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APPENDIXS

APPENDIX A

Preparation for protein determination (Lowry *et al.*, 1951)

Solution A (0.5% copper sulfate, 1% sodium citrate)

Copper sulfate	0.5	g
Sodium citrate	1.0	g

Dissolved in deionized water to the total volume of 100 ml

Solution B (2% sodium carbonate, 1 N sodium hydroxide)

Sodium carbonate	20	g
Sodium hydroxide	4	g

Dissolved in deionized water to the total volume of 1 liter

Solution C

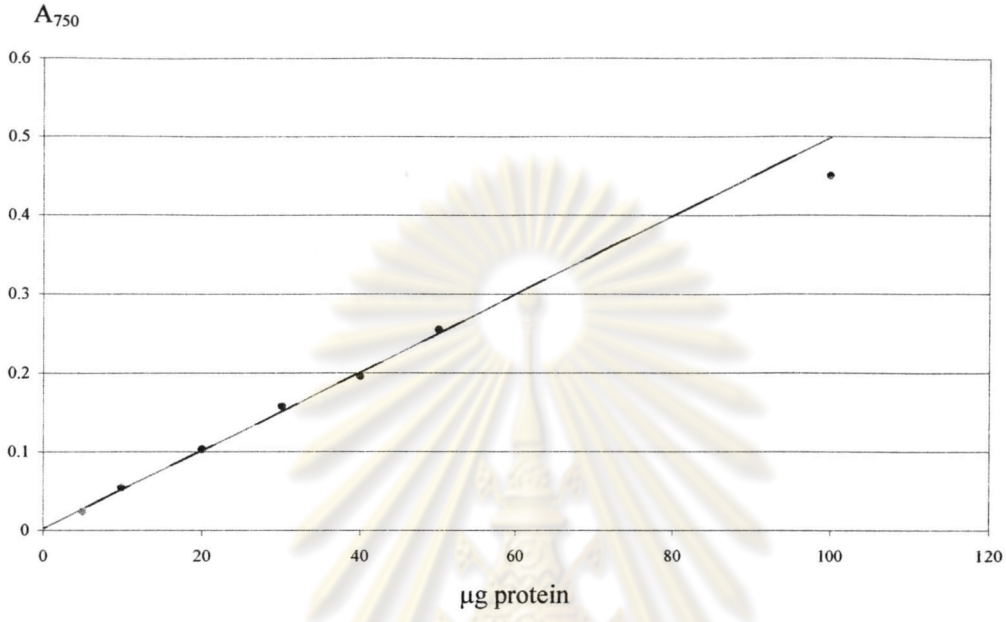
Solution A	1	ml
Solution B	50	ml

Solution D (1:1 diluted Folin-Ciocalteu phenol reagent)

Folin-Ciocalteu phenol reagent	10	ml
Deionized water	10	ml

APPENDIX B

Calibration curve for protein concentration determined by Lowry method



APPENDIX C

Preparation for SDS-polyacrylamide gel electrophoresis

1. Stock solutions

2 M Tris-HCl (pH 8.8)

Tris (hydroxymethly)-aminomethane	24.2	g

Adjusted pH to 8.8 with 1 M HCl and volume to 100 ml with deionized water

1 M Tris-HCl (pH 6.8)

Tris (hydroxymethly)-aminomethane	24.2	g

Adjusted pH to 8.8 with 1 M HCl and volume to 100 ml with deionized water

10 % SDS (W/V)

Sodium dodecyl sulfate (SDS)	10	g

Dissolved in deionized water to the total volume of 100 ml

50 % glycerol (V/V)

100% Glycerol	50	ml
Deinoized water	50	ml

1% bromophenol blue (W/V)

Bromophenol blue	100	mg

Brought to 10 ml with deionized water and stirred until dissolved and filtered aggregated dye.

2. Working solutions

Solution A (30% (W/V) acrylamide, 0.8 % (W/V) bis-acrylamide)

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

Dissolved in deionized water to the total volume of 100 ml and stirred until completely dissolved.

