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APPENDICES

Appendix A

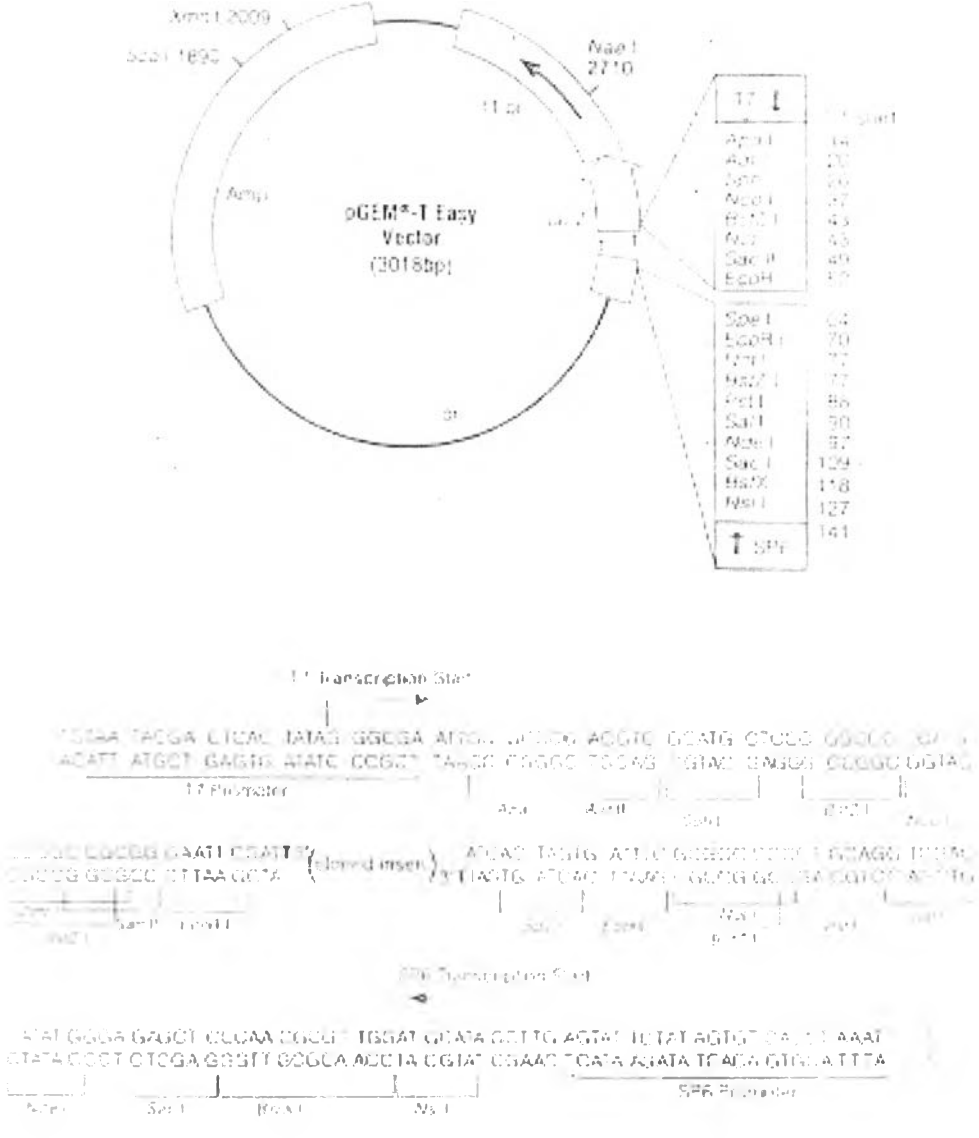

Figure A.1 Map of the pGEM®-T easy vector and multiple cloning sites.

Appendix B

CL140Contig1

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defense27Contig1

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CAGGATGGGCAAGATATGAAGTCCCAGACTTTCCTGAGGCAATGCTGAATTGGTACGCAGTCAATCGAA
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GG

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Piti10

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Piti11

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Piti6(F)

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Piti6

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Figure A.2 Nucleotide sequence of Mn-SOD of *P. monodon* from cDNA library of *P.monodon*

CL67Contig1

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CL67Contig2

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CL67Contig3

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phrase1-CL78CONTIG1

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phrase1-CL78CONTIG2

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phrase1-CL78CONTIG3

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Figure A.3 Nucleotide sequence of AK of *P. monodon* from cDNA library of *P.monodon*

```
cytochrome      ATGACATCATCTCACGGACACCACGCTTTCCATTTAGTAGATATAAGACCATGACCACTT
Cu              -----ACTAGTGATTGGTAAGCATGGTTTCCATTTAGTAGATATAAGACCATGACCACTT
                     * *       **  * ** * **********************************

cytochrome      ACAGGTTCAATTAGAGCTATAATATTAACCACAGGACTAGTTAAATGATTTCATCAATTT
Cu              ACAGGTTCAATTAGAGCTATAATATTGACTACGGGATTAGTTAAATGATTTCATCAATTT
                ************************** ** ** *** ************************

cytochrome      AACCCTGACCTTTTATTTTTAGGAATTATTGCGACAACTCTTACTATAATTCAATGATGA
Cu              AATCCTGACCTTTTATTTTTAGGAATTATTGCAACAACTCTTACTATAATTCAATGATGA
                ** ****************************** ****************************

cytochrome      CGAGATATTACGCGAGAAGGTACTTACCAAGGATTACATACTAAAGCAGTTACTATCGGC
Cu              CGAGATATTACACGAGAAGGTACCTATCAAGGGTTACATACGAAAGCAGTTACTATCGGT
                ********** ********** ** ***** ******** ****************

cytochrome      CTTCGATGGGGTATAATCTTGTTTATTACTTCAGAAG-TATTATTTTTTTTCTCTTTCTT
Cu              CTTCGATGAGGTATAATCTTATTTATTACCTCAGAAGGTATTATTTTTTTTCTCTTTCTT
                ******** ********** ******** ******* ************************

cytochrome      TTGAGC--TTTTTTCCACAGAA-GATTATCTCCTAACGTAGAAGTAGGTAGTTGTTGACC
Cu              TTGAGCCTTTTTTTTCACAGAAAGATTATCTCCTAATGTAGAA-----------------
                ******  ****** ******* ************* ******

cytochrome      TCCTGCAGGAATTCAAACCTTTAACCCTTTTCAAATTCCTCTCCTTAATACAGCAATCCT
Cu              ------------------------------------------------------------
```

Figure A.4 Alignment of nucleotide sequence of Cu/Zn product from DNA sequencing with cytochrome c oxidase subunit 3 of *P. monodon*.

Appendix C

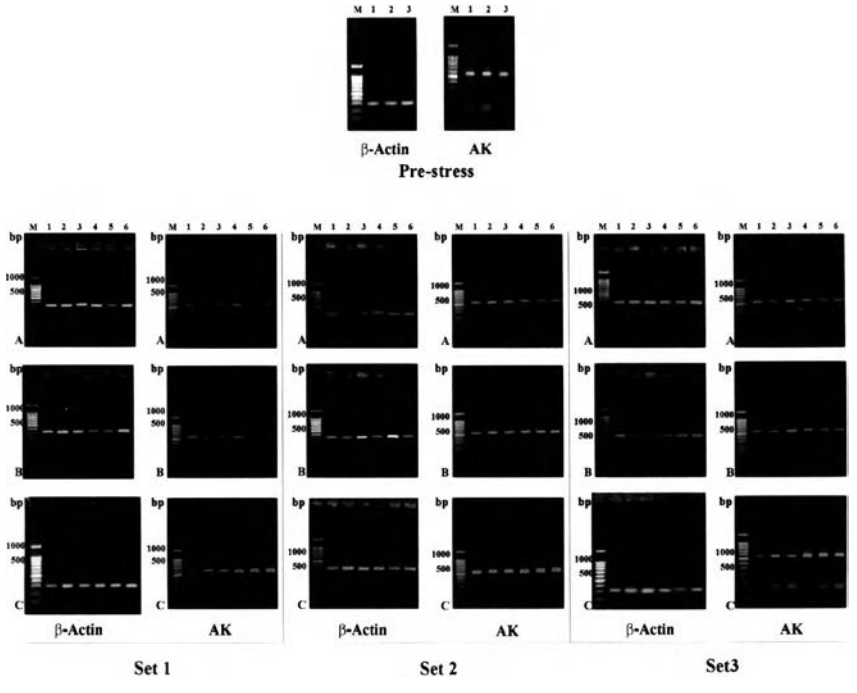

Figure A.5 The expression level of AK gene transcripts in haemocyte of pre-stress shrimp and between control and stress with salinity change in comparison with β-actin gene. Samples were obtained from 3 shrimps (Set 1, Set 2 and Set 3, respectively) and analyzed by 1.2% agarose gel electrophoresis. A, B, and C are the result of at 15, 30 and 45ppt of stress shrimp. Lane M is 100 bp markers, Lane 1- 6 represent 2, 6, 12, 24, 48, and 72h of stress with salinity change.

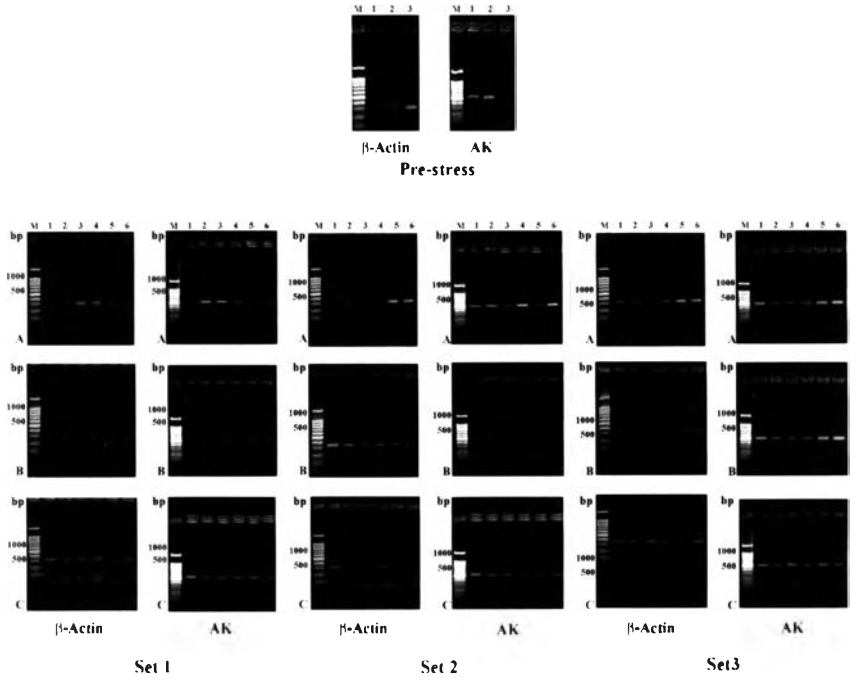

Figure A.6 The expression level of AK gene transcripts in gill of pre-stress shrimp and between control and stress with salinity change in comparisons with β-actin gene. Samples were obtained from 3 shrimps (Set 1, Set 2 and Set 3, respectively) and analyzed by 1.2% agarose gel electrophoresis. A, B, and C are the result of at 15, 30 and 45ppt of stress shrimp. Lane M is 100 bp markers, Lane 1- 6 represent 2, 6, 12, 24, 48, and 72h of stress with salinity change.

