ลักษณะสมบัติระดับโมเลกุลของไซยาโนแบคทีเรีย *Synechococcus* sp. และสาหร่ายขนาดเล็ก *Chlorella* spp. และ *Scenedesmus* spp. ที่แยกได้ในประเทศไทย

นางสาวนนทิชา แจ่มกังวาล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาจุลชีววิทยาทางอุตสาหกรรม ภาควิชาจุลชีววิทยา
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2547

ISBN 974-53-2117-6

MOLECULAR CHARACTERIZATION OF CYANOBACTERIA *Synechococcus* sp. AND MICRO-ALGAE *Chlorella* spp. AND *Scenedesmus* spp. ISOLATED IN THAILAND

Miss Nonticha Jamkangwan

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Industrial Microbiology
Department of Microbiology
Faculty of Science
Chulalongkorn University
Academic Year 2004

ISBN 974-53-2117-6

Thesis Title MOLECULAR CHARACTERIZATION OF CYANOBACTERIA *Synechococcus* sp. AND MICRO-ALGAE *Chlorella* spp. AND *Scenedesmus* spp. ISOLATED IN THAILAND

By Miss Nonticha Jamkangwan

Department Microbiology

Thesis Advisor Associate Professor Kanjana Chansa-ngavej, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillments for the Master's Degree

……………………………………………Dean of Faculty of Science
(Professor Piamsak Menasveta, Ph.D.)

THESIS COMMITTEE

…………………………………………… Chairperson
(Associate Professor Dr. Pairoh Pinphanichakarn)

……………………………………………..Thesis Advisor
(Associate Professor Kanjana Chansa-ngavej, Ph.D.)

………………………………………………Member
(Associate Professor Yuwadee Peerapornpisal, Ph. D.)

…………………………………………… Member
(Associate Professor Wipa Chungjatupornchai, Ph. D.)

นนทิชา แจ่มกังวาล : ลักษณะสมบัติระดับโมเลกุลของไซยาโนแบคทีเรีย *Synechococcus* sp. และ
สาหร่ายขนาดเล็ก *Chlorella* spp. และ *Scenedesmus* spp. ที่แยกได้ในประเทศไทย
(MOLECULAR CHARACTERIZATION OF CYANOBACTERIA *Synechococcus* sp.
AND MICRO-ALGAE *Chlorella* spp. AND *Scenedesmus* spp. ISOLATED IN THAILAND)
อาจารย์ที่ปรึกษา : รศ. ดร. กาญจนา ชาญสง่าเวช,130 หน้า ISBN 974-53-2117-6

ไซยาโนแบคทีเรียและสาหร่ายขนาดเล็กเป็นจุลินทรีย์ที่มีศักยภาพเชิงพาณิชย์ แต่ยังไม่มีการนำมาใช้อย่างแพร่หลายในการผลิตผลิตภัณฑ์ที่มีประโยชน์ เป็นที่ทราบกันดีว่าเมื่อเก็บรักษาและนำจุลินทรีย์มาใช้ในอุตสาหกรรมต่างๆอย่างต่อเนื่องจะเกิดการเปลี่ยนแปลงสารพันธุกรรม วัตถุประสงค์ของการทดลองนี้เพื่อหาลักษณะสมบัติระดับโมเลกุลของ *Synechococcus* sp., *Chlorella* spp., และ *Scenedesmus* spp. ที่แยกในประเทศไทย โดยหาลายพิมพ์ดีเอ็นเอด้วยวิธี RAPD-PCR , ลำดับนิวคลีโอไทด์ของ 16S rDNA, และ โพรไฟล์ของโปรตีนภายในเซลล์ระยะ mid-log, ระยะ early stationary และระยะ late stationary ได้แยกและฝากเชื้อบริสุทธิ์ 1 สายพันธุ์ของ *Synechococcus* sp., 6 สายพันธุ์ของ *Chlorella* spp., และ 9 สายพันธุ์ของ *Scenedesmus* spp. ที่ Bangkok MIRCEN ภายใต้รหัส TISTR 8867; TISTR 8852 ถึง TISTR8857 และ TISTR8858 ถึง TISTR 8866 โพรไฟล์ของโปรตีนภายในเซลล์ ได้จากการแยกแถบโปรตีนโดยวิธี SDS-PAGE ผลการทดลองบ่งชี้ได้ว่า ไพรเมอร์ที่ใช้ในการหาลำดับนิวคลีโอไทด์ของ 16S rDNA ของแบคทีเรียแกรมลบ *E.coli* ได้แก่ ไพรเมอร์ 27f, 343r, 519r, 787r, 907r, 1100r, 1241f, 1385r และ 1492r สามารถนำมาใช้ในการหาลายพิมพ์ดีเอ็นเอโดยวิธี RAPD-PCR (ไพรเมอร์ 27f, 343r, 1100r, 1492r และ CRL-7) และสามารถใช้ไพรเมอร์ทั้ง 9 ชนิดในการหาลำดับนิวคลีโอไทด์ของ 16S rDNA ของไซยาโนแบคทีเรีย *Synechococcus* sp. TISTR 8867 คลอโรพลาสต์ของสาหร่ายสีเขียวขนาดเล็ก *Chlorella* sp. TISTR 8852 และ *Scenedesmus* sp. TISTR 8859 ผลการแยกโปรตีนโดยวิธี SDS-PAGE ชี้ให้เห็นว่าเซลล์ที่การเจริญระยะต่างๆของ *Synechococcus* sp. TISTR 8867, *Chlorella* sp. TISTR 8852, และ *Scenedesmus* sp. TISTR 8859 มีโปรตีนโพรไฟด์คล้ายกัน พบพอลิเปปไทด์ 46, 16.5, 15 และ 14 กิโลดาลตัน มากใน *Chlorella* sp. TISTR 8852 และพบพอลิเปปไทด์ 23 กิโลดาลตันมากใน *Synechococcus* sp. TISTR 8867 และ *Scenedesmus* sp. TISTR 8859 พอลิเปปไทด์ 43 กิโลดาลตัน อาจเฉพาะเจาะจงต่อไซยาโนแบคทีเรียซึ่งเป็นโพรคาริโอต ข้อมูลลักษณะสมบัติระดับโมเลกุลที่พบในงานวิจัยนี้ ไม่เพียงแต่เป็นประโยชน์ต่อการประยุกต์ในอุตสาหกรรม แต่ยังมีส่วนเสริมความก้าวหน้างานวิจัยด้านพันธุศาสตร์ระดับโมเลกุลของไซยาโนแบคทีเรียและสาหร่ายขนาดเล็กในประเทศไทย

ภาควิชา จุลชีววิทยา ลายมือชื่อนิสิต..
สาขาวิชา จุลชีววิทยาทางอุตสาหกรรม ลายมือชื่ออาจารย์ที่ปรึกษา..
ปีการศึกษา 2547

##4672288023 : MAJOR INDUSTRIAL MICROBIOLOGY
KEY WORDS : DNA FINGERPRINTS, INTRACELLULAR PROTEIN PROFILES, *Synechococcus* spp.,
Chlorella spp., *Scenedesmus* spp.

NONTICHA JAMKANGWAN: MOLECULAR CHARACTERIZATION OF CYANOBACTERIA
Synechococcus sp. AND MICRO-ALGAE *Chlorella* spp. AND *Scenedesmus* spp. ISOLATED IN
THAILAND
THESIS ADVISOR : ASSOCIATE PROFESSOR KANJANA CHANSA-NGAVEJ, Ph.D. 130 pp.
ISBN 974-53-2117-6

Cyanobacteria and micro-algae are micro-organisms whose potential is largely untapped. It is well-known that upon storage and continuous utilization of micro-organisms in industries, changes in genetic materials occur. The aims of the experiments are to characterize local isolates of *Synechococcus* sp., *Chlorella* spp., and *Scenedesmus* spp. by RAPD-PCR fingerprinting and 16S rDNA sequencing and obtain intracellular protein profiles of mid-log phase, early stationary phase, and late stationary phase cells. Pure cultures of one strain of *Synechococcus* sp., six strains of *Chlorella* spp., and nine strains of *Scenedesmus* spp. were obtained and deposited at Microbiological Resources Center (Bangkok MIRCEN) under the following codes : TISTR 8867;TISTR 8852 to TISTR 8857;and TISTR 8858 to TISTR 8866. Intracellular protein profiles were obtained by SDS-PAGE. Results revealed the following primers (27f, 343r, 519r, 787r, 907r, 1100r, 1241f, 1385r, and 1492r) which were normally used to obtain Gram negative *E. coli* 16S rDNA sequence could be used to obtain RAPD-PCR fingerprints of all isolated cultures (primers 27f, 343r, 1100r, 1492r and CRL-7). 16S rDNA sequences of *Synechococcus* sp. TISTR 8867, and chloroplast 16S rDNA of *Chlorella* sp. TISTR 8852 and *Scenedesmus* sp.TISTR 8859 were also obtained with the use of all the nine primers. SDS-PAGE separation of proteins indicated cells of *Synechococcus* sp. TISTR 8867, *Chlorella* sp. TISTR 8852, and *Scenedesmus* sp. TISTR 8859 at different stages of growth exhibited similar intracellular protein profiles. Polypeptides 46, 16.5, 15 and 14 kDa were found in abundance in *Chlorella* sp. TISTR 8852 while more polypeptide 23 kDa was found in *Synechococcus* sp. TISTR 8867 and *Scenedesmus* sp. TISTR 8859. Polypeptide 43 kDa may be specific for prokaryotic cyanobacteria. Molecular data obtained in this research are not only useful for industrial applications but also contribute to the advancement of molecular genetics research on cyanobacteria and micro-algae in Thailand.

Department Microbiology Student's signature……………………………………..
Field of study Industrial Microbiology Advisor's signature…………………………..................
Academic year 2004

Acknowledgements

I wish to express sincere thanks and gratitude to my thesis advisor, Associate Professor Dr Kanjana Chansa-ngavej, for her tireless efforts as well as valuable advice and comments throughout the course of this study.

I would also like to thank Associate Professor Dr. Pairoh Pinphanichkarn for serving as the thesis committee chairperson and Associate Professor Dr Yuwadee Peerapornpisal, Associate Professor Dr Wipa Chungjatupornchai for serving as thesis committee members and their recommendations for the research.

Special thanks are given to student members in laboratory 404, all friends, and all staff members in the Department of Microbiology, especially, Mr.Weerasak Jungfungprinya, for their help and friendship during my study.

The last, but most important, is my sincere and deepest gratitude to my parents and everyone in my family for their great love, constant support, understanding and heartfelt encouragement extended throughout my study.

Contents

Page

Abstract (in Thai)……………………………………………………………………………..iv

Abstract (in English)…………………………………………………………………………v

Acknowledgements…………………………………………………………………….....vi

Content of Figures…………………………………………………………………………ix

Content of Tables…………………………………………………………………………..xi

Chapters

I Introduction……………………………………………………………………...1

II Literature Survey………………………………………………………………..3

Molecular characterization of cyanobacteria and micro-algae……………4

Molecular characterization of *Synechococcus* spp…………………………5

Molecular chracterization of *Chlorella* spp. and *Scenedesmus* spp……...8

Research on molecular characterization of cyanobacteria and micro-algae in Thailand…………………………………………………...9

III Materials & Methods……………………………………………………………10

Sample collection and isolation of cultures………………………………...10

RAPD-PCR fingerprinting………………………………………………..10

16S rDNA sequencing……………………………………………………12

Determination of changes in protein profiles………..............................13

IV Results……………………………………………………………………………15

V Discussion…………………………………………………………………………48

VI Conclusion……………………………………………………………………..…..50

References…………………………………………………………………………………59

Appendices………………………………………………………………………………..64

Appendix A: Cyanobacteria and Micro-algae Growth Media……… ……………………64

Appendix B: Chemicals and Solutions…………………………………………………......66

Appendix C: 16S rDNA Sequences……………………………………………………….69

- First determination for *Synechococcus* sp. NJ7………………………………...70

  Chlorella sp. NJ26……………………….....................79

*Scenedessmus*sp. NJ8 ………………………..............88

- Second determination for *Synechococcus* sp. NJ7…………………………….97

Chlorella sp. NJ26 ………………………………….106

Scenedessmus sp. NJ8…………………………….115

- Comparisons between sequences obtained from the first and the second determinations.…………………………………………………………………124

Biography…………………………………………………………………………………...130

Content of Figures

Page

Figure 2.1 Diagram of a general *rrn* operon……………………………………………….5

Figure 2.2 Predicted secondary structure of the ITS region of the rRNA operon………...6

Figure 2.3 Predicted secondary structures of the 16S-23S rRNA ITS……………………..7

Figure 2.4 A dendrogram constructed from 434 nucleotide positions…………………….8

Figure 4.1 Representative morphology of *Synechococcus* sp……………………………16

Figure 4.2 RAPD-PCR fingerprints of isolates NP4, NJ7, NJ21, NJ22, and NJ28………17

Figure 4.3 Representative morphology of isolates NJ1, NJ3, NJ5, NJ26, NJ27, NJ29, NJ31, NJ41, and NJ43…………………………………… ………………………18

Figure 4.4 RAPD-PCR fingerprints of the isolates NJ1, NJ3, NJ5, NJ26, NJ27, NJ29, NJ31, NJ41, NJ43, and N-686……………………………………..……….19

Figure 4.5 Representative morphology of isolates NJ9, NP10, NJ19, NJ35, and NJ37……………………………………………………………………………20

Figure 4.6 RAPD-PCR fingerprints of NJ9, NP10, NJ19, NJ35, and NJ37 ……………....21

Figure 4.7 Morphology of isolates NJ24 and NJ30..…………………………………….22

Figure 4.8 RAPD-PCR fingerprints of NJ24 and NJ30…….………………………………..22

Figure 4.9 Cell morphology of isolates NJ34, NJ46, NJ49, and NJ50……………………23

Figure 4.10 RAPD-PCR fingerprints of isolates NJ34, NJ46, NJ49, and NJ50…..……..24

Figure 4.11 Representative morphology of *Scenedesmus* sp. NP2, NP6, NJ14, NJ18 and NJ39……………………………………………………………………..25

Figure 4.12 RAPD-PCR fingerprints of *Scenedesmus* spp. NP2, NP6, NJ14, NJ18 and NJ39……………………………………………………………………26

Figure 4.13 Morphology of *Scenedesmus quadricauda* isolates NJ23, NJ40, and NJ45…………………………………………………………………………27

Figure 4.14 RAPD-PCR fingerprints of *Scenedesmus quadricauda* isolates NJ23, NJ40, and NJ45……………………………………………………………28

Figure 4.15 Morphology of isolates NJ8, NJ12, NJ20, NJ25, NJ42, NJ47, and NJ48……………………………………………………………………………29

Figure 4.16 RAPD-PCR fingerprints of isolates NJ8, NJ12, NJ20, NJ25, NJ42, NJ47, and NJ48…………………..……………………………………………….30

Figure 4.17 Cell morphology of the reference strain, *Synechococcus* sp. NIES-946…..31

Figure 4.18 Cell morphology of the reference strain, *Chlorella vulgaris* var *vulgaris* NIES-686…………………………………………………………………………..32

Figure 4.19 Cell morphology of the reference strain, *Scenedesmus dimorphus* NIES-93……………………………………………………………………………32

Figure 4.20 Summary of RAPD-PCR fingerprints of 16 strains when 27f was used as the primer………………...33

Figure 4.21 Summary of RAPD-PCR fingerprints of 16 strains when 343r was used as the primer ……………………………………………………………………...34

Figure 4.22 Summary of RAPD-PCR fingerprints of 16 strains when 1100r was used as the primer ……………………………………………………………………...34

Figure 4.23 Summary of RAPD-PCR fingerprints of 16 strains when 1492r was used as the primer ……………………………………………………………………. . .35

Figure 4.24 Amplified 16S rDNA products…………………………………………….35

Figure 4.25 16S rDNA sequence of *Synechococcus* sp. NJ7 (TISTR 8867) …………...37

Figure 4.26 Homology comparison between 16S rDNA of *Synechococcus* sp. NJ7 (TISTR 8867) and data in GenBank………...39

Figure 4.27 16S rDNA sequence of *Chlorella* sp. NJ26 (TISTR 8852) ………………….40

Figure 4.28 Homology comparison between 16S rDNA of *Chlorella* sp. NJ26 (TISTR 8852) and data in GenBank …………………………………………..42

Figure 4.29 16S rDNA sequence of *Scenedesmus* sp. NJ8 (TISTR 8859)……………..43

Figure 4.30 Homology comparison between 16S rDNA of *Scenedesmus* sp NJ8 (TISTR 8859) and data in GenBank ……. ……………………………………45

Figure 4.31 Growth curves of *Synechococcus* sp.NJ7 (TISTR 8867), *Chlorella* sp. NJ26 (TISTR 8852), and *Scenedesmus* sp. NJ8 (TISTR 8859)……………46

Figure 4.32 SDS-PAGE of intracellular protein profiles…………………………………...47

Content of Tables

Page

Table 4.1 Locations of freshwater bodies where samples were collected for the identification of cyanobacteria and micro-algae……………………………15

Table 4.2 Summary of homology comparisons of 16S rDNA sequences………………36

CHAPTER I

INTRODUCTION

At present cyanobacteria and micro-algae have not been widely used on a commercial scale. One reason may be because it is relatively difficult to isolate pure cultures of cyanobacteria and micro-algae for use in industries. Commercially available products from cyanobacteria and micro-algae are usually those that are cultivated as mixed cultures of cyanobacteria or micro-algae and other microbial identities. Examples are the production of single-celled *Spirulina* in open ponds for supplementary food and feed and the production of β carotene by *Dunaliella bardawil* along the coast of South Australia (Borowitzka, 1986; Becker, 1994).

Identification of cyanobacteria and micro-algae is usually carried out by observing their morphology under the microscope. However, in some cases cyanobacteria and micro-algae isolated from different water bodies have similar morphology with differences only in size. These micro-organisms may or may not be the same species. The observations have given rise to the concept of “cryptic species” which refers to various genotypic strains within the same phenotypic “species” (Cassamatta et al., 2003). It has been suggested that both morphology and molecular characterization should be taken into account when identifying cyanobacteria and micro-algae and that there might be “local strains” rather than “global strains”.

Several molecular techniques have been used to characterize cyanobacteria and micro-algae. However, no attempt has so far been made to employ sequencing primers which are used in the sequencing of 16S rDNA of bacteria to characterize 16S rDNA of cyanobacteria and chloroplats of micro-algae.

The aims of the experiments are to obtain pure cultures of cyanobacteria and micro-algae and to use bacterial 16S rDNA sequencing primers to characterize local isolates of *Synechococcus* sp., *Chlorella* spp. and *Scenedesmus* spp. by RAPD-PCR fingerprinting and 16S rDNA sequencing in order provide more evidence of the presence of cryptic species in *Chlorella* spp., and *Scenedesmus* spp., In addition, intracellular protein profiles of mid-log phase, early stationary phase, and late stationary phase cells will also be obtained. RAPD-PCR fingerprints may be used in the monitoring of changes in genetic materials of these microorganisms which result in changes in industrial performance.

CHAPTER II

LITERATURE SURVEY

Cyanobacteria and micro-algae are commonly found in soils and water bodies as well as snow-covered areas and hot springs. Identification of these micro-organisms is usually carried out by observing their morphology under the microscope and using picture keys contributed by Desikachary, T. V. (1959), or Prescott (1970) or John et al.(2003) or referring to published articles including Stanier et al. (1971), or Rippka et al. (1979). However, sometimes cyanobacteria and micro-algae that are isolated from different water bodies have similar morphology with differences only in size. These micro-organisms may or may not be the same species. The observations have given rise to the concept of “cryptic species” which refers to various genotypic strains in the same phenotypic “species”. In 2003, Cassamatta et al isolated 12 morphotypes which fitted the description of *Phormidium retzii* from various freshwater bodies in Canada, The US, Mexico, and Costa Rica, for use as a model in the study of cryptic species. Seven primers purchased from Operon Technologies, Inc., USA, were used to obtain RAPD-PCR (Random Amplified Polymorphic DNA-PCR) fingerprints of the morphotypes. Numerical allocation of 1 was used to represent the presence of a PCR product (a DNA fragment) in fingerprint of one morphotypes. Number 0 was allocated for the absence of the DNA fragment in the fingerprints of other morphotypes. Analysis of similarity matrix with the program Ny.Syt V.2 showed that *P. retzii* which were isolated from different geographic areas belonged to the same cluster and the strains that were isolated from nearby locations belonged to different clusters. Genetic similarity was not found to depend on the distance of freshwater bodies. In addition 16S rDNA sequences of eight of the *P. retzii* strains revealed that three of the strains might belong to the same species based on the similarity index among pair-wise comparisons of 16S rDNA data of more than 0.975. The authors concluded that there might be more than one species among the 12 strains of *P. retzii*. It was suggested that both morphology and genetic characterization should be taken into account when cyanobacteria were identified and that there might be “local strains” rather than “global strains”.

Molecular characterization of cyanobacteria and micro-algae

Several molecular techniques have been used to characterize cyanobacteria and micro-algae. Techniques such as PCR have mostly been used to characterize toxic cyanobacteria *Microcystis aeruginosa* which produce hepato-toxins, microcystins (Neilan, 1995). Baker et al. (2002) reported that the use of the phycocyanin intergenic spacer (PC-IGS) between *cpc B* and *cpc A* which encode the β and α subunits of phycocyanin was specific for the detection of cyanobacteria *Microcystis aeruginosa* in algal blooms. Moreover, toxigenic strains of *M.aeruginosa* were detected by the presence of an approximately 1300 bp PCR product when primers for the amplification of the N-methyl tranferase (NMT) domain of the microcystin synthetase gene, *mcyA*, in the microcystin biosynthetic pathway were used.

Neilan et al. (1997b) and Otsuka et al. (1999) used the 16S-23S rRNA internal transcribed spacer region (ITS) for the differentiation among toxic and non-toxic *M. aeruginosa* isolates. Rouhiainen et al. (1995) utilized repetitive heptamers (5'GGGGACTGGGGACTGGGGACTGGGG3') as a probe to perform Southern blot hybridization with *Hind*III or *Hinc*II digested genomes of 15 strains of *Anabaena* spp. and 2 strains of *Nostoc* spp. The hybridization results indicated that the RFLP patterns obtained could be used to discriminate *Anabaena* spp. which produced hepato-toxins from those that produced neurotoxins. The authors could also discriminate *Nostoc* sp. which produced hepato-toxins from the non-producing strain. 16S rDNA sequences as well as sequences of *rpo* c1 which encodes RNA polymerase have been used to identify different genotypic strains of *Anabaena circinalis*, *Microcystis aeruginosa*, and *Synechococcus* spp. (Fergusson & Saint, 2000; Neilan et al., 1997a; Toledo & Palenik, 1997). Nubel et al. (1997) reported that comparisons between 16S rDNA sequences could lead to the finding if cyanobacteria occurring in nature were the same strains as those cultivated in the laboratory. This comparison is possible because when environmental conditions change, 16S rDNA sequences do not change. In 1997 Nubel et al.(1997) performed multiple alignments on 16S rDNA sequences of all the 174 cyanobacteria strains deposited in GenBank (Benson et al., 1997) and in the Ribosome Database Project (Maidak et al., 1997) to obtain cyanobacterial specific primers

CYA106F and CYA359F which were interchangeable and the reverse primer (CYA781R) was found to be a solution of equal molarity between CYA781R (a) and CYA781R (b).

Molecular characterization of *Synechococcus* spp.

In 2002, Rocap et al. used 16S-23S ribosomal DNA internal transcribed spacer sequences (ITS) to resolve *Synechococcus* ecotypes.

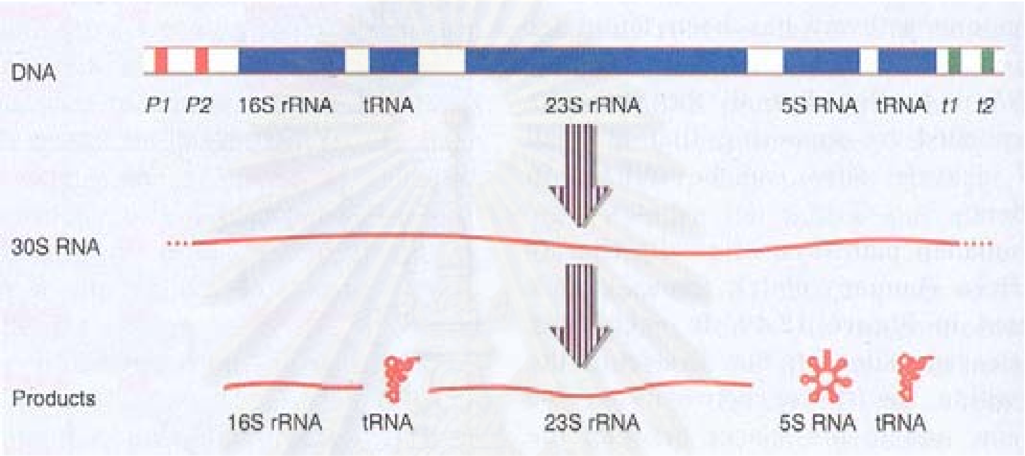
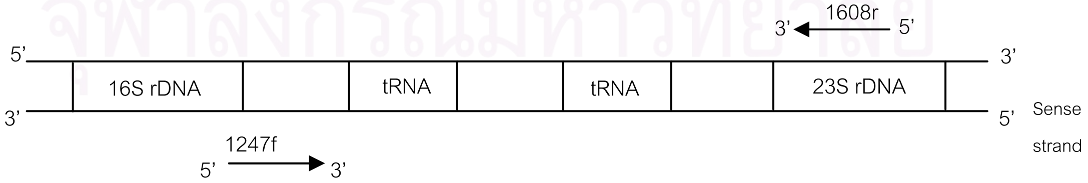


Figure 2.1 Diagram of a general *rrn* operon (Lewin, 1997)

In most eubacteria, Rocap et al. (2002) reported that genes for rRNA are organized in operon, with gene encoding the 16S, 23S, and 5S rRNAs separated by internal transcribed spacer (ITS) regions as shown in Figure 2.1. Primers 16S-1247f and 23S-1608r were used to amplify 16S rDNA-23S rDNA internal transcribed spacers of several strains of *Synechococcus* spp. The PCR products were sequenced using Big Dye Terminator Sequencing Kit and ABI 310 Genetic Analyzer (Perkin-Elmer).



Secondary structures and complete 16S rDNA-23S rDNA internal transcribed spacer sequences of *Synechococcus* strain WH 8102 and other strains were obtained as shown in the following figures.

Rocap et al. (2002) reported that differences in the ITS sequences as observed in the different secondary structures of *rrn* operons as shown in Figure 2.2 to Figure 2.3 indicated that part of the conserved ITS sequences could be used as primers for RAPD-PCR fingerprinting.

Figures 2.2 and 2.3 indicated that the ITS regions of 7 strains of *Synechococcus* contained tRNAs for Isoleucine and for Alanine.

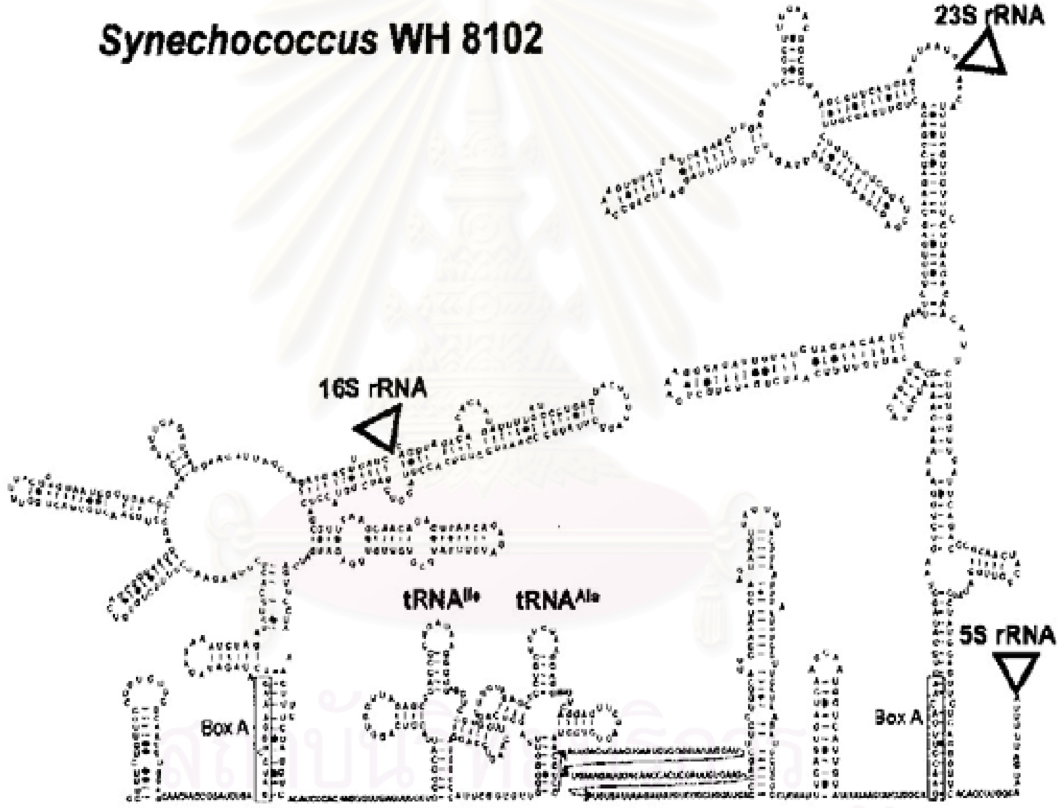
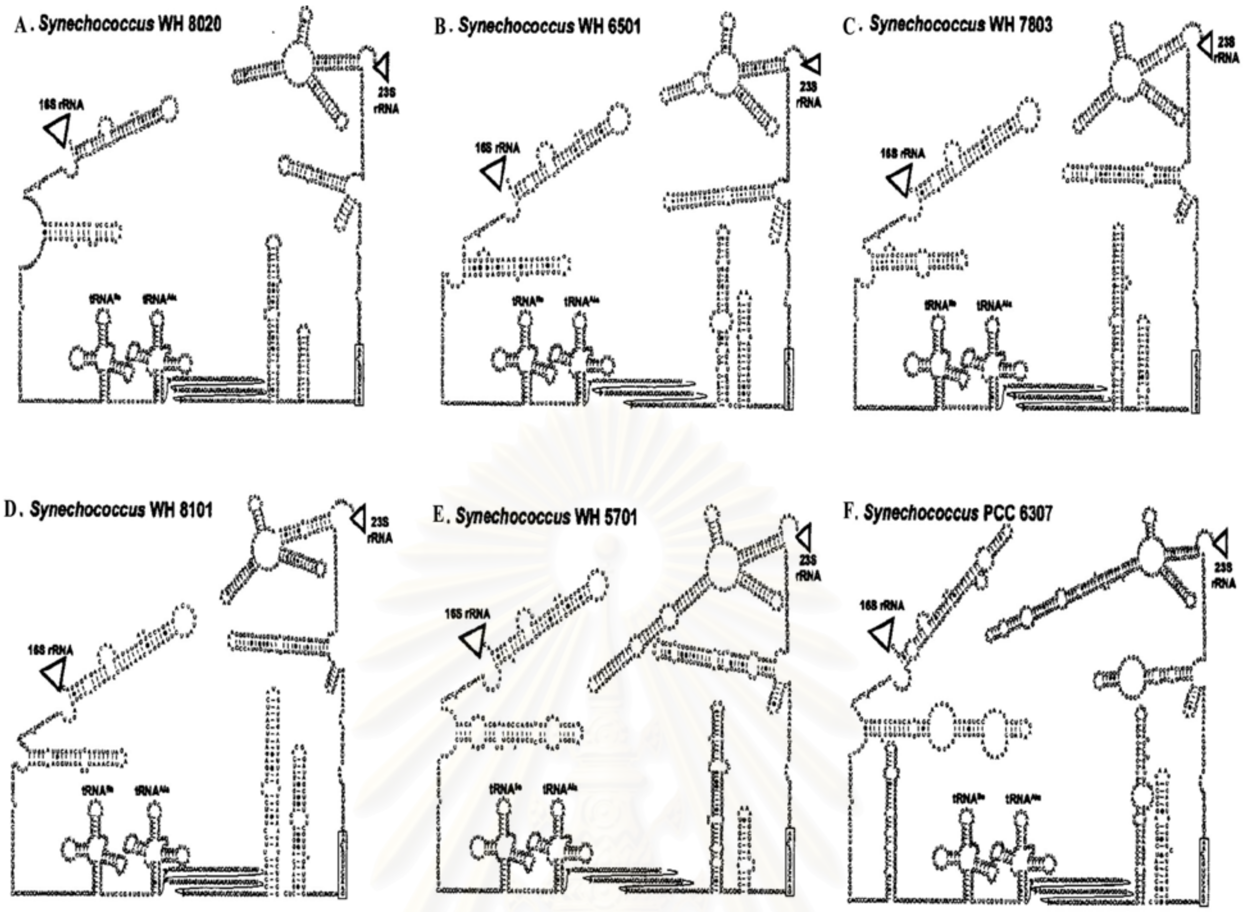


Figure 2.2 Predicted secondary structure of the ITS region of the rRNA operon in *Synechococcus* strain WH 8102. Locations of the 16S rRNA, 23S rRNA, and 5S rRNA are represented by triangles conserved in most bacterial ITS sequences, as well as genes for tRNA isoleucine and alanine typical of cyanobacterial ITS sequences. Sequence corresponding to the box A motif is enclosed in a rectangle. The 5' region of the tRNA^{Ala}-23S rRNA spacer (between the tRNA^{Ala} and the box A loop) for which no structure was inferred is shown in three rows of text to save space. (Rocap et al., 2002)



A. *Synechococcus* WH 8020

B. *Synechococcus* WH 6501

C. *Synechococcus* WH 7803

D. *Synechococcus* WH 8101

E. *Synechococcus* WH 5701

F. *Synechococcus* PCC 6307

Figure 2.3 Predicted secondary structures of the 16S-23S rRNA ITS in six *Synechococcus* strains identified by Rocap et al. (2002), (A) marine cluster A *Synechococcus* strain WH 8020, (B) marine cluster A *Synechococcus* strain WH 6501, (C) marine cluster A *Synechococcus* strain WH 7803, (D) marine cluster B *Synechococcus* strain WH 8101, (E) marine cluster B *Synechococcus* strain WH 5701, (F) *Synechococcus* strain PCC 6307. (Rocap et al, 2002).

Alignment of 434 nucleotide positions in the 16S-23S rDNA spacer (not including nucleotides of tRNAs) for phylogenetic analyses revealed that there were many ecotypes of marine *Synechococcus* spp. thriving in different micro-environments as shown in Figure 2.4 (Rocap et al., 2002)

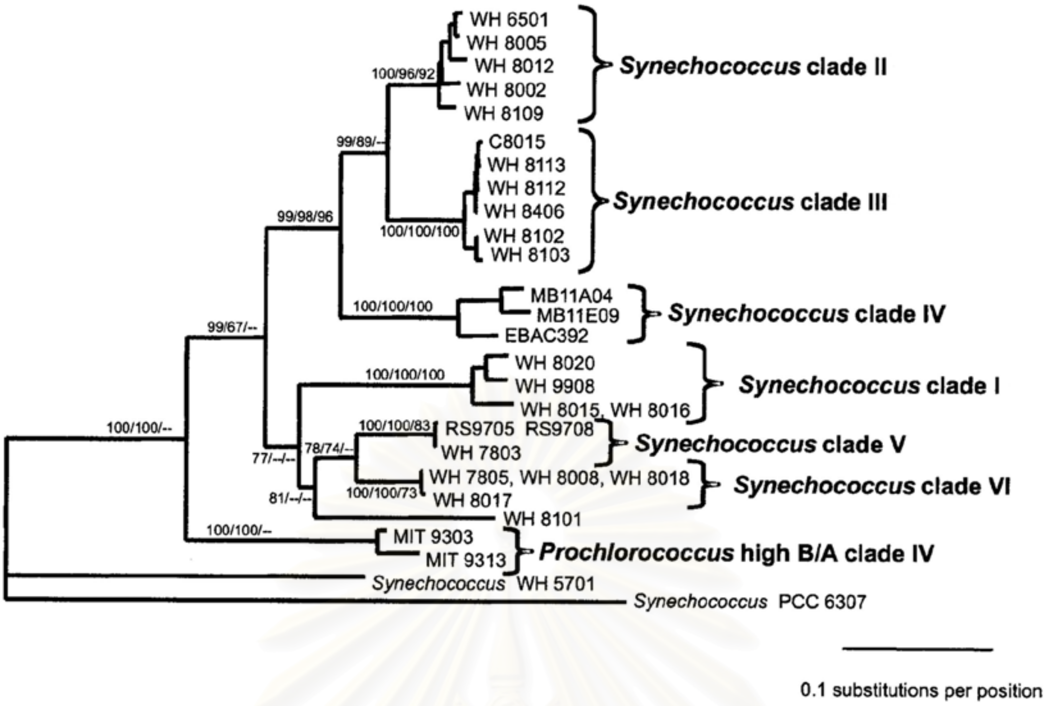


Figure 2.4 A dendrogram constructed from 434 nucleotide positions of the 16S-23S rDNA spacer regions. (Rocap et al., 2002)

Molecular chracterization of *Chlorella* spp. and *Scenedesmus* spp.

From the literature survey, most of the research conducted with green micro-algae *Chlorella* spp. and *Scenedesmus* spp. has been on algal physiology and applications such as metal accumulation (Costa et al., 1994; Jie et al., 2001), effects of pollutants and/or herbicides/pesticides on growth (Ma, 2000; Ma et al., 2002), effects of nutrients/ inhibitors/ light regimes on cell growth (Zachleder et al., 2002), design of reactors for cultivation of cells for useful products (Mundt et al., 2001), enzymes purification, characterization of genes encoding enzymes and genetic engineering (Dawson et al., 1997), immobilized *Scenedesmus quadricauda* for long-term storage and for application for water quality control in fish culture (Chen, 2001).

There are three sources of small subunit ribosomal RNA genes in eukaryotic algae including the green algae *Chlorella* spp. and *Scenedesmus* spp. These are 16S rRNA genes in chloroplasts and mitochondria as well as the 18S rRNA genes. In *Chlorella ellipsoidea* 18S rDNA, Group I intron has been reported (GenBank accession no X 63520).

Huss et al (1999) studied the biological taxonomy and molecular phylogeny of the genus *Chlorella sense* Lato (Chlorophyta). 18S rRNA gene sequences of *Chlorella* *ellipsoidea* Gerneck (GenBank accession no X 63520, Huss et al, 1992), *Chlorella fusca* var. *vacuslata* Shihira & Krauss (GenBank accession no X 56104, Huss & Sogin, 1990) *Scenedesmus obliquus* (Turp.) Keitzing (GenBank accession no X 56103) have been reported. Wu et al. (2001) reported on the identification of *Chlorella* spp. isolates using ribosomal DNA sequences.

Burja et al. (2001) reported that PCR primers specific for the amplification of 16S rRNA genes from cyanobacteria as proposed by Nubel et al. (1997) could be used to amplify a 578-bp fragment of the 16S rRNA gene of *Chlorella vulgaris* (SDC1)'s chloroplast. It is not surprising that primers specific for the amplification of cyanobacteria were found to amplify a section of the green alga's chloroplast 16S rDNA since green algal chloroplasts have long been speculated to originate from cyanobacterial-like ancestor (Urbach et al., 1992). Wakasugi et al. (1997) reported a complete a nucleotide sequence of the chloroplast genome from *Chlorella vulgaris*.

Research on molecular characterization of cyanobacteria and micro-algae in Thailand

In Thailand there have not been many studies on molecular characterization of cyanobacteria and micro-algae (Abstract books of the First and the Second National Conferences on Algae and Plankton, 2003, 2005).

Somphong and Peerapornpisal (2004) classified cyanobacteria from some hot spring areas in Thailand by morphological criteria and will amplify 16S RNA genes by using forward primer CYA106F with GC clamp and reverse primer CYA781R(a) for DGGE (Denaturing Gradient Gel Electrophoresis) analysis of the cyanobacteria composition. Phitaktansakul et al. (2004) amplified small subunit rDNA of *Chlorella* spp., *Chlamydomonas* sp., *Euglena* sp. and *Scenedesmus* spp. by using NS3 and NS6 primers and the partial (approximately 600 bp) sequences were used to identify the species with a range of 84% - 97% homology with sequences deposited at GenBank (NCBI).The practice of using only a partial sequence of small subunit rDNA to compare homology with deposited sequences at NCBI with low percent homology is not acceptable as a molecular means for identification at the molecular level (Fox et al., 1992).

CHAPTER III

MATERIALS AND METHODS

3.1 Sample collection and isolation of cultures

Freshwater samples were collected from several locations listed in Table 4.1. Water temperature and pH were recorded. Cyanobacteria and micro-algae in the samples were collected by centrifugation of water samples at 3,000 rpm for 10 minutes at room temperature. Cell pellets were observed under a compound microscope for preliminary identification. Single colony isolation was performed by repeated streaking of green colonies on fresh agar plates containing BG-11 medium (Rippka et al., 1979). Cultures were maintained either on agar slants or in liquid culture and incubated at 25° C with continuous illumination of 3,000 lux. Cyanobacteria or micro-algal isolates were grown in 3 ml BG-11 in test-tubes and incubated at 25° C under continuous illumination of 3,000 lux. Cultures at 3,6,9,12 days were taken for microscopic observation and photography. Cultures were identified based on morphology using keys (Prescott, 1970; Rippka et al., 1979) and RAPD-PCR fingerprints.

3.2 RAPD-PCR fingerprinting of cyanobacteria and micro-algae

3.2.1 Isolation of Chromosomal DNA

Cells were scrapped from Petri dishes containing BG-11 medium to eppendorf tube (two plates per tube). 400 μl 25mM Tris-EDTA buffer pH 7.5, 20μl 10% SDS and 0.2 g sterilized glass beads were added to the cell pellet, mixed by vortexing two minutes for three times. The mixture was centrifuged at 12,000 rpm, 4° C for 10 minutes. The supernatant was transferred to a fresh eppendorf tube. One volume of Phenol:Chloroform:Isoamyl alcohol 25:24:1(v/v/v) was added to the solution which was gently mixed by inverting the eppendorf tube. The mixture was centrifuged at 12,000 rpm, 4° C for 10 minutes. The supernatant was transferred to a fresh eppendrof tube. One volume of Chloroform:Isoamyl alcohol 24:1 (v/v) was added to the solution which was gently mixed by inverting the eppendorf tube. The mixture was centrifuged at 12,000 rpm, 4° C for 10 minutes. The supernatant was transferred to a fresh eppendrof tube. 0.1 volume of 3M sodium acetate, pH 5.2 and 2.5 volumes of absolute ethanol were added to the solution which was gently mixed and incubated in -70° C for 15 minutes before centrifugation at 12,000 rpm, 4° C for 15 minutes. The precipitate was washed with 70% ice-cold ethanol and air dried in a laminar flow hood . 25 μl high-purity distilled water was added to dissolve the nucleic acid precipitate at room temperature overnight. Quantity of isolated DNA was determined by absorbance at 260 nm and quality of isolated chlomosomal DNA was checked by OD_{260}/ OD_{280} ratios and 0.8% agarose gel electrophoresis by standard methods (Sambrook & Russel, 2001).

3.2.2 RAPD-PCR fingerprinting

Each of the primers used in RAPD-PCR was from the collection of primers used to determine 16S rDNA of *E.coli* as reported by Blackall(1999) as follows:

Sequences of the primers from 5’ end to 3’ end with numbers in brackets indicating positions in the 16S rDNA of *E. coli* as given by Blackall (1999) were as follows :

27f (9-27)	GAGTTTGATCCTGGCTCAG
343r (343-357)	CTGCTGCCTCCCGTA
519r (519-536)	GTATTACCGCGGCTGCTG
787r (787-803)	CTACCAGGGTATCTAAT
907r (907-926)	CCGTCAATTCATTTGAGTTT
1100r (1100 -1115)	AGGGTTGCGCTCGTTG
1241f (1224-1241)	TACACACGTGCTACAATG
1385r (1385-1401)	CGGTGTGTACAAGGCCC
1492r (1492-1512)	ACGGCTACCTTGTTACGACTT

In addition, primer CRL-7 as reported by Mathis & McMillin(1996) was also used. Sequence of CRL-7 was as follows:

CRL-7 : 5’ GCCCGCCGCC 3’

Each primer was used in RAPD-PCR fingerprinting in the PCR mixture was as follows: 10x PCR buffer 5.00 μl, 50mM $MgCl_2$ 1.00 μl, 10mM dNTPs 1.50 μl, 10 μM primer 1.50 μl, DNA template(60-100 ng) 4.00 μl, *Taq* polymerase (5U. μl^{-1}) 0.25 μl. High quality double distilled water 36.75 μl, Total volume 50.00 μl. PCR program: 94 °C, 4 minutes, 94 °C, 30 seconds, 45 °C, 60 seconds, 72 °C, 120 seconds for 30 cycles, 72 °C, 10 minutes.

PCR products were separated by 1.25% agarose gel electrophoresis by standard method (Sambrook & Russel, 2001). RAPD-PCR fingerprints were stained with Ethidium bromide and photographed on a UV transilluminator (Bio-rad). 1 kb plus DNA ladder (Invitrogen) was used molecular size marker. Reference strains were obtained from National Institute for Environmental Studies (NIES), Tsukuba, Japan, as *Synechococcus* sp. NIES-946, *Chlorella vulgaris* var. *vulgaris* NIES-686, and *Scenedesmus dimorphus* NIES-93. All isolated cyanobacteria and micro-algae which were different strains were deposited with Bangkok MIRCEN (Microbiological Resources Centre) under the code TISTR followed by numbers.

3.3 16S rDNA sequencing

3.3.1 Amplification of 16S rDNA fragments

16S rDNA fragments of *Synechococcus* sp. TISTR 8867, *Chlorella* sp. TISTR 8852 and *Scenedesmus* sp. TISTR 8859 were obtained by PCR amplification by using 27f and 1492r as the forward and the reverse primers. Nucleotide sequences of 27f and 1492r were as reported by Blackall (1999) as follows :

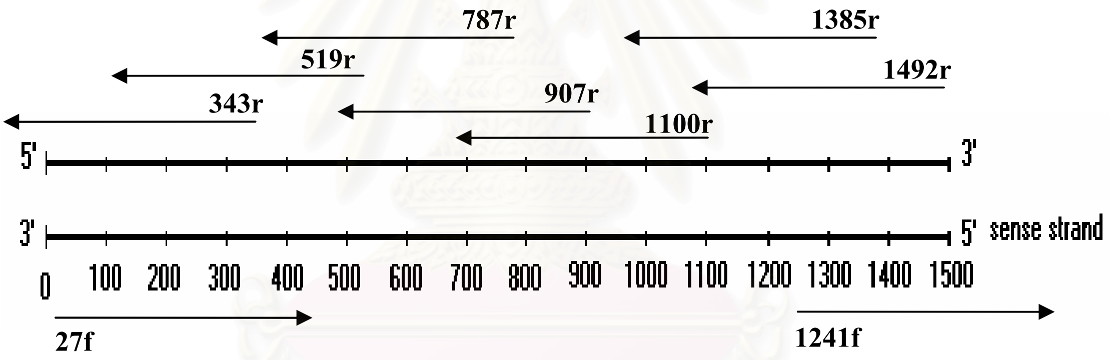
27f : 5'GAGTTTGATCCTGGCTCAG3',1492r : 5'ACGGCTACCTTGTTACGACTT3'

Contents of the PCR mixture for the PCR run were as indicated below. PCR program was set as described by Blackall (1999) as follows : 10x PCR buffer 5.00 μl, 1.5mM $MgCl_2$ 1.50 μl, 10mM dNTP 1.00 μl, 27f (200 ng. μl^{-1}) 0.50 μl, 1492r (200 ng. μl^{-1}) 0.50 μl, DNA template(60-100 ng) 1.00 μl, *Taq* polymerase (5U. μl^{-1}) 0.25 μl, High quality double distilled water 40.25 μl, Total volume 50.00 μl. PCR program: 95 °C 30 minutes, 95 °C 60 seconds, 48 °C 60 seconds, 72 °C 120 seconds for 30 cycles, 48 °C 60 seconds,72 °C 300 seconds for 1 cycle.

Each PCR product was separated by 1.25% agarose gel electrophoresis, viewed and photographed on a UV transilluminator (Bio-rad). The expected molecular size of the 16S rDNA was approximately 1,500 bp.

3.3.2 Sequencing of 16S rDNA fragments

Tubes containing 16S rDNA PCR products obtained in Section 3.3.1 were sent to the BioService Unit, National Center for Genetic Engineering and Biotechnology for sequencing by the ABI PRISM (Dye terminator Cycle Sequencing Kit (Applied Biosystems). The nine primers as indicated in Section 3.2.2 were supplied with the samples. Direct sequencing of 16S rDNA as suggested by Dorch & Stackebrandt (1992) was performed twice for the following three strains: *Synechococcus* sp. NJ7 (TISTR 8867), *Chlorella* sp. NJ26 (TISTR 8852) and *Scenedesmus* sp. NJ8 (TISTR 8859).



The BioEdit program (http://www.mbio.ncsu.edu) was used to analyse overlapping sequences of amplified fragments in order to get sequences of the sense strand. Complete sequences were compared with available sequences deposited in GenBank using the NCBI BLAST program.

3.4 Determination of protein profiles

Seed culture was prepared by inoculating one loop of *Synechococcus* sp. NJ7 (TISTR 8867) into 50 ml of BG-11 medium, pH 7.4 and one loop of *Chlorella* sp. NJ26 (TISTR 8852) or *Scenedesmus* sp. NJ8 (TISTR 8859) into 50 ml of Bold' Basal Medium (BBM), pH 6.8. Medium composition of BBM medium was as reported by Stein (1973).

The cultures were grown at 150 rpm, 40°C until mid log phase. Five ml of each seed culture were inoculated into a set of 45 ml BG-11 or BBM medium. Cultures were grown at 150 rpm, 40°C until mid log phase as determined by measurement of carotenoids at wavelength 450 nanometer. Intracellular proteins were extracted by harvesting cells at 12,000 rpm, 15 min at 4°C. Cell pellet was washed twice with extraction buffer (0.5 M Tris HCl, pH 7.0). Two to three volumes of sterilized glass beads (Sigma G-9143) were added to the cell pellet which was suspended in 80 µl extraction buffer, vortexed at top speed for 40 seconds, left on ice then the vortexing was repeated 9 more times with tubes on ice after each vortexing. Contents were centrifuged at 12,000 rpm, 40 minutes at 4°C. Concentrations of soluble proteins in the supernatant were determined by the Bradford method (Bradford, 1976) using the protein dye assay (BIO-RAD) with Bovine Serum Albumin as the standard. Soluble proteins were separated by SDS-PAGE as described by Laemmli (1970) with 10% separating gel and 50 µg protein per well. Proteins were stained by Silver stain kit (BIO-RAD) according to the manufacturer ‘s instruction.

CHAPTER IV

RESULTS

4.1 Sample collection and isolation of cultures

Table 4.1 Locations of freshwater bodies where samples were collected for the isolation of cyanobacteria and micro-algae including list of the isolates.

Collection dates	Places	Temp. (°C)	pH	Isolates
October,01	Physics Building, Chulalongkorn University	28	6.8	NJ1
December,01	Medium used in growing soybeans, Tab Bld., Chulalongkorn University	28	6.8	NP2
January,02	Tab Building , Chulalongkorn University	28	6.8	NJ3
January,02	Clock Tower, Chulalongkorn University	28	6.8	NP4
February,02	Physics Building, Chulalongkorn University	28	6.8	NJ5
November,02	Pond at 23/8 Soi Lardprao I,Bangkok	29	6.8	NP6
May,02	Clock Tower, Chulalongkorn University	28	6.8	NJ7
December,01	Lumpini Park,Bangkok	28	6.6	NJ8
February,02	Physics Building, Chulalongkorn University	27	6.8	NJ9
June,03	Female Students Dormitory, Chulalongkorn University	28	6.8	NP10
December,02	Lumpini Park,Bangkok	28	6.6	NJ11
August,03	Lumpini Park,Bangkok	28	6.6	NJ12
October,03	Lumpini Park,Bangkok	28	6.6	NJ13
November,01	Medium used in growing soybeans, Tab Bld., Chulalongkorn University	28	6.8	NJ14
May,03	Lumpini Park,Bangkok	28	6.6	NJ15
February,03	Lumpini Park,Bangkok	28	6.6	NJ16
October,03	Lumpini Park,Bangkok	27	6.6	NJ17
May,03	Pond at 23/8 Soi Lardprao I,Bangkok	29	6.8	NJ18
November,02	Physics Building, Chulalongkorn University	28	6.8	NJ19
December,02	Lumpini Park,Bangkok	28	6.6	NJ20
June,03	Physics Building, Chulalongkorn University	28	6.8	NJ21
September,03	Clock Tower, Chulalongkorn University	27	6.8	NJ22
June,03	Lumpini Park,Bangkok	28	6.6	NJ23
March,03	Faculty of Pharmacy, Chulalongkorn University	28	6.8	NJ24
February,03	Lumpini Park,Bangkok	28	6.6	NJ25
May,03	Bangkae Nai Road,Singburi Province	28	6.8	NJ26
October,01	Biology I Building, Chulalongkorn University	28	6.8	NJ27
September,03	Physics Building, Chulalongkorn University	29	6.8	NJ28
March,03	Female Students Dormitory, Chulalongkorn University	28	6.8	NJ29
November,02	Faculty of Pharmacy, Chulalongkorn University	28	6.8	NJ30
May,03	Biology I Building, Chulalongkorn University	28	6.8	NJ31
December,02	Lumpini Park,Bangkok	28	6.6	NJ32
November,01	Lumpini Park,Bangkok	29	6.6	NJ33
May,03	Golden Jubrilee Building,Kasetsart University	29	6.8	NJ34
June,03	Golden Jubrilee Building,Kasetsart University	29	6.8	NJ35
February,02	Lumpini Park,Bangkok	29	6.6	NJ36
February,03	Golden Jubrilee Building,Kasetsart University	28	6.8	NJ37
June,02	Kok Hirun Temple, Ayudhaya Province	28	6.8	NJ38
October,02	Golden Jubrilee Building,Kasetsart University	28	6.8	NJ39

March,02	Paholyothin 32 Road, Bangkok	28	6.8	NJ40
March,03	Female Students Dormitory, Chulalongkorn University	28	6.8	NJ41
October,03	Lumpini Park,Bangkok	28	6.6	NJ42
October,03	Ancient City , Ayudhaya Province	27	6.8	NJ43
September,03	Santiparb Park,Victory Monument ,Bangkok	28	6.8	NJ44
September,03	Kok Hirun Temple, Ayudhaya Province	28	6.8	NJ45
June,03	Golden Jubrilee Building,Kasetsart University	28	6.8	NJ46
September,03	Santiparb Park,Victory Monument ,Bangkok	28	6.8	NJ47
September,03	Santiparb Park,Victory Monument ,Bangkok	28	6.8	NJ48
August,03	Paholyothin 32 Road, Bangkok	28	6.8	NJ49
August,03	Paholyothin 32 Road, Bangkok	28	6.8	NJ50

4.2 Identification of cyanobacteria and micro-algae based on cell morphology and RAPD-PCR fingerprints

Synechococcus sp.

Based on morphology of cells grown in BG11 medium at 25°C under 3,000 Lux continuous light intensity, the following isolates may be the same *Synechococcus* strain: NP4, NJ7, NJ21, NJ22, and NJ28.

Representative morphology of these isolates was shown in Figure 4.1. RAPD-PCR fingerprints of these isolates when CRL-7 or 27f or 343r or 1100r or 1492r was used as the primer are shown in Figure 4.2.

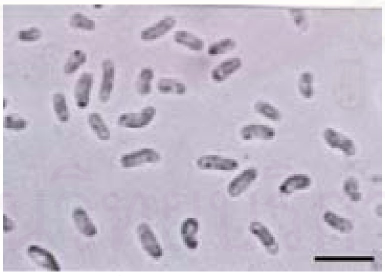


Figure 4.1 Representative morphology of *Synechococcus* sp. NP4, NJ7, NJ21, NJ22, and NJ28 grown for 9 days in BG-11 medium under 3,000 lux light intensity. Bar indicates 30 µm.

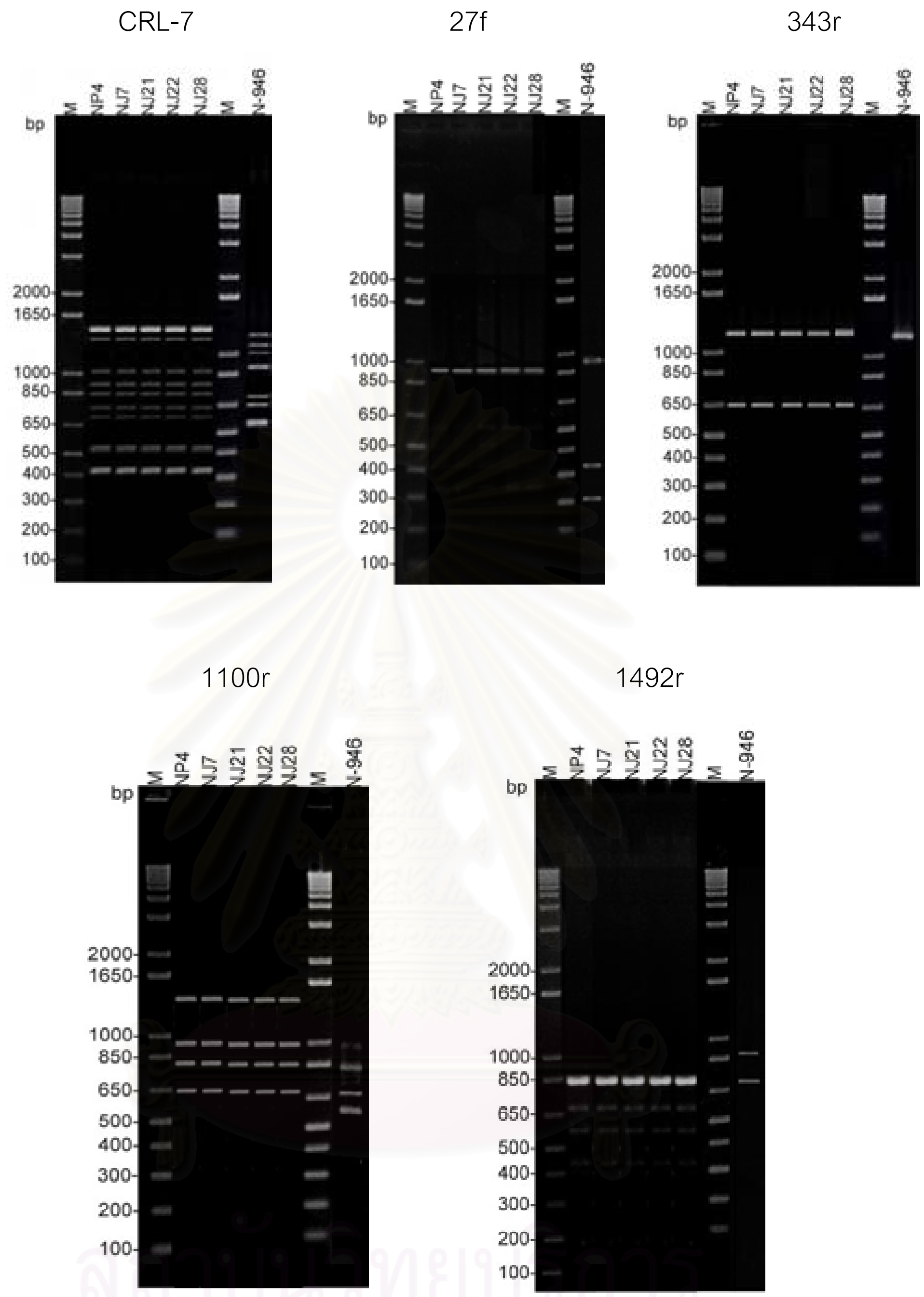
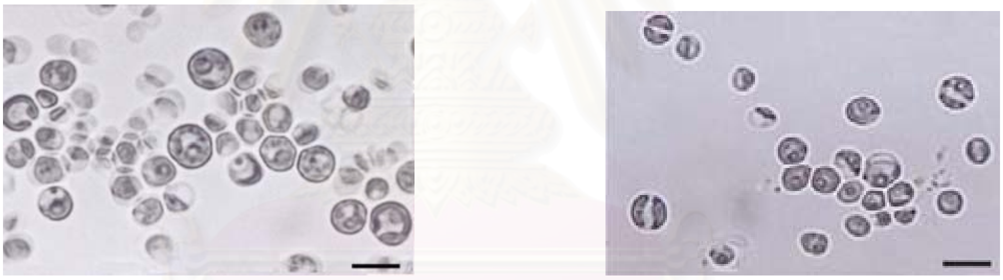


Figure 4.2 RAPD-PCR fingerprints of isolates NP4, NJ7, NJ21, NJ22, and NJ28 when CRL-7 or 27f or 343r or 1100r or 1492r was used as the primer. Lanes M were molecular size markers. Lanes N-946 indicated RAPD-PCR fingerprints of *Synechococcus* sp. NIES-946.