Solution B (4X separating gel buffer: 1.5 M Tris-HCl (pH 8.8), 0.4% SDS)

2 M Tris-HCl (pH 8.8)	75	ml

10% SDS	4	ml
Deionized water	21	ml

Solution C (4X stacking gel buffer: 0.5 M Tris-HCl (pH 6.8), 0.4% SDS)

1 M Tris-HCl (pH 6.8)	50	ml
10% SDS	4	ml
Deionized water	46	ml

10% ammonium persulfate

Ammonium persulfate	0.5	g

Dissloved in deionized water to the total volume of 5 ml

Electrophoresis buffer (25 mM Tris, 192 mM Glycine, 0.1% SDS)

Tris (hydroxymethly)-aminomethane	3	g
Glycine	14.4	g
SDS	1	g

Dissolved in deionized water to the total volume of 1 litre

5X Sample buffer (60 mM Tris-Hcl (pH 6.8), 25% glycerol, 2% SDS, 14.4 mM 2-mercaptoethanol, 0.1% bromophenol blue)

1 M Tris-HCl (pH 6.8)	0.6	ml
50 % Glycerol	5	ml
10 % SDS	2	ml
2-Mercaptoethanol	0.5	ml
1% Bromopenol blue	1	ml
Deionized water	0.9	ml

3. SDS-PAGE

10 % Separating gel

Solution A	3.3	ml
Solution B	2.5	ml
Deionized water	4.2	ml
10 % Ammonium persulfate	50	µl
TEMED	5	µl

5 % Stacking gel

Solution A	0.67	ml
Solution C	1.0	ml
Deionized water	2.3	ml
10 % Ammonium persulfate	30	µl
TEMED	5	µl

APPENDIX D

Preparation for phenol-sulfuric acid (PAS) staining solution

Fixative solution (7.5 % acetic acid)

Glacial acetic acid	15	ml
Deionized water	185	ml

Schiff's reagent

- Dissolve 1 g of Basic fuchsin in 200 ml of boiling deionized water, stir for 5 minutes and cool to 50 °C
- Filter and add 20 ml of 1 N HCl
- Mix and cool to 25 °C
- Add 1 g of sodium or potassium metabisulphite
- Let the solution stand in the dark for 12-24 hours.
- Add 2 g of activated charcoal, shake for 1 minute, filter and store at room temperature.

0.2 % aqueous periodic acid

Periodic solution		
Deionized water		

Destaining solution

(10% acetic acid for normal staining)

Acetic acid	10	ml
Deionized water	90	ml

(0.05 N HCl, 0.5 % metabisulphite for fasten staining)

1 N HCl	5	ml
Metabisulphite	0.5	g
Deionized water	95	ml

APPENDIX E

Preparation for isoelectric focusing gel electrophoresis

Monomer-ampholyte solution

30 % Acrylamide solution	0.9	ml
1.0 % Bis-acrylamide solution	1.25	ml
Ampholyte pH 3-10	0.243	ml
Deionized water	1.39	ml
50 % Sucrose	1.186	ml
EMED	2	µl
0.02 M Ammonium persulfate	39.5	µl

Fixative solution

Sulfosalicylic acid	4	ml
Trichloroacetid acid	12.5	ml
Methanol	30	ml

Staining solution

Ethanol	27	ml
Acetic acid	10	ml
Coomassie brilliant blue R-250	0.04	g
$CuSO_4$	0.5	g
Deionized water	63	ml

Dissolve $CuSO_4$ in water before adding the ethanol.

Destaining solution

First destaining solution

Ethanol	12	ml
Acetic acid	7	ml
$CuSO_4$	0.5	g
Deionized water	81	ml

Dissolve $CuSO_4$ in water before adding the ethanol.

