REFERENCES

Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K., and Watson, J. D. 1983. *Molecular Biology of the Cell.* New York : Garland.

Alkami Quick Guide™, 1999. Available from: http://www.Alkami.com

Anger, B. and Bernatchez, L. 1997. Complex evolution of a salmonid microsatellite locus and its consequences in inferring allelic divergence from size information. *Mol. Biol. Evol.* 14: 230-238.

Arrathrakorn, S. 2001. *Breeding ecology, and agriculture field utilization of green peafowl Pavo muticus Linnaeus, 1766 at Doi Phu Nang national park.* Master's Thesis, Department of Biology, Graduate School, Chulalongkorn University.

Arrathrakorn, S., and Meckwichai, W. 2000. Foraging ecology of green peafowl in breeding season at Doi Phu Nang national park, Phrayao province. *26th Congress on science and technology of Thailand.* 26: 322.

Attippahakun, W. and Attippahakun, M. (eds.) 1996. *PCR Technology* Mahidol University.

Avise, J. C. 1994. *Molecular Markers, Natural History and Evolution.* New York: Chapman & Hall, Inc.

Baratti, M., Alberti, A., Groenen, M., Veenendaal, T. and Fulgheri, F. D. 2001. Polymorphic microsatellites developed by cross-species amplification in common pheasant breeds. *Animal Genetics* 32: 222-225.

Boongsong, L., and Philip, D. R. 1991. *A guide to the birds of Thailand.* 101.

Boripat, S. 1997. *MitochondrialDNA sequence variation within and between red jungle fowl (Gallus gallus gallus* and *Gallus gallus padiceus) in Thailand.*

Master's Thesis, Department of Biology, Graduate School, Chulalongkorn University.

Britten R. J., and Kohne, D. E. 1968. Repeated sequences in DNA. Hundreds of thousands of copies of DNA sequences have been incorporated into the genomes of higher organisms. *Science* 161: 529–40.

Brooker, A. L., Cook, D., Bentzen, P., Wright, J. M. and Doyle, R. W. 1994. Organization of microsatellites differs between mammals and cold-water teleost fishes. *Can. J. Fish. Aquat. Sci.* 51: 1959-1966.

Brondani, C., Brondani, R. P. V., Garrido, L. R. and Ferreira, M. E. 2000. Development of microsatellite markers for the genetic analysis of *Magnaporthe grisea*. *Genetics and Molecular Biology* 23(4): 753-762.

Brown, W. M., George, M. J, and Wilson, A. C. 1979. Rapid evolution of mitochondrial DNA. *Proc. Natl. cad. Sci.* 76: 1967-1971.

Budowle, B., Giusti, A. M., Waye, J. S., Baechtel, F. S., Fourney, R. M., Adams, D. E., Presley, L. A., Deadman, H. A. and Monson, K. L. 1991. Fixed BIN analysis for statistical evaluation of continuous distributions of allelic data from VNTR loci for use in forensic comparisons. *Am. J. Hum. Genet.* 48: 841-855.

Carlson, J. E., Tulsieram, L. K. Glaubitz, J. C., Luk, V. W. K., Kauffeldt, C., Rutledge, R., 1991. Segregation of random amplified DNA markers in F1 progeny of conifers. *Theor. Appl. Genet.* 83: 194-200.

Cheng, H. H., Levin, I., Vallejo, R. L., Khatib, H. Dodgson, J. B., Crittenden, L. B. and Hillel, J. 1995. Development of a Genetic Map of the Chicken with Markers of High Utility. *Poultry Science* 74: 1855-1874.

Cheng, H. H. and Crittenden, L. B. 1994. Microsatellite markers for genetic mapping in the chicken. *Poultry Science* 73: 539-546.

CITES, 1996. Checklist of CITES species. World Conservation Monitoring centre. United Kingdom.

Cooper, A. 1994. DNA from museum specimens. *Ancient DNA* Springer- Verlag.

Crooijmans, R. P. M. A., Dijkhof, R. J. M., Poel, J. J., and Groenen, M. A. M. 1997. New microsatellite markers in chicken optimized for automatesd fluorescent genotyping. *Animal Genetics* 28: 427-437.

Crooijmans, R. P. M. A., Kampen, A. J. A., Poel, J. J., and Groenen, M. A. M. 1993. Highly polymorphic microsatellite markers in poultry. *Animal Genetics* 24: 441-443.

Devis, L. G., Kuehl, W. M., and Battey, J. F. 1994. *Basic Method in Molecular Biology* 2nd ed. Connecticut: Appleton and Lauge.

David, H. M., Moritz, C., and Mable, B. K. 1996. *Molecular Systematics* 2nd ed. Sunderland MA: Sinauer.

Deepak, S., Appa Rao, K. B. C., Singh, H. P., and Totey, S. M. 1998. Randomly amplified polymorphic DNA (RAPD) for evaluating genetic relationships among varieties of guinea fowl. *Genetic Analysis* 14: 125-128.

Delacour, J. 1977. *The Pheasant of the World.* England: The World Association.

Desjardins, P., and Morais, R. 1990. Sequence and gene organization of the chicken mitochondrial genome. A novel gene order in higher vertebrates. *Journal of Molecular Biology and Evolution* 212: 599-634.

Don, R. H., Cox, P. T., Wainwright, B. J., Baker, K, and Matticj, J. S. 1991. 'Touchdown' PCR to circumvent spurious priming during gene amplification, *Nucleic Acids Research* 19(14): 4008.

Edward, S. V. 1993. Long-distance gene flow in a cooperative breeder detected in genealogis of mitochondrial DNA sequence. *Proceeding Royal Society London series B* 252: 177-185.

Faculty of Science, Chulalongkorn University. 1998. *Ecological assessment of KaengSua Ten Dam Project (executive summary)* Thailand: Office of Environment Policy and Planing, Ministry of Science, Technology and Environment.

Ferraris, J. D., and Palumbi, S. R. 1996. *Molecular Zoology: Advances, Strategies, and Protocols* New York: John Wiley and Sons.

Fleischer, R. C. 1983. A comparison of theoretical and electrophoretic assessments of genetic structure in population of the house sparrow (Passer domesticus). *Evolution* 37: 1001-1009.

Gammow, G. 1954. *Molecular Genetic Markers.* Conference at ministry of Public Health.

Galvin, P., McGregor, D. and Cross, T. 1995a. A single locus minisatellite as a hypervariable genetic marker in gadoid species. *Aquaculture* 137 (1-4): 31-40.

Galvin, P., Sadusky, T., McGregor, D. and Cross, T. 1995b. Population genetics of Atlantic cod using amplified single locus minisatellite VNTR analysis. *J. Fish Biol.* 47(Suppl. A): 200-208.

Georges, M. 1991. Hypervariable minisatellites and their use in animal breeding. pp. 89-112. Cited in Schook, L.B., Lewin. H.A. and McLaren, D.G. *eds.* Gene-Mapping Techniques and Applications. Marcel Dekker, Inc. New York.

Gerloff, U., Schlötterer, C., Rassmann, K., Rambold, I., Hohmann. G., Fruth, B., and Tautz, D. 1995. Amplification of hypervariable simple sequence repeats

(microsatelites) from excremental DNA of wild living bonobos (Pan paniscus). *Mol. Ecol.* 4: 515.

Gibbs. M., Dawson, D. A., McCamley, C., Wardle, A. F., Armour, J. A. L., and Burke, T. 1997. Chicken microsatellite markers isolated from libraries enriched for simple tandem repeats. *Animal Genetics* 28: 401-417.

Grosberg, R. K. 1996. *Characterization of Genetic Structure and Genealogies Using RAPD-PCR Markers: A Random Primer for the Novice and Nervous.* Cited in Ferraris, J. D., and Palumbi, S. R. *Molecular Zoology: Advances, Strategies, and Protocols* New York: John Wiley and Sons.

Hearne, C. M., Ghosh, S. and Todd, J. A. 1992. Microsatellites for linkage analysis of genetic traits. *Trend. Genet.* 8: 288-294.

Hillis, D. M., Moritz, C., and Mable, B. K. 1996. *Molecular Systematics.* 2nd ed. Sunderland MA: Sinauer.

Hoelzel, A. R. (eds) 1998. *Molecular Genetic Analysis of Population.* 2nd ed. New York: IRL

Hoelzel, A.R., and Green, A. 1998. *PCR protocols* pp. 201-235. Cited in Hoelzel, A.R. (ed.) *Molecular Genetic Analysis of Population.* 2nd ed. New York: IRL

Humphrey, R. S. and Bain, J. R. 1990. *Endangered Animals of Thailand.* Florida: Sandhill Crane Press.

Hunter, R. and Markert, C., 1957. Histochemical demonstration of enzymes separated be zone electrophoresis in starch gels. *Science* 125: 1294-1295.

Irwin, D. M., Kocher, T. D., and Wilson, A. C. 1991. Evolution of cytochrom B gene in mammals. *Journal of Molecular and Evolution* 32: 128-144.

IUCN, 1988. IUCN Red Data List of Treated Animal. Gland. Switzerland: IUCN.

Ivanna, T., Wlasiuk, G., and Lessa, E. P. 2002. Evalution of polymorphism in ten microsatellite loci in Uruguayan sheep flocks. *Genetics and Molecular Biology* 25(1): 37-41.

Jeffreys, A. J., Wilson, V., and Thein, S. L. 1985. Hypervariable minisatellite regions in human DNA. *Nature* 314: 67–73.

Jeffreys, A. J., Wilson, V. and Keyte, J. 1988. Amplification of human minisatellites by the polymerase chain reaction: Towards DNA fingerprinting of single cells. *Nucl. Acids Res.* 16: 10953-10971.

Jeffreys, A. J., MacLeod, A., Tamaki, K., Neil, D. L., and Monckton, D. G. 1991. Minisatellite repeat coding as a digital approach to DNA typing. *Nature* 354: 204-209.

Jeffreys, A. J., Tamaki, K., macLeod, A., Monckton, D. G., Neil, D. L. and Armour, J. A. L. 1994. Complex gene conversion events in germline mutation at human minisatellites. *Nat. Genet.* 6(2): 136-145.

Joan, D. F., and Stephen, R. P. (eds) 1996. *Molecular zoology advances, strategies, and protocols* New York: Wiley.

Johannesson, H. S., Johannesson, K. H. P., and Stenlid, J. 2000. Development of primers sets to amplify fragments of conserved genes for use in population studies of the fungus *Daldinia loculata*. *Molecular Ecology* 9: 375-377.

Karnsomdee, P. 1999. *Genetic variation of Thai native fowls Gallus gallus domesticus based on microsatellite DNA.* Master's Thesis, Program of Biotechnology, Graduate School, Chulalongkorn University.

Khatib, H., Genislav, E., Critenden, L. B., Bamstead, N., and Sollor, M. 1993. Sequence-Tagged microsatellite site as marker in chicken reference and resource populations. *Animal Genetics* 24: 355-362.

Khatib, H., and Gruenbaum,Y. 1996. Chicken Red Blood Cell as a substance for direct PCR. *Animal Genetics* 27: 53-54.

Khatib, H., 1997. Chicken Red Blood Cell as a substance for direct PCR. *Animal Genetics* 27: 58-61.

Klinbunga, S., Ampayup, P., Tassanakajon, A., Jarayabhand, P., and Yoosukh, W. 2001. Genetic Diversity and Molecular Markers of Cupped Oysters (*Genera Crassostrea, Saccostrea,* and *Striostrea*) in Thailand Revealed by Randomly Amplified Polymorphic DNA Analysis. *Mar. Biotechnol.* 3: 133-144.

Lewis, P. O. and Snow, A. A. 1992. Deterministic paternity exclusion using RAPD markers. *Mol. Ecol.* 1: 155-160.

O'Reilly, P., and Wright, J. M. 1995. The evolving technology of DNA fingerprinting and its application to fisheries and aquaculture. *J. Fish biol.* 47(supplA): 29-55.

Quinn, T. W. 1992. The genetic legacy of Mother Goose phylogeographic patterns of lesser snow goose *Chen caerulescens* maternal lineages. *Molecular Ecology*1: 105-117.

Quinn, T. W., and Wilson, A. C. 1993. Sequence evolution in and ground the mitochondrial control region in birds. *Journal of Molecular and Evolution* 37: 417-425.

McGowan, P. *et al.* 1999. A review of the status of the Green Peafowl *Pavo muticus* and recommendations for future action. *Bird Conservation International* 9: 331-348.

Marshall, H. D., and Baker, A. J. 1997. Structural conservation and variation in the mitochondrial control region of fringilline finches *(Fringilla spp.)* and the greenfinch *(Carduelis chloris)*. *Molecular Biology and Evolution* 14:173-184.

Marion, S., 2004. History of peafowl in captivity. Available from: http://www.peafowl.org/

Mary, V., Stephen, C., Lougheed. and Peter, T.B. 2002. Conservation genetics of the loggerhead shrike (*Lanius ludovicianus*) in central and eastern North America. *Conservation Genetics* 3: 1–13.

Meckvichai, W., Malaivijitnond, S., and Tirawatanapong, T. 1997. *Cytochrome b variation in pheasants and jungle fowls* Chulalongkorn University Research symposium Bangkok.

