REFERENCES

Akratanakul, P. 1990. Beekeeping in Asia. *FAO Agricultural Services Bullentin 68/4.* Food and Agricultural Organization of the United Nation. Rome, Italy.

Albert, S., Bhattacharya, D., Klaudiny, J., Schmitzova and Simuth, J. 1999a. The family of major royal jelly proteins and its evolution. *J. Mol. Evol.* 49:290-297.

Albert, S., Klaudiny, J., Simuth, J. 1999b. Molecular characterization of MRJP3, highly polymorphic protein of honeybee (*Apis mellifera*) royal jelly. *Insect. Biochem. Molec. Biol.* 29 : 427-434.

Albert, S. and Schmitz, J. 2002. Characterization of major royal jelly protein-like DNA sequences in *Apis dorsata. J. Apic. Res.* 41(3-4) : 75-82.

Albert, S. and Klaudiny, J. 2004. The MRJP/YELLOW protein family of *Apis mellifera*: Identification of new members in the EST library. *J. Insect Physiol.* 50(1) : 51-59.

Albertova, V., Su, S., Brockmann, A., Gadau, J., and Albert, S. 2005. Organization and potential function of the *mrjp*3 locus in four honeybee species. *J. Agric. Food. Chem.* 53 : 8075-8081.

Antinelli, J.F., Zeggane, S., Davico, R., Rognone, C., Faucon, J.P. and Lizzani, L. 2003. Evaluation of (*E*)-10-hydroxydec-2-enoic acid as a freshness parameter for royal jelly. *Food. Chem.* 80 : 85-89.

Bariliak, I.R. Berdyshev G.D., Dugan A.M. 1996. The antimutagenic action of apiculture products. *Tsitol Genet.* 30(6) : 48-55.

Bilikova, K., Kluadiny, J., and Simuth, J. 1999. Characterization of the basic major royal jeily protein MRJP2 of honeybee (*Apis mellifera*) and its preparation by heterologous expression in *E. coli. Biológia, Bratislava.* 54/6 : 733-739.

Bilikova, K., Wu, G., Simuth, J. 2001. Isolation of peptide fraction from honeybee royal jelly as antifaulbrood factor. *Apidologie.* 32 : 275-283.

Bilikova, K., Hanes, J., Nordhoff, E., Saenger, W., Klaudiny, J. and Simuth, J. 2002. Apisimin, a new serine-valine-rich peptide from honeybee (*Apis mellifera* L.) royal jelly : purification and molecular characterization. *FEBS Lett.* 528: 125-129.

Brouwers, E.V.M. 1982. Measurement of hypopharyngeal gland activity in the honeybee. *J. Apic. Res.* 21(4) : 193-198.

Celi, F.S., Zenilman, M.E., Shuldiner, R. 1993. A rapid and versatile method to synthesize internal standards for competitive PCR. *Nucleic. Acids. Res.* 21(4) : 1047.

Cenphakdee, K. 2003. *Cloning and sequencing of cDNA encoding major royal jelly protein family 4 and 5 of Apis cerana in Thailand.* Master's Thesis, Department of Biochemistry, Faculty of Science, Chulalongkorn University.

Cho, Y.T. 1977. Studies on royal jelly and abnormal cholesterol and triglycerides. *Am. Bee J.* 117 : 36-38.

Chung H.W. 2001. Reverse transcriptase PCR (RT-PCR) and quantitative-competitive PCR (QC-PCR). *Exp. Mol. Med.* 33(1 Suppl) : 85-97.

Corona, M., Estrada, E. and Zurita, M. 1999. Differential expression of mitochondrial genes between queens and workers during caste determination in the honey bee *Apis mellifera. J. Exp. Biol.* 202: 929-938.

Crailsheim, K. 1992. The flow of jelly within a honeybee colony. *J. Comp. Physiol. [B].* 162 : 681-689.

Diviacco, S., Norio, P., Sentilin, L., Menzo, S., Clementi, M., Biamonti, G., Riva, S., Falaschi, A., and Giacca, M. 1992. A novel procedure for quantitative polymerase chain reaction by coamplification of competitive templates. *Gene.* 122 : 313-320.

Elder, P., French, C., Subramaniam, M., Schmidt, L., and Getz, M. 1988. Evidence that the functional β-actin gene is single copy in most mice and is associated with 5′sequences capable of conferring serum-and cycloheximide-dependent regulation. *Mol. Cell. Biol.* 8 : 480-485.

Evans, J.D. and Wheeler, D.E. 1999. Differential gene expression between developing queens and workers in the honey bee, *Apis mellifera. Proc. Natl. Acad. Sci. USA.* 96 : 5575-5580.

Evans, J.D., Wheeler, D.E. 2001. Gene expression and the evolution of insect polyphenisms. *Bioessays.* 23 : 62-68.

Fontana, R., Mendes, M.A., de Souza, B.M., Konno, K. 2004. Jelleines: a family of antimicrobial peptides from the royal jelly of honeybees (*Apis mellifera*). *Peptides.* 25(6) : 919-928.

Fujiwara, S., Imai, J., Fujiwara, J., Yaeshima, T., Kawashima, T. and Kobayashi, K. 1990. A potent antibacterial protein in royal jelly. Purification and determination of the primary structure of royalisin. *J. Biol. Chem.* 265 : 11333-11337.

Gilliland, G., Perrin, S., Blanchard, K., and Bunn, H.F.. 1990. Analysis of cytokine mRNA and DNA detection and quantitation by competitive polymerase chain

reaction. *Proc. Natl. Acad. Sci. USA.* 87 : 2725-2729.

Hanes, J., Simuth, J. 1992. Identification and partial characterization of the major royal jelly protein of the honey bee (*Apis mellifera* L.). *J. Apic. Res.* 31 : 22-26.

Haydak, M.H. 1970. Honey bee nutrition. *Annu. Rev. Entomol.* 15 : 143-156.

Henco, K., and Heibey, M. 1990. Quantitative PCR: the determination of template copy numbers by temperature gradient gel electrophoresis (TGGE). *Nucleic Acids Res.* 18 : 6733-6734.

Hoegh, A.M., and Hviid, T.V. 2001. Design of an internal DNA standard for competitive RT-PCR using partial intron sequence and an artificial linker sequence. *BioTechniques.* 31(4) : 730-734.

