IV. RESULTS

1. Amplification of *C. trachomatis* DNA by PCR

Among 50 *C. trachomatis* positive samples randomly chosen in this study,40 samples were indicated positive by the Gen Probe DNA hybridization test and 10 were culture positive. Those samples were subsequently confirmed for the presence of *C. trachomatis* by PCR amplification using the Nest2 and Nest4 primers. All samples could be readily amplified, and produced a clear single band of approximate 350 bp on 1 % agarose gel eletrophoresis (Fig.4).

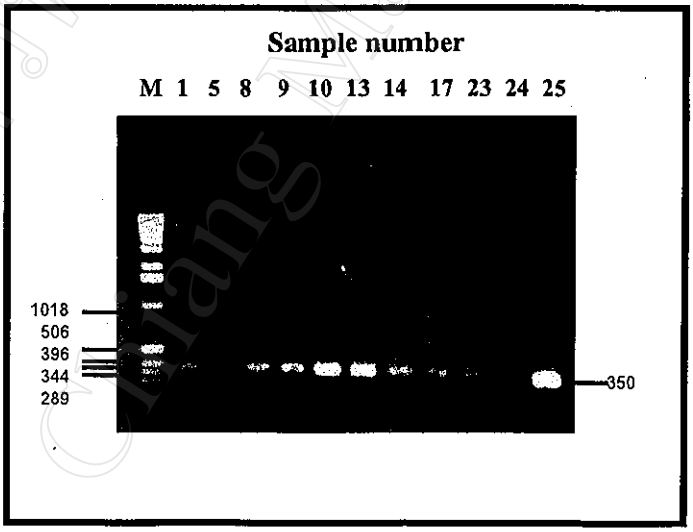


Figure 4. The amplification of *C. trachomatis* DNA from clinical samples by PCR. Lane M = DNA marker.

2. Genotyping of *C. trachomatis* by RFLP analysis

Genotyping of *C. trachomatis* was carried out by means of the RFLP analysis of the VD4-MOMP gene. The VD4 region, flanked by Nest2 and Nest4 primers, was amplified and then subjected to the restriction digestion with *Alu* I, *Hind* III, *Dde* I and *EcoR* II. The digested fragments were then visualized in 2 % agarose gel electrophoresis. The profile of the RFLP was analysed by a comparison of the fragment sizes of the RFLP of 18 serotypes reference strain *C. trachomatis*, generated from the computer analysis (Table 2). Among the 50 samples analyzed, genotype D/Da/L1 was identified predominately in 16 (32 %). Genotype F was the second most frequently found, 9 out of 50 (18 %), whereas an ocular genotype B/Ba was unexpectedly found in as high as 12 %. Genotype K, H/J/Ia, G and E were found in a decreasing order of 10 %, 10 %, 6 % and 2 %, respectively. Since the nucleotide sequence of the VD4 region of genotype D, Da and L was different in only a few bases, it was not in the recognition site of the restriction endonuclease used. This made it impossible to distinguish between those genotypes from this RFLP. Also, genotype B and the type variant Ba, exhibit the same RFLP pattern. However, due to the identical nucleotide sequence in the VD4 region, they were not differentiated. In addition, there were 5 samples (10%) that could not be typed directly. This might be because of the present low copy number of *C. trachomatis* in the original samples. In this study, genotypes A, C, I, L2, L2a and L3 were not detected. The overall genotype distribution was summarized in Table 3. However, genotypes D/Da/L1 and F, the most prevalent genital genotypes found in this study, accounted for 50 % of those identified, whereas, genotype E which was reported to be the most prevalent in several countries, was found in only 2 %. The RFLP patterns of genotypes identified were shown in Figure 5-8.

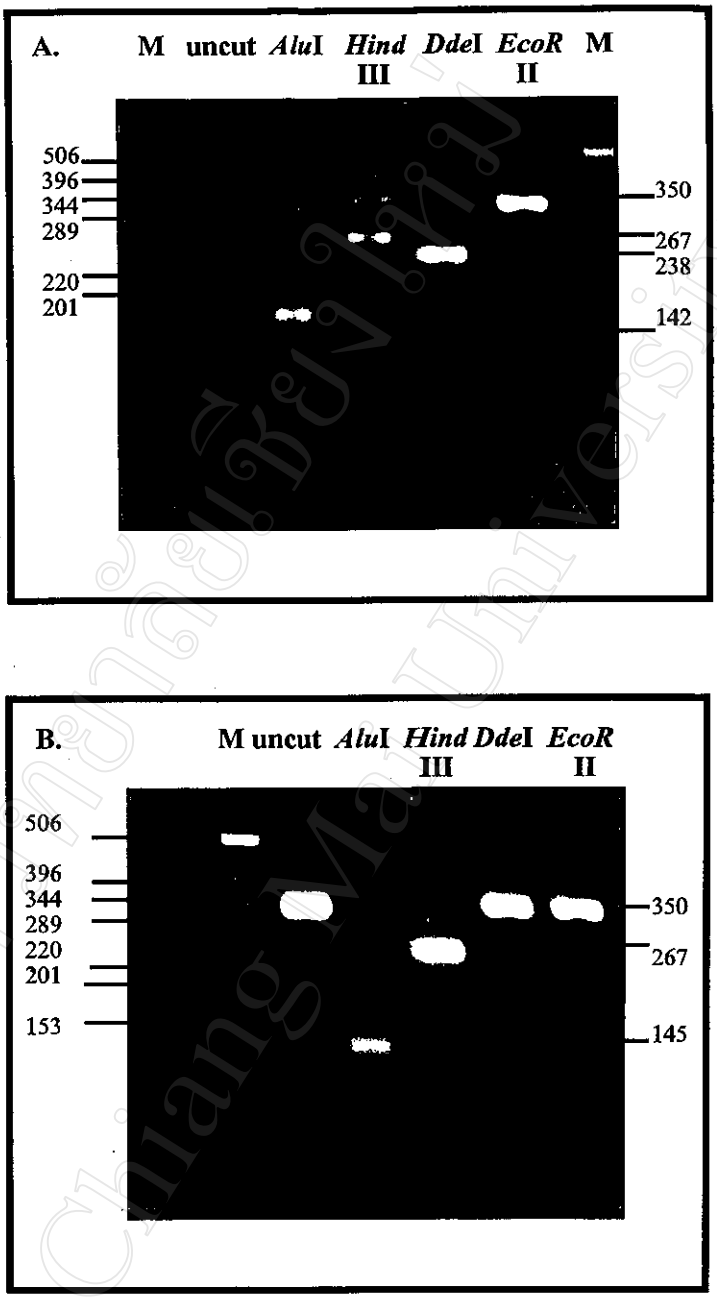


Figure 5. Illustration of the PCR-RFLP pattern after digestion by *Alu*I, *Hind*III, *Dde*I and *EcoR*II.

A = serotypes B/Ba B = serotypes D/Da/L1

Lane M = DNA marker Uncut = No restriction enzyme

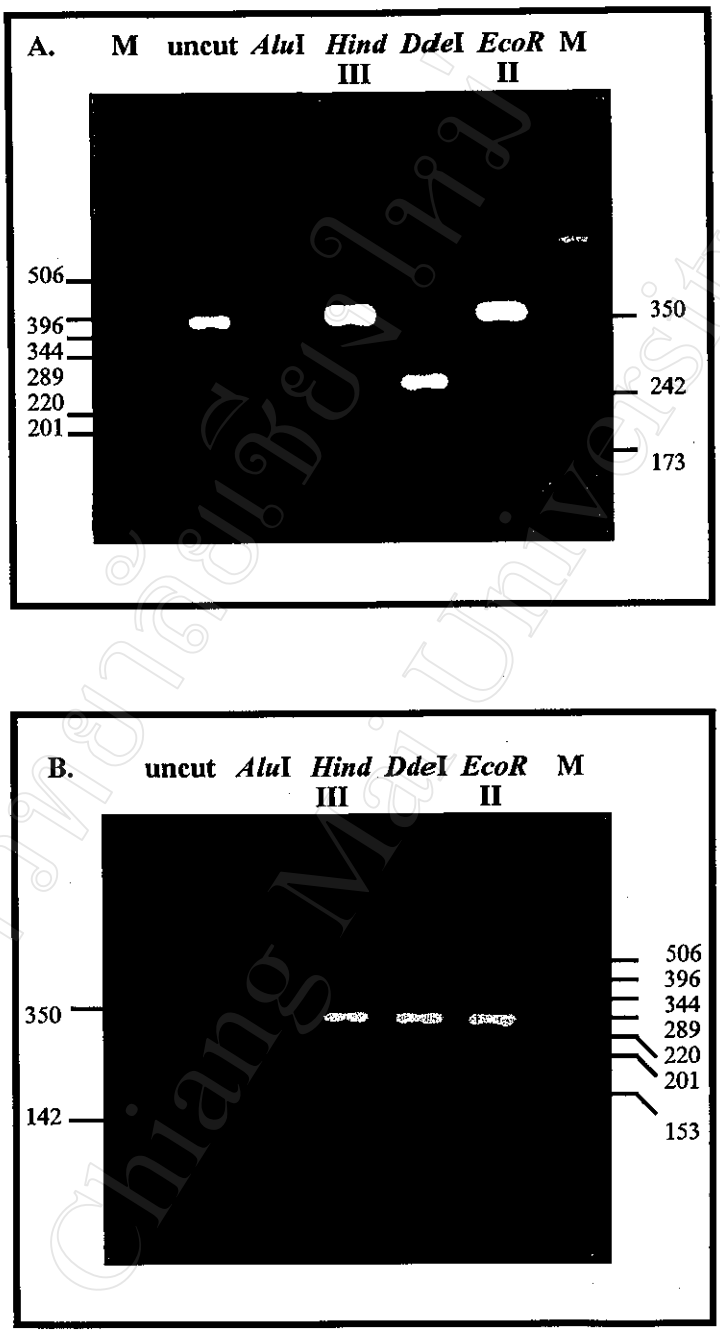


Figure 6. Illustration of the PCR-RFLP pattern after digestion by *Alu*I, *Hind*III, *Dde*I and *EcoR*II.

A = serotype K B = serotype E

Lane M = DNA marker Uncut = No restriction enzyme

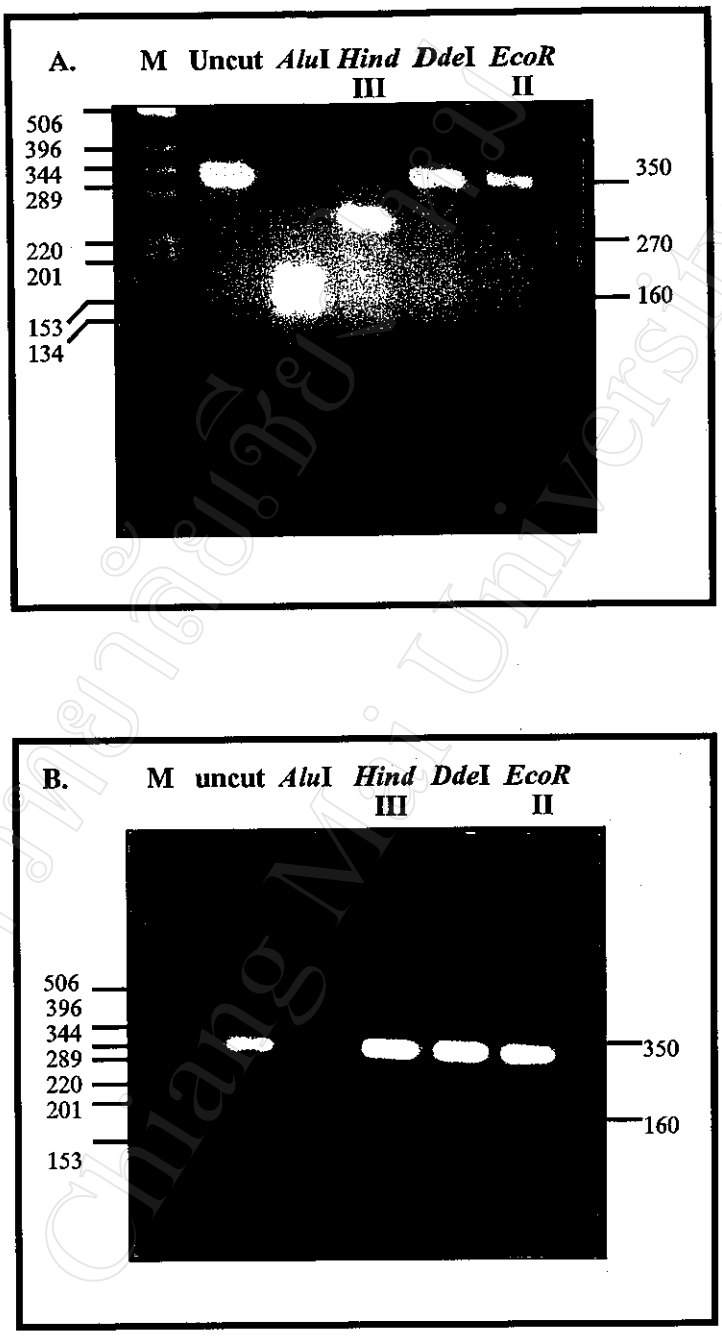


Figure 7. Illustration of the PCR-RFLP pattern after digestion by *Alu*I, *Hind*III, *Dde*I and *EcoR*II.