Miligan, B.G. 1998. *Total DNA isolation.* pp. 29-64. Cited in Hoelzel, A.R. (ed.) *Molecular Genetic Analysis of Population.* 2nd ed. New York: IRL

Meckvichai, W., Sripong, U. and Srikwan, S. 2001. *Progressive Report the 3rd Biodiversity Research Tranning Programm.* 1-14.

Meyer, A. 1994. *DNA technology and phylogeny of fish.* pp. 219-249. Cited in Beaumont, A.R., (ed.). *Genetic and Evolution of Aquatic Organisms.* Chapman & Hall, London.

Mila, B., Girman, D. J. Kimura, M., and Smith, T. B. 2000. Genetic evidence for the effect of postglacial population expansion on thephylogeography of a North American songbird. *Proceeding Royal Society London Biological Science* 267(1447): 1033-1040.

Moore, S. S., Sargeant, L. L., King, T. J. Mattick, J. S., Georges, M., and Hetzel. J. S. 1991. The conservation of dinucleotide microsatellite among mammalian genomes allows the use of heterologous PCR primer pairs in closely related species. *Genomics* 10: 654-660.

Natasha, R. L. and Peter, B. S. S. 2000. Polymorphic microsatellite merkers in the ornate dragon lizard, *Cternophorus ornatus. Molecular Ecology* 9: 365-378.

Nei, M. 1987. *Molecular Evolutionary Genetics.* New York, Columbia University Press. 511.

Nei, M. and Li, W. H. 1979. Mathematical model for studying genetic variation in terms of restriction endonuclease. *Proc. Natl. Acad. Sci. USA.* 76: 5269-5273.

Nicholas, I. M., Clark, S., Tim, B., and Woodruff, D. S. 1997. Microsatellite variation and microevolution in the critically endangered San Clemente Island loggerhand shrike (*Lanius ludovicianus mearnsi*). *Proc. R. Soc. Lond.* B. 264: 869-875.

Paetkau, D., and Strobeck, C. 1994. Microsatellite analysis of genetic variation in black bear populations. *Mol. Ecol.* 3: 489-495.

Palumbi, S. R.1996. *Nucleic Acids II: The Polymerase Chain Reaction.* pp.205-247. Cited in Hoelzel, A.R. (eds) *Molecular Genetic Analysis of Population.* 2nd ed. New York: IRL

Park, L. K. and Moran, P. 1994. Developments in molecular genetic techniques in fisheries. *Rev. Fish Biol. Fish* 4: 272-299.

Piyachokanakul, P. 2002. *Genome and molecular markers: RAPD and RFLP.* Bangkok: Kasetsart university press.

Plubcharoensook, P. 2000. *Genetic variation of green peafowls Pavo muticus in northern Thailand.* Master's Thesis, Program of Biotechnology, Graduate School, Chulalongkorn University.

Pongsomboon, S. 2002. *Isolation and characterization of tri- tetranucleotide microsatellite sequences for application to DNA typing in the black tiger prawn.* Doctor of Philosophy's Thesis, Program of Biotechnology, Graduate School, Chulalongkorn University.

Ponsena, P. 1998. *Biological characteristics and breeding behavior of green peafowl (Pavo muticus Linn.) In Huai Kha Kaeng wildlife sanctuary*. Master's Thesis, Department of Forest Biology, Graduate School, Kasetsart University.

Primmer, C. R., Mùller, A.. P., and Ellegren, H. 1996. A widerange survey of cross-species microsatellite amplification in birds. *Molec. Ecol.* 5: 365-378.

Randi, E. 2001. WPA/Birdlife/species survival commission (ssc) Pheasant Specialist Group. *WPA News* 65.

Rattanawiboon, N. 1986. *Social behaviour of greenpeafowl (Pavo muticus) in Chiang Mai zoo*. Master's Thesis, Graduate School, Chiang Mai University.

Riedy, M. F., Hamilton, W. J. and Aquadro, C. F. 1992. Excess of non-parental bands in offspring from known pedigrees assayed using RAPD PCR. *Nucl. Acids Res.* 20: 918.

Reinartz, G. E., Karron, J. D., Phillips, R. B., and Weber, J. L. 2000. Pattern of microsatellite polymorphism in the range restricted bonobo (Panpaniscus): considerations for inter specific comparison with chimpanzees (*P. troglodytes*). *Mol. Ecol.* 9: 315-328.

Red Data Book threatened birds of Asia. 2003. *Birdlife International.* Available from: http://www.rdb.or.id/

Rojanadelok, P., Phumpakpun, N., Kutin, U., Naksatit, N., Prayulsit, T., and Sukmasaung, R. 1986. *Distribution range and some behavioral characteristic of green peafowl in Huai Kha Khang wildlife sanctuary*. 120-150.

Sambrook, J 1989. *Molecular Cloninglaboratory manual* vol.1 New York: Cold Spring Harbor.

Sanger-sam, J., Tanguay, L., and Riggs, A. D. 1989. Use of Chelex to improve the PCR signal from a small amount number of cell. *Amplification* 3: 11.

Schlötterer, C. 1998. *Microsatellites.* pp.237-261. Cited in Hoelzel, A.R. (eds) *Molecular Genetic Analysis of Population.* 2nd ed. New York: IRL

Scott, M. P., Haymes, K. M. and Williams S. M. 1993. Parentage analysis using RAPD PCR. *Nucl. Acids. Res.* 20: 5493

Somchai, P., 1993. *Studying of disperse and least of green peafowl In Huai Kha Kaeng wildlife sanctuary in 1992-1993.* Special problem, Department of Forest Biology, Graduate School, Kasetsart University.

Stephen, A. K. 1996. *Application of AnomymousbNuclear Loci to Conservation Biology.* P. 38 Cited in Smith, T. B., and Wayne, R. K. (eds) *Molecular Genetic Approaches in Conservation* New York: Oxford University Press.

Subramanian, K. S., and John, M. C. 2001. Roosting and nesting habits of free ranging Indian peafowl(Pavo cristatus) in southern Tamil Nadu. *Zoos' Print Journal.* 16(7): 537-538. (abstract)

Sukapan, T. 2000. *Morphological comparison between subadult male and female of green peafowls.* Senoir project, Department of Biology, Chulalongkorn University.

Taberlet, P., Meyer, A., and Bouvet, J. 1992. Unusual mitochondrial DNA polymorphism in two local populations of blue tit *Parus caeruleus. Molecular Ecology* 1(1): 27-36.

Tassanakajon, A., Pongsomboon, S., Jarayabhand, P., Klinbunga, S., and Boonsaeng, V. 1998. Genetic structure in wild populations of black tiger shrimp (*Penaeus monodon*) using randomly amplified polymorphic DNA analysis. *J. Mar. Biotechnol.* 6: 249-254.

Taylor, E. B., Beacham, T. D. and Kaeriyama, M. 1994. Population structure and identification of North Pacific Ocean chum salmon (*Oncorhynchus keta*)

revealed by an analysis of minisatellite DNA variation. *Can. J. Fish. Aquat. Sci.* 51: 1430-1442.

Tautz, D. 1989. Hypervariability of Simple sequences as a general source for polymorphic DNA markers. *Nucl Acids Res.* 17: 6463-6471.

Tautz, D. and Renz, M. 1984. Simple sequences are ubiquitons repetitivecomponents of eukaryotic genomes. *Nucl Acids Res.* 12(10): 4127-4138.

Thera, W. 1997. The studies of Ecology and The strategies of Habitat management of Green Peafowl(*Pavo muticus*) at Yom watrershed. Song District, Phare Province and Vhiang Muan and Pong District, Phayao Province. Proposal in seminar ENID 695 subject. Mahidol University.

Thorsten, B. H. R. 2000. Five microsatellite loci in eelgrass *Zostera marina* and a test of cross-species amplification in *Z. noltii* and *Z. japonica*. *Molecular Ecology* 9: 371-373.

Traxler, B., Brem, G., Müller, M., and Achmann, R. 2000. Polymorphic DNA microsatellites in the domestic pigeon, *Columba livia* var. *domestica*. *Molecular Ecology* 9: 366.

Valdes, A. M., Slatkin, M. and Freimer, M. B. 1993. Allele frequencies at microsatellite loci: the stepwise mutation model revisited. *Genetics* 133: 737-749.

Wahls, W. P., Wallace, L. J., and Moore, P. D. 1990. Hypervariable minisatellite DNA is a hot spot for homologous recombination in human-cells. *Cell* 60: 95–103.

Walsh, P. S., Metzger, D. A. and Higuchi, R. 1991. Chelex 100 as medium for simpleExtraction of DNA for PCR-based typing from forensic material. *Biotechniques* 10: 506-513.

Ward, R. D. and Grewe, P. M. 1995. Appraisal of molecular geneticin fisheries. p. 29-54. Cited in Carvalho, G.R. and Pitches, T.J. (eds). *Molecular genetic techniques in fisheries.* Great Britain: Chapman Hall.

Wayre, P. 1969. *A guide to the pheasants of the world.* London: Poul Hamlyn

Weber, J. L. and May, P. E. 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Am. J. Hum. Genet.* 44: 388-396.

Welsh, J., and McClelland, M. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucl Acids Res.* 18: 7213-7218.

Wenick, P. W., Baker, A. J., and Tilanus, M. G. J. 1993. Hypervariable control region sequence reveal global population structuring in a lone-distance migrant shorebird, the dulin *(Calidris alphina). Proceeding National Academic Science USA* 90: 94-98.

Williams, J. G., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers *Nucl Acids Res.* 18(22): 6531-6535

Wolff, R. K., Pleatke, R., Jeffreys, A. J. and White, R. 1989. Unequal crossingover between homologous chromosomes is not the major mechanism involved in the generation of new alleles at VNTR loci. *Genomics* 5: 382-384.

Woodruff, D. S. 1990. Genetic and demography in the conservation of biodiversity. *Journal of Science Society Thailand.* 16: 117-132.

Wright, J. 1993. *DNA fingerprinting of fishes.* p. 57-91. Cited in Hochachka, P. W., and Mommsen, T., (eds.) *Biochemistry and Molecular biology of Fishes.* New York : Elsevier.

Wright, J. M., and Bentzen, P. 1994. Microsatellites: genetic markers for the future. *Rev. Fish Biol. Fish.* 4:384-388.

Wright, J. M. and Bentzen, P. 1995. *Microsatellites: genetic markers for this future.* pp.117-122. Cited in Carvalho, G.R. and Pitcher, T.J. (eds.). *Molecular genetic in fisheries*. Great Britain: Chapman & Hall.

Yasuyuki, I., Osamu, M., and Syuiti, A. 2000. Isolation and characterization of microsatellite loci in the Japanese marsh warbler *Locustella pryeri*. *Molecular Ecology* 9: 373-375.

Zhou, U., and Lamont, S. J. 1999. Genetic characterization of biodiversity in highly inbred chicken lines by microsatellite markers. *Animal Genetics* 30: 256-264.

Zane, L., Bargelloni, L., and Patarnello, T. 2002. Strategies for microsatellite isolation: a review. *Molecular Ecology* 11(1): 1-16.

APPENDICES

APPENDIX I: Sampling data

Table A *P. m. imperator* data from Khao Soi Dao wildlife research and breeding station.

Number	Code of samples (RFD.THA)	Number of cage	Sex	Weight (kg)	Age	Date of collection
1	R0324	16	M	7.0	10 yrs	11-1-2003
2	R0119	1	M	4.5	1yrs3mths	24-5-2003
3	R0336	1	M	6.1	6 yrs	24-5-2003
4	R0481	2	M	3.7	1yrs3mths	24-5-2003
5	R0491	1	M	3.7	1yrs3mths	24-5-2003
6	R0500	1	M	3.3	1yrs3mths	24-5-2003
7	R0323	16	F	4.8	1 yrs	11-1-2003
8	R0348	1	F	5.1	6 yrs	24-5-2003
9	R0483	3	F	3.3	1yrs3mths	24-5-2003
10	R0490	3	F	4.0	1yrs3mths	24-5-2003
11	R0496	2	F	3.4	1yrs3mths	24-5-2003
12	M1482	1	F	3.2	1yrs3mths	24-5-2003
13[a]	R0305	4	F	6 kg	10 yrs	11-1-2003

a: Female Cambodia green peafowl

Table B Sampling sites, sex and date collection at other sources.

Number	Sampling site	Sex	Date of collection
1	Doi Phu Nang National Park	M	12-5-2001
2	Wieng Lor Wildlife Sanctuary	M	12-5-2001
3	Sri Nan National Park	M	11-5-2002
4	Huay Kha Kaeng Wildlife Sanctuary	M	26-1-2002
5	Huay Kha Kaeng Wildlife Sanctuary	F	18-4-2002
6	Huay Hong Krai Royal Project	M	13-5-2001
7	Phatthalung wildlife and breeding station	M	14-10-2002
8	Phatthalung wildlife and breeding station	F	14-10-2002

APPENDIX II : DNA extraction protocol

A. Chelex 100® DNA extraction method

1. Cut a small piece filter paper containing blood stain (approximately 2-3 mm^2) and place in a sterile labeled microcentrifuge tube.
2. Add 1 ml of sterile double distilled water into a sterile 1.5 ml microcentrifuge tube, incubate at room temperature for 15-30 minutes and mixed gently.
3. Spin a microcentrifuge for 2-3 minutes at 10,000 to 15,000 rpm.
4. Remove supernatant and discard.
5. Add 5 % Chelex to a final volume of 200 µl. [a]
6. Add 20 µl Proteinase K (5mg/ml)and incubate at 56°C for 40 minutes
7. Add 20 µl Proteinase K again and incubate at 56°C for 1 hr or overnight.
8. Vortex at high speed (DNA to avoid shearing) for 5-10 seconds or mix gently. Then boiled in boiling water bath for 8 minutes.
9. Spin in a microcentrifuge for 2-3 minutes at 10,000 to 15,000 rpm.
10. Transfer supernatant into a new sterile microcentrifuge tube and discard fabric substrate and Chelex.
11. Store the sample at either -20^{0}C for long period or 4^{0}C for short period used. To re-use the sample in further amplification, repeat the vortexing and centrifugation steps before removing an aliquot.

