การแปรผันทางพันธุกรรมของนกยูงไทย *Pavo muticus* ในภาคเหนือของประเทศไทย

นางสาวภัทรา พลับเจริญสุข

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GENETIC VARIATION OF GREEN PEAFOWLS *Pavo muticus* IN NORTHERN THAILAND

Miss Pattra Plubcharoensook

A Thesis Submitted in Partial Fulfillment of the Requirements

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ภัทรา พลับเจริญสุข : การแปรผันทางพันธุกรรมของนกยูงไทย *Pavo muticus* ในภาคเหนือของประเทศไทย. (GENETIC VARIATION OF GREEN PEAFOWLS *Pavo muticus* IN NORTHERN THAILAND) อ. ที่ปรึกษา : รศ. วีณา เมฆวิชัย, 93 หน้า. ISBN 974-13-1148-6

ความแปรผันทางพันธุกรรมของประชากรนกยูงไทย *Pavo muticus* 2 แห่งในภาคเหนือของประเทศไทย คือที่อุทยานแห่งชาติดอยภูนาง และเขตรักษาพันธุ์สัตว์ป่าเวียงลอ โดยศึกษาลำดับนิวคลีโอไทด์ของดีลูปในไมโทคอนเดลียล ยาว 330 bp พบว่า ภายในประชากรนกยูงที่อุทยานแห่งชาติดอยภูนางมี variable site ทั้งหมด 26 ตำแหน่ง โดยเป็น informative site 14 ตำแหน่ง transition 16 ตำแหน่ง transvertion 8 ตำแหน่ง และมีค่า Genetic distance ระหว่าง 0.0000 - 0.0513 ส่วนภายในประชากรนกยูงที่เขตรักษาพันธุ์สัตว์ป่าเวียงลอ พบว่ามี variable site 7 ตำแหน่งซึ่งเป็น transition ทั้งหมด และมี Genetic distance ระหว่าง 0.0000 - 0.0219 และเมื่อศึกษาลำดับนิวคลีโอไทด์ระหว่างประชากรทั้งสองพบว่ามี variable site ทั้งหมด 24 ตำแหน่ง เป็น transition 16 ตำแหน่ง และ transversion 8 ตำแหน่ง ส่วน Genetic distance อยู่ระหว่าง 0.0000-0.0547 ความหลากหลายทางพันธุกรรมของนกยูงที่อุทยานแห่งชาติดอยภูนางสูงกว่าเขตรักษาพันธุ์สัตว์ป่าเวียงลอ โดยมี genetic diversity เท่ากับ 1.92 และ 0.22 ตามลำดับ ซึ่งชี้ให้เห็นว่าประชากรนกยูงที่อุทยานแห่งชาติดอยภูนางมีการแปรผันทางพันธุกรรมมากกว่านกยูงที่เขตรักษาพันธุ์สัตว์ป่าเวียงลอ เมื่อศึกษาความสัมพันธ์ทางพันธุกรรมเชิงวิวัฒนาการโดยใช้ Parsimony method พบว่าประชากรนกยูงที่อุทยานแห่งชาติดอยภูนางกลุ่มหนึ่งยังคงมีความสัมพันธ์กัน และประชากรนกยูงที่อุทยานแห่งชาติดอยภูนางอีกกลุ่มแยกต่างออกไป โดยมีดัชนีคอนซิสเทนซีเท่ากับ 0.943 และดัชนีรีเทนชั่นเท่ากับ 0.853

ภาควิชา……-……ลายมือชื่อนิสิต……

สาขาวิชา…..เทคโนโลยีทางชีวภาพ……ลายมือชื่ออาจารย์ที่ปรึกษา……

ปีการศึกษา……2543……..ลายมือชื่ออาจารย์ที่ปรึกษาร่วม……..

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Genetic variation of *Pavo muticus* between Doi Phu Nang National Park and Wieng Lor Wildlife Sunctuary populations by 330 bp by mitochondrial D-loop nucleotide sequence. It is found there are 26 variable site, 14 informative sites, 16 transition sites and 8 transversion sites in Doi Phu Nang National Park population. And its genetic distance ranges between 0.0000 and 0.0513. Meanwhile, there are 7 variable sites and transition in Wieng Lor Wildlife sanctuary population, and its Genetic distance ranges between 0.0000 and 0.0219. According to the Nucleotide sequence study between the two populations, there are 24 variable sites, 16 transition sites and 8 transversion sites and their Genetic distance is between 0.0000 - 0.0547. Genetic diversity of *Pavo muticus* within Doi Phu Nang National Park and Nucleotide diversity within Wieng Lor Wildlife Sunctuary is 1.92 and 0.22 respectively. These result reveal that genetic variation of Green Peafowls from Doi Phu Nang National Park is higher than that of Wieng Lor Wildlife Sunctuary. The analysis of phylogenetic relationship using parsimony approach found that one group of population of Doi Phu Nang and Wieng Lor Wildlife Sanctuary are still to related, and another group of Green Peafowls of Doi Phu Nang National Park has been diverged. The consistency index (CI) is 0.949 and Retention index (RI) is 0.853.

Department…….. Student's signature…….
Field of study……Biotechnology…….. Advisor's signature……
Academic year……2000…… Co-advisor's signature……..

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List of Abbreviations

ATGC	nucleotide containing the base Adenine, Thymine, Cytosine and Guanine
bp	base pairs
dNTP	deoxyribonucleotide triphosphate containing the base Adenine, Thymine, Cytosine, and Guanine
DNA	deoxyribonucleic acid
EDTA	ethylinediamine tetraacetic acid
mM	millimolar
ng	nanogram
MgCl2	Magnesium Chloride
rpm	revolution per minute
SDS	Sodium Dodecyl Sulphate
TBE	Tris / Borate / EDTA buffer
µl	microliter
µM	micromolar

Chapter 1

Introduction

Peafowls have been famous in arts and letters for several tens of centuries. Not only in India and in China, but also around the Mediteranean Sea (Delacour, 1977). They have been admired for beauty so they are hunted for flesh. Recently, they became one of the most popular and graceful additions to parks and gardens (Wayre, 1969).

There are three species of Peafowls, the Congo Peafowls (*Afropavo Conginsis*), live in congo, central africa, the Indian Peafowls (*Pavo cristatus*) from India, often called Blue Peafowls, and the Green Peafowls (*Pavo muticus*) which live in southeast Assam through Burma and Thailand to the China sea and Southwards to the Malaysian Peninsula and Java. The characteristics of two Asian Peafowls are similar but Green Peafowls are bigger than Blue Peafowls. Furthermore, the feather of Blue Peafowl's body coverts are blue and of it's wings are light black and white. The other's feather is brown. The facial skin of Blue Peafowls are white while Green Peafowl's facial skin of Blue Peafowl is white while Green Peafowls's facial skin is blue and yellow. Blue Peafowls's crest is fan shaped while Green Peafowl's crest is erect standed (Wayre, 1969).

The classification of Green peafowl is :

Class Aves
Subclass Neonithes
Order Galliformes
Suborder Galli
Family Phasianidae
Subfamily Pavoninae
Genus Pavo
Species *Pavo muticus*
Subspecies *Pavo muticus imperator*

(Ponsena, 1988)

There are three subspecies of Green Peafowls; *Pavo muticus specifer* distributed in the western Burma. *Pavo muticus imperator* live in eastern Burma, Thailand and Indo-China. In the southern part of Thailand from Kra and Java were founded *Pavo muticus muticus* (Wayre, 1969).

There is only one species of *Pavo muticus* in Thailand. There are two subspecies, the Indo-Chinese green peafowl (*Pavo muticus imperator*) and the javenese green peafowl (*Pavo muticus muticus*). The former is distributed in the north to Kra and the latter is found in Kra to the south (Deignan, 1963).

Green Peafowls (*Pavo muticus*) were classified as an endangered species reflected from small population sizes in Thailand. Furthermore, it is one of the important about beautiful and well known bird of Southeast Asia including Thailand. Once they were found through out Thailand below nine hundred meter except in the central valley of Chao Phraya and Southeastern of the northwest (Humprey and Bain, 1990). Overhunting threatens to eliminate this species from Thailand. Hunters intensively trap it for the feather and pet trade and vellagers take it as food as well as collect eggs from the forest. Not only its' habitats were destroyed but were also separated in to small areas. Currently, IUCN (International Union For The Conservation of Nature and Natural 1998) classified as vulnerable species. In Thailand, Green Peafowls have been classified as a protected animal and endangered species (OEPP, 1997). Furthermore, they were classified into Appendix II by CITES (The Conservation on International Trade in Endanger Species of World Fauna and Flora 1996). Green Peafowls were founded in small individual at the following national parks (NP) and wildlife sanctuaries (WS): Phu khieo WS, Phu Wa Ws, Phu Miang-Phu Thong WS, Salawin WS and Khun Yuan WS (Humphrey and Bain, 1990). The largest population of Green Peafowls are found at Huai Kha Khaeng about three to four hundred (Ponsena, 1988).

The wildlife conservation required the informations about ecology, biology and behavior for species survival. In addition, genetic variation in population level

is the important information in a long run. Because genetic variation is the essential quality for adaptation to survival. At present, there is no report about genetic variation of Green peafowls in Thailand yet. Then, it should be necessary to study this information so that it would to be laid plans for conservation and maintain the populations.

Recently, mitochondrial DNA were useful for studying genetic variation among species, populations and individuals. Mitochondrial DNA has high mutation rate (Brown *et al*, 1979). Furthermore, the different regions of the mitochondrial genome evolve at different rates (Saccone *et al*, 1991). Mitochondrial DNA is materanally inherited in most species and does not recombine (Hayashi *et al*, 1985). The animal mitochondrial DNA (mtDNA) is a small circular molecule of 16,000 base pairs. There is no repetitive DNA, spacer or intron. The mitochondrial DNA encodes 13 mRNAs, 22 tRNAs, control region (D-loop) containing initiation sites for replication and transcription, cytochrome b, NADH dehydrogenase, three subunits of cytochrome c oxidase (*CO I,II,III*) and 2 rRNAs.

When polymerase chain reaction (PCR) has been developed. The small amount of DNA from the field such as bloodstain, hair, feather can be amplified at specific DNA region such as mitochondrial DNA. Furthermore, To detect variation in nucleotide sequencing is a powerful tool assess an intraspecificly phylogenetic pattern in many animal species (Avise, 1994).

Objective

1. To study genetic variation within and between two populations of Green Peafowls (*Pavo muticus*) in northern Thailand by D-loop sequences.
2. To get partial D-loop sequences of Green Peafowls.

Anticipated benefit

1. For basic knowledge on genetic variation within and between two populations of Green peafowls in northern Thailand.

2. For knowledge on the present status of genetic variation of the Green Peafowls Of two populations in northern Thailand.
3. To applied this information for planning wildlife management to conserve Green Peafowls in length.

Chapter 2

Literature Reviews

2.1 The Characteristic of Green Peafowls

The male Green Peafowl is larger and more colorful than the Indian Peafowl. Its brilliant green crest is composed of a long narrow tuft of feathers and its plumage is even more colorful. The neck, breast and mantle are scale-appearant. Each feather are bright blue and are with a broad metallic green border. The train is of a brighter emerald green. The wing coverts are bright metallic blue and green, and the bare facial skin is pale blue and yellow. The mantle, back and tail of the Green Peafowls are the same as in the Indian Peafowls, but only are more brilliant and coppery. The primaries are bright chestnut, the dark green abdomen and the grey vent and under tail coverts. The secondaries are blackish-brown on the inner web and dark blue and green on the outer. The female peafowl is similar to the male, but are only slightly duller. There is no train in female but it is replaced by short greenish-brown feathers with buff. The female can be distinguished from the young males by the brown patch instead of bluish-black loral one between an eye and the bill. Also, distinguished by the primaries, the female's chestnut speck are bright and brown, whereas the male's chestnut is pure.

Green Peafowls live in the same habitat like Indian Peafowls. Green Peafowls are even more wary and less prone to live near human habitation. They are often found in a jungle, usually in the vicinity of a river or open clearing. The flock is small, except during the breeding season. Adult males fight to defend their territory and band of female (Wayre, 1969).

Figure 2.1 Green Peafowl (*Pavo muticus*)

2.2 *Pavo muticus imperator* (An Indo-Chinese green peafowl)

Males are similar to *Pavo muticus muticus* but are not quite brilliant generally. The fringes of the neck, upper back, and breast feathers are more coppery, but are not so golden green. Lower breast and flanks are duller and darker. Mantle and back are slightly more bluish and less golden. Wing coverts and the outer web of secondaries are bluer and little duller, and less green on the borders.

Females differs from *Pavo muticus muticus* in having the borders of the breast feathers. They are more heavily marked with buff, less green, and the wing-coverts are less brilliant (Delacour,1977).

2.3 Distribution of Green peafowl

Once, Green Peafowls had been found through out Thailand below 900 meter except in the central Valley of the Chaophraya and the southeastern provinces. At present, this bird is classified as one of the threatened species. The decreasing of its population is due to habitat destruction, environmental pollution and hunting. (Rojanadelok *et. al*, 1986).

They have been reported in many areas, but about 300 peafowls are only confirmed in Hwai Kha Kaeng wildlife sanctuary (Collar *et al*, 1994). According to a report on ecological effects of Kaeng Sua Ten Dam project, there are one of two possibly surviving wild population of Green Peafowls in the north of Thailand at Doi Phu Nang National Park. Furthermore, Green Peafowls had been found at Wieng Lor wildlife sanctuary which these two areas are bounded (Meckvichai *et al*, 2001).

2.5 Biology of Green peafowl

The Green Peafowls often flock into a small group of 3-5 birds (Ponsena, 1988). It uses a wide variety of habitats, including an open forest which

is preferable, a riverbank, a coastal scrub, a teak, a tea and coffee gardens, a forest edge and clearing, an area with dense secondary growth near shifting agriculture, and others. (Humphrey and Bain, 1990). It can fly weakly so it spend most of its time on the ground looking for food or perching. It is omnivorous. It likes to eating berries, pears, and other fruits, including rice-grain and seedling such as grass seed. Also, it can eat crickets, dragonflies, small moth, etc., and frogs and lizards, etc. (Humphrey and Bain, 1990). At Doi Phu Nang National Park, they can be also fed on *Heteropgon contortus*, *Antidesma ghesimbia , Onchna integerrina , Vegna mungo and Zea mays* (Arrathrakorn and Meckvichai, 2000).

Peafowls are polygamous, so four or five females may be mated to one male. Moreover, it has specially behavioral characteristic. During the breeding season, the dominant male will move to sand bars along the main stream and create a breeding territory. It tries to defend its territory from other males. A females usually moves in its flock ranging from 2 to 6 individuals. Their feeding range at this season may cover 2 to 4 male‘s territories. The male uses calling signals and displays to induce females to come into his territory. Mating usually occurs in the morning and in the late afternoon. A mated begin to lay eggs at 22 months of age (Humphrey and Bain, 1990). A Female usually lays 3-5 eggs in a shallow hole dug on the ground. The female incubates the eggs for approximately 28 days by herself. After hatching, the young chicks follow the mother, even though they are capable of foraging on their own. Arrathrakorn and Meckvichai (2000) reported that a breeding season of green peafowl at Doi Phu Nang is from January to April. They create breeding territories on the top of the hill and also found at the bottom of the hill. The clutch sizes are for 4-6 eggs. The nestling and hatching are abundantly found in May.

Green peafowls have a good sense of seeing and bearing. They usually can run away from human from far distance. It prefers to run away from an enemy but, for sudden alarm, they will run for a short distance and then fly up into the air (Rojahadelok *et.al* , 1986).

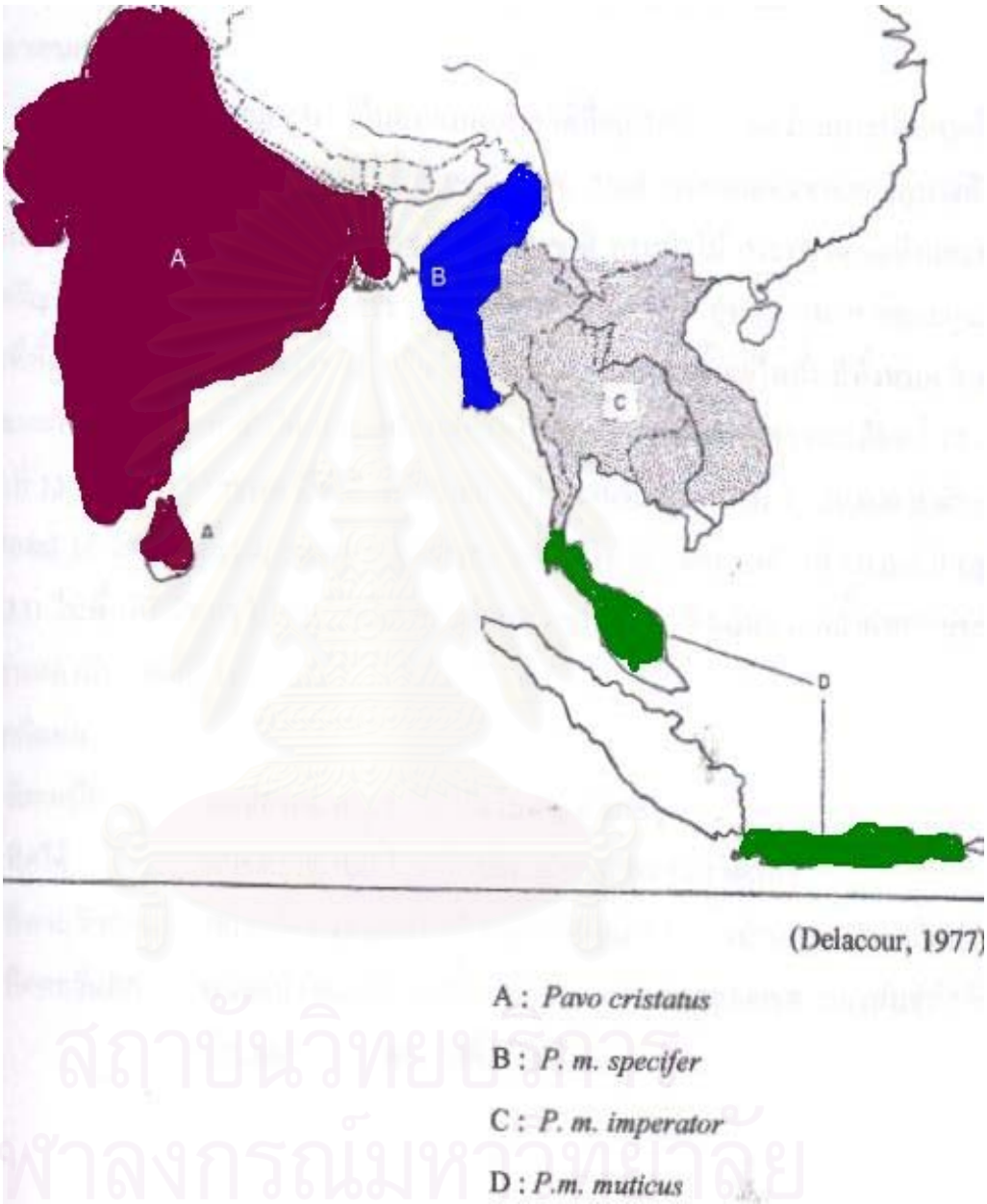

Figure 2.2 Map showing geographic distribution of *P. cristatus* and 3 subspecies of *P. muticus*.

2.6 The areas of this study

Doi Phu Nang National Park

Doi Phu Nang National Park. is located in Prayao Province. The area is about 840 km^2. It covers the area about 840 km^2. It locates in Prayao covering within three district which are Dok Khum Tai, Pong and Chiang Muan. Fishing cat is the only extirpated species of this area. Furthermore, there are many vulnerable species such as foxes, wild cats, and green peafowls (Arrathrakorn, 1998). Doi Phu Nang National park boundary is connected to Wieng Lor wildlife sanctuary in the north, Mae Yom National Park in the south, Doi Pha Chung wildlife sanctuary in the east and Wieng Lor wildlife sanctuary in the west (Phungsawade, 1997).

Weing Lor wildlife Sunctuary

It is located in Prayao Province. The total area is 371 km^2. It is between $19^{\circ}\ 4'$ N to $19^{\circ}\ 28'$ N in latitude and $100^{\circ}\ 3'$ E to $100^{\circ}\ 19'$ E in longtitude. Wieng Lor wildlife sunctuary is connected to Doi Phu Nang in the south. Wieng Lor wildlife Sunctuary is a source of Yom and Eing river, so this area is very rich or suitable for wildlife and plants. Furthermore, it is s residence of green peafowls which one the important wildlife (Wieng Lor WS, 1998).

2.6 Genetic Variation

The loss of genetic variation is caused by reduced population size and habitat fragmentation which are important for biodiversity conservation. Genetic variation is a highly desirable characteristic (Woodruff, 1990). Genetic variation can be monitored directly and indirectly in a number of ways. Studies of allozyme variation have been the most commonly employed approach during the last twenty years. In this technique, the allelic variants of soluble enzymes and other proteins

that can be visualized biochemically on a gel after electrophoresis are counted directly. More recently, there are several molecular genetic approaches to monitoring genetic variation and determine relationships between individual birds, population, species including mitochondrial DNA (mtDNA), restriction fragment length polymorphism (RFLP) analyses, whole genomic DNA-fingerprinting and direct sequencing of mtDNA and nuclear DNA loci. Such techniques facilitate very fine detail analysis of microevolutionary process. (Avise, 1994).

2.7 Genetic in the conservation of Biodiversity

The late 1980's, all studying DNA level variation required the large amount of tissue or blood and the large-scale extraction of high molecular weight DNA. Furthermore, collecting tissue samples from free-ranging animals was difficult. The development of the polymerase chain reaction (PCR) technique enables investigators to amplify very small amounts of tissue, Thus eliminating the need for large blood or tissue samples which are difficult to handle in the field (Woodruff, 1990). DNA can be extracted and amplified from nanogram samples of pulp from feathers and microgram amounts of tissue from museum skin or preserved specimen (Kocher *et al*, 1989). Appropriate molecular genetic markers can be chosen by matching the level of innate variability of a gene to the level of ecological of evolutionary resolution requied (Hillis et al, 1996). Mitochondrial DNA is an information molecule for defining species and subspecies boundaries, interspecific and intraspecific phylogeny and phylogeography (Palumbi & Wilson, 1990, Edward *et al*, 1991, lrwin *et al*,1991). Beside that it has potential for use in population level studies.

2.8 Mitochondrial DNA

The control region is the primary non-coding region, and is responsible for the regulation of heavy (H) and light (L) strand transcription and H-strand replication (Figure 2.3)

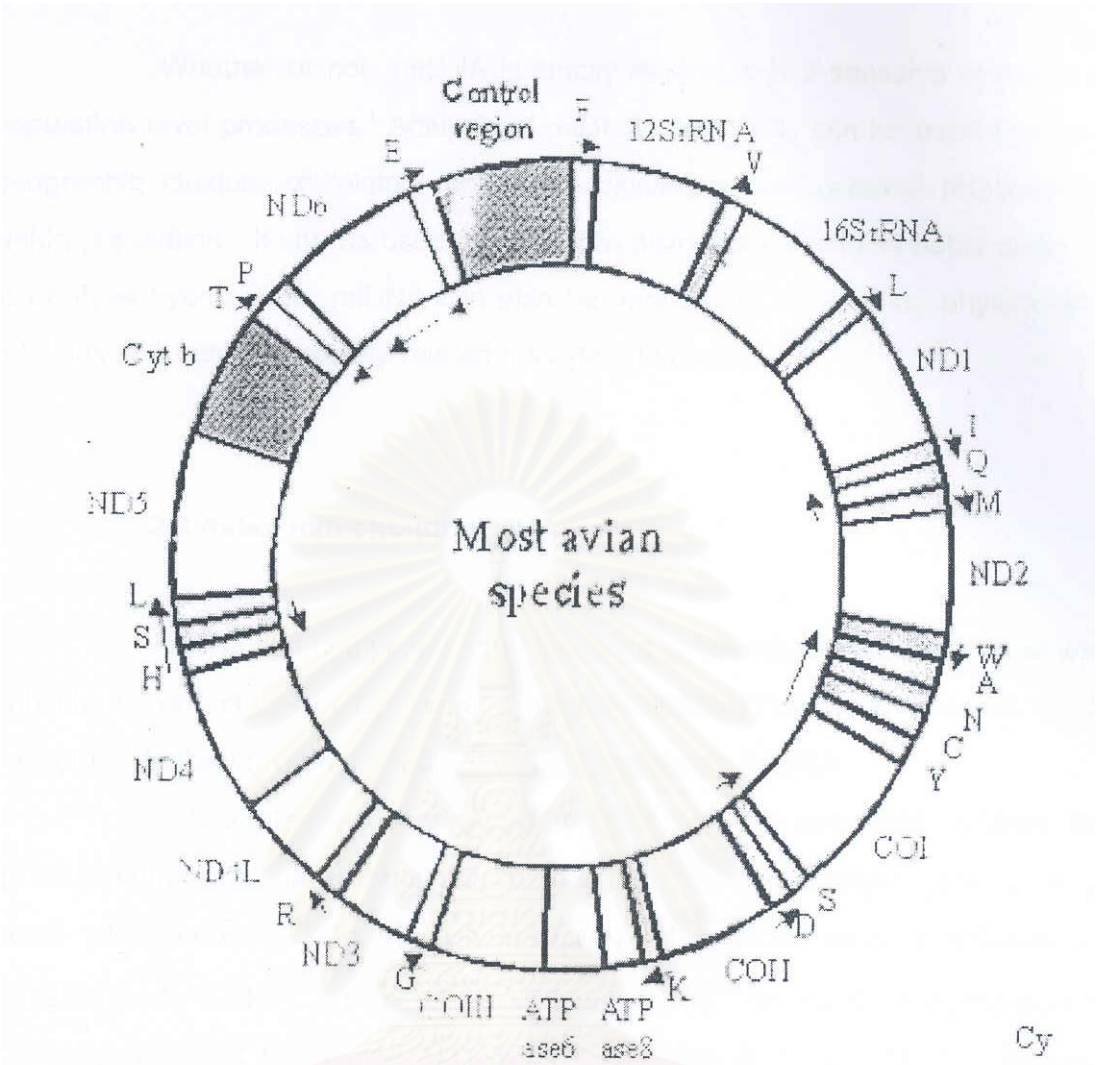

Figure 2.3 Mitochondrial genomes of birds. The outer circle represents the heavy (H) strand and the inner circle represents the light (L) strand. Polarity of transcription and the transcribed strand is shown with arrowheads.

As a molecular marker, mitochondrial DNA has many advantages. It evolves faster than nuclear DNA (Brown *et al*, 1982). Different regions of the mitochondrial genome evolve at different rates (Saccone *et al*, 1991). This allows suitable regions to be chosen for the study. Mitochondrial DNA is maternally inherited in most species and does not recombine (Hayashi *et al*, 1985). In addition, mitochondrial DNA is simple, it has 500-1000 copies per cell instead of only one copies per haploid genome.

Whether or not mtDNA is strictly neutral, it is a sensitive indicator of population level processes. Analysis of mtDNA divergence can be used to reveal geographic clusters of related molecules (individuals) or maternal relationships within population. It can be used also to trace historical events like bottlenecks, or to analyse hybrid zone, mtDNA can also be very useful in resolving phylogenetic relationships between closely related taxa (Moritz *et al*, 1987).

2.9 Avian mitochondrial DNA

The first complete sequence of an avian mitochondrial genome was published from chickens by Desjardins and Moriais (1990). It showed highly conserved features when compared to other vertebrate MtDNA.

Since many features are the same in all the vertebrate mtDNAs, the avian genome has some remakable differences. First, the avian gene order is novel when compared to the mammalian's. The ND5 gene is followed by cytochrome b, tRNAThr and tRNAPro, ND6 and tRNAGlu in the 5'→3' direction of the avian L-strand (Desjadins & Morais 1990, Quinn & Wilson, 1993). Second, the L-strand replication origin that is found between tRNACys and tRNAAsn in other vertebrates is absent in the avian genome (Desjadins & Morais, 1990).

2.9.1 Cytochrome b

Cytochrome b is one of the cytochromes involved in the electron transport system. It is the only cytochrome coded by mitochondrial DNA. The cytochrome b gene is the most widely used gene for phylogenetic. Although it evolves slowly in terms of nonsynonymous substitutions (Irwin *et al*, 1991). Cytochrome b is variable enough to study at a population level, and conserved enough to clarify phylogenetic relationships in deeper details.

2.9.2 Control region of mtDNA

The mtDNA control region is the only large non-coding region in avian mitochondria. It varies from 1,044 - 1,227 bp in *Gallus domesticus* (Desjadin & Morais, 1990). It contains the heavy strand replication origin. This region is divided into three domains identified by Desjadeans and Morais 1990 from *Gallus domesticus* and by Quinn and Wilson (1993). The first domain at the 5' end of the control region contains C-stretch and high variation. C-stretch is specific to the 5' terminus of the avian control region. It is present in various forms at least in Anatidae, Phasianidae and Poridae (Quinn & Wilson 1993, Desjadins & Morais 1990). The central domain is the most conserved. The most variable part is usually the third domain at the 3' end of the control region. Also, it is highly variable in other birds (Wenick *et al*,1993). This variability has lead to the expanding usage of control region sequence to examine questions ranging from population structures to phylogenetic relationships. This region has already been proven to be quite a powerful tool in elucidating the global population structures in shorebirds (Wenick *et al,* 1993) and fringilline finches (Marshall and Baker, 1997), DNA polymorphism in two local populations of blue tit *Parus caeruleus* (Taberlet *et al*, 1992), phylogeography studies of Nearctic songbirds (Milai *et al*, 2000) in revealing recent mixing of maternal lineages in snow geese (Quinn, 1992) and inevaluating gene flow between social groups and populations in babblers (Edwards, 1993). Moreover, this region can be used in geographic analysis of many species such as song sparrow (Adam *et al*, 1998), blue chaffinch (Pestano *et al*, 2000).

