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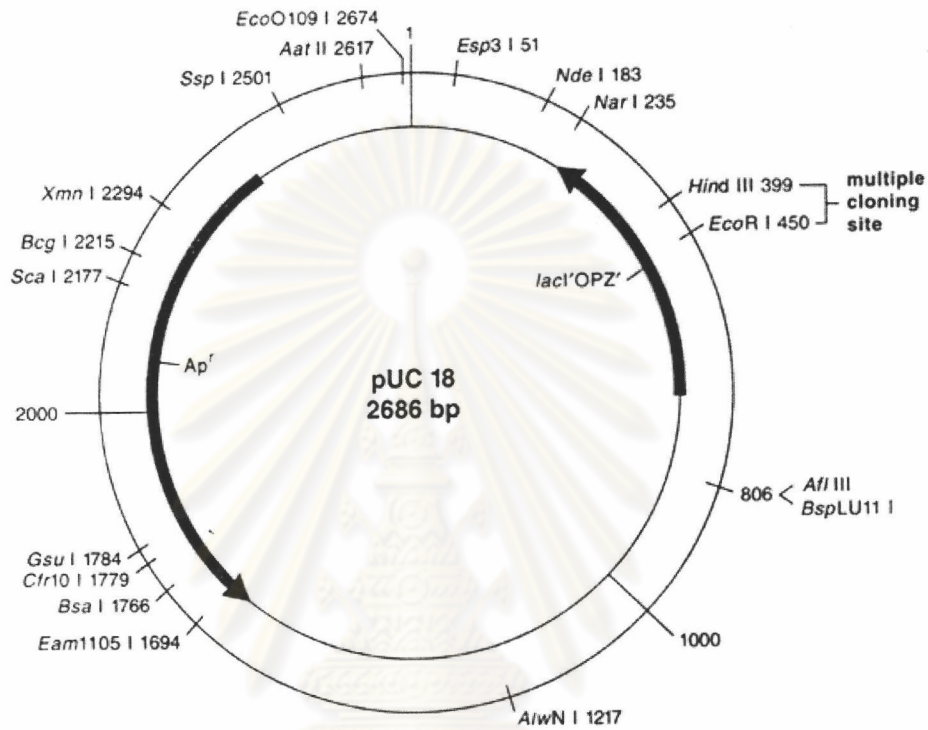
# APPENDICES

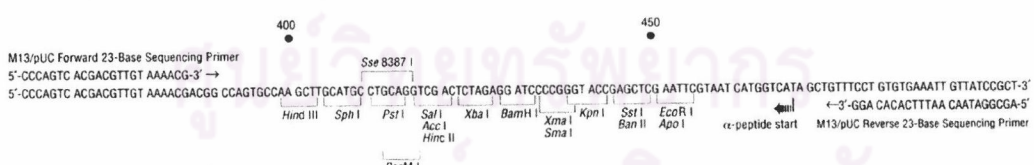
# APPENDIX A

## Restriction map of pUC18




The sequence has not been confirmed by sequence analysis. It was assembled from the known sequence of fragments used to construct the vector.

**pUC18 multiple cloning site and primer binding regions: 364-500**



# APPENDIX B

## QIAquick gel extraction kit protocol

1. The DNA fragment from the agarose gel was excised with a clean and sharp scalpel.
2. The gel slice was weighed in a colorless tube. Then, 3 volumes of buffer QG was added to 1 volume of gel (100 mg ~ 100 µl).
3. The tube was incubated at 50 °C and mixed by vortexing the tube every 2-3 minutes until the gel slice had completely dissolved.
4. After the gel slice had dissolved completely, 1 gel volume of isopropanol was added to the sample and mixed.
5. QIAquick spin column was placed in a provided 2-ml collection tube.
6. To trap DNA, the sample was applied to the QIAquick column and centrifuged at 10,000 rpm for 1 minute.
7. The flow-through was discarded and QIAquick column was placed back in the same collection tube.
8. Then, 0.5 ml of buffer QG was added to QIAquick column and centrifuged at 10,000 rpm for1 minute.
9. Buffer PE 0.75 ml was added to QIAquick column to wash and further centrifuged at 10,000 rpm for 1 minute.
10. The flow-through was discarded and QIAquick column was centrifuged at 10,000 rpm for an additional 1 minute.
11. Finally, 50 µl of buffer EB (10 mM Tris-Cl, pH 8.5) was added to elute DNA and centrifuged at 10,000 rpm for 1 minute.

# APPENDIX C

## Preparation of *E. coli* competent cells for electroporation (Dower, 1988)

1. A fresh overnight culture of *E. coli* JM 109 was inoculated into 1 liter of LB broth with 1 volume of overnight culture to 100 volume of LB broth.
2. Cells were grown to log phase at 37 °C with vigorous shaking. The $OD_{600}$ was about 0.5 to 0.8.
3. To harvest, the culture was chilled on ice for 15 to 30 minutes, and then centrifuged at 8,000 x g for 15 minutes at 4 °C.
4. The cells were washed with 1 liter of cold water, were spun down and washed again with 0.5 liter of cold water.
5. After the centrifugation, cells were resuspended in approximately 20 ml of 10% glycerol in distilled water and centrifuged at 8,000 x g for 15 minutes at 4 °C.
6. The cell pellets were resuspended to a final volume of 2 to 3 ml in 10 % glycerol. This suspension was stored at –70 °C until used.

# APPENDIX D

## Bradford Solution

1. Bradford Stock Solution

   100 ml of 95 % ethanol

   200 ml of 85 % phosphoric acid

   350 mg Serva Blue G

2. Bradford Working Buffer

   425 ml of distilled water

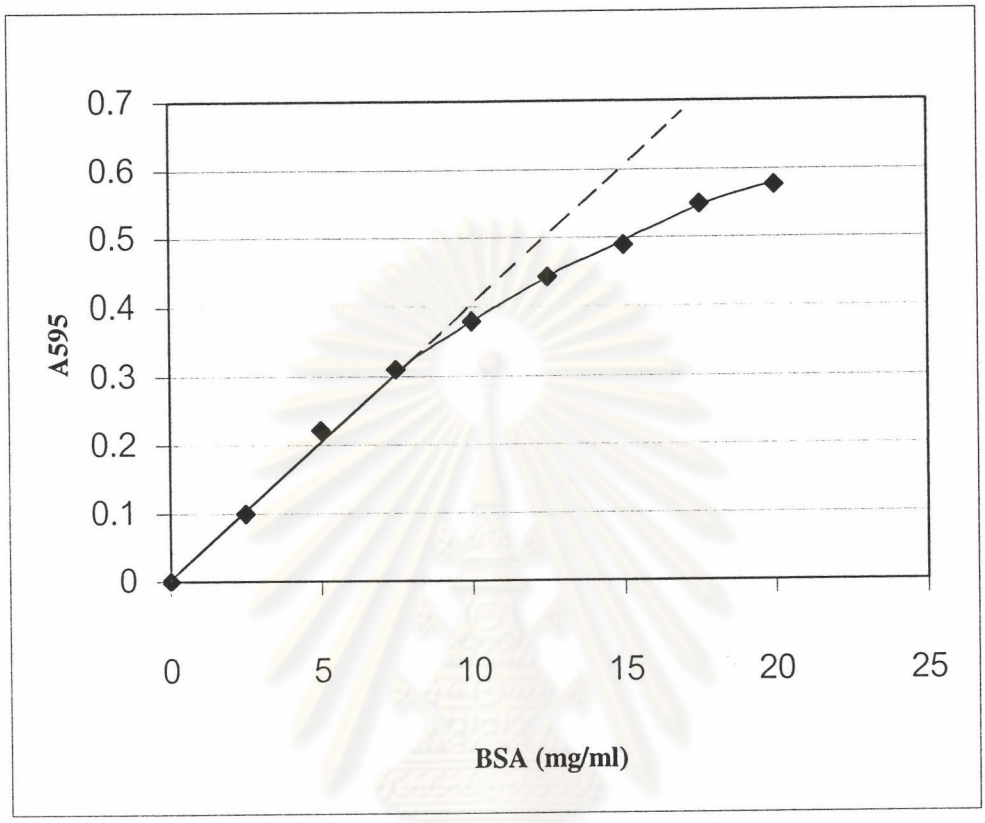
   15 ml of 95 % ethanol

   30 ml of 88 % phosphoric acid

   30 ml of Bradford Stock Solution

# APPENDIX E

## Standard curve for protein determination by Bradford's method



# APPENDIX F

## Polyacrylamide gel electrophoresis solution

### Discontinuous Nondenaturing Gel Electrophoresis

**1) Solution A** (Acrylamide Stock Solotion), 100 ml

*30 % Acrylamide, 0.8 % Bis-acrylamide*

Distilled water was added to an Erlenmeyer flask contained 30 g acrylamide and 0.8 g bis-acrylamide powder to make 100 ml and stirred until completely dissolved.

**2) Solution B** (4 x Separating Buffer), 100 ml

*1.5 M Tris-HCl (pH 8.8)*

18.2 g of Tris was added to 40 ml $H_2O$. Then adding HCl to adjusted solution to pH 8.8 and make 100 ml with $H_2O$.

**3) Solution C** (4 x Stacking Buffer), 100 ml

*0.5 M Tris (pH 6.8)*

6.0 g of Tris-HCl was added to 40 ml $H_2O$. Then adding HCl to adjusted solution to pH 6.8 and make 100 ml with $H_2O$.

**4) 10 % Ammonium Persulfate**, 5 ml

0.5 g of ammonium persulfate was dissolved in 5 ml $H_2O$

**5) Electrophoresis Buffer**, 1 liter

3.0 g and 14.4 g of Tris (25 mM) and glycine (192 mM) were dissolved in 1 liter $H_2O$

**6) 5 x Sample Buffer**, 10 ml

1.4 ml of $H_2O$ containing 3.1 ml 1M Tris-HCl, pH 6.8 (312.5 mM), 5 ml glycerol (50 %) and 0.5 ml 1% bromophenol blue (0.05 %)

**7) Protein staining solution**

**- Coomassie Gel Stain**, 1 liter, contained 1.0 g Coomassie Blue R-250, 450 ml methanol, 450 ml $H_2O$ and 100 ml glacial acetic acid

**- Coomassie Gel Destain**, 1 liter, contained 100 ml methanol, 100 ml glacial acetic acid and 800 ml $H_2O$

**8) Enzyme activity staining solution** contained 4.25 mM Tris-HCl, pH 8.5, 40 μM L-phenylalanine, 50 μM $NAD^+$, 250 μg phenazine methosulfate and 2.5 mg nitroblue tetrazolium in 10 ml distilled water

# APPENDIX G

## Description of *Brevibacillus brevis* (Buchanan, 1974 and Shida, 1996).

Brevi.ba.cil'lus. L.adj. *brevis*, short; L. dim. N. *bacillus*, small rod; M. L. masc. N. *Brevibacillus*, short, small rod.

The recognition of this species depends largely on: (1) the elliptical spore which distends the sporangium into a spindle-shaped or clavate body; when liberated, its surface shows considerable stainability; (2) acid formation from glucose may be positive, weak or absent; it is not detectable in glucose peptone water.

Minimal nutritional requirement of most strains is a mixture of amino acids without vitamins. Aerobic. The inclusion of strains having quite different temperature growth ranges suggests that the species is heterogeneous in spite of the phenetic evidence indicating that it is homogenous.

Has been isolated chiefly from soil and foods.

The G+C content of the DNA in mole percent is reported to be 42.5 - 47.0 ($T_m$).

### The characteristics of *Brevibacillus brevis*.

| Characteristic | *Brevibacillus brevis* |
|---|---|
| Spore | |
| - Shape | E |
| - Distends sporangium distinctly | + |
| - Dominant position | CT |
| Product of action on glucose | |
| - Acid | + or – |
| - Gas | - |
| - Acetoin | - |
| Easily stainable body attached to one side of spore | - |
| Motility | + |
| Temperature for growth, C | |
| - Maximum | 40 – 60 |
| - Minimum | 10 - 35 |
| Catalase activity | + |
| Hydrolysis of starch | - |
| Production of | |
| - Alkaline reaction in V-P broth | + |
| - Indole | - |
| $NO_3^-$ to $NO_2^-$ | d |
| Decomposition of | |
| - Casein | + |
| - Tyrosine | + |
| Deamination of phenylalanine | - |

The symbols used are: E, elliptical or cylindrical; CT, central to terminal, variation within or between strains.

+ = positive for 90-100 % of strains; - = negative for 90-100 % of strains; d = reactions differ, positive for 11-89 % of strains.

# APPENDIX H

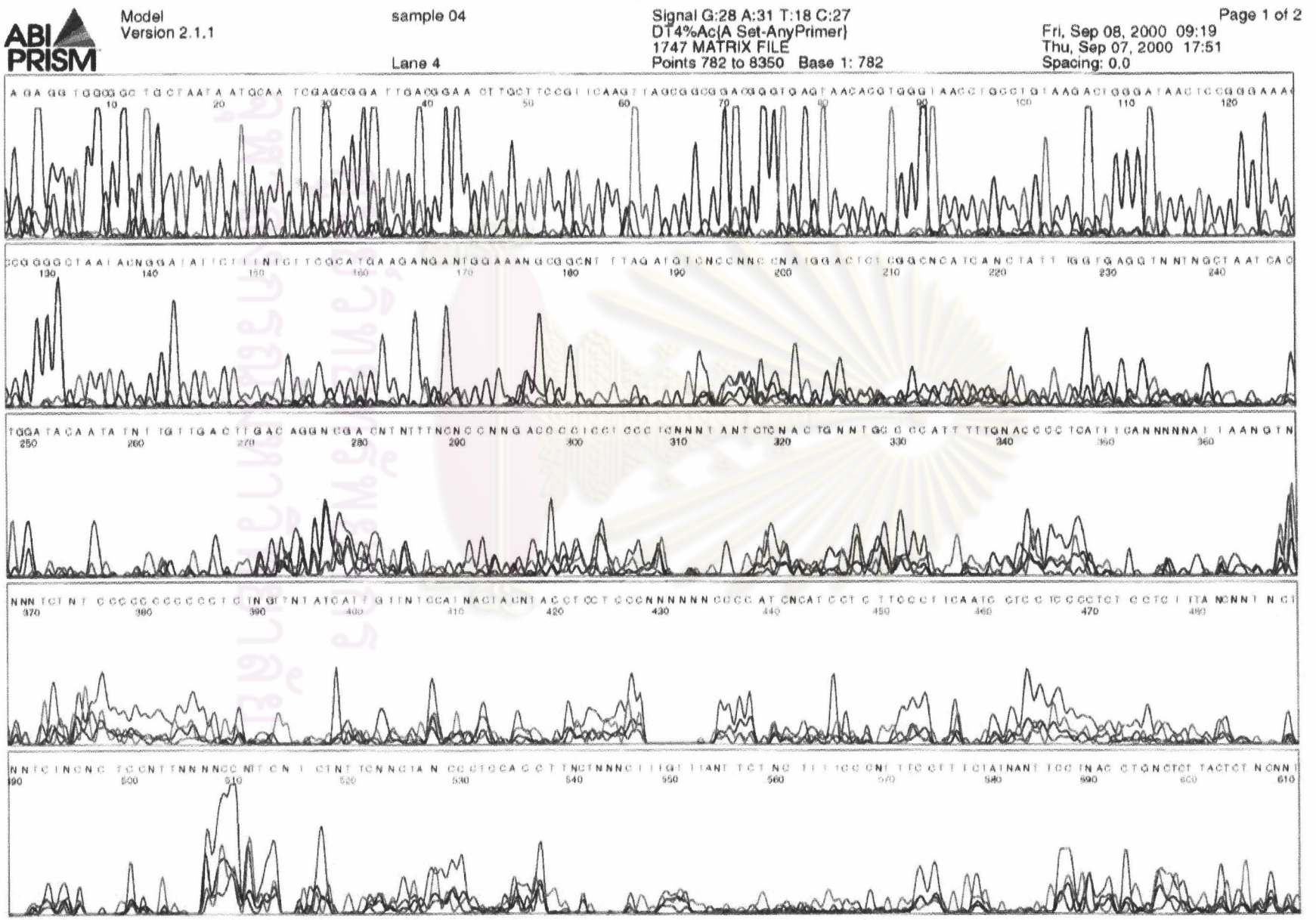
**The DNA sequencing profile of the 16S rRNA gene fragment from thermotolerant bacterial strain BC1.**

(a) = The DNA sequencing profile of the 16S rRNA gene fragment from thermotolerant bacterial strain BC1 using the sense primer A.

(b) = The DNA sequencing profile of the 16S rRNA gene fragment from thermotolerant bacterial strain BC1 using the sense primer D.