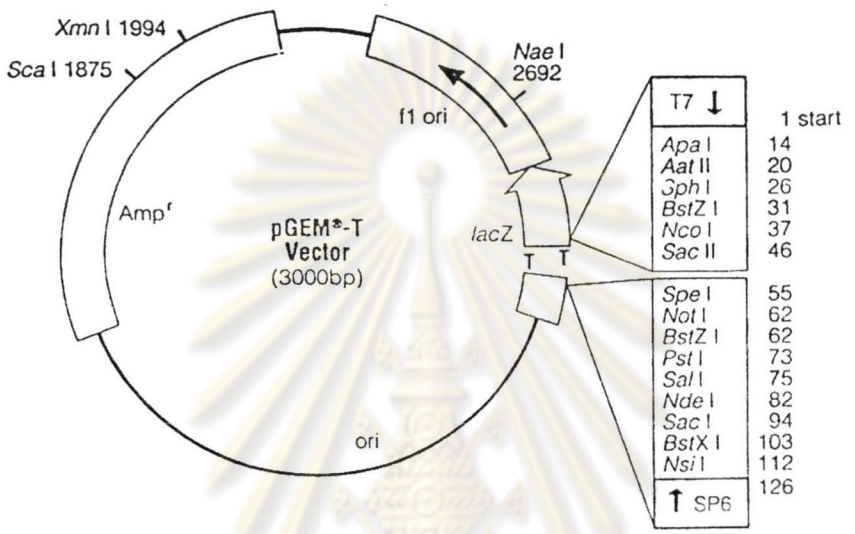
Second destaining solution

Ethanol	25	ml

Acetic acid	7	ml
Deionized water	68	ml

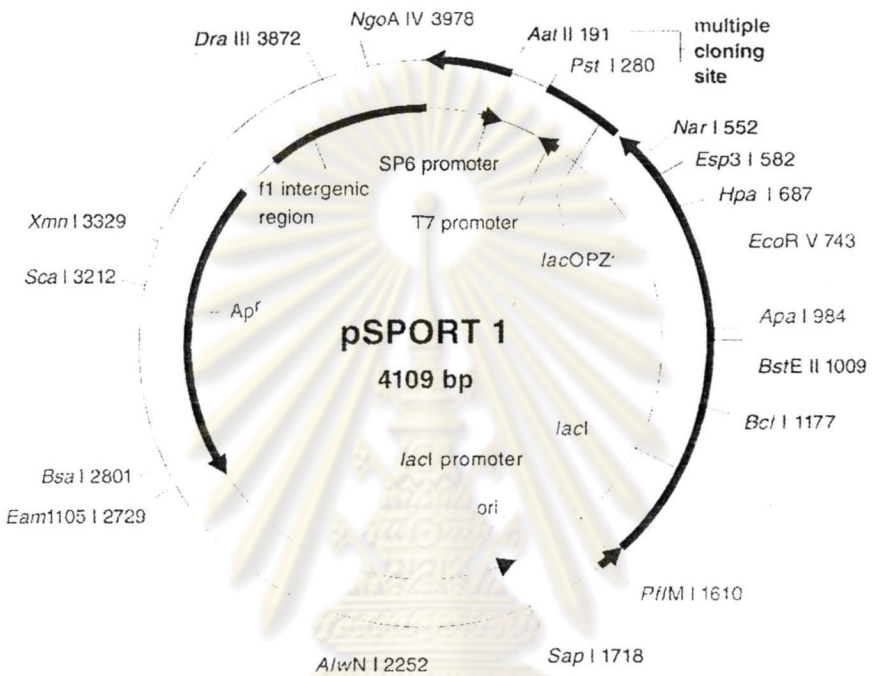
APPENDIX F

Restriction map and multiple cloning site of pGEM®-T Easy vector



APPENDIX G

Restriction map and multiple cloning site of pSPORT 1 vector



APPENDIX H

Nucleotide sequences of AmMRJP1 deposited in the GenBank.

```
DEFINITION  Apis mellifera major royal jelly protein MRJP1 (MRJP1) mRNA,
            complete cds.
ACCESSION   AF000633
VERSION     AF000633.1  GI:3676301

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TLERHNIRTVAQSDETLQMIASMKIKEALPHVPIFDRYINREYILVLSNKMQKMVNND
FNFDDVNFRIMNANVNELILNTRCENPDNDRTPFKISIHL"

BASE COUNT      451 a    285 c    265 g    428 t      1 others
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      121 tctttaaaca aatcattacc catccttcac gaatggaaat tctttgatta tgatttcggt
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```

APPENDIX I

Nucleotide sequences of AmMRJP2 deposited in the GenBank.

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            complete cds.
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VERSION     AF000632.1  GI:3676299

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DN"

BASE COUNT      539 a    266 c    291 g    448 t
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      661 agaccataaa ggtgatgctt taatcgtcta ccaaaatgcc gatgattcct tccatcgatt
      721 gacttccaac actttcgact acgatcccag atatgccaaa atgacgatcg atggagaaag
      781 tttcacactg aaaaatggaa tttgtggaat ggctcttagt cccgtgacga acaatcttta
      841 ttacagtcct ctcgcttctc acggtttgta ttatgttaac acggcaccat ttatgaaatc
      901 acaatttgga gaaaataacg tccaatacca aggatccgaa gatattttga acacgcaatc
      961 attggctaaa gcagtatcga aaaatggcgt cctcttcgtc ggacttgtag gtaattcagc
     1021 tgttggctgc tggaacgagc atcaatcact tcagagacaa aatttagaaa tggtcgctca