A = serotype F B = serotype G

Lane M = DNA marker Uncut = No restriction enzyme

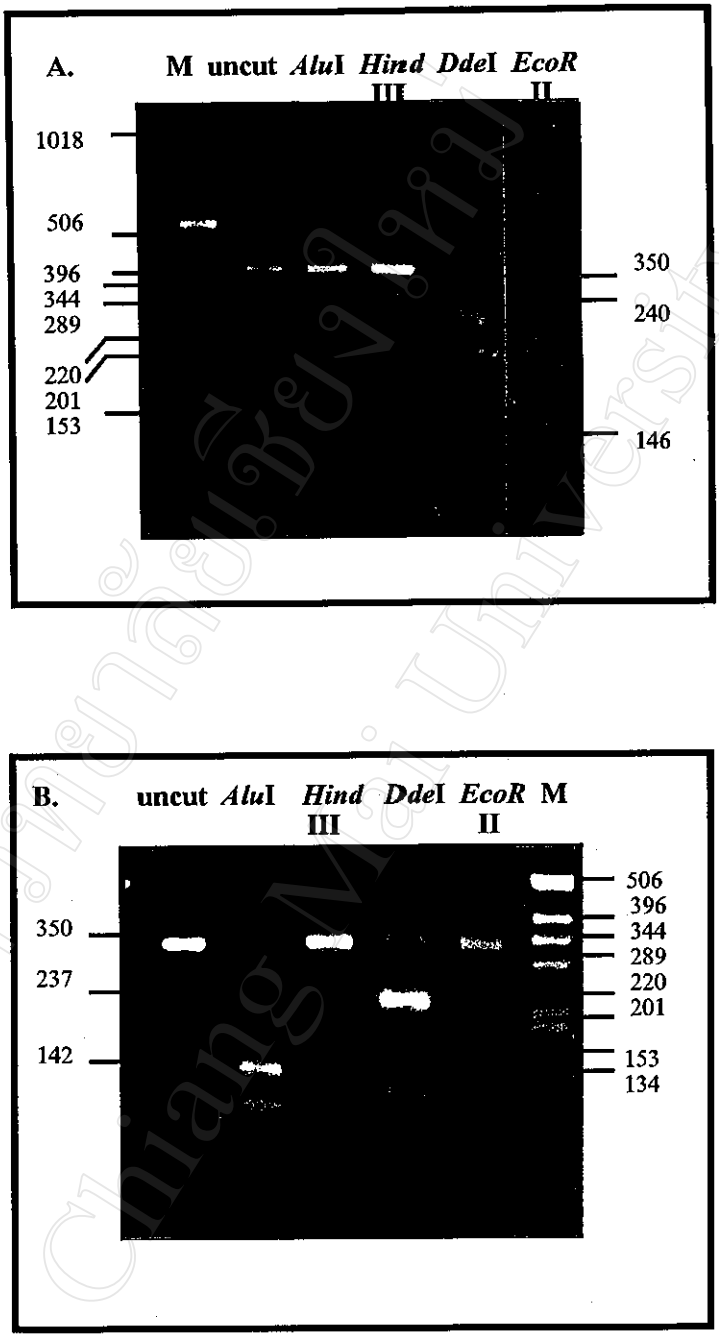


Figure 8. Illustration of the PCR-RFLP pattern after digestion by *Alu*I, *Hind*III, *Dde*I and *EcoR*II.

A = serotype H/J/Ia B = serotype L2 (positive control)

Lane M = DNA marker Uncut = No restriction enzyme

Table 3. Genotypes distribution as determined by PCR-RFLP genotyping of *C. trachomatis* VD4-MOMP gene, detected in Chiang Mai.

Serogroup	RFLP genotype	No. of samples n = 50	Percentage (%)
B complex	B/Ba	6	12.0
	D/Da/L1	16	32.0
	E	1	2.0
Intermediate	F	9	18.0
	G	3	6.0
C complex	H/J/Ia	5	10.0
	K	5	10.0
-	Unidentified	5	10.0

3. Genotyping of *C. trachomatis* by nucleotide sequence analysis

The RFLP technique was not reliable in identifying mixed infection. As shown by this study and in others, the RFLP could not always differentiate the type variants, especially those that occurred outside the recognition sequence. To ensure that the genotypes were classified correctly from the RFLP patterns, the nucleotide sequencing of samples, including the samples that could not be identified in RFLP analysis, were determined.

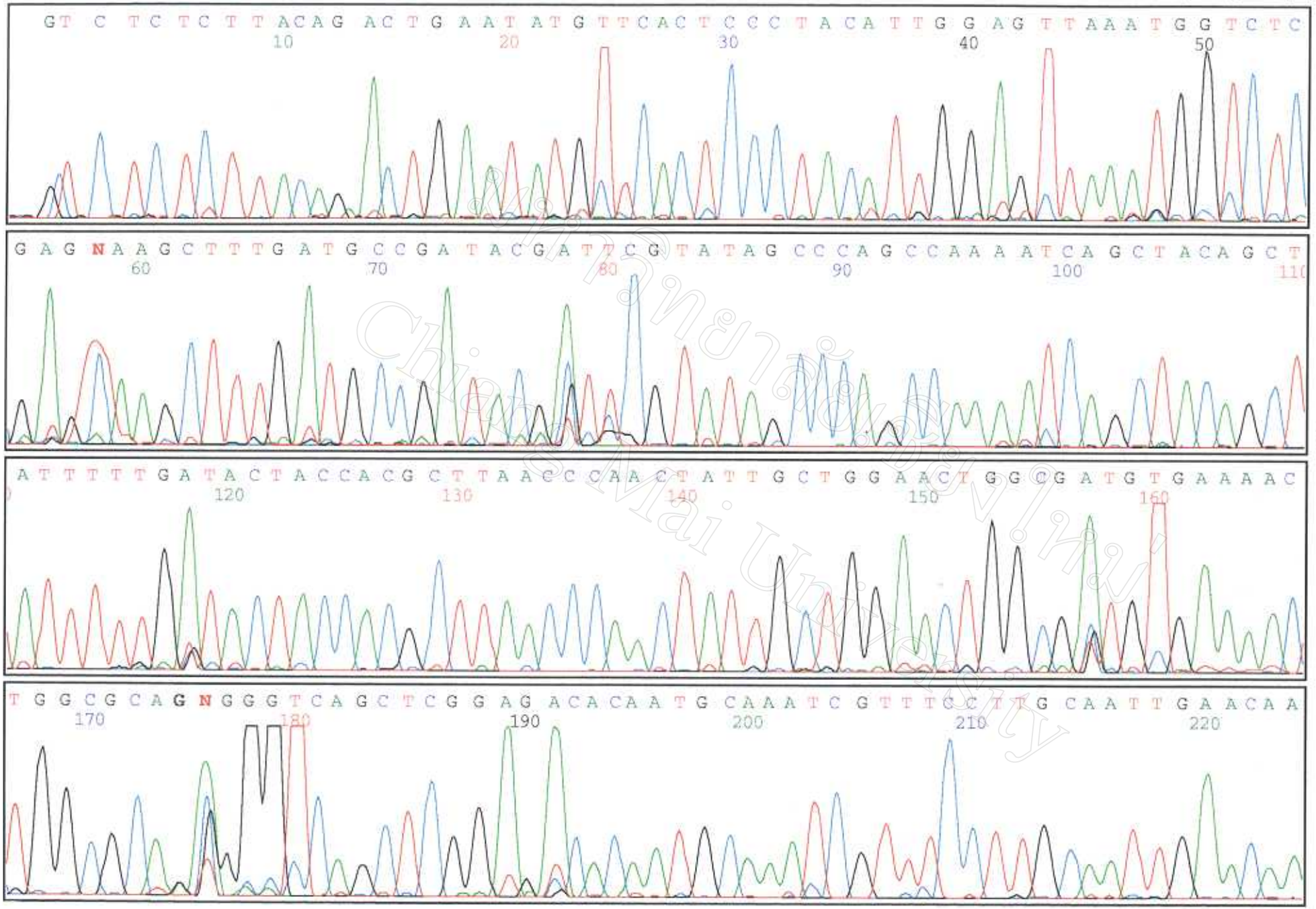
The primer Nest2 was used to sequence forward through the VD4 region. If necessary, the primer Nest4 was used to sequence backwards. The electrophoregram of nucleotide sequences in the VD4-MOMP gene of the samples were shown in Figure 9-15. In the electrophoregram, each of the four different colored curves indicated the fluorescence intensity of a particular dye that was linked to the specific ddNTP involved in the termination of the primer extension reaction (green, red, black and blue were linked with ddATP, ddTTP, ddGTP, and ddCTP, respectively). The 3'-terminal base of each terminated oligonucleotide was identified by the fluorescence liberated from the gel, and then detected and recorded by the device. The data were analyzed by computer programmes ABI 310 data collection version 3.0 and ABI 310 DNA sequencing version 2.2.

In analysis of the genotype, the resulting nucleotide sequences were compared to the prototype sequences obtained from the GenBank or those reported by Yuan *et al.* (16) and Dean *et al.* (21). The genotypes of *C. trachomatis* resulting from nucleotide sequencing of the VD4 DNA were in complete agreement with the RFLP analysis (Table 4). There were 6 samples shown as genotypes B/Ba (Fig.11 and 19), 21 (including 5 samples not identified by RFLP pattern) as genotype D variant (Fig.9 and 17), 1 as genotype E (Fig. 24), 9 as genotype F (Fig.10 and 18) , 3 as genotype G (Fig.14 and 22), 4 as genotype H/Ia (Fig.13 and 21), 1 as genotype J (Fig.15 and 23) and 5 as genotype K (Fig.12 and 20). Genotype D and F were still the most prevalent

genotypes and accounted for 60 % of those identified in this study (Table 5). Among the 16 samples identified by RFLP patterns as a group of genotypes; D/Da/L1 were all genotype D after the sequence analysis. However, a comparison of the VD4 nucleotide sequences of the D samples with the prototype D/UW-3 showed that the D samples were not identical to the prototype, and they were called D variant in this study. The same genotype K, as identified by the RFLP technique, all agreed with nucleotide sequencing. Nevertheless, their nucleotide sequence of the VD4 region were not identical to the prototype K/UW-31. They were all identified as K variants. Among 5 samples identified by RFLP patterns as genotypes H/J/Ia, 1 was genotype J while the rest were H or Ia. Indeed, all H, Ia and J genotypes identified in this study had identical VD4 sequences to the prototypes H/UW-4, Ia/UW-202, J/UW-36, respectively. The conclusion of genotype distribution as determined by the RFLP and nucleotide sequencing ,was shown in Table 5 and Figure 16.

Figure 9. The electrophoregram of the nucleotide sequence in VD4-MOMP gene of *C. trachomatis* genotype D, detected in Chiang Mai by automated DNA sequencer. The letter numbers indicate the positions of the bases in the DNA segment being sequenced.

PRISM Version 2.1.1 Lane 4 Points 1466 to 5360 Base 1: 1466 Spacing: 9.75(0)



PRISM Version 2.1.1 Lane 4 Points 1466 to 5360 Base 1: 1466 Spacing: 9.75{ 0l}

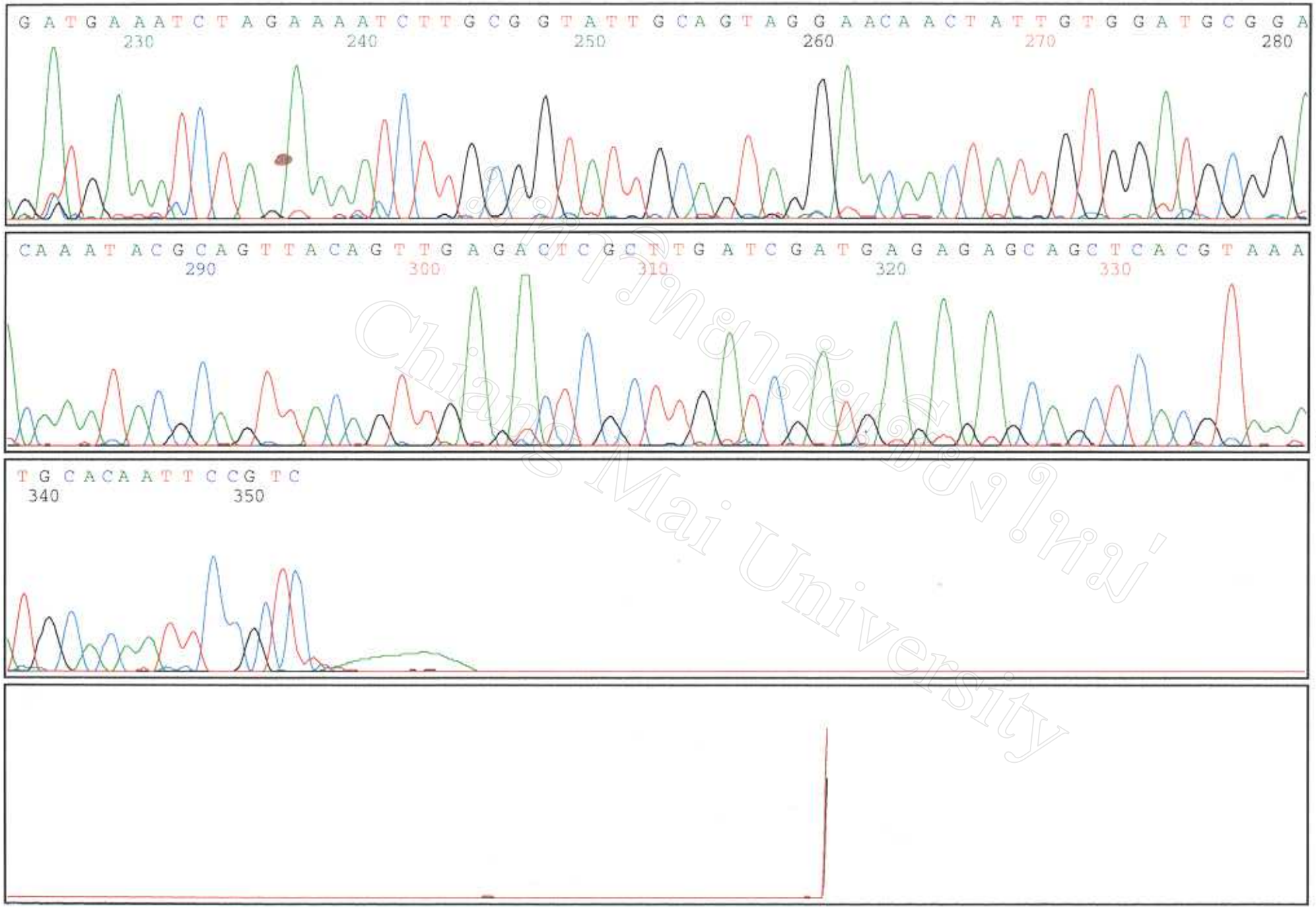
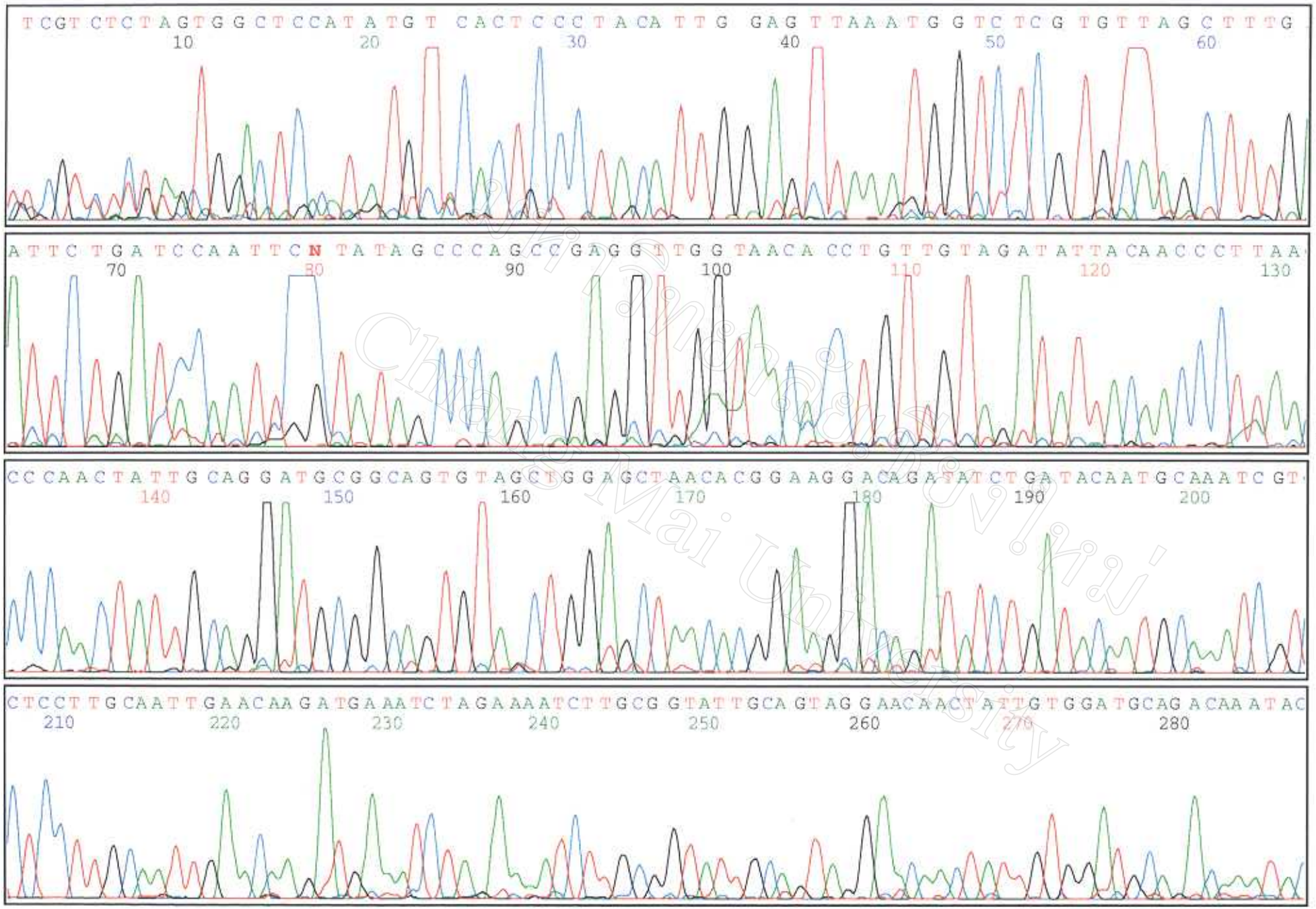