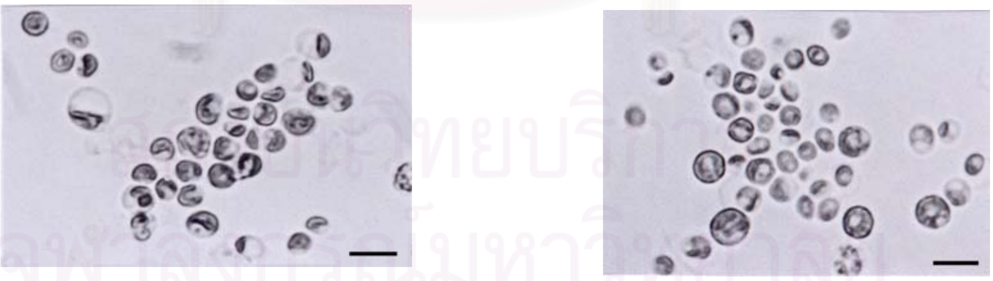
RAPD-PCR fingerprints as shown in Figure 4.2 show that isolates NP4, NJ7, NJ21, NJ22, and NJ28 belong to the same *Synechococcus* strain which is different from *Synechococcus* sp. NIES-946 obtained from the National Institute for Environmental studies (NIES). The isolated *Synechococcus* strain was deposited at Bangkok MIRCEN under the code TISTR 8867. The results indicated that primers which are usually used in the determination of 16S rDNA sequence of *E.coli* can be used to determine 16S rDNA sequence of *Synechocoocus* sp. NJ7 (TISTR 8867) because there were annealings between the target DNA from *Synechocoocus* sp. NJ7 (TISTR 8867) and some of the primers.

Chlorella spp.

Based on cell morphology, isolates NJ1, NJ3, NJ5, NJ26, NJ27, NJ29, NJ31, NJ41, NJ43 could be the same strains. All cells were spherical with diameters ranging from 7 µm to 20 µm (Figure 4.3)

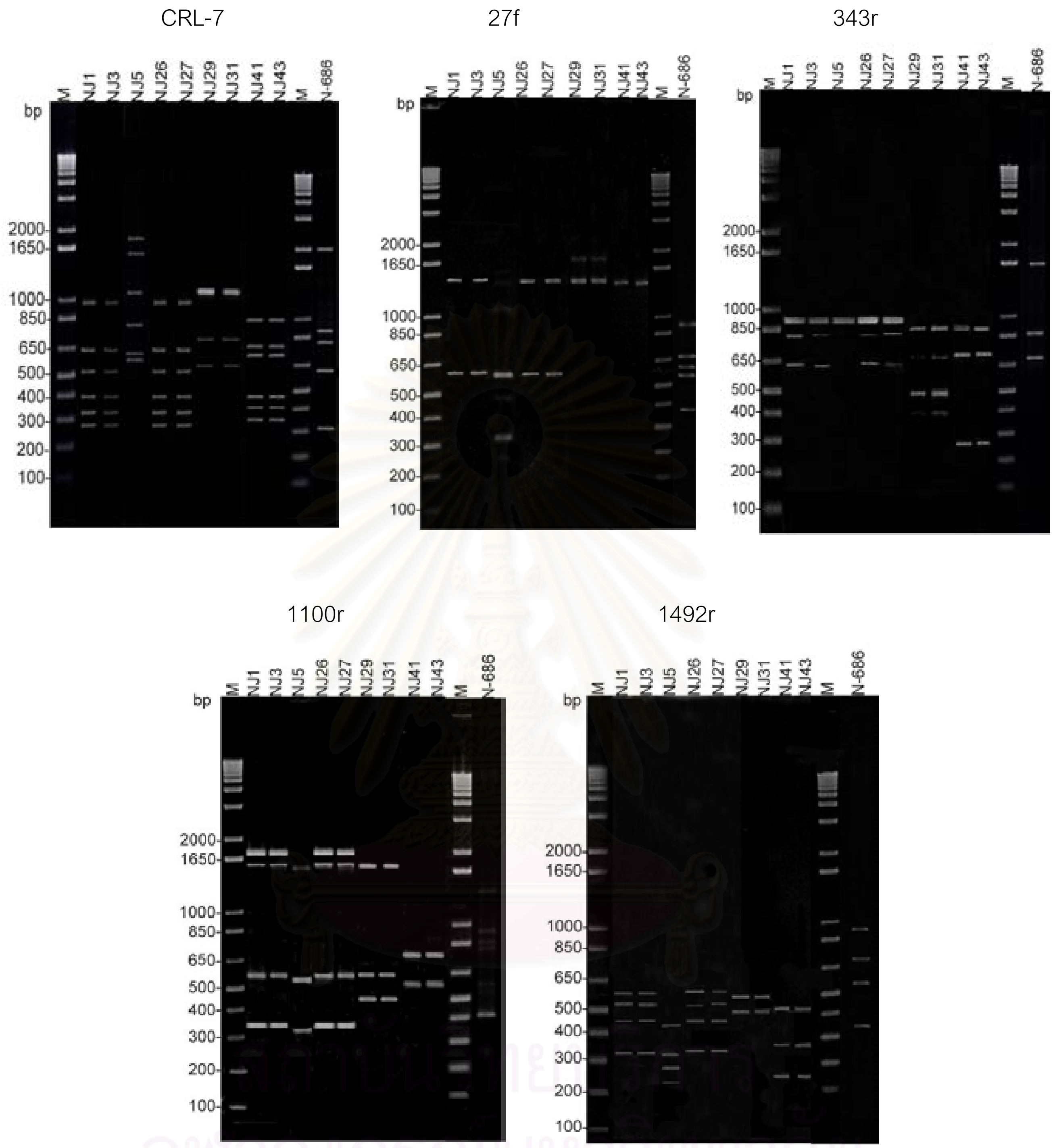


9 days 9days



12days 12 days

Figure 4.3 Representative morphology of Isolates NJ1, NJ3, NJ5, NJ26, NJ27, NJ29, NJ31,NJ41, NJ43 grown in BG-11 medium for 9-12 days under 3,000 lux light intensity. Bar indicates 30 µm.



CRL-7

27f

343r

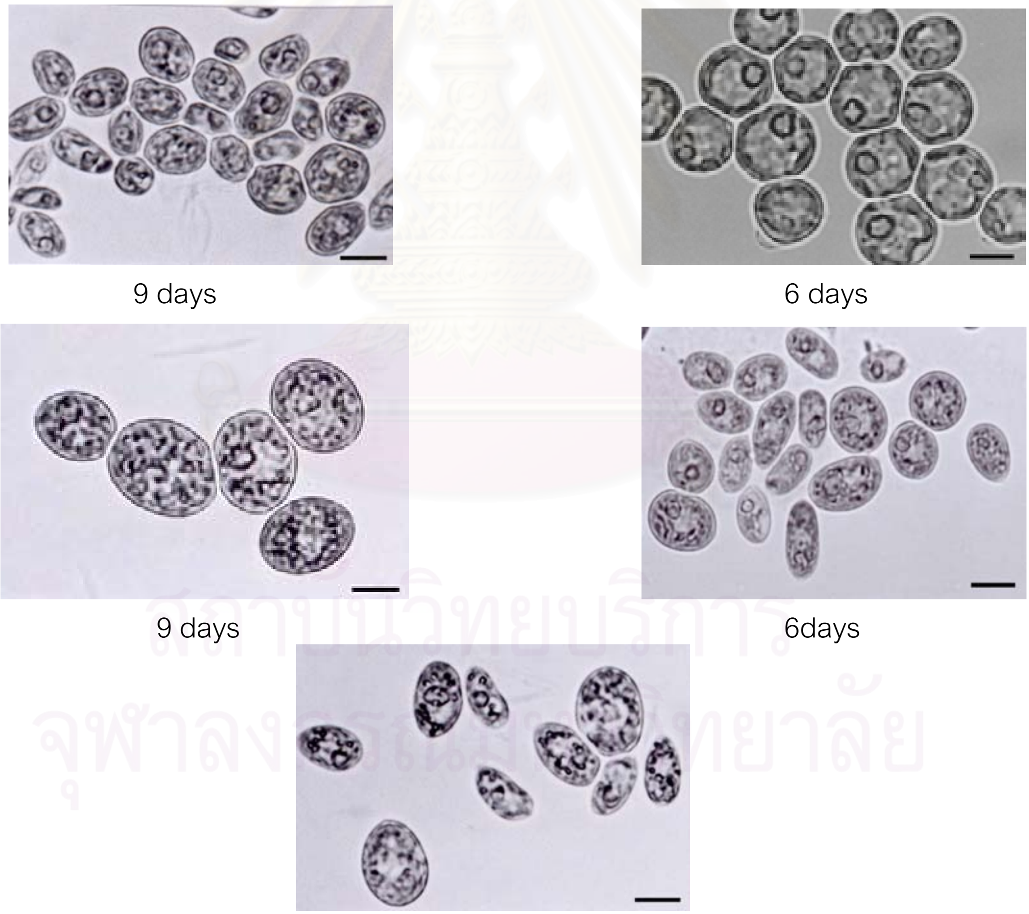
1100r

1492r

Figure 4.4 RAPD-PCR fingerprints of the isolates NJ1, NJ3, NJ5, NJ26, NJ27, NJ29, NJ31, NJ41, NJ43 and N-686 when either CRL-7 or 27f or 343r or 1100r or 1492r was used as the primer. Lanes M molecular size markers. Lane N-686 indicated RAPD-PCR fingerprints of *Chlorella vulgaris* var. *vulgaris* NIES-686.

RAPD-PCR fingerprints showed that NJ1, NJ3, NJ26, NJ27 were the same strain which was deposited at MIRCEN under the code *Chlorella* sp, TISTR 8852 ; NJ29, NJ31 were the same strain with the code *Chlorella* sp. TISTR 8854, NJ41, NJ43 were the same strain with the code *Chlorella* sp. TISTR 8855. NJ5 was another *Chlorella* strain with the code *Chlorella* sp. TISTR 8853. All of the four different *Chlorella* strains were not *Chlorella vulgaris* var. *vulgaris* NIES-686.

Figure 4.5 showed that isolates NJ9, NP10, NJ19, NJ35, and NJ37 might be the same strains due to similar cell morphology. Cells were ovoid with length 30 μm and width 15 μm. One pyrenoid was clearly observed in each cell. However RAPD-PCR fingerprints revealed that isolates NJ9 and NJ19 were the same strain which was deposited at MIRCEN as TISTR 8856 and isolates NP10, NJ35, NJ37 were the same strain which is being sent to deposit at MIRCEN (Figure 4.6).



9 days

6 days

9 days

6days

12 days

Figure 4.5 Representative morphology of Isolates NJ9, NP10, NJ19, NJ35, and NJ37grown for 6-9 days in BG-11 medium at 28°C under 3,000 lux light intensity. Bar indicates 30 μm.

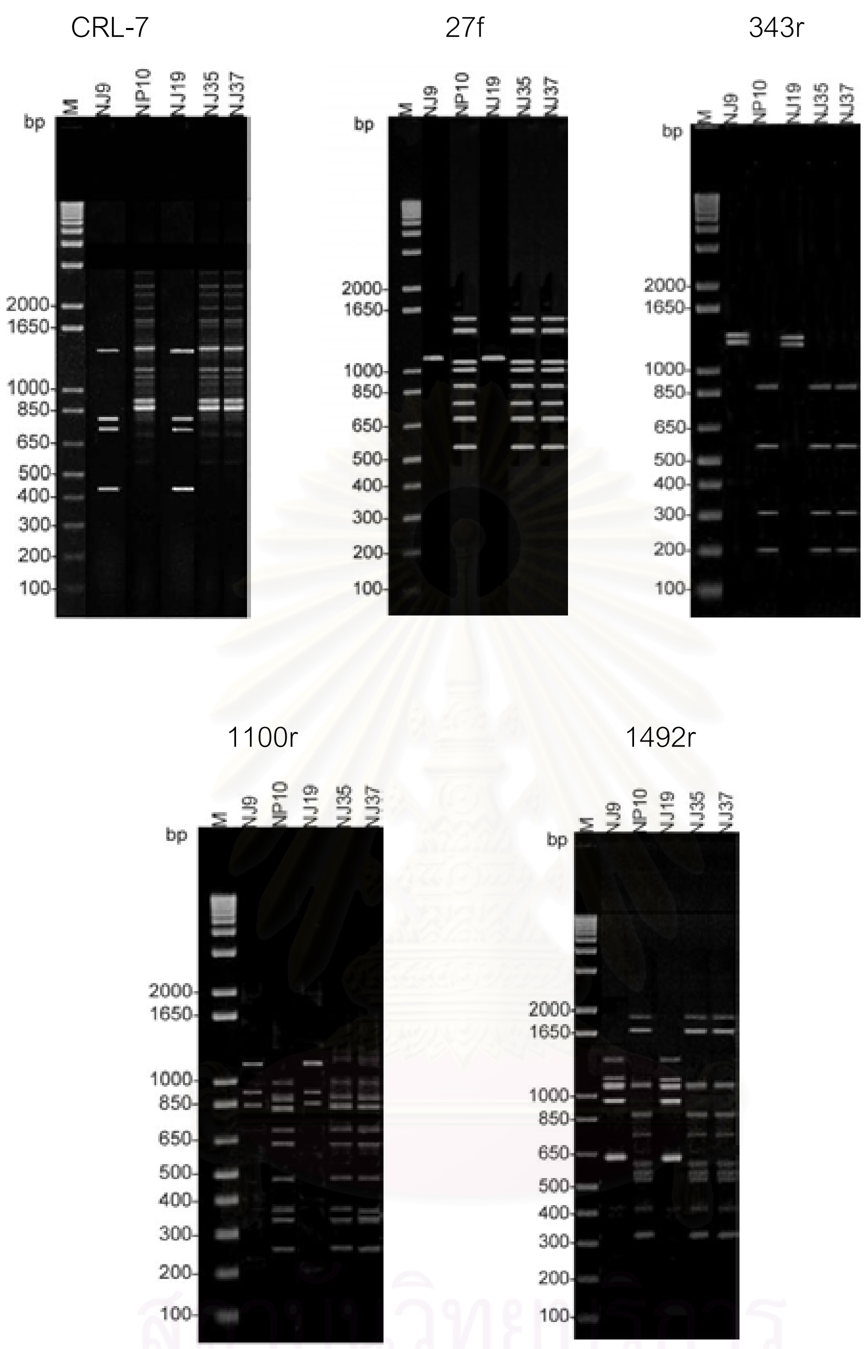
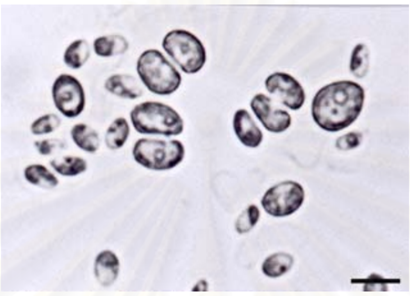


Figure 4.6 RAPD-PCR fingerprints of NJ9, NP10, NJ19, NJ35, and NJ37 with either CRL-7 or 343r or 1100r or 1492r as the primer. Lanes M were molecular size markers.

Figure 4.7 indicated that isolates NJ24 and NJ30 might be the same strain because cells of both isolates were small and ovoid with average length 15-40 μm and width 10-20 μm. RAPD-PCR fingerprints with either CRL-7 or 27f or 343r or 1100r or

1492r revealed that the two isolates were the same strain (Figure 4.8).The isolates were tentatively identified as *Chlorella* sp. but we were advised by MIRCEN's curator (personal communication) that the strain might be *Tetrachlorella* sp. Closer microscopic examination of cell morphology indicated the appearance of groups of two to four cells former colonies similar to *Scenedesmus* sp. The identify of this strain is under investigation.



12 days

Figure 4.7 Morphology of isolates NJ24 and NJ30. Bar indicates 30 μm.

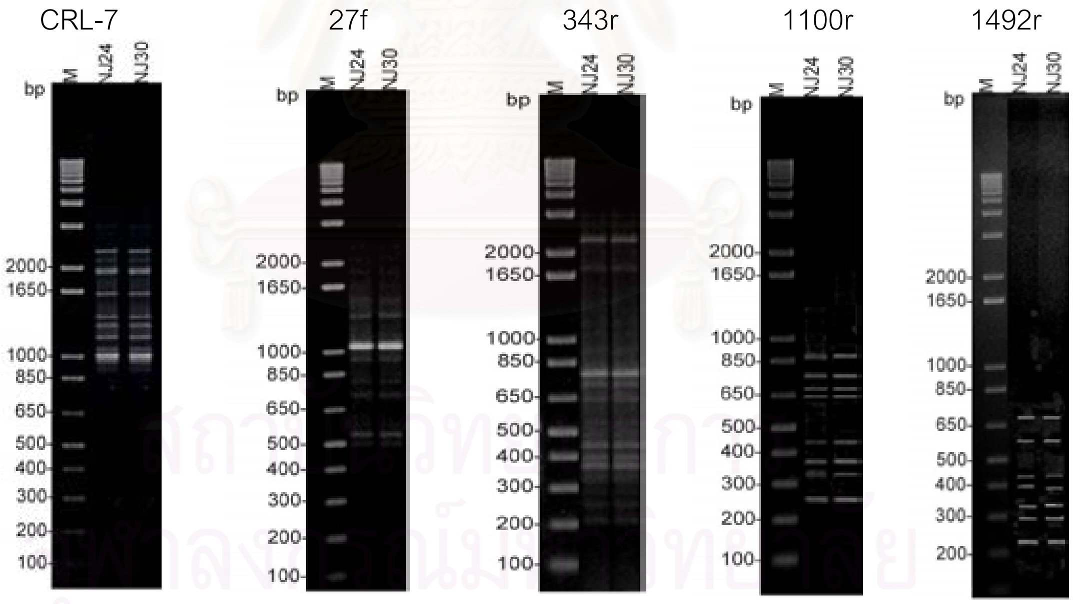
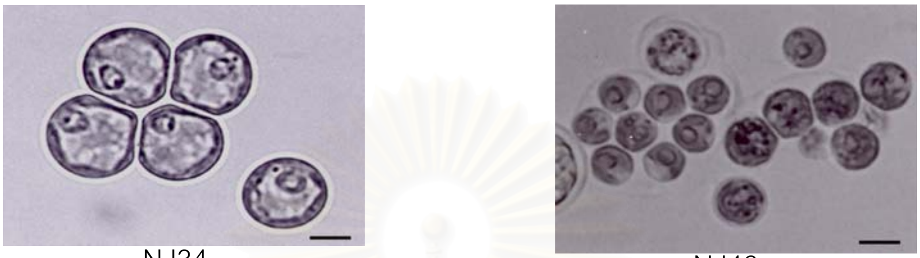


Figure 4.8 RAPD-PCR fingerprints of NJ24 and NJ30 with either CRL-7 or 27f or 343r or 1100r or 1492r was used as the primer.

Cell morphology of isolates NJ34, NJ46, NJ49, and NJ50 showed that the isolates might be the same strain became of the presence of spherical cells with various sizes ranging from 10-15 μm to 60 μm in diameter (Figure 4.9). RAPD-PCR fingerprints with either CRL-7 or 27f or 343r or 1100r or 1492r as the primer as shown in Figure 4.10 showed that isolates NJ34 and NJ46 were the same strain. The isolates NJ 34 and NJ46 were deposited at MIRCEN as *Chlorella* sp. TISTR 8857. The isolates NJ49 and NJ50 were the same strain. Which was tentatively identified as *Chloococcum* sp. by a MIRCEN's curator.



NJ34

6 days

NJ46

3 days

NJ46

9 days

NJ46

12 days

NJ49

6days

NJ50

3days

NJ50

9 days

NJ50

12 days

Figure 4.9 Cell morphology of isolates NJ34, NJ46, NJ49, and NJ50 grown for 3, 6, 9, 12 days in BG-11 at 25°C, 3,000 lux light intensity. Bar indicates 30 μm.

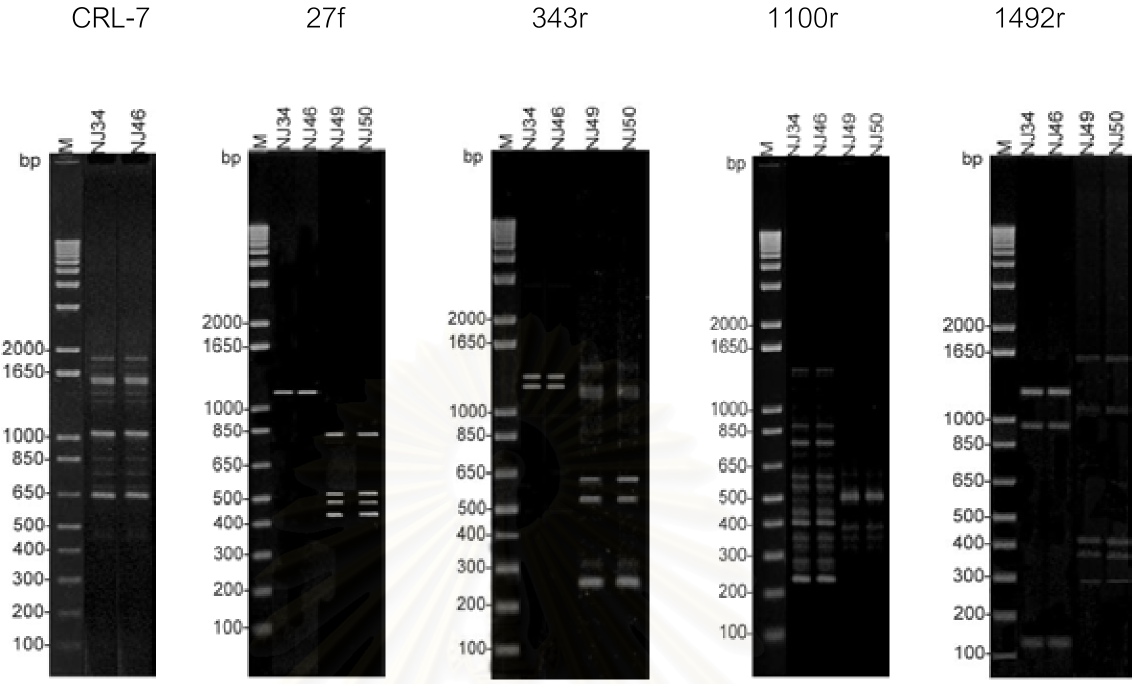


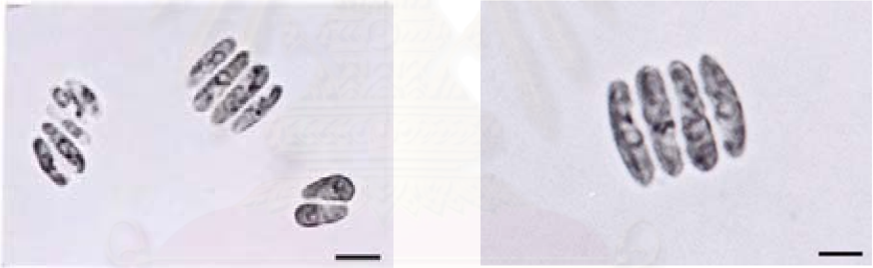
Figure 4.10 RAPD-PCR fingerprints of isolates NJ34, NJ46, NJ49, and NJ50 with either CRL-7 or 27f or 343r or 1100r or 1492r was used as the primer.

On closer observations some cells of isolates NJ49 and NJ50 appeared to be larger with average diameter of 60 μm which divided to smaller multiple daughter cells. The identify of this strain is under investigation. (Figure 4.9).

Scenedesmus spp.

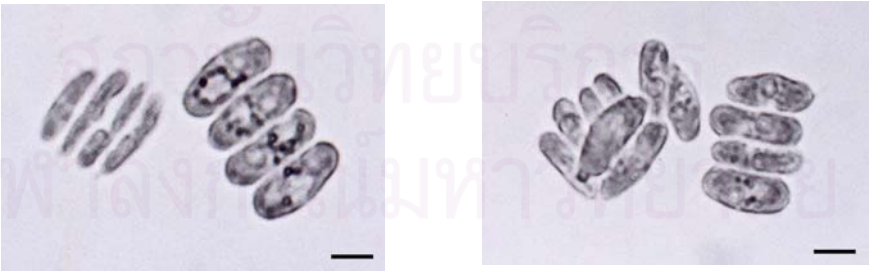
The following isolates of *Scenedesmus* spp. NP2, NP6, NJ14, NJ18, NJ39 may be the same strain. Figure 4.11 show morphology of these isolates. However, RAPD-PCR fingerprints indicated that NP6 and NJ18 were the same strain which was deposited at MIRCEN as *Scenedesmus* sp. TISTR 8861; NP2, NJ14, and NJ39 were different strains (Figure 4.12) which were deposited at MIRCEN as *Scenedesmus* sp. TISTR 8862, *Scenedesmus* sp. TISTR 8863, *Scenedesmus* sp. TISTR 8858 respectively.

NP2 (12 days)



NP6 (9 days)

NP14 (12 days)



NJ18 (12 days)

NJ39 (12 days)

Figure 4.11 Morphology of *Scenedesmus* spp. NP2, NP6, NJ14, NJ18, NJ39 grown for 9-12 days in BG-11 medium at 25°C, 3000 lux light intensity. Bar indicates 30 µm.

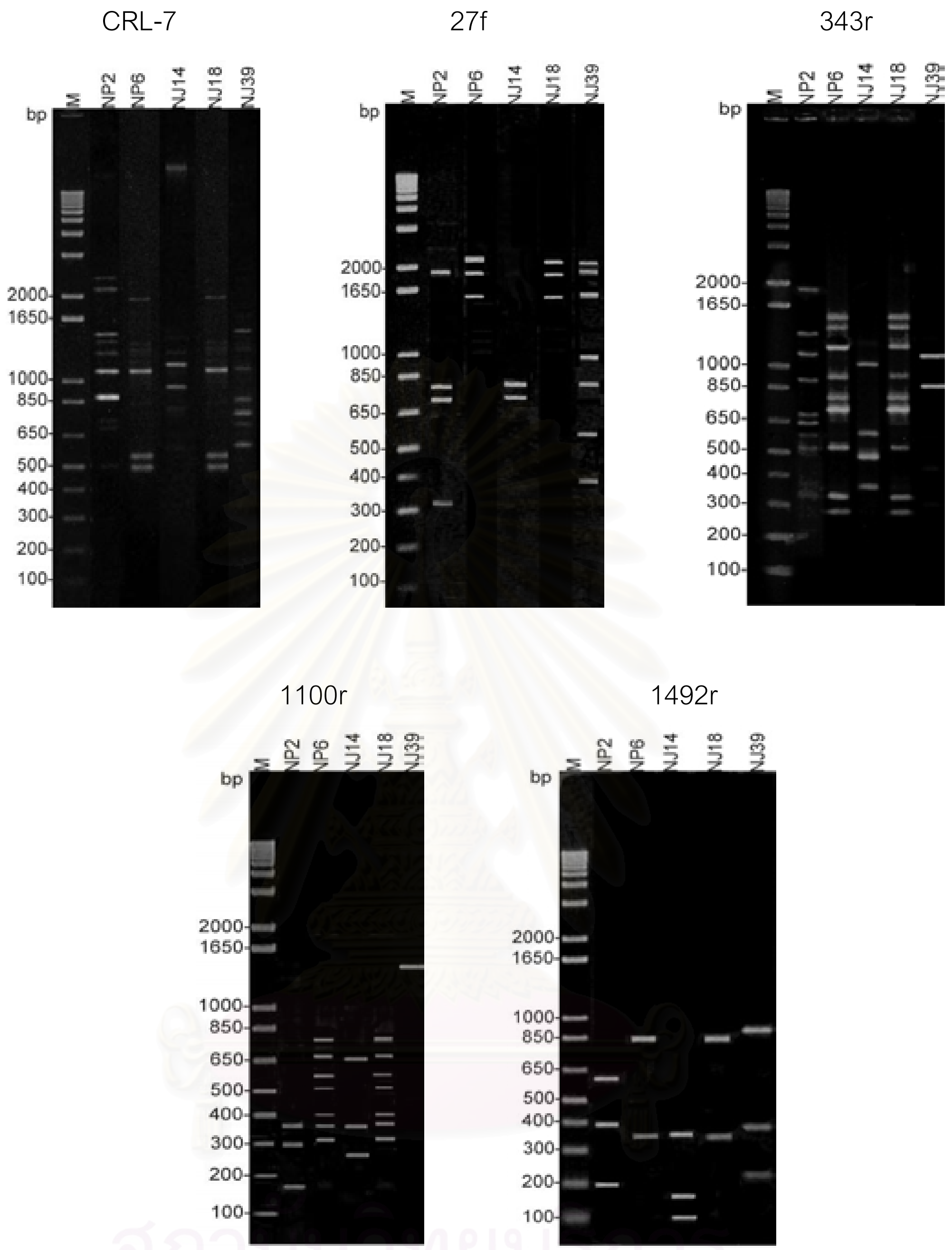
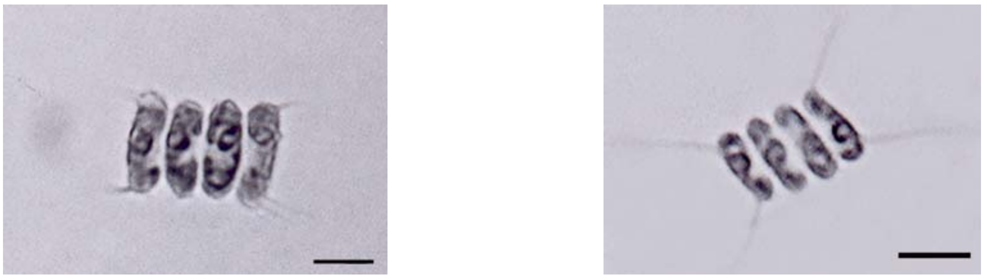


Figure 4.12 RAPD-PCR fingerprints of *Scenedesmus* spp. NP2, NP6, NJ14, NJ18, and NJ39 using either CRL-7 or 27f or 343r or 1100r or 1492r as the primer.

Based on morphology *Scenedesmus quadricauda* isolate NJ23 may be the same strain as isolate NJ45 while NJ40 might be a different strain. However, RAPD-PCR fingerprints revealed that the three isolates were there different strains (Figures 4.13, 4.14). The strains were deposited at MIRCEN as *Scenedesmus* sp. TISTR 8864, *Scenedesmus* sp. TISTR 8866, *Scenedesmus* sp. TISTR 8865 respectively.



NJ23

12 days

NJ45

12 days

NJ40

3 days

Figure 4.13 Morphology of *Scenedesmus quadricauda* isolates NJ23, NJ40, and NJ45 grown for 3 and 12 days in BG-11 medium at 25°C, 3000 lux light intensity. Bar indicates 30 µm.

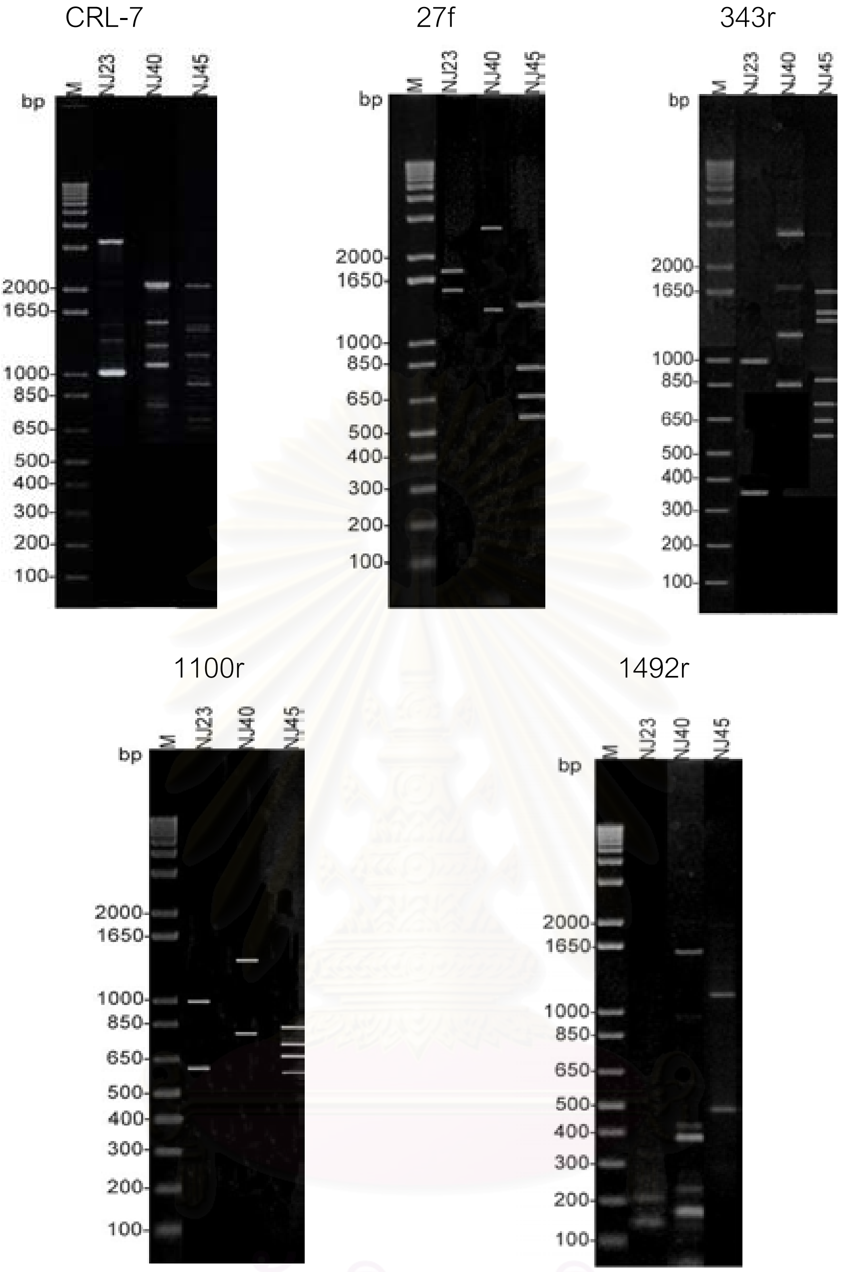
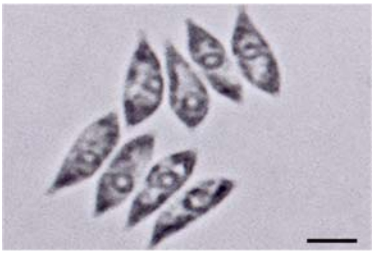


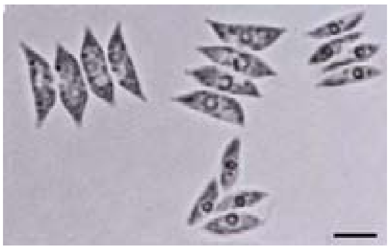
Figure 4.14 RAPD-PCR fingerprints of *Scenedesmus quadricauda* isolates NJ23, NJ40 and NJ45 when CRL-7 or 27f or 343r or 1100r or 1492r was used as the primer.

From morphology, *Scenedesmus* spp. isolates NJ8, NJ12, NJ20, NJ25, NJ42, NJ47, NJ48 may be the same strain (Figure 4.15). RAPD-PCR fingerprints as shown in Figure 4.16 revealed that the isolates except NJ42 were the same strain which was deposited at MIRCEN as *Scenedesmus* sp. TISTR 8859. Isolate NJ42 was deposited at MIRCEN as *Scenedesmus* sp. TISTR 8860. The cells were sometimes observed to contain 4 cells. Cell length 30-40 μm; Cell width 10 μm.



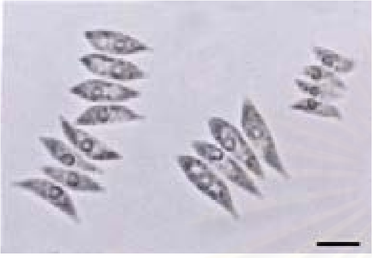
NJ8

9 days



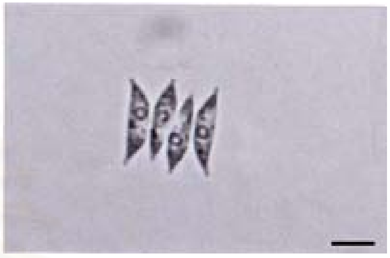
NJ12

9 days



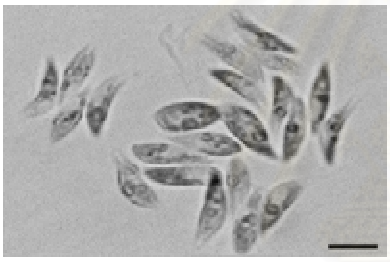
NJ20

9 days



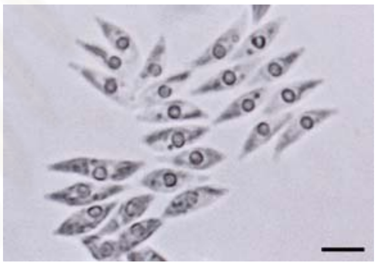
NJ25

9 days



NJ42

12 days



NJ47

12 days

NJ48

12 days

Figure 4.15 Morphology of isolates NJ8, NJ12, NJ20, NJ25, NJ42, NJ47 and NJ48 grown for 9 and 12 days in BG-11 medium at 25°C, 3000 lux light intensity. Bar indicates 30 µm.

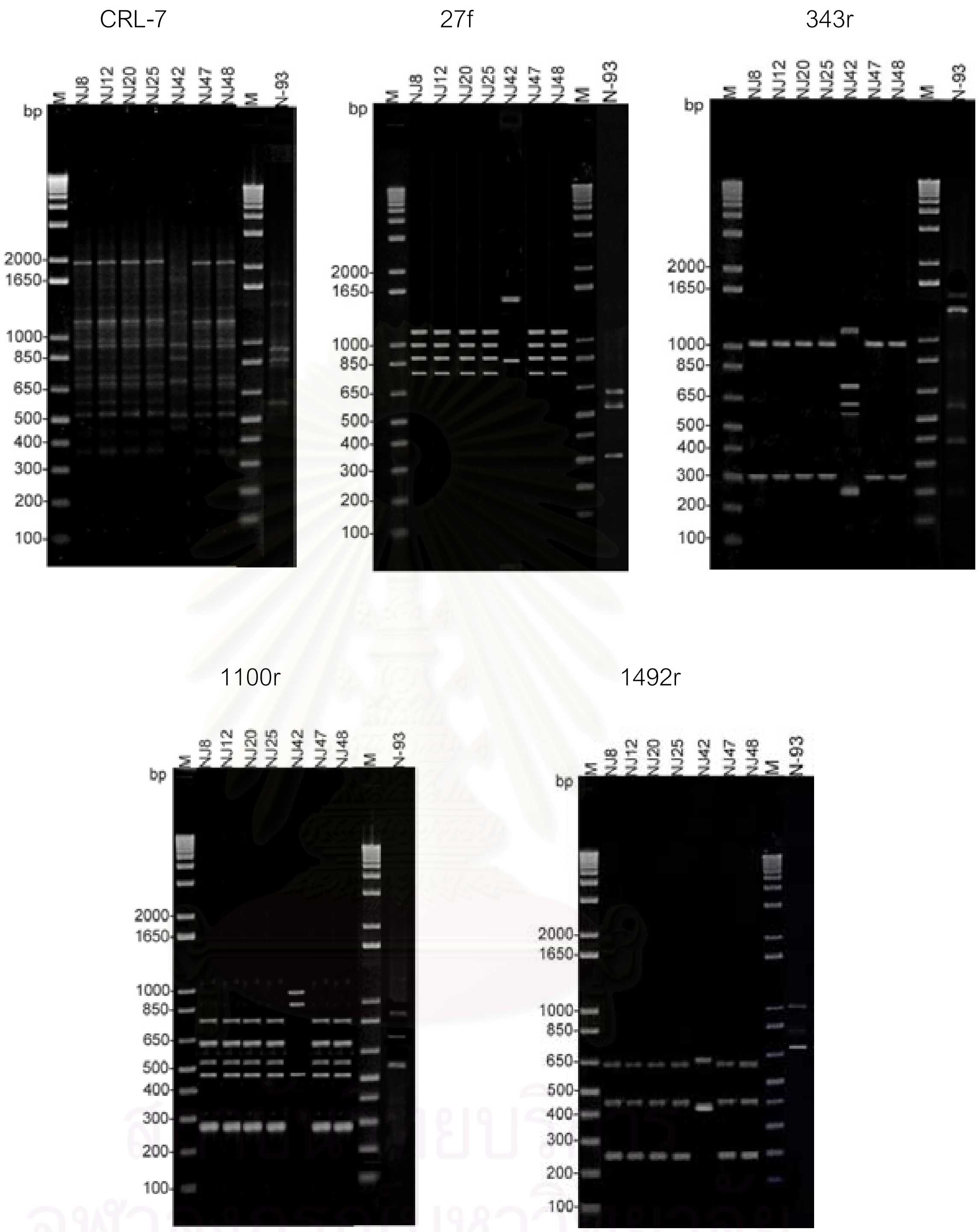
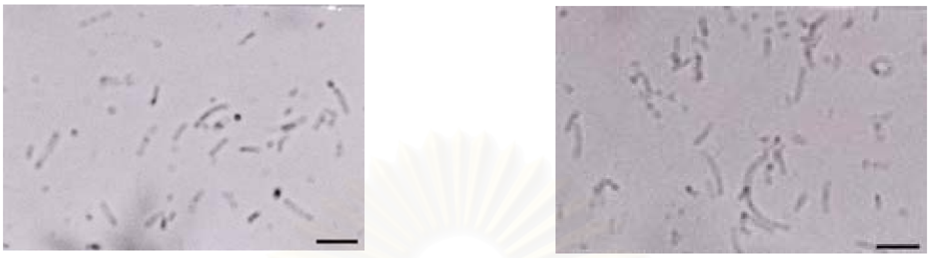


Figure 4.16 RAPD-PCR fingerprints of isolates NJ8, NJ12, NJ20, NJ25, NJ42, NJ47, NJ48 with either CRL-7 or 27f or 343r or 1100r or 1492r as the primer. Lanes M were molecular size markers. Lane N-93 indicated RAPD-PCR fingerprints of *Scenedesmus dimorphus* NIES -93

Figure 4.17 to 4.19 show cell morphology of the reference strains obtained from NIES. RAPD-PCR fingerprints of each strain were previously shown in Figure 4.2, 4.4, and 4.16.



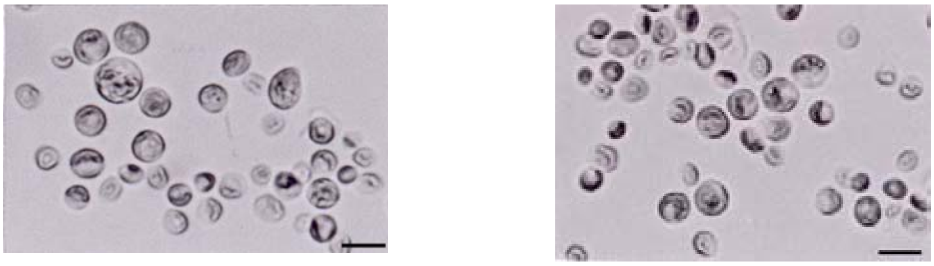
3 days

6 days

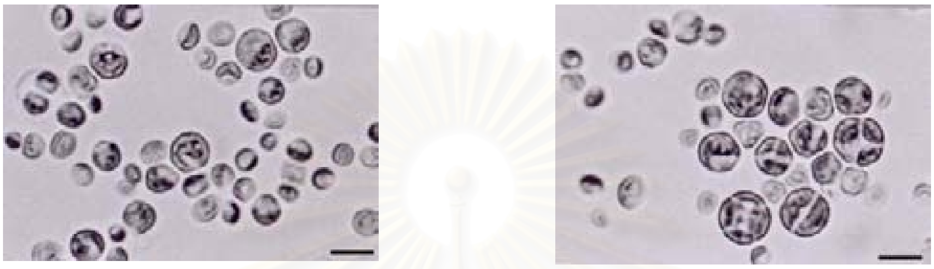
9 days

12 days

Figure 4.17 Cell morphology of the reference strain, Synechococcus sp. NIES-946 grown for 3, 6, 9, and 12 days in BG-11 medium at 25°C, 3,000 lux light intensity. Bar indicates 30 μm.

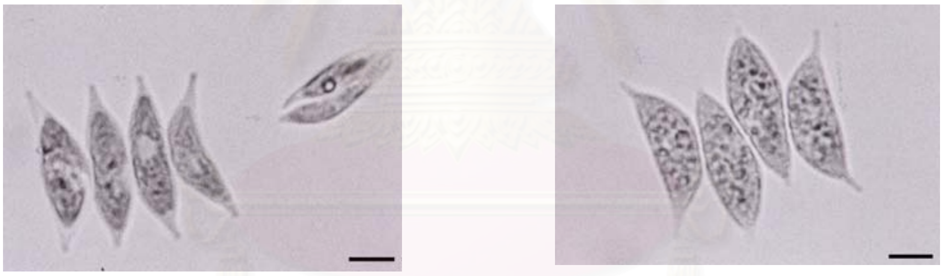


3 days 6 days



9 days 12 days

Figure 4.18 Cell morphology of the reference strain, *Chlorella vulgaris* var. *vulgaris* NIES-686 grown for 3, 6, 9, and 12 days in BG-11 medium at 25°C, 3,000 lux light intensity. Bar indicates 30 μm.



3 days 6 days

9 days 12 days

Figure 4.19 Cell morphology of the reference strain, *Scenedesmus dimorphus* NIES-93 grown for 3, 6, 9, and 12 days in BG-11 medium at 25°C, 3,000 lux light intensity. Bar indicates 30 μm.

Figures 4.20 to 4.23 indicated that there were no PCR product bands that were specific to either *Synechococcus* spp. or Chlorella spp. or *Scenedesmus* spp. The results also indicated that the isolated *Synechococcus* sp. *Chlorella* spp. and *Scenedesmus* spp. were different strains from the reference strains

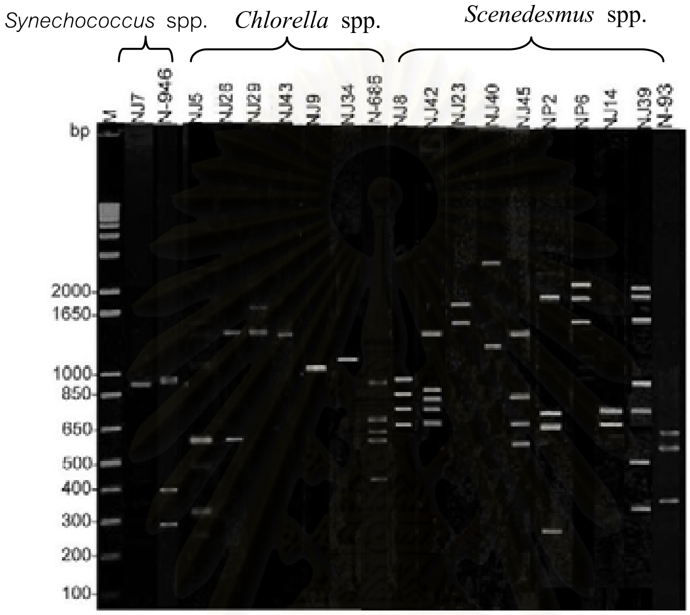


Figure 4.20 Summary of RAPD-PCR fingerprints of 16 strains of isolated *Synechococcus* sp., *Chlorella* spp. and *Scenedesmus* spp. as well as reference strains *Synechococcus* sp. NIES-946, *Chlorella vulgaris* var. *vulgaris* NIES-686, and *Scenedesmus dimorphus* NIES-93 when 27f was used as the primer.

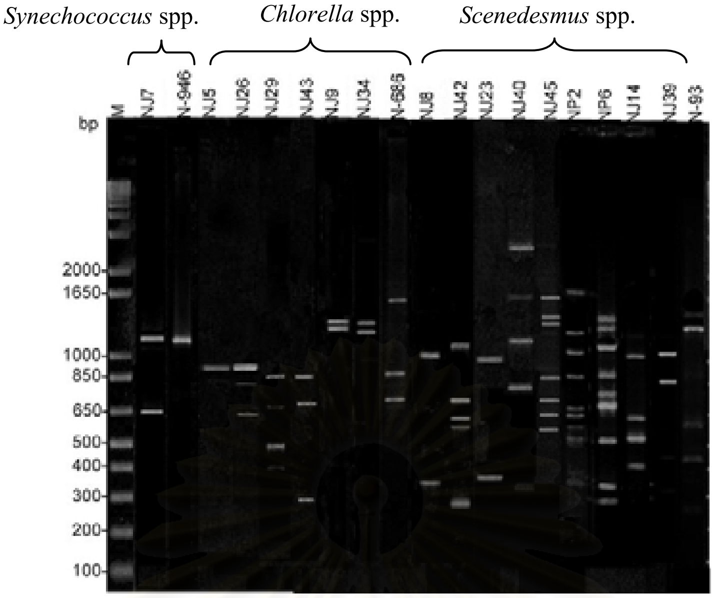


Figure 4.21 Summary of RAPD-PCR fingerprints of 16 strains of isolated *Synechococcus* sp., *Chlorella* spp. and *Scenedesmus* spp. as well as reference strains *Synechococcus* sp. NIES-946, *Chlorella vulgaris* var. *vulgaris* NIES-686, and *Scenedesmus dimorphus* NIES-93 when 343r was used as the primer.

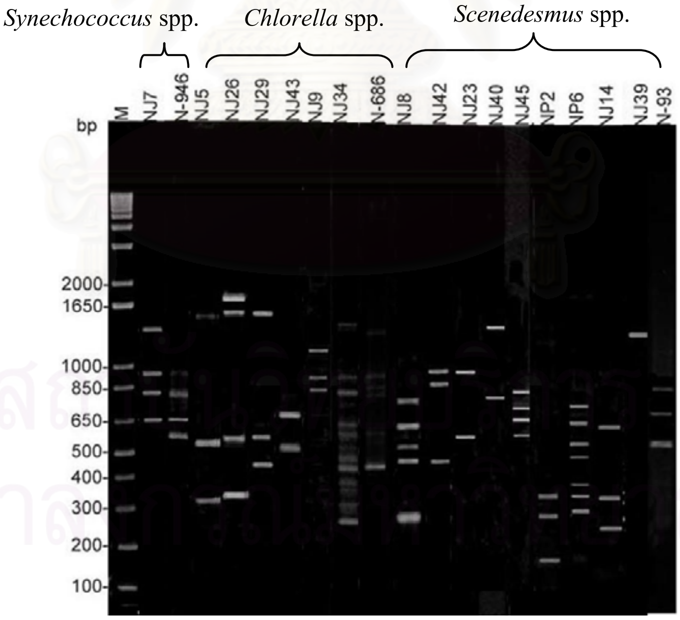


Figure 4.22 Summary of RAPD-PCR fingerprints of 16 strains of isolated *Synechococcus* sp., *Chlorella* spp. and *Scenedesmus* spp. as well as reference strains *Synechococcus* sp. NIES-946, *Chlorella vulgaris* var. *vulgaris* NIES-686, and *Scenedesmus dimorphus* NIES-93 when 1100r was used as the primer.

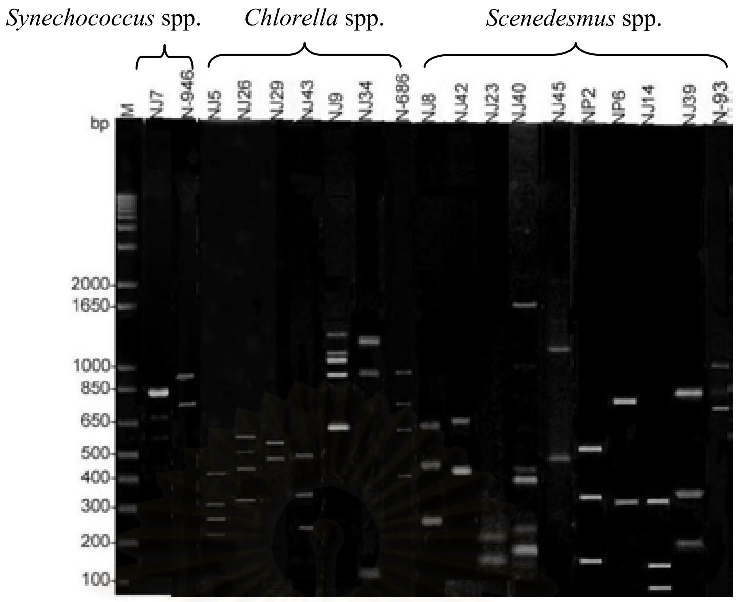


Figure 4.23 Summary of RAPD-PCR fingerprints of 16 strains of isolated *Synechococcus* sp., *Chlorella* spp. and *Scenedesmus* spp. as well as reference strains *Synechococcus* sp. NIES-946, *Chlorella vulgaris* var. *vulgaris* NIES-686, and *Scenedesmus dimorphus* NIES-93 when 1492r was used as the primer.

16S rDNA sequence

Figure 4.24 showed amplified products of 16S rDNA of *Synechococcus* sp.NJ 7 (TISTR 8867), *Chlorella* sp. NJ26 (TISTR 8852) and *Scenedesmus* sp. NJ 8(TISTR 8859). Figure 4.25-4.30 show 16S rDNA sequence of the three strains and their homology comparisons with data deposited in GenBank. Table 4.2 summarises the results of the homology comparisons.

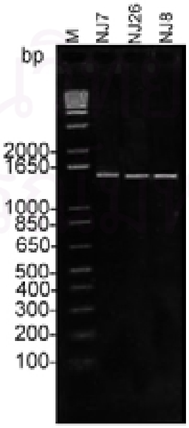
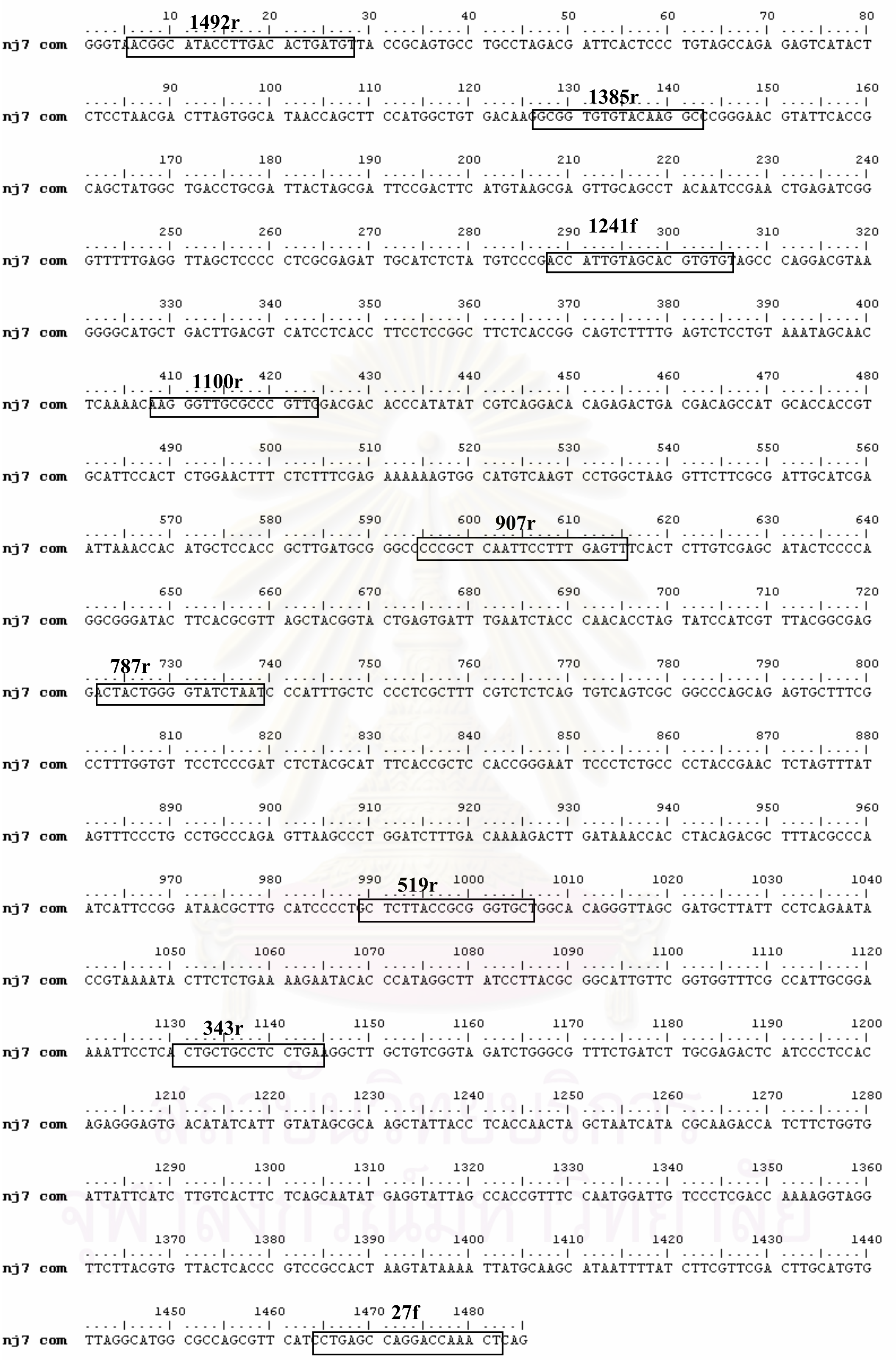


Figure 4.24 Amplified 16S rDNA products when DNA of *Synechococcus* sp. TISTR 8867, *Chlorella* sp. TISTR 8852 and *Scenedesmus* sp. TISTR 8859 were used as target DNA. Molecular size of the products was 1,500 bp as expected.

Table 4.2 Summary of homology comparisons of 16S rDNA sequences obtained by the use of NCBI's BLAST program.

Strain	number of nucleotides	percent homology	Strain in GenBank
Synechococcus sp. NJ7 (TISTR 8867)	1485	one partial sequence Homology=584/608 (96%)	*Koliella spiculiformis*
Chlorella sp.NJ26 (TISTR 8852)	1520	one partial sequence Homology=1273/1328(95%)	*Chlorella sorokiniana*
Scenedesmus sp.NJ28 (TISTR 8859)	1470	one partial sequence Homology=1167/1213(96%)	*Scenedesmus obliquus*



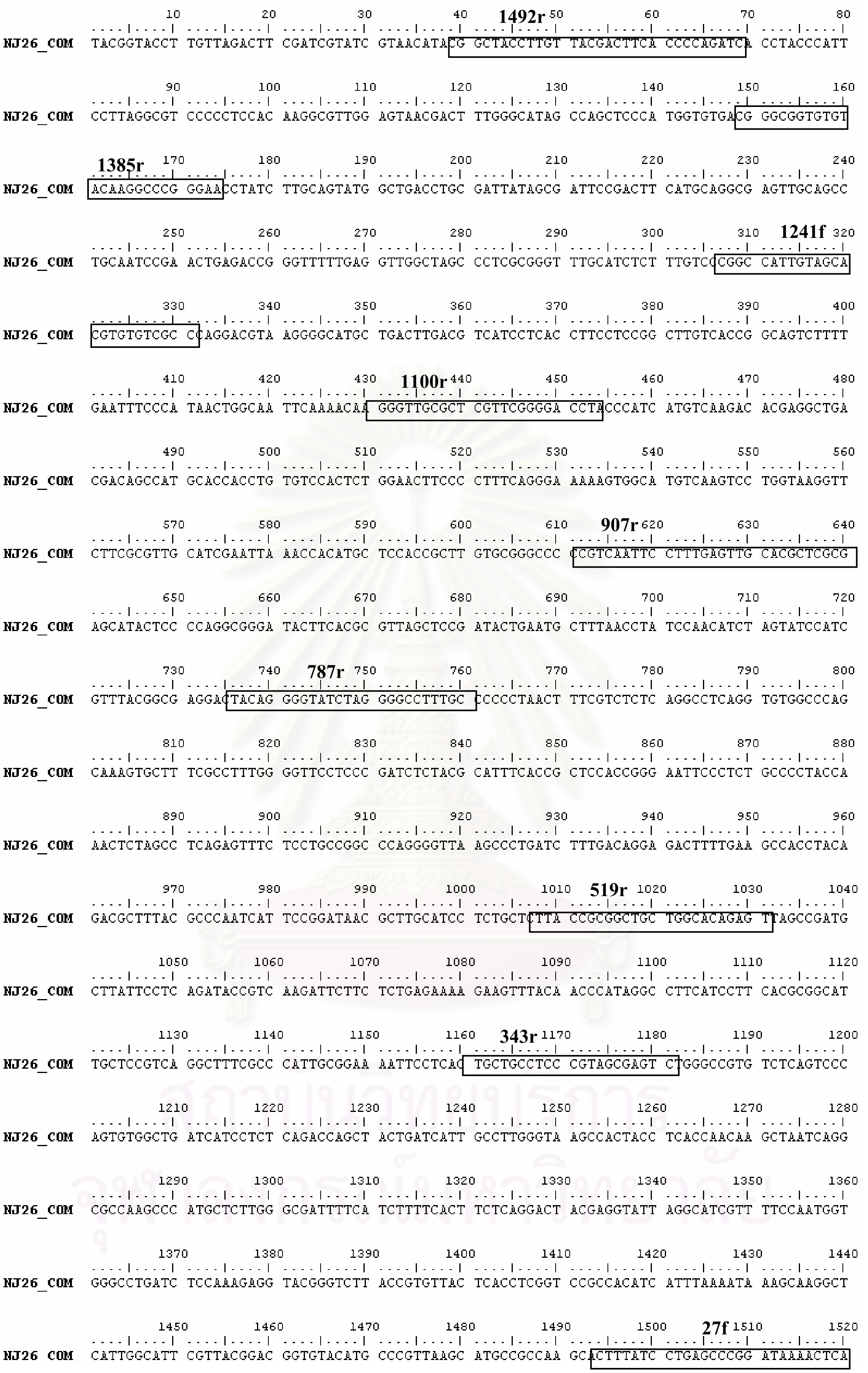
```
                 10         20         30         40         50         60         70         80
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 com  GGGTAACGGC ATACCTTGAC ACTGATGTTA CCGCAGTGCC TGCCTAGACG ATTCACTCCC TGTAGCCAGA GAGTCATACT

                 90        100        110        120        130        140        150        160
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 com  CTCCTAACGA CTTAGTGGCA TAACCAGCTT CCATGGCTGT GACAAGGCGG TGTGTACAAG GCCCGGGAAC GTATTCACCG

                170        180        190        200        210        220        230        240
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 com  CAGCTATGGC TGACCTGCGA TTACTAGCGA TTCCGACTTC ATGTAAGCGA GTTGCAGCCT ACAATCCGAA CTGAGATCGG

                250        260        270        280        290        300        310        320
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 com  GTTTTTGAGG TTAGCTCCCC CTCGCGAGAT TGCATCTCTA TGTCCCGACC ATTGTAGCAC GTGTGTAGCC CAGGACGTAA

                330        340        350        360        370        380        390        400
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 com  GGGGCATGCT GACTTGACGT CATCCTCACC TTCCTCCGGC TTCTCACCGG CAGTCTTTTG AGTCTCCTGT AAATAGCAAC

                410        420        430        440        450        460        470        480
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 com  TCAAAACAAG GGTTGCGCCC GTTGGACGAC ACCCATATAT CGTCAGGACA CAGAGACTGA CGACAGCCAT GCACCACCGT

                490        500        510        520        530        540        550        560
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 com  GCATTCCACT CTGGAACTTT CTCTTTCGAG AAAAAAGTGG CATGTCAAGT CCTGGCTAAG GTTCTTCGCG ATTGCATCGA

                570        580        590        600        610        620        630        640
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 com  ATTAAACCAC ATGCTCCACC GCTTGATGCG GGCCCCCGCT CAATTCCTTT GAGTTTCACT CTTGTCGAGC ATACTCCCCA

                650        660        670        680        690        700        710        720
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 com  GGCGGGATAC TTCACGCGTT AGCTACGGTA CTGAGTGATT TGAATCTACC CAACACCTAG TATCCATCGT TTACGGCGAG

                730        740        750        760        770        780        790        800
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 com  GACTACTGGG GTATCTAATC CCATTTGCTC CCCTCGCTTT CGTCTCTCAG TGTCAGTCGC GGCCCAGCAG AGTGCTTTCG

                810        820        830        840        850        860        870        880
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 com  CCTTTGGTGT TCCTCCCGAT CTCTACGCAT TTCACCGCTC CACCGGGAAT TCCCTCTGCC CCTACCGAAC TCTAGTTTAT

                890        900        910        920        930        940        950        960
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 com  AGTTTCCCTG CCTGCCCAGA GTTAAGCCCT GGATCTTTGA CAAAAGACTT GATAAACCAC CTACAGACGC TTTACGCCCA

                970        980        990       1000       1010       1020       1030       1040
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 com  ATCATTCCGG ATAACGCTTG CATCCCCTGC TCTTACCGCG GGTGCTGGCA CAGGGTTAGC GATGCTTATT CCTCAGAATA

               1050       1060       1070       1080       1090       1100       1110       1120
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 com  CCGTAAAATA CTTCTCTGAA AAGAATACAC CCATAGGCTT ATCCTTACGC GGCATTGTTC GGTGGTTTCG CCATTGCGGA

               1130       1140       1150       1160       1170       1180       1190       1200
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 com  AAATTCCTCA CTGCTGCCTC CTGAAGGCTT GCTGTCGGTA GATCTGGGCG TTTCTGATCT TGCGAGACTC ATCCCTCCAC

               1210       1220       1230       1240       1250       1260       1270       1280
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 com  AGAGGGAGTG ACATATCATT GTATAGCGCA AGCTATTACC TCACCAACTA GCTAATCATA CGCAAGACCA TCTTCTGGTG

               1290       1300       1310       1320       1330       1340       1350       1360
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 com  ATTATTCATC TTGTCACTTC TCAGCAATAT GAGGTATTAG CCACCGTTTC CAATGGATTG TCCCTCGACC AAAAGGTAGG

               1370       1380       1390       1400       1410       1420       1430       1440
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 com  TTCTTACGTG TTACTCACCC GTCCGCCACT AAGTATAAAA TTATGCAAGC ATAATTTTAT CTTCGTTCGA CTTGCATGTG

               1450       1460       1470       1480
         ....|....| ....|....| ....|....| ....|....| ....|
nj7 com  TTAGGCATGG CGCCAGCGTT CATCCTGAGC CAGGACCAAA CTCAG
```

Figure 4.25 16S rDNA sequence of *Synechococcus* sp.NJ 7 (TISTR 8867). Sequences of primers are shown in boxes.

```
Query= NJ_7 com  (1485 letters)

                                                                 Score    E
Sequences producing significant alignments:                      (bits) Value

gi|18642513|gb|AF278746.1|  Koliella spiculiformis 16S small...   908   0.0
gi|2224352|dbj|AB001684.1|  Chlorella vulgaris C-27 chloropl...   728   0.0
gi|11468|emb|X16579.1|CHCVSSRN  Chlorella vulgaris chloropla...   728   0.0
gi|45385169|gb|AY553213.1|  Auxenochlorella protothecoides 1...   728   0.0
gi|1232075|dbj|D11347.1|CHLCPV16S  Chlorella vulgaris (strai...   720   0.0
                                  Alignments

☐
   >gi|18642513|gb|AF278746.1|   Koliella spiculiformis 16S small subunit
ribosomal RNA gene, partial
            sequence; chloroplast gene for chloroplast product
          Length = 801

 Score =  908 bits (458), Expect = 0.0
 Identities = 584/608 (96%), Gaps = 16/608 (2%)
 Strand = Plus / Minus

Query: 457  ctgacgacagccatgcaccacc-gtgcattccactctggaactttctctttcgagaaaaa 515
            |||||||||||||||||||||| |||   |||||||||||||||||||||||||||||||
Sbjct: 660  ctgacgacagccatgcaccacctgtg---tccactctggaactttctctttcgagaaaaa 604


Query: 516  agtggcatgtcaagtcctggctaaggttcttcgcgattgcatcgaattaaaccacatgct 575
            |||||||||||||||||||| |||||||||||||| ||||||||||||||||||||||||
Sbjct: 603  agtggcatgtcaagtcctgg-taaggttcttcgcg-ttgcatcgaattaaaccacatgct 546


Query: 576  ccaccgcttgatgcgggcccccgctcaattcctttgagtttcactcttgtcgagcatact 635
            |||||||||| |||||||||||| ||||||||||||||||||||||||| ||||||||||
Sbjct:  545 ccaccgcttg-tgcgggcccccg-tcaattcctttgagtttcactcttg-cgagcatact 489


Query: 636  ccccaggcgggatacttcacgcgttagctacggtactgagtgatttgaatctacccaaca 695
            ||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||||
Sbjct: 488  ccccaggcgggatacttcacgcgttagctacggtactgaatgatttgaatctacccaaca 429


Query: 696  cctagtatccatcgtttacggcgaggactactggggtatctaatcccatttgctcccctc 755
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 428  cctagtatccatcgtttacggcgaggactactggggtatctaatcccatttgctcccctc 369


Query: 756  gctttcgtctctcagtgtcagtcgcggcccagcagagtgctttcgcctttggtgttcctc 815
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 368  gctttcgtctctcagtgtcagtcgcggcccagcagagtgctttcgcctttggtgttcctc 309


Query: 816  ccgatctctacgcatttcaccgctccaccgggaattccctctgcccctaccgaactctag 875
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 308  ccgatctctacgcatttcaccgctccaccgggaattccctctgcccctaccgaactctag 249


Query: 876  t-ttatagtttc-cctgcctgcccagagttaagccctggatctttgacaaaagacttgat 933
            | | |||||||| |||||||||||||||||||||||| ||||||||||| |||||||| |
Sbjct: 248  tctaatagtttctcctgcctgcccagagttaagccct-gatctttgacagaagacttggt 190


Query: 934  aaaccacctacagacgctttacgcccaatcattccggataacgcttgcatcccctgctct 993
            |||||||||||||||||||||||||||||||||||||||||||||||||||| ||| |||
Sbjct: 189  aaaccacctacagacgctttacgcccaatcattccggataacgcttgcatcctctg-tct 131
```

```
Query: 994  taccgcgggtgctggcacagggttag-cgatgcttattcctcagaataccgt-aaaatac 1051
            |||||||| ||||||||||| ||||| ||||||||||||||||| ||||||| ||||| |
Sbjct: 130  taccgcggctgctggcacagagttagccgatgcttattcctcag-ataccgtcaaaattc 72


Query: 1052 ttctctga 1059
            ||||||||
Sbjct: 71   ttctctga 64
```

Figure 4.26 Result of homology comparison between 16S rDNA of *Synechococcus* sp. NJ 7 (TISTR 8867) and data in GenBank.



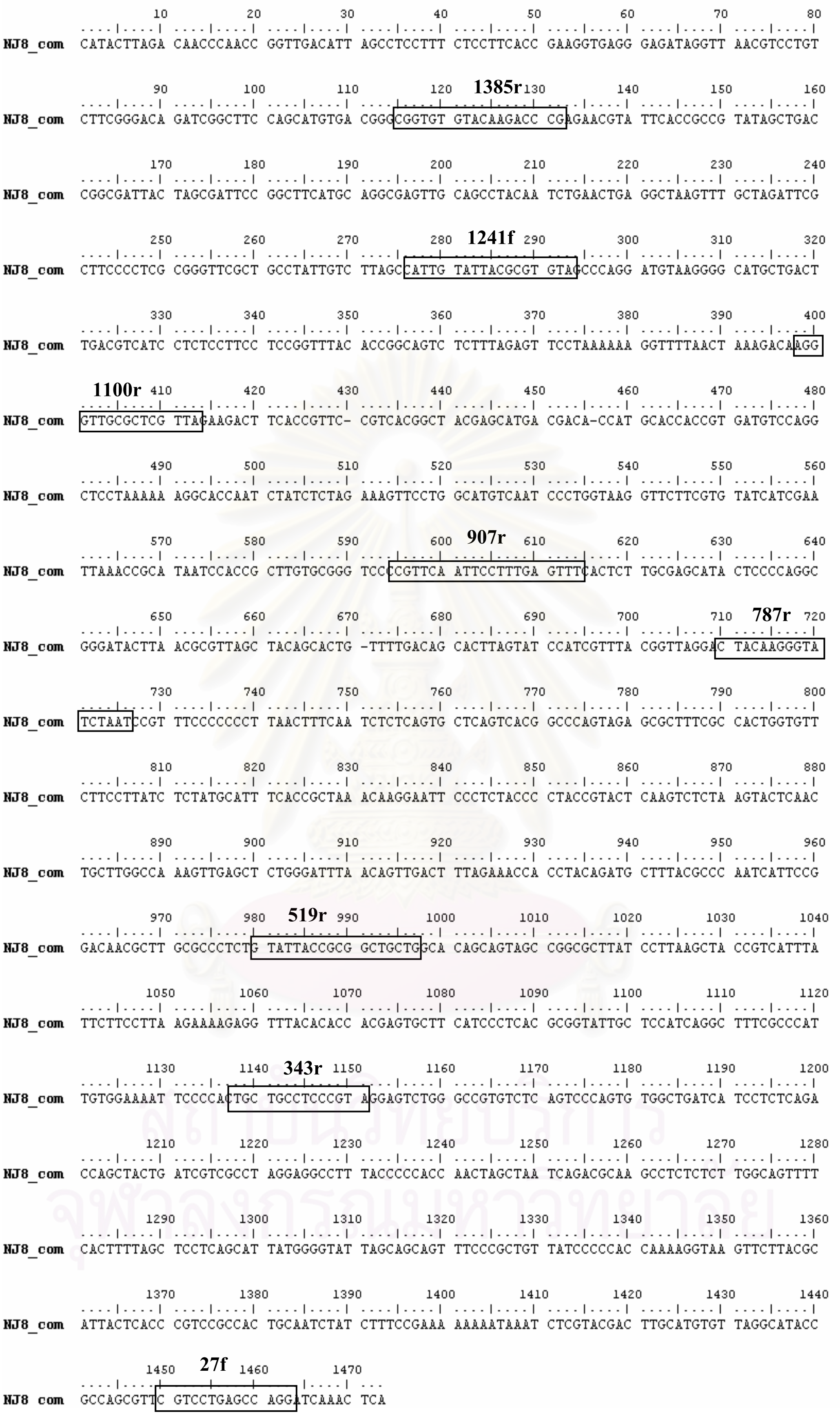
```
                   10         20         30         40   1492r 50         60         70         80
           ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26_COM   TACGGTACCT TGTTAGACTT CGATCGTATC GTAACATACG GCTACCTTGT TACGACTTCA CCCCAGATCA CCTACCCATT

                   90        100        110        120        130        140        150        160
           ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26_COM   CCTTAGGCGT CCCCCTCCAC AAGGCGTTGG AGTAACGACT TTGGGCATAG CCAGCTCCCA TGGTGTGACG GGCGGTGTGT

           1385r  170        180        190        200        210        220        230        240
           ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26_COM   ACAAGGCCCG GGAACCTATC TTGCAGTATG GCTGACCTGC GATTATAGCG ATTCCGACTT CATGCAGGCG AGTTGCAGCC

                  250        260        270        280        290        300        310  1241f 320
           ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26_COM   TGCAATCCGA ACTGAGACCG GGTTTTTGAG GTTGGCTAGC CCTCGCGGGT TTGCATCTCT TTGTCCCGGC CATTGTAGCA

                  330        340        350        360        370        380        390        400
           ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26_COM   CGTGTGTCGC CCAGGACGTA AGGGGCATGC TGACTTGACG TCATCCTCAC CTTCCTCCGG CTTGTCACCG GCAGTCTTTT

                  410        420        430  1100r 440        450        460        470        480
           ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26_COM   GAATTTCCCA TAACTGGCAA TTCAAAACAA GGGTTGCGCT CGTTCGGGGA CCTACCCATC ATGTCAAGAC ACGAGGCTGA

                  490        500        510        520        530        540        550        560
           ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26_COM   CGACAGCCAT GCACCACCTG TGTCCACTCT GGAACTTCCC CTTTCAGGGA AAAAGTGGCA TGTCAAGTCC TGGTAAGGTT

                  570        580        590        600        610   907r 620        630        640
           ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26_COM   CTTCGCGTTG CATCGAATTA AACCACATGC TCCACCGCTT GTGCGGGCCC CCGTCAATTC CTTTGAGTTG CACGCTCGCG

                  650        660        670        680        690        700        710        720
           ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26_COM   AGCATACTCC CCAGGCGGGA TACTTCACGC GTTAGCTCCG ATACTGAATG CTTTAACCTA TCCAACATCT AGTATCCATC

                  730        740   787r 750        760        770        780        790        800
           ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26_COM   GTTTACGGCG AGGACTACAG GGGTATCTAG GGGCCTTTGC CCCCCTAACT TTCGTCTCTC AGGCCTCAGG TGTGGCCCAG

                  810        820        830        840        850        860        870        880
           ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26_COM   CAAAGTGCTT TCGCCTTTGG GGTTCCTCCC GATCTCTACG CATTTCACCG CTCCACCGGG AATTCCCTCT GCCCCTACCA

                  890        900        910        920        930        940        950        960
           ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26_COM   AACTCTAGCC TCAGAGTTTC TCCTGCCGGC CCAGGGGTTA AGCCCTGATC TTTGACAGGA GACTTTTGAA GCCACCTACA

                  970        980        990       1000       1010  519r 1020       1030       1040
           ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26_COM   GACGCTTTAC GCCCAATCAT TCCGGATAAC GCTTGCATCC TCTGCTCTTA CCGCGGCTGC TGGCACAGAG TTAGCCGATG

                 1050       1060       1070       1080       1090       1100       1110       1120
           ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26_COM   CTTATTCCTC AGATACCGTC AAGATTCTTC TCTGAGAAAA GAAGTTTACA ACCCATAGGC CTTCATCCTT CACGCGGCAT

                 1130       1140       1150       1160  343r 1170       1180       1190       1200
           ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26_COM   TGCTCCGTCA GGCTTTCGCC CATTGCGGAA AATTCCTCAC TGCTGCCTCC CGTAGCGAGT CTGGGCCGTG TCTCAGTCCC

                 1210       1220       1230       1240       1250       1260       1270       1280
           ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26_COM   AGTGTGGCTG ATCATCCTCT CAGACCAGCT ACTGATCATT GCCTTGGGTA AGCCACTACC TCACCAACAA GCTAATCAGG

                 1290       1300       1310       1320       1330       1340       1350       1360
           ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26_COM   CGCCAAGCCC ATGCTCTTGG GCGATTTTCA TCTTTTCACT TCTCAGGACT ACGAGGTATT AGGCATCGTT TTCCAATGGT

                 1370       1380       1390       1400       1410       1420       1430       1440
           ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26_COM   GGGCCTGATC TCCAAAGAGG TACGGGTCTT ACCGTGTTAC TCACCTCGGT CCGCCACATC ATTTAAAATA AAGCAAGGCT

                 1450       1460       1470       1480       1490       1500   27f 1510       1520
           ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26 COM   CATTGGCATT CGTTACGGAC GGTGTACATG CCCGTTAAGC ATGCCGCCAA GCACTTTATC CTGAGCCCGG ATAAAACTCA
```

Figure 4.27 16S rDNA sequence of *Chlorella* sp.NJ 26 (TISTR 8852). Sequences of primers are shown in boxes.

```
Query= NJ26_com (1520 letters)

                                                              Score    E
Sequences producing significant alignments:                      (bits) Value

gi|12547|emb|X65689.1|CSSSRRNAX  C.sorokiniana plastid DNA s...  2072   0.0
gi|2224352|dbj|AB001684.1|  Chlorella vulgaris C-27 chloropl...  1947   0.0
gi|11468|emb|X16579.1|CHCVSSRN  Chlorella vulgaris chloropla...  1947   0.0
gi|45385169|gb|AY553213.1|  Auxenochlorella protothecoides 1...  1947   0.0
gi|1232075|dbj|D11347.1|CHLCPV16S  Chlorella vulgaris (strai...  1931   0.0
                                                  Alignments

☐
    >gi|12547|emb|X65689.1|CSSSRRNAX   C.sorokiniana plastid DNA small subunit
(16S-like) ribosomal RNA
          Length = 1493

 Score = 2072 bits (1045), Expect = 0.0
 Identities = 1273/1328 (95%), Gaps = 18/1328 (1%)
 Strand = Plus / Minus

Query: 37   tacggctaccttgttacgacttcaccccagatcacctacccattccttaggcgtccccct 96
             |||||||||||||||||||||||||||||| |||| || || | ||||||||||||||||
Sbjct: 1464 tacggctaccttgttacgacttcaccccag-tcac-tagccctgccttaggcgtccccct 1407


Query: 97   ccacaaggcgttggagtaacgactttgggcatagccagctcccatggtgtgacgggcggt 156
             |||||||| |||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1406 ccacaagg-gttggagtaacgactttgggcatagccagctcccatggtgtgacgggcggt 1348


Query: 157  gtgtacaaggcccgggaacctatctt--gcagtatggctgacctgcgatta-tagcgatt 213
             ||||||||||||||||||| |||     ||||||||||||||||||||||| ||||||||
Sbjct: 1347 gtgtacaaggcccgggaacgtattcaccgcagtatggctgacctgcgattactagcgatt 1288


Query: 214  ccgacttcatgcaggcgagttgcagcctgcaatccgaactgagaccgggttttgaggtt 273
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1287 ccgacttcatgcaggcgagttgcagcctgcaatccgaactgagaccgggttttgaggtt 1228


Query: 274  ggctagccctcgcgggtttgcatctctttgtcccggccattgtagcacgtgtgtcgccca 333
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1227 ggctagccctcgcgggtttgcatctctttgtcccggccattgtagcacgtgtgtcgccca 1168


Query: 334  ggacgtaaggggcatgctgacttgacgtcatcctcaccttcctccggcttgtcaccggca 393
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1167 ggacgtaaggggcatgctgacttgacgtcatcctcaccttcctccggcttgtcaccggca 1108


Query: 394  gtcttttgaatttcccataactggcaattcaaaacaagggttgcgctcgttcggggacct 453
             |||||||||||||||||||||||||||||||||||||||||||||||||||  ||||| |
Sbjct: 1107 gtcttttgaatttcccataactggcaattcaaaacaagggttgcgctcgttgcgggactt 1048


Query: 454  a-cccatcatgtcaagacacgaggctgacgacagccatgcaccacctgtgtccactctgg 512
             | |||| ||| |||||||||||| |||||||||||||||||||||||||| |||||||||
Sbjct: 1047 aacccaacatctcaagacacgag-ctgacgacagccatgcaccacctgtgyccactctgg 98


Query: 513  aacttcccctttcagggaaaaagtggcatgtcaagtcctggtaaggttcttcgcgttgca 572
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 988  aacttcccctttcagggaaaaagtggcatgtcaagtcctggtaaggttcttcgcgttgca 929
```

```
Query: 573  tcgaattaaaccacatgctccaccgcttgtgcgggcccccgtcaattcctttgagttgca 632
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||
Sbjct: 928  tcgaattaaaccacatgctccaccgcttgtgcgggcccccgtcaattcctttgagtttca 869


Query: 633  cgctcgcgagcatactccccaggcgggatacttcacgcgttagctccgatactgaatgct 692
            | || |||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 868  ctcttgcgagcatactccccaggcgggatacttcacgcgttagctccgatactgaatgct 809


Query: 693  ttaacctatccaacatctagtatccatcgtttacggcgaggactacaggggtatctaggg 752
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 808  ttaacctatccaacatctagtatccatcgtttacggcgaggactacaggggtatctaatc 749


Query: 753  gcctttgcccccctaactttcgtctctcaggcctcaggtgtggcccagcaaagtgctttc 812
             | ||||| |||||  ||||||||||||| |  |||| | |||||||||| |||||||||
Sbjct: 748  ccttttgctcccctcgctttcgtctctca-gtgtcagttatggcccagcagagtgctttc 690


Query: 813  gcctttggggttcctcccgatctctacgcatttcaccgctccaccgggaattccctctgc 872
            |||||||| ||||||||||| |||||||||||||||||||||||||||||||||||||||
Sbjct: 689  gcctttggtgttcctcccgakctctacgcatttcaccgctccaccgggaattccctctgc 630


Query: 873  ccctaccaaactctagcctcagagtttctcctgccggcccaggggttaagccctgatctt 932
            ||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||
Sbjct: 629  ccctaccaaactctagcctcagagtttctcctgccggccca-gggttaagccctgatctt 571


Query: 933  tgacaggagacttttgaagccacctacagacgctttacgcccaatcattccggataacgc 992
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 570  tgacaggagacttttgaagccacctacagacgctttacgcccaatcattccggataacgc 511


Query: 993  ttgcatcctctgctcttaccgcggctgctggcacagagttagccgatgcttattcctcag 1052
            |||||||||||| |||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 510  ttgcatcctctg-tcttaccgcggctgctggcacagagttagccgatgcttattcctcag 452

Query: 1053 ataccgtcaagattcttctctgagaaaagaagtttacaacccataggccttcatccttca 1112
            |||||||||| |||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 451  ataccgtcaaaattcttctctgagaaaagaagtttacaacccataggccttcatccttca 392


Query: 1113 cgcggcattgctccgtcaggctttcgcccattgcggaaaattcctcactgctgcctcccg 1172
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 391  cgcggcattgctccgtcaggctttcgcccattgcggaaaattcctcactgctgcctcccg 332


Query: 1173 tagcgagtctgggccgtgtctcagtcccagtgtggctgatcatcctctcagaccagctac 1232
            ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 331  tag-gagtctgggccgtgtctcagtcccagtgtggctgatcatcctctcagaccagctac 273


Query: 1233 tgatcattgccttgggtaagccactacctcaccaacaagctaatcaggcgccaagcccat 1292
            ||||||||||||| ||||||||| ||||||||||||||||||||||| || |||||||||
Sbjct: 272  tgatcattgcctt-ggtaagccattacctcaccaacaagctaatcagccg-caagcccat 215


Query: 1293 gctcttgggcgattttcatcttttcacttctcaggactacgaggtattaggcatcgtttt 1352
             ||||| ||||||||||||| ||||||||||||| | ||||||||||||| ||||| |||
Sbjct: 214  -ctctt-ggcgattttcatc-tttcacttctcagcaatacgaggtattagccatcg-ttt 159


Query: 1353 ccaatggt 1360
            ||||||||
Sbjct: 158  ccaatggt 151
```

Figure 4.28 Result of homology comparison between 16S rDNA of *Chlorella* sp.NJ 26 (TISTR 8852) and data in GenBank.



```
                 10         20         30         40         50         60         70         80
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_com  CATACTTAGA CAACCCAACC GGTTGACATT AGCCTCCTTT CTCCTTCACC GAAGGTGAGG GAGATAGGTT AACGTCCTGT

                 90        100        110        120 1385r 130        140        150        160
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_com  CTTCGGGACA GATCGGCTTC CAGCATGTGA CGGGCGGTGT GTACAAGACC CGAGAACGTA TTCACCGCCG TATAGCTGAC

                170        180        190        200        210        220        230        240
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_com  CGGCGATTAC TAGCGATTCC GGCTTCATGC AGGCGAGTTG CAGCCTACAA TCTGAACTGA GGCTAAGTTT GCTAGATTCG

                250        260        270        280 1241f 290        300        310        320
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_com  CTTCCCCTCG CGGGTTCGCT GCCTATTGTC TTAGCCATTG TATTACGCGT GTAGCCCAGG ATGTAAGGGG CATGCTGACT

                330        340        350        360        370        380        390        400
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_com  TGACGTCATC CTCTCCTTCC TCCGGTTTAC ACCGGCAGTC TCTTTAGAGT TCCTAAAAAA GGTTTTAACT AAAGACAAGG

           1100r 410        420        430        440        450        460        470        480
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_com  GTTGCGCTCG TTAGAAGACT TCACCGTTC- CGTCACGGCT ACGAGCATGA CGACA-CCAT GCACCACCGT GATGTCCAGG

                490        500        510        520        530        540        550        560
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_com  CTCCTAAAAA AGGCACCAAT CTATCTCTAG AAAGTTCCTG GCATGTCAAT CCCTGGTAAG GTTCTTCGTG TATCATCGAA

                570        580        590        600  907r  610        620        630        640
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_com  TTAAACCGCA TAATCCACCG CTTGTGCGGG TCCCGTTCA ATTCCTTTGA GTTTCACTCT TGCGAGCATA CTCCCCAGGC

                650        660        670        680        690        700        710  787r 720
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_com  GGGATACTTA ACGCGTTAGC TACAGCACTG -TTTTGACAG CACTTAGTAT CCATCGTTTA CGGTTAGGAC TACAAGGGTA

                730        740        750        760        770        780        790        800
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_com  TCTAATCCGT TTCCCCCCCT TAACTTTCAA TCTCTCAGTG CTCAGTCACG GCCCAGTAGA GCGCTTTCGC CACTGGTGTT

                810        820        830        840        850        860        870        880
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_com  CTTCCTTATC TCTATGCATT TCACCGCTAA ACAAGGAATT CCCTCTACCC CTACCGTACT CAAGTCTCTA AGTACTCAAC

                890        900        910        920        930        940        950        960
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_com  TGCTTGGCCA AAGTTGAGCT CTGGGATTTA ACAGTTGACT TTAGAAACCA CCTACAGATG CTTTACGCCC AATCATTCCG

                970        980  519r  990       1000       1010       1020       1030       1040
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_com  GACAACGCTT GCGCCCTCTG TATTACCGCG GCTGCTGGCA CAGCAGTAGC CGGCGCTTAT CCTTAAGCTA CCGTCATTTA

               1050       1060       1070       1080       1090       1100       1110       1120
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_com  TTCTTCCTTA AGAAAAGAGG TTTACACACC ACGAGTGCTT CATCCCTCAC GCGGTATTGC TCCATCAGGC TTTCGCCCAT

               1130       1140 343r 1150       1160       1170       1180       1190       1200
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_com  TGTGGAAAAT TCCCCACTGC TGCCTCCCGT AGGAGTCTGG GCCGTGTCTC AGTCCCAGTG TGGCTGATCA TCCTCTCAGA

               1210       1220       1230       1240       1250       1260       1270       1280
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_com  CCAGCTACTG ATCGTCGCCT AGGAGGCCTT TACCCCCACC AACTAGCTAA TCAGACGCAA GCCTCTCTCT TGGCAGTTTT

               1290       1300       1310       1320       1330       1340       1350       1360
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_com  CACTTTTAGC TCCTCAGCAT TATGGGGTAT TAGCAGCAGT TTCCCGCTGT TATCCCCCAC CAAAAGGTAA GTTCTTACGC

               1370       1380       1390       1400       1410       1420       1430       1440
         ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_com  ATTACTCACC CGTCCGCCAC TGCAATCTAT CTTTCCGAAA AAAAATAAAT CTCGTACGAC TTGCATGTGT TAGGCATACC

               1450  27f  1460       1470
         ....|....| ....|....| ....|....| ...
NJ8 com  GCCAGCGTTC GTCCTGAGCC AGGATCAAAC TCA
```

Figure 4.29 16S rDNA sequence of *Scenedesmus* sp.NJ 8 (TISTR 8859). Sequences of primers are shown in boxes.

```
Query= NJ8_com (1470 letters)

                                                                Score    E
Sequences producing significant alignments:                     (bits) Value

gi|15011445|gb|AF394206.1|AF394206  Scenedesmus obliquus 16S...  1907   0.0
gi|28881833|emb|AJ548895.1|USC548895  uncultured Scenedesmus...  1372   0.0
gi|28881832|emb|AJ548894.1|USC548894  uncultured Scenedesmus...  1150   0.0
gi|28881831|emb|AJ548893.1|USC548893  uncultured Scenedesmus...   971   0.0
gi|22266264|emb|AJ427435.1|UAL427435  Uncultured algae chlor...   942   0.0
                                 Alignments

☐
   >gi|15011445|gb|AF394206.1|AF394206   Scenedesmus obliquus 16S ribosomal
RNA gene, partial sequence;
            chloroplast gene for chloroplast product
          Length = 1220

 Score = 1907 bits (962), Expect = 0.0
 Identities = 1167/1213 (96%), Gaps = 21/1213 (1%)
 Strand = Plus / Minus

Query: 187  atgcaggcgagttgcagcctacaatctgaactgaggctaagtttgctagattcgcttccc 246
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1217 atgcaggcgagttgcagcctacaatctgaactgaggctaagtttgctagattcgcttccc 1158


Query: 247  ctcgcgggttcgctgcctattgtcttagccattgtattacgcgtgtagcccaggatgtaa 306
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1157 ctcgcgggttcgctgcctattgtcttagccattgtattacgcgtgtagcccaggatgtaa 1098


Query: 307  ggggcatgctgacttgacgtcatcctctccttcctccggtttacaccggcagtctcttta 366
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 1097 ggggcatgctgacttgacgtcatcctctccttcctccggtttacaccggcagtctcttta 1038


Query: 367  gagttcctaaaaaaggttttaactaaagacaagggttgcgctcgttagaagacttcaccg 426
            |||||||   ||| ||||||||||||||||||||||||||||||||||||||||| ||
Sbjct: 1037 gagttcc---aaatggttttaactaaagacaagggttgcgctcgttagaagacttaactc 981


Query: 427  ttc-cgtcacggctacgagcatgacgaca-ccatgcaccaccgtgatgtccaggctccta 484
            | | | ||||||| |||||| |||||||| |||||||||||| || |||||||| |||||
Sbjct: 980  tacacctcacggc-acgagc-tgacgacagccatgcaccacc-tg-tgtccaggttccta 925


Query: 485  aaaaaggcaccaatctatctctagaaagttcctggcatgtcaatccctggtaaggttctt 544
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 924  aaaaaggcaccaatctatctctagaaagttcctggcatgtcaatccctggtaaggttctt 865


Query: 545  cgtgtatcatcgaattaaaccgcataatccaccgcttgtgcgggtccccgttcaattcct 604
            |||||||||||||||||||||||||||||||||| |||||||||||||||| ||||||||
Sbjct: 864  cgtgtatcatcgaattaaaccgcataatccaccg-ttgtgcgggtccccgt-caattcct 807


Query: 605  ttgagtttcactcttgcgagcatactccccaggcgggatacttaacgcgttagctacagc 664
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 806  ttgagtttcactcttgcgagcatactccccaggcgggatacttaacgcgttagctacagc 747


Query: 665  actgtttt-gacagcacttagtatccatcgtttacggttaggactacaagggtatctaat 723
            |||||||| |||||||||||||||||||||||||| ||||||||||||||||||||||||
Sbjct: 746  actgttttgacagcacttagtatccatcgtttacagttaggactacaagggtatctaat 687
```

```
Query: 724  ccgtttnnnnnnnttaactttcaatctctcagtgctcagtcacggcccagtagagcgctt 783
            || |||        ||||||||| |||||||||| ||||| ||||||||| |||||||||
Sbjct: 686  ccttttcgctcccctaactttca-tctctcagtg-tcagttacggcccagaagagcgctt 629


Query: 784  tcgccactggtgttcttccttatctctatgcatttcaccgctaaacaaggaattccctct 843
            |||||| ||||||||||||||||||||||||||||||||| |||||||||||||||||||
Sbjct: 628  tcgccaatggtgttcttccttatctctatgcatttcaccg-taaacaaggaattccctct 570


Query: 844  acccctaccgtactcaagtctctaagtactcaactgcttggccaaagttgagctctggga 903
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 569  acccctaccgtactcaagtctctaagtactcaactgcttggccaaagttgagctctggga 510


Query: 904  tttaacagttgactttagaaaccacctacagatgctttacgcccaatcattccggacaac 963
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 509  tttaacagttgactttagaaaccacctacagatgctttacgcccaatcattccggacaac 450


Query: 964  gcttgcgccctctgtattaccgcggctgctggcacagcagtagccggcgcttat-cctta 1022
            |||||| |||||||||||||||||| ||||||||||| ||||||||| |||||| |||||
Sbjct: 449  gcttgcaccctctgtattaccgcgg-tgctggcacag-agtagccggtgcttattcctta 392


Query: 1023 agctaccgtcatttattcttccttaagaaaagaggtttacacaccacgagtgcttcatcc 1082
            || |||||||||| ||||||||||||||||||||||||||||||||||| ||||||||||
Sbjct: 391  ag-taccgtcattgattcttccttaagaaaagaggtttacacaccacga-tgcttcatcc 334


Query: 1083 ctcacgcggtattgctccatcaggctttcgcccattgtggaaaattccccactgctgcct 1142
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 333  ctcacgcggtattgctccatcaggctttcgcccattgtggaaaattccccactgctgcct 274


Query: 1143 cccgtaggagtctgggccgtgtctcagtcccagtgtggctgatcatcctctcagaccagc 1202
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 273  cccgtaggagtctgggccgtgtctcagtcccagtgtggctgatcatcctctcagaccagc 214


Query: 1203 tactgatcgtcgcctagg-aggcctttaccccccaccaactagctaatcagacgcaagcct 1261
            ||||||||||||||| || |||||||||||||||||||||||||||||||||||||||||
Sbjct: 213  tactgatcgtcgccttgggaggcctttaccccccaccaactagctaatcagacgcaagcct 154


Query: 1262 ctctcttggcagttttcacttttagctcctcagcattatggggtattagcagcagtttcc 1321
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 153  ctctcttggcagttttcacttttagctcctcagcattatggggtattagcagcagtttcc 94


Query: 1322 cgctgttatcccccaccaaaaggtaagttcttacgcattactcacccgtccgccactgca 1381
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct: 93   cgctgttatcccccaccaaaaggtaagttcttacgcattactcacccgtccgccactgca 34


Query: 1382 atctatctttccg 1394
            ||||| |||||||
Sbjct: 33   atctacctttccg 21
```

Figure 4.30 Result of homology comparison between 16S rDNA of *Scenedesmus* sp. NJ8 (TISTR 8859) and data in GenBank.

Protein Profiles

Figure 4.31 showed growth curves of *Synechococccus* sp. NJ 7 (TISTR 8867) cultured in BG-11, *Chlorella* sp. NJ26 (TISTR 8852) and *Scenedesmus* sp. NJ 8(TISTR 8859) cultured in BBM medium at 150 rpm, 38-42° C and 1000 lux. Mid log phase cells were obtained after cultivation for 7 days. Their intracellular protein profiles were shown in Figure 4.32.

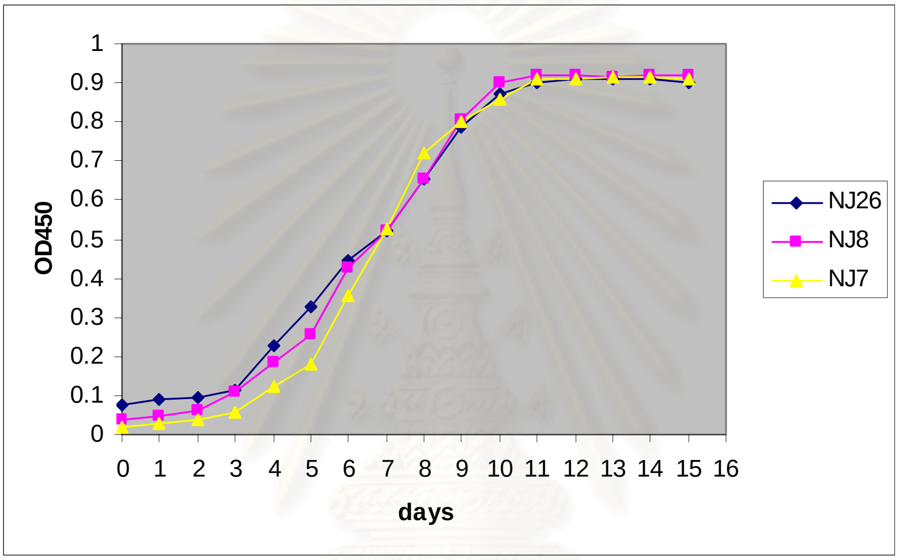


Figure 4.31 Growth curves of *Synechococcus* sp. NJ 7 (TISTR 8867) cultured in BG-11 , *Chlorella* sp. NJ 26 (TISTR 8852), and *Scenedesmus* sp.NJ 8 TISTR 8859 cultured in BBM medium at 150 rpm, 38-42° C and 1000 lux. Mid log phase cells were obtained after cultivation for 7 days.

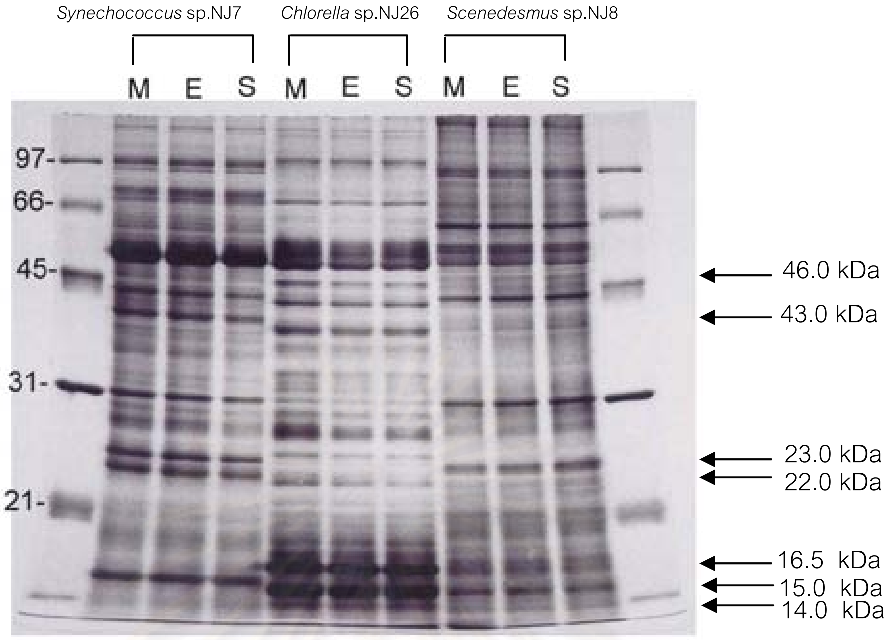


Figure 4.32 SDS-PAGE of intracellular protein profiles of Mid-log(M), Early stationary phase (E) and Late stationary phase cells (S) of *Synechococcus* sp. NJ 7 (TISTR 8867), *Chlorella* sp.NJ 26 (TISTR 8852) and *Scenedesmus* sp.NJ 8 (TISTR 8859).

Intracellular protein profiles of the prokaryotic *Synechococcus* sp. NJ7 (TISTR 8867) and eukaryotic *Chlorella* sp. NJ26 (TISTR 8852) and *Scenedesmus* sp. NJ8 (TISTR 8859) indicated that polypeptide patterns from cells of different stages of growth of the three strains were the same. However, some polypeptides such as the 43 kDa, the 46 kDa, the 23 kDa may be specific for *Synechococcus* sp. NJ7 (TISTR 8867) *Chlorella* sp. NJ26 (TISTR 8852) and *Scenedesmus* sp. NJ8 (TISTR 8859) respectively. In addition, the 22 kDa polypeptide was more abundant in *Synechococcus* sp. NJ7 (TISTR 8867) and *Scenedesmus* sp. NJ8 (TISTR 8859) while polypeptides 16.5 kDa, 15 kDa, 14 kDa were more abundant in *Chlorella* sp. NJ26 (TISTR 8852).

CHAPTER V

DISCUSSION

The isolation of just one strain of *Synechococcus* sp. NJ7(TISTR 8867) from freshwater ponds at the Clock Tower and the Physics building, Chulalongkorn University (Table 4.1, Figures 4.1 and 4.2) was not as expected. Many isolates of *Synechococcus* spp. had been reported from hot springs, in the Northern part of Thailand. Sompong et al.(2005) reported the presence of three *Synechococcus* spp. (*S. lividus*, *S. bigranulatus* and *Synechococcus* sp.) in hot springs in nine districts of Chiangmai in the Northern part of Thailand. The most abundant forms which dominated in the 60°C - 80°C rang were *Synechococcus* sp. and *S. lividus* while all the three *Synechococcus* spp. were found across the 40°C - 80°C temperature range.

It remains to be seen if more *Synechococcus* sp. isolates are obtained from extreme environments such as hot springs and marine environments when compared to freshwater bodies. More freshwater *Synechococcus* strains could lead to molecular characterization which, in turns, would lead to comparisons of genotypes or ecotypes of *Synechococcus* spp. found in mild and in extreme environments. Sompong et al. (2005) stated that genotypic characters are more sensitive measures of diversity in cyanobacteria compared to morphotypic characters or morphotypes.

Different RAPD-PCR fingerprints for *Chlorella* spp. and *Scenedesmus* spp. isolates of similar morphology indicated there were cryptic species in these micro-algae.

It is of interest to note that pure cultures of cyanobacteria and micro-algae obtained were either unicellular (*Synechococcus* sp., *Chlorella* spp.) or colonial type which at times were unicellular (*Scenedesmus* spp.) Figures 4.11 showed colonial *Scenedesmus* spp. which became unicellular upon prolonged growth. It is envisaged that unicellular cells render themselves isolatable while filamentous strains are relatively harder to isolate as pure cultures. The reason is because of more surface area for attachment by contaminating bacteria. Future work could include the use of finely-drawn Pasteur pipettes for single cell isolation and repeated washings in order to isolate cyanobacteria and micro-algae of diverse morphologies. RAPD-PCR fingerprints with the set of primers normally used to determine 16S rDNA sequence of *E. coli* yielded satisfactory results. Isolates with similar morphology could be determined if they were the same strains based on identical RAPD-PCR fingerprints. However, 16S rDNA sequences could not be used to identify the strains of *Synechococcus* sp. NJ7 (TISTR 8867), *Chlorella* sp. NJ26 (TISTR 8852), *Scenedemus* sp. NJ8 (TISTR 8859) due to the unavailability of full sequences deposited at GenBank. Fox et al, (1992) reported that sequence analysis of the 16S rRNA gene was a powerful method for assigning strains to species, provided 16S rDNA sequences of the species were deposited in the database. Homology comparisons with 16S rDNA sequences in GenBank indicated that the sequences obtained were the first reported of full 16S rDNA sequences for *Synechococcus* sp. NJ7 (TISTR 8867), *Chlorella* sp. NJ26 (TISTR 8852), *Scenedemus* sp. NJ8 (TISTR 8859). The sequences will soon be deposited with GenBank.

Based on the collage of RAPD-PCR fingerprints of all the 16 isolated strains as shown in Figures 4.20-4.23 , no DNA fragment specific to each of the genera was detected. In addition all the isolated strains were not *Synechococcus* sp. NIES-946, *Chlorella vulgaris* var. *vulgaris* NIES-686, *Scenedesmus dimorphus* NIES-93 which were reference strains obtained from the Institute for Environmental studies (NIES), Japan.

All of 16 isolated strains are available from both the Bangkok MIRCEN (Microbiological Resources Center) and the Department of Microbiology, Faculty of Science, Chulalongkorn University. When the strains are requested, both the strains and their RAPD-PCR fingerprints will be distributed. The RAPD-PCR fingerprints will be useful for industries to keep track on changes in genetic materials of the algal strains upon industrial utilization.

RAPD-PCR fingerprints could be used in monitoring changes in genetic materials of these cyanobacterium and micro-algae upon storage or continuous uses. The 16S rDNA sequence data obtained will be useful for the determination of cryptic species in *Synechococcus* spp., *Chlorella* spp., and *Scenedesmus* spp. N-terminal amino acid sequences of polypeptides such as the 46 and 16.5 kDa may lead to the design of primers specific for the detection of *Chlorella* spp. and eukaryotic micro-algae respectively.

CHAPTER VI

CONCLUSION

In conclusion, RAPD-PCR fingerprints of 1 *Synechococcus* sp. strain, 9 *Chlorella* spp. strains, and 6 *Scenedesmus* spp. strains were obtained as summarized in this chapter.

Three 16S rDNA sequences and SDS-PAGE intracellular protein profiles of mid-log, early stationary and late stationary phases cells of the following strains were obtained: *Synechococcus* sp. NJ7 (TISTR 8867), *Chlorella* sp. NJ26 (TISTR 8852) and *Scenedesmus* sp. NJ8 (TISTR 8859). The complete 16S rDNA sequences were the first reported full sequences which will be deposited with GenBank. The protein profiles indicated a 46 kDa polypeptide may be specific for *Chlorella* spp.and a 16.5 kDa polypeptide may be specific for eukaryotic *Chlorella* spp. and *Scenedesmus* spp.

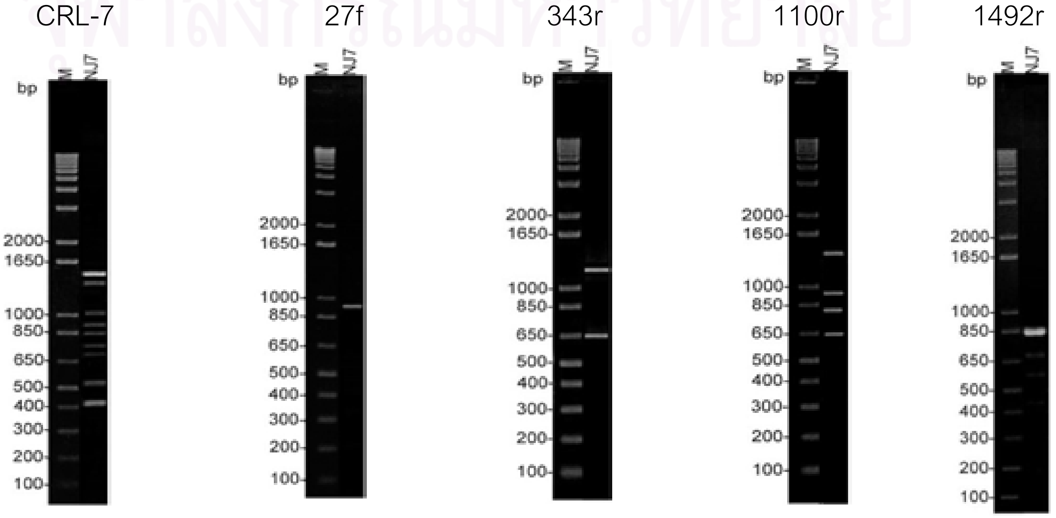
The isolated strains deposited with Bangkok MIRCEN were given TISTR codes as indicated in the following summary.

Synechococcus sp. NJ7 (TISTR 8867)

Morphology in BG-11 at 25°C

3 days 6 days 9 days 12 days

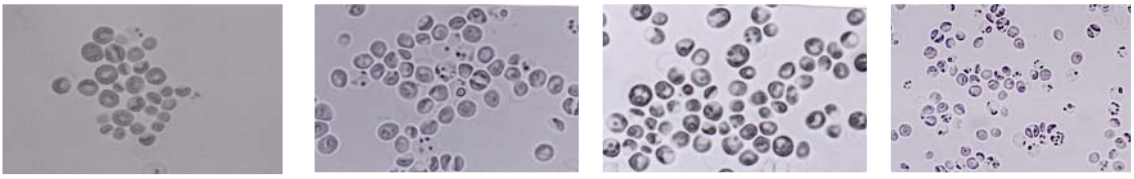
RAPD-PCR fingerprints



CRL-7 27f 343r 1100r 1492r

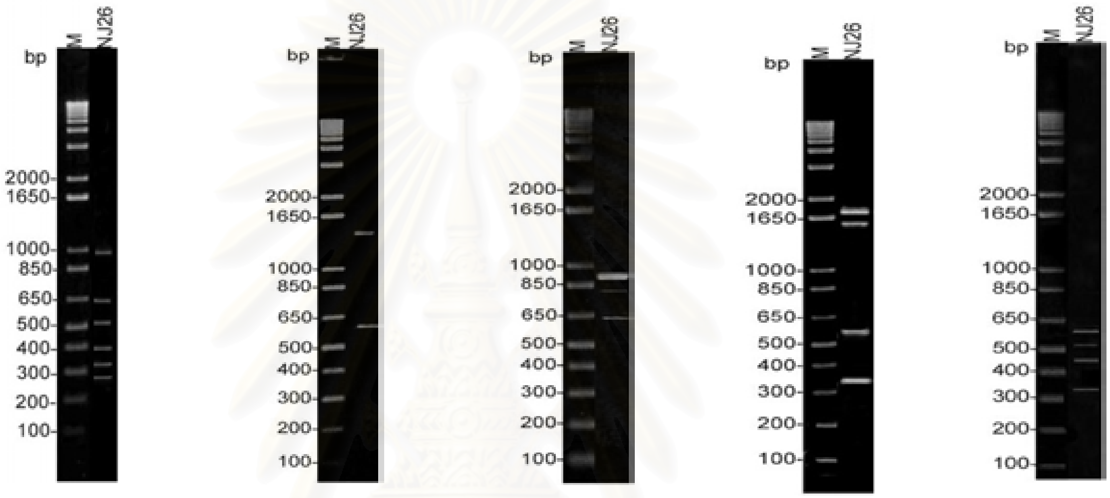
Chlorella sp. NJ26 (TISTR 8852)

Morphology in BG-11 at 25°C



3 days 6 days 9 days 12 days

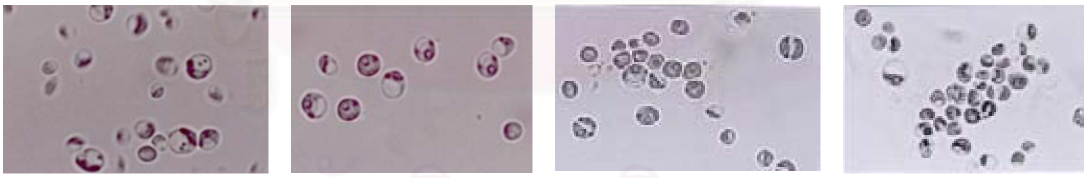
RAPD-PCR fingerprints



CRL-7 27f 343r 1100r 1492r

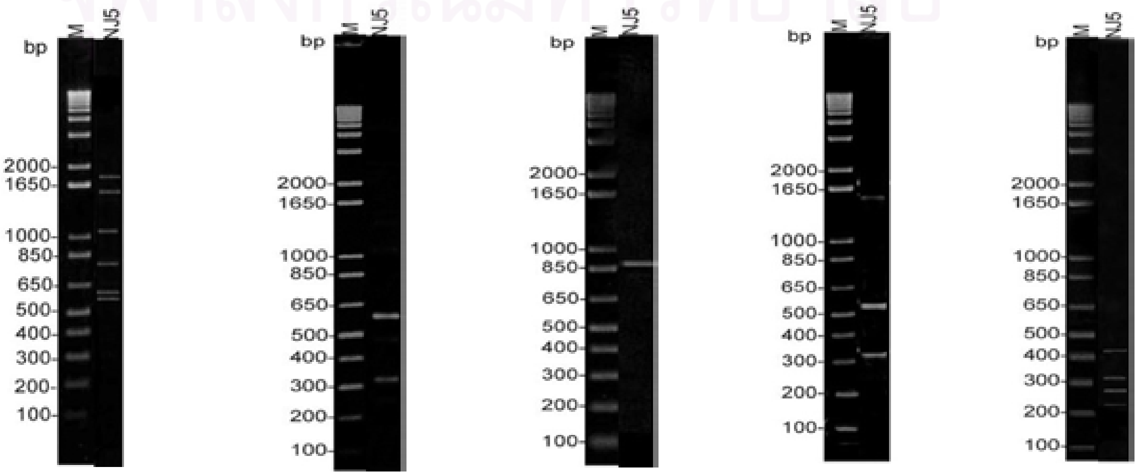
Chlorella sp. NJ5 (TISTR 8853)

Morphology in BG-11 at 25°C



3 days 6 days 9 days 12 days

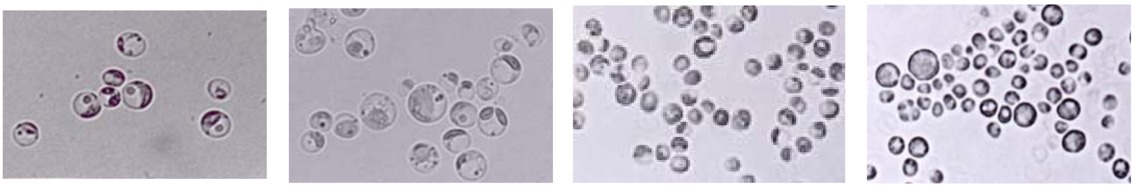
RAPD-PCR fingerprints



CRL-7 27f 343r 1100r 1492r

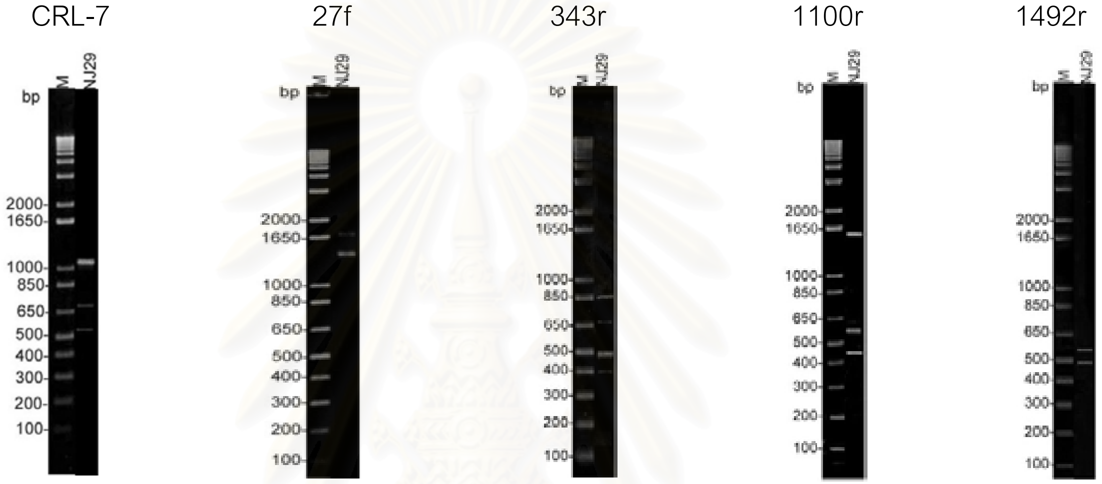
Chlorella sp. NJ29 (TISTR 8854)

Morphology in BG-11 at 25°C



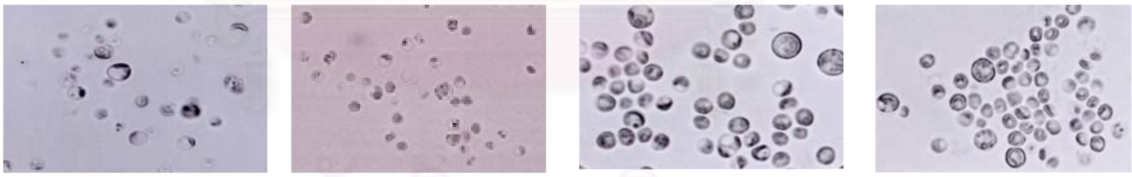
3 days 6 days 9 days 12 days

RAPD-PCR fingerprints



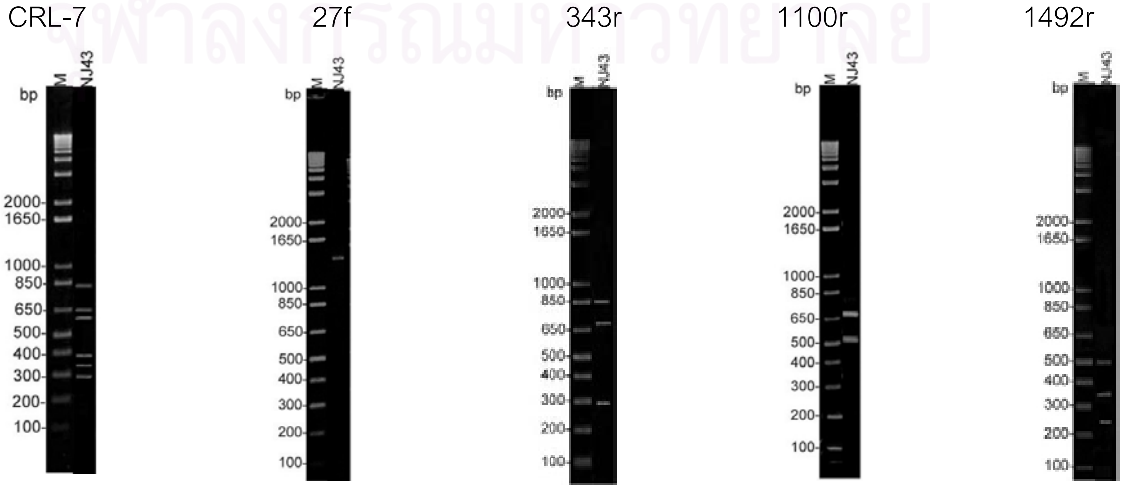
Chlorella sp. NJ43 (TISTR 8855)