     1081 aaatgacaga acacttcaaa tgatcgcagg tatgaaaatt aaggaagagc ttccacattt
     1141 cgtaggaagc aacaaacctg taaaggatga atatatgtta gttttaagta acagaatgca
     1201 gaaaatagta aatgatgatt ttaatttcga cgatgtaaac ttccgaattt tgggtgcaaa
     1261 tgtaaaggaa ttaataagaa atactcattg cgtaaataac aatcagaatg ataacattca
     1321 aaatactaac aatcagaatg ataacaatca gaagaataac aagaaaaatg ctaacaatca
     1381 aaagaataac aatcagaatg ataattaagt tggtcgtttt tcaaaattgc attaaaatca
     1441 attaattatg atgtaaacta aatatctttt gaaatatttt ctcaatataa accaaatatt
     1501 ttgtaaaaat ctttttaaat tatattataa atgaataaaa tatt
```

APPENDIX J

Nucleotide sequences of AmMRJP3 deposited in the GenBank.

```
DEFINITION  A.mellifera mRNA for royal jelly protein (RJP57-1).
ACCESSION   Z26318
VERSION     Z26318.1  GI:1113118

translation="MTKWLLLVVCLGIACQDVTSAAVNHQRKSANNLAHSMKVIYEWK
HIDFDFGSDERRDAAIKSGEFDHTKNYPFDVDRWRDKTFVTIERNNGVPSSLNVVTNK
KGKGGPLLRPYPDWSFAKYEDCSGIVSAFKIAVDKFDRLWVLDSGLVNNNQPMCSPKL
LTFDLKTSKLVKQVEIPHNIAVNATTGMGELVSLAVQAIDRTNTMVYIADEKGEGLIM
YQNSDDSFHRLTSNTFDYDPRYTKLTVAGESFTVKNGIYGIALSPVTNNLYYSPLLSH
GLYYVDTEQFSNPQYEENNVQYEGSQDILNTQSFGKVVSKNGVLFLGLVGNSGIACVN
EHQVLQRESFDVVAQNEETLQMIVSMKIMENLPQSGRINDPEGNEYMLALSNRMQKII
NNDFNFNDVNFRILGANVDDLMRNTRCGRYHNQNAGNQNADNQNADNQNANNQNADNQ
NANKQNGNRQNDNRQNDNKQNGNRQNDNKQNGNRQNDNKQNGNRQNGNKQNDNKQNGN
RQNDNKRNGNRQNDNQNNQNDNNRNDNQVHHSSKLH"

BASE COUNT      701 a    289 c    339 g    501 t
ORIGIN
        1 gtcaattgga aaatatctgt attatcctag aaaaatgaca aagtggttgt tgctggtggt
       61 gtgccttggt atagcttgtc aagatgtaac aagcgcagct gtgaatcatc aaagaaaatc
      121 tgcaaataat ttggcacatt ctatgaaagt gatctacgaa tggaaacaca ttgattttga
      181 tttcggtagc gatgaaagaa gagatgctgc gattaaatct ggcgaatttg atcacacaaa
      241 aaattatcct ttcgatgtgg acagatggcg tgataagaca tttgtcacca tagaaaggaa
      301 caatggtgta ccttcttctt tgaacgtggt aactaataaa aagggcaaag gtggacctct