Figure 10. The electrophoregram of the nucleotide sequence in VD4-MOMP gene of *C. trachomatis* genotype F, detected in Chiang Mai by automated DNA sequencer. The letter numbers indicate the positions of the bases in the DNA segment being sequenced.


Version 3.0b1
Lane 3
Points 1460 to 5000 Base 1: 1460
Spacing: 9.75(9.75)
TCGT CTCTAGTGGCTCCATATGT CACTCCCTACATTG GAG TTAAATGGTCTCG TGTTAGCTTTG
10 20 30 40 50 60
ATTC TGATCCAATTCN TATAGCCCAGCCGAGGTTGG TAACA CCTGTTGTAGATATTACAACCCT TAA
70 80 90 100 110 120 130
CC CAACTATTG CAGGATGCGGCAGTGTAGCTGGAGCTAACACGGAAGGACAGATCTGATACAATGCAAATC GT
140 150 160 170 180 190 200
CTCCTTGCAATTGAACAAGATGAAATCTAGAAAATCTTGCG GTATTGCA GTAG GAACAACTATTGTGGATGCAG ACAAATAC
210 220 230 240 250 260 270 280

PRISM Version 3.0B1 Lane 3 Points 1460 to 5000 Base 1: 1460 Spacing: 9.75(9.75)

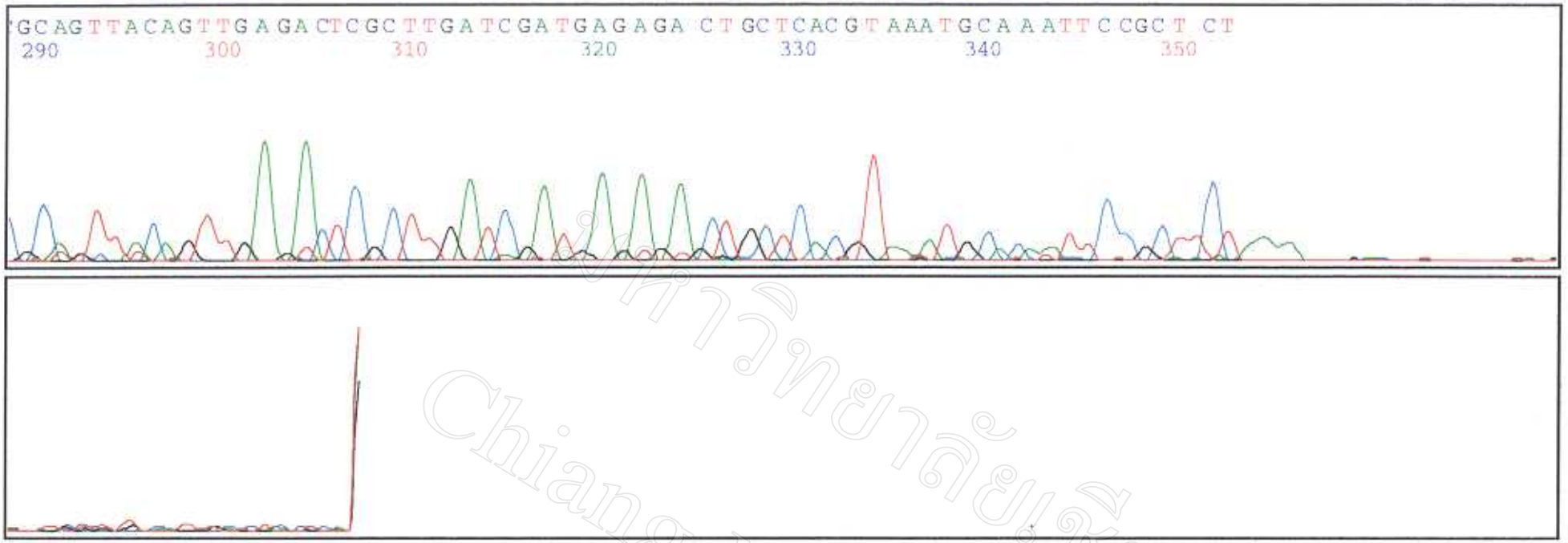


Figure 11. The electrophoregram of the nucleotide sequence in VD4-MOMP gene of *C. trachomatis* genotypes B/Ba, detected in Chiang Mai by automated DNA sequencer. The letter numbers indicate the positions of the bases in the DNA segment being sequenced.

PCR product F32
Lane 3
Install Seq A
Points 1499 to 5640 Base 1: 1499
Wed, Mar 12, 1997 12:26:1 M
Spacing: 9.84 ABI-CE1

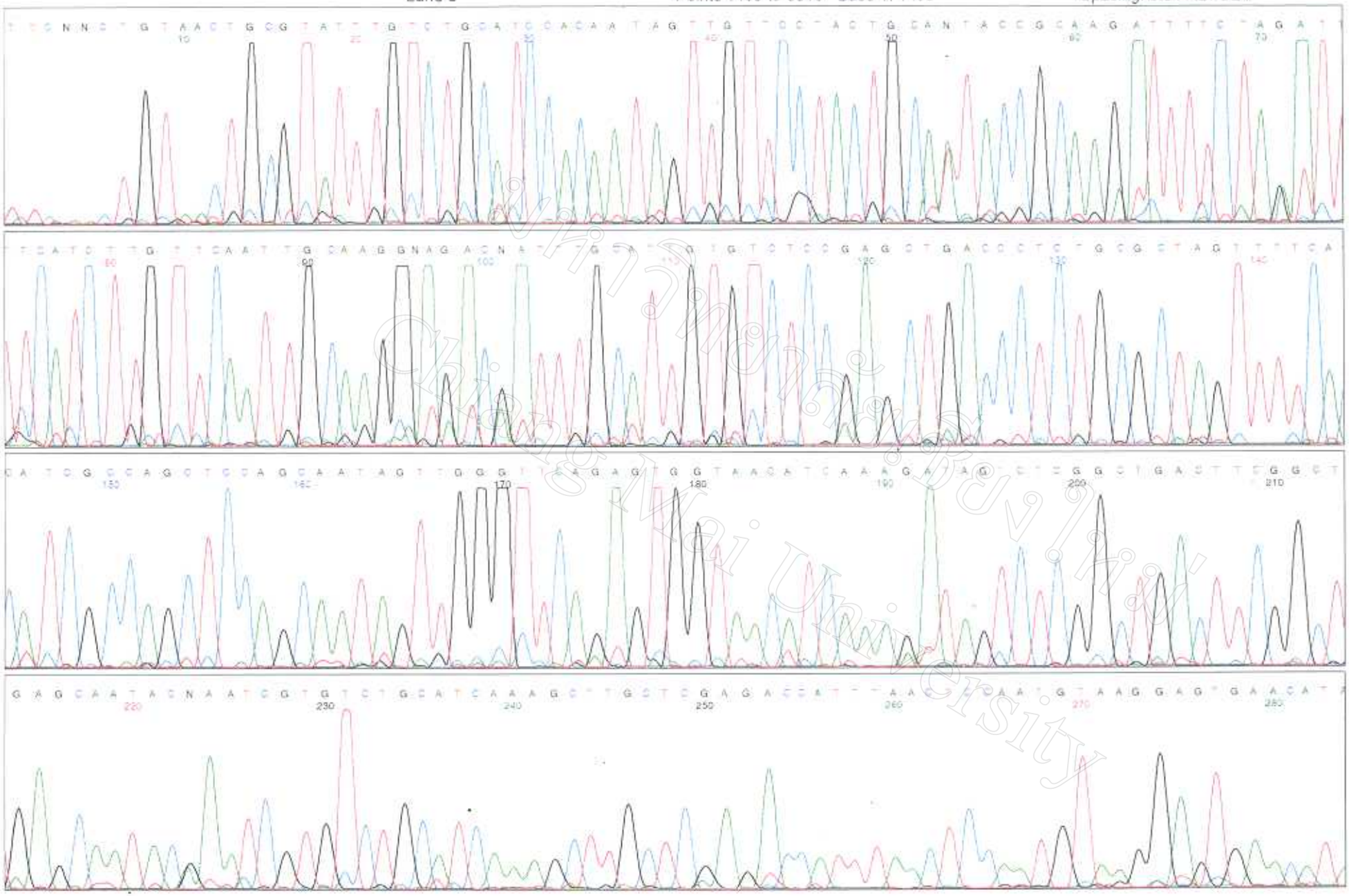
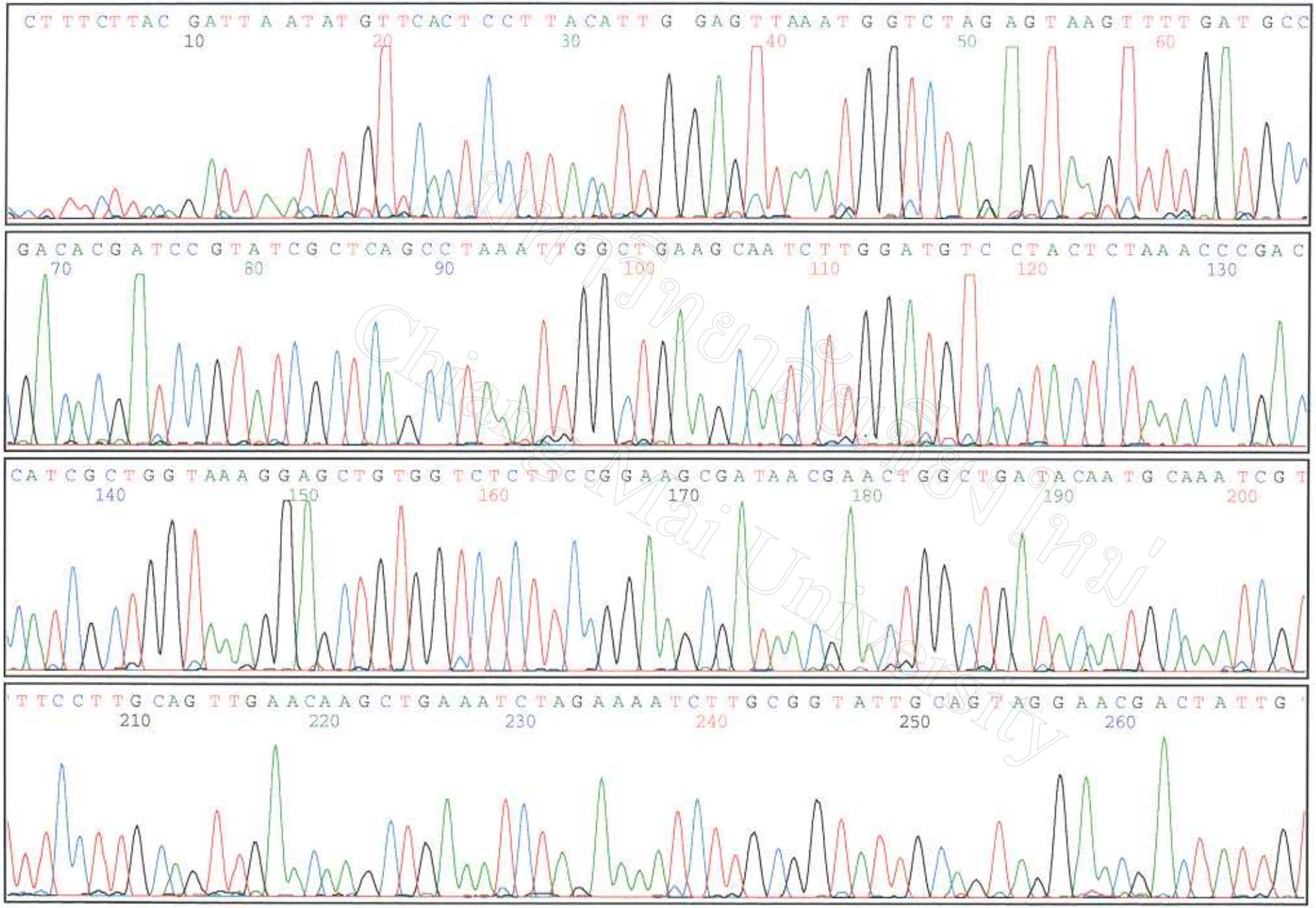


Figure 12. The electrophoregram of the nucleotide sequence in VD4-MOMP gene of *C. trachomatis* genotype K, detected in Chiang Mai by automated DNA sequencer. The letter numbers indicate the positions of the bases in the DNA segment being sequenced.