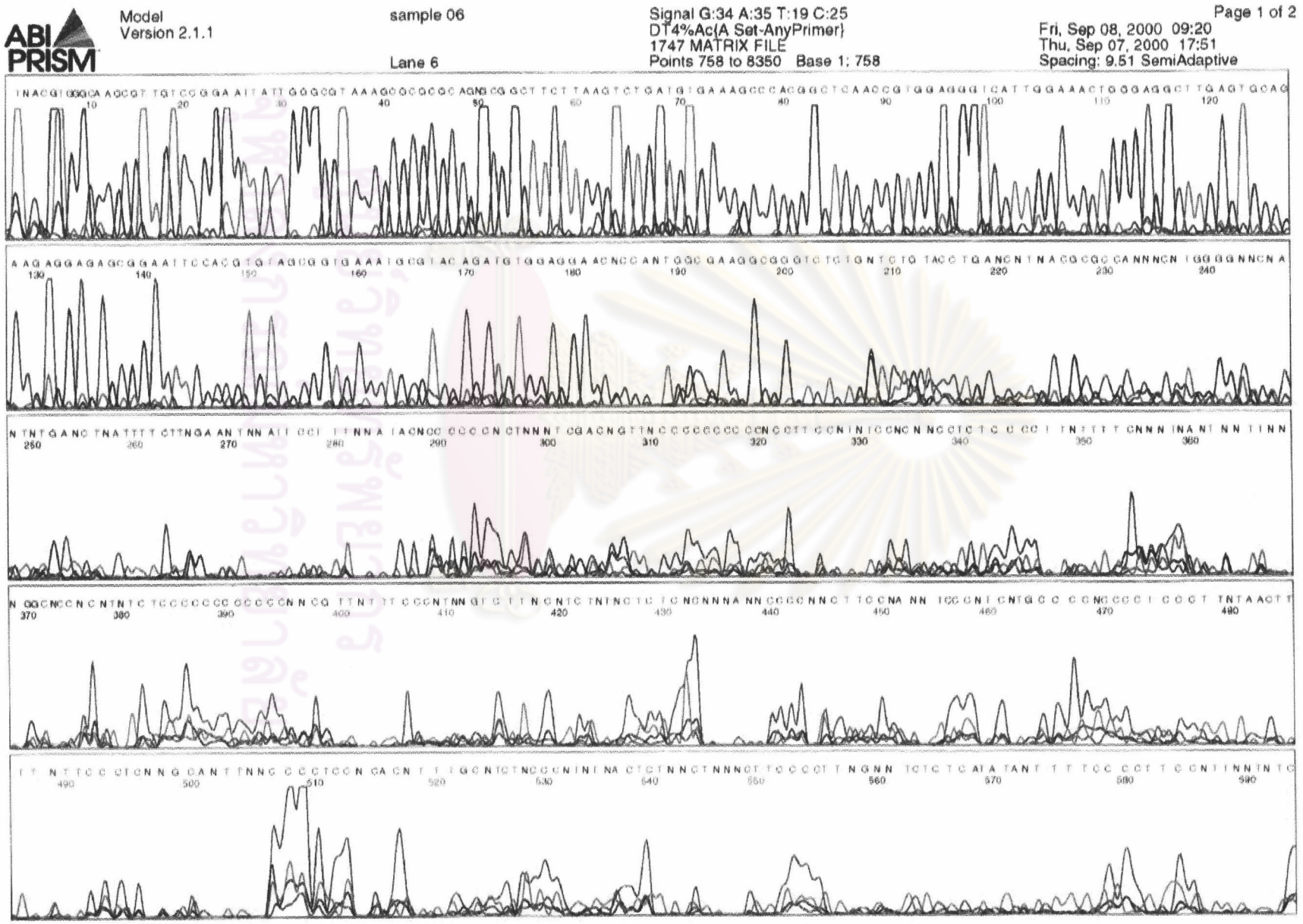
(c) = The DNA sequencing profile of the 16S rRNA gene fragment from thermotolerant bacterial strain BC1 using the antisense primer D'.

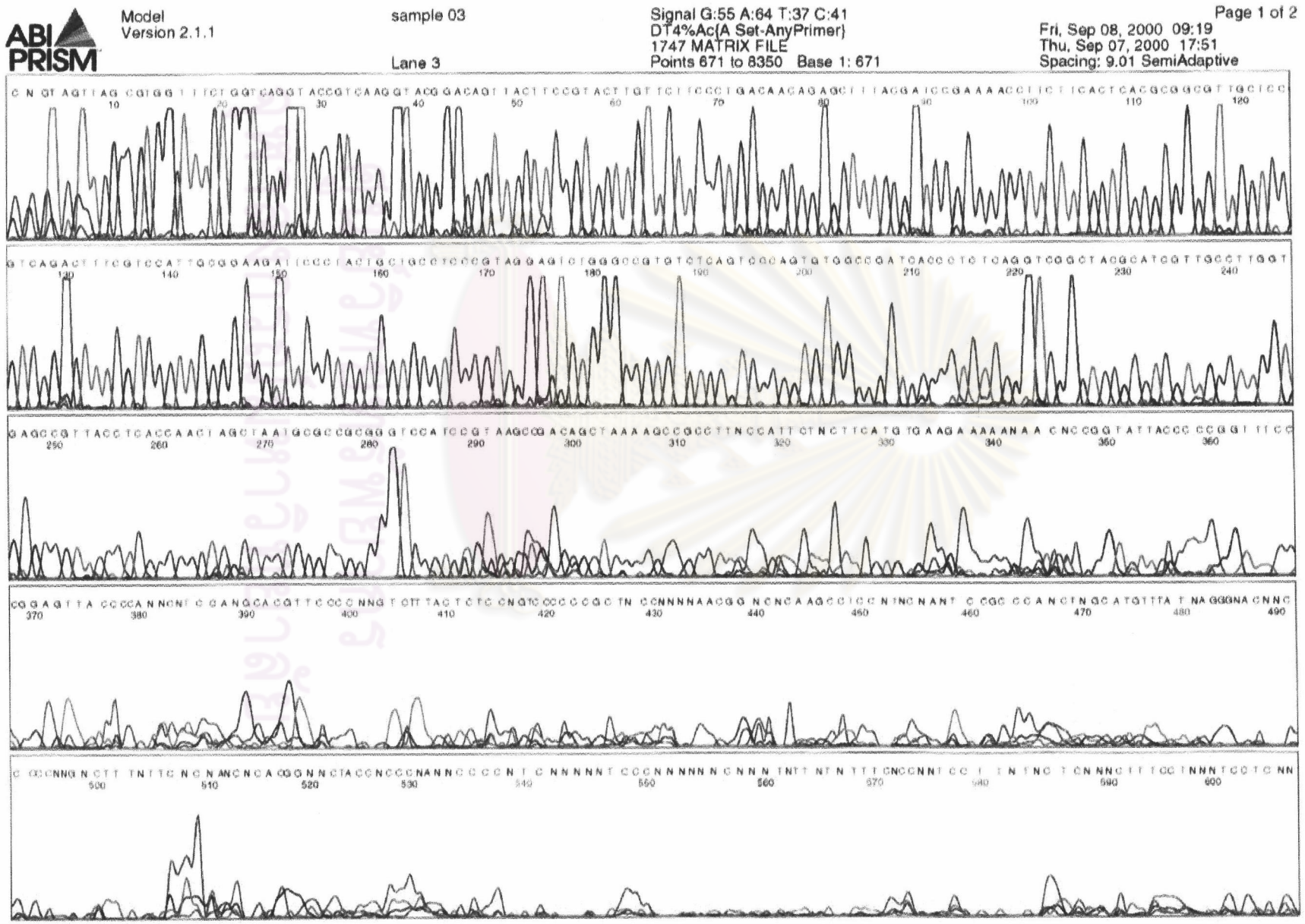
(d) = The DNA sequencing profile of the 16S rRNA gene fragment from thermotolerant bacterial strain BC1 using the sense primer F.


ABI PRISM
Model
Version 2.1.1
sample 04
Lane 4
Signal G:28 A:31 T:18 C:27
DT4%Ac(A Set-AnyPrimer)
1747 MATRIX FILE
Points 782 to 8350 Base 1: 782
Page 1 of 2
Fri, Sep 08, 2000 09:19
Thu, Sep 07, 2000 17:51
Spacing: 0.0

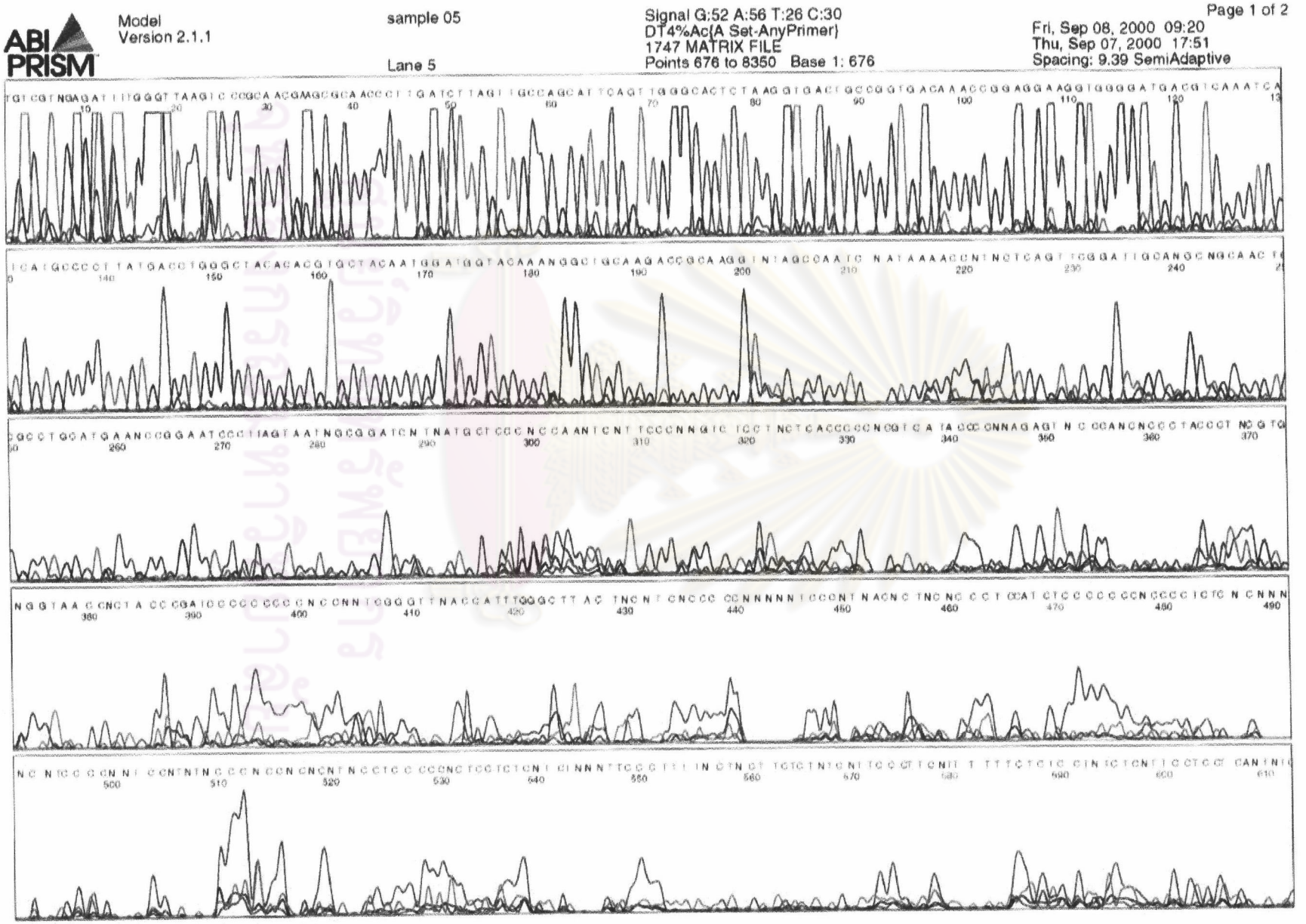
(a)

(g)


ABI PRISM
Model
Version 2.1.1
sample 06
Lane 6
Signal G:34 A:35 T:19 C:25
DT4%Ac(A Set-AnyPrimer)
1747 MATRIX FILE
Points 758 to 8350 Base 1: 758
Page 1 of 2
Fri, Sep 08, 2000 09:20
Thu, Sep 07, 2000 17:51
Spacing: 9.51 SemiAdaptive


ABI PRISM
Model
Version 2.1.1
sample 03
Lane 3
Signal G:55 A:64 T:37 C:41
DT4%Ac{A Set-AnyPrimer}
1747 MATRIX FILE
Points 671 to 8350 Base 1: 671
Page 1 of 2
Fri, Sep 08, 2000 09:19
Thu, Sep 07, 2000 17:51
Spacing: 9.01 SemiAdaptive

(c)

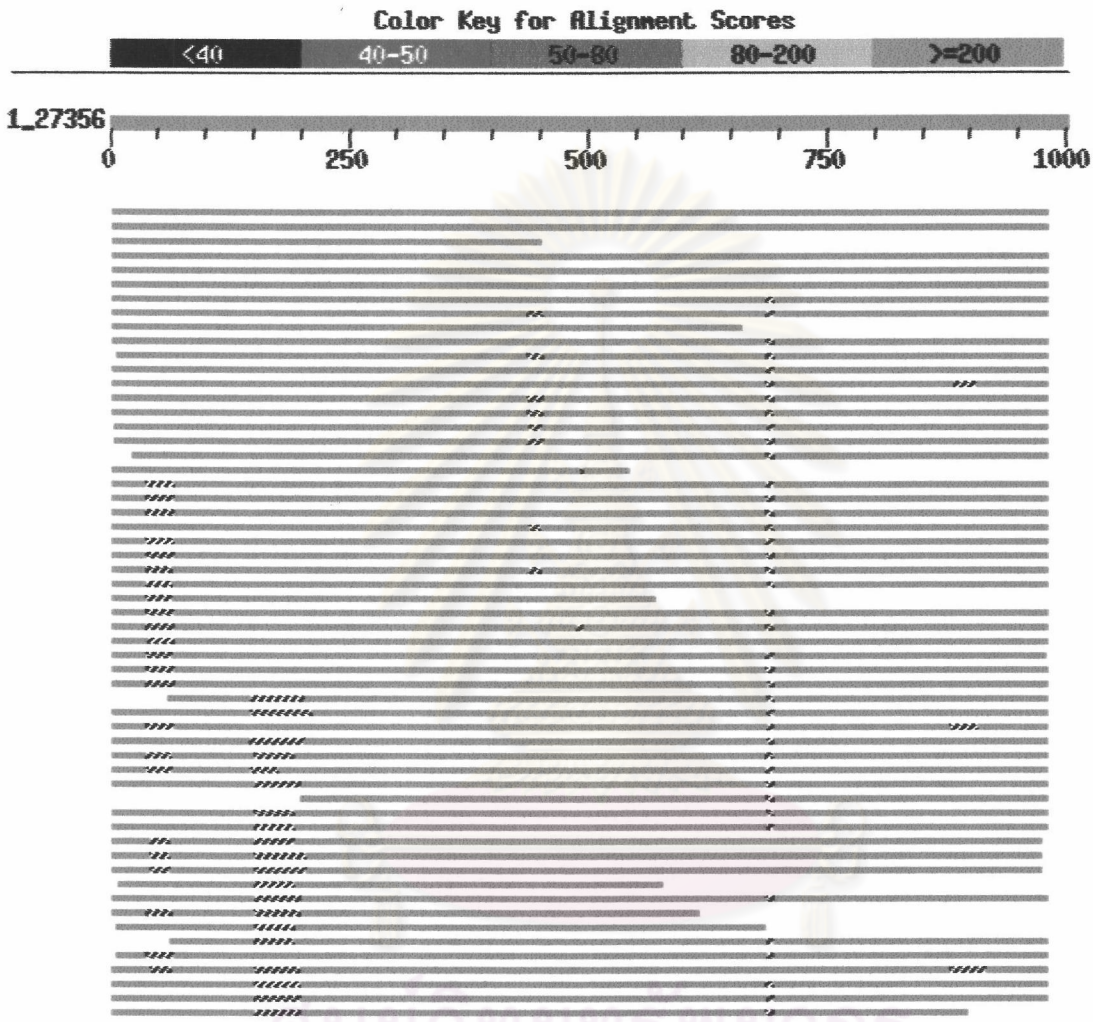

ABI PRISM
Model
Version 2.1.1
sample 05
Lane 5
Signal G:52 A:56 T:26 C:30
DT4%Ac(A Set-AnyPrimer)
1747 MATRIX FILE
Points 676 to 8350 Base 1: 676
Page 1 of 2
Fri, Sep 08, 2000 09:20
Thu, Sep 07, 2000 17:51
Spacing: 9.39 SemiAdaptive

(p)