Horikoshi, T., Danenberg, K.D., Stadlbauer, T.H.W., Volkenandt, M., Shea, L.C.C., Aigner, K., Gustavsson, B., Leichman, L., Frosing, R., Ray, M., Gibson, N.W., Spears, C.P., and Danenberg, P.V. 1992. Quantitation of thymidylate synthase, dihydrofolate reductase, and DT-diaphoroase gene expression in human tumors using the polymerase chain reaction. *Cancer Res.* 52 : 108-116.

Howe, S.R., Dimick, P.S., Benton, A.W. 1985. Composition of freshly harvested and commercial royal jelly. *J. Apic. Res.* 24 : 52-61.

Huang, Z.-Y., Otix, G.W., and Teal, P.E.A. 1989. Nature of brood signal activating the protein synthesis of hypopharyngeal gland in honeybee, *Apis mellifera* (Apidae : Hymenoptera). *Apidologie.* 20 : 455-464.

Iannuzzi J. 1990. Royal jelly: mystery food. *Am. Bee J.* 130 : 532-662.

Imjongjirak, C., Klinbunga, S., and Sittipraneed, S. 2005. Cloning, expression and

genomic organization of genes encoding major royal jelly protein 1 and 2 of the honey bee (*Apis cerana*). *J. Biochem. Mol. Biol.* 38(1) : 49-57.

Johansson, T.S.K. 1995. Royal jelly. *Bee World.* 36 : 3-13.

Judova, J., Klaudiny, J., and Simuth, J. 1998. Preparation of combinant abundant protein MRJP1 of royal jelly. *Biológia, Bratislava.* 53 : 777-784.

Kamakura, M., Suenobu, N., and Fukushima, M. 2001a. Fitty-seven-kDa protein in royal jelly enhances proliferation of primary cultured rat hepatocytes and increases albumin production in the absence of serum. *Biophys. Res. Commun.* 282 : 865-874.

Kamakura M., Mitani, N., Fukuda, T., and Fukushima, M. 2001b. Antifatigue effect of fresh royal jelly in mice. *J. Nutr. Sci. Vitaminol (Tokyo).* 47 : 394-401.

Karaali, A., Meydanoglu, F. and Eke, D. 1988. Studies on composition, freeze drying and storage of Turkish royal jelly. *J. Apic. Res.* 27(3) : 182-5.

Kattz, H.H. and Knecht, D. 1990. Patterns of larval food production by hypopharyngeal glands in adult worker honey bees. *Apidologie.* 21 : 457-468.

Khan, I., Tabb, T., Garfield, R.E., and Grover, A.K. 1992. Polymerase chain reaction assay of mRNA using 28S rRNA as internal standard. *Neurosci. Lett.* 136 : T18.

Kimura, Y., Kajiyama, S., Kanaeda, J., Izukawa, T., Yonekura, M. 1996. N-linked sugar chain of 55-kDa royal jelly glycoprotein. *Biosci. Biotech. Biochem.* 60(12) : 2099-2102.

Kinoshita, T., Imamura, J., Nagai, H., and Shimotohno, K. 1992. Quantification of gene expression over a wide range by the polymerase chain reaction. *Anal. Biochem.* 206 : 231-235.

Kludiny, J., Hanes, J., Kulifajova, J., Albert, S., and Simuth, J. 1994a. Molecular cloning of two cDNAs from the head of the nurse honeybee (*Apis mellifera* L.) for coding related proteins of royal jelly. *J. Apic. Res.* 33(2) : 105-111.

Klaudiny, J., Kulifajova, J., Crailsheim, K., Simuth, J. 1994b. New approach to the study of division of labour in the honeybee colony (*Apis mellifera* L.). *Apidologie*. 25 : 596-600.

Kohno, K., Okamoto, I., Sano, O., Arai, N., Iwaki, K., Ikeda, M. and Kurimoto, M. 2004. Royal jelly inhibits the production of proinflammatory cytokines by activated macrophages. *Biosci. Biotech. Biochem.* 68(1) : 138-145.

Koya-Miyata, S., Okamoto, I., Ushio, S., Iwaki, K.,et al. 2004. Identification of a collagen production-promoting factor from an extract of royal jelly and its possible mechanism. *Biosci. Biotech. Biochem.* 68 : 767-773.

Krell, R. 1996. Value-Added products from beekeeping. *FAO Agricultural services bullentin No. 124*. Food and Agricultural Organization of the United Nation. Rome, Italy.

Kubo, T., Sasaki, M., Nakamura, J., Sasagawa, H., Ohashi, K., Takeucha, H. and Natori, S. 1996. Change in the expression of hypopharyngeal-gland proteins of the worker honeybee (*Apis mellifera* L.) with age and/or role. *J. Biochem.* 119(2) : 291-5.

Kucharski, R., Maleszka, R., Hayward, D.C., and Ball, E.E. 1998. A royal jelly protein is expressed in a subset of Kenyon cells in the mushroom bodies of the honey bee brain. *Naturwissenschaften.* 85 : 343-346.

Kucharski R, and Maleszka R. 2002. Evaluation of differential gene expression during behavioral development in the honeybee using microarrays and

northern blots. *Genome Biology.* 3(2) : 0007.1-0007.9.

Laidlaw, H.H. Jr. and Eckert, J.E. 1962. *Queen rearing.* California : University of California Press.

Lercker, G., Capella, P., Conte, L.S., Ruinji, F. and Giordani, G. 1981. Components of royal jelly. I. Identification of organic acid. *Lipids.* 16 : 912-919.

Lercker, G., Capella, P., Conte, L.S., Ruinji, F. and Giordani, G. 1982. Components of royal jelly: II. The lipid fraction, hydrocarbons and sterolds. *J. Apic. Res.* 21(3) : 178-184.

Leung, R., Ho, A., Chan, J., Choy, D. and Lai, C.K. 1997. Royal jelly consumption and hypersensitivity in the community. *Clin. Exp. Allergy.* 27(3) : 333-6.

Lin, Y.H. 2004. Honeybees make a comeback. *Tzu Chi.* 11(1) : 3-10.

Loitsch, S.M., Kippenberger, S., Dauletbaev, N., Wagner, T.O.F., and Bargon, J. 1999. Reverse transcription-competitive multiplex PCR improves quantification of mRNA in clinical samples-Application to the low abudance *CFTR* mRNA. *Clin. Chem.* 45 : 619-624.

Malecova, B., Ramser, J., O'Brien, J.K., Janitz, M., Judova, J., Lehrach, H., Simuth, J. 2003. Honeybee (*Apis mellifera* L.) *mrjp* gene family: computational analysis of putative promoters and genomic structure of *mrjp* 1, the gene coding for the most abundant protein of larval food. *Gene.* 303 : 165-175.

Murphy, L.D., Herzog, C. E., Rudick, J.B., Fojo, A.T., and Bates, S.E. 1990. Use of the polymerase chain reaction in the quantitation of *mdr*-1 gene expression. *Biochemistry.* 29 : 10351-10356.

Nagai, T., and Inoue, R. 2004. Preparation and the functional properties of water extract and alkaline extract of royal jelly. *Food Chem.* 84 : 181-186.

Naiem, E.-S., Hrassnigg, N, and Crailsheim, K. 1999. Nurse bees support the physiological development of young bees (*Apis mellifrea* L.). *J. Comp. Physiol. [B].* 169 : 271-279.

Nakayama, H., Yokoi, H., and Fujita, J. 1992. Quantification of mRNA by non-radioactive RT-PCR and CCD imaging system. *Nucleic. Acids. Res.* 20 : 4939.

Ohashi, K., Sawata, M., Takeuchi, H., Natori, S., and Kubo, T. 1996. Molecular cloning of cDNA and analysis of expression of the gene for α-glucosidase from the hypopharyngeal gland of the honeybee *Apis mellifera* L. *Biochem. Biophys. Res. Commun.* 221 : 380-385.

Ohashi, K., Natori, S., Kubo, T. 1997. Change in the mode of gene expression of the hypopharyngeal gland cells with an age-dependent role change of the worker honeybee *Apis mellifera* L. *Eur. J. Biochem.* 249 : 797-802.

Ohashi, K., Natori, S., and Kubo, T. 1999. Expression of amylase and glucose oxidase in the hypopharyngeal gland with an age-dependent role change of the worker honeybee (*Apis mellifera* L.). *Eur. J. Biochem.* 265 : 127-133.

Oka, H, Emori, Y., Kobayashi, N, Hayashi, Y, and Nomoto, K. 2001. Suppression of allergic reactions by royal jelly in association with the restoration of macrophage function and the improvement of Th1/Th2 cell responses. *Int. Immunopharmacol.* 1(3) : 531-32.

Okamoto, I., Taniguchi, Y., Kunikata, T., Kohno, K., Iwaki, K., Ikeda, M. and Kurimoto, M. 2003. Major royal jelly protein 3 modulates immune responses *in vitro* and *in vivo*. *Life Sci.* 73 : 2029-2045.

Page Jr., R.E. and Peng C.Y.-S. 2001. Aging and development in social insects with

emphasis on the honeybee, *Apis mellifera* L. *Exp. Gerontol.* 36 : 695-771.

Palma, M.S. 1992. Composition of freshly harvested Brazilian royal jelly: identification of carbohydrates from sugar fraction. *J. Apic. Res.* 31(1) : 42-44.

Pannetier, C., Delassus, S., Farche, S., Saucier, C., and Kourilshy, P. 1993. Quantitative titration of nucleic acids by enzymatic amplification reactions run to saturation. *Nucleic Acids Res.* 21 : 577-583.

Pothichot, S. and Wongsiri, S. 1993. Attempts in queen rearing of *Apis cerana* larvae in *Apis mellifera* colonies and *Apis mellifera* larvae in *Apis cerana* colonies. *Asian Apicul.* 128-133.

Robinson, G.E. 1991. Hormonal and genetic control of honeybee division of labour. In L. J. Goodman and R.C. Fisher (eds.), *The behaviour and physiology of bees*, pp.14-27. Melksham : Redwood Press.

Ruttner, F. 1988. *Biogeography and Taxonomy of Honey Bee*. Berlin : Springer-Verlag, pp.120-166.

Sanguandeekul, R. and Nimachailool, P. 1993. *Chemical composition and antibacterial action of royal jelly in Thailand.* In L.J. connor, T., Rinderer, H.A. Sylvester S. and Wongsiri (eds.). Asian Apiculture chesshire : Wicwas Press. 327-332 pp.

Sano, O., Kunikata, T., Kohno, K., Iwaki, K., Ikeda, M., and Kurimoto, M. 2004. Characterization of royal jelly proteins in both Africanized and European honeybees (*Apis mellifera*) by two-dimensional gel electrophoresis. *J. Agr. Food Chem.* 52(1) : 15-20.

Schmidt, J.O. and Buchmann, S.L. 1992. *Other products of the hive*. In J.M. Graham,

ed., The hive and the honeybee, pp. 927-988. USA : Dadant & Sons.

Schmitzova, J., Klaudiny, J., Albert, S., Schroder, W., Schreckkengost, W., Hanes, J., Judova., J. and Simuth, J. 1998. A family of major royal jelly proteins of the honeybee *Apis mellifera* L. *Cell. Mol. Life Sci.* 54 : 1020-1030.

Siebert, P.D., and Larrick, J.W. 1993. PCR MIMICs : competitive DNA fragments for use as internal standards in quantitative PCR. *BioTechniques.* 14 : 244-249.

Simuth J. 2001. Some properties of the main protein honeybee (*Apis mellifera* L.) royal jelly. *Apidologie.* 32 : 69-80.

Simuth, J., Bilikova, K., Kovacova, E., Kuzmova, Z., and Schroder, W. 2004. Immunochemical approach to detection of adulteration in honey: physiologically active royal jelly protein stimulating TNF-α release is a regular component of honey. *J. Agr. Food Chem.* 52 : 2154-2158.

Smith, D.R. 1991. Mitochondrial DNA and honey bee biogeography. In D.R. Smith (ed), *Diversity in the gene Apis*, pp. 131-176. Oxford : Westview Press.

Srisuparbh, D. 2002. *Characterization and cDNA cloning of major royal jelly protein of Apis cerana in Thailand.* Ph.D. Thesis, Department of Biochemistry, Faculty of Science, Chulalongkorn university.

Srisuparbh, D., Klinbunga, S., Wongsiri, S. and Sittipraneed, S. 2003. Isolation and characterization of major royal jelly cDNAs and proteins of the honey bee (*Apis cerana*). *J. Biochem. Mol. Biol.* 36 (6) : 572-579.

Su, S., Albert, S., Chen, S., and Zhong, B. 2005. Molecular cloning and analysis of four cDNAs from the heads of *Apis cerana cerana* nurse honeybees coding for major royal jelly proteins. *Apidologie.* 36 : 389-401.

Sver, L., Orsolic, N., Tadic, Z., Njari, B., Valpotic, I. and Basic, I. 1996. A royal jelly as new potential immunomodulator in rats and mice. *Comp. Immun. Microbiol. Infect. Dis.* 19(1) : 31-38.

Takenaka, T. 1982. Chemical composition for royal jelly. *Honeybee-Science.* 3 : 69-74.

Takenaka, T. and Echigo, T. 1983. Proteins and peptides in royal jelly. *Nippon Nogeik. Kaishi.* 57 : 1203-1209.

Takenaka, T. and Takenaka, Y. 1996. Royal jelly from *Apis cerana japonica* and *Apis mellifera. Biosci. Biotech. Biochem.* 60(3) : 518-520.

Tamura, T., Fiji, A. and Kuboyama, N. 1985. Effect of royal jelly on experimental. *Proceeding of the XXXth International Apicultural Congress*, Nagoya, Japan. Pp. 474-477.

Thien, F.C., Leung, R., Baldo, B.A., Weiner, J.A., Plomly, R. and Czarny, D. 1996. Asthma and anaphylaxis induced by royal jelly. *Clin. Exp. Allergy.* 26(2) : 216-222.

Trongnipatt, N. 2002. *Chemical composition of royal jelly and screening of cDNA library of mamdibular gland of Apis cerana by DNA sequencing.* Master's Thesis, Department of Biochemistry, Faculty of Science, Chulalongkorn University.

Vanden Heuval, J., Tyson, F., and Bell, D. 1993. Construction of recombinant RNA templates for use as internal standards in quantitative RT-PCR. *BioTechniques.* 14 : 395-398.

Verma, L.R. 1992. Species and genetic diversity in Himalayan honeybee. In L.R. Verma (ed), *Honeybees in mountain agriculture*, pp. 39-50. Colorado :

Westview Press.

Vittek, J. 1995. Effect of royal jelly on serum lipids in experimental animals and humans with atherosclerosis. *Experientia.* 51 : 927-935.

Wang, A.M., Doyle, M.V., and Mark, D.F. 1989. Quantitation of mRNA by the polymerase chain reaction. *Proc. Natl. Acad. Sci. USA.* 86 : 9719-9721.

Watanabe, K., Shinmoto, H., Kobori, M., Tsushida, T., Shinokara, K., Kanaeda, J., and Yonekura, M. 1996. Growth stimulation with honeybee royal jelly DIII protein of lymphocytic cell lines in a serum-free medium. *Biotechnol. Tech.* 10 : 959-962.

Whitfield, C.W., Band, M.R., Bonaldo, M.F., Kumar, C.G., Liu, L., Pardinas, J.R., Robertson, H.M., Soares, M.B., Robinson, G.E. 2002. Annotated expressed sequence tags and cDNA microarrays for studies of brain and behavior in the honey bee. *Genome Res.* 12(4) : 555-566.

Wong, M.L., and Medrano, J.F. 2005. Real-time PCR for mRNA quantitation. *BioTechniques.* 39(1) : 75-85.

Wongsiri, S. 1989. *Biology of honeybees (in Thai).* Bangkok : Ton-or.

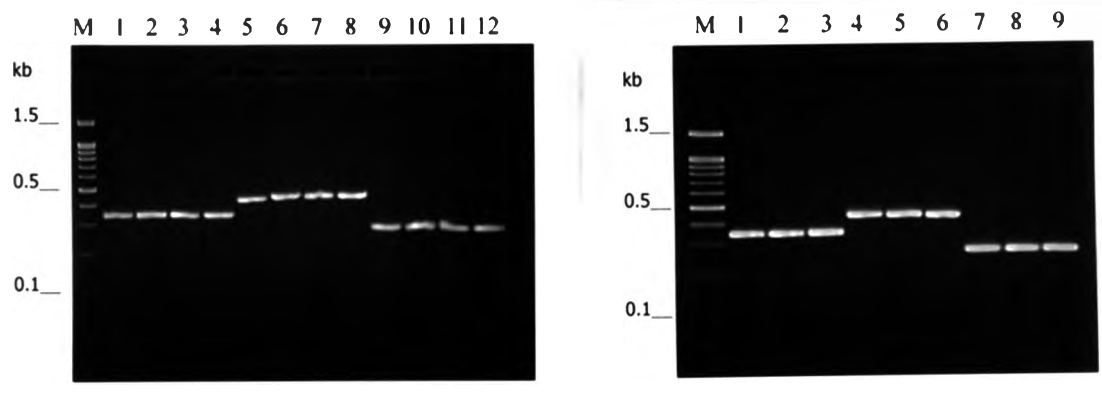
Wongsiri, S., Ringerer, T.E. and Sylvester, H.A. 1990. *Biodiversity of honey bee in Thailand.* Bee Biology Research Unit. Chulalongkorn University.

Wongsiri, S., Chanchao, C., Deowanish, S., Aemprapa, S., Chaiyawong, T., Petersen, S. and Leepitakrat, S. 2000. Honey bee diversity and beekeeping in Thailand. *Bee World.* 81(1) : 20-29.

Yonei, Y., Shibagaki, K., Tsukada, N., Nagasu, N., Inagaki, Y., Miyamoto, K., Suzuki, O. and Kiryu, Y. 1997. Case report: haemorrhagic colitis associated with royal jelly intake. *J. Gastroenterol. Hepatol.* 12(7) : 495-9.

APPENDICES

Appendix A



(a) (b)

I. Agarose gel electrophoresis showing an optimization of primers concentration, at 1.5 mM $MgCl_2$ concentration (a), and optimization of $MgCl_2$ concentration, at a constant primers concentration of 0.15 μM (b), for AcMRJP1-3 quantification using 28S rRNA and genomic DNA competitor as internal standard.

Lane M = 100 bp DNA ladder

Panel a, Lane 1-4 = Amplification products using 0.1, 0.15, 0.20, and 0.25 μM of AcMRJP1 primers, respectively.

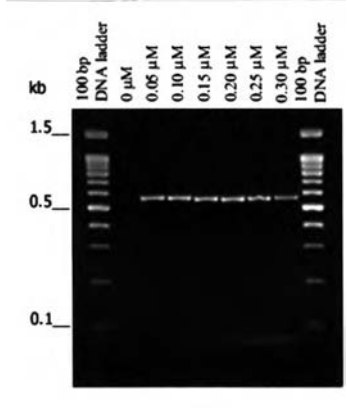
Lane5-8 = Amplification products using 0.1, 0.15, 0.20, and 0.25 μM of AcMRJP2 primers, respectively.