PRISM Version 3.0b1 Lane 2 Points 1150 to 5280 Base 1: 1150 Spacing: 12.64(12.64)



PRISM Version 3.0b1 Lane 2

Points 1150 to 5280 Base 1: 1150

Thu, Jun 19, 1997 9:38 PM

Spacing: 12.64(12.64)

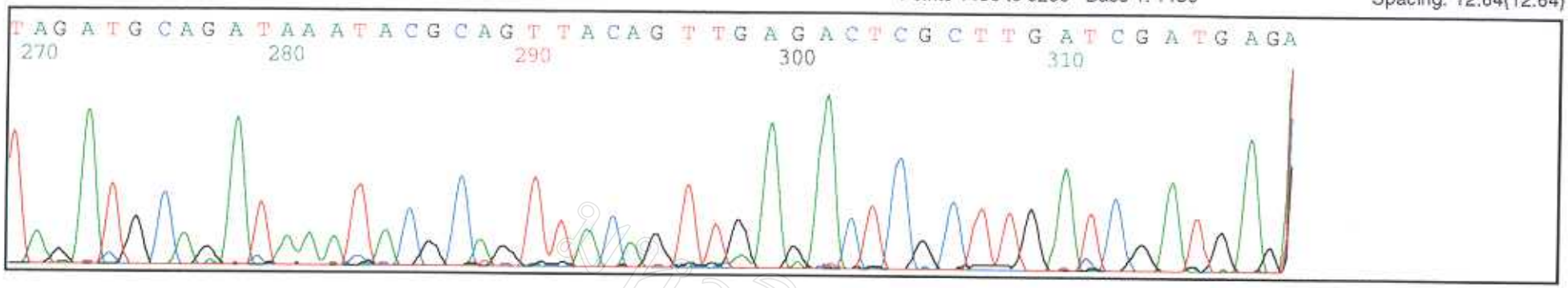
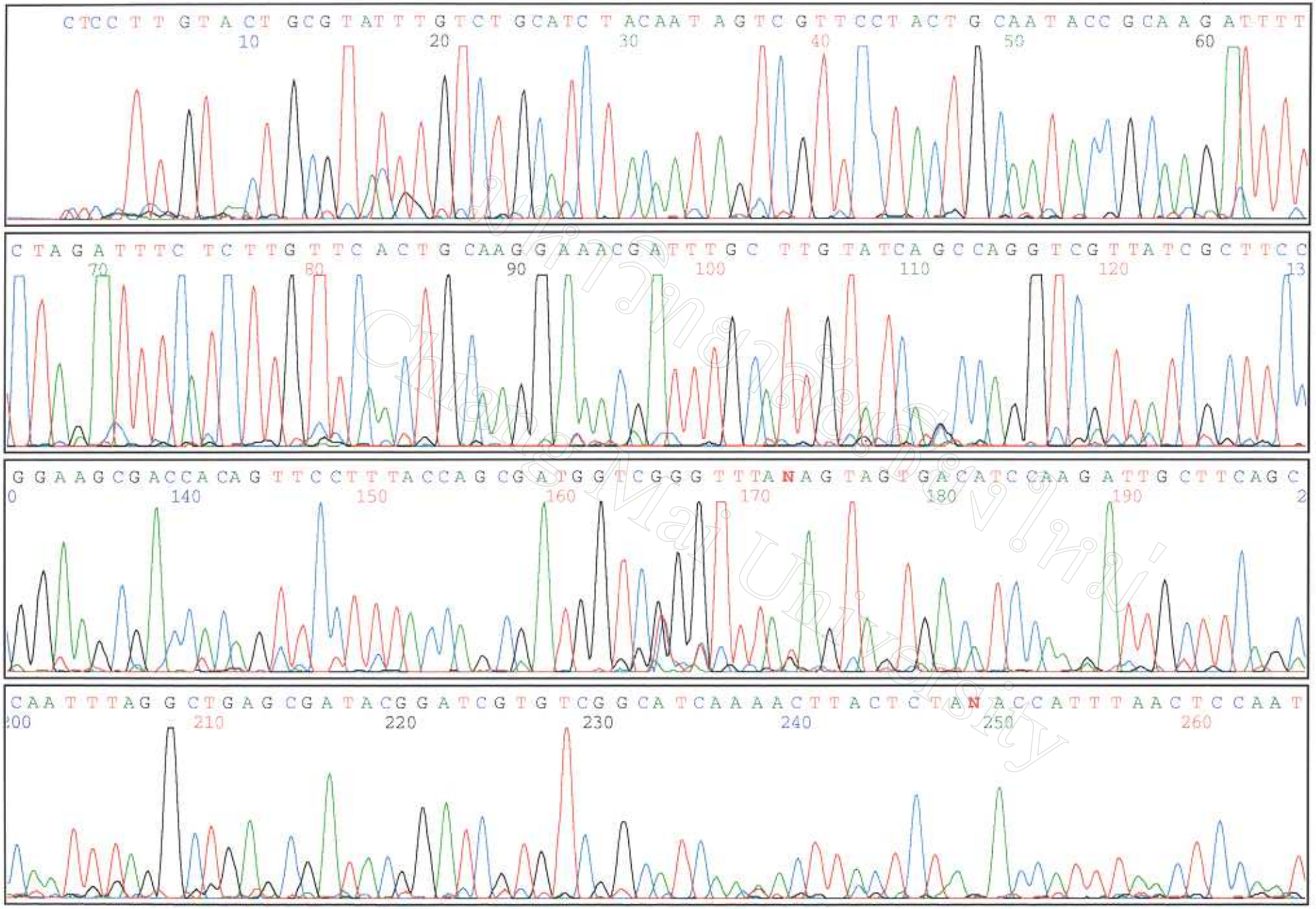


Figure 13. The electrophoregram of the nucleotide sequence in VD4-MOMP gene of *C. trachomatis* genotypes H/Ia, detected in Chiang Mai by automated DNA sequencer. The letter numbers indicate the positions of the bases in the DNA segment being sequenced.

PRISM Version 3.0b1 Lane 2 Points 1100 to 5500 Base 1: 1100 Spacing: 12.80(12.80)



56

PRISM Version 3.0b1 Lane 2 Points 1100 to 5500 Base 1: 1100 Spacing: 12.80(12.80)

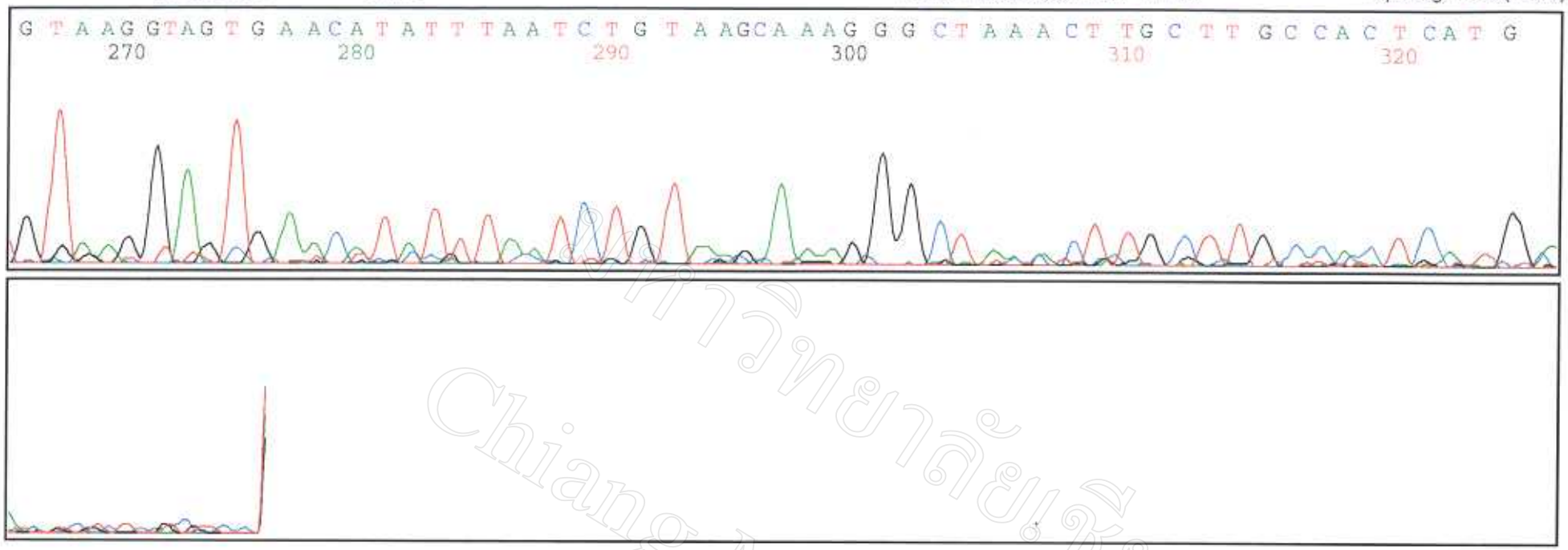


Figure 14. The electrophoregram of the nucleotide sequence in VD4-MOMP gene of *C. trachomatis* genotype G, detected in Chiang Mai by automated DNA sequencer. The letter numbers indicate the positions of the bases in the DNA segment being sequenced.

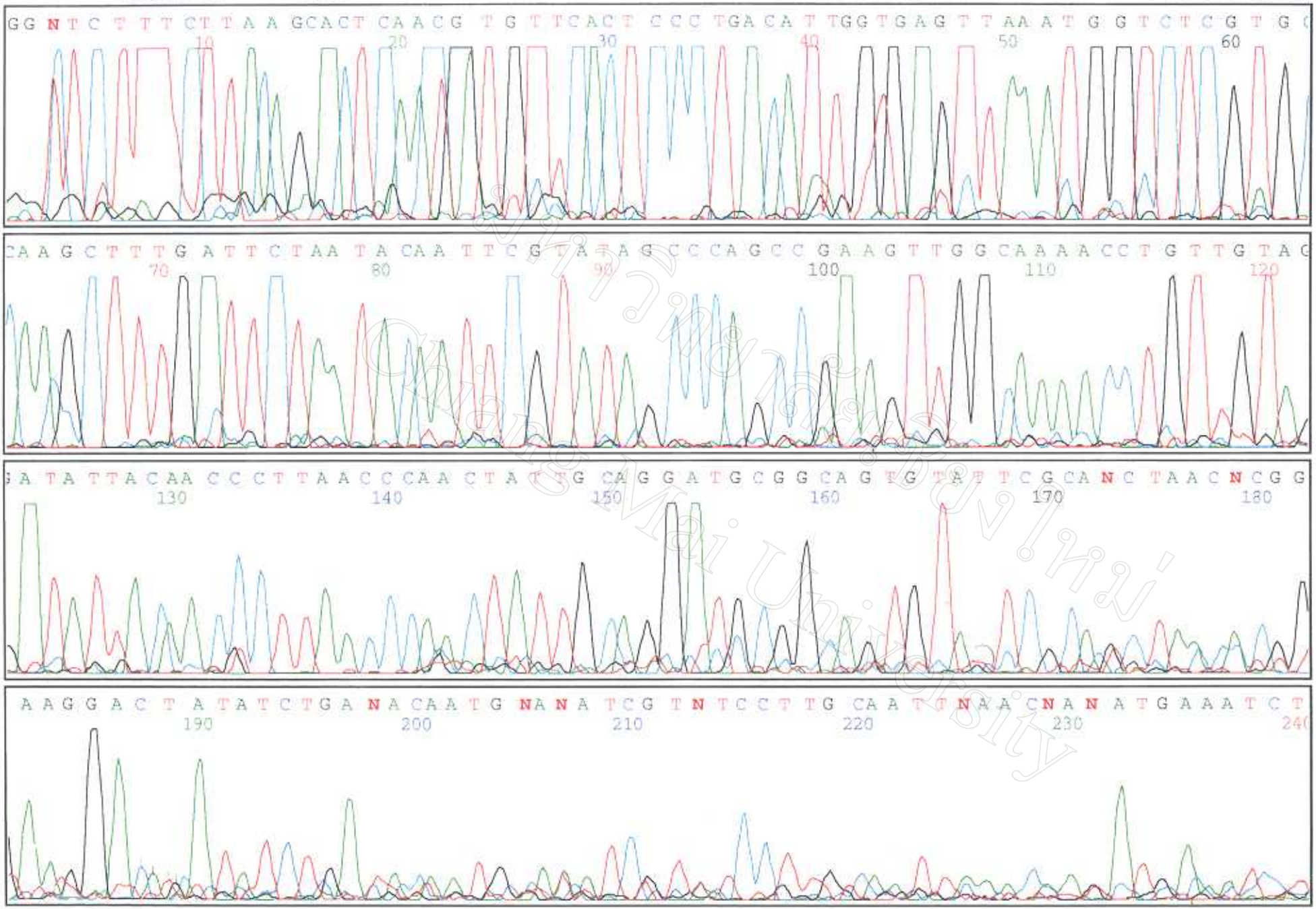
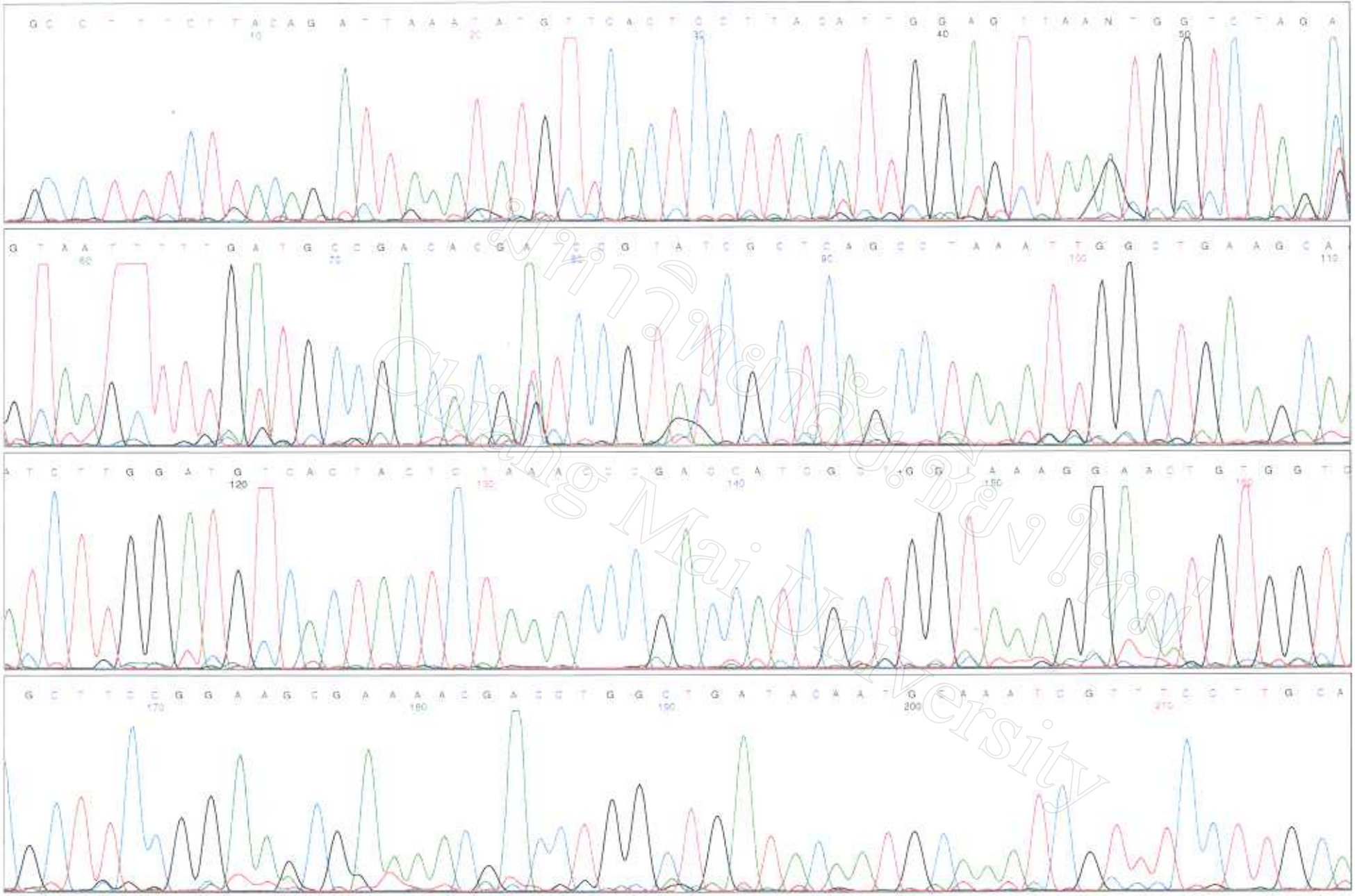


Figure 15. The electrophoregram of the nucleotide sequence in VD4-MOMP gene of *C. trachomatis* genotype J, detected in Chiang Mai by automated DNA sequencer. The letter numbers indicate the positions of the bases in the DNA segment being sequenced.