# APPENDIX I

**Blast result of 16S rRNA gene sequence from thermotolerant bacterial strain BC1**

## Distribution of 593 Blast Hits on the Query Sequence




| Sequences producing significant alignments: | Score (bits) | E Value |
|---|---|---|
| gi\|456616\|emb\|X77790.1\|BB16SRR B.badius (ATCC 14574) 16S rR... | 942 | 0.0 |
| gi\|39349\|emb\|X60610.1\|BBAD16S B.badius 16S ribosomal RNA | 900 | 0.0 |
| gi\|20069538\|emb\|AJ438292.1\|BSP438292 Bacillus sp. R-1168 pa... | 854 | 0.0 |
| gi\|15076637\|dbj\|AB066346.1\|AB066346 Bacillus sp. TH64 gene ... | 692 | 0.0 |
| gi\|15076628\|dbj\|AB066337.1\|AB066337 Bacillus sp. TP81 gene ... | 692 | 0.0 |
| gi\|15076627\|dbj\|AB066336.1\|AB066336 Bacillus sp. TP50 gene ... | 692 | 0.0 |
| gi\|16517855\|gb\|AF423263.1\|AF423263 Uncultured soil bacteriu... | 674 | 0.0 |
| gi\|14326032\|gb\|AF326373.1\| Bacillus sp. SG-1 16S ribosomal ... | 654 | 0.0 |
| gi\|4220578\|emb\|AJ011423.1\|UBA011423 unidentified bacterium ... | 654 | 0.0 |
| gi\|16517891\|gb\|AF423299.1\|AF423299 Uncultured soil bacteriu... | 650 | 0.0 |
| gi\|14326029\|gb\|AF326370.1\| Bacillus sp. PL-30 16S ribosomal... | 648 | 0.0 |
| gi\|14326031\|gb\|AF326372.1\| Bacillus sp. SD-18 16S ribosomal... | 646 | 0.0 |
| gi\|15215555\|gb\|AY043084.1\| Bacillus circulans 16S ribosomal... | 646 | 0.0 |
| gi\|457645\|dbj\|D16268.1\|BAC16SRR03 Bacillus firmus 16S rRNA ... | 646 | 0.0 |
| gi\|14326020\|gb\|AF326361.1\| Bacillus sp. MB-12 16S ribosomal... | 640 | 0.0 |
| gi\|14326027\|gb\|AF326368.1\| Bacillus sp. PL-21 16S ribosomal... | 636 | e-179 |
| gi\|1220197\|gb\|U49078.1\|BSU49078 Bacillus sporothermodurans ... | 636 | e-179 |

| | | | |
|---|---|---|---|
| gi\|15215556\|gb\|AY043085.1\| | Bacillus benzoevorans 16S riboso... | 613 | e-172 |
| gi\|11907564\|gb\|AF114719.1\|AF114719 | Bacillus sp. m38b 16S ri... | 605 | e-170 |
| gi\|14326023\|gb\|AF326364.1\| | Bacillus sp. MB-7 16S ribosomal ... | 599 | e-168 |
| gi\|14326018\|gb\|AF326359.1\| | Bacillus sp. MB-1 16S ribosomal ... | 599 | e-168 |
| gi\|9368344\|emb\|AJ276808.1\|BSP276808 | Virgibacillus picturae ... | 599 | e-168 |
| gi\|19548708\|gb\|AF483625.1\| | Bacillus aquaemaris strain TF-12... | 595 | e-167 |
| gi\|14326021\|gb\|AF326362.1\| | Bacillus sp. MB-3 16S ribosomal ... | 583 | e-163 |
| gi\|16973333\|emb\|AJ315060.1\|VSP315060 | Virgibacillus picturae... | 583 | e-163 |
| gi\|7209541\|dbj\|AB021194.1\|AB021194 | Bacillus niacini gene fo... | 573 | e-160 |
| gi\|7209529\|dbj\|AB021182.1\|AB021182 | Bacillus carboniphilus g... | 573 | e-160 |
| gi\|15789010\|gb\|AY037578.1\| | Uncultured soil bacterium clone ... | 571 | e-160 |
| gi\|19069925\|emb\|AJ316297.1\|VPI316297 | Virgibacillus picturae... | 569 | e-159 |
| gi\|12830434\|emb\|AJ309562.1\|BNI309562 | Bacillus nitritophilus... | 569 | e-159 |
| gi\|6318160\|emb\|AJ229238.1\|BSA229238 | Bacillus from anoxic bu... | 567 | e-159 |
| gi\|2326376\|emb\|Y07605.1\|BS16SRM09 | Uncultured bacterium DA01... | 555 | e-155 |
| gi\|12830432\|emb\|AJ309559.1\|BLI309559 | Bacillus litoralis par... | 551 | e-154 |
| gi\|3925805\|dbj\|AB020192.1\|AB020192 | Bacillus sp. DNA for 16S... | 545 | e-152 |
| gi\|19568797\|gb\|AF479371.1\| | Glacial ice bacterium SB100-8-1-... | 535 | e-149 |
| gi\|396502\|emb\|X62178.1\|BANCIMB | B.aminovorans NCIMB 8292 (T)... | 529 | e-147 |
| gi\|19568773\|gb\|AF479347.1\| | Glacial ice bacterium G200-N5 16... | 527 | e-147 |
| gi\|7415828\|dbj\|AB034720.1\|AB034720 | Uncultured compost bacte... | 527 | e-147 |
| gi\|10444086\|gb\|AF281158.1\|AF281158 | Bacillus kangii 16S ribo... | 525 | e-146 |
| gi\|1711250\|dbj\|D88778.1\|D88778 | Bacillus sp. gene for 16S ri... | 525 | e-146 |
| gi\|16973337\|emb\|AJ315064.1\|BSP315064 | Bacillus sp. 19496 16S... | 513 | e-142 |
| gi\|2326384\|emb\|Y07578.1\|BSY07578 | Uncultured bacterium DA115... | 513 | e-142 |
| gi\|15789023\|gb\|AY037591.1\| | Uncultured soil bacterium clone ... | 509 | e-141 |
| gi\|2664287\|emb\|Y15712.1\|MEY15712 | Macrococcus equipercicus 1... | 509 | e-141 |
| gi\|2664288\|emb\|Y15713.1\|MCY15713 | Macrococcus carouselicus 1... | 509 | e-141 |
| gi\|3892880\|emb\|Y15714.1\|MBY15714 | Macrococcus bovicus 16S rR... | 509 | e-141 |
| gi\|7209536\|dbj\|AB021189.1\|AB021189 | Bacillus lentus gene for... | 509 | e-141 |
| gi\|457649\|dbj\|D16272.1\|BAC16SRR07 | Bacillus lentus 16S rRNA ... | 509 | e-141 |
| gi\|39476\|emb\|X60616.1\|BFI16S | B.firmus 16S ribosomal RNA | 507 | e-141 |
| gi\|3411027\|gb\|AF018046.1\|AF018046 | Uncultured bacterium I-11... | 505 | e-140 |
| gi\|4220577\|emb\|AJ011422.1\|UBA011422 | unidentified bacterium ... | 505 | e-140 |
| gi\|1088408\|dbj\|D78315.1\|BAC16SRRG | Bacillus lentus strain JC... | 504 | e-139 |
| gi\|2706435\|emb\|AJ222833.1\|BAJ833 | Bacterial sp. 16S rRNA gen... | 500 | e-138 |
| gi\|16517841\|gb\|AF423249.1\|AF423249 | Uncultured soil bacteriu... | 498 | e-138 |
| gi\|16517797\|gb\|AF423204.1\|AF423204 | Uncultured soil bacteriu... | 498 | e-138 |
| gi\|9864168\|gb\|AF286486.1\|AF286486 | Bacillus sp. VAN35 16S ri... | 498 | e-138 |
| gi\|4585728\|emb\|AJ237708.1\|BMA237708 | Bacillus marinus 16S rR... | 498 | e-138 |
| gi\|7415827\|dbj\|AB034719.1\|AB034719 | Uncultured compost bacte... | 498 | e-138 |
| gi\|7209537\|dbj\|AB021190.1\|AB021190 | Bacillus marinus gene fo... | 498 | e-138 |
| gi\|8572542\|gb\|AF227844.2\|AF227844 | Bacillus sp. 76992 16S ri... | 496 | e-137 |
| gi\|14326028\|gb\|AF326369.1\| | Bacillus sp. PL-26 16S ribosomal... | 494 | e-137 |
| gi\|14326026\|gb\|AF326367.1\| | Bacillus sp. PL-16 16S ribosomal... | 494 | e-137 |
| gi\|3328011\|gb\|AF071856.1\|AF071856 | Bacillus sp. 171544 16S r... | 494 | e-137 |
| gi\|12275965\|gb\|AF275714.1\|AF275714 | Unidentified Hailaer sod... | 494 | e-137 |
| gi\|12056335\|emb\|AJ295684.1\|UBA295684 | Bacterium IrT-RS2 part... | 494 | e-137 |
| gi\|2293104\|emb\|AJ000983.1\|UB16SDA34 | Uncultured bacterium DA... | 494 | e-137 |
| gi\|5834511\|emb\|Y14693.1\|BBY14693 | Bacillus benzoevorans 16S ... | 494 | e-137 |
| gi\|7415820\|dbj\|AB034712.1\|AB034712 | Uncultured compost bacte... | 494 | e-137 |
| gi\|11414967\|dbj\|AB043854.1\|AB043854 | Bacillus sp. N6 gene fo... | 494 | e-137 |
| gi\|19568777\|gb\|AF479351.1\| | Glacial ice bacterium G200-T19 1... | 490 | e-135 |
| gi\|14326030\|gb\|AF326371.1\| | Bacillus sp. PL-7 16S ribosomal ... | 490 | e-135 |
| gi\|7415825\|dbj\|AB034717.1\|AB034717 | Uncultured compost bacte... | 490 | e-135 |
| gi\|10242129\|gb\|AF252320.1\|AF252320 | Uncultured bacterium pPD... | 488 | e-135 |
| gi\|15076632\|dbj\|AB066341.1\|AB066341 | Bacillus sp. TAT112 gen... | 488 | e-135 |
| gi\|19908353\|gb\|AY082367.1\| | Uncultured Bacillus sp. clone DG... | 486 | e-134 |
| gi\|19879238\|gb\|AY028328.1\| | Bacillus sp. ES20 16S ribosomal ... | 486 | e-134 |
| gi\|19879237\|gb\|AY028327.1\| | Bacillus sp. amh-4467 16S riboso... | 486 | e-134 |
| gi\|19568798\|gb\|AF479372.1\| | Glacial ice bacterium SB100-9-5-... | 486 | e-134 |
| gi\|19568762\|gb\|AF479336.1\| | Glacial ice bacterium G500K-16 1... | 486 | e-134 |
| gi\|15183058\|gb\|AY039415.1\| | Soil bacterium S76M1 16S ribosom... | 486 | e-134 |
| gi\|15183018\|gb\|AY039400.1\| | Earthworm burrow bacterium B6D1 ... | 486 | e-134 |
| gi\|8925909\|gb\|AF221062.1\|AF221062 | Bacillus sp. YKJ-11 16S r... | 486 | e-134 |

| | | | |
|---|---|---|---|
| gi\|14009321\|gb\|AY030327.1\| | Bacillus pumilus strain KL-052 1... | 486 | e-134 |
| gi\|12698847\|gb\|AF329473.1\|AF329473 | Bacillus sp. LMG 19636 1... | 486 | e-134 |
| gi\|9652393\|gb\|AF288735.1\|AF288735 | Bacillus pumilus 16S ribo... | 486 | e-134 |
| gi\|8778111\|gb\|AF270793.1\|AF270793 | Bacillus subtilis N5 16S ... | 486 | e-134 |
| gi\|16973340\|emb\|AJ315067.1\|BSP315067 | Bacillus sp. 19499 16S... | 486 | e-134 |
| gi\|7862189\|gb\|AF260751.1\|AF260751 | Bacillus pumilus strain F... | 486 | e-134 |
| gi\|7862181\|gb\|AF260745.1\|AF260745 | Bacillus pumilus strain I... | 486 | e-134 |
| gi\|7862180\|gb\|AF260744.1\|AF260744 | Bacillus pumilus strain I... | 486 | e-134 |
| gi\|4761973\|gb\|AF128759.1\|AF128759 | Soil bacterium is11 16S r... | 486 | e-134 |
| gi\|12830433\|emb\|AJ309561.1\|BLI309561 | Bacillus litoralis par... | 486 | e-134 |
| gi\|2293105\|emb\|AJ000981.1\|UB16SDA36 | Uncultured bacterium DA... | 486 | e-134 |
| gi\|407872\|emb\|Z26892.1\|SC16SRRNF | S.caseolyticus gene for 16... | 486 | e-134 |
| gi\|2664286\|emb\|Y15711.1\|MCY15711 | Macrococcus caseolyticus 1... | 486 | e-134 |
| gi\|10129890\|dbj\|AB048252.1\|AB048252 | Bacillus pumilus gene f... | 486 | e-134 |
| gi\|1199941\|dbj\|D83359.1\|STA16SRR07 | Staphylococcus caseolyti... | 486 | e-134 |
| gi\|3970889\|dbj\|AB020208.1\|AB020208 | Bacillus pumilus DNA for... | 486 | e-134 |
| gi\|9864166\|gb\|AF286484.1\|AF286484 | Bacillus sp. VAN23 16S ri... | 484 | e-134 |
| gi\|3328013\|gb\|AF071858.1\|AF071858 | Bacillus sp. 115898 16S r... | 484 | e-134 |

**Reference**:

Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schäffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.

# APPENDIX J

## Alignment of 16S rRNA gene sequence from thermotolerant bacterial strain BC1 and related organisms.

```
CLUSTAL X (1.64b) multiple sequence alignment


BC1             ------------------------AGAGGCTGGCGGCGTGCCTAATACATGCAAGTCGAG
BBA             GAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAG
BSM             GAGAGTTNGATCCTGGCTCAGGACGAACGCTNGCGGCGTGCCTAATACATGCAAGTCGAG
BCI             GAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTNATACATGCAAGTCGAG
BSI             GAGAGTTTNATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAG
BIN             GAGAGTTNGATCCTGGCTCAGGACGAACGCTGGCGGCATGCCTAATACATGCAAGTCGAG
BAZ             GAGAGTTTNANCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTNANACNTNCNAGTCGAG
BBR             GAGAGTTTGATCCTGGCTCAGGACGAACGCTNGNGGCGTGCCTAATACATGCAAGTCGAG
                                      * *** * *** ***** * ** * * *******

BC1             CGGACTTGACGGAAGCTTGCTTCCGTTCAAGTTAGCGGCGGACGGGTGAGTAACACGTGG
BBA             CGGACTTGACGGAAGCTTGCTTCCGTTCAAGTTAGCGGCGGACGGGTGAGTAACACGTGG
BSM             CGGACTTTCAAGAAGCTTGCTTTT-TGAAAGTTAGCGGCGGACGGGTGAGTAACACGTGG
BCI             CGGACTTTAA--AAGCTTGCTN---TTNAAGTNAGCGGCGGACGGGTGAGTAACACGTGG
BSI             CG-AATCGATGGGAGCTTGCTCCC--TGAGATNAGCGGCGGACGGGTGAGTAACACGTGG
BIN             CGAATGATGAAGAAGCTTGCTNCT-TCTGATTCAGCGGCGGACGGGTGAGTAACACGTGG
BAZ             CGGATGATAAAGGAGCTTGCTCCT-TTAGATTCAGCGGCGGACGGGTNAGTNANNCGTGG
BBR             CG-----------AGGGT-CTTC---GGACCCTAGCGGCGGACGGGTGAGTAACACGTAG
                **           **  * **              ************** *** *  *** *

BC1             GTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATATTCTT
BBA             GTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATATTCTT
BSM             GCAACCTGCCTGCAAGACGGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAATATC
BCI             GCAACCTGCCTGTAAGACTGGGATAACTTCGGGAAACCGGAGCTNATACCGGATNATCCT
BSI             GCAACCTGCCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGATACGTTC
BIN             GCAACCTGCCCTGTAGATTGGGATAACTCCGGGAAACCGGNGCTNATACCGAATAATCCA
BAZ             GCAACCTGCCTGTAAGACTGGGATAACTTCGGGAAACCGGAGCTNATACCGGATAACTCT
BBR             GCAACCTGCCTCTCAGACCGGGATAACATAGGGAAACTTATGCTAATACCGGATAGGTTT
                * ********    ***  ********   *******    *** ****** **

BC1             TTTCTTCGCATGAAGAAGACTGGAAACGCGGCTTTTAACTGTCGCTTACGGATGGACCCG
BBA             TTTCTTCGCATGAAGAAGAATGGAAAGGCGGCTTTTAACTGTCACTTACAGATGGACCCG
BSM             TTCCTTCGCATGAAGGAAGGTTGAAA-GCGGC----AACTGCCGCTTGCAGATGGGCCCG
BCI             NTTCCTCTCATGAGGAAAAGCTGAAAGACGGTTTA-CGCTGTCACTTACAGATGGGCCCG
BSI             TNTTCTCGCATGAGAGAAGATGGAAAGACGGTTTA-CGCTGTCACTAATAGATNGGCCNN
BIN             TTTCCTCTCATGGGGAAATGTTAAAAGACGGTTTC-GGCTNTCACTNCNGGATNGGCCCG
BAZ             TCGAACCTCATGGTTNGAAGATAAAAGATGGTTTC-GGCTATCACTTACNGANGGGCCCG
BBR             NTGGATCGCATGATCCGAAAAGAAAAGGCGGCTTC-GGCTGTCACTGGGAGATGGGCCTG
                      * ****          ***  **      ** * **    **  * **
```