Lane9-12 = Amplification products using 0.1, 0.15, 0.20, and 0.25 μM of AcMRJP3 primers, respectively.

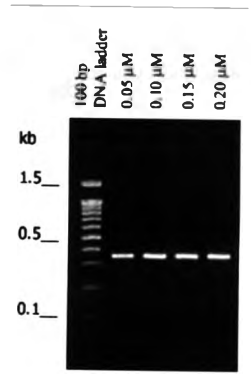
Panel b, Lane 1-3 = Amplification products of AcMRJP1 primers using 1.0, 1.5, and 2.0 mM of $MgCl_2$, respectively.

Lane 4-6 = Amplification products of AcMRJP2 primers using 1.0, 1.5, and 2.0 mM of $MgCl_2$, respectively.

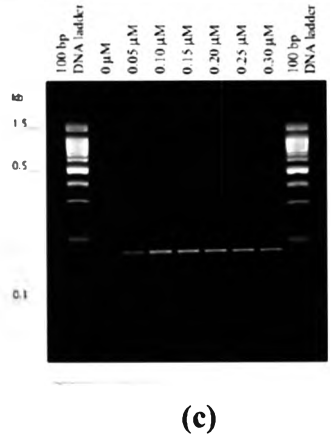
Lane7-9 = Amplification products of AcMRJP3 primers using 1.0, 1.5, and 2.0 mM of $MgCl_2$, respectively.



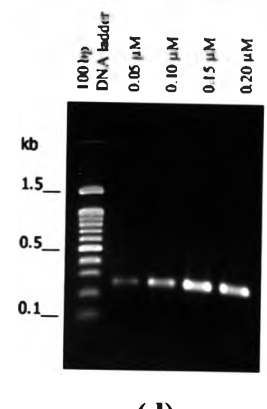
(a)



(b)



(c)



(d)

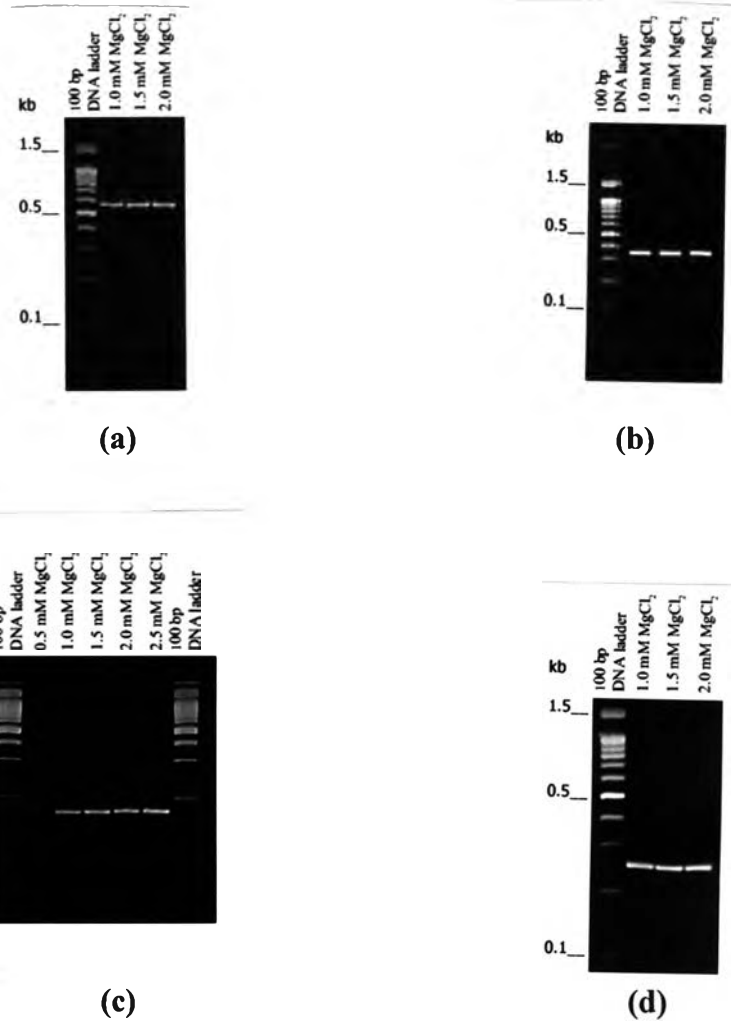
II. Agarose gel electrophoresis showing an optimization of primers concentration, at 1.5 mM $MgCl_2$ concentration for quantification using 28S rRNA as internal standard

Panel a = AcMRJP5 primers

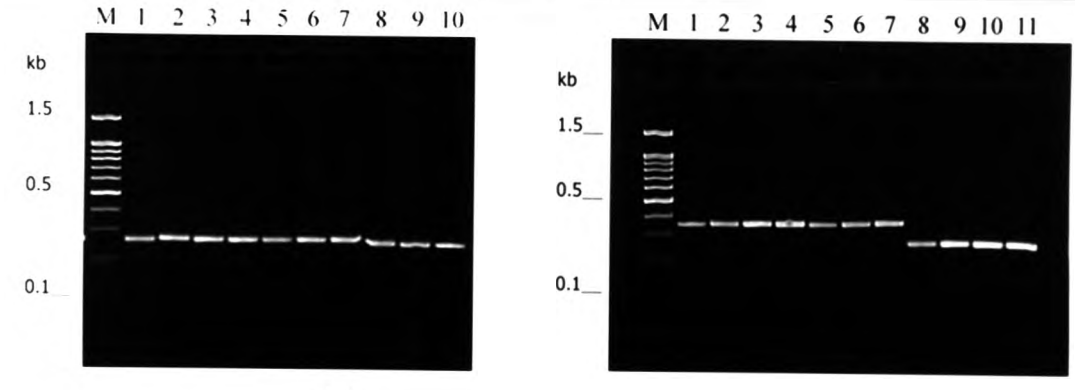
Panel b = AcMRJP6 primers

Panel c = AcApisimin primers

Panel d = 28S rRNA primers



III. Agarose gel electrophoresis showing an optimization of $MgCl_2$ concentration, at a 0.10 μM constant primers concentration of AcMRJP5 (a), at a 0.15 μM for AcMRJP6 (b), AcApisimin (c) and 28S rRNA (d) for quantification using 28S rRNA as internal standard.