Chapter 3

Material and Method

3.1 Materials

3.1.1 Equipments

- Disposable syringe tuberculin® 1.0 ml with needle gauge number 25
- Whatman® filter paper (number 1)
- Whatman Laboratory sealing film
- Automatic Micropipette P10 ,P20 ,P200 and P1000 (Gilson Medical Electronic)
- Microcentrifuge tube 0.2 ,0.5 and 1.0 ml. (Treff® switzerland)
- Micropipette tip P10,P20,P200 and P1000 (Treff® switzerland)
- Ice-box (Scientific plastic Co.,Ltd.,)
- Electronic clock timer Model CT-30 (Canon Co.,Ltd.,)
- Surgical knife, scissors and forceps
- Disposable Gloves (Meditrate)
- Polaroid film 677 (Polaroid)
- Polaroid DS-34 camera (Polaroid)
- Dessicator
- Autoclave
- Waterbath (Uni-Bath model RU-2, Sakura Finetecnical Co.,Ltd.,)
- Centrifuge (Eppendorf model 5410)
- Minicentrifuge
- Incubator (TaiTec Microincubator M-36)
- Shaker (MS 1 minishaker)
- Larminar flow hood with UV Light (Model DFL 120)
- PCR Thermal cycler : omnigene (Hybrid)
- PCR Thermal cycler:Perkin-Elmer 2400 (PE Applied Biosystem)
- ABI 310 Genetic Analyzer (Perkin Elmer, Applied Biosystem)
- Mupid Electrophoresis (ADVANCE Co., Ltd.,)

- Ultra-lum UV transluminator
- -20oC Freezer (Sanyo Co., Ltd.,)
- pH meter sp-7 (SunTex Digital pH meter)
- Balance (Sartorius)
- ABI Prism 310 Capillaries, 47 cm. x 50 (m i.d. (for rapid sequencing with Pop-6) (PE Applied Biosystem)
- Smartspec 3000 spectrophotometer (Biorad laboratory)

3.1.2.Chemicals

- Chelex® 100 (BioRad laboratory)
- Absolute ethanol (Merck)
- Sodium acetate (Merck)
- 100 mM dATP ,dTTP, dCTP, dGTP (Promega)
- Tris-(Hydroxymethyl) aminomethane (Promega)
- Boric acid (Biorad Laboratory)
- EDTA (Biorad Laboratory)
- Loading Dye (Promega corporation)
- Agarose (Promega)
- λ DNA (Promega)
- Big-Dye Terminator Cycle Sequencing (PE Applied Biosystem)
- Phi x 174 HinfI Marker (Promega)
- D-Loop primer (BSU)
- Ethidium bromide (Etbr)
- Mineral oil (Sigma)
- Geneclean spin kit (Bio 101 , Inc)
- Qiagen purification kit (QIAGEN)
- Performance optimized polymer 6 with TSR for the ABI Prism 310
- Genetic Analyzer (PE Applied Biosystems)

3.1.3 Enzymes

- *Taq* DNA Polymerase (Promega)
- Proteinase K (Promega)

3.2 Methods

3.2.1 Sample collection

Green Peafowls specimens had been collected 14 individuals from Doi Phu Nang National Park and 8 individuals from Wieng Lor wildlife Sanctuary between 1997 -1999. Blood was collected by radial venipuncture from branchial vein, with a tuburaclin® syringe with needle gauge number 25. Blood of 0.1-0.2 ml was dropped on a piece of Whatman® filter paper, air dried and placed into labeled paper bag for each sample. A feather was collected by cutting at the end and placed into a labeled paper bag. Both blood stain and feathers were kept in descicators.

3.2.2 Total DNA Extraction

The most common method of DNA extraction can be divided into two methods. They can be used to extract DNA from bloodstain for DNA template in PCR amplification.

Chelex® extraction method

Chelex is a polyvalent chelating agent in resin form. it is used routinely to assay a small number of cells and amount of DNA. Heating over boiling point condition may help to disrupt cell membranes, which it may also help to assure completed denaturation of the DNA template and separate DNA from cell (Sanger-Sam et al, 1989). This method is easy, inexpensive, less time-consuming and reduce contamination chance. Protocol of this method is below.

Total DNA was extracted from bloodstain and feathers using Chelex extraction medium (Sanger-Sam *et al*,1989; Walsh *et al*; 1991). Bloodstain of 2×2 mm^2 was cut and immersed in 1.5 ml eppendorf tubes containing 1,000 μl of

sterile distilled water, Then mixed gently. It was incubated at room temperature for 15-30 minutes, then spined in microcentrifuge at 10,000 -15,000 x g for 2-3 minutes and carefully remove supernatant and discard. Take the filter paper out off the eppendorf tube, then add 5-10 % Chelex® 100 to final volume of 200 µl. The sample was incubated at 56 °C for 15-30 minutes and vortexed again at 14,000 for 5-10 s, incubated at 100°C for 8-10 minutes and centrifuged for 2-3 minutes at 14,000xg in microcentrifuge. DNA extraction was kept at –20°C and used in amplification.

The single feather tips were washed with 70% ethanol and sterile water. Then 5-10 mm of the end of the tips was sliced off with a sterile razor blade and transferred to a 1.5 microcentrifuge tube containing 300 µl sterile 10% Chelex® 100. Sample were vortexed and incubated at 56°C overnight. After that, the sample was vortexed again then, incubated at 100°C for 8 minutes and centrifuged at 14,000 rpm for 2-3 minutes in microcentrifuge. DNA extracts were kept at -20oC.

3.2.3 *In vitro* Amplification of D-loop using the Polymerase Chain Reaction (PCR)

- Selection of Polymerase Chain Reaction Primers

Two D-loop primers were designed from sequences reported in genebank (http://www.ncbi.nlm.nih.gov). Primer 3 program was used in primer design for this study (http://www.genome.wi.mit.edu). The designed primer sequences is in Table 1. Oligonucleotide primers were synthesized by Bioservice Unit (BSU) of National Science and Technology Development Agency (NSTDA), Thailand.

Table 2.1 The oligonucleotide primers used for PCR amplification of D-loop primer.

Oligonucleotide sequence	Length	Tm (°C)
5'GGGGG TATAC TATGC ATAAT CGTG'3	24	60.67
5'AAAGA ATGGG CCTGA AGCTA GT'3	22	60.61

- Polymerase Chain Reaction (PCR)

Fragment at 330 bp of the D-loop was amplified. The amplification reaction as performed in 50 μl reaction mixture containing 20 mM Tris-HCl (pH 8), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol, 2 mM $MgCl_2$, 200 μM each of dATP, dGTP, dTTP and dCTP, 0.4 μM of each different primers, 30-50 ng of DNA from chelex extraction, and 1 unit of *Taq* Polymerase (Promega). The amplification was performed in omnigene PCR Thermal cycler (Hybaid) for predenaturation at 94°C of 3 minutes and then 35 cycles of denaturation at 94°C for 30 seconds, annealing at 57°C for 1 minutes, extension at 72°C for 1 minutes and the final extension at 72°C for 10 minutes. The amplification product was electrophoretically analysed by agarose gel electrophoresis.

- PCR product analysis by 1.5 % agarose gel electrophoresis

The size of PCR products and the size markers (Phix174 Hinfl) are appeared after electrophoresis. One primer pair provide a single expected band of PCR product. The positive band was observed under ultraviolet light.

3.2.4 PCR product purification

The PCR products were purified by Bio 101 Geneclean spining kit. This kit eliminates the small size DNA such as primers, dNTP and others. Protocol for Geneclen spining kit was used as follow (Bio 101, 1998):

Add DNA solution to 400 of GC spin Glassmilk in Spin Filter.

a Shake to mix Geneclean spin glass milk and add 400 μl to spin filter.

b Add DNA Solution (300 μl maximum/filter). Incubate at room temperature for 5 minutes. Invent a tube every minute to prevent settling of glassmilk.

c Spin liquid out of spin filter into a catch tube.

Wash with GENECLEN SPIN WASH

a Add 50 μl of GENECLEN SPIN NEW WASH to the spin filter.

b Spin at 14,000 rpm for 30 seconds or until spin filter is emptied of wash .

c Optional: Repeat wash.

d Empty a catch tube and spin for 1 minutes to dry pellet.

Elute DNA with GENECLEN SPIN Elution Solution.

a Transfer spin filter to a catch tube .

b Add 10-25 μl geneclean spin elution solution to the filter and resuspend glassmilk by flicking the tube or by gently vortexing 1-2 seconds.

C Spin 30 seconds to transfer eluted DNA to a catch tube. A second elution can increase 10-15% yield.

d Discard the spin filter and cap the tube. DNA in solution is ready to use without further manipulation.

3.2.5 Measurement of DNA concentration

The concentration of PCR product was measured at 260 nm by UV absorbance with smartspec 3000 spectrophotometer. Double strand DNA at concentration of 50 μg/ml have an absorbance of 1.0 at 260/280 nanometer. For DNA sequencing, the DNA amount of 30 – 90 ng is required.

3.2.6 Direct Sequencing of D-loop region by automatic Sequencer Cycle Sequencing (Perkin Elmer ABI PRISM Big-Dye terminator protocol, 1998).

a) To prepare a reaction mixture

A reaction mixture was combined as follow :

- Terminator Ready Reaction Mix	4	μl
- Template	30-90	ng
- D-loop primer (5 pmol)	1	μl
- Dionized water	q.s	

For a total volume of 10 μl, this reaction mix was sequenced by a GeneAmp PCR System 2400. The cycle program consists of 25 cycles of denaturation step at 96°C for 10 seconds, primer annealing step at 50°C for 5 seconds , and extension step at 60°C for 4 minutes, then extension products were purified..

b) To precipitate Extension products by Ethanol/Sodium Acetate

The protocol was used as follow:

1. For each sequencing reaction, prepare a 1.5 ml microcentrifuge tube contain the following :
   - 1.0 μl of 3 M Sodium acetate (NaOAc), pH 4.6
   - 50 μl of 95 % Ethanol (EtOH)
2. Pipet the entire contents of each extension reaction into a tube of sodium acetate and Ethanol mixture. Mix throughly.
3. Vortex the tubes and leave at room temperature for 15 minutes to precipitate the extension products. Precipitation time under 15 minutes will result in the loss of very short extension products.
4. Spin the tubes in a microcentrifuge at 14,000 rpm for 20 minutes.
5. Carefully aspirate the supernatant with a pipette tip and discard. The supernatant must be removed completely, as unincorporated dye terminators are dissolved in them. The more residual supernatant left in the tube, the more unincorporated dye terminators will remain in the samples.
6. Rinse the pellet with 250 μl of 70 % Ethanol
7. Vortex briefly.
8. Spin for 5 minutes in a microcentrifuge at maximum speed. Again, carefully aspirate the supernatant and discard.
9. Dry the pellet in a vacuum centrifuge for 10 – 15 minutes , or lids open in a heat block or thermal cycler at 90° C for 1 minutes.

C) Electrophoresis on the ABI PRISM 310 was used as follow:

1. Resuspend each sample pellet in 12-25 μl of template suppression reagent. Vortex and spin the samples.
2. Heat the samples at 95oC for 2 minutes to denature , then chill on ice.
3. Vortex and spin the samples again. Place on ice until ready to loading the sample.

3.2.7 Data analysis

Sequence reading by an automate sequencer was illegible, so it is needed to be re - alphabetical by eye again. From sequence Navigator comparision, all sequences were aligned visually using the program clustal W (http://www.ebi.ac.uk/clustalw). The correct alignment of the sequences is fundamental to identify homologous characters.

Genetic distance

The most common way to evaluate the degree of sequence dissimilarity is to calculate pairwise genetic distance. The distance is the estimation of the number of nucleotide substitution per nucleotide site between two sequence. The two distance methods used in this study are the Jukes-Cautor distance and Kimura's two parameter distance (Kimura, 1980) by MEGA.

The genetic distance between sequence (d) is often given simply as the percentage difference, when d is small or corrected for multiple substitutions at a given site as follows (Kimura two parameter).

$$d = (1/2) \ln [1-2P-Q] + 1/4 \ln [1/(1-2Q)]$$

P and Q are the proportional difference between two sequences.

Nucleotide diversity within a population

The average number of nucleotide substitutions within a population was calculated by

$$\pi = n/(n-1)\sum_{ij} x_i x_j d_{ij}$$

where n is the number of sequences sampled and d_{ij} is the number of nucleotide substitution per site between the i^{th} and j^{th} genotype. The x_i and x_j values are the sample freqrencies of the i^{th} and j^{th} genotype within population.

Phylogenetic analysis

Aligned sequences were defined haplotype and analysed based on the parsimony method by using PAUP (Vers. 3.1.1 Swofford, 1991) and MEGA (Ver. 1.02 Kumar *et al*., 1993). Phylogenetic analyses were performed using maximum parsimony. The analysis was conducted using heuristic search option. The trees produced by were run through 100 replication bootstapping analysed for statistic proof.

Chapter 4

Results

4.1 DNA extraction

The total DNA from two populations of *Pavo muticus* specimens were extracted from bloodstains and feathers by 5% chelex extraction method. The quality of extracted DNA was visualized by 1% agarose gel. The result shows that the quality of DNA extraction from bloodstains was higher than the feather. Figure 4.1 shows quality of DNA extraction from bloodstains and feathers

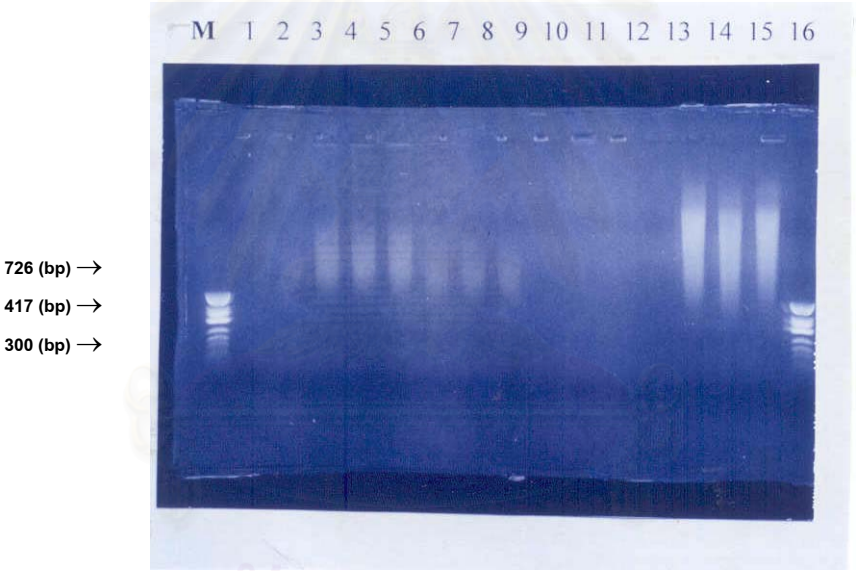

Figure 4.1 DNA extraction from the extraction of bloodstain and feather of *Pavo muticus* on 1% agarose that stained with Ethidium bromide.

lane M and 16 : Phix174 Hinf I standard marker

lane 1-2 and 9-12 : total DNA extraction of *P. muticus* from feather by 5% chelex extraction method

Lane 3-8 and 13-15 : total DNA extraction of *P. muticus* from bloodstain by 5 % chelex extraction method

4.2 PCR product of D-loop region

PCR product of D-loop region from 14 samples of *Pavo muticus* from Doi Phu Nang National Park and 9 samples of Wieng Lor Wildlife sanctuary were successfully amplification and seperated by 1.5% agarose gel electrophoresis shows in Figure 4.2

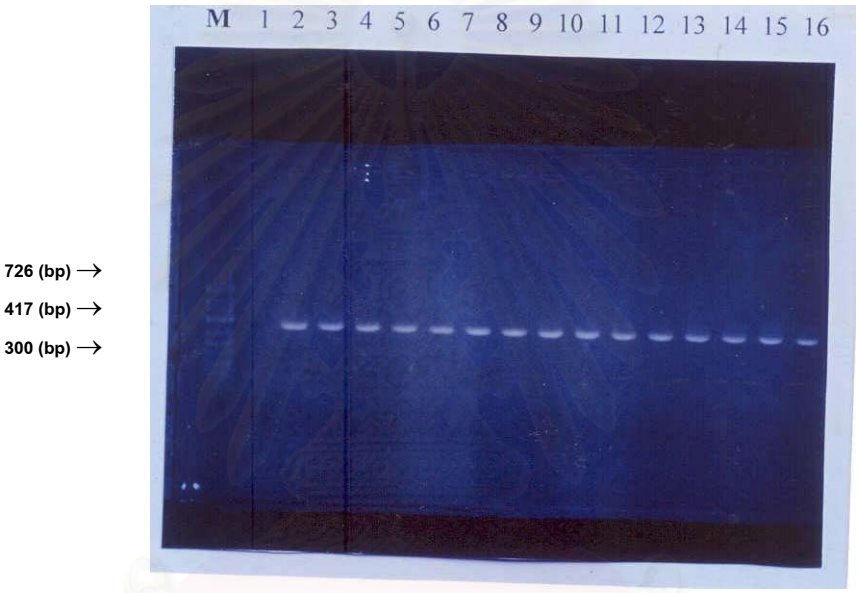

Figure 4.2 PCR product from D-loop region of *Pavo muticus* from Doi Phu Nang National Park seperated by 1.5% agarose gel electrophoresis.

Lane M : Phix174 Hinf I standard marker

Lane 1 : Negative control

Lane 2 : Positive control

Lane 3-10 : PCR product from D-loop region of *P. muticus* from bloodstains

Lane 11-16 : PCR product from D-loop region of *P.muticus* from feathers.

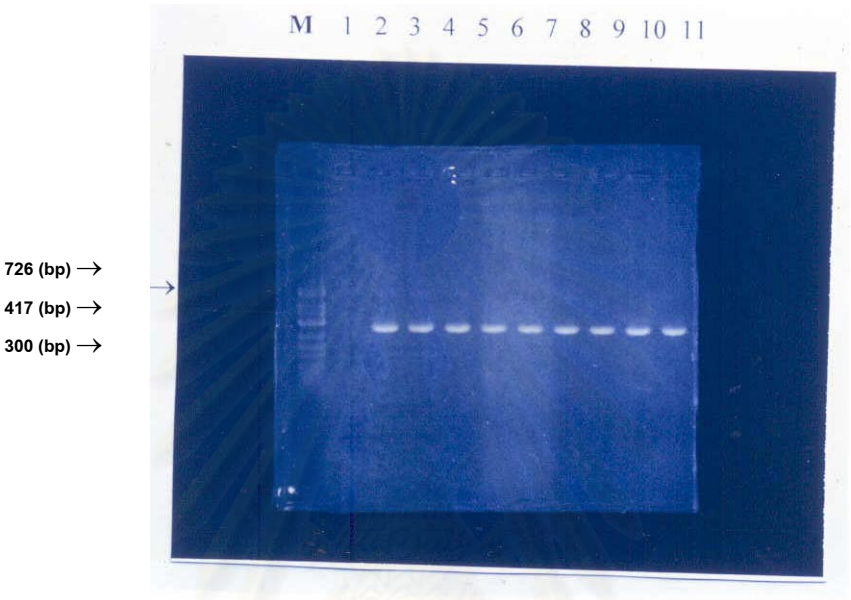

Figure 4.3 PCR product from D-loop region of *Pavo muticus* from Wieng Lor Wildlife sanctuary separated by 1.5% agarose gel electrophoresis.

Lane M : Phix 174 Hinf I standard marker

Lane 1 : Negative control

Lane 2 : Positive control

Lane 3-10 : PCR product from D-loop region of *P. muticus* from feathers.

4.3 Direct sequencing by an automated Sequencer

DNA concentration about 50 ng is the best quantity for a successful reaction mixture. The excess dye must be removed from the successful sequence completely.

4.4 Data analysis

Partial sequences of D-loop region were determined from all samples. At least 333 bp of D-loop were readable. The sequences were compared to *Gallus domesticus* sequences by Desjardins and Morais (1990) at L 55 to L 382 positions which is in the domain I of control region. The Alignment of *P. muticus* sequences with two sequences outgroups species, *Coturnix coturnix japonica*, and *Gallus gallus* are shown in Figure 4.4. There are eight haplotypes of *Pavo muticus* from Doi Phu Nang National Park population. The first haplotype (Pm1a) is peafowla11. The second haplotype is peafowla11. The third (Pm3a) is compose of peafowla10, a12, a8. The fourth (Pm4a) is peafowl is peafowl a9. The fifth (Pm5a) is peafowla13. Then, Pm69 ompose of peafowla2, a6 and a7. Pm7a is peafowl a4. Finally Pm8a is compose of peafowl a1, a3, a5. Meanwhile, there are three haplotypes in Wieng lor Wildlife Sanctuary population, the first haplotype (Pm3b) compose of pefowlb1, peafowl b2, peafowl b3, peafowlb5, peafowl b6 and peafowl b8. The second (Pm9b) is peafowl b4 then, Pmb haplotype is peafowl B7.

Twenty six variable sites from 14 individuals of *P. muticus* from Doi Phu Nang National Park population (Table 4.1). Sixteen nucleotide substitutions were transitions. Eight were transversions between intrapopulation. Fourteen sites were phylogenetically informatives. The genetic distance within this population is between 0.000-0.0513. The sequence divergence varies from 0 to 5.13 %.

Within eight individuals of *P. muticus* from Wieng Lor Wildlife sanctuary population (Table 4.1), the variable site were seven. All were transitions. The

genetic distance within this population is between 0.000 - 0.0219. The sequence divergence varies from 0 to 2.19%

The variable site between populations were 24 transitions and seven transversions. The genetic distance between two populations are between 0.0000 - 0.0547.

The nucleotide diversity within Doi Phu Nang National Park was 1.92 and nucleotide diversity within Wieng Lor Wildlife Sanctuary was 0.22

Genetic distances by Kimura's two parameter method are shown in Table 4.2. The maximum parsimony analysis yields the strict consensus tree shown in Figure 4.5. The tree was confirmed by more than 80% of the bootstrap replication performance. the tree has a consistency index (CI) of 0.947, homoplasy index (HI) of 0.053, excluding uninformative character, CI = 0.821 and HI =0.179. The retention index (RI) = 0.908.