(continued)

BC1 = Thermotolerant bacterial strain BC1, BBA = *Bacillus badius*, BSM = *Bacillus smithii*, BCI = *Bacillus circulans*, BSI = *Bacillus simplex*, BIN = *Bacillus insolitus*, BAZ = *Bacillus azotoformans* and BBR = *Brevibacillus brevis*

# APPENDIX J (continued)

## Alignment of 16S rRNA gene sequence from thermotolerant bacterial strain BC1 and related organisms.

(continued)

```
BC1             CGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCT
BBA             CGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCT
BSM             CGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCT
BCI             CGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCT
BSI             NGGCGCATTAGCTAGTTNGTGAGGTAATGGCTCACCAAGGCGACGATGCGTAGCCGACCT
BIN             CGGCGCNTTAGCTNGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCT
BAZ             CGGCGCNTTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCT
BBR             CGGCGCATTAGCTAGTTGGTGGGGTAACGGCCTACCAAGGCGACGATGCGTAGCCGACCT
                 ***** ****** *** *** ***** ***  ******** ******************

BC1             GAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCA
BBA             GAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCA
BSM             GAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCA
BCI             GAGAGGGTGATCGGCNACNCTGGGACTGAGACNCGGCCCAGACTCCTACGGGAGGCAGCA
BSI             GAGAGGGTNATCGGCCACACTGGGACTNAGACACGGCCCAGACTCCTACGGGAGGCAGCA
BIN             GAGAGGGTNATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCA
BAZ             GAGAGGGTNATCGGCCACNCTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCNGCA
BBR             GAGAGGGTGACCGGACACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCA
                ******** * ***  ** ******** **** ********************** ***

BC1             GTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGAAGAAG
BBA             GTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCNGCGTGAGTGAAGAAG
BSM             GTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGCGAAGAAG
BCI             GTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCNACGCCGCGTGAGTGATGAAG
BSI             GTAGGGAATCTTCCGCAATGGACGNAAGTCTGACGGAGCAACGCCGCGTGAACGAAGAAG
BIN             GTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAATGCCGCGTGAGTGAAGAAG
BAZ             GTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCNACGCCGCGTGAGCGATGAAG
BBR             GTAGGGAATTTTCCACAATGGACGGAAGTCTGATGGAGCAACGCCGCGTGAACGATGAAG
                ********* **** ********* ******** ***** * ** ******  ** ****

BC1             GTTTTCGGATCGTAAAGCTCTGTTGTCAGGGAAGAACAAGTACGGAAGTAACTGT-CCGT
BBA             GTTTTCGGATCGTAAAGCTNTGTTGTCAGGGAAGAACAAGTACGGAAGTAACTNT-CNGT
BSM             GTCTTCGGATCGTAAAGCTNTNTTGTCAGGGAAGAACAAGTACCGTTCGAACAGGGCGGT
BCI             GTNNTCGGATCGTAAAACTCTGTTGTTAGGGAAGAACAAGTACNAGAGTNACTNC-TGGT
BSI             GCCTTCGGGTCGTAAAGTTCTGTNGNNAGGGAAGAACAAGTACCAGAGTAACTGCNNNGT
BIN             GTTTTCGGATCGTAAAACTCTGTTGTGAGGGAAGAACAAGTACGAGAGTNACTNC-TNGT
BAZ             GCCTNCGGGTCGTNAAGCTNTGTTGTTNGGGAAGAACAAGTACCAGT-TNACTGC-TNGT
BBR             GNCTTCGGATTGTAAAGTTCTGTTGTTAGGGACGAATAAGTACCGNTCGAATAGGGCGGT
                *  *** * ** **  * * * *  **** *** ******      *       **
```

(continued)

BC1 = Thermotolerant bacterial strain BC1, BBA = *Bacillus badius*, BSM = *Bacillus smithii*, BCI = *Bacillus circulans*, BSI = *Bacillus simplex*, BIN = *Bacillus insolitus*, BAZ = *Bacillus azotoformans* and BBR = *Brevibacillus brevis*

# APPENDIX J (continued)

## Alignment of 16S rRNA gene sequence from thermotolerant bacterial strain BC1 and related organisms.

(continued)

```
BC1             ACCTTGACGGTACCTGACCAGAAAGCCACGGCTAACTACGGG-----------------
BBA             NCCTNGACGGTACCTNACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATA
BSM             NCCTNNACGGTACCTNACCAGAAAGCCACGGCTNACTACGTGCCAGCAGCCGCGGTNATA
BCI             GCCTNGACGGTACCTNACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATA
BSI             NCCTNGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTNATA
BIN             ACCTTGACGGTACCTCATTAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTNATA
BAZ             NCCTTGACGGTACCTAACGAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTNATA
BBR             ACCTTGACGGTACCTGACGAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATA
                 ***  ********* *  ************** ****** *

BC1             --------GCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGCTTCTTA
BBA             CGTAGGTGGCAAGCGTTNTCCGGAATTATTGGGCGTNAAGCGCGCGCAGGCGGCTNCTTA
BSM             CGTAGGTGGCNAGCGTTNTCCGGAATTATTGGGCGTNAAGCGCGCGCAGGCGGTCTCTNN
BCI             CGTAGGTGGCNAGCGTTNTCCGGAATTATTGGGCGTNAAGCGCGCGCAGGCGGTCCTNNN
BSI             CGTAGGTGGCNAGCGTTGTCCGGAATTATTGGGCGTNAAGCGCGCGCAGGTGGTTCCTNN
BIN             CGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTNAAGCGCGCGCAGGCGGTCCTTTA
BAZ             CGTAGGTGGCNAGCGTTGTCCGGATTTATTGGGCGTNAAGCGCGCGCNGGCGGTTCTTTA
BBR             CGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGCGCGCAGGCGGCTATGTA
                        ** ****** ****** ********** ********** ** **

BC1             AGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGGCTTGA
BBA             AGTCTNATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATNGGAAACTGGGAGGCTTGA
BSM             AGTCTNATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTNGA
BCI             AGTCTNATGTGAAAGCCCACGGCTNAACCGTGGAGGGTCATTGGAAACTGGGGNACTNGA
BSI             AGTCTNATGTGAAAGCCCACGGCTNAACCGTNGAGGGTCATTGGAAACTGGGGNACTTGA
BIN             AGTCTNATGTGAAATCCCACGGCTCAACCGTGGAAGGTCATTGGAAACTGGGGGACTTGA
BAZ             AGTCTNATGTGAAAGCCCNCNGCTCAACCGGGGAGGGTCNTTGGAAACTGGGGNACTTGA
BBR             AGTCTGGTGTTAAAGCCCGGGGCTCAACCCCGGTTCG-CATCGGAAACTGTGTAGCTTGA
                ***** *** *** ***   *** ****   *   * * * ******** *   ** **

BC1             GTGCAGAAGAGGAGAGCGGAATTCCACGTGTAGCGGTGAAATGCGTACAGATGTGGAGGA
BBA             GTGCAGAAGAGGAGAGCGGAATTCCACGTGTAGCGGTNAAATGCGTAGAGATGTGGAGGA
BSM             GTGCAGAAGAGGAGAGCGGAATTCCACGTGTAGCGGTNAAATGCGTAGAGATGTGGAGGA
BCI             GTGCAGAAGAGAAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGA
BSI             GTGCAGAAGAGGAAAGTGGAATTCCAAGTGTAGCGGTNAAATGCGTAGAGATTTGGAGGA
BIN             GTACAGAAGAGGAAAGTGGAATTCCAAGTGTAGCGGTNAAATGCGTAGAGATTTGGAGGA
BAZ             GTGCNGAAGAGAAGAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGA
BBR             GTGCAGAAGAGGAAAGCGGTATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGA
                ** * ****** * ** ** ****** ********** ********* **** *******
```

(continued)

BC1 = Thermotolerant bacterial strain BC1, BBA = *Bacillus badius*, BSM = *Bacillus smithii*, BCI = *Bacillus circulans*, BSI = *Bacillus simplex*, BIN = *Bacillus insolitus*, BAZ = *Bacillus azotoformans* and BBR = *Brevibacillus brevis*

# APPENDIX J (continued)

## Alignment of 16S rRNA gene sequence from thermotolerant bacterial strain BC1 and related organisms.

(continued)

```
BC1             ACACCAGTGGCGAAGGCGGGTCTCTGTT--------------------------------
BBA             ACACCAGTGGCGAAGGCGGCTCTCTNGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGG
BSM             ACACCAGTGGCGAAGGCGGCTCTCTNGTCTGTAACTGACGCTGAGGCGCGNAAGCGTGGG
BCI             ACACCAGTGGCGAAGGCGACTNTNTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGG
BSI             ACACCAGTGGCGAAGGCGACTNTCTNGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGG
BIN             ACACCAGTGGCGAAGGCGACTNTCTNGTCTGTAACTGACGCTGAGGCGCGNAAGCGTGGG
BAZ             ACACCAGTGGCGAAGGCGGCTNTNTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGG
BBR             ACACCAGTGGNGANGGCGGNTTTCTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGG
                ********** ** ****  * * *  *

BC1             ------------------------------------------------------------
BBA             GAGCGAACAGGATTAGATACCCTNGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTN
BSM             GAGCGAACAGGATTAGATACCCTNGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTA
BCI             GAGCAAACAGGATTAGATACCCTNGTAGTCCACGCCGTNAACGATGAGTGCTNAGTGTTA
BSI             GAGCAAACAGGATTAGATACCCTNGTNGTCCACGCCGTAAACGATGAGTGCTAAGTGTTA
BIN             GAGCAAACAGGATTAGATACCCTNGTAGTCCACGCCGTAAACGATGAGTGCTNAGTGTTA
BAZ             GAGCAAACAGGATTAGATACCCTNGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTG
BBR             GAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTNCTAGGTGTTG

BC1             ------------------------------------------------------------
BBA             GAGGGTTTCCGCCCTTCAGTGCTGCA-CTAACGCATTAAGCACTCCGCCTNGGGAGTACG
BSM             GAGGGCTTCCACCCTTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACG
BCI             GAGGGTTTCCGCCCNNTAGTGCTGCAGCAAACGCATTAAGCACTCCGCCTGGGGAGTACG
BSI             GAGGGTNTCNGCCCTNTAGTGCTNCA-CTAACGCATTAAGCACTCCGCCTNGGGAGTACG
BIN             GGGGGTTTCCGCCCCTTAGTGCTGCA-NTAACGCATTAAGCACTCCGCCTNGGGAGTACG
BAZ             GAGGGTTTCCGCCCTTCAGTGCTGCA-NTAACGCATTAAGCACTCCGCCTGGGGAGTACG
BBR             GGGGTTTCAATACCCTCAGTGCCGCAGCTAACGCAATAAGCACTCCNCCTGGGGAGTACG

BC1             ------------------------------------------------------------
BBA             GCCGCAAGGCTNAAACTCNAAGGAATTNACGGGGNCC-GCACAAGCGGTGGAGCATGTNG
BSM             GCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCC-GCACAAGCGGTGGAGCATGTGG
BCI             GCCGCAAGGCTNAAACTCAAAGGAATTGACGGGGGCC-GCACAAGCGGTGGAGCATGTGG
BSI             GCCGCAAGGCTNAAACTCAAAGGAATTGACGGGGGCC-GCACAAGCGGTGGAGCATGTNG
BIN             GTCGCAAGACTNAAACTCAAAGGAATTGACGGGGGCC-GCACAAGCGGTNGAGCATGTNG
BAZ             GTCGCAAGACTNAAACTCAAAGGAATTGACGGGGGCC-GCACAAGCGGTGGAGCATGTGG
BBR             CTCGCAAGAGTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGG
```

(continued)

BC1 = Thermotolerant bacterial strain BC1, BBA = *Bacillus badius*, BSM = *Bacillus smithii*, BCI = *Bacillus circulans*, BSI = *Bacillus simplex*, BIN = *Bacillus insolitus*, BAZ = *Bacillus azotoformans* and BBR = *Brevibacillus brevis*

# APPENDIX J (continued)

## Alignment of 16S rRNA gene sequence from thermotolerant bacterial strain BC1 and related organisms.

(continued)

```
BC1             ------------------------------------------------------------
BBA             TTTAATTCGAAGNAACGCGAAGAACCTNACCAGGTCTTGACATCCC-GCTNACCNGTCTG
BSM             TTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCT-TCGCTACCTCTAG
BCI             TTTAATTCGAAGNAACGCGAAGAACCTTACCAGGTCTTGACATCCT-CTGACACTCCTAG
BSI             TTTAATTCGAAGNAACGCGAAGAACCTTACCAGGTCTTGACATCCT-CTGACAACCCTAG
BIN             TTTAATTCGAAGNAACGCGAAGAACCTNACCAGGTCTTGACATCCCACTGACCGGTCTAG
BAZ             TTTAATNCGAAGCAACGCNAAGAACCTTACCAGGTCTTGACNTCNT-CTGACNATCCTAG
BBR             TTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCCNCTGACCGCTCTGG

BC1             ------------------------------------------------------------
BBA             GAGACAGGCCNTTCTTCGGGGACAGCGGNGACAGGTGGAGCATNGTNGTCGTCAGCTCGT
BSM             AGATAGAGGGTTCCTTCGGGGGACGGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGT
BCI             AGATAGGACGTN--NTCGGGGNACAGAGTGACAGGTGGNGCATGGTNGTCGTCAGCTNGT
BSI             AGATAGGN-NTNTCTTCGGGGNACAGAGTGACAGGTGGNGCATNGTNGTCGTCAGCTCGT
BIN             AGATAGATCTTT-CTTCGGGGNCAGTGGTGACAGGTGGNGCATGGTTGTCGTCAGCTNGT
BAZ             AGATAGGACTT--CTTCGGGGN-CAGAATGACNGGTGGNGCATNGTTGTCGTCAGCTCGT
BBR             AGACAGAGCTTCCCTTCGGGGCAGCG-GTGACAGGTGGTGCATGGTTGTCGTCAGCTCGT

BC1             GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCA
BBA             GTCGTGAGATGTTGGGTNAAGTCCCGCAACGAGCGCAACCCTNNATCTTAGTTGCCAGCA
BSM             GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTNGACNTNAGTTGCCAGCA
BCI             GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTNGATCTTAGTTGCCAGCA
BSI             GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTNGATCTTAGTTGCCAGCA
BIN             GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCA
BAZ             GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTGCCAGCA
BBR             GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCTTTAGTTGNCAGCA
                ****************** ************************     * ***** *****

BC1             TTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGT
BBA             TTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGT
BSM             TTCAGTTGGGCACTCTAAGGTNACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGT
BCI             TTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGT
BSI             TTCAGTTGGGCACTCTAAGGTNACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGT
BIN             TTCAGTTGGGCACTCTAAGGTGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGT
BAZ             TTCAGTTGGGCACTCTAAGGAGACTGCCGGTGACNAACCGGAGGAAGGCNGGGATGACGT
BBR             TTCAGTTGGGCACTCTAGAGAGACTGCCGTCGACAAGACGGAGGAAGGCGGGGATGACGT
                ****************  *  *******  **  *  **********  **********
```

(continued)

BC1 = Thermotolerant bacterial strain BC1, BBA = *Bacillus badius*, BSM = *Bacillus smithii*, BCI = *Bacillus circulans*, BSI = *Bacillus simplex*, BIN = *Bacillus insolitus*, BAZ = *Bacillus azotoformans* and BBR = *Brevibacillus brevis*