(a) (b)

IV. Agarose gel electrophoresis showing an optimization of primers and $MgCl_2$ concentration for AcMRJP4-6 amplification for quantification using genomic DNA competitor as internal standard.

Lane M = 100 bp DNA ladder

Panel a, Lane 1-4 = Amplification products using AcMRJP5 primers concentration as 0.05, 0.10, 0.15 and 0.20 μM, respectively at a constant $MgCl_2$ of 1.5 mM.

Lane 5-7 = Amplification products using 1.0, 1.5, and 2.0 mM of $MgCl_2$ at constant 0.15 μM AcMRJP5 primers concentration.

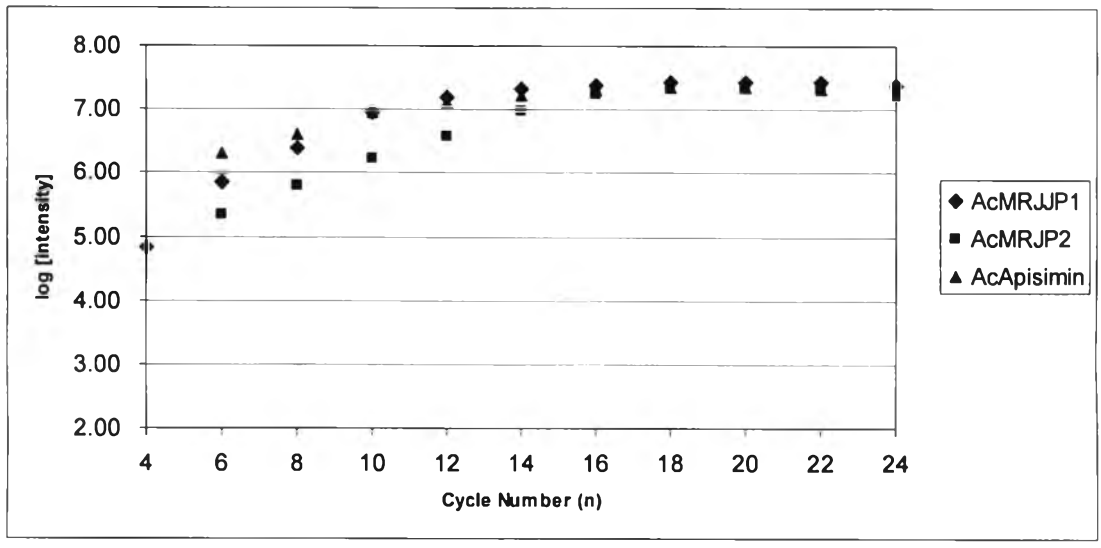
Lane 8-10 = Amplification products using 1.0, 1.5, and 2.0 mM of $MgCl_2$ at constant 0.15 μM AcMRJP4 primers concentration.

Panel b, Lane 1-4 = Amplification products using AcMRJP6 primers concentration as 0.05, 0.10, 0.15 and 0.20 μM, respectively at a constant $MgCl_2$ of 1.5 mM.

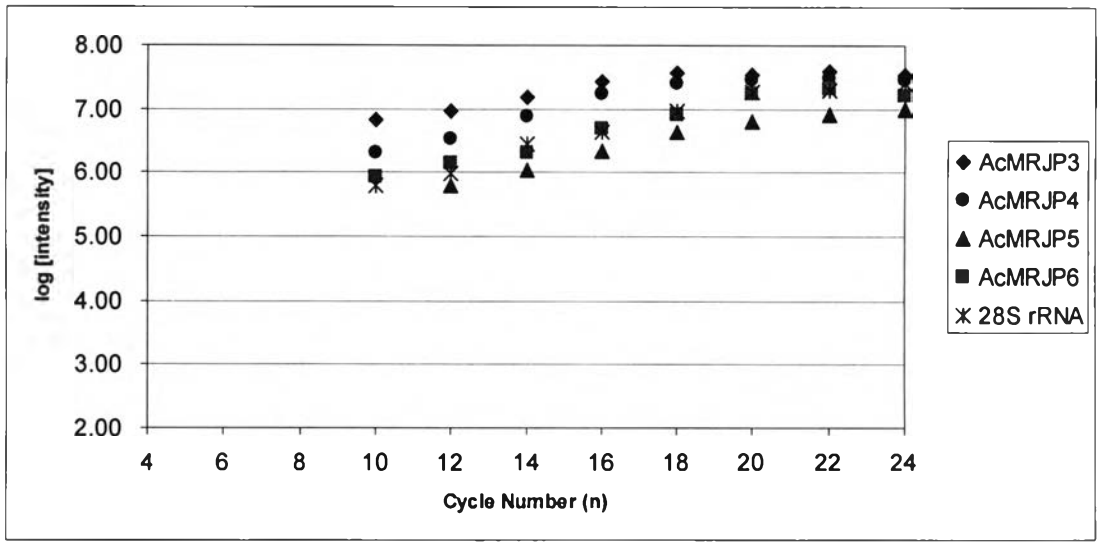
Lane 5-7 = Amplification products using 1.0, 1.5, and 2.0 mM of $MgCl_2$ at constant 0.15 μM AcMRJP6 primers concentration.

Lane 8-11 = Amplification products using AcMRJP4 primers concentration as 0.05, 0.10, 0.15 and 0.20 μM, respectively at a constant $MgCl_2$ of 1.5 mM.