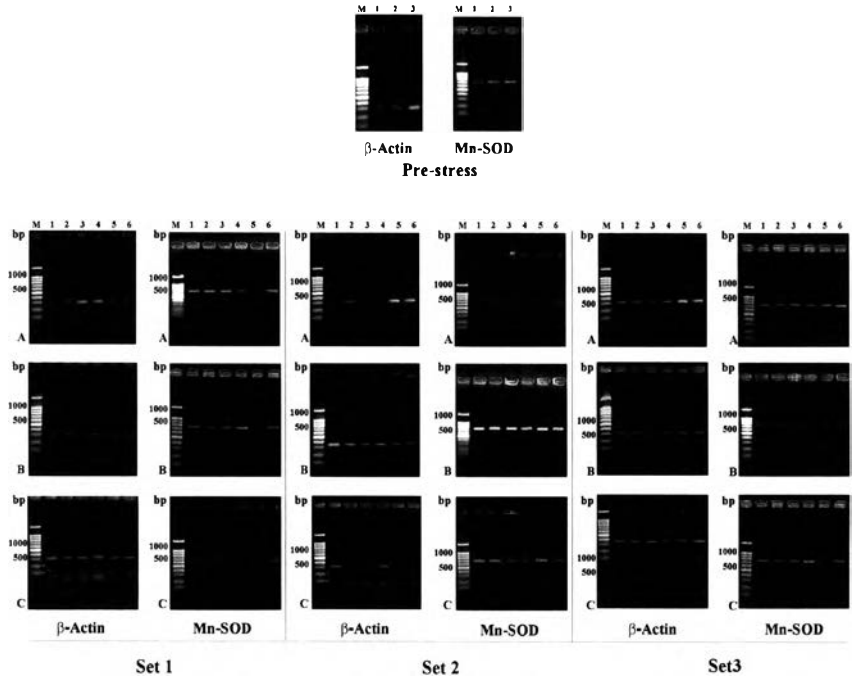

Figure A.7 The expression level of Mn-SOD gene transcripts in gill of pre-stress shrimp and between control and stress with salinity change in comparisons with β-actin gene. Samples were obtained from 3 shrimps (Set 1, Set 2 and Set 3, respectively) and analyzed by 1.2% agarose gel electrophoresis. A, B, and C are the result of at 15, 30 and 45ppt of stress shrimp. Lane M is 100 bp markers, Lane 1- 6 represent 2, 6, 12, 24, 48, and 72h of stress with salinity change.

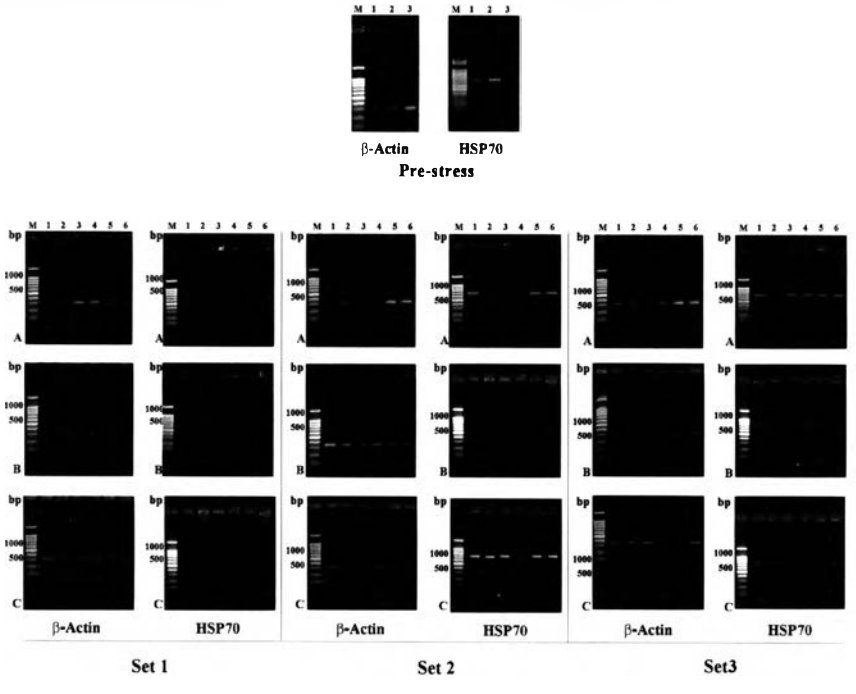

Figure A.8 The expression level of HSP70 gene transcripts in gill of pre-stress shrimp and between control and stress with salinity change in comparisons with β-actin gene. Samples were obtained from 3 shrimps (Set 1, Set 2 and Set 3, respectively) and analyzed by 1.2% agarose gel electrophoresis. A, B, and C are the result of at 15, 30 and 45ppt of stress shrimp. Lane M is 100 bp markers, Lane 1- 6 represent 2, 6, 12, 24, 48, and 72h of stress with salinity change.

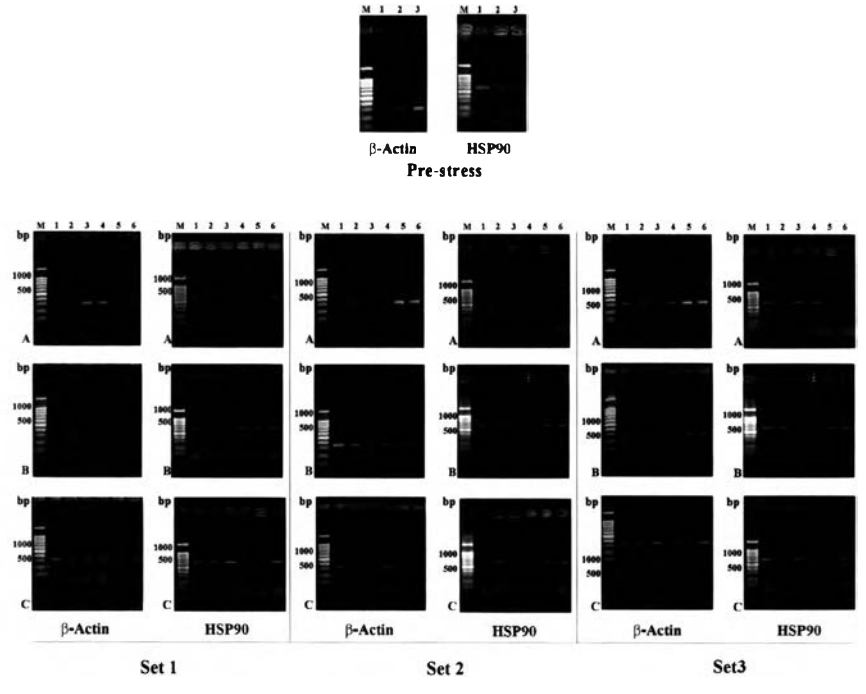

Figure A.9 The expression level of HSP90 gene transcripts in gill of pre-stress shrimp and between control and stress with salinity change in comparisons with β-actin gene. Samples were obtained from 3 shrimps (Set 1, Set 2 and Set 3, respectively) and analyzed by 1.2% agarose gel electrophoresis. A, B, and C are the result of at 15, 30 and 45ppt of stress shrimp. Lane M is 100 bp markers, Lane 1- 6 represent 2, 6, 12, 24, 48, and 72h of stress with salinity change.

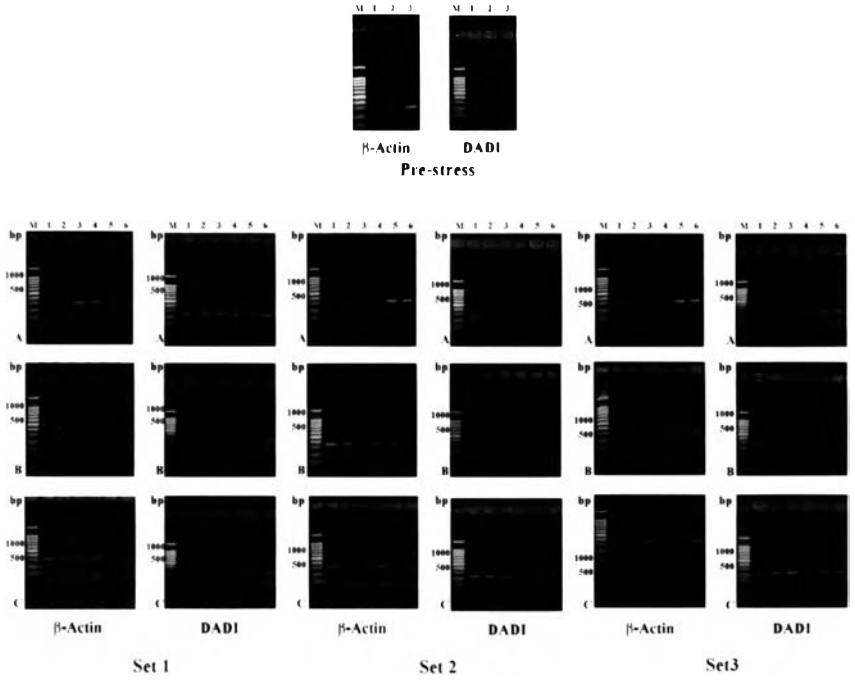

Figure A.10 The expression level of DADI gene transcripts in gill of pre-stress shrimp and between control and stress with salinity change in comparisons with β-actin gene. Samples were obtained from 3 shrimps (Set 1, Set 2 and Set 3, respectively) and analyzed by 1.2% agarose gel electrophoresis. A, B, and C are the result of at 15, 30 and 45ppt of stress shrimp. Lane M is 100 bp markers, Lane 1- 6 represent 2, 6, 12, 24, 48, and 72h of stress with salinity change.

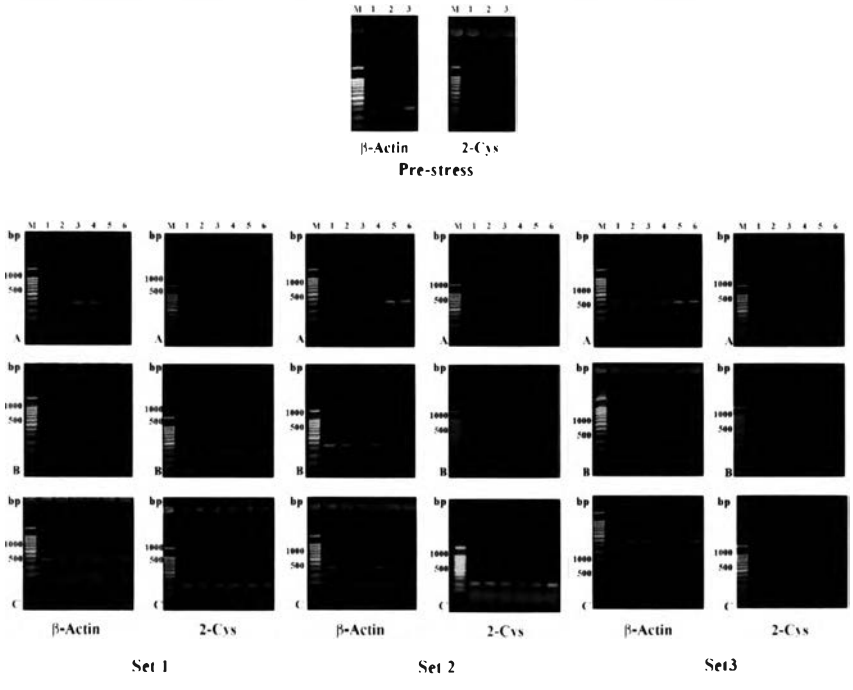

Figure A.11 The expression level of Thioredoxin peroxidase gene transcripts in gill of pre-stress shrimp and between control and stress with salinity change in comparisons with β-actin gene. Samples were obtained from 3 shrimps (Set 1, Set 2 and Set 3, respectively) and analyzed by 1.2% agarose gel electrophoresis. A, B, and C are the result of at 15, 30 and 45ppt of stress shrimp. Lane M is 100 bp markers, Lane 1- 6 represent 2, 6, 12, 24, 48, and 72h of stress with salinity change.