      361 tctacgacca tatcctgatt ggtcgtttgc caaatacgaa gattgctctg gaattgtgag
      421 cgctttcaaa attgcggtcg acaaatttga cagattatgg gttctggact caggtcttgt
      481 caataataat caacctatgt gctctccaaa attgttaacc tttgatctga aaacctcaaa
      541 attggttaag caagtcgaga taccacataa tattgccgta aacgccacca caggaatggg
      601 agaattagtt tcactagctg ttcaagctat agatcgtacg aatactatgg tgtacatagc
      661 agacgaaaaa ggcgaaggtt taatcatgta tcaaaactcc gacgattcct tccatcgatt
      721 gacttccaat actttcgatt acgatcccag atataccaaa ttgacagtcg ctggagaaag
      781 tttcacagtg aaaaatggaa tttatggaat tgcacttagt cccgtgacga acaatcttta
      841 ttacagccct cttctttctc acggtttgta ttatgttgat acggaacaat tcagcaatcc
      901 acaatatgaa gaaaataacg tgcaatatga aggatctcaa gatattttga acactcaatc
      961 attcggtaaa gtagtatcga aaaatggcgt ccttttcttg ggactcgtgg gtaattcagg
     1021 tattgcctgc gtgaatgaac atcaagtact tcagagagaa agttttgatg ttgtcgctca
     1081 gaatgaagag acacttcaaa tgatcgttag tatgaaaatc atggaaaatc ttccacaatc
     1141 cggcagaatt aatgatcctg aaggcaatga atatatgttg gctttgagta acagaatgca
     1201 aaaaataata aacaatgatt ttaatttcaa cgacgtaaat ttccgaattt tgggtgcgaa
     1261 tgtagatgac ttaatgagaa acactcgttg cggaagatat cacaatcaga atgctggcaa
     1321 tcagaatgct gacaatcaga atgctgacaa tcagaatgct aacaatcaga atgctgataa
     1381 tcagaatgct aacaaacaaa atggtaatag acaaaatgat aacagacaga atgataacaa
     1441 gcaaaatggt aacagacaga atgataacaa gcaaaatggt aacagacaga atgataacaa
     1501 gcaaaatggt aacagacaaa atggtaacaa acagaatgat aacaagcaaa atggtaacag
     1561 acagaatgat aacaagagga atggtaacag gcaaaatgat aatcaaaata atcagaatga
     1621 taataatcga aatgataatc aagttcatca ttcttcaaaa ttacattaaa tcaatcaatt
     1681 atcaattaaa atcaattaat taagatgtaa atcaaattat tttttaaaat attttttcga
     1741 tgtaaacaaa attttgtaaa atctttcatt atattataaa taaataaaat aaatatcgtt
     1801 ttcgcataaa aaaaaaaaaa aaaaaaaaaa
```