[a] When pipetting Chelex solutions, the resin beads must be distributed evenly in the solution. This can be accomplished by gently stirring a small quantity (e.g., 10 ml) of the stock solution in a beaker while removing the necessary quantity with a pipette. A large-bore pipette tip (e.g., a standard 1000 µl pipette tip) should be used.

B. Phenol/chloroform DNA extraction

1. Cut a small piece of filter paper containing bloodstain (approximately 2-3 mm^2) and place in a sterile labeled 1.5 ml microcentrifuge tube.
2. Add 1.0 ml of TE buffer and incubate at room temperature for 30-60 minutes.
3. Add 25 µl of 10% SDS and 25 µL of Proteinase K (5mg/ml)and incubate at room temperature for 10 minutes and gentle mix by inversion.
4. Add 1 volume of SS-phenol and 1 volume of chloroform (approximately 250 µl) and mix this sample by repeated inversion (do not vortex)
5. Centrifuge each tube for one minute at 10,000 rpm.
6. Remove upper aqueous layer (do not disturb interphase) and transfer to a new sterile1.5 ml microcentrifuge tube.
7. Add 1/10 th volume of 3 M sodium acetate pH 7.0
8. Add at 2.5 volumes of 95% ethanol and incubate at -20° C overnight.
9. Spin at 10,000 to 15,000 rpm for 10 minutes.
10. Discard supernatant carefully (do not disturb the pellet). To remove residual salt, wash pellet in 300 µl or more of 80% ethanol. Gently mix by inversion.
11. Spin at 10,000 to 15,000 rpm for 2 minutes and discard the supernatant and air-dried.
12. Add 200 µl of TE buffer and store at -20°C until further needed.

C. QIAamp® DNA extraction

1. Place 3 punched-out circles from a dried blood spot into a sterile labeled 1.5 ml microcentrifuge tube and adds 180 µl of Buffer ATL.
2. Incubate at 85 ^{0}C for 10 min. Briefly centrifuge to remove drops from inside the lid.
3. Add 20 µl Proteinase K stock, mix by vortexing, and incubate at 37 ^{0}C for 1 hr. Briefly centrifuge to remove drops from inside the lid.
4. Add 200 µl Buffer AL to the sample, mix thoroughly by vortexing, and incubate at 70^{0}C for 10 min. Briefly centrifuge to remove drops from inside the lid.
5. Add 200 µl ethanol (96-100%) to the sample, and mix thoroughly by vortexing. Briefly centrifuge to remove drops from inside the lid.
6. Carefully apply the mixture from step 5 to the QIAamp Spin Column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Spin Column in a clean 2 ml collection tube, and discard the tube containing the filtrate.
7. Carefully open the QIAamp Spin Column and add 500 µl Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Spin Column in a clean 2 ml collection tube, and discard the collection tube containing the filtrate.
8. Carefully open the column and add 500 µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min. Continued directly with step 9, or to eliminate any

chance of possible Buffer AW2 carryover, perform step 8a, and then continue with step 9.

8a (Optional): Place the QIAamp Spin Column in a new 2 ml collection tube and discard the collection tube with the filtrate. Centrifuge at full speed for 1 min.

9. Place the QIAamp Spin Column in a clean 1.5 ml microcentrifuge tube and discard the collection tube containing the filtrate. Carefully open the QIAamp Spin Column and add 150 µl Buffer AE or distilled water. Incubate at room temperature for 1 ml, and then centrifuge at 6000 x g (8000 rpm) for 1 min.

APPENDIX III : Quality determination and size estimation of PCR products

A. Quality determination of DNA

Description previously was used followed:

1. 1% agarose was weighed out and heated to dissolve in an appropriate volume of 1 X TBE buffer.
2. Melted agarose was poured into the gel mould that the comb was already inserts to gel mould. When the gel completely set (the gel had cooled and solidified), the comb was gently removed.
3. The gel submerged in the gel chamber containing an enough of 1X TBE buffer that covered the gel to a depth about 1-2 mm. Each of extracted DNA was prepared for loading by mixing loading dye buffer (0.25% bromophenol blue and 25% Ficoll in water) and mixed well.
4. The samples were applied into the wells slowly that used Phi X 174 -λ DNA digested with *Hinf* I , was loaded into a well for served as a DNA standard.
5. A gel bath was connected to a power supply and turned on (the gel was run at 100 volt), then DNA migrated into the gel toward the anode. When bromophenol blue had migrated about three-fourths (about 40 mins) of a gel distance turned off the power supply and stain the gel with 0.25 μg/ml ethidium bromide.
6. The gel was destained in deionise distilled water for 5-10 minutes to leach out unbound 1 % ethidium bromide.
7. It was placed on a long wavelength UV. transluminator and photographed using Polaroid 667 film or Fuji 3000B film. The exposure time was usually about 10-15 seconds.

B. Size estimation of microsatellite PCR products and gel preparation

I. Glass Preparation

1. Clean both glasses with soft detergent and water.
2. Rinse with distilled water, dry and clean with ethanol (95%)
3. Let them dry using Kimwipes.

II. Gel Preparation

1. Mix the reagents in a beaker : 8% polyacrylamide gel is set by following Appendix III
2. Leave them until the urea is completely dissolved.

(** setting up the plates can be done while urea is dissolving.)

3. Add 10 µl TEMED and 10%APS 100 µl
4. Swirl beaker continuously while adding for it to mix well.

III. Plate setup

Before setting up plates, ensure no liquid or particles on their surface.

1. Put the spacers on the side of big glass.
2. Put the small glass on top.
3. Pour gel softly and consistently, don't stop.
4. Once the gel fills the space between glasses (without bubbles) put the combs in.
5. Leave to polymerize 2.5-3 hrs (18 hrs max)
6. If leaving for more than 8 hours put a wet Kimwipe® on the border of the gel, where the comb is, to prevent over drying.

IV. Loading PCR products

1. The gel submerged in the gel chamber containing an enough of 1X TBE buffer.
2. The samples were applied into the wells slowly that used Phi X 174 -λ DNA digested with *Hinf* I , was loaded into a well for served as a DNA standard.
3. A gel bath was connected to a power supply and turned on (the gel was run at 100 volt), then DNA migrated into the gel toward the anode. When Orange G had migrated about three-fourths (about 2 hours) of a gel distance turned off the power supply and stain the gel with silver nitrate staining.

C. Size estimation of RAPD PCR products

Description previously was used followed:

1. An approximate amount of 1.5% agarose was weighed out and heated to dissolve in an appropriate volume of 1 X TBE buffer.
2. Melted agarose was poured into the gel mould that the comb was already inserts to gel mould. When the gel completely set (the gel had cooled and solidified), the comb was gently removed.
3. The gel submerged in the gel chamber containing an enough of 1X TBE buffer that covered the gel to a depth about 1-2 mm. Each of RAPD PCR product was prepared for loading.
4. Ten microlitres of PCR products were mixed with 2 µl of the loading dye.
5. The samples were applied into the wells slowly that used 100 bp DNA ladders, was loaded into a well for served as a DNA standard.
6. A gel bath was connected to a power supply and turned on (the gel was run at 80 volt), then DNA migrated into the gel toward the anode. When bromophenol blue moved to approximately 0.5 mm from the bottom of the gel (about 1.30 hrs), turned off the power supply and stain the gel with 0.25 µg/ml ethidium bromide.
6. The gel was destained in deionise distilled water for 5-10 minutes to leach out unbound ethidium bromide.
7. It was placed on a long wavelength UV. transluminator and photographed using polaroid 667 film or Fuji 3000B film. The exposure time was usually about 10-15 seconds.

APPENDIX IV: Units and reagent preparation protocol

UNIT IN CALCULATION

M (molar) = containing mole of solute in one liter of solution

mol(mole) = the base unit amount *

1M	=	1 mol / l
	=	1 mmol / ml
	=	1 µmol / µl
1mM	=	1 mmol / l
	=	1 µmol / ml
	=	1 nmol / µl
1µM	=	1 µmol / l
	=	1 nmol / ml
	=	1 pmol / µl

* The base unit anount of pure substance in the International System of Units that contains the same number of elementary entitles as there are atoms in exactly 12 grams of the isotope carbon 12.

1. Agarose

1.1) 1 % Agarose

An enough amount of ingredients for a 100 ml gel composed of:

- Agarose 1.0 gm
- 1 X TBE buffer 100.0 ml

1.2) 1.5 % Agarose

Preparing 1.5 % agarose is like 1 % agarose but 1.5 gram of agarose is used to dissolve in 100 ml 1xTBE buffer.

How to applied the description previously is used follow:

1. • For 1% Agarose, agarose powder about 0.30 gm is mixed into 1 X TBE buffer 30 ml.

   • For 1.5% Agarose,agarose powder about 0.75 gm is mixed into 1 X TBE buffer 30 ml.
2. The agarose solution is solubilized by heating in a microwave oven.
3. The solubilized agarose allow for cool to 50-60 °C before pour into a gel mould.
4. Prepare gel mould for set the gel. When time is finished, the dissolved gel is transferred about 25-50 ml
5. The soluble gel is poured into the gel mould which the comb is already inserted to the gel mould.
6. When the gel has completely setting. The comb was removed.
7. The gel is transferred into a gel chamber containing an enough of 1X TBE buffer that covered the gel to about 1-2 mm depth.

2. 8% Polyacrylamide gel

An enough amount of ingredients for a 10 ml gel composed of:

- 7M urea	4.20	gm
- 10X TBE buffer	1.00	ml
- 30% stock acrylamide solution (Bio-rad®, acrylamide monomer: bis-acrylamide = 29: 1)	2.67	ml
- distilled water for added up to	10.00	ml

How to use the description previously is used follow:

1. The dissolved ingredients are added 10% APS 100 µl (fresh prepared) and TEMED 10 µl before used.
2. Then the ingredients are poured into the gel apparatus. The comb is inserted into the upper and pointing out for making a sharp cut edged of the gel.
3. The polymerization process is allowed to complete for 2.5 hours or the gel is set for overnight.
4. The comb is then removed that urea and small pieces of gel are flushed out of the wells prior to loading PCR products.

Remark:

Handling of the Reagent - The solution is as toxic as the compounds. Wear gloves at all times when handling the reagent and reagent should be prepared in Fume hood.

3. 10X Tris Boric EDTA buffer (10XTBE)

An enough amount of ingredients for a 1000 ml composed of:

- Tris aminomethane	108	gm
- Boric acid	50.4	gm
- EDTA	7.44	gm

The solution is prepared as follow:

1. Tris, Boric and EDTA are mixed into volumetric flask 1000 ml.
2. Double distilled water is added up 1000 ml.
3. Solutions stirred until completely dissolve.
4. Store in room temperature and use 10X TBE for acrylamide, while use 1X TBE for running electrophoresis.

4. 40% Methanol

The solution is prepared as follow: Methanol is poured about 400 ml into 1000 ml cylinder. Double distilled water was added up to 1000 ml, and mixed gently.

5. 1 M Nitric acid

The solution is prepared as follow:

1. 2 N of Nitric acid is prepared for stock solution and poured into 500 ml cylinder about 62.9 ml for 1 M working solution (prepared in hood).
2. Deionized water is added up to 500 ml.
3. Mix gently and kept at room temperature.

6. 0.2% Silver nitrate

The solution was prepared as follow:

Silver nitrate 0.2 gm is prepared for 100 ml total volume working solution in 500 ml bottle (freshly prepared and mixed gently).

7. Developer solution (3% Sodium carbonate and 40% formaldehyde)

The solution was prepared as follow:

1. Sodium carbonate 3 gm is prepared and added double distilled water about 100 ml
2. The solution is mixed gently for dissolve.
3. 40% formaldehyde about 50 µl is added into the solution, before use.

8. Stop solution (0.1 M Citric acid or 20% acetic acid)

The solution was prepared as follow:

- 0.1 M Citric acid

1 M Citric acid is prepared for stock solution and used 0.1 M for working stop reaction.

- 20% Acetic acid

10 ml. Of Glacial acetic acid is poured into 500 ml cylinder and added double distilled water up to 500 ml. Mixed gently.

9. 6X loading dye

The Blue / orange 6x loading dye supplied with a composition of

- 15 % Ficoll® 400
- 0.03 % Bromophenol Blue
- 0.03 % Xylene Cyanol FF
- 0.4 % Orange G
- 10 mM Tri-HCl (pH 7.5)
- 50 mM EDTA

The Xylene Cyanol FF migrates at approximate 4 kb, Bromphenol Blue at approximately 300 bp and Orange G at approximate 50 bp in 0.5 % to 1.5 % agarose gels.

