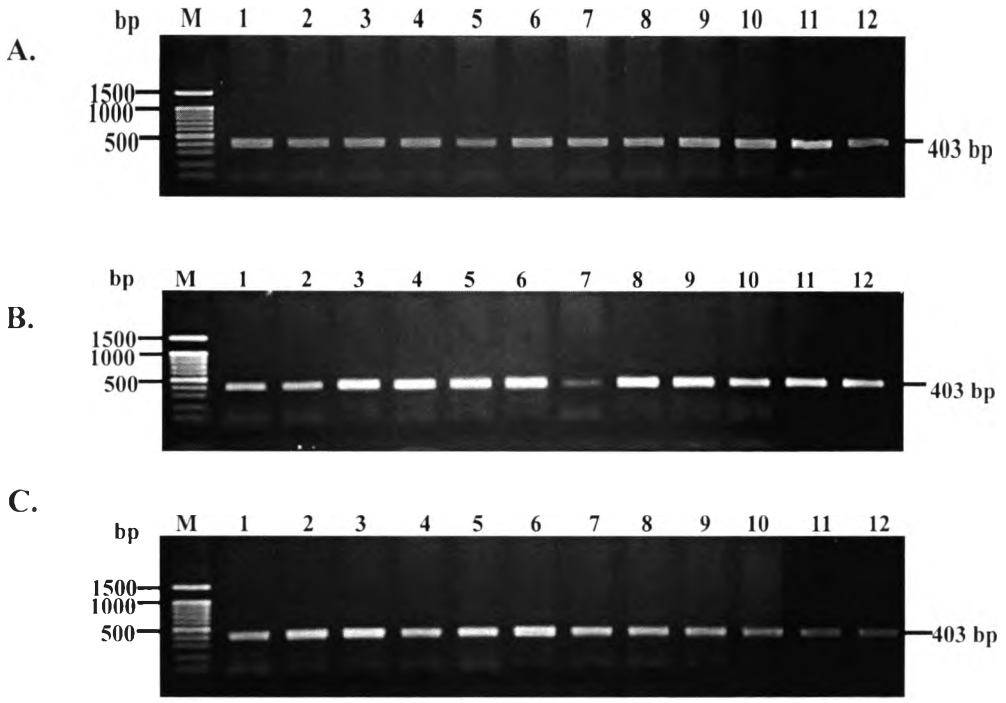


Figure 3.5 A 1.5% ethidium bromide-stained agarose gel showing the amplification result of the *PmCyC* gene segment against genomic DNA of juvenile *P. monodon*. Lanes 1-12 (A) = genomic DNA of 3-month-old juveniles (BUM03 sample). Lanes 1–12 (B) = genomic DNA of 3-month-old juveniles (SNP3A sample), and Lanes 1 – 12 (C) = genomic DNA of 5-month-old juveniles (PM05 sample). Lanes M are a 100 bp DNA ladder.

3.2.3 *PmCdc25*

A 285 bp fragment was obtained from amplification of *PmCdc25* using genomic DNA of 3-month-old (BUM03 and SNP3A) and 5 month-month-old (PM05) juveniles (Figure 3.6). Sizes of the amplification products from genomic DNA and the expected product from cDNA were identical suggesting that the amplified gene segment did not contain the intron. No obvious length polymorphism was observed from the amplification of *PmCdc25* across different sample sets of juvenile shrimp

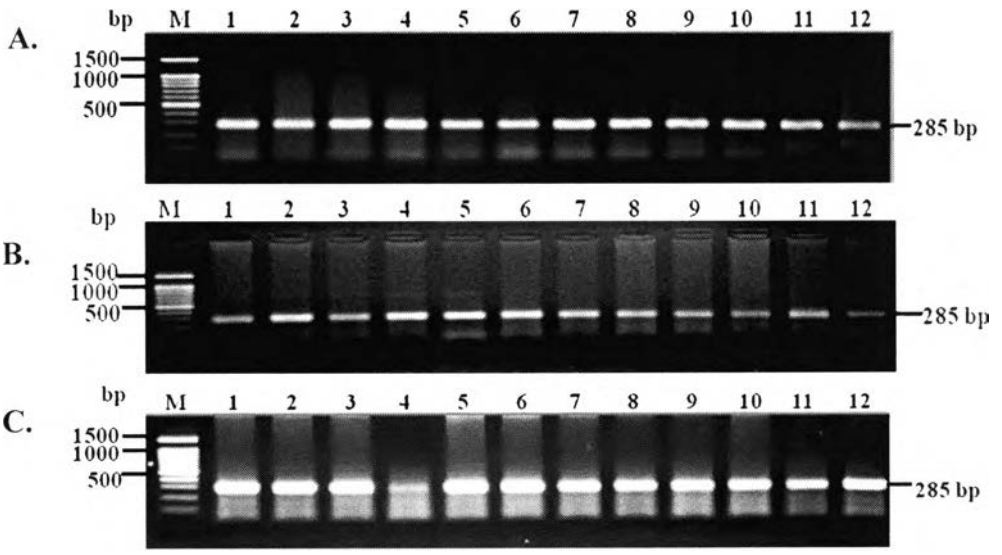


Figure 3.6 A 1.5% ethidium bromide-stained agarose gel showing the amplification result of *PmCdc25* gene segment against genomic DNA of juvenile *P. monodon* using primer cdc25-F/R. Lanes 1-12 (A) = genomic DNA of 3-month-old juveniles (BUM03 sample). Lanes 1-12 (B) = genomic DNA of 3-month-old juveniles (SNP3A sample), and Lanes 1-12 (C) = genomic DNA of 5-month-old juveniles (PM05 sample). Lanes M are a 100 bp DNA ladder.

3.3 Identification of polymorphic SSCP patterns of *PmCnn1*, *PmCyC* and *PmCdc25* and their relationships with growth parameters of *P. monodon*

3.3.1 *PmCnn1*

SNP by SSCP analysis in the $PmCnn1_{530}$ gene segment (530 bp) generated from primers Cnn1-F/R were examined. Three polymorphic patterns were observed in the SNP3A sample while a monomorphic pattern was found in the BUM03 sample. For 5-month-old shrimp (PM05), four different patterns were observed (Figure 3.7). In addition, PCR-SSCP of $PmCnn1_{425}$ was also carried out using Cnn1-F3/R3 primers (N = 69, 151 and 79 for BUM03, SNP3A and PM05, respectively). Five, five and two polymorphic patterns were found in the BUM03, SNP3A and PM05 samples, respectively (Figure 3.8). A summary for the number of SSCP patterns found in different set of samples is shown in Table 3.1.

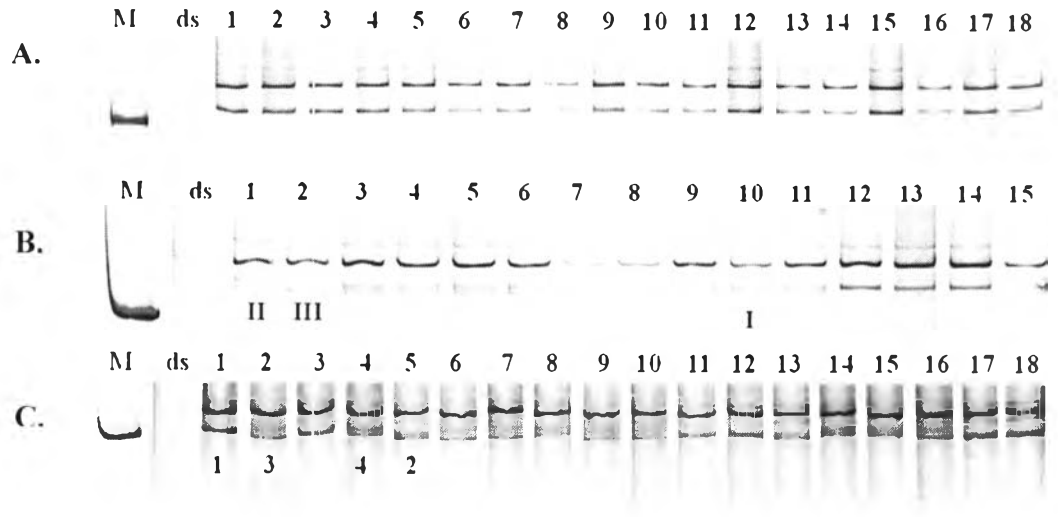


Figure 3.7 SSCP patterns of the *PmCnn1*$_{530}$ (primer Cnn1-F/R) gene segment amplified from genomic DNA of the BUM03 (lanes 1-18, A), SNP3A (lanes 1-15, B) and PM05 (lanes 1-18, C) samples. One pattern were observed in BUM03 (lanes 1-18, B), three SSCP patterns were observed in SNP3A (I, lane 10 and 12-14; II, lane 1, 3-6, 9, 11 and 15 and III, lane 2 and 7-8, A), and four patterns were observed in PM05 (1, lanes 1, 3 and 11-12; 2, lanes 5, 13 and 17-18; 3, lanes 2, 6-7, 9-10 and 16 and 4, lanes 4, 8 and 14-15). Lanes M are a 100 bp DNA marker. ds = non-denatured PCR product (double strand control).

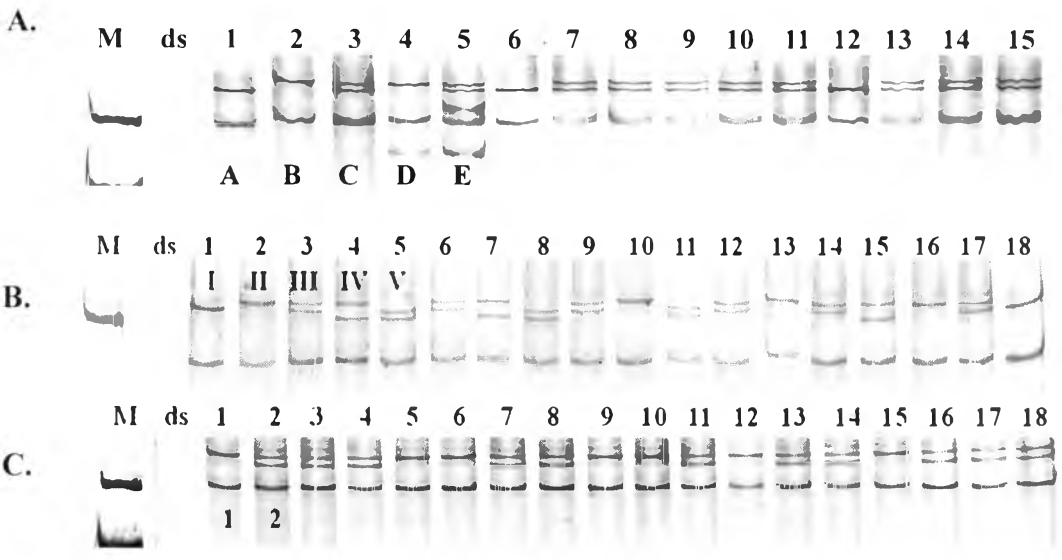


Figure 3.8 SSCP patterns of the *PmCnn1*$_{425}$ (primer Cnn1-F3/R3) gene segment amplified from genomic DNA of the BUM03 (A, lanes 1-15), SNP3A (B, lanes 1-18) and PM05 samples (C, lanes 1-18). Five patterns were found in BUM03 (A, lanes 1, 6 and 12; B, lane 2; C, lanes 3, 7-11, and 13-15; D, lanes 4 and E, lanes 5). Five SSCP patterns were also observed in SNP3A (I, lanes 1 and 18; II, lanes 2, 10, 13 and 16; III, lanes 3, 6, 9, 12, 14 and 17; IV, lanes 4, 7 and 15 and V, lanes 5, 8 and 11, A). Two patterns were observed in PM05 (1, lanes 1, 5-6, 9-10, 12 and 15 and 2, lanes 2-4, 7-8, 11, 13-14 and 16-18, C). Lanes M are a 100 bp DNA marker. ds = non-denatured PCR product (double strand control).

Table 3.1 A summary of PCR-SSCP of *PmCnn1*, *PmCyC* and *PmCdc25* gene segments of *P. monodon* in this study

Gene	Primer name	Expectd size (bp)	Observed size (bp)	No. of SSCP pattern		
				SNP3A	**BUM03**	**PM05**
PmCnn1	Cnn1-F/R	316	530	3 (I, II and III)	Monomorphism (A)	3 (1, 2, 3 and 4)
	Cnn1-F3/R3	119	425	5 (I, II, III, IV and V)	5 (A, B, C, D and E)	2 (1 and 2)
PmCyC	CyC-F/R	280	403	3 (I, II and III)	4 (A, B, C and D)	2 (1 and 2)
PmCdc25	Cdc25-F/R	280	280	2 (I and II)	Monomorphism (A)	2 (1 and 2)

Relationships between SSCP patterns and growth parameters of examined shrimp were statistically tested. For $PmCnn1_{530}$, SNP3A shrimp exhibiting patterns I and II ($P < 0.05$) had a greater average BW and TL than those carrying genotype III (Table 3.2). However, the average HP weight and HSI was not significantly different among shrimp with different SSCP patterns ($P > 0.05$). When male and female shrimp were tested separately, only female SNP3A juveniles carrying patterns I and II ($P < 0.05$) had a greater average BW than those carrying pattern III (Table 3.2).

Disregarding sexes, the PM05 shrimp possessing different SSCP patterns did not show significant different average BW and TL ($P > 0.05$). When data between different sexes of shrimp were analyzed separately, male shrimp with SSCP patterns I showed a greater average BW and TL than those exhibiting different patterns ($P < 0.05$) (Table3.3).

For $PmCnn1_{425}$, the BUM03 shrimp with patterns B and C had a significantly greater average BW and TL than those with patterns D and E (Table 3.4; $P < 0.05$). When sex of shrimp are considered, the average BW and TL of male juveniles exhibiting different SSCP patterns were not statistically different ($P > 0.05$). In contrast, female BUM03 shrimp having pattern C showed a greater average TL than those carrying pattern A ($P < 0.05$).

For the SNP3A sample ($PmCnn1_{425}$), shrimp exhibiting pattern I showed the greatest average BW and TL compared with those carrying other patterns of *PmCnn1* ($P < 0.05$). Shrimp with patterns IV also showed a greater average BW and TL than those carrying pattern II (Table 3.5) but were not different from those with patterns III and V ($P > 0.05$). Similarly, shrimp with pattern I had a greater average HPW than those with the remaining patterns except pattern IV. In addition, shrimp with SSCP patterns IV showed a greater HPW than those carrying patterns II and III ($P < 0.05$). However, the average HSI was not significantly different among shrimp with different SSCP patterns ($P > 0.05$).

Table 3.2 Relationships between SSCP patterns of *PmCnn1*$_{530}$ and growth parameters of 3-month-old juveniles (primers Cnn1-F/R, SNP3A; *N* =156)

Pattern	*N*	Average BW ± SD (g)	Average TL ± SD (cm)	Average HP weight ± SD (g)	Average HSI± SD (%)
Disregarding sexes					
I	51	13.81±6.31[a]	11.68±1.86[a]	0.44±0.20[a]	3.31±0.73[a]
II	81	13.01±5.99[a]	11.41±1.76[a]	0.42±0.19[a]	3.34±0.59[a]
III	24	9.99±4.52[b]	10.55±1.66[b]	0.33±0.17[a]	3.34±0.83[a]
Male					
I	20	10.79±5.20[a]	10.83±1.67[a]	0.36±0.16[a]	3.45±0.64[a]
II	28	10.51±4.53[a]	10.90±1.52[a]	0.34±0.14[a]	3.33±0.59[a]
III	12	9.35±5.19[a]	10.24±1.94[a]	0.34±0.23[a]	3.53±1.12[a]
Female					
I	31	15.75±6.27[a]	12.23±1.79[a]	0.49±0.20[a]	3.21±0.77[a]
II	53	14.32±6.28[a]	11.68±1.83[a]	0.47±0.19[a]	3.34±0.59[a]
III	12	10.61±3.86[b]	10.86±1.34[a]	0.33±0.11[a]	3.15±0.32[a]

The same superscripts indicate non-significant differences between shrimp carrying different SSCP patterns ($P > 0.05$).

Table 3.3 Relationships between SSCP patterns of *PmCnn1*$_{530}$ and growth parameters of 5-month-old juveniles (primers Cnn1-F/R, PM05; *N* =97)

Pattern	*N*	Average BW ± SD (g)	Average TL ± SD (cm)
Disregarding sexes			
1	21	35.41±8.85[a]	15.61±1.51[a]
2	24	33.42±8.26[a]	15.35±1.26[a]
3	28	30.40±8.55[a]	14.97±1.47[a]
4	24	33.28±9.94[a]	15.46±1.37[a]
Male			
1	9	36.98±8.65[a]	16.13±1.58[a]
2	4	29.16±5.29[ab]	14.65±0.76[b]
3	11	26.05±6.96[b]	14.26±1.08[b]
4	10	26.96±6.96[b]	14.64±1.18[b]
Female			
1	12	34.24±9.18[a]	15.22±1.39[a]
2	20	34.28±8.58[a]	15.49±1.30[a]
3	17	33.22±8.46[a]	15.43±1.52[a]
4	14	37.79±9.43[a]	16.05±1.21[a]

The same superscripts indicate non-significant differences between shrimp carrying different SSCP patterns ($P > 0.05$).

When sexes for shrimp were considered, male SNP3A juvenile exhibiting patter I showed the greatest average BW and TL compared with those with other patterns. Nevertheless, the average BW and TL of shrimp with genotype patterns II, III, IV and V were not statistically different ($P > 0.05$). The average HPW and HSI was not significantly different among male SNP3A shrimp with different SSCP patterns ($P > 0.05$). In female SNP3A shrimp, similar results were observed for the average BW and TL. In contrast, the HPW of shrimp exhibiting pattern I was significantly greater than others. In addition, female SNP3A shrimp with pattern IV had a greater average HP weight than those with pattern II ($P < 0.05$).

For the PM05 sample, shrimp exhibiting different SSCP patterns did not showed different growth parameters when data were analyzed with and without consideration of sexes of examined shrimp ($P > 0.05$) (Table 3.6).

Table 3.4 Relationships between SSCP patterns of *PmCnn1*$_{425}$ and growth parameters of 3-month old juveniles (primers Cnn1-F3/R3, BUM03; $N = 79$)

Genotype	*N*	Average BW ± SD (g)	Average TL ± SD (cm)
Disregarding sexes			
A	21	12.26±4.00^{ab}	11.58±1.02^{ab}
B	3	14.74±3.00^{a}	12.26±1.10^{a}
C	46	14.82±3.06^{a}	12.35±0.83^{a}
D	5	10.03±0.77^{b}	11.02±0.31^{b}
E	4	9.42±0.55^{b}	10.90±0.25^{b}
Male			
	11	11.44±2.99^{a}	11.46±0.88^{a}
B	-	-	-
C	14	11.97±2.94^{a}	11.64±0.88^{a}
D	4	10.37±0.14^{a}	11.15±0.12^{a}
E	4	9.42±0.55^{a}	10.90±0.25^{a}
Female			
A	10	13.16±4.88^{a}	11.72±1.20^{b}
B	3	14.74±3.00^{a}	12.26±1.10^{ab}
C	32	16.06±2.18^{a}	12.67±0.59^{a}
D	1	-	-
E	-	-	-

The same superscripts indicate non-significant differences between shrimp carrying different SSCP patterns ($P > 0.05$).