Version 2.1.1
Primer N2,DNA 6µl
PCR Product P33
Lane 3
DT5%CEHV(A Set-AnyPrimer)
Install Seq A
Points 1430 to 5360 Base 1: 1430
Sat, Mar 15, 1997 1:24 PM
Sat, Mar 15, 1997 11:29 AM
Spacing: 9.84 ABI-CE1

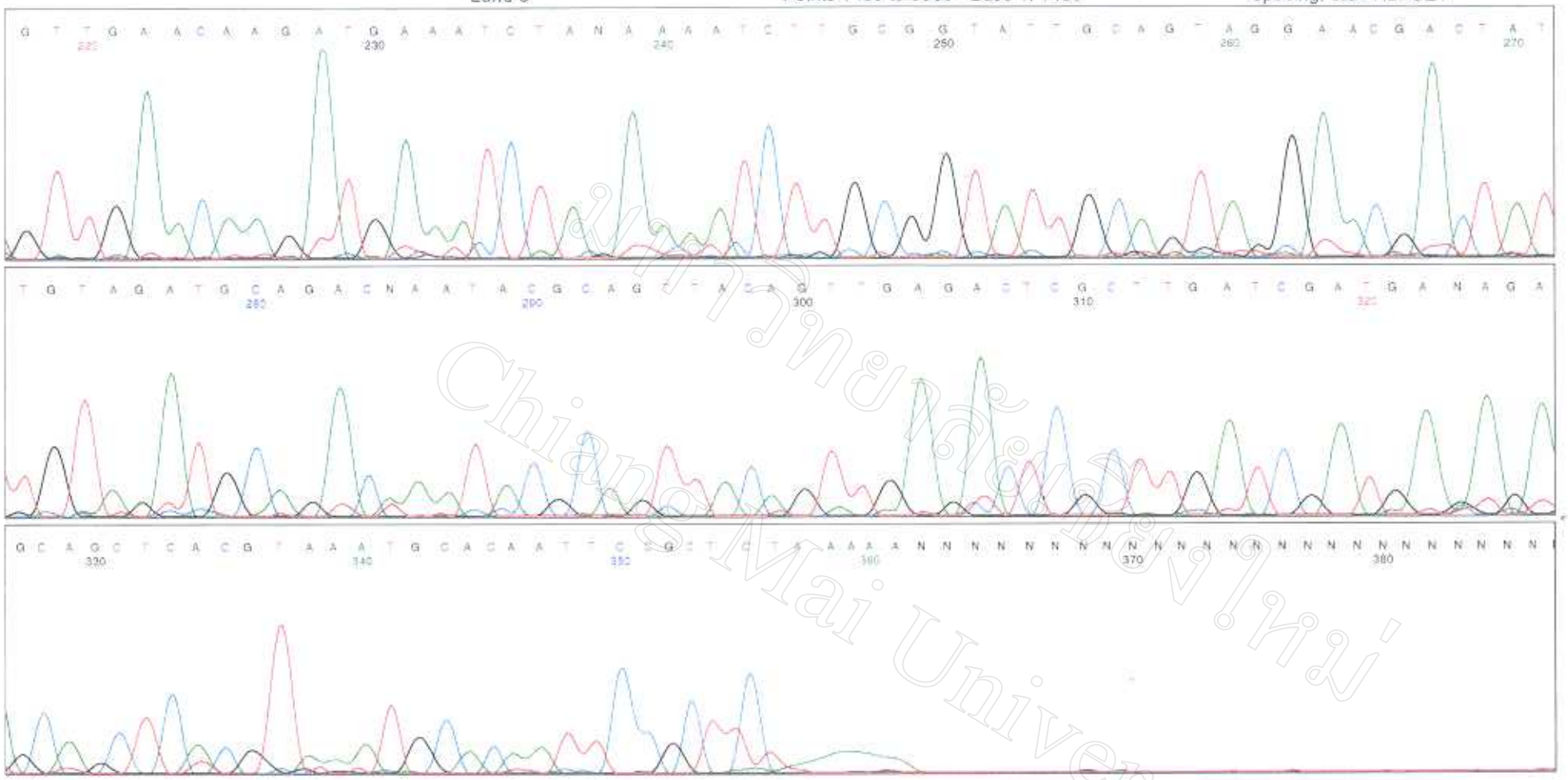


Table 4. Comparison of genotypes of PCR-RFLP and nucleotide sequencing analysis of *C. trachomatis* VD4-MOMP gene.

RFLP		nucleotide sequencing of VD4-MOMP gene	
Genotype	No.of samples n = 50	Genotype	No.of samples n = 50
B/Ba	6	B/ Ba prototypes	6
D/Da/L1	16	D variant	16
E	1	E prototype	1
F	9	F prototype	9
G	3	G prototype	3
H/J/Ia	5	H/ Ia prototypes	4
		J prototype	1
K	5	K variant	5
Unidentified	5	D variant	5

Table 5. The conclusion of *C. trachomatis* genotypes distribution as determined genotyping by PCR-RFLP and nucleotide sequencing analysis of VD4-MOMP gene, detected in Chiang Mai

Serogroup	RFLP and nucleotide sequencing genotype	No. of samples n = 50	Percentage (%)
B complex	B/ Ba prototypes	6	12.0
	D variant	21	42.0
	E prototype	1	2.0
Intermediate	F prototype	9	18.0
	G prototype	3	6.0
C complex	H/ Ia prototypes	4	8.0
	J prototype	1	2.0
	K variant	5	10.0

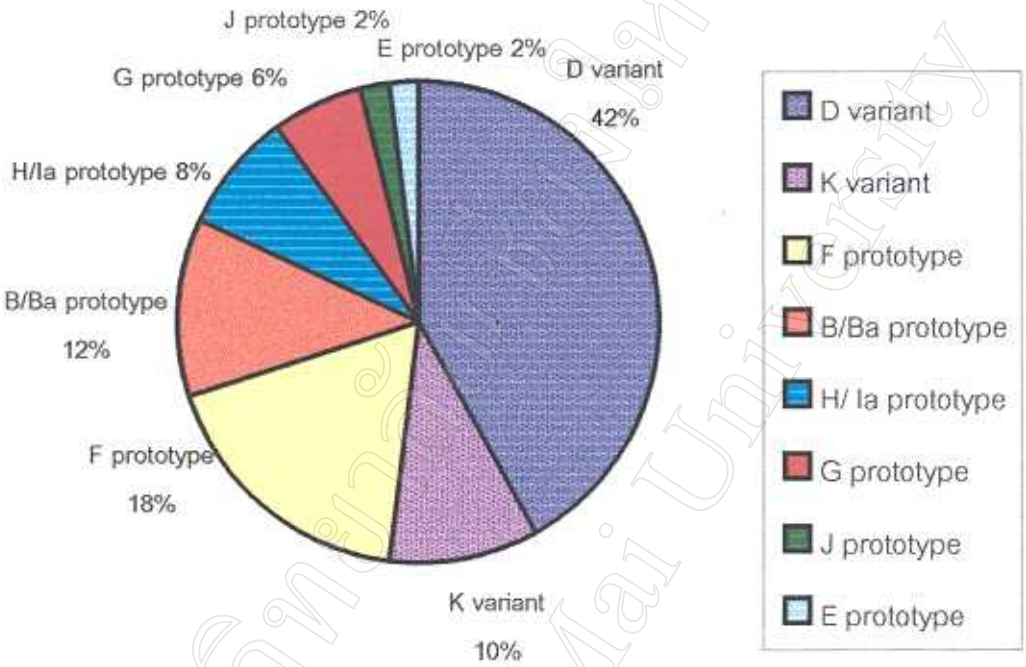


Figure 16. The distribution of *C. trachomatis* genotype after PCR-RFLP and nucleotide sequencing analysis, detected in Chiang Mai. The genotype distributions of *C. trachomatis*, demonstrated by pie chart: serotype D variant = 42%, K variant = 10%, F prototype = 18%, B/Ba prototype = 12%, H/Ia prototype = 8%, G prototype = 6%, J prototype = 2% and E prototype = 2%.

4. Analysis of nucleotide sequence polymorphism of the VD4-MOMP gene

To determined the extent of nucleotide sequence polymorphism in the VD region of the MOMP gene, the nucleotide sequences of the VD4 DNA were analyzed in comparison to the prototype sequences. Among the 50 *C. trachomatis* sequence analyses, 24 (48 %) had sequences identical to their prototypes, whereas, 26 (52%), genotypes D and K showed sequence variations (Table 6 and Fig.17-24). Among those with the sequence variation, one or two nucleotide substitutions was the most common form.

As compared to the prototype D/UW-3, all genotype D identified in this study had one nucleotide substitution at the nucleotide position 979; guanine was substituted by adenine. This observation was identical to those previously reported D/B-185 and other D variants (15,44). This transition resulted in an amino acid substitution that was threonine to alanine, and leading to the loss of immune reactivity to subgroup-specific monoclonal antibody BB-11 (45). The nucleotide sequences of the VD4 DNA of these D, other D variants and the prototype were compared and shown in Figure 17. However, these D variants were not detected by the RFLP analysis since the major band (145 bp) in the RFLP pattern of D variants (Fig. 5B) and prototype (Table 2) was not different. Even though, the mutation that occurred at nucleotide position 979 resulted in the loss of one of the *Alu*I sites (Fig. 25), but this was not shown in the RFLP pattern.

Comparison of the VD4 nucleotide sequences of the K samples with the prototype K/UW-31 showed that the K samples were not identical to the prototype. There was one substitution at nucleotide 973; adenine was substituted by guanine. This substitution resulted in the transition of an amino acid; alanine to threonine (Fig.20). The K variants observed here were identical to previously reported K/Nairobi and other K variants (15,34,40,46). This point mutation was not in the restriction sites of the enzymes used in this study (Fig.26). Therefore, this change did not effect the pattern of the RFLP.

In general, genotype B and the type variant Ba were both serologically and genetically different. The nucleotide sequence differences between those B and Ba were observed only in the VD1 and VD2, but not in the VD4 region. As compared to the prototype B/TW-5, three nucleotide substitutions in the VD1 and two in the VD2 were observed in the Ba. The nucleotide sequence analysis was extended to the VD1 and VD2 region in order to differentiate B from the Ba genotype. However, only 2 from 6 samples originally identified as genotype B/Ba by RFLP and VD4 nucleotide sequencing were successfully sequenced through those VDs, dut to the limitation of the samples obtained. The electrophoregram of nucleotide sequences in the VD1-2 MOMP gene of these samples was shown in Figure 27. When the VD1 and VD 2 sequences of the samples were compared to those of B/TW-5 and Ba/AP2 prototypes, the sequence of the samples was identical to the Ba/AP2 , apart from only one substitution at nucleotide 511 in the VD2; adenine was substituted by guanine. This transition resulted in an amino acid; changing from serine to glycine, as shown in Figure 28 and 29. However, they had both VD sequences identical to the Ba/583/OT and Ba/809/OT isolated in Tunisia, Ba/J160/OT strain isolated from a patient with trachoma in Egypt (32), and 2 Ba from genital samples in Canada that were previously obtained by Yang *et al.* (34). Those strains were known as the Ba variants.

Table 6. The nucleotide sequence polymorphism of the VD4-MOMP gene of *C. trachomatis*, detected in Chiang Mai.

Nucleotide sequence polymorphism of VD4-MOMP	Genotype	No.of samples n = 50	Percentage (%)
Variant		26	52
	D variant	21	
	K variant	5	
Prototype		24	48
	F prototype	9	
	B/Ba prototypes	6	
	H/ Ia prototypes	4	
	G prototype	3	
	J prototype	1	
	E prototype	1	

Serotype	Strain	Nucleotide sequence of VD4-MOMP gene of *C. trachomatis*
		928 ↓ 979 ↓ 1017 ↓
D prototype	D/UW-3 (ref.)	TCAGCTACAGCTATT TTTGATACTACCACGCTTAACCCAACTATTGCTGGAGCTGGCGATGTGAAAACTGGCGCAGAGGGTCAGCTCGGA
		Ala
D variant	spec#P3/Cx Chiang Mai	... ... A... ... ...
		Thr
D variant	spec#P7/Cx Chiang Mai	... ... A... ... ...
		Thr
D variant	spec#P10/Cx Chiang Mai	... ... A... ... ...
		Thr
D variant	spec#P11/Cx Chiang Mai	... ... A... ... ...
		Thr
D variant	spec#P13/Cx Chiang Mai	... ... A... ... ...
		Thr
D variant	spec#P18/Cx Chiang Mai	... ... A... ... ...
		Thr
D variant	spec#P24/Cx Chiang Mai	... ... A... ... ...
		Thr
D variant	spec#P26/Cx Chiang Mai	... ... A... ... ...
		Thr
D variant	spec#P27/Cx Chiang Mai	... ... A... ... ...
		Thr
D variant	spec#P31/Cx Chiang Mai	... ... A... ... ...
		Thr
D variant	spec#P34/Cx Chiang Mai	... ... A... ... ...
		Thr

Figure 17. Nucleotide and amino acid sequence comparison of the VD4-MOMP gene of the prototype D/UW-3, the genital D strain in this study and respective variants. The nucleotide substitutions are double underlined and the codons having nucleotide substitutions are underlined.