Appendix B



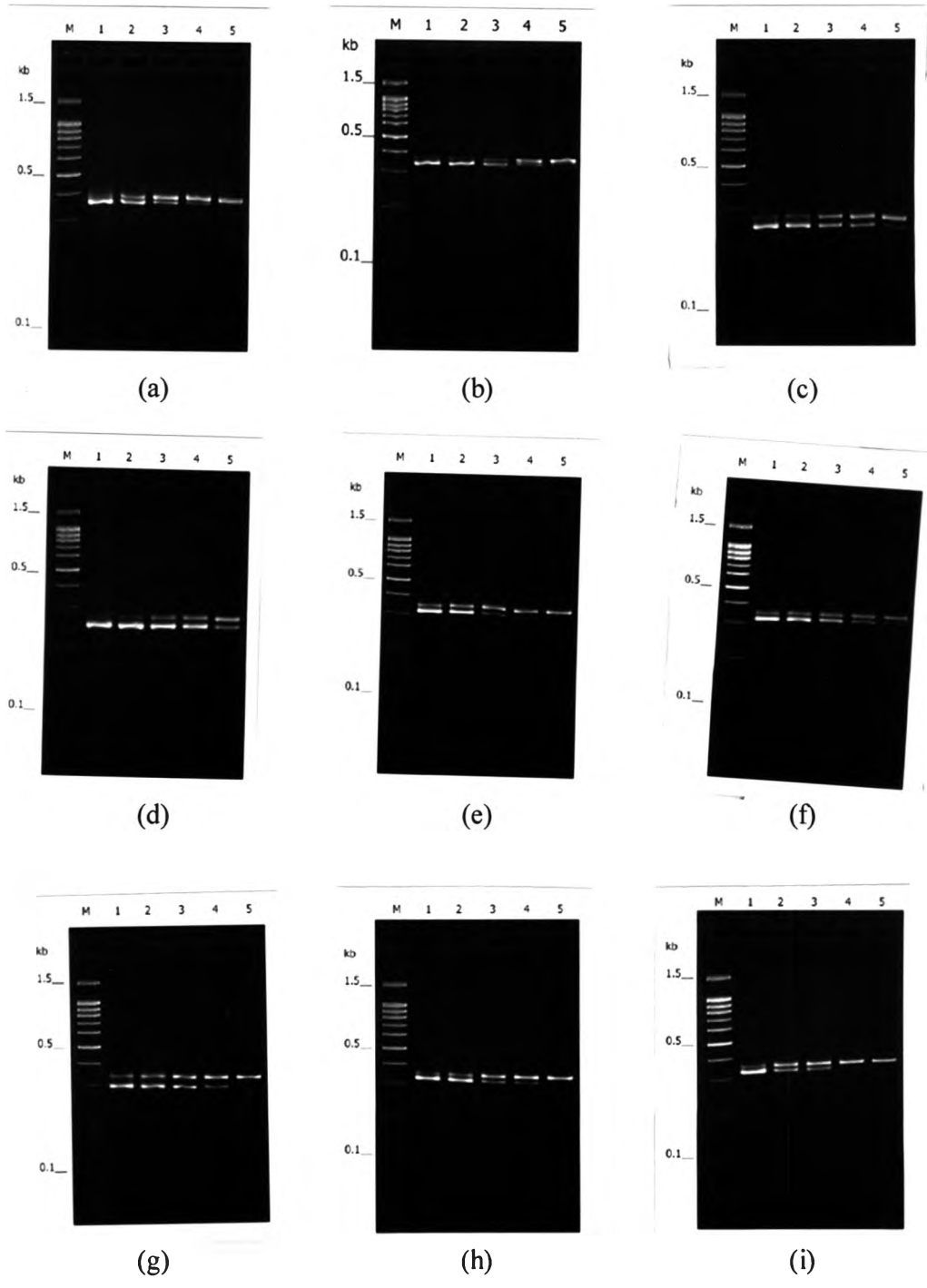
(a)



(b)

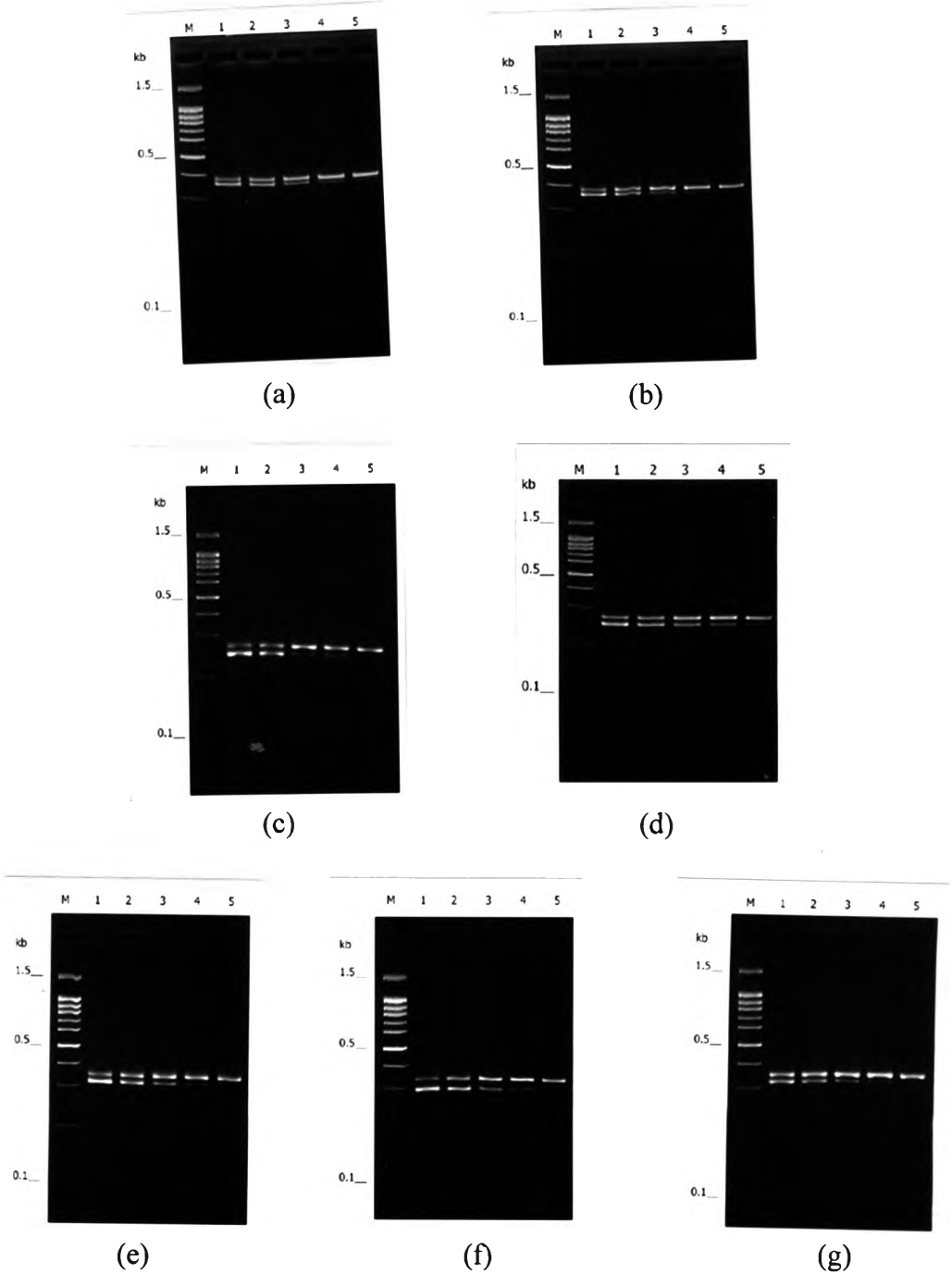
I. The relationship between the log of the intensity of PCR product and a number of consecutive amplification cycles (n)