CLUSTAL W (1.81) multiple sequence alignment

```
peafowla2     GGGGGTAAACTATGCATAATCGTGCATACATTTATATACCACATACATTATGGTCACAGT 60
peafowla6     GGGGGTAAACTATGCATAATCGTGCATACATTTATATACCACATACATTATGGTCACAGT 60
peafowla7     GGGGGTAAACTATGCATAATCGTGCATACATTTATATACCACATACATTATGGTCACAGT 60
peafowla4     CCCGGTAAACTATGCATAATCGTGCATACATTTATATACCACATACATTATGGTCACAGT 60
peafowla3     GGGGGTAAACTATGCATAATCGTGCATACATTTATATACCACATACATTATGGTCACAGT 60
peafowla1     GGGGGTAAACTATGCATAATCGTGCATACATTTATATACCACATACATTATGGTCACAGT 60
peafowla5     GGGGGTAAACTATGCATAATCGTGCATACATTTATATACCACATACATTATGGTCACAGT 60
peafowla10    GGGGGTAAACTATGCATAATCGTGCATACATT-ATAT-CCACATACATTATGGTCACAGT 58
peafowlb2     GGGGGTAAACTATGCATAATCGTGCATACATTTATATACCACATACATTATGGTCACAGT 60
peafowla11    GGGGGTAAACTATGCATCATCGTGCATACATT-ATATACCACATACATTATGGTCACAGT 59
peafowla14    GGGGGTAAAC---ATGATATCGTGCATACATT-ATATACCACATACATTATGGTCACAGT 56
peafowla12    GGGGGTAAACTATGCATAATCGTGCATACATTTATATACCACATACATTATGGTCACAGT 60
peafowlb3     GGGGGTAAACTATGCATAATCGTGCATACATTTATATACCACATACATTATGGTCACAGT 60
peafowlb1     GGGGGTAAACTATGCATAATCGTGCATACATTTATATACCACATACATTATGGTCACAGT 60
peafowla8     GGGGGTAA-C-ATGCA--ATCGTGCATACATTTATATACCACATACATTATGGTCACAGT 56
peafowlb8     GGGGGTAA-CTATGCAT-ATCGTGCATACATT-ATATACCACATACATTATGGTCACAGT 57
peafowlb5     GGGGGTAA-CTATGCATAATCGTGCATACATTTATATACCACATACATTATGGTCACAGT 59
peafowlb6     GGGGGTAA-CTATGCATAATCGTGCATACATT-ATAT-CCACATACATTATGGTCACAGT 57
peafowlb7     GGGGGTAA-CTATGCATAATCGTGCATACATTTATATACCACATATATTATGGTCACAGT 59
peafowla9     GGGGGTAA-CTATGCATAATCGTGCATACATTTATATACCACATACATTATGGTCACAGT 59
peafowla13    GGGGGTAAACTATGCATAATCGTGCATACATTTATATACCACATATATTATGGTCACAGT 60
peafowlb4     GGGGGTAAACTATGCATAATCGTGCATACATTTATATACCACATATATTATGGTCACAGT 60
coturnix      GGGGGTATACTATGCATAATCGTGCATACATTTATATTCCACATATACTATGGTACCGGT 60
chicken       GGGGGTATACTATGCATAATCGTGCATACATTTATATACCACATATATTATGGTACCGGT 60
                 ****  *        ************** **** ******* * ******  * **

peafowla2     AATACATACTATATACGTACTAAACCCATTATATGTAGACGGACATTACACTATCCTCCC 120
peafowla6     AATACATACTATATACGTACTAAACCCATTATATGTAGACGGACATTACACTATCCTCCC 120
peafowla7     AATACATACTATATACGTACTAAACCCATTATATGTAGACGGACATTACACTATCCTCCC 120
peafowla4     AATACATACTATATACGTACTAAACCCATTATATGTAGACGGACATTACACTATCCTCCC 120
peafowla3     AATACATACTATATACGTACTAAACCCATTATATGTAGACGGACATTACACTATCCTCCC 120
peafowla1     AATACATACTATATACGTACTAAACCCATTATATGTAGACGGACATTACACTATCCTCCC 120
peafowla5     AATACATACTATATACGTACTAAACCCATTATATGTAGACGGACATTACACTATCCTCCC 120
peafowla10    AATACATACTATATACGTACTAAACCCATTATATGTAGACGGACATTACACTATCTTCCC 118
peafowlb2     AATACATACTATATACGTACTAAACCCATTATATGTAGACGGACATTACACTATCTTCCC 120
peafowla11    AATACATACTATATACGTACTAAACCCATTATATGTAGACGGACATTACACTATCTTCCC 119
peafowla14    AATACATACTATATACGTACTAAACCCATTATATGTAGACGGACATTACACTATCTTCCC 116
peafowla12    AATACATACTATATACGTACTAAACCCATTATATGTAGACGGACATTACACTATCTTCCC 120
peafowlb3     AATACATACTATATACGTACTAAACCCATTATATGTAGACGGACATTACACTATCTTCCC 120
peafowlb1     AATACATACTATATACGTACTAAACCCATTATATGTAGACGGACATTACACTATCTTCCC 120
peafowla8     AATACATACTATATACGTACTAAACCCATTATATGTAGACGGACATTACACTATCTTCCC 116
peafowlb8     AATACATACTATATACGTACTAAACCCATTATATGTAGACGGACATTACACTATCTTCCC 117
peafowlb5     AATACATACTATATACGTACTAAACCCATTATATGTAGACGGACATTACACTATCTTCCC 119
peafowlb6     AATACATACTATATACGTACTAAACCCATTATATGTAGACGGACATTACACTATCTTCCC 117
peafowlb7     AATACATACTATATACGTACTAAACCCATTATATGTAGACGGACATTACACTATCTTCCC 119
peafowla9     AATACATACTATATACGTACTAAACCCATTATATGTAGACGGACATTACACTATTCTCCC 119
peafowla13    AATACATACTATATACGTACTAAACCCATTATATGTAGACGGACATTACACTATCCTCCC 120
peafowlb4     AATACATACTATATACGTACTAAACCCATTATATGTAGACGGACATTACACTATCCTCCC 120
coturnix      AATATATATTATATACGTACTAAACCCATTATATGTATACGGGCATTACA-TATTGTCCC 119
chicken       AATATATACTAT-TATGTACTAAACCCATTATATGTATACGGGCATTAACCTATATTCCA 119
              **** *** *** ** ********************** **** *****   ***  ***

peafowla2     CATTTATCCCCACGTTCAACCAATGCATGCTT-CCAGACATAAGACCTACACCTACCTAT 179
peafowla6     CATTTATCCCCACGTTCAACCAATGCATGCTT-CCAGACATAAGACCTACACCTACCTAT 179
peafowla7     CATTTATCCCCACGTTCAACCAATGCATGCTT-CCAGACATAAGACCTACACCTACCTAT 179
peafowla4     CATTTATCCCCACGTTCAACCAATGCATGCTTTCCAGACATAACACCTACACCTACCTAT 180
peafowla3     CATTTATCCCCACGTTCAACCAATGCATGCTT-CCAGACATAACACCTACACTTACCTAT 179
peafowla1     CATTTATCCCCACGTTCAACCAATGCATGCTTTCCAGACATAACACCTACACTTACCTAT 180
peafowla5     CATTTATCCCCACGTTCAACCAATGCATGCTT-CCAGACATAACACCTACACTTACCTAT 179
peafowla10    CATTTATCCCCACGTTCAACCAATGCATACTC-CTAGACATAACACCTATACTTACCCAT 177
peafowlb2     CATTTATCCCCACGTTCAACCAATGCATACTC-CTAGACATAACACCTATACTTACCCAT 179
peafowla11    CATTTATCCCCACGTTCAACCAATGCATACTC-CTAGACATAACACCTATACTTACCCAT 178
peafowla14    CATTTATCCCCACGTTCAACCAATGCATACTC-CTAGACATAACACCTATACTTACCCAT 175
peafowla12    CATTTATCCCCACGTTCAACCAATGCATACTC-CTAGACATAACACCTATACTTACCCAT 179
peafowlb3     CATTTATCCCCACGTTCAACCAATGCATACTC-CTAGACATAACACCTATACTTACCCAT 179
peafowlb1     CATTTATCCCCACGTTCAACCAATGCATACTC-CTAGACATAACACCTATACTTACCCAT 179
peafowla8     CATTTATCCCCACGTTCAACCAATGCATACTC-CTAGACATAACACCTATACTTACCCAT 175
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peafowlb8     CATTTATCCCCACGTTCAACCAATGCATACTC-CTAGACATAACACCTATACTTACCCAT 176
peafowlb5     CATTTATCCCCACGTTCAACCAATGCATACTC-CTAGACATAACACCTATACTTACCCAT 178
peafowlb6     CATTTATCCCCACGTTCAACCAATGCATACTC-CTAGACATAACACCTATACTTACCCAT 176
peafowlb7     CATTTATCCCCACGTTCAACCAATGCATACTC-CTAGACATAACACCTATACTTACCCAT 178
peafowla9     CATTTATCCCCACGTTCAACCAATGCATACTC-CTAGACATAACACCTATACTTACCCAT 178
peafowla13    CATTTATCCCCACGTTCAACCAATGCATACTC-CTAGACATAACACCTATACTTACCCAT 179
peafowlb4     CATTTATCCCCACGTTCAACCAATGCATACTC-CTAGACATAACACCTATACTTACCCAT 179
coturnix      CATTTCTCCCCATGTACA-TTAGTGCATGCTC-CAAGACATAAACCATACGTTCACCTAG 177
chicken       CATTTCTCCCAATGTCCATTCTATGCATGATC-TAGGACATA-CTCATTTACCCTCCCCA 177
              ***** **** * ** **     *****  *     ******   * *       **


peafowla2     TCCCTGCTCACAGA--ACCCACAAGTCACC-TAACTATGAATGGTCACAGGACATAACGT 236
peafowla6     TCCCTGCTCACAGA--ACCCACAAGTCACC-TAACTATGAATGGTCACAGGACATAACGT 236
peafowla7     TCCCTGCTCACAGA--ACCCACAAGTCACC-TAACTATGAATGGTCACAGGACATAACGT 236
peafowla4     TCCCTGCTCACA-A--ACCCACAAGTCACC-TAACTATGAATGGTCACAGGACATAACGT 236
peafowla3     TCCCTGCTCACA-A--ACCCACAAGTCACC-TAACTATGAATGGTCACAGGACATAACGT 235
peafowla1     TCCCTGCTCACA-A--ACCCACAAGTCACC-TAACTATGAATGGTCACAGGACATAACGT 236
peafowla5     TCCCTGCTCACACA--ACCCACAAGTCACC-TAACTATGAATGGTCACAGGACATAACGT 236
peafowla10    TCCCTGCTCCCACA--ACCCACAAGCCACC-TAACTATGAATGGTCACAGGACATAACGT 234
peafowlb2     TCCCTGCTCCCACA--ACCCACAAGCCACC-TAACTATGAATGGTCACAGGACATAACGT 236
peafowla11    TCCCTGCTCCCACA--ACCCACAAGCCACC-TAACTATGAATGGTCACAGGACATAACGT 235
peafowla14    TCCCTGCTCCCACA--ACCCACAAGCCACC-TAACTATGAATGGTCACAGGACATAACGT 232
peafowla12    TCCCTGCTCCCACA--ACCCACAAGCCACC-TAACTATGAATGGTCACAGGACATAACGT 236
peafowlb3     TCCCTGCTCCCACA--ACCCACAAGCCACC-TAACTATGAATGGTCACAGGACATAACGT 236
peafowlb1     TCCCTGCTCCCACA--ACCCACAAGCCACC-TAACTATGAATGGTCACAGGACATAACGT 236
peafowla8     TCCCTGCTCCCACA--ACCCACAAGCCACC-TAACTATGAATGGTCACAGGACATAACGT 232
peafowlb8     TCCCTGCTCCCACA--ACCCACAAGCCACC-TAACTATGAATGGTCACAGGACATAACGT 233
peafowlb5     TCCCTGCTCCCACA--ACCCACAAGCCACC-TAACTATGAATGGTCACAGGACATAACGT 235
peafowlb6     TCCCTGCTCCCACA--ACCCACAAGCCACC-TAACTATGAATGGTCACAGGACATAACGT 233
peafowlb7     TCCCTGCTCCCACA--ACCCACAAGCCACC-TAACTATGAATGGTCACAGGACATAACGT 235
peafowla9     TCCCTGCTCCCACA--ACCCACAAGCCACC-TAACTATGAATGGTCACAGGACATAACGT 235
peafowla13    TCCCTGCTTCCACA--ACCCACAAGCCACC-TAACTATGAATGGTCACAGGACATAACGT 236
peafowlb4     TCCCTGCTTCCACA--ACCCACAAGCCACC-TAACTATGAATGGTCACAGGACATAACGT 236
coturnix      TAATAGACTTTCCA---CTAACAGGACACCATAACTATGAATGGTTGCAGGACATAA-GC 233
chicken       TAGACAGTTCCAAACCACTATCAAGCCACC-TAACTATGAATGGTTACAGGACATAAATC 236
              *          *   *   ** * **** **************  **********


peafowla2     TCATATTACAGCTC-TTCCCCATTTGGTTATGCTCGACGTACCAGATGGATTTATTGATC 295
peafowla6     TCATATTACAGCTC-TTCCCCATTTGGTTATGCTCGACGTACCAGATGGATTTATTGATC 295
peafowla7     TCATATTACAGCTC-TTCCCCATTTGGTTATGCTCGACGTACCAGATGGATTTATTGATC 295
peafowla4     TCATATTACAGCTC-TTCCCCATTTGGTTATGCTCGACGTACCAGATGGATTTATTGATC 295
peafowla3     TCATATTACAGCTC-TTCCCCATTTGGTTATGCTCGACGTACCAGATGGATTTATTGATC 294
peafowla1     TCATATTACAGCTC-TTCCCCATTTGGTTATGCTCGACGTACCAGATGGATTTATTGATC 295
peafowla5     TCATATTACAGCTC-TTCCCCATTTGGTTATGCTCGACGTACCAGATGGATTTATTGATC 295
peafowla10    TCATATTACAGCTC-TCCCCCATTTGGTTATGCTCGACGTATCAGATGGATTTATTGATC 293
peafowlb2     TCATATTACAGCTC-TCCCCCATTTGGTTATGCTCGACGTATCAGATGGATTTATTGATC 295
peafowla11    TCATATTACAGCTC-TCCCCCATTTGGTTATGCTCGACGTATCAGATGGATTTATTGATC 294
peafowla14    TCATATTACAGCTC-TCCCCCATTTGGTTATGCTCGACGTATCAGATGGATTTATTGATC 291
peafowla12    TCATATTACAGCTC-TCCCCCATTTGGTTATGCTCGACGTATCAGATGGATTTATTGATC 295
peafowlb3     TCATATTACAGCTC-TCCCCCATTTGGTTATGCTCGACGTATCAGATGGATTTATTGATC 295
peafowlb1     TCATATTACAGCTC-TCCCCCATTTGGTTATGCTCGACGTATCAGATGGATTTATTGATC 295
peafowla8     TCATATTACAGCTC-TCCCCCATTTGGTTATGCTCGACGTATCAGATGGATTTATTGATC 291
peafowlb8     TCATATTACAGCTC-TCCCCCATTTGGTTATGCTCGACGTATCAGATGGATTTATTGATC 292
peafowlb5     TCATATTACAGCTC-TCCCCCATTTGGTTATGCTCGACGTATCAGATGGATTTATTGATC 294
peafowlb6     TCATATTACAGCTC-TCCCCCATTTGGTTATGCTCGACGTATCAGATGGATTTATTGATC 292
peafowlb7     TCATATTACAGCTC-TCCCCCATTTGGTTATGCTCGACGTATCAGATGGATTTATTGATC 294
peafowla9     TCATATTACAGCTC-TCCCCCATTTGGTTATGCTCGACGTATCAGATGGATTTATTGATC 294
peafowla13    TCATATTACAGCTC-TTCCCCATTTGGTTATGCTCGACGTATCAGATGGATTTATTGATC 295
peafowlb4     TCATATTACAGCTC-TTCCCCATTTGGTTATGCTCGACGTATCAGATGGATTTATTGATC 295
coturnix      TTACTAAATACTTAGCTCCCCATTTGGTTATGCTAGACGTACCAGATGGATTTATTGATC 293
chicken       TCACTCTCATGTTCTCCCCCCAACAAGTCAC-CTAACTAT---GAATGG--TTACAGGAC 290
              * *         *    ****     ** *  **     *     ****  ***  *  *


peafowla2     GTACACCTCACGAGAGA-TCAGCAACCCCTGCCCGT 330
peafowla6     GTACACCTCACGAGAGA-TCAGCAACCCCTGCCCGT 330
peafowla7     GTACACCTCACGAGAGA-TCAGCAACCCCTGCCCGT 330
peafowla4     GTACACCTCACGAGAGA-TCAGCAACCCCTGCCTGT 330
peafowla3     GTACACCTCACGAGAGA-TCAGCAACCCCTGCCTGT 329
peafowla1     GTACACCTCACGAGAGA-TCAGCAACCCCTGCCTGT 330
peafowla5     GTACACCTCACGAGAGA-TCAGCAACCCCTGCCTGT 330
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peafowla10    GTACACCTCACGAGAGA-TCAGCAACCCCTGCCTGT 328
peafowlb2     GTACACCTCACGAGAGA-TCAGCAACCCCTGCCTGT 330
peafowla11    GTACACCTCACGAGAGA-TCAGCAACCCCTGCCTGT 329
peafowla14    GTACACCTCACGAGAGA-TCAGCAACCCCTGCCTGT 326
peafowla12    GTACACCTCACGAGAGA-TCAGCAACCCCTGCCTGT 335
peafowlb3     GTACACCTCACGAGAGA-TCAGCAACCCCTGCCTGT 330
peafowlb1     GTACACCTCACGAGAGA-TCAGCAACCCCTGCCTGT 330
peafowla8     GTACACCTCACGAGAGA-TCAGCAACCCCTGCCTGT 326
peafowlb8     GTACACCTCACGAGAGA-TCAGCAACCCCTGCCTGT 350
peafowlb5     GTACACCTCACGAGAGA-TCAGCAACCCCTGCCTGT 329
peafowlb6     GTACACCTCACGAGAGA-TCAGCAACCCCTGCCTGT 330
peafowlb7     GTACACCTCACGAGAGA-TCAGCAACCCCTGCCTGT 329
peafowla9     GTACACCTCACGAGAGA-TCAGCAACCCCTGCCTGT 329
peafowla13    GTACACCTCACGAGAGA-TCAGCAACCCCTGCCTGT 330
peafowlb4     GTACACCTCACGAGAGA-TCAGCAACCCCTGCCTGT 330
coturnix      GTACACCTCACGAGAGAATCACCAACCCCTGTCTGT 330
chicken       ATACATTTAACTACCATGTT-CTAACCCAT-TTGGT 328
               ****  * ** *     *      *** *     *
```

Figure 4.4 Multiple sequence of 330 bp partial D-loop region from two populations of *Pavo muticus* at Doi Phu Nang National Park and Wieng Lor Wildlife Sanctuary population and *Cotunix cotunix* and *Gallus gallus* as an out groups. Stars indicated identityfications of the sequence.

Table4.1 Haplotype of D-loop region (330 bp) of *P. muticus* from Doi Phu Nang National Park and Wieng Lor Wildlife Sanctuary which are shown only variable sites.

	Nucleotide position																										
										1	1	1	1	1	1	1	1	1	1	1	2	2	2	3
				1	1	1	1	1	4	1	1	4	5	6	6	6	7	7	8	8	9	0	5	7	2
Haplotype	1	2	3	4	5	6	7	8	6	5	6	9	2	4	3	9	2	7	8	9	2	3	2	7	8
Pm1a	G	G	G	G	C	A	T	C	C	C	T	A	C	T	C	T	T	C	C	C	C	C	T	T	
Pm2a	.	.	.	A	T	G	A	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Pm3a	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Pm4a	.	.	.	.	.	.	.	A	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	.	
Pm5a	.	.	.	.	.	.	.	A	T	.	C	.	.	.	.	.	.	T	.	.	.	.	.	.	
Pm6a	.	.	.	.	.	.	.	A	.	.	C	G	T	C	G	C	C	T	.	A	G	T	T	C	C
Pm7a	C	C	C	.	.	.	.	A	.	.	C	G	T	C	.	C	C	T	.	A	-	T	T	C	.
Pm8a	.	.	.	.	.	.	.	A	.	.	C	G	T	C	.	C	.	T	.	A	.	T	T	C	.
Pm3b	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Pm9b	.	.	.	.	.	.	.	A	T	.	C	.	.	.	.	.	.	T	T	.	.	.	.	.	
Pm10b	.	.	.	.	.	.	.	A	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	

Note:

Dots indicated identical nucleotides with Pm1a

Pm1a-Pm8a = *Pavo muticus* haplotype from Doi Phu Nang National Park

Pm9b-Pm10b = *Pavo muticus* haplotype from Wieng Lor Wildlife Sanctuary.

Pm1a = peafowla11, Pm2a = peafowla14, Pm3a = peafowla8,a10,a12

Pm4a = peafowla9, Pm5a = peafowla13, Pm6a = peafowla2, a6, a7

Pm7a = peafowla4, Pm8a = peafowla1, a3, a5

Pm3b = peafowlb1, b2, b3, b5, b6, b8

Pm9b = peafowlb4, Pm10b = peafowlb7

Table 4.2 Genetic distance estimated by Kimura two parameter method among 22 samples of *Pavo muticus* imperator and *Cotunix cotunix japonica* is an out group which were obtained from 333 base pair of D-loop sequence

	A2	A6	A7	A4	A1	A3	A5	A11	A14	B3	A12	B2	B1	B6	B8	A10	A8	B5	B7	A9	A13	B4	CC
A2	-	0.0000	0.0000	0.0156	0.0156	0.0125	0.0283	0.0481	0.0481	0.0448	0.0448	0.0481	0.0448	0.0448	0.0448	0.0448	0.0448	0.0448	0.0547	0.0448	0.0415	0.0447	0.2147
A6		-	0.0000	0.0156	0.0156	0.0125	0.0283	0.0481	0.0481	0.0448	0.0448	0.0481	0.0448	0.0448	0.0448	0.0448	0.0448	0.0448	0.0547	0.0448	0.0415	0.0447	0.2147
A7			-	0.0156	0.0156	0.0125	0.0283	0.0481	0.0481	0.0448	0.0448	0.0481	0.0448	0.0448	0.0448	0.0448	0.0448	0.0448	0.0547	0.0448	0.0415	0.0447	0.2147
A4				-	0.0188	0.0156	0.0316	0.0513	0.0513	0.0480	0.0480	0.0513	0.0480	0.0480	0.0480	0.0480	0.0480	0.0480	0.0579	0.0480	0.0447	0.0479	0.2223
A1					-	0.0031	0.0188	0.0382	0.0382	0.0414	0.0414	0.0447	0.0414	0.0414	0.0414	0.0414	0.0414	0.0414	0.0513	0.0414	0.0381	0.0413	0.2139
A3						-	0.0156	0.0350	0.0350	0.0382	0.0382	0.0414	0.0382	0.0382	0.0382	0.0382	0.0382	0.0382	0.0480	0.0382	0.0349	0.0381	0.2099
A5							-	0.0413	0.0412	0.0446	0.0446	0.0479	0.0446	0.0446	0.0446	0.0446	0.0446	0.0446	0.0545	0.0446	0.0413	0.0445	0.2220
A11								-	0.0000	0.0031	0.0031	0.0062	0.0031	0.0031	0.0031	0.0031	0.0031	0.0031	0.0124	0.0093	0.0188	0.0220	0.2235
A14									-	0.0031	0.0031	0.0062	0.0031	0.0031	0.0031	0.0031	0.0031	0.0031	0.0093	0.0093	0.0188	0.0220	0.2235
B3										-	0.0000	0.0031	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0093	0.0062	0.0157	0.0188	0.2194
A12											-	0.0031	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0124	0.0062	0.0157	0.0188	0.2194
B2												-	0.0031	0.0031	0.0031	0.0031	0.0031	0.0031	0.0093	0.0093	0.0188	0.0220	0.2235
B1													-	0.0000	0.0000	0.0000	0.0000	0.0000	0.0093	0.0062	0.0157	0.0188	0.2194
B6														-	0.0000	0.0000	0.0000	0.0000	0.0093	0.0062	0.0157	0.0188	0.2194
B8															-	0.0000	0.0000	0.0000	0.0093	0.0062	0.0157	0.0188	0.2194
A10																-	0.0000	0.0000	0.0093	0.0062	0.0157	0.0188	0.2194
A8																	-	0.0000	0.0093	0.0062	0.0157	0.0188	0.2194
B5																		-	0.0093	0.0062	0.0157	0.0188	0.2194
B7																			-	0.0156	0.0188	0.0219	0.2231
A9																				-	0.0157	0.0188	0.2151
A13																					-	0.0031	0.2022
BA																						-	0.2062
CC																							-

Remark : A1 - A14 = *Pavo muticus* from Doi Phu nang National Park , B1 - B8 = *Pavo muticus* from Wieng Lor Wildlife Sunctuary.

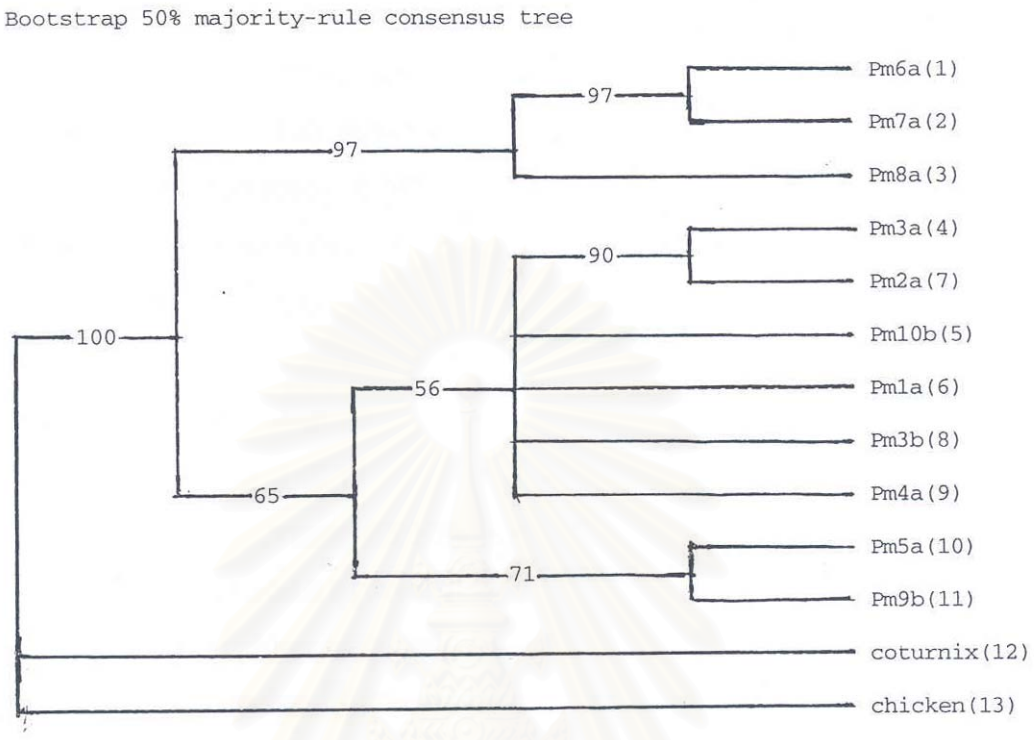

Figure 4.5 Strict concensus tree derived from 15 parsimony tree of 330 bp of *Pavo muticus* indicated the percentage of bootstrap replicates out of 100 that support each branch.

Note : out group = Cotunix and chicken

Pm1a - Pm8a = *Pavo muticus* haplotype 1-8 from Doi Phu Nang National Park

Pm3b = *Pavo muticus* haplotype 3 from Wieng Lor Wildlife Sanctuary

Pm9b - Pm10b = *Pavo muticus* haplotype 9-10 from Wieng Lor Wildlife Sanctuary

Chapter 5

Discussion

DNA from feathers and bloodstain of *Pavo muticus imperator* from Doi Phu Nang Nationnal Park and Wieng Lor Wildlife Sunctuary were successfully extracted by using Chelex@ 100 extraction medium (Sanger-Sam *et al*, 1989; Walsh *et al*, 1991). This extraction method is easy, cheap, and less time consuming. It involves fewer opportunities for DNA contamination and loss of DNA than traditional phenol/chloroform extraction (Sambrook *et al*, 1989). Furthermore, this method involves no organic solvent and does not alliquote in to several tubes (Walsh *et al*, 1991). The advantage of chelex extraction method is capable to extract small amount of degraded DNA in samples such as bloodstains, feathers, hairs or even museum skins or preserved specimen. Although they are in very small amount, DNA can be extracted by this method and sucessfully amplified (Cooper, 1994). Although phenol/chloroform extraction is successful in recovering high molecular weight DNA. Chelex @100 is still more efficient than proteinase K and phenol-chloroform extraction because this method includes the use of high salt concentration and excess proteinase K digestion (Walsh *et al*, 1991).

Furthermore, chelex extraction can be amplified even small quantities of short target DNA sequence of specific oligonucleotide primers. In general PCR can not amplify a long chain DNA but it also can amplify a difficult template DNA such as degraded DNA (Walsh *et al*, 1991).

One or two, or three tips of primary, or secondary, or train feathers are enough to harvest DNA for amplification. The result shows that concentration of the extracted DNA from feathers was lower than from bloodstains. Even, it could not be detected with 1% agarose gel but it can be detected by UV spectrophotometer at 260 nm. This method can also be used in many avian species such as Hazel grouse in Japan (Yoshiyuki *et al*, in press), Red Junglefowl subspecies of *Gallus gallus spadiceus* (Begthaisong, 1998), and American Woodcock, *Scolopax minor* (Alyson *et al*, inpress).

The primers in this study were designed from D-loop gene (Fumihito *et al*, 1995), and covered 365 bp of D-loop region in Domain I. In this study, Polymerase Chain Reaction (PCR) is successfully amplified by using 5% chelex extraction. The most importance thing for amplification succeeded is based on a concentration of DNA template, it should be between 30-50 ng. Supposing that the concentration is too high or too low, the amplification may not be succeeded. A result from this research disagreed with a previous study by Boripat in 1997 that chelex $^{@}$ 100 extraction can not be used to provide DNA amplified D-loop region in Red *Gallus gallus gallus* and jungle fowl *Gallus gallus spadiceus.*

Furthermore, this extraction method can provide DNA from red jungle fowl *Gallus gallus spadiceus* for microsatellite technique (Begthaisong,1998). Meckvichai (1997) succeeded in using chelex$^{@}$100 extracts for cytochrome b gene amplification.

A cycle sequencing reaction for automated sequencing is based on the dideoxynucleotide chain-termination method of Sanger *et al*, 1977. There are three important processes. First, The concentration of template is the critical composition. The concentration of template should be ranged from 40 to 50 nanogram which was the same as recommended in ABI PRISM BigDye terminator cycle sequencing Ready Reaction kit (30-90 ng). Second, the specific primer is needed for automated sequencer. Third, DNA is precipitated by Ethanol and Sodium Acetate. Excess dye has to be removed completely because it can interfere sequencing reaction which will result in unclear

From the DNA sequence analysis, it is shown that *Pavo muticus imperator* within Doi Phu Nang National Park population are higher divergent and more variable than *Pavo muticus imperator* within Wieng Lor Wildlife sanctuary population. From a progressive report of Wina and colleague, (2001) found that the number of *Pavo muticus imperator* in Wieng Lor Wildlife Sanctuary population is about three times less than in Doi Phu Nang population, therefore, it is possible that the population size should be involved in genetic variation within population. It is found that nucleotide sequences from the study of Fumihito and colleague, (1995) are

more similar to Wieng Lor Wildlife Sanctuary population than to Doi Phu Nang National Park population. There are only 2 difference site.

From a phylogenetic tree based on the nucleotide sequence using Parsimony method to find the optimal tree by heuristic method and 100 bootstrap frequency, two populations of *Pavo muticus imperator* are divided into two groups. The first group composites some Green peafowls at Doi Phu Nang National Park only (Pm6a, Pm7a, and Pm8a haplotype). The results coincides with the result from a bootstrap 50% majority consensus tree. It shows that this group is to be diverged from another group.

The second group is composed of populations from both Doi Phu Nang National Park and Wieng Lor Wildlife Sanctuary (Pm3a, Pm2a, Pm1a, Pm4a, Pm5a, haplotype and Pm3b, Pm9b, Pm10b haplotype). All of the samples in this group did not separated clearly. In addition, there are some Green Peafowls in this group begins to be diverged. It is possible that both areas are located closely and two populations can still be involved. Furthermore, these population were separated not for a long time by geographical barriers. There are moutains and roads separating two populations. These populations still share more character of ancestors, because in the former, these two populations may be in continuous areas. At present, both populations were separated by two roads. Furthermore, there are many villages along two side of the road so the two populations of Green Peafowls can not to be in the habit of visiting but the some section of roads are the forests. Therefore, Green Peafowls still related. The first one is a road from Amphor Chiang Muan to Amphor Dokkhumtai and the second are from Amphor Chiang Muan to Amphor Chun. Although both populations live in different river basin; Doi Phu nang population is in Yom basin and Wieng Lor population is in Eing basin. However, in the past both populations used to be in one population. Therefore, these populations still share some of the character. As in previous study about population such as *Fringilla teydea* (blue chaffinch) by using 767 basepair of control region analyse by maximum parsimony. The tree is clearly supported the separated of two population maternal lineage (Pestano *et al*, 2000). Furthermore, Ava and Felix et al

(1992) studied in *Poecilia reticulata* (The Trinidad guppy) about 465 base pairs of control region. Found that The maximum parsimony shown the four populations of the drainage comprised one dichotomy group, and the second group included all other populations.

Genetic distances of these two populations supports the pasimonious infer phylogenetic tree. The range of genetic distances within Doi Phu Nang population and Wieng Lor population are 0.0000 - 0.0513 and 0.0000 - 0.0219 respectively and between population is 0.0000 - 0.00547 but the genetic distance between population is closed to the genetic distance within population of Doi Phu Nang National Park. The nucleotide diversity within Doi Phu Nang National Park (π = 1.92) is higher than nucleotide diversity within Wieng Lor Wildlife Sanctuary (π = 0.22). These value within Doi Phu Nang National Park are higher than Wieng Lor Wildlife Sanctuary, it may be population size of Wieng Lor Wildlife Sanctuary less than Doi Phu Nang National Park.

From this study partial D-loop nucleotide sequence of *Pavo muticus imperator* (330 base pairs) can be classified genetic variation in population level at Doi Phu Nang and Wieng Lor population. Even D-loop is very variable. It may be two populations are closed areas and still contact together. It is different from *Fringilla teydea* (blue chaffinch) which are separated to each population by D-loop sequence. It is may be the life cycle span of finch is shorter or more than Green Peafowls and the areas of this species populations are long distance.

However, this study found that both populations begin separate into subpopulations. In the future, two populations may be completely separated. If people who live along these two roads expand the area. Green Peafowls from these two populations can not move and flow back and forward. Then, the conservation may by considered because metapopulation or habitat fragmentation may occur.

Chapter 6

Conclusion and Recommendations

Conclusion

Genetic variation of *Pavo muticus* between Doi Phu Nang National Park and Wieng Lor Wildlife Sanctuary populations by 330 bp D-loop nucleotide sequences. Doi Phu Nang National Park has 26 variable site, 14 informative site, 16 transition site and 8 transversion site. Genetic distance were 0.0000-0.0513.

Within Wieng Lor Wildlife Sanctuary population has 7 variable site and transition. Genetic distance were ranged 0.0000-0.0219.

Genetic diversity of *Pavo muticus* of Doi Phu Nang National Park and Wieng Lor Wildlife Sanctuary is 1.92 and 0.22 respectively.

From a phylogenetic tree based on the D-loop sequence using parsimony method, two populations of *Pavo muticus* are divided into two groups. The first group to be diverged. It is composed some Green peafowls at Doi Phu Nang National Park only. the second groups is composed of populations from both Doi Phu Nang National Park and Wieng Lor Wildlife Sanctuary but, the partial of two populations begin to be diverged.

Recommendations

From the result, the Green Peafowls from Doi Phu Nang National Park should be maintained because this population has high genetic variation.

In the future , researcher should study genetic variation of Green Peafowls that far away from Doi Phu Nang National Park and Wieng Lor Wildlife Sanctuary. So would to know about using D-loop in population study.

Researcher will increase the number of Green Peafowls specimens from both Doi Phu Nang National Park and Wieng Lor Wildlife Sanctuary so that this information has good better about genetic variation for wildlife management in the future.

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APPENDICES I

Reagent preparation protocol

1) 1% Agarose gel

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

- Agarose 1.0 gm
- 1xTBE buffer 100.0 ml.

How to applied the description previously in use is as followed:

1. Mix 1 gm of agarose powder into 30 ml of 1xTBE buffer.
2. Cook the agarose resuspension in a microwave for 2 minutes.
3. Prepare a gel mould to set a gel. When time is finished, add 0.2 μl of 1% Ethidium Bromide into the about 25-50 ml of dissolved gel. The gel is mixed.
4. Poure the soluble gel into the gel mould, which the comb is already inserted to the gel mould.
5. When the gel has completely been cooled and solidifed; removed the comb. Transfer gel into a gel chamber containing enough volume of 1XTBE buffer that will covers the gel about 1-2 mm above.

Remark : Preparing 1.5 % Agarose is like 1 % Agarose but 1.5 gram of agarose is used to dissolved in 100 ml 1X TBE buffer.

2) 10X TBE buffer (Tris Boric EDTA buffer)

An enough amount of ingredients for a 1,000 ml is composed of

- Tris aminomethane 108.00 gm
- Boric acid 50.40 gm
- EDTA 7.44 gm

The buffer is prepared as followed :

1. Tranfer the exact amount of Tris, Boric acid and EDTA into a 1,000 Volumetric flask
2. Add double distilled water up to 1,000 ml
3. Stir the solution until completely dissolved.