Serotype	Strain	Nucleotide sequence of VD4-MOMP gene of *C. trachomatis*
		928 ↓ 979 ↓ 1017 ↓
D prototype	D/UW-3 (ref.)	TCAGCTACAGCTATT TTTGATACTACCACGCTTAACCCAACTATTGCTGGA<u>GC</u>TGGCGATGTGAAAACTGGCGCAGAGGGTCAGCTCGGA
		Ala
D variant	spec#P45/Cx chiang mai	... ... **A**.. ... ...
		Thr
D variant	spec#P58/Cx chiang mai	... ... **A**.. ... ...
		Thr
D variant	spec#P78/Cx chiang mai	... ... **A**.. ... ...
		Thr
D variant	spec#P79/Cx chiang mai	... ... **A**.. ... ...
		Thr
D variant	spec#S1/Cx chiang mai	... ... **A**.. ... ...
		Thr
D variant	spec#S9/Cx chiang mai	... ... **A**.. ... ...
		Thr
D variant	spec#S17/Cx chiang mai	... ... **A**.. ... ...
		Thr
D variant	spec#U1/Ur chiang mai	... ... **A**.. ... ...
		Thr
D variant	spec#U3 chiang mai	... ... **A**.. ... ...
		Thr
D variant	spec#U4 chiang mai	... ... **A**.. ... ...
		Thr
D variant	D/B-185 Sayada'95	... ... **A**.. ... ...
		Thr

Figure 17. (Continue) Nucleotide and amino acid sequence comparison of the VD4-MOMP gene of the prototype D/UW-3, the genital D strain in this study and respective variants. The nucleotide substitutions are double underlined and the codons having nucleotide substitutions are underlined.

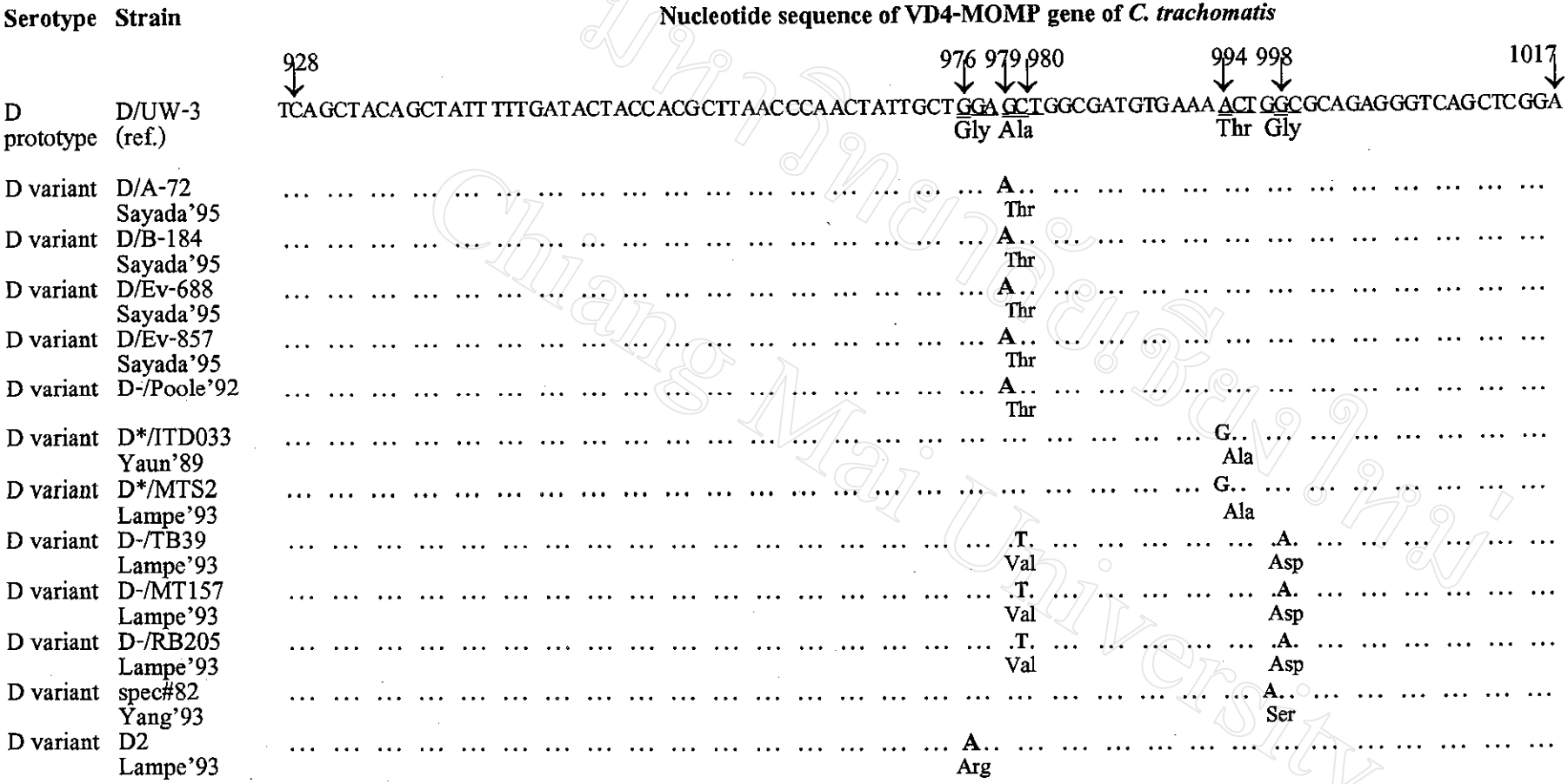


Figure 17. (Continue) Nucleotide and amino acid sequence comparison of the VD4-MOMP gene of the prototype D/UW-3, the genital D strain in this study and respective variants. The nucleotide substitutions are double underlined and the codons having nucleotide substitutions are underlined.

Serotype	Strain	Nucleotide sequence of VD4-MOMP gene of *C. trachomatis*
		928 ↓ 1020 ↓
F prototype	F/IC-CAL3 (ref.)	TTGGTAACACCTGTTGTAGATATTACAACCCTTAACCCAACTATTGCAGGATGCGGCAGTGTAGCTGGAGCTAACACGGAAGGACAGATATCT
F prototype	spec#P12 Chiang Mai	... ...
F prototype	spec#P28 Chiang Mai	... ...
F prototype	spec#P71 Chiang Mai	... ...
F prototype	spec#P72 Chiang Mai	... ...
F prototype	spec#P76 Chiang Mai	... ...
F prototype	spec#P113 Chiang Mai	... ...
F prototype	spec#P190 Chiang Mai	... ...
F prototype	spec#S7 Chiang Mai	... ...
F prototype	spec#S25 Chiang Mai	... ...

Figure 18. Nucleotide and amino acid sequence comparison of the VD4-MOMP gene of the prototype F/IC-CAL3 and the genital F strain in this study.

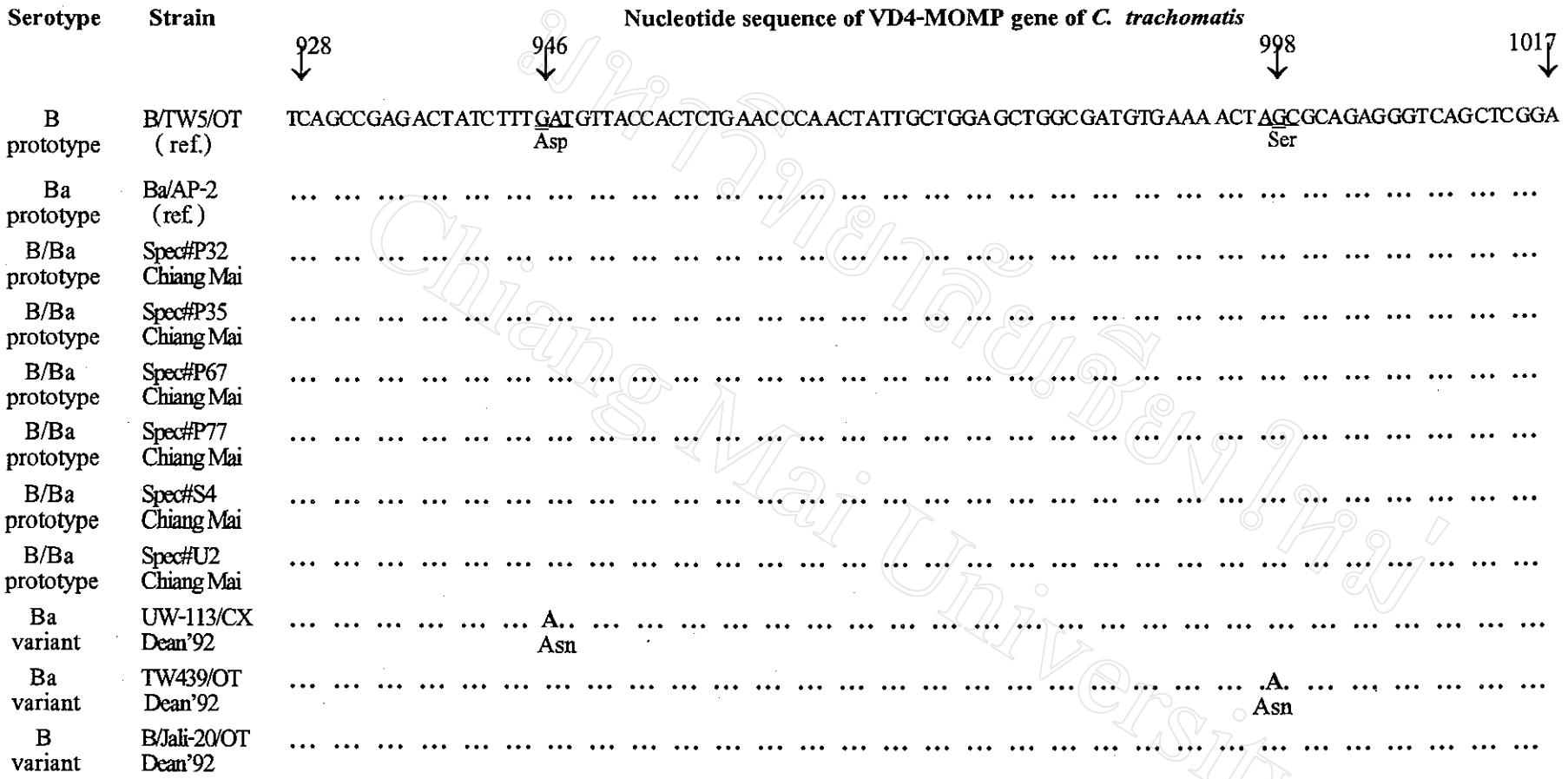


Figure 19. Nucleotide and amino acid sequence comparison of the VD4-MOMP gene of the prototype B/TW5/OT, Ba/AP-2 and the genital B/Ba strain in this study and respective variants. The nucleotide substitutions are double underlined and the codons having nucleotide substitutions are underlined.

Serotype	Strain	Nucleotide sequence of VD4-MOMP gene of *C. trachomatis* (928 ↓ … 973 ↓ … 1020 ↓)
K prototype	UW-31/Cx (ref.)	TTGGCTGAAGCAATCTTGGATGTCACTACTCTAAACCCGACCATC<u>ACT</u>GGTAAAGGAGCTGTGGTCTCTTCCGGAAGCGATAACGAACTGGCT Thr
K variant	spec#P16 Chiang Mai	... ... G.. ... ... Ala
K variant	spec#S3 Chiang Mai	... ... G.. ... ... Ala
K variant	spec#S5 Chiang Mai	... ... G.. ... ... Ala
K variant	spec#S10 Chiang Mai	... ... G.. ... ... Ala
K variant	spec#S24 Chiang Mai	... ... G.. ... ... Ala
K variant	spec#79 Yang'93	... ... G.. ... ... Ala
K variant	K/Nairobi Brunham'94	... ... G.. ... ... Ala
K variant	K/UW-31/Cx Poole'92	... ... G.. ... ... Ala
K variant	K/UW-31 Stothard'98	... ... G.. ... ... Ala

Figure 20. Nucleotide and amino acid sequence comparison of the VD4-MOMP gene of the prototype K/UW-31/Cx, the genital K strain in this study and respective variants. The nucleotide substitutions are double underlined and the codons having nucleotide substitutions are underlined.

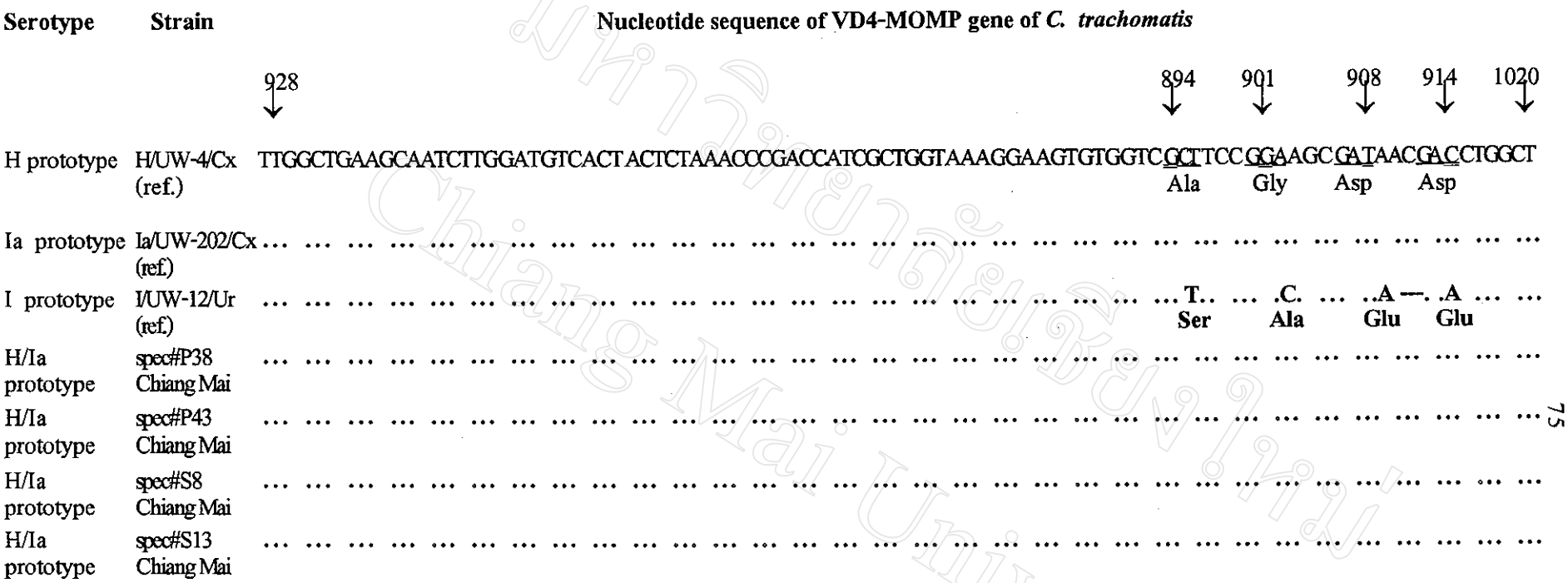


Figure 21. Nucleotide and amino acid sequence comparison of the VD4-MOMP gene of the prototype H/UW-4/Cx, Ia/UW-202 /Cx, I/UW-12/Ur, the genital H/Ia strain in this study and respective variants. The nucleotide substitutions are double underlined and the codons having nucleotide substitutions are underlined.