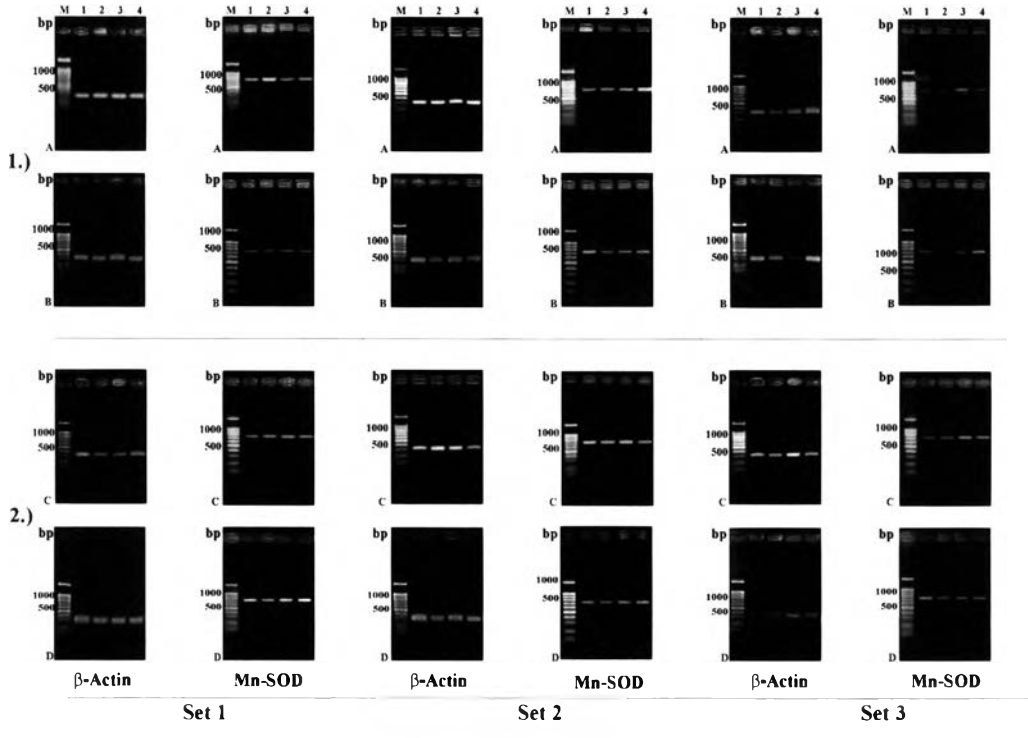

Figure A.12a The expression level of Mn-SOD gene transcripts in haemocyte from *P.monodon* of tank1 (1) and Tank2 (2) between control and stress with *V.harveyi* exposure in comparison with β-actin gene. Samples were obtained from 3 shrimps and analyzed by 1.2% agarose gel electrophoresis. A=Cotrol1, B= *Vibrio.*1, C=Control2 and D= *Vibrio*2 are the result at control and stress shrimp. Lane M is 100 bp markers, Lane 1-6 represent 0, 6, 12, 24, 48, and 72h of stress shrimp with *V.harveyi*.

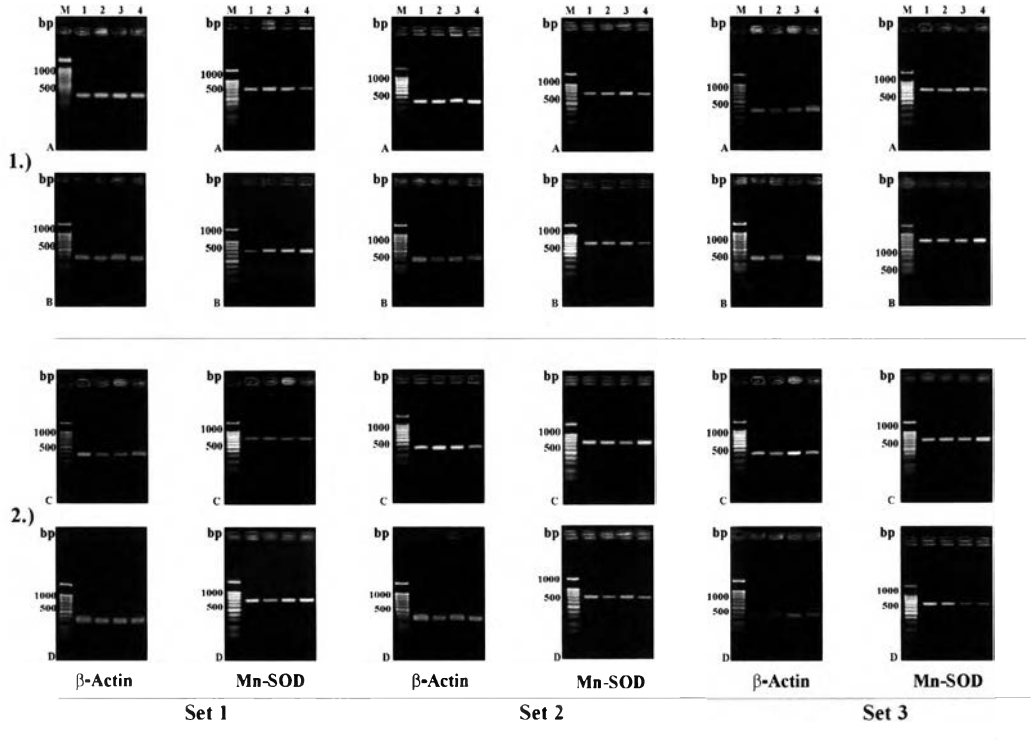

Figure A.12b The expression level of Mn-SOD gene transcripts in gill from *P.monodon* of tank1 (1) and Tank2 (2) between control and stress with *V.harveyi* exposure in comparison with β-actin gene. Samples were obtained from 3 shrimps and analyzed by 1.2% agarose gel electrophoresis. A=Cotrol1, B= *Vibrio.*1, C=Control2 and D= *Vibrio*2 are the result at control and stress shrimp. Lane M is 100 bp markers, Lane 1-6 represent 0, 6, 12, 24, 48, and 72h of stress shrimp with *V.harveyi*.

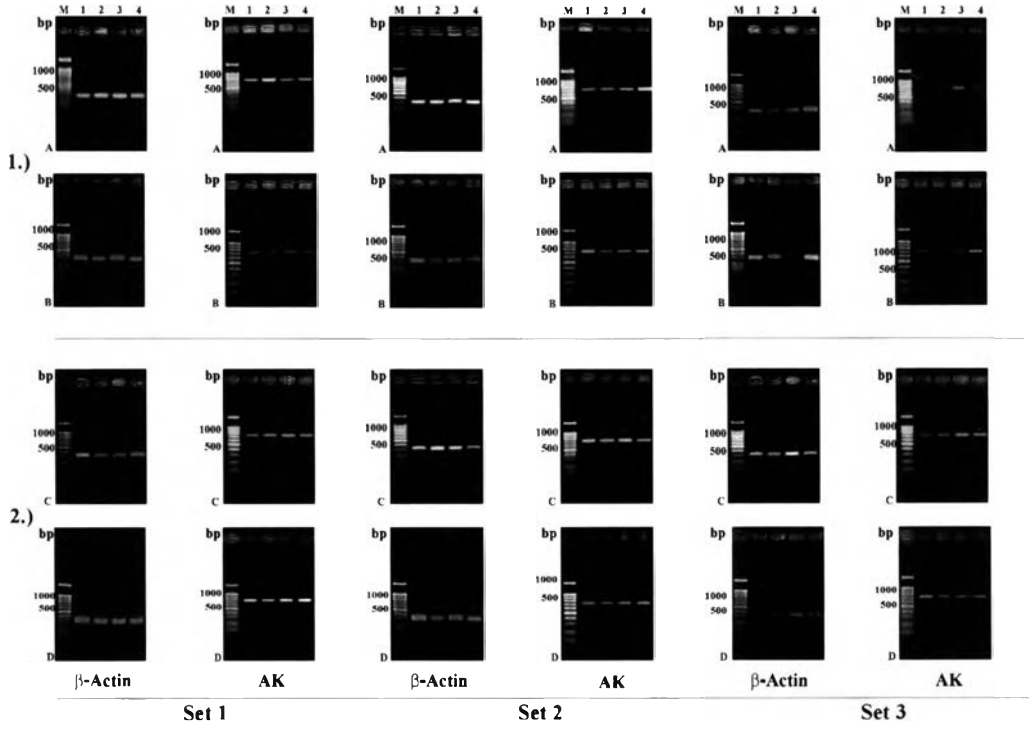

Figure A.13a The expression level of AK gene transcripts in haemocyte from *P.monodon* of tank1 (1) and Tank2 (2) between control and stress with *V.harveyi* exposure in comparison with β-actin gene. Samples were obtained from 3 shrimps and analyzed by 1.2% agarose gel electrophoresis. A=Cotrol1, B= *Vibrio.*1, C=Control2 and D= *Vibrio*2 are the result at control and stress shrimp. Lane M is 100 bp markers, Lane 1-6 represent 0, 6, 12, 24, 48, and 72h of stress shrimp with *V.harveyi.*

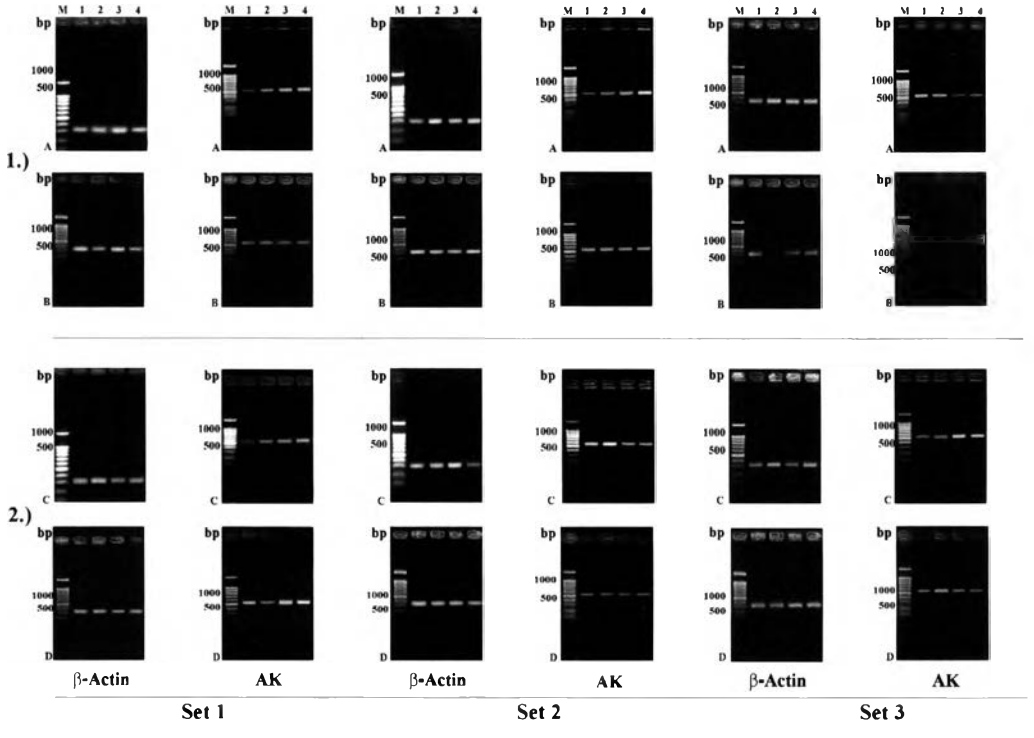

Figure A.13b The expression level of AK gene transcripts in gill from *P.monodon* of tank1 (1) and Tank2 (2) between control and stress with *V.harveyi* exposure in comparison with β-actin gene. Samples were obtained from 3 shrimps and analyzed by 1.2% agarose gel electrophoresis. A=Cotrol1, B= *Vibrio.*1, C=Control2 and D= *Vibrio*2 are the result at control and stress shrimp. Lane M is 100 bp markers, Lane 1-6 represent 0, 6, 12, 24, 48, and 72h of stress shrimp with *V.harveyi*.

