

## REFERENCES

- Ashburner, M. 1982. The effects of heat shock and other stress on gene activity : an introduction. 1-9 pp. *in* Schlesinger, M.J., Ashburner, M., Tissers (eds) Heat Shock : From bacteria to man. New York : Cold Spring Harbor Laboratory Press.
- Babst, M., Hennecke, H., Fischer, H-M. 1996. Two different mechanisms are involved in the heat shock regulation of chaperonin gene expression in *Bradyrhizobium japonicum*. Mol. Microbiol. 19(4) : 827-839.
- Blackall, L.L. 1999. Workshop on Molecular Biology Techniques. September 22-24 and 26-28, 1999. Thaksin University, Songkla, Thailand. p. 23, j1-j9.
- 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.
- Chen, W.X., Yan, G.H., Li, J.L. 1988. Numerical taxonomic study of fast-growing soybean rhizobia and a proposal that *Rhizobium fredii* be assigned to *Sinorhizobium* gen.nov. Int. J. Syst. Bacteriol. 38(4) : 392-397.
- de Bruijn, F.J. 1992. Use of repetitive (repetitive extragenic palindromic and enterobacterial repetitive consensus) sequences and the polymerase chain reaction to fingerprint the genomes of *Rhizobium meliloti* isolates and other soil bacteria. Appl. Environ. Microbiol. 58 (7) : 2180-2187.
- Department of Agricultural Extension. 2001. Areas for soybean cultivation in different parts of Thailand. 9 pp.
- Dorsch, M., Stackebrandt, E. 1992 Some modifications in the procedure of direct sequencing of PCR amplified 16S rDNA. J. Microbiol. Meth. 16 : 271-279.
- Elkan, G.H., Bunn, C.R. 1992 The Rhizobia. In Balows, A., Truper, H.G., Dworkin, M., Harder, W., Schleifer, K-H (eds). The Prokaryotes. 2<sup>nd</sup> Edition. Chapter 107. p.2197-2213. New York : Springer-Verlag.
- Estrada-de Los Santos, Bustillos-Gistales,R., Cahallero-Mellado.2001. *Burholderia* sp. A genes rich in plant-associated nitrogen-fixer with experimental and geographic distribution. Appl. Environ. Microbiol. 67(6): 2790-2789

- Fischer, H.M., Babst, M., Kasper, T., Acuna, G., Arigoni, F., Hennecke, H. 1993. One member of a *groESL*-like chaperonin multigene family in *Bradyrhizobium japonicum* is co-regulated with symbiotic nitrogen fixation genes. *EMBO J.* 12 : 2901-2912.
- Heuer, H., Krsek, M., Baher, P., Smalla, K., Wellington, E.M.H. 1997. Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. *Appl. Environ. Microbiol.* 63:3233-3241.
- Gillis, M., Van Van, T., Bardin, R., Goor, M., Hebbar P., Willems, A., Segers, P., Kersters, K., Heulin, T., Perandez, M.P. 1995. Polyphasic taxonomy in the genus *Burkholderia* leading to an amended prescription of the genus and proposition of *Burkholderia vietnamiensis* sp. nov. for N<sub>2</sub>-fixing isolates from rice in Vietnam. *Int. J. Syst. Bacteriol.* 45 : 2: 274-289.
- Glover, T., Mitchell, K. 1998. *An Introduction to Biostatistics*. 1<sup>st</sup> International edition. p.203-206.USA : McGraw-Hill .
- Haukka, K., Lindstrom, K., Young, J.P.W. 1998. Three phylogenetic groups of *nodA* and *nifH* genes in *Sinorhizobium* and *Mesorhizobium* isolates from leguminous trees grouping in Africa and Latin America. *Appl. Environ. Microbiol.* 64 (2) : 419-426.
- Heuer, H., Krsek, M., Baker, P., Smalla, K., Wellington, E.M.H. 1997. Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. *Appl. Environ. Microbiol.* 63 : 3233-3241.
- Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T., Williams, S.T. 1994. *Bergey's Determinative Bacteriology*. 9<sup>th</sup> Edition. p. 95, 97. Philadelphia : Lippincott Williams & Wilkins.
- Jordan, D.C. 1982. Transfer of *Rhizobium japonicum* Buchanan 1980 to *Bradyrhizobium* gen. nov., a genus of slow growing, root nodule bacteria from leguminous plants. *Int. J. Syst. Bacteriol.* 32 : 136-139.
- Judd, A.K., Schneider, M., Sadowsky, M. J., de Bruijn, F. J. 1993. Use of repetitive sequences and the polymerase chain reaction technique to classify genetically

- related *Bradyrhizobium japonicum* serocluster 123 strains. Appl. Environ. Microbiol. 59(6) : 1702-1708.
- Keyser, H.H., Bohlool B.B., Hu, T.S. and Weber, D.F. 1982. Fast-growing rhizobia isolated from root nodules of soybean. Science. 215:1631-1632.
- Kosslak, R.M., Bookland, R., Barkai, J., Paaren, H.E., Applebaum, E.R. 1987. Induction of *Bradyrhizobium japonicum* common nod genes by isoflavones isolated from *Glycine max*. Proceedings of the National Academy of Science, USA. 84 : 7428-7432.
- Kündig, C., Hennecke, H., Göttfert, M. 1993. Correlated physical and genetic map of the *Bradyrhizobium japonicum* 110 genome. J. Bacteriol. 175 : 613-622.
- Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227 : 680-685.
- Li, D., Hubbell, D.H. 1969. Infection thread formation as a basis of nodulation specificity in *Rhizobium*-strawberry clover associations. Can. J. Microbiol. 15 : 1133-1136.
- Lin, C.C., Chen, Y.C., Song, S.C., Lin, L.P. 1999. Flavonoids as inducers of extracellular proteins and expolysaccharides of *Sinorhizobium fredii*. Biol Fertil Soils. 30 : 83-89.
- Mathis, J.N., McMillen, 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.
- Minder, A.C., Fischer, H-M., Hennecke, H., Narberhaus, F. 2000. Role of Hrc A and CIRCE in the heat shock regulatory network of *Bradyrhizobium japonicum*. J Bacteriol. 182 : 14-22.
- Minder, A.C., Narberhaus, F., Babst, M., Hennecke, H., Fischer, H.-M. 1997. The *dnaKJ* operon belongs to the  $\sigma^{32}$  -dependent class of heat shock genes in *Bradyrhizobium japonicum*. Mol. Genet. 254 : 195-206.
- Moulin, L., Munive, A., Dreyfuss. B., Boivin-Masson, C. 2001. Nodulation of legumes by member of the B-subclass of Proteobacteria. Nature. 411 : 948-950.
- Mullis, K. B., and F.A. Falloona. 1987. Specific synthesis of DNA in vitro via a polymerase catalyzed chain reaction. Methods Enzymol. 155 : 335-350.

- Münchbach, M., Nocker, A., Narberhaus, F. 1999. Multiple small heat shock proteins in rhizobia. J. Bacteriol. 18 (1) : 83-90.
- Nocker, A., Hausherr, T., Balsiger, S., Krstulovic, N-P., Hennecke, H., Narberhaus, F. 2001. A mRNA-based thermosensor controls expression of rhizobial heat shock genes. Nucleic Acids Res. 29 (23) : 4800-4807.
- Richardson, A.E., Viccars, L.A., Watson, J.M., Gibson, A.H. 1995. Differentiation of *Rhizobium* strains using the polymerase chain reaction with random and directed primers. Soil Biol. Biochem. 27 : 515-524.
- Salles,J.F.,De Souza,F.A.,van Elsas.J.D.2002.Molecular method to assess the diversity of *Burkholderia* species in environmental samples. Appl.Environ.Microbiol.68 (4):1595-1603
- Sambrook, J., Russell, D.W. 2001. Molecular Cloning : A Laboratory Manual, 3<sup>rd</sup> Ed. New York : Cold Spring Harbor Laboratory Press. Book1.
- Sanchez-Contreras, M., Lloret. J., Martin, M.,Villacieros, M., Bonilla, I.,Bivilla,R.2002.PCR use of highly conserved DNA regions for identification of *Sinorhizobium meliloti*. Appl.Environ.Microbiol.66(8):3621-3623
- Schneider, S.H. 1989. The Greenhouse Effect : Science and Policy. Science 243 : 771-781.
- Schofield, P.R., Watson, J.M. 1985. Conservation of *nif*-and species-specific domains within repeated promoter sequences from fast-growing *Rhizobium* species. Nucleic Acids Res. 13 (10) : 3407-3418.
- Scholla, M.H., and Elkan, G.H.1984. *Rhizobium fredii* sp. nov., a fast-growing species that effectively nodulates soybeans. Int. J.Syst. Bacteriol. 34:484-486.
- Singleton, P., Sainsbury, D. 1988. Dictionary of Microbiology and Molecular Biology. 2 nd Edition. p.394. Singapore : John Wiley & Sons.
- Somasegaran, P. and Hoben, H. J. 1994. Handbook for Rhizobia : Methods in legume-rhizobium Technology. p. 340, 370-1.New York : Springer Verlag.
- Suwat Saengkerdsub. 2000. Effects of initial pH on hydrogenase activity and protein patterns of acid-tolerant *Bradyrhizobium japonicum*. Master of Science Thesis in Industrial Microbiology. Faculty of Science, Chulalongkorn University. 103 pp.

- Trân Van, V., Berge, O., Ke, S.N., Balandreau, J., Heulin, T. 2000. Repeated beneficial effects of rice inoculation with a strain of *Burkholderia vietnamensis* on early and late yield components in low fertility sulphate acid soils of Vietnam. Plant and Soil 218 : 273-284.
- Viallard, V., Poirier, I., Cournoyer, B., Haurat, J., Wiebkin, S., Ophel-Keller, K., Balandreau, J. 1998. *Burkholderia graminis* sp. nov., a rhizospheric *Burkholderia* species, and reassessment of [Pseudomonas] *Rhenazinum*, [Pseudomonas] *pysrocinia* and [Pseudomonas] glathei as *Burkholderia*. Int. J.Syst. Bacteriol. 48 (2) : 549-563.
- Voet, D., Voet, J.G. 1995. Biochemistry. 2<sup>nd</sup> Edition.p.201-203. New York : John Wiley & Sons.
- Young.C.C.,Cheng,K.T.1998. Genetic diversity of fast-and slow-growing soybean rhizobia determined by random amplified polymorphic DNA analysis. Biol. Fertil. Soils 26 : 254-256.
- Zuber,U.,Schunmann,W.1994,CIRCE,a novel heat shock element involved in regulation of heat shock operon *dnaK* of *Bacillus subtilis*. J. Bacteriol.176(5):1359-1363.



## APPENDIXES

ศูนย์วิทยทรัพยากร  
จุฬาลงกรณ์มหาวิทยาลัย

## Appendix A

### Bacterial Growth Media and Plant Nutrient Solutions

Preparation of all bacterial growth media and plant nutrient solutions are as described by Somasegaran and Hoben (1994) unless otherwise stated.

#### **Yeast Extract Mannitol Broth (YMB)**

Mannitol	10.0 g
K <sub>2</sub> HPO <sub>4</sub>	0.5 g
MgSO <sub>4</sub> .7H <sub>2</sub> O	0.2 g
NaCl	0.1 g
Yeast extract	0.5 g
Deionized water	1.0 g

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

#### **Yeast Extract Mannitol Agar (YMA)**

YMB	1 liter
Agar	15 g

Agar was added to 1 liter of YMB. The solution was shaken to suspend the agar then autoclaved at 121°C for 15 min. After autoclaving, the medium was shaken to ensure even mixing of melted agar with medium before pouring onto petri dishes and left to solidify.

#### **YMA with Congo Red**

Congo Red stock solution : 250 mg of Congo Red dissolved in 100 ml of deionized water. 10 ml of Congo Red stock solution were added to 1 liter of YMA. The final Congo Red concentration was 25 µg.ml<sup>-1</sup>. The medium was autoclaved at 121°C for 15 min.

### N-free Nutrient Solutions

Stock Solutions	Chemicals	g/liter
1	CaCl <sub>2</sub> .2H <sub>2</sub> O	294.1
2	KH <sub>2</sub> PO <sub>4</sub>	136.1
3	FeC <sub>6</sub> H <sub>5</sub> O <sub>7</sub> .3H <sub>2</sub> O	6.7
	MgSO <sub>4</sub> .7H <sub>2</sub> O	123.3
	K <sub>2</sub> SO <sub>4</sub>	87.0
	MnSO <sub>4</sub> .H <sub>2</sub> O	0.338
4	H <sub>3</sub> BO <sub>3</sub>	0.247
	ZnSO <sub>4</sub> .7H <sub>2</sub> O	0.288
	CuSO <sub>4</sub> .5H <sub>2</sub> O	0.100
	CoSO <sub>4</sub> .7H <sub>2</sub> O	0.056
	Na <sub>2</sub> MoO <sub>4</sub> .7H <sub>2</sub> O	0.048

Warm water was used to prepare stock solutions to get the ferric-citrate into solution. Ten liters of full-strength plant culture solution were prepared as follows :

- To 5 liters of water, add 5 ml of each stock solution and mix.
- Dilute to 10 liters by adding another 5 liters of water.
- Adjust pH to either 5.0 or 6.8 with 1 N HCl
- For positive control treatment, 0.05% KNO<sub>3</sub> was added to give final N concentration of 70 ppm.

จุฬาลงกรณ์มหาวิทยาลัย

## Appendix B

### Chemicals and Solutions

#### 1. Solutions for DNA extraction (Gibco BRL)

##### Saline-EDTA solution

15 mM NaCl, 10 mM EDTA, pH 8.0

0.9 g NaCl, 0.29 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.

##### DNAzol

DNAzol solution (Gibco BRL) was used according to manufacturer's instruction.

#### 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<sub>2</sub>O

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 persulfate

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

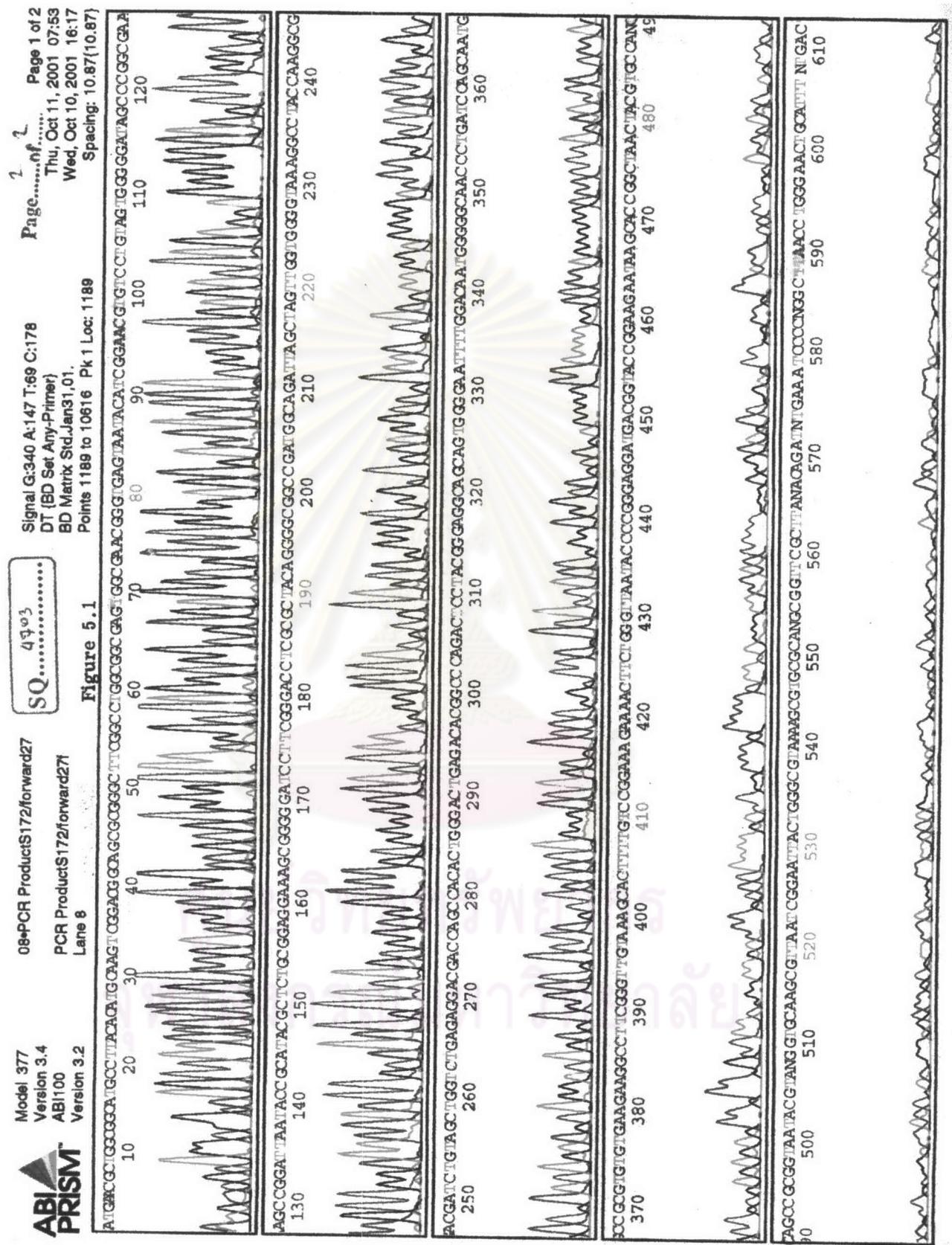
## Appendix C

### Raw Data

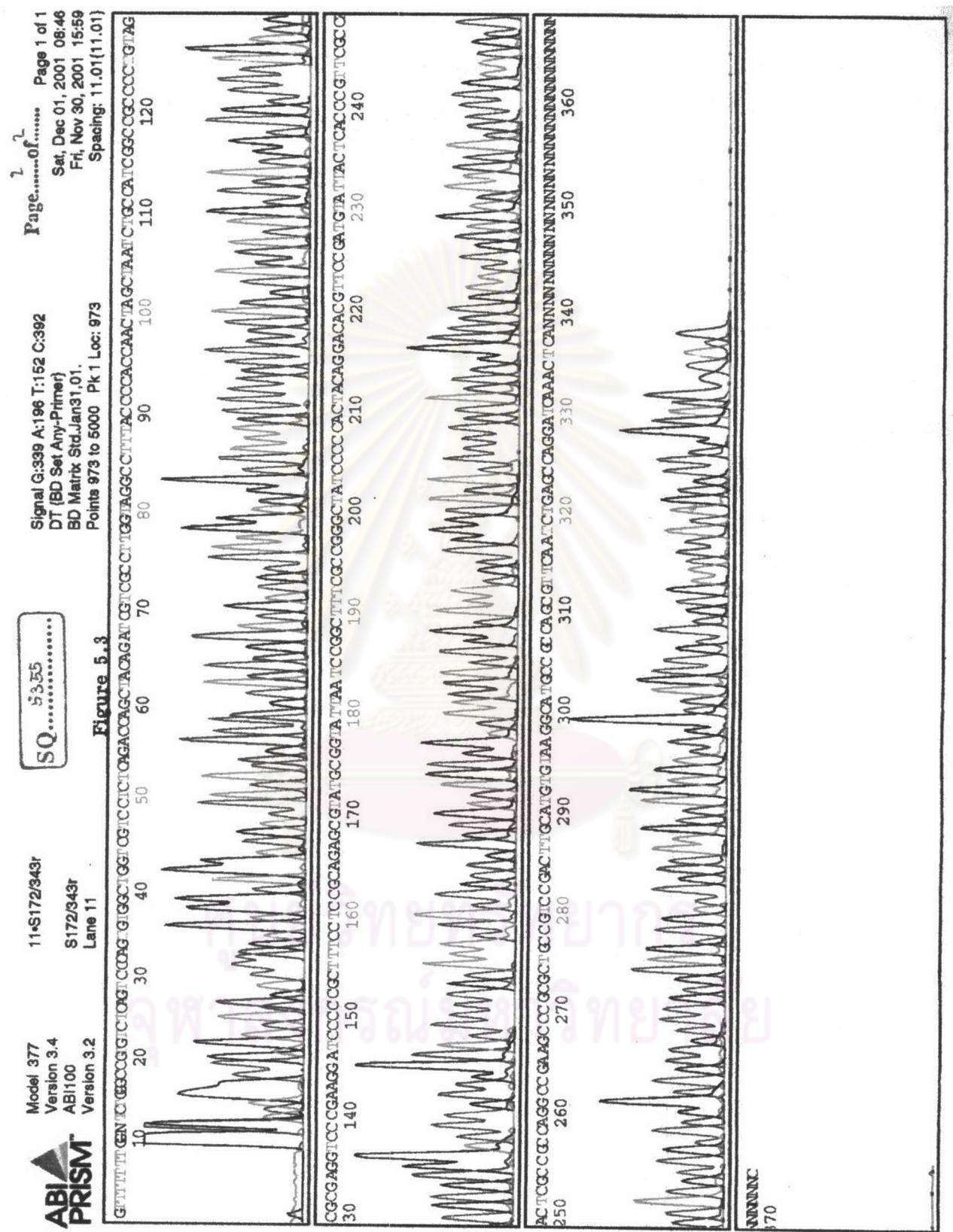
1. Figures 5.1-5.6, 5.7-5.12, 5.13-5.18 showed 16S rDNA sequences of strains S172, S173 and S174 respectively.
2. Comparisons of 16S rDNA sequences of isolates S172, S173, and S174 with data in GenBank using the BLAST program (NCBI).
3. Duncan's Mutiple Range Test (Glover & Mitchell, 1998)

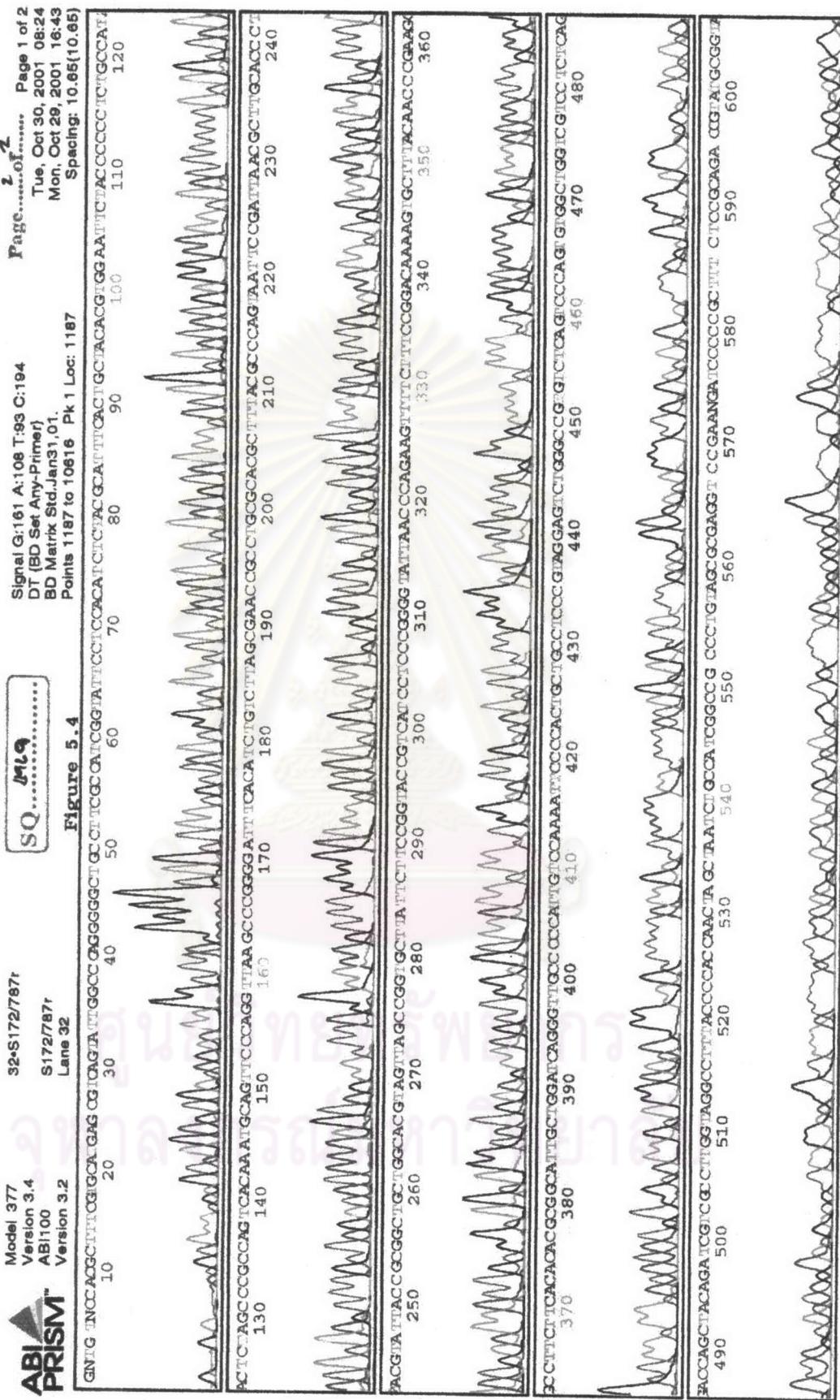
Duncan's Mutiple Range Test was performed as follows :

- 1.Means of plant dry weight were placed in order of magnitide.
- 2.The standard error of the means was calculated .
- 3.The values of  $r_{\alpha}$  ( $p$ ,  $f$ ) were obtained from Duncan's table.
- 4.Any two means that were "p" apart and which differed by more than  $R_p$  were declared to be significantly different.









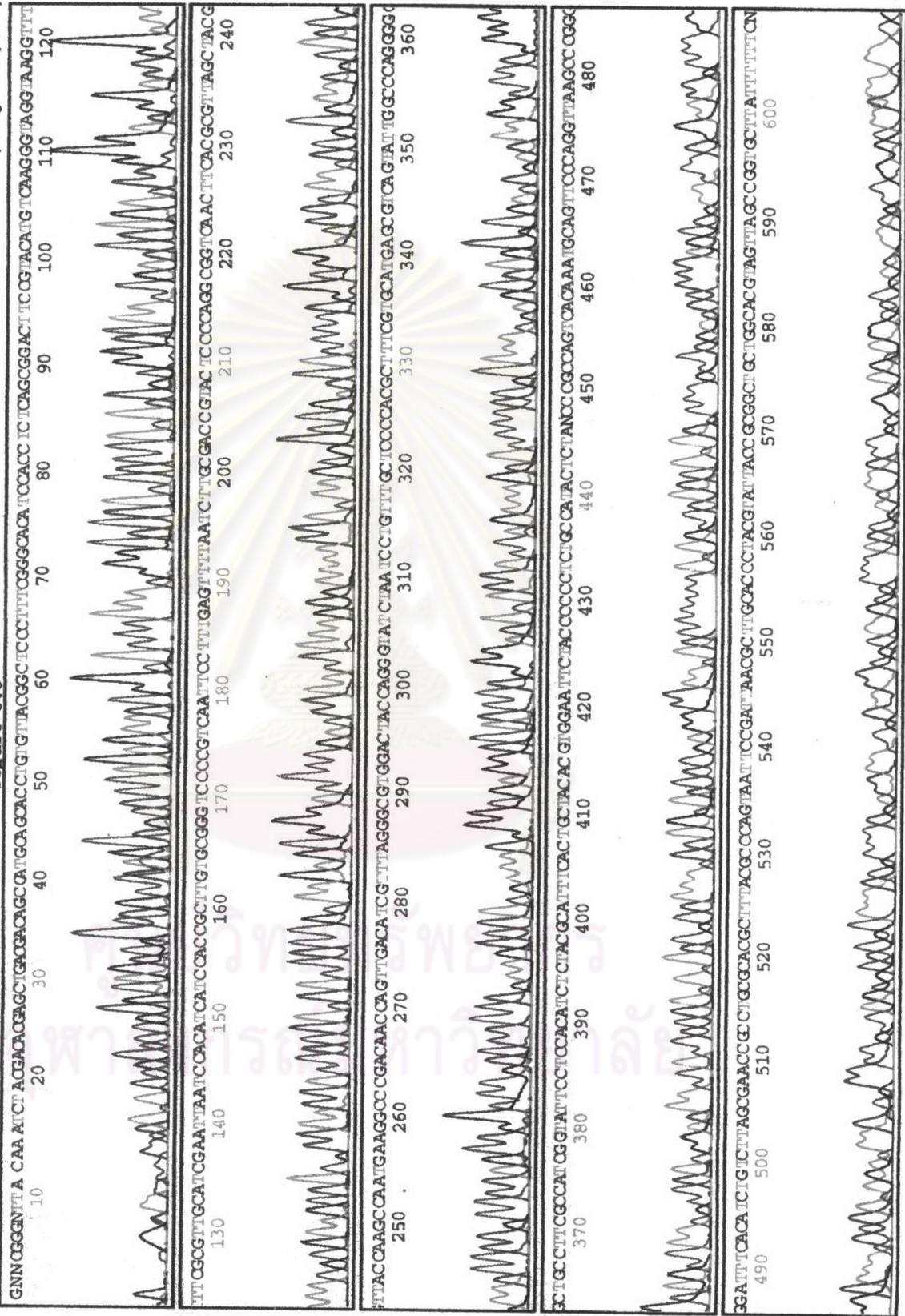
**ABI PRISM®**  
Model 377  
Version 3.4  
AB1100  
S172/1100r  
35-S172/1100r

SO...4440

Signal G:205 A:124 T:98 C:206  
DT (BD Set Any-Primer)  
BD Matrix StdJan31.01.

Points 1004 to 10616 Pk 1 Loc: 100

Figure 5.5





Model 377  
Version 3.4  
ABI100  
Version 3.2



09•PCR ProductS173:forward277  
SQ.....  
L...49.0P

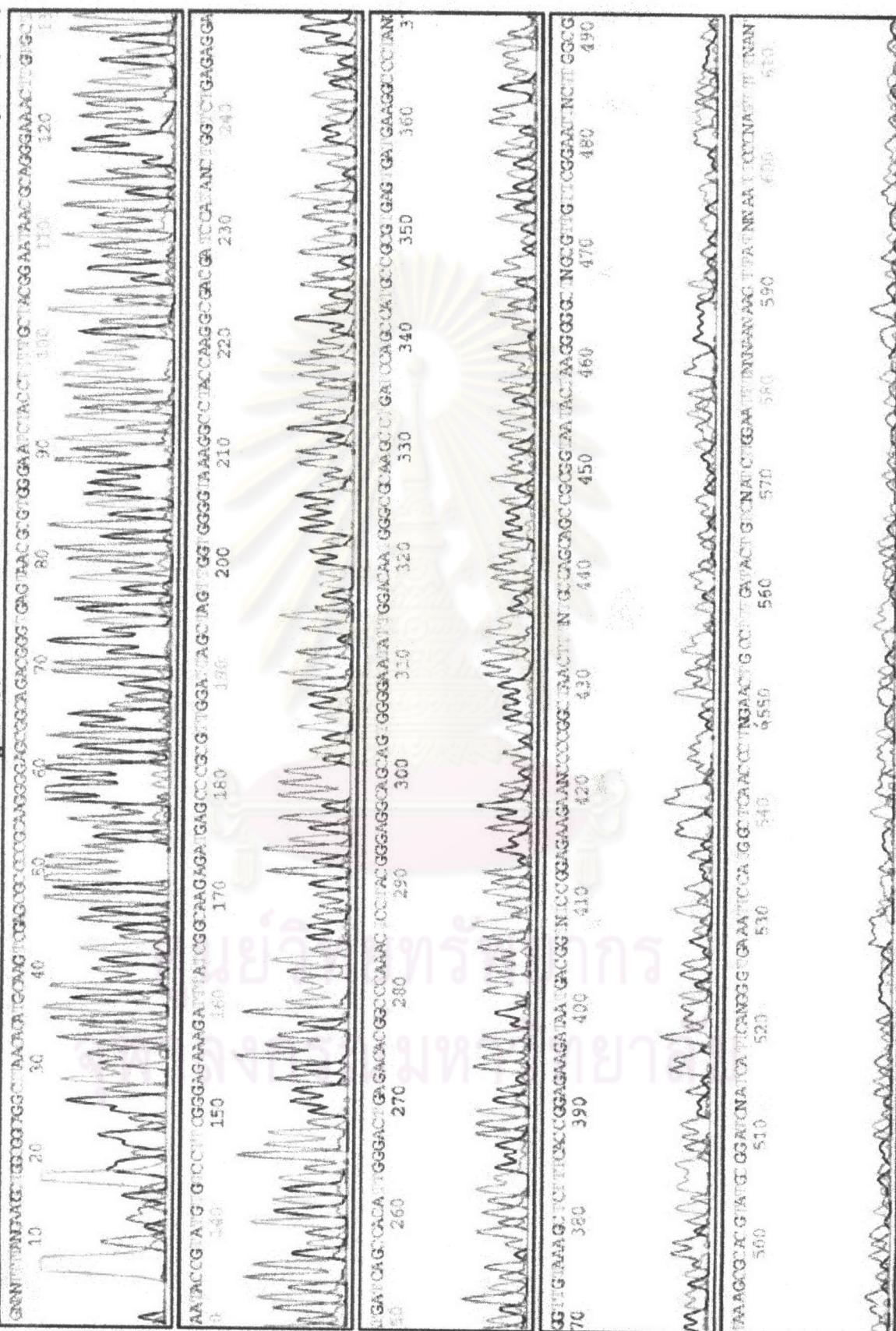
PCR ProductS173:forward277

Lane 9

Version 3.2

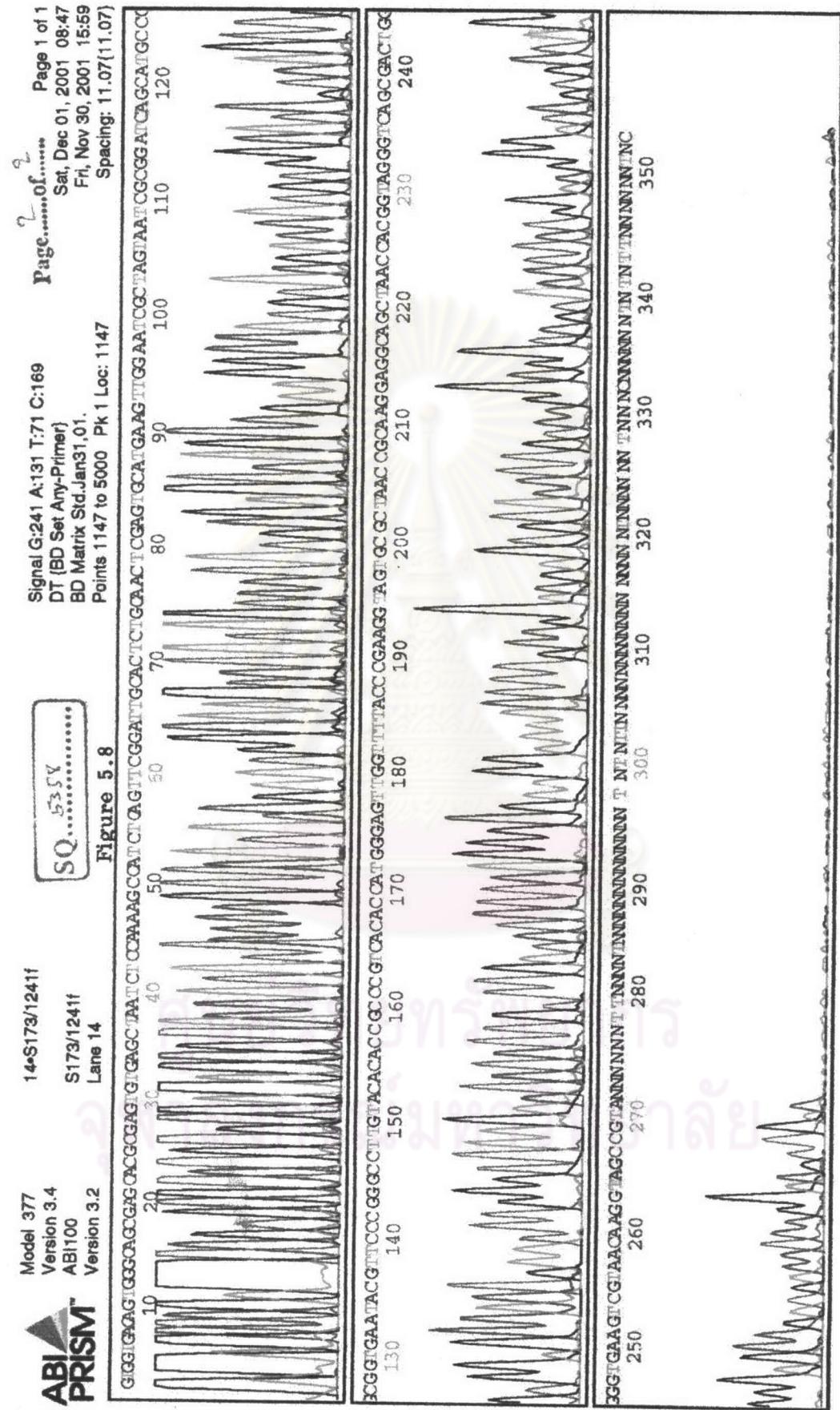
Signal G:116 A:54 T:30 C:60  
DT (BD Set Any-Primer)  
BD Matrix Std Jan31.91.  
Points 1001 to 10616 Pk 1 Loc: 1001:  
Spacing: 10.82(10.82)

**Figure 5.7**

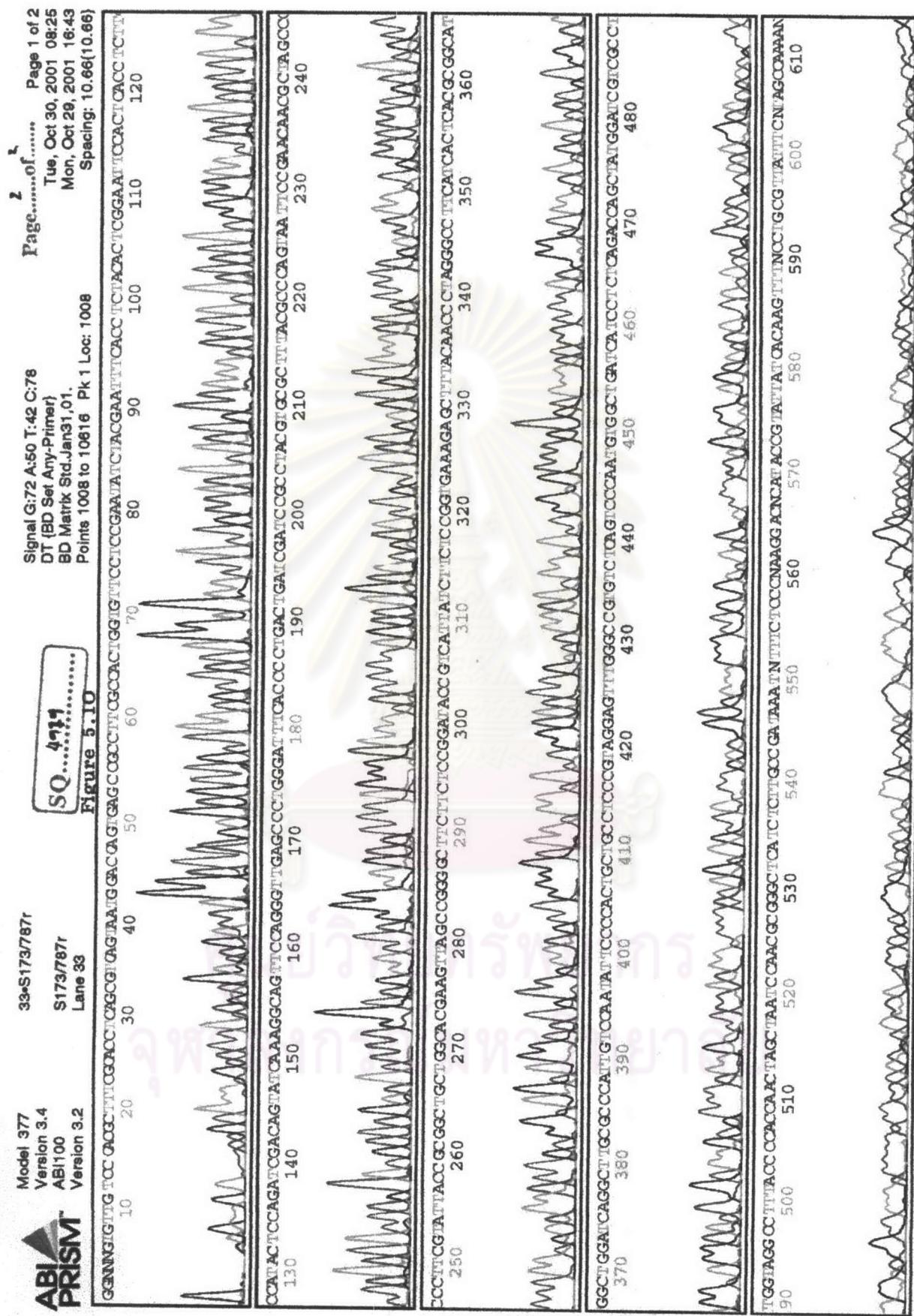


Page.....of..... Page 1 of 2  
Thu, Oct 11, 2001 07:53  
Wed, Oct 10, 2001 16:17

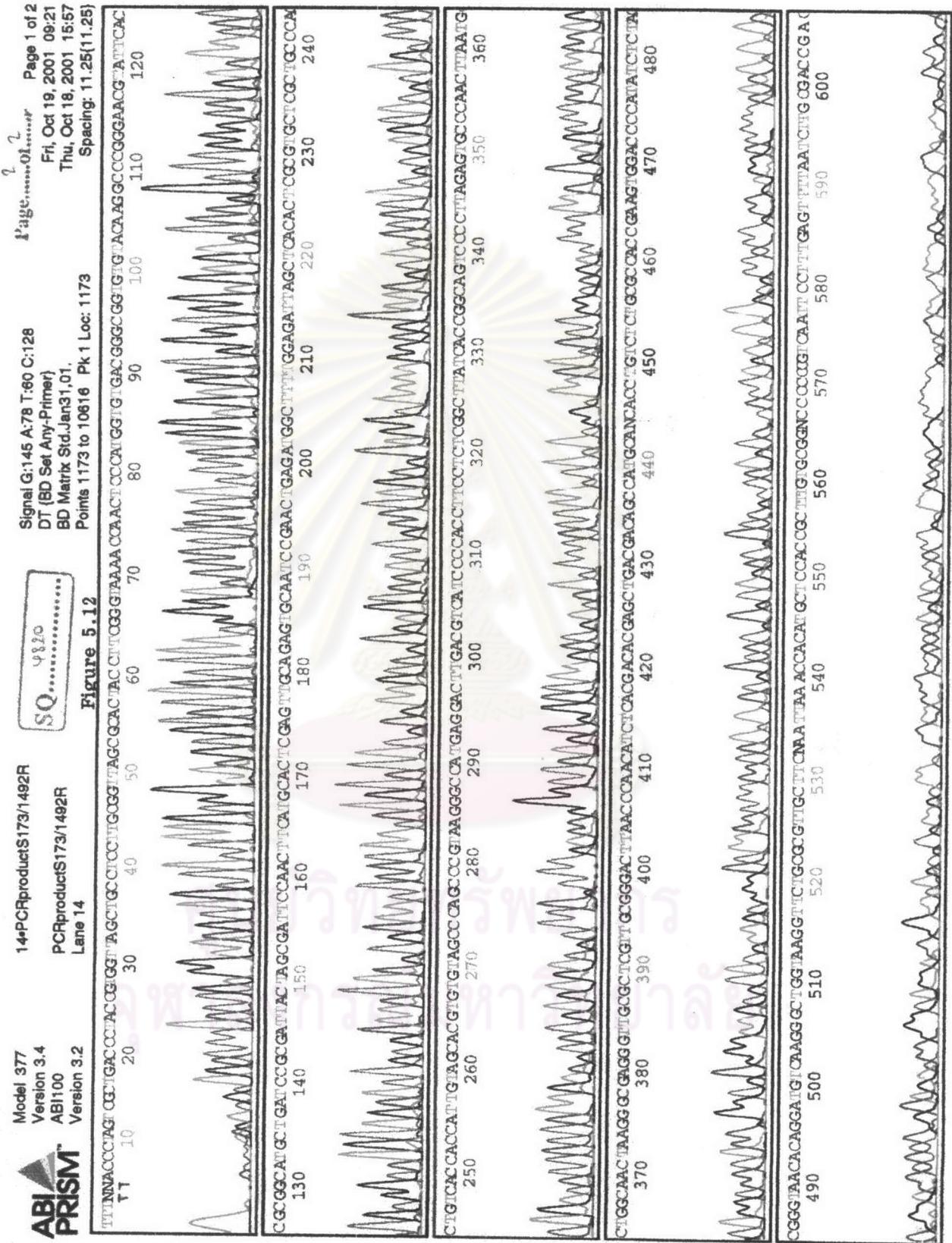
Page.....of..... Page 1 of 2  
Thu, Oct 11, 2001 07:53  
Wed, Oct 10, 2001 16:17











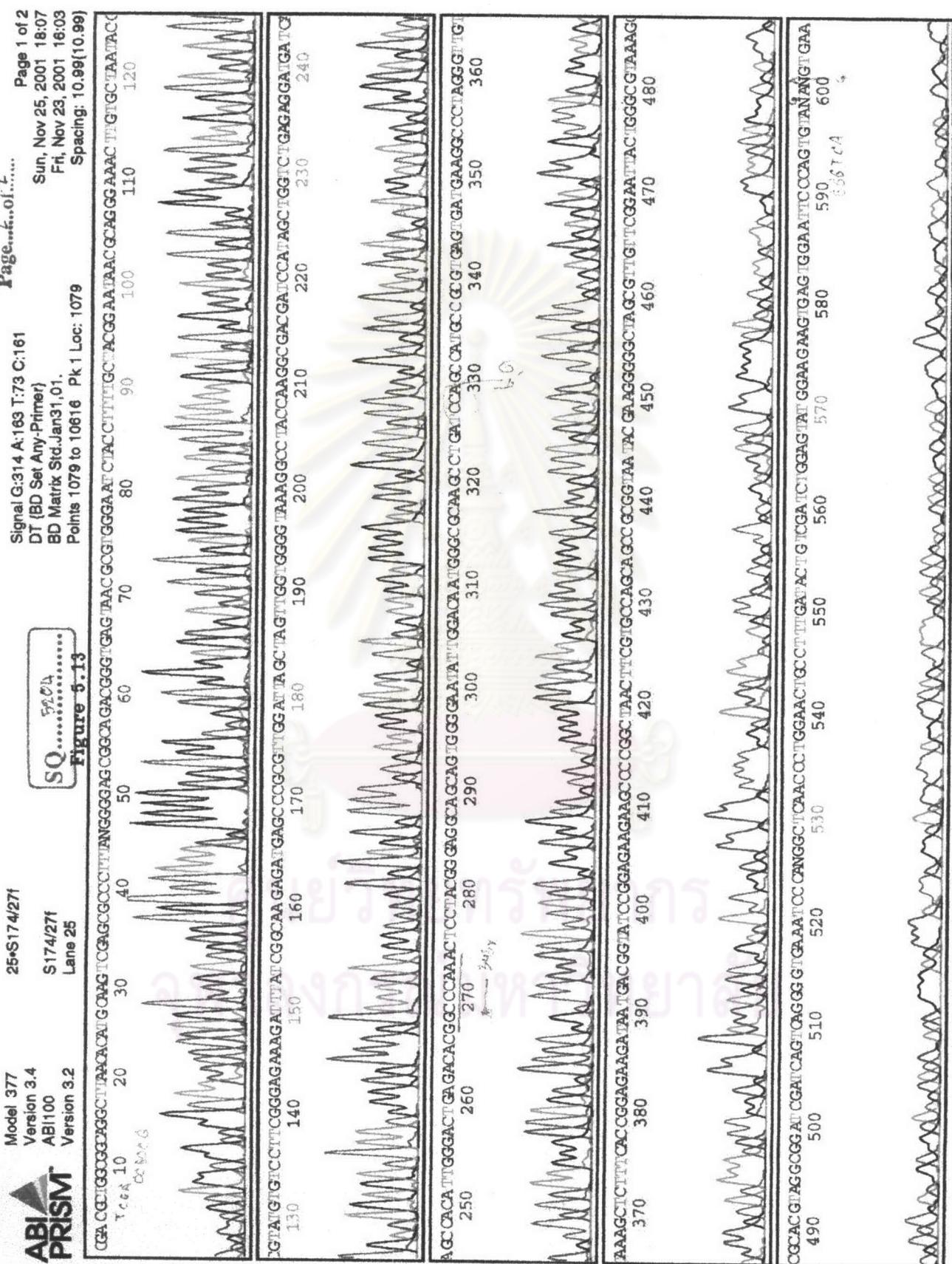
**ABI PRISM®**  
Model 377  
Version 3.4  
ABI100  
Version 3.2

25-S174/271

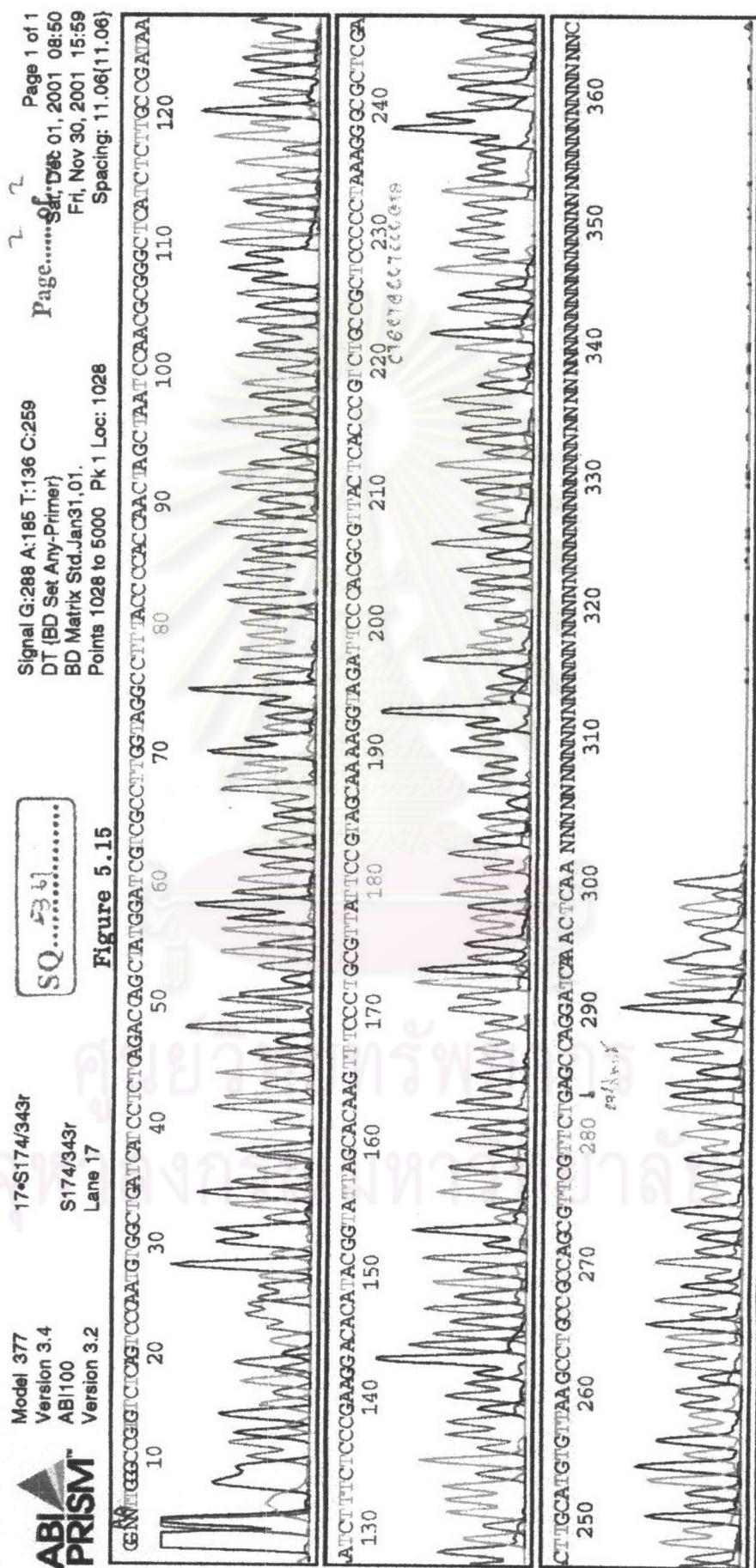
S174/27f  
Lane 25

**SQ.....5204.....**

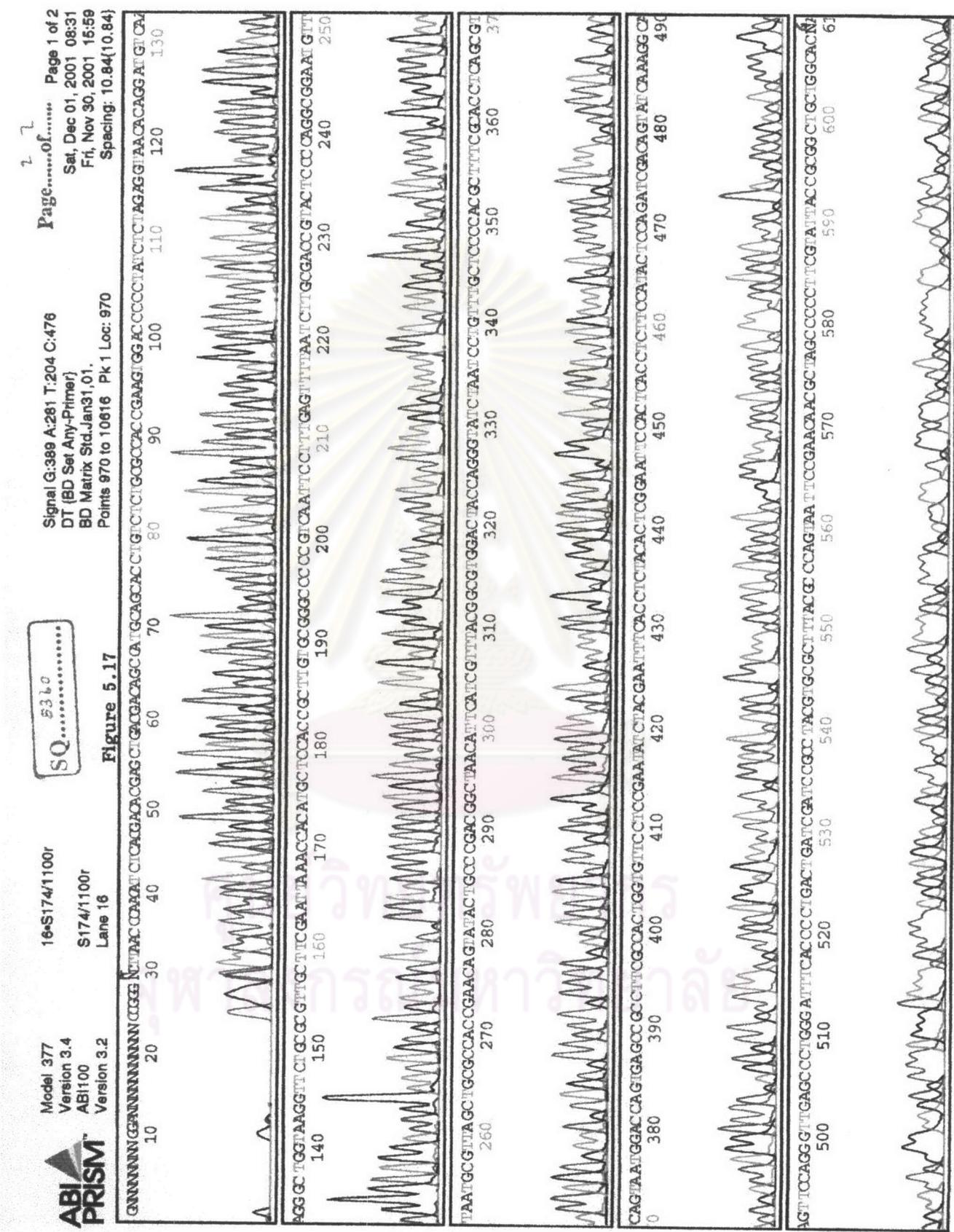
**Figure 3-13**

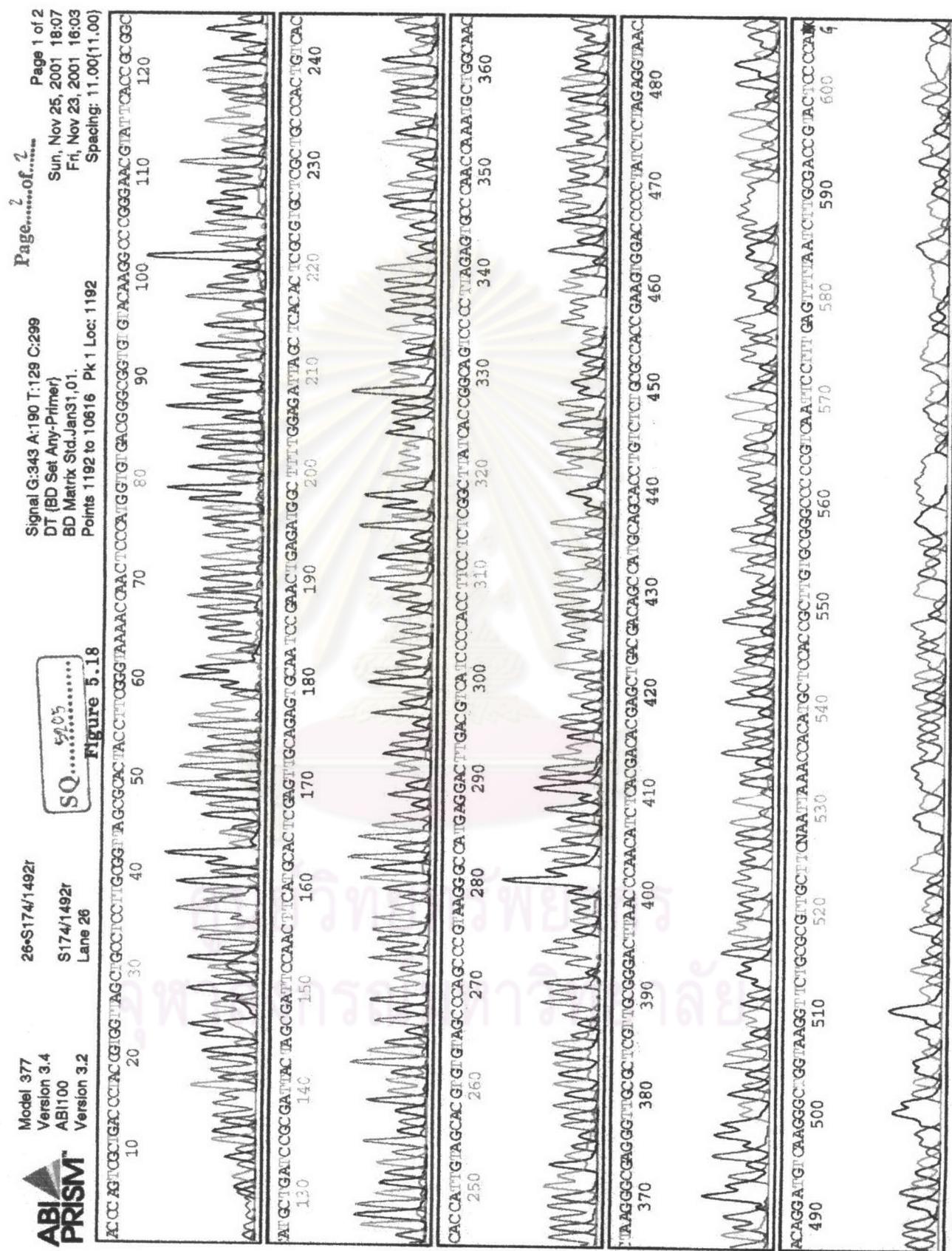












Query= gi|S172| (1444 letters)

Sequences producing significant alignments:	Score	E	(bits)	Value
gi 3273472 gb U96941.1 U96941 Burkholderia graminis 16S rib...	1053	0.0		
gi 3273471 gb U96940.1 U96940 Burkholderia graminis 16S rib...	1053	0.0		
gi 3273470 gb U96939.1 U96939 Burkholderia graminis 16S rib...	1053	0.0		
gi 9280413 gb AF247493.1 AF247493 Burkholderia sp. C4.7 16S...	1045	0.0		
gi 17863929 gb AF448046.1 AF448046 Burkholderia sp. SOD-7 1...	1039	0.0		
gi 2623537 gb U91570.1 BCU91570 Burkholderia caryophylli 16...	1031	0.0		
gi 17863928 gb AF448045.1 AF448045 Burkholderia sp. SOD-6 1...	1013	0.0		
gi 21070363 gb AF508806.1  Burkholderia sp. TNFYE-5 16S rib...	1005	0.0		
gi 18249661 dbj AB066231.1  Burkholderia sp. PJ310 gene for...	1001	0.0		
gi 12718590 emb AJ292643.1 UEU292643 uncultured eubacterium...	997	0.0		

#### Alignments

>gi|3273472|gb|U96941.1|U96941 Burkholderia graminis 16S ribosomal RNA gene, partial sequence  
Length = 1584

Score = 1053 bits (531), Expect = 0.0

Identities = 534/535 (99%)

Strand = Plus / Minus

Query: 524 acgacacgagctgacgacagccatgcagcacctgtttacggctcccttcggcacatc 583  
 |||||||  
 Sbjct: 1025 acgacacgagctgacgacagccatgcagcacctgtttacggctcccttcggcacatc 966

Query: 584 cacctctcagcgacttccgtacatgtcaaggtaggtaagggttttcgcgttgcatcga 643  
 |||||||  
 Sbjct: 965 cacctctcagcgacttccgtacatgtcaaggtaggtaagggttttcgcgttgcatcga 906

Query: 644 attaatccacatcatccaccgcttgtgcgggtcccgtaattcctttagtttatct 703  
 |||||||  
 Sbjct: 905 attaatccacatcatccaccgcttgtgcgggtcccgtaattcctttagtttatct 846

Query: 704 tgcgaccgtactccccaggcggtcaactcacgcgttagctacgttaccaagccatgaa 763  
 |||||||  
 Sbjct: 845 tgcgaccgtactccccaggcggtcaactcacgcgttagctacgttaccaagccatgaa 786

Query: 764 ggcccgacaaccaggtaggtgacatcggtttagggcggtggactaccagggttatctaattctgttt 823  
 |||||||  
 Sbjct: 785 ggcccgacaaccaggtaggtgacatcggtttagggcggtggactaccagggttatctaattctgttt 726

Query: 824 gctccccacgcttcgtcatgagcgtcagtattggccagggggctgcctcgccatcg 883  
 |||||||  
 Sbjct: 725 gctccccacgcttcgtcatgagcgtcagtattggccagggggctgcctcgccatcg 666

Query: 884 gtattcctccacatctctacgcattcactgctacacgttgaattctaccccccgtcc 943  
 |||||||  
 Sbjct: 665 gtattcctccacatctctacgcattcactgctacacgttgaattctaccccccgtcc 606

Query: 944 atactctatcccgccagtcaaaatgcagttccaggtaagccgggattcacatct 1003  
 |||||||  
 Sbjct: 605 atactctagccgcccagtcaaaatgcagttccaggtaagccgggattcacatct 546

Query: 1004 gtcttagcgaaccgcctgcgcacgccttacgcccagaattccgattaacgcttg 1058  
 |||||||  
 Sbjct: 545 gtcttagcgaaccgcctgcgcacgccttacgcccagaattccgattaacgcttg 491

Score = 795 bits (401), Expect = 0.0  
 Identities = 444/457 (97%), Gaps = 1/457 (0%)  
 Strand = Plus / Minus

Query: 1 caccctacgtattaccgcggctgtggcacgttagtttagccgggtctattctccggtaac 60  
 |||||||  
 Sbjct: 490 caccctacgtattaccgcggctgtggcacgttagtttagccgggtctattctccggtaac 431

Query: 61 cgtagtccgggtttaaccagaagtttcttccggacaaaagtgtttacaac 120  
 |||||  
 Sbjct: 430 cgtagtccgggtttaaccagaagtttcttccggacaaaagtgtttacaac 371

Query: 121 ccgaaggccttcacacacgcggcattgtggatcagggttgcggccattgtccaaaa 180  
 |||||||  
 Sbjct: 370 ccgaaggccttcacacacgcggcattgtggatcagggttgcggccattgtccaaaa 311

Query: 181 ttccccactgtgcctccgtaggagtctggccgtgtctcagtcccagtgtggctggc 240  
 |||||||  
 Sbjct: 310 ttccccactgtgcctccgtaggagtctggccgtgtctcagtcccagtgtggctggc 251

Query: 241 gtcctctcagaccagactacagatcgtgccttggtaggccttacccaccaactagcta 300  
 |||||||  
 Sbjct: 250 gtcctctcagaccagactacagatcgtgccttggtaggccttacccaccaactagcta 191

Query: 301 atctgccatcgcccccgtagcgcgaggcccgaaggatccccgttcctccgca 360  
 |||||||  
 Sbjct: 190 atctgccatcgcccccgtagcgcgaggcccgaaggatccccgttcctccgca 131

Query: 361 gagcgtatcggttataatccggcttcgcggctatccccactacaggacacgttcc 420  
 |||||||  
 Sbjct: 130 gagcgtatcggttataatccggcttcgcggctatccccactacaggacacgttcc 71

Query: 421 gatgtattactcaaccgttcgccactcgccagg 457  
 |||||  
 Sbjct: 70 gatgtattactc-acccgttcgccactcgccaccagg 35

Score = 753 bits (380), Expect = 0.0  
 Identities = 387/388 (99%), Gaps = 1/388 (0%)  
 Strand = Plus / Minus

Query: 1057 tgaatcctaccgtggtagccgtcccttgcggttagactatgccacttctggtaaaacc 1116  
 |||||||  
 Sbjct: 1421 tgaatcctaccgtggtagccgtcccttgcggttagacta-gccacttctggtaaaacc 1363

Query: 1117 cactccatggtgtgacggcggtgttacaagacccggaaacgtattaccgcggcatg 1176  
 |||||  
 Sbjct: 1362 cactccatggtgtgacggcggtgttacaagacccggaaacgtattaccgcggcatