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APPENDICES

APPENDIX A: Preparation of solutions

1. LB broth

Tryptone	10.0	g
NaCl	5.0	g
Yeast extracts	5.0	g

pH is adjusted to 7.0 by 1 M NaOH before making the volume up to 100 ml with dd-H_2O. The broth is finally autoclaved.

2. LB plate with ampicillin/IPTG/X-GAL

LB plate gradient is combined as described in 1. The mixture is supplemented with 0.5 mM IPTG and 80 μg/ml X-GAL before pouring plates. Alternatively, 100 μl of 100 mM IPTG and 20 μl of 50 mg/ml X-GAL can be spread on the surface of an LB-amplicillin plate and allowed to absorb for 30 min at 37°C before use.

3. SOC medium

Tryptone	2.0	g
1 M NaCl	1.0	ml
Yeast extracts	0.5	g
1 M KCl	0.25	ml
2 M Mg^{2+} stock	1.0	ml
2 M glucose	1.0	ml

Add Tryptone, Yeast extract, NaCl and KCl to 97 ml of distilled water. Stir to dissolve. Autoclave and cool to room temperature. Add 2 M of Mg^{2+} stock and 2 M of glucose, each to a final concentration of 20 mM. Bring to 100 ml with dd-H_2O. Filter the complete medium through a 0.2 μm filter unit. The final pH should be 7.0.

4. Marine broth pH 7.3

Peptone	5.0	g
Yeast extract	1.0	g
Ferric Citrate	0.1	g
NaCl	20.0	g

Adjusted pH to be 7.3 by 1 M HCl and adjusted volume to 1,000 ml by dd-H_2O and autoclave.

5. Coomassie stain solution

Coomassie brilliant blue R-250	0.25	g
Methanol	45.0	ml
Acetic acid	10.0	ml
d-H_2O adjust volume to	45.0	ml

6. De-staining solution

Methanol	400	ml
Acetic acid	100	ml
d-H_2O adjust volume to	500	ml

7. 1xPBS (Phosphate buffer saline)

Na_2HPO	40.72	g
KH_2PO_4	0.12	g
KCl	0.10	g
NaCl	0.40	g

Adjust volume to be 1,000 ml by dd-H_2O and autoclave.

8. Colour reagent

Thiourea	0.75	g
Gracial acetic acid	470	ml
O-toluidine	30	ml

Mix and cover with aluminum foil and store at room temperature.

9. 1xTBE buffer (Tris borate EDTA)

Tris	108	g
Boric acid	55	g
EDTA	9.3	g

Adjust volume to 1,000 ml by d-H_2O and autoclave.

10. Gel loading dye

bromophenol blue	0.25 %
xylene cyanol FF	0.25 %
ficoll	15.0 %

11. 1.5 M Tris-HCl, pH 8.8

Tris (hydroxymethyl)-aminometane	18.17	g

Adjust pH to 8.8 by 1 M HCl and adjust volume to 100 ml with dd-H_2O

12. 0.5 M Tris-HCl, pH 6.8

Tris (hydroxymethyl)-aminometane	6.06	g

Adjust pH to 6.8 by 1 M HCl and adjust volume to 100 ml with dd-H_2O

13. Staining solution

Silver nitrate	1.5	g
Formaldehyde	2.25	ml

Adjust volume to 1,500 ml with dd-H_2O

14. Low Molecular Weight markers (LMW, Amersham Biosciences): consists of

Phosphorylase b, rabbit muscle	97	kDa
Albumin, bovine serum	66	kDa
Ovelbumin, chicken egg white	45	kDa
Carbonic anhydrase, bovine erythrocyte	30	kDa
Trypsin inhibitor, soybean	20	kDa
∝-lactalbumin, bovine milk	14.4	kDa

15. 30% Acrylamide and 0.8% bis-acrylamide

Acrylamide	29.2	g
N, N'-methylene-bis-acrylamide	0.8	g

Adjust volume to 100 ml with dd-H_2O

16. 5x Loading buffer

1 M Tris-HCl, pH 6.8	0.6	ml
Glycerol	5.0	ml
10% (w/v) SDS	2.0	ml
2-mercaptoethanol	0.5	ml
1% Bromophenol blue	0.5	g

One part of sample buffer is added to four parts of sample. The mixture is heated for 5 min in boiling H_2O before loading to the gel.

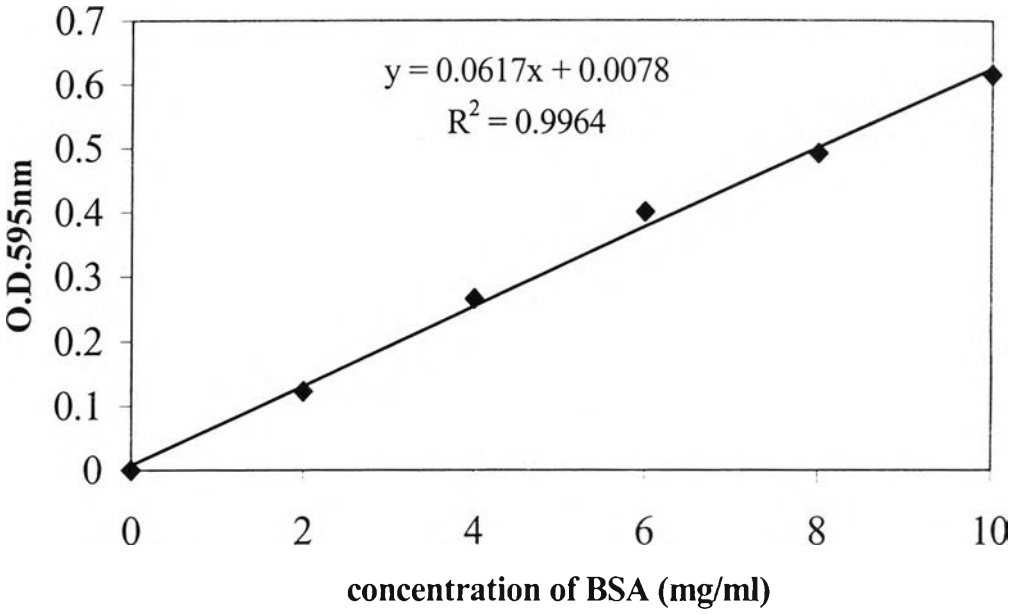
17. Electrophoresis buffer (25 mM Tris, 192 mM glycine and 0.1% SDS)