Appendix C



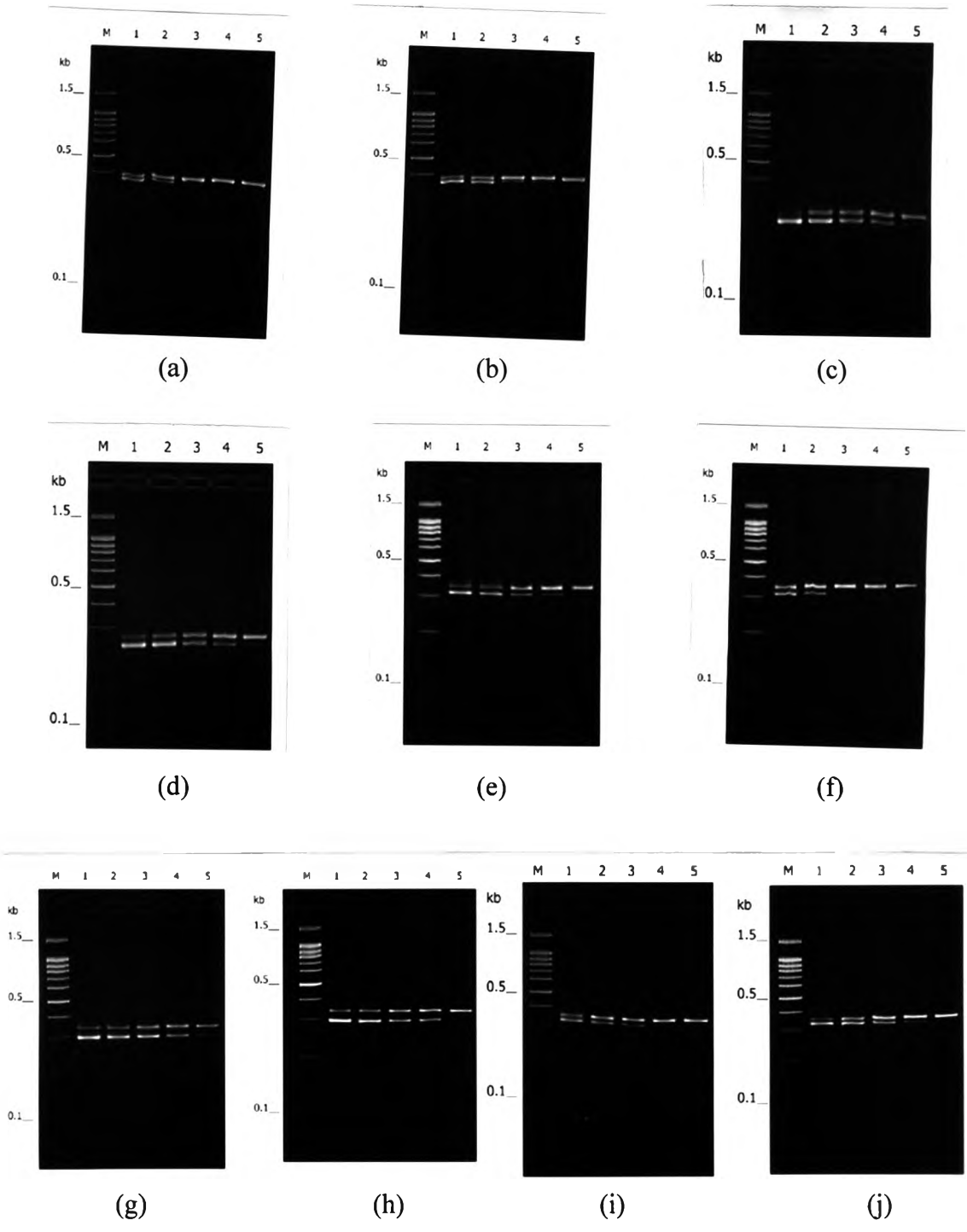
I. Agarose gel electrophoresis for quantification of AcMRJPs mRNA in hypopharyngeal gland of *A. cerana* 5-10 days nurse bees at the constant of the target co-amplified in the presence of cDNA competitor.

- For AcMRJP2, at the constant 1 ng (a) and 0.5 ng (b) of the target.
- For AcMRJP3, at the constant 10 ng (c) and 4 ng (d) of the target.
- For AcMRJP4, at the constant 10 ng (e) and 5 ng (f) of the target.
- For AcMRJP5, at the constant 40 ng (g) of the target.
- For AcMRJP6, at the constant 20 ng (h) and 10 ng (i) of the target.



II. Agarose gel electrophoresis for quantification of AcMRJPs mRNA in hypopharyngeal gland of *A. cerana* 11-15 days nurse bees at the constant of the target co-amplified in the presence of cDNA competitor.

- For AcMRJP2, at the constant 4 ng (a) and 2 ng (b) of the target.
- For AcMRJP3, at the constant 10 ng (c) and 4 ng (d) of the target.
- For AcMRJP4, at the constant 10 ng (e) of the target.
- For AcMRJP5, at the constant 40 ng (f) of the target.
- For AcMRJP6, at the constant 20 ng (g) of the target.



III. Agarose gel electrophoresis for quantification of AcMRJPs mRNA in hypopharyngeal gland of *A. cerana* forager bees at the constant of the target co-amplified in the presence of cDNA competitor.

- For AcMRJP2, at the constant 10 ng (a) and 5 ng (b) of the target.
- For AcMRJP3, at the constant 20 ng (c) and 40 ng (d) of the target.
- For AcMRJP4, at the constant 20 ng (e) and 40 ng (f) of the target.
- For AcMRJP5, at the constant 40 ng (g) and 60 ng (h) of the target.
- For AcMRJP6, at the constant 10 ng (i) and 5 ng (j) of the target.

BIOGRAPHY

Miss Uraiwan Yimprasert was born on April 4, 1976. She graduated with the Bachelor degree of Science from the Department of Associated Medical Science at KhonKaen University in 1998. She has studied for the degree of Master of Science at the department of Biochemistry, Chulalongkorn University since 2002.