Table 3.5 Relationships between SSCP patterns of *PmCnn1*$_{425}$ and growth parameters of 3-month-old juveniles (primers Cnn1-F3/R3, SNP3A; $N = 151$)

Pattern	*N*	Average BW ± SD (g)	Average TL ± SD (cm)	Average HP weight ± SD (g)	Average HSI± SD (%)
Disregarding sexes					
I	13	17.81±5.46^{a}	12.97±1.46^{a}	0.55±0.18^{a}	3.15±0.24^{a}
II	25	9.96±4.77^{c}	10.51±1.66^{c}	0.33±0.17^{c}	3.36±0.82^{a}
III	42	11.28±5.49^{bc}	10.93±1.69^{bc}	0.36±0.17^{c}	3.27±0.60^{a}
IV	30	14.44±6.43^{b}	11.88±1.81^{b}	0.47±0.20^{ab}	3.40±0.64^{a}
V	41	12.77±5.58^{bc}	11.38±1.74^{bc}	0.41±0.19^{bc}	3.33±0.70^{a}
Male					
I	6	14.98±5.46^{a}	12.20±1.65^{a}	0.48±0.19^{a}	3.26±0.19^{a}
II	13	9.09±4.58^{b}	10.23±1.78^{b}	0.33±0.20^{a}	3.55±1.08^{a}
III	17	9.76±3.62^{b}	10.58±1.37^{b}	0.30±0.18^{a}	3.14±0.52^{a}
IV	11	12.55±5.91^{ab}	11.61±1.73^{ab}	0.31±0.12^{a}	3.58±0.59^{a}
V	14	9.42±4.06^{b}	10.39±1.36^{b}	0.35±0.16^{a}	3.42±0.35^{a}
Female					
I	7	20.23±4.47^{a}	13.64±0.92^{a}	0.61±0.15^{a}	3.05±0.25^{a}
II	12	10.89±4.99^{b}	10.81±1.52^{b}	0.34±0.14^{c}	3.15±0.31^{a}
III	25	12.32±6.33^{b}	11.16±1.87^{b}	0.40±0.19^{bc}	3.36±0.64^{a}
IV	19	15.53±6.62^{b}	12.04±1.88^{b}	0.50±0.21^{ab}	3.30±0.65^{a}
V	27	14.08±6.33^{b}	11.90±1.71^{b}	0.46±0.20^{abc}	3.28±0.83^{a}

The same superscripts indicate non-significant differences between shrimp carrying different SSCP patterns ($P > 0.05$).

Table 3.6 Relationships between SSCP patterns of *PmCnn1*$_{425}$ and growth parameters of 5-month-old juveniles (primers Cnn1-F3/R3, PM05; $N = 69$)

Pattern	*N*	Average BW ± SD (g)	Average TL ± SD (cm)
Disregarding sexes			
1	35	30.91±10.71^{a}	14.95±1.65^{a}
2	34	31.69±10.76^{a}	15.03±1.74^{a}
Male			
1	17	26.50±9.21^{a}	14.42±1.44^{a}
2	13	29.90±9.69^{a}	15.02±1.66^{a}
Female			
1	18	34.54±11.07^{a}	15.45±1.71^{a}
2	21	32.80±11.45^{a}	15.04±1.83^{a}

The same superscripts indicate non-significant differences between shrimp carrying different SSCP patterns ($P > 0.05$).

3.3.2 *PmCyC*

PCR-SSCP was also applied to determine SNP polymorphism in the amplified *PmCyC* gene segment of BUM03 (N = 57), SNP3A (N = 145) and PM05 (N = 66). Four, three and two polymorphic SSCP patterns were found in respective sample sets (Figure 3.9).

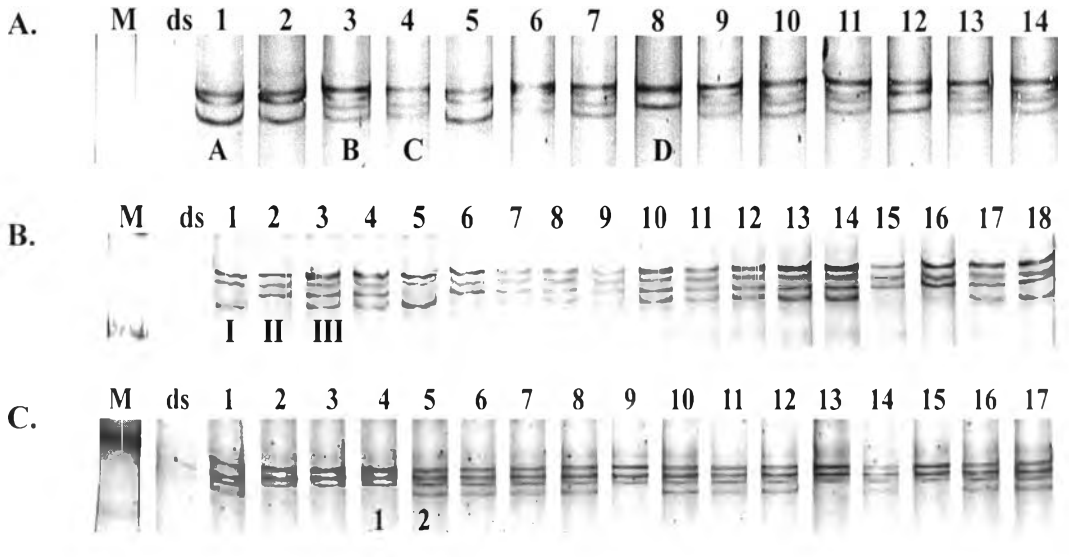


Figure 3.9 SSCP patterns of the *PmCyC* gene segment amplified from genomic DNA of the BUM03 (A, lanes 1-14), SNP3A (B, lanes 1-18) and PM05 (C, lanes 1-17) samples. Four SSCP patterns were observed in the BUM03 sample (A, lanes 1-2, 5 and 12; B, lanes 3, 6-7, 9-11 and 13-14; C, lanes 4 and D, lanes 8; panel A), three patterns were found in the SNP3A sample (I, lanes 1 and 5; II, lanes 2, 6 and 15-16 and III, lanes 3-4, 7-14 and 17-18, panel B) and two patterns were observed in the PM05 sample (1, lane 2-4, 9, 13 and 15 and 2, lane 1, 5-8, 10-12, 14 and 16-17, panel C). Lanes M is a 100 bp DNA marker. ds = non-denatured PCR product (double strand control).

Relationships between domesticated shrimp carrying different SSCP patterns of *PmCyC* and their growth parameters were statistically examined. For the BUM03 sample, shrimp exhibiting different SSCP patterns did not showed different growth parameters when data were analyzed with and without consideration of sexes of examined shrimp ($P > 0.05$) (Table 3.7).

For the SNP3A sample, shrimp having pattern II had a greater average BW and HPW than those with patterns I and III (Table 3.8; $P < 0.05$). In addition, shrimp exhibiting this SSCP pattern also showed a greater average TL than those with pattern I ($P < 0.05$) but not with pattern III ($P > 0.05$). When sexes of examined shrimp were considered, male SNP3A shrimp having pattern II had a greater average BW than those carrying pattern I ($P < 0.05$). The result was similar for female SNP3A juveniles ($P < 0.05$).

Like the BUM03 sample, 5-month-old juveniles (PM05) carrying different SSCP patterns did not reveal different BW and TL ($P > 0.05$) (Table 3.9).

Table 3.7 Relationships between SSCP patterns of *PmCyC* and growth parameters of 3-month old juveniles (BUM03, $N = 57$)

Pattern	*N*	Average BW ± SD (g)	Average TL ± SD (cm)
Disregarding sexes			
A	4	13.34±3.30[a]	12.05±1.19[a]
B	7	12.40±3.02[a]	11.72±0.79[a]
C	18	13.45±4.50[a]	11.91±1.07[a]
D	28	12.43±3.07[a]	11.68±0.92[a]
Male			
A	1	-	-
B	2	11.24±2.04[a]	11.00±0.28[a]
C	11	10.49±2.18[a]	11.43±0.63[a]
D	10	10.49±2.18[a]	11.22±0.70[a]
Female			
A	3	14.59±2.66[a]	12.46±1.05[a]
B	5	13.45±2.94[a]	12.02±0.74[a]
C	7	16.92±5.25[a]	12.67±1.22[a]
D	18	13.51±3.00[a]	11.95±0.95[a]

The same superscripts indicate non-significant differences between shrimp carrying different SSCP patterns ($P > 0.05$).

Table 3.8 Relationships between SSCP patterns of *PmCyC* and growth parameters of 3-month old juveniles (SNP3A, $N = 145$)

Pattern	*N*	Average BW ± SD (g)	Average TL ± SD (cm)	Average HP weight ± SD (g)	Average HSI± SD (%)
Disregarding sexes					
I	17	9.78±4.00^{b}	10.55±1.40^{b}	0.35±0.15^{b}	3.57±0.68^{a}
II	71	14.38±6.08^{a}	11.79±1.80^{a}	0.46±0.18^{a}	3.33±0.72^{a}
III	57	11.31±4.74^{b}	11.02±1.58^{ab}	0.37±0.17^{b}	3.31±0.63^{a}
Male					
I	6	8.16±2.95^{b}	10.33±1.15^{a}	0.29±0.09^{a}	3.58±0.25^{a}
II	25	12.24±5.58^{a}	11.27±1.84^{a}	0.41±0.17^{a}	3.48±0.91^{a}
III	26	9.22±3.92^{ab}	10.45±1.47^{a}	0.30±0.15^{a}	3.30±0.60^{a}
Female					
I	11	10.66±4.29^{b}	10.83±1.49^{a}	0.38±0.17^{a}	3.57±0.84^{a}
II	46	15.54±6.08^{a}	12.07±1.73^{a}	0.49±0.18^{a}	3.25±0.59^{a}
III	31	13.06±4.71^{ab}	11.51±1.51^{a}	0.43±0.17^{a}	3.32±0.66^{a}

The same superscripts indicate non-significant differences between shrimp carrying different SSCP patterns ($P > 0.05$).

Table 3.9 Relationships between SSCP patterns of *PmCyC* and growth parameters of 5-month old juveniles (PM05, $N = 66$)

Pattern	*N*	Average BW ± SD (g)	Average TL ± SD (cm)
Disregarding sexes			
1	31	33.68±8.07^{a}	15.40±1.17^{a}
2	35	32.37±7.25^{a}	15.30±1.14^{a}
Male			
1	8	29.12±7.06^{a}	14.82±1.26^{a}
2	16	30.66±8.12^{a}	15.08±1.24^{a}
Female			
1	23	35.26±7.93^{a}	15.60±1.10^{a}
2	19	33.82±6.29^{a}	15.50±1.05^{a}

The same superscripts indicate non-significant differences between shrimp carrying different SSCP patterns ($P > 0.05$).

3.3.3 *PmCdc25*

SNP by SSCP analysis of a 285 bp fragment of the *PmCdc25* gene segment in the BUM03 (N = 35), SNP3A (N = 145) and PM05 (N = 70) samples were examined and one, two and two SSCP patterns were observed in these sample sets, respectively.

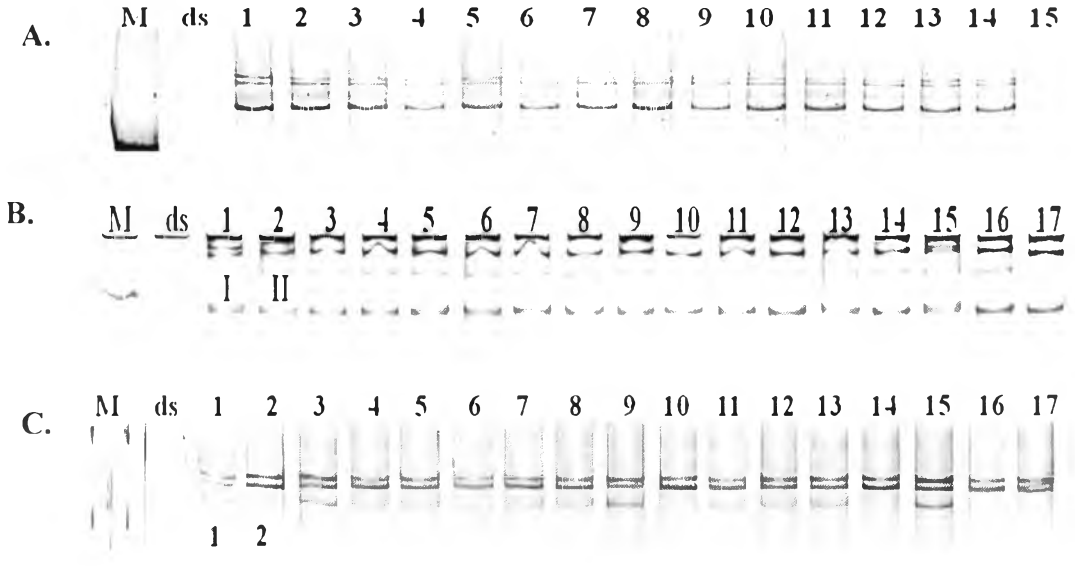


Figure 3.10 SSCP patterns of the *PmCdc25* gene segment amplified from genomic DNA of the BUM03 (lanes 1-15, A), SNP3A (lanes 1-17, B), and PM05 (lanes 1-17, C) samples. One SSCP pattern was observed in BUM03 (lanes 1-17, panel A), two patterns was found in SNP3A (I, lanes 1 and 15; II, lanes 2-14 and 16-17, panel B) and two patterns were observed in PM05 (1, lanes 1 and 3-9, 11-13 and 15; 2, lanes 2, 10, 14 and 16, panel C). Lanes M is a 100 bp DNA marker. ds = non-denatured PCR product (double strand control).

For the SNP3A sample, *P. monodon* juvenile with pattern I had a greater average BW, TL and HPW than those with pattern II ($P < 0.05$). However, shrimp exhibiting different SSCP patterns did not show a significant difference in HSI ($P > 0.05$). When sexes of examined shrimp were considered, results were consistent in both male and female juveniles. ($P < 0.05$) (Table 3.10). The PM05 shrimp carrying different SSCP patterns did not reveal different BW and TL neither sexes of shrimp were regarded nor disregarded ($P > 0.05$) (Table 3.11).

Table 3.10 Relationships between SSCP patterns of *PmCdc25* and growth parameters of 3-month old juveniles (SNP3A, $N = 144$)

Pattern	*N*	Average BW ± SD (g)	Average TL ± SD (cm)	Average HP weight ± SD (g)	Average HSI± SD (%)
Disregarding sexes					
I	26	19.48±5.30[a]	12.76±1.95[a]	0.56±0.17[a]	3.20±0.58[a]
II	117	11.10±5.23[b]	10.96±1.57[b]	0.37±0.16[b]	3.34±0.61[a]
Male					
I	7	17.43±5.21[a]	12.87±1.60[a]	0.54±0.18[a]	3.49±0.78[a]
II	50	8.88±3.75[b]	10.26±1.37[b]	0.31±0.14[b]	3.18±0.43[a]
Female					
I	20	20.28±5.13[a]	13.29±1.48[a]	0.63±0.17[a]	3.29±0.67[a]
II	67	12.74±5.56[b]	11.32±1.68[b]	0.41±0.18[b]	3.18±0.56[a]

The same superscripts indicate non-significant differences between shrimp carrying different SSCP patterns ($P > 0.05$).

Table 3.11 Relationships between SSCP genotypes of *PmCdc25* and growth parameters of 5-month old juveniles (PM05, $N = 70$)

Pattern	*N*	Average BW ± SD (g)	Average TL ± SD (cm)
Disregarding sexes			
1	33	32.49±9.54[a]	15.45±1.16[a]
2	37	32.05±7.19[a]	15.36±1.13[a]
Male			
1	9	28.14±7.73[a]	14.98±1.27[a]
2	17	30.88±8.55[a]	15.23±1.23[a]
Female			
1	19	34.67±7.45[a]	15.70±1.11[a]
2	25	33.96±6.79[a]	15.65±1.06[a]

The same superscripts indicate non-significant differences between shrimp carrying different SSCP patterns ($P > 0.05$).

3.4 Identification and characterization of SNP in *PmCnn1*, *PmCyC* and *PmCdc25* gene segment by DNA sequencing

The amplified *PmCnn1*, *PmCyC* and *PmCdc25* gene segment was cloned. SNPs in the amplified region was sequenced and statistically tested. Results were compared with PCR-SSCP analysis

3.4.1 SNP in the *PmCnn1* gene segment

3.4.1.1 *PmCnn1* generated from Cnn1-F/R

The PCR product of ten individuals representing each SSCP pattern of *PmCnn1* amplified from primer Cnn1-F/R (*PmCnn1*$_{530}$) were cloned and sequenced. A total of 6 SNP positions (including 2 indels) were observed from multiple sequence alignments of the *PmCnn1*$_{530}$ gene segment (Figure 3.11). All of these were located in the intron region and corresponding to SSCP genotypes I, II and III, respectively. Genotypes of each SNP were statistically tested using one way ANOVA ($N = 30$).

Shrimp exhibiting SSCP pattern I of *PmCnn1*$_{530}$ possessed composite SNP diplotypes of $G/G_{209}T/T_{210}$-/-$_{212}$-/-$_{213}C/C_{218}G/G_{240}$ while those with pattern III possessed alternative homozygotic states; $A/A_{209}A/A_{210}G/G_{212}T/T_{213}T/T_{218}A/A_{240}$. Shrimp having SSCP pattern II possessed heterozygtic states of diplotype $(G/A)_{209}(T/A)_{210}(-/G)_{212}(-/T)_{213}(C/T)_{218}(G/A)_{240}$ at these loci.

The results from analysis of relationships between genotypes of each SNP of *PmCnn1*$_{530}$ and growth parameter indicate that shrimp with G/G_{209} and $(G/A)_{209}$, T/T_{210} and $(T/A)_{210}$, -/-$_{212}$ and $(-/G)_{212}$, -/-$_{213}$ and $(-/T)_{213}$, C/C_{218} and $(C/T)_{218}$ and G/G_{240} and $(G/A)_{240}$ had greater average BW, TL and HPW than those with A/A_{209}, A/A_{210}, G/G_{212}, T/T_{213}, T/T_{218} and A/A_{240} ($P < 0.05$). No SNP exhibited a significant relationship with HSI of examined shrimp (Table 3.12). Results were consistent when the data was inferred for 156 individuals of the SNP3A sample ($P< 0.05$, Table 3.13).

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calponin1-III_202        GGACTTGTATGTTTCGGTTCAAAAGGCTAATGTTTTTTTATTGACCTTTCTTTGCACTGA  240
calponin1-III_126        GGACTTGTATGTTTCGGTTCAAAAGGCTAATGTTTTTTTATTGACCTTTCCTTGCACTGA  240
calponin1-III_008        GGACTTGTATGTTTCGGTTCAAAAGGCTAATGTTTTTTTATTGACCTTTCTTTGCACTGA  240
calponin1-III_42         GGACTTGTATGTTTCGGTTCAAAAGGCTAATGTTTTTTTATTGACCTTTCTTTGCACTGA  240
calponin1-III_082        GGACTTGTATGTTTCGGTTCAAAAGGCTAATGTTTTTTTATTGACCTTTCTTTGCACTGA  240
calponin1-III_091        GGACTTGTATGTTTCGGTTCAAAAGGCTAATGTTTTTTTATTGACCTTTCTTTGCACTGA  240
calponin1-III_100        GGACTTGTATGTTCCGGTTCAAAAGGCTAATGTTTTTTTATTGACCTTTCTTTGCACTGA  240
calponin1-III_131        GGACTTGTATGTTTCGGTTCAAAAGGCTAATGTTTTTTTATTGACCTTTCTTTGCACTGA  240
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calponin1-I_018       TATCTAGTAATGTGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
calponin1-I_163       TATCTAGTAATGTGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
calponin1-I_207       TATCTAGTAATGCGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAGTTAGAGGGGGTTC  300
calponin1-I_040       TATCTAGTAATGTGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
calponin1-I_172       TATCTAGTAATGTGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
calponin1-I_150       TATCTAGTAATGTGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
calponin1-I_25        TATCTAGTAATGTGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
calponin1-I_12        TATCTAGTAATGTGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
calponin1-I_138       TATCTAGTAATGTGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
calponin1-I_320       TATCTAGTAATGTGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
calponin1-II_021      TATCTAGTAATGTGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
calponin1-II_2        TATCTAGTAATGTGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
calponin1-II_009      TATCTAGTAATGTGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
calponin1-II_160      TATCTAGTAATGTGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
calponin1-II_201      TATCTAGTAATGTGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
calponin1-II_220      TATCTAGTAATGTGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
calponin1-II_211      TATCTAGTAATGTGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
calponin1-II_127      TATCTAGTAATGTGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
calponin1-II_046      TATCTAGTAATGTGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
calponin1-II_153      TATCTAGTAATGTGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
calponin1-III_26      TATCTAGTAATGTGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
calponin1-III_152     TATCTAGTAATGTGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
calponin1-III_202     TATCTAGTAATGTGTTTGTTTTTAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
calponin1-III_126     TATCTAGTAATGTGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
calponin1-III_008     TATCTAGTAATGTGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
calponin1-III_42      TATCTAGTAATGTGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
calponin1-III_082     TATCTAGTAATGTGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
calponin1-III_091     TATCTAGTAATGTGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
calponin1-III_100     TATCTAGTAATGTGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
calponin1-III_131     TATCTAGTAATGTGTTTGTTTTAAGTCTTCGTCGTCATCATCAGTAATTAGAGGGGGTTC  300
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calponin1-I_018       TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACCCCTCAAGCCCGGGTCAGATCAAG  360
calponin1-I_163       TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGTCAGATCAAG  360
calponin1-I_207       TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGTCAGATCAAG  360
calponin1-I_040       TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGTCAGATCAAG  360
calponin1-I_172       TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGTCAGATCAAG  360
calponin1-I_150       TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGTCAGATCAAG  360
calponin1-I_25        TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGTCAGATCAAG  360
calponin1-I_12        TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGTCAGATCAAG  360
calponin1-I_138       TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGTCGGATCAAG  360
calponin1-I_320       TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGTCAGATCAAG  360
calponin1-II_021      TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGTCAGATCAAG  360
calponin1-II_2        TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGTCAGATCAAG  360
calponin1-II_009      TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGTCAGATCAAG  360
calponin1-II_160      TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGTCAGATCAAG  360
calponin1-II_201      TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGTCAGATCAAG  360
calponin1-II_220      TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGTCAGATCAAG  360
calponin1-II_211      TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGTCAGATCAAG  360
calponin1-II_127      TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGTCAGATCAAG  360
calponin1-II_046      TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGCCAGATCAAG  360
calponin1-II_153      TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGTCAGATCAAG  360
calponin1-III_26      TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGTCAGATCAAG  360
calponin1-III_152     TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGTCAGATCAAG  360
calponin1-III_202     TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGTCAGATCAAG  360
calponin1-III_126     TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGTCAGATCAAG  360
calponin1-III_008     TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGTCAGATCAAG  360
calponin1-III_42      TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGTCAGATCGAG  360
calponin1-III_082     TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGTCAGATCAAG  360
calponin1-III_091     TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGTCAGATCAAG  360
calponin1-III_100     TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGTCAGATCAAG  360
calponin1-III_131     TCATTCATTTACTTTGTTCCCAACAGGGTGATTAACGCCCTCAAGCCCGGTCAGATCAAG  360
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calponin1-I_018         AAGATCCAGACCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGAG  420
calponin1-I_163         AAGATCCAGACCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGAG  420
calponin1-I_207         AAGATCCAGACCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGAG  420
calponin1-I_040         AAGATCCAGACCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGAG  420
calponin1-I_172         AAGATCCAGACCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGAG  420
calponin1-I_150         AAGATCCAGACCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGAG  420
calponin1-I_25          AAGATCCAGACCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGAG  420
calponin1-I_12          AAGATCCAGACCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGAG  420
calponin1-I_138         AAGATCCAGACTTCCGCCATGGCATTCAAGTGCATGGAAAGCATCAACGCCTTTGTGGAG  420
calponin1-I_320         AAGATCCAGACCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGAG  420
calponin1-II_021        AAGATCCAGACCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGAG  420
calponin1-II_2          AAGATCCAGACCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGAG  420
calponin1-II_009        AAGATCCAGACCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGAG  420
calponin1-II_160        AAGATCCAGACCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGAG  420
calponin1-II_201        AAGATCCAGACCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGAG  420
calponin1-II_220        AAGATCCAGACCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGAG  420
calponin1-II_211        AAGATCCAGACCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGAG  420
calponin1-II_127        AAGATCCAGACCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGAG  420
calponin1-II_046        AAGATCCAGACCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGAG  420
calponin1-II_153        AAGATCCAGACCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGAG  420
calponin1-III_26        AAGATCCAGACCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGAG  420
calponin1-III_152       AAGATCCAGACCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGAG  420
calponin1-III_202       AAGATCCAGACCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGAG  420
calponin1-III_126       AAGATCCAGACCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGAG  420
calponin1-III_008       AAGATCCAGACCTCCGCCATGGCATCCAAGTGCATGGAAAACATCAACGCCTTTGTGGAG  420
calponin1-III_42        AAGATCCAGACCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGAG  420
calponin1-III_082       AAGATCCAGACCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGAG  420
calponin1-III_091       AAGATCCAGACCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGGG  420
calponin1-III_100       AAGGTCCAGGCCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGAG  420
calponin1-III_131       AAGATCCAGACCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGAG  420
                        ***.*****.* ************ ************.*****************.*

calponin1-I_018         GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAA  480
calponin1-I_163         GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAA  480
calponin1-I_207         GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAA  480
calponin1-I_040         GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGAACGTCGACCTCTGGGAA  480
calponin1-I_172         GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAA  480
calponin1-I_150         GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAA  480
calponin1-I_25          GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAA  480
calponin1-I_12          GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAA  480
calponin1-I_138         GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAA  480
calponin1-I_320         GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAA  480
calponin1-II_021        GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAA  480
calponin1-II_2          GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAA  480
calponin1-II_160        GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAA  480
calponin1-II_201        GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAA  480
calponin1-II_220        GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAA  480
calponin1-II_211        GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAA  480
calponin1-II_127        GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAA  480
calponin1-II_046        GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAA  480
calponin1-II_153        GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAA  480
calponin1-III_26        GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAA  480
calponin1-III_152       GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAA  480
calponin1-III_202       GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCCGGGAA  480
calponin1-III_126       GAAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAA  480
calponin1-III_008       GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAA  480
calponin1-III_42        GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAA  480
calponin1-III_009       GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAA  480
calponin1-III_082       GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAA  480
calponin1-III_091       GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAA  480
calponin1-III_100       GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAA  480
calponin1-III_131       GGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAA  480
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calponin1-I_018       CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-I_163       CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-I_207       CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-I_040       CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-I_172       CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-I_150       CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-I_25        CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-I_12        CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-I_138       CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-I_320       CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-II_021      CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-II_2        CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-II_009      CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-II_160      CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-II_201      CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-II_220      CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-II_211      CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-II_127      CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-II_046      CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-II_153      CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-III_26      CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-III_152     CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-III_202     CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-III_126     CGGCAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-III_008     CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-III_42      CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-III_082     CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-III_091     CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-III_100     CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
calponin1-III_131     CGACAGAACCTTAACTCTGTTGTTATCTGCTTGC------------------  530
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Figure 3.11 Multiple sequence alignments of the *PmCnn1*$_{530}$ gene segment amplified from genomic DNA of representative individuals of 3-month-old juveniles (SNP3A) exhibiting SSCP patterns I, II and III, respectively. Dashes referred to the primer sequences. SNPs are highlight. Intronic region is italicized.

Table 3.12 Relationships between SNPs of the *PmCnn1*$_{530}$ gene segment and growth parameters of the SNP3A sample considering for specimens that were sequenced (N = 30)

SSCP pattern	N	SNP position						Growth parameters			
		209	210	212	213	218	240	BW (g)	TL (cm)	HP (g)	HSI (%)
I	10	G/G	T/T	-/-	-/-	C/C	G/G	15.41±4.9[a]	12.24±1.38[a]	0.50±0.18[a]	3.29±0.48[a]
II	10	G/A	T/A	-/G	-/T	C/T	G/A	15.39±4.4[a]	12.30±1.38[a]	0.54±0.17[a]	3.52±0.49[a]
III	10	A/A	A/A	G/G	T/T	T/T	A/A	9.72±4.99[b]	10.41±1.88[b]	0.33±0.18[b]	3.26±0.47[a]

The same letters indicate that the expression levels were not significantly different ($P > 0.05$).
BW = average body weight, TL = average total length, HP = average hepatopancreatic weight, HSI = average hepatosomatic index (%)

Table 3.13 Relationships between SNPs of the *PmCnn1*$_{530}$ gene segment and growth parameters of the SNP3A sample considering for specimens inferred for overall specimens examined by SSCP (N = 156)

SSCP pattern	N	SNP position						Growth parameters			
		209	210	212	213	218	240	BW (g)	TL (cm)	HP (g)	HSI (%)
I	51	G/G	T/T	-/-	-/-	C/C	G/G	13.81±6.31[a]	11.67±1.87[a]	0.44±0.20[a]	3.31±0.73[a]
II	81	G/A	T/A	-/G	-/T	C/T	G/A	13.01±6.00[a]	11.42±1.77[a]	0.43±0.19[a]	3.34±0.59[a]
III	24	A/A	A/A	G/G	T/T	T/T	A/A	9.99±4.53[b]	10.55±1.66[b]	0.34±0.18[a]	3.34±0.83[a]

The same letters indicate that the expression levels were not significantly different ($P > 0.05$).
BW = average body weight, TL = average total length, HP = average hepatopancreatic weight, HSI = average hepatosomatic index (%)

3.4.1.2 *PmCnn1*$_{425}$ generated from primers Cnn1-F3/R3

The amplified *PmCnn1*$_{425}$ gene fragment was also sequenced. The PCR product of five individuals representing each SSCP patterns was cloned and sequenced (N = 25). A total of 6 SNPs located in the intron were found (Figure 3.12). Composite SNPs were generated from these SNP positions and can be categorized into 3 SNP $D1_{425}$-/-$_{291}$-/-$_{292}$-/-$_{293}$A/A$_{294}$T/T$_{298}$-/-$_{315}$; $D2_{425}$ G/G$_{291}$T/T$_{292}$G/G$_{293}$C/C$_{294}$G/G$_{298}$G/G$_{315}$ and $D3_{425}$(-/G)$_{291}$(-/T)$_{292}$(-/G)$_{293}$(A/C)$_{294}$ (T/G)$_{298}$ (-/G)$_{315}$. These diplotypes correspond to shrimp exhibiting SSCP patterns I+V, II+IV and III, respectively.

On the basis of sequenced individuals, results from statistical analysis did not indicate that examined shrimp carrying different SNP genotypes exhibit different growth parameters (body weight, total length, hepatopancreatic weight and HSI, N = 25, $P > 0.05$, Table 3.14).

When the data was inferred covering overall individuals previously analyzed by SSCP (N = 151). Juvenile shrimp exhibiting -/-$_{291}$, -/-$_{292}$, -/-$_{293}$, A/A$_{294}$, T/T$_{298}$, and -/-$_{315}$ showed a greater average BW and HP than those carrying (-/G)$_{291}$, (-/T)$_{292}$, (-/G)$_{293}$, (A/C)$_{294}$, (T/G)$_{298}$ and (-/G)$_{315}$ ($P < 0.05$). Moreover, those with -/-$_{291}$, -/-$_{292}$, -/-$_{293}$, A/A$_{294}$, T/T$_{298}$, or -/-$_{315}$ showed a greater average TL than those carrying G/G$_{291}$ or (-/G)$_{291}$, T/T$_{292}$ or (-/T)$_{292}$, G/G$_{293}$ or (-/G)$_{293}$, C/C$_{294}$ or (A/C)$_{294}$, G/G$_{298}$ or (T/G)$_{298}$ and G/G$_{315}$ or (-/G)$_{315}$ ($P < 0.05$). Considering relationships between diplotypes and growth parameters, those having diplotype $D1_{425}$ possessed a greater average BW and HPW than those having $D3_{425}$ and an average TL than those having $D2_{425}$ ($P < 0.05$, Table 3.15).

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calponin1F3R3_I_150       --------------------TGTAGCATTACTTGCTACCATTTGTAATTGTAGTCTGTAT  60
calponin1F3R3_I_104       --------------------TGTAGCATTACTTGCTACCATTTGTAATTGTAGTCTGTAT  60
calponin1F3R3_I_207       --------------------TGTAGCATTACTTGCTACCATTTGTAATTGTAGTCTGTAT  60
calponin1F3R3_I_102       --------------------TGTAGCATTACTTGCTACCATTTGTAATTGTAGTCTGTAT  60
calponin1F3R3_I_134       --------------------TGTAGCATTACTTGCTACCATTTGTAATTGTAGTCTGTAT  60
calponin1F3R3_II_082      --------------------TGTAGCATTACTTGCTACCATTTGTAATTGTAGTCTGTAT  60
calponin1F3R3_II_113      --------------------TGTAGCATTACTTGCTACCATTTGTAATTGTAGTCTGTAT  60
calponin1F3R3_II_131      --------------------TGTAGCATTACTTGCTACCATTTGTAATTGTAGTCTGTAT  60
calponin1F3R3_II_124      --------------------TGTAGCATTACTTGCTACCATTTGTAATTGTAGTCTGTAT  60
calponin1F3R3_II_005      --------------------TGTAGCATTACTTGCTACCATTTGTAATTGTAGTCTGTAT  60
calponin1F3R3_III_008     --------------------TGTAGCATTACTTGCTACCATTTGTAATTGTAGTCTGTAT  60
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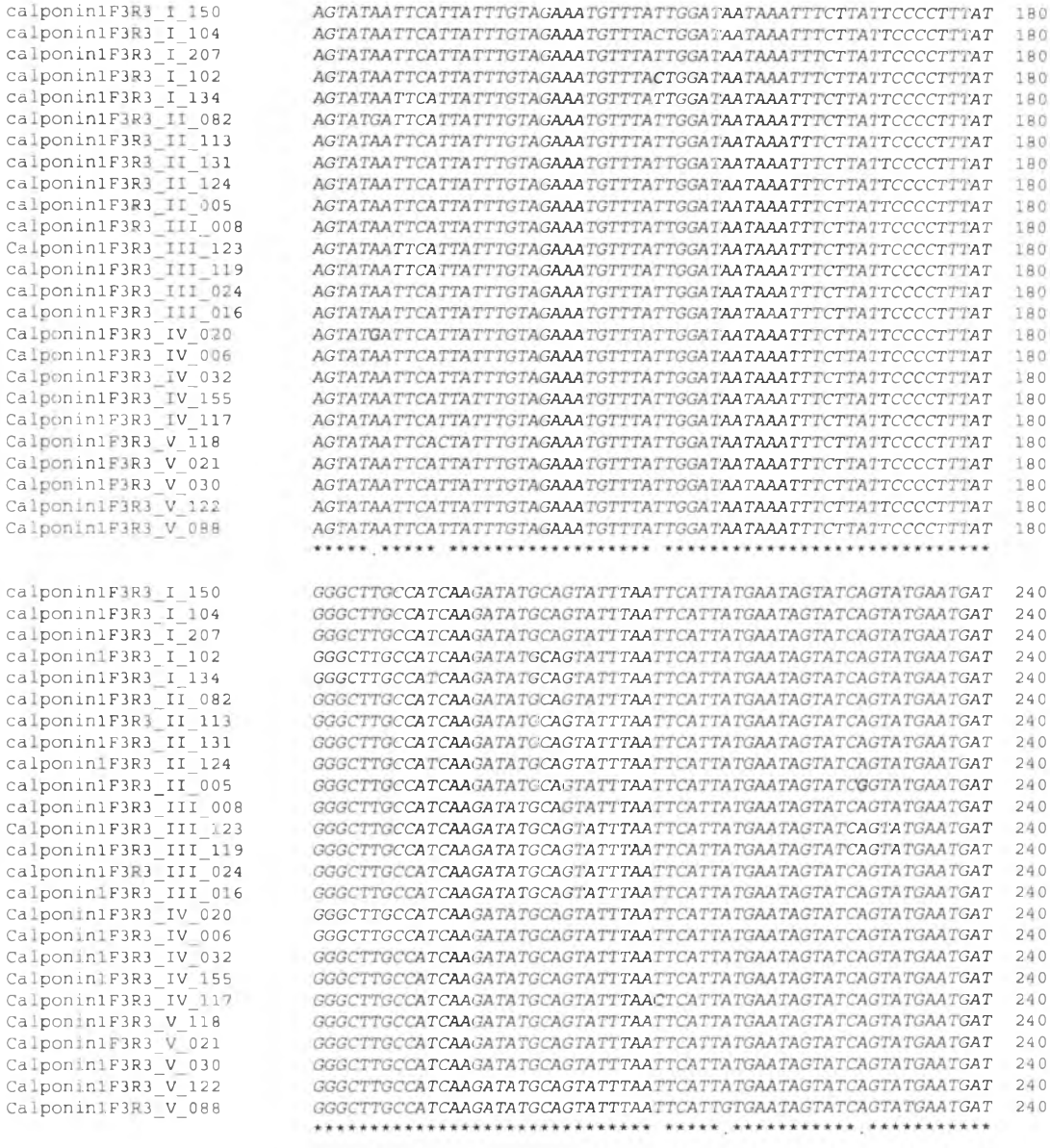
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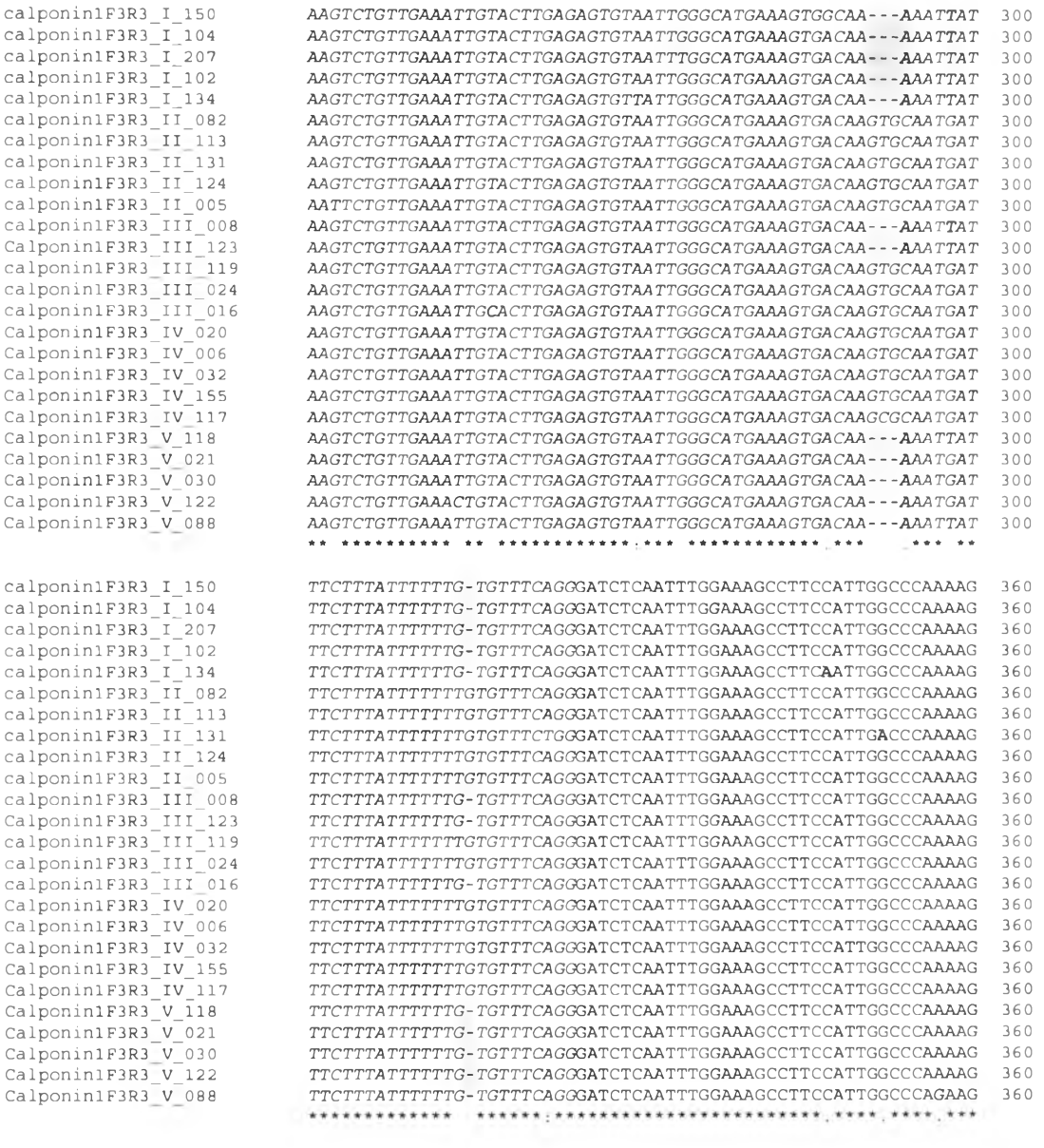
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Figure 3.12 Multiple sequence alignments of the *PmCnn1*$_{425}$ gene segment (primers Cnn1-F3/R3) amplified from genomic DNA of representative individuals of 3-month-old juveniles (SNP3A, N = 5 for each SSCP pattern) exhibiting SSCP patterns I, II, III, IV and V, respectively. Dashes referred to the primer sequences. SNPs are highlighted. An intronic region is italicized.