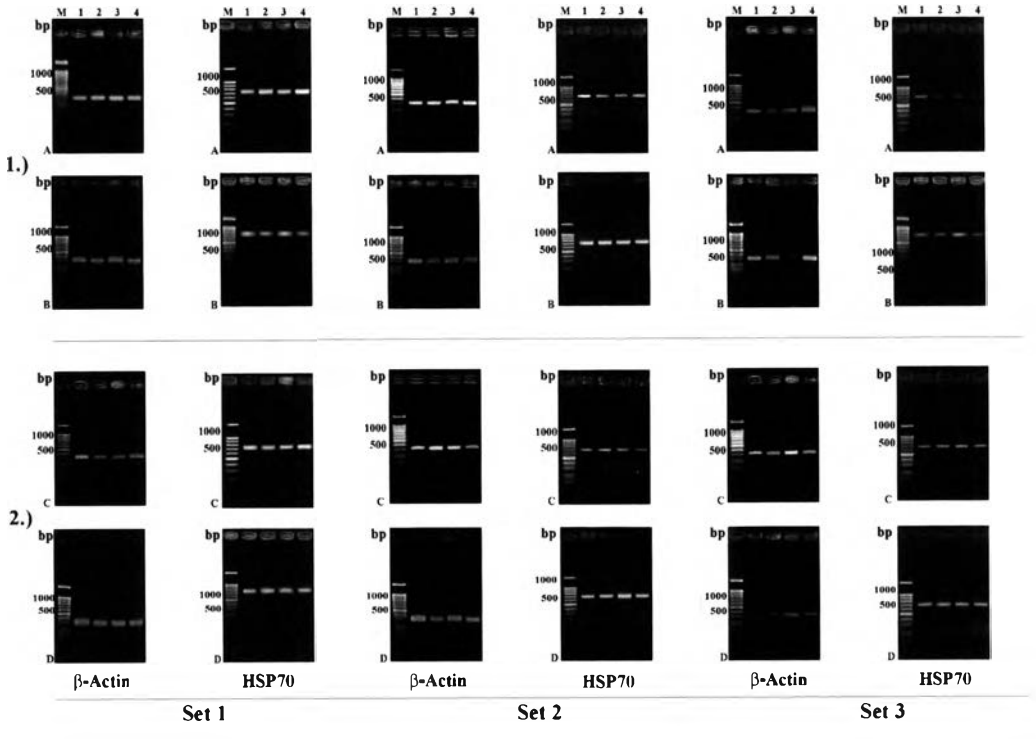

Figure A.14a The expression level of HSP70 gene transcripts in haemocyte from *P.monodon* of tank1 (1) and Tank2 (2) between control and stress with *V.harveyi* exposure in comparison with β-actin gene. Samples were obtained from 3 shrimps and analyzed by 1.2% agarose gel electrophoresis. A=Cotrol1, B= *Vibrio*.1, C=Control2 and D= *Vibrio*2 are the result at control and stress shrimp. Lane M is 100 bp markers, Lane 1-6 represent 0, 6, 12, 24, 48, and 72h of stress shrimp with *V.harveyi*.

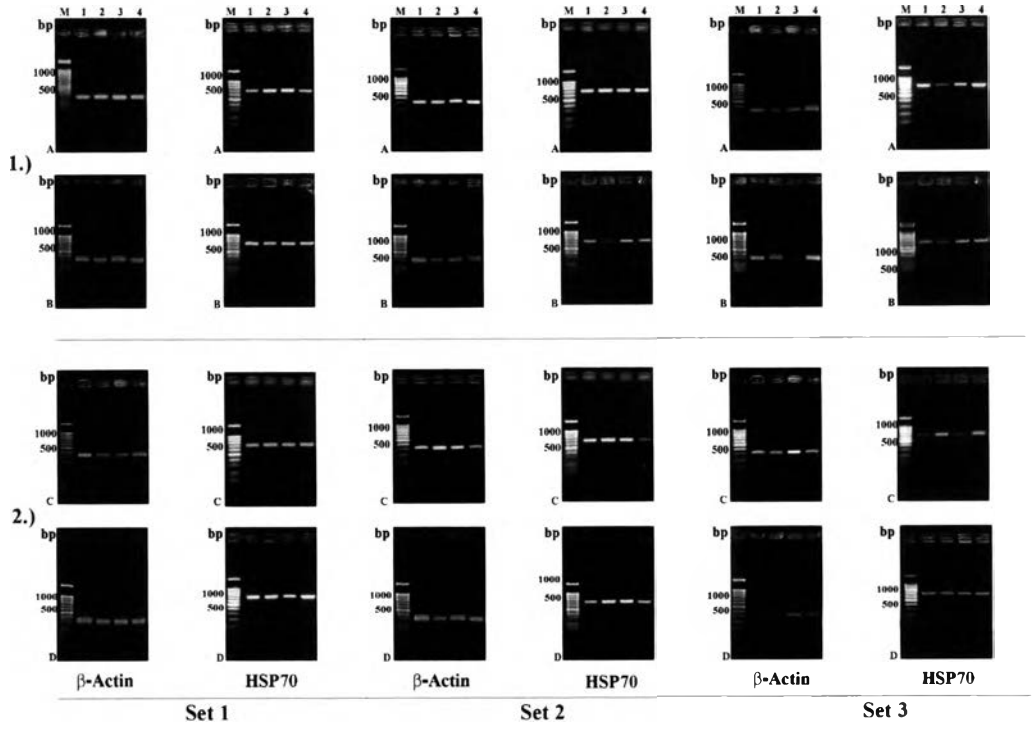

Figure A.14b The expression level of HSP70 gene transcripts in haemocyte from *P.monodon* of tank1 (1) and Tank2 (2) between control and stress with *V.harveyi* exposure in comparison with β-actin gene. Samples were obtained from 3 shrimps and analyzed by 1.2% agarose gel electrophoresis. A=Cotrol1, B= *Vibrio*.1, C=Control2 and D= *Vibrio*2 are the result at control and stress shrimp. Lane M is 100 bp markers, Lane 1-6 represent 0, 6, 12, 24, 48, and 72h of stress shrimp with *V.harveyi*.

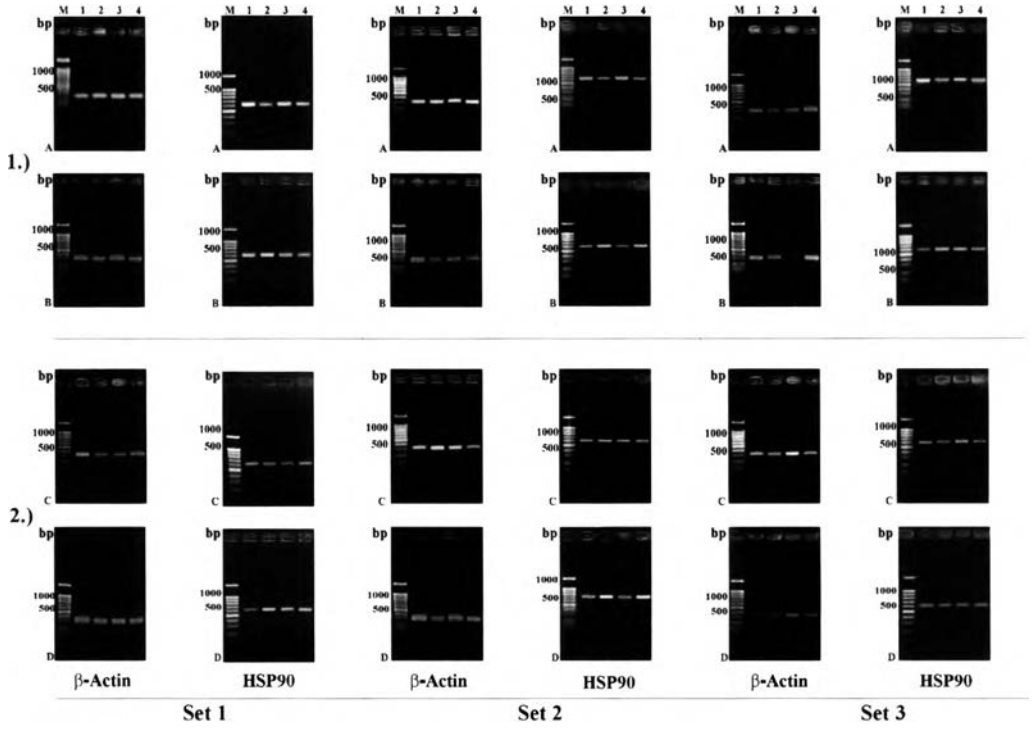

Figure A.15a The expression level of HSP90 gene transcripts in haemocyte from *P.monodon* of tank1 (1) and Tank2 (2) between control and stress with *V.harveyi* exposure in comparison with β-actin gene. Samples were obtained from 3 shrimps and analyzed by 1.2% agarose gel electrophoresis. A=Cotrol1, B= *Vibrio*.1, C=Control2 and D= *Vibrio*2 are the result at control and stress shrimp. Lane M is 100 bp markers, Lane 1-6 represent 0, 6, 12, 24, 48, and 72h of stress shrimp with *V.harveyi*.

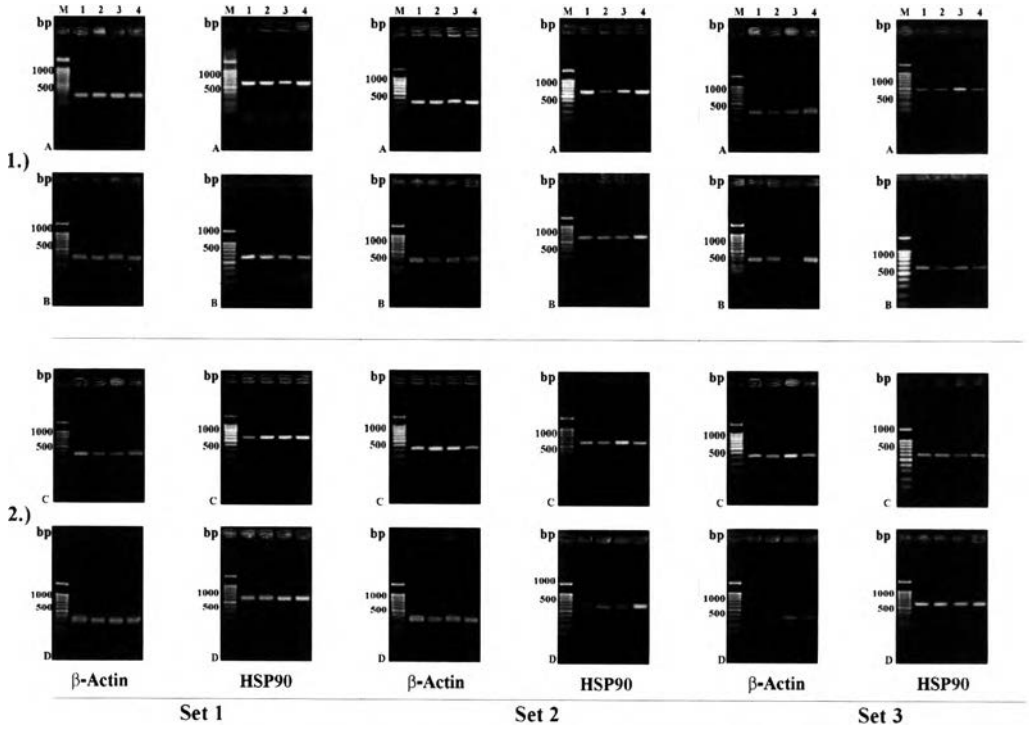

Figure A.15b The expression level of HSP90 gene transcripts in gill from *P.monodon* of tank1 (1) and Tank2 (2) between control and stress with *V.harveyi* exposure in comparison with β-actin gene. Samples were obtained from 3 shrimps and analyzed by 1.2% agarose gel electrophoresis. A=Cotrol1, B= *Vibrio.*1, C=Control2 and D= *Vibrio*2 are the result at control and stress shrimp. Lane M is 100 bp markers, Lane 1-6 represent 0, 6, 12, 24, 48, and 72h of stress shrimp with *V.harveyi*.