APPENDIX K

Nucleotide sequences of AmMRJP4 deposited in the GenBank.

```
DEFINITION A.mellifera mRNA for royal jelly protein (RJP57-2).
ACCESSION Z26319
VERSION Z26319.1 GI:433530

Translation ="MTKWLLLMVCLGIACQNIRGGVVRENSSGKNLTNTLNVIHKWKY
LDYDFDNDERRQAAIQSGEYDRTKNYPLDVDQWHNKTFLAVIRYNGVPSSLNVVSDKT
GNGGRLLQPYPDWSFAKYEDCSGIVSAHKIAIDEYERLWVLDSGLVNNTQPMCSPKLF
AFDLNTSQLLKQVEIPHDVATTGKGELVSLTVQAMDSTNTMVYMVDNKNTLIIYQNAD
DSFHRLSSHTLNHNSDKMSDQQENLTLKEVDNKVYGMALSPVTHNLYYNSPSSENLYY
VNTESLMKSENQGNDVQYERVQDVFDSQLTVKAVSKNGVLLFGLANNTLSCWNEHQSL
DRQNIDVVARNEDTLQMVVSMKIKQNVPQSGRVNNTQRNEYLLALSDRNQNVLNNDLN
LEHVNFQILGANVNDLIRNSRCANFDNQDNNHYNHNHNQARHSSKSDNQNNNQHNDQA
HHSSKSNNRHNNND"

BASE COUNT 560 a 301 c 285 g 466 t
ORIGIN
        1 gtcacttgta aaatatttgt aatatcctag aaaaaaaatg acaaaatggt tgctgttgat
       61 ggtatgcctt ggcatagctt gtcaaaatat tagaggtggc gttgttcgag aaaattcctc
      121 gggaaaaaac ttgacaaata cgttgaacgt gattcacaaa tggaagtatc tcgattatga
      181 tttcgataac gacgaaagga ggcaagctgc gattcaatct ggcgaatatg atcgtacaaa
      241 aaattatcct cttgacgtcg atcaatggca caacaagact tttctcgctg taataagata
      301 caatggtgtg ccttcctctt tgaacgtggt atctgacaaa actggcaacg gtggacgact
      361 tctacaaccg tatcctgatt ggtcatttgc caagtacgaa gattgctctg gaatcgtgag
      421 cgctcataaa attgctatcg acgaatatga gagattgtgg gttctggatt cgggtctcgt
      481 caataatacg caacccatgt gttctccaaa actgttcgct tttgatctta atacctcgca
      541 attgctcaag caagtcgaga taccgcacga tgttgccacc acaggaaagg gcgaattagt
      601 atctttaact gttcaagcta tggattcgac aaatactatg gtgtacatgg tagacaacaa
      661 aaatactttg atcatctacc aaaatgccga tgattctttt catcgattgt cttcccacac
      721 tttgaatcac aactctgaca aaatgtcaga tcaacaagaa aatctcacct tgaaagaagt
      781 agacaacaaa gtttatggaa tggcacttag tcccgtgacg cataatcttt attacaattc
      841 tccgtcttct gagaatttgt attatgttaa cacagaatcg ttaatgaaat cggaaaatca
      901 aggaaatgac gtgcaatatg aaagagtcca agacgttttc gacagtcaat taaccgttaa
      961 agcagtatcg aaaaatggcg tactcctttt cggactcgcg aataatactc ttagttgctg
     1021 gaacgagcat cagtcacttg acagacaaaa tatcgatgtc gtagctcgaa atgaggacac
     1081 gcttcaaatg gtcgttagta tgaagattaa gcaaaacgtt ccacaatctg gcagagttaa
     1141 taatacgcaa agaaatgaat atttgttggc tttaagcgac agaaaccaga acgtgctaaa
     1201 caacgatctt aatctcgaac acgtgaactt ccaaattttg ggcgctaacg taaacgactt
     1261 gatacggaat agtcgttgcg caaattttga caatcaggat aataatcact ataatcataa
     1321 tcataatcaa gctcgtcatt cttcaaaatc tgacaatcag aataacaatc aacataacga
     1381 tcaagctcat cattcttcaa agtctaacaa tcggcataac aataacgatt aagctcatca
     1441 tttttcaaaa tttgataatc agaataacaa tcagaataac gattaatata ataatcaatt
     1501 ttatcattct ttaaaatctg ttaattaatc ttttctcgat gtaagtcaaa tattttaaaa
     1561 aaatttcatt acattataaa acgataaaat aaatatcgtt tttttgcata at
```

APPENDIX L

Nucleotide sequences of AmMRJP5 deposited in the GenBank.

DEFINITION Apis mellifera major royal jelly protein MRJP5 (MRJP5) mRNA, complete cds.
ACCESSION AF004842
VERSION AF004842.1 GI:4101571

translation="MTTWLLLVVCLGIACQGITSVTVRENSPRKLANSMNVIHEWKYL
DYDFGSDERRQAAMQSGEYDHTKNYPFDVDQWRGMTFVTVPRYKGVPSSLNVISEKIG
NGGRLLQPYPDWSWANYKDCSGIVSAYKIAIDKFDRLWILDSGIINNTQPMCSPKLHV
FDLNTSHQLKQVVMPHDIAVNASTGNGGLVSLVVQAMDPVNTIVYMADDKGDALIVYQ
NSDESFHRLTSNTFDYDPKYIKMMDAGESFTAQDGIFGMALSPMTNNLYYSPLSSRSL
YYVNTKPFMKSEYGANNVQYQGVQDIFNTESIAKIMSKNGVLFFGLMNNSAIGCWNEH
QPLQRENMDMVAQNEETLQTVVAMKMMHLPQSNKMNRMHRMNRVNRVNRMDRMDRIDR
MDRMDRMDTMDTMDRIDRMDRMDRIDRIDRMHTMDTMDTMDRTDKMSSMDRMDRMDRV
DRMDTMDRTDKMSSMDRMDRMDRVDTMDTMDTMDRMDRMDRMDRMDRMDRMDTMDRTD
KMSRIDRMDKIDRMDRMDRTNRMDRMNRMNRQMNEYMMALSMKLQKFINNDYNFNEVN
FRILGANVNDLIMNTRCANSDNQNNNQNKHNN"