# APPENDIX J (continued)

## Alignment of 16S rRNA gene sequence from thermotolerant bacterial strain BC1 and related organisms.

(continued)

```
BC1             CAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAAAGG
BBA             CAAATCATCATGCCCCTTATGACCTNGGCTACACACGTGCTACAATGGATGGTACAAAGG
BSM             CAAATCATCATGCCCNNNNATGACCTNGGCTACACACGTGCTACAATGGATGGTACAAAGG
BCI             CAAATCATCATGCCCCTTATGACCTNGGCTACACACGTGCTACAATGGATGGTACAAAGG
BSI             CAAATCATCATGCCCCTTATGACCTNGGCTACACACGTGCTACAATGGATGGTACAAAGG
BIN             NAAATCATCATGCCCCTTATGACCTNGGCTACACACGTGCTACAATGGACGGTACAGAGG
BAZ             CNAATCATCATGCCCCTNNTGACCTNGGCTACNCNCGTGCNNCNATGGANGNTACNNAGG
BBR             CAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGTTGGTACAACGG
                  *************    ****** ****** * *****  * ****  * ***   **

BC1             GCTGCAAGACCGCAAGGTTTAGTCCAATCGATAAAACCATCCTCAGTTCGGATTGCAGGC
BBA             GCTGCAAGACCGCAAGGTTTAGCCAATCCCATAAAACCATTCTCAGTTCGGATTGCAGGC
BSM             GTCGCGAAACCGCGAGGTGGAGCTAATCCCAAAAAACCATTCTCAGTTCGGATTGCAGGC
BCI             GCAGCAAAACCGCGAGGTCGAGCAAATCCCATAAAACCATTCTCAGTTCGGATTGTAGGC
BSI             GCTGCAAACCTGCGAAGGTNAGCGAATCCCATAAAGCCATNCTCAGTTCGGATTGTAGGC
BIN             GTCGCAACCCCGCGAGGGTGAGCTAATCCCATAAAACCGTNCTCAGTTCGGATTGTAGGC
BAZ             GNNGCNAANNNNNGAGGNTGAGCCAAT-CCATAAAGCCATTCTCAGTTCGGATTGTAGGC
BBR             GATGCTACCTCGCGAGAGGACGCNAATCTCTTAAAACCAATCTCAGTTCGGATTGTAGGC
                *  ** *       *     *   *      *** **   ************** ****

BC1             CGCAACTCGCCTGCATGAAGCCGGAATCCCCAGTAATCGCGGATCCATGATGCTCCCGCC
BBA             TGCAACTCGCCTNCATGAAGCCGGAATCGCTAGTAATCGCGGATC-AGCATGCCGCGGTN
BSM             TGCAACTCGCCTGCATGAAGCCGGAATCGCTAGTAATCGCGGATC-AGCATGCCGCGGTN
BCI             TGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGATC-AGCATGCCGCGGTG
BSI             TGCAACTCGCCTNCATGAAGCCGGAATCGCTNGTAATCGCGGATC-AGCATGCCGCGGTN
BIN             TGCAACTCGCCTNCATGAAGCCGGAATCGCTNGTAATCGTGGATC-AGCATGCCACGGTG
BAZ             TGCAACTCGCCTACATGAAGCCGGAATCGCTAGTAATCGCGGATC-AGCATGCCGCGGTG
BBR             TGCAACTCGCCTACATGAAGTCGGAATCGCTAGTAATCGCGGATC-AGCATGCCGCGGTG
                 *********** *******  ****** *  ******* ***** *  ****  * *

BC1             AACT----------------------------------------------------
BBA             AATACGTTCCCGGGCCTNGTACACACCGNNCGTCACACCACGAGAGTTTGCAACACCC
BSM             AATACGTTCCCGGGCCTNGTACNCACNGCNCGTCACACCACGAGAGTTTGCAACACCC
BCI             AATACGTTCCCGGGCNTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCC
BSI             AATACGTTCCCGGGCNTNGTACACACCGCNCGTNACACCACGAGAGTTNGTAACACCC
BIN             AATACGTTCCCGGGCCTNGTACACACCGCNCGTCACACCACGAGAGTTTGTAACACNC
BAZ             AATACGTTCCCGGGCCTTGTACACACCGCNCGTCNCACCACGAGAGTTTGTAACACCC
BBR             AATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGGGAGTTTGCAACACCC
                **
```

BC1 = Thermotolerant bacterial strain BC1, BBA = *Bacillus badius*, BSM = *Bacillus smithii*, BCI = *Bacillus circulans*, BSI = *Bacillus simplex*, BIN = *Bacillus insolitus*, BAZ = *Bacillus azotoformans* and BBR = *Brevibacillus brevis*

# APPENDIX K

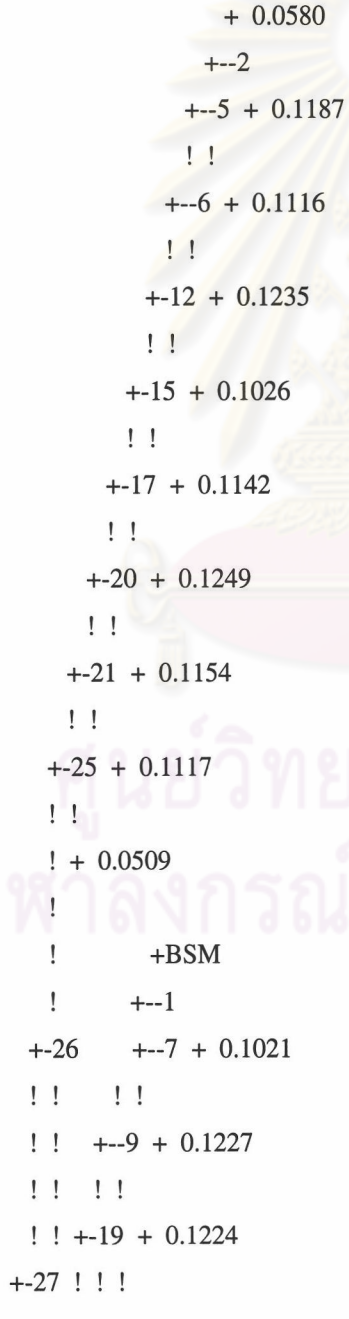
**Evolutionary distance values of 16S rRNA gene sequence from thermotolerant bacterial strain BC1 and related organisms.**

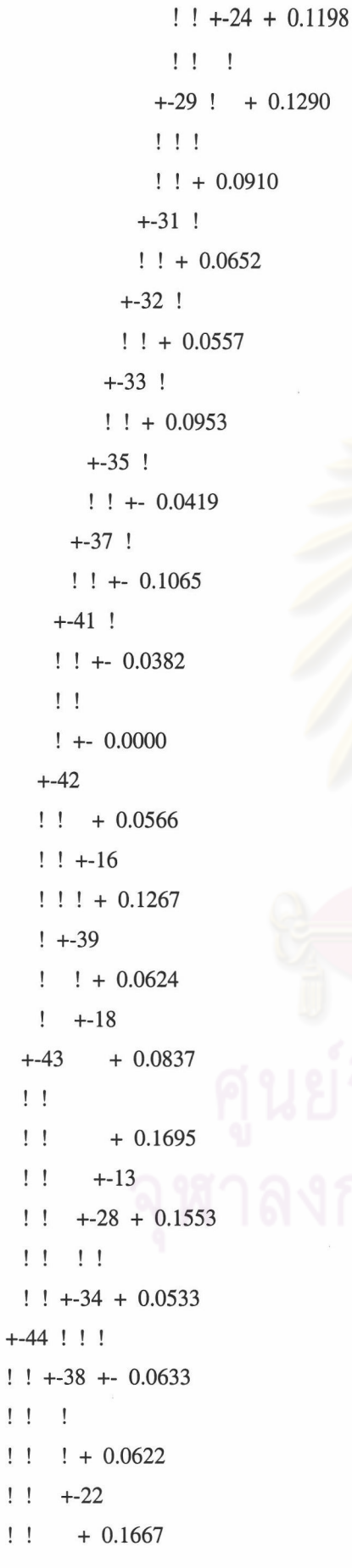
46 Populations

Neighbor-Joining/UPGMA method version 3.572c

UPGMA method

Negative branch lengths allowed





```
 ! !
 ! ! +BC1
 ! +--3
 !   + 0.0391
-45
 !     + 0.1079
 !   +-10
 !   ! + 0.0845
 !   !
 ! +-36    + 0.1205
 ! ! ! +--4
 ! ! ! ! + 0.0583
 ! ! ! !
 ! ! +-30      +BFU
 ! !   !   +-11
 +-40    ! +-14 + 0.0968
   !   ! ! !
   !   +-23 + 0.0755
   !     !
   !     + 0.0977
   !
   ! + 0.0961
   +--8
     + 0.0852
```

| Between | And | Length |
|---|---|---|
| 45 | 44 | 0.00239 |
| 44 | 43 | 0.00612 |
| 43 | 42 | 0.00446 |
| 42 | 41 | 0.00206 |
| 41 | 37 | 0.00471 |
| 37 | 35 | 0.00177 |
| 35 | 33 | 0.00263 |
| 33 | 32 | 0.00495 |
| 32 | 31 | 0.00215 |
| 31 | 29 | 0.00215 |
| 29 | 27 | 0.00410 |
| 27 | 26 | 0.00627 |

| | | |
|---|---|---|
| 26 | 25 | 0.00232 |
| 25 | 21 | 0.00432 |
| 21 | 20 | 0.00000 |
| 20 | 17 | 0.00000 |
| 17 | 15 | 0.00000 |
| 15 | 12 | 0.00000 |
| 12 | 6 | 0.00000 |
| 6 | 5 | 0.00000 |
| 5 | 2 | 0.00000 |
| 2 | 0.0580 | 0.00000 |
| 2 | 0.1187 | 0.00000 |
| 5 | 0.1116 | 0.00000 |
| 6 | 0.1235 | 0.00000 |
| 12 | 0.1026 | 0.00000 |
| 15 | 0.1142 | 0.00000 |
| 17 | 0.1249 | 0.00000 |
| 20 | 0.1154 | 0.00000 |
| 21 | 0.1117 | 0.00000 |
| 25 | 0.0509 | 0.00432 |
| 26 | 24 | 0.00664 |
| 24 | 19 | 0.00000 |
| 19 | 9 | 0.00000 |
| 9 | 7 | 0.00000 |
| 7 | 1 | 0.00000 |
| 1 | SM | 0.00000 |
| 1 | 0.1021 | 0.00000 |
| 7 | 0.1227 | 0.00000 |
| 9 | 0.1224 | 0.00000 |
| 19 | 0.1198 | 0.00000 |
| 24 | 0.1290 | 0.00000 |
| 27 | 0.0910 | 0.01291 |
| 29 | 0.0652 | 0.01701 |
| 31 | 0.0557 | 0.01917 |
| 32 | 0.0953 | 0.02132 |
| 33 | 0.0419 | 0.02627 |
| 35 | 0.1065 | 0.02890 |
| 37 | 0.0382 | 0.03067 |
| 41 | 0.0000 | 0.03538 |
| 42 | 39 | 0.00277 |

| | | |
|---|---|---|
| 39 | 16 | 0.03467 |
| 16 | 0.0566 | 0.00000 |
| 16 | 0.1267 | 0.00000 |
| 39 | 18 | 0.03467 |
| 18 | 0.0624 | 0.00000 |
| 18 | 0.0837 | 0.00000 |
| 43 | 38 | 0.00815 |
| 38 | 34 | 0.00586 |
| 34 | 28 | 0.01363 |
| 28 | 13 | 0.01425 |
| 13 | 0.1695 | 0.00000 |
| 13 | 0.1553 | 0.00000 |
| 28 | 0.0533 | 0.01425 |
| 34 | 0.0633 | 0.02788 |
| 38 | 22 | 0.03374 |
| 22 | 0.0622 | 0.00000 |
| 22 | 0.1667 | 0.00000 |
| 44 | 3 | 0.04801 |
| 3 | BC1 | 0.00000 |
| 3 | 0.0391 | 0.00000 |
| 45 | 40 | 0.01556 |
| 40 | 36 | 0.00461 |
| 36 | 10 | 0.03024 |
| 10 | 0.1079 | 0.00000 |
| 10 | 0.0845 | 0.00000 |
| 36 | 30 | 0.01226 |
| 30 | 4 | 0.01798 |
| 4 | 0.1205 | 0.00000 |
| 4 | 0.0583 | 0.00000 |
| 30 | 23 | 0.01798 |
| 23 | 14 | 0.00000 |
| 14 | 11 | 0.00000 |
| 11 | BFU | 0.00000 |
| 11 | 0.0968 | 0.00000 |
| 14 | 0.0755 | 0.00000 |
| 23 | 0.0977 | 0.00000 |
| 40 | 8 | 0.03485 |
| 8 | 0.0961 | 0.00000 |
| 8 | 0.0852 | 0.00000 |

# APPENDIX L

## Description of *Bacillus badius* (Buchanan, 1974).

ba.di'us. L. adj. *badius* chestnut brown.

Distinguished from *Brevibacillus brevis* by growth in 5 % NaCl broth; rods are greater in diameter (0.8-1.2 μm) and are not distended by the spore; free spores show little surface stainability.

The type strain of Bacillus badius grows as chains of rods with blunt or flat ends, and its colony has a folded hair structure and rhizoid outgrowths. Other strains appear to differ from the type culture only in the absence of chains and the production of smooth colonies.

Has been isolated infrequently from feces, dust, marine sources, foods and antacids.

The G+C content of the DNA in mole percent is reported to be 45.2 and 50.0 ($T_m$).

### The characteristics of *Bacillus badius.*

| Characteristic | *Bacillus badius* |
|---|---|
| Spore | |
| - Shape | E |
| - Distends sporangium distinctly | - |
| - Dominant position | CT |
| Acid from glucose | - |
| Anaerobic growth | - |
| Growth at 3C | - |
| Catalase activity | + |
| Hydrolysis of starch | - |
| Production of | |
| - Acetylmethylcarbinol | v |
| - Lactic acid | v |
| $NO_3^-$ to $NO_2^-$ | d |
| Decomposition of | |
| - Casein | v |
| - Tyrosine | v |
| Deamination of phenylalanine | v |

The symbols used are: E, elliptical or cylindrical; CT, central to terminal, variation within or between strains.

+ = positive for 90 – 100 % of strains; - = negative for 90 – 100 % of strains; d = reactions differ, positive for 11 – 89 % of strains; v = variable reaction..

# APPENDIX M

## The DNA sequencing profiles of the phenylalanine dehydrogenase gene from *Bacillus badius* BC1

(a) = The DNA sequencing profile of the internal gene fragment amplification using N1xC2 as PCR primers; sense sequencing primer N1.

(b) = The DNA sequencing profile of the internal gene fragment amplification using N1xC2 as PCR primers; antisense sequencing primer C2.

(c) = The DNA sequencing profile of the internal gene fragment amplification using N1xC1 as PCR primers; antisense sequencing primer C1.

(d) = The DNA sequencing profile of the internal gene fragment amplification using N1xC1 as PCR primers; sense sequencing primer N1.

(e) = The DNA sequencing profile of the internal gene fragment amplification using N2xC2 as PCR primers; antisense sequencing primer C2.

(f) = The DNA sequencing profile of the internal gene fragment amplification using N2xC2 as PCR primers; sense sequencing primer N2.

(g) = The DNA sequencing profile of the first 5'-terminal gene fragment amplification using chromosomal DNA digested with *Spe*I as PCR template and Phe-N1xCassette C1 as PCR primers; antisense primer Phe-N1.

(h) = The DNA sequencing profile of the second 5'-terminal gene fragment amplification using chromosomal DNA digested with *Spe*I as PCR template and Phe-N2xCassette C2 as PCR primers; antisense sequencing primer Phe-N2.