Tris (hydroxymethyl)-aminometane	6.06	g
Glycine	14.4	g
SDS	1.2	g

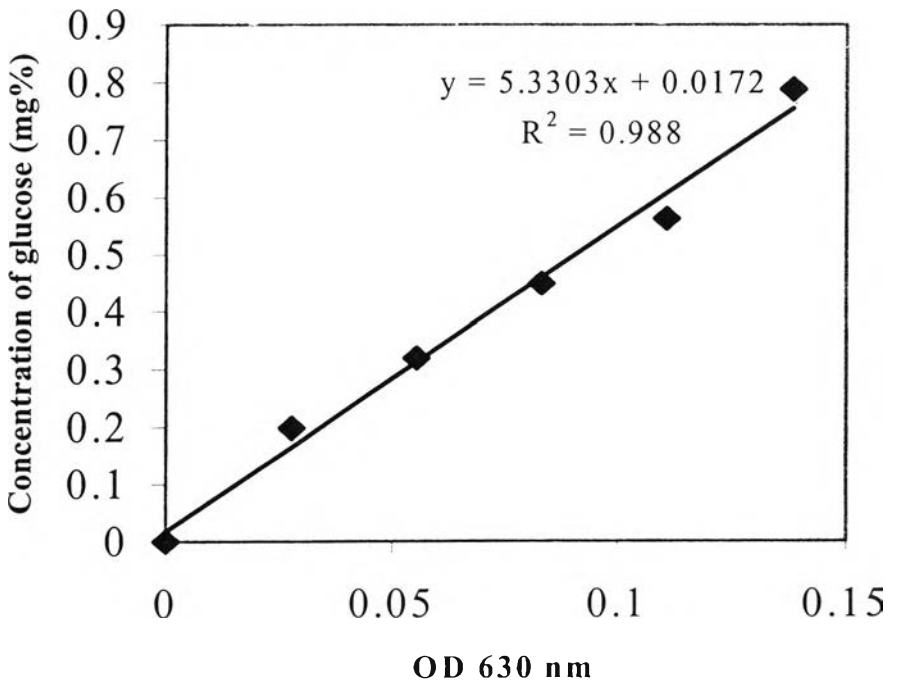
Adjust volume to be 1,000 ml with dd-H_2O

APPENDIX B

1. Standard curve for protein determination by Bradford's method

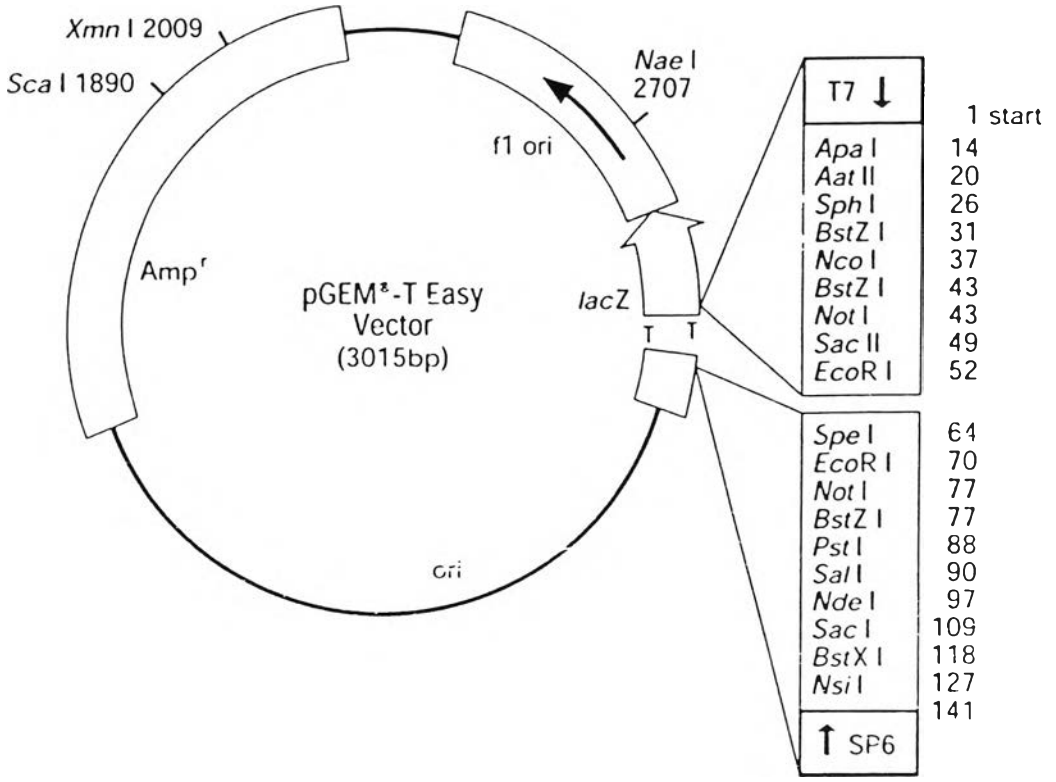


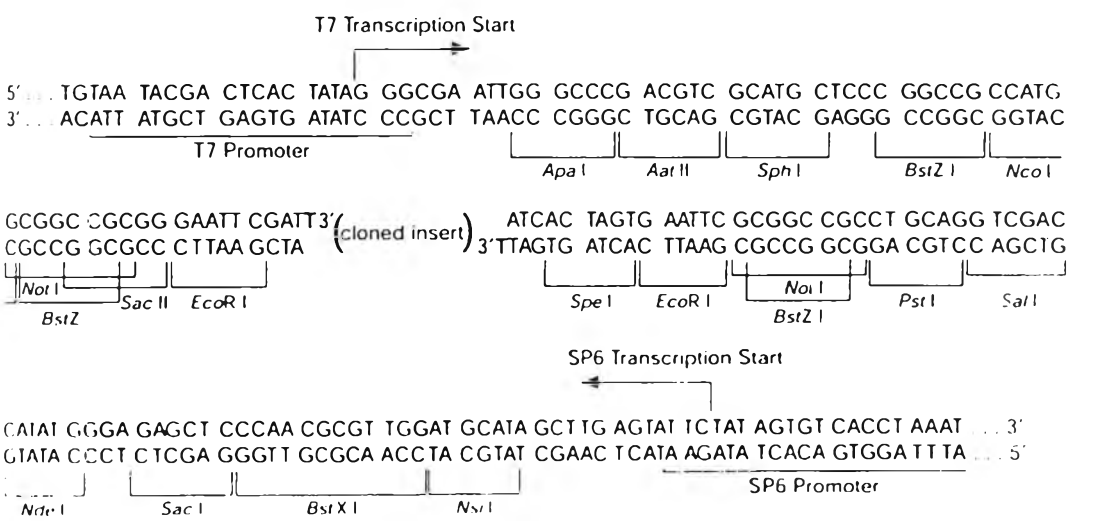
2. Standard curve for glucose determination