Morphology in BG-11 at 25°C



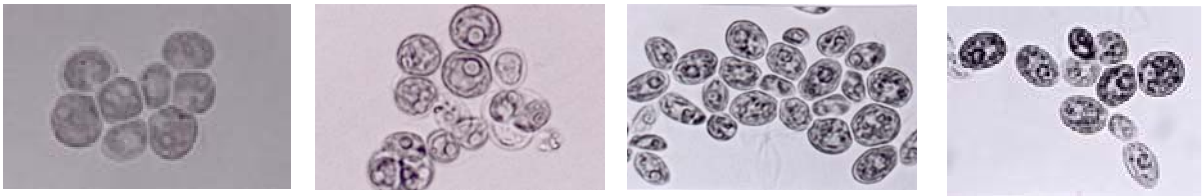
3 days 6 days 9 days 12 days

RAPD-PCR fingerprints



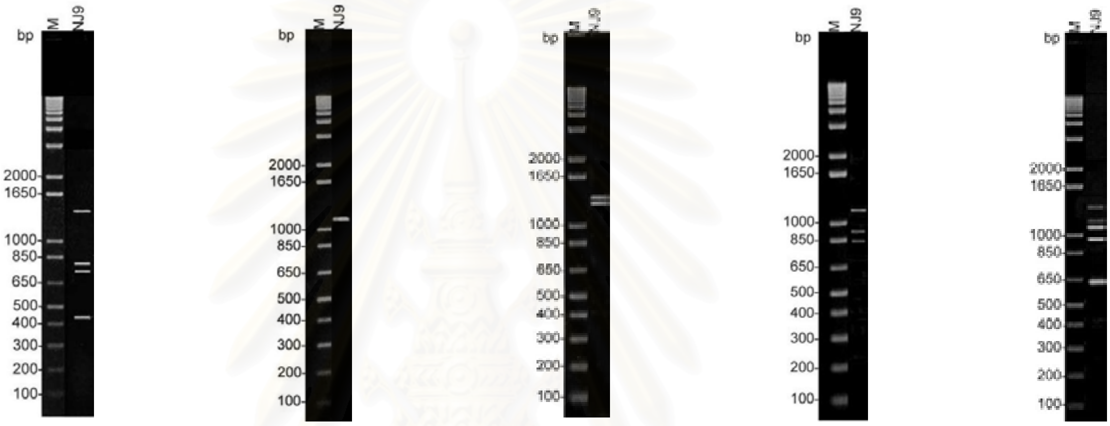
Chlorella sp. NJ9 (TISTR 8856)

Morphology in BG-11 at 25°C



3 days 6 days 9 days 12 days

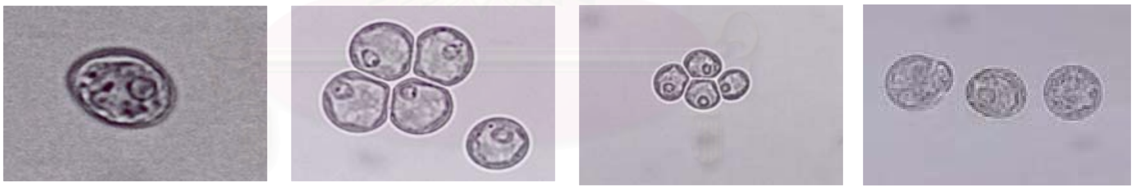
RAPD-PCR fingerprints



CRL-7 27f 343r 1100r 1492r

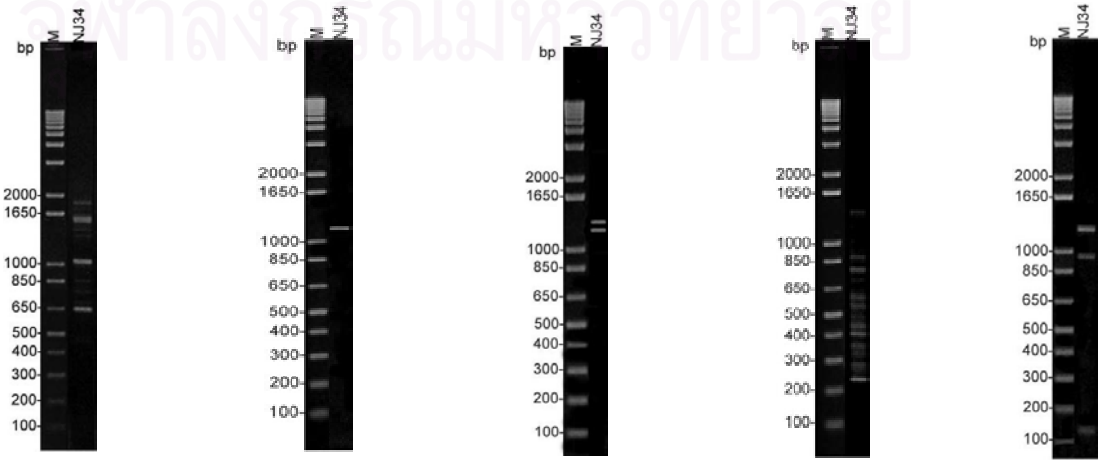
Chlorella sp. NJ34 (TISTR 8857)

Morphology in BG-11 at 25°C



3 days 6 days 9 days 12 days

RAPD-PCR fingerprints



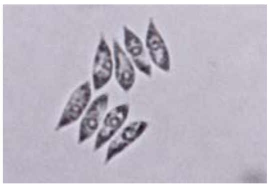
CRL-7 27f 343r 1100r 1492r

Scenedesmus sp. NJ8 (TISTR 8859)

Morphology in BG-11 at 25°C


3 days


6 days


9 days


12 days

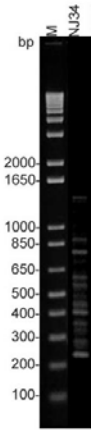
RAPD-PCR fingerprints

CRL-7

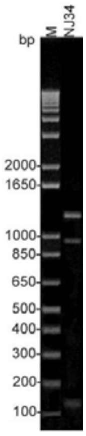
27f

343r

1100r



1492r

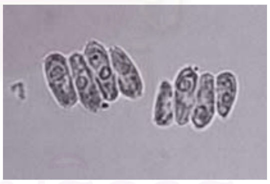


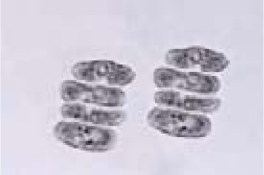
Scenedesmus sp. NJ39 (TISTR 8858)

Morphology in BG-11 at 25°C


3 days

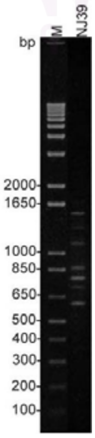

6 days


9 days

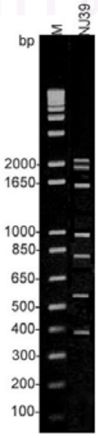

12 days

RAPD-PCR fingerprints

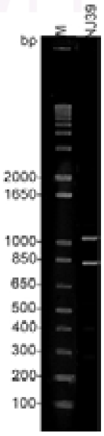
CRL-7



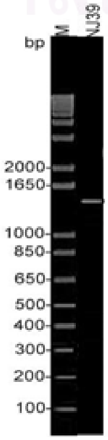
27f



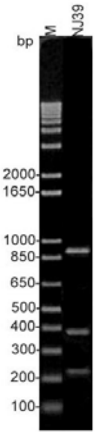
343r



1100r

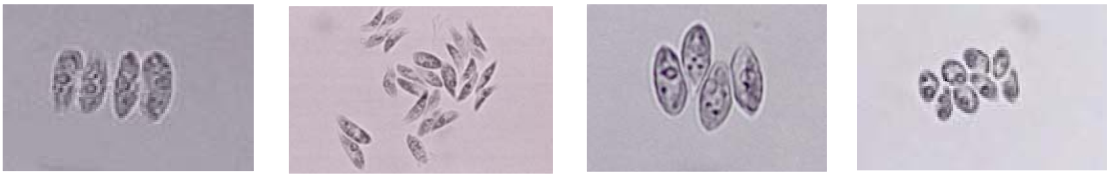


1492r



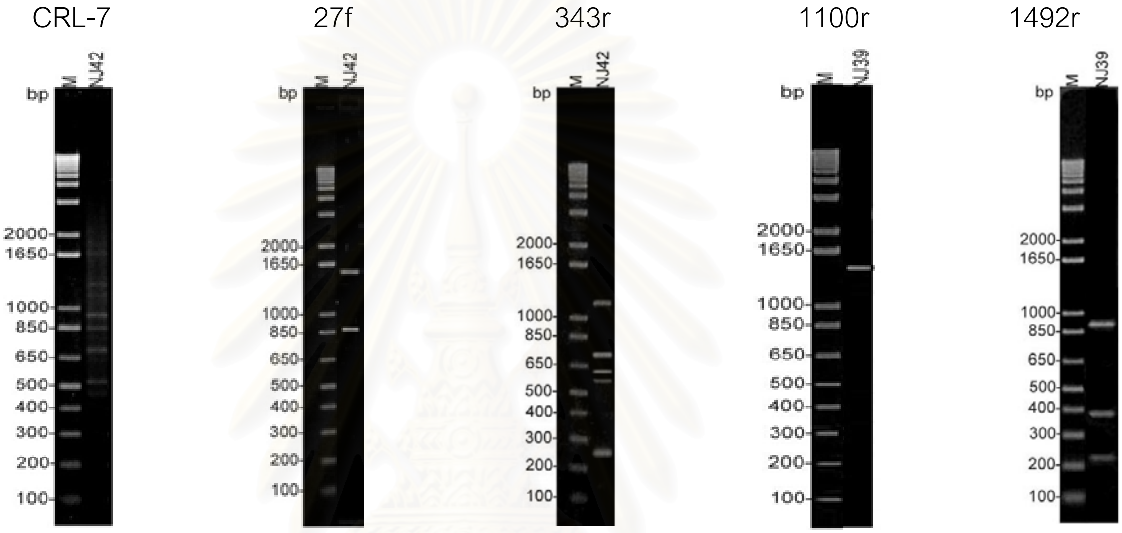
Scenedesmus sp. NJ42 (TISTR 8860)

Morphology in BG-11 at 25°C



3 days 6 days 9 days 12 days

RAPD-PCR fingerprint

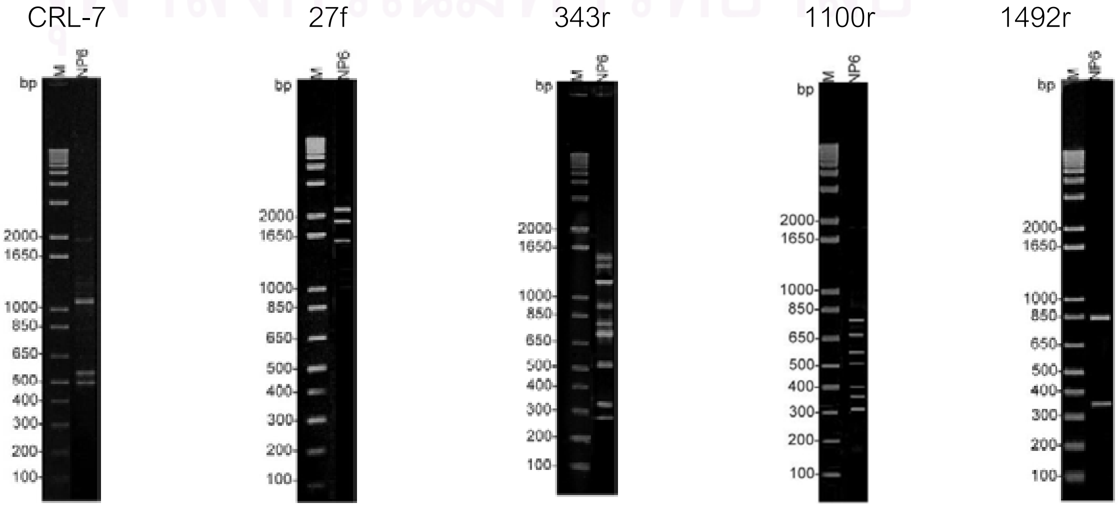


Scenedesmus sp.NP6 (TISTR 8861)

Morphology in BG-11 at 25°C

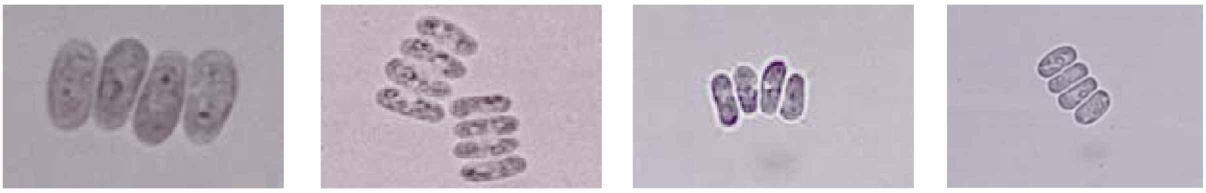
3 days 6 days 9 days 12 days

RAPD-PCR fingerprints



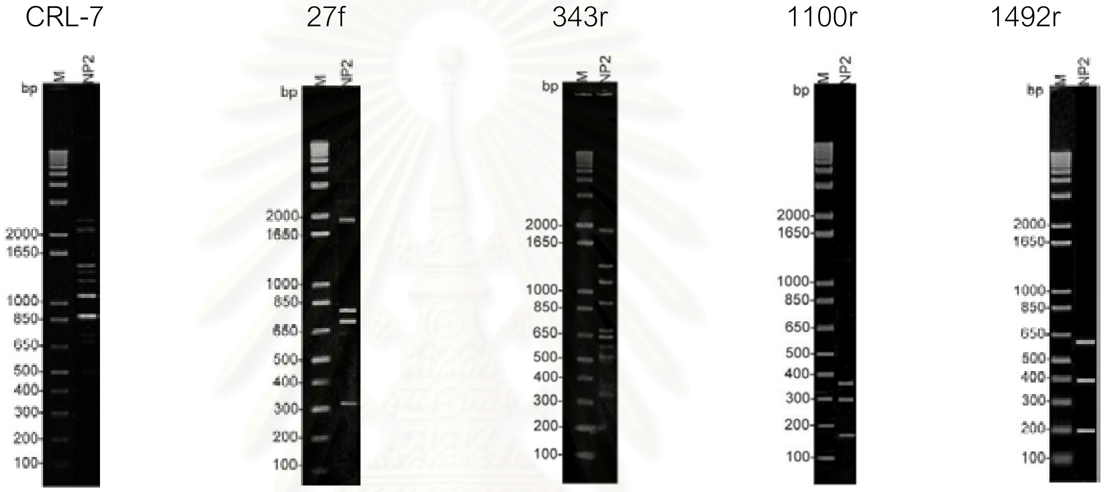
Scenedesmus sp. NP2 (TISTR 8862)

Morphology in BG-11 at 25°C



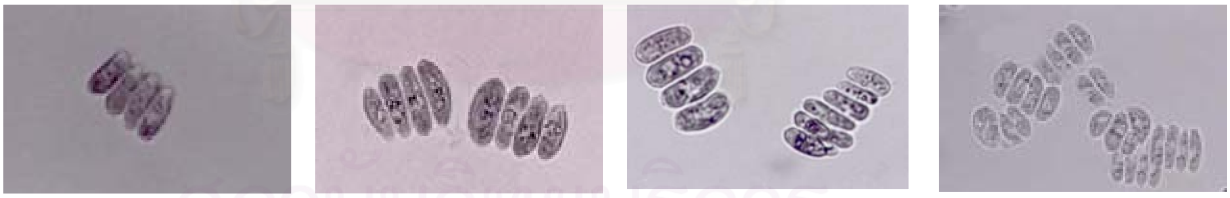
3 days 6 days 9 days 12 days

RAPD-PCR fingerprints



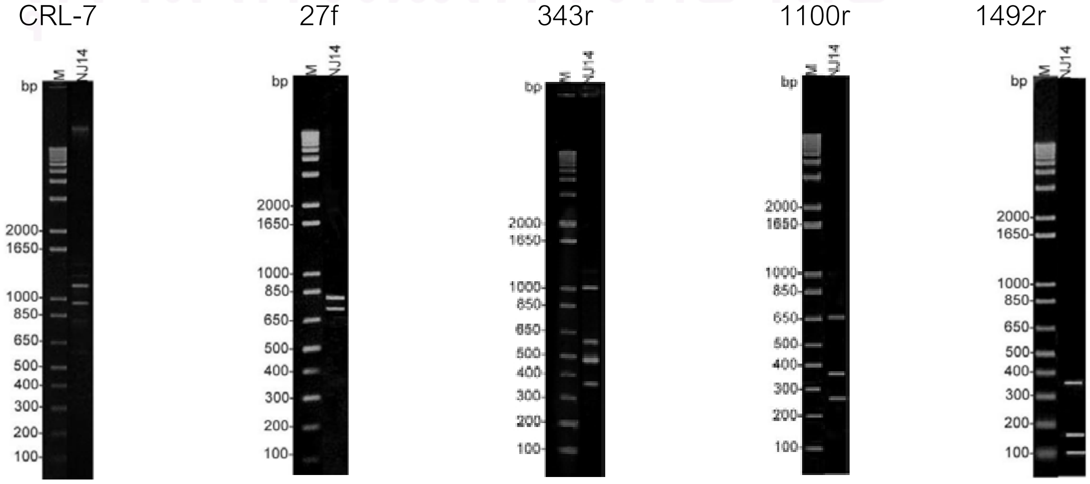
Scenedesmus sp. NJ14 (TISTR 8863)

Morphology in BG-11 at 25°C



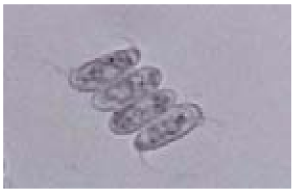
3 days 6 days 9 days 12 days

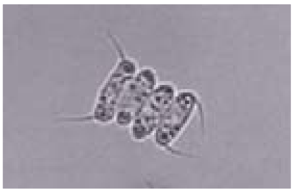
RAPD-PCR fingerprints

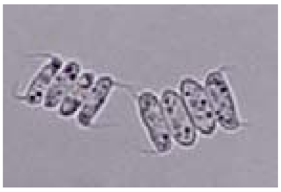


Scenedesmus sp. NJ23 (TISTR 8864)

Morphology in BG-11 at 25°C


3 days


6 days

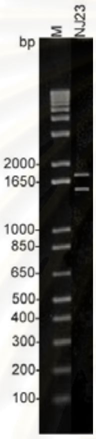

9 days


12 days

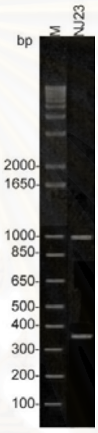
RAPD-PCR fingerprints

CRL-7

27f

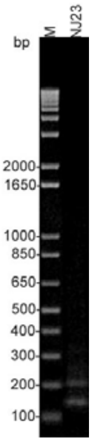


343r



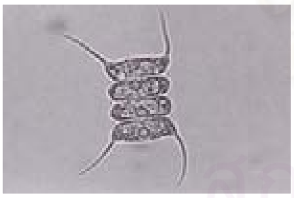
1100r

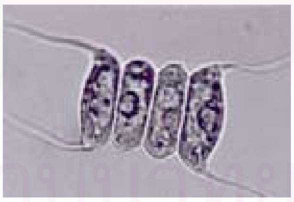
1492r



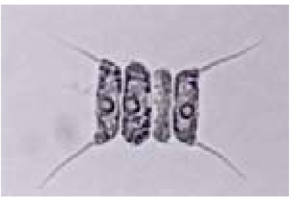
Scenedesmus sp. NJ40 (TISTR 8865)

Morphology in BG-11 at 25°C


3 days

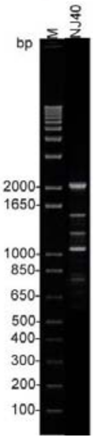

6 days


9 days

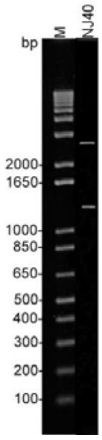

12 days

RAPD-PCR fingerprints

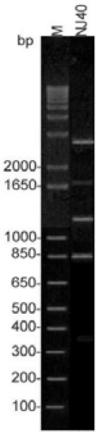
CRL-7



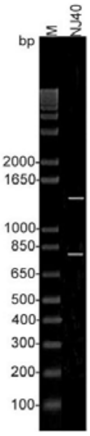
27f



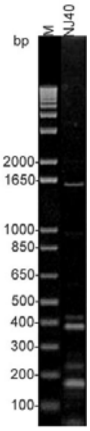
343r



1100r

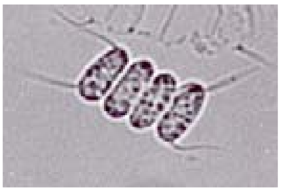


1492r

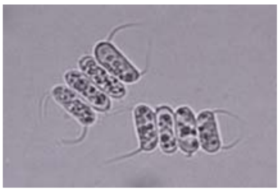


Scenedesmus sp. NJ45 (TISTR 8866)

Morphology in BG-11 at 25°C



3 days



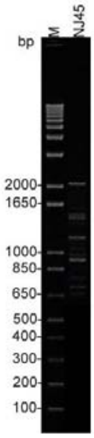
6 days

9 days

12 days

RAPD-PCR fingerprints

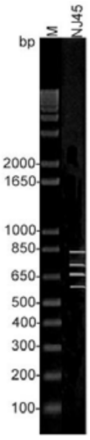
CRL-7



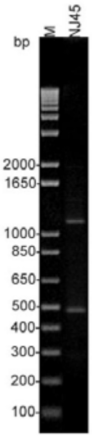
27f

343r

1100r



1492r



References

Baker, J. A., Entsch, B., Neilan, B. A., and Mckay, D. B. 2002. Monitoring changing toxigenicity of a cyanobacterial bloom by molecular methods. Appl. Environ. Microbiol. 68 : 6070-6076.

Becker, E. W. 1994. Applications of Algae. In: Becker , E. W. (ed) Microalgal Biotechnology and Microbiology. London: Cambridge University Press. p 250-260.

Benson, D. A., Boguski, M. S., Lipman, D. J., and Ostell, J. 1997. GenBank. Nucleic Acids Res. 25 :1-6.

Blackall, L. L. 1999. Workshop on Molecular Biology Techniques. September 22-24 and 26-28, 1999. Thaksin University, Songkla, Thailand. p. 23, j1-j9.

Borowitzka, M. A. 1986. Micro-algae as sources of fine chemicals. Microbiol. Sci. 3 : 372-375.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem 72 : 248-254.

Burja, A. M., Tamagnimi, P., Bustard, M. T., and Wright, P. C. 2001. Identification of the green alpa, *chlorella vulgaris* (SDC1) using cyanobacterial derived 16S rDNA primers : targeting the chloroplast. FEMS Microbiol lett. 202 : 195-203.

Cassamatta, D. A., Vis, M. L., and Sheath, R.G. 2003. Cryptic species in cyanobacterial systematics: a case study of *Phormidium retzii* (Oscillatoriales) using RAPD molecular makers and 16S rDNA sequence data. Aquatic Botany. 77: 295-309.

Chen, Y. C., 2001. Immobilized micro-alga *Scenedesmus quadricauda* (Chlorophyta, Chlorococcales) for long-term storage and for application for water quality control in fish culture. Aquaculture 195(1-20 : 71-80.

Costa, A. C. A. Telees, E. M. F., and Leite, S. G. F. 1994. Accumulation of cadmium from moderately concentrated cadmium solutions by *Chlorella* and *Scenedesmus* strains. Rev. Mirobiol. 25(1) : 42-45.

Dawson, H. N., Burlingame, R., and Cannons, A. C. 1997. Stable transformation of *Chlorella* : Rescue of nitrite reductase-deficient mutants with the nitrate reductase gene. Curr. Microbiol. 35(6) : 356-362.

Desikachary, T. V. 1959. Cyanophyta. New Delhi : Indian Council of Agricultural Research. 621 pp.

Dorsch, M., and E. Stackebrandt. 1992. Some modifications in the procedure of direct sequencing of PCR amplified 16S rDNA. J. Microbiol. Meth. 16: 27 271-279.

Fergusson, K. M., and Saint, C. P. 2000. Molecular phylogeny of *Anabaena circinalis* and its identification in environmental samples by PCR. Appl. Environ. Microbiol. 66 : 4145-4148.

Fox, G. E., Wisotzkey, J. D., and Jurtshuk, P. 1992. How close is close: 16S rRNA sequence identity may not be sufficient to guarantee species identity. Int. J. Syst. Bacteriol. 42: 166-170.

Huss, V. A. R., Frank, C., Hartmann, E. C., Hirmer, M., Kloboucek, A., Seidel, B. M., Wenzeler, P., and Kessler, E. 1999. Biological taxonomy and molecular phylogeny of the genus *Chlorella* sensu Lato (Chlorophyta). J. Phycol.35: 587-598.

Huss, V. A. R., Seidel, B. And Kessler, E. 1992. Sequence announcements. Pl. Mol. Biol. 22 : 557-560.

Huss, V. A. R., and Sogin, M. L. 1990. Phylogenetic position of some *Chlorella* species within the Chlorococcales based upon complete small subunit ribosomal RNA sequences. J. Mol. Evol. 31 : 432-442.

Jie, N., Zhang, Q. And Yao, G. 2001. Study on the adsorption of vanadium(v) with *Scenedesmus oblfiquus*. Bull. Environ. Contam. Toxicol. 67(3) : 431-437.

John, D.M., Whitton, B. A., and Brook, A.J. 2000. The Freshwater Algal Flora of the ritish Isles. An Identification (eds). Guide to Freshwater and Terrestrial Algae.London : University of Cambridge Press. 702 pp.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227 : 680-685.

Lewin, B., 1997. Genes VI. London : Oxford University Press, p. 388.

Maidak, B. L., Olson, G. J., Larsen, N., Overbeck, R., McCaughey, M. J., Overbeek, R., and Woese, C. R. 1997. The RDP (Ribosomal Database Project). Nucleic Acids Res. 25 : 109-110.

Ma, J., 2000. Differential sensitivity to 30 herbicides among populations of two green algae *Scenedesmus obliquus* and *Chlorella pyrenoidosa* Bull. Environ. Contam. Toxicol. 68(2) : 275-281.

Ma, J., Zheng, R., Xu, L, and Wang, S. 2002. Differential sensitivity of two green algae, *Scenedesmus* obliquus and *Chlorella pyrenoidosa*, to 12 pesticides. Ecotoxiol. Environ. Saf. 52(1) : 57-61.

Mathis, J. N., and McMillin, D. E. 1996. Detection of genetic variation in *Bradyrhizobium japonicum* USDA 110 variants using DNA fingerprints generated with GC rich arbitrary PCR primers. Plant and Soil. 186 : 81-85.

Mundt, S., Krcitlow, S., Nowotny, A., and Effmert, U. 2001. Biochemical and pharmacological investigations of selected cyanobacteria. Int. J. Hyg. Environ. Heaith. 203: 327-334.

Neilan, B. A. 1995. Identification and phylogenetic analysis of toxigenic cyanobacteria by multiplex randomly amplified polymorphic DNA PCR. Appl. Environ. Microbiol. 61: 2286-2291.

Neilan, B. A., Jacobs, D., Del Dot , T., Blackall, L. L., Hawkins, P. R., Cox., P. T., and Goodman , A. E., 1997a. rRNA sequences and evolutionary relationships among toxic and non-toxic cyanobacteria of the genus *Microcystis*. Int. J. Syst. Bacteriol. 47: 693-697.

Neilan , B. A., Stuart, J. L., Goodman, A. E., Cox, P. T., and Hawkins, P. R. 1997b. Specific amplification and restriction polymorphisms of the cyanobacterial rRNA operon spacer region. Syst. Appl. Microbiol. 20: 612-621.

Nubel, U., Garcia-Pichel, F., and Muyzer, G. 1997c. PCR primers to amplify 16S rRNA genes from cyanobacteria. Appl. Environ. Microbiol. 63: 3327-3332.

Otsuka, S., Suda, S., Li, R. H., Watanabe, M., Oyaizu, H., Matsumoto, S., and Watanabe, M. M. 1999. Phylogenetic relationships between toxic and non-toxic strains of the genus *Microcystis* based on 16S to 23S internal transcribed spacer sequence. FEMS Microbiol. Lett. 172 : 15-21.

Phitaktansakul, R., Apanich, N., Seubsuk, P.,, Khosachan, S., and Uvairong, H. 2004. Gene Relation and Identification of Fresh water Algae using SSU rDNA sequences. J. Sci. Ref. Chula. Univ. (section T) 3: 19-26 (in thai)

Prescott, G. W. 1970. How to Know the Freshwater Algae. 3rd Edition. Iowa: Wm. C. Brown, 293 pp.

Rasmussen, U., and Svenning, M. M. 1998. Fingerprinting of cyanobacteri based on PCR with primers derived from short and long tandemly repeated repetitive sequences. Appl. Environ. Microbiol. 64:265-272.

Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M., and Stanier, R. Y. 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol. 111:1-61.

Rocap, G., Disel, D. L., Waterbury, J. B., Chisholm, S. W. 2002. Resolution of *Prochlorococcus* and *Synechococcus* ecotypes by using 16S-23S ribosomal DNA internal transcribed spacer sequences. Appl. Environ. Microbiol. 68(3): 1180-1191.

Rouhiainen , L., Sivonen , K., Buikema , W. J., and Haselkorn , R. 1995. Characterization of toxin-producing cyanobacteria by using an oligonucleotide probe containing a tandemly repeated heptamer. J. Bacteriol. 177: 6021-6026.

Sambrook, J., and Russel, D. W. 2001. Molecular Cloning: A Laboratory Manual, 3rd Ed. New York: Cold Spring Harbor Laboratory Press. Book1.

Stenier, R. Y., Kunisawa, R. W., Mandel, M. and Cohen-Bazire, G. 1971. Purification and properties of unicellular blue-green algae (Order *Chroococcales*). Bacteriol. Rev. 35: 171-205.

Stein, J. 1973. Handbook of Phycological Methods: Culture Methods and Growth Measurements. London: Cambridge University Press. 448 pp.

Sompong, U., and Peerapornpisal, Y. 2004. Morphological and genetic criteria in taxonomic classification of Blue-green algae in some hot spring areas of of Thailand. J. Sci. Res. Chula. Univ. (Section T) 3: 19-26 (in Thai).

Sompong, U., Hawkins, P. R., Besley, C., Peerapornpisal, Y. 2005. The distribution of cyanobacteria across physical and chemical gradients in hot springs in northern Thailand. FEMS Microbiol Ecol. (in press) online publication January 4, 2005 (www.FEMS-Microbiology.org).

Toledo, G., and Palenik, B. 1997. *Synechococcus* diversity in the California current as seen by RNA polymerase (*rpoC1*) gene sequences of isolated strains. Appl. Environ. Microbiol. 63 : 4298-4303.

Urbach, E., Robertson, D. L., and Chisholm, S. W. 1992. Multiple evolutionary origins of prochlorophytes with the cyanobacterial radiation. Nature. 355: 267-269.

Wakasugi, T., Nagai, T., Kapoor, M., Sugita, M., Ito, M., Ito, S., Tsudzuki, J., Nakashima, K., Tsudzuki, T., Suzuki, Y., Hamada, A., Ohta, T., Inamura, A., Yoshinaga, K., and Sugiura, M. 1997. Complete nucleotide sequence of the chloroplast genome from the green alga *Chlorella vulgaris*: The existence of genes possibly involved in chloroplast division. Proc. Natl. Acad Sci. USA. 94: 5967-5872.

Wu, H. L., Hseu, R. S., and Lin, L. P. 2001. Identification of *Chlorella* spp. isolates using ribosomal DNA sequences. Bot. Bull. Acad. Sin. 42: 115-121.

Zachleder, V., Bisova, K., Vitora, M., Kubin, S., and Hendrychova, J. 2002. Variety of cell cycle patterns in the alga Scenedesmus quadricauda (chlorophyta) as revealed by application of illumination regimes and inhibitors. Eur. J. Phycol. 37(3): 361-371.

Zheng, W., Song, T., Bao, X., Bergman, B., and Rasmussen, U. 2002. High cyanobacterial diversity in coralloid roots of cycads revealed by PCR fingerprinting. FEMS. Microbiol. Ecol. 40: 215-222.

Appendices

Appendix A

CYANOBACTERIA AND MICRO-ALGAE GROWTH MEDIA

BG-11 medium (Rippka et al,1979)

Trace metal mix A5

H_3BO_3	2.86 mg
$MnCl_2.4H_2O$	1.81 mg
$ZnSO_4.7H_2O$	0.222 mg
$Na_2MoO_4.2H_2O$	0.390 mg
$CuSO_4.5H_2O$	0.079 mg
$Co(NO_3)_2.6H_2O$	0.050 mg
Deionized water	1 liter
$NaNO_3$	1.5 g
K_2HPO_4	0.04 g
$MgSO_4.7H_2O$	0.075 g
$CaCl_2.2H_2O$	0.036 g
Citric acid	0.006 g
Ferric ammonium citrate	0.006 g
EDTA (disodium ma0.001gnesium salt)	0.001 g
Na_2CO_3	0.02 g
Trace metal mix A5	1.00 ml
Deionized water	1 liter

pH of medium was adjusted to 7.4 with 0.1 N NaOH. The medium was autoclaved at 121°C for 15 min.

Bold’s Basal Medium (BBM) (stein, 1973)

Trace metal mix A5	1 ml
H_3BO_3	2.86 mg
$MnCl_2.4H_2O$	1.81 mg
$ZnSO_4.7H_2O$	0.222 mg
$Na_2MoO_4.2H_2O$	0.390 mg
$CuSO_4.5H_2O$	0.079 mg
$Co(NO_3)_2.6H_2O$	0.050 mg
Deionized water	1 liter
KH_2PO_4	0.175 g
$CaCl_2.2H_2O$	0.025 g
$MgSO_4.7H_2O$	0.075 g
$NaNO_3$	0.250 g
K_2HPO_4	0.075 g
NaCl	0.025 g
Na_2EDTA	0.010 g
KOH	6.2 mg
$FeSO_4.7H_2O$	4.98 mg
H_2SO_4(conc.)	1 µl
Deionized water	1 liter

pH of medium was adjusted to 6.8 with 0.1 N NaOH. The medium was autoclaved at 121°C for 15 min.

Appendix B

CHEMICALS AND SOLUTIONS

1. Solutions for DNA extraction (Gibco BRL)

TE buffer (10 mM Tris-HCl, 1 mM EDTA. pH 7.5)

0.12 g Tris-HCl, 0.037 g EDTA were added to distilled water. The final volume was made to 100 ml. 0.1 N NaOH was used to adjust pH to 8.0 before autoclaving at 121°C for 15 min.

10% SDS

Dissolve 10 g SDS in 90 ml water with gentle stirring and bring to 100 ml with distilled water

Phenol:Chloroform:Isoamyl alcohol 25: 24: 1 (v/v/v)

3 M Sodium acetate

24.61 g Sodium acetate was added 100 ml distilled water.

Absolute ethanol

70% Ethanol

2. Solutions for SDS-PAGE (Bio-rad)

Stock solutions

A. Acrylamide/bis (30% T, 2.67%C)

87.6 g acrylamide (29.2 g/100 ml)

2.4 g N'N'-bis-methylene-acrylamide (0.8 g/100 ml)

Make to 300 ml with deionized water. Filter and store at 4°C in the dark (30 days maximum).

B. 1.5 M Tris-HCl, pH 8.8

27.23 g Tris base (18.15 g/100 ml)

80 ml deionized water

Adjust to pH 8.8 with 6N HCl. Make to 150 ml with deionized water and store at 4°C

C. 0.5 M Tris-HCl, pH 6.8

6 g Tris base

60 ml deionized water

Adjust to pH 6.8 with 6N HCl. Make to 100 ml with deionized water and store at 4°C

D. 10% SDS

Dissolve 10 g SDS in 90 ml water with gentle stirring and bring to 100 ml with ddH_2O

E. Sample buffer (SDS reducing buffer) (store at room temperature)

Deionized water	3.8 ml
0.5 M Tris-HCl, pH 6.8	1.0 ml
Glycerol	0.8 ml
10% (w/v) SDS	1.6 ml
2-mercaptoethanol	0.4 ml
1 % (w/v) bromophenol blue	0.4 ml

Dilute the sample at least 1:4 with sample buffer, and heat at 95°C for 4 minutes

F. 5X electrode (running buffer), pH 8.3

Tris base	9.0 g	(15 g/l)
Glycine	43.2 g	(72 g/l)
SDS	3.0 g	(5 g/l)

Make to 600 ml with deionized water.

Store at 4°C. Warm to room temperature before use if precipitation occurs. Dilute 60 ml 5X stock with 240 ml deionized water for one electrophoretic run.

G. 10% Ammonium persulphate

One milliliter of aqueous 10% (w/v) Ammonium persulphate stock solution was prepared and stored at 4 C. Ammonium persulphate decomposes slowly, and fresh solutions were prepared weekly.

H. Protein molecular weight standard (Bio-rad)

Rabbit muscle phosphorylase b	97 kDa
Bovine serum albumin (BSA)	66 kDa
Hen egg white ovalbumin	45 kDa
Ovine carbonic anhydrase	31 kDa
Soybean trypsin	21 kDa
Hen egg white lysozyme	14 kDa

Appendix C

16S rDNA SEQUENCES

- First determination for *Synechococcus* sp. NJ7 (TISTR 8867)
  Chlorella sp. NJ26 (TISTR 8852)
  Scenedessmus sp. NJ8 (TISTR 8859)

- Second determination for *Synechococcus* sp. NJ7 (TISTR 8867)
  Chlorella sp. NJ26 (TISTR 8852)
  Scenedessmus sp. NJ8 (TISTR 8859)

- Comparison between sequences obtained from two determinations

ABI PRISM

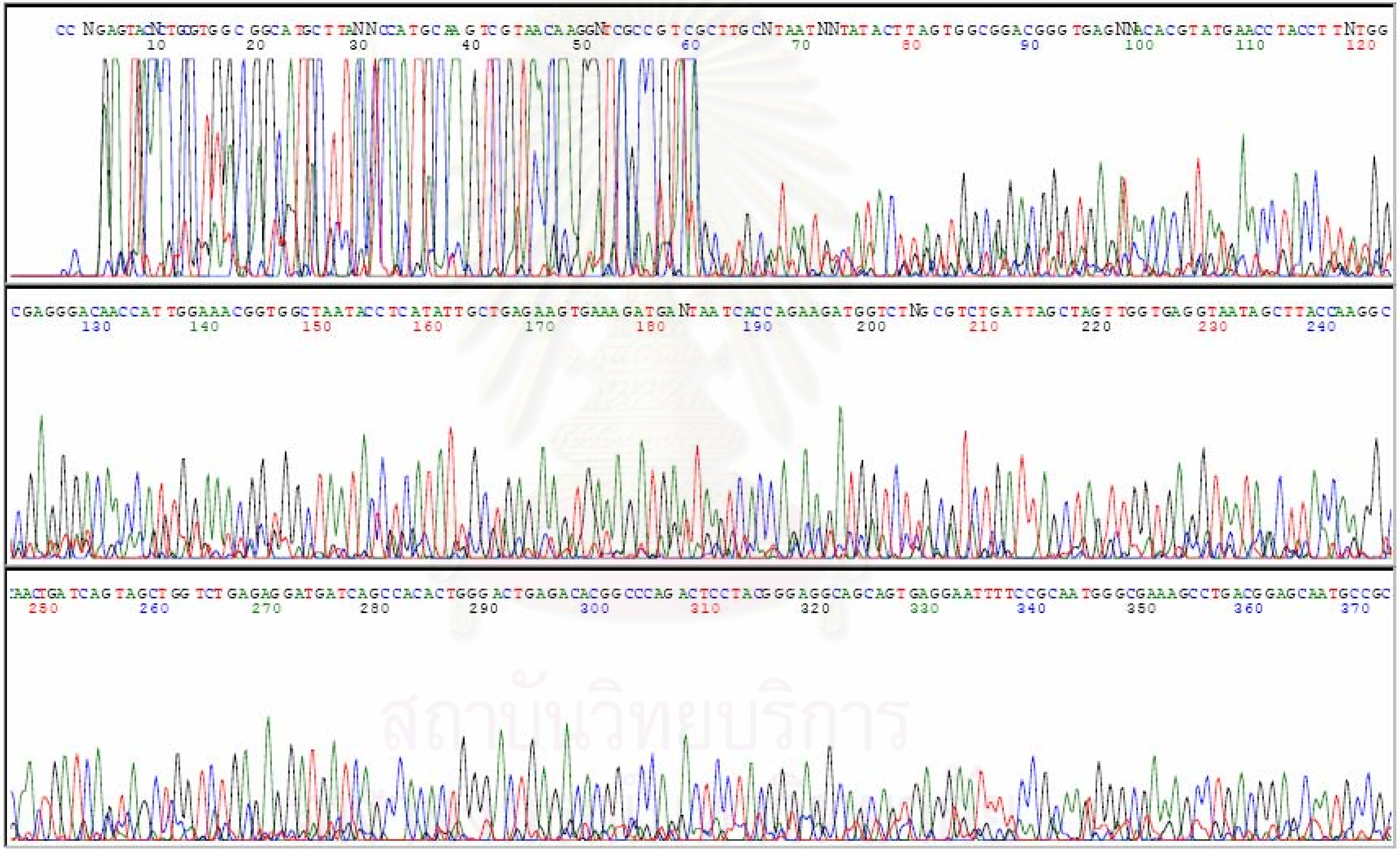
Model 3100
Version 3.7
Basecaller-3100APOP6SNJ7_27f
BC 1.5.0.0

30-08-04A_F06_NJ7_27f_12.ab1

Cap 12

Signal G:392 T:531 A:893 C:287
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106   Pk 1 Loc: 250

Page 1 of 3
Wed, Sep 01, 2004 8:08 AM
Mon, Aug 30, 2004 11:35 PM
Spacing: 11.01{11.01}

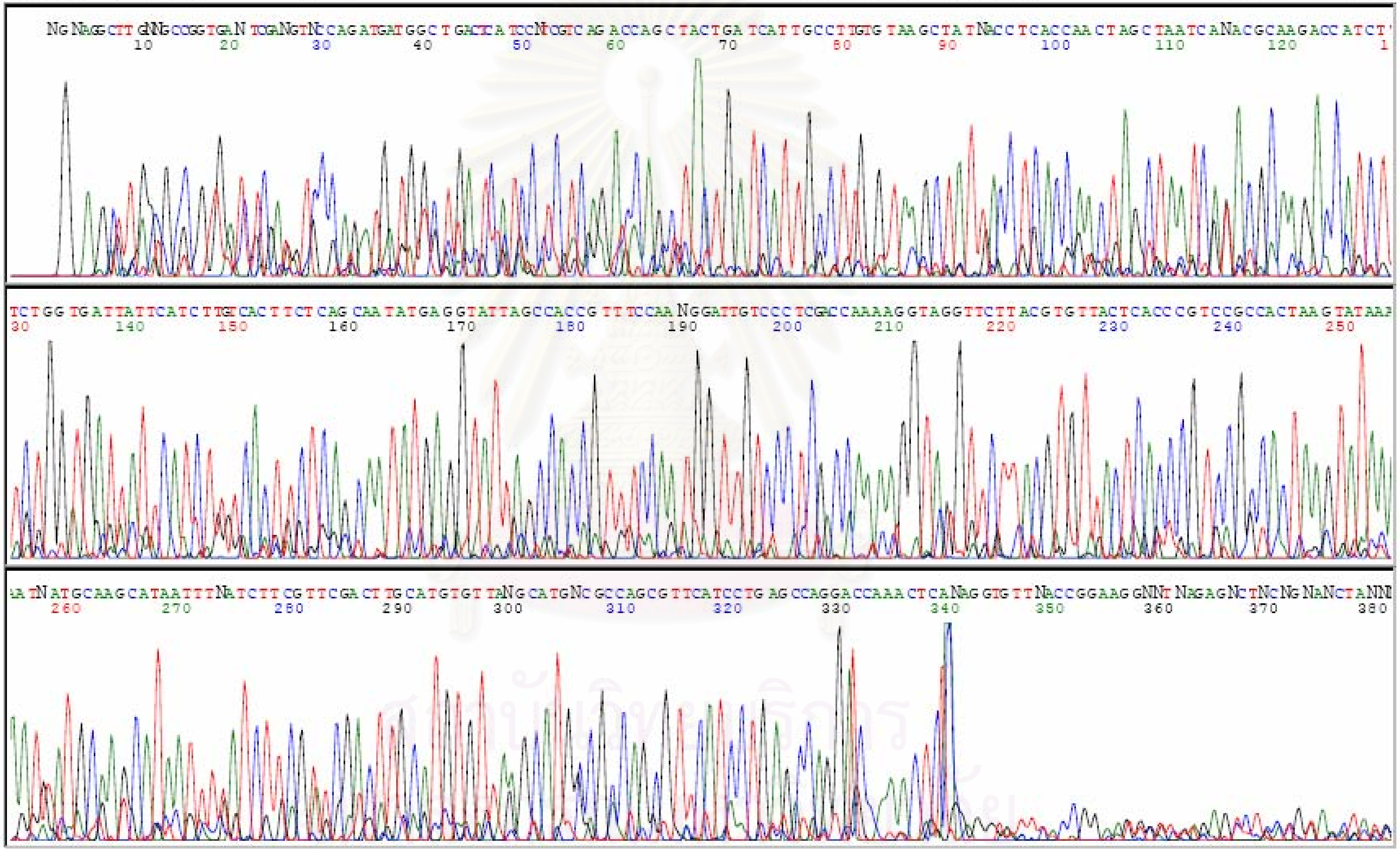


ABI PRISM

Model 3100 30-08-04A_H06_NJ7_343r_16.ab1
Version 3.7
Basecaller-3100APOP6SNJ7_343r
BC 1.5.0.0 Cap 16

Signal G:224 T:390 A:535 C:201
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

Page 1 of 3
Wed, Sep 01, 2004 8:09 AM
Mon, Aug 30, 2004 11:35 PM
Spacing: 11.25{11.25}

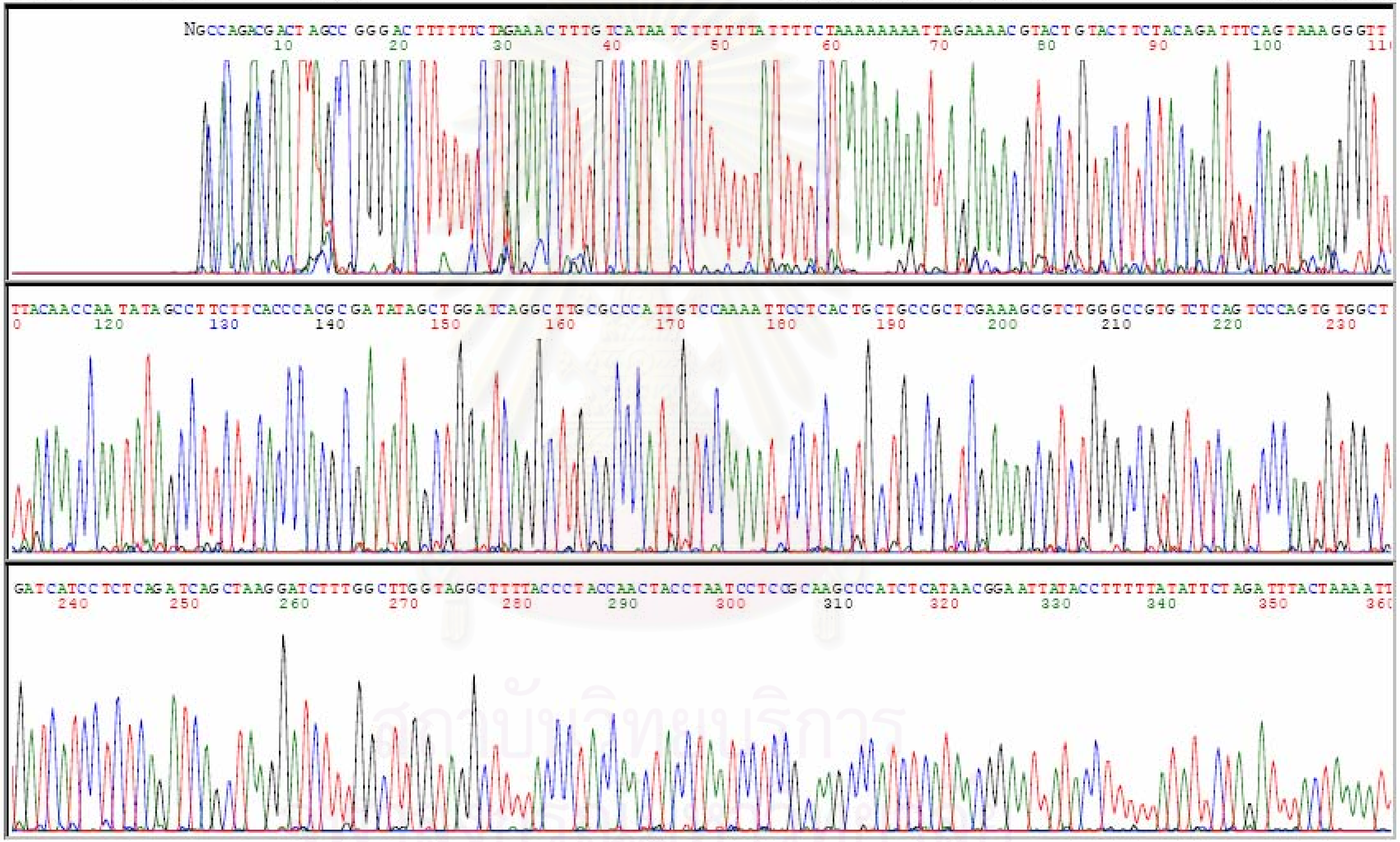


ABI PRISM

Model 3100 8-2-05A_E04_NJ7_519r_10.ab1
Version 3.7
Basecaller-3100APOP6SNJ7_519r
BC 1.5.0.0 Cap 10

Signal G:147 T:314 A:441 C:136
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

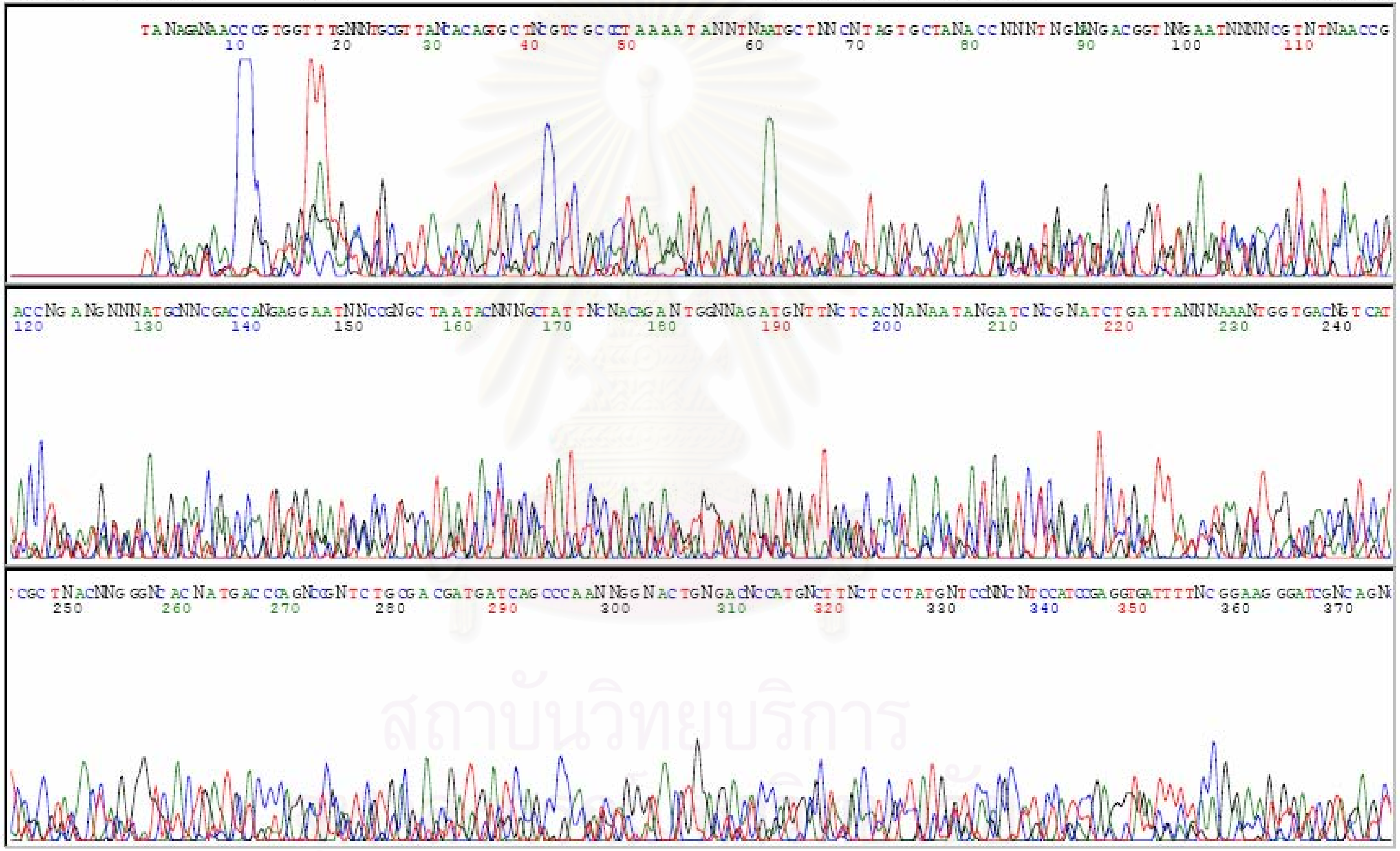
Page 1 of 3
Wed, Feb 09, 2005 8:18 AM
Tue, Feb 08, 2005 8:24 PM
Spacing: 11.77{11.77}



Model 3100 10-09-04_E06_NJ7_787r_10.ab1
Version 3.7
Basecaller-3100APOP6SNJ7_787r
BC 1.5.0.0 Cap 10

Signal G:72 T:98 A:163 C:46
DT3100POP6{ET}50cm.mob
demo_3100
Points 200 to 10106 Pk 1 Loc: 200

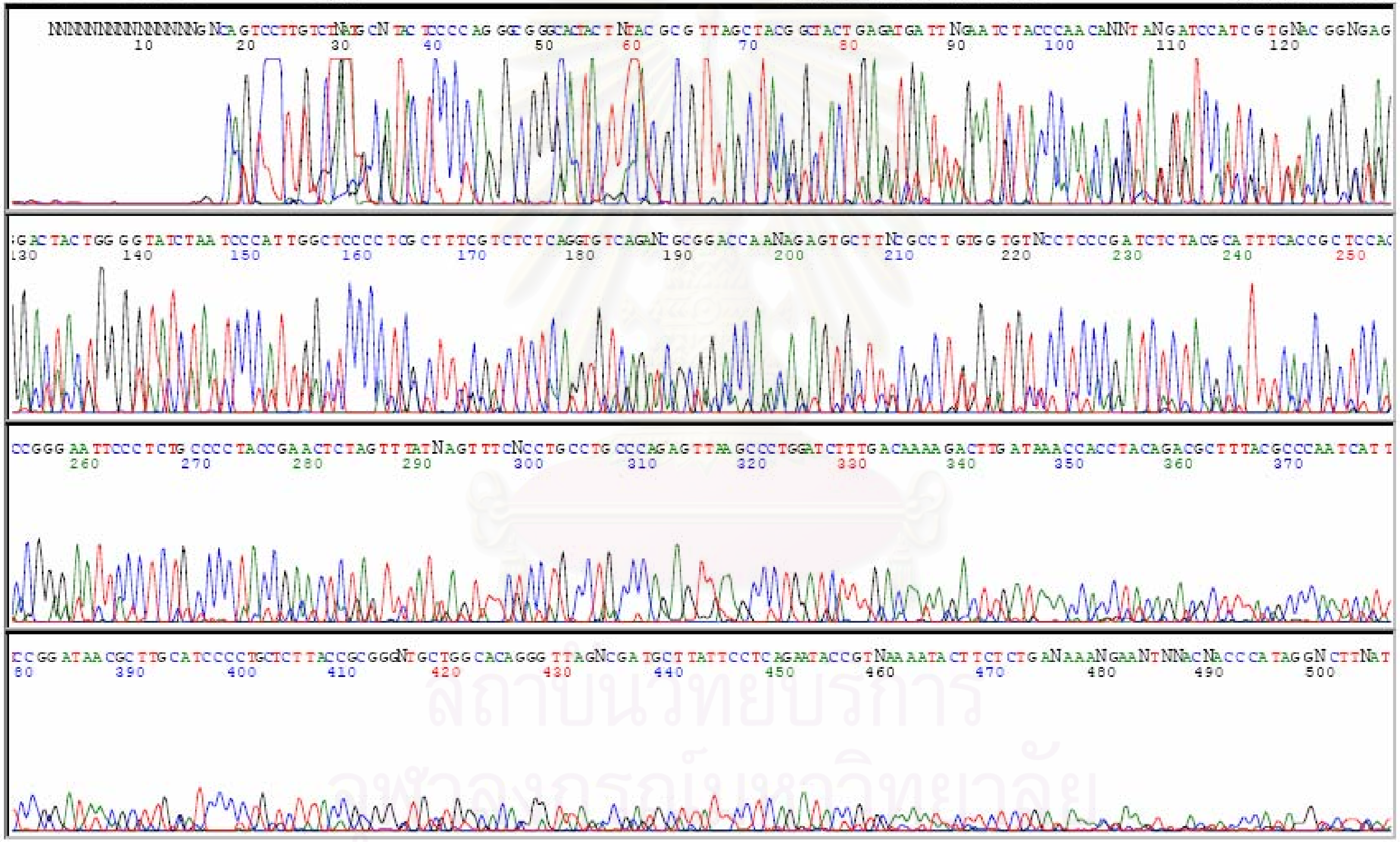
Page 1 of 3
Fri, Sep 10, 2004 3:40 PM
Fri, Sep 10, 2004 12:16 PM
Spacing: 11.91{11.91}

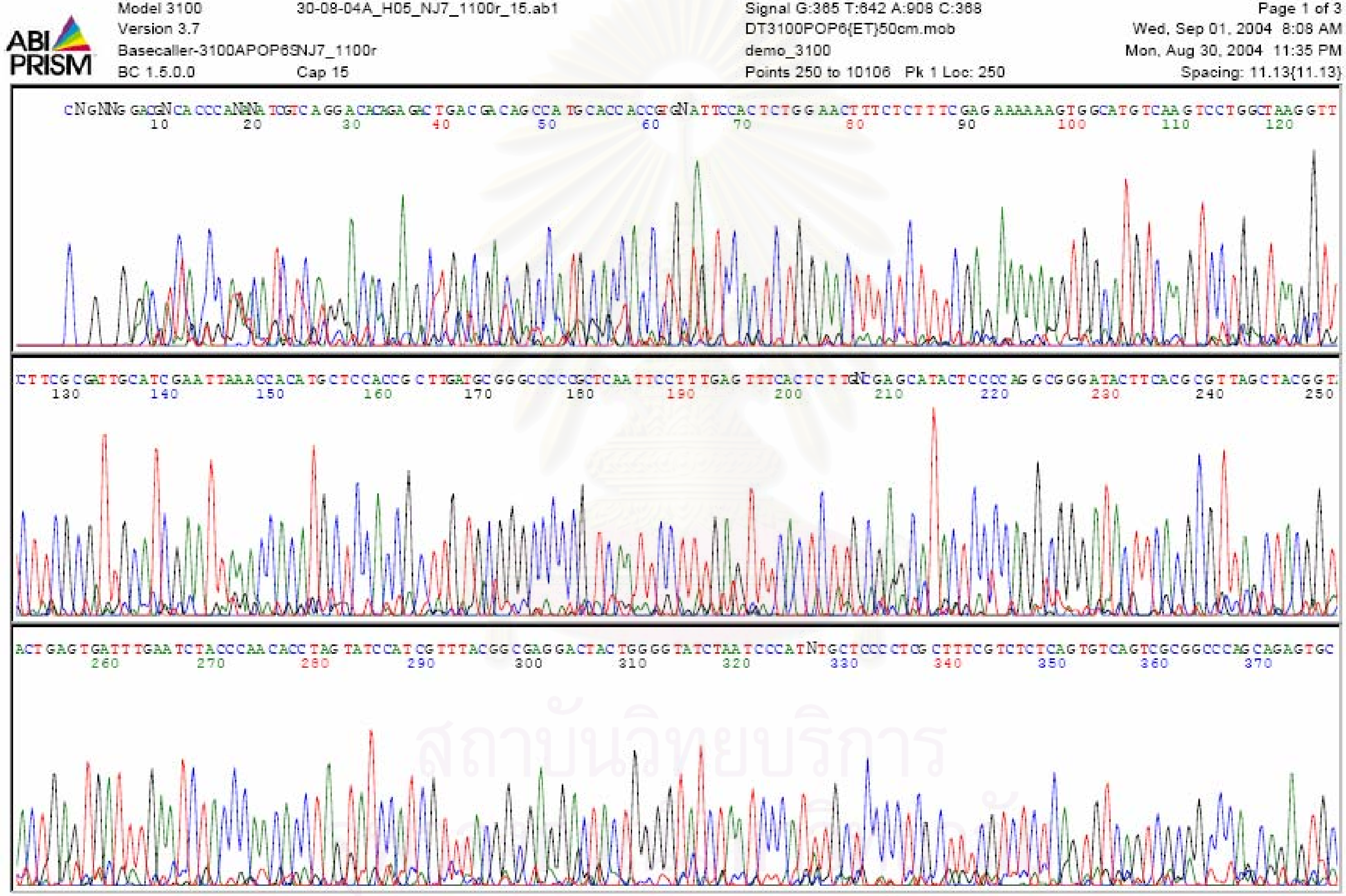


Model 3100 8-2-05A_F03_NJ7_907r_11.ab1
Version 3.7
Basecaller-3100APOP6SNJ7_907r
BC 1.5.0.0 Cap 11

Signal G:25 T:37 A:48 C:23
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

Page 1 of 3
Wed, Feb 09, 2005 8:18 AM
Tue, Feb 08, 2005 8:24 PM
Spacing: 11.64{11.64}





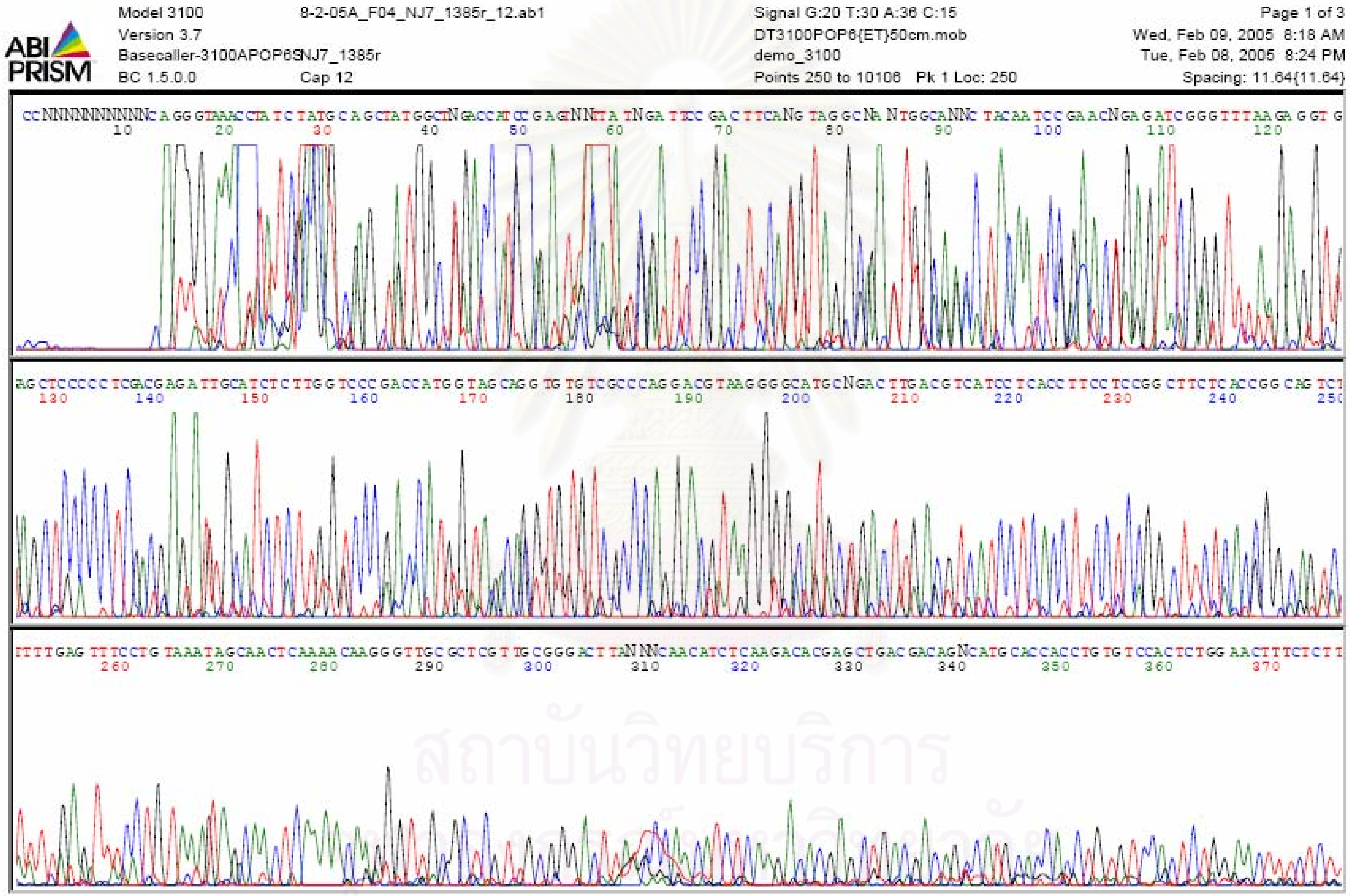
Model 3100
Version 3.7
Basecaller-3100APOP6SNJ7_1100r
BC 1.5.0.0

30-08-04A_H05_NJ7_1100r_15.ab1

Cap 15

Signal G:365 T:642 A:908 C:368
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

Page 1 of 3
Wed, Sep 01, 2004 8:08 AM
Mon, Aug 30, 2004 11:35 PM
Spacing: 11.13{11.13}



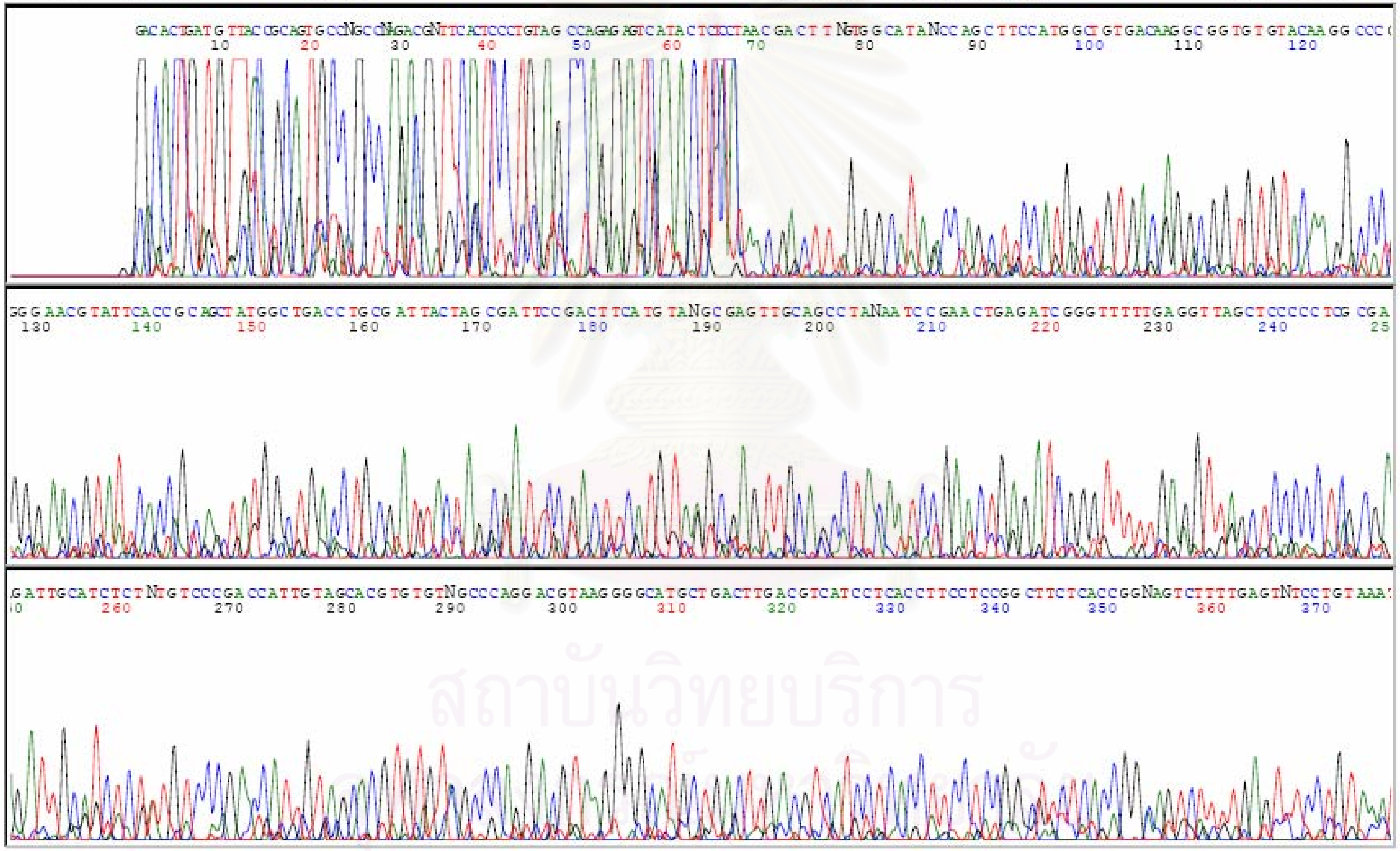
Model 3100
Version 3.7
Basecaller-3100APOP6SNJ7_1385r
BC 1.5.0.0

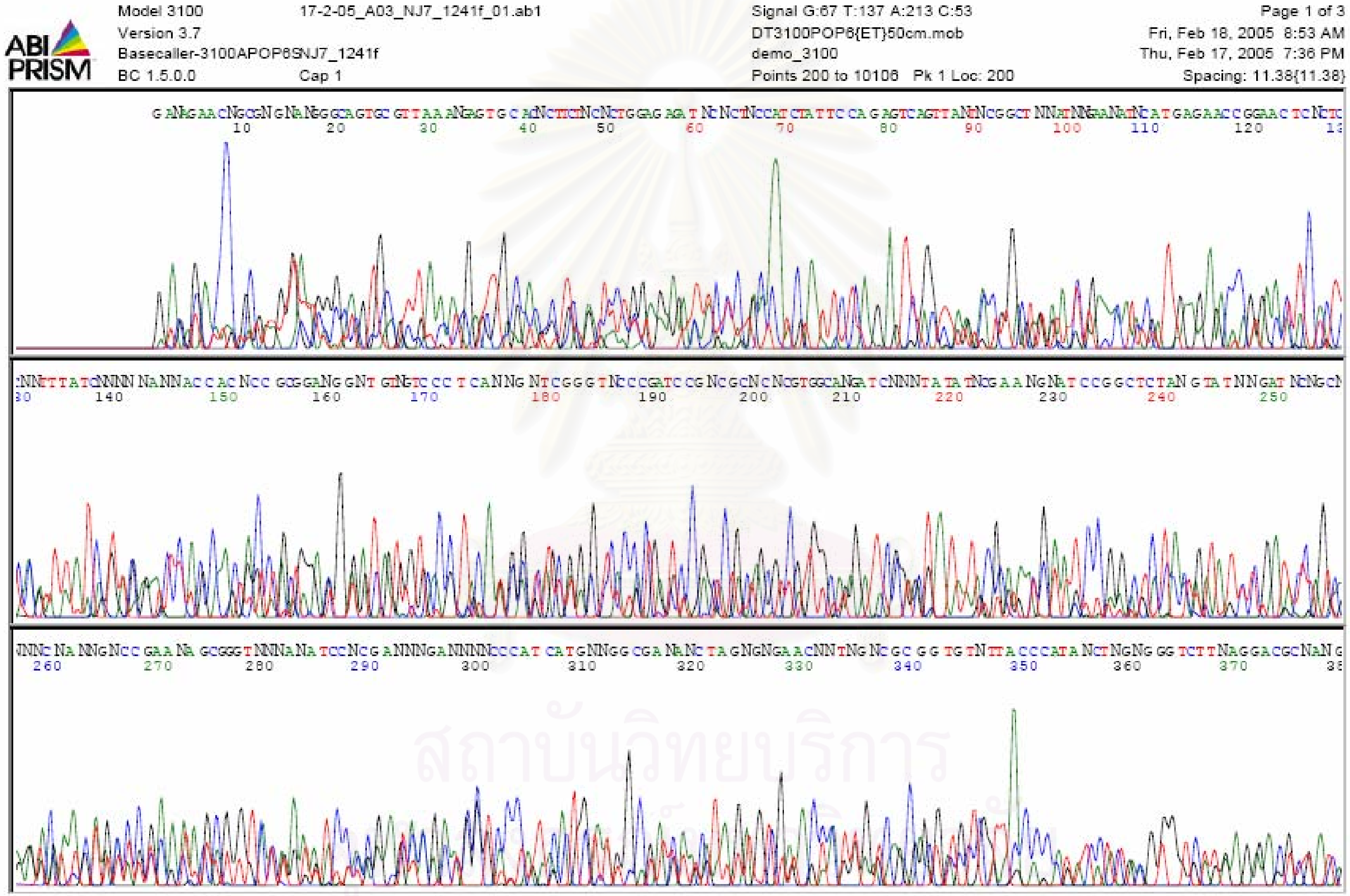
8-2-05A_F04_NJ7_1385r_12.ab1
Cap 12

Signal G:20 T:30 A:36 C:15
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

Page 1 of 3
Wed, Feb 09, 2005 8:18 AM
Tue, Feb 08, 2005 8:24 PM
Spacing: 11.64{11.64}

```
Model 3100            30-08-04A_G06_NJ7_1492r_14.ab1        Signal G:344 T:587 A:724 C:324                    Page 1 of 3
Version 3.7                                                 DT3100POP6{ET}50cm.mob                 Wed, Sep 01, 2004  8:08 AM
Basecaller-3100APOP6SNJ7_1492r                              demo_3100                              Mon, Aug 30, 2004  11:35 PM
BC 1.5.0.0            Cap 14                                Points 250 to 10106   Pk 1 Loc: 250      Spacing: 11.01{11.01}
```





Model 3100
17-2-05_A03_NJ7_1241f_01.ab1
Version 3.7
Basecaller-3100APOP6SNJ7_1241f
BC 1.5.0.0
Cap 1

Signal G:67 T:137 A:213 C:53
DT3100POP6{ET}50cm.mob
demo_3100
Points 200 to 10106   Pk 1 Loc: 200

Page 1 of 3
Fri, Feb 18, 2005 8:53 AM
Thu, Feb 17, 2005 7:36 PM
Spacing: 11.38{11.38}

ABI PRISM

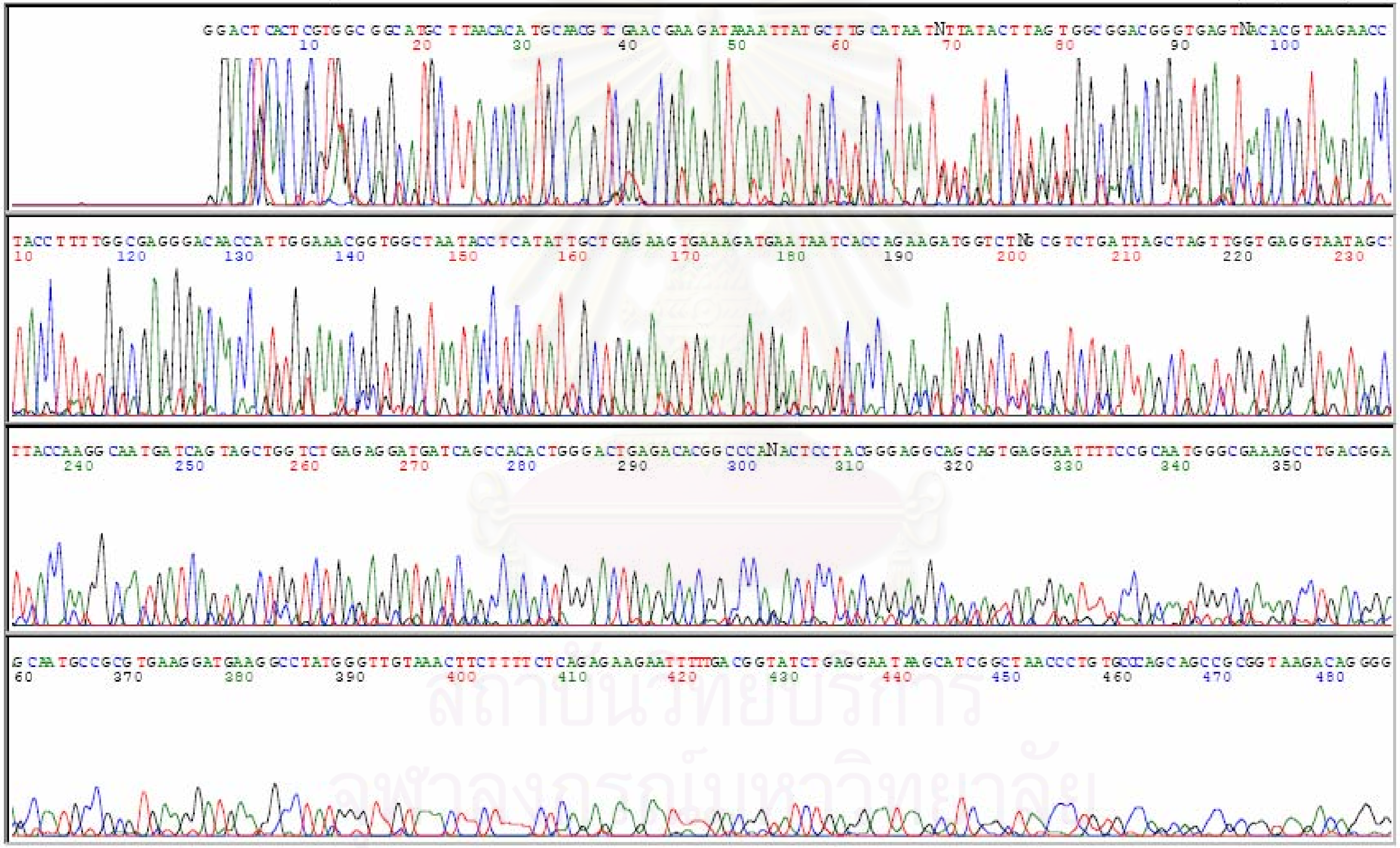
Model 3100
Version 3.7
Basecaller-3100APOP6SNJ7_27f
BC 1.5.0.0

29-3-05B_G06_NJ7_27f_14.ab1

Cap 14

Signal G:58 T:82 A:149 C:31
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

Page 1 of 2
Fri, Apr 01, 2005 1:10 PM
Wed, Mar 30, 2005 10:24 AM
Spacing: 11.91{11.91}

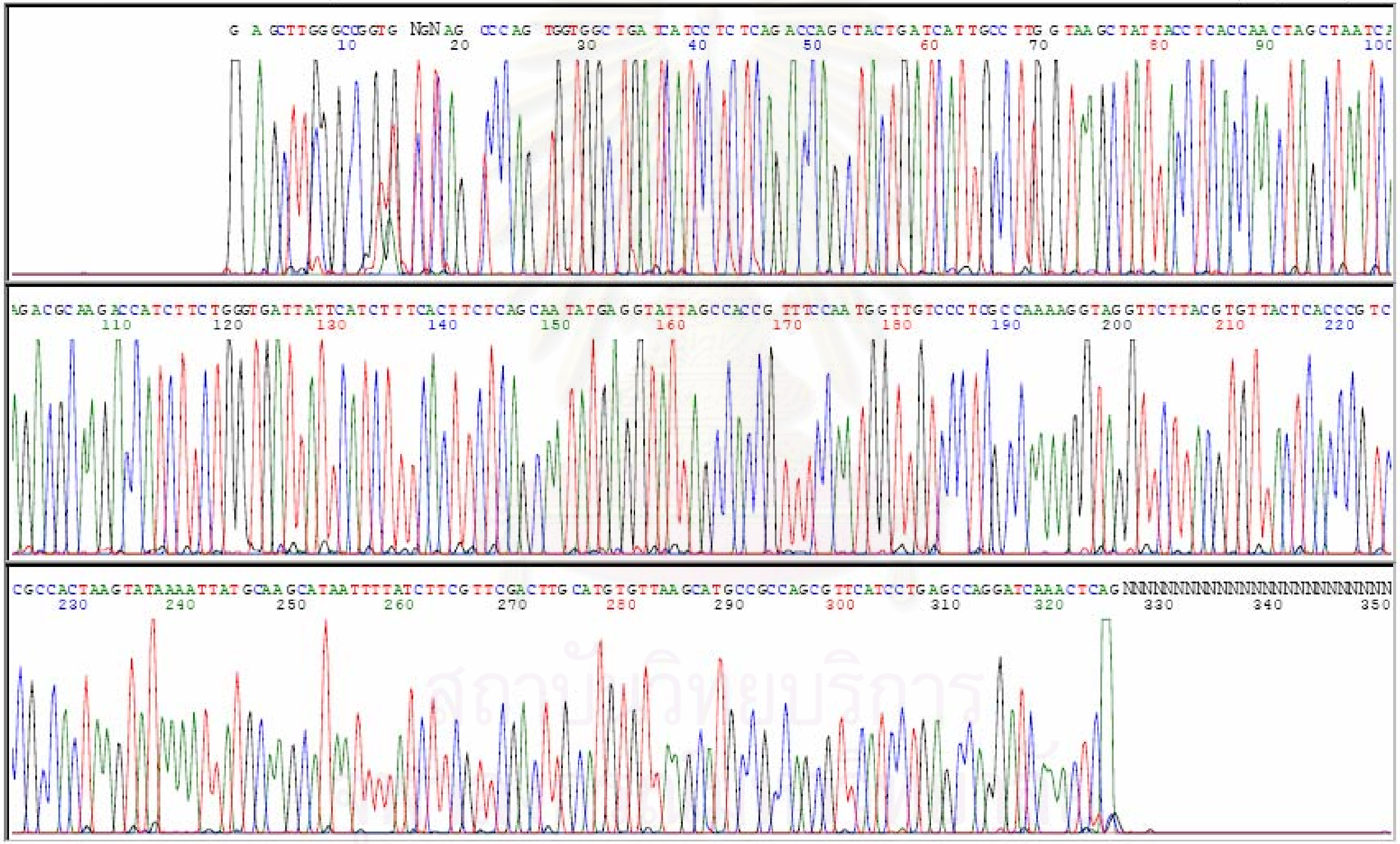


ABI PRISM

Model 3100 31-3-48A_G01_NJ7_343r_13.ab1
Version 3.7
Basecaller-3100APOP6SNJ7_343r
BC 1.5.0.0 Cap 13

Signal G:41 T:83 A:93 C:40
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

Page 1 of 3
Fri, Apr 01, 2005 1:22 PM
Thu, Mar 31, 2005 6:30 PM
Spacing: 11.77{11.77}



ABI PRISM

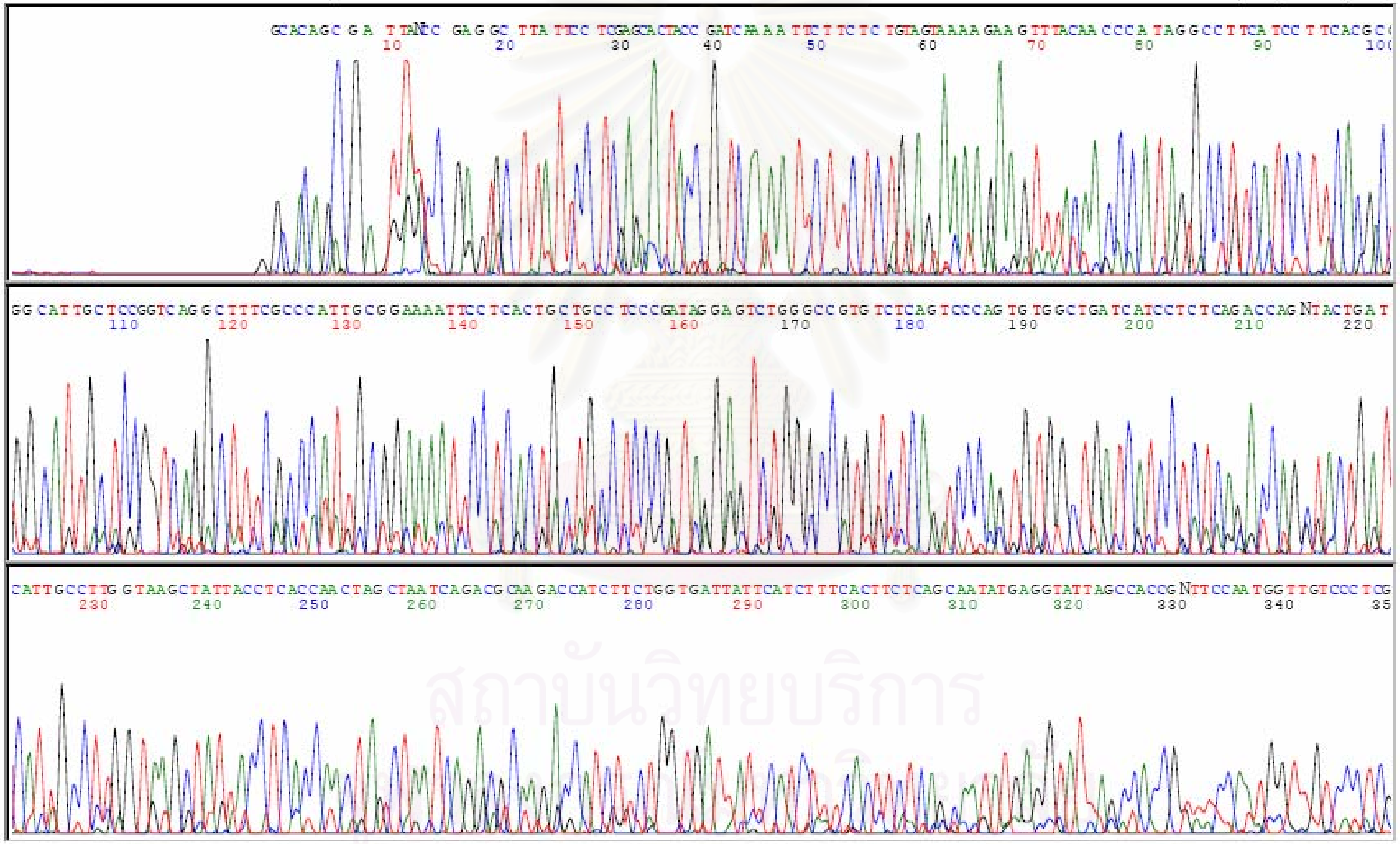
Model 3100
Version 3.7
Basecaller-3100APOP6SNJ7_519r
BC 1.5.0.0

31-3-48A_E01_NJ7_519r_09.ab1

Cap 9

Signal G:16 T:31 A:37 C:17
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106   Pk 1 Loc: 250

Page 1 of 3
Fri, Apr 01, 2005 1:12 PM
Thu, Mar 31, 2005 6:30 PM
Spacing: 11.91{11.91}



ABI PRISM

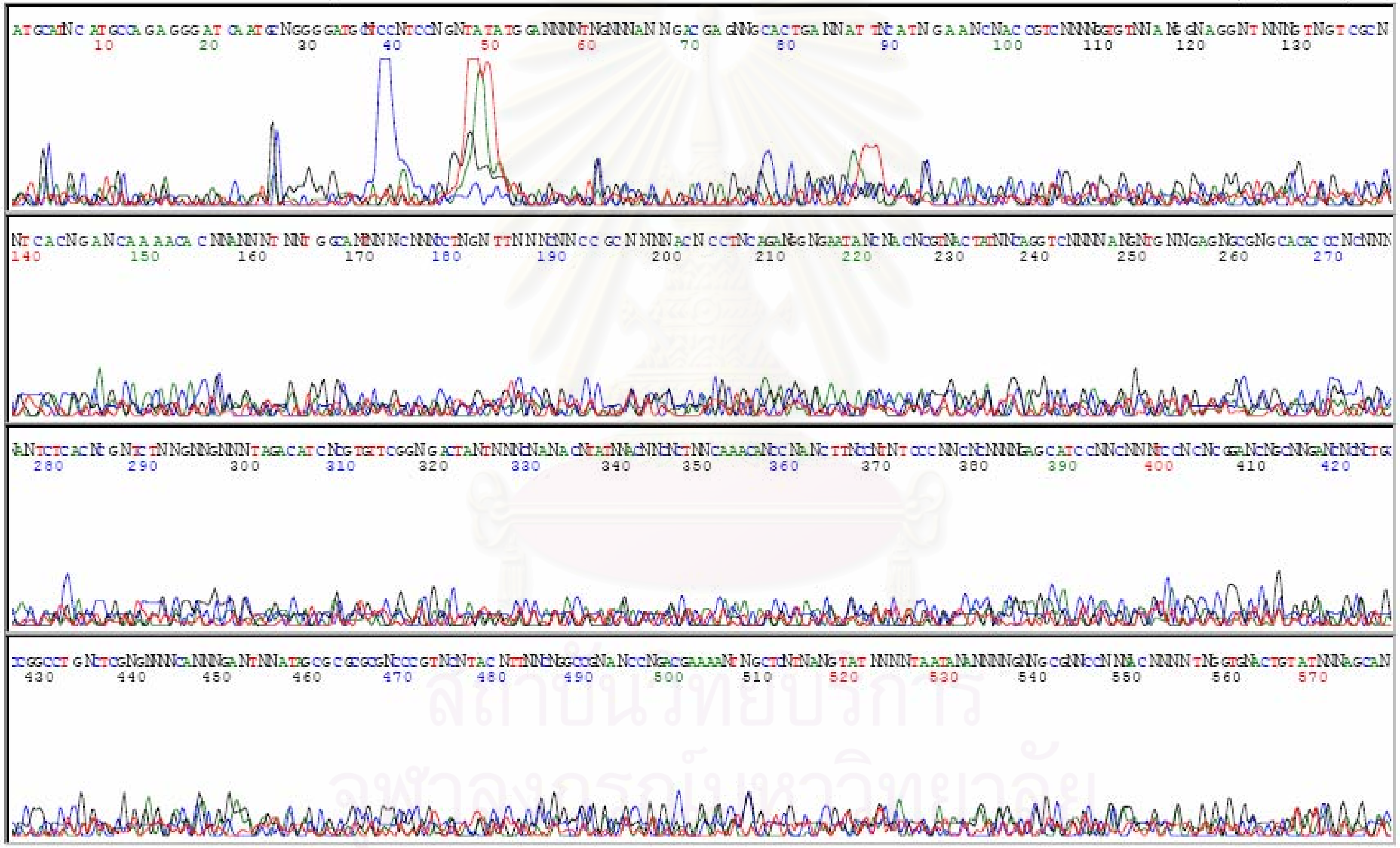
Model 3100
Version 3.7
Basecaller-3100APOP6SNJ7_787r
BC 1.5.0.0

31-3-48A_F01_NJ7_787r_11.ab1

Cap 11

Signal G:3 T:5 A:4 C:3
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106   Pk 1 Loc: 250

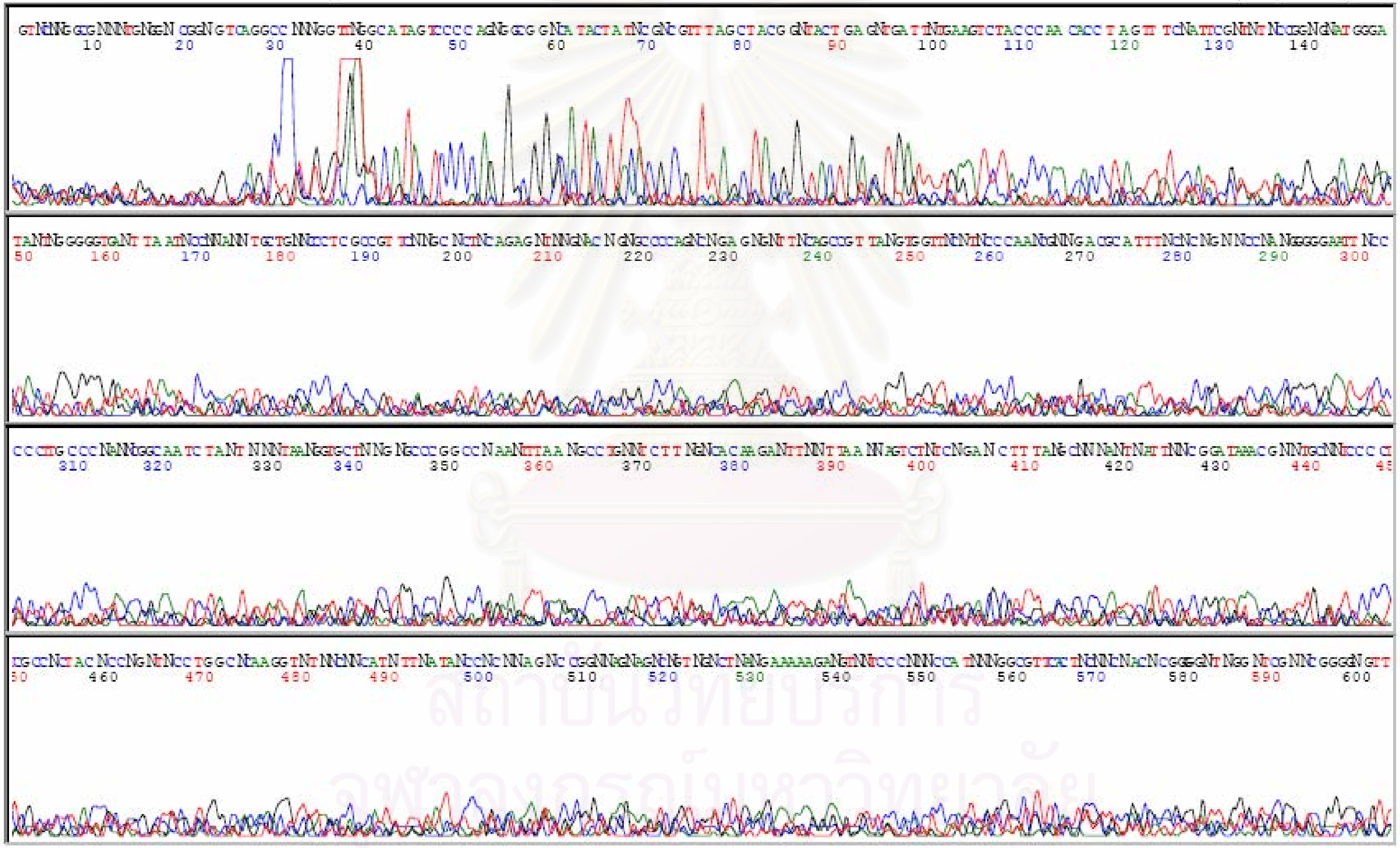
Page 1 of 3
Fri, Apr 01, 2005 1:22 PM
Thu, Mar 31, 2005 6:30 PM
Spacing: 10.45{10.45}



Model 3100 31-3-48A_G02_NJ7_907r_14.ab1
Version 3.7
Basecaller-3100APOP6SNJ7_907r
BC 1.5.0.0 Cap 14

Signal G:5 T:7 A:6 C:4
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

Page 1 of 2
Fri, Apr 01, 2005 1:22 PM
Thu, Mar 31, 2005 6:30 PM
Spacing: 15.28{15.28}



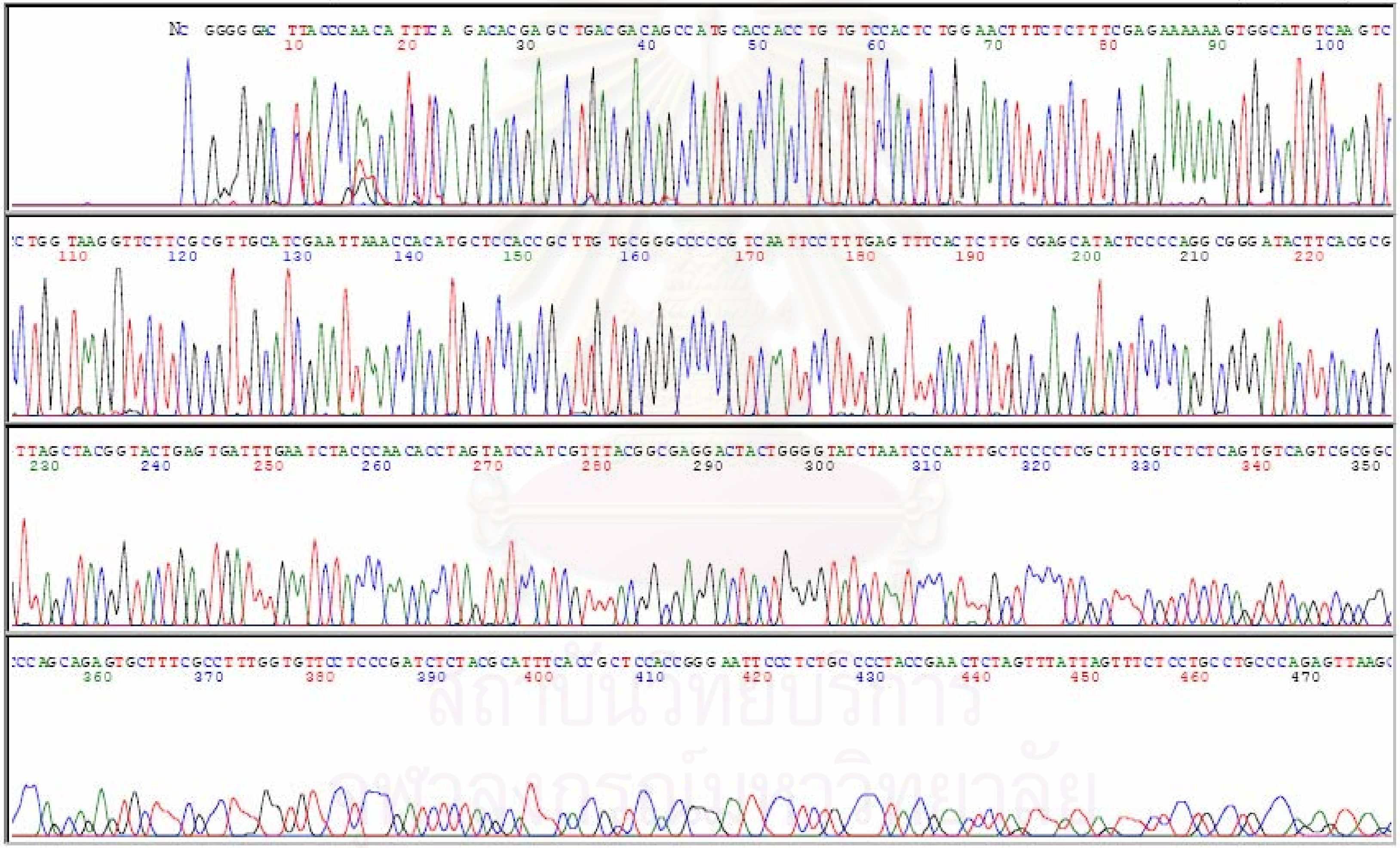
Model 3100
Version 3.7
Basecaller-3100APOP6SNJ7_1100r
BC 1.5.0.0

31-3-48A_E02_NJ7_1100r_10.ab1

Cap 10

Signal G:72 T:125 A:152 C:76
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

Page 1 of 2
Fri, Apr 01, 2005 1:22 PM
Thu, Mar 31, 2005 6:30 PM
Spacing: 11.91{11.91}



ABI PRISM

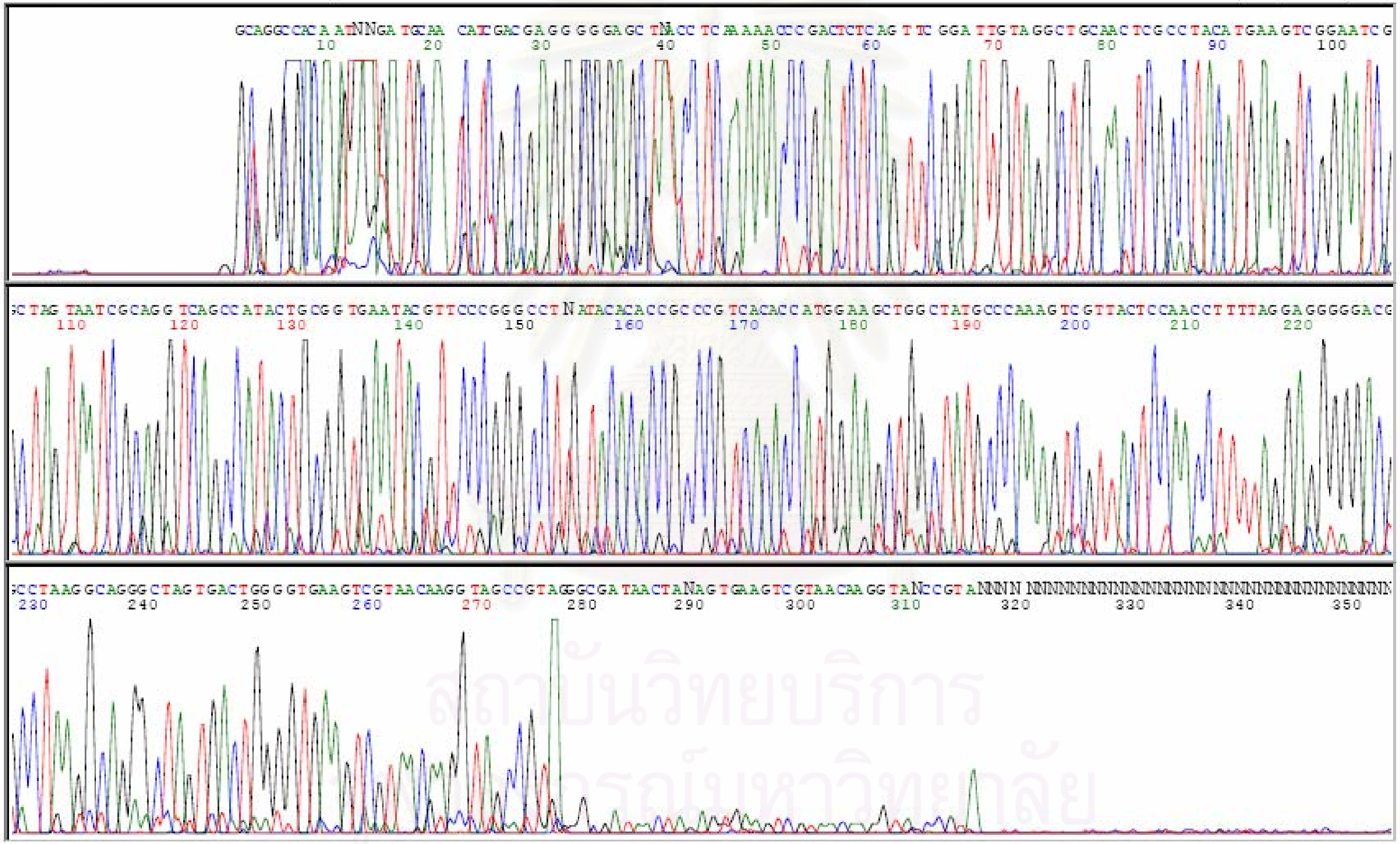
Model 3100
Version 3.7
Basecaller-3100APOP6SNJ7_1241f
BC 1.5.0.0

31-3-48A_F02_NJ7_1241f_12.ab1

Cap 12

Signal G:34 T:46 A:63 C:25
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

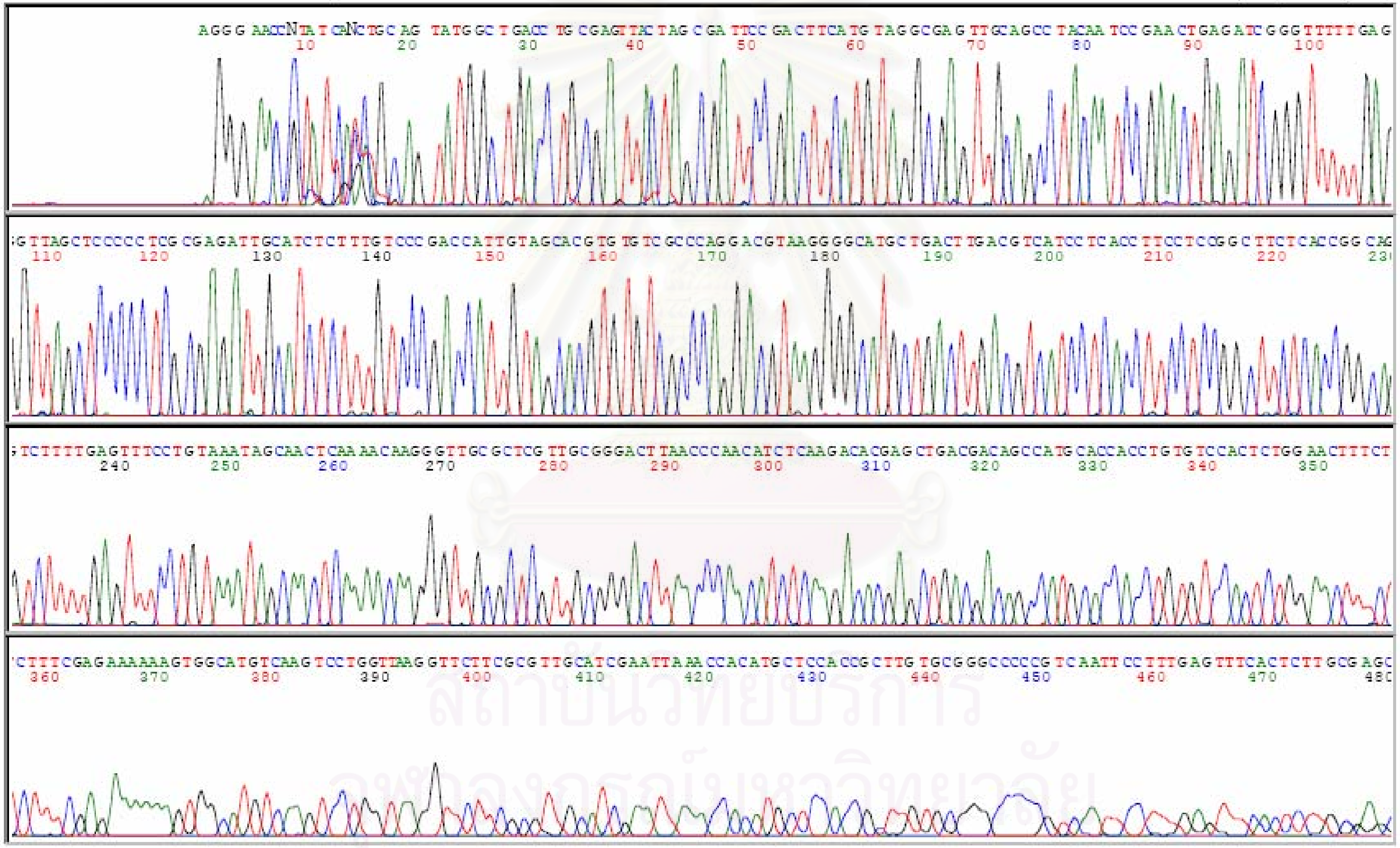
Page 1 of 3
Fri, Apr 01, 2005 1:22 PM
Thu, Mar 31, 2005 6:30 PM
Spacing: 11.77{11.77}



Model 3100 31-3-48A_D02_NJ7_1385r_08.ab1
Version 3.7
Basecaller-3100APOP6SNJ7_1385r
BC 1.5.0.0 Cap 8

Signal G:34 T:51 A:56 C:28
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

Page 1 of 2
Fri, Apr 01, 2005 1:12 PM
Thu, Mar 31, 2005 6:30 PM
Spacing: 12.05{12.05}



ABI PRISM

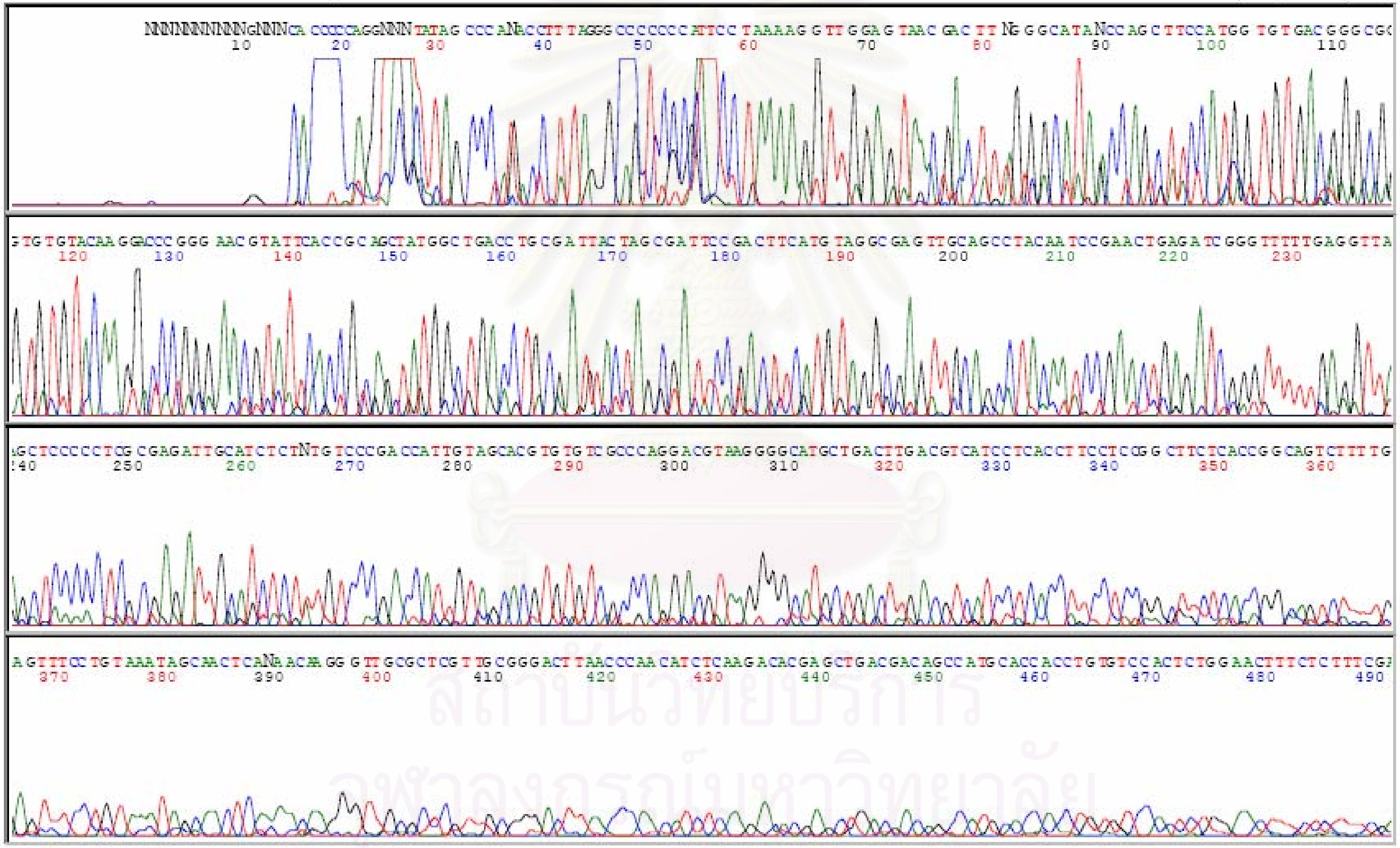
Model 3100
Version 3.7
Basecaller-3100APOP6SNJ7_1492r
BC 1.5.0.0

31-3-48A_D01_NJ7_1492r_07.ab1

Cap 7

Signal G:59 T:97 A:96 C:68
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106   Pk 1 Loc: 250

Page 1 of 2
Fri, Apr 01, 2005 1:12 PM
Thu, Mar 31, 2005 6:30 PM
Spacing: 11.91{11.91}

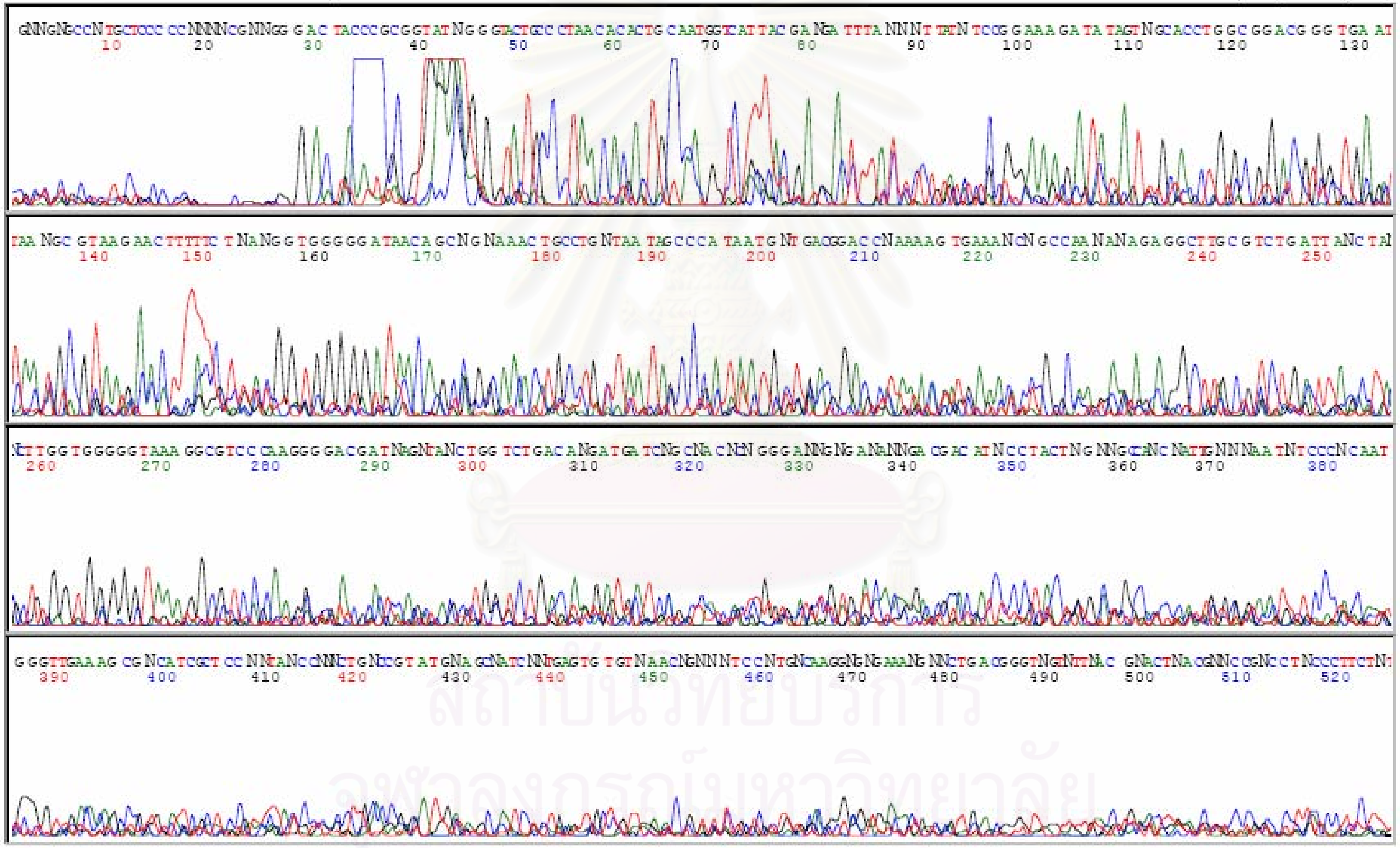


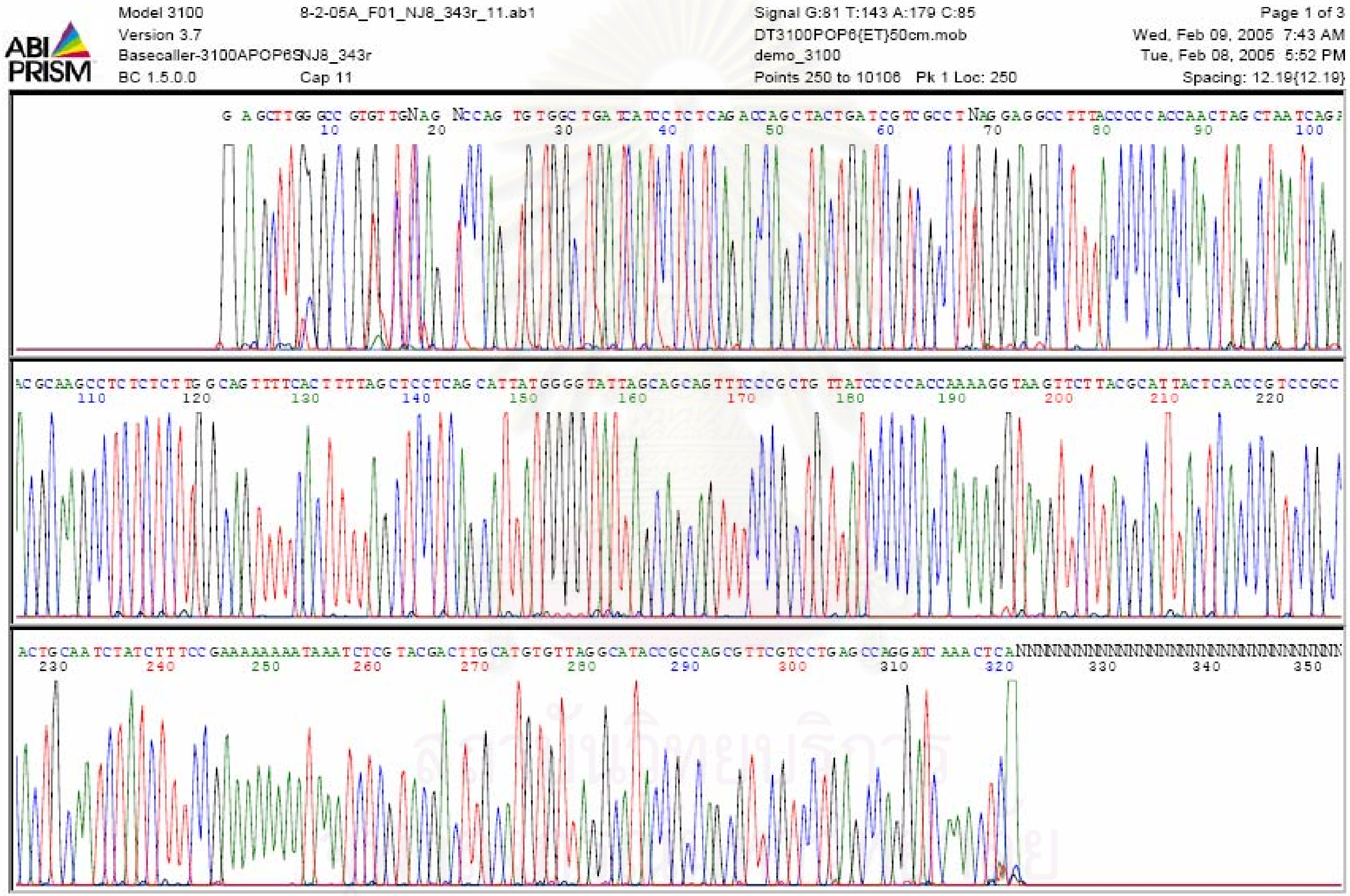
ABI PRISM

Model 3100 8-2-05A_D02_NJ8_27f_08.ab1
Version 3.7
Basecaller-3100APOP6SNJ8_27f
BC 1.5.0.0 Cap 8

Signal G:6 T:8 A:9 C:5
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

Page 1 of 2
Wed, Feb 09, 2005 7:43 AM
Tue, Feb 08, 2005 5:52 PM
Spacing: 12.49{12.49}





Model 3100 8-2-05A_F01_NJ8_343r_11.ab1
Version 3.7
Basecaller-3100APOP6SNJ8_343r
BC 1.5.0.0 Cap 11

Signal G:81 T:143 A:179 C:85
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