10. 10 % Ammonium Persulfate

- 0.1 gm of APS
- DDW up to 1000 µl. The solution is mixed gently for dissolve.

11. 4.5% MetaPhor

An enough amount of ingredients for a 40 ml gel composed of:

- MetaPhor 1.8 gm
- 1 X TBE buffer 40.0 ml

Preparation MetaPhor is like low melting agarose. Swirl until the gel is dissolved by using low temperature. Beware bubbles in the gel.

APPENDIX V: Silver Nitrate staining method

A. Silver nitrate staining

1. Transfer the gel into the clean staining chamber.
2. Then the gel was fixed in 40 % methanol and 10 % acetic acid for 12 minutes or until loading dye was dissolved. When finished, discard solution.
3. The gel was rinsed in double distilled water (DDW) and discard.
4. Prepared suitable physical condition of the gel by soaked in nitric acid for 5 minutes. When finished, discard solution.
5. The gel was soaked in DDW for 2 minutes, 2-3 times and discards.
6. The gel was soaked in 0.2 % silver nitrate for 16 minutes that this solution was fresh prepared. Softly and thoroughly shake. When finish then discard solution.
7. The gel was rinsed in DDW and discard.
8. Then the gel was soaked in developer solution. Three percents sodium carbonate, 37 % formaldehyde 100 µl and 1% sodium thiosulfate 10 µl was added before used. When PCR products band were occurred, discard solution.
9. Then stop reaction, the gel was soaked in 0.1 M. citric acid or 20 % acetic acid for 1 minute, discard solution and soaked the gel in DDW for 5 minutes, discard solution.
10. Transfer the gel into to fine hard paper, wrapped with by saran wrap, dried the gel by dryer and the gel was dried at 80°C about 45 minutes, or wrap the gel by cellophane and air-dried for over night. Labeled the gel and take a photograph.

B. Optional silver staining

The following protocol was used based on the staining improvements. The desilver and impregnation steps were modified. (Alexander Binder)

Step	Time	Reagents
1. Fix	30 min	10 % acetic acid (v/v)
2. Rinse	3 x 2 min	DDW
3. Impregnate	20 – 30 min	0.1% $AgNO_3$ + 100µl 37% formaldehyde(100ml)
4. Rinse	20 s	DDW
Thoroughly wash the gel surface and back of the gel.		
5. Develop (visual control)	2 – 5 min	2.5 % $NaCO_3$ + 100 µl 37 % formaldehyde(100ml) + 20 µl $Na_2S_2O_3.5H_2O$ (2mg/litre)
6. Stop	10 min	2 % (g/v) glycine + 0.5 % EDTA disodium salt solution
7. Impregnate	10 min	5 % (v/v) glycerol
8. Dry	Overnight	Room temperature

Table C Procedure for silver staining of nucleic acids

APPENDIX VI: Primer sequence data

Table D Microsatellite primer sequences

Primer	Name	Sequences	Size (bp)	Tm (°C)
1	ADL 23A	5'-CTT CTA TCC TGG GCT TCT GA-3'	20	52
	ADL 23B	5'-CCT GGC TGT GTA TGT GTT GC-3'	20	47
2	MCW 87A	5'-ATT TCT GCA GCC AAC TTG GAG-3'	21	62
	MCW 87B	5'-CTC AGG CAG TTC TCA AGA ACA-3'	21	62
3	MCW 240A	5'-CAA AAC CGG TGT CAC CTA CTG-3'	21	64
	MCW 240B	5'-GGT TAT TTC TTC AGT GAC TTC C-3'	22	62
4	LEI 73A	5'-CCA TAT CAT TTG TCA AGC ACC-3'	21	60
	LEI 73B	5'-AAT TCC TGA CCT CCA TGA TAC-3'	21	60
5	ADL 37A	5'-ATG CCC CAA ATC TCA ACT CT-3'	20	58
	ADL 37B	5'-TCT CTA AAA TCC AGC CCT AA-3'	20	56
6	HUJ 1A	5'-CCA TCC GCT TAT ACA GAG CAC A-3'	22	66
	HUJ 1B	5'-CCC TTT GTT AAC AAC TAC TGC A-3'	22	62
7	HUJ 2A	5'-CAT CTC ACA GAG CAG CAG TG-3'	20	62
	HUJ 2B	5'-GAA TCC TGG ATG TCA AAG CC-3'	20	60
8	HUJ 7A	5'-CAT AAA CTA AAG TCT CAA CAC-3'	21	56
	HUJ7B	5'-TTC TTC CAC ACA TCC TTG CTA-3'	21	60
9	LEI 92A	5'-GAT CTA CAT TTG TGC AGT GTC-3'	21	60
	LEI 92B	5'-TCC TTG GTC TGA CTC TCC ATG-3'	21	64
10	LEI 126A	5'-GTC AGA GAA GGA AGA TAC ATC-3'	21	60
	LEI 126B	5'-CTA ACT ACA ATG CCTG GAA TGC-3'	21	60
11	MCW 305A	5'-TCA GAA ACA AAG CAG GAG CTG-3'	21	52
	MCW 305B	5'-TGA CAT CTT TCA AAC GAG ACC-3'	21	49
12	LEI 80A	5'-GTT AGA GCC ATA CAG AAA CTT C-3'	22	45
	LEI 80B	5'-ATC ACA CAA GCT TTC TTC CTG-3'	21	49
13	LEI 136A	5'-CAT TTG TAA CAA GTG CAC GTG-3'	21	49
	LEI 136B	5'-TCA GCT CTC CTA GAC CTA GTG-3'	21	45
14	HUJ 12A	5'-GTC TCA TGC TAT GAG AGT GG-3'	20	60
	HUJ 12B	5'-CCT CTG GTT GAA TCA GTC TG3'	20	60

Table D Microsatellite primer sequences (continued)

Primer	Name	Sequences	Size (bp)	Tm (°C)
15	ADL 102A	5'-TTC CAC CTT TCT TTT TTA TT-3'	20	50
	ADL 102B	5'-GCT CCA CTC CCT TCT AAC CC-3'	20	64
16	ADL 136A	5'-TGT CAA GCC CAT CGT ATC AC-3'	20	60
	ADL 136B	5'-CCA CCT CCT TCT CCT GTT CA-3'	20	62
17	ADL 158A	5'-TGG CAT GGT TGA GGA ATA CA-3'	20	58
	ADL 158B	5'-TAG GTG CTG CAC TGG AAA TC-3'	20	60
18	ADL 171A	5'-ACA GGA TTC TTG AGA TTT TT-3'	20	52
	ADL 171B	5'-GGT CTT AGC AGT GTT TGT TT-3'	20	56
19	ADL 172A	5'-CCC TAC AAC AAA GAG CAG TG-3'	20	60
	ADL 172B	5'-CTA TGG AAT AAA ATG GAA AT-3'	20	50
20	ADL 176A	5'-TTG YGG ATT CTG GTG GTA GC-3'	20	60
	ADL 176B	5'-TTC TCC CGT AAC ACT CGT CA-3'	20	60
21	ADL 181A	5'-CCA GTG AAA TTC ATC CTT TT-3'	20	54
	ADL 181B	5'-CAA TCT TTT GTG GGG TAT GG-3'	20	58
22	ADL 210A	5'-ACA GGA GGA TAG TCA CAC AT-3'	20	58
	ADL 210B	5'-GCC AAA AAG ATG AAT GAG TA-3'	20	54
23	ADL 267A	5'-AAA CCT CGA TCA GGA AGC TA-3'	20	58
	ADL 267B	5'-GTT ATT CAA AGC CCC ACC AC-3'	20	60

Table E RAPD primer sequences

Primer	Sequences	Primer	Sequences
1	CCT GGG CTT C	31	CCG GCC TTC C
2	CCT GGG CTT G	32	GGG GCC TTA A
3	CCT GGG CTT A	33	CCGGCT GGA A
4	CCT GGG CTT G	34	CCG GCC CCA A
5	CCT GGG TTC C	35	CCG GGG TTA A
6	CCT GGG CCT A	36	CCC CCC TTA G
7	CCT GGG GGT T	37	CCG GGG TTT T
8	CCT GGC GGT A	38	CCG GGG AAA A
9	CCT GCG CTT A	39	TTA ACC GGG C
10	GGG GGG ATT A	40	TTA CCT GGG C
11	CCC CCC TTT A	41	TTA ACC GGG G
12	CCT GGG TCC A	42	TTA ACC CGG C
13	CCT GGG TGC A	43	AAA ACC GGG C
14	CCT GGG TTT C	44	TTA CCC CGG C
15	CCT GGG TTT G	45	TTA ACC CCG G
16	GGT GGC GGG A	46	TTA AGG GGG C
17	CCT GGG CCT C	47	TTC CCC AAG C
18	GGG CCG TTTA	48	TTA ACG GGG A
19	GCC CGG TTT A	49	TTC CCC GAG C
20	TCC GGG TTT G	50	TTC CCC GCG C
21	ACC GGG TTT C	51	CTA CCC GTG C
22	CCC TTG GGG G	52	TTC CCG GAG C
23	CCC GCC TTC C	53	CTC CCT GAG C
24	ACA GGG GTG A	54	GTC CCA GAG C
25	ACA GGG CTC A	55	TCC CTC GTG C
26	TTT GGG CCC A	56	TGC CCC GAG C
27	TTT GGG GGG A	57	TTC CCC GAG G
28	CCG GCC TTA A	98	ATC CTG CCA G
29	CCG GCC TTA C	99	ATC CCC TGG G
30	CCG GCC TTA G	100	ATC GGG TCC G

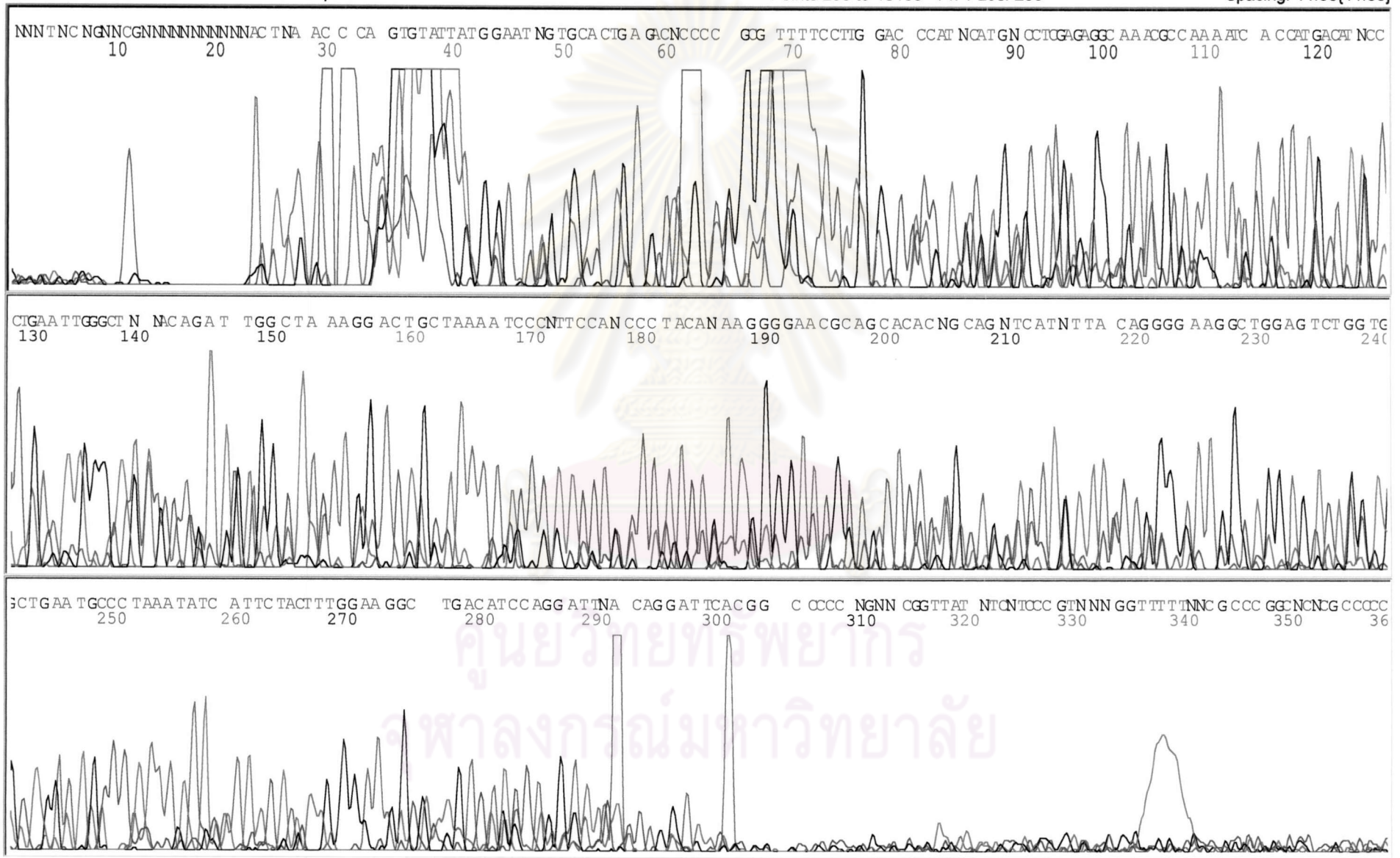
APPENDIX VII: Electrofluorogram of microsatellite sequences