ORIGIN

```
        1 tacactgcgt tctcttgaaa ctgtcgtttg caaaatattt gcagcatcca agaacaatga
       61 caacttggtt gttgctggtc gtgtgccttg gcatagcttg tcaaggtatc acaagcgtca
      121 ctgttcgaga aaattctccg agaaagttgg caaattcgat gaacgtgatt cacgaatgga
      181 agtatctcga ttatgatttt ggtagcgacg aaaggaggca agctgcgatg caatctggcg
      241 agtatgacca tacgaaaaat tatcccttcg atgtcgatca atggcgtggt atgacttttg
      301 taaccgtacc aagatacaaa ggtgtacctt cttctttgaa cgtgatatct gagaaaattg
      361 gcaacggtgg acgacttcta caaccgtatc ctgattggtc gtgggcgaac tataaagatt
      421 gctctggaat agtgagcgct tacaaaattg cgatcgacaa gttcgacaga ttgtggattc
      481 tggactcagg tattatcaat aatactcaac ccatgtgttc accaaaattg catgtctttg
      541 atctcaatac ctcacatcag cttaagcaag ttgtgatgcc gcacgatatt gccgtaaatg
      601 ccagcacagg gaatggggga ctcgtatcac tagttgttca agctatggat cctgtgaata
      661 ctatcgtgta tatggcagat gacaaaggtg atgctttaat cgtctaccaa aattctgacg
      721 aatctttcca tcgattgact tccaacactt tcgattacga tcccaaatat atcaaaatga
      781 tggacgcggg agaaagtttc acagcgcaag atggaatttt tggaatggca ctcagtccca
      841 tgacaaacaa tctttattac agccctcttt cttctcgcag tttgtattat gttaatacaa
      901 aaccattcat gaaatcagaa tatggagcaa ataacgtaca atatcaaggt gtccaagata
      961 ttttcaacac tgaatcgatt gctaaaataa tgtcgaaaaa tggcgttctc tttttcggcc
     1021 tcatgaataa ttcagctatt ggttgttgga acgagcatca accacttcag agagaaaata
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     1201 atagaatgga tagaatggat agaatagata ggatggatag gatggatagg atggatacaa
     1261 tggatacaat ggatagaata gataggatgg ataggatgga tagaatagat aggatagata
     1321 ggatgcatac aatggataca atggatacaa tggatagaac agataagatg agtagcatgg
     1381 ataggatgga tagaatggat agggtggata ggatggatac aatggataga acagataaga
     1441 tgagtagcat ggataggatg gatagaatgg atagggtgga tacaatggat acaatggata
     1501 caatggatag aatggatagg atggatagga tggatagaat ggatagaatg gataggatgg
     1561 atacaatgga tagaacagat aagatgagta ggatagatag aatggataaa atagatagaa
     1621 tggataggat ggataggaca aatagaatgg atagaatgaa taggatgaat agacaaatga
     1681 atgaatatat gatggcttta agtatgaaat tacagaaatt tataaataat gattataatt
     1741 tcaacgaagt aaacttccga attttgggtg caaatgtaaa cgatttaata atgaatactc
     1801 gttgtgcaaa ttctgacaat cagaataaca atcaaaataa gcataataat taagatgatc
     1861 gttctttata ttaaaatctg ttaatcagtc ttttctcgat ataaaccaaa tattctttaa
     1921 aatttcttta tattataaat gaataaaata aatatttttg catgat
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BIOGRAPHY

Mrs. Duangporn (Sihanuntavong) Srisuparbh was born on September 11, 1972 in Bangkok, Thailand. She graduated with Bachelor degree of Science in Biochemistry and Master degrees of Science in Biotechnology, from Chulalongkorn University. She has been enrolled in Ph.D. Biochemistry, Faculty of Science, Chulalongkorn University since 1999. During this study, she has been awarded the travel fellowship for oral presentation at the 6th Graduate Congress, Singapore on December 1st, 2001 and for poster presentation at the 16th FAOBMB Symposium, Taipei on 20th-22th, 2002.