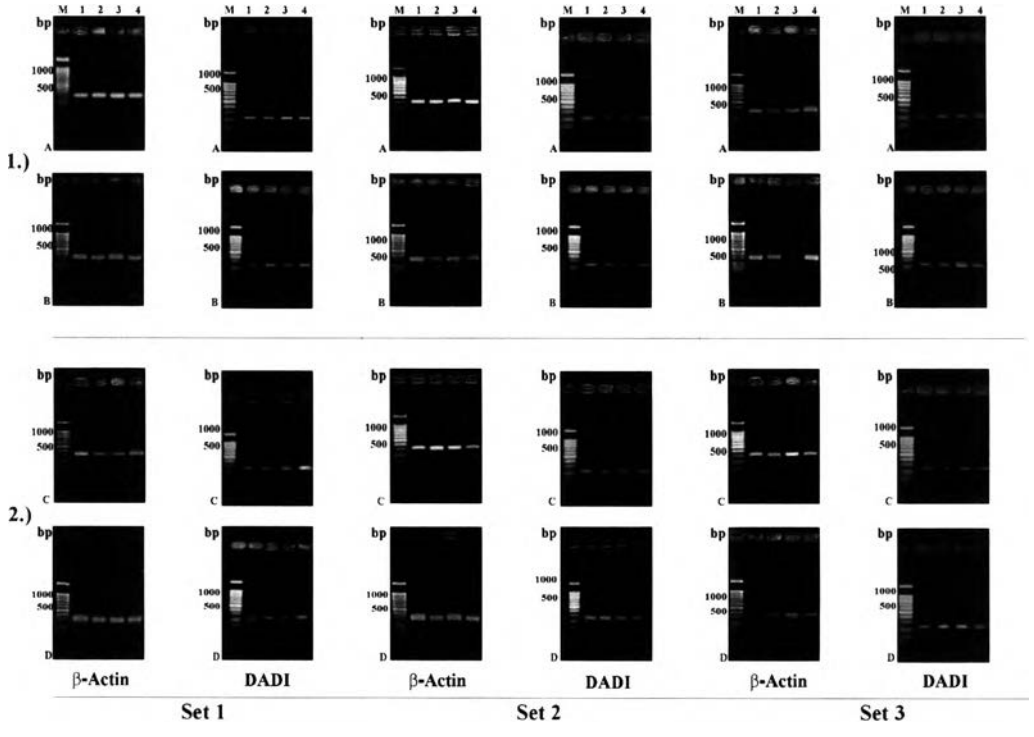

Figure A.16a The expression level of DADI gene transcripts in haemocyte from *P.monodon* of tank1 (1) and Tank2 (2) between control and stress with *V.harveyi* exposure in comparison with β-actin gene. Samples were obtained from 3 shrimps and analyzed by 1.2% agarose gel electrophoresis. A=Cotrol1, B= *Vibrio*.1, C=Control2 and D= *Vibrio*2 are the result at control and stress shrimp. Lane M is 100 bp markers, Lane 1-6 represent 0, 6, 12, 24, 48, and 72h of stress shrimp with *V.harveyi*.

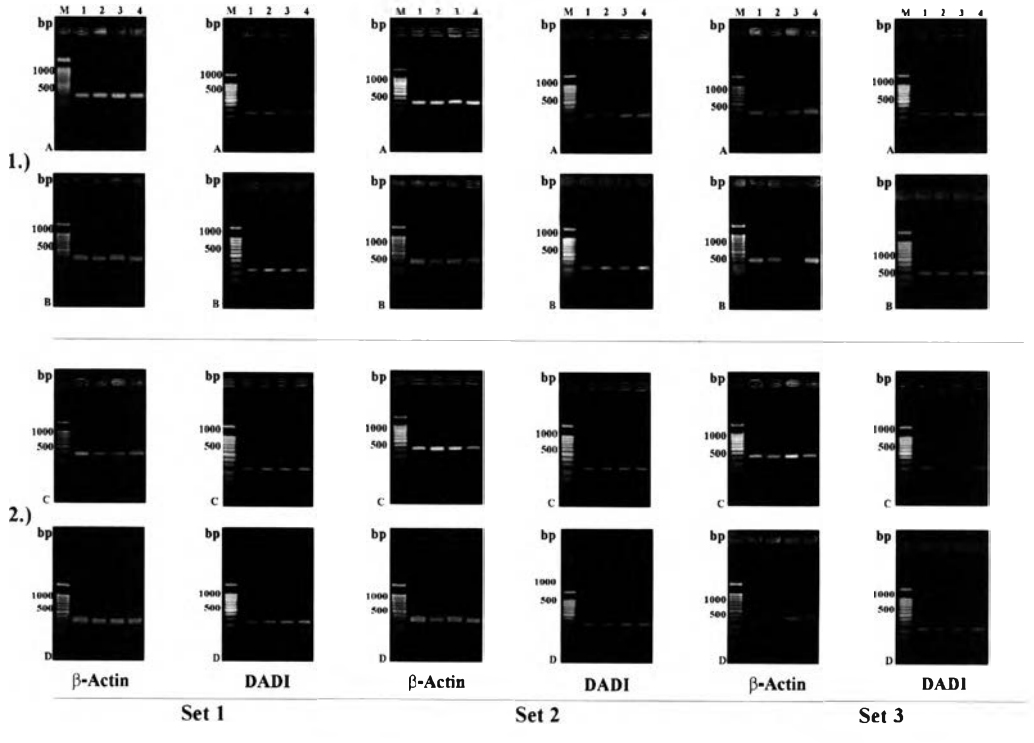

Figure A.16b The expression level of DADI gene transcripts in gill from *P.monodon* of tank1 (1) and Tank2 (2) between control and stress with *V.harveyi* exposure in comperison with β-actin gene. Samples were obtained from 3 shrimps and analysed by 1.2% agarose gel electrophoresis. A=Cotrol1, B= *Vibrio.*1, C=Control2 and D= *Vibrio*2 are the result at control and stress shrimp. Lane M is 100 bp markers, Lane 1-6 represent 0, 6, 12, 24, 48, and 72h of stress shrimp with *V.harveyi*.

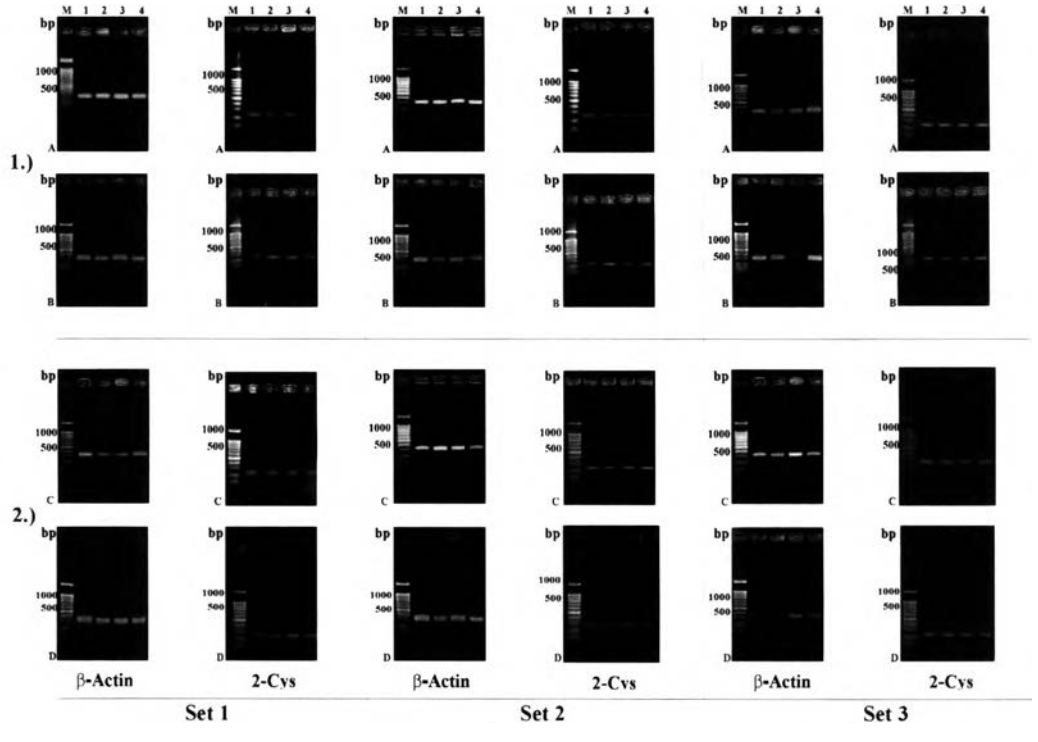

Figure A.17a The expression level of Thioredoxin peroxidase gene transcripts in haemocyte from *P.monodon* of tank1 (1) and Tank2 (2) between control and stress with *V.harveyi* exposure in comparison with β-actin gene. Samples were obtained from 3 shrimps and analyzed by 1.2% agarose gel electrophoresis. A=Cotrol1, B= *Vibrio*.1, C=Control2 and D= *Vibrio*2 are the result at control and stress shrimp. Lane M is 100 bp markers, Lane 1-6 represent 0, 6, 12, 24, 48, and 72h of stress shrimp with *V.harveyi*.

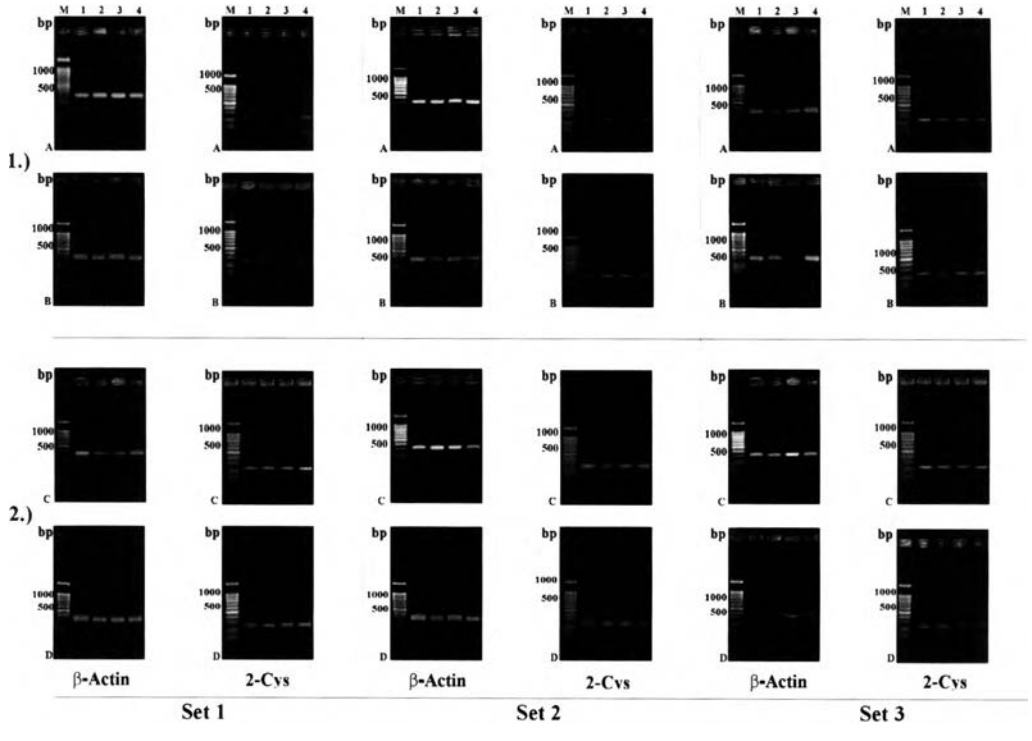

Figure A.17b The expression level of Thioredoxin peroxidase gene transcripts in gill from *P.monodon* of tank1 (1) and Tank2 (2) between control and stress with *V.harveyi* exposure in comparison with β-actin gene. Samples were obtained from 3 shrimps and analyzed by 1.2% agarose gel electrophoresis. A=Cotrol1, B= *Vibrio.*1, C=Control2 and D= *Vibrio*2 are the result at control and stress shrimp. Lane M is 100 bp markers, Lane 1-6 represent 0, 6, 12, 24, 48, and 72h of stress shrimp with *V.harveyi.*