Table 3.14 Relationships between SNPs of the *PmCnn1*$_{425}$ gene segment and growth parameters of the SNP3A sample considering for specimens that were sequenced (N = 25)

SSCP pattern	N	SNP position						Growth parameters			
		291	292	293	294	298	315	BW (g)	TL (cm)	HPW (g)	HSI (%)
I+V	10	-/-	-/-	-/-	AA	T/T	-/-	13.56±6.05[a]	11.66±2.06[a]	0.43±0.20[a]	3.16±0.47[a]
II+IV	10	G/G	T/T	G/G	C/C	G/G	G/G	10.48±6.40[a]	10.53±2.19[a]	0.35±0.23[a]	3.18±0.43[a]
III	5	-/G	-/T	-/G	A/C	T/G	-/G	7.52±2.84[a]	9.66±1.39[a]	0.26±0.17[a]	3.30±0.58[a]

The same letters indicate that the expression levels were not significantly different (P > 0.05).
BW = average body weight, TL = average total length, HP = average hepatopancreatic weight, HSI = average hepatosomatic index (%)

Table 3.15 Relationships between SNPs of the *PmCnn1*$_{425}$ gene segment and growth parameters of the SNP3A sample inferred for 151 individuals

SSCP pattern	N	SNP position						Growth parameters			
		291	292	293	294	298	315	BW (g)	TL (cm)	HPW (g)	HSI (%)
I+V	51	-/-	-/-	-/-	AA	T/T	-/-	14.42±6.01[a]	11.90±1.76[a]	0.46±0.19[a]	3.28±0.65[a]
II+IV	58	G/G	T/T	G/G	C/C	G/G	G/G	12.10±6.10[ab]	11.17±1.86[b]	0.40±0.20[ab]	3.39±0.70[a]
III	42	-/G	-/T	-/G	A/C	T/G	-/G	11.29±5.50[b]	10.93±1.70[b]	0.36±0.17[b]	3.28±0.60[a]

The same letters indicate that the expression levels were not significantly different (P > 0.05).
BW = average body weight, TL = average total length, HP = average hepatopancreatic weight, HSI = average hepatosomatic index (%)

3.4.2 SNP in the *PmCyC* gene segment

The *PmCyC* gene segment was 403 bp in length covering the intron sequence of 123 bp. Multiple sequence alignment of the amplified gene segment of 10 individuals of each SSCP patterns revealed 5 substitutions. Of these, three SNP including A/G_{31}, G/A_{379}, and T/C_{382} were located in the exon region, which caused synonymous mutation. Two SNP including T/C_{134} and T/C_{188} were located in the intron region (Figure 3.13). All SNP except position 134 can distinguish different genotypes of *PmCyC*. (corresponding to SSCP genotypes I, II and III, respectively).

Five SNPs revealed that genotype I possessed homozygotic states with a string of SNP of $A/A_{31}C/C_{134}T/T_{188}G/G_{379}T/T_{382}$, genotype II possessed alternative homozygotic states; $G/G_{31}(C/T)_{134}C/C_{188}A/A_{379}C/C_{382}$ and genotype III possessed heterozygtic states; $(A/G)_{31}$ $(C/T)_{134}(T/C)_{188}(G/A)_{379}(T/C)_{382}$ at these loci.

Statistical analysis indicated that results from both examined shrimp (N = 30) and inferred for 145 individuals shrimp were significantly related with growth parameters (except HSI) of shrimp ($P < 0.05$, Table 3.16 and 3.17). Where individuals with each of 4 SNPs of genotype II except position C/T_{134} had significantly faster growth rate than those with each of the 4 SNPs of genotype I and III.

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cyclinc_I_038      --------------------TCGACAGTCAAGACTTGATACAAGAGCGCCAGGCTGACCT  60
cyclinc_I_122      --------------------TCGACAGTCAAGACTTGATACAAGAGCGCCAGGCTGACCT  60
cyclinc_I_025      --------------------TCGACAGTCAAGACTTGATACAAGAGCGCCAGGCTGACCT  60
cyclinc_I_235      --------------------TCGACAGTCAAGACTTGATACAAGAGCGCCGGGCTGACCT  60
cyclinc_I_030      --------------------TCGACAGTCAAGACTTGATACAAGAGCGCCAGGCTGACCT  60
cyclinc_I_113      --------------------TCGACAGTCAAGACTTGATACAAGAGCGCCAGGCTGACCT  60
cyclinc_II_116     --------------------TCGACAGTCAGGACTTGATACAAGAGCGCCAGGCTGACCT  60
cyclinc_II_097     --------------------TCGACAGTCAGGACTTGATACAAGAGCGCCAGGCTGACCT  60
cyclinc_II_012     --------------------TCGACAGTCAGGACTTGATACAAGAGCGCCAGGCTGACCT  60
cyclinc_II_203     --------------------TCGACAGTCAGGACTTGATACAAGAGCGCCAGGCTGACCT  60
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cyclinc_II_161     --------------------TCGACAGTCAGGACTTGATACAAGAGCGCCAGGCTGACCT  60
cyclinc_II_227     --------------------TCGACAGTCAGGACTTGATACAAGAGCGCCAGGCTGACCT  60
cyclinc_III_096    --------------------TCGACAGTCAAGACTTGATACAAGAGCGCCAGGCTGACCT  60
cyclinc_III_117    --------------------TCGACAGTCAGGACTTGATACAAGAGCGCCAGGCTGACCT  60
cyclinc_III_129    --------------------TCGACAGTCAGGACTTGATACAAGAGCGCCAGGCTGACCT  60
cyclinc_III_032    --------------------TCGACAGTCAGGACTTGATACAAGAGCGCCAGGCTGACCT  60
cyclinc_III_088    --------------------TCGACAGTCAGGACTTGATACAAGAGCGCCAGGCTGACCT  60
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cyclinc_III_005    --------------------TCGACAGTCAGGACTTGATACAAGAGCGCCAGGCTGACCT  60
cyclinc_III_027    --------------------TCGACAGTCAGGACTTGATACAAGAGCGCCAGGCTGACCT  60
cyclinc_III_041    --------------------TCGACAGTCAGGACTTGATACAAGAGCGCCAGGCTGACCT  60
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cyclinc_I_235       TCAGCAACTTGGTGAATCACTCAAGCTTAAACAACAGGTCATCGCAACTGCCACATGCTT  300
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cyclinc_III_032     TCAGCAACTTGGTGAATCACTCAAGCTTAAACAACAGGTCATCGCAACTGCCACATGCTT  300
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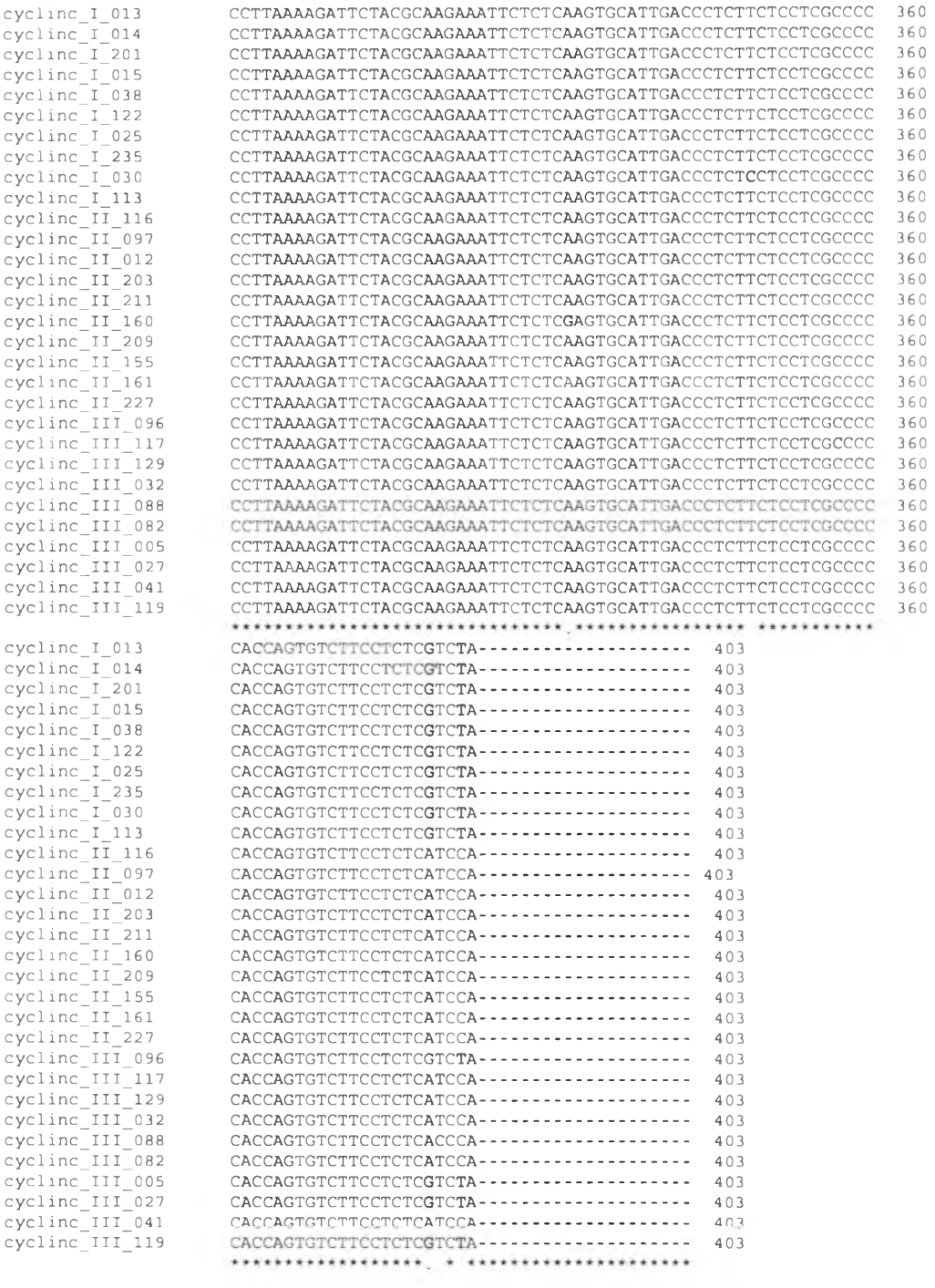

Figure 3.13 Multiple sequence alignments of the *PmCyC* gene segment amplified from genomic DNA of representative individuals of 3-month-old juveniles (SNP3A, N = 10 for each SSCP pattern) exhibiting SSCP patterns I, II and III, respectively. Dashes referred to the primer sequences. SNPs are highlighted. Intron is illustrated in italics.

Table 3.16 Relationships between SNPs of the *PmCyC* gene segment and growth parameters of the SNP3A sample considering for specimens that were sequenced ($N = 30$)

SSCP pattern	N	SNP position					Growth parameters			
		31**	134*	188*	379**	382**	BW (g)	TL (cm)	HP (g)	HSI (%)
I	10	A/A	C/C	T/T	G/G	T/T	11.96±3.85^{b}	11.29±1.39^{b}	0.41±0.16^{b}	3.40±0.37^{a}
II	10	G/G	C/T	C/C	A/A	C/C	19.60±6.06^{a}	13.20±1.64^{a}	0.59±0.19^{a}	3.10±0.66^{a}
III	10	G/A	C/T	C/T	G/A	T/C	9.00±3.65^{b}	10.26±1.43^{b}	0.29±0.13^{b}	3.16±0.40^{a}

**= exon, *= intron. The same letters indicate that the expression levels were not significantly different ($P > 0.05$).
BW = average body weight, TL = average total length, HP = average hepatopancreatic weight, HSI = average hepatosomatic index (%).

Table 3.17 Relationships between SNPs of the *PmCyC* gene segment and growth parameters of the SNP3A sample inferred for overall specimens ($N = 145$)

SSCP pattern	N	SNP position					Growth parameters			
		31**	134*	188*	379**	382**	BW (g)	TL (cm)	HP (g)	HSI (%)
I	10	A/A	C/C	T/T	G/G	T/T	9.78±4.00^{b}	10.55±1.40^{b}	0.35±0.16^{b}	3.58±0.68^{a}
II	10	G/G	C/T	C/C	A/A	C/C	14.39±6.09^{a}	11.89±1.80^{a}	0.46±0.18^{a}	3.34±0.73^{a}
III	10	G/A	C/T	C/T	G/A	T/C	11.31±4.74^{b}	11.03±1.58^{ab}	0.38±0.18^{b}	3.32±0.63^{a}

The same letters indicate that the expression levels were not significantly different ($P > 0.05$).
BW = average body weight, TL = average total length, HP = average hepatopancreatic weight, HSI = average hepatosomatic index (%). **= exon, *= intron

3.4.3 SNP in the *PmCdc25* gene segment

The PCR product (285 bp) of ten individuals representing each SSCP pattern of *PmCdc25* were amplified, cloned ad sequenced. Only one SNP located in the exonic region resulted in a synonymous mutation was found (N = 20) similar as the genotypic patterns found from SSCP analysis (Figure 3.14).

Analysis between relationships of SNP within *PmCdc25* (position 243) and growth parameters was carried out using independent-sample t-test. Results showed that *P. monodon* juveniles (SNP3A) with SNP genotype A/C_{243} had significantly greater average BW, TL and HPW ($P < 0.05$) but not HSI (Table 3.18; $P > 0.05$) than those carrying C/C_{243}. Results were similar when the data was inferred for 144 individuals previously analyzed by PCR-SSCP (Table 3.19; $P > 0.05$).