Serotype	Strain	Nucleotide sequence of VD4-MOMP gene of *C. trachomatis* (928 ↓ … 1003 ↓ … 1020 ↓)
G prototype	G/UW-57 (ref.)	TTGGCAAAACCTGTTGTAGATATTACAACCCTTAACCCAACTATTGCAGGATGCGGCAGTGTAGTCGCAGCTAAC TCG GAAGGACAGATATCT Ser
G prototype	spec#P17 Chiang Mai	... ...
G prototype	spec#P48 Chiang Mai	... ...
G prototype	spec#S14 Chiang Mai	... ...
G prototype	spec#P72 Chiang Mai	... ...
G Variant	Ga,Morre'98	... ... G.. ... ... Ala
G Variant	G'/Poole'92	... ... G.. ... ... Ala
G Variant	G-/Poole'92	... ... **TT.** ... ... Leu

Figure 22. Nucleotide and amino acid sequence comparison of the VD4-MOMP gene of the prototype G/UW-57, the genital G strain in this study and respective variants. The nucleotide substitutions are double underlined and the codons having nucleotide substitutions are underlined.

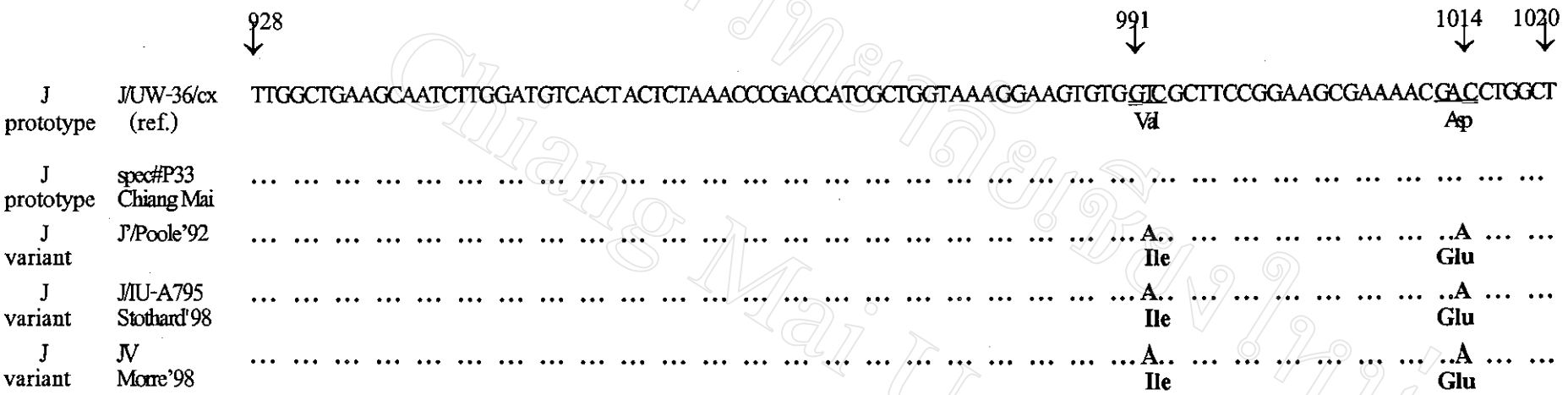


Figure 23. Nucleotide and amino acid sequence comparison of the VD4-MOMP gene of the prototype J/UW/36, the genital J strain in this study and respective variants. The nucleotide substitutions are double underlined and the codons having nucleotide substitutions are underlined.

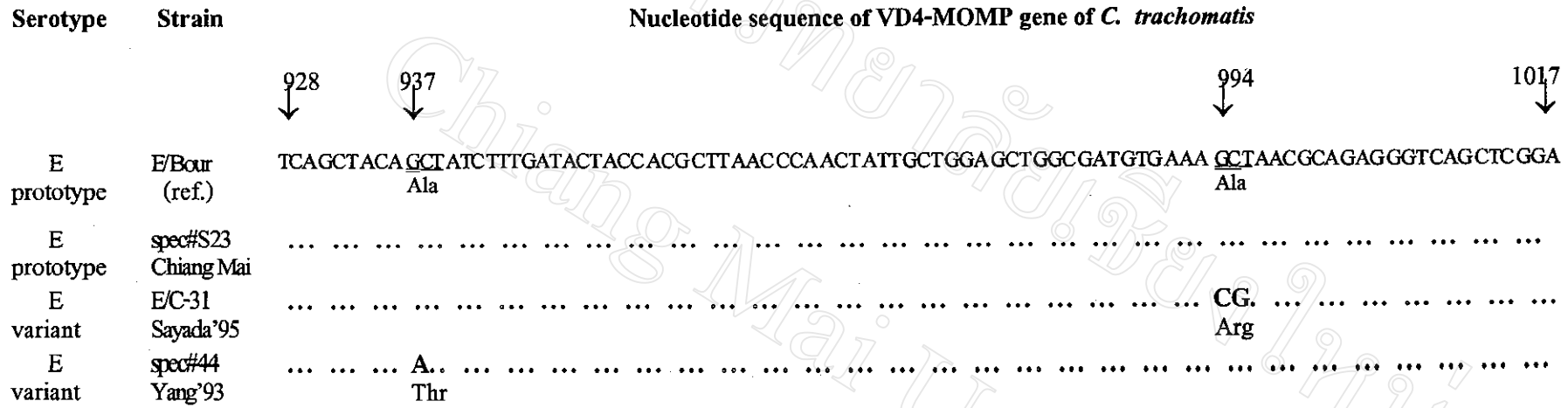


Figure 24. Nucleotide and amino acid sequence comparison of the VD4-MOMP gene of the prototype E/Bour, the genital E strain in this study and respective variants. The nucleotide substitutions are double underlined and the codons having nucleotide substitutions are underlined.

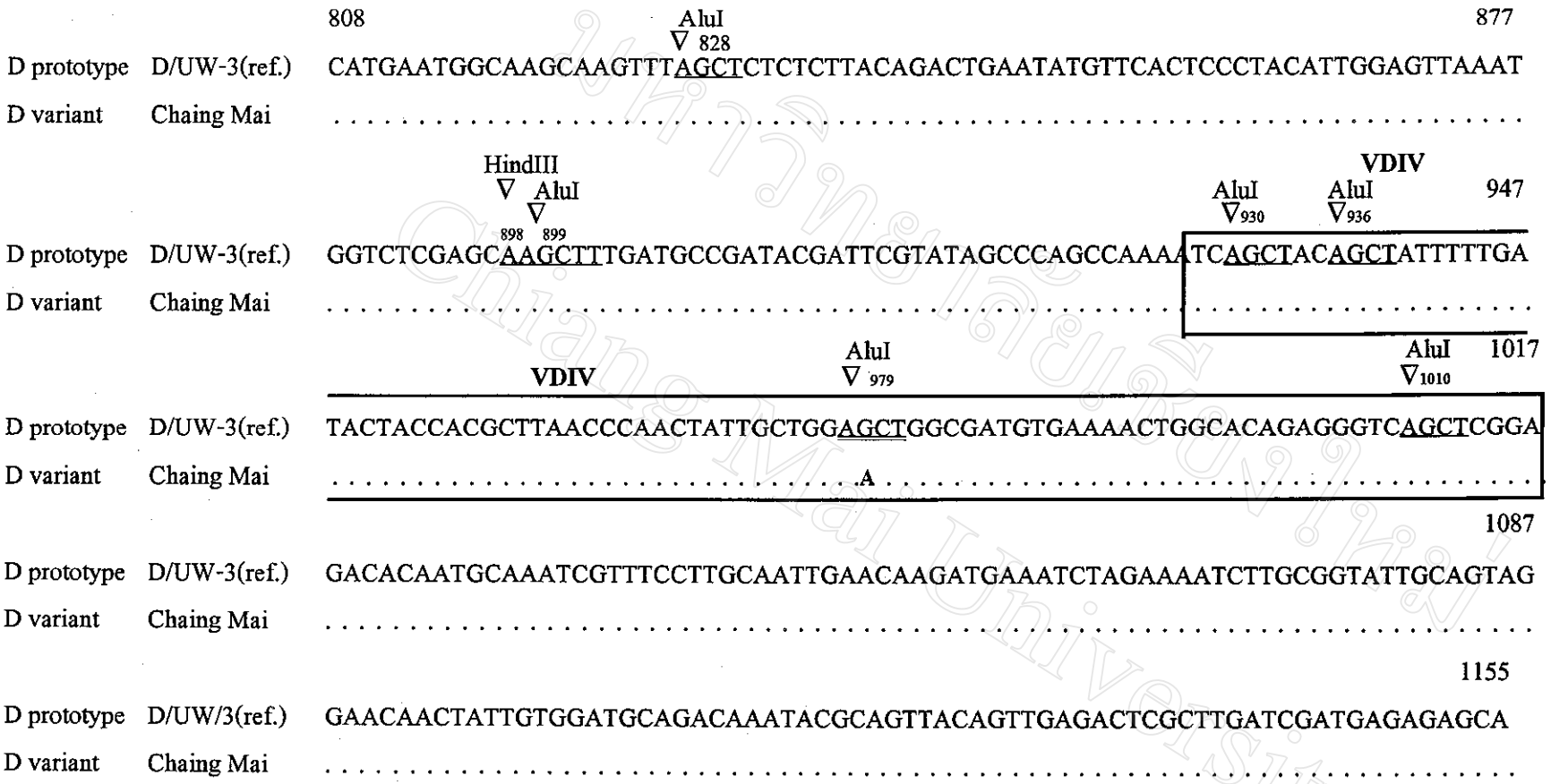


Figure 25. Comparison of the Nest2-Nest 4 nucleotide sequence and the restriction sites from the prototype D/UW-3 and the D variant, found in this study. The restrictions sites are underlined and double underlined showed the restriction site with nucleotide substitution. The VD4-MOMP gene is in the box.

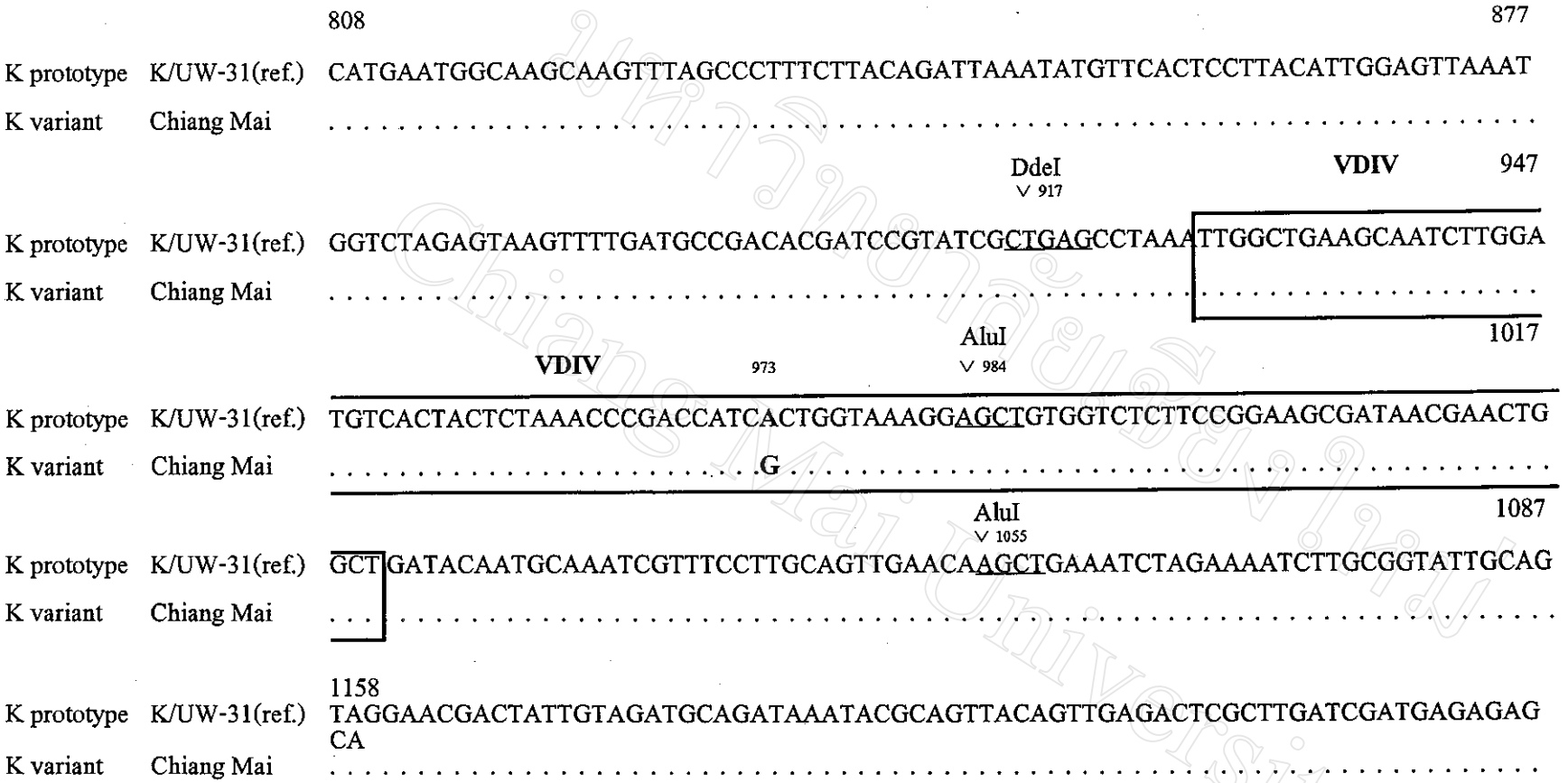
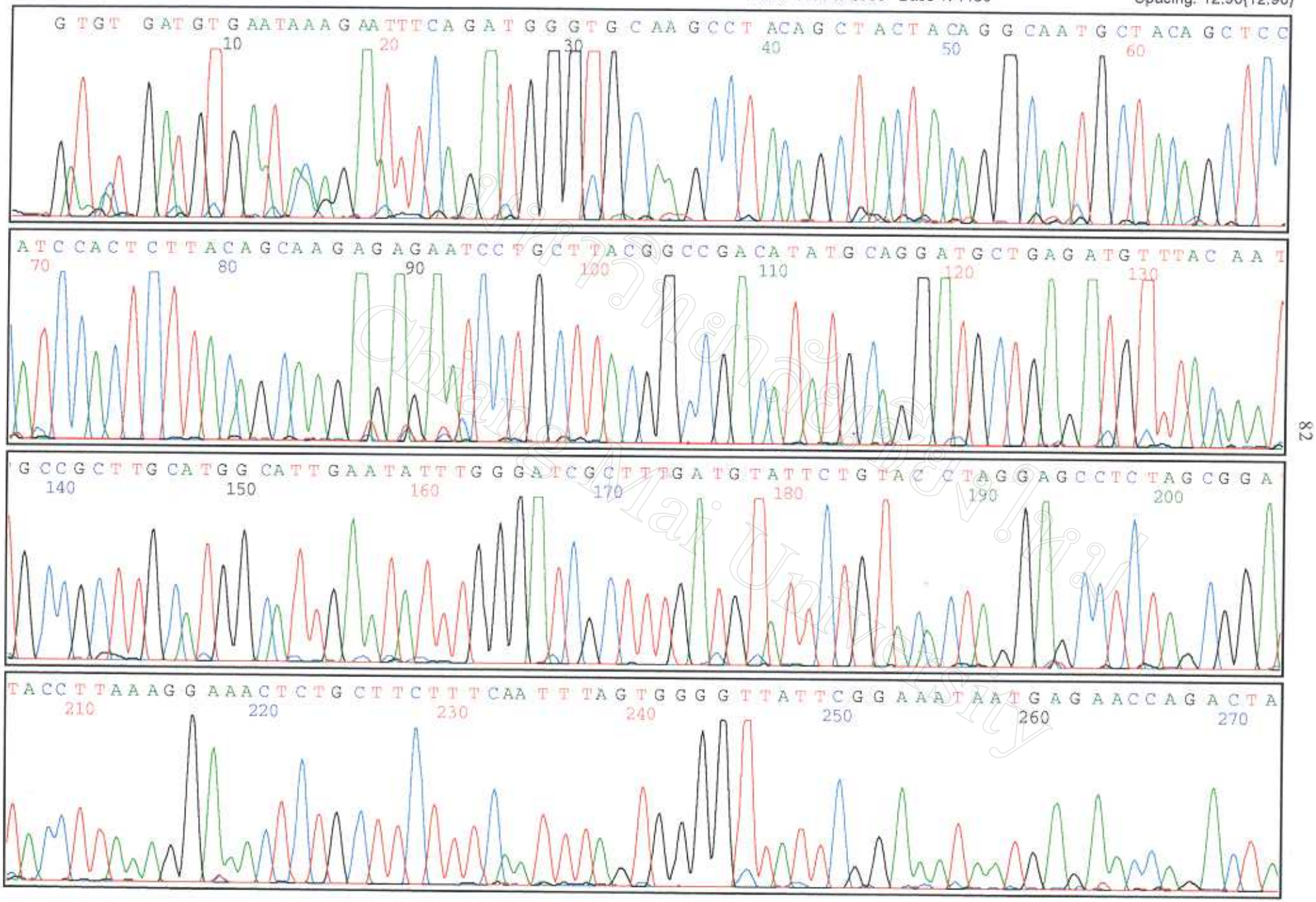