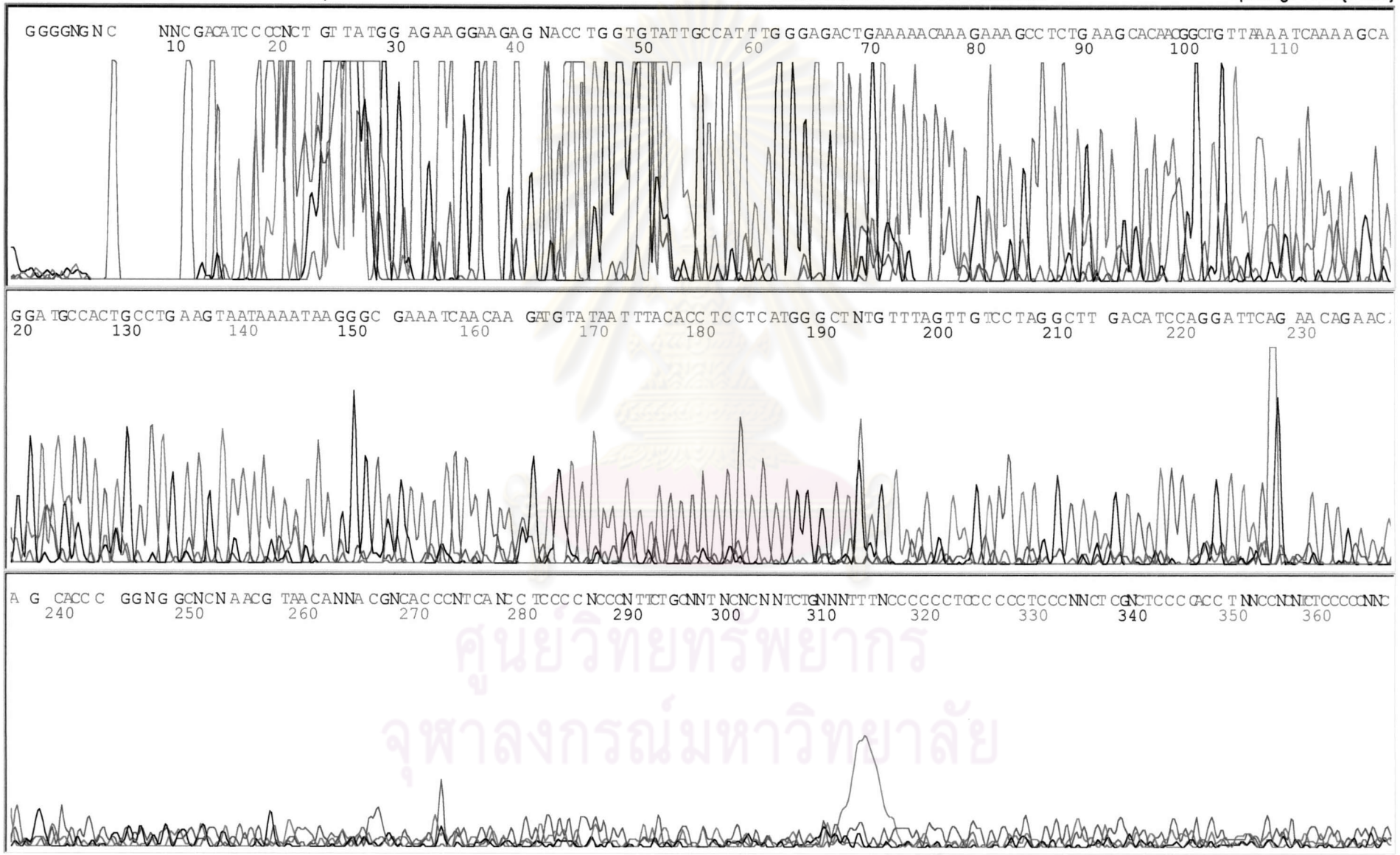
The DNA sequencing profiles from BSU, Thailand as follow by:

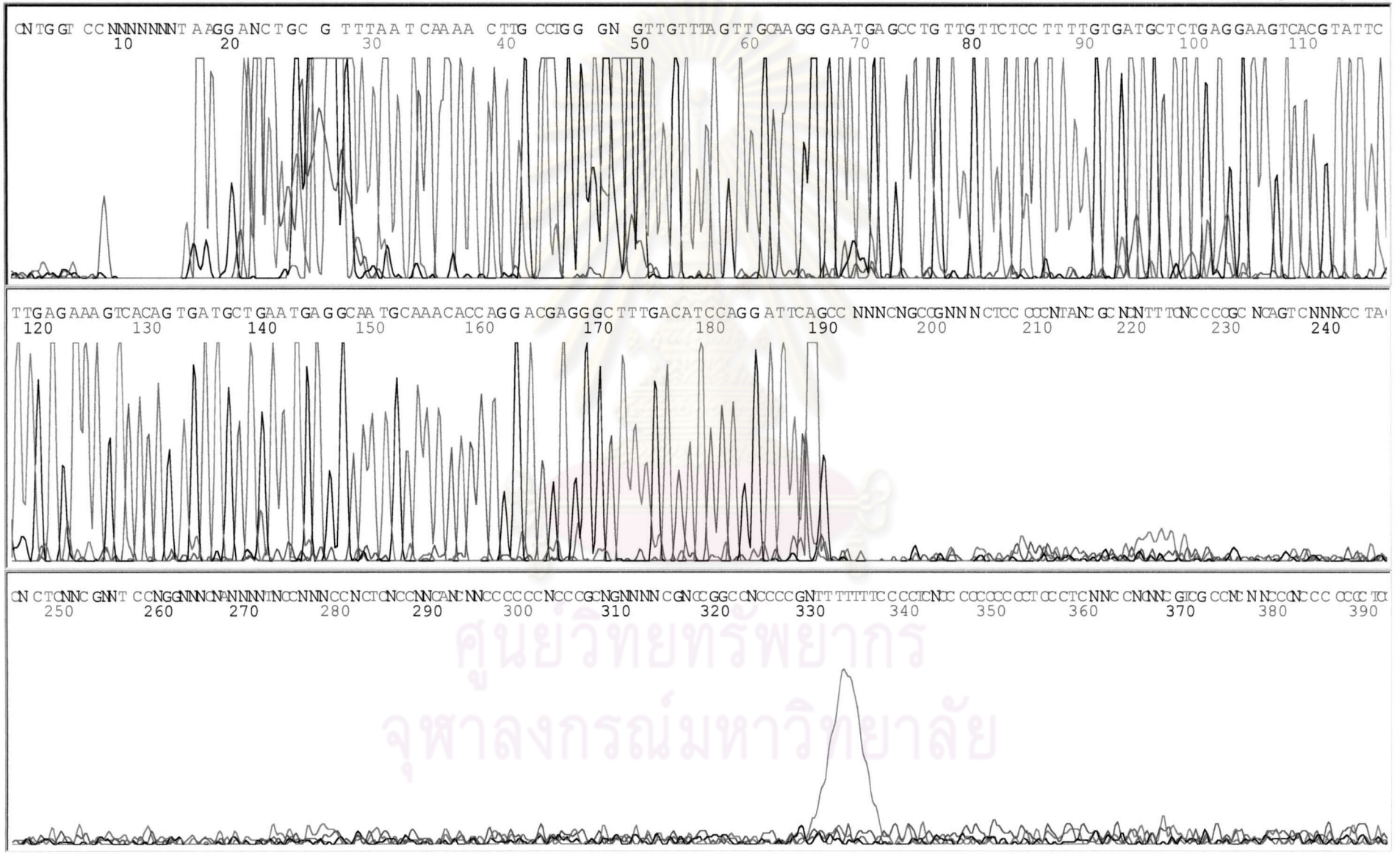
The DNA sequencing profile of *P. m. imperator* (HUJ2-1) at the HUJ2 locus
The DNA sequencing profile of *P. m. imperator* (HUJ2-2) at the HUJ2 locus
The DNA sequencing profile of *P. m. imperator* (HUJ2-3) at the HUJ2 locus
The DNA sequencing profile of *P. m. imperator* (HUJ2-4) at the HUJ2 locus
The DNA sequencing profile of *P. m. imperator* (HUJ2-5) at the HUJ2 locus
The DNA sequencing profile of *P. m. imperator* (HUJ2-6) at the HUJ2 locus
The DNA sequencing profile of *P. m. imperator* (HUJ2-7) at the HUJ2 locus
The DNA sequencing profile of *P. m. imperator* (LEI80) at the LEI80 locus

The DNA sequencing profiles from Macrogen, Korea as follow by:

The DNA sequencing profile of *P. m. imperator* (ADL23-1) at the ADL23 locus
The DNA sequencing profile of *P. m. imperator* (ADL23-2) at the ADL23 locus
The DNA sequencing profile of *P. m. imperator* (ADL23-3) at the ADL23 locus
The DNA sequencing profile of *P. m. imperator* (ADL23-4) at the ADL23 locus
The DNA sequencing profile of *P. m. imperator* (ADL23-5) at the ADL23 locus
The DNA sequencing profile of *G. Gallus* (ADL23-14) at the ADL23 locus
The DNA sequencing profile of *G. Gallus* (ADL23-15) at the ADL23 locus