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cdc25_II_120     ----------GCCCTGCACTTCCCGGAGACGTACCTGCTGGAGGGCGGC  60
cdc25_II_083     ----------GCCCTGCACTTCCCGGAGACGTACCTGCTGGAGGGCGGC  60
cdc25_II_049     ----------GCCCTGCACTTCCCGGAGACGTACCTGCTGGAGGGCGGC  60
cdc25_II_062     ----------GCCCTGCACTTCCCGGAGACGTACCTGCTGGAGGGCGGC  60
cdc25_II_005     ----------GCCCTGCACTTCCCGGAGACGTACCTGCTGGAGGGCGGC  60
                 **********************************************************

cdc25_I_002      TACAAGGCCTTCTTCGAGGCGTACCCCGACCTGTGCACGCCCCACGAGTACGTGCGGATG  120
cdc25_I_135      TACAAGGCCTTCTTCGAGGCGTACCCCGACCTGTGCACGCCCCACGAGTACGTGCGGATG  120
cdc25_I_217      TACAAGGCCTTCTTCGAGGCGTACCCCGACCTGTGCACGCCCCACGAGTACGTGCGGATG  120
cdc25_I_207      TACAAGGCCTTCTTCGAGGCGTACCCCGACCTGTGCACGCCCCACGAGTACGTGCGGATG  120
cdc25_I_023      TACAAGGCCTTCTTCGAGGCGTACCCCGACCTGTGCACGCCCCACGAGTACGTGCGGATG  120
cdc25_I_161      TACAAGGCCTTCTTCGAGGCGTACCCCGACCTGTGCACGCCCCACGAGTACGTGCGGATG  120
cdc25_I_187      TACAAGGCCTTCTTCGAGGCGTACCCCGACCTGTGCACGCCCCACGAGTACGTGCGGATG  120
cdc25_I_012      TACAAGGCCTTCTTCGAGGCGTACCCCGACCTGTGCACGCCCCACGAGTACGTGCGGATG  120
cdc25_I_209      TACAAGGCCTTCTTCGAGGCGTACCCCGACCTGTGCACGCCCCACGAGTACGTGCGGATG  120
cdc25_I_155      TACAAGGCCTTCTTCGAGGCGTACCCCGACCTGTGCACGCCCCACGAGTACGTGCGGATG  120
cdc25_II_017     TACAAGGCCTTCTTCGAGGCGTACCCCGACCTGTGCACGCCCCACGAGTACGTGCGGATG  120
cdc25_II_034     TACAAGGCCTTCTTCGAGGCGTACCCCGACCTGTGCACGCCCCACGAGTACGTGCGGATG  120
cdc25_II_082     TACAAGGCCTTCTTCGAGGCGTACCCCGACCTGTGCACGCCCCACGAGTACGTGCGGATG  120
cdc25_II_009     TACAAGGCCTTCTTCGAGGCGTACCCCGACCTGTGCACGCCCCACGAGTACGTGCGGATG  120
cdc25_II_024     TACAAGGCCTTCTTCGAGGCGTACCCCGACCTGTGCACGCCCCACGAGTACGTGCGGATG  120
cdc25_II_120     TACAAGGCCTTCTTCGAGGCGTACCCCGACCTGTGCACGCCCCACGAGTACGTGCGGATG  120
cdc25_II_083     TACAAGGCCTTCTTCGAGGCGTACCCCGACCTGTGCACGCCCCACGAGTACGTGCGGATG  120
cdc25_II_049     TACAAGGCCTTCCTCGAGGCGTACCCCGACCTGTGCACGCCCCACGAGTACGTGCGGATG  120
cdc25_II_062     TACAAGGCCTTCTTCGAGGCGTACCCCGACCTGTGCACGCCCCACGAGTACGTGCGGATG  120
cdc25_II_005     TACAAGGCCTTCTTCGAGGCGTACCCCGACCTGTGCACGCCCCACGAGTACGTGCGGATG  120
                 ************ ********************************************
```

```
cdc25_I_002       CTGGACGCGAACTTCGCCGAGGAGCTGAAGATTCACCGCGGCAAGTCGAAGTCGTGGGCG  180
cdc25_I_135       CTGGACGCGAACTTCGCCGAGGAGCTGAAGATTCACCGCGGCAAGTCGAAGTCGTGGGCG  180
cdc25_I_217       CTGGACGCGAACTTCGCCGAGGAGCTGAAGATTCACCGCGGCAAGTCGAAGTCGTGGGCG  180
cdc25_I_207       CTGGACGCGAACTTCGCCGAGGAGCTGAAGATTCACCGCGGCAAGTCGAAGTCGTGGGCG  180
cdc25_I_023       CTGGACGCGAACTTCGCCGAGGAGCTGAAGATTCACCGCGGCAAGTCGAAGTCGTGGGCG  180
cdc25_I_161       CTGGACGCGAACTTCGCCGAGGAGCTGAAGATTCACCGCGGCAAGTCGAAGTCGTGGGCG  180
cdc25_I_187       CTGGACGCGAACTTCGCCGAGGAGCTGATGATTCACCGCGGCAAGTCGAAGTCGTGGGCG  180
cdc25_I_012       CTGGACGCGAACTTCGCCGAGGAGCTGAAGATTCACCGCGGCAAGTCGAAGTCGTGGGCG  180
cdc25_I_209       CTGGACGCGAACTTCGCCGAGGAGCTGAAGATTCACCGCGGCAAGTCGAAGTCGTGGGCG  180
cdc25_I_155       CTGGACGCGAACTTCGCCGAGGAGCTGAAGATTCACCGCGGCAAGTCGAAGTCGTGGGCG  180
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cdc25_II_034      CTGGACGCGAACTTCGCCGAGGAGCTGAAGATTCACCGCGGCAAGTCGAAGTCGTGGGCG  180
cdc25_II_082      CTGGACGCGAACTTCGCCGAGGAGCTGAAGATTCACCGCGGCAAGTCGAAGTCGTGGGCG  180
cdc25_II_009      CTGGACGCGAACTTCGCCGAGGAGCTGAAGATTCACCGCGGCAAGTCGAAGTCGTGGGCG  180
cdc25_II_024      CTGGACGCGAACTTCGCCGAGGAGCTGAAGATTCACCGCGGCAAGTCGAAGTCGTGGGCG  180
cdc25_II_120      CTGGACGCGAACTTCGCCGAGGAGCTGAAGATTCACCGCGGCAAGTCGAAGTCGTGGGCG  180
cdc25_II_083      CTGGACGCGAACTTCGCCGAGGAGCTGAAGATTCACCGCGGCAAGTCGAAGTCGTGGGCG  180
cdc25_II_049      CTGGACGCGAACTTCGCCGAGGAGCTGAAGATTCACCGCGGCAAGTCGAAGTCGTGGGCG  180
cdc25_II_062      CTGGACGCGAACTTCGCCGAGGAGCTGAAGATTCACCGCGGCAAGTCGAAGTCGTGGGCG  180
cdc25_II_005      CTGGACGCGAACTTCGCCGGGGAGCTGAAGATTCACCGCGGCAAGTCGAAGTCGTGGGCG  180
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cdc25_I_002       GCCGAGAACAAGCAGGCCAAGAGCAGAAGCACGCTACCTCGCACGGGACTCAAGAGACTG  240
cdc25_I_135       GCCGAGAACAAGCAGGCCAAGAGCAGAAGCACGCTACCTCGCACGGGACTCAAGAGACTG  240
cdc25_I_217       GCCGAGAACAAGCAGGCCAAGAGCAGAAGCACGCTACCTCGCACGGGACTCAAGAGACTG  240
cdc25_I_207       GCCGAGAACAAGCAGGCCAAGAGCAGAAGCACGCTACCTCGCACGGGACTCAAGAGACTG  240
cdc25_I_023       GCCGAGAACAAGCAGGCCAAGAGCAGAAGCACGCTACCTCGCACGGGACTCAAGAGACTG  240
cdc25_I_161       GCCGAGAACAAGCAGGCCAAGAGCAGAAGCACGCTACCTCGCACGGGACTCAAGAGACTG  240
cdc25_I_187       GCCGAGAACAAGCAGGCCAAGAGCAGAAGCACGCTACCTCGCACGGGACTCAAGAGACTG  240
cdc25_I_012       GCCGAGAACAAGCAGGCCAAGAGCAGAAGCACGCTACCTCGCACGGGACTCAAGAGACTG  240
cdc25_I_209       GCCGAGAACAAGCAGGCCAAGAGCAGAAGCACGCTACCTCGCACGGGACTCAAGAGACTG  240
cdc25_I_155       GCCGAGAACAAGCAGGCCAAGAGCGGAAGCACGCTACCTCGCACGGGACTCAAGAGACTG  240
cdc25_II_017      GCCGAGAACAAGCAGGCCAAGAGCAGAAGCACGCTACCTCGCACGGGACTCAAGAGACTG  240
cdc25_II_034      GCCGAGAACAAGCAGGCCAAGAGCAGAAGCACGCTACCTCGCACGGGACTCAAGAGACTG  240
cdc25_II_082      GCCGAGAACAAGCAGGCCAAGAGCAGAAGCACGCTACCTCGCACGGGACTCAAGAGACTG  240
cdc25_II_009      GCCGAGAACAAGCAGGCCAAGAGCAGAAGCACGCTACCTCGCACGGGACTCAAGAGACTG  240
cdc25_II_024      GCCGAGAACAAGCAGGCCAAGAGCAGAAGCACGCTACCTCGCACGGGACTCAAGAGACTG  240
cdc25_II_120      GCCGAGAACAAGCAGGCCAAGAGCAGAAGCACGCTACCTCGCACGGGACTCAAGAGACTG  240
cdc25_II_083      GCCGAGAACAAGCAGGCCAAGAGCAGAAGCACGCTACCTCGCACGGGACTCAAGAGACTG  240
cdc25_II_049      GCCGAGAACAAGCAGGCCAAGAGCAGAAGCACGCTACCTCGCACGGGACTCAAGAGACTG  240
cdc25_II_062      GCCGAGAACAAGCAGGCCAAGAGCAGAAGCACGCTACCTCGCACGGGACTCAAGAGACCG  240
cdc25_II_005      GCCGAGAACAAGCAGGCCAAGAGCAGAAGCACGCTACCTCGCACGGGACTCAAGAGACTG  240
                  ************************************************************

cdc25_I_002       GGATTATGATGACTGCCCTGTCCT----------------------  285
cdc25_I_135       GGATTATGATGACTGCCCTGTCCT----------------------  285
cdc25_I_217       GGATTATGATGACTGCCCTGTCCT----------------------  285
cdc25_I_207       GGATTATGATGACTGCCCTGTCCT----------------------  285
cdc25_I_023       GGCTTATGATGACTGCCCTGTCCT----------------------  285
cdc25_I_161       GGATTATGATGACTGCCCTGTCCT----------------------  285
cdc25_I_187       GGATTATGATGACTGCCCTGTCCT----------------------  285
cdc25_I_012       GGCTTATGACGACTGCCCTGTCCT----------------------  285
cdc25_I_209       GGATTATGATGACTGCCCTGTCCT----------------------  285
cdc25_I_155       GGCTTATGATGACTGCCCTGTCCT----------------------  285
cdc25_II_017      GGCTTATGATGACTGCCCTGTCCT---------------------- 285
cdc25_II_034      GGCTTATGATGACTGCCCTGTCCT----------------------  285
cdc25_II_082      GGCTTATGATGACTGCCCTGTCCT----------------------  285
cdc25_II_009      GGCTTATGATGACTACCCTGTCCT----------------------  285
cdc25_II_024      GGCTTATGATGACTGCCCTGTCCT----------------------  285
cdc25_II_120      GGCTTATGATGACTGCCCTGTCCT----------------------  285
cdc25_II_083      GGCTTATGATGACTGCCCTGTCCT----------------------  285
cdc25_II_049      GGCTTATGATGACTGCCCTGTCCT----------------------  285
cdc25_II_062      GGCTTATGATGACTGCCCTGTCCT----------------------  285
cdc25_II_005      GGCTTATGATGACTGCCCTGTCCT----------------------  285
                  **.****** ****.****************************
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Figure 3.14 Multiple sequence alignments of the *PmCdc25* gene segment amplified from genomic DNA of representative individuals of 3-month-old juveniles (SNP3A, $N = 10$ for each SSCP pattern) exhibiting SSCP patterns I and II, respectively. Dashes refered to the primer sequences. SNPs are highlighted. Intron is illustrated in italics.

Table 3.18 Relationships between SNP of *PmCdc25* gene segment and growth parameters of the SNP3A sample considering for specimens that were sequenced ($N = 20$)

SSCP pattern	*N*	SNP position 243	Growth parameters			
			BW (g)	TL (cm)	HP (g)	HSI (%)
I	10	A/C	20.14±3.53[a]	13.31±0.82[a]	0.62±0.16[a]	3.54±0.42[a]
II	10	C/C	9.33±2.71[b]	10.48±1.16[b]	0.33±0.08[b]	3.12±0.70[a]

The same letters indicate that the expression levels were not significantly different ($P > 0.05$).
BW = average body weight, TL = average total length, HP = average hepatopancreatic weight, HSI = average hepatosomatic index (%)

Table 3.19 Relationships between SNP of *PmCdc25* gene segment and growth parameters of the SNP3A sample inferred for overall specimens ($N = 144$)

SSCP pattern	*N*	SNP position 243	Growth parameters			
			BW (g)	TL (cm)	HP (g)	HSI (%)
I	26	A/C	19.49±5.30[a]	12.77±1.96[a]	0.57±0.18[a]	3.21±0.58[a]
II	177	C/C	11.10±5.23[b]	10.96±1.38[b]	0.37±0.16[b]	3.34±0.62[a]

The same letters indicate that the expression levels were not significantly different ($P > 0.05$).
BW = average body weight, TL = average total length, HP = average hepatopancreatic weight, HSI = average hepatosomatic index (%)

3.5 Development of PCR-RFLP for detection of SNP in *Calponin1 and Cyclin C* of the giant tiger shrimp *Penaeus monodon*

In this study, SNPs in $PmCnn1_{530}$ and *PmCyC* of 3-months old *P. monodon* were screened based on SSCP and DNA sequencing. Three different SSCP patterns of $PmCnn1_{530}$ were found. There are 6 SNPs in the intron region of $PmCnn1_{F/R}$ including G/A_{209}, T/A_{210}, $-/G_{212}$, $-/T_{213}$, C/T_{218} and G/A_{240}. Multiple sequence alignments between shrimp carrying different SSCP patterns of a particular gene suggested that SNP found in *PmCnn1* and *PmCyC* can be simply detected using PCR-RFLP. For $PmCnn1_{530}$, polymorphic SNP, G/A_{240} allows the development of a PCR–RFLP using *Eco* RV. The expected digestion profiles are illustrated by Figure 3.15.

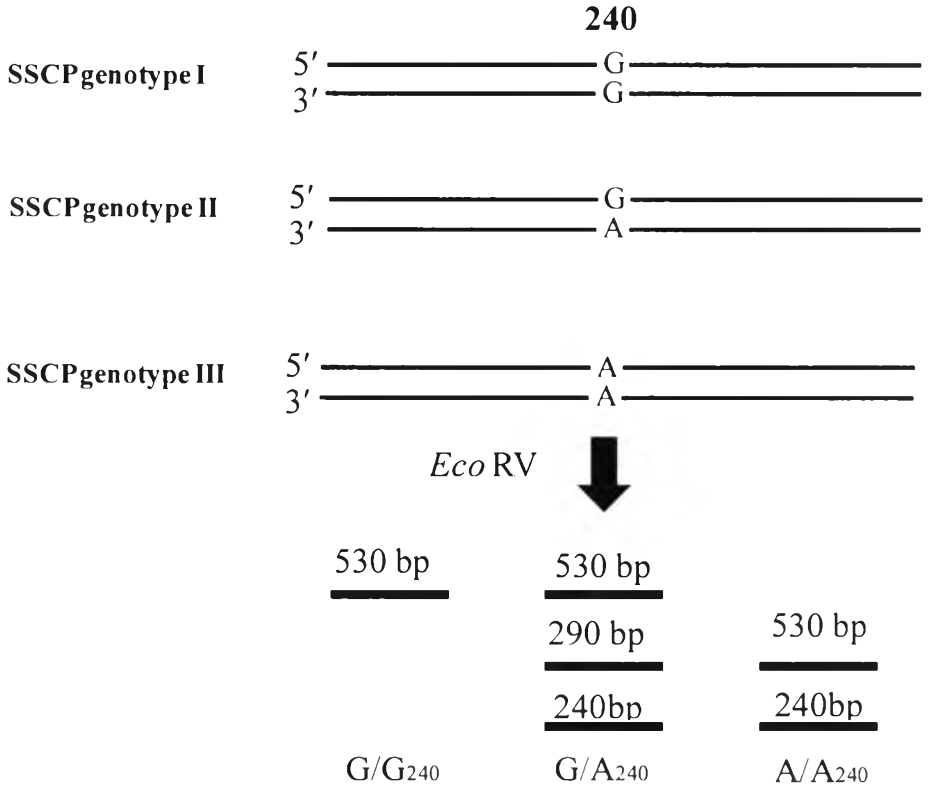

Figure 3.15 Schematic illustration of the expected RFLP profiles of $PmCnn1_{530}$ after digested with *Eco* RV.

PCR-RFLP was then carried out against the amplified *PmCnn*$_{530}$ gene segment of 60 individuals of 3-month-old juveniles (SNP3A) restricted with *Eco* RV. As expected, three restriction patterns were found including a single band of 530 bp (corresponding to GG_{240}) three bands of 530, 290 and 240 bp (corresponding to G/A_{240}) and two bands of 290 and 240 bp (corresponding to A/A_{240}) (Figure 3.16).

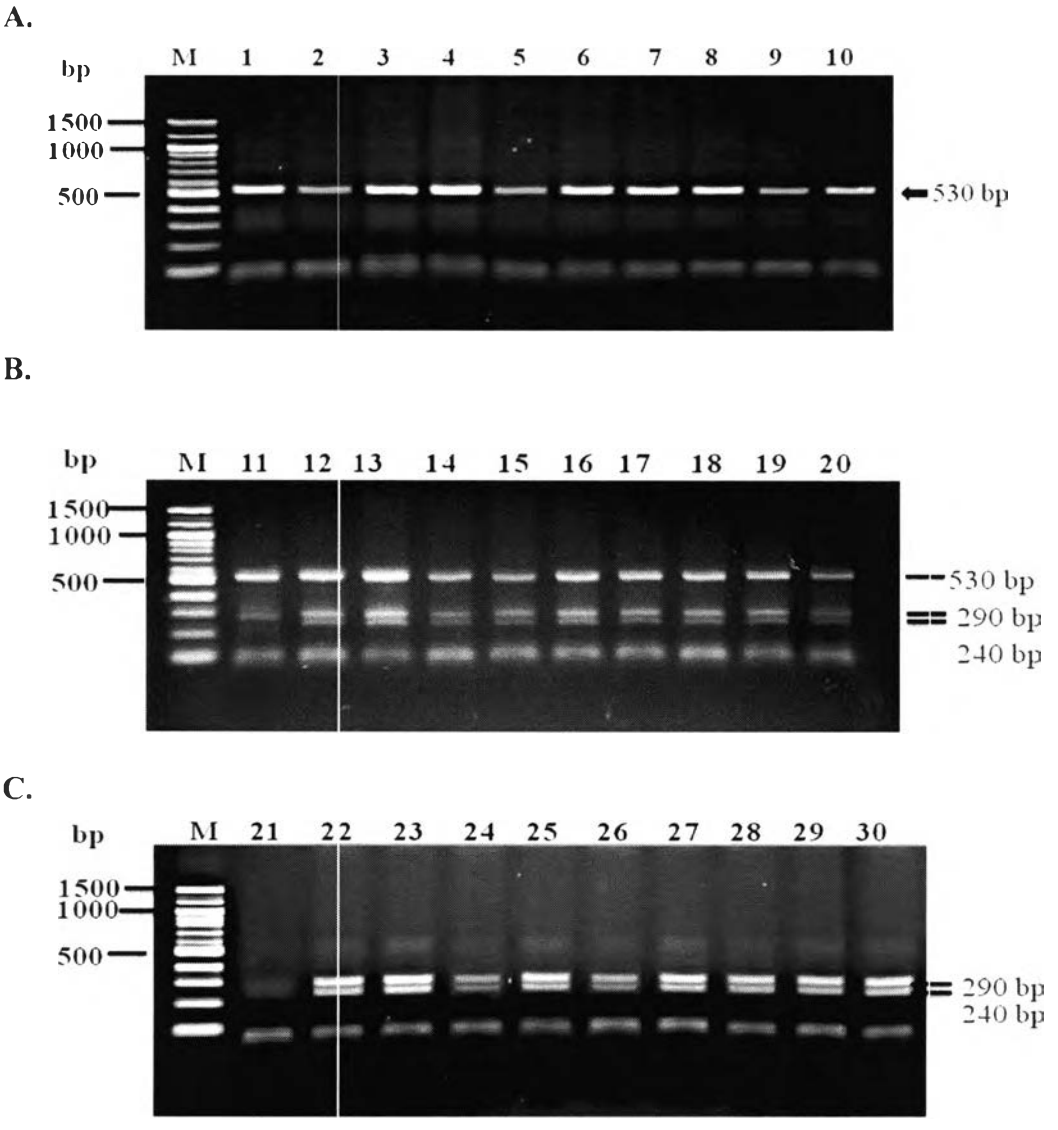

Figure 3.16 PCR-RFLP of the *PmCnn1*$_{530}$ gene segment digested with *Eco* RV. Three digestion patterns are found: A = undigested 530-bp PCR product (SSCP pattern I), B = restriction fragments of 530, 290 and 240 bp (SSCP pattern II), C = restriction fragments of 290 and 240 bp (SSCP pattern III).