4. Store the solution at room temperature and dilute into 1X TBE for Agarose gel preparation.

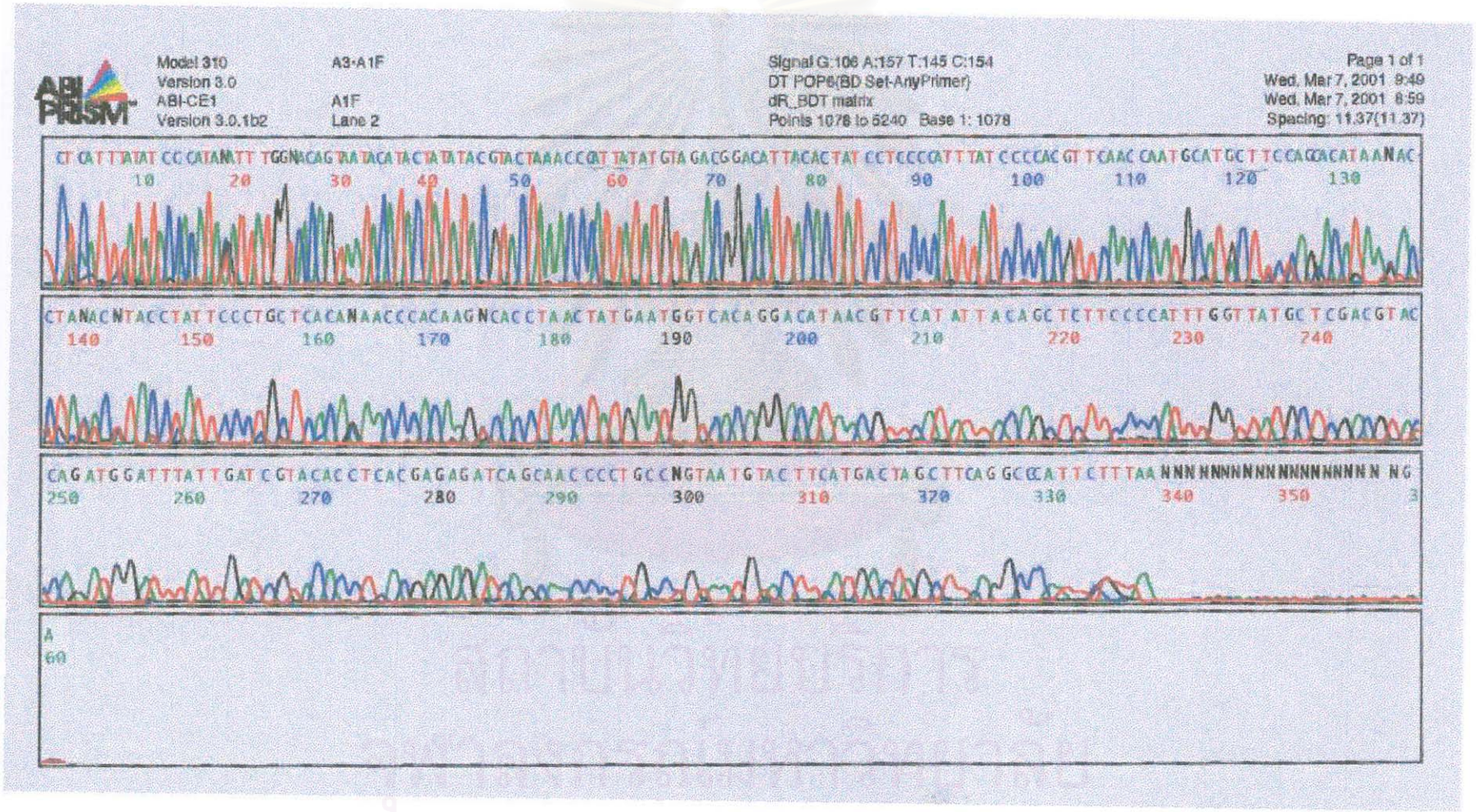
2) 3M NaOAc pH 4.6 (20 ml) M.W = 136

- Dissolve 8.16 g Sodium Acetate in double distilled water
- Add any acetic acid
- Adjust pH with glacial acetic acid to be 4.6
- Add H2O to 20 ml

APPENDICES II

Electropherogram and D-loop sequences

From ABI 310 Genetic Analyzer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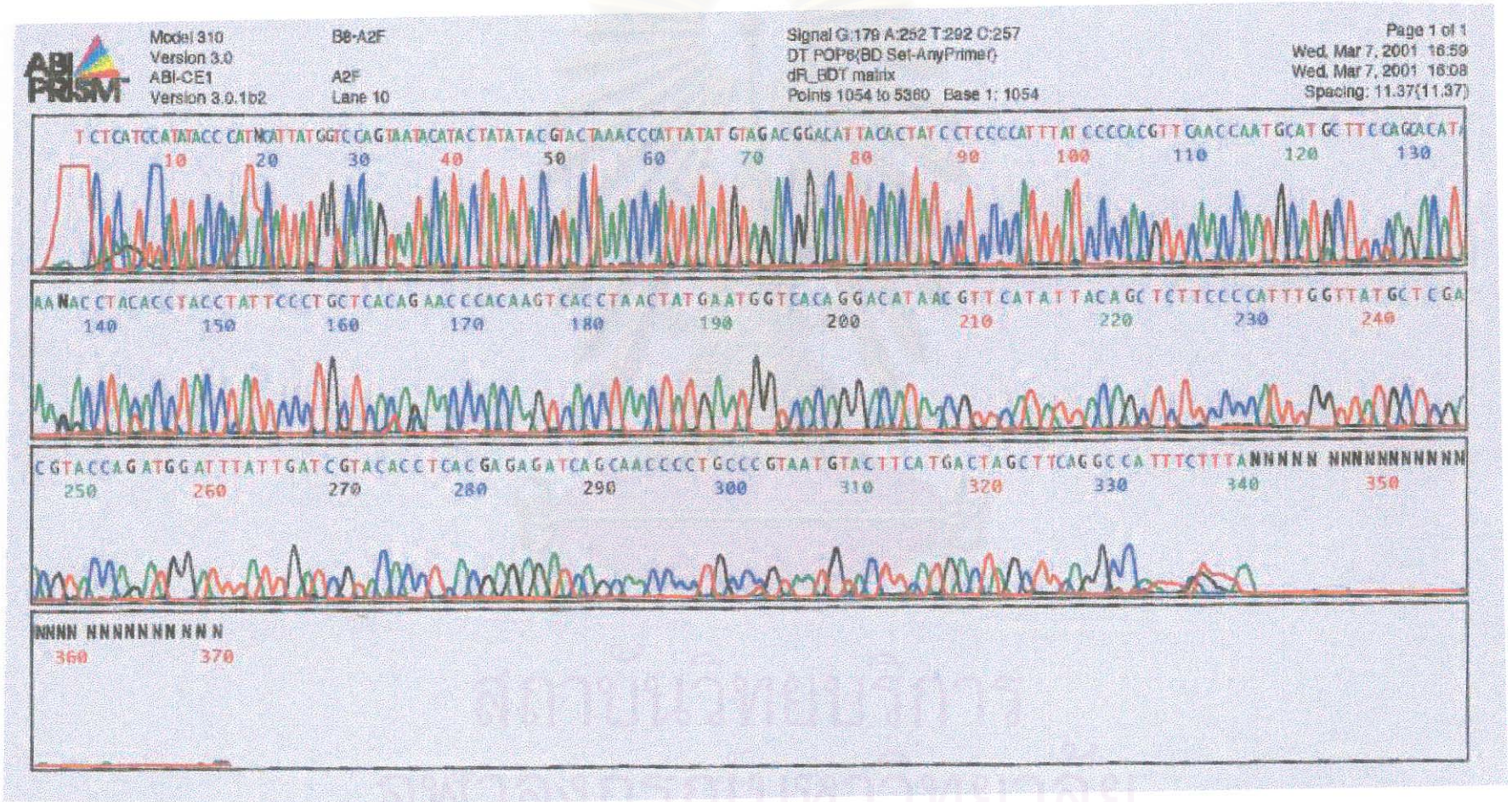
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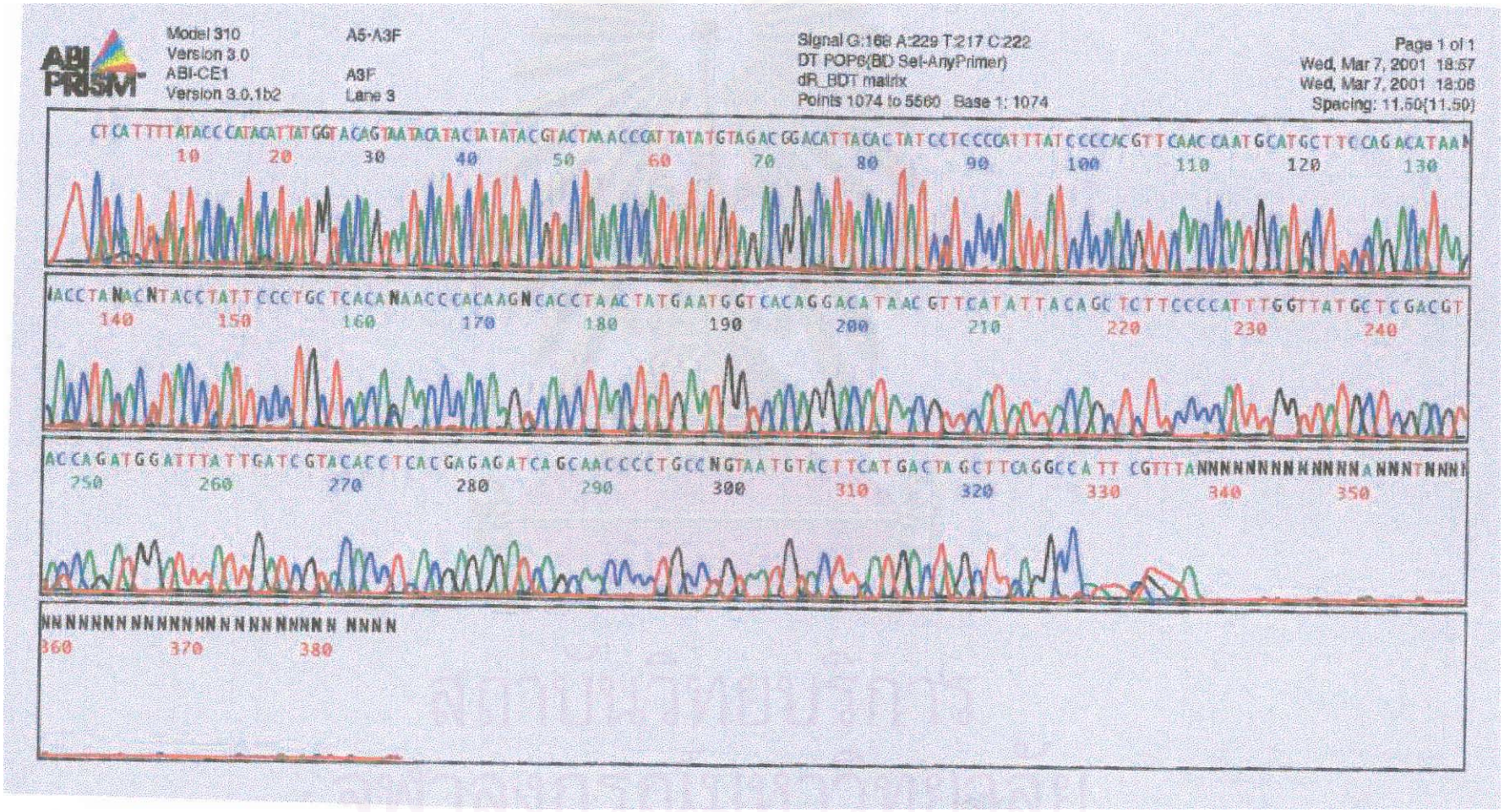
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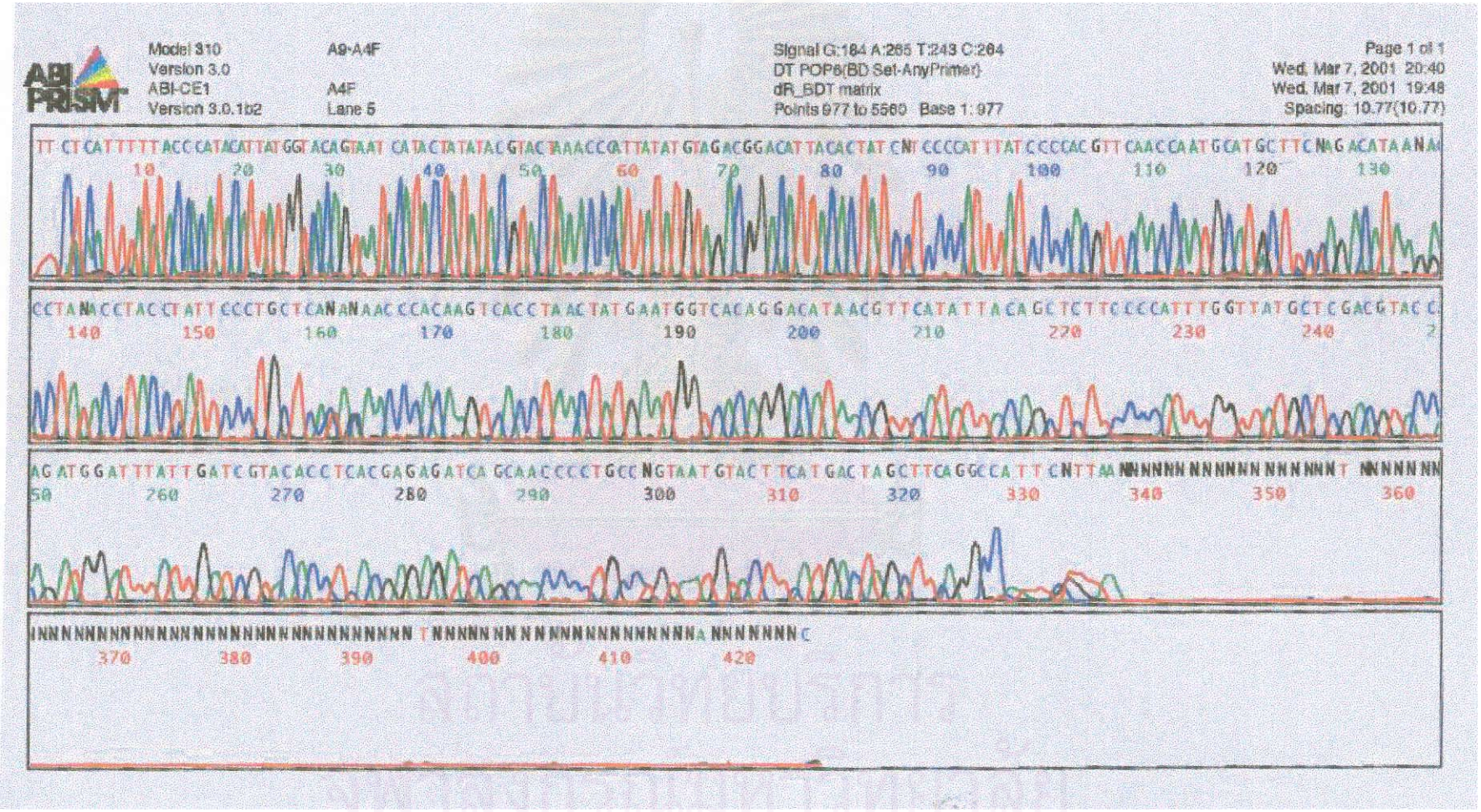
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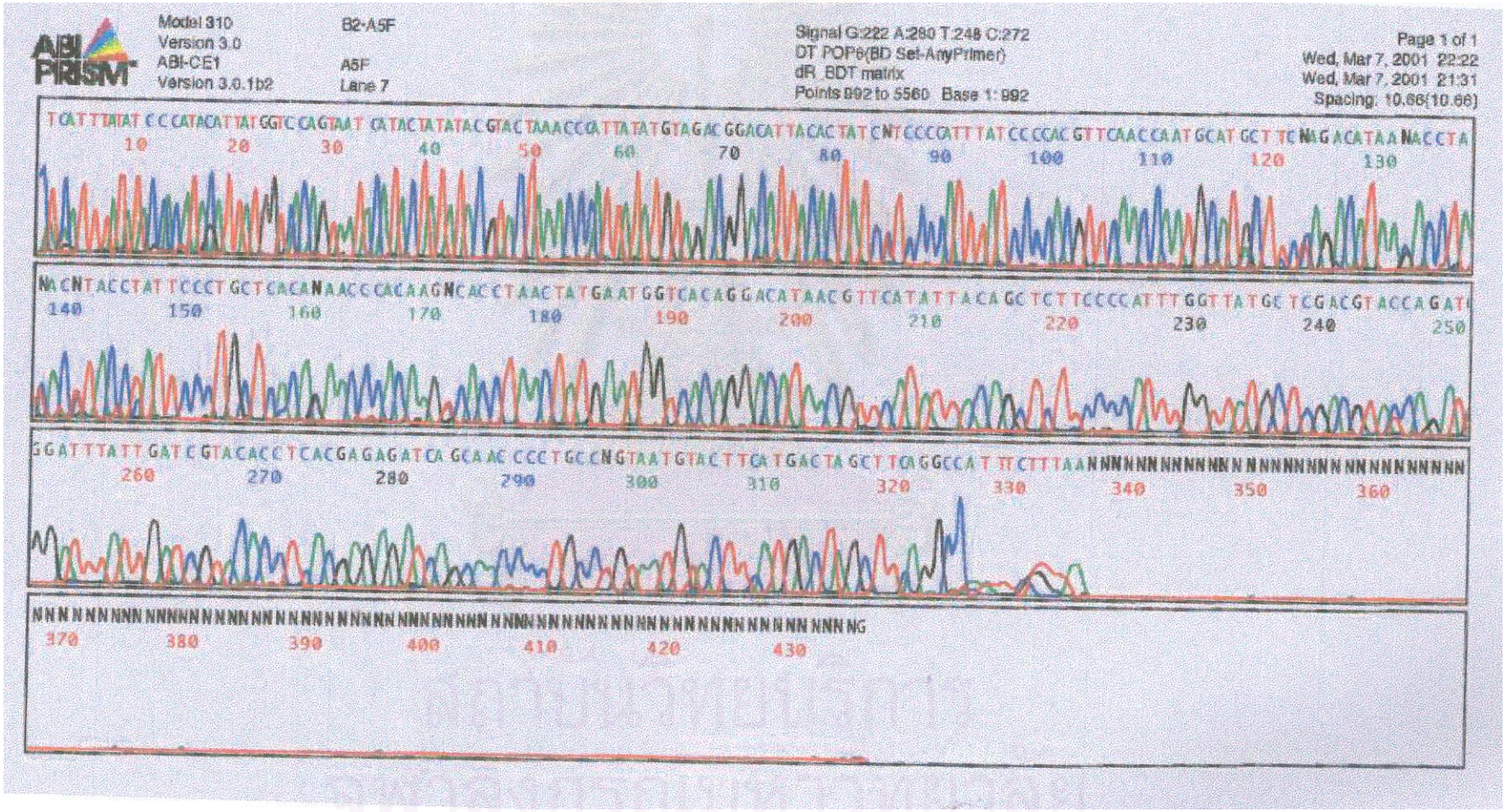
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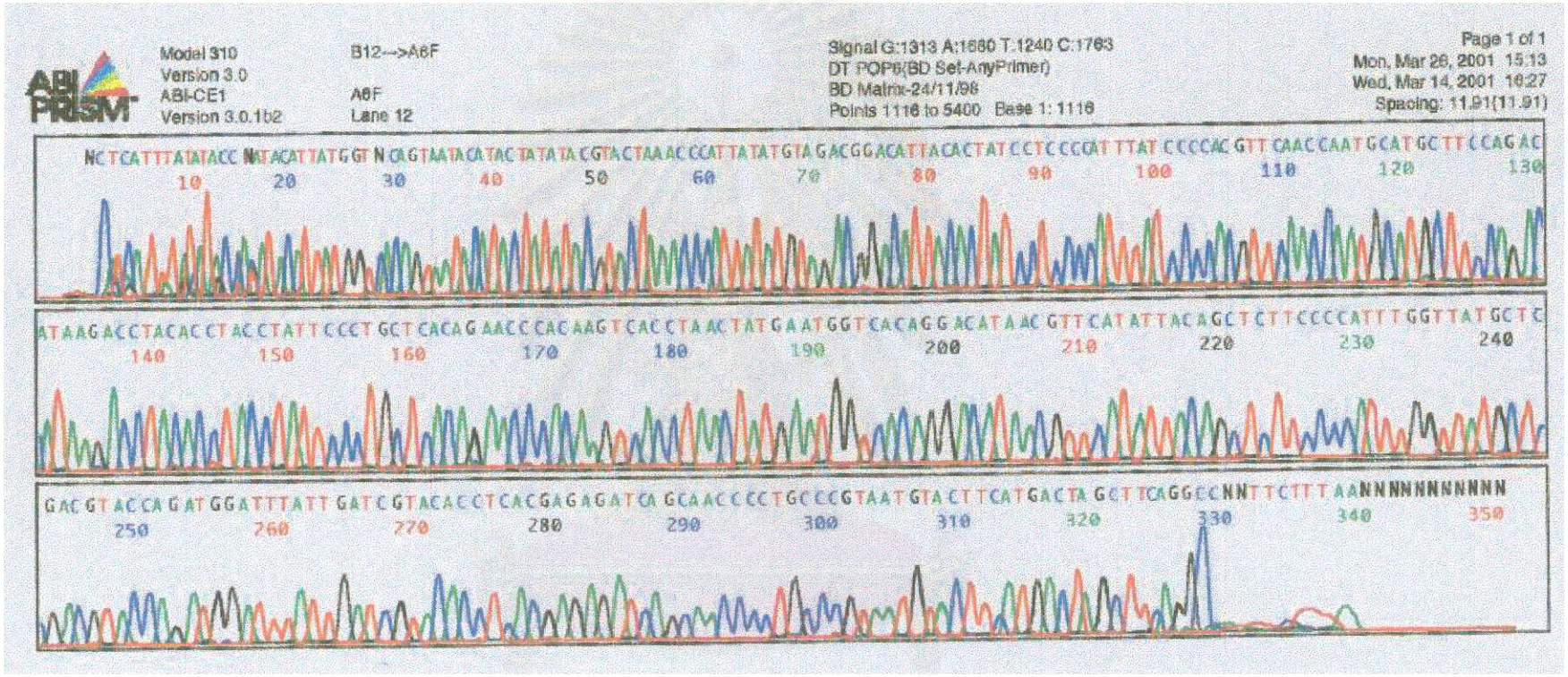