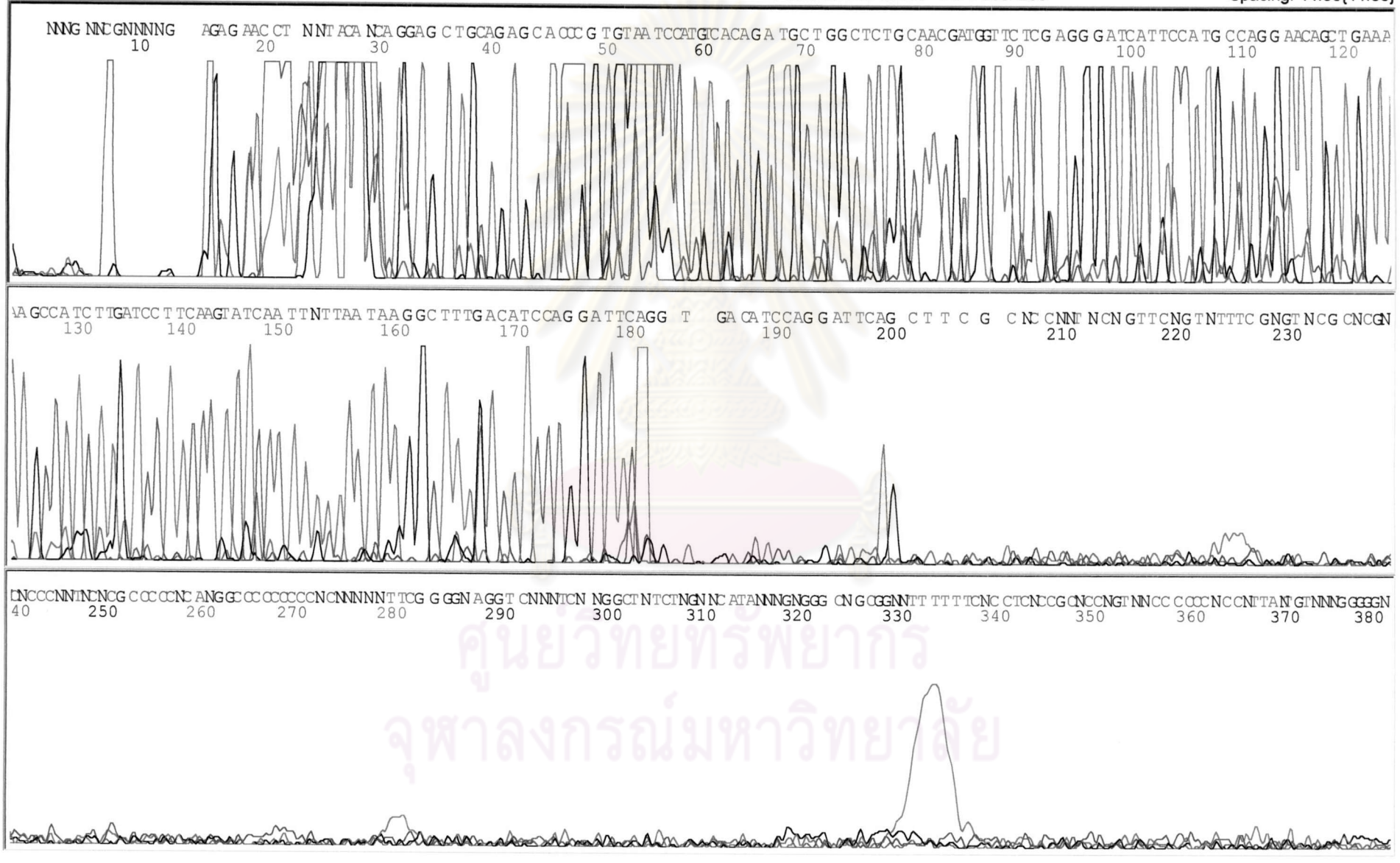
ABI PRISM

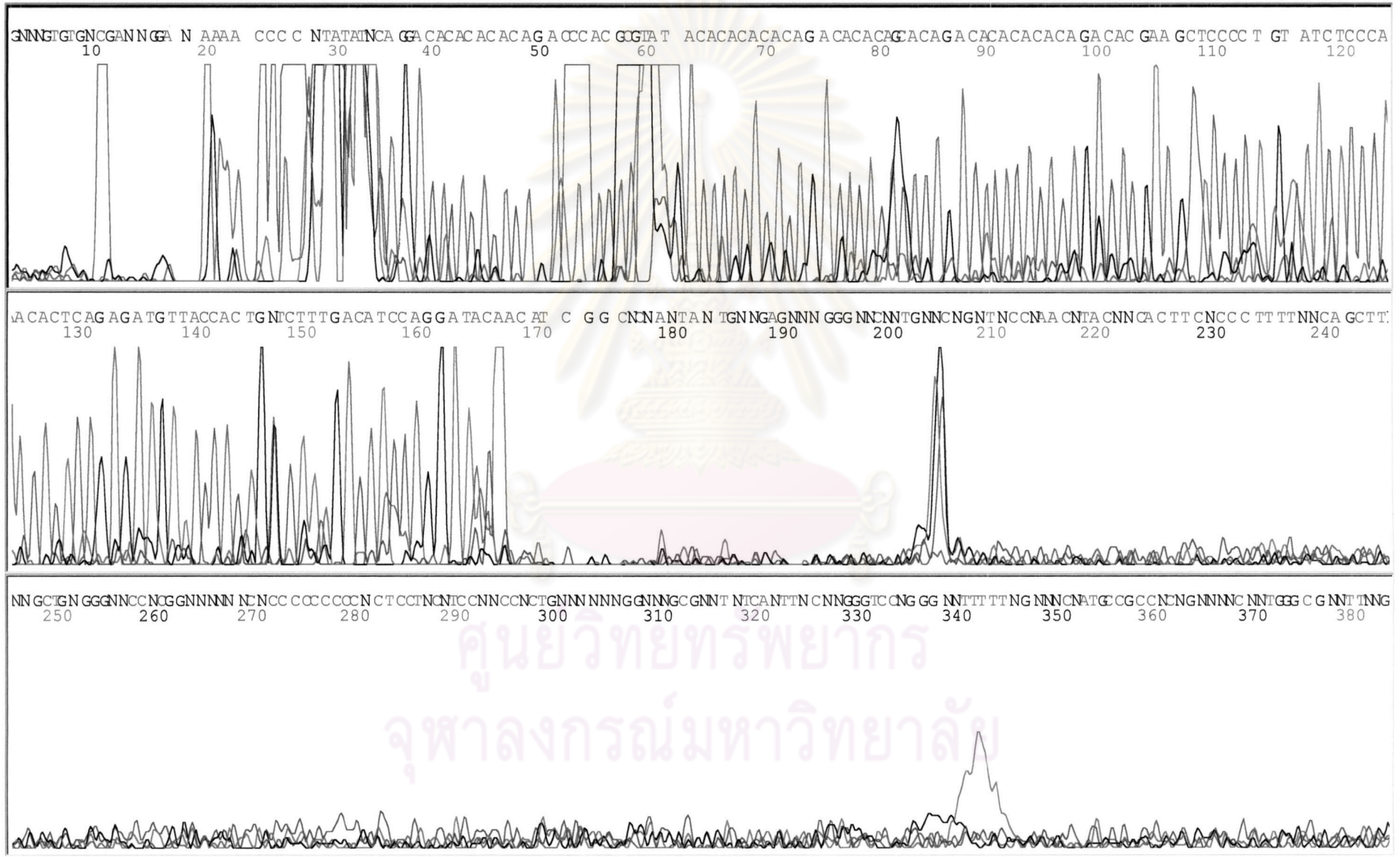
Model 3100
Version 3.7
Basecaller-3100APOP6
BC 1.5.0.0

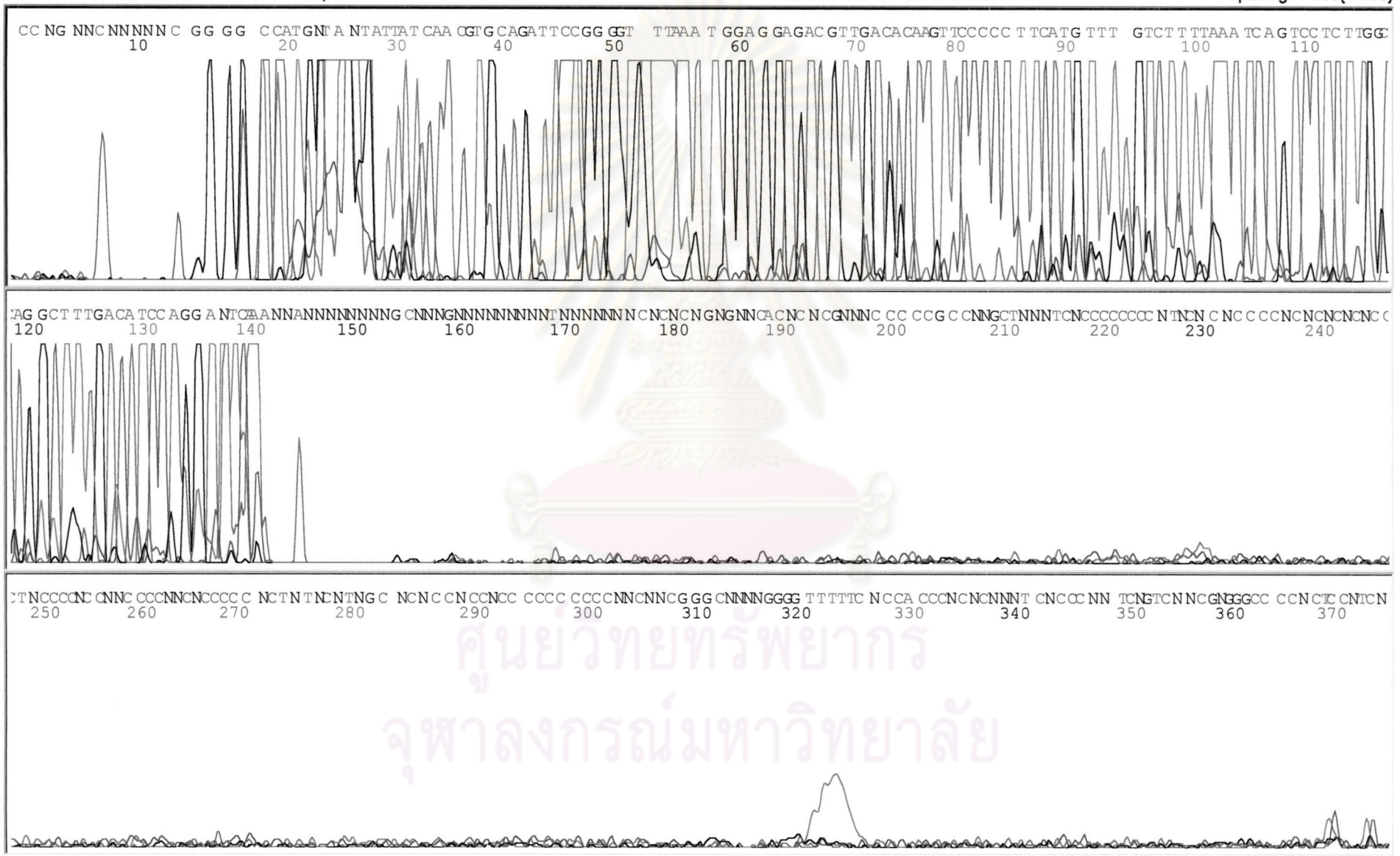
16-03-04_C10_HUJ2-4_HUJ2-1_06.ab1
HUJ2-4_HUJ2-1
Cap 6

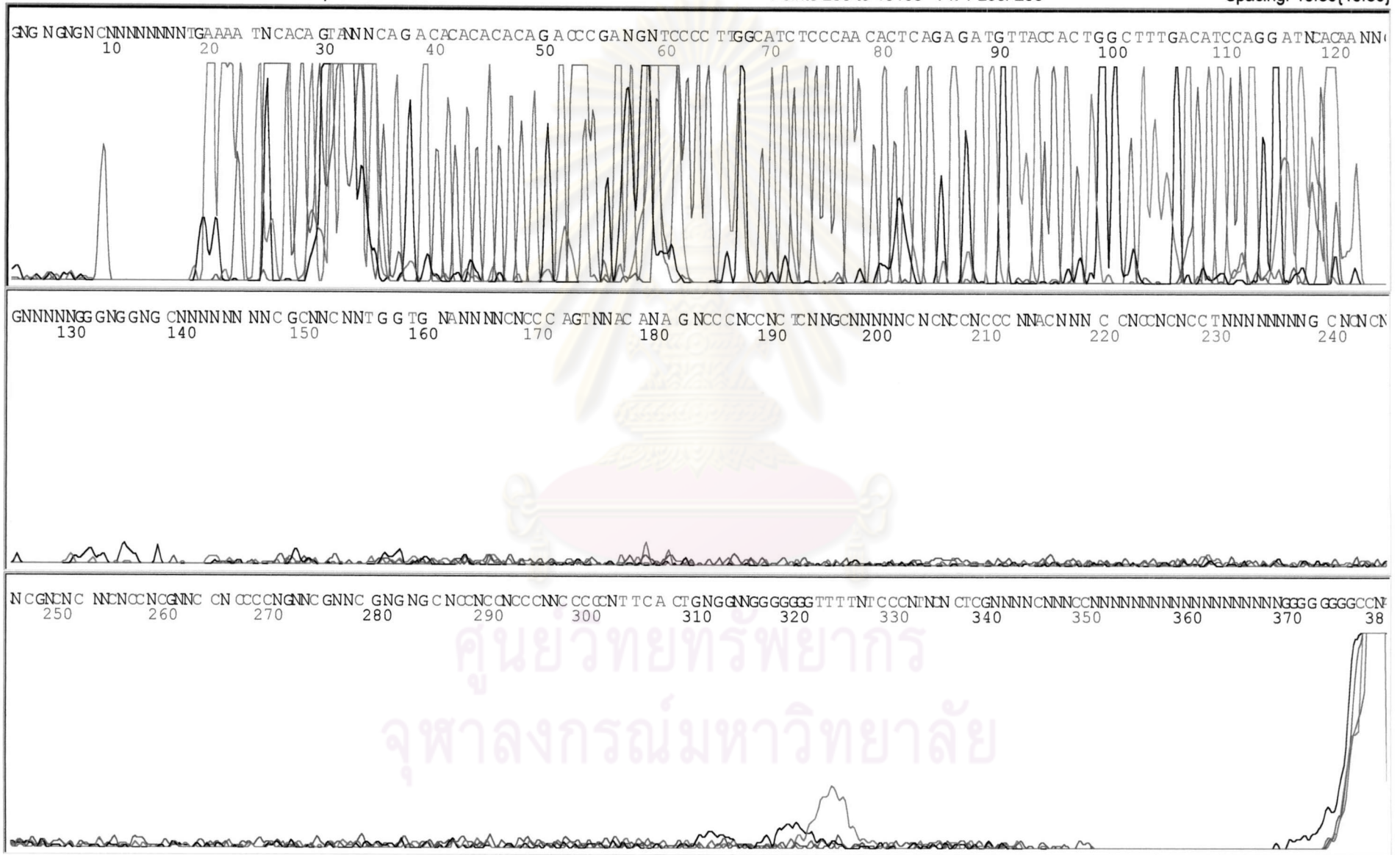
Signal G:26 T:53 A:37 C:29
DT3100POP6{ET}50cm.mob
demo_3100
Points 200 to 10106 Pk 1 Loc: 200