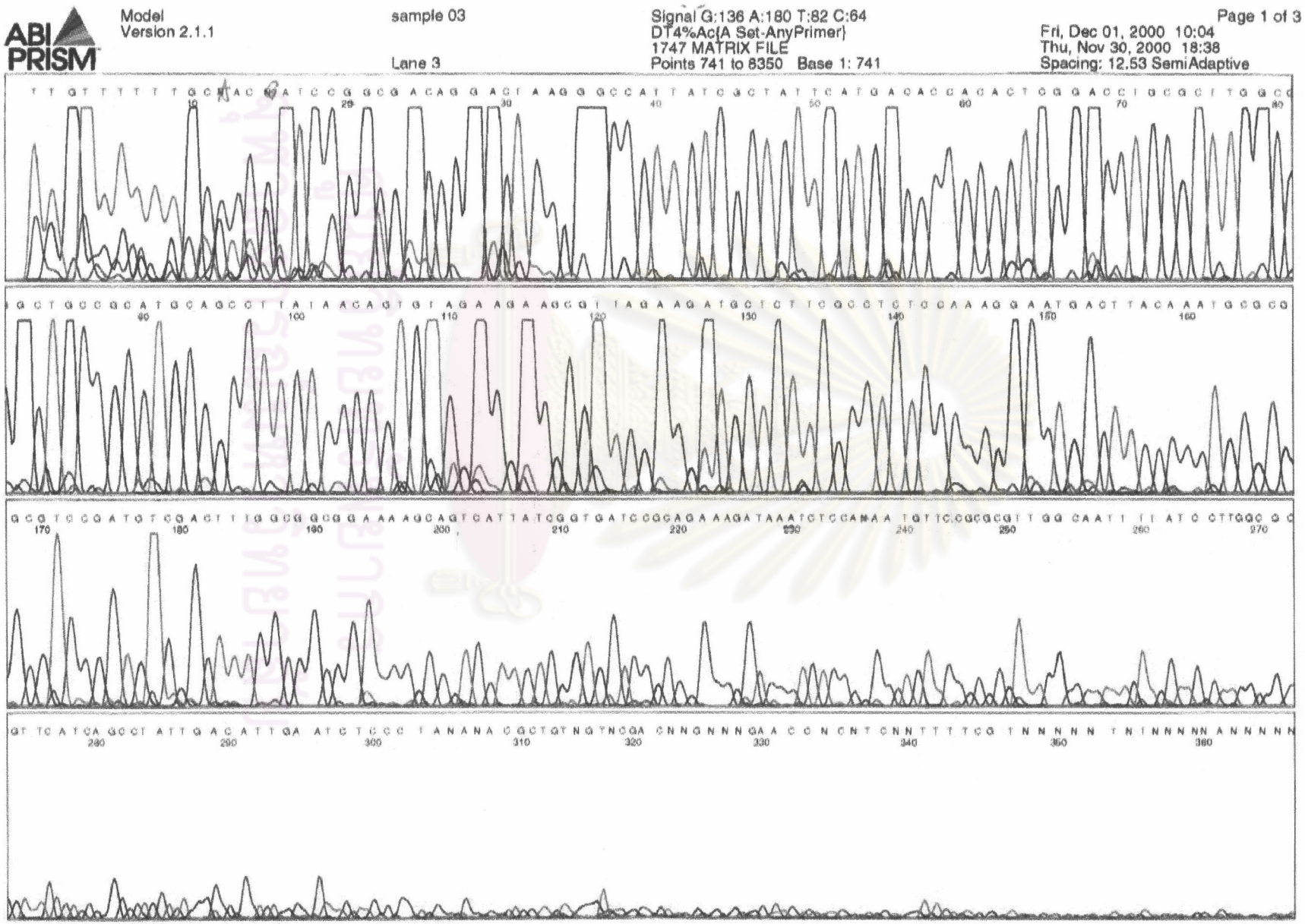
(i) = The DNA sequencing profile of the first 3'-terminal gene fragment amplification using chromosomal DNA digested with *Pst*I as PCR template and Phe-C1xCassette C1 as PCR primers; sense sequencing primer Phe-C1.

(j) = The DNA sequencing profile of the second 3'-terminal gene fragment amplification using chromosomal DNA digested with *Pst*I as PCR template and Phe-C2xCassette C2 as PCR primers; sense sequencing primer Phe-C2.

(k) = The DNA sequencing profile of the second 3'-terminal gene fragment amplification using chromosomal DNA digested with *Pst*I as PCR template and Phe-C2xCassette C2 as PCR primers; sense sequencing primer Phe-C1.

(l) = The DNA sequencing profile of the first 3'-terminal gene fragment amplification using chromosomal DNA digested with *Pst*I as PCR template and Phe-C1xCassette C1 as PCR primers; sense sequencing primer Phe-C3.

(a)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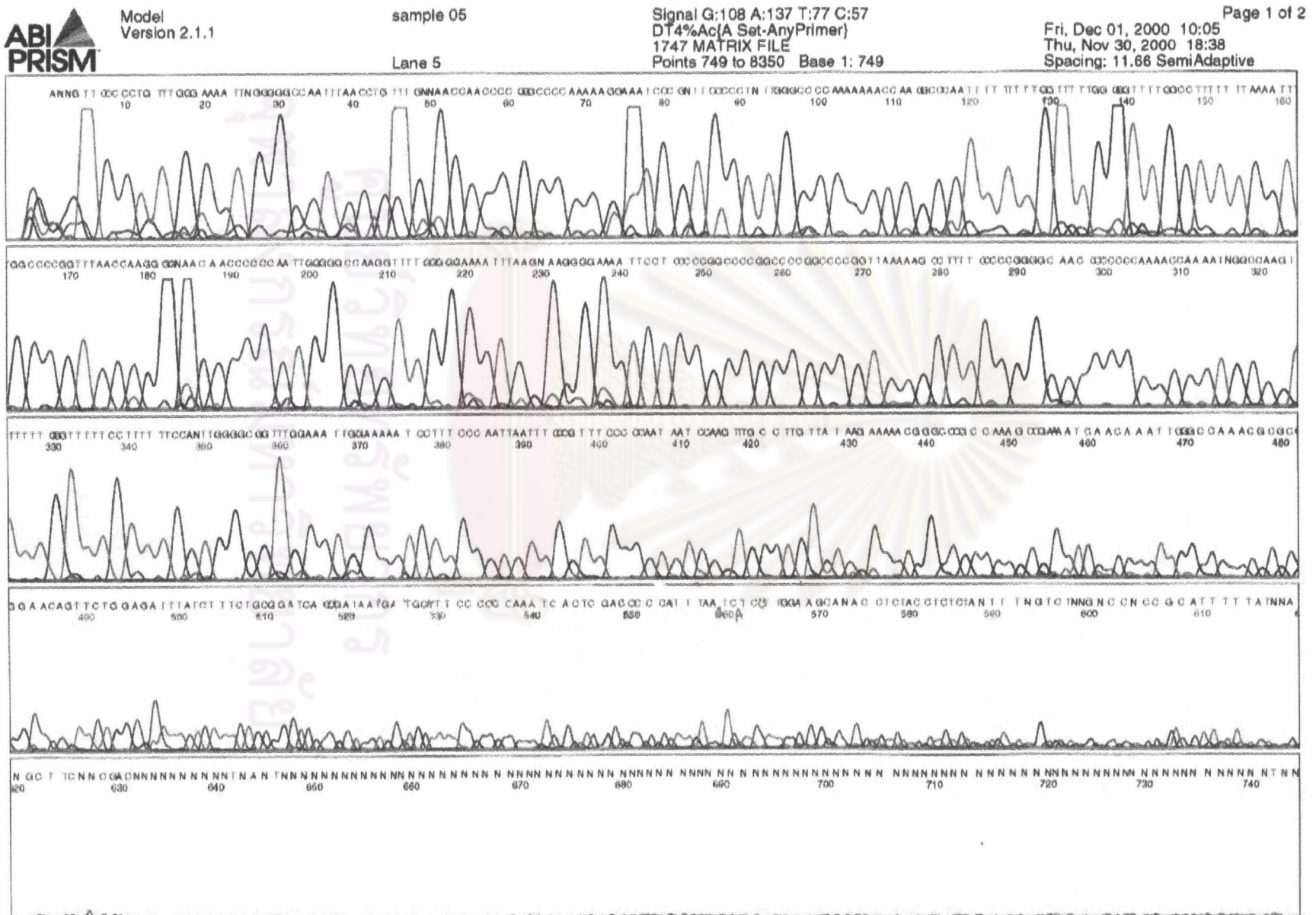
(b)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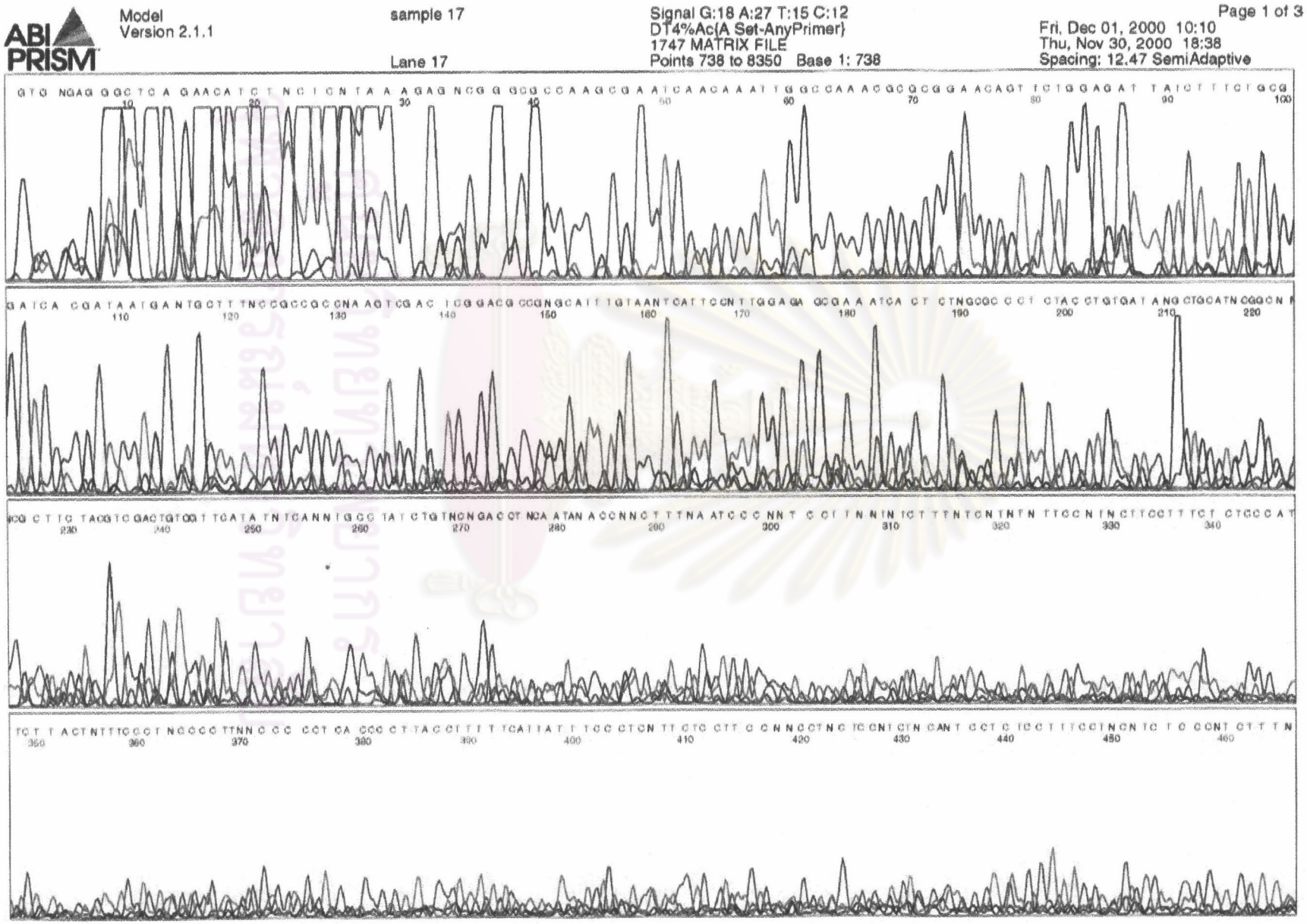
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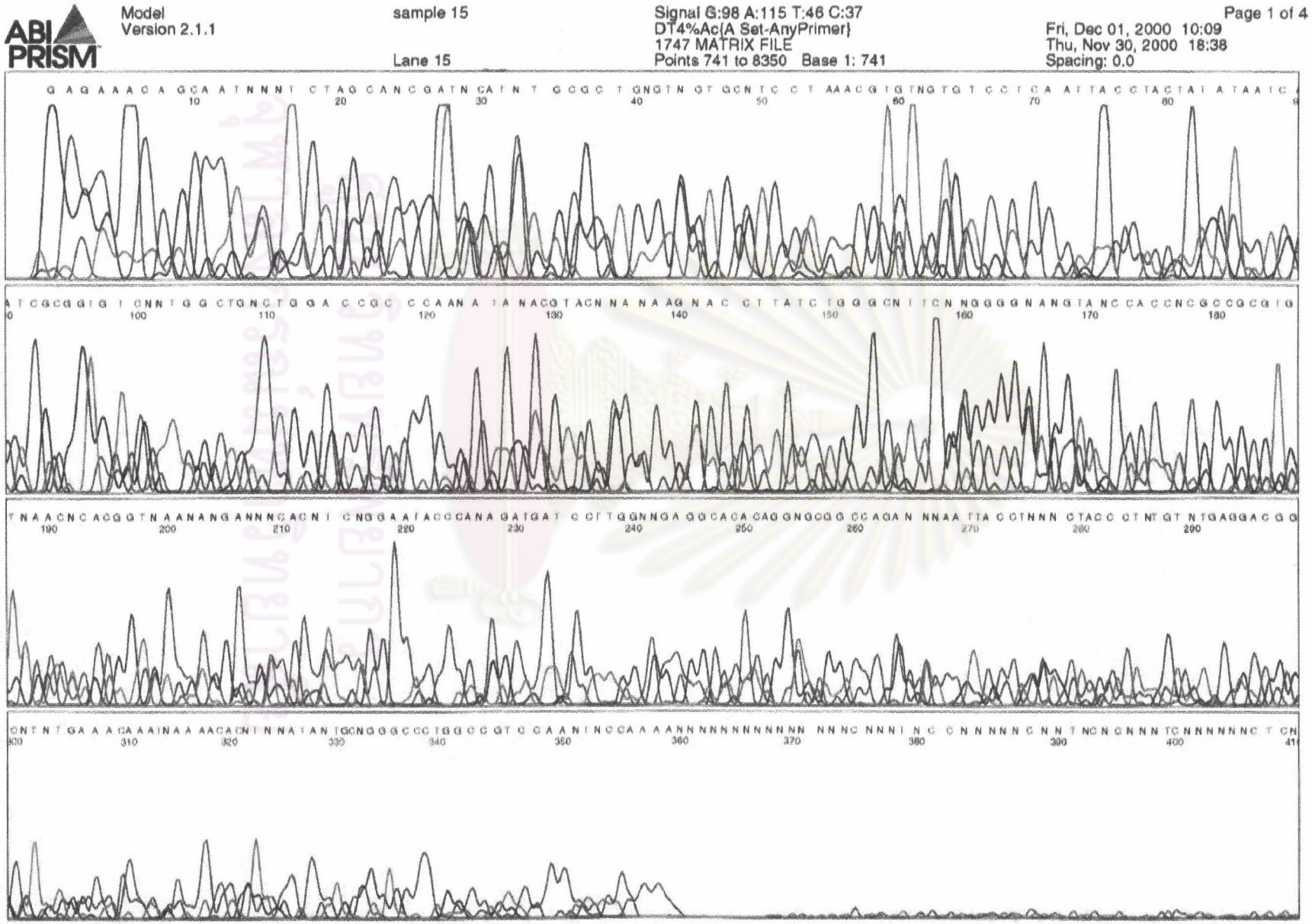
(c)