For the *PmCyC* gene segment, three different SSCP patterns were found across all examined individuals. Multiple sequence alignments between individuals representing each patterns (N = 30) revealed 5 SNP positions within the examined gene region. Of these, three SNP including A/G_{31}, G/A_{379}, and T/C_{382} were located in the exon region while two SNP including T/C_{134} and T/C_{188} were located in the intron region. Sequence analysis suggested that T/C_{382} can be simply detected by PCR-RFLP using *Dde* I (Figure 3.17). Bioinformatic analysis indicated that an undigested 403 bp band, 2 bands of 381 and 22 bp and 3 bands of 403, 381 and 22 bp should be observed from C/C_{382}, T/T_{382} and C/T_{382} genotypes when digested with *Dde* I (CTNAG), respectively.

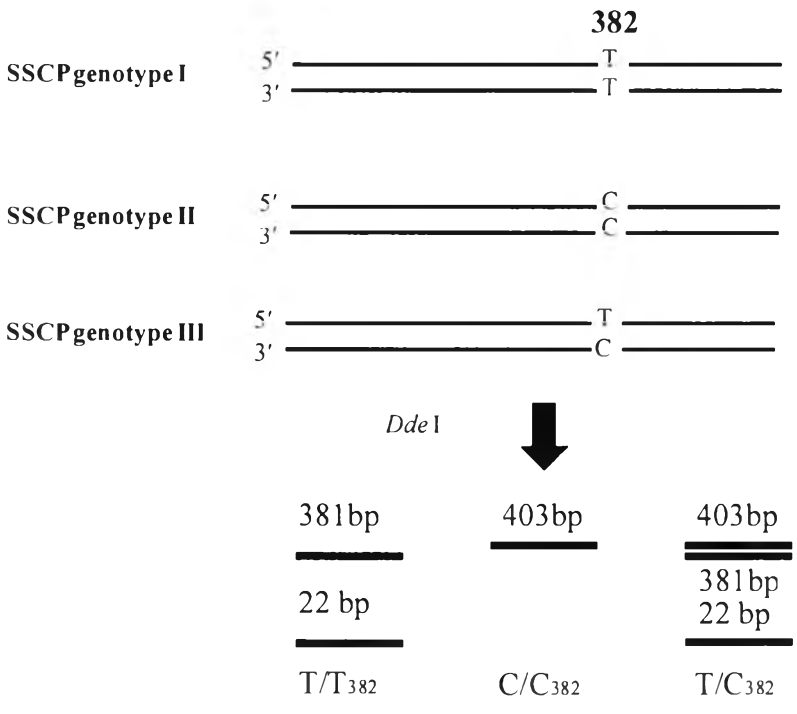

Figure 3.17 Schematic illustration of the expected RFLP profiles of *PmCyC* after digested with *Dde* I.

A 403 bp fragment obtained from amplification of *PmCyC* using genomic DNA of 24 individuals of *P. monodon* juveniles (SNP3A) was further examined by RFLP analysis. As expected, three PCR-RFLP genotypes were found including a single band of 403 bp which represents C/C_{382}, two bands of 403 and 381 bp which represents T/T_{382} and a single band of 381 which represents C/T_{382} (Figure 3.18). Notably, the 22 bp band was missing from agarose gel elcctrophoresis due to its small size.

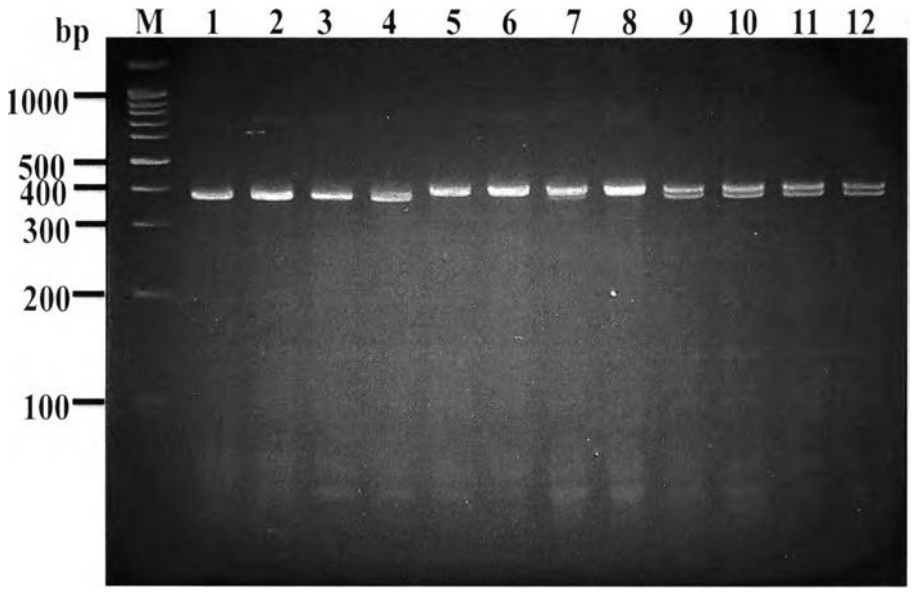

Figure 3.18 PCR-RFLP from digestion of the amplified *PmCyC* gene segment with *Dde* I. Lanes 1-4 are a homozygous T/T_{382} genotype (SSCP pattern I). Lanes 5-8 are a homozygous C/C_{382} genotype (SSCP pattern II). Lanes 9-12 are heterozygous C/T_{382} genotype (SSCP pattern III). Lane M is a 100 bp DNA ladder.

3.6 Isolation and characterization of the full-length cDNA of *P. monodon cyclin C (PmCyC)* using Rapid Amplification of cDNA Ends-Polymerase Chain Reaction (RACE-PCR)

3.6.1 RNA extraction and first strand synthesis

The quantity and quality of total RNA was evaluated. Agarose gel electrophoresis indicated large-size total RNA with a few discrete bands (Figure 3.19). The ovarian mRNA was purified and large amount of mRNA was obtained (30 - 50 μg from 500 μg total RNA). The purified mRNA was subjected to the synthesis of 5′ and 3′ RACE-PCR template.

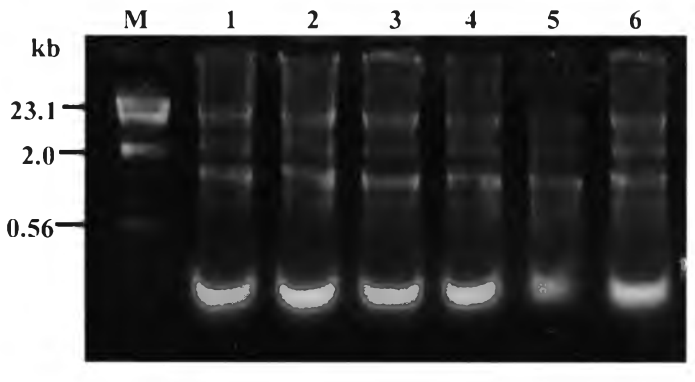

Figure 3.19 A 0.8% ethidium bromide-stained agarose gel showing the quality of RNA extracted from ovaries of *P. monodon* broodstock (lanes = 1 - 6) of *P. monodon.* Lanes M is λ/*Hin*d III marker.

3.6.2 Isolation of the full length cDNA of *PmCyC*

A homologue of *PmCyC* was initially obtained from EST analysis of the hepatopancreas cDNA library (clone no. HC-N-N01-13007-LF). This EST clone contains an insert of 693 bp (Figure 3.20A). Sequence similarity analysis using Blast*X* showed that it significantly matched *g1/s-specific cyclin C* of *Tribolium castaneumat* (XP_968481.1, *E*-value = 7e-119; Figure 3.20B).

A.

CCAGAATTTCCTTCTTTTTCTTCGGGATTTCTAGGATTTTCTGGACTAAAAACATCTTACTT
TATCCTATGGAATGGCAGGGAATTTTTGGCAGAGCGCACACTTCCAACAATGGCTCCTCGAC
AGTCAGGACTTGATACAAGAGCGCCAGGCTGACCTAGAGGTGCTGTCTGAAGAAGAGTACAT
GAAGATTATGACCTTCTTTGCTAACTTTATTCAGCAACTTGGTGAATCACTCAAGCTTAAAC
AACAGGTCATCGCAACTGCCACATGCTTCCTTAAAAGATTCTACGCAAGAAATTCTCTCAAG
TGCATTGACCCTCTTCTCCTCGCCC**CCACCAGTGTCTTCCTCTCATCCA**AGGTTGAGGAGTT
TGGGGTCATCTCCAACAGCAGATTAATTTCCACTTGCCAAACTATTGTAAAGAACAAGTTTG
CTTATGCGTACACAACAGAATTTCCATATCGGACTAACCACATTTTGGAATGTGAGTTTTAC
CTCCTGGAGAGTATGGACTGTTGTCTCATTGTATATCAGCCATACAGACCATTGGTGCAATA
CATGCAGGACCTAGGAGGAGAAGGGGAAGTGCTGCAACTAGCTTGGAGGATTGTAAATGATT
CCCTTCGCACAGATGTCTGTCTTCTGTTTCCCCCCTATGAAATTGCATTATCCTGTATCCAT
ATGGCATGTGT

B.

```
>    ref|XP_968481.1|  PREDICTED: similar to g1/s-specific cyclin c [Tribolium
castaneum]
 gb|EEZ99604.1|  hypothetical protein TcasGA2_TC002120 [Tribolium castaneum]
Length=266

 GENE ID: 656888 LOC656888 | similar to g1/s-specific cyclin c
[Tribolium castaneum] (10 or fewer PubMed links)

 Score =  345 bits (886),  Expect = 7e-119
 Identities = 159/206 (77%), Positives = 187/206 (91%), Gaps = 0/206 (0%)
 Frame = +2

Query  74   MAGNFWQSAHFQQWLLDSQDLIQERQADLEVLSEEEYMKIMTFFANFIQQLGESLKLKQQ  253
            MAGNFWQS+H QQWLLD QDLI+ERQ DL++L+EEEY KI  FFA+ IQ LGE LKL+QQ
Sbjct  1    MAGNFWQSSHHQQWLLDKQDLIRERQHDLQLLTEEEYQKIFIFFASVIQTLGEQLKLRQQ  60

Query  254  VIATATCFLKRFYARNSLKCIDPLLLAPTSVFLSSKVEEFGVISNSRLISTCQTIVKNKF  433
            VIATAT + KRFYA+NSLKCIDPLLLAPT +FL+SKVEEFGVISNSRLI+TCQT++KNKF
Sbjct  61   VIATATVYFKRFYAKNSLKCIDPLLLAPTCIFLASKVEEFGVISNSRLITTCQTVIKNKF  120

Query  434  AYAYTTEFPYRTNHILECEFYLLESMDCCLIVYQPYRPLVQYMQDLGGEGEVLQLAWRIV  613
            +YAY+ EFPYRTNHILECEFYLLE++DCCLIVYQPYRPL+Q +QD+G E ++L LAWRIV
Sbjct  121  SYAYSQEFPYRTNHILECEFYLLENLDCCLIVYQPYRPLLQLVQDMGQEDQLLTLAWRIV  180

Query  614  NDSLRTDVCLLFPPYEIALSCIHMAC  691
            NDSLRTDVCLL+PPY+IA+ C+ +AC
Sbjct  181  NDSLRTDVCLLYPPYQIAIGCLQIAC  206
```

Figure 3.20 (A) Partial cDNA sequence of *PmCyC* from hepatopancreas cDNA library (clone no. HC-N-N01-13007-LF). Primers for 3′ RACE-PCR of *PmCyC* is illustrated in boldface and underlined. (B) BlastX analysis of similarity of the original EST.

3′ RACE-PCR of *PmCyC* was carried out and the discrete amplification bands were obtained using a 3′CyC-F primer. The amplification product was 1200 bp in length (Figure 3.21). The fragment was cloned and sequenced. The obtained sequence was searched against previously deposited data in GenBank using Blast X. Its closest similarity was *g1/s-specific cyclin C* of *Tribolium castaneumat* (XP_968481.1, *E*-value = 4e-93; Figure 3.23). Nucleotide sequences from original EST and 3′RACE-PCR were assembled (Figure 3.22). The full-length cDNA of *PmCyC* were obtained.

The full-length cDNA of *PmCyC* was 1443 bp containing an open reading frame (ORF) of 804 bp corresponding to a polypeptide of 267 amino acids. The 5′UTR and 3′UTR were 73 and a 542 bp (exclude the poly A tail, Figure 3.24). Its closest match was *g1/s-specific cyclin C* of *Tribolium castaneumat* (XP_968481.1, *E*-value = 8e-148). The deduced PmCyC protein contained 2 predicted cyclin domains located at amino acid positions 46-144 (*E*-value = 2.22e-12) and 157-236 (*E*-value = 8.48e-09) (Figure 3.25). The predicted molecular weight (MW) and theoretical isoelectric point (p*I*) of the deduced PmCyC protein were 31.35 kDa and 5.36, respectively.

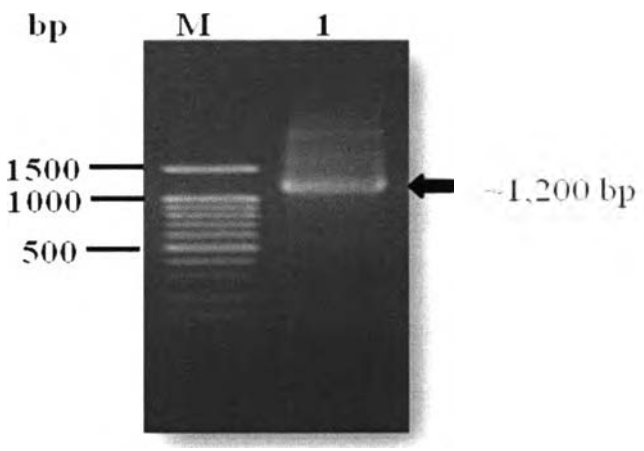

Figure 3.21 A 1.5% ethidium bromide-stained agarose gel showing the amplification result of a 3′ RACE-PCR of *PmCyC* (lane 1). Lane M is a 100 bp DNA ladder.

CCAGAATTTCCTTCTTTTTCTTCGGGATTTCTAGGATTTTCTGGACTAAAAACATCTTACTT
TATCCTATGGAATGGCAGGGAATTTTTGGCAGAGCGCACACTTCCAACAATGGCTCCTCGAC
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GAAGATTATGACCTTCTTTGCTAACTTTATTCAGCAACTTGGTGAATCACTCAAGCTTAAAC
AACAGGTCATCGCAACTGCCACATGCTTCCTTAAAAGATTCTACGCAAGAAATTCTCTCAAG
TGCATTGACCCTCTTCTCCTCGCCCCCACCAGTGTCTTCCTCTCATCCAAGGTTGAGGAGTT
TGGGGTCATCTCCAACAGCAGATTAATTTCCACTTGCCAAACTATTGTAAAGAACAAGTTTG
CTTATGCGTACACAACAGAATTTCCATATCGGACTAACCACATTTTGGAATGTGAGTTTTAC
CTCCTGGAGAGTATGGACCGTTGTCTCATTGTATATCAGCCATACAGACCATTGGTGCAATA
CATGCAGGACCTAGGAGGAGAAGGGGAAGTGCTGCAACTAGCTTGGAGGATTGTAAATGATT
CCCTTCGCACAGATGTCTGTCTTCTGTTTCCCCCCTATGAAATTGCATTATCCTGTATCCAT
ATGGCATGCGTCGTCCATCAGAAGGATTGCAAGCAGTGGTTTGCTGAACTGAACACTGACCT
GGACCGGCTCATGGAGATCACTAGGTACATTCTCAACTTGTATGAACTCTGGAAATCATATG
ATGAGCGCAAGGAGATCCAGGCTCTCCTCCAGAAGATGCCTAAACCCAATACCCAGCCTGTC
CCCCGGTGATTGATGCACCCTGGCTTAGGGTCAAGTTATGGAGGAGAGACATGTGTCAGGGA
TCTGTTCCTTCCAATATATAGGGTTAAGAAATAAGAGGGTCCTATTCTATTTTGCGGATGTT
GATGCATAACATTTTGACTCAGTGTTATGGTATTTTGTGGTTATTATAACCTCAACCGGGCT
AATTTCTTAATTGGCTCTTTATGTAGTAATTTTATTTTTATTTTCCTATACAGAGTGGTGCC
TGGCATTTTGTGACAAATTTTTTATTATTATTGTTATTATTATTAATTTATTGAGTCATTGA
TAATTTTAGTTAGTCTTTTTAGAATTGATGCCATGCAGGACTTTGATGCAGAAACTTGTATG
ATTAGGTTACATTTGAAATATCTTATAAAGAAAGGAACATTAAAAAGATAATATCAAAATGT
ATAAAATTAGATGCAAGAGAACATTTTAAGTTTCTAAAAGTGGTTTCATATTCATAACATAT
TTCCTAATTCCCCTTTCGCTTATTTCTGTGTCAAGTGTGTCTAATAAAGAACTCCAAAAAAA
AAAAAAAAAAAAAAAA

Figure 3.22 Assembled nucleotide sequences of nucleotide sequences from EST and 3′ RACE-PCR (highlighted). The original EST sequence is shown in boldface and the 3′CyC primer is underlined.

```
>    ref|XP_968481.1|  PREDICTED:  similar  to  g1/s-specific  cyclin  c
[Tribolium castaneum]

 gb|EEZ99604.1|  hypothetical protein TcasGA2_TC002120 [Tribolium castaneum]
Length=266

 GENE ID: 656888 LOC656888 | similar to g1/s-specific cyclin c
[Tribolium castaneum] (10 or fewer PubMed links)

 Score =  287 bits (734),  Expect = 4e-93
 Identities = 128/176 (73%), Positives = 156/176 (89%), Gaps = 0/176 (0%)
 Frame = +2

Query  83   TSVFLSSKVEEFGVISNSRLISTCQTIVKNKFAYAYTTEFPYRTNHILECEFYLLESMDR  262
            T +FL+SKVEEFGVISNSRLI+TCQT++KNKF+YAY+ EFPYRTNHILECEFYLLE++D
Sbjct  89   TCIFLASKVEEFGVISNSRLITTCQTVIKNKFSYAYSQEFPYRTNHILECEFYLLENLDC  148

Query  263  CLIVYQPYRPLVQYMQDLGGEGEVLQLAWRIVNDSLRTDVCLLFPPYEIALSCIHMACVV  442
            CLIVYQPYRPL+Q +QD+G E ++L LAWRIVNDSLRTDVCLL+PPY+IA+ C+ +ACV+
Sbjct  149  CLIVYQPYRPLLQLVQDMGQEDQLLTLAWRIVNDSLRTDVCLLYPPYQIAIGCLQIACVI  208
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```
Query  443  HQKDCKQWFAELNTDLDRLMEITRYILNLYELWKSYDERKEIQALLQKMPKPNTQP  610
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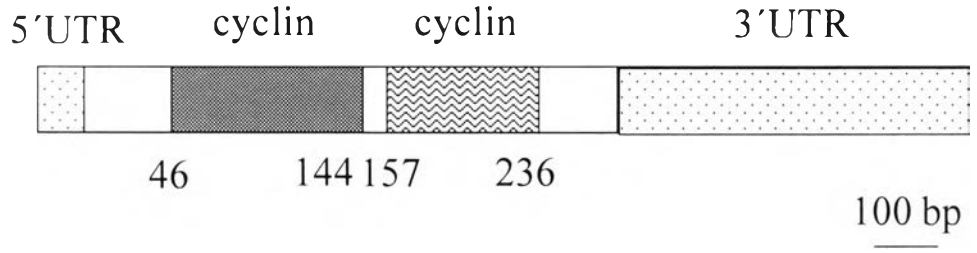
Sbjct  209  LQKDHKAWFAELNVDIERIQEIARYVINLFELWKTYDEKKEIQGLLNKMPKPKPAP  264
```