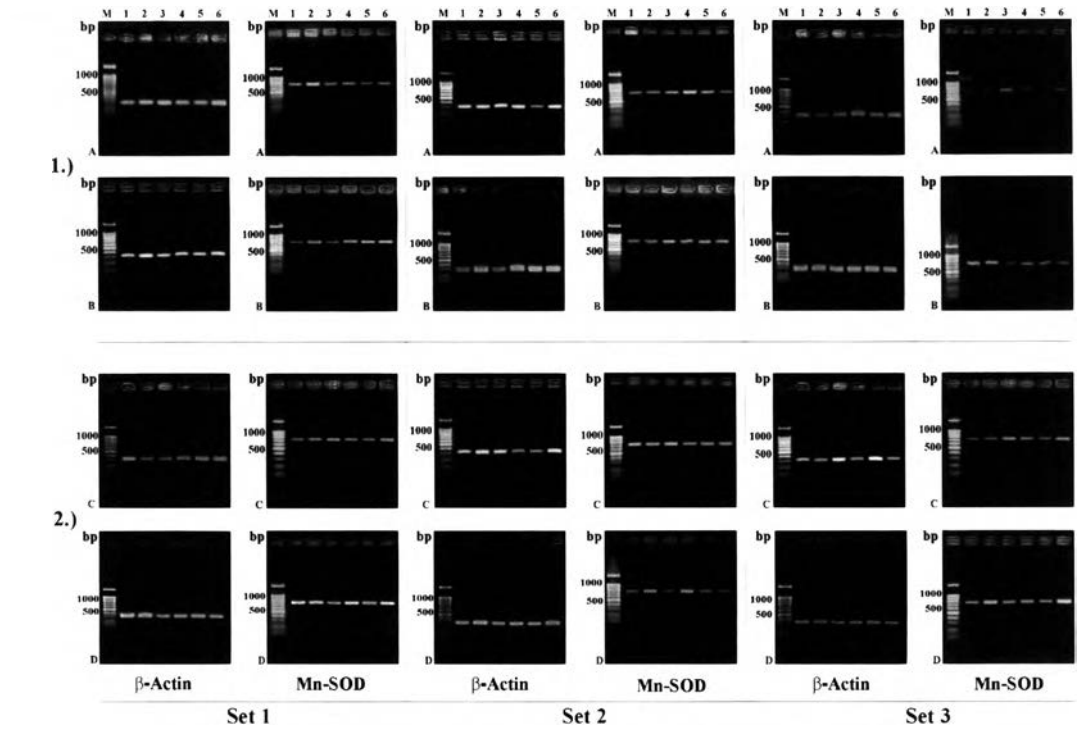

Figure A.18a The expression level of Mn-SOD gene transcripts in haemocyte from *P.monodon* of tank1 (1) and Tank2 (2) between control and stress with handling stress in comparison with β-actin gene. Samples were obtained from 3 shrimps and analyzed by 1.2% agarose gel electrophoresis. A=Cotrol1, B=Handling1, C=Control2 and D=Handling2 are the result at control, handling stress shrimp. Lane M is 100 bp markers, Lane 1-6 represent 0, 6, 12, 24, 48, and 72h of stress shrimp with handling stress.

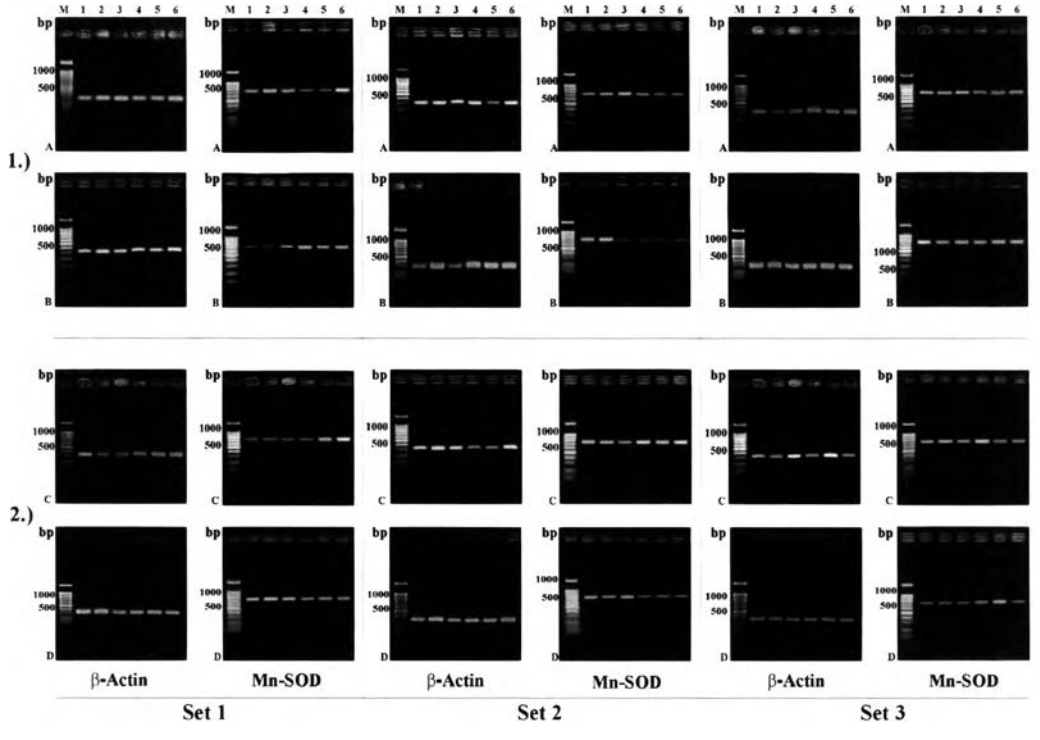

Figure A.18b The expression level of Mn-SOD gene transcripts in gill from *P.monodon* of tank1 (1) and Tank2 (2) between control and stress with handling stress in comparison with β-actin gene. Samples were obtained from 3 shrimps and analyzed by 1.2% agarose gel electrophoresis. A=Cotrol1, B=Handling1, C=Control2 and D=Handling2 are the result at control, handling stress shrimp. Lane M is 100 bp markers, Lane 1-6 represent 0, 6, 12, 24, 48, and 72h of stress shrimp with handling stress.

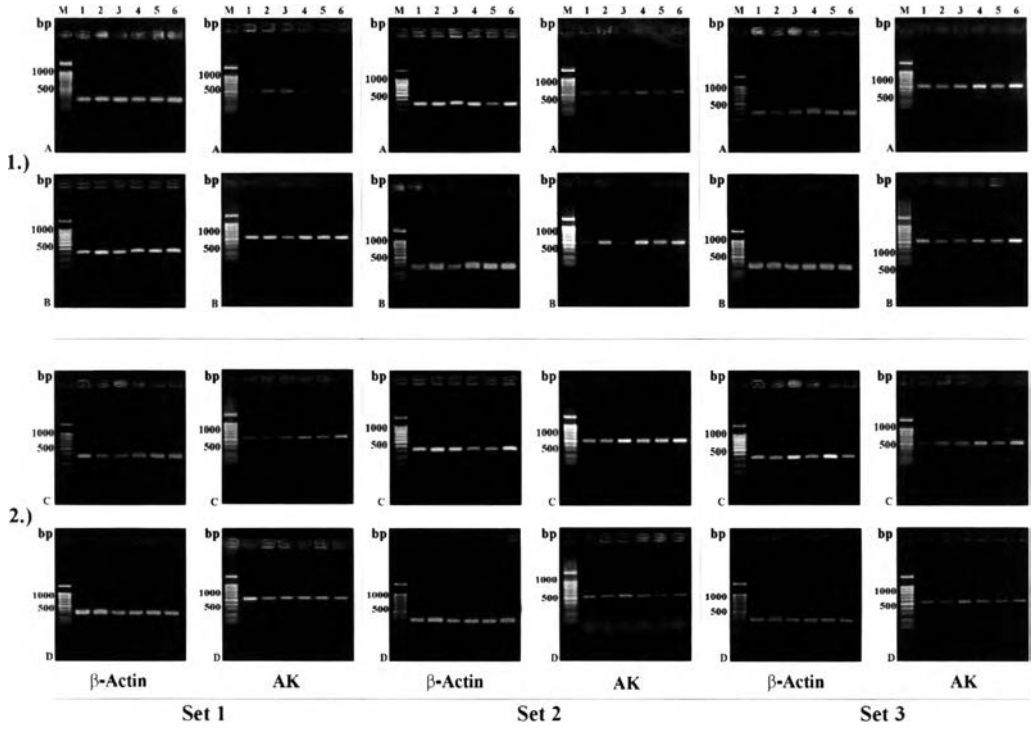

Figure A.19a The expression level of AK gene transcripts in haemocyte from *P.monodon* of tank1 (1) and Tank2 (2) between control and stress with handling stress in comparison with β-actin gene. Samples were obtained from 3 shrimps and analyzed by 1.2% agarose gel electrophoresis. A=Cotrol1, B=Handling1, C=Control2 and D=Handling2 are the result at control, handling stress shrimp. Lane M is 100 bp markers, Lane 1-6 represent 0, 6, 12, 24, 48, and 72h of stress shrimp with handling stress.

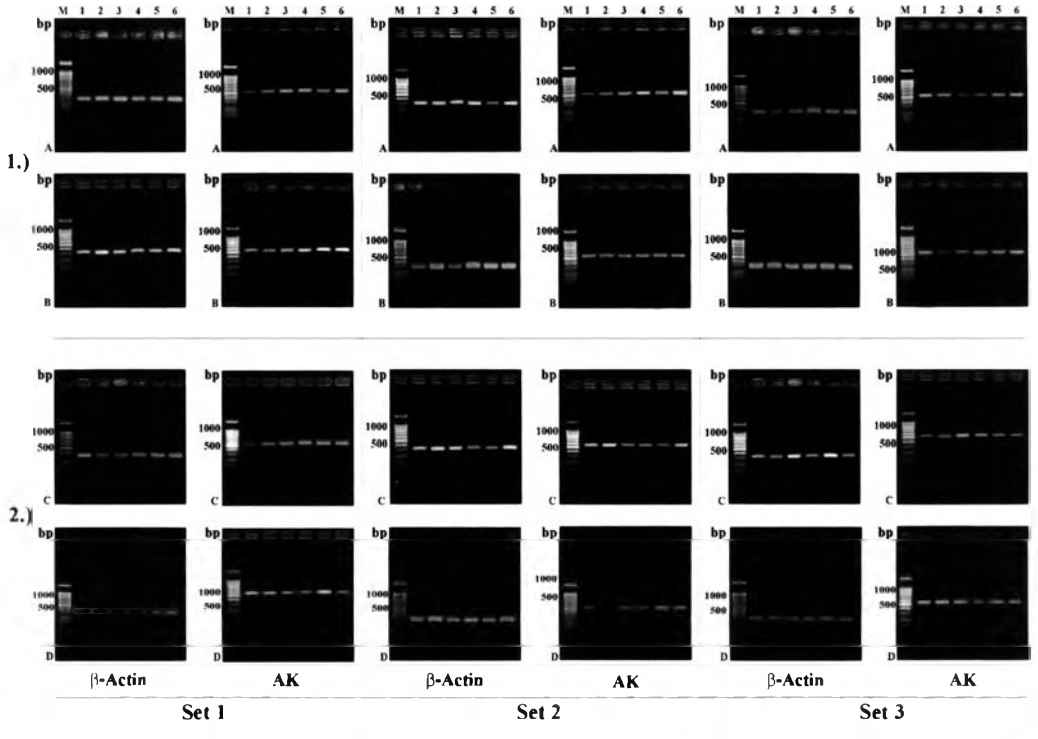

Figure A.19b The expression level of AK gene transcripts in gill from *P.monodon* of tank1 (1) and Tank2 (2) between control and stress with handling stress in comparison with β-actin gene. Samples were obtained from 3 shrimps and analyzed by 1.2% agarose gel electrophoresis. A=Cotrol1, B=Handling1, C=Control2 and D=Handling2 are the result at control, handling stress shrimp. Lane M is 100 bp markers, Lane 1-6 represent 0, 6, 12, 24, 48, and 72h of stress shrimp with handling stress.