APPENDIX C

Physical map of pGEM®-T easy vector and multiple cloning sites





APPENDIX D

Nucleotides and deduced amino acid sequences

ATTGGGCGATCCGACCACTCTTCACATGTGCAAGGTCCCCTGTTTAGACCATTCGGACCAGG
TGCCTGTTTGCAATTTCTGTTTCAAGGACACTATTACCCTGTCCCTTTGGGTTCAGTCTAGG
ACGGGATCCTGATATTGATTCTGATTCAGGATCCCAACCCTCAGACAGGCCGTGCCAGGTTA
TCTTTGGCACGACCCGAACCCTCACGAGGCTTGGTGATATATACATGTGTCCATCCTGACAC
ATGTTCTTCAGAGGTCAATAGAGCCAAAGATCCTCATCCTTGATTATCACTGAAGGGAAGTC
GTCGATGACATCCTCTTCACATATGTCTTCATCATAAGCCCCGTTAAGGAACGCTGCAGCCA
TACAGGTCTGCTCATGTC**CTCGCAGCTT**

Figure D.1 Nucleotide and deduced amino acid sequences of clone 1. Bold letters indicate the regions of primers.

ATTGGGCAGATCAGTTTTCCGTGTTTTTTTTCAGAAAATAGGATCAGAAGTCATACATCCGG
TGTTCTGTCTGACAAGTTACGAGTTTATTGTTCCTGAATGTAGAGTTAAATTTGTGAATGNA
CGCCATGCTGAGGAGTGTTGTTCAGCCACGTAGATTCACGTGTGTGTGTGCGTGTGTGTGTG
TGTGTGTGTGTGTGTGTGTGTTTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTA
TGTGTGTATGTGTGTGTGTGTGTGTGTGTACATACGTTTGTGTATGTGTAGATATGTGAA
GGAATGAAACCTACAATGCATGACATGCATGGAGAGAGGAACGAGTTTTGAAAACTTCCTAT
AGCTTCATCCCCGCCACTCCATTGCC**CTCGCAGCTT**

Figure D.2 Nucleotide and deduced amino acid sequences of clone 2. Bold letters indicate the regions of primers.

AGGCCGCTTAGAAAAGTGAAACAACAAAGCAGACAGTCAACATTCTCAGCCGTTCGCGCTTC
AAGACATGTAGATTTCAGTAGCTCCTTGTAATTGAGGTACGATTCACTTCCTTGCATGTCGT
TTACTCCTCGTTTAACCTGTTAATACGTTTCGTGTAACTAAATAGCTAGTTCTTACTTCCAT
GTTTTGCTTTCTACATTGTTTTTATGTGAATAACTAGTTACTATCGTTTTGCTCTGTCAGTG
AATAACTTGTAACTACTTTGATTTGTTTTTTCTAAACTGTTTTTGCTCTGAAGTAAATAACT
AGTTACTCCTTCGAATAGGACCTTAAACAAACACATTTTTACTCTGCTGTCCTCCAGGATTA
TATCCGTTAAAAGCCCCATTCAGAATGCCGGGAAAAAAATGCAAATTTTTTTAACGGTTATT
TCCACCAGCTAATTTCCCCTTGCCTCT**TAAGCGGCCT**

Figure D.3 Nucleotide and deduced amino acid sequences of clone 3. Bold letters indicate the regions of primers.

ATTGGGCGATCATAGTTTTCCATCCCGTTCTGTATGTTTAGCAGTTCTAAGTCACTTCTGCC
CATATTGAGATCTTTGTTCAGGGGTATACAAAGGCAGAAACCTTTGTCTACCCTTGCTTCTC
TTTCCCTCTATCTTTCCTATTAACACTAAGTTTTCCAATCCTTTACATTTCATTTTTTTTAA
CGGTAGGTTCATGTCTGAGCCACCGTAGTCACAGTATGATACTTAATTGTCGGTTTCATGTT
GTGATGCTCTTGGAGTGAGTACGTGGTAGGGTCCCCAGTTCCTTTCCACGGAGAGGCATA**TA**
AGCGGCCT

Figure D.4 Nucleotide and deduced amino acid sequences of clone 4. Bold letters indicate the regions of primers.

AGGCCGCTTAGAAAAGTGAAACAACAAAGCAGACAGTCAACATTCTCAGCCGTTCGCGCTTC
AAGACATGTAGATTTCAGTAGCTCCTTGTAATTGAGGTACGATTCACTTCCTTGCATGTCGT
TTACTCCTCGTTTAACCTGTTAATACGTTTCGTGTAACTAAATAGCTAGTTCCTACTTCCAT
GTTTTGCTTTCTACATTGTTTTTATGTGAATAACTAGTTACTATCGTTTTGCTCTGTCAGTG
AATAACTTGTAACTACTTTGATTTGTTTTTTCTAAACTGTTTTTGCTCTGAAGTAAATAACT
AGTTACTCCTTCGAATAGGACCTTAAACAAACACATTTTTACTCTGCTGTCCTCCAGGATTA
TATCCGTTAAAAGCCCCATTCAGAATGCCGGGAAAAAAATGCAAATTTTTTTAACGGTTATT
TCCACCAGCTAATTTCCCCTTGCCTCT**TAAGCGGCCT**

Figure D.5 Nucleotide and deduced amino acid sequences of clone 5. Bold letters indicate the regions of primers.