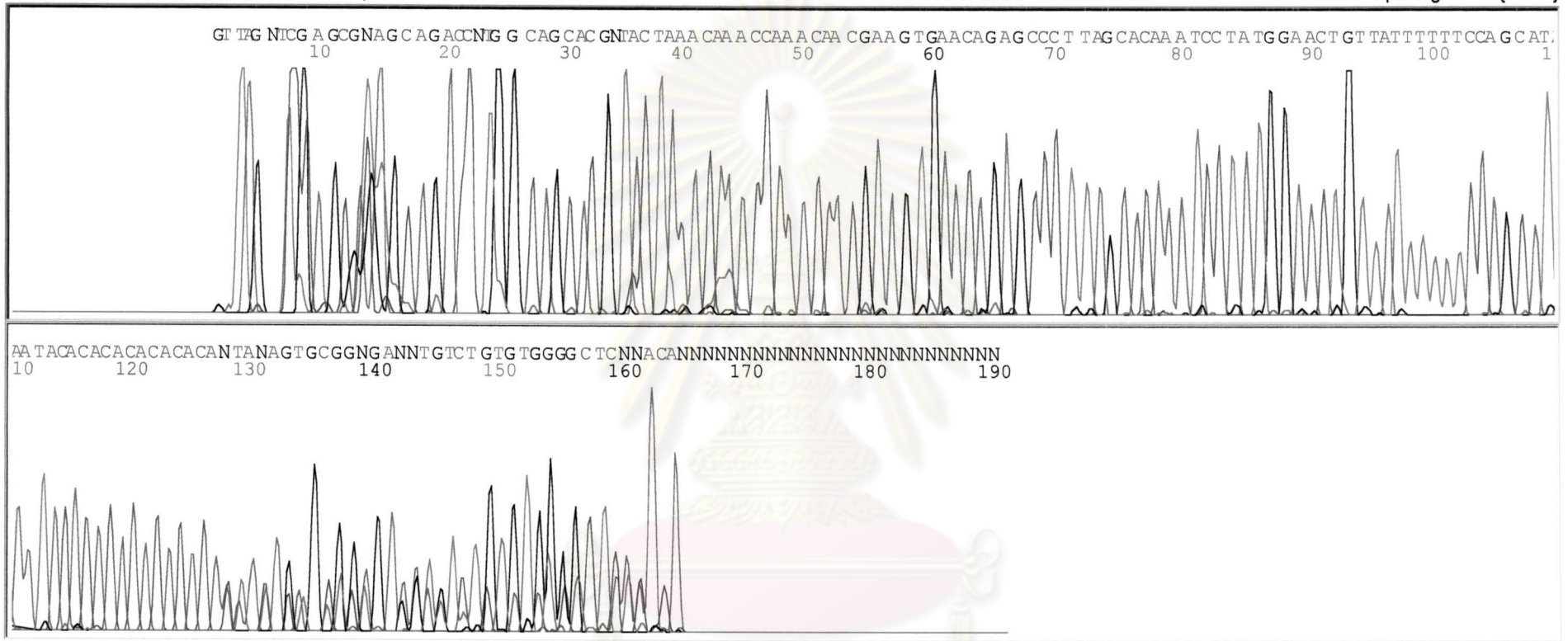
Wed, Mar 17, 2004 8:01 AM
Tue, Mar 16, 2004 4:36 PM
Spacing: 11.50{11.50}