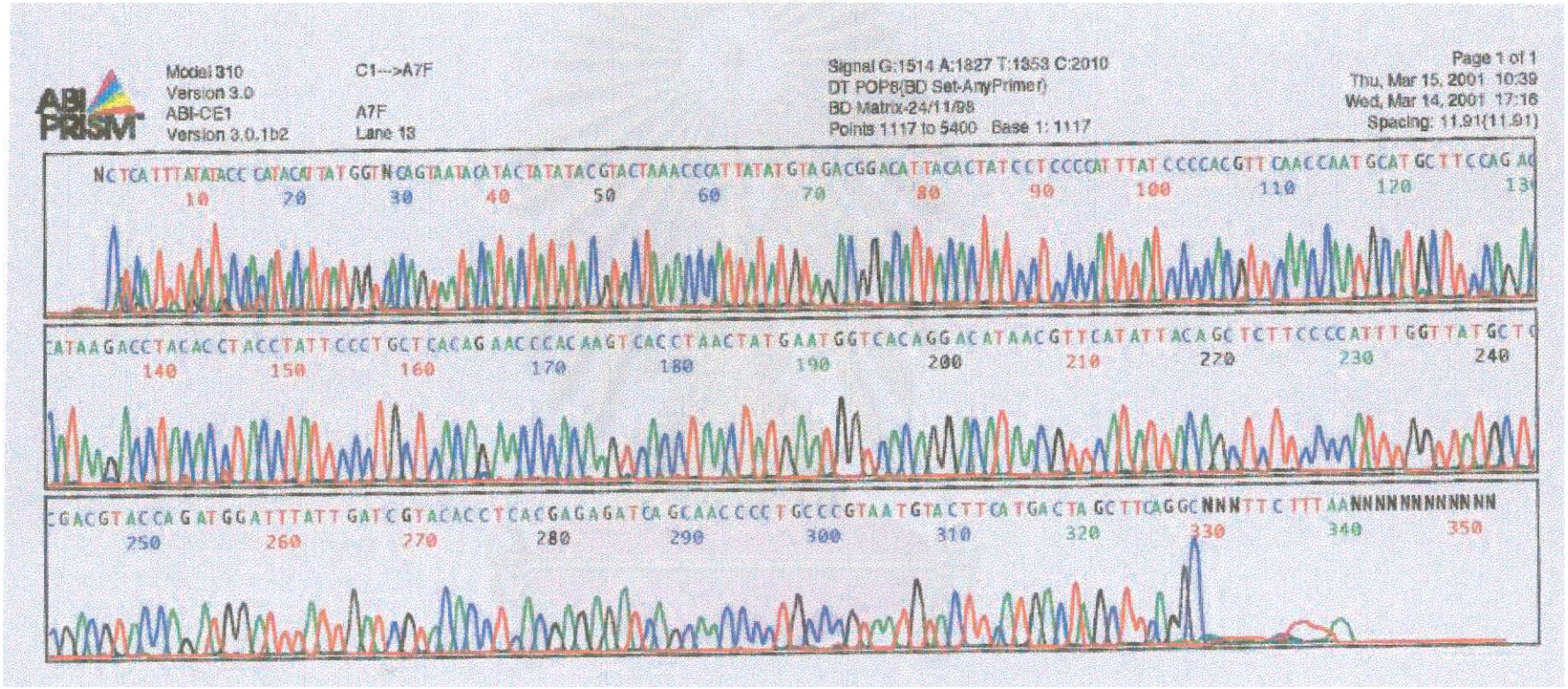

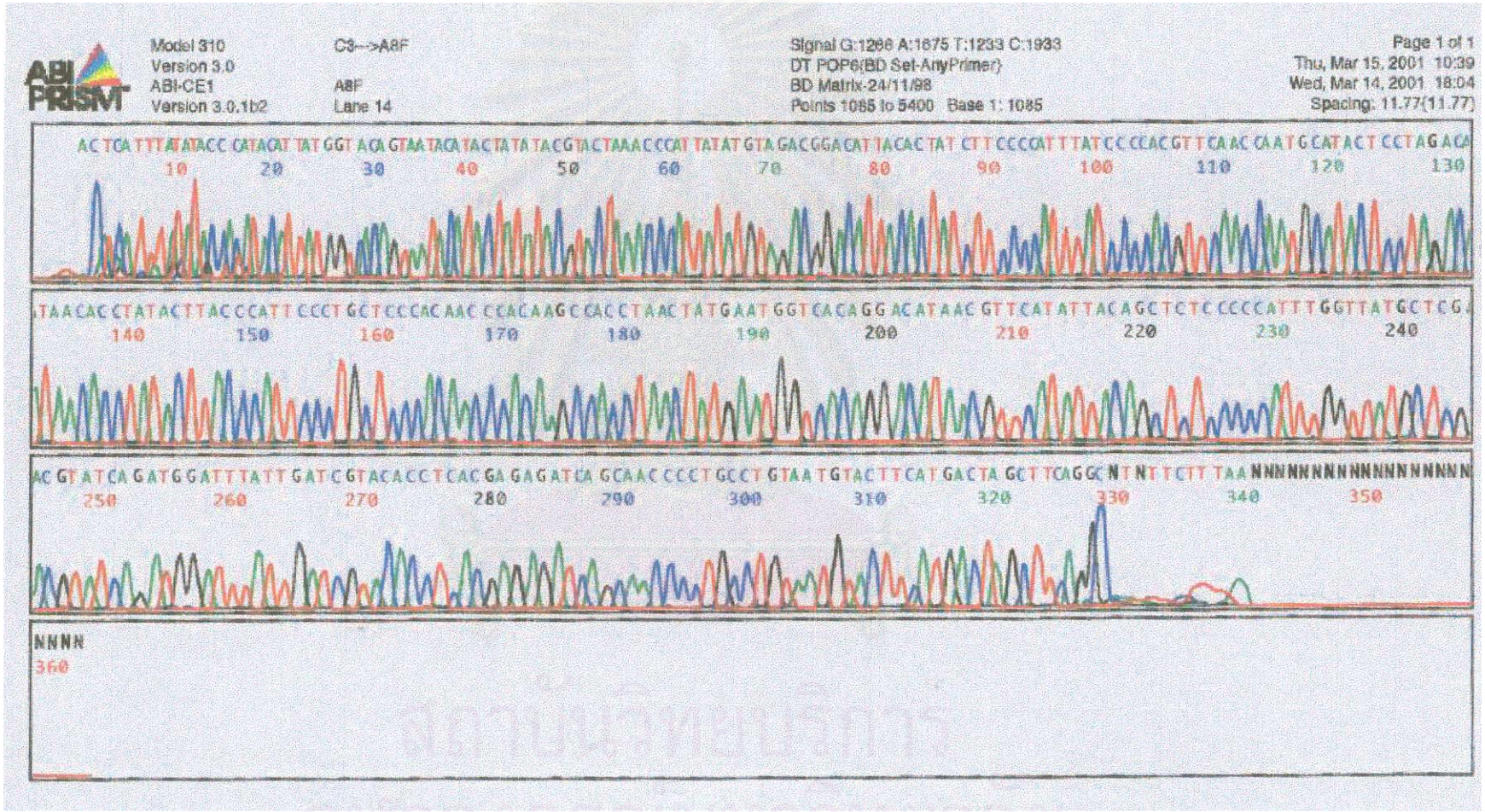

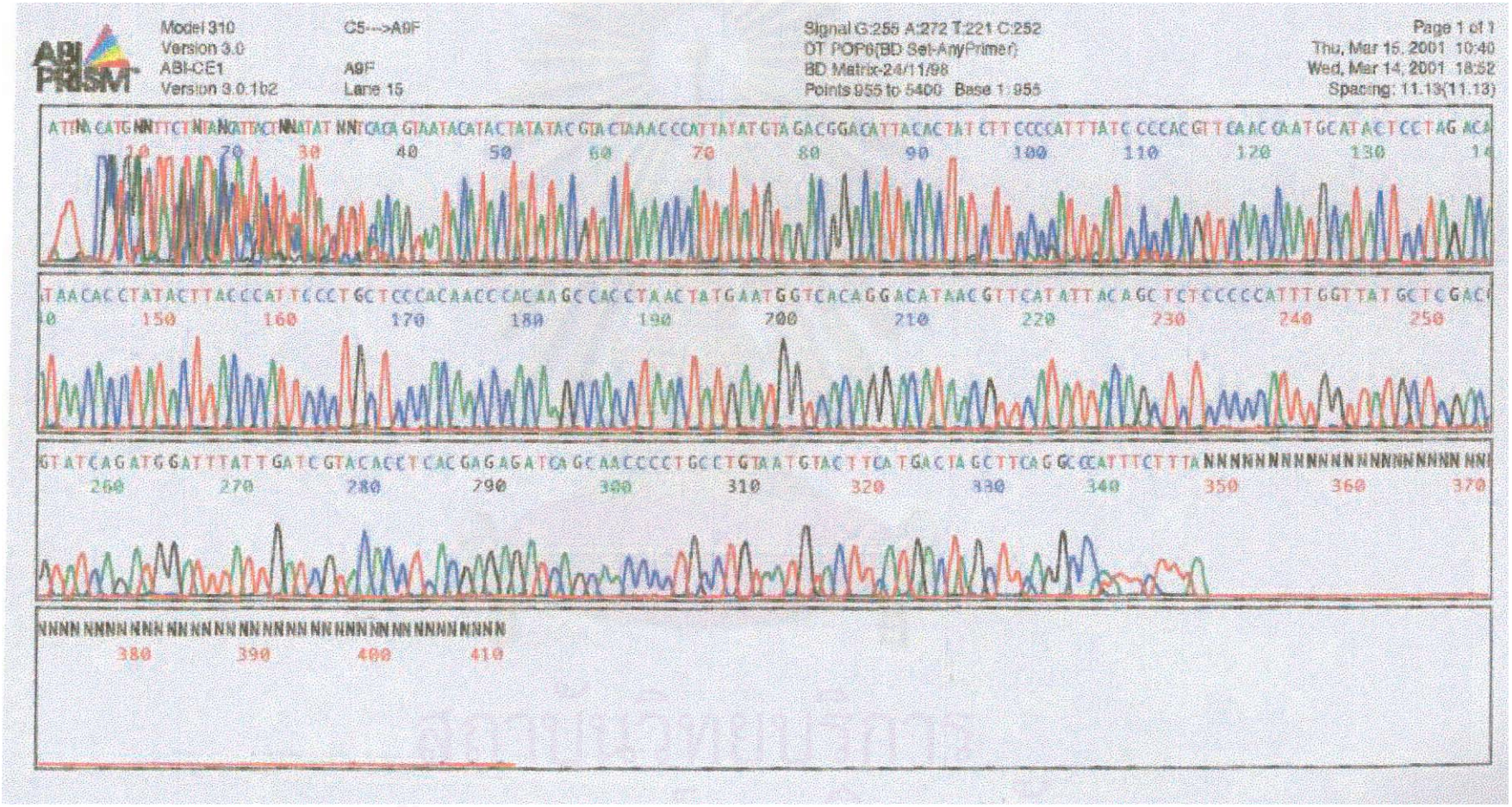

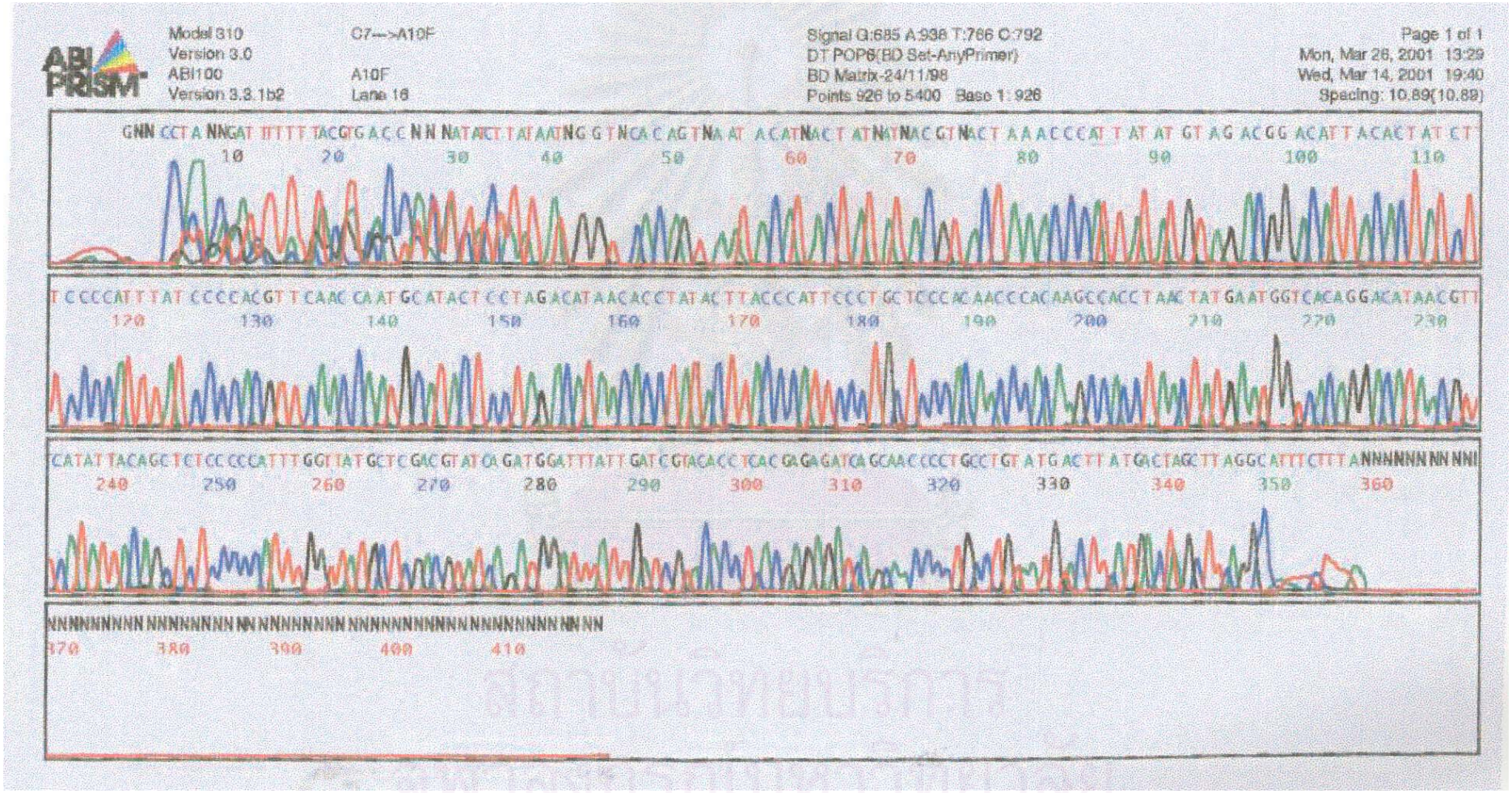
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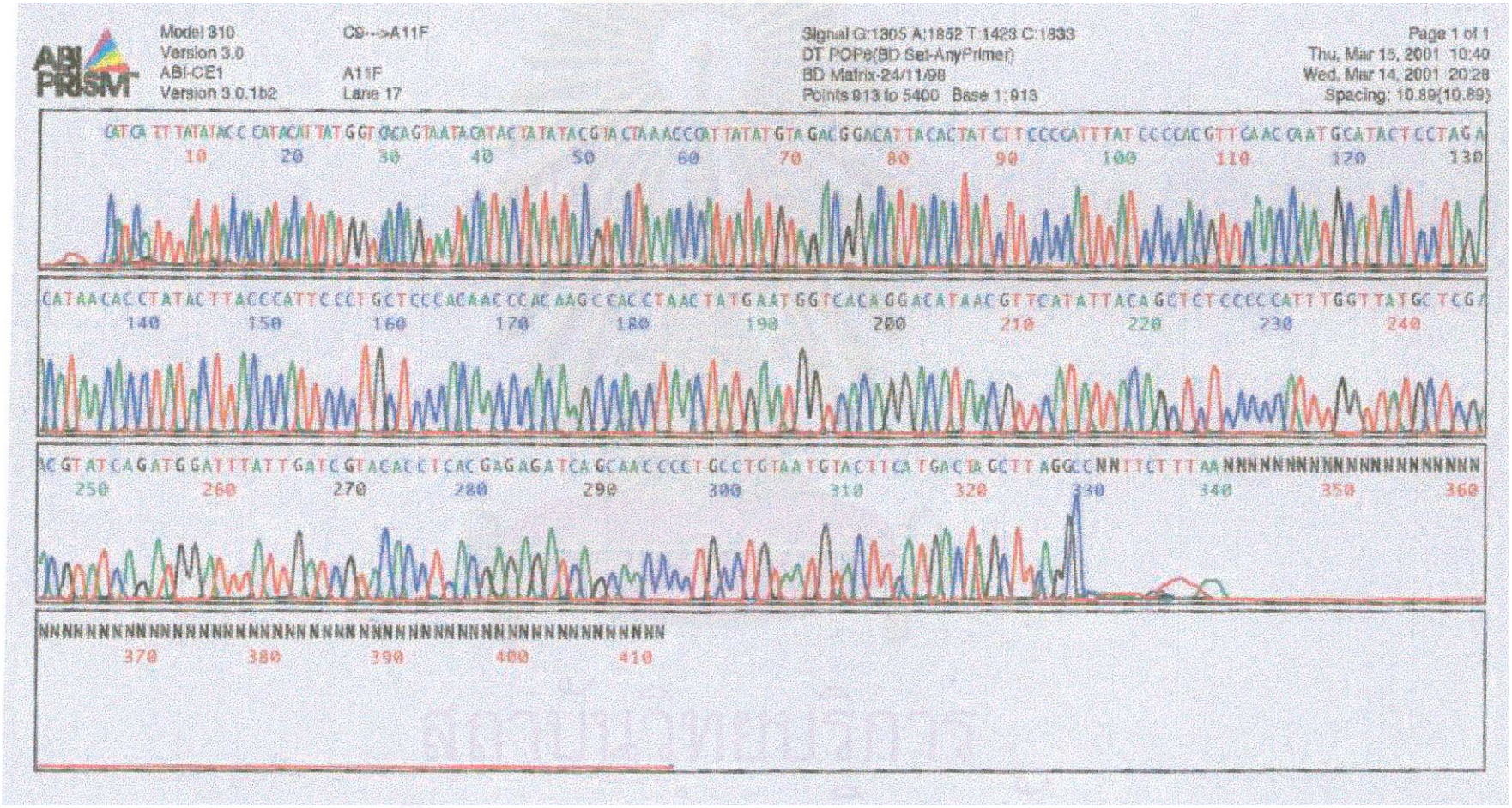

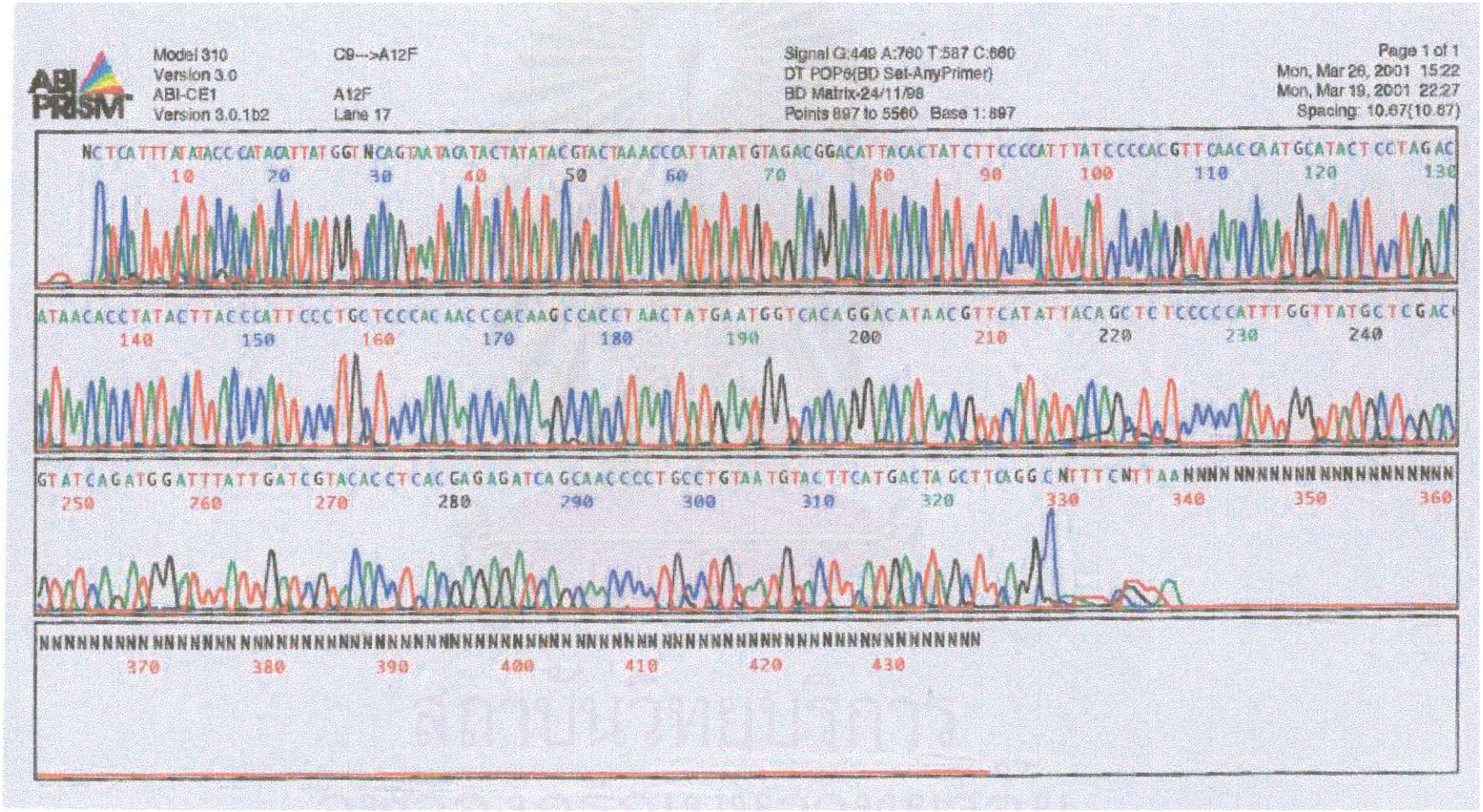
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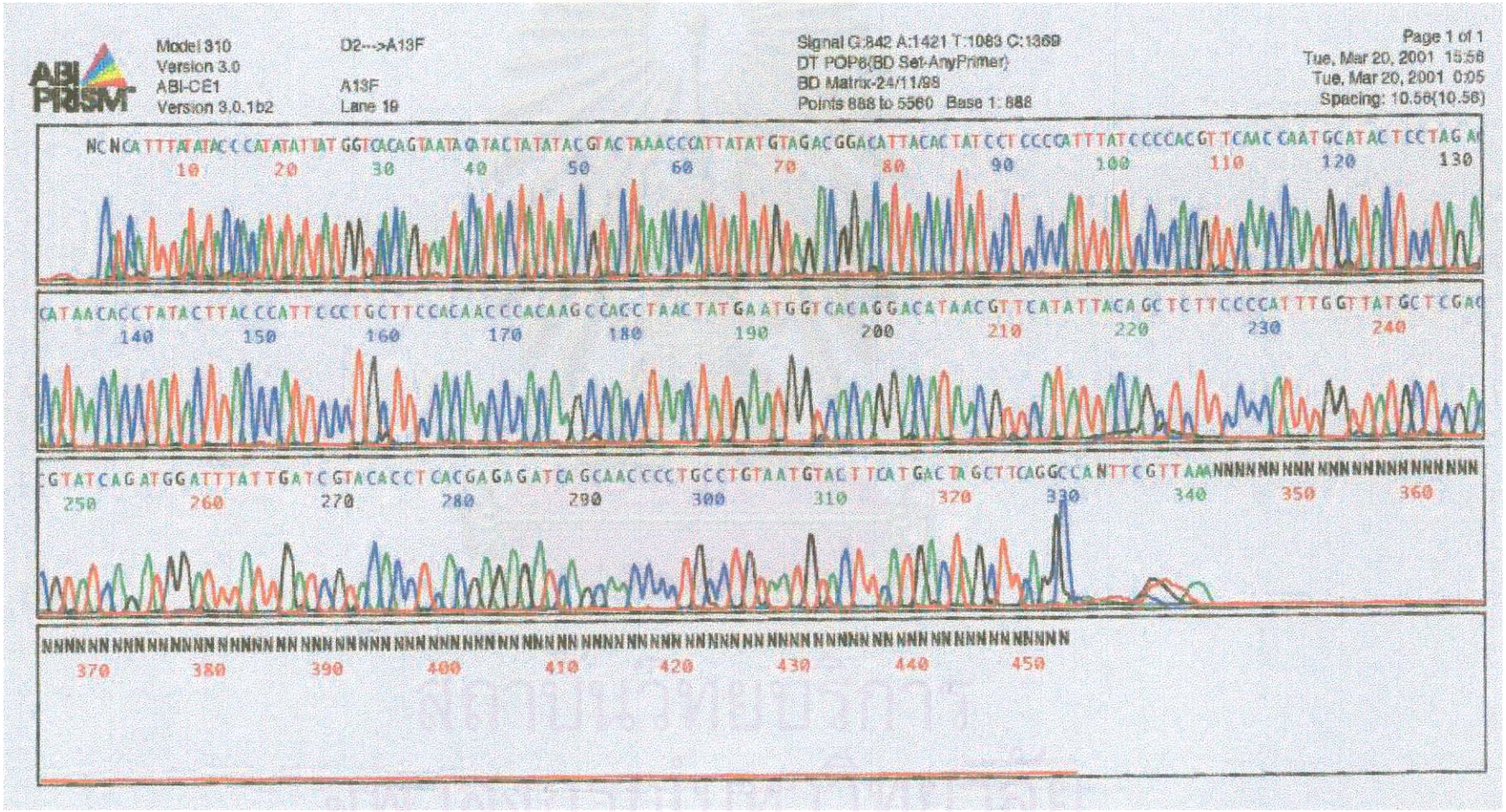
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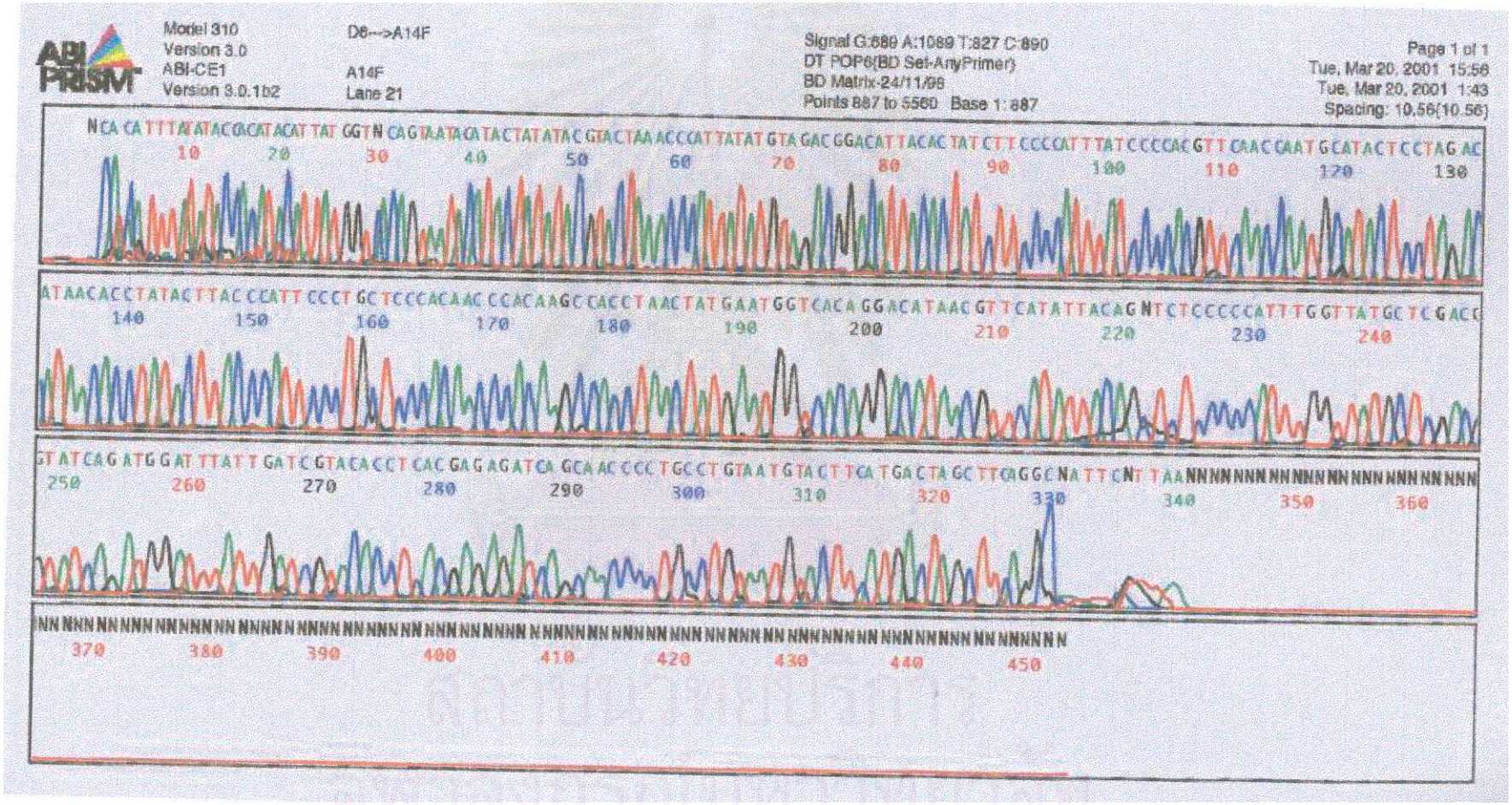
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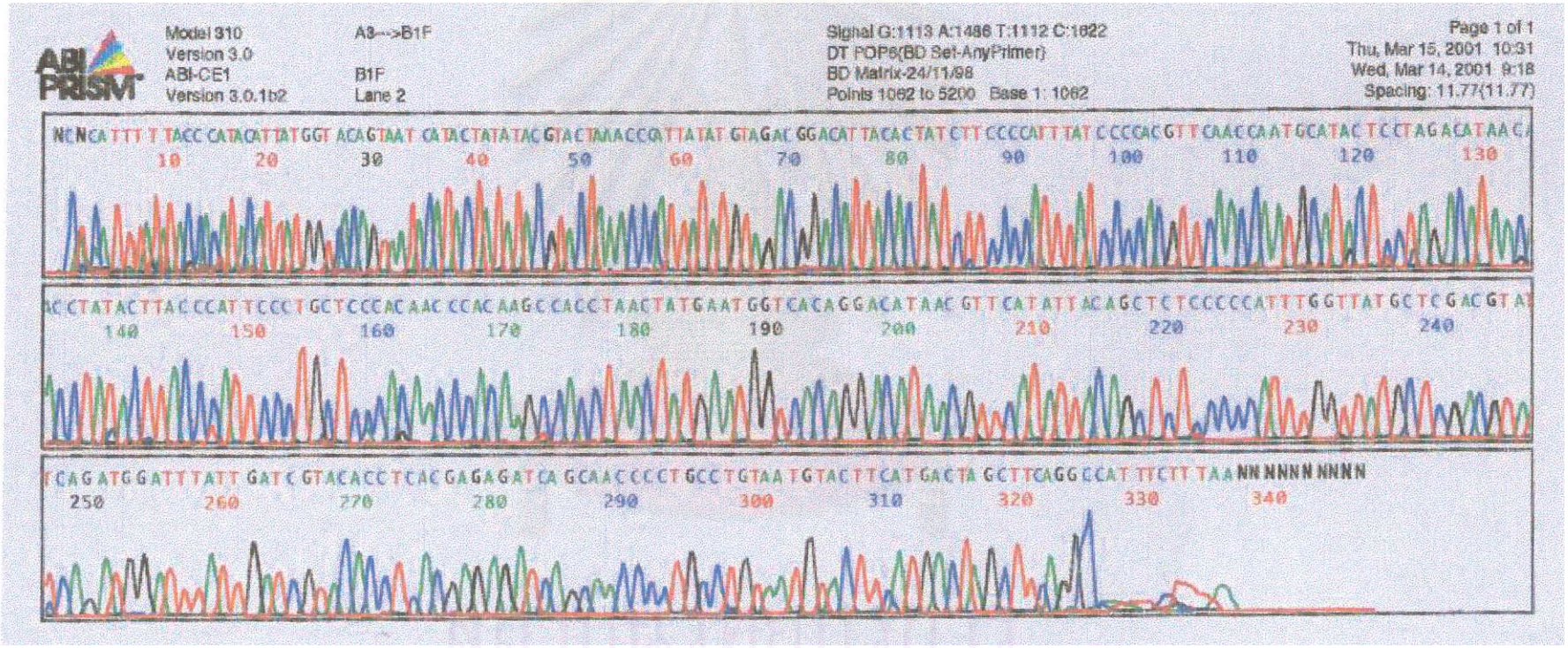

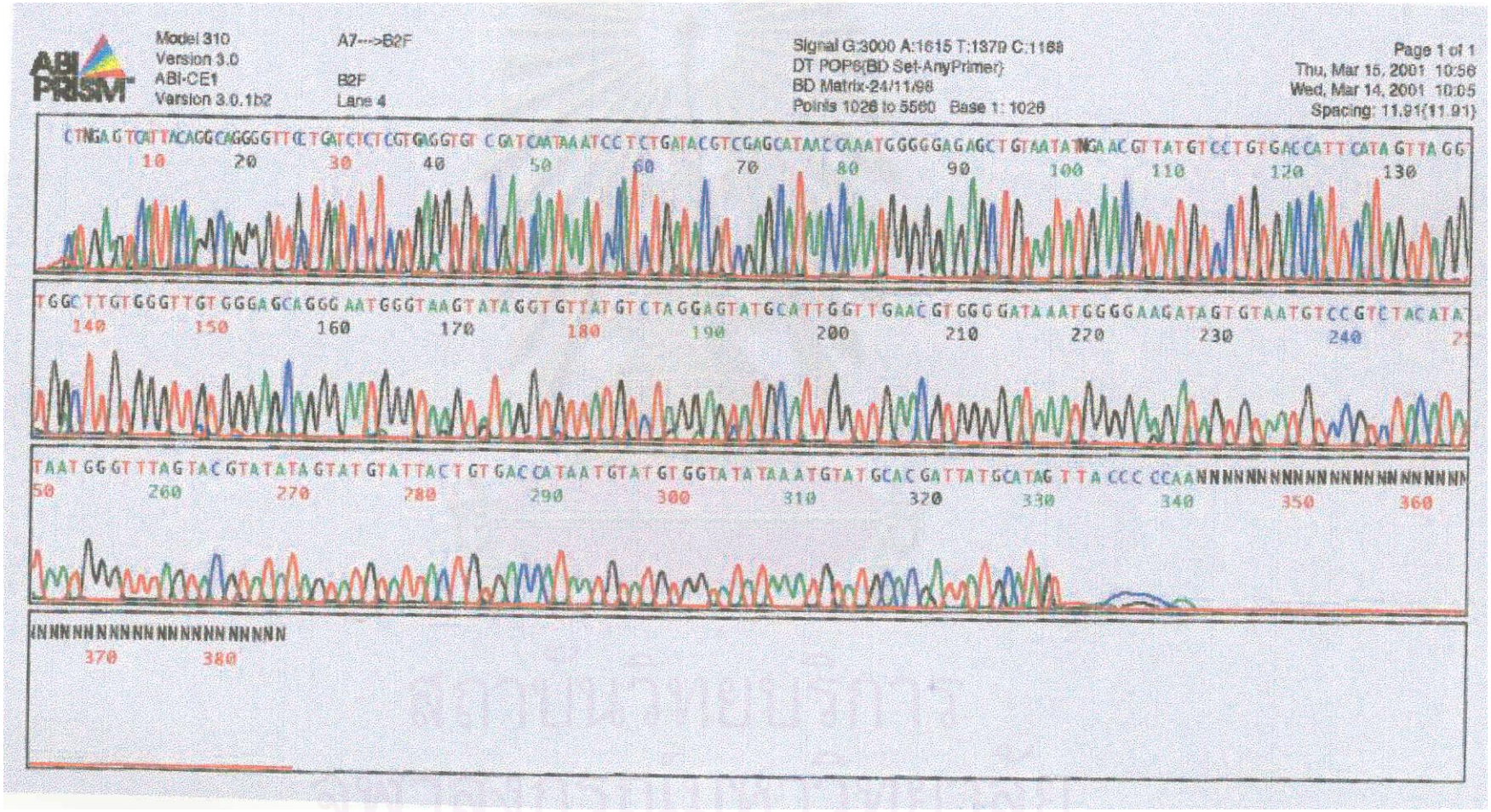
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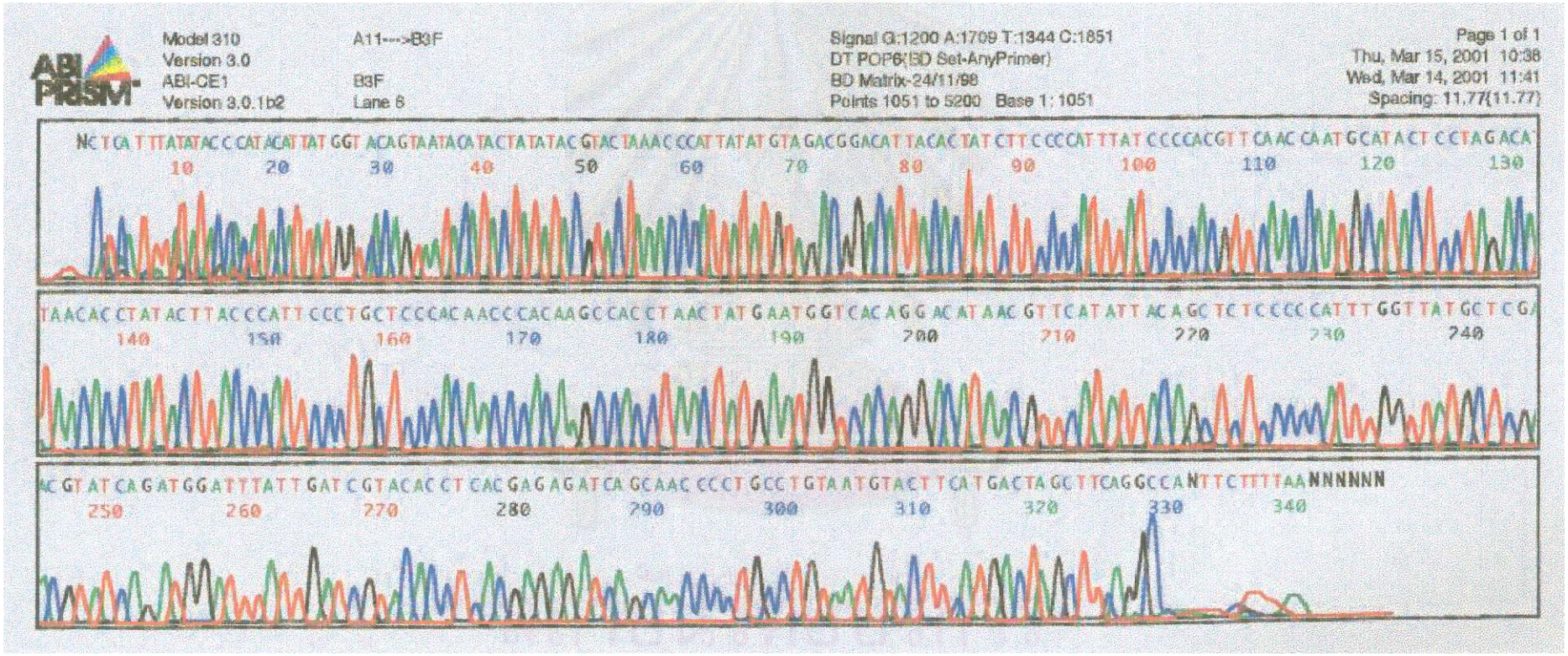