(p)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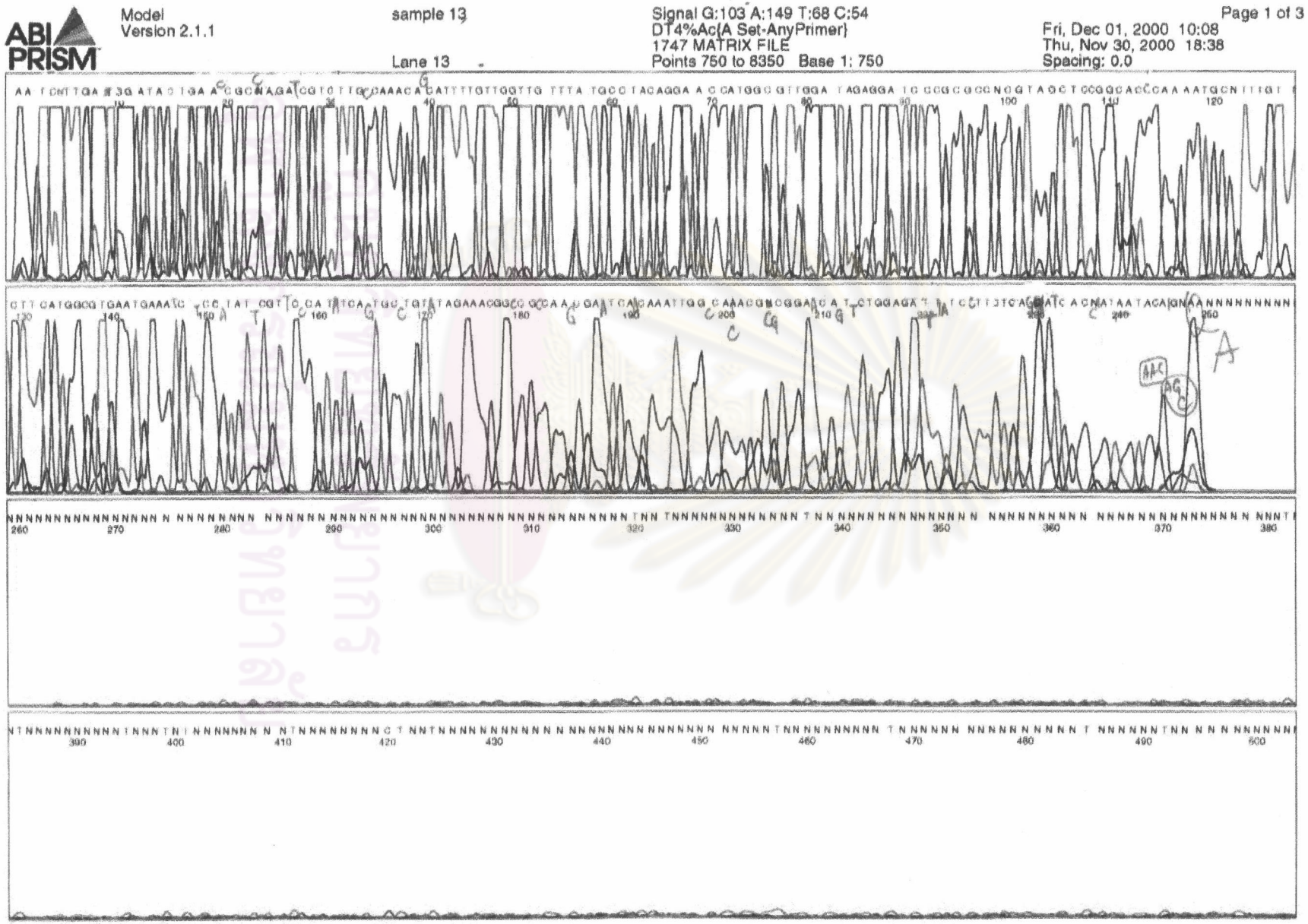
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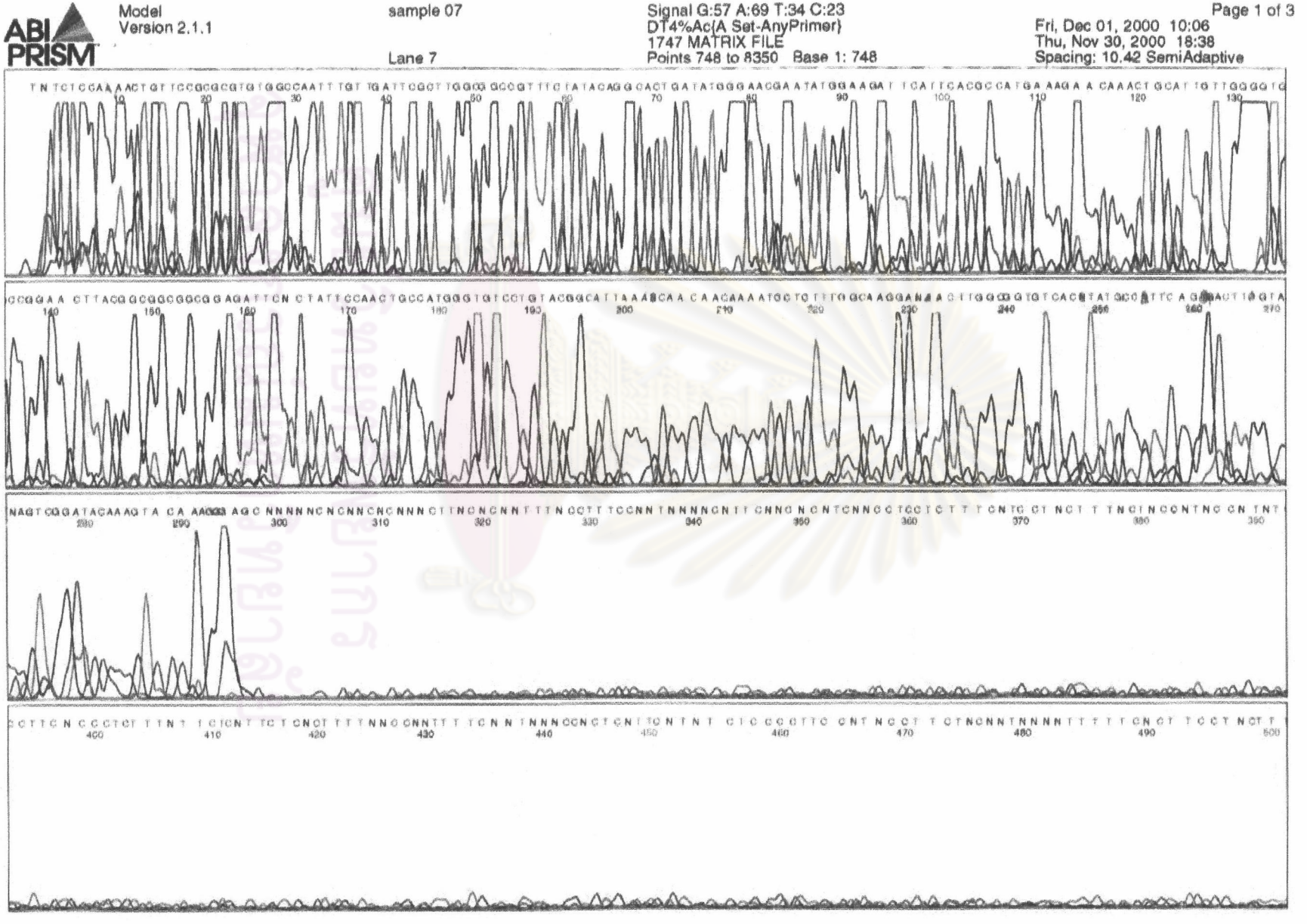
(e)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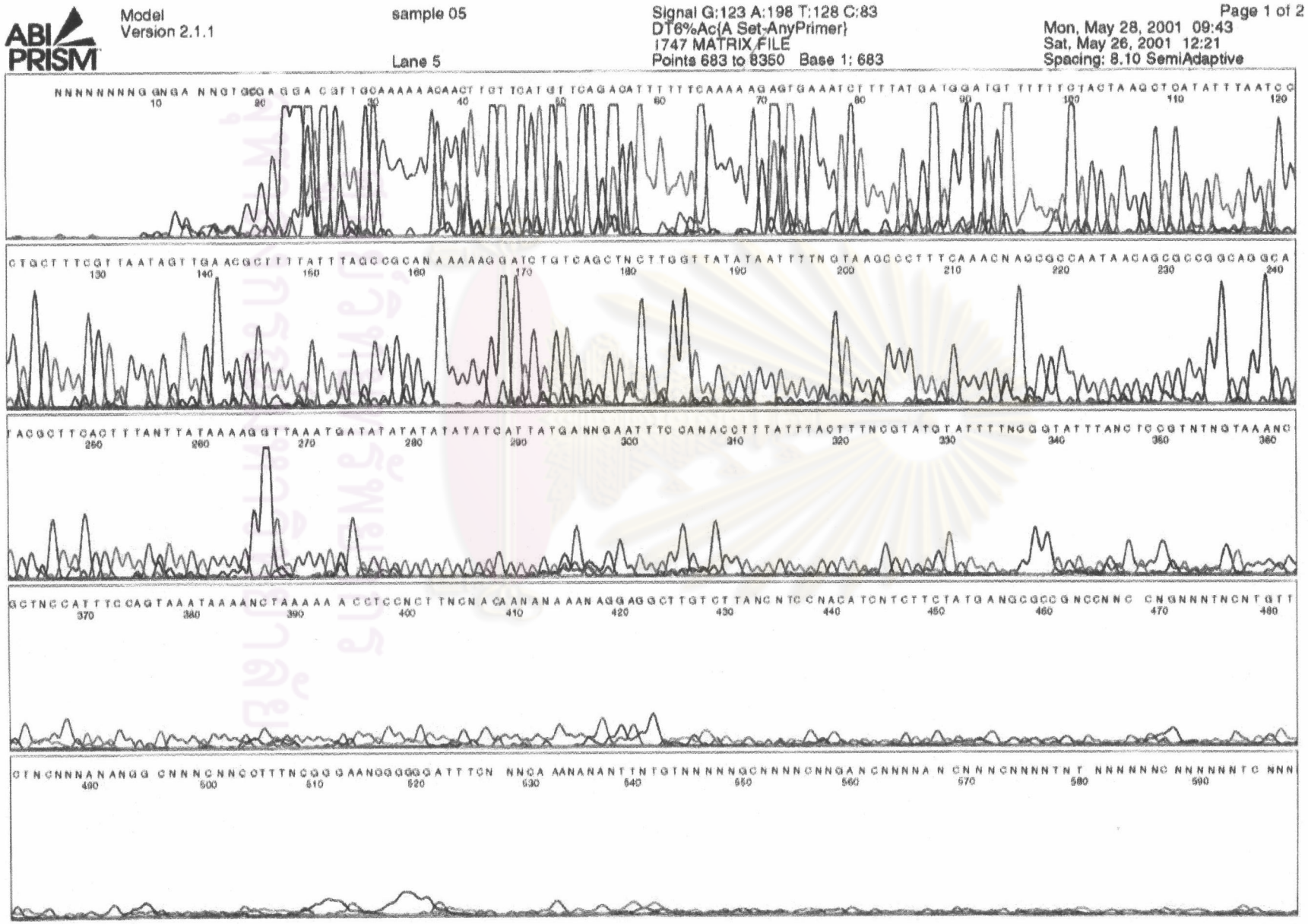
Lane 7
Signal G:57 A:69 T:34 C:23
DT4%Ac{A Set-AnyPrimer}
1747 MATRIX FILE
Points 748 to 8350 Base 1: 748
Page 1 of 3
Fri, Dec 01, 2000 10:06
Thu, Nov 30, 2000 18:38
Spacing: 10.42 SemiAdaptive

(F)

(g)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sample 05
Lane 5
Signal G:123 A:198 T:128 C:83
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Points 683 to 8350 Base 1: 683
Page 1 of 2
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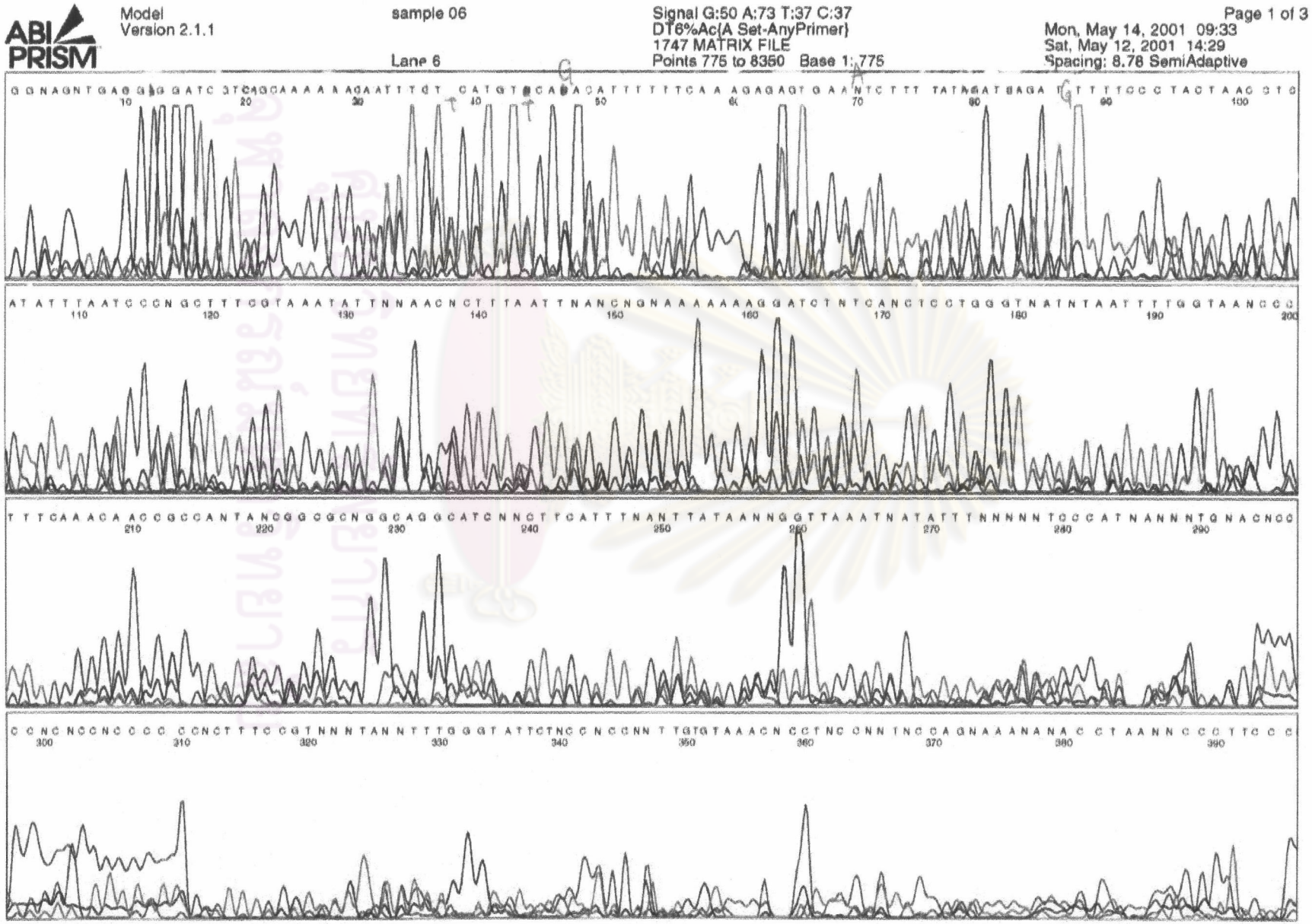
Sat, May 26, 2001 12:21
Spacing: 8.10 SemiAdaptive