Figure 3.23 3′ RACE of *cyclin C* was searched against data in the GenBank using Blast*X* and the closest homologues was *g1/s-specific cyclin C* of *Tribolium castaneumat* (4e-93, XP_968481.1).

```
CCAGAATTTCCTTCTTTTTCTTCGGGATTTCTAGGATTTTCTGGACTAAAAACATCTTAC 60
TTTATCCTATGGAATGGCAGGGAATTTTTGGCAGAGCGCACACTTCCAACAATGGCTCCT 120
                M  A  G  N  F  W  Q  S  A  H  F  Q  Q  W  L  L 16
CGACAGTCAGGACTTGATACAAGAGCGCCAGGCTGACCTAGAGGTGCTGTCTGAAGAAGA 180
  D  S  Q  D  L  I  Q  E  R  Q  A  D  L  E  V  L  S  E  E  E 36
GTACATGAAGATTATGACCTTCTTTGCTAACTTTATTCAGCAACTTGGTGAATCACTCAA 240
  Y  M  K  I  M  T  F  F  A  N  F  I  Q  Q  L  G  E  S  L  K 56
GCTTAAACAACAGGTCATCGCAACTGCCACATGCTTCCTTAAAAGATTCTACGCAAGAAA 300
  L  K  Q  Q  V  I  A  T  A  T  C  F  L  K  R  F  Y  A  R  N 76
TTCTCTCAAGTGCATTGACCCTCTTCTCCTCGCCCCCACCAGTGTCTTCCTCTCATCCAA 360
  S  L  K  C  I  D  P  L  L  L  A  P  T  S  V  F  L  S  S  K 96
GGTTGAGGAGTTTGGGGTCATCTCCAACAGCAGATTAATTTCCACTTGCCAAACTATTGT 420
  V  E  E  F  G  V  I  S  N  S  R  L  I  S  T  C  Q  T  I  V 116
AAAGAACAAGTTTGCTTATGCGTACACAACAGAATTTCCATATCGGACTAACCACATTTT 480
  K  N  K  F  A  Y  A  Y  T  T  E  F  P  Y  R  T  N  H  I  L 136
GGAATGTGAGTTTTACCTCCTGGAGAGTATGGACTGTTGTCTCATTGTATATCAGCCATA 540
  E  C  E  F  Y  L  L  E  S  M  D  C  C  L  I  V  Y  Q  P  Y 156
CAGACCATTGGTGCAATACATGCAGGACCTAGGAGGAGAAGGGGAAGTGCTGCAACTAGC 600
  R  P  L  V  Q  Y  M  Q  D  L  G  G  E  G  E  V  L  Q  L  A 176
TTGGAGGATTGTAAATGATTCCCTTCGCACAGATGTCTGTCTTCTGTTTCCCCCCTATGA 660
  W  R  I  V  N  D  S  L  R  T  D  V  C  L  L  F  P  P  Y  E 196
AATTGCATTATCCTGTATCCATATGGCATGTGTCGTCCATCAGAAGGATTGCAAGCAGTG 720
  I  A  L  S  C  I  H  M  A  C  V  V  H  Q  K  D  C  K  Q  W 216
GTTTGCTGAACTGAACACTGACCTGGACCGGCTCATGGAGATCACTAGGTACATTCTCAA 780
  F  A  E  L  N  T  D  L  D  R  L  M  E  I  T  R  Y  I  L  N 236
CTTGTATGAACTCTGGAAATCATATGATGAGCGCAAGGAGATCCAGGCTCTCCTCCAGAA 840
  L  Y  E  L  W  K  S  Y  D  E  R  K  E  I  Q  A  L  L  Q  K 256
GATGCCTAAACCCAATACCCAGCCTGTCCCCCGGTGATTGATGCACCCTGGCTTAGGGTC 900
  M  P  K  P  N  T  Q  P  V  P  R  *                        267
AAGTTATGGAGGAGAGACATGTGTCAGGGATCTGTTCCTTCCAATATATAGGGTTAAGAA 960
ATAAGAGGGTCCTATTCTATTTTGCGGATGTTGATGCATAACATTTTGACTCAGTGTTAT 1020
GGTATTTTGTGGTTATTATAACCTCAACCGGGCTAATTTCTTAATTGGCTCTTTATGTAG 1080
TAATTTTATTTTTATTTTCCTATACAGAGTGGTGCCTGGCATTTTGTGACAAATTTTTTA 1140
TTATTATTGTTATTATTATTAATTTATTGAGTCATTGATAATTTTAGTTAGTCTTTTTAG 1200
AATTGATGCCATGCAGGACTTTGATGCAGAAACTTGTATGATTAGGTTACATTTGAAATA 1260
TCTTATAAAGAAAGGAACATTAAAAAGATAATATCAAAATGTATAAAATTAGATGCAAGA 1320
GAACATTTTAAGTTTCTAAAAGTGGTTTCATATTCATAACATATTTCCTAATTCCCCTTT 1380
CGCTTATTTCTGTGTCAAGTGTGTCTAATAAAGAACTCCAAAAAAAAAAAAAAAAAAAAA 1440
AAA                                                            1443
```

Figure 3.24 The full-length cDNA and deduced amino acid sequences of *PmCyC*. The putative start (ATG) and stop (TGA) codons are in boldface and underlined. Two predicted cyclin domains (positions 46-144 and 157-236, *E*-value = 2.22e-12 and 8.48e-09) are highlighted.



Domain	Position	*E*-value
Cyclin	46-144	2.22e-1.26
Cyclin	157-236	8.48e-09

Figure 3.25 Diagram illustrating the full-length cDNA of *PmCyC*. The 2 cyclin domains were found in the deduced amino acid sequence of *PmCyC*. The scale bar is 100 bp in length.

3.7 Expression levels of *PmCnn1* and *PmCdc25* transcripts in hepatopancreas of *P. monodon* juveniles (SNP3A) carrying different SSCP patterns analyzed by quantitative real-time PCR

The expression level of *PmCnn1* and *PmCdc25* in shrimp carrying different SSCP patterns (and SNPs) was examined using quantitative real-time PCR. The standard curves of *PmCnn1*, *PmCdc25* and *EF-1α* were constructed from a 10-fold dilution covering 10^3-10^8 copy numbers of these genes. High R^2 values and efficiency of amplification of examined transcripts were found (Figure 3.26). Therefore, these standard curves were acceptable to be used for quantitative estimation of the mRNA levels of examined genes.

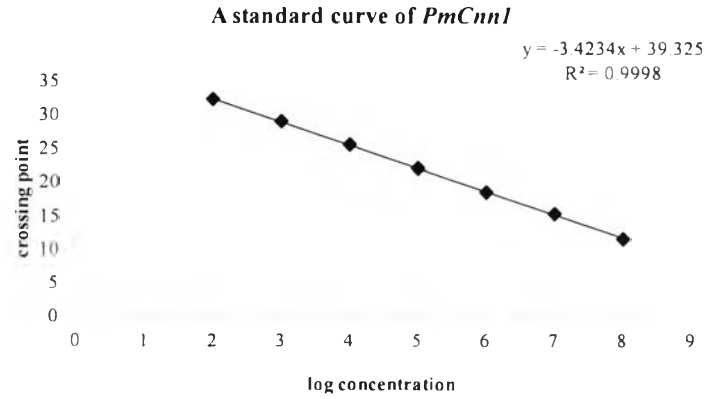
3.7.1 *PmCnn1*

SSCP analysis of *PmCnn1* revealed that three SSCP patterns were found in the SNP3A sample for which shrimp with patterns I and II had a greater average BW, TL and HPW than those with pattern III ($P < 0.05$). Differences between the expression level of *PmCnn1* in shrimp carrying different SSCP patterns were statistically examined. The expression level of *PmCnn1* in shrimp exhibiting genotypes III was significantly greater than those exhibiting genotypes I and II ($P < 0.05$; Figure 3.27).

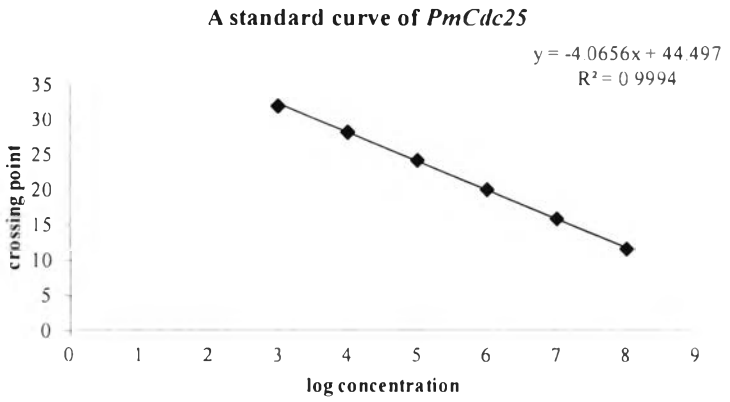
3.7.2 *PmCdc25*

SSCP analysis of *PmCdc25* gene revealed that two SSCP patterns were found in the SNP3A sample for which shrimp with pattern I had a greater average BW, TL and HPW than those with pattern II ($P < 0.05$). Quantitative real-time PCR was carried out to determine whether shrimp having different SSCP pattern showed differences in the expression level of *PmCdc25*. Results indicated that the expression level of *PmCdc25* in shrimp exhibiting genotypes I was significantly greater than those exhibiting genotypes II ($P < 0.05$; Figure 3.28).

A.



B.



C.

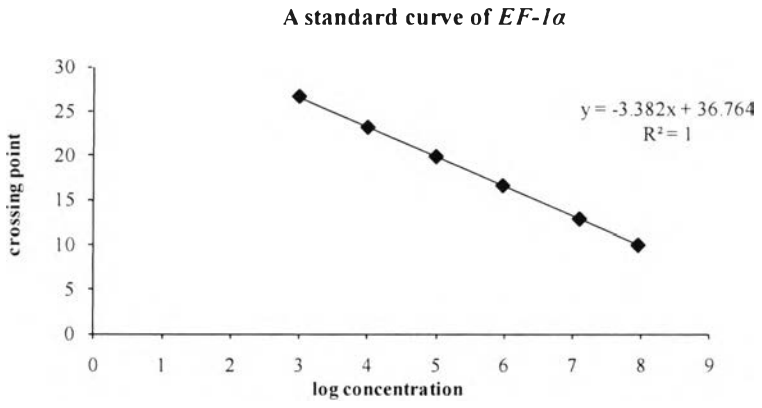


Figure 3.26 Standard amplification curves of *PmCnn1* (A; amplification efficiency = 1.951, error = 0.00950), *PmCdc25* (B; amplification efficiency = 1.980, error = 0.0197) and *EF-1α* (C; amplification efficiency = 1.969, error = 0.00609)

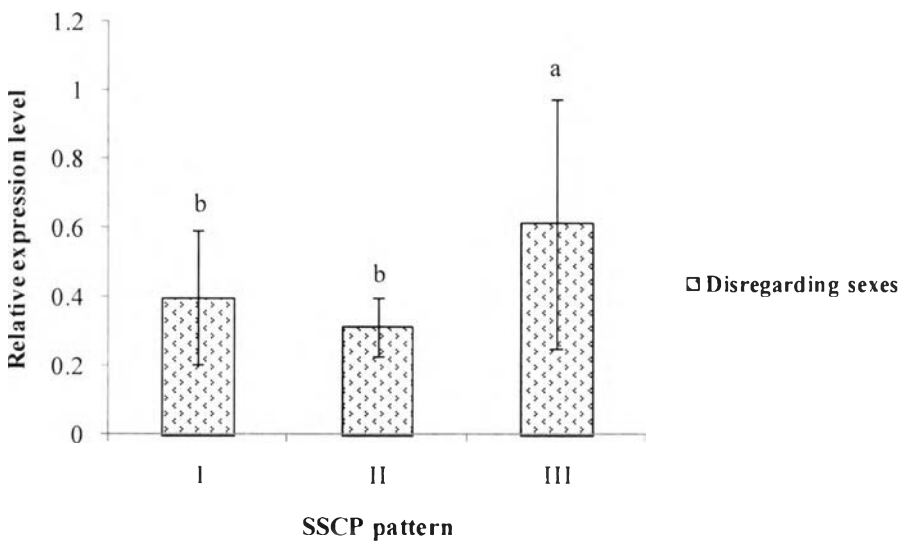


Figure 3.27 Histograms showing relationships between the relative expression level of *PmCnn1* in hepatopancreas of shrimp carrying different SSCP patterns (3-month-old juveniles; SNP3A; $N = 29$). Expression levels were measured as the absolute copy number of *PmCnn1* mRNA (500 ng template) and normalized by that of *EF-1α* mRNA (5 ng template).The same letters above the bars reveal non-significant differences between groups of samples ($P > 0.05$).

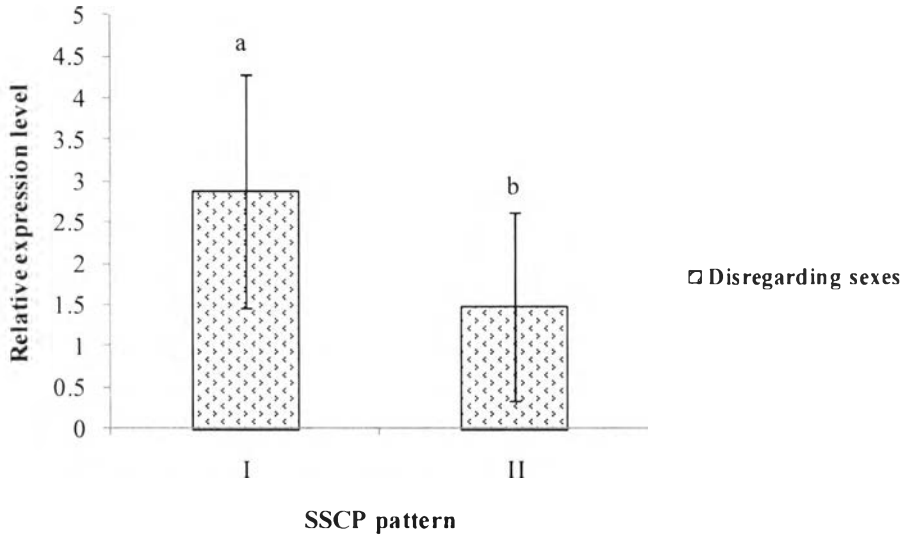


Figure 3.28 Histograms showing relationships between the relative expression level of *PmCdc25* in hepatopancreas of juvenile shrimp carrying different SSCP patterns (SNP3A; N = 30). Expression levels were measured as the absolute copy number of *PmCdc25* mRNA (500 ng template) and normalized by that of *EF-1α* mRNA (5 ng template).

3.8 *In vitro* expression of recombinant PmCnn1 proteins in a bacterial expression system

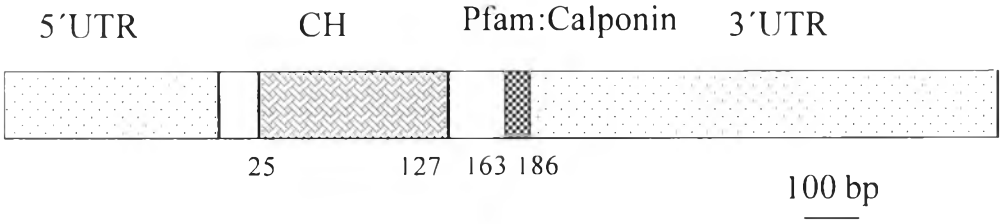
3.8.1 Construction of the recombinant plasmid

Recombinant plasmid carrying the entire ORF of *PmCnn1* were prepared for *in vitro* expression of the corresponding proteins. Previously, an EST covering the complete ORF of *PmCnn1* was isolated (Tassanakajon et al., 2006) (Figure 3.29). Moreover, the genomic DNA sequence of *PmCnn1* was further characterized by genome walking analysis (Buaklin, 2005). The predicted CH and Calponin domains were found in the deduced amino acid sequence of *PmCnn1* (Figure 3.30).

```
AAAACCCTGTACAGTTATCTATACAATATGAAGCGTCAGATAAATGTTACCAGATATATT 60
TAACCTCCAGTCTGTTGGAAGAATATATCTTGGCCGGAAATGTGAACCCAGACACACTAC 120
AGTTCGACCCTTGTTGTTAAAGGTGCACTAGAGTAAAAGAAGGTTAATTTCCCCGTTTGT 180
TGGCATCAGATTCCTTTGGTTTTGGGTTAAAGCAGATAGAGCACCATCTATGGATTTTCT 240
TCCCCCATCTCTAGTGCACTCTTTTTGGGTCGACTTTCCTTCCTCCGTTGCTTTCTCTCG 300
CGTATCTTCGAATTACTTCGTGCCATGTAATGCAATTCAGTGATAAGTTTGGGCATTCCT 360
TTTAGTATTGTATGTAACTCTCACAATGAACCGTGCTACCAAGTCCGGAATCGCTGCCGA 420
                        M  N  R  A  T  K  S  G  I  A  A  E  12
GGCTCAGGCTAAGGTCAACGCAAAGTACAGCGAAGAGCAGGCCGCCGAGTGCTTGGAATG 480
 A  Q  A  K  V  N  A  K  Y  S  E  E  Q  A  A  E  C  L  E  W  32
GATCGCCATCATCACGAGCGCCGACATCAGCAAGTCTGGAGACGCCGACAATTTCTACGA 540
 I  A  I  I  T  S  A  D  I  S  K  S  G  D  A  D  N  F  Y  E  52
GACCTTGAAGAATGGACAGCTGTTGTGCCAGGTGATTAACGCCCTCAAGCCCGGTCAGAT 600
 T  L  K  N  G  Q  L  L  C  Q  V  I  N  A  L  K  P  G  Q  I  72
CAAGAAGATCCAGACCTCCGCCATGGCATTCAAGTGCATGGAAAACATCAACGCCTTTGT 660
 K  K  I  Q  T  S  A  M  A  F  K  C  M  E  N  I  N  A  F  V  92
GGAGGGAGCTAAGGCCTGTGGGGTGCCCACTCAGGAGACCTTCCAGACCGTCGACCTCTG 720
 E  G  A  K  A  C  G  V  P  T  Q  E  T  F  Q  T  V  D  L  W  112
GGAACGACAGAACCTTAACTCTGTTGTTATCTGCTTGCAGTCTCTGGGCAGGAAGGGATC 780
 E  R  Q  N  L  N  S  V  V  I  C  L  Q  S  L  G  R  K  G  S  132
TCAATTTGGAAAGCCTTCCATTGGCCCAAAAGAGTCTGAGAAGAATGTCCGTCACTTCAC 840
 Q  F  G  K  P  S  I  G  P  K  E  S  E  K  N  V  R  H  F  T  152
CGAGGAGCAGCTCAGGGCTTCTGAGGGCATCGTCAACCTGCAGTATGGCTCCAACAAGGG 900
 E  E  Q  L  R  A  S  E  G  I  V  N  L  Q  Y  G  S  N  K  G  172
TGCCACTCAGTCTGGCATGTCCTTCGGCAATACTCGCCACATGTAAAAGCAGTCTTTGTA 960
 A  T  Q  S  G  M  S  F  G  N  T  R  H  M  *                 186
GACTTTCACTTTCACTTCATTTTTTAAAAAAAGTAGTTCAACATAATTCATCATGCTTCT 1020
AATATGTTCCAATATATAATAGCGGGGAGGATTTCTTTTATATATAAAAATAAAAACTGA 1080
AAAAAATGCATTGGCAGTGGTATGCCTAGAAAAGGAATTTTTACAACTGCAGTCTTAGGC 1140
AAAGAAATGAATGTAAAAAAGGATGAAATCAGACATGTATCACTTGACCAATAGGTTGCT 1200
ACAATTTTTATTACATTGCATAGGAACTGGTAATAATGAAGCGAAGTCTCAAGGCCAGAG 1260
AAAATGCTTTAAAGTTCTCACTGAAACCAGAATTAAATATTTTAGTGCAAGCTGATGAGT 1320
AGCACCATTAGCTCATTTCCAAATTGATGCATTTTCAATACACATCACATATTTGTTTTA 1380
ACTGAAAACTGAAGGCGTAGATACATTATCAAAGAAAAATTATCCATCCAGGCTTTTTTC 1440
ATATTTTACTAATTTGTAAGCTTATTATAGTACAATTTATACAGATATAAGTGTTATACA 1500
TTATGCAGTATAAAATGTATTTAAATAATGTTATTCTATGAAAAAGAAATTCATATATAT 1560
GAAAAGTATTCCTTATTTATCATAGTTGCAGCTCATCTGTAGCAATATAGTGAAATGAAT 1620
ATTGTTTCATTTTTCTTTCCTTTTTTTATTGGAGTCATATTCTCTTTATTTTACGTTACC 1680
ATTGTATGTGTGGTATGAAGTTTTATTTTCTTTTCCTAATAGAGAAATTATAGTCTTGTT 1740
TGAGCTGTCACATTCCAGTTTGAGAGAAATTTTGTATGGAAATGAAATAAAAAGTTCAAT 1800
ACTAAAAAAAAAAAAAAAAAAC                                       1822
```

Figure 3.29 The full-length cDNA and deduced amino acids of *PmCnn1* (1822 bp with an ORF of 561 bp encoding a polypeptide of 186 amino acids). The start and stop codons are illustrated in boldface. The poly A additional signal site are underlined. The Calponin homology domain (CH; 7.49e-24, position 25-127) and Calponin domain (Pfam:Calponin; 1.10e-06, position 163-186) are highlighted (Buaklin, 2005).