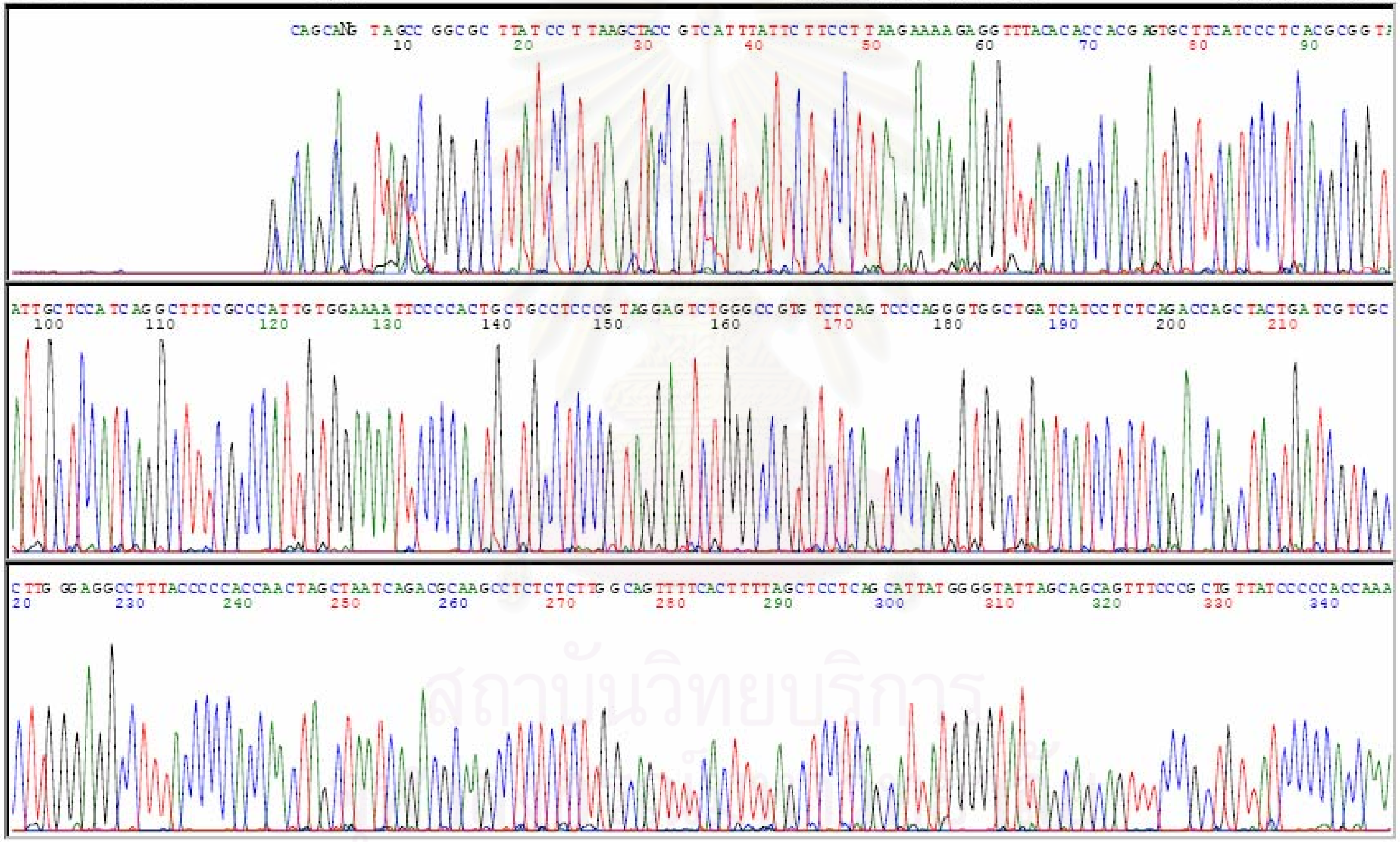
Page 1 of 3
Wed, Feb 09, 2005 7:43 AM
Tue, Feb 08, 2005 5:52 PM
Spacing: 12.19{12.19}

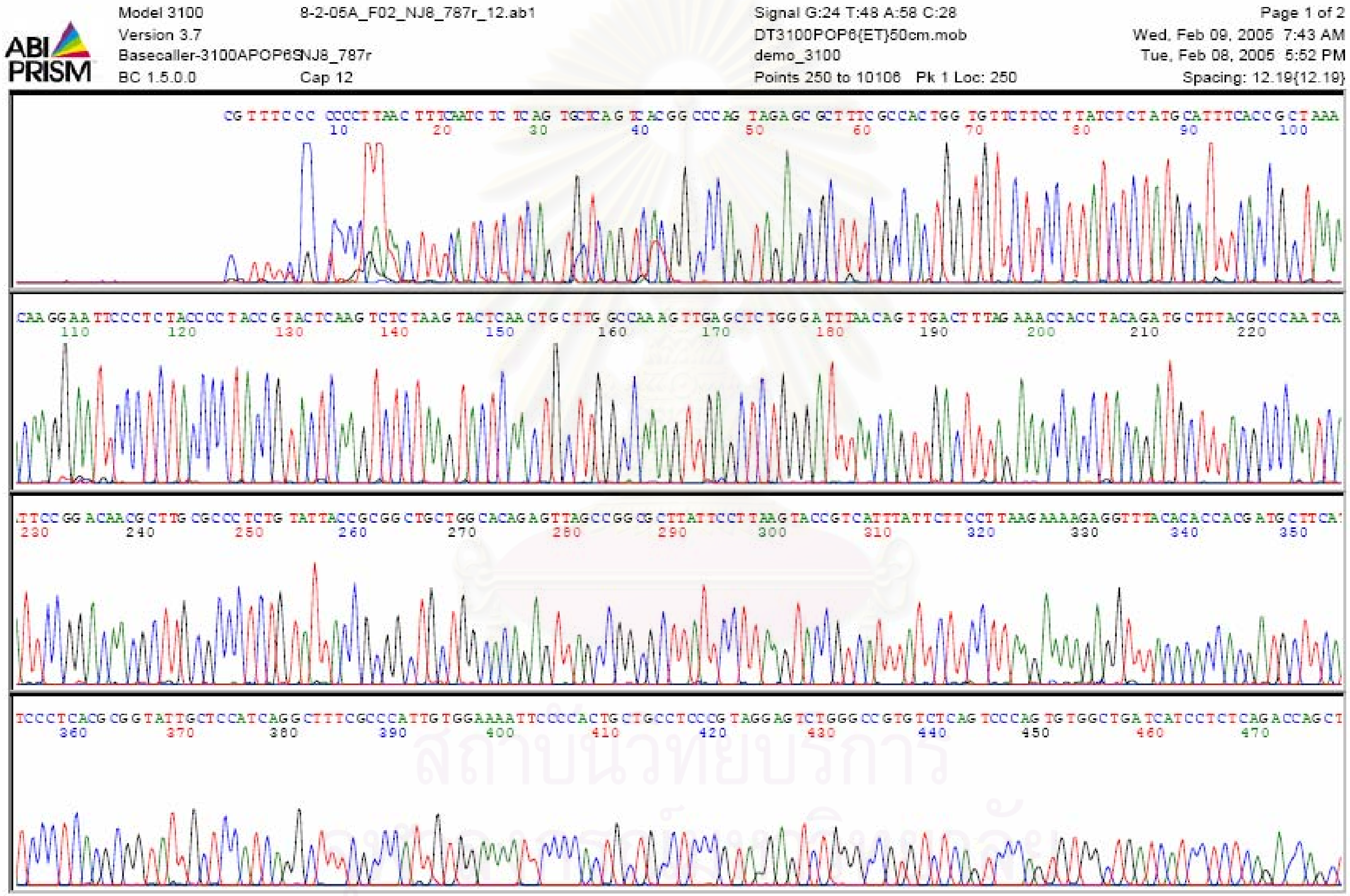
ABI PRISM

Model 3100 8-2-05A_G02_NJ8_519r_14.ab1
Version 3.7
Basecaller-3100APOP6SNJ8_519r
BC 1.5.0.0 Cap 14

Signal G:21 T:39 A:48 C:22
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

Page 1 of 3
Wed, Feb 09, 2005 7:43 AM
Tue, Feb 08, 2005 5:52 PM
Spacing: 12.49{12.49}





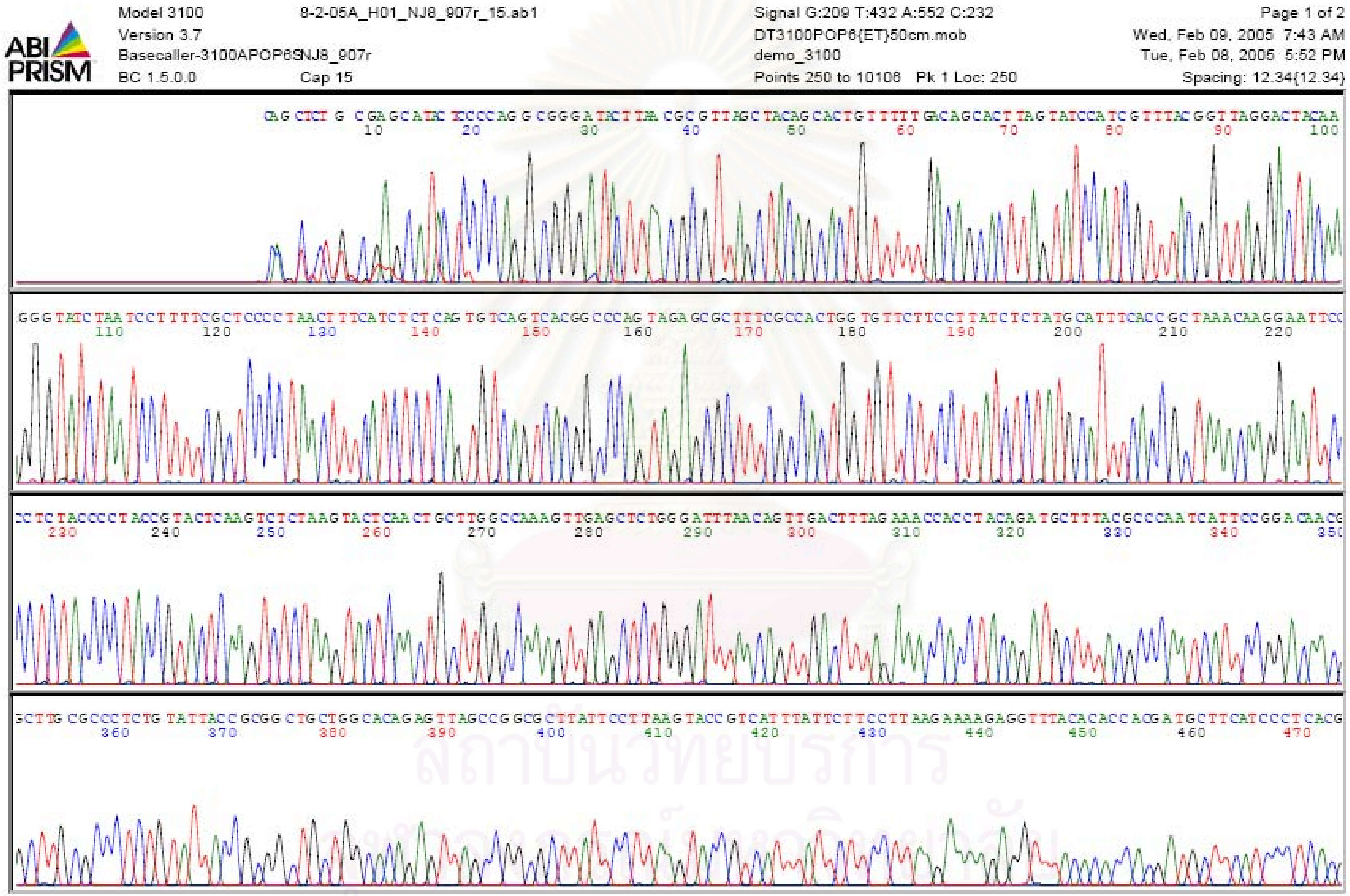
ABI PRISM

Model 3100
Version 3.7
Basecaller-3100APOP6SNJ8_787r
BC 1.5.0.0

8-2-05A_F02_NJ8_787r_12.ab1
Cap 12

Signal G:24 T:48 A:58 C:28
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106   Pk 1 Loc: 250

Page 1 of 2
Wed, Feb 09, 2005 7:43 AM
Tue, Feb 08, 2005 5:52 PM
Spacing: 12.19{12.19}



Model 3100 8-2-05A_H01_NJ8_907r_15.ab1
Version 3.7
Basecaller-3100APOP6SNJ8_907r
BC 1.5.0.0 Cap 15

Signal G:209 T:432 A:552 C:232
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

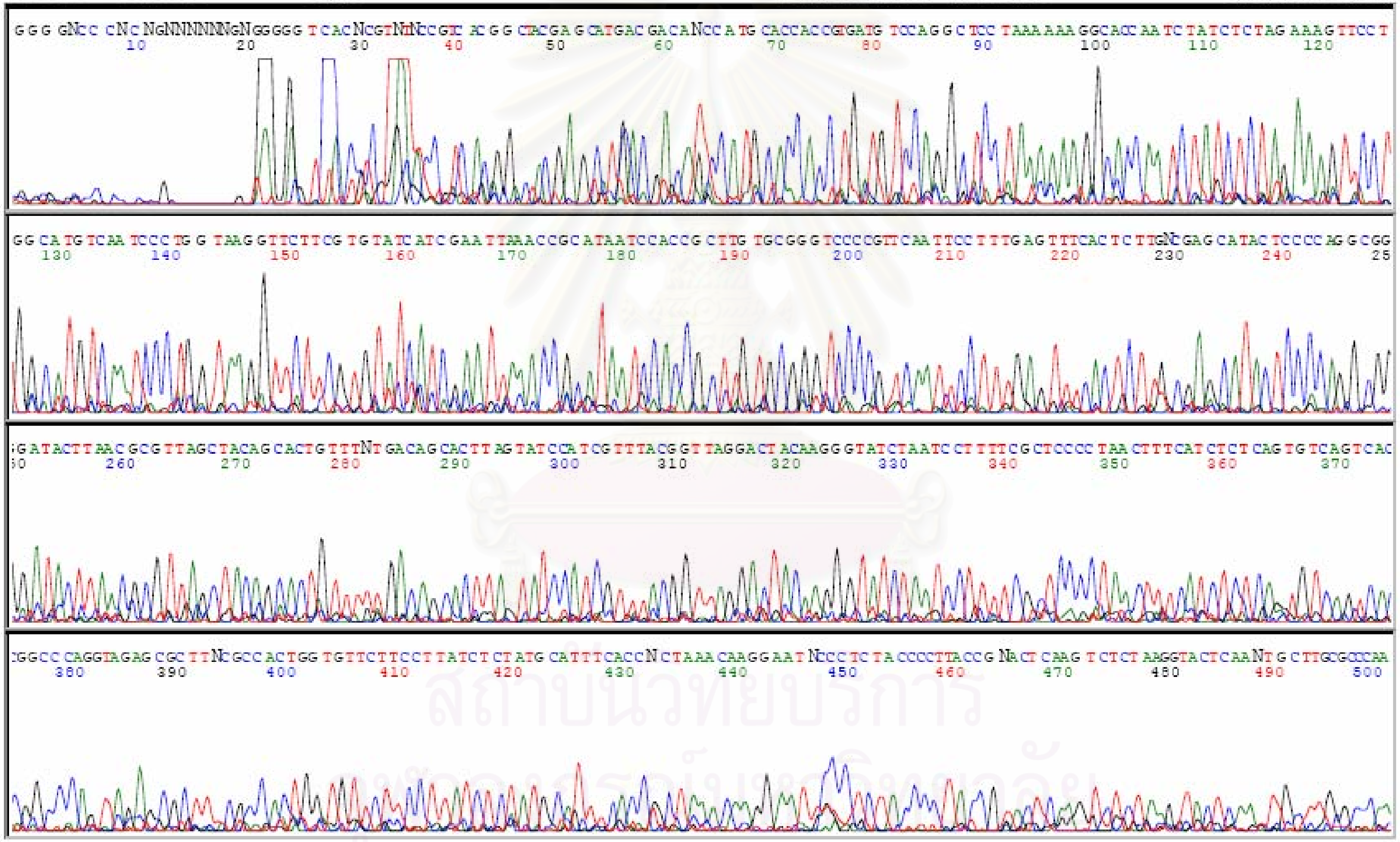
Page 1 of 2
Wed, Feb 09, 2005 7:43 AM
Tue, Feb 08, 2005 5:52 PM
Spacing: 12.34{12.34}

ABI PRISM

Model 3100 8-2-05A_G01_NJ8_1100r_13.ab1
Version 3.7
Basecaller-3100APOP6SNJ8_1100r
BC 1.5.0.0 Cap 13

Signal G:7 T:10 A:12 C:6
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

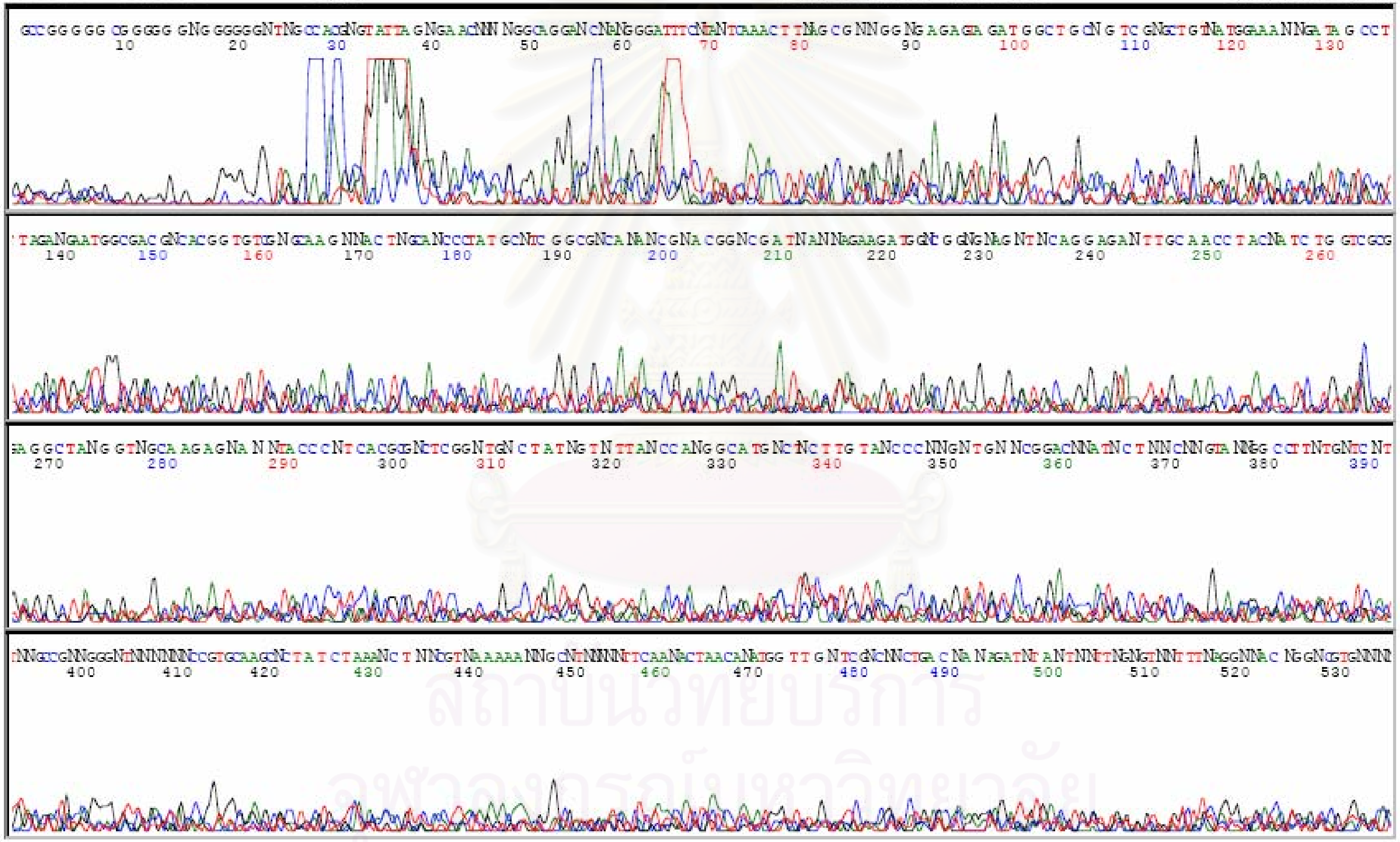
Page 1 of 2
Wed, Feb 09, 2005 7:43 AM
Tue, Feb 08, 2005 5:52 PM
Spacing: 12.19{12.19}

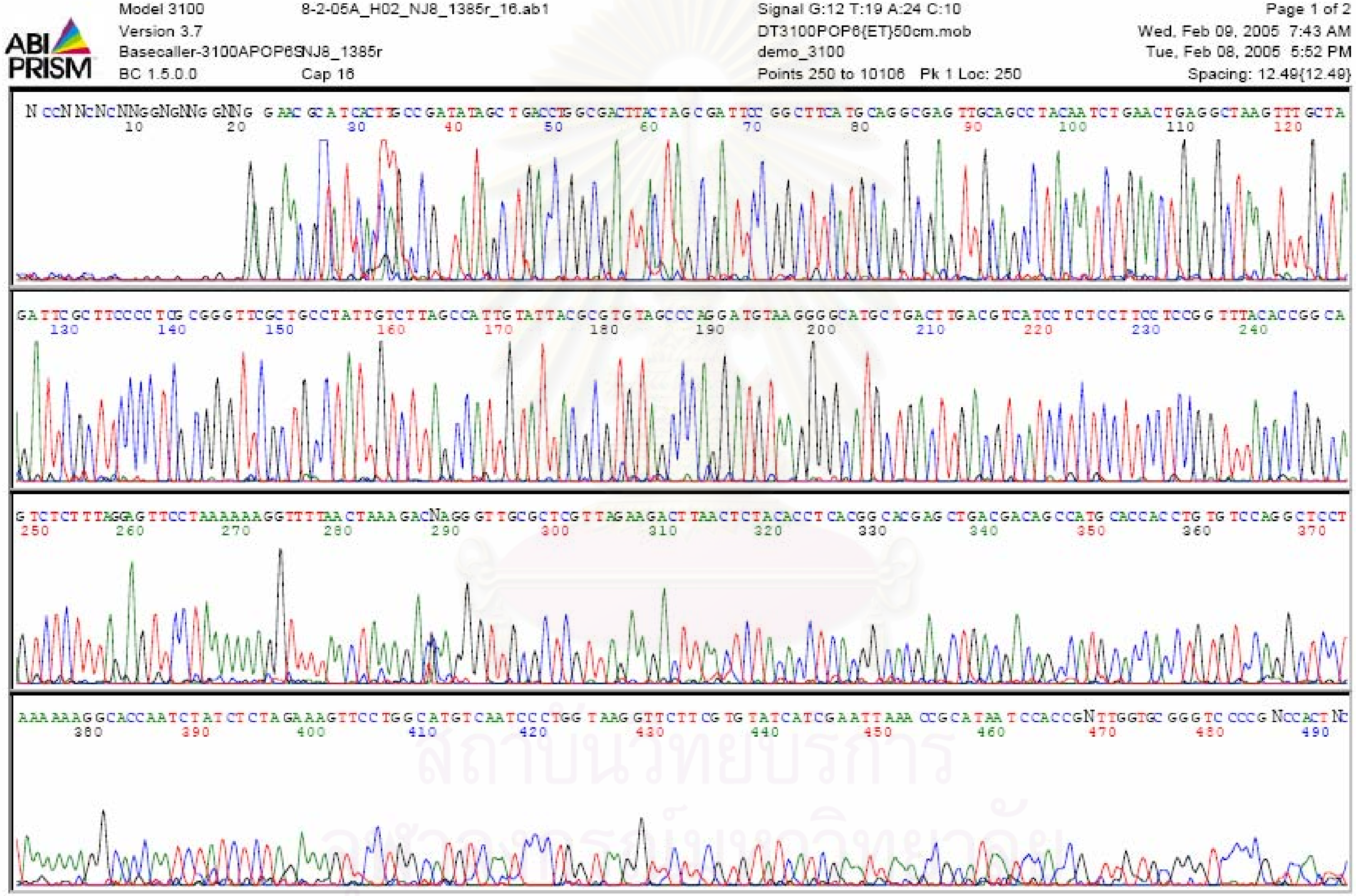


Model 3100 8-2-05A_E02_NJ8_1241f_10.ab1
Version 3.7
Basecaller-3100APOP6SNJ8_1241f
BC 1.5.0.0 Cap 10

Signal G:5 T:7 A:7 C:4
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

Page 1 of 2
Wed, Feb 09, 2005 7:43 AM
Tue, Feb 08, 2005 5:52 PM
Spacing: 12.19{12.19}





Model 3100
Version 3.7
Basecaller-3100APOP6SNJ8_1385r
BC 1.5.0.0

8-2-05A_H02_NJ8_1385r_16.ab1
Cap 16

Signal G:12 T:19 A:24 C:10
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

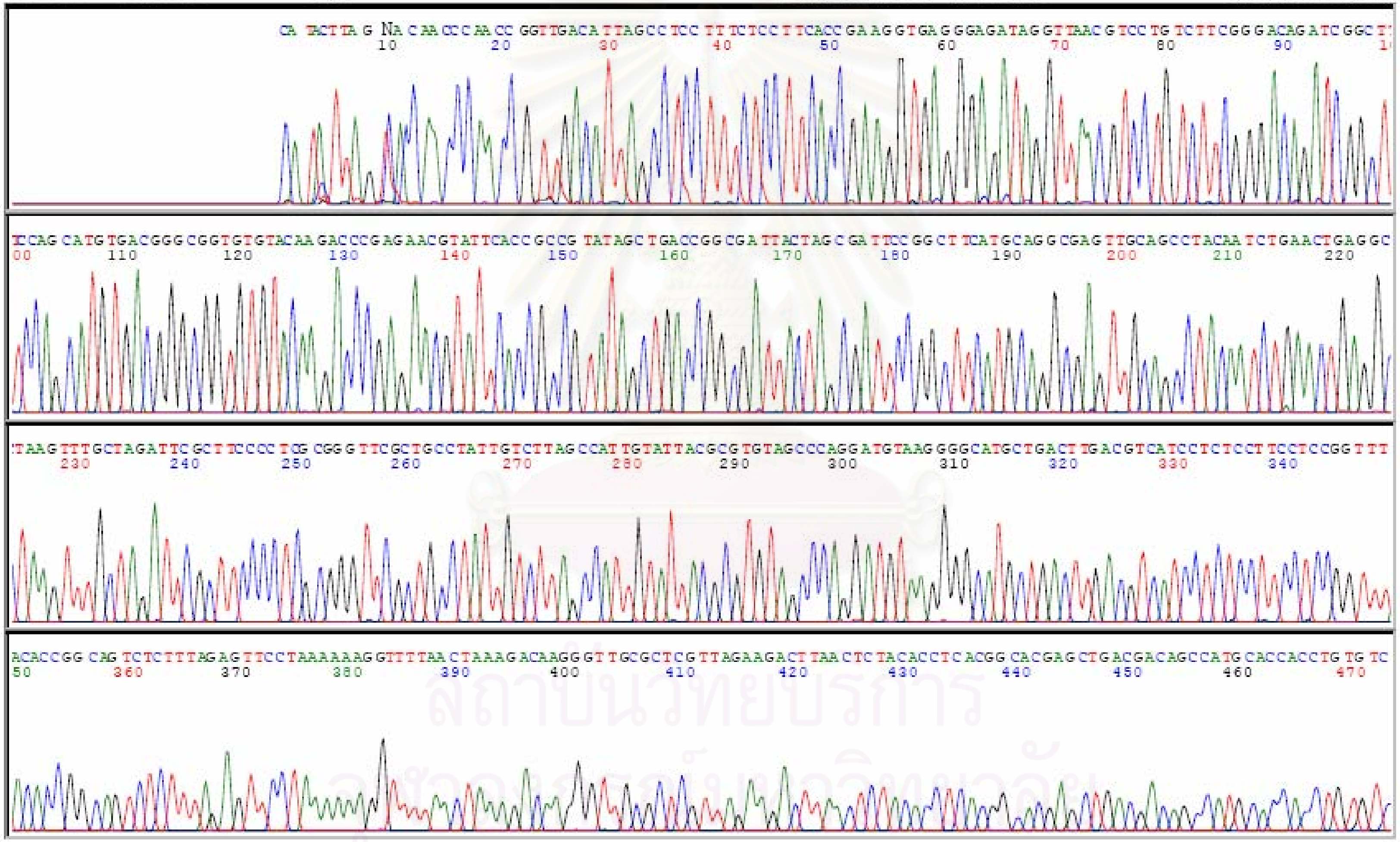
Page 1 of 2
Wed, Feb 09, 2005 7:43 AM
Tue, Feb 08, 2005 5:52 PM
Spacing: 12.49{12.49}

ABI PRISM

Model 3100 8-2-05A_E01_NJ8_1492r_09.ab1
Version 3.7
Basecaller-3100APOP6SNJ8_1492r
BC 1.5.0.0 Cap 9

Signal G:297 T:456 A:598 C:246
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

Page 1 of 2
Wed, Feb 09, 2005 7:43 AM
Tue, Feb 08, 2005 5:52 PM
Spacing: 12.19{12.19}

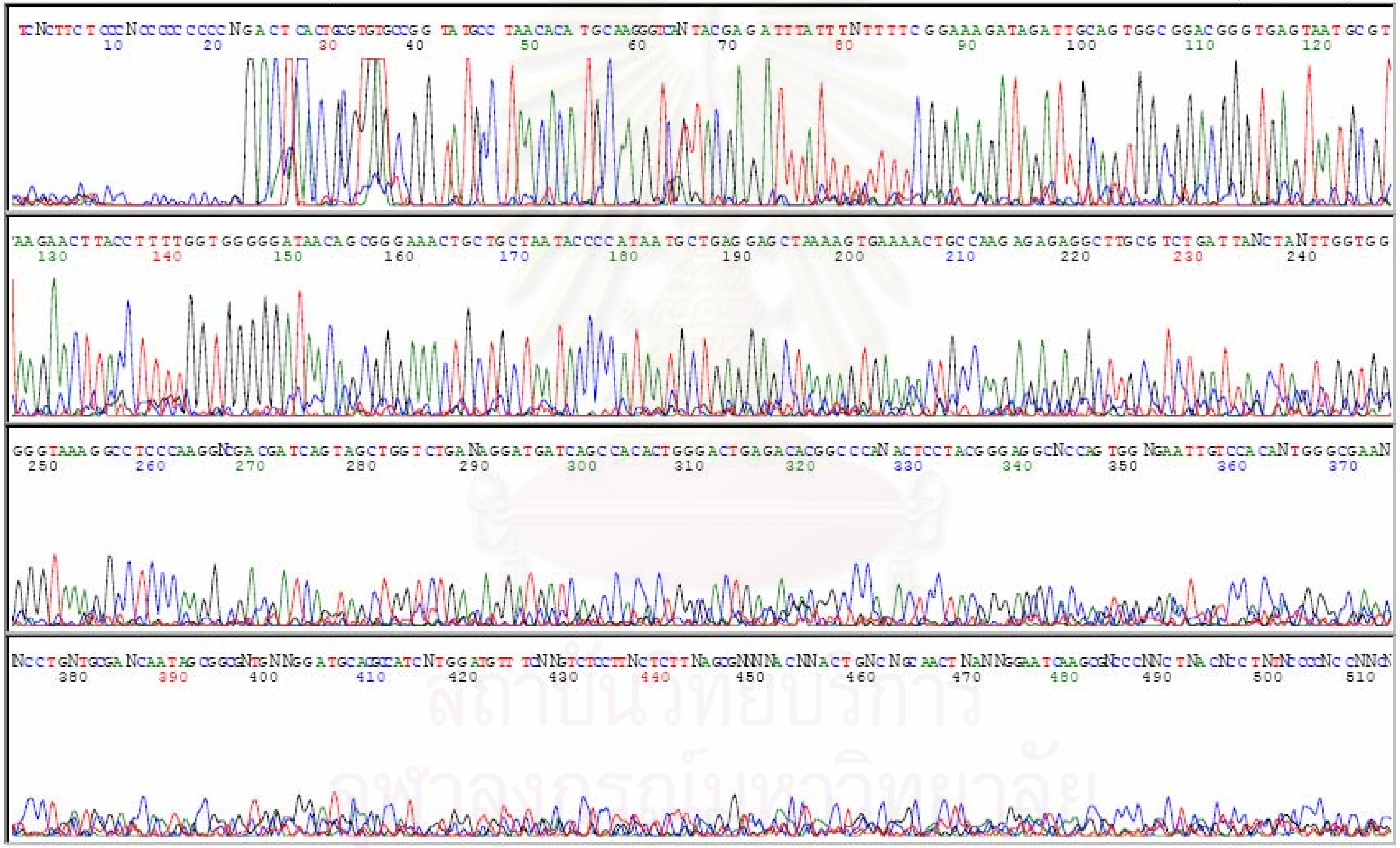


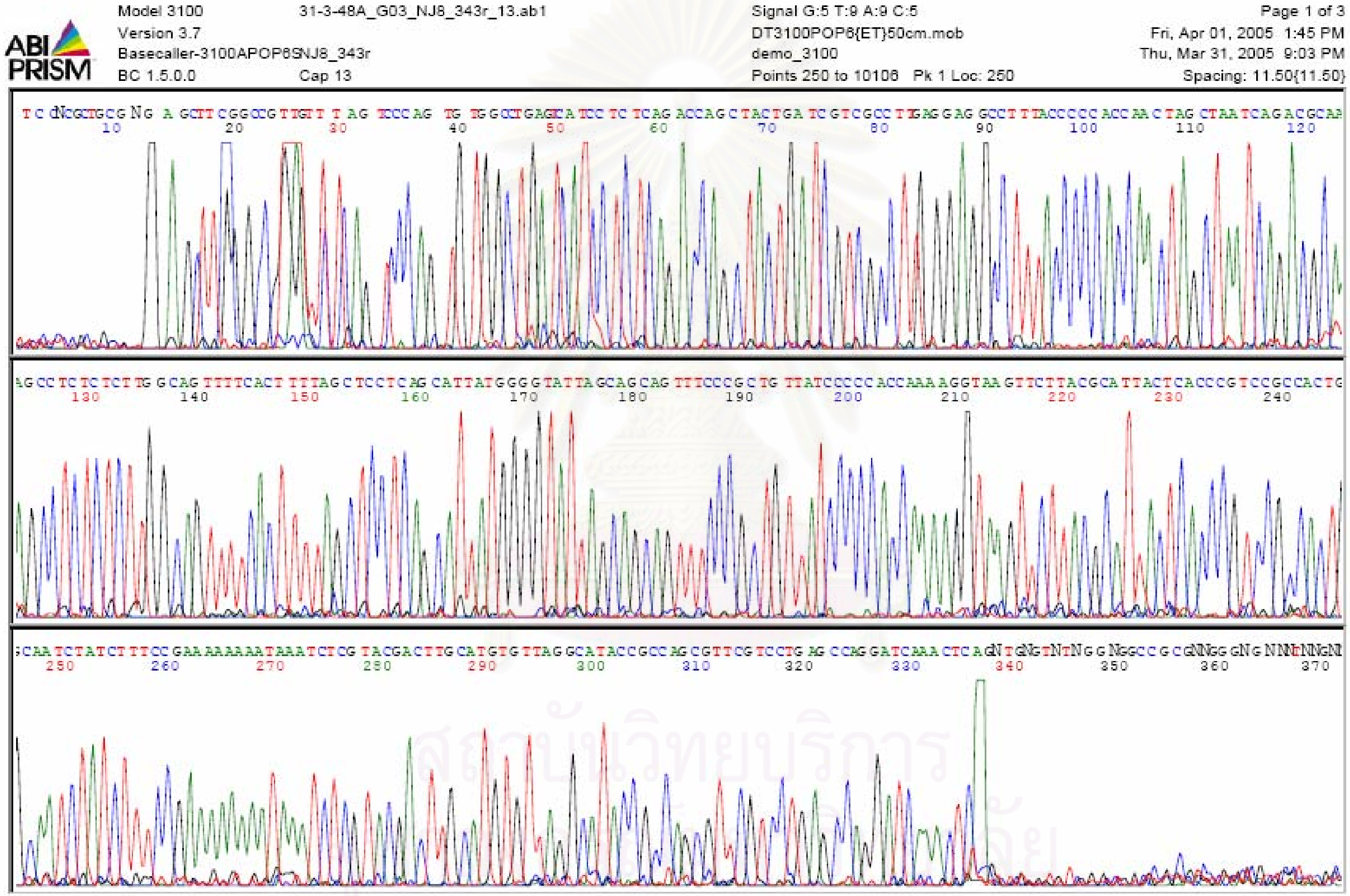
Model 3100
Version 3.7
Basecaller-3100APOP6SNJ8_27f
BC 1.5.0.0

29-3-05B_H08_NJ8_27f_16.ab1
Cap 16

Signal G:7 T:9 A:12 C:5
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

Page 1 of 3
Fri, Apr 01, 2005 1:10 PM
Wed, Mar 30, 2005 10:24 AM
Spacing: 11.91{11.91}

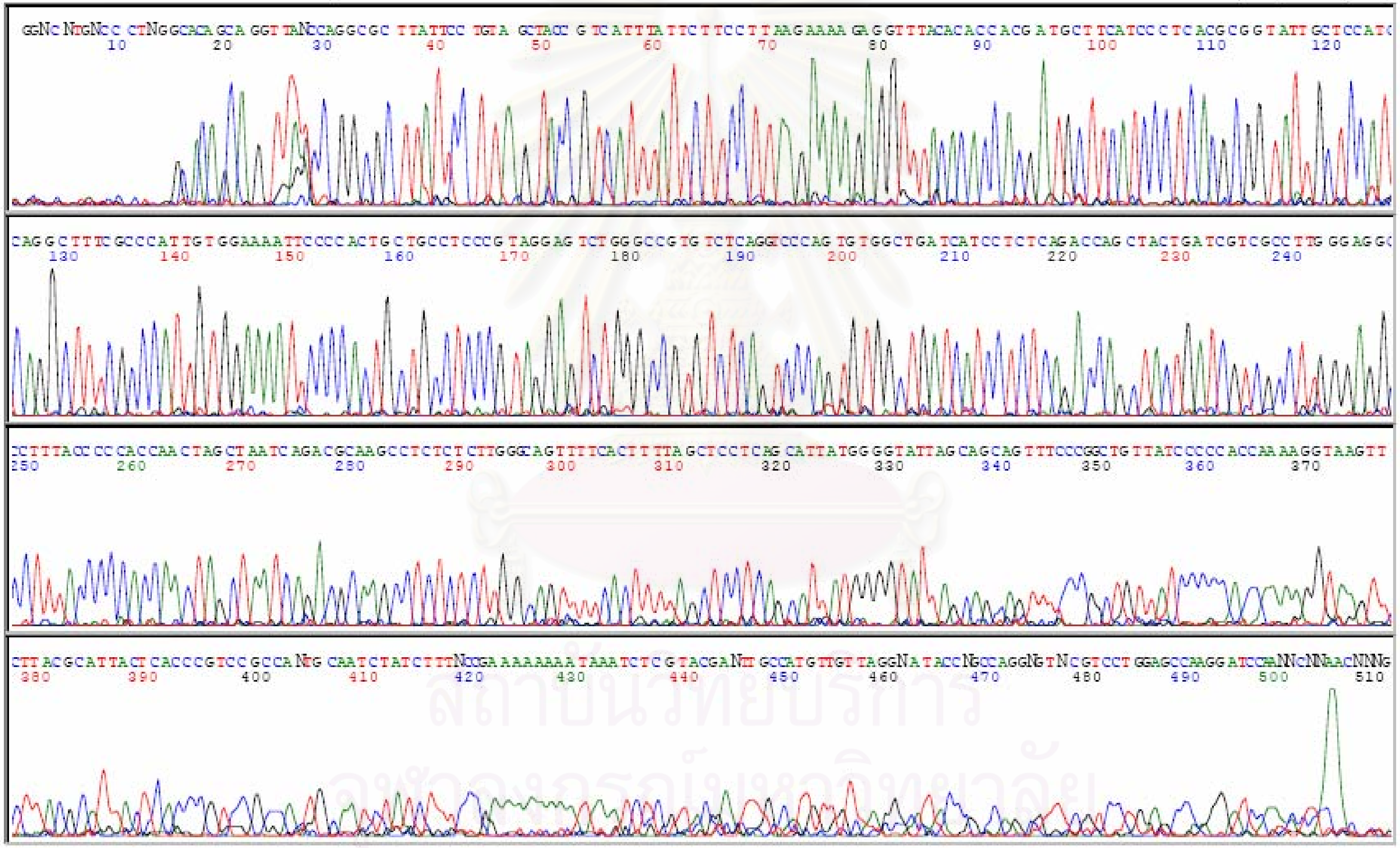




Model 3100 31-3-48A_E03_NJ8_519r_09.ab1
Version 3.7
Basecaller-3100APOP6SNJ8_519r
BC 1.5.0.0 Cap 9

Signal G:5 T:9 A:10 C:5
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

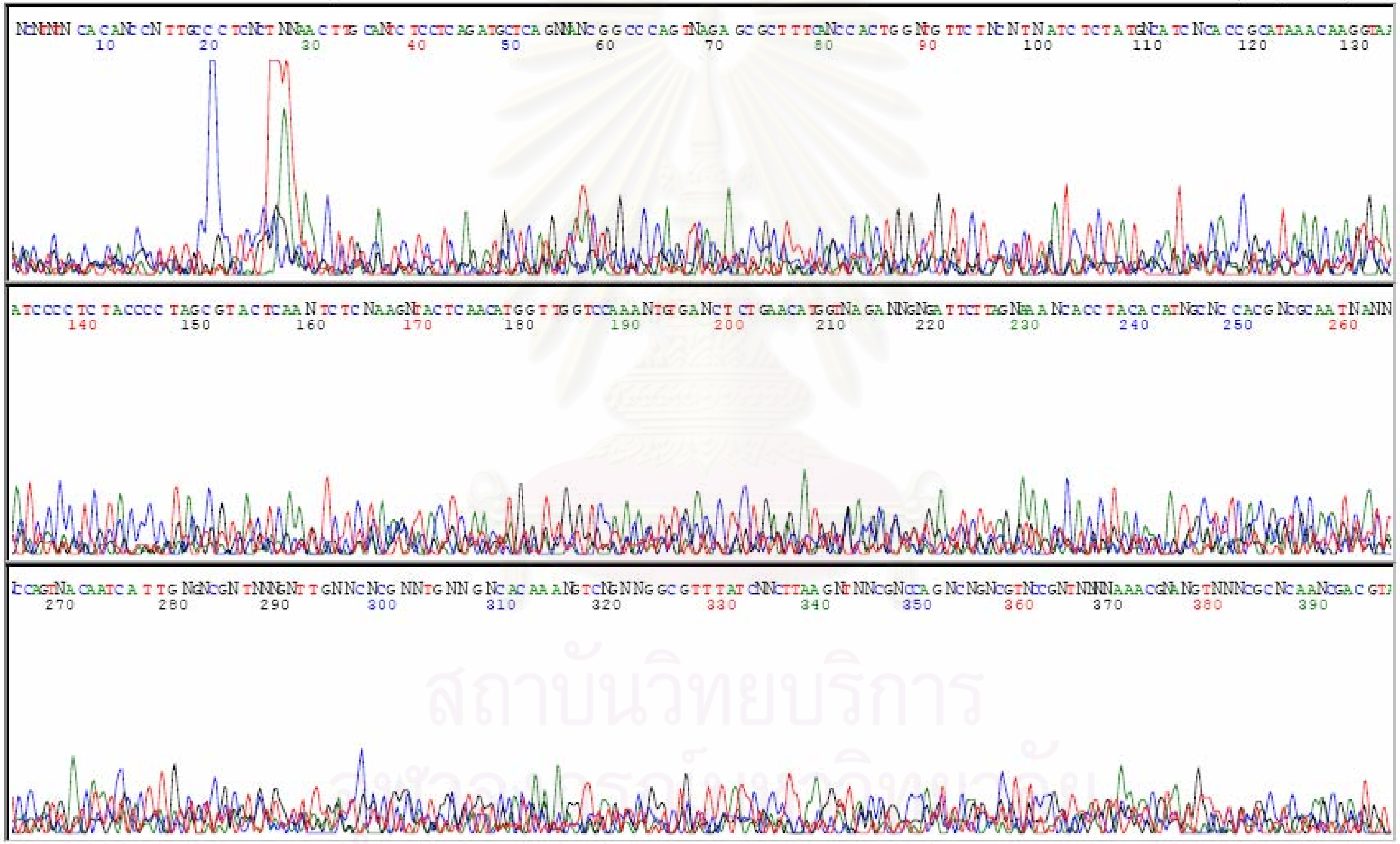
Page 1 of 3
Fri, Apr 01, 2005 1:45 PM
Thu, Mar 31, 2005 9:03 PM
Spacing: 11.77{11.77}



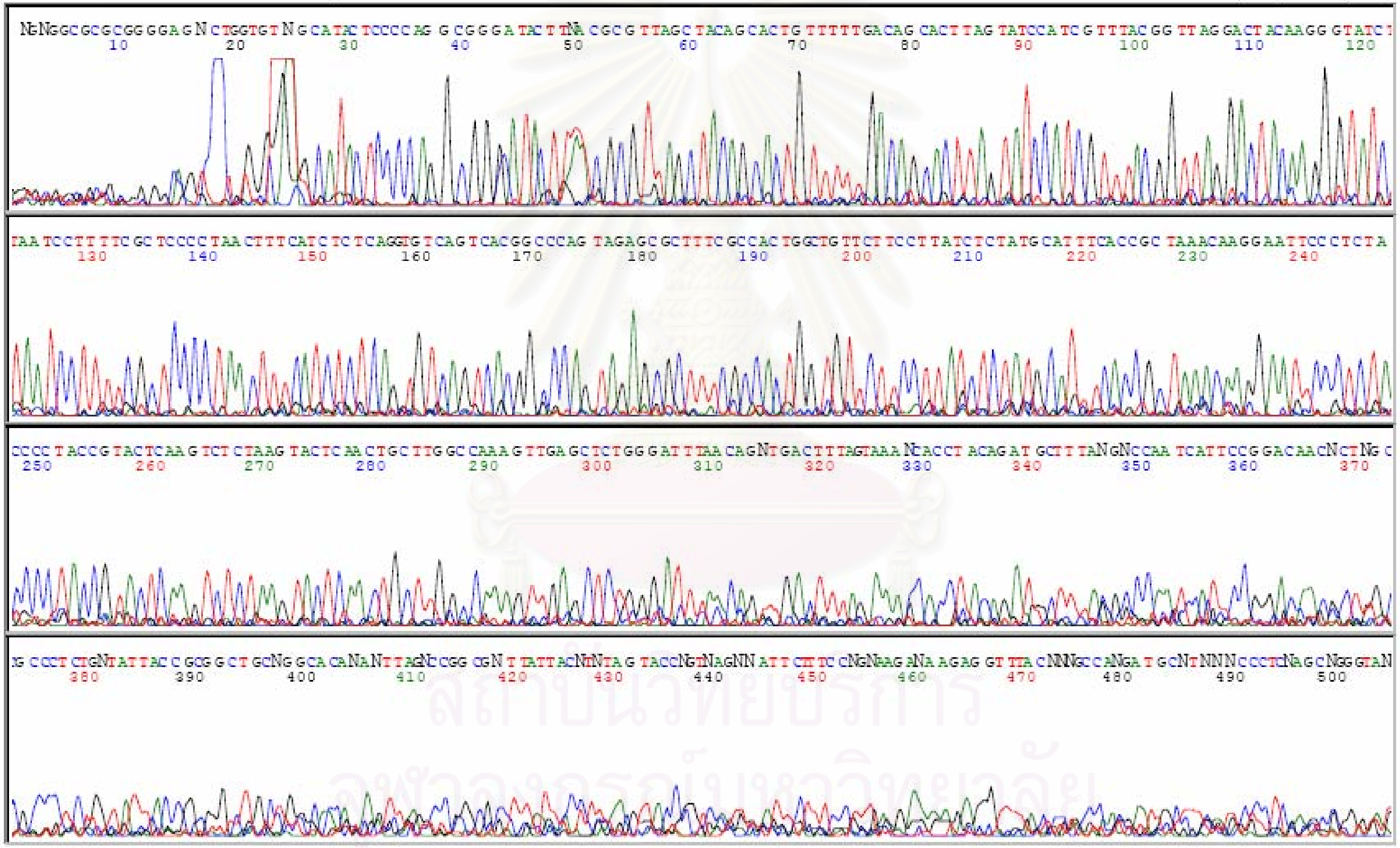
Model 3100 31-3-48A_F03_NJ8_787r_11.ab1
Version 3.7
Basecaller-3100APOP6SNJ8_787r
BC 1.5.0.0 Cap 11

Signal G:3 T:5 A:4 C:3
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

Page 1 of 3
Fri, Apr 01, 2005 1:45 PM
Thu, Mar 31, 2005 9:03 PM
Spacing: 11.50{11.50}



Model 3100	31-3-48A_G04_NJ8_907r_14.ab1	Signal G:5 T:8 A:7 C:4	Page 1 of 3
Version 3.7		DT3100POP6{ET}50cm.mob	Fri, Apr 01, 2005 1:45 PM
Basecaller-3100APOP6SNJ8_907r		demo_3100	Thu, Mar 31, 2005 9:03 PM
BC 1.5.0.0	Cap 14	Points 250 to 10106 Pk 1 Loc: 250	Spacing: 11.50{11.50}



ABI PRISM

Model 3100
Version 3.7
Basecaller-3100APOP6SNJ8_1100r
BC 1.5.0.0

31-3-48A_E04_NJ8_1100r_10.ab1

Cap 10

Signal G:3 T:5 A:4 C:3
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106   Pk 1 Loc: 250

Page 1 of 3
Fri, Apr 01, 2005 1:45 PM
Thu, Mar 31, 2005 9:03 PM
Spacing: 15.28{15.28}

Model 3100
Version 3.7
Basecaller-3100APOP6SNJ8_1241f
BC 1.5.0.0

31-3-48A_F04_NJ8_1241f_12.ab1

Cap 12

Signal G:3 T:5 A:4 C:3
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

Page 1 of 3
Fri, Apr 01, 2005 1:45 PM
Thu, Mar 31, 2005 9:03 PM
Spacing: 16.00{16.00}

ABI PRISM

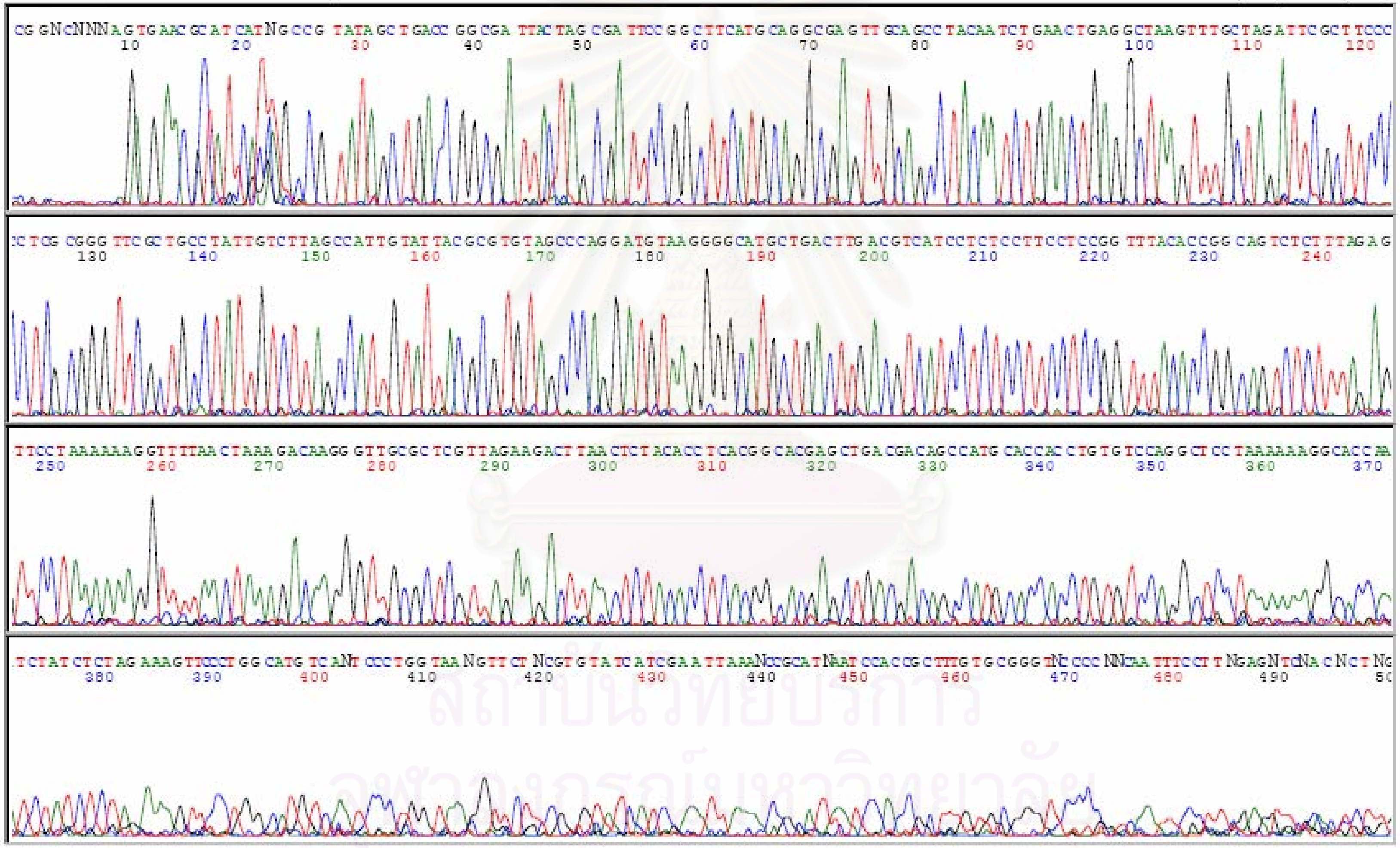
Model 3100
Version 3.7
Basecaller-3100APOP6SNJ8_1385r
BC 1.5.0.0

31-3-48A_D04_NJ8_1385r_08.ab1

Cap 8

Signal G:7 T:10 A:10 C:5
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106   Pk 1 Loc: 250

Page 1 of 3
Fri, Apr 01, 2005 1:45 PM
Thu, Mar 31, 2005 9:03 PM
Spacing: 11.64{11.64}



ABI PRISM

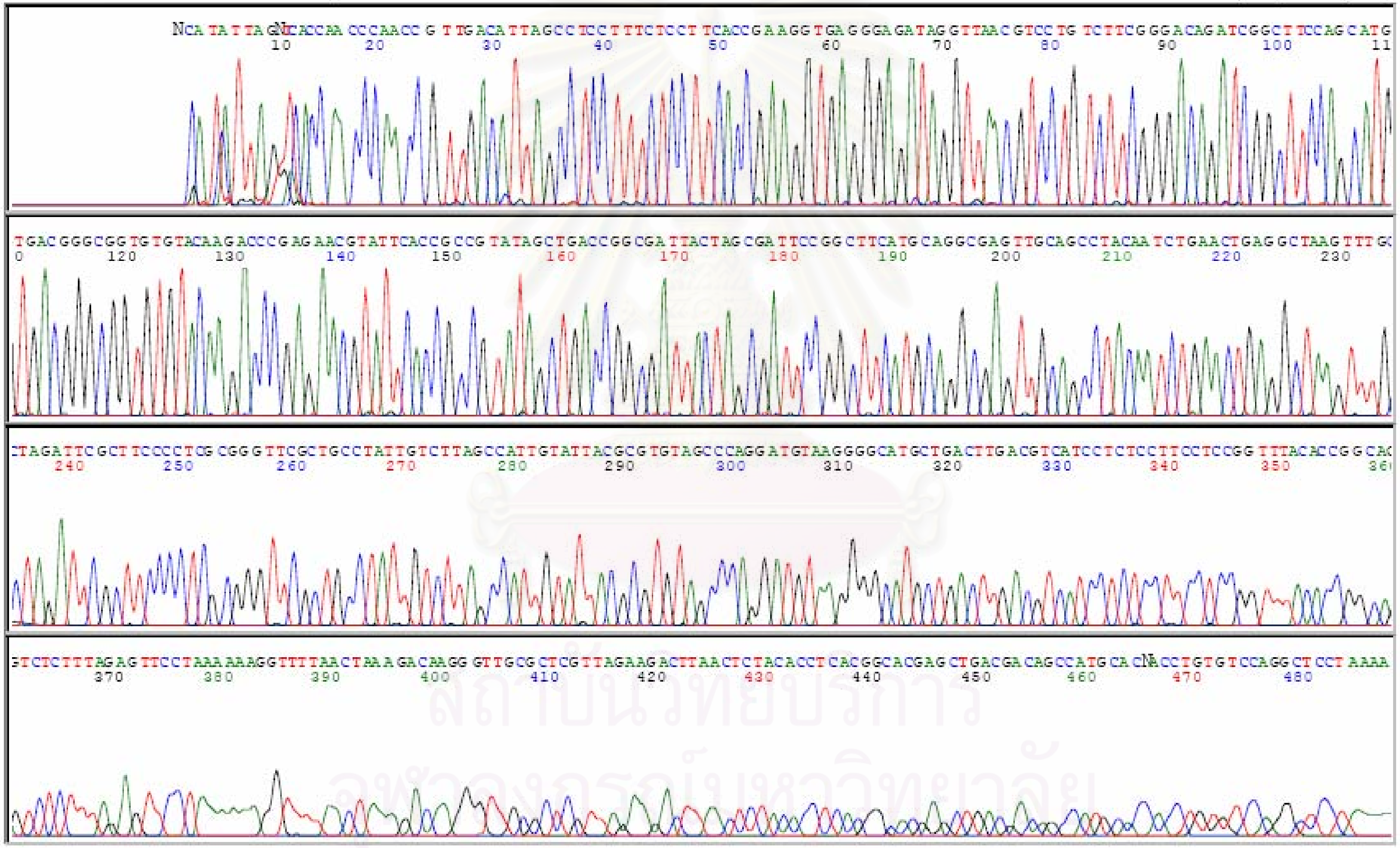
Model 3100
Version 3.7
Basecaller-3100APOP6SNJ8_1492r
BC 1.5.0.0

31-3-48A_D03_NJ8_1492r_07.ab1

Cap 7

Signal G:76 T:120 A:144 C:66
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

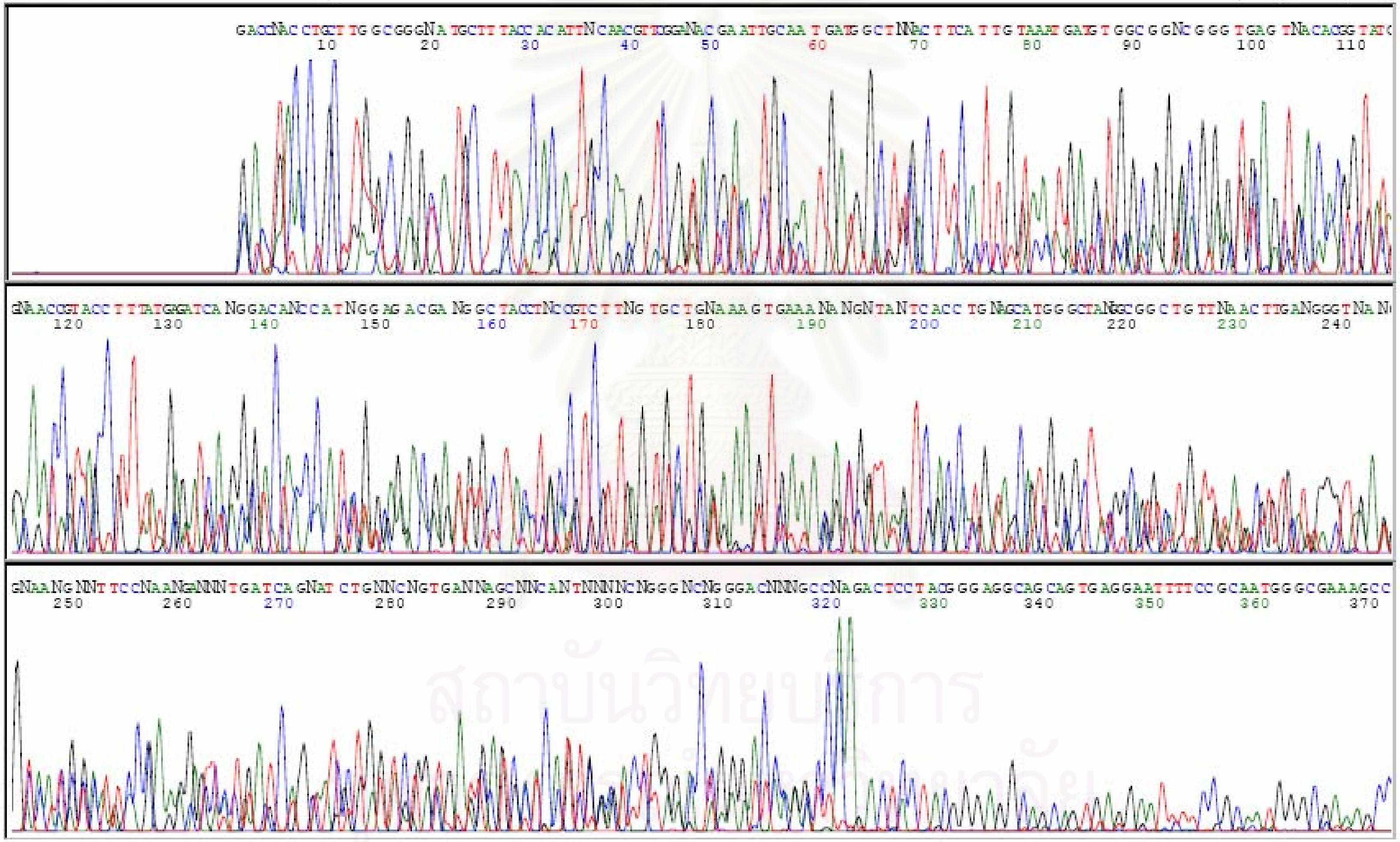
Page 1 of 2
Fri, Apr 01, 2005 1:45 PM
Thu, Mar 31, 2005 9:03 PM
Spacing: 11.64{11.64}