(b)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sample 06
Lane 6
Signal G:50 A:73 T:37 C:37
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Points 775 to 8350 Base 1: 775
Page 1 of 3
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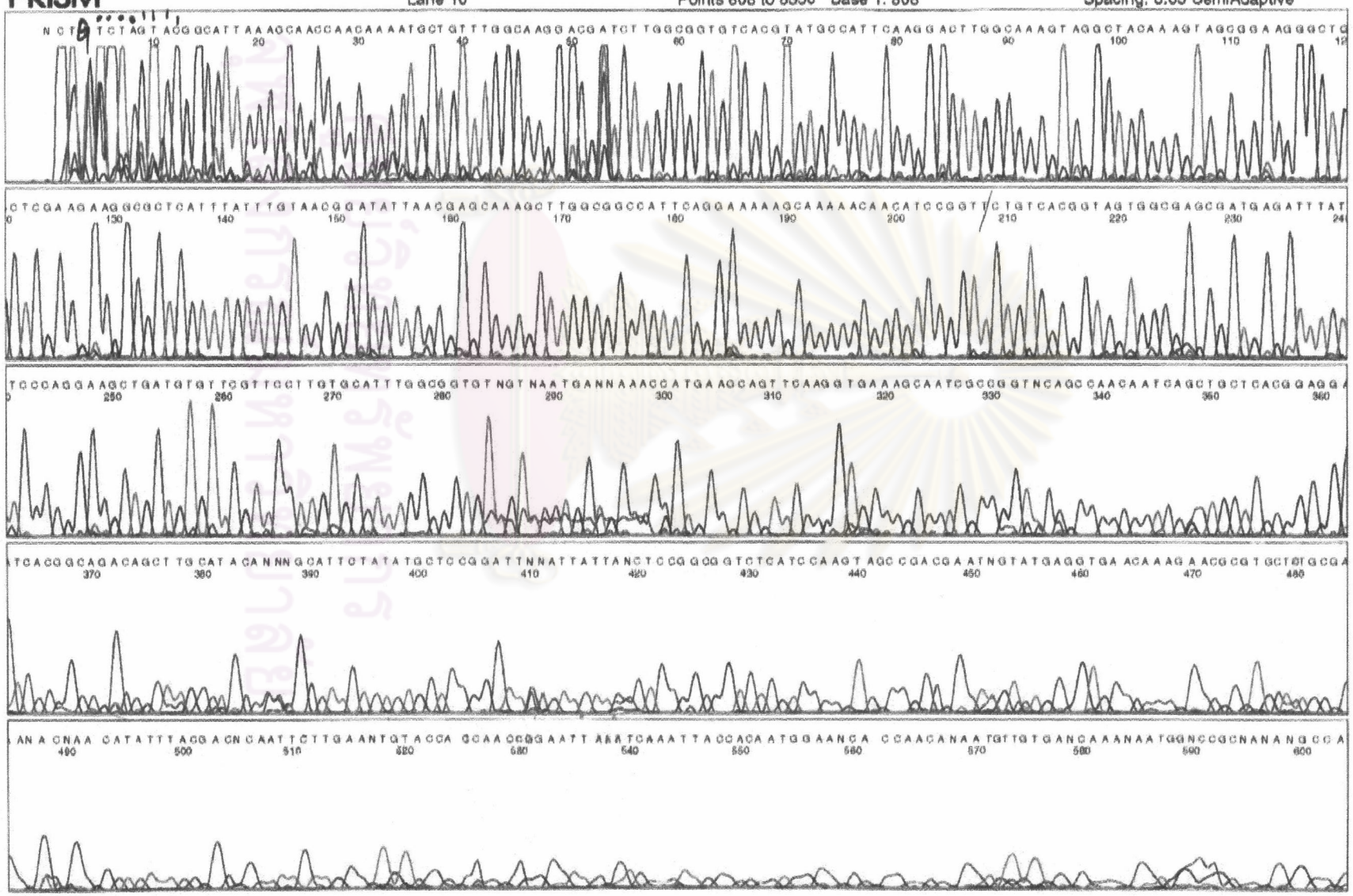
Sat, May 12, 2001 14:29
Spacing: 8.78 SemiAdaptive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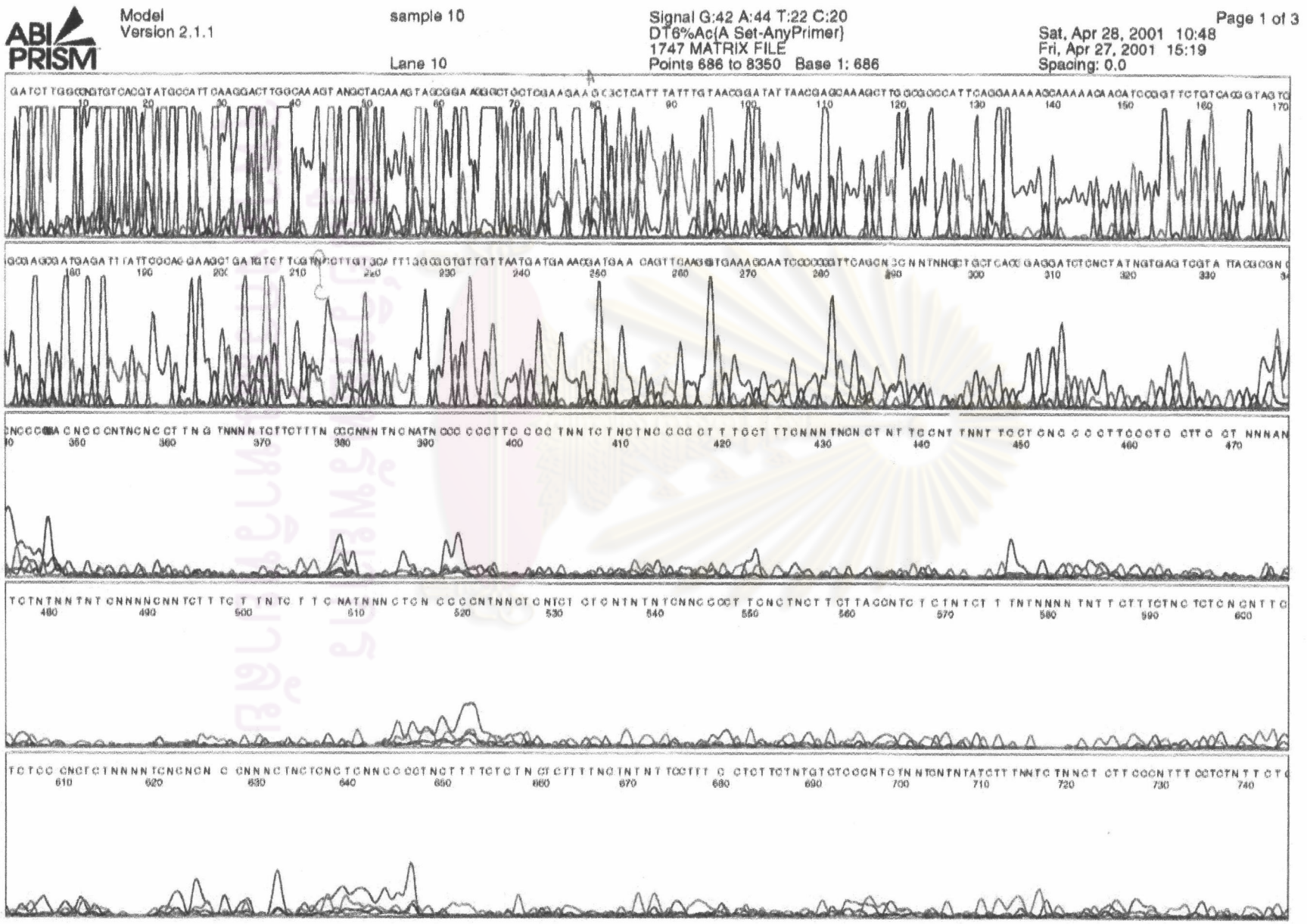
Model
Version 2.1.1
sample 10
Lane 10
Signal G:171 A:257 T:112 C:98
DT6%Ac(A Set-AnyPrimer)
1747 MATRIX FILE
Points 808 to 8350 Base 1: 808
Page 1 of 2
Mon, May 14, 2001 09:34
Sat, May 12, 2001 14:29
Spacing: 8.65 SemiAdaptive

(ii)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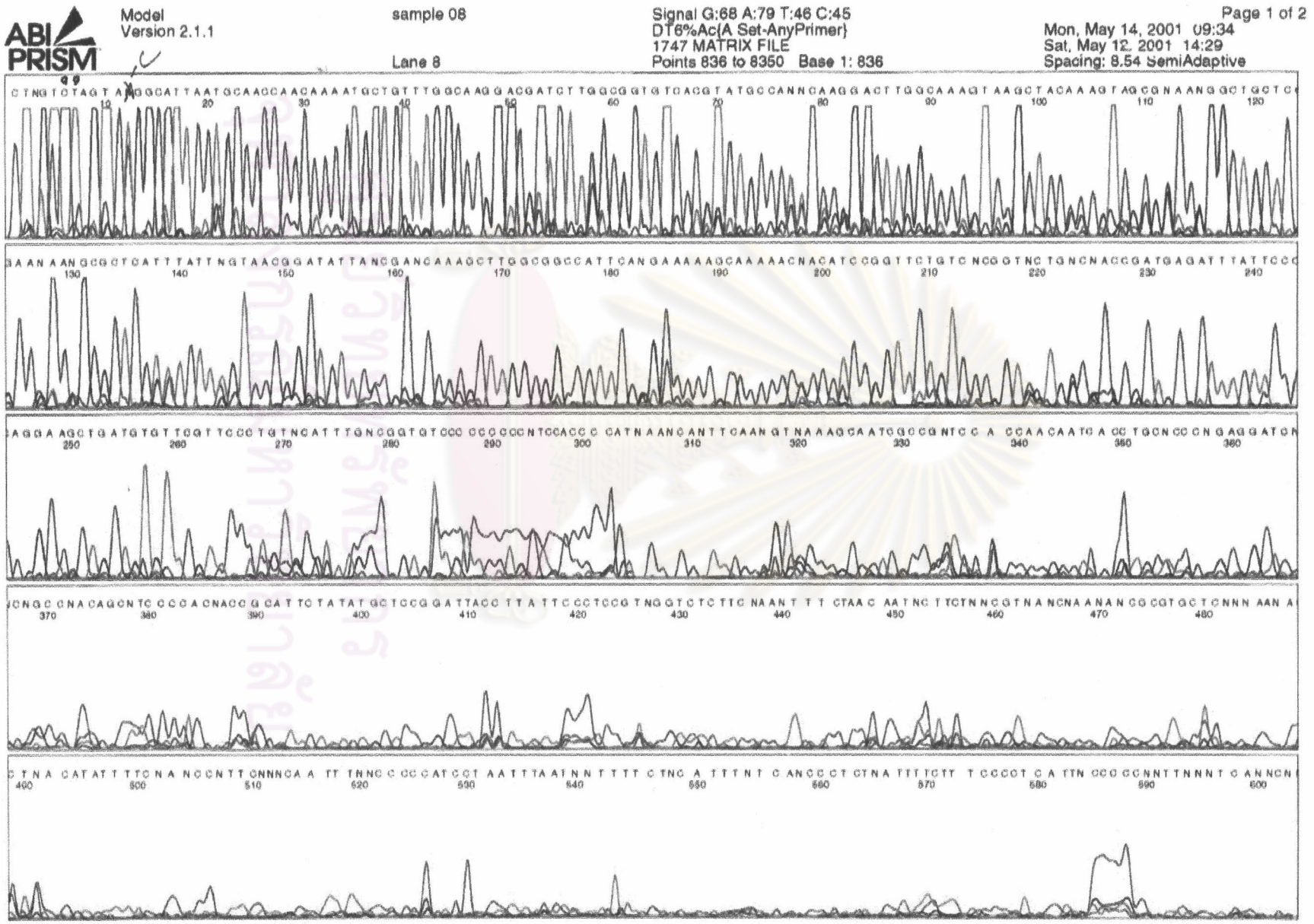
Model
Version 2.1.1
sample 10
Lane 10
Signal G:42 A:44 T:22 C:20
DT6%Ac(A Set-AnyPrimer)
1747 MATRIX FILE
Points 686 to 8350 Base 1: 686
Page 1 of 3
Sat, Apr 28, 2001 10:48
Fri, Apr 27, 2001 15:19
Spacing: 0.0

160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Model
Version 2.1.1
sample 08
Lane 8
Signal G:68 A:79 T:46 C:45
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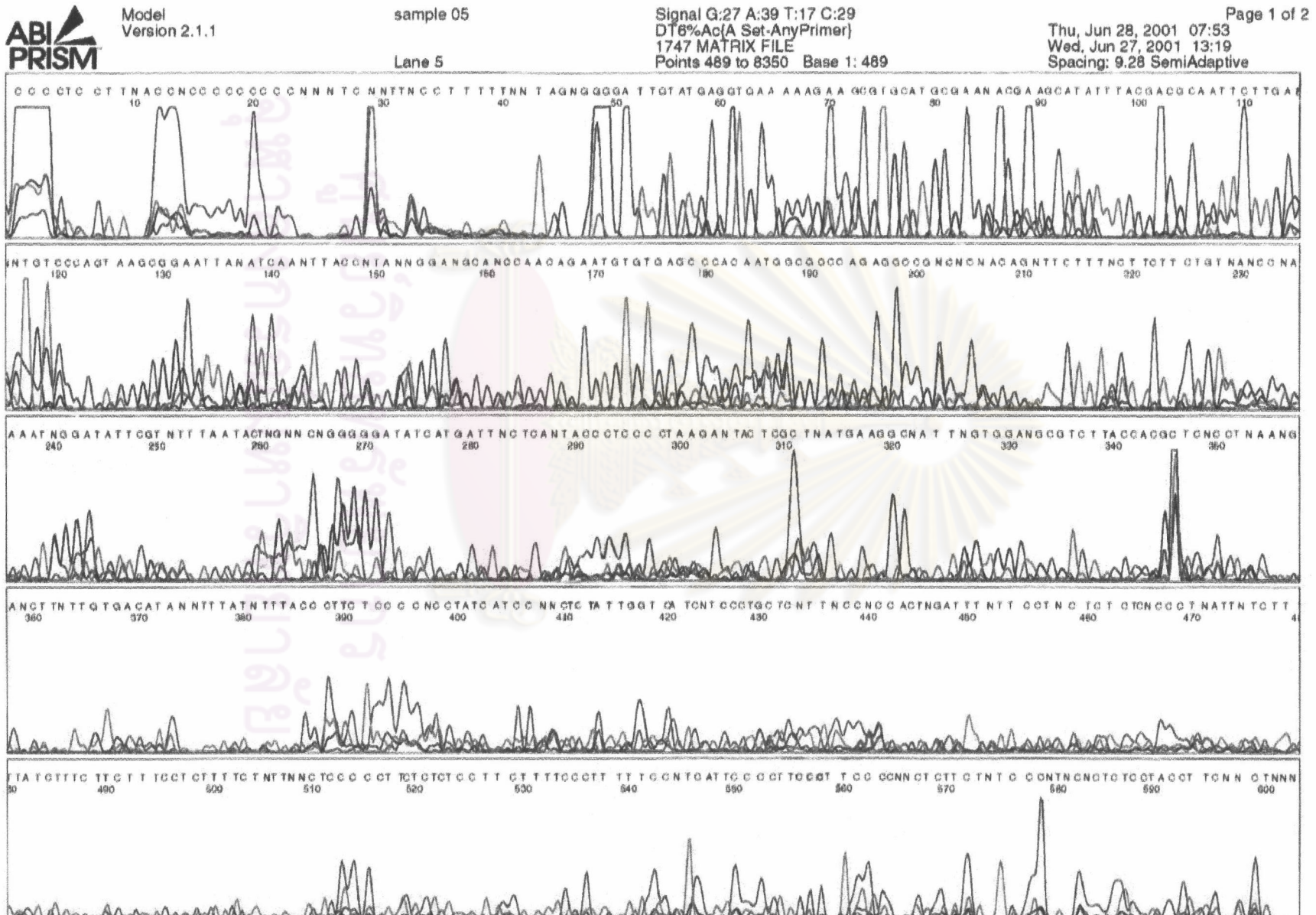
1747 MATRIX FILE
Points 836 to 8350 Base 1: 836
Page 1 of 2
Mon, May 14, 2001 09:34
Sat, May 12, 2001 14:29
Spacing: 8.54 SemiAdaptive

(k)


ABI PRISM
Model
Version 2.1.1
sample 05
Lane 5
Signal G:27 A:39 T:17 C:29
DT6%Ac(A Set-AnyPrimer)
1747 MATRIX FILE
Points 489 to 8350 Base 1: 489
Page 1 of 2
Thu, Jun 28, 2001 07:53
Wed, Jun 27, 2001 13:19
Spacing: 9.28 SemiAdaptive

(H)

# APPENDIX N

**Comparison of phenylalanine dehydrogenase properties from *Bacillus badius* (Asano *et al.*, 1987) and *Bacillus badius* BC1 (Leksakorn, 2001)**

| Properties | *Bacillus badius* | BC1 |
|---|---|---|
| Native molecular weight | 335,000 | 358,000 |
| Subunit molecular weight | 41,350 | 44,500 |
| Structure | octamer | octamer |
| Isoelectric point (pI) | 3.5 | ND |
| pH optimum | | |
| Oxidative deamination | 10.4 | 10.7 |
| Reductive amination | 9.4 | 8.3 |
| Inhibitors | $AgNO_3$, $HgCl_2$, *p*-chloromercuribenzoate | $AgNO_3$, $HgCl_2$, $FeCl_3$ |
| Substrate specificity (% relative activity) | | |
| *Oxidative deamination* | | |
| L-phenylalanine | 100 | 100 |
| L-tyrosine | 9 | 0 |
| L-tryptophan | 4 | 3 |
| L-methionine | 8 | 4 |
| L-valine | 4 | 2 |
| L-leucine | 3 | 0 |
| L-isoleucine | 0.2 | 0 |
| L-norvaline | 5 | ND |
| L-norleucine | 19 | ND |
| L-phenylalaninamide | 9 | ND |
| L-phenylalaninol | 9.4 | ND |

Remark: ND = Not determined

# APPENDIX N (continued)

**Comparison of phenylalanine dehydrogenase properties from *Bacillus badius* (Asano *et al.*, 1987) and *Bacillus badius* BC1 (Leksakorn, 2001)**

| Properties | *Bacillus badius* | BC1 |
|---|---|---|
| L-phenylalanine methyl ester | 38 | ND |
| *p*-fluoro-DL-phenylalanine | 34 | 11 |
| *m*-fluoro-DL-phenylalanine | 11 | 5 |
| *o*-fluoro-DL-phenylalanine | 2 | 0 |
| α-amino-β-phenylbutanoate | ND | 8 |
| D-amino acids | ND | 0 |
| *Reductive amination* | | |
| phenylpyruvate | 100 | 100 |
| *p*-hydroxyphenylpyruvate | 53 | 0 |
| α-ketovalerate | 12 | 3 |
| α-ketocaproate | ND | 12 |
| α-ketoisovalerate | ND | 5 |
| α-ketoisocaproate | ND | 4 |
| α- ketobutyrate | 3 | 0 |
| α-ketohexanoate | 31 | ND |
| α-keto-γ-methylthiobutyrate | 16 | 0 |
| α-keto-γ-methylvalerate | 4 | 0 |
| α-keto-γ-methylpentanoate | 13 | ND |

Remark: ND = Not determined

# APPENDIX N (continued)

**Comparison of phenylalanine dehydrogenase properties from *Bacillus badius* (Asano *et al.*, 1987) and *Bacillus badius* BC1 (Leksakorn, 2001)**

| Properties | *Bacillus badius* | BC1 |
|---|---|---|
| Apparent *Km* (mM) | | |
| L-phenylalanine | 0.088 | 0.59 |
| $NAD^+$ | 0.15 | 0.28 |
| NADH | 0.21 | 0.067 |
| phenylpyruvate | 0.106 | 0.33 |
| ammonia | 127 | 200 |

# BIOGRAPHY

Miss Jittima Charoenpanich was born on November 29, 1977. She graduated with the degree of Bachelor of Science from the department of Biochemistry at Chulalongkorn University in 1998. She has studied for the degree of Master of Science at the Department of Biochemistry, Chulalongkorn University since 1999.