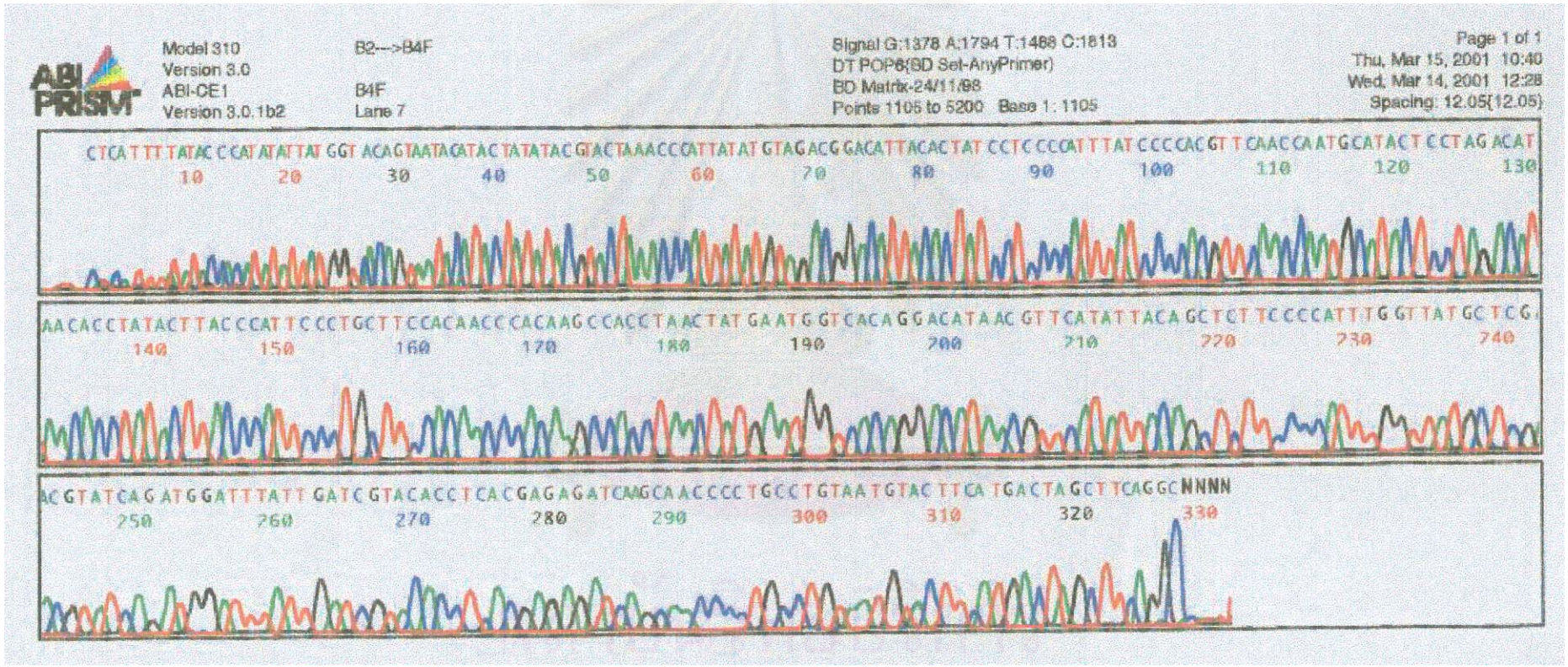

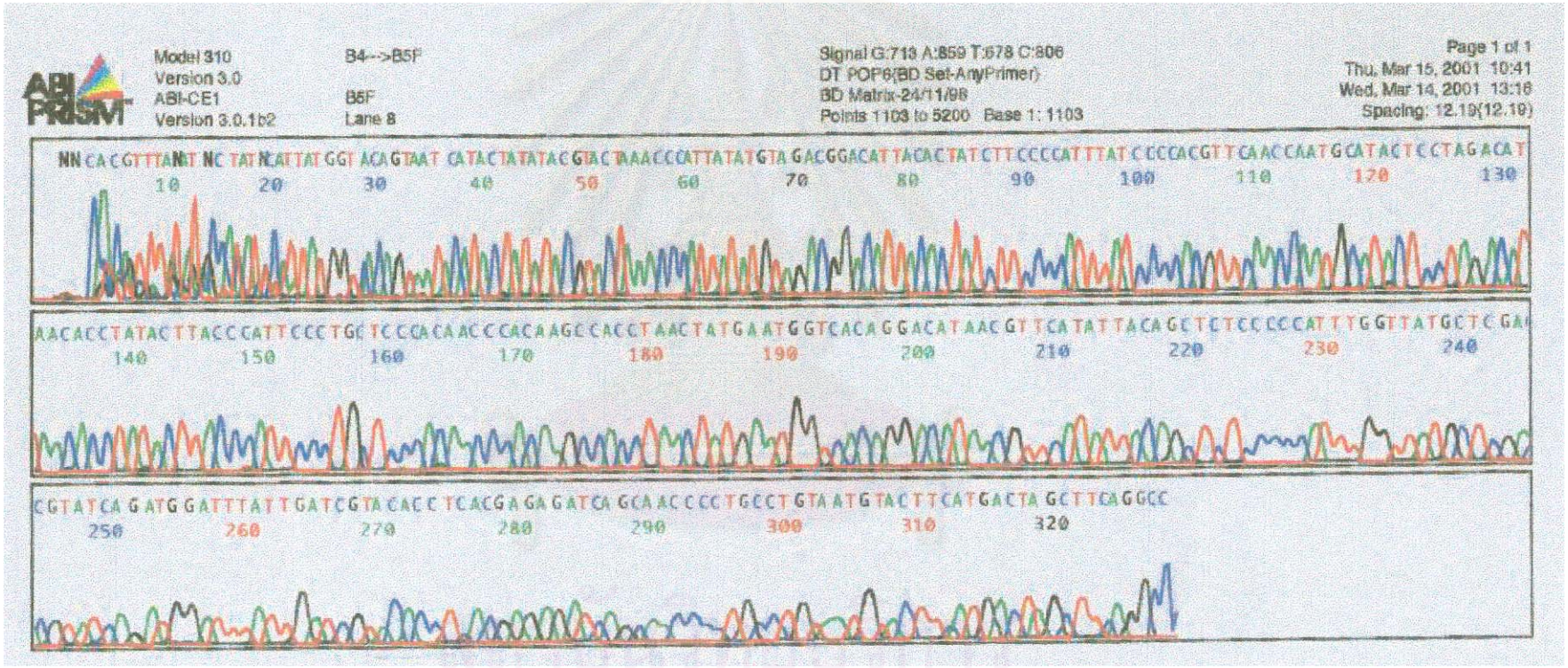

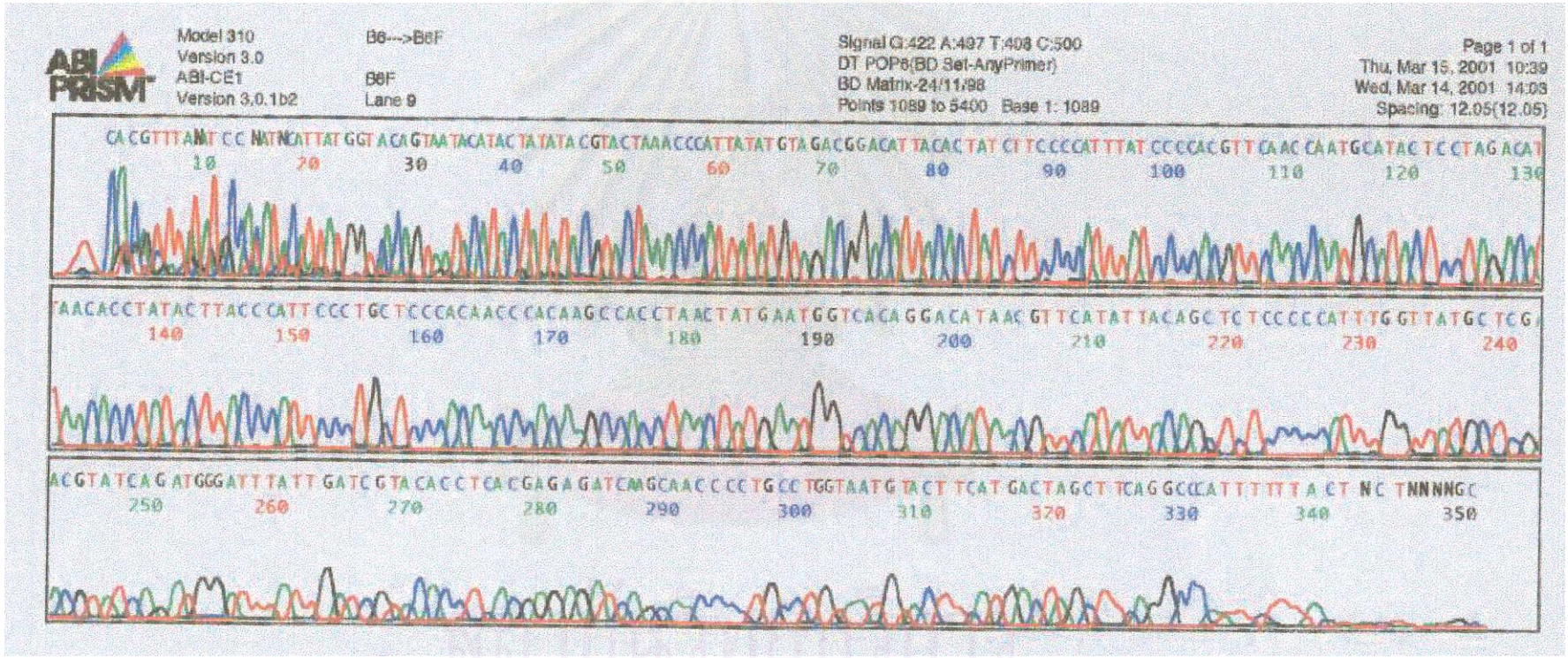

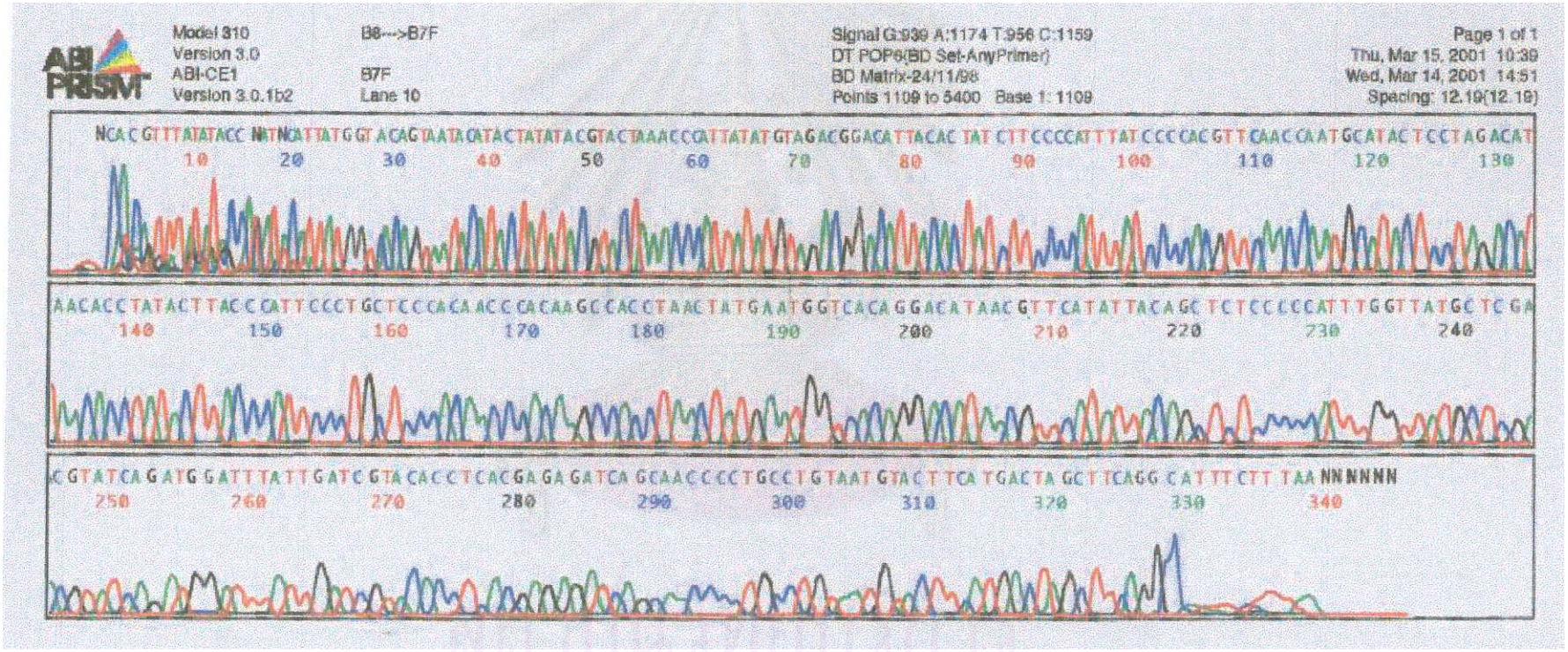

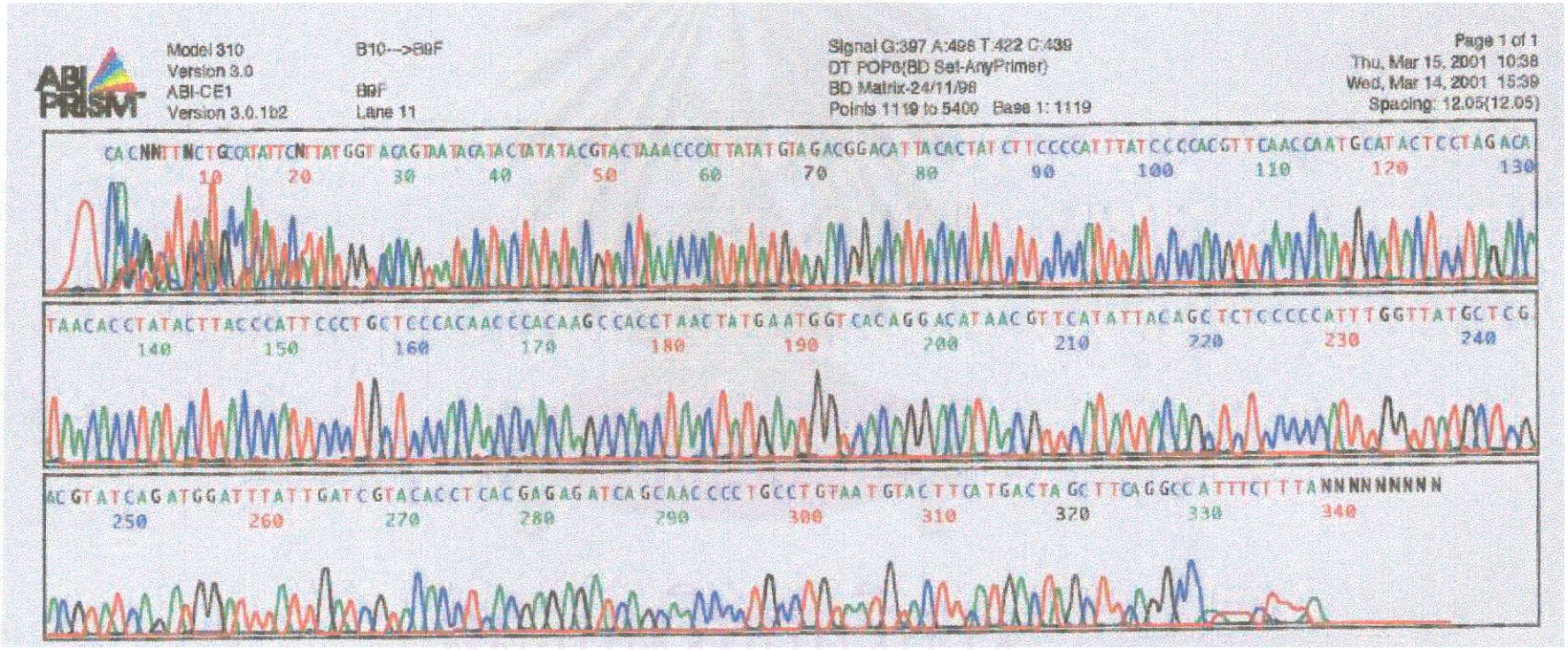

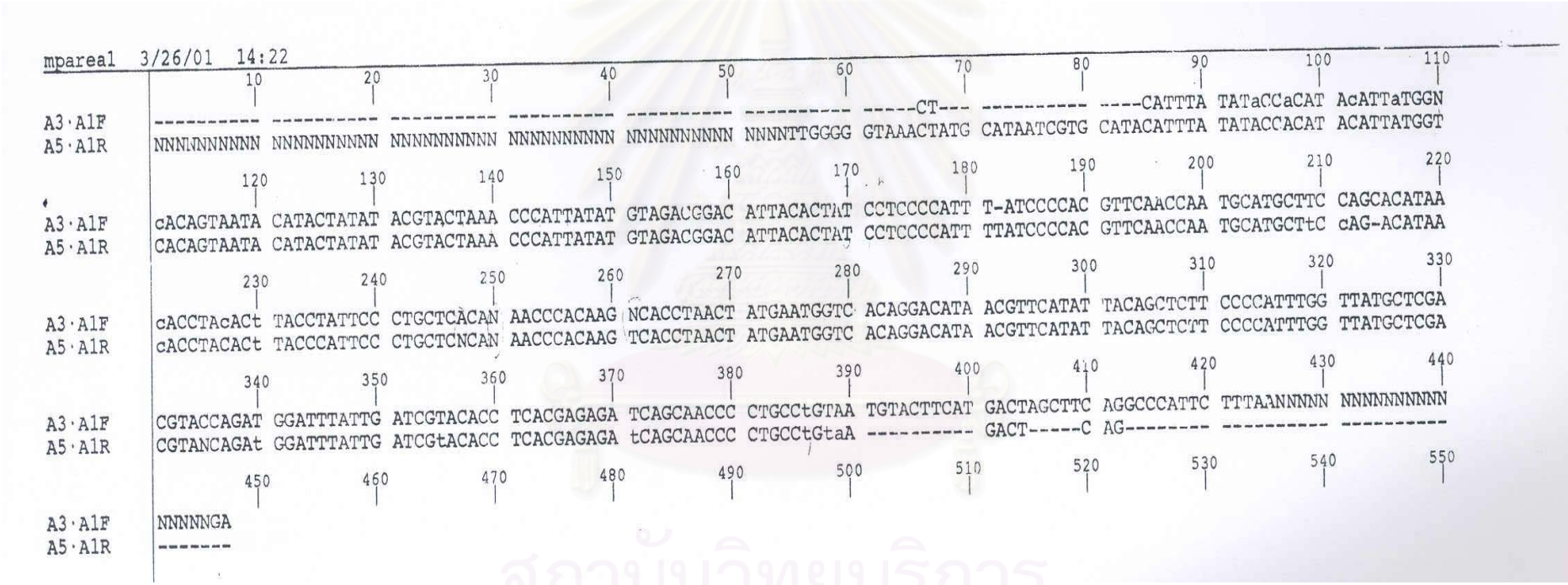

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A5·A1R   CACAGTAATA CATACTATAT ACGTACTAAA CCCATTATAT GTAGACGGAC ATTACACTAT CCTCCCCATT TTATCCCCAC GTTCAACCAA TGCATGCTtC cAG-ACATAA

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A5·A1R   CGTANCAGAt GGATTTATTG ATCGtACACC TCACGAGAGA tCAGCAACCC CTGCCtGtaA ---------- GACT-----C AG-------- ---------- ----------

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A5·A1R   -------
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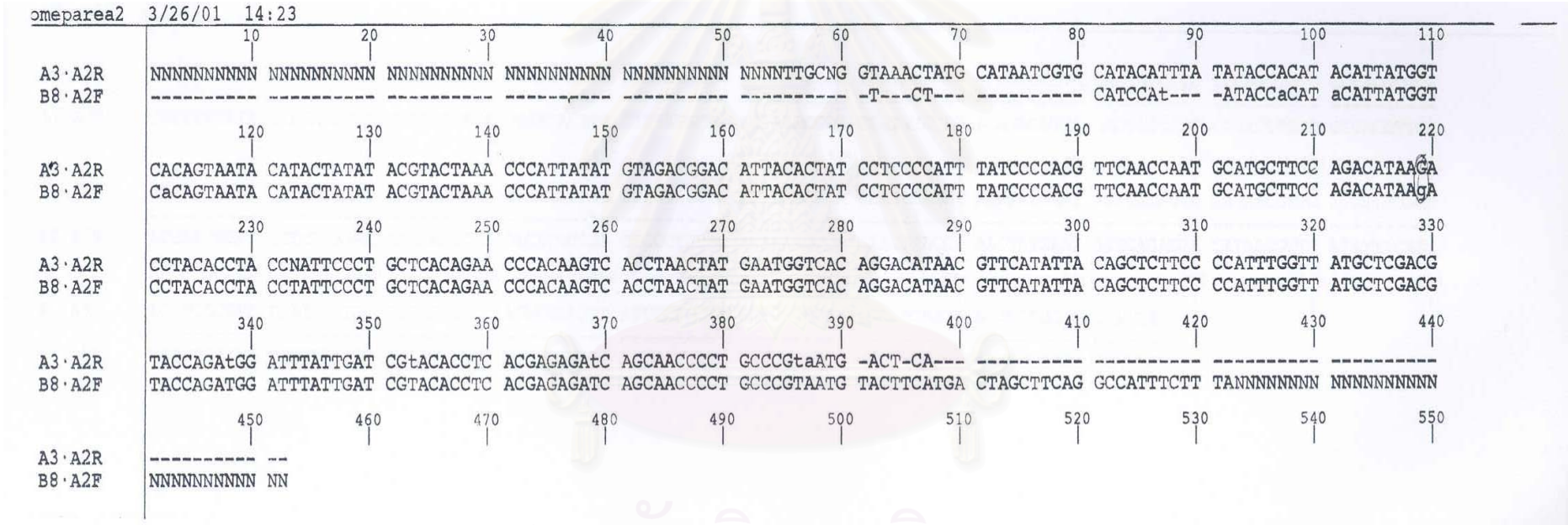

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B8·A2F     ---------- ---------- ---------- ---------- ---------- ---------- -T---CT--- ---------- CATCCAt--- -ATACCaCAT aCATTATGGT

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                    230        240        250        260        270        280        290        300        310        320        330
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B8·A2F     CCTACACCTA CCTATTCCCT GCTCACAGAA CCCACAAGTC ACCTAACTAT GAATGGTCAC AGGACATAAC GTTCATATTA CAGCTCTTCC CCATTTGGTT ATGCTCGACG

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B8·A2F     NNNNNNNNNN NN
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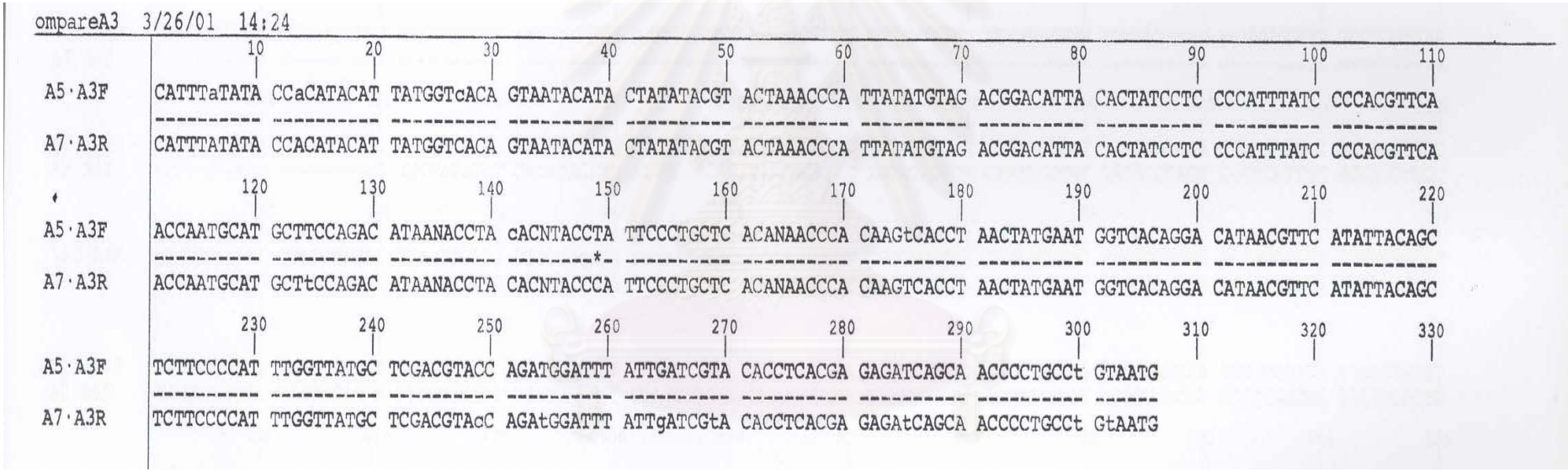

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          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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          ---------- ---------- ---------- --------*- ---------- ---------- ---------- ---------- ---------- ---------- ----------
A7·A3R    ACCAATGCAT GCTtCCAGAC ATAANACCTA CACNTACCCA TTCCCTGCTC ACANAACCCA CAAGTCACCT AACTATGAAT GGTCACAGGA CATAACGTTC ATATTACAGC

                   230       240       250       260       270       280       290       300       310       320       330
A5·A3F    TCTTCCCCAT TTGGTTATGC TCGACGTACC AGATGGATTT ATTGATCGTA CACCTCACGA GAGATCAGCA ACCCCTGCCt GTAATG
          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ------
A7·A3R    TCTTCCCCAT TTGGTTATGC TCGACGTAcC AGAtGGATTT ATTgATCGTA CACCTCACGA GAGAtCAGCA ACCCCTGCCt GtAATG
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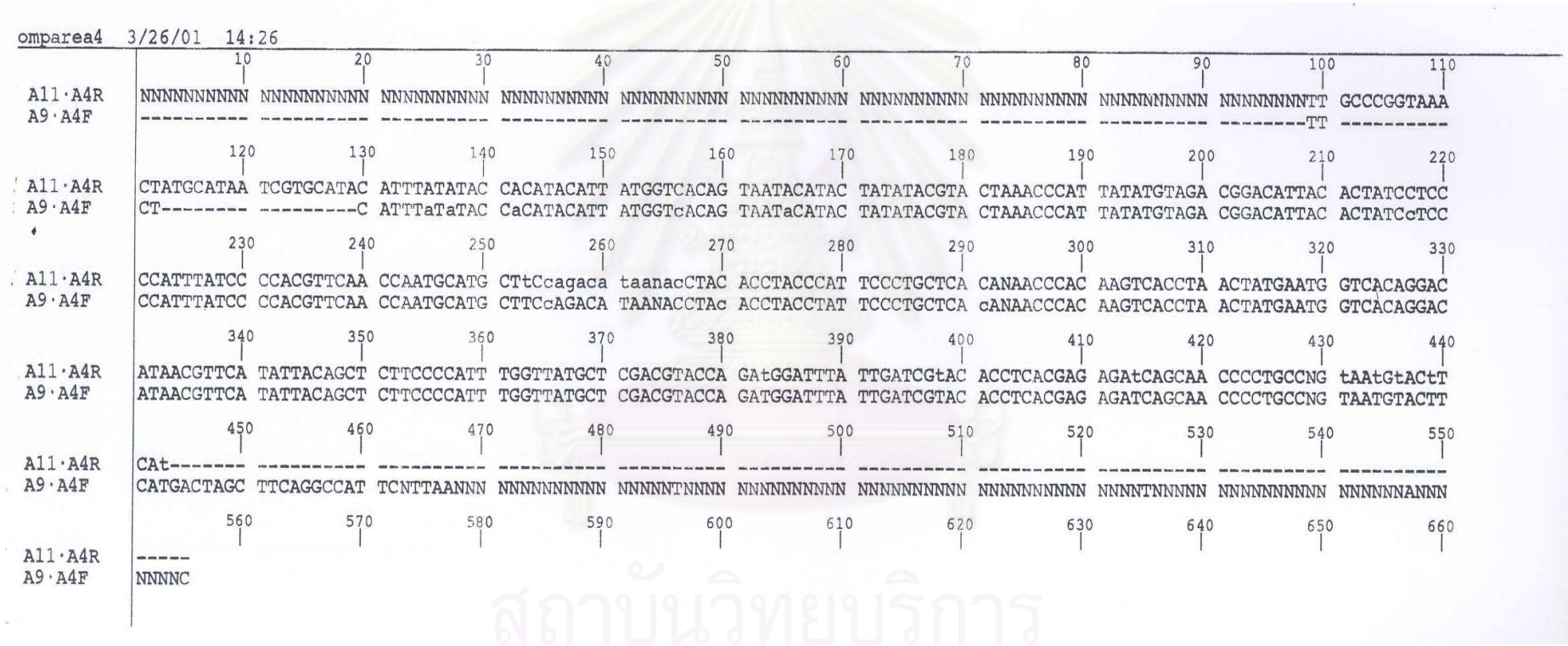