Model 3100 8-2-05A_A03_NJ26_27f_01.ab1
Version 3.7
Basecaller-3100APOP6SNJ26_27f
BC 1.5.0.0 Cap 1

Signal G:74 T:80 A:164 C:32
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

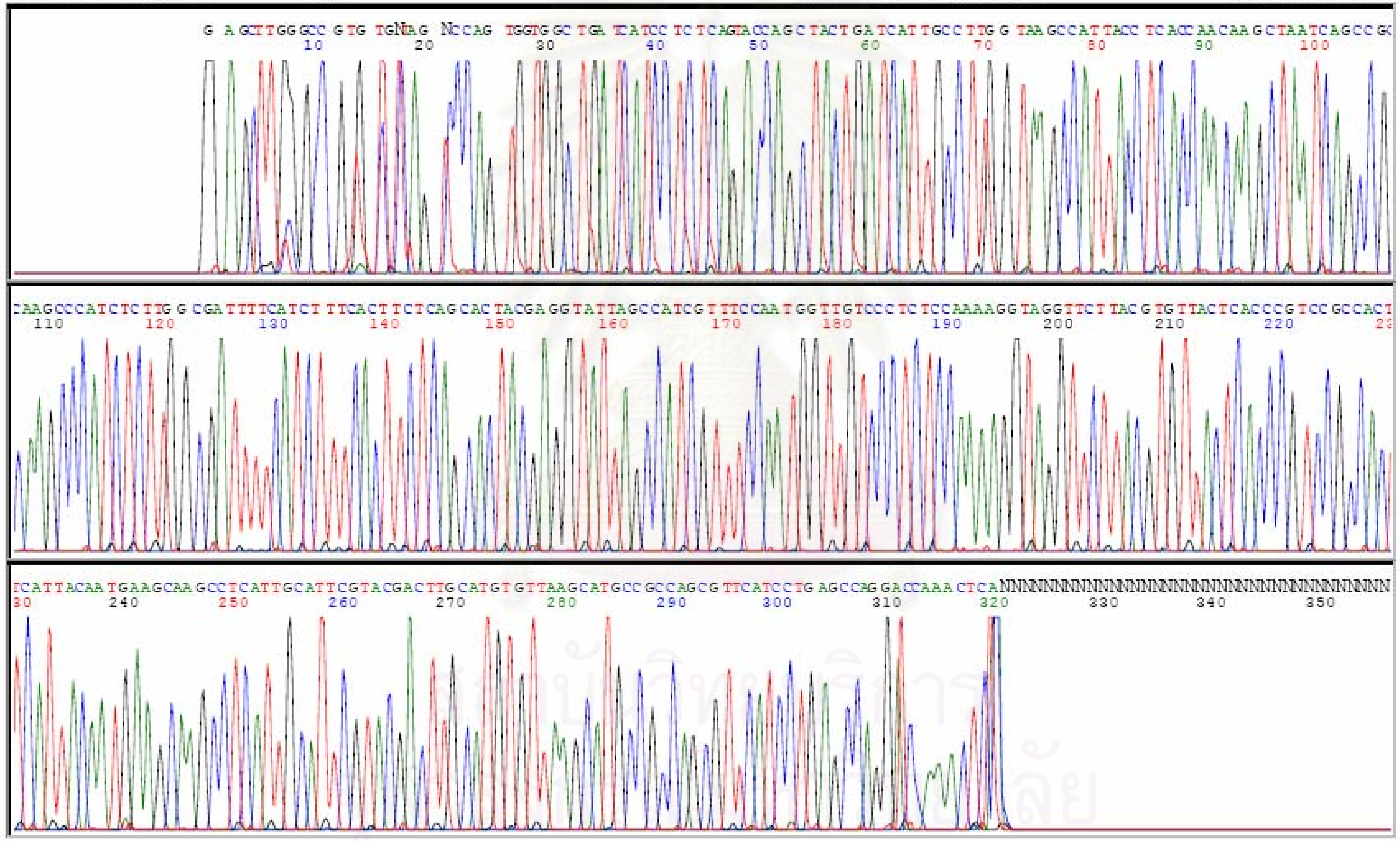
Page 1 of 3
Wed, Feb 09, 2005 8:18 AM
Tue, Feb 08, 2005 8:24 PM
Spacing: 12.05{12.05}



Model 3100 8-2-05A_B04_NJ26_343r_04.ab1
Version 3.7
Basecaller-3100APOP6SNJ26_343r
BC 1.5.0.0 Cap 4

Signal G:248 T:447 A:521 C:258
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

Page 1 of 3
Wed, Feb 09, 2005 8:18 AM
Tue, Feb 08, 2005 8:24 PM
Spacing: 12.19{12.19}



ABI PRISM

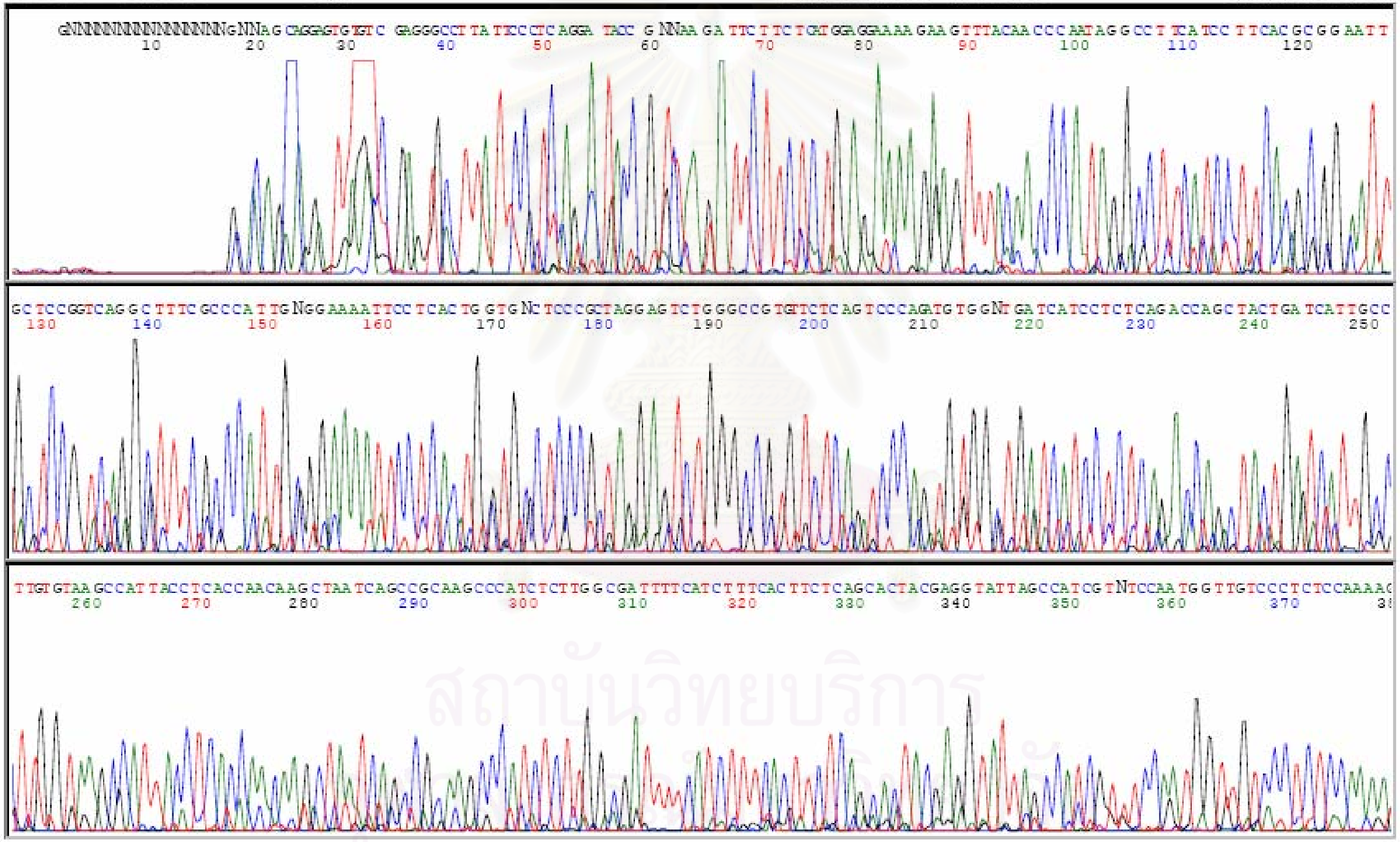
Model 3100
Version 3.7
Basecaller-3100APOP6SNJ26_519r
BC 1.5.0.0

8-2-05A_D03_NJ26_519r_07.ab1

Cap 7

Signal G:11 T:21 A:25 C:12
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106   Pk 1 Loc: 250

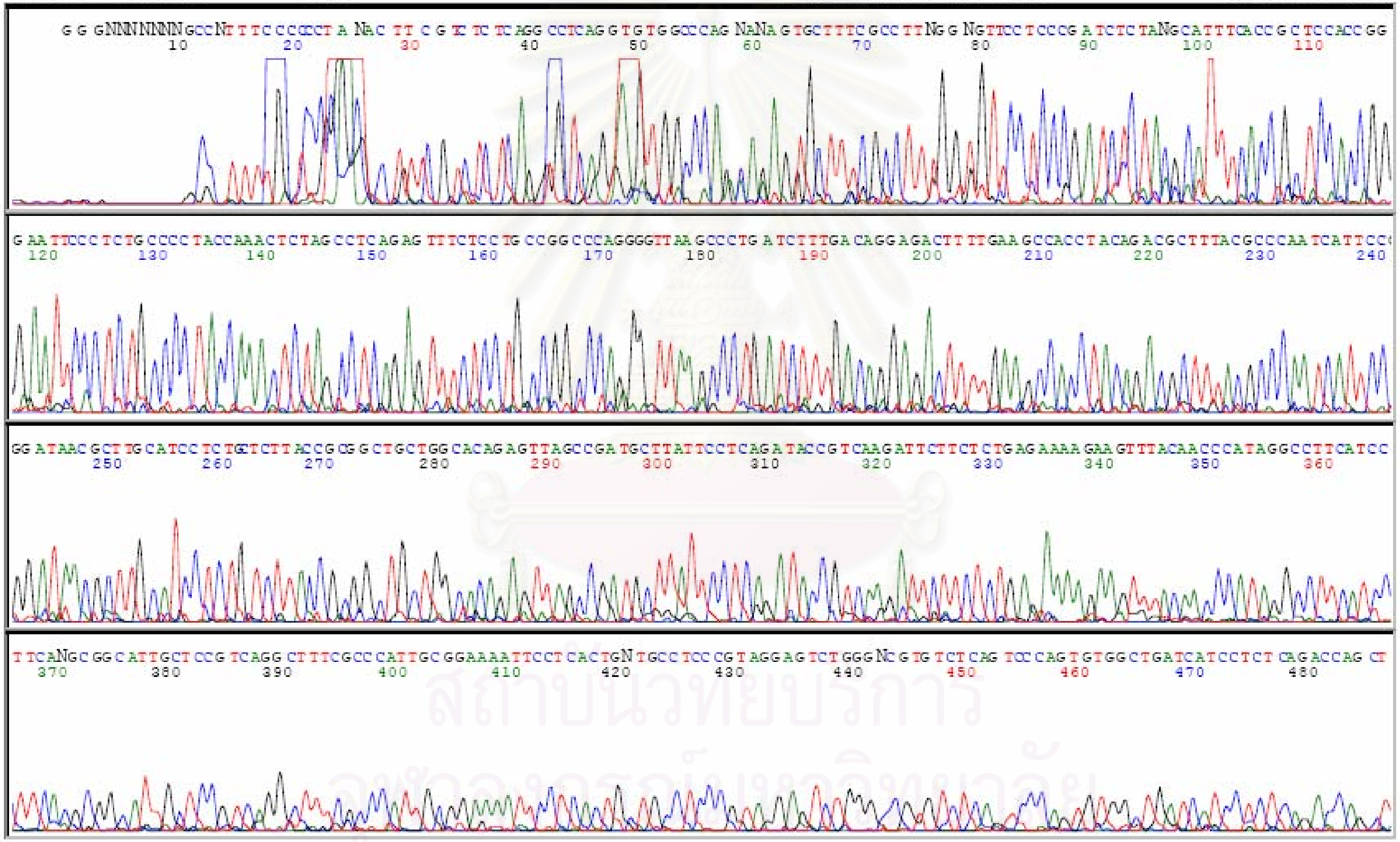
Page 1 of 3
Wed, Feb 09, 2005 8:18 AM
Tue, Feb 08, 2005 8:24 PM
Spacing: 11.91{11.91}



Model 3100 8-2-05A_C03_NJ26_787r_05.ab1
Version 3.7
Basecaller-3100APOP6SNJ26_787r
BC 1.5.0.0 Cap 5

Signal G:14 T:28 A:25 C:16
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

Page 1 of 2
Wed, Feb 09, 2005 8:18 AM
Tue, Feb 08, 2005 8:24 PM
Spacing: 12.05{12.05}



ABI PRISM

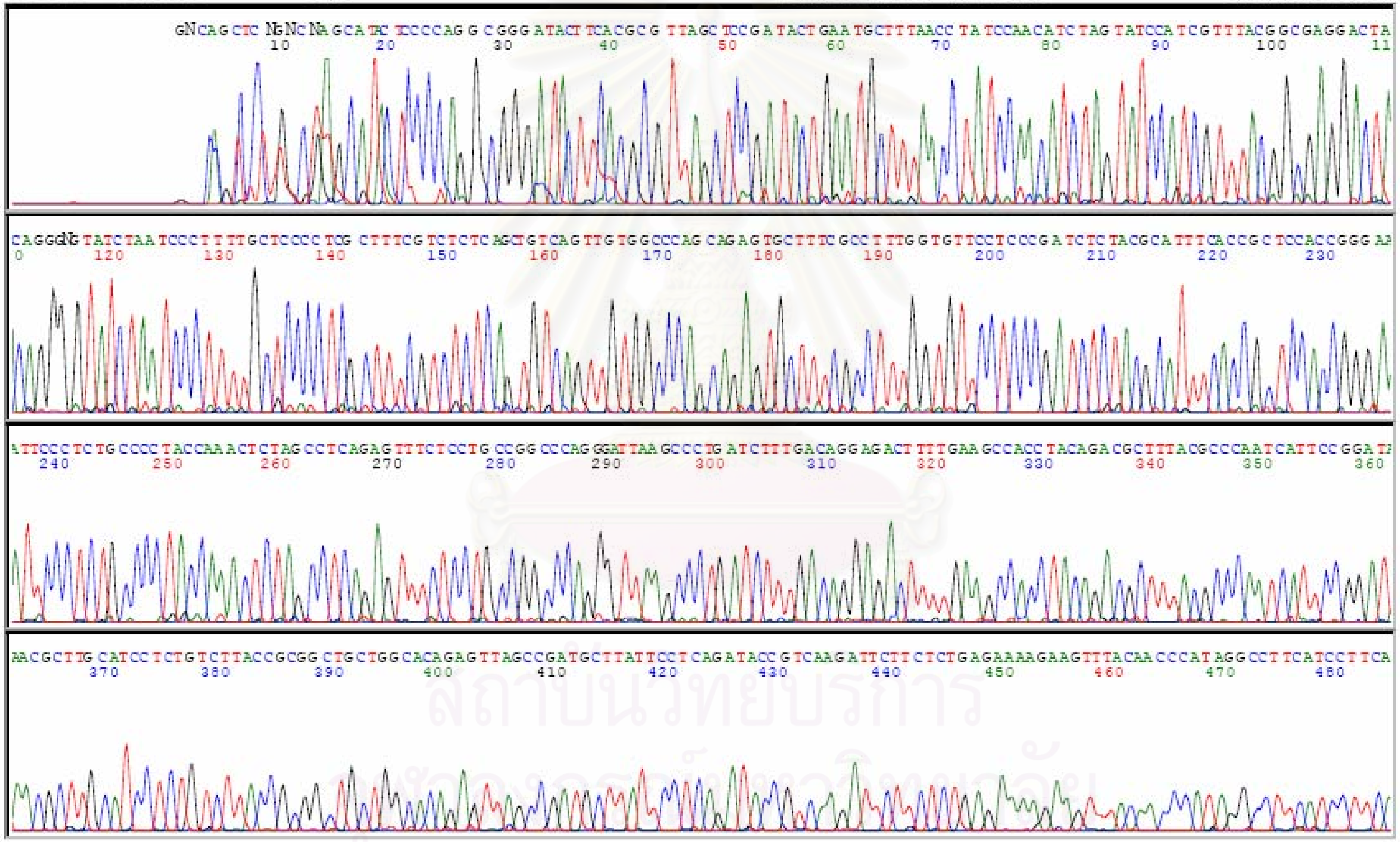
Model 3100
Version 3.7
Basecaller-3100APOP6SNJ26_907r
BC 1.5.0.0

8-2-05A_D04_NJ26_907r_08.ab1

Cap 8

Signal G:28 T:53 A:57 C:31
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

Page 1 of 3
Wed, Feb 09, 2005 8:18 AM
Tue, Feb 08, 2005 8:24 PM
Spacing: 11.91{11.91}



ABI PRISM

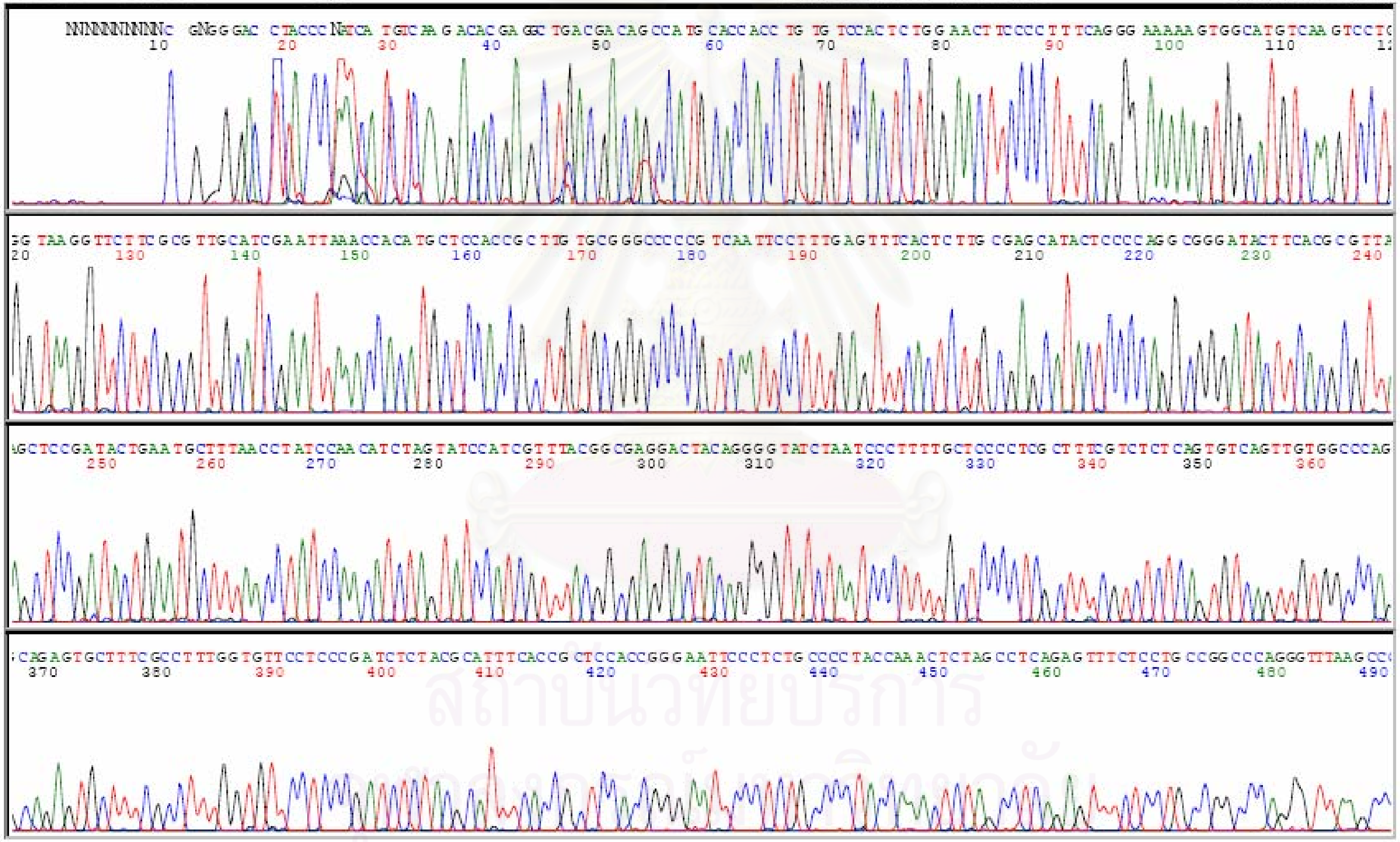
Model 3100
Version 3.7
Basecaller-3100APOP6SNJ26_1100r
BC 1.5.0.0

8-2-05A_C04_NJ26_1100r_06.ab1

Cap 6

Signal G:19 T:32 A:37 C:20
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106   Pk 1 Loc: 250

Page 1 of 2
Wed, Feb 09, 2005 8:18 AM
Tue, Feb 08, 2005 8:24 PM
Spacing: 12.19{12.19}



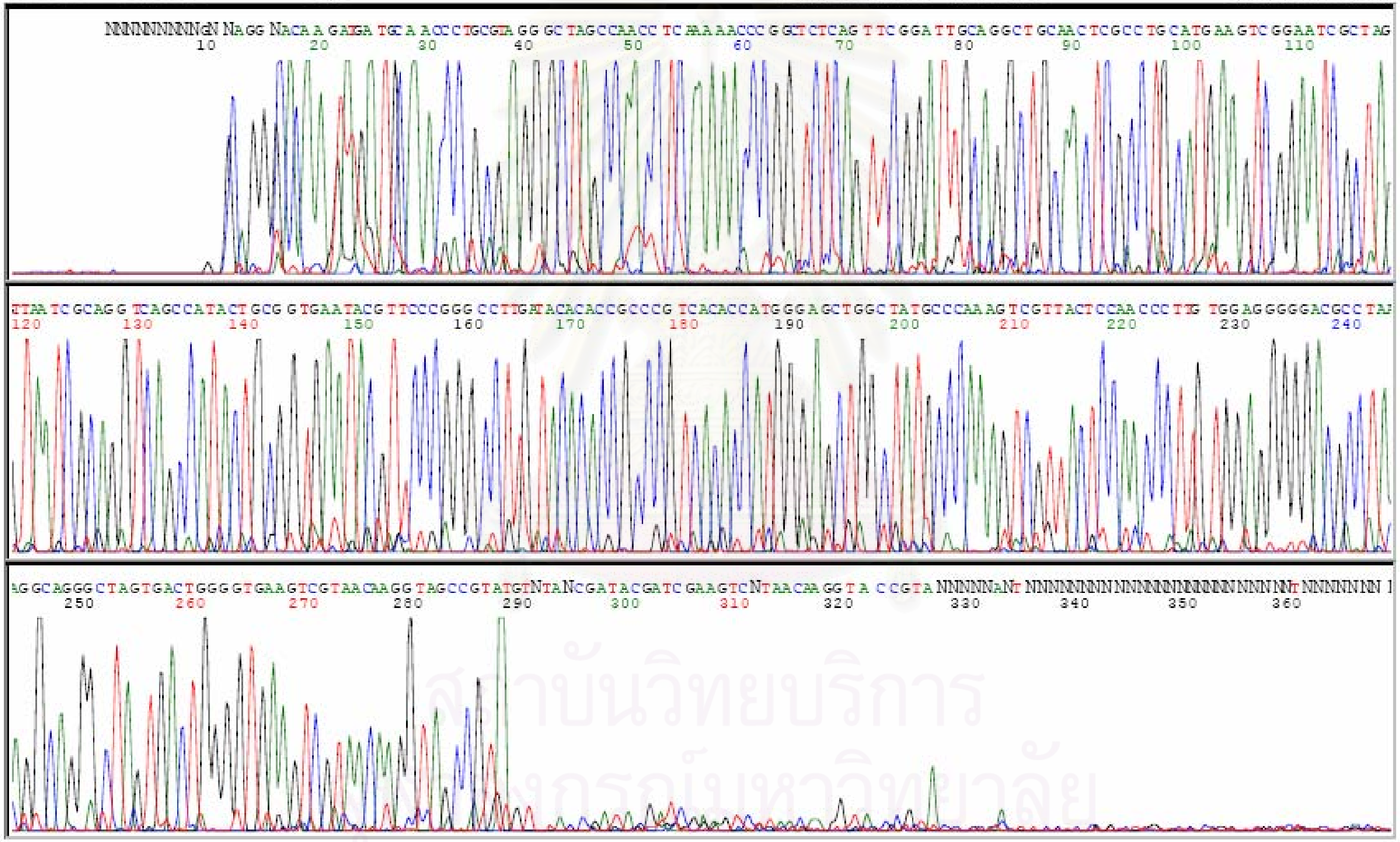
Model 3100
Version 3.7
Basecaller-3100APOP6SNJ26_1241f
BC 1.5.0.0

8-2-05A_B03_NJ26_1241f_03.ab1

Cap 3

Signal G:34 T:33 A:59 C:22
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

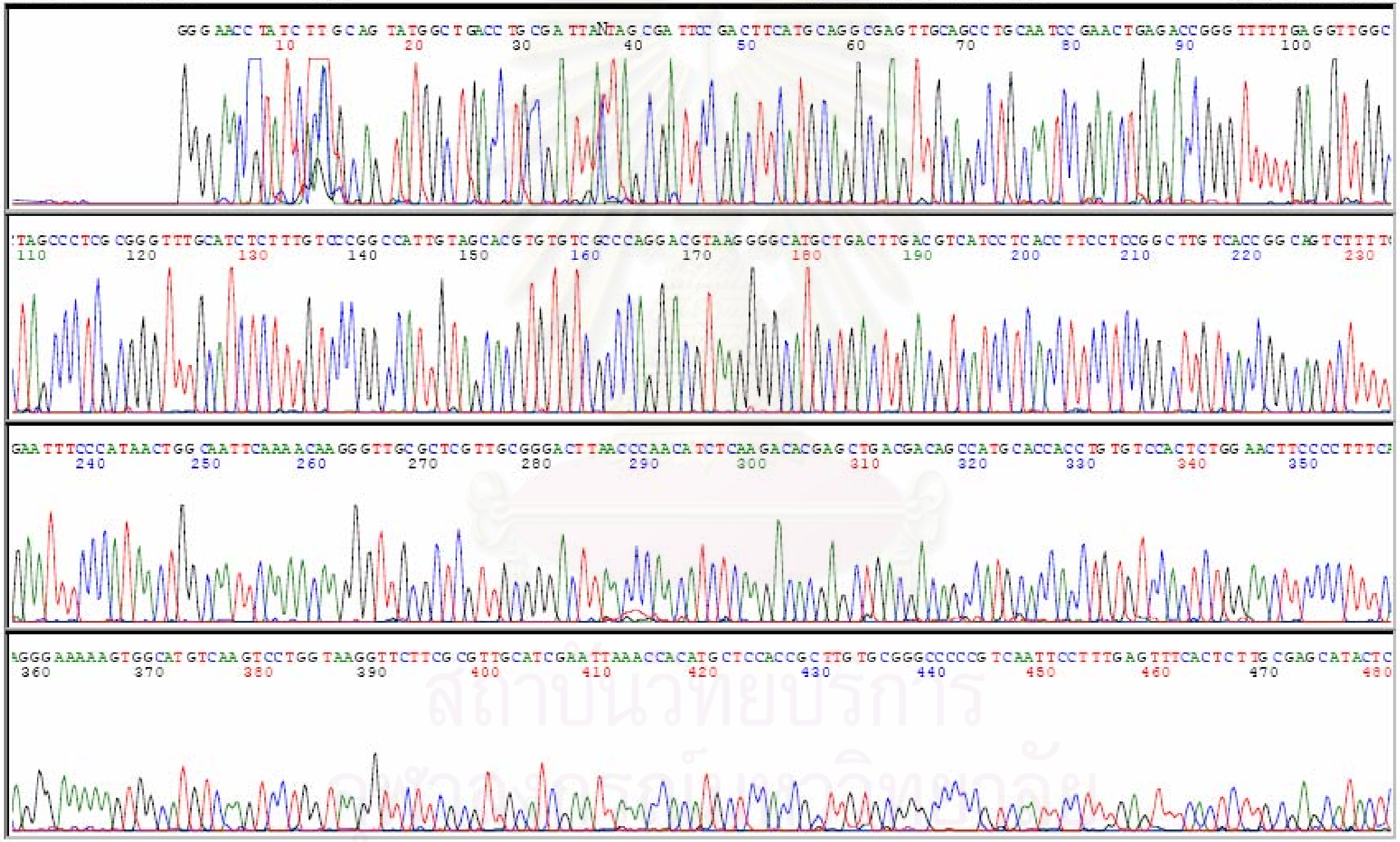
Page 1 of 3
Wed, Feb 09, 2005 8:18 AM
Tue, Feb 08, 2005 8:24 PM
Spacing: 12.05{12.05}



Model 3100 8-2-05A_E03_NJ26_1385r_09.ab1
Version 3.7
Basecaller-3100APOP6SNJ26_1385r
BC 1.5.0.0 Cap 9

Signal G:19 T:28 A:28 C:16
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

Page 1 of 3
Wed, Feb 09, 2005 8:18 AM
Tue, Feb 08, 2005 8:24 PM
Spacing: 11.64{11.64}



ABI PRISM

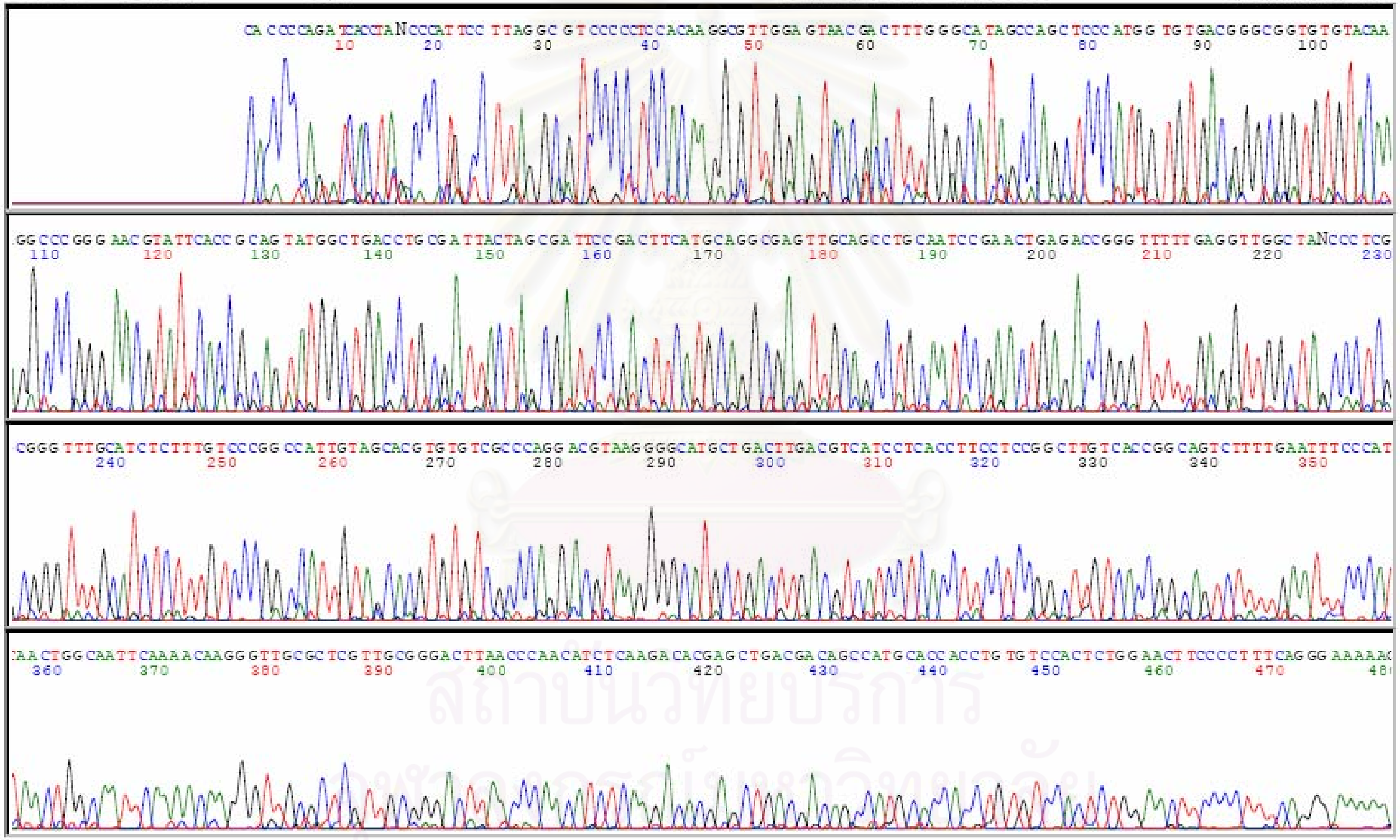
Model 3100
Version 3.7
Basecaller-3100APOP6SNJ26_1492r
BC 1.5.0.0

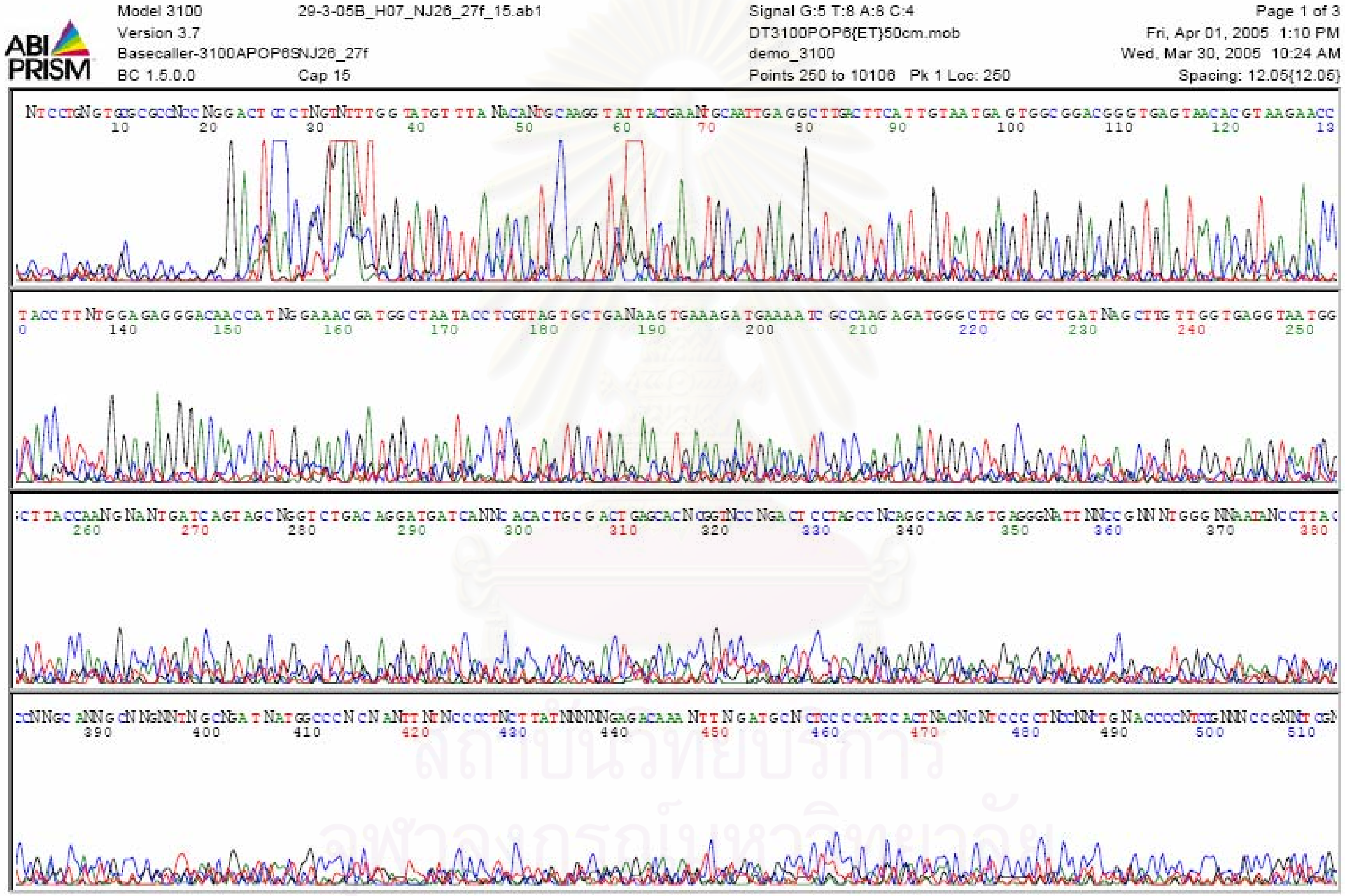
8-2-05A_A04_NJ26_1492r_02.ab1

Cap 2

Signal G:737 T:1030 A:1314 C:600
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

Page 1 of 2
Wed, Feb 09, 2005 8:18 AM
Tue, Feb 08, 2005 8:24 PM
Spacing: 12.19{12.19}





Model 3100
Version 3.7
Basecaller-3100APOP6SNJ26_27f
BC 1.5.0.0

29-3-05B_H07_NJ26_27f_15.ab1
Cap 15

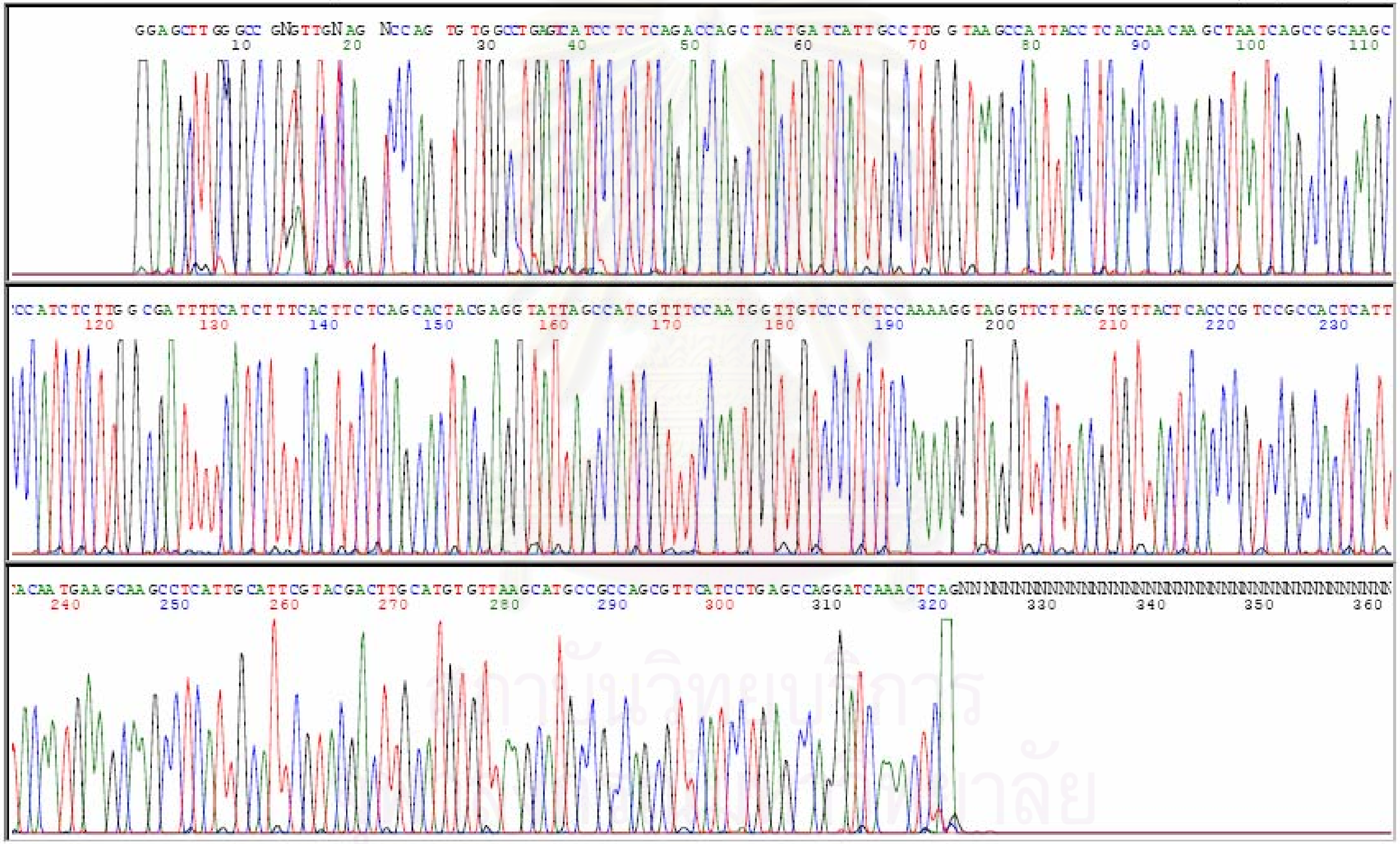
Signal G:5 T:8 A:8 C:4
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

Page 1 of 3
Fri, Apr 01, 2005 1:10 PM
Wed, Mar 30, 2005 10:24 AM
Spacing: 12.05{12.05}

Model 3100 31-3-48A_C03_NJ26_343r_05.ab1
Version 3.7
Basecaller-3100APOP6SNJ26_343r
BC 1.5.0.0 Cap 5

Signal G:73 T:136 A:152 C:75
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 200

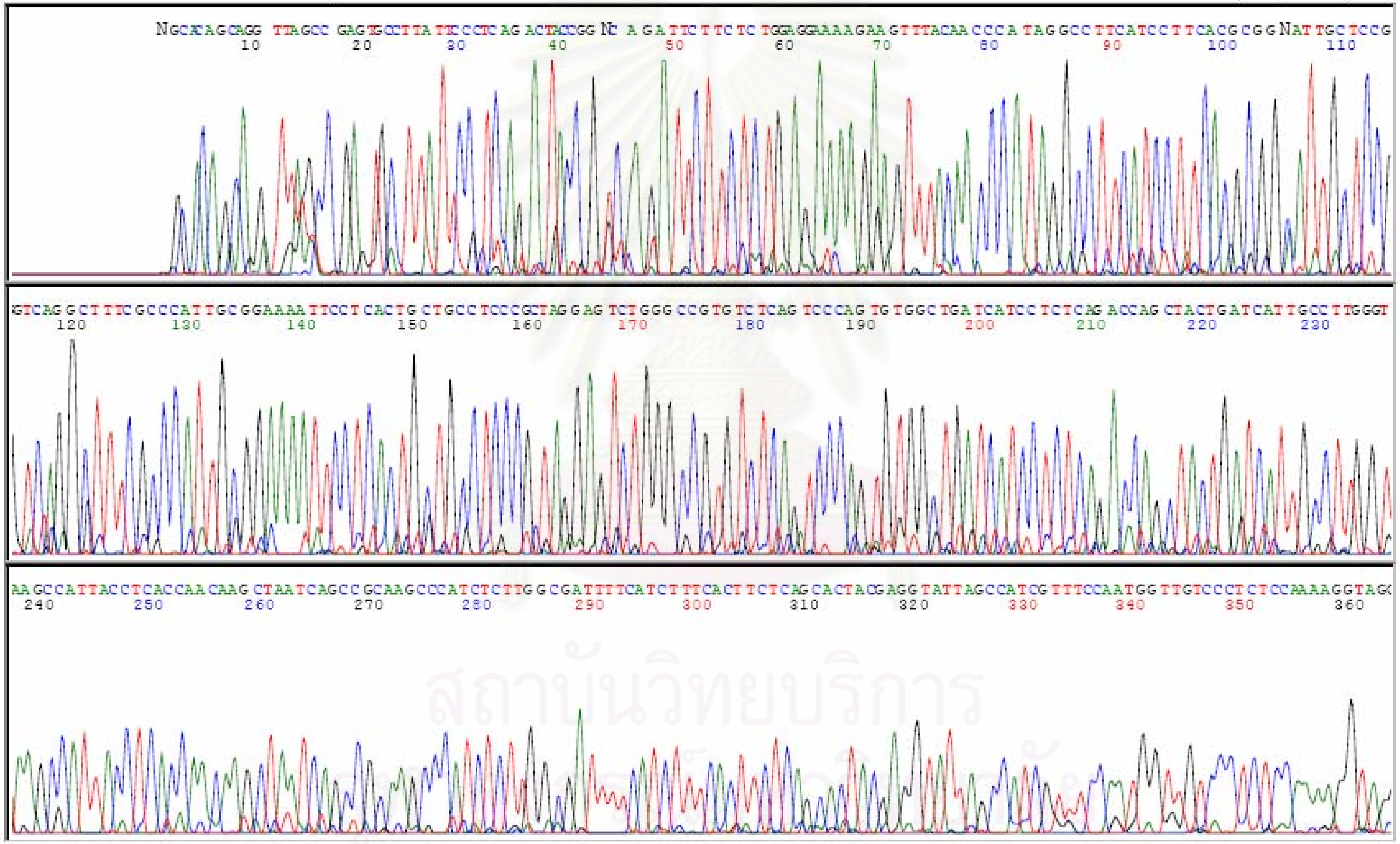
Page 1 of 3
Fri, Apr 01, 2005 1:45 PM
Thu, Mar 31, 2005 9:03 PM
Spacing: 11.77{11.77}

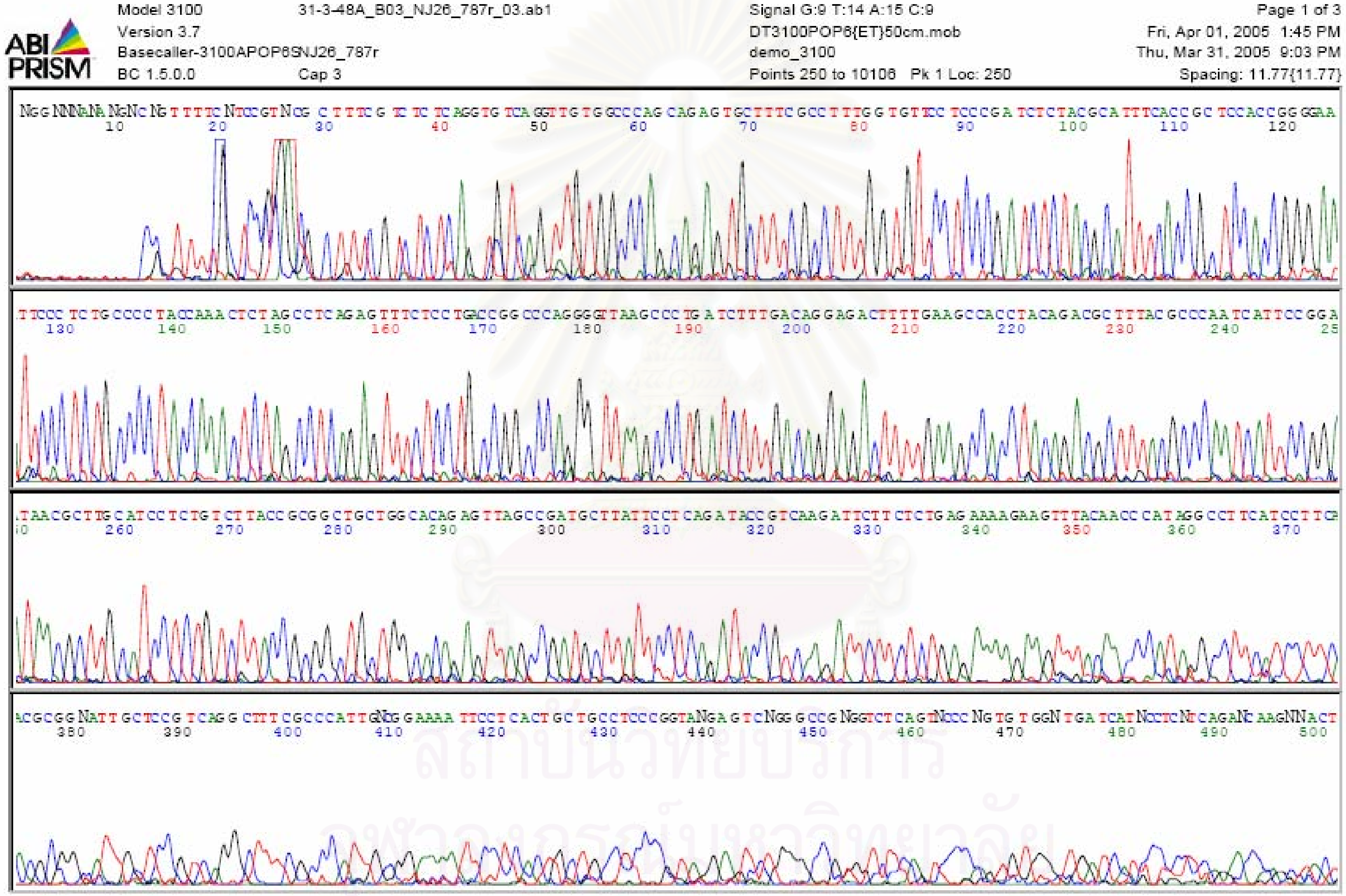


Model 3100 31-3-48A_A03_NJ26_519r_01.ab1
Version 3.7
Basecaller-3100APOP6SNJ26_519r
BC 1.5.0.0 Cap 1

Signal G:41 T:76 A:93 C:44
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

Page 1 of 3
Fri, Apr 01, 2005 1:22 PM
Thu, Mar 31, 2005 9:03 PM
Spacing: 11.91{11.91}





Model 3100
Version 3.7
Basecaller-3100APOP6SNJ26_787r
BC 1.5.0.0

31-3-48A_B03_NJ26_787r_03.ab1

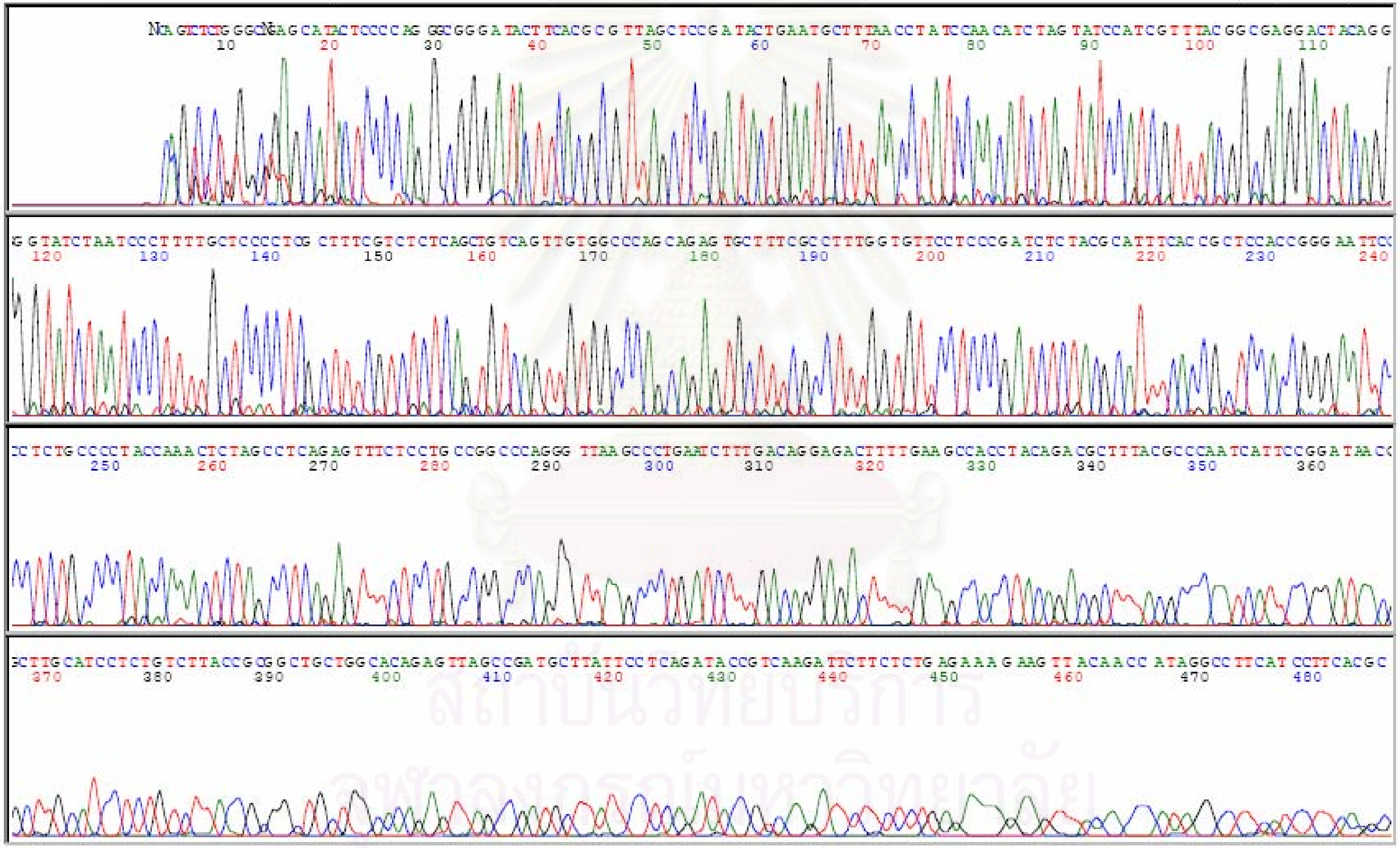
Cap 3

Signal G:9 T:14 A:15 C:9
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

Page 1 of 3
Fri, Apr 01, 2005 1:45 PM
Thu, Mar 31, 2005 9:03 PM
Spacing: 11.77{11.77}

ABI PRISM

Model 3100 31-3-48A_C04_NJ26_907r_08.ab1 Signal G:49 T:95 A:98 C:59 Page 1 of 2
Version 3.7 DT3100POP6{ET}50cm.mob Fri, Apr 01, 2005 1:45 PM
Basecaller-3100APOP6SNJ26_907r demo_3100 Thu, Mar 31, 2005 9:03 PM
BC 1.5.0.0 Cap 6 Points 250 to 10106 Pk 1 Loc: 250 Spacing: 11.91{11.91}



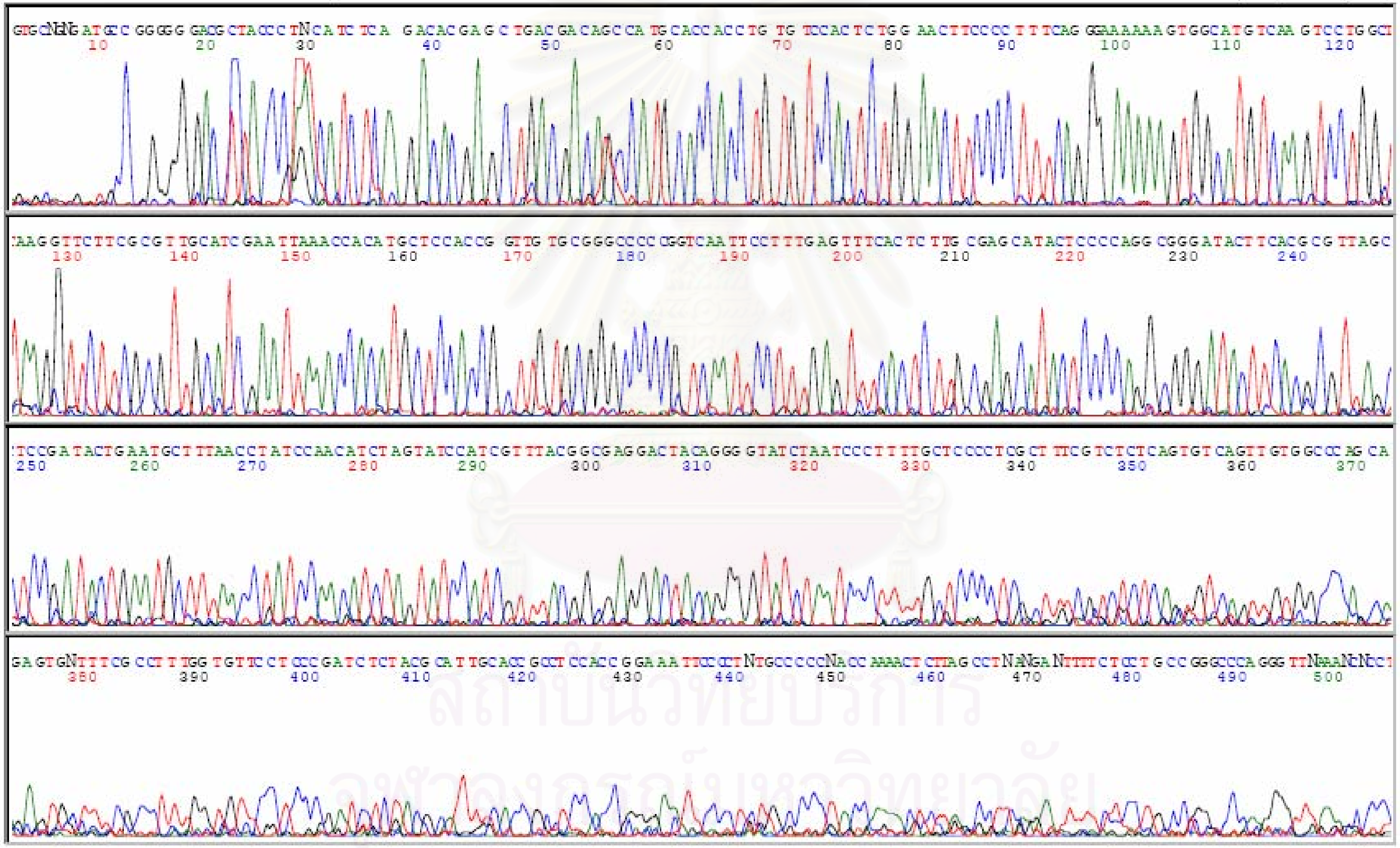
ABI PRISM

Model 3100
Version 3.7
Basecaller-3100APOP6SNJ26_1100r
BC 1.5.0.0

31-3-48A_A04_NJ26_1100r_02.ab1
Cap 2

Signal G:7 T:10 A:11 C:6
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

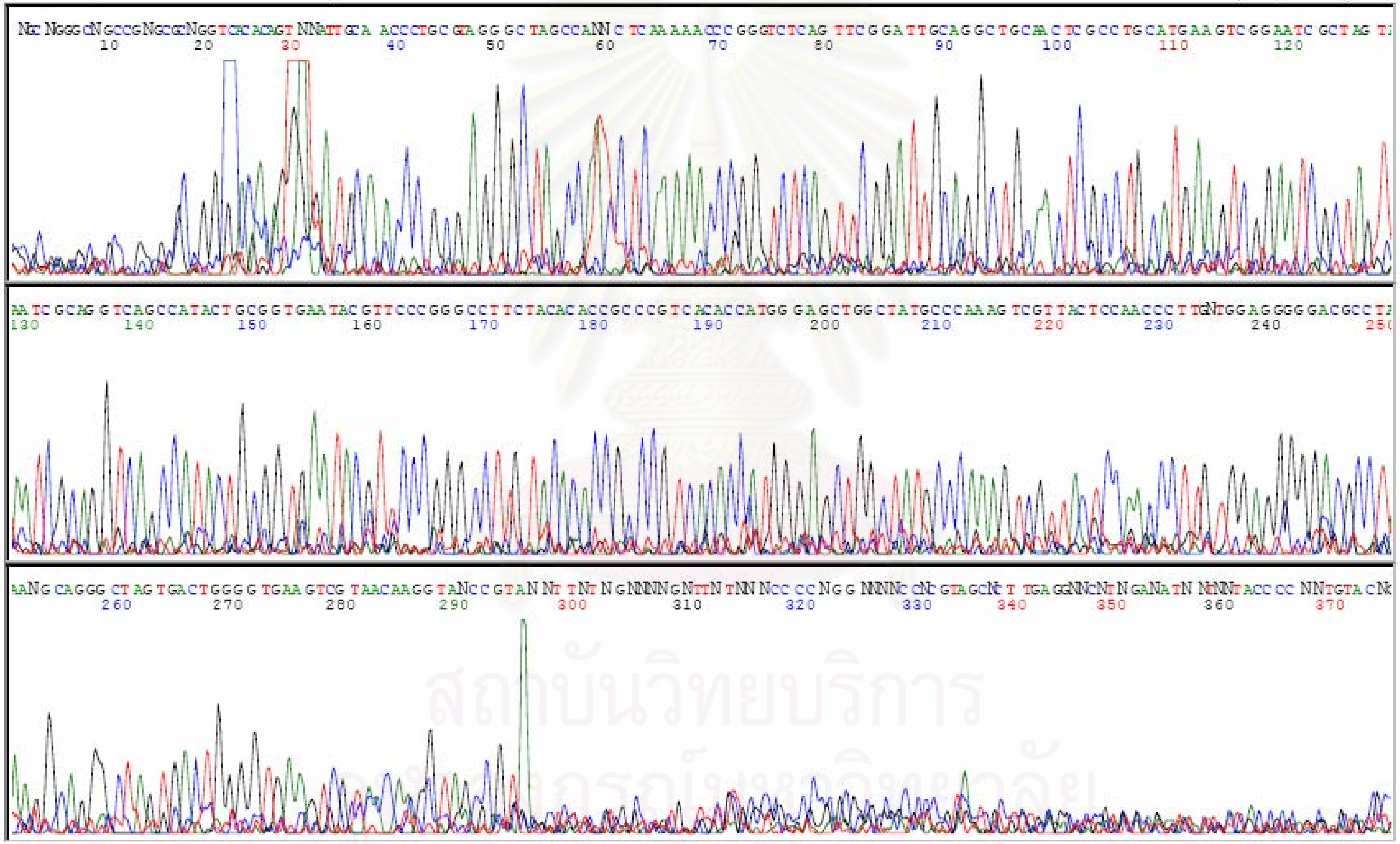
Page 1 of 3
Fri, Apr 01, 2005 1:22 PM
Thu, Mar 31, 2005 9:03 PM
Spacing: 12.05{12.05}

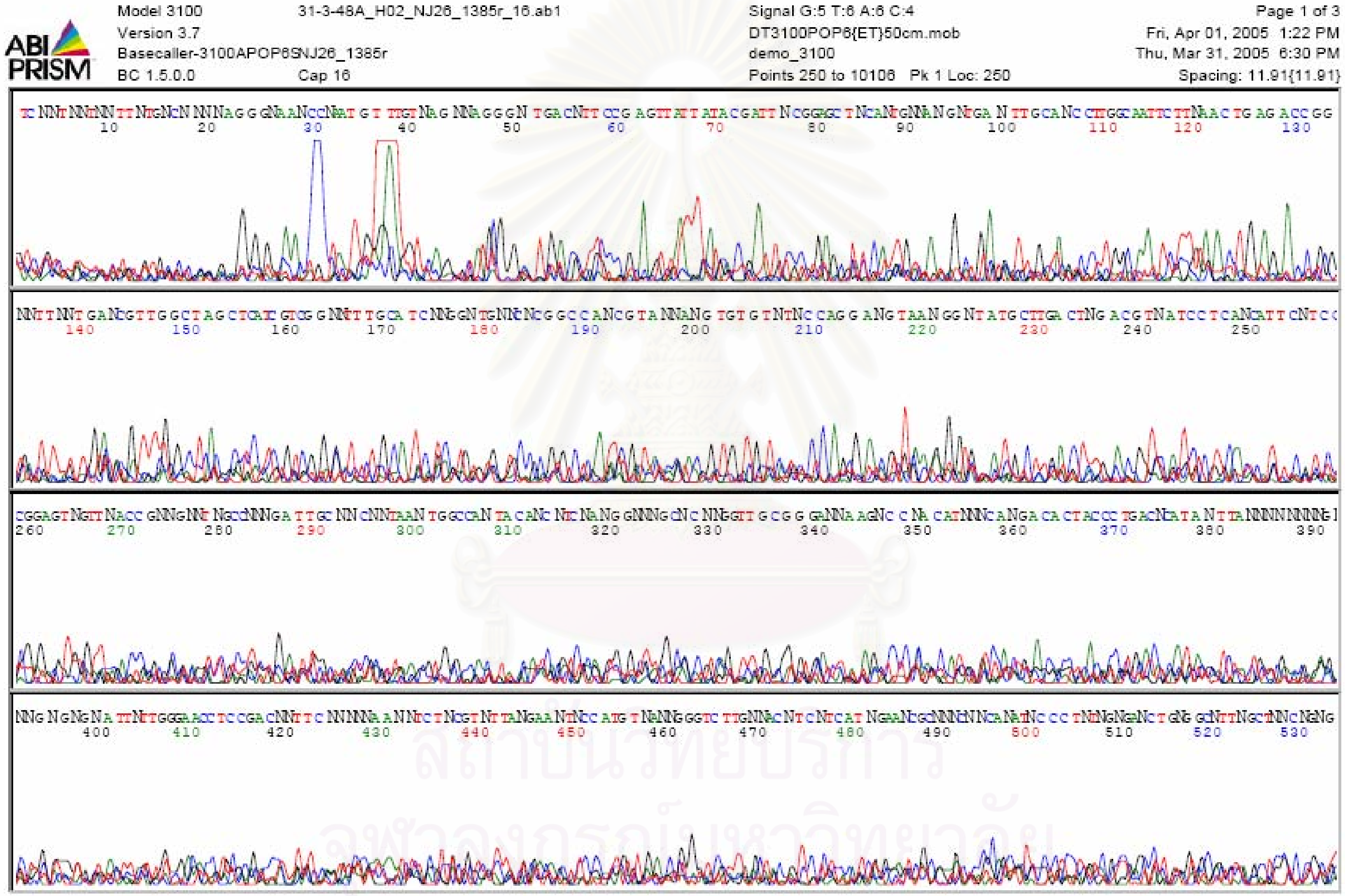


Model 3100 31-3-48A_B04_NJ26_1241f_04.ab1
Version 3.7
Basecaller-3100APOP6SNJ26_1241f
BC 1.5.0.0 Cap 4

Signal G:4 T:5 A:6 C:3
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

Page 1 of 3
Fri, Apr 01, 2005 1:45 PM
Thu, Mar 31, 2005 9:03 PM
Spacing: 11.77{11.77}





Model 3100 31-3-48A_H02_NJ26_1385r_16.ab1

Version 3.7

Basecaller-3100APOP6SNJ26_1385r

BC 1.5.0.0 Cap 16

Signal G:5 T:6 A:6 C:4

DT3100POP6{ET}50cm.mob

demo_3100

Points 250 to 10106 Pk 1 Loc: 250

Page 1 of 3

Fri, Apr 01, 2005 1:22 PM

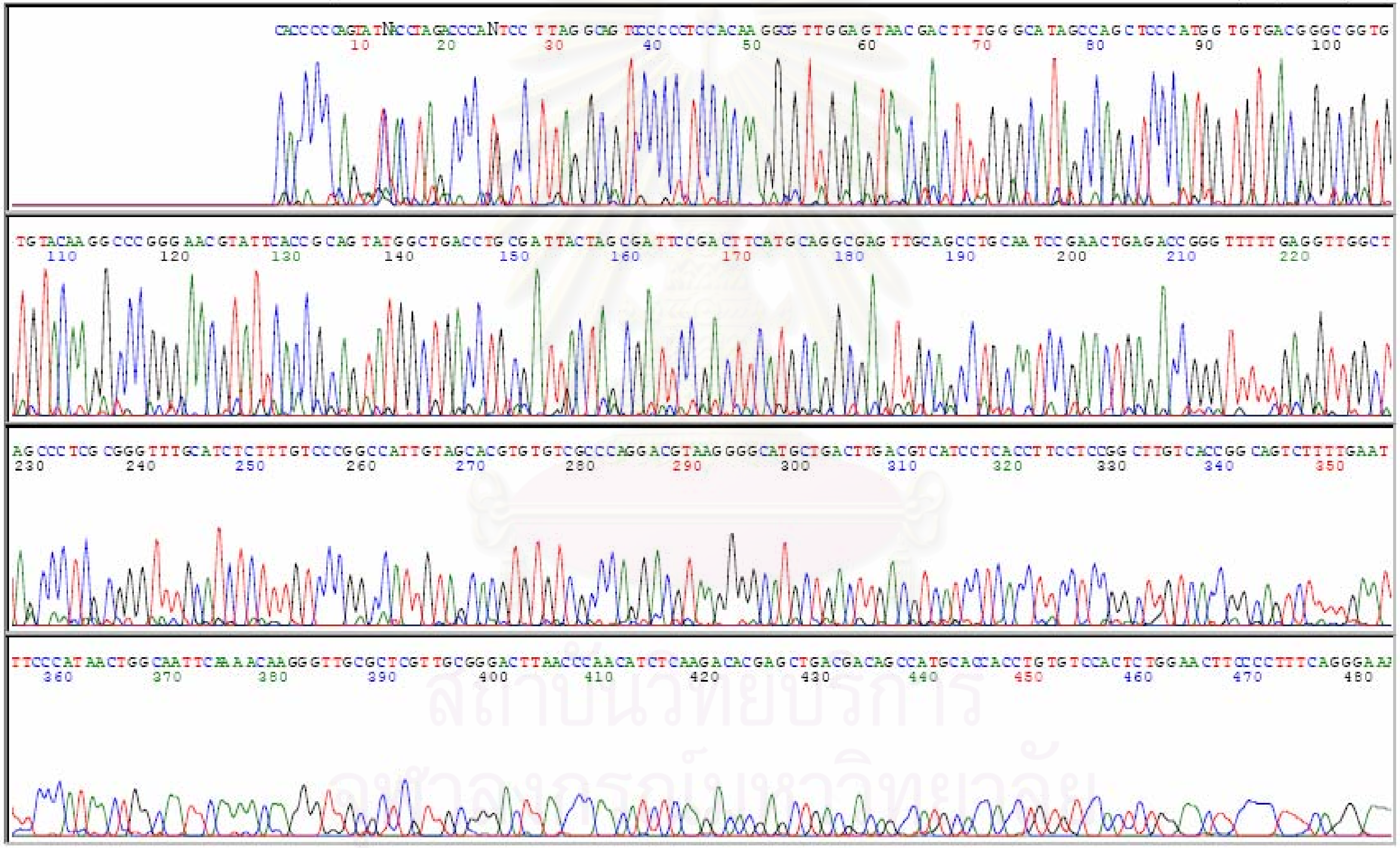
Thu, Mar 31, 2005 6:30 PM

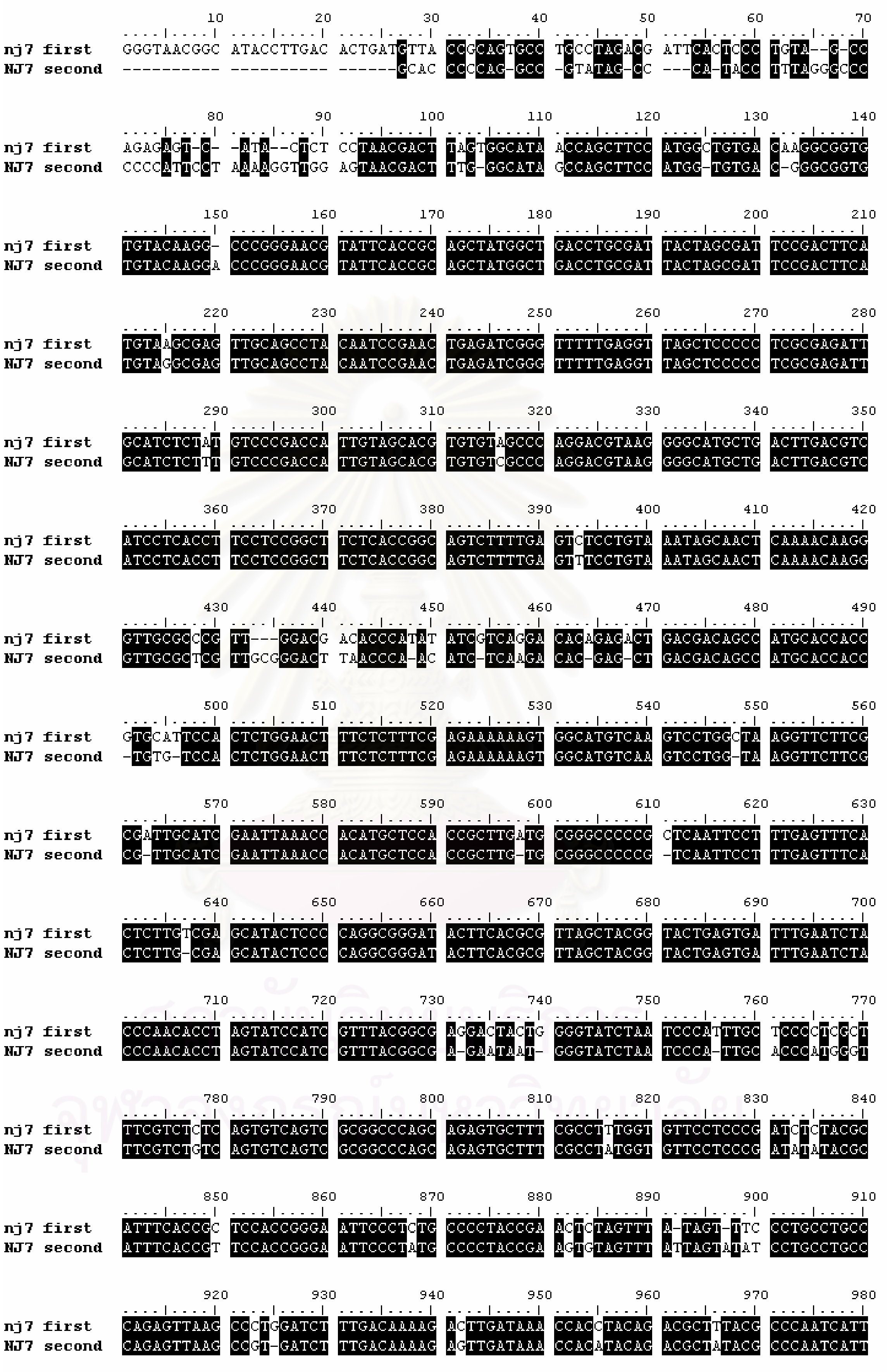
Spacing: 11.91{11.91}

Model 3100 31-3-48A_H01_NJ26_1492r_15.ab1
Version 3.7
Basecaller-3100APOP6SNJ26_1492r
BC 1.5.0.0 Cap 15

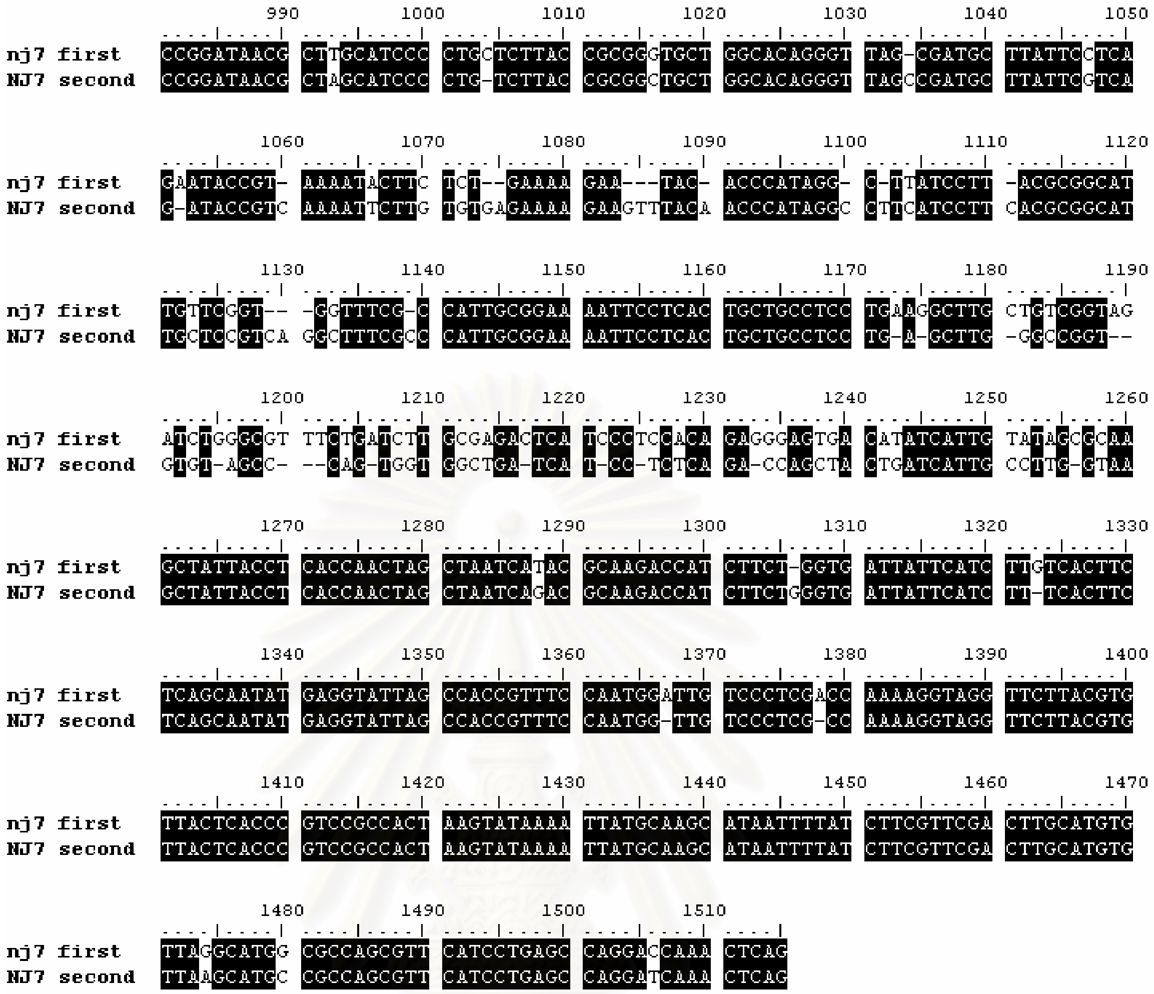
Signal G:92 T:125 A:151 C:77
DT3100POP6{ET}50cm.mob
demo_3100
Points 250 to 10106 Pk 1 Loc: 250

Page 1 of 2
Fri, Apr 01, 2005 1:22 PM
Thu, Mar 31, 2005 6:30 PM
Spacing: 11.91{11.91}

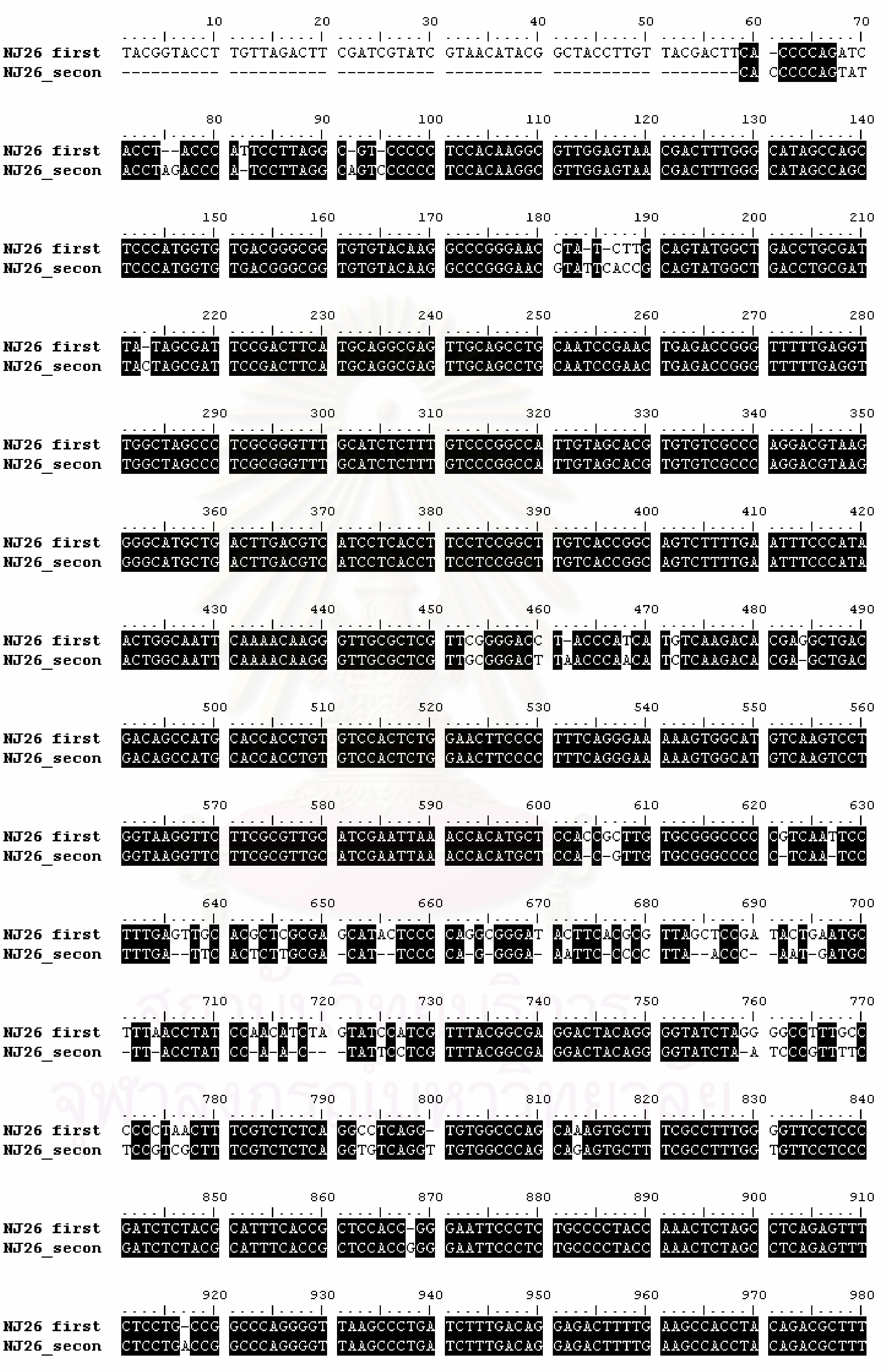




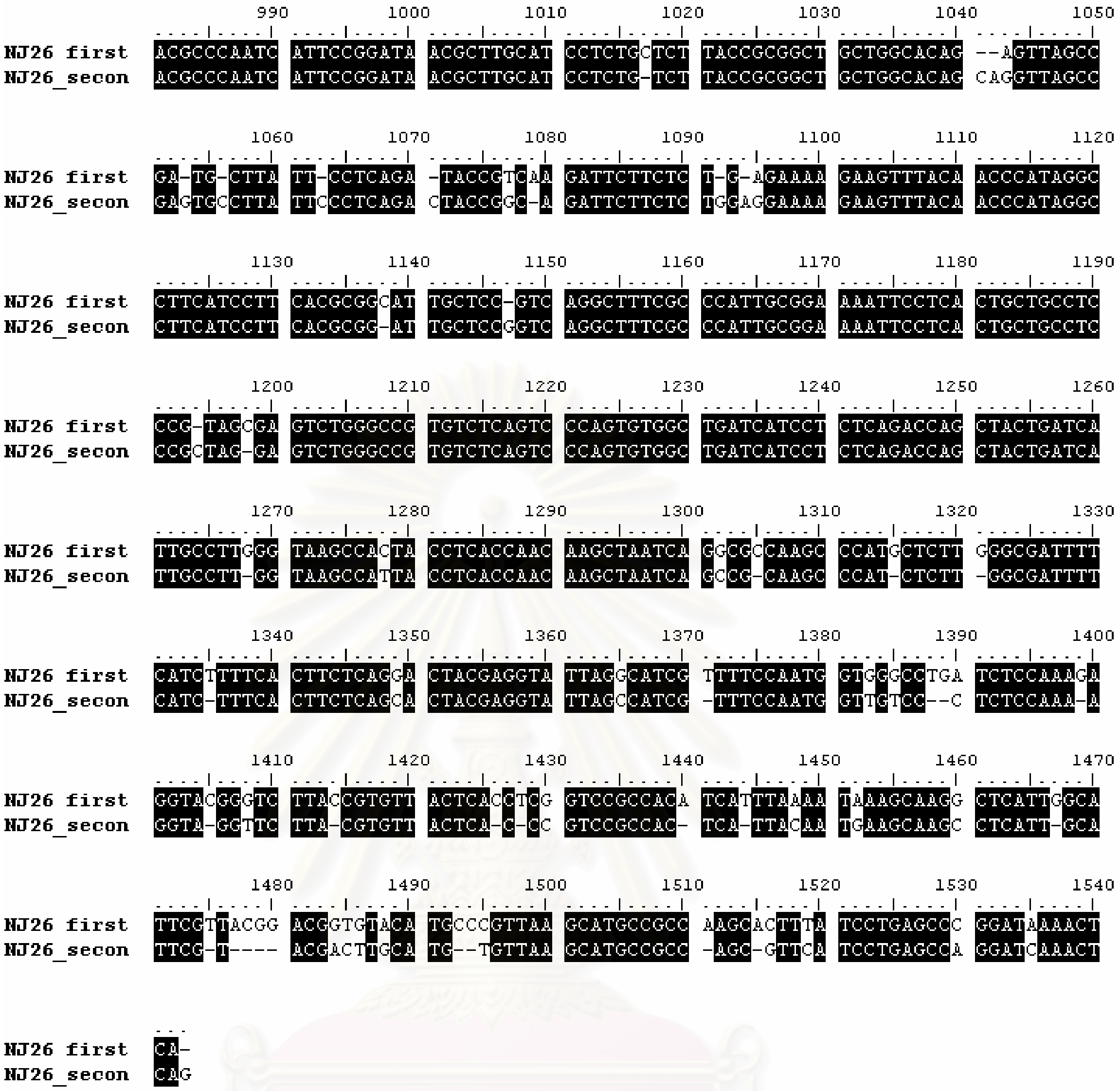
```
                      10         20         30         40         50         60         70
              ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 first     GGGTAACGGC ATACCTTGAC ACTGATGTTA CCGCAGTGCC TGCCTAGACG ATTCACTCCC TGTA--G-CC
NJ7 second    ---------- ---------- ------GCAC CCCCAG-GCC -GTATAG-CC ---CA-TACC TTTAGGGCCC

                      80         90        100        110        120        130        140
              ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 first     AGAGAGT-C- -ATA--CTCT CCTAACGACT TAGTGGCATA ACCAGCTTCC ATGGCTGTGA CAAGGCGGTG
NJ7 second    CCCCATTCCT AAAAGGTTGG AGTAACGACT TTG-GGCATA GCCAGCTTCC ATGG-TGTGA C-GGGCGGTG

                     150        160        170        180        190        200        210
              ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 first     TGTACAAGG- CCCGGGAACG TATTCACCGC AGCTATGGCT GACCTGCGAT TACTAGCGAT TCCGACTTCA
NJ7 second    TGTACAAGGA CCCGGGAACG TATTCACCGC AGCTATGGCT GACCTGCGAT TACTAGCGAT TCCGACTTCA

                     220        230        240        250        260        270        280
              ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 first     TGTAAGCGAG TTGCAGCCTA CAATCCGAAC TGAGATCGGG TTTTTGAGGT TAGCTCCCCC TCGCGAGATT
NJ7 second    TGTAGGCGAG TTGCAGCCTA CAATCCGAAC TGAGATCGGG TTTTTGAGGT TAGCTCCCCC TCGCGAGATT

                     290        300        310        320        330        340        350
              ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 first     GCATCTCTAT GTCCCGACCA TTGTAGCACG TGTGTAGCCC AGGACGTAAG GGGCATGCTG ACTTGACGTC
NJ7 second    GCATCTCTTT GTCCCGACCA TTGTAGCACG TGTGTCGCCC AGGACGTAAG GGGCATGCTG ACTTGACGTC

                     360        370        380        390        400        410        420
              ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 first     ATCCTCACCT TCCTCCGGCT TCTCACCGGC AGTCTTTTGA GTCTCCTGTA AATAGCAACT CAAAACAAGG
NJ7 second    ATCCTCACCT TCCTCCGGCT TCTCACCGGC AGTCTTTTGA GTTTCCTGTA AATAGCAACT CAAAACAAGG

                     430        440        450        460        470        480        490
              ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 first     GTTGCGCCCG TT---GGACG ACACCCATAT ATCGTCAGGA CACAGAGACT GACGACAGCC ATGCACCACC
NJ7 second    GTTGCGCTCG TTGCGGGACT TAACCCA-AC ATC-TCAAGA CAC-GAG-CT GACGACAGCC ATGCACCACC

                     500        510        520        530        540        550        560
              ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 first     GTGCATTCCA CTCTGGAACT TTCTCTTTCG AGAAAAAAGT GGCATGTCAA GTCCTGGCTA AGGTTCTTCG
NJ7 second    -TGTG-TCCA CTCTGGAACT TTCTCTTTCG AGAAAAAAGT GGCATGTCAA GTCCTGG-TA AGGTTCTTCG

                     570        580        590        600        610        620        630
              ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 first     CGATTGCATC GAATTAAACC ACATGCTCCA CCGCTTGATG CGGGCCCCCG CTCAATTCCT TTGAGTTTCA
NJ7 second    CG-TTGCATC GAATTAAACC ACATGCTCCA CCGCTTG-TG CGGGCCCCCG -TCAATTCCT TTGAGTTTCA

                     640        650        660        670        680        690        700
              ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 first     CTCTTGTCGA GCATACTCCC CAGGCGGGAT ACTTCACGCG TTAGCTACGG TACTGAGTGA TTTGAATCTA
NJ7 second    CTCTTG-CGA GCATACTCCC CAGGCGGGAT ACTTCACGCG TTAGCTACGG TACTGAGTGA TTTGAATCTA

                     710        720        730        740        750        760        770
              ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 first     CCCAACACCT AGTATCCATC GTTTACGGCG AGGACTACTG GGGTATCTAA TCCCATTTGC TCCCCTCGCT
NJ7 second    CCCAACACCT AGTATCCATC GTTTACGGCG A-GAATAAT- GGGTATCTAA TCCCA-TTGC ACCCATGGGT

                     780        790        800        810        820        830        840
              ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 first     TTCGTCTCTC AGTGTCAGTC GCGGCCCAGC AGAGTGCTTT CGCCTTTGGT GTTCCTCCCG ATCTCTACGC
NJ7 second    TTCGTCTGTC AGTGTCAGTC GCGGCCCAGC AGAGTGCTTT CGCCTATGGT GTTCCTCCCG ATATATACGC

                     850        860        870        880        890        900        910
              ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 first     ATTTCACCGC TCCACCGGGA ATTCCCTCTG CCCCTACCGA ACTCTAGTTT A-TAGT-TTC CCTGCCTGCC
NJ7 second    ATTTCACCGT TCCACCGGGA ATTCCCTATG CCCCTACCGA AGTGTAGTTT ATTAGTATAT CCTGCCTGCC

                     920        930        940        950        960        970        980
              ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
nj7 first     CAGAGTTAAG CCCTGGATCT TTGACAAAAG ACTTGATAAA CCACCTACAG ACGCTTTACG CCCAATCATT
NJ7 second    CAGAGTTAAG CCGT-GATCT TTGACAAAAG AGTTGATAAA CCACATACAG ACGCTATACG CCCAATCATT
```



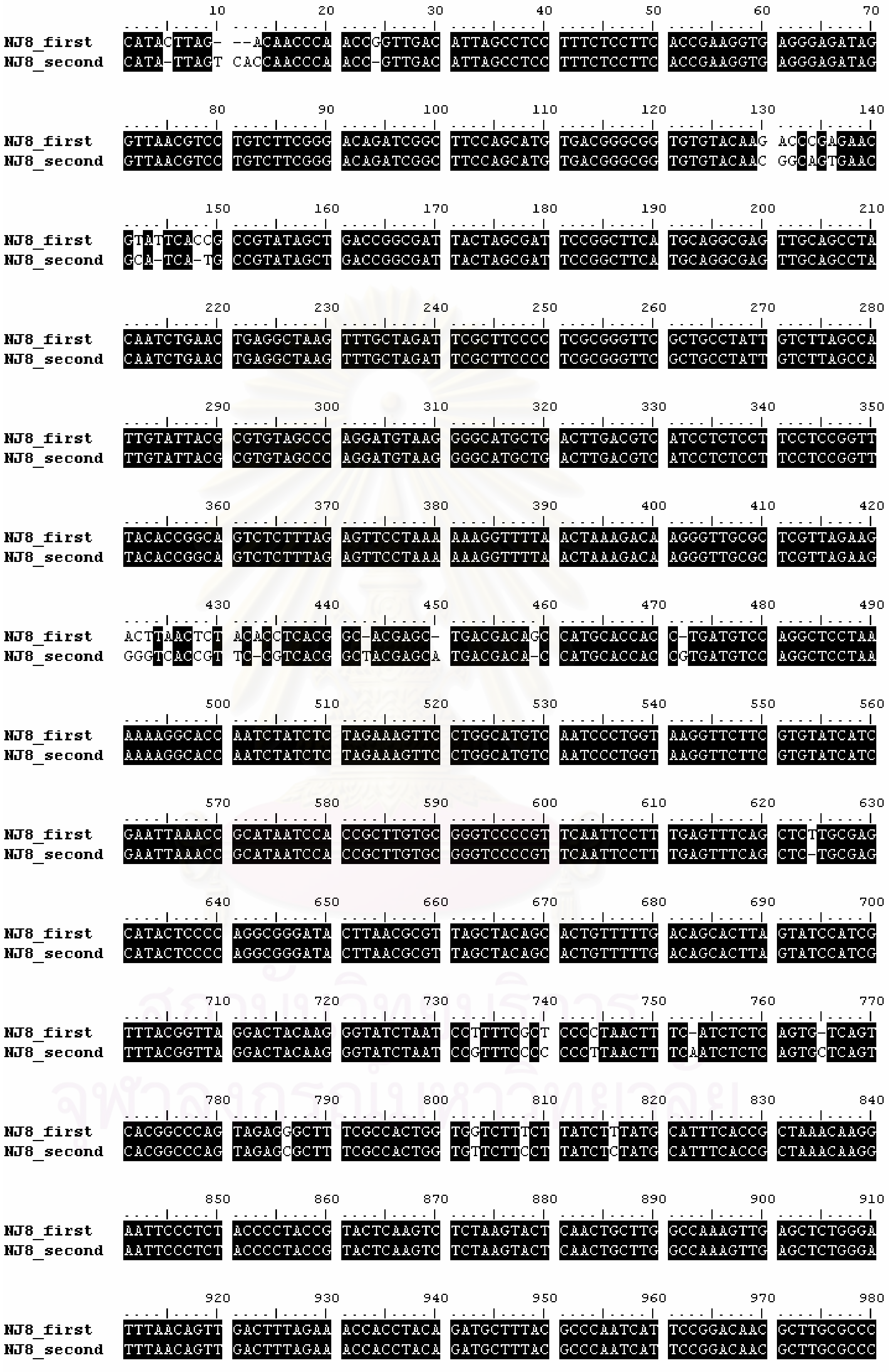
```
                   990       1000       1010       1020       1030       1040       1050
            ----|----| ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
nj7 first   CCGGATAACG CTTGCATCCC CTGCTCTTAC CGCGGGTGCT GGCACAGGGT TAG-CGATGC TTATTCCTCA
NJ7 second  CCGGATAACG CTAGCATCCC CTG-TCTTAC CGCGGCTGCT GGCACAGGGT TAGCCGATGC TTATTCGTCA

                  1060       1070       1080       1090       1100       1110       1120
            ----|----| ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
nj7 first   GAATACCGT- AAAATACTTC TCT--GAAAA GAA---TAC- ACCCATAGG- C-TTATCCTT -ACGCGGCAT
NJ7 second  G-ATACCGTC AAAATTCTTG TGTGAGAAAA GAAGTTTACA ACCCATAGGC CTTCATCCTT CACGCGGCAT

                  1130       1140       1150       1160       1170       1180       1190
            ----|----| ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
nj7 first   TGTTCGGT-- -GGTTTCG-C CATTGCGGAA AATTCCTCAC TGCTGCCTCC TGAAGGCTTG CTGTCGGTAG
NJ7 second  TGCTCCGTCA GGCTTTCGCC CATTGCGGAA AATTCCTCAC TGCTGCCTCC TG-A-GCTTG -GGCCGGT--

                  1200       1210       1220       1230       1240       1250       1260
            ----|----| ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
nj7 first   ATCTGGGCGT TTCTGATCTT GCGAGACTCA TCCCTCCACA GAGGGAGTGA CATATCATTG TATAGCGCAA
NJ7 second  GTGT-AGCC- --CAG-TGGT GGCTGA-TCA T-CC-TCTCA GA-CCAGCTA CTGATCATTG CCTTG-GTAA

                  1270       1280       1290       1300       1310       1320       1330
            ----|----| ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
nj7 first   GCTATTACCT CACCAACTAG CTAATCATAC GCAAGACCAT CTTCT-GGTG ATTATTCATC TTGTCACTTC
NJ7 second  GCTATTACCT CACCAACTAG CTAATCAGAC GCAAGACCAT CTTCTGGGTG ATTATTCATC TT-TCACTTC

                  1340       1350       1360       1370       1380       1390       1400
            ----|----| ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
nj7 first   TCAGCAATAT GAGGTATTAG CCACCGTTTC CAATGGATTG TCCCTCGACC AAAAGGTAGG TTCTTACGTG
NJ7 second  TCAGCAATAT GAGGTATTAG CCACCGTTTC CAATGG-TTG TCCCTCG-CC AAAAGGTAGG TTCTTACGTG

                  1410       1420       1430       1440       1450       1460       1470
            ----|----| ----|----| ----|----| ----|----| ----|----| ----|----| ----|----|
nj7 first   TTACTCACCC GTCCGCCACT AAGTATAAAA TTATGCAAGC ATAATTTTAT CTTCGTTCGA CTTGCATGTG
NJ7 second  TTACTCACCC GTCCGCCACT AAGTATAAAA TTATGCAAGC ATAATTTTAT CTTCGTTCGA CTTGCATGTG

                  1480       1490       1500       1510
            ----|----| ----|----| ----|----| ----|----| ----|
nj7 first   TTAGGCATGG CGCCAGCGTT CATCCTGAGC CAGGACCAAA CTCAG
NJ7 second  TTAAGCATGC CGCCAGCGTT CATCCTGAGC CAGGATCAAA CTCAG
```



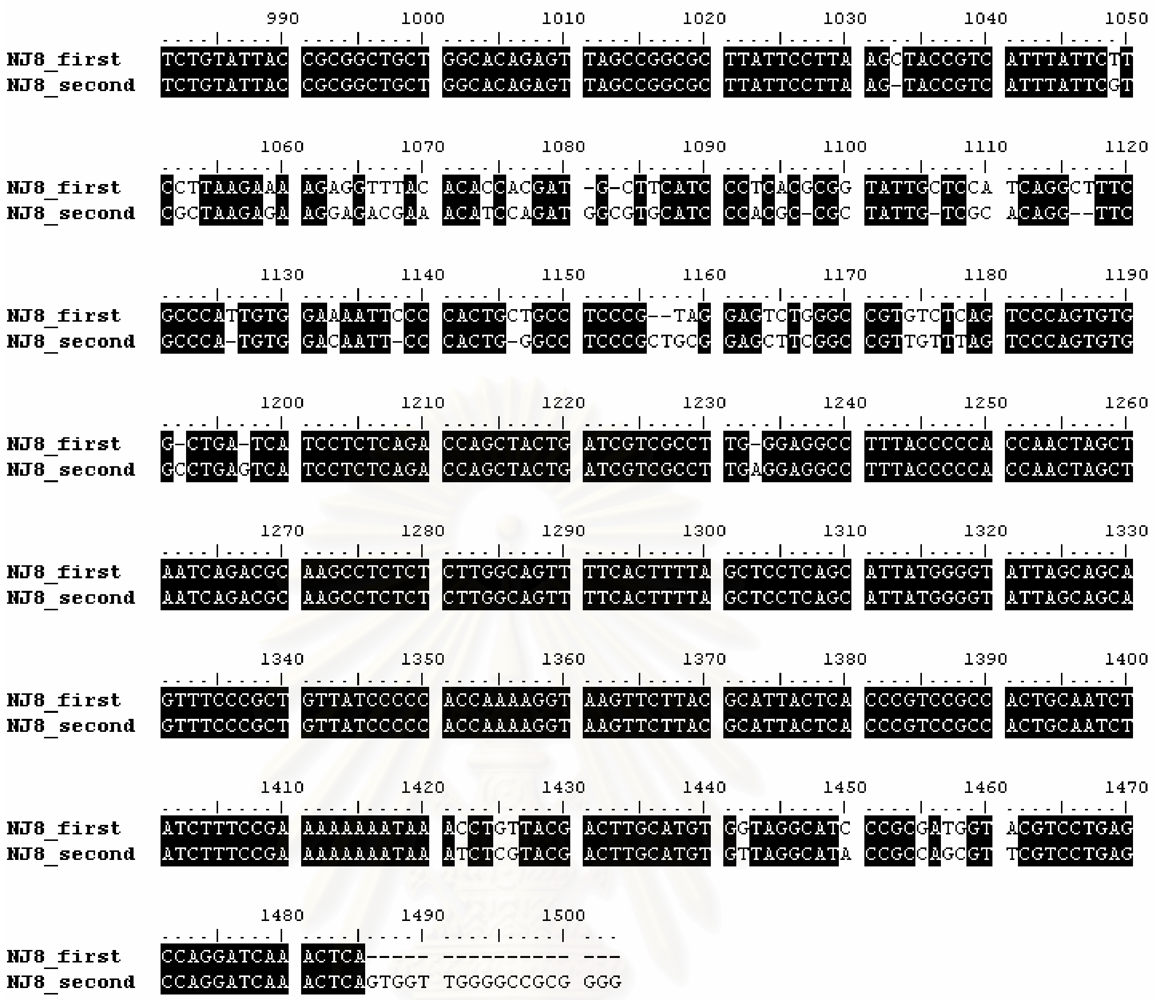
```
                    10         20         30         40         50         60         70
            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26 first  TACGGTACCT TGTTAGACTT CGATCGTATC GTAACATACG GCTACCTTGT TACGACTTCA -CCCCAGATC
NJ26_secon  ---------- ---------- ---------- ---------- ---------- --------CA CCCCCAGTAT

                    80         90        100        110        120        130        140
            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26 first  ACCT--ACCC ATTCCTTAGG C-GT-CCCCC TCCACAAGGC GTTGGAGTAA CGACTTTGGG CATAGCCAGC
NJ26_secon  ACCTAGACCC A-TCCTTAGG CAGTCCCCCC TCCACAAGGC GTTGGAGTAA CGACTTTGGG CATAGCCAGC

                   150        160        170        180        190        200        210
            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26 first  TCCCATGGTG TGACGGGCGG TGTGTACAAG GCCCGGGAAC CTA-T-CTTG CAGTATGGCT GACCTGCGAT
NJ26_secon  TCCCATGGTG TGACGGGCGG TGTGTACAAG GCCCGGGAAC GTATTCACCG CAGTATGGCT GACCTGCGAT

                   220        230        240        250        260        270        280
            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26 first  TA-TAGCGAT TCCGACTTCA TGCAGGCGAG TTGCAGCCTG CAATCCGAAC TGAGACCGGG TTTTTGAGGT
NJ26_secon  TACTAGCGAT TCCGACTTCA TGCAGGCGAG TTGCAGCCTG CAATCCGAAC TGAGACCGGG TTTTTGAGGT

                   290        300        310        320        330        340        350
            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26 first  TGGCTAGCCC TCGCGGGTTT GCATCTCTTT GTCCCGGCCA TTGTAGCACG TGTGTCGCCC AGGACGTAAG
NJ26_secon  TGGCTAGCCC TCGCGGGTTT GCATCTCTTT GTCCCGGCCA TTGTAGCACG TGTGTCGCCC AGGACGTAAG

                   360        370        380        390        400        410        420
            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26 first  GGGCATGCTG ACTTGACGTC ATCCTCACCT TCCTCCGGCT TGTCACCGGC AGTCTTTTGA ATTTCCCATA
NJ26_secon  GGGCATGCTG ACTTGACGTC ATCCTCACCT TCCTCCGGCT TGTCACCGGC AGTCTTTTGA ATTTCCCATA

                   430        440        450        460        470        480        490
            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26 first  ACTGGCAATT CAAAACAAGG GTTGCGCTCG TTCGGGGACC T-ACCCATCA TGTCAAGACA CGAGGCTGAC
NJ26_secon  ACTGGCAATT CAAAACAAGG GTTGCGCTCG TTGCGGGACT TAACCCAACA TCTCAAGACA CGA-GCTGAC

                   500        510        520        530        540        550        560
            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26 first  GACAGCCATG CACCACCTGT GTCCACTCTG GAACTTCCCC TTTCAGGGAA AAAGTGGCAT GTCAAGTCCT
NJ26_secon  GACAGCCATG CACCACCTGT GTCCACTCTG GAACTTCCCC TTTCAGGGAA AAAGTGGCAT GTCAAGTCCT

                   570        580        590        600        610        620        630
            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26 first  GGTAAGGTTC TTCGCGTTGC ATCGAATTAA ACCACATGCT CCACCGCTTG TGCGGGCCCC CGTCAATTCC
NJ26_secon  GGTAAGGTTC TTCGCGTTGC ATCGAATTAA ACCACATGCT CCA-C-GTTG TGCGGGCCCC C-TCAA-TCC

                   640        650        660        670        680        690        700
            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26 first  TTTGAGTTGC ACGCTCGCGA GCATACTCCC CAGGCGGGAT ACTTCACGCG TTAGCTCCGA TACTGAATGC
NJ26_secon  TTTGA--TTC ACTCTTGCGA -CAT--TCCC CA-G-GGGA- AATTC-CCCC TTA--ACCC- -AAT-GATGC

                   710        720        730        740        750        760        770
            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26 first  TTTAACCTAT CCAACATCTA GTATCCATCG TTTACGGCGA GGACTACAGG GGTATCTAGG GGCCTTTGCC
NJ26_secon  -TT-ACCTAT CC-A-A-C-- -TATTCCTCG TTTACGGCGA GGACTACAGG GGTATCTA-A TCCCGTTTTC

                   780        790        800        810        820        830        840
            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26 first  CCCCTAACTT TCGTCTCTCA GGCCTCAGG- TGTGGCCCAG CAAAGTGCTT TCGCCTTTGG GGTTCCTCCC
NJ26_secon  TCCGTCGCTT TCGTCTCTCA GGTGTCAGGT TGTGGCCCAG CAGAGTGCTT TCGCCTTTGG TGTTCCTCCC

                   850        860        870        880        890        900        910
            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26 first  GATCTCTACG CATTTCACCG CTCCACC-GG GAATTCCCTC TGCCCCTACC AAACTCTAGC CTCAGAGTTT
NJ26_secon  GATCTCTACG CATTTCACCG CTCCACCGGG GAATTCCCTC TGCCCCTACC AAACTCTAGC CTCAGAGTTT

                   920        930        940        950        960        970        980
            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26 first  CTCCTG-CCG GCCCAGGGGT TAAGCCCTGA TCTTTGACAG GAGACTTTTG AAGCCACCTA CAGACGCTTT
NJ26_secon  CTCCTGACCG GCCCAGGGGT TAAGCCCTGA TCTTTGACAG GAGACTTTTG AAGCCACCTA CAGACGCTTT
```



```
                       990       1000       1010       1020       1030       1040       1050
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26 first      ACGCCCAATC ATTCCGGATA ACGCTTGCAT CCTCTGCTCT TACCGCGGCT GCTGGCACAG --AGTTAGCC
NJ26_secon      ACGCCCAATC ATTCCGGATA ACGCTTGCAT CCTCTG-TCT TACCGCGGCT GCTGGCACAG CAGGTTAGCC

                      1060       1070       1080       1090       1100       1110       1120
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26 first      GA-TG-CTTA TT-CCTCAGA -TACCGTCAA GATTCTTCTC T-G-AGAAAA GAAGTTTACA ACCCATAGGC
NJ26_secon      GAGTGCCTTA TTCCCTCAGA CTACCGGC-A GATTCTTCTC TGGAGGAAAA GAAGTTTACA ACCCATAGGC

                      1130       1140       1150       1160       1170       1180       1190
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26 first      CTTCATCCTT CACGCGGCAT TGCTCC-GTC AGGCTTTCGC CCATTGCGGA AAATTCCTCA CTGCTGCCTC
NJ26_secon      CTTCATCCTT CACGCGG-AT TGCTCCGGTC AGGCTTTCGC CCATTGCGGA AAATTCCTCA CTGCTGCCTC

                      1200       1210       1220       1230       1240       1250       1260
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26 first      CCG-TAGCGA GTCTGGGCCG TGTCTCAGTC CCAGTGTGGC TGATCATCCT CTCAGACCAG CTACTGATCA
NJ26_secon      CCGCTAG-GA GTCTGGGCCG TGTCTCAGTC CCAGTGTGGC TGATCATCCT CTCAGACCAG CTACTGATCA

                      1270       1280       1290       1300       1310       1320       1330
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26 first      TTGCCTTGGG TAAGCCACTA CCTCACCAAC AAGCTAATCA GGCGCCAAGC CCATGCTCTT GGGCGATTTT
NJ26_secon      TTGCCTT-GG TAAGCCATTA CCTCACCAAC AAGCTAATCA GCCG-CAAGC CCAT-CTCTT -GGCGATTTT

                      1340       1350       1360       1370       1380       1390       1400
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26 first      CATCTTTTCA CTTCTCAGGA CTACGAGGTA TTAGGCATCG TTTTCCAATG GTGGGCCTGA TCTCCAAAGA
NJ26_secon      CATC-TTTCA CTTCTCAGCA CTACGAGGTA TTAGCCATCG -TTTCCAATG GTTGTCC--C TCTCCAAA-A

                      1410       1420       1430       1440       1450       1460       1470
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26 first      GGTACGGGTC TTACCGTGTT ACTCACCTCG GTCCGCCACA TCATTTAAAA TAAAGCAAGG CTCATTGGCA
NJ26_secon      GGTA-GGTTC TTA-CGTGTT ACTCA-C-CC GTCCGCCAC- TCA-TTACAA TGAAGCAAGC CTCATT-GCA

                      1480       1490       1500       1510       1520       1530       1540
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ26 first      TTCGTTACGG ACGGTGTACA TGCCCGTTAA GCATGCCGCC AAGCACTTTA TCCTGAGCCC GGATAAAACT
NJ26_secon      TTCG-T---- ACGACTTGCA TG--TGTTAA GCATGCCGCC -AGC-GTTCA TCCTGAGCCA GGATCAAACT

                ...
NJ26 first      CA-
NJ26_secon      CAG
```



```
                    10         20         30         40         50         60         70
            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_first   CATACTTAG- --ACAACCCA ACCGGTTGAC ATTAGCCTCC TTTCTCCTTC ACCGAAGGTG AGGGAGATAG
NJ8_second  CATA-TTAGT CACCAACCCA ACC-GTTGAC ATTAGCCTCC TTTCTCCTTC ACCGAAGGTG AGGGAGATAG

                    80         90        100        110        120        130        140
            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_first   GTTAACGTCC TGTCTTCGGG ACAGATCGGC TTCCAGCATG TGACGGGCGG TGTGTACAAG ACCCGAGAAC
NJ8_second  GTTAACGTCC TGTCTTCGGG ACAGATCGGC TTCCAGCATG TGACGGGCGG TGTGTACAAC GGCAGTGAAC

                   150        160        170        180        190        200        210
            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_first   GTATTCACCG CCGTATAGCT GACCGGCGAT TACTAGCGAT TCCGGCTTCA TGCAGGCGAG TTGCAGCCTA
NJ8_second  GCA-TCA-TG CCGTATAGCT GACCGGCGAT TACTAGCGAT TCCGGCTTCA TGCAGGCGAG TTGCAGCCTA

                   220        230        240        250        260        270        280
            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_first   CAATCTGAAC TGAGGCTAAG TTTGCTAGAT TCGCTTCCCC TCGCGGGTTC GCTGCCTATT GTCTTAGCCA
NJ8_second  CAATCTGAAC TGAGGCTAAG TTTGCTAGAT TCGCTTCCCC TCGCGGGTTC GCTGCCTATT GTCTTAGCCA

                   290        300        310        320        330        340        350
            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_first   TTGTATTACG CGTGTAGCCC AGGATGTAAG GGGCATGCTG ACTTGACGTC ATCCTCTCCT TCCTCCGGTT
NJ8_second  TTGTATTACG CGTGTAGCCC AGGATGTAAG GGGCATGCTG ACTTGACGTC ATCCTCTCCT TCCTCCGGTT

                   360        370        380        390        400        410        420
            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_first   TACACCGGCA GTCTCTTTAG AGTTCCTAAA AAAGGTTTTA ACTAAAGACA AGGGTTGCGC TCGTTAGAAG
NJ8_second  TACACCGGCA GTCTCTTTAG AGTTCCTAAA AAAGGTTTTA ACTAAAGACA AGGGTTGCGC TCGTTAGAAG

                   430        440        450        460        470        480        490
            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_first   ACTTAACTCT ACACCTCACG GC-ACGAGC- TGACGACAGC CATGCACCAC C-TGATGTCC AGGCTCCTAA
NJ8_second  GGGTCACCGT TC-CGTCACG GCTACGAGCA TGACGACA-C CATGCACCAC CGTGATGTCC AGGCTCCTAA

                   500        510        520        530        540        550        560
            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_first   AAAAGGCACC AATCTATCTC TAGAAAGTTC CTGGCATGTC AATCCCTGGT AAGGTTCTTC GTGTATCATC
NJ8_second  AAAAGGCACC AATCTATCTC TAGAAAGTTC CTGGCATGTC AATCCCTGGT AAGGTTCTTC GTGTATCATC

                   570        580        590        600        610        620        630
            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_first   GAATTAAACC GCATAATCCA CCGCTTGTGC GGGTCCCCGT TCAATTCCTT TGAGTTTCAG CTCTTGCGAG
NJ8_second  GAATTAAACC GCATAATCCA CCGCTTGTGC GGGTCCCCGT TCAATTCCTT TGAGTTTCAG CTC-TGCGAG

                   640        650        660        670        680        690        700
            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_first   CATACTCCCC AGGCGGGATA CTTAACGCGT TAGCTACAGC ACTGTTTTTG ACAGCACTTA GTATCCATCG
NJ8_second  CATACTCCCC AGGCGGGATA CTTAACGCGT TAGCTACAGC ACTGTTTTTG ACAGCACTTA GTATCCATCG

                   710        720        730        740        750        760        770
            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_first   TTTACGGTTA GGACTACAAG GGTATCTAAT CCTTTTCGCT CCCCTAACTT TC-ATCTCTC AGTG-TCAGT
NJ8_second  TTTACGGTTA GGACTACAAG GGTATCTAAT CCGTTTCCCC CCCTTAACTT TCAATCTCTC AGTGCTCAGT

                   780        790        800        810        820        830        840
            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_first   CACGGCCCAG TAGAGGGCTT TCGCCACTGG TGGTCTTTCT TATCTTTATG CATTTCACCG CTAAACAAGG
NJ8_second  CACGGCCCAG TAGAGCGCTT TCGCCACTGG TGTTCTTCCT TATCTCTATG CATTTCACCG CTAAACAAGG

                   850        860        870        880        890        900        910
            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_first   AATTCCCTCT ACCCCTACCG TACTCAAGTC TCTAAGTACT CAACTGCTTG GCCAAAGTTG AGCTCTGGGA
NJ8_second  AATTCCCTCT ACCCCTACCG TACTCAAGTC TCTAAGTACT CAACTGCTTG GCCAAAGTTG AGCTCTGGGA

                   920        930        940        950        960        970        980
            ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_first   TTTAACAGTT GACTTTAGAA ACCACCTACA GATGCTTTAC GCCCAATCAT TCCGGACAAC GCTTGCGCCC
NJ8_second  TTTAACAGTT GACTTTAGAA ACCACCTACA GATGCTTTAC GCCCAATCAT TCCGGACAAC GCTTGCGCCC
```



```
                       990       1000       1010       1020       1030       1040       1050
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_first       TCTGTATTAC CGCGGCTGCT GGCACAGAGT TAGCCGGCGC TTATTCCTTA AGCTACCGTC ATTTATTCTT
NJ8_second      TCTGTATTAC CGCGGCTGCT GGCACAGAGT TAGCCGGCGC TTATTCCTTA AG-TACCGTC ATTTATTCGT

                      1060       1070       1080       1090       1100       1110       1120
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_first       CCTTAAGAAA AGAGGTTTAC ACACCACGAT -G-CTTCATC CCTCACGCGG TATTGCTCCA TCAGGCTTTC
NJ8_second      CGCTAAGAGA AGGAGACGAA ACATCCAGAT GGCGTGCATC CCACGC-CGC TATTG-TCGC ACAGG--TTC

                      1130       1140       1150       1160       1170       1180       1190
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_first       GCCCATTGTG GAAAATTCCC CACTGCTGCC TCCCG--TAG GAGTCTGGGC CGTGTCTCAG TCCCAGTGTG
NJ8_second      GCCCA-TGTG GACAATT-CC CACTG-GGCC TCCCGCTGCG GAGCTTCGGC CGTTGTTTAG TCCCAGTGTG

                      1200       1210       1220       1230       1240       1250       1260
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_first       G-CTGA-TCA TCCTCTCAGA CCAGCTACTG ATCGTCGCCT TG-GGAGGCC TTTACCCCCA CCAACTAGCT
NJ8_second      GCCTGAGTCA TCCTCTCAGA CCAGCTACTG ATCGTCGCCT TGAGGAGGCC TTTACCCCCA CCAACTAGCT

                      1270       1280       1290       1300       1310       1320       1330
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_first       AATCAGACGC AAGCCTCTCT CTTGGCAGTT TTCACTTTTA GCTCCTCAGC ATTATGGGGT ATTAGCAGCA
NJ8_second      AATCAGACGC AAGCCTCTCT CTTGGCAGTT TTCACTTTTA GCTCCTCAGC ATTATGGGGT ATTAGCAGCA

                      1340       1350       1360       1370       1380       1390       1400
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_first       GTTTCCCGCT GTTATCCCCC ACCAAAAGGT AAGTTCTTAC GCATTACTCA CCCGTCCGCC ACTGCAATCT
NJ8_second      GTTTCCCGCT GTTATCCCCC ACCAAAAGGT AAGTTCTTAC GCATTACTCA CCCGTCCGCC ACTGCAATCT

                      1410       1420       1430       1440       1450       1460       1470
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
NJ8_first       ATCTTTCCGA AAAAAAATAA ACCTGTTACG ACTTGCATGT GGTAGGCATC CCGCGATGGT ACGTCCTGAG
NJ8_second      ATCTTTCCGA AAAAAAATAA ATCTCGTACG ACTTGCATGT GTTAGGCATA CCGCCAGCGT TCGTCCTGAG

                      1480       1490       1500
                ....|....| ....|....| ....|....| ...
NJ8_first       CCAGGATCAA ACTCA----- ---------- ---
NJ8_second      CCAGGATCAA ACTCAGTGGT TGGGGCCGCG GGG
```

Biography

Miss Nonticha Jamkangwan was born on November 17, 1980. She obtained a Bachelor of Science Degree in Microbiology from Chulalongkorn University, Bangkok, Thailand, in 2002.

Publications

1) Nonthicha Jamkangwan, Wanvisa Boonsri, Charasporn Suralai, Sirikarn Charoenbhakdi, Patima Permpoonpattana and Kanjana Chansa-ngavej. 2002. RAPD-PCR fingerprints of cyanobacteria and micro-algae. Proceedings of the 14th Annual Meeting of the Thai Society for Biotechnology. 3 pages. CD-ROM format.

Presentation at Scientific Conferences

1) นนทิชา แจ่มกังวาล และ กาญจนา ชาญสง่าเวช. 2546.ความหลากหลายด้านสารพันธุกรรมตามที่ปรากฏในลายพิมพ์ดีเอ็นเอของ *Chlorella* spp., *Scenedesmus* spp. และ *Synechococcus* spp. หนังสือรวมบทคัดย่อการประชุมวิชาการ คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย. ครั้งที่ 11 : หน้า 8.
2) นนทิชา แจ่มกังวาล และ กาญจนา ชาญสง่าเวช. 2546. การใช้ RAPD-PCR หาลายพิมพ์ดีเอ็นเอของไซยาโนแบคทีเรีย *Synechococcus* spp. และสาหร่ายสีเขียว *Chlorella* spp. และ *Scenedesmus* spp. หนังสือรวมบทคัดย่อการประชุมวิชาการสาหร่ายและแพลงก์ตอนแห่งชาติ ครั้งที่ 1: หน้า 29.
3) นนทิชา แจ่มกังวาล และ กาญจนา ชาญสง่าเวช. 2548. ลายพิมพ์ดีเอ็นเอ ลำดับนิวคลีโอไทด์ 16S rDNA และโพรไฟล์ของโปรตีนภายในเซลล์ของ *Synechococcus* sp. NJ7, *Chlorella* sp. NJ26, และ *Scenedesmus* sp. NJ8. หนังสือรวมบทคัดย่อการประชุมวิชาการ คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย. ครั้งที่ 13 : หน้า 12.
4) Kannasut, P., Klinkumoun, S., Jamkangwan, N., Niyomrit, S., and Chansa-ngavej, K. 2005. On-line Identification of Cyanobacteria and Micro-algae. Abstract Book, The 2nd National Conference on Algae and Plankton. March 23-25, 2005. Chiang Mai, Thailand. P. C-09.