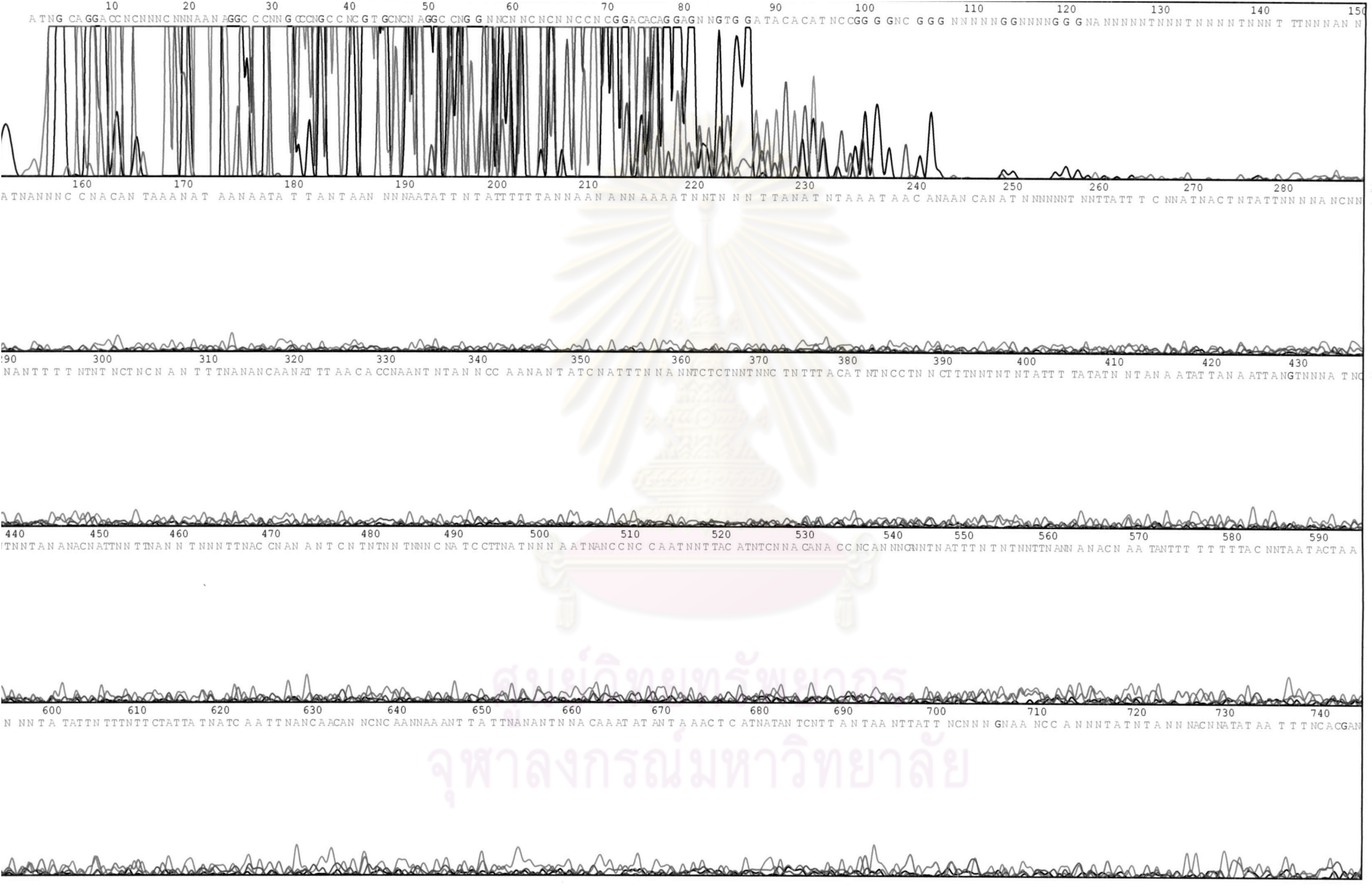


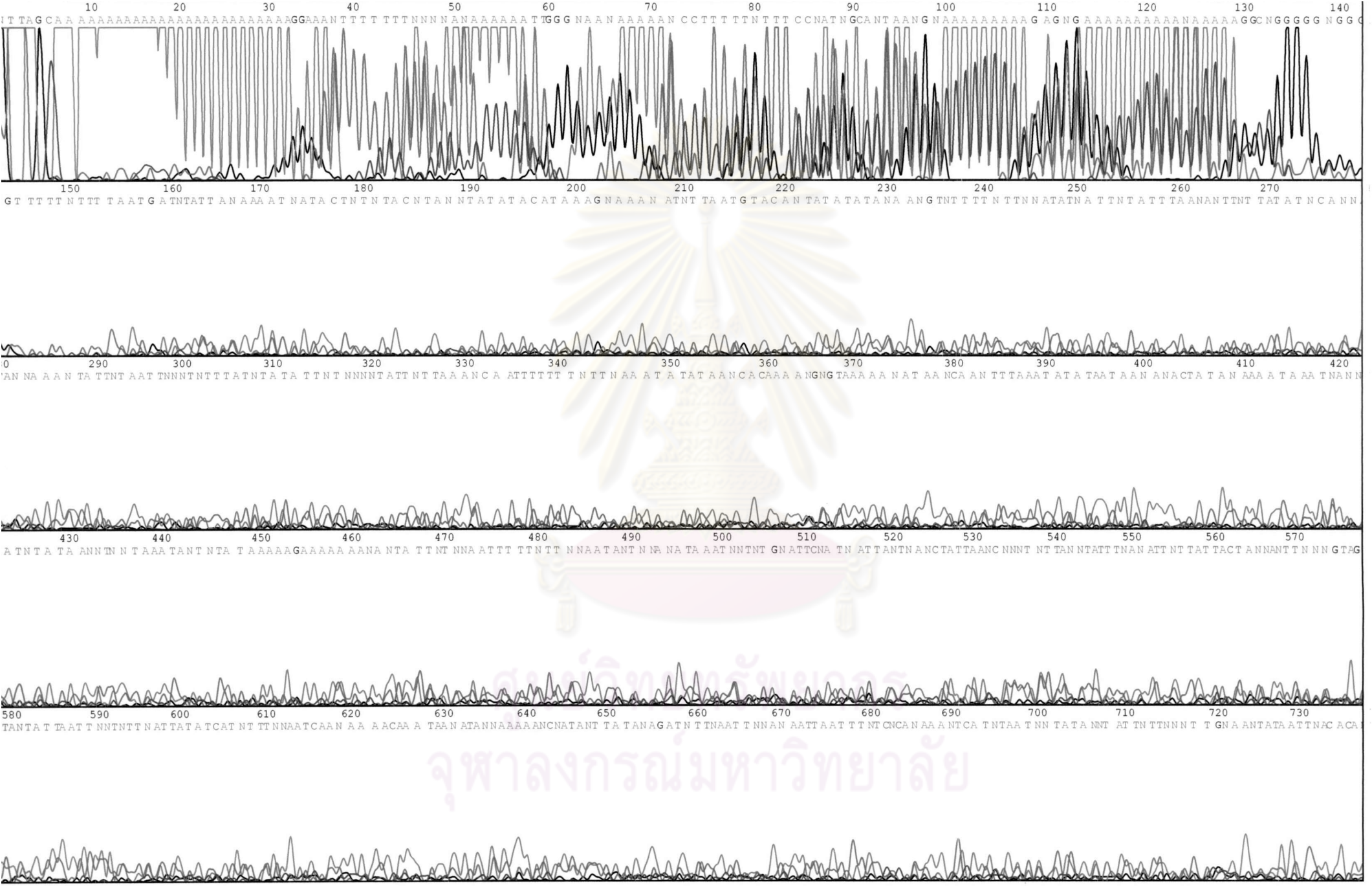


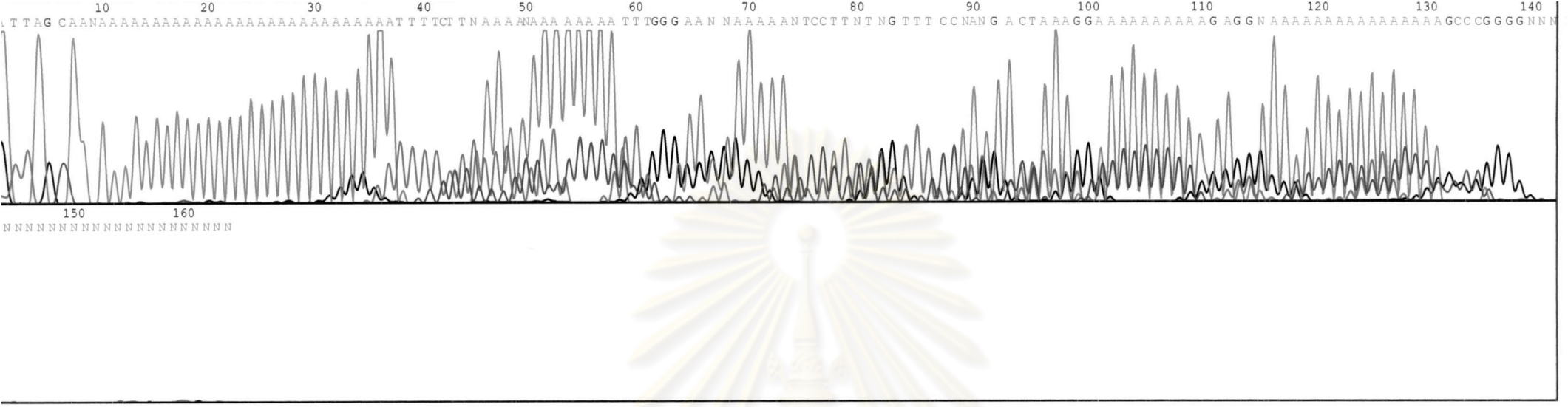


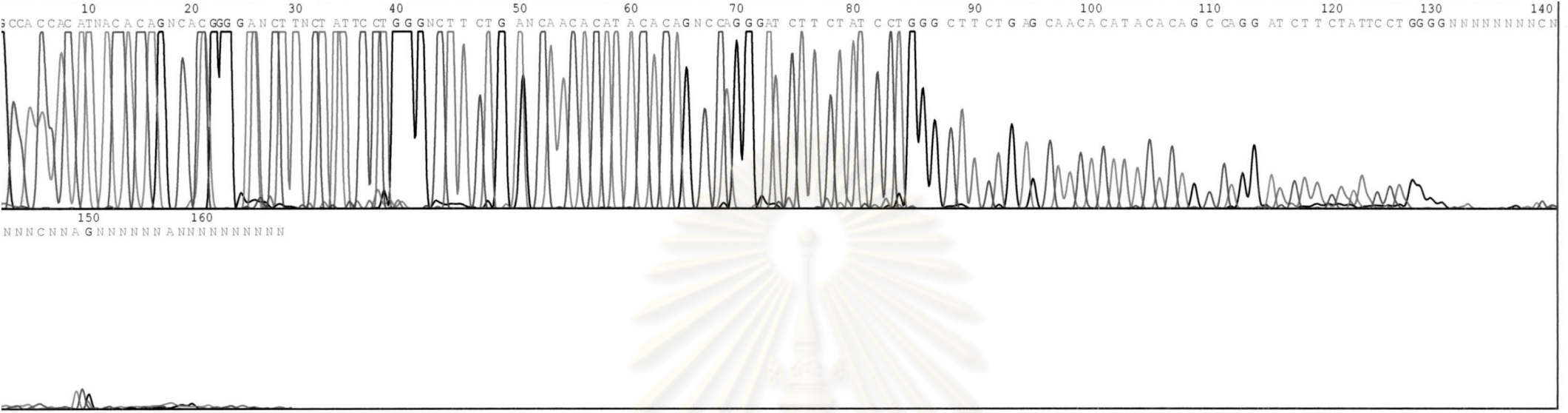


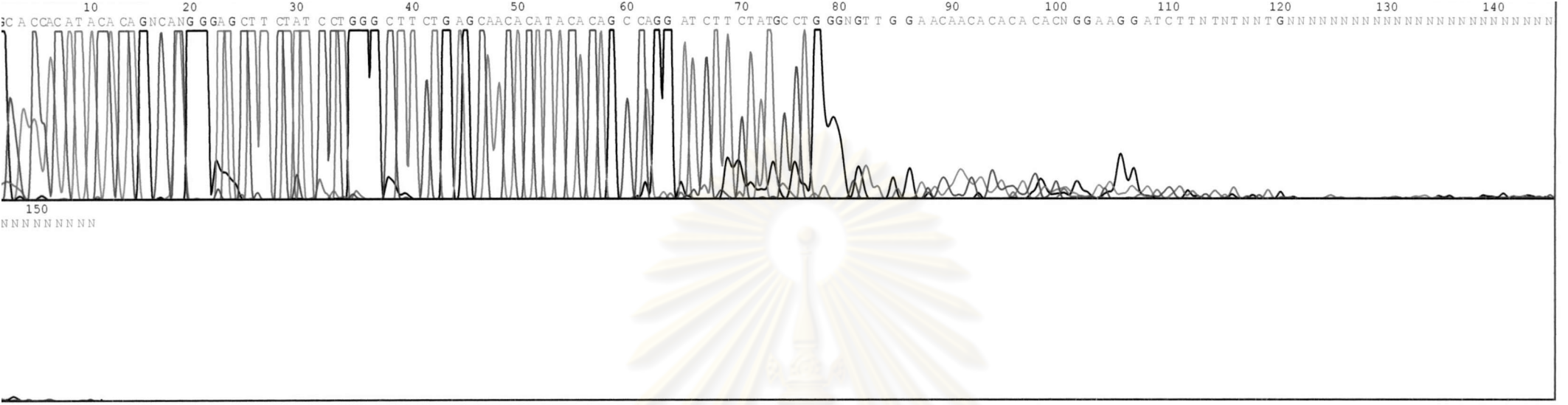


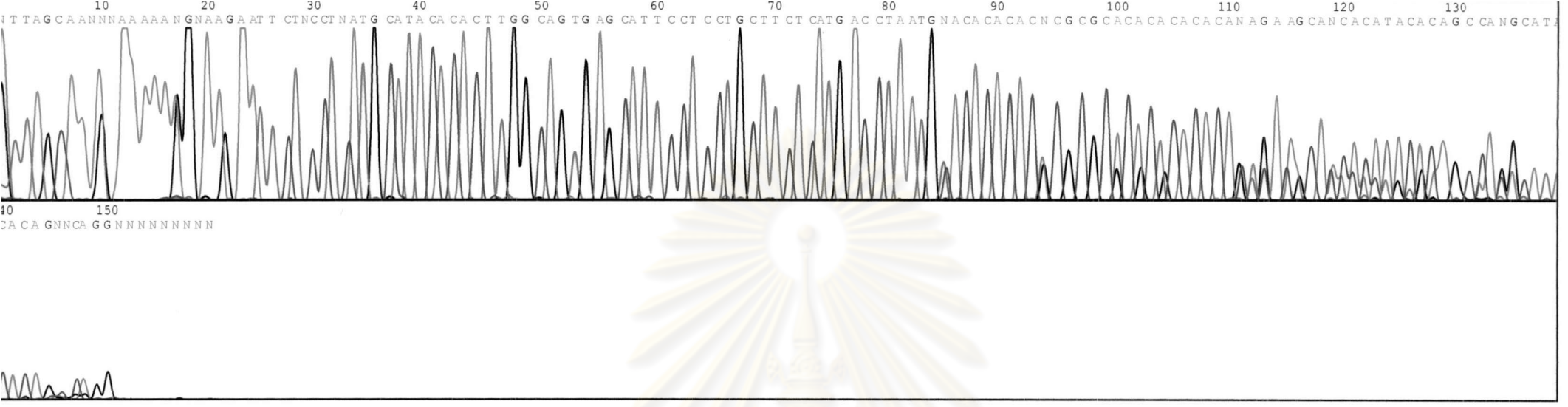


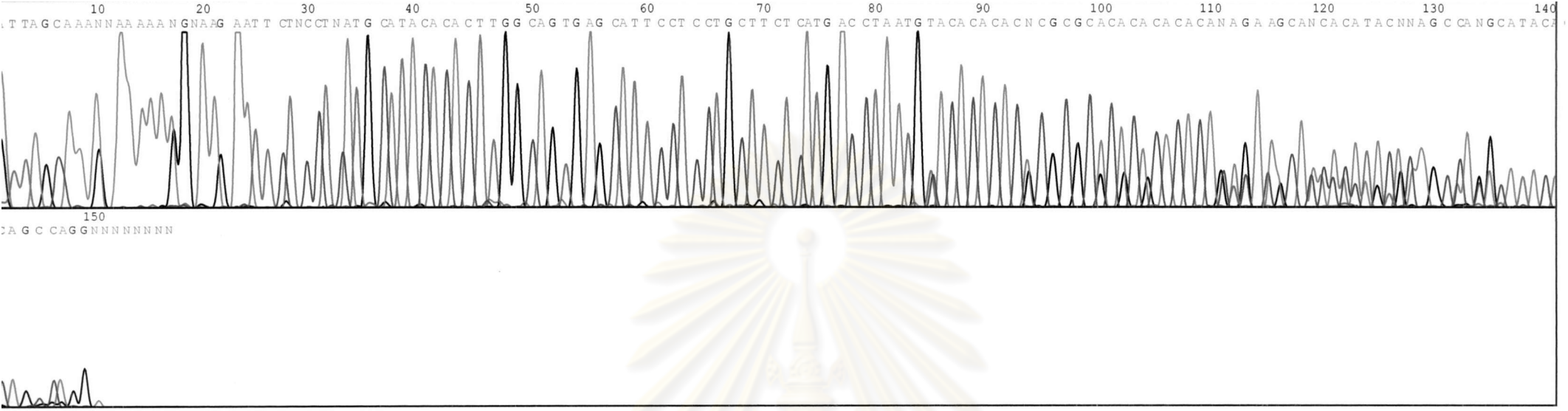

10 20 30 40 50 60 70 80 90 100 110 120 130 140
150 160 170 180 190 200 210 220 230 240 250 260 270
290 300 310 320 330 340 350 360 370 380 390 400 410 420
430 440 450 460 470 480 490 500 510 520 530 540 550 560 570
580 590 600 610 620 630 640 650 660 670 680 690 700 710 720 730











APPENDIX VIII : Hazardous chemicals and safety data sheets

The following hazardous chemicals in this study are shown below:-

Acetic Acid - *Corrosive*

Major Hazards Corrosive to the skin and eyes; vapor or mist is very irritating and can be destructive to the eyes, mucous membranes, and respiratory system; ingestion causes internal irritation and severe injury.

Acrylamide – *Toxic*

Acrylamide – *Toxic* Toxic. Both Acrylamide and Bisacrylamide are neurotoxic and can be absorbed through the skin. Wear gloves and a face mask when weighing out these two compounds. Weigh out in a fume cupboard. Wash hand prior to removing gloves. Wash any exposed skin that comes in contact with these compounds IMMEDIATELY.

Major Hazards

Ammonium Persulfate – *Harmful*

Major Hazards Harmful if swallowed. Very destructive of mucous membranes. May cause dermatitis. May cause irritation. May cause sensitization. Safety glasses, adequate ventilation.

Bisacrylamide (N,N'-Methylenebisacrylamide) - *Harmful*

Major Hazards Harmful if swallowed. Safety mask and gloves.

Boric Acid – *Harmful*

Major Hazards May be harmful or act as an irritant by inhalation. Cough. Sore throat. May be absorbed! Redness. May be absorbed through injured skin. The substance irritates the eyes, the skin and the respiratory tract. The substance may cause effects on the gastrointestinal tract, liver and kidneys.

Chloroform – *Poison*

Major Hazards Poison. May be fatal if inhaled or swallowed. Possible carcinogen. Readily absorbed through the skin. Acts as a defatting agent in contact with skin. Harmful if splashed into the eye. Chronic exposure may cause liver and kidney damage.

Ethanol – *Flammable liquid*

Major Hazards

The acute toxicity of ethanol is very low. Ingestion of ethanol can cause temporary nervous system depression with anesthetic effects such as dizziness, headache, confusion, and loss of consciousness

Formaldehye – *Flammable liquid and vapor*

Major Hazards

May cause allergic skin reaction. May cause central nervous system depression. This substance has caused adverse reproductive and fetal effects in animals. May cause liver and kidney damage. Cannot be made non-poisonous. Potential cancer hazard. Respiratory sensitizer. Contains formaldehye. Causes eye and skin irritation. Causes digestive and respiratory tract irritation. **Danger!** May be fatal or cause blindness if swallowed. Vapor harmful.

Hydrochloric Acid - *Strong Corrosive*

Major Hazards

Corrosive. Inhalation of vapor is harmful. Ingestion may be fatal. Liquid can cause severe damage to skin and eyes. Don't contact it directly.

Methanol – *Highly Flammable liquid*

Major Hazards

Low acute toxicity. The acute toxicity of methanol by ingestion, inhalation, and skin contact is low. Ingestion of methanol or inhalation of high concentrations can produce headache, drowsiness, blurred vision, nausea, vomiting, blindness, and death. In humans, 60 to 250 mL is reported to be a lethal dose. Prolonged or repeated skin contact can cause irritation and inflammation; methanol can be absorbed through the skin in toxic amounts. Contact of methanol with the eyes can cause irritation and burns. Methanol is not considered to have adequate warning properties. Methanol has not been found to be carcinogenic in humans.

Nitric Acid – *Highly corrosive*

Major Hazards

Highly corrosive to the eyes, skin, and mucous membranes; powerful oxidizing agent that ignites on contact or reacts explosively with many organic and inorganic substances. Contact with the eyes can cause serious long-term damage.Concentrated and moderately concentrated solutions are very corrosive and can cause serious skin damage.Fumes from concentrated nitric acid are very damaging if inhaled.Always wear safety glasses. Do not allow even dilute nitric acid solution to come into contact with your skin. Ensure that good ventilation is available, especially if using concentrated acid. If you need to use gloves, butyl rubber, neoprene, or polyethylene are suitable for handling solutions at concentrations of up to 70%

Phenol – *Corrosive. Combustible liquid and vapor*

Major Hazards

Toxic. Harmful if swallowed, inhaled, or absorbed through the skin. May cause severe respiratory and digestive tract irritation with possible burns. Causes severe eye and skin burns. May cause liver and kidney damage. May cause reproductive and fetal effects. Eye contact may result in permanent eye damage. May cause central nervous system effects.

Storage: Keep away from heat and flame. Keep away from sources of ignition. Keep from contact with oxidizing materials. Store in a cool, dry, well-ventilated area away from incompatible substances.

Silver Nitrate – *Poison*

Major Hazards

1) Poisonous if swallowed or inhaled
2) Skin contact with silver nitrate solid or solutions is likely to leave silver stains on the skin. These develop slowly over a period of hours and are initially brown, but darken gradually to black. Once the stains become apparent, they cannot usually be removed with soap and water, but gradually disappear as new skin grows.
3) Inorganic nitrates are oxidizers and may react vigorously with reducing agents.

Wear safety glasses. Do not breathe dust. Do not allow solution or solid to come into contact with the skin.

Sodium Acetate – *Irritant*

Major Hazards

May be harmful by ingestion, inhalation or through skin absorption. May act as an irritant.

Sodium Thiosufate – *Irritant*

Major Hazards

May cause eye and skin irritation. May cause respiratory and digestive tract irritation.

TEMED - *Corrosive*

Synonyms: N,N,N',N'-tetramethylethylenediamine, tetramethylethylenediamine,1,2-di-(dimethylamino) ethane, Propamine D, Tetrameen, TMEDA

TEMED - *Corrosive* (continued)

Major Hazards

Highly flammable,Harmful by inhalation and if swallowed,Causes burns. Keepaway from sources of ignition – no smoking.In case ofcontact with eyes,rinse immediately with plenty of water ane seek medical advice.wear sutible protective clothing, gloves and eye/facee protection. IN CASE OF ACCIDENT OR IF YOU FEEL UNWELL, SEEK MEDICAL ADVICE IMMEDIATELY

Symbols about safety

C Corrosive
F Highly flammable

Biography

Miss Waree Wutthivikaikan was born on 15th of July 1978 in Bangkok, Thailand. She graduated from bachelor's degree of Science in Biochemistry in 1999 from Department of Biochemistry, Faculty of Science, Chulalongkorn University. She continued her graduated study for Master's Degree of Science in Biotechnology program at Chulalongkorn University in 2003.