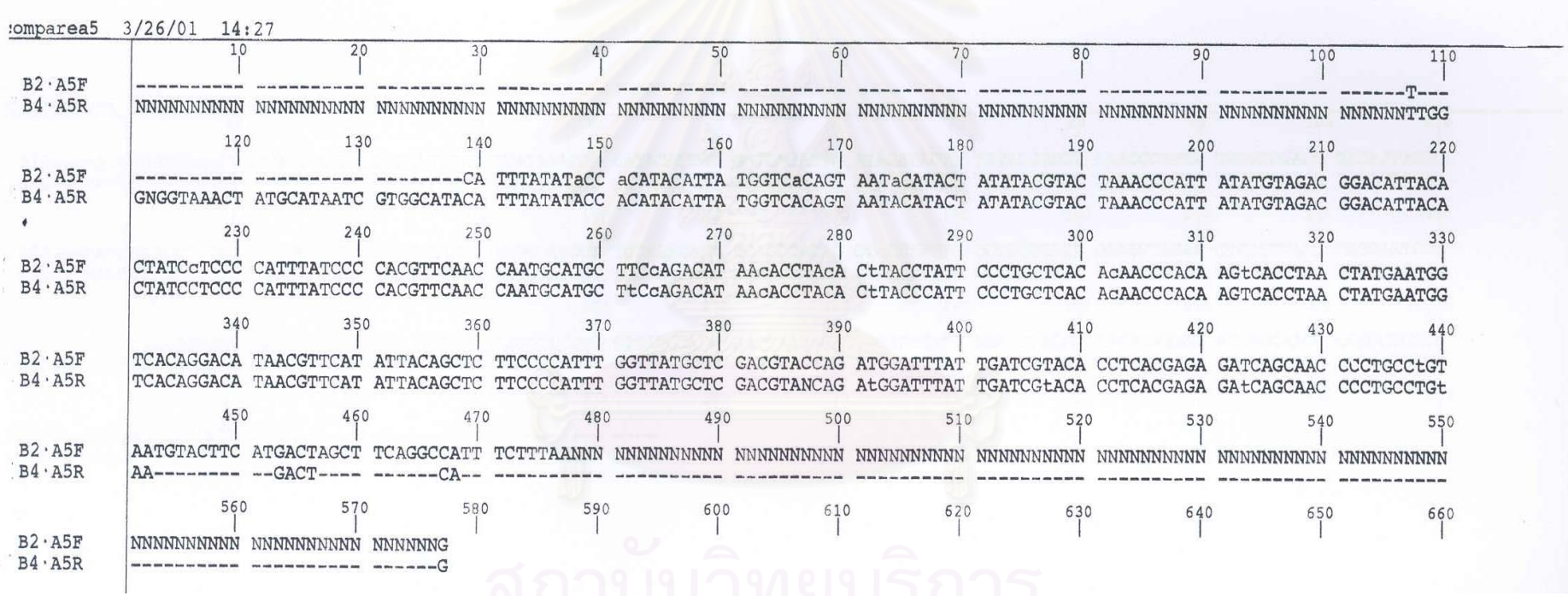

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                    10         20         30         40         50         60         70         80         90         100        110
B2·A5F      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ------T---
B4·A5R      NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNTTGG

                    120        130        140        150        160        170        180        190        200        210        220
B2·A5F      ---------- ---------- --------CA TTTATATaCC aCATACATTA TGGTCaCAGT AATaCATACT ATATACGTAC TAAACCCATT ATATGTAGAC GGACATTACA
B4·A5R      GNGGTAAACT ATGCATAATC GTGGCATACA TTTATATACC ACATACATTA TGGTCACAGT AATACATACT ATATACGTAC TAAACCCATT ATATGTAGAC GGACATTACA

                    230        240        250        260        270        280        290        300        310        320        330
B2·A5F      CTATCcTCCC CATTTATCCC CACGTTCAAC CAATGCATGC TTCcAGACAT AAcACCTAcA CtTACCTATT CCCTGCTCAC AcAACCCACA AGtCACCTAA CTATGAATGG
B4·A5R      CTATCCTCCC CATTTATCCC CACGTTCAAC CAATGCATGC TtCcAGACAT AAcACCTACA CtTACCCATT CCCTGCTCAC AcAACCCACA AGTCACCTAA CTATGAATGG

                    340        350        360        370        380        390        400        410        420        430        440
B2·A5F      TCACAGGACA TAACGTTCAT ATTACAGCTC TTCCCCATTT GGTTATGCTC GACGTACCAG ATGGATTTAT TGATCGTACA CCTCACGAGA GATCAGCAAC CCCTGCCtGT
B4·A5R      TCACAGGACA TAACGTTCAT ATTACAGCTC TTCCCCATTT GGTTATGCTC GACGTANCAG AtGGATTTAT TGATCGtACA CCTCACGAGA GAtCAGCAAC CCCTGCCTGt

                    450        460        470        480        490        500        510        520        530        540        550
B2·A5F      AATGTACTTC ATGACTAGCT TCAGGCCATT TCTTTAANNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
B4·A5R      AA-------- --GACT---- ------CA-- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

                    560        570        580        590        600        610        620        630        640        650        660
B2·A5F      NNNNNNNNNN NNNNNNNNNN NNNNNNG
B4·A5R      ---------- ---------- ------G
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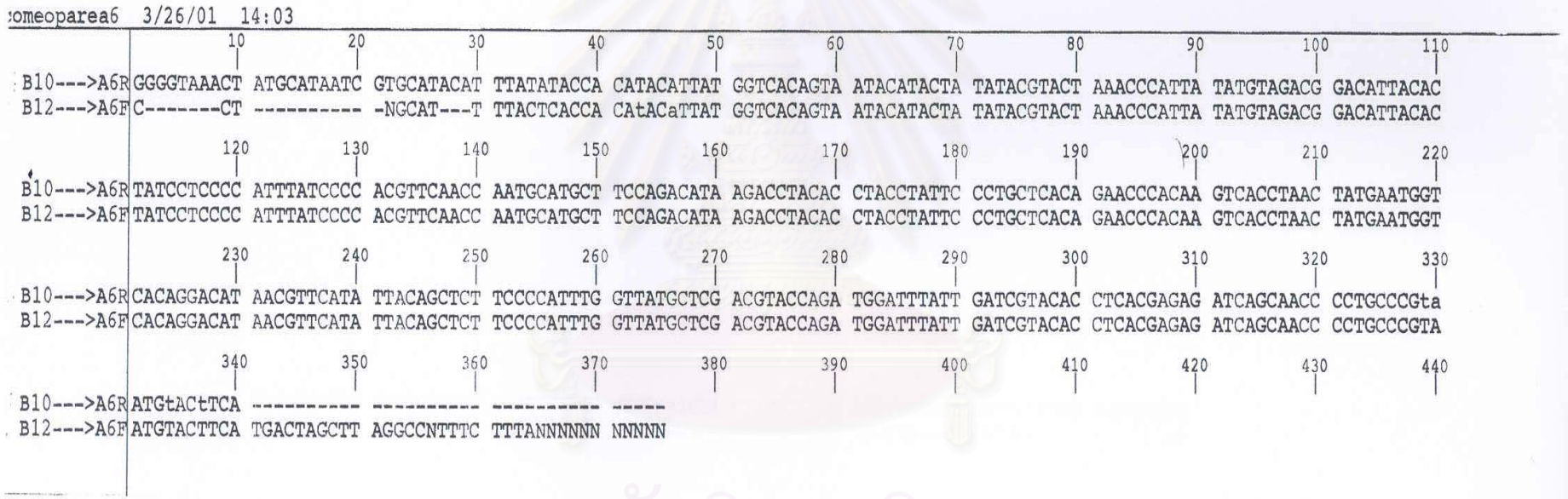

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                        10         20         30         40         50         60         70         80         90         100        110
 B10--->A6R GGGGTAAACT ATGCATAATC GTGCATACAT TTATATACCA CATACATTAT GGTCACAGTA ATACATACTA TATACGTACT AAACCCATTA TATGTAGACG GACATTACAC
 B12--->A6F C--------CT ---------- -NGCAT---T TTACTCACCA CAtACaTTAT GGTCACAGTA ATACATACTA TATACGTACT AAACCCATTA TATGTAGACG GACATTACAC

                        120        130        140        150        160        170        180        190        200        210        220
 B10--->A6R TATCCTCCCC ATTTATCCCC ACGTTCAACC AATGCATGCT TCCAGACATA AGACCTACAC CTACCTATTC CCTGCTCACA GAACCCACAA GTCACCTAAC TATGAATGGT
 B12--->A6F TATCCTCCCC ATTTATCCCC ACGTTCAACC AATGCATGCT TCCAGACATA AGACCTACAC CTACCTATTC CCTGCTCACA GAACCCACAA GTCACCTAAC TATGAATGGT

                        230        240        250        260        270        280        290        300        310        320        330
 B10--->A6R CACAGGACAT AACGTTCATA TTACAGCTCT TCCCCATTTG GTTATGCTCG ACGTACCAGA TGGATTTATT GATCGTACAC CTCACGAGAG ATCAGCAACC CCTGCCCGta
 B12--->A6F CACAGGACAT AACGTTCATA TTACAGCTCT TCCCCATTTG GTTATGCTCG ACGTACCAGA TGGATTTATT GATCGTACAC CTCACGAGAG ATCAGCAACC CCTGCCCGTA

                        340        350        360        370        380        390        400        410        420        430        440
 B10--->A6R ATGtACtTCA ---------- ---------- ---------- -----
 B12--->A6F ATGTACTTCA TGACTAGCTT AGGCCNTTTC TTTANNNNNN NNNNN
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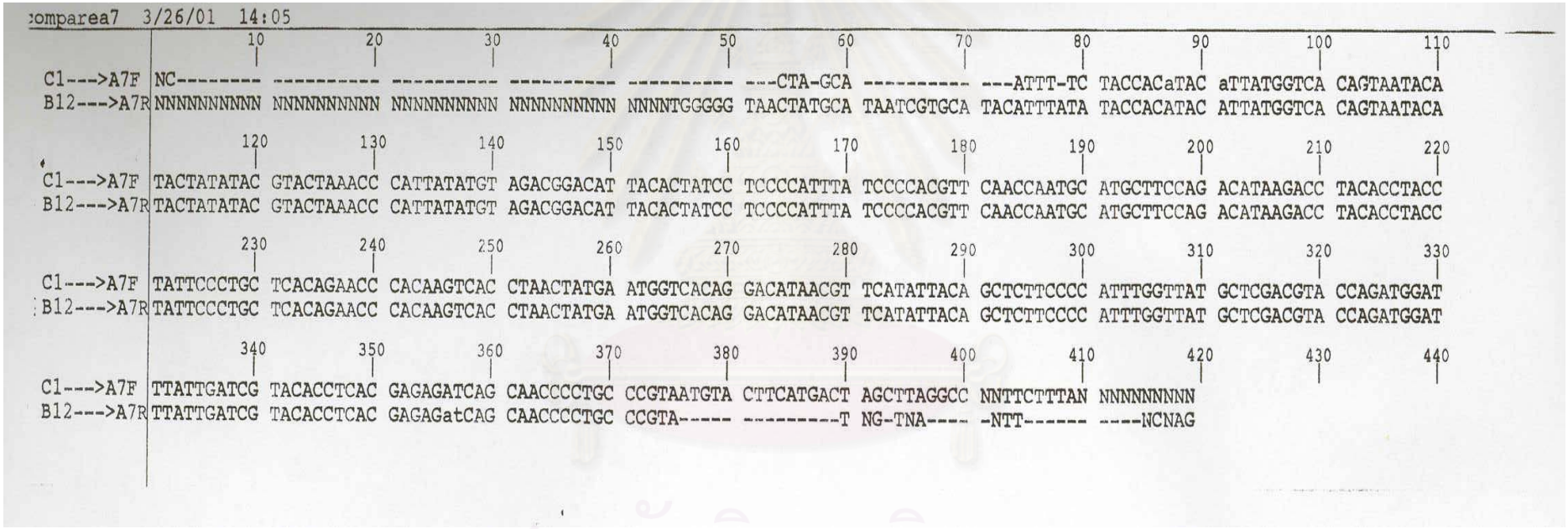

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                        10        20        30        40        50        60        70        80        90        100       110
C1--->A7F  NC--------- ---------- ---------- ---------- ---------- ---CTA-GCA ---------- ---ATTT-TC TACCACaTAC aTTATGGTCA CAGTAATACA
B12--->A7R NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNTGGGGG TAACTATGCA TAATCGTGCA TACATTTATA TACCACATAC ATTATGGTCA CAGTAATACA

                        120       130       140       150       160       170       180       190       200       210       220
C1--->A7F  TACTATATAC GTACTAAACC CATTATATGT AGACGGACAT TACACTATCC TCCCCATTTA TCCCCACGTT CAACCAATGC ATGCTTCCAG ACATAAGACC TACACCTACC
B12--->A7R TACTATATAC GTACTAAACC CATTATATGT AGACGGACAT TACACTATCC TCCCCATTTA TCCCCACGTT CAACCAATGC ATGCTTCCAG ACATAAGACC TACACCTACC

                        230       240       250       260       270       280       290       300       310       320       330
C1--->A7F  TATTCCCTGC TCACAGAACC CACAAGTCAC CTAACTATGA ATGGTCACAG GACATAACGT TCATATTACA GCTCTTCCCC ATTTGGTTAT GCTCGACGTA CCAGATGGAT
B12--->A7R TATTCCCTGC TCACAGAACC CACAAGTCAC CTAACTATGA ATGGTCACAG GACATAACGT TCATATTACA GCTCTTCCCC ATTTGGTTAT GCTCGACGTA CCAGATGGAT

                        340       350       360       370       380       390       400       410       420       430       440
C1--->A7F  TTATTGATCG TACACCTCAC GAGAGATCAG CAACCCCTGC CCGTAATGTA CTTCATGACT AGCTTAGGCC NNTTCTTTAN NNNNNNNNN
B12--->A7R TTATTGATCG TACACCTCAC GAGAGatCAG CAACCCCTGC CCGTA----- ---------T NG-TNA---- -NTT------ ----WCNAG
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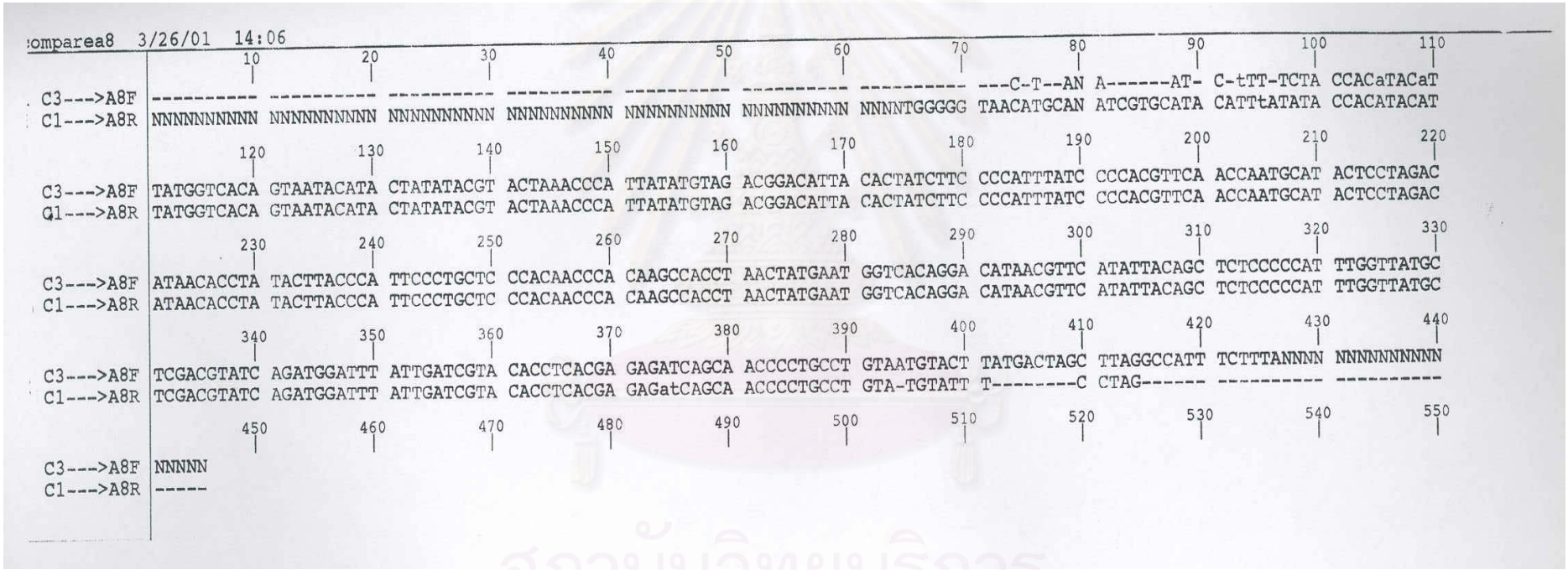

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                        10        20        30        40        50        60        70        80        90        100       110
                        |         |         |         |         |         |         |         |         |         |         |
. C3--->A8F  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---C-T--AN A------AT- C-tTT-TCTA CCACaTACaT
 C1--->A8R  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNTGGGGG TAACATGCAN ATCGTGCATA CATTtATATA CCACATACAT

                        120       130       140       150       160       170       180       190       200       210       220
                        |         |         |         |         |         |         |         |         |         |         |
 C3--->A8F  TATGGTCACA GTAATACATA CTATATACGT ACTAAACCCA TTATATGTAG ACGGACATTA CACTATCTTC CCCATTTATC CCCACGTTCA ACCAATGCAT ACTCCTAGAC
 C1--->A8R  TATGGTCACA GTAATACATA CTATATACGT ACTAAACCCA TTATATGTAG ACGGACATTA CACTATCTTC CCCATTTATC CCCACGTTCA ACCAATGCAT ACTCCTAGAC

                        230       240       250       260       270       280       290       300       310       320       330
                        |         |         |         |         |         |         |         |         |         |         |
 C3--->A8F  ATAACACCTA TACTTACCCA TTCCCTGCTC CCACAACCCA CAAGCCACCT AACTATGAAT GGTCACAGGA CATAACGTTC ATATTACAGC TCTCCCCCAT TTGGTTATGC
 C1--->A8R  ATAACACCTA TACTTACCCA TTCCCTGCTC CCACAACCCA CAAGCCACCT AACTATGAAT GGTCACAGGA CATAACGTTC ATATTACAGC TCTCCCCCAT TTGGTTATGC

                        340       350       360       370       380       390       400       410       420       430       440
                        |         |         |         |         |         |         |         |         |         |         |
 C3--->A8F  TCGACGTATC AGATGGATTT ATTGATCGTA CACCTCACGA GAGATCAGCA ACCCCTGCCT GTAATGTACT TATGACTAGC TTAGGCCATT TCTTTANNNN NNNNNNNNNN
 C1--->A8R  TCGACGTATC AGATGGATTT ATTGATCGTA CACCTCACGA GAGatCAGCA ACCCCTGCCT GTA-TGTATT T--------C CTAG------ ---------- ----------

                        450       460       470       480       490       500       510       520       530       540       550
                        |         |         |         |         |         |         |         |         |         |         |
 C3--->A8F  NNNNN
 C1--->A8R  -----
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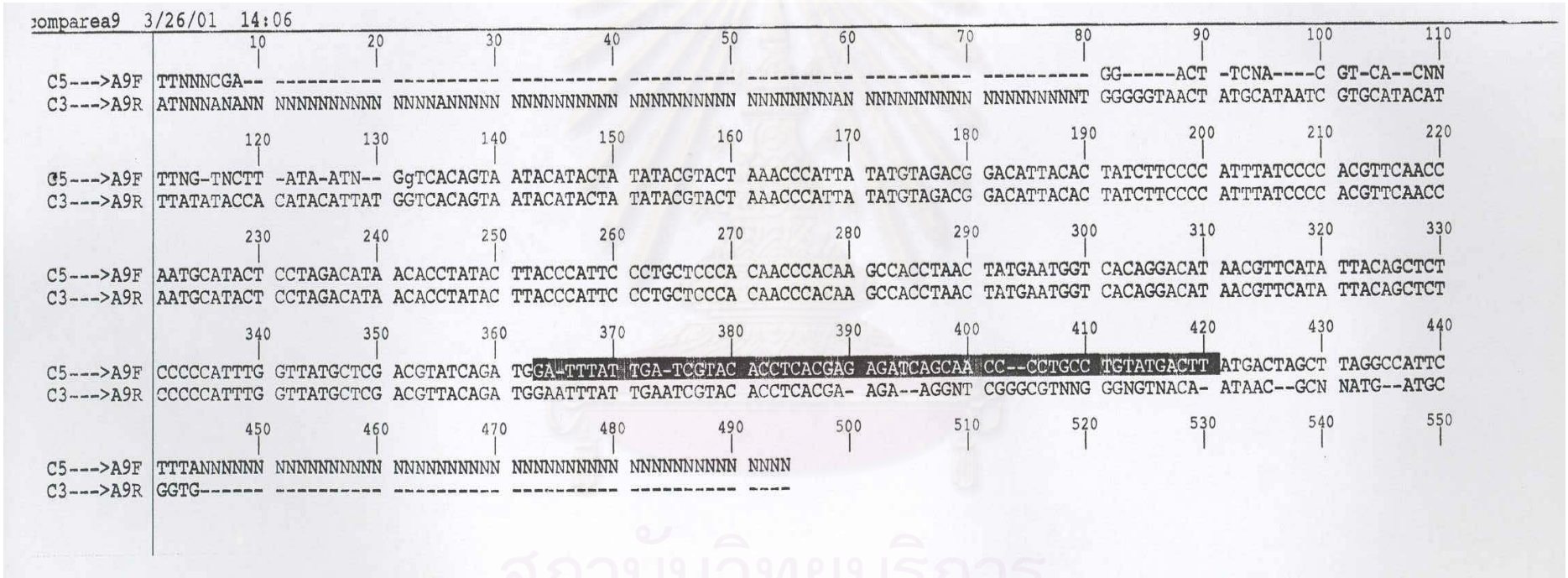

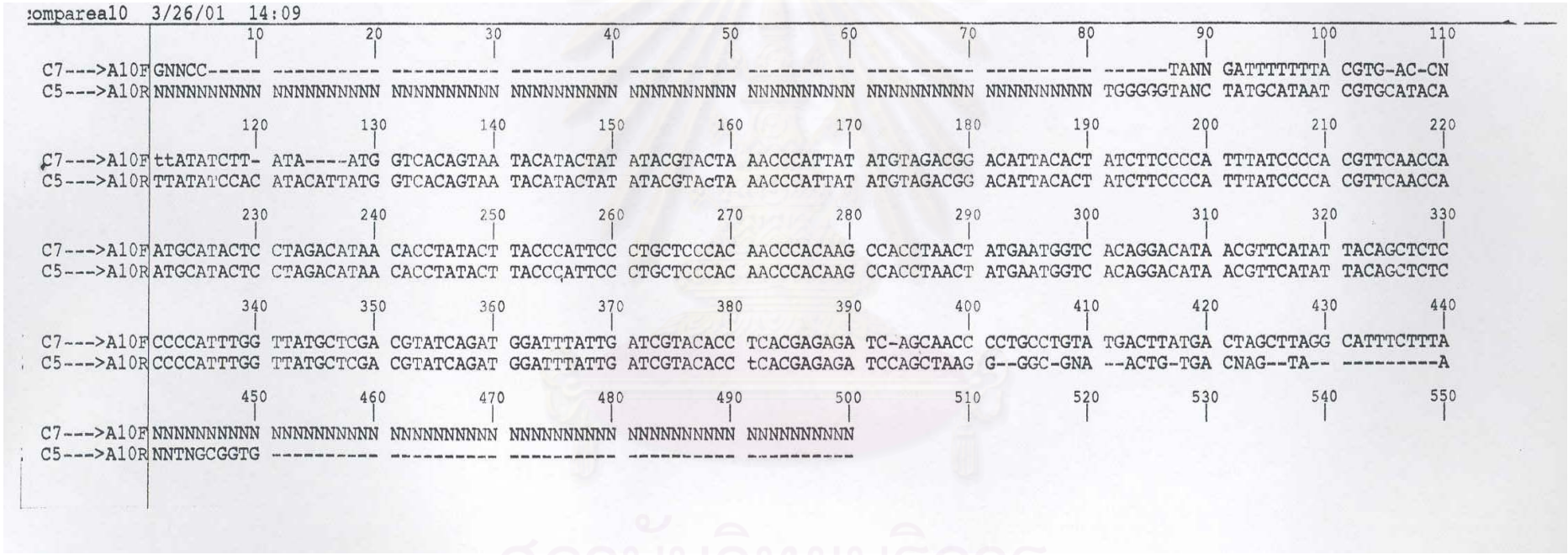

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                        10        20        30        40        50        60        70        80        90       100       110
C7--->A10F GNNCC----- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ------TANN GATTTTTTTA CGTG-AC-CN
C5--->A10R NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN TGGGGGTANC TATGCATAAT CGTGCATACA

                       120       130       140       150       160       170       180       190       200       210       220
C7--->A10F ttATATCTT- ATA----ATG GTCACAGTAA TACATACTAT ATACGTACTA AACCCATTAT ATGTAGACGG ACATTACACT ATCTTCCCCA TTTATCCCCA CGTTCAACCA
C5--->A10R TTATATCCAC ATACATTATG GTCACAGTAA TACATACTAT ATACGTAcTA AACCCATTAT ATGTAGACGG ACATTACACT ATCTTCCCCA TTTATCCCCA CGTTCAACCA

                       230       240       250       260       270       280       290       300       310       320       330
C7--->A10F ATGCATACTC CTAGACATAA CACCTATACT TACCCATTCC CTGCTCCCAC AACCCACAAG CCACCTAACT ATGAATGGTC ACAGGACATA ACGTTCATAT TACAGCTCTC
C5--->A10R ATGCATACTC CTAGACATAA CACCTATACT TACCCATTCC CTGCTCCCAC AACCCACAAG CCACCTAACT ATGAATGGTC ACAGGACATA ACGTTCATAT TACAGCTCTC

                       340       350       360       370       380       390       400       410       420       430       440
C7--->A10F CCCCATTTGG TTATGCTCGA CGTATCAGAT GGATTTATTG ATCGTACACC TCACGAGAGA TC-AGCAACC CCTGCCTGTA TGACTTATGA CTAGCTTAGG CATTTCTTTA
C5--->A10R CCCCATTTGG TTATGCTCGA CGTATCAGAT GGATTTATTG ATCGTACACC tCACGAGAGA TCCAGCTAAG G--GGC-GNA --ACTG-TGA CNAG--TA-- ---------A

                       450       460       470       480       490       500       510       520       530       540       550
C7--->A10F NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
C5--->A10R NNTNGCGGTG ---------- ---------- ---------- ---------- ----------
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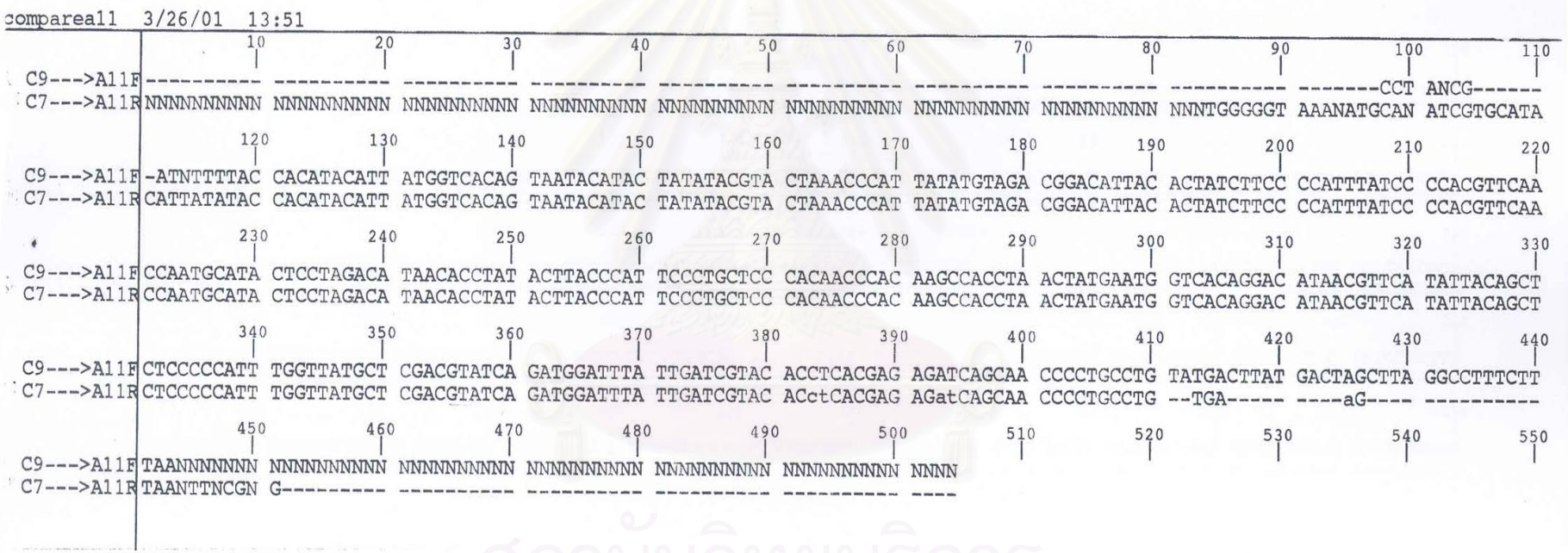