A pair of primers overhang with *Bam* HI and *Xho* I-6His was designed to amplify the complete ORF of *PmCnn1* using *Pfu* DNA polymerase (Figure 3.31). The amplification product was analyzed by agarose gel electrophoresis and the target product was eluted from the gel. The gel-eluted PCR product was digested with *Bam* HI and *Xho* I and ligated with pET-29a and transformed into *E. coli* JM109.



Domain	Position	*E*-value
CH	25-127	7.49e-24
Calponin	163-186	1.10e-06

Figure 3.30 Diagram illustrating the deduced PmCnn1 protein. The predicted CH and Calponin domains were found in the deduced amino acid sequence of *PmCnn1*. The scale bar is 100 bp in length.

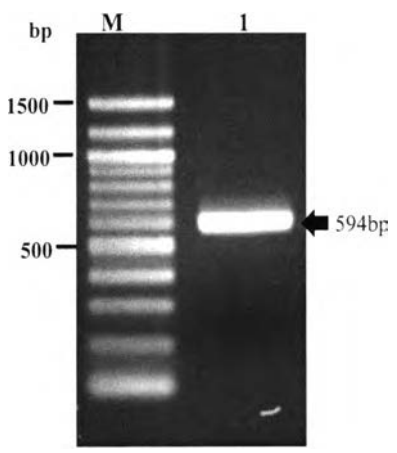


Figure 3.31 A 1.5% ethidium bromide stained agarose gel showing the complete ORF of *PmCnn1* amplified by specific primer overhang with *Bam* HI and *Xho* I-6His using the first strand cDNA from hepatopancrease as the template. Lane M is a 100 bp DNA ladder.

Plasmid DNA of the positive clone was sequenced to confirm the orientation of the recombinant clones and nucleotide sequence was analyzed by Blast X (Figures 3.32A and B). Plasmid DNA was extracted from a clone carrying the correct direction of *PmCnn1* and transformed into *E. coli* BL21-CodonPlus (DE3)-RIPL competent cells.

A.

GGATCCATGAACCGTGCTACCAAGTCCGGAATCGCTGCCGAGGCTCAGGCTAAGGTCAACGC
AAAGTACAGCGAAGAGCAGGCCGCCGAGTGCTTGGAATGGATCGCCATCATCACGAGCGCCG
ACATCAGCAAGTCTGGAGACGCCGACAATTTCTACGAGACCTTGAAGAATGGACAGCTGTTG
TGCCAGGTGATTAACGCCCTCAAGCCCGGTCAGATCAAGAAGATCCAGACCTCCGCCATGGC
ATTCAAGTGCATGGAAAACATCAACGCCTTTGTGGAGGGAGCTAAGGCCTGTGGGGTGCCCA
CTCAGGAGACCTTCCAGACCGTCGACCTCTGGGAACGACAGAACCTTAACTCTGTTGTTATC
TGCTTGCAGTCTCTGGGCAGGAAGGGATCTCAATTTGGAAAGCCTTCCATTGGCCCAAAAGA
GTCTGAGAAGAATGTCCGTCACTTCACCGAGGAGCAGCTCAGGGCTTCTGAGGGCATCGTCA
ACCTGCAGTATGGCTCCAACAAGGGTGCCACTCAGTCTGGCATGTCCTTC**GGCAATACTCGC**
CACATGCATCATCATCATCATCATTAACTCGAG

B.

gb|ADD20603.1| calponin [Glossina morsitans morsitans]

Length=188

Score = 293 bits (751), Expect = 3e-98, Method: Compositional matrix adjust.
Identities = 138/187 (74%), Positives = 158/187 (84%), Gaps = 0/187 (0%)
Frame = +2

```
Query  5    SMNRATKSGIAAEAQAKVNAKYSEEQAAECLEWIAIITSADISKSGDADNFYETLKNGQL  184
            S+NRA KSG AAEAQ K+N+KYSEE A ECLEWI  IT   I+ SGD DNF+E LK+G L
Sbjct  2    SVNRAPKSGFAAEAQRKINSKYSEELAQECLEWIKTITGEPINASGDMDNFFEVLKDGVL  61

Query  185  LCQVINALKPGQIKKIQTSAMAFKCMENINAFVEGAKACGVPTQETFQTVDLWERQNLNS  364
            LC++ N L+PG IKKI  S MAFKCMENI+AF+E AK  GVPTQETFQ+VDLWERQNLNS
Sbjct  62   LCKLANCLQPGVIKKINESKMAFKCMENISAFLECAKNLGVPTQETFQSVDLWERQNLNS  121

Query  365  VVICLQSLGRKGSQFGKPSIGPKESEKNVRHFTEEQLRASEGIVNLQYGSNKGATQSGMS  544
            VVICLQSLGRK   FGKPSIGPKE++KNVRHFTEEQLRA + +++LQYGSNKGA QSG++
Sbjct  122  VVICLQSLGRKAHHFGKPSIGPKEADKNVRHFTEEQLRAGQNVISLQYGSNKGANQSGIN  181

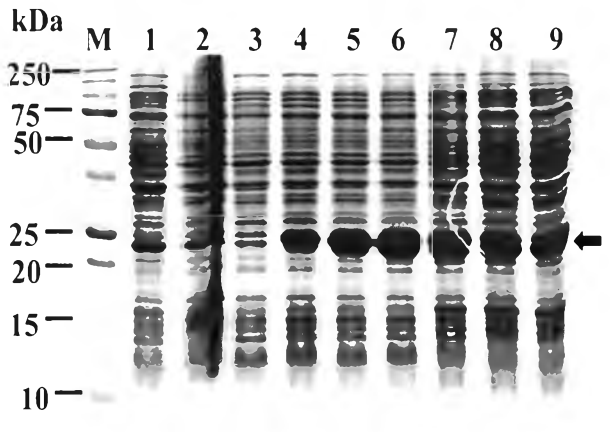
Query  545  FGNTRHM  565
            FGNTRHM
Sbjct  182  FGNTRHM  188
```

Figure 3.32 Nucleotide sequence of a recombinant plasmid containing the calponin domain sequence of *PmCnn1*. Primer sequences are highlighted (A). The result of similarity analysis using blastX is illustrated (B).

3.8.2 *In vitro* expression of recombinant proteins

A recombinant clone containing *PmCnn1* (expected molecular mass of 21.12 kDa) was selected and the expression profile of the corresponding recombinant protein was examined at 0, 1, 2, 3, 6, 12 and 24 hours after induced by 1 mM IPTG. An induced recombinant protein (approximately 21 kDa) was observed between 1-24 hours after induction where the expressed rPmCnn1 protein was gradually increased at 1-3 hour post treatment with 1 mM IPTG and it was decreased from 6-24 hours post IPTG induction (Fig. 3.33).

A.



B.

Figure 3.33 A 15% SDS-PAGE (A) and Western blot analysis (B) showing *in vitro* expression of rPmCnn1 after induced with 1 mM IPTG for 0, 1, 2, 3, 6, 12 and 24 hr, respectively (lanes 3-9). Lanes 1-2 = *E. coli* BL21-CodonPlus and *E. coli* BL21-CodonPlus containing pET29a vector.

The expression profile of 3 recombinant *PmCnn1*clones was further confirmed at 3 and 6 hours post IPTG induction. The results were consistent and the expression level of rPmCnn1 at 3 hours were clearly great than that at 6 hours post induction.

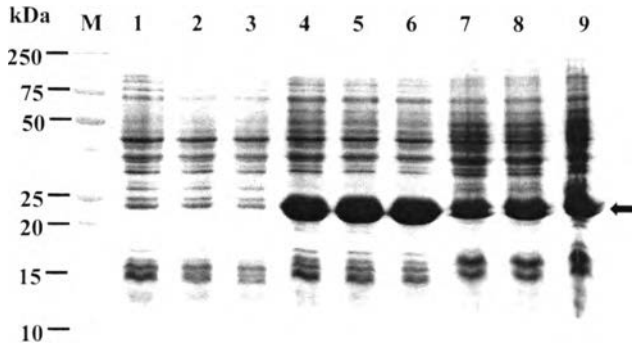


Figure 3.34 A 15% SDS-PAGE showing the rPmCnn1 protein overexpressed at 0 (lanes 1-3), 3 (lanes 4-6) and 6 (lanes 7-9) hours post induction by IPTG.

Moreover, an aliquot of the IPTG-induced culture (at 37°C for 3 hours, OD = 1) of a recombinant *PmCnn1* clone was collected. The cells were disrupted. The soluble and insoluble protein fractions were analyzed by 15% SDS-PAGE. The rPmCnn1 was mainly expressed in both soluble and insoluble forms (Figure 3.35).

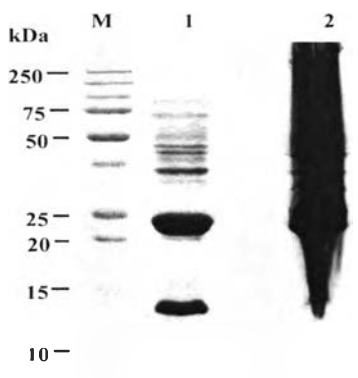


Figure 3.35 A 15% SDS-PAGE showing expression of rPmCnn1 in the soluble (lane 1) and insoluble (lane 2) fractions, after a recombinant clone was induced by IPTG 3 hours at 37°C (1 mM).

3.8.3 Purification of recombinant proteins

The recombinant PmCnn1 protein was purified from the soluble fractions (Fig. 3.36). A single band of rPmCnn1 protein was obtained from the eluted fractions (Fig. 3.37). The purified rPmCnn1 protein from the fraction 5 of 150 mM imidazole and fraction 1-4 of 500 mM imidazole was concentrated and size-fractionated by 15%

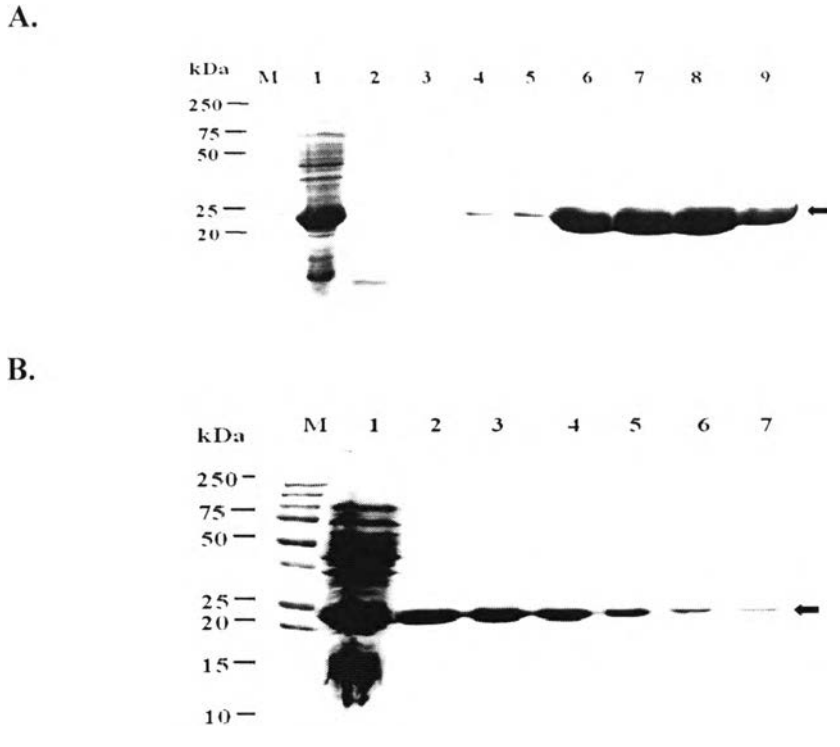


Figure 3.36 (A) A 15% SDS-PAGE of purified rPmCnn1 protein. The recombinant clone was cultured at 37°C and induced with 1 mM IPTG for 3 hours. Lane 1 is the crude recombinant protein. Lanes 2 and 3 are fractions 1 and 6 from the 20 mM imidazole (pH 7.4) washing solution. Lanes 4-5 are fractions 3 and 5 of 40 mM imidazole washing solution. Lanes 6-7 are fractions 3 and 5 of 80 mM imidazole washing solution. Lanes 8-9 are fractions 3 and 5 of 150 mM imidazole washing solution. (B) A 15% SDS-PAGE of purified recombinant PmCnn1 protein. The recombinant clone was cultured at 37°C and induced with 1 mM IPTG for 3 hours. Lane 1 is a recombinant protein after pass through the column. Lanes 2-7 are eluted fractions 1-6 from the 500 mM imidazole elution buffer, respectively.

SDS-PAGE. The gel-purified rPmCnn1 was excised from the gel and electroeluted. The purified rPmCalponin1 protein (2 mg) was sent to Faculty of Associated Medical Sciences, Chiengmai University, for the production of the polyclonal antibody in rabbit.

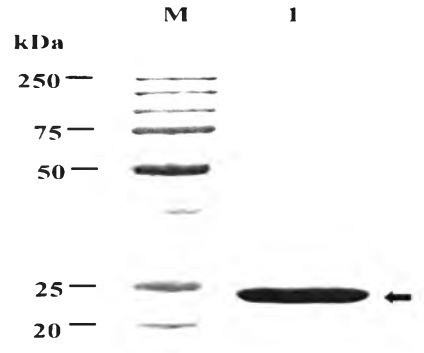


Figure 3.37 A 15% SDS-PAGE showing the gel-eluted rPmCnn1 protein used for the production of polyclonal antibody.

3.8.4 The production of polyclonal antibodies againts recombinant PmCnn1

Anti-rPmCnn1 polyclonal antibody (PAb) was successfully produced in rabbits. The titer of anti-rPmCnn1 PAb was high after the five immunizations (1:32000 with OD_{450} = 0.36 against 1 μg of purified rPmCnn1; Table 3.2). Rabbit was sacrificed and the serum was collect, filtrated through 0.22 μM membrane and kept at -20°C.

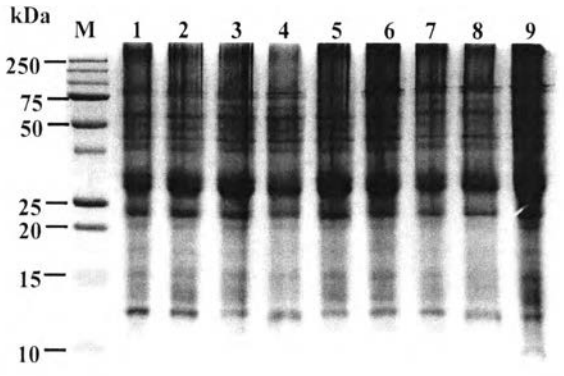
Table 3.20 Titers of polyclonal antibody using an indirect ELISA assay (OD_{450}) after rabbits was immunized three times with rPmCnn1 protein

Dilution of serum	Pre-immunized serum	Uncoated pre-immunized	Immunized serum	Uncoated immunized
1:500	0.017	0.014	2.152	0.239
1:2000	0.005	0.005	1.668	0.111
1:8000	0.003	0.003	1.051	0.056
1:32000	0.005	0.007	0.36	0.016
	0.005	0.001	0.000	
	Conjugate control		Blank	

Positive control: Serum rabbit anti-subtilisinA (1:2000)		
Coated	1.794	
Uncoated	0.018	
Conjugate control	0.003	0.001

Western blot analysis revealed a single discrete band of approximately 21 kDa suggesting that this protein is not glycosylated after translation. The preliminary results indicated that the band intensity of large, medium and small sizes of the SNP3A sample was not different (Figure 3.38).

A.



B.

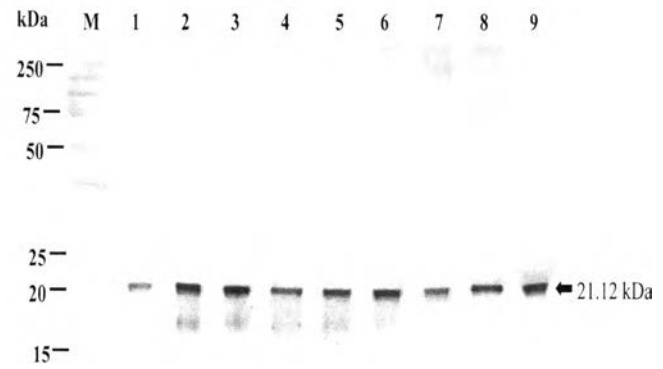


Figure 3.38 A 15% SDS-PAGE (A) and Western blot analysis (B) of the extracted total protein from hepatopancreas of juvenile shrimp having large (lanes 1-3), medium (lanes 4-6) and small (lanes 7-9) sizes. Juveniles (3-month-old) were cultured together in the same earth pond.