ATTGGGCGATCCGACCACTCTTCACATGTGCAAGGTCCCCTGTTTAGACCATTCGGACCAGG
TGCCTGTTTGCAATTTCTGTTTCAAGGACACTATTACCCTGCCCCTTTGGGTTCAGTCTAGG
AAGGAATCCTGATATTGATTCTGATTCAGGATCCCAACCCTCAGACAGGCCATGCCAGGTTA
TCTTCGGCACGACCCGAACCTTCACGAGGCTTGGTGATATATACATGTGTCCATCCTGACAC
ATGTTCTTGAGAGGTCAATAGAGCCAAAGATCCTCATCCTTGGTTATCACTGGAGGGAAGTC
ATCGATGACATCCTCTTCACATATGTCTTCATCATAAGCCCCGGTTTAAGGGAACCGGCCTT
GGAAGGCCATTACCAGGGTCCGTTCATTGTC**CTCGCAGNTT**

Figure D.6 Nucleotide and deduced amino acid sequences of clone 6. Bold letters indicate the regions of primers.

ATTGGGCGATCAAGCCTCAGCAATATCATTAGCATTTGCCTCATATTATCTCTAGTGCCTGA
AGTTTCTCCTTCATACCTATCTTGGCATTAAAATCTCTCATAATTATTTAATTATTGTATTT
TCCTCTATCGCAGGCTAAATGTATATCTCCATAGGAGCCCTCTATTTCTTTATCAACCTTGG
CTACAGGTTGGGACGTAAACTTTAAGTTGTACCTATTGTTTAGTTTTATTGTTACTGTAGCT
ACTCTCTCGCTTACACAACAGAATTCCACGATATTCTTTTCTAGGCGTTTGTGAACATGTCC
ATCTCTTGGTACTTTCTGTTCTTCTCCAAGTCTTCAAACTTCACAGAGGCCTACAATATCGT
ATTTAATGAAGGGAAGTTCTCCAAGCAATGCCAGTGAGTTGGATTCAGTTCTC**ATCGCCCAA**
T

Figure D.7 Nucleotide and deduced amino acid sequences of clone 7. Bold letters indicate the regions of primers.

ATTGGGCGATACAAACAGACAAATAGAGTTCCATGAAATACAGAATAAGAAAATAAGAACAT
TGAAATGTTTGTCCTTCACTGTCTTTCACAGACTTAAAGAATCTTTTCCTTTGTGCAGCAAC
TATATGTAGTCATTATATCGAGGTGTTCATGTCTACAGGTAAATATAGAAAATATAAAAACG
AAAATGAAAGAAGTGAAAACACCTAACACGTTACCATAGCAATCCAAACGGCTAATGGCAAC
TCTTCGGGTTGACAATATGGCAGGTGGGCACGGGCATTCTCGATTCACAACTCACGAATAGA
AACAGTCTAAGAAGGGGACTATAGCTTCTTAGATAAAAATGGCTGCTGACTGGGGATCTGCA
GCCGCTACGCTGTC**ATCGCCCAAT**

Figure D.8 Nucleotide and deduced amino acid sequences of clone 8. Bold letters indicate the regions of primers.

ATTGGGCGATCAAGCCTCAGCAATATCATTAGCATTTGCCTCATATTATCTCTAGTGCCTGA
AGTTTCTCCTTCATACCTATCTTGGCATTAAAATCTCTCATAATTATTTAATTATCGTATTT
TCCTCTATCGCTGGCTAAATGTATATCTCCATAGAAGCCCTCTATTTCTTTATTACTTTGGC
TACAGGTTGGGACGTAAACTTTAAGTTGTACCTATTGTTTAGTTTTATTGTTACTGTAGCTA
CTCCCTCGCTTACACAACAGAATTCCACGATATTCTTTTCTAGGCGTTTGTGAACATGTCCA
TCTTTTAGTACTTTCTGTTCTTCTCCAAGTCTTCAAACTTCACAGAGGCCTACAATATCGTA
TTTAATGAAGGGAAGTTCTCCAAGCAATGCCAGTAAGTTGGATTCAGTTCTC**ATCGCCCAAT**

Figure D.9 Nucleotide and deduced amino acid sequences of clone 9. Bold letters indicate the regions of primers.

ATTGGGCGATGTCATTTACACGTGAATATGTAAGTGTATTTGGAATTATTCATCCCAGGGTA
ACACTTATTAATTTCACAATGATGTAGTTTTCGACGCGTTACTCATTACCGAGTATACCATG
TTATCAAAATCTACATTTGGCTAATACCTGTATTGGAGCCTTTAATTCCCTGGCCCCTNAAA
AANTGGCTTTCCCGNATGGGTTATATAACCCGCGTTGGGGCTCGTGTANTCATCNGTGCGCG
CGNGAGAGATGCATCTACGTTACTATTANTCCTTACTATTATCATCATGTACGCGCCTGTAT
GATCGAGTGCTCGGCCCTCGCGNTCAGTAAAGTATC**AGCCATACTA**

Figure D.10 Nucleotide and deduced amino acid sequences of clone 10. Bold letters indicate the regions of primers.

ATTGGGCGATCATGATTACAGTCTTACATATATCTGAATTTCCGTAAGATCCTTTAAAAAAA
ATCTACGGATTTGGTTCGCGAAAATTGAAGTATCTCTATACGGGTATCAGGCAACCCACTTT
TGCACCTGTATAGACTCAGCCTATCACAAGGAATCATCCCAAGCTCCTGGACTAAAATCTTA
ATCATTCCTATACCTTAAAGCTTATTACTTGGACCAATTAACCAGCGGCCCAATTTGNNAAA
GGNTATTCTTGGAACCAGGCTATATGTGTACCGCGCNNTAATGTGTNAATGTATATCCCCAC
AAACGTGTATGGATTTCTCC**CAGGCGTCGA**

Figure D.11 Nucleotide and deduced amino acid sequences of clone 11. Bold letters indicate the regions of primers.

ATTGGGCGATGGAGGCGAAGATGTAATTCCAATTCCCATCTATTGTTTGGGAATAAAGCCCA
TCAAGATTGAATTGAAATTTTTGATAATTTGACTTTGATTTAGATGACTCCCAGTCTGTTTG
TTTATACCGGAAAGATCGTCTTTCTTTGTTTAGTATTGGTGATGTTAAAATTTTAATTATTG
TGGGTATGTGGTCTGAACCAACGTTCTGACCGGTTTGTAGGTGTGTGTGGTTCCAGAGAACG
AGAT**CAGGGCGTCG**

Figure D.12 Nucleotide and deduced amino acid sequences of clone 12. Bold letters indicate the regions of primers.

ATTGGGCGATGTATGTGCAGGTGTTGATTCTTTTGAGTCTTTTATCTTGTCCTTATTTTACT
TCTACGTTTTCTTGAGTTCCCCCGTTTTTCCAGTTTTCCGATTCTTTTTTCTTGATTTCCTG
CTATCTGTTTTACTCATTTTAGTGTTTTATTCACGATGTTTTAATCTTAACTAGTATCTGTT
TTCTTGTGGTTTAACATATTAGAACCTGAAGAGTTAGACACGGGGACACAGTCACTTTAACG
AATCTAGGGTTGGCAGTACTGCAGCACTATACCCTAACCCCCCTCCCCCCACCACTAGC**CAG**
GGCGTCG

Figure D.13 Nucleotide and deduced amino acid sequences of clone 13. Bold letters indicate the regions of primers.

ATTGGGCGATCTAACCCTGCCAATTCCTATCTGGGAACTGACTTCTAATCTTCCTAGTTAAG
TACGACTGTCACAAACAAGGCCGCTTGACCTATTCCAGGCAGGACGAACAGTTGAATATAGG
TATACTCCCGCTGCAGATTGTTGTATTATGTGGATCTAATGGCAATTTTGCAACAAATTACA
ACTCAGTACAGCTCTCTACAGACCACCTTCCATCGACAGCGTATCTTTAGTGTTGACGCATC
CC**CTCGACGC**

Figure D.14 Nucleotide and deduced amino acid sequences of clone 14. Bold letters indicate the regions of primers.

AATTGGNGCGGATATNATTCTATACAATCTCTTTCCCCACAACACGCTCTAGAGATACGCCC
ATATAGTGACACAATATCACTTACCACTAAATATATTTTTTTTGCAAGGCCATTTTCAAGGT
TAATTCCCAAACCATTCCACCACCCAACCCATGAGAAATCAACCACCAAACATTATCACTAA
CCACAAAGACCACTTATTGTATGAGACACCACCCTCAGGCACACATGCACGGTGAATCAGTG
GTACGACCTGTGTTCTCGCTGAGCCCGAACGACCTAACTGC**CCTCGACGC**

Figure D.15 Nucleotide and deduced amino acid sequences of clone 15. Bold letters indicate the regions of primers.

ATTGGGCGATGTCATTTACACGTGAATATGTAAGTGTATTTGGAATTATTCATCCCAGGGTA
ACACTTATTAATTTCACAATGATGTAGTTTTCGACGCGTTACTCATTACCGAGTATACCATG
CTATCAAAATCTACATTTGGCTAATACCTGTACTGAGCTTTATTCCTGCCCCTAAGAAGTTG
CTTTTCCTGATTGTTTTATAACCGCTTGGCTTGATAATATTGTGCTTGCTTGAGAGATCAGT
CTAGATTTCTCTAATATTATCATTTGTACCGTATTATGATCTGGGCCTCGGTTCAGTTAGT**A**
TCGCCCAAT

Figure D.16 Nucleotide and deduced amino acid sequences of clone 16. Bold letters indicate the regions of primers.

ATTGGGCGATACTAACTGAACCGAGGCTAGATCATAATACGGTACAAATGATAATATTAGAG
AAATCTAGGTTGATCTCTCAAGCAAGCACAATATTATCAGGCCAAGCGGTTATAAAACAATC
CGGAAAAGCAACTTCTTAGGGGCAGGAATAAAGCTCAGTACAGGTATTAGCCAAATGTAGAT
TTTGATAACATGGTATACTCGGTAATGAGTAACGCGTCGAAAACTACATCATTGTGAAATTA
ATAATTGTTACCCTGGGATGAATAATTCCAAATACACTTACATATTCACGTGTAAATGAC**AT**
CGCCCAAT

Figure D.17 Nucleotide and deduced amino acid sequences of clone 17. Bold letters indicate the regions of primers.

ATTGGGCGATGTCATTTACACGTGAATATGTAAGTGTATTTGGAATTATTCATCCCAGGGTA
ACAATTATTAATTTCACAATGATGTAGTTTTCGACGCGTTACTCATTACCGAGTATACCATG
TTATCAAAATCTACATTTGGCTAATACCTGTACTGAGCTTTATTCCTGCCCCTAAGAAGTTG
CTTTTCCGGATTGTTTTATAACCGCTTGGCCTGATAATATTGTGCTTGCTTGAGAGATCAAT
CTAGATTTCTCTAATATTATCATTTGTACCGTATTATGATCTAGCCTCGGTTCAGTTAGT**AT**
CGCCCAAT

Figure D.18 Nucleotide and deduced amino acid sequences of clone 19. Bold letters indicate the regions of primers.

ATTGGGCGATGCAGGCGAAGATGTAATTCCAATGTCCTTCTATTGTTTGGGAATCAAGCCCT
TCAAGACTAAATTCAAAGTTATGATTATCTAATTTAGATTTAAACGACTCCCAGTCTGCTTG
TTTATACCGGAAAGATCGTCTTTCTTTGTTTACTATTGGTGATGTGGAAATCTATAATATGA
TAGGTATGTGGTCTGAACCAACGTTCGGACCGGGTTGTAGGTGTGTGTGATAGGCAAGTGTT
GCTCTGAT**CAGGGCGTCG**

Figure D.19 Nucleotide and deduced amino acid sequences of clone 20. Bold letters indicate the regions of primers.

ATTGGGCGATGAAAGTTCTATTGGCACGGTGAGCAGCATAATACACTTGCATGGCCAGACTG
TTTGAGTCACAGTCCACAGTCCCACAGACTTTATTCTGATCATTTAAGTTACTAAAATTGAG
ACCTCCCTTAAGGACAAAGTATACTGGAGGTCCAACACTTAGGTACTTTCCTAGATACTCAA
AATATTTTTGTAGGTACGAGTCTTCTGGCATAGACAATTCTTGATCAAGACCTATGTCAATC
ATTGGCAGAACAGCAATGCTCGAGAACAGCCATCCTACAAACAAGACCACCACAACTGGTCT
GGCAATGCGCGACAAAAGCATGGGAGCATAGACGAGTTTGAAGAATTTCTGTATGGGGCCCT
CAGGTGACGTCCCTTCCTTGTTTGAT**CCCTCGACGC**

Figure D.20 Nucleotide and deduced amino acid sequences of clone 21. Bold letters indicate the regions of primers.

ATTGGGCGATCTAACCCTGCCAATTCCCATCTGGGAACTGACTTCTAATCTTCCTAGTTAAG
TACGACAGTCACAAACAAGGCCGCTTGACCTATTCCAGGCAGGACGAACAGTTGAATATAGG
TATACTCCCGCTGCAGATTGTTGTATTATGTGGATCTAATGGCAATTTTGCAACAAATTACA
ACTCAGTACAGCTCTCTACAGACCACCTTCCATCGACAGCGTATCTTTAGTGTTGACGCATC
CCCTCGACGC

Figure D.21 Nucleotide and deduced amino acid sequences of clone 22. Bold letters indicate the regions of primers.

AGGCCGCTTAGAAAAGTGAAACAACAAAGCAGACAGTCAACATTCTCAGCCGTTCGCGCTTC
AAGACATGTAGATTTCAGTAGCTCCTTGTAATTGAGGTACGATTCACTTCCTTGCATGTCGT
TTACTCCTCGTTTAACCTATTAATACGTTTTGTGTAACTAAATAGCTAGTTCCTACTTCCAT
GTTTTGCTTTCTACATTGTTTTAATGTGAATAACTAGTTACTATCGTTTGGCTTTGTCAGTG
AATAACTTGTAACTACTTTGATTTGTTTTGTTTTCTAAACTGTTTTTGCTTTGAAGTAAATA
ACTAGTTACTCCTTCGAATAGGACCTTAAACAAACACATTTTTACTCTGCTGTCCTCCAGGA
TTATATCCGTT**AAAAGCCCCA**

Figure D.22 Nucleotide and deduced amino acid sequences of clone 24. Bold letters indicate the regions of primers.

AACGGGCAGCCGACGTGGTAGCGCTGTTGGTGTGTGGTGTGAAGGGTCAGTCATCATATGTT
TGCTGGAGTCAGGGATTCACTGCCACTATCCGAAATGCAGTCTATGGGGCGCTTGCTGGTGA
TGGCGAGAGGGAAGTCAGTTGAGTGTAATGCGTCAGCTTCGTTCGGCAATGCTCGATGCTGG
TGTGGGACAAGCGCTAGCCAGTGATCCATAGTCGGGTAATCATCAACGTCATCTTCGCTGAT
CGGCAAAACCGTCTCGGCATCAACAAATTTGAAGGTCCTGAGTAATCCATACCTCCCCCTCT
GCAGCGCGCGAGGGGGAGGCACTGACGTCACGAGCGGGTATGCAATGACGGGCAAGCACGTT
ACGCCCAGCTCACTGCGCATGCGCAGGAGCGGTAGCCTCGAGCAGGGAAGGCTTGTACCCGA
CGAGTTTATTTCAAATCTCCAGGCTAGGTCTACACCCTCTTCCTGGGGCACTAATACGTTCT
CTGGGTCTTACTAGTATCCTGTGGAACGAATAGTTG**CTGCCCGTT**

Figure D.23 Nucleotide and deduced amino acid sequences of clone 28. Bold letters indicate the regions of primers.

ATTGGGCGATACACCGCATGCATAATAAAATGACGTGGGATCCGACTGTAGCAAACAGATAC
GGATTTGTCAATAAAACGTTAAAACCCGAAATCCACGATGAAGTGTATAATGTAGTACCATC
ATCGGGGGGAAGGGATGGGGAAATATTTCAATCCATACAATATGACTTTTTGTATGGAATAA
TCACAAAACAATATCTAAAAAATCTACGCTCTGATTTCAAAGCACGTGATTACGAAACCCAA
CATAATCCGACCTGAAATTGAGTGGGATGTTGATGTACGATATTTTCGCCTATACCTCAC**AT**
CGCCCAAT

Figure D.24 Nucleotide and deduced amino acid sequences of clone 29. Bold letters indicate the regions of primers.

ATTGGGCGATGTCATTTACACGTGAATATGTAAGTGTATTTGGAATTATTCATCCCAGGGTA
ACACTTATTAATTTCACAATGATGTAGTTTTCGACGCGTTACTCATTACCGAGTATACCATG
TTATCAAAATCTACATTTGGCTAATACCTGTACTGAGCTTTATTCCTGCCCCTAAGAAGTTG
CTTTTCCTGATTGTTTTATAACCGCTTGGCCTGATAATATTGTGCTTGCTTGAGAGATCAAT
CTAGATTTCTCTAATATTATCATTTGTACCGTATTATGATCTGGCCTCGGTTCAGTTAGT**AT**
CGCCCAAT

Figure D.25 Nucleotide and deduced amino acid sequences of clone 30. Bold letters indicate the regions of primers.

ACTAGTGATTGCGTCGAGGGCAATTAAACGACAAGCTGTGAGGGCATCCAGGTTGTTGCAGT
TTTCTGTTGCAAGTTTTCTAGCAAGAGATGCAATGGCTTTGCAAAGGTCATTTGCAGCTGAC
CCAAACTTGCTGCTGCTCAGGATGGTTTTCCATGCCTTTGCGTCGAGTCCAGAAGGTCCAGC
TGCACCATGTGTATGCAATGCATGTGTTCTTATC**TTTTCGCCGG**

Figure D.26 Nucleotide and deduced amino acid sequences of clone 52. Bold letters indicate the regions of primers.

ACTAGTGATTATTGGGCGATACACCGCATGCATAATAAAATGACGTGGGATCCGACTGTAGC
AAACAGATACGGATTTGTCAATAAAACGTTGAAACCCGATATCCACGATGAAGTGTATAATG
TAGTACCATCATCGGGGGGAAGGGACGGGGAAATATTTCAATCCATACAATATGACTTTTTG
TATGGAATAATCACAAAACAATATCTAGAAAATCTACGCTCTGATTTCAAAGCACGTGATTA
CGAAACCCAACATAATCCGACCTGAAATTGAGTGGGATGTTGANTGTACGATATTGTCGCCT
NTACCTCACAGTCGC**CCAATAATCG**

Figure D.27 Nucleotide and deduced amino acid sequences of clone 58. Bold letters indicate the regions of primers.