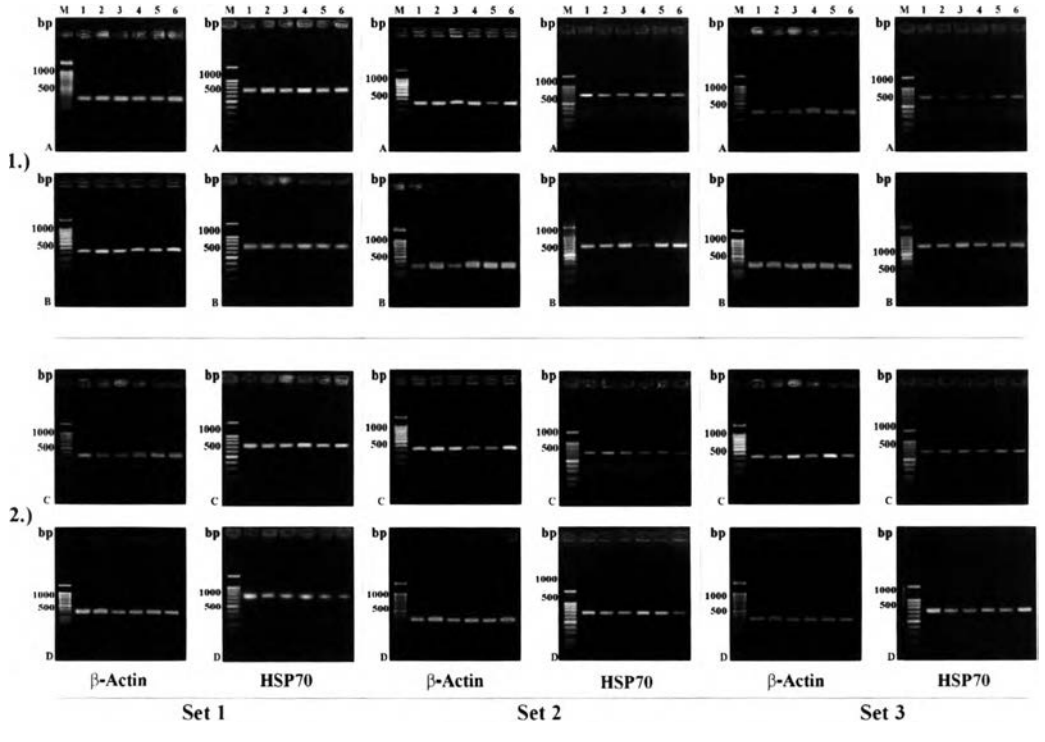

Figure A.20a The expression level of HSP70 gene transcripts in haemocyte from *P.monodon* of tank1 (1) and Tank2 (2) between control and stress with handling stress in comparison with β-actin gene. Samples were obtained from 3 shrimps and analyzed by 1.2% agarose gel electrophoresis. A=Cotrol1, B=Handling1, C=Control2 and D=Handling2 are the result at control, handling stress shrimp. Lane M is 100 bp markers, Lane 1-6 represent 0, 6, 12, 24, 48, and 72h of stress shrimp with handling stress.

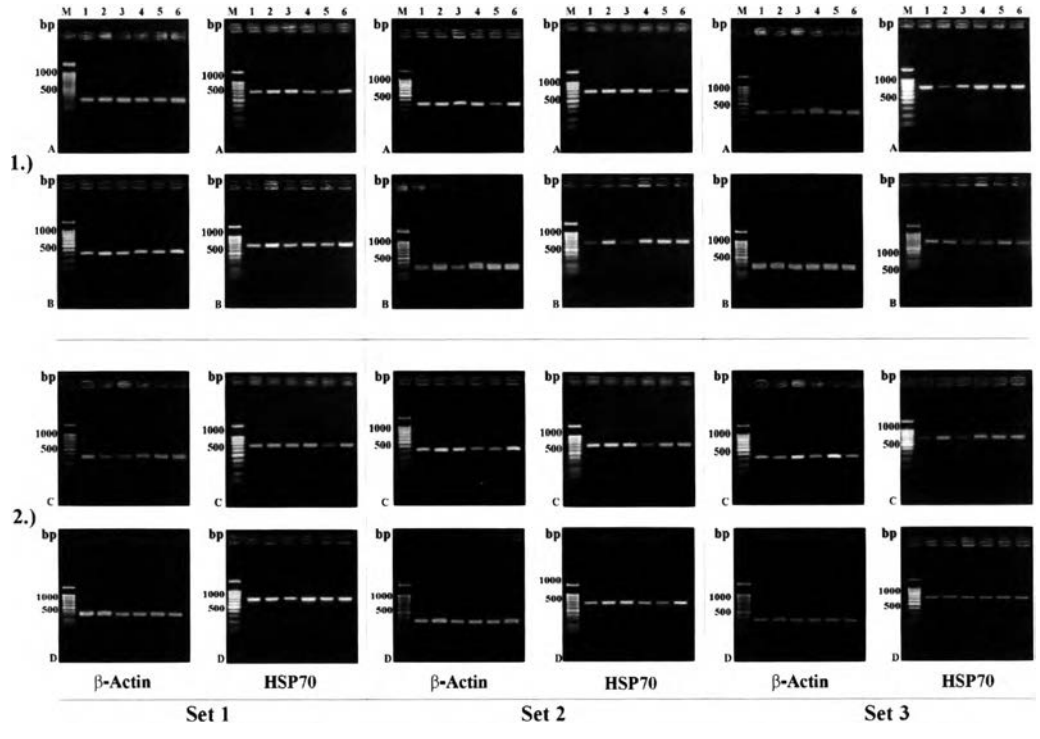

Figure A.20b The expression level of HSP70 gene transcripts in gill from *P.monodon* of tank1 (1) and Tank2 (2) between control and stress with handling stress in comparison with β-actin gene. Samples were obtained from 3 shrimps and analyzed by 1.2% agarose gel electrophoresis. A=Cotrol1, B=Handling1, C=Control2 and D=Handling2 are the result at control, handling stress shrimp. Lane M is 100 bp markers, Lane 1-6 represent 0, 6, 12, 24, 48, and 72h of stress shrimp with handling stress.

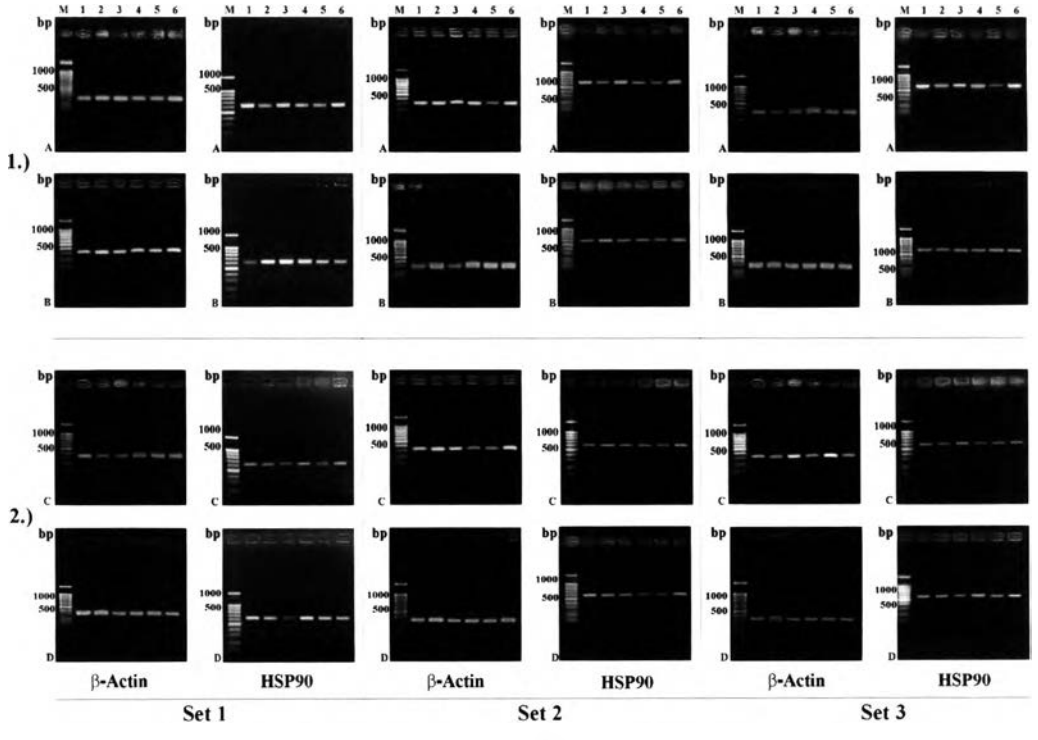

Figure A.21a The expression level of HSP90 gene transcripts in haemocyte from *P.monodon* of tank1 (1) and Tank2 (2) between control and stress with handling stress in comparison with β-actin gene. Samples were obtained from 3 shrimps and analyzed by 1.2% agarose gel electrophoresis. A=Cotrol1, B=Handling1, C=Control2 and D=Handling2 are the result at control, handling stress shrimp. Lane M is 100 bp markers, Lane 1-6 represent 0, 6, 12, 24, 48, and 72h of stress shrimp with handling stress.

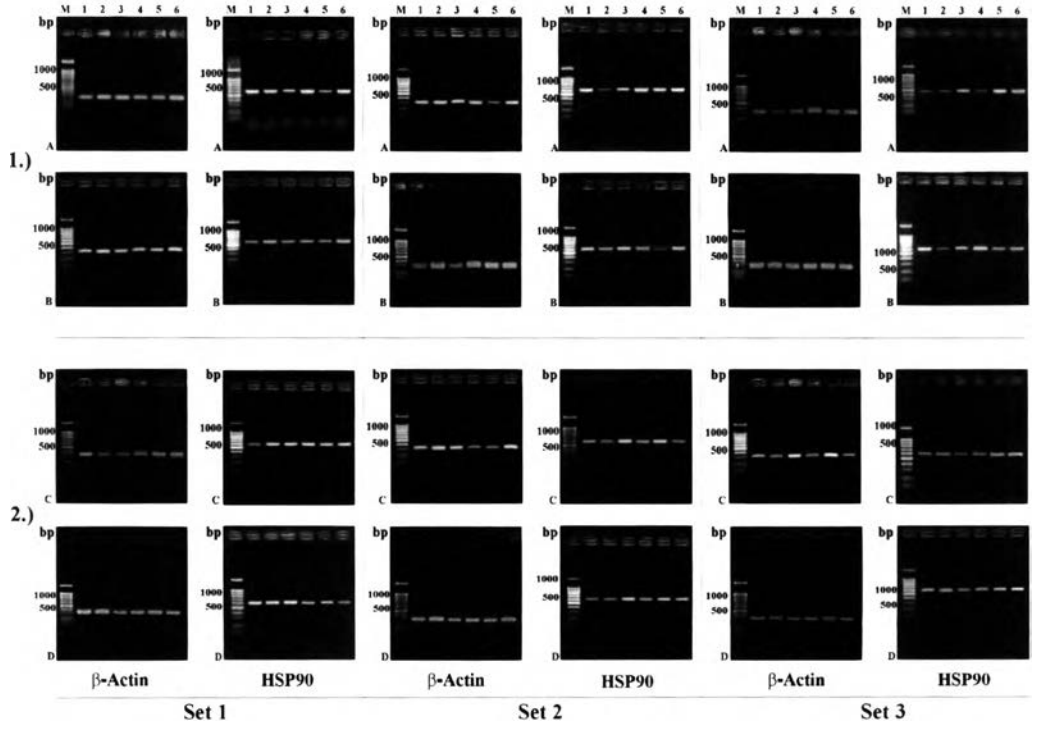

Figure A.21b The expression level of HSP90 gene transcripts in gill from *P.monodon* of tank1 (1) and Tank2 (2) between control and stress with handling stress in comparison with β-actin gene. Samples were obtained from 3 shrimps and analyzed by 1.2% agarose gel electrophoresis. A=Cotrol1, B=Handling1, C=Control2 and D=Handling2 are the result at control, handling stress shrimp. Lane M is 100 bp markers, Lane 1-5 represent 0, 6, 12, 24, 48, and 72h of stress shrimp with handling stress.