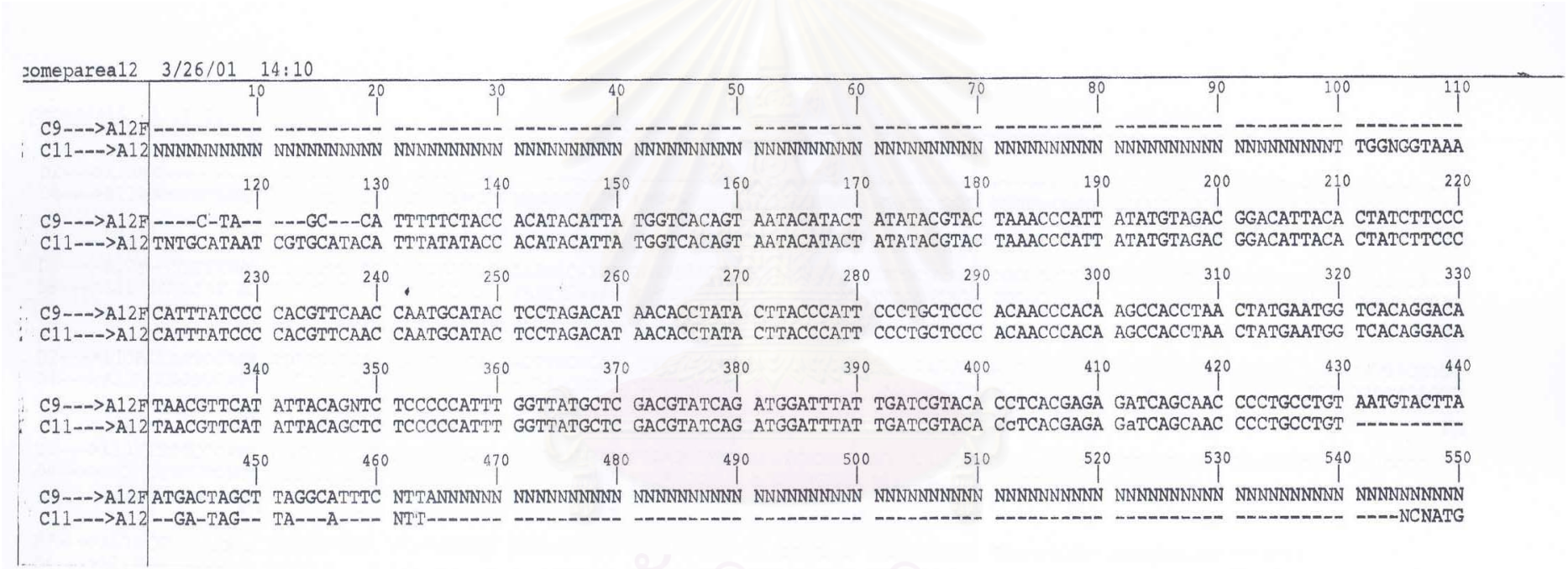

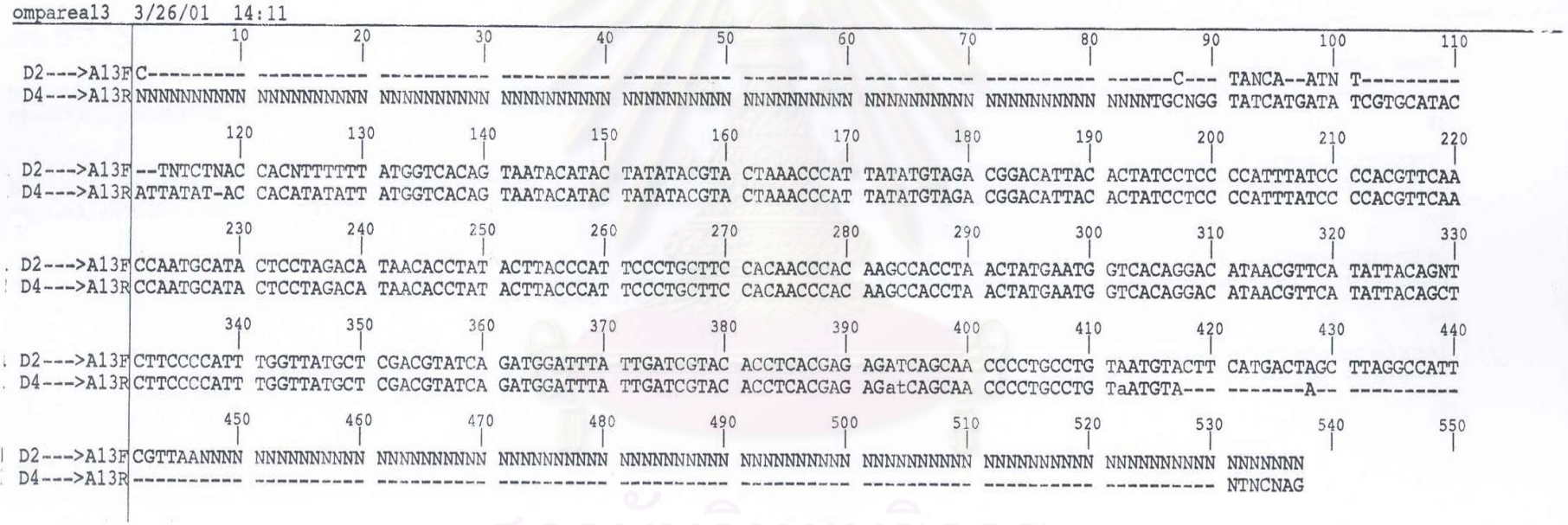

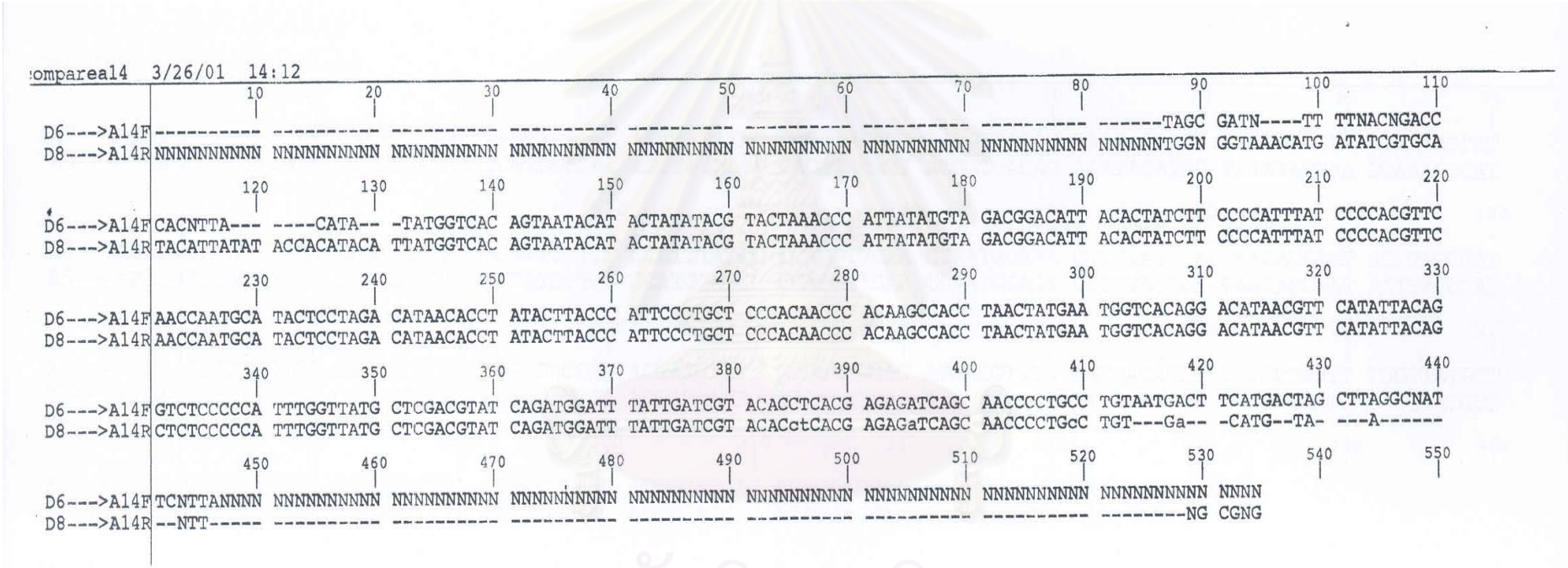

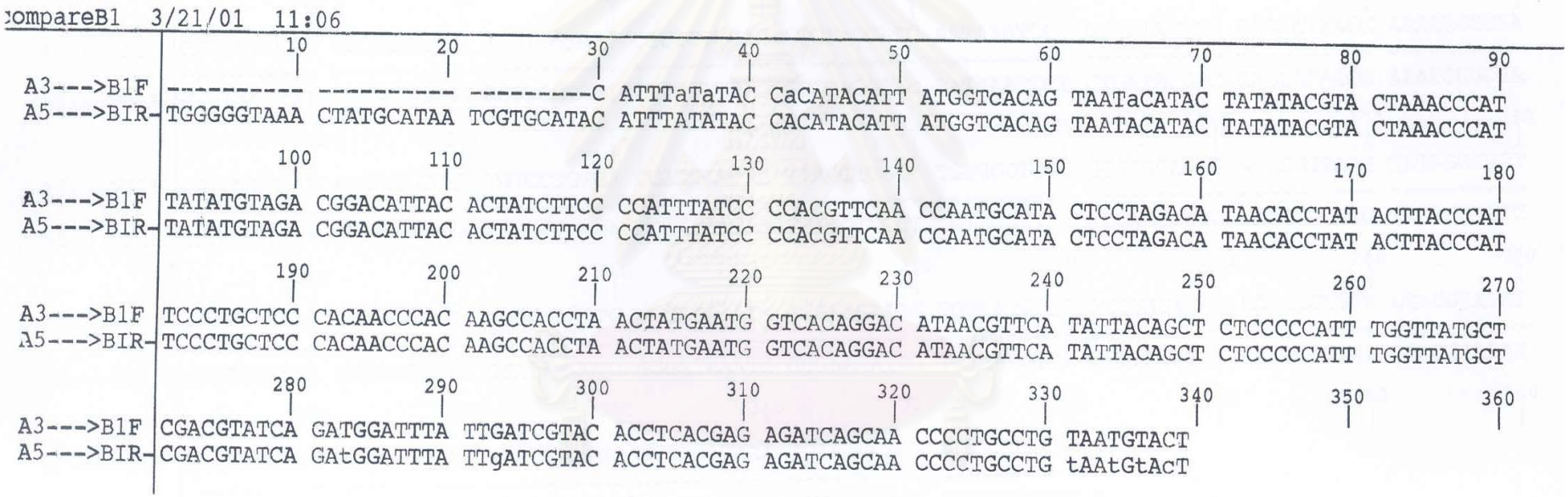

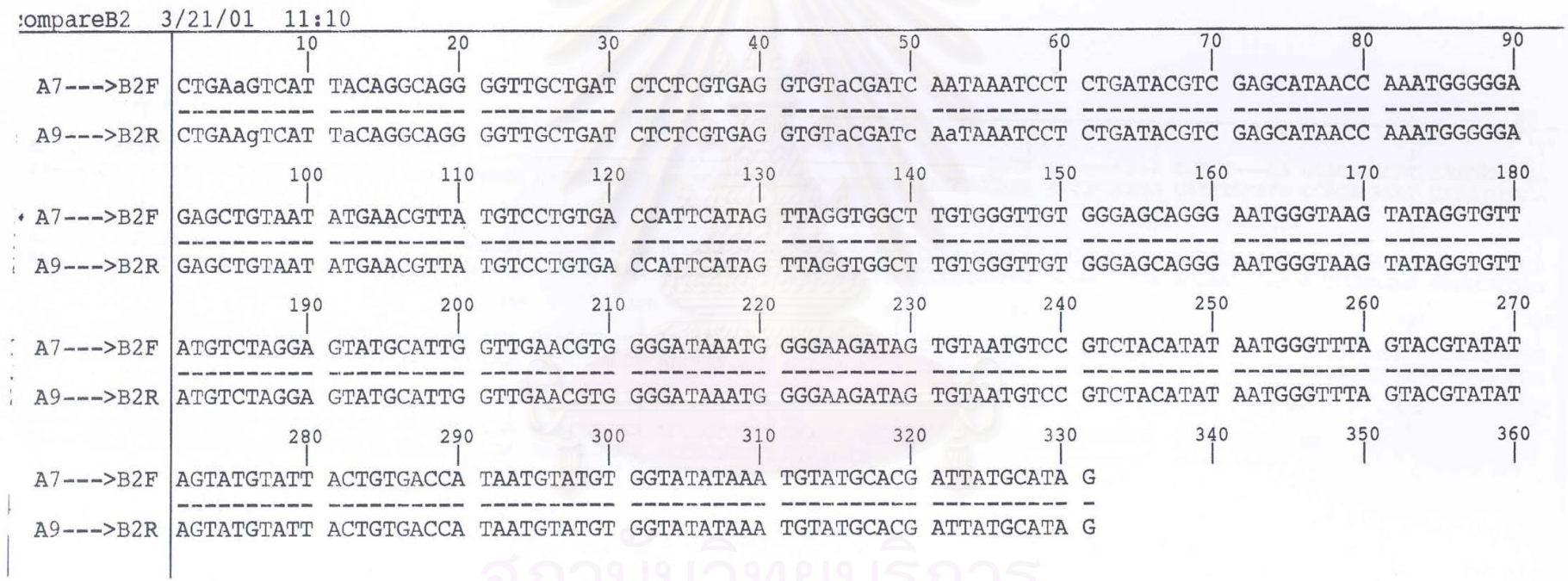

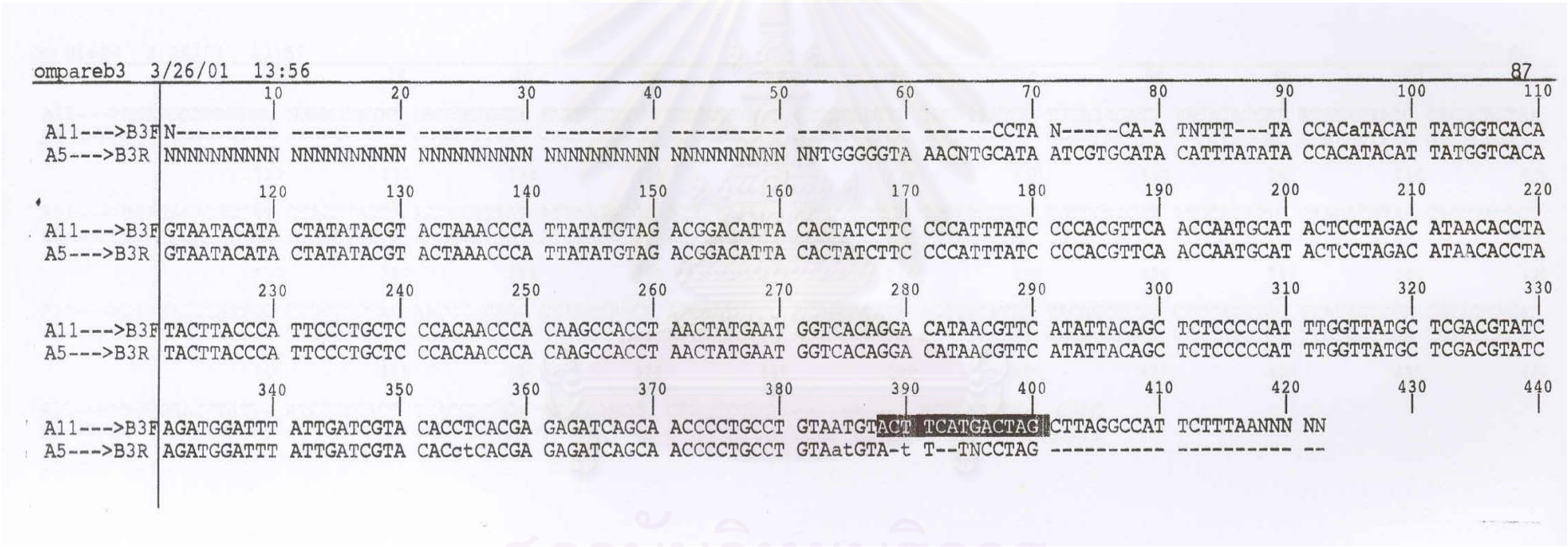

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A11--->B4R NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNTG GGGGTAACTA TGCATAATCG TGCATACATT TATATACCAC ATATATTATG GTCACAGTAA
B2--->B4F  ---------- ---------- ---------- ---------- ---------- ---------- --C------- ----TACATT T-TCTACCAC ATaTaTTATG GTCACAGTAA

                   120        130        140        150        160        170        180        190        200        210        220
A11--->B4R TACATACTAT ATACGTACTA AACCCATTAT ATGTAGACGG ACATTACACT ATCCTCCCCA TTTATCCCCA CGTTCAACCA ATGCATACTC CTAGACATAA CACCTATACT
B2--->B4F  TACATACTAT ATACGTACTA AACCCATTAT ATGTAGACGG ACATTACACT ATCCTCCCCA TTTATCCCCA CGTTCAACCA ATGCATACTC CTAGACATAA CACCTATACT

                   230        240        250        260        270        280        290        300        310        320        330
A11--->B4R TACCCATTCC CTGCTTCCAC AACCCACAAG CCACCTAACT ATGAATGGTC ACAGGACATA ACGTTCATAT TACAGCTCTT CCCCATTTGG TTATGCTCGA CGTATCAGAT
B2--->B4F  TACCCATTCC CTGCTTCCAC AACCCACAAG CCACCTAACT ATGAATGGTC ACAGGACATA ACGTTCATAT TACAGCTCTT CCCCATTTGG TTATGCTCGA CGTATCAGAT

                   340        350        360        370        380        390        400        410        420        430        440
A11--->B4R GGATTTATTG ATCGTACACC TCACGAGAGa tCAGCAACCC CTGCCTGTAT ---------- AGTAACTTN- -CNAG
B2--->B4F  GGATTTATTG ATCGTACACC TCACGAGAGA TCAGCAACCC CTGCCTGTAT GTACTTCATG ACTAGCTTAG GCNTT
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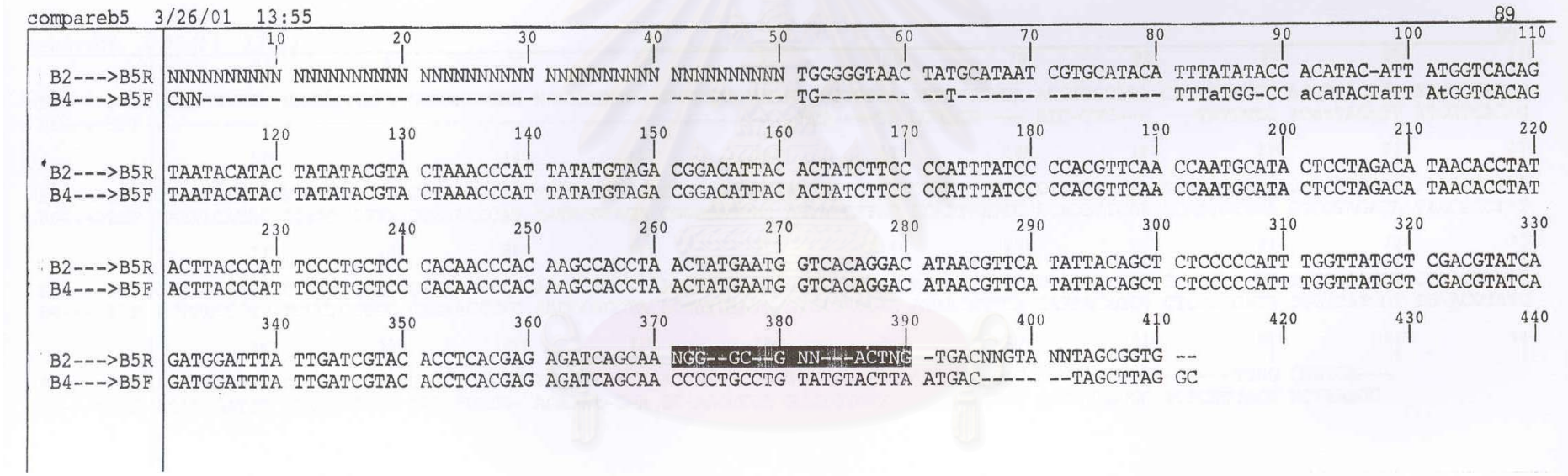

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                   10         20         30         40         50         60         70         80         90        100        110
B4--->B6R  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNGG GNNNNANNAN NNNTGGGGTA ACTATGCATA ATCGTGCATA CATTATATCC ACAT-ACATT ATGGTCACAG
B6--->B6F  ---------- ---------- ---------- ---------- ---------- -TTT----TN CCNACG---- ATC-TTC--- ---TNTGNCC ACatTACaTT ATGGTCACAG

                  120        130        140        150        160        170        180        190        200        210        220
B4--->B6R  TAATACATAC TATATACGTA CTAAACCCAT TATATGTAGA CGGACATTAC ACTATCTTCC CCATTTATCC CCACGTTCAA CCAATGCATA CTCCTAGACA TAACACCTAT
B6--->B6F  TAATACATAC TATATACGTA CTAAACCCAT TATATGTAGA CGGACATTAC ACTATCTTCC CCATTTATCC CCACGTTCAA CCAATGCATA CTCCTAGACA TAACACCTAT

                  230        240        250        260        270        280        290        300        310        320        330
B4--->B6R  ACTTACCCAT TCCCTGCTCC CACAACCCAC AAGCCACCTA ACTATGAATG GTCACAGGAC ATAACGTTCA TATTACAGCT CTCCCCCATT TGGTTATGCT CGTACGTATC
B6--->B6F  ACTTACCCAT TCCCTGCTCC CACAACCCAC AAGCCACCTA ACTATGAATG GTCACAGGAC ATAACGTTCA TATTACAGCT CTCCCCCATT TGGTTATGCT CG-ACGTATC

                  340        350        360        370        380        390        400        410        420        430        440
B4--->B6R  AGATGGATTT ATTGATCGTA CACCTCACGG AGAAGTaNCN AGCNAANGGN TG---G---G ACTGG-TGAC NAGC--AN-- ------TTAG CNGTGA---
B6--->B6F  AGATGGATTT ATTGATCGTA CACCTCACG- AGAGAt-C-A GC-AACCCCT GCCTGTATGA CTTCATGACT AGCTTAGGCC ATTCTTTACT NCTNANGC
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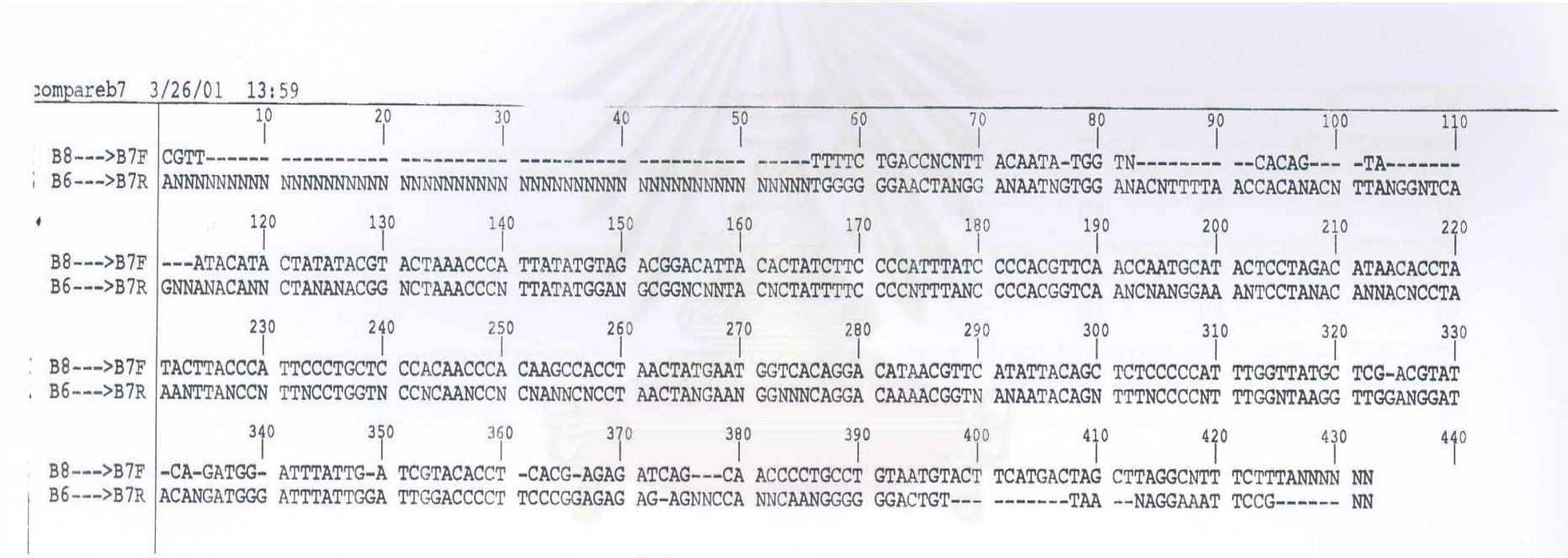

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                    10        20        30        40        50        60        70        80        90        100       110
B8--->B7F  CGTT------ ---------- ---------- ---------- ---------- -----TTTTC TGACCNCNTT ACAATA-TGG TN-------- --CACAG--- -TA-------
B6--->B7R  ANNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNTGGGG GGAACTANGG ANAATNGTGG ANACNTTTTA ACCACANACN TTANGGNTCA

                    120       130       140       150       160       170       180       190       200       210       220
B8--->B7F  ---ATACATA CTATATACGT ACTAAACCCA TTATATGTAG ACGGACATTA CACTATCTTC CCCATTTATC CCCACGTTCA ACCAATGCAT ACTCCTAGAC ATAACACCTA
B6--->B7R  GNNANACANN CTANANACGG NCTAAACCCN TTATATGGAN GCGGNCNNTA CNCTATTTTC CCCNTTTANC CCCACGGTCA ANCNANGGAA ANTCCTANAC ANNACNCCTA

                    230       240       250       260       270       280       290       300       310       320       330
B8--->B7F  TACTTACCCA TTCCCTGCTC CCACAACCCA CAAGCCACCT AACTATGAAT GGTCACAGGA CATAACGTTC ATATTACAGC TCTCCCCCAT TTGGTTATGC TCG-ACGTAT
B6--->B7R  AANTTANCCN TTNCCTGGTN CCNCAANCCN CNANNCNCCT AACTANGAAN GGNNNCAGGA CAAAACGGTN ANAATACAGN TTTNCCCCNT TTGGNTAAGG TTGGANGGAT

                    340       350       360       370       380       390       400       410       420       430       440
B8--->B7F  -CA-GATGG- ATTTATTG-A TCGTACACCT -CACG-AGAG ATCAG---CA ACCCCTGCCT GTAATGTACT TCATGACTAG CTTAGGCNTT TCTTTANNNN NN
B6--->B7R  ACANGATGGG ATTTATTGGA TTGGACCCCT TCCCGGAGAG AG-AGNNCCA NNCAANGGGG GGACTGT--- -------TAA --NAGGAAAT TCCG------ NN
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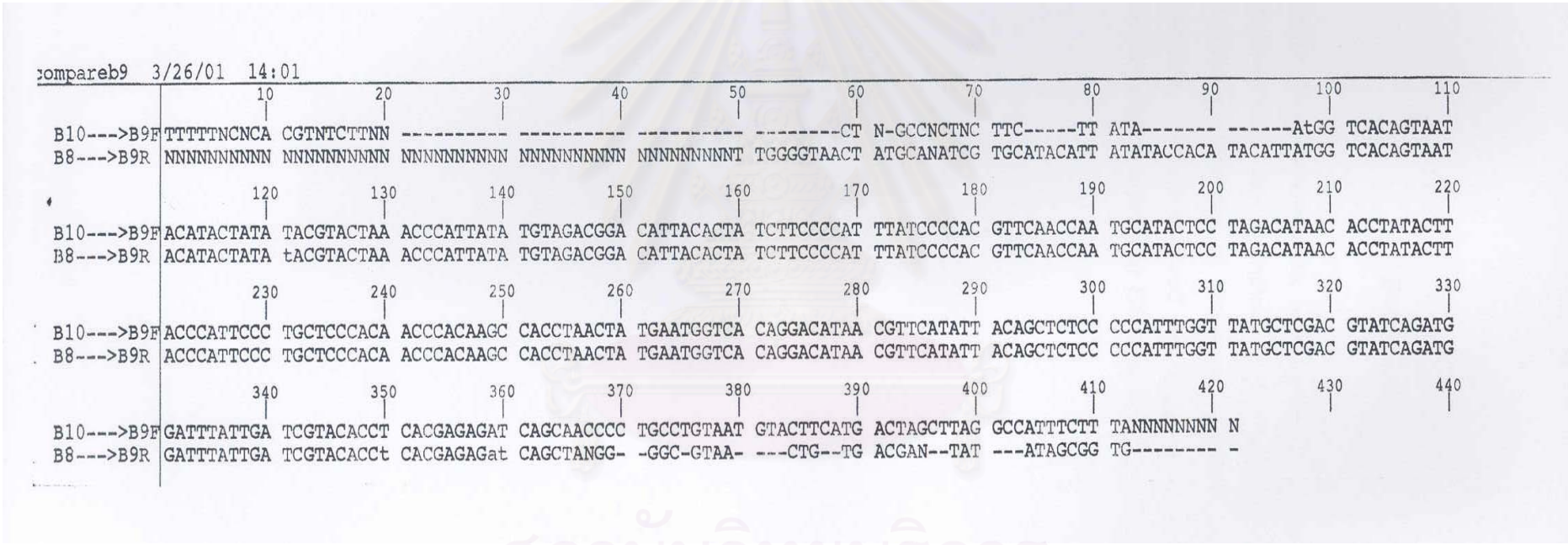

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compareb9  3/26/01  14:01

                        10        20        30        40        50        60        70        80        90       100       110
                        |         |         |         |         |         |         |         |         |         |         |
B10--->B9F TTTTTNCNCA CGTNTCTTNN ---------- ---------- ---------- --------CT N-GCCNCTNC TTC-----TT ATA------- ------AtGG TCACAGTAAT
B8--->B9R  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNT TGGGGTAACT ATGCANATCG TGCATACATT ATATACCACA TACATTATGG TCACAGTAAT

                       120       130       140       150       160       170       180       190       200       210       220
                        |         |         |         |         |         |         |         |         |         |         |
B10--->B9F ACATACTATA TACGTACTAA ACCCATTATA TGTAGACGGA CATTACACTA TCTTCCCCAT TTATCCCCAC GTTCAACCAA TGCATACTCC TAGACATAAC ACCTATACTT
B8--->B9R  ACATACTATA tACGTACTAA ACCCATTATA TGTAGACGGA CATTACACTA TCTTCCCCAT TTATCCCCAC GTTCAACCAA TGCATACTCC TAGACATAAC ACCTATACTT

                       230       240       250       260       270       280       290       300       310       320       330
                        |         |         |         |         |         |         |         |         |         |         |
B10--->B9F ACCCATTCCC TGCTCCCACA ACCCACAAGC CACCTAACTA TGAATGGTCA CAGGACATAA CGTTCATATT ACAGCTCTCC CCCATTTGGT TATGCTCGAC GTATCAGATG
B8--->B9R  ACCCATTCCC TGCTCCCACA ACCCACAAGC CACCTAACTA TGAATGGTCA CAGGACATAA CGTTCATATT ACAGCTCTCC CCCATTTGGT TATGCTCGAC GTATCAGATG

                       340       350       360       370       380       390       400       410       420       430       440
                        |         |         |         |         |         |         |         |         |         |         |
B10--->B9F GATTTATTGA TCGTACACCT CACGAGAGAT CAGCAACCCC TGCCTGTAAT GTACTTCATG ACTAGCTTAG GCCATTTCTT TANNNNNNNN N
B8--->B9R  GATTTATTGA TCGTACACCt CACGAGAGat CAGCTANGG- -GGC-GTAA- ---CTG--TG ACGAN--TAT ---ATAGCGG TG-------- -
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Biography

Miss Pattra Plubcharoensook was born on 6th of April 1975 in Bangkok, Thailand. She graduated from bachelor' degree of Science in Biology in 1996 from Department of Biology, Faculty of Science, Silpakorn University, Prarajchawang Sanamchun Campus. She continued her gradiated study for Master' Degree of Science in Biotechnology program at Chulalongkorn University in 1977.