ACTAGTGATTATTGGGCGATAACATATGATACCAAATCTTAAGACATCAACAACAATAATAA
AGATAATAGCCAGTGATCCATAGTCGGGTAATCATCAACGTCATCTTCGCTGATCGGCAAAA
CCGTCTCGGCATCAACAAATTTGAAGGTCCTGAGTAATCCATACCTCCCCCTCTGCAGCGCG
CGAGGGGGAGGCACTGACGTCACGAGCGGGTATGCAATGACGGGCAA**G**CACGTTACGCCCAG
CTCACTGCGCATGCGCAGGAGCGTAATAATTAATGTTGCTGCTGATGATTACAATGATATTG
ATAATGATATCAACAACG**GTAATAGTAA**

Figure D.28 Nucleotide and deduced amino acid sequences of clone 62. Bold letters indicate the regions of primers.

Penaeus monodon clone TUZX4-6:86 microsatellite sequence

Length =517

Score = 149 bits (75)

Expect = 3e-33

Identities = 90/95 (94%)

Strand = Plus / Plus

```
Query: 171    ttttaacggtaggttcatgtctgagccaccgtagtcacagtatgatacttaattgtcggt 230
              ||||||||||||||||||||||||||| |||| ||||||| |||||||||||||| | |
Sbjct: 168    ttttaacggtaggttcatgtctgagccgccgtggtcacagcatgatacttaattgtagtt 227

Query: 231    ttcatgttgtgatgctcttggagtgagtacgtggt 265
              ||||||||||||||||||||||||||||||||||
Sbjct: 228    ttcatgttgtgatgctcttggagtgagtacgtggt 262
```

Figure D.29 The results of blast in GENBANK database and the high significant alignment of a deduced amino acid sequence of clone 4. The website (http://www.ncbi.nlm.nih.gov/blast) was used in this analysis.

Penaeus (Litopenaeus) vannamei microsatellite TUMXLv10.221 sequence

Length = 600

Score = 93.7 bits (47)

Expect =3e-16

Identities = 83/95 (87%)

Strand = Plus / Plus

```
Query: 196 tttaagttgtacctattgtttagttttattgttactgtagctactccctcgcttacacaa 255
           |||||||||||||||||||||||||||| |||| | ||| ||||  |||||||||| |
Sbjct: 235 tttaagttgtacctattgtttagttttattattacagaagccactctttcgcttacacta 294

Query: 256 cagaattccacgatattcttttctaggcgtttgtg 290
           ||||||| ||||| |||||||||| | |||||||
Sbjct: 295 tagaattctacgatgttcttttctaagagtttgtg 329
```

Figure D.30 The results of blast in GENBANK database and the high significant alignment of a deduced amino acid sequence of clone 9. The website (http://www.ncbi.nlm.nih.gov/blast) was used in this analysis.

ENSANGP00000010415 [*Anopheles gambiae* str. PEST]

Length = 1548

Score = 146 bits (369)

Expect = 1e-34

Identities = 68/125 (54%)

Positives = 91/125 (72%) Frame = -2

```
Query: 375  GTSPEGPIQKFFKLVYAPMLLSRIARPVVVVLFVGWLFSSIAVLPMIDIGLDQELSMPED196
            G   EG + KFFK +Y P+++ R     V+++F GWL SSIAV P IDIGLDQELSMP D
Sbjct: 776  GNIGEGLLYKFFKSIYVPFVMKRPVRVAVMIVFFGWLCSSIAVAPHIDIGLDQELSMPGD 835

Query: 195  SYLQKYFEYLGKYLSVGPPVYFVLKGGLNFSNLNDQNKVCGTVDCDSNSLAMQVYYAAHR 16
            S++ KYF YL +YLS+GPPVYFV+K GLN+S +NDQN +CG   C ++SL+ Q+Y A++
Sbjct: 836  SFVLKYFRYLQQYLSIGPPVYFVVKNGLNYSTMNDQNLICGGQYCNLDSLSTQLYIASKQ 895

Query: 15   ANRTF 1
               T+
Sbjct: 896  PQSTY 900
```

Niemann-Pick type C1 disease protein [*Oryctolagus cuniculus*]

Length = 1286

Score = 128 bits (322)

Expect = 3e-29

Identities = 63/120 (52%)

Positives = 85/120 (70%)

Frame = -2

```
Query: 363  EGPIQKFFKLVYAPMLLSRIARPVVVVLFVGWLFSSIAVLPMIDIGLDQELSMPEDSYLQ 184
            E   ++FFK  Y+P+LL    RP+V ++FVG L  SIAVL  ++IGLDQ LSMP+DSY+
Sbjct: 823  ESYLFRFFKNSYSPLLLKDWMRPIVIAVFVGVLSFSIAVLNKVEIGLDQSLSMPDDSYVV 882

Query: 183   YFEYLGKYLSVGPPVYFVLKGGLNFSNLNDQNKVCGTVDCDSNSLAMQVYYAAHRANRT 4
             YF+ LG+YL  GPPVYFVL+ G N+++L  QN VCG  +CD++SL  Q++ AA   N T
Sbjct: 883  DYFKSLGQYLHAGPPVYFVLEEGHNYTSLQGQNMVCGGLGCDNDSLVQQIFNAAQLDNYT 942
```

Figure D.31 The results of BLAST from GENBANK database and the high significant alignment of a deduced amino acid sequence of clone 21 (http://www.ncbi.nlm.nih.gov/blast)

APPENDIX E

Table E.1 Protein concentration in haemolymph thermal treatment for 6 h

(hours)	15°C	Control (27°C)	30°C	33°C	35°C
	13.95	50.00	80.60	53.40	73.40
	20.91	47.13	52.20	77.40	68.40
	12.95	41.62	63.80	64.20	53.94
	17.33	49.25	70.60	93.60	99.60
0	13.56	39.25	64.80	76.20	79.80
	28.81	39.69	66.40	72.96	75.03
	6.34	49.66	61.40	93.00	59.80
	12.25	42.75	69.60	54.20	78.20
	24.26	46.63	91.80	54.60	85.40
3	9.42	39.34	53.60	90.40	72.20
	24.56	48.69	64.68	70.64	72.88
	13.05	43.51	88.80	66.20	54.60
	23.35	35.63	*61.60*	*52.60*	*62.20*
	9.42	40.29	62.60	70.20	54.80
6	23.15	46.43	58.60	69.20	98.40
	21.03	41.48	60.48	63.76	66.12
	16.87	47.26	68.40	67.00	62.20
	17.65	37.55	79.00	72.60	56.20
	18.00	44.26	62.80	69.40	70.20
12	30.42	39.96	71.40	81.40	93.80
	20.23	38.98	68.12	68.64	69.00
	25.17	42.20	60.60	75.20	82.20
	27.78	39.32	59.40	83.40	41.80
	36.84	42.41	73.20	48.80	57.80
24	13.86	39.77	58.60	69.80	89.80
	26.94	44.03	66.32	65.88	66.60
	22.44	43.88	51.60	71.00	55.80
	31.18	33.26	52.00	67.00	86.20
	20.22	38.66	76.20	48.60	62.20
72	29.31	37.24	72.60	54.30	45.80

Table E.2 Glucose concentration in haemolymph thermal treatment for 6 h

(hours)	15°C	Control (27°C)	30°C	33°C	35°C
	46.85	45.12	35.24	35.21	25.65
	20.91	27.5	25.69	30.15	30.33
	33.43	30.58	50.01	35.4	20.56
	19.12	40.01	20.69	40.26	37.51
0	23.59	25.67	30.14	30.58	55.01
	23.59	35.03	160.14	70.44	125.03
	34.33	22.51	30.99	75.65	55.26
	24.49	30.98	60.14	60.11	60.45
	37.91	40.01	30.59	45.63	45.7
3	12.86	32.56	20.98	70.32	90.11
	19.12	35.22	35.47	115.01	90.2
	23.59	30.54	35.67	75.34	90.47
	27.17	40.11	30.12	95.74	85.96
	100.52	40.68	25.26	65.11	105.51
6	19.12	25.12	10.25	80.02	85.03
	21.80	20.98	95.02	90.17	100.01
	19.12	25.47	120.14	100.03	80.19
	24.49	60.05	65.22	70.68	115.32
	22.70	30.24	40.8	90.03	105.12
12	24.49	50.11	55.48	95.05	90.94
	45.06	35.48	150.11	85.44	95.21
	40.59	30.29	65.28	90.04	100.34
	47.74	17.56	85.67	70.55	7.5.55
	43.27	45.13	5.97	60.33	85.64
24	30.75	40.65	30.56	95.84	80.88
	43.27	50.14	65.15	45.62	40.43
	45.06	20.69	40.55	60.25	60.51
	23.59	30.54	60.01	50.55	50.02
	36.12	30.68	37.53	35.44	45.06
72	11.07	40.16	60.33	55.28	90.81

Table E.3 Protein concentration of the haemolymph from heat-induced shrimps after *vibrio* exposure

hours	sample	control	heat induced	un-induced
0	1	46.6	63.3	49.1
	2	70	75.9	49.3
	3	44.6	60.2	42.4
	4	62.3	73.2	35.7
	5	32.9	64.3	64.2
	6	52.4	57.4	66.2
3	1	46.6	73.2	50.1
	2	33.7	67.4	60.5
	3	60.5	64.2	52.6
	4	46.7	71.9	44.2
	5	66.9	61.7	67.8
	6	64.7	71.7	47.6
6	1	51.5	74.2	52.6
	2	46.5	69.6	66.9
	3	60.9	58.8	53.9
	4	44.7	76.9	47.9
	5	71.1	88.7	67.8
	6	42.5	67.2	52.3
12	1	22.9	82.9	69.9
	2	64.9	72.9	68.4
	3	61.5	76.1	70.5
	4	52.8	64.6	52.7
	5	52.2	69.7	67.9
	6	50.1	52.1	47.8
24	1	59.1	73.5	42.5
	2	60.8	60.3	43.9
	3	30.6	71.7	68
	4	50.9	48.2	52.9
	5	58.1	74.5	53.7
	6	59.1	76.5	64.6
72	1	32.7	72.7	63.4
	2	47.2	50.5	49.5
	3	62.1	61.4	51.9
	4	55.4	56.7	62
	5	62.9	71.1	31.9
	6	42.7	64.9	53.1
120	1	36.1	58.8	63.8
	2	62.8	45.4	47.9
	3	53.2	66.7	56.2
	4	67.6	60.9	45.4
	5	41.3	64.7	42.1
	6	58.1	52.6	60.8
168	1	60.4	73.4	58.2
	2	73.9	35.5	61.6
	3	49.31	55.3	54.3
	4	36.3	34.2	30.6
	5	47.9	65.8	61.2
	6	40.9	43.3	52.6

Table E.4 Glucose concentration of the haemolymph from heat-induced shrimps after *vibrio* exposure.

hours	sample	control	heat induced	un-induced
0	1	32.54	43.27	40.59
	2	40.59	45.06	49.53
	3	37.91	44.17	34.33
	4	27.17	43.27	48.64
	5	38.80	54.01	42.38
	6	39.69	32.54	36.12
3	1	46.85	37.91	39.69
	2	25.38	46.85	42.38
	3	32.54	56.69	34.33
	4	42.38	59.37	ɔ1.32
	5	26.28	46.85	38.80
	6	39.69	55.80	37.01
6	1	35.22	53.11	40.59
	2	33.43	58.48	35.22
	3	50.43	46.85	34.33
	4	34.33	61.16	31.64
	5	41.48	62.06	50.43
	6	27.17	62.95	34.33
12	1	31.64	63.85	33.43
	2	48.64	53.11	39.69
	3	30.75	57.58	26.28
	4	41.48	53.11	39.69
	5	30.75	73.69	45.96
	6	33.43	66.53	47.74
24	1	46.85	48.64	38.80
	2	34.33	43.27	34.33
	3	29.85	49.53	32.54
	4	28.96	52.22	45.06
	5	35.22	55.80	43.27
	6	37.01	65.64	29.85
72	1	33.43	51.32	42.38
	2	35.22	48.64	38.80
	3	41.48	42.38	29.85
	4	33.43	37.91	47.74
	5	29.85	50.43	37.01
	6	52.22	45.06	33.43
120	1	28.07	28.96	43.27
	2	45.06	43.27	48.64
	3	45.06	31.64	41.48
	4	28.07	53.11	28.96
	5	36.12	38.80	31.64
	6	25.38	28.07	34.33
168	1	35.22	37.01	43.27
	2	25.38	33.43	34.33
	3	41.48	36.12	29.85
	4	37.91	41.48	38.80
	5	46.85	42.38	37.91
	6	24.49	28.96	32.54

BIOGRAPHY

Miss Kanchana Doungpunta was born on July 23, 1978 in Rayong. She graduated with the Bachelor Degree of Science in Department of Biology, Facultly of Science, Burapha University in 2000. She has been a graduate student in the Master' s Degree in Biotechnology program, Facultly of Science, Chulalongkorn University since 2004.

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