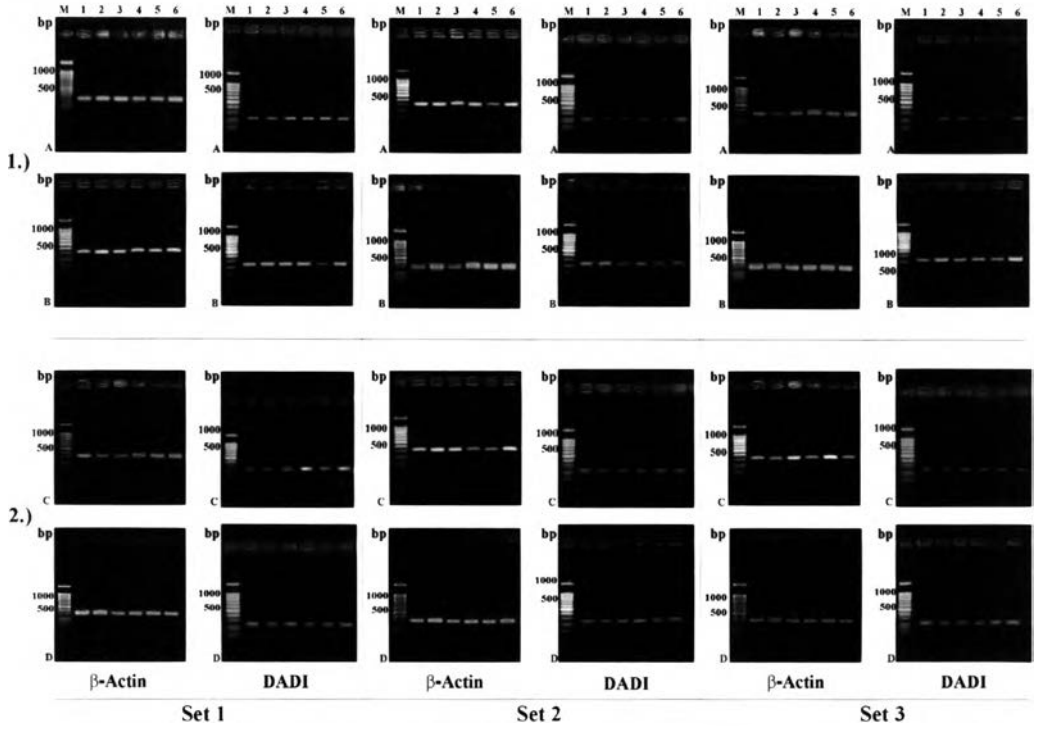

Figure A.22a The expression level of DADI gene transcripts in haemocyte from *P.monodon* of tank1 (1) and Tank2 (2) between control and stress with handling stress in comparison with β-actin gene. Samples were obtained from 3 shrimps and analyzed by 1.2% agarose gel electrophoresis. A=Cotrol1, B=Handling1, C=Control2 and D=Handling2 are the result at control, handling stress shrimp. Lane M is 100 bp markers, Lane 1-6 represent 0, 6, 12, 24, 48, and 72h of stress shrimp with handling stress.

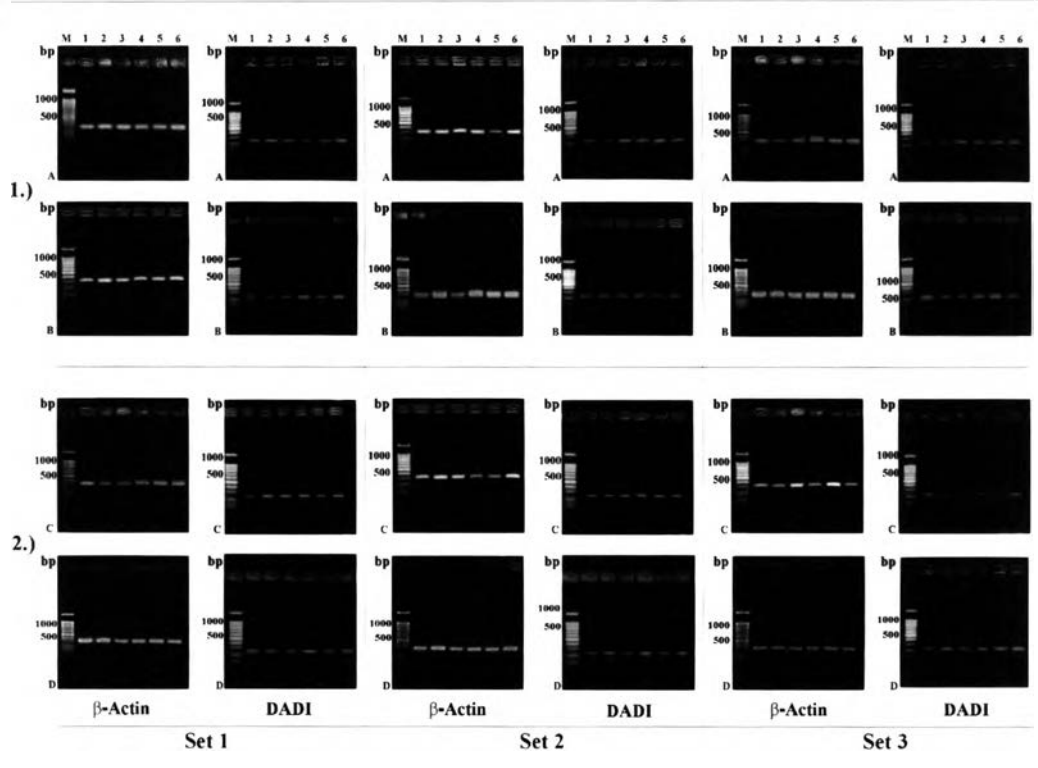

Figure A.22b The expression level of DADI gene transcripts in gill from *P.monodon* of tank1 (1) and Tank2 (2) between control and stress with handling stress in comparison with β-actin gene. Samples were obtained from 3 shrimps and analyzed by 1.2% agarose gel electrophoresis. A=Cotrol1, B=Handling1, C=Control2 and D=Handling2 are the result at control, handling stress shrimp. Lane M is 100 bp markers, Lane 1-6 represent 0, 6, 12, 24, 48, and 72h of stress shrimp with handling stress.

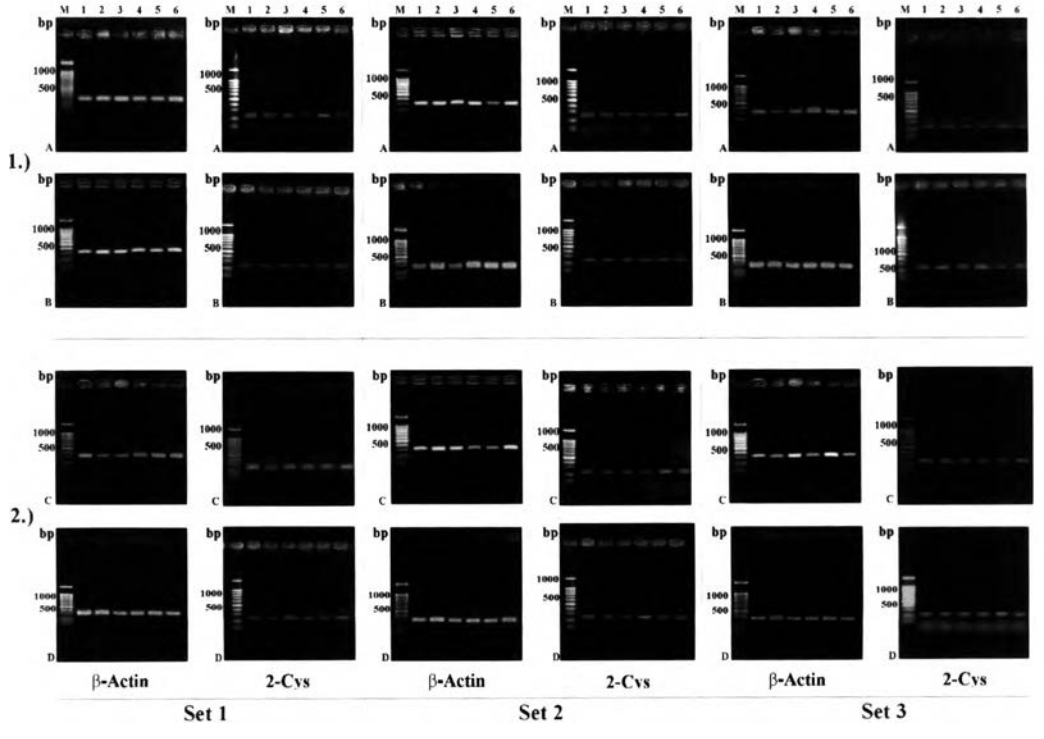

Figure A.23a The expression level of Thioredoxin peroxidase gene transcripts in haemocyte from *P.monodon* of tank1 (1) and Tank2 (2) between control and stress with handling stress in comparison with β-actin gene. Samples were obtained from 3 shrimps and analyzed by 1.2% agarose gel electrophoresis. A=Cotrol1, B=Handling1, C=Control2 and D=Handling2 are the result at control, handling stress shrimp. Lane M is 100 bp markers, Lane 1-6 represent 0, 6, 12, 24, 48, and 72h of stress shrimp with handling stress.

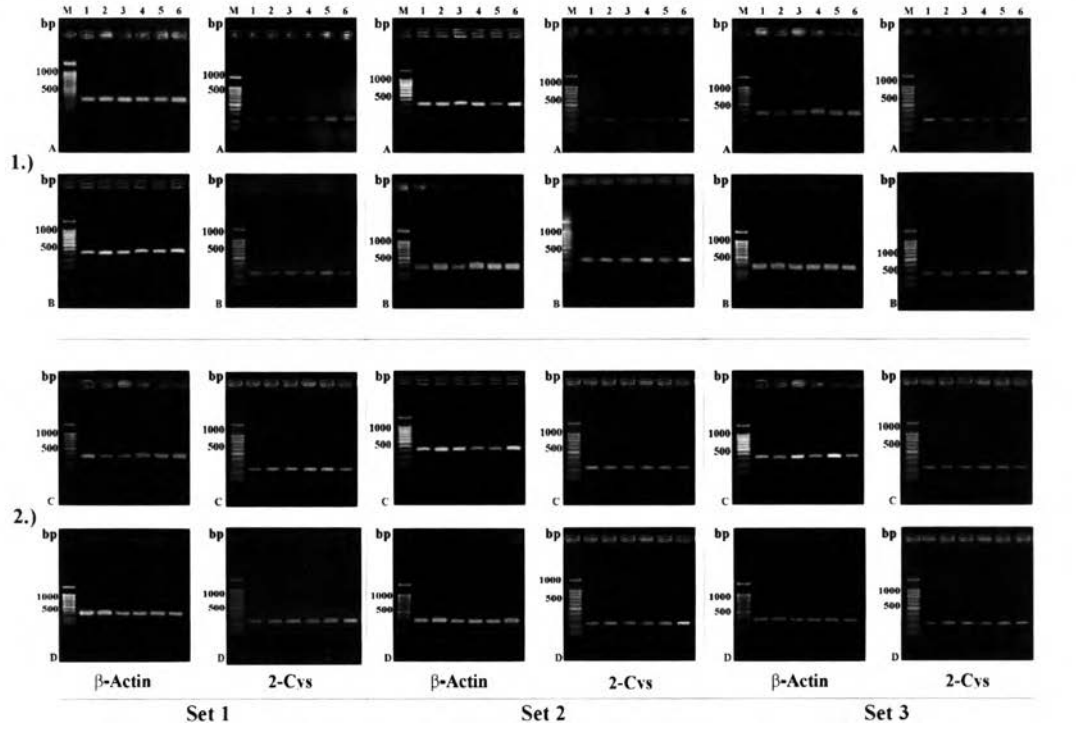

Figure A.23b The expression level of Thioredoxin peroxidase gene transcripts in gill from *P.monodon* of tank1 (1) and Tank2 (2) between control and stress with handling stress in comparison with β-actin gene. Samples were obtained from 3 shrimps and analyzed by 1.2% agarose gel electrophoresis. A=Cotrol1, B=Handling1, C=Control2 and D=Handling2 are the result at control, handling stress shrimp. Lane M is 100 bp markers, Lane 1-6 represent 0, 6, 12, 24, 48, and 72h of stress shrimp with handling stress.

Appendix D

Publication from this thesis

1. Sansook Boonseub, Narongsak Puanglarp and Paimsak Menasveta (2004). Molecular cloning and identification of superoxide dismutase and arginine kinase variants in black tiger shrimp, *Penaeus monodon*. 30th Congress on Science and technology of Thailand (Illustration).

Biography

Police Sub-Lieutenant Sansook Boonseub was born on July 27, 1979 in the province of Nakorn-Panom, Thailand. She graduated with the degree of Bachelor of Science in Biochemistry from faculty of Science at Khonkaen University in 2000. In 2002, she entered the Master program of Biotechnology at Chulalongkorn University.