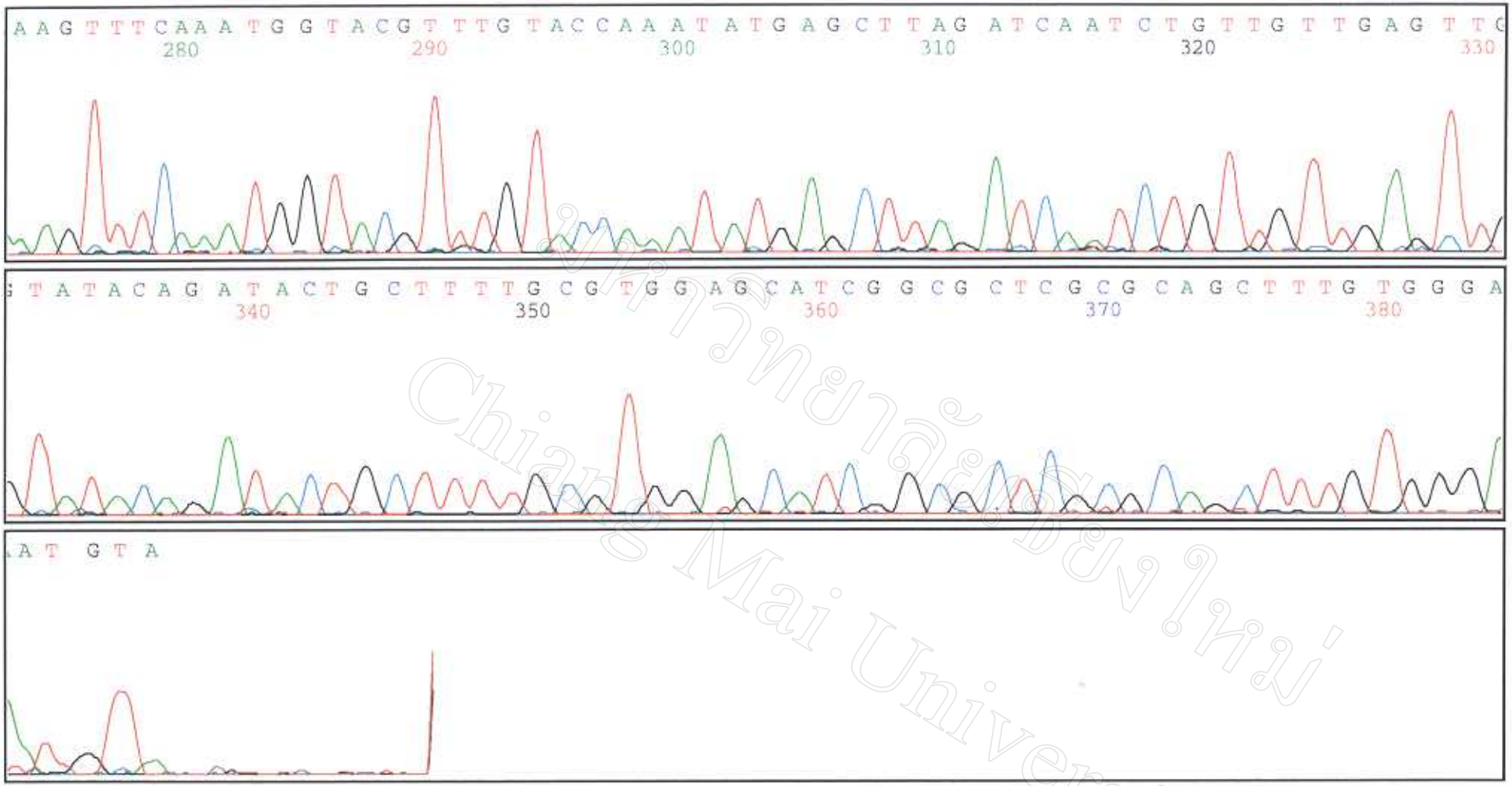
Figure 26. Comparison of the Nest2-Nest4 amplification sequences and the restriction sites from the prototype K/UW-31 and the K variant, found in this study. The restriction sites are underlined. The VD4-MOMP gene is in the box.

Figure 27. The electrophoregram of the nucleotide sequence in VD1-2 MOMP gene of *C. trachomatis* genotype Ba, detected in Chiang Mai by automated DNA sequencer. The letter numbers indicate the positions of the bases in the DNA segment being sequenced.

PRISM Version 3.0b1 Lane 3 Points 1130 to 6300 Base 1: 1130 Spacing: 12.96{12.96}



PRISM Version 3.0b1 Lane 3 Points 1130 to 6300 Base 1: 1130 Spacing: 12.96(12.96)



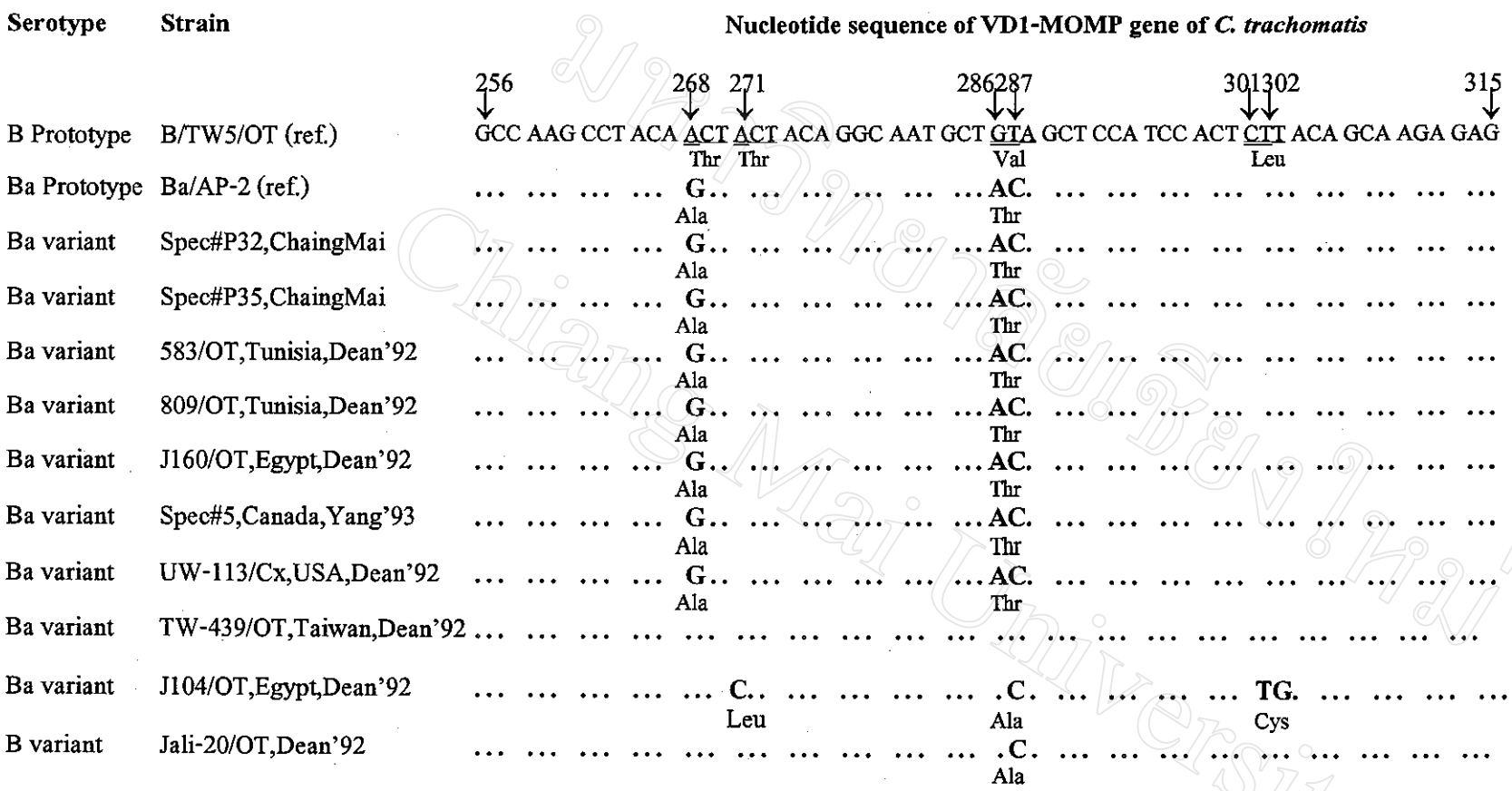


Figure 28. Nucleotide and amino acid sequence comparison of the VD1-MOMP gene of the prototype B/TW5/OT, Ba/AP-2, the genital Ba strain in this study and respective variants. The nucleotide substitutions are double underlined and the codons having nucleotide substitutions are underlined.

Serotype	Strain	481										511	514		522								546
B Prototype	B/TW5/OT(ref.)	AAT	AAT	GAG	AAC	CAG	ACT	AAA	GTT	TCA	AAT	GGT Gly	GCG Ala	TTT	GTA Val	CCA	AAT	ATG	AGC	TTA	GAT	CAA	TCT
Ba Prototype	Ba/AP-2 (ref.)	...	...	...	...	...	...	...	...	...	...	A.. Ser	A.. Thr	...	...	...	...	...	...	...	...	...	...
Ba variant	Spec#P32,ChaingMai	...	...	...	...	...	...	...	...	...	...	...	A.. Thr	...	...	...	...	...	...	...	...	...	...
Ba variant	Spec#P35,ChaingMai	...	...	...	...	...	...	...	...	...	...	...	A.. Thr	...	...	...	...	...	...	...	...	...	...
Ba variant	583/OT,Tunisia,Dean'92	...	...	...	...	...	...	...	...	...	...	...	A.. Thr	...	...	...	...	...	...	...	...	...	...
Ba variant	809/OT,Tunisia,Dean'92	...	...	...	...	...	...	...	...	...	...	...	A.. Thr	...	...	...	...	...	...	...	...	...	...
Ba variant	J160/OT,Egypt,Dean'92	...	...	...	...	...	...	...	...	...	...	...	A.. Thr	...	...	...	...	...	...	...	...	...	...
Ba variant	Spec#5/Cx,Canada,Yang'93	...	...	...	...	...	...	...	...	...	...	...	A.. Thr	...	...	...	...	...	...	...	...	...	...
Ba variant	UW-113/Cx,USA,Dean'92	...	...	...	...	...	...	...	...	...	...	A.. Ser	A.. Thr	...	...	...	...	...	...	...	...	...	...
Ba variant	TW-439/OT,Taiwan,Dean'92	...	...	...	...	...	...	...	...	...	...	...	A.. Thr	...	..G Val	...	...	...	...	...	...	...	...
Ba variant	J104/OT,Egypt,Dean'92	...	...	...	...	...	...	...	...	...	...	...	A.. Thr	...	...	...	...	...	...	...	...	...	...
B variant	Jali-20/OT,Dean'92	...	...	...	...	...	...	...	...	...	...	...	A.. Thr	...	...	...	...	...	...	...	...	...	...

Figure 29 . Nucleotide and amino acid sequence comparison of the VD2-MOMP gene of the prototype B/TW5/OT, Ba/AP-2, the genital Ba strain in this study and respective variants. The nucleotide substitutions are double underlined and the codons having nucleotide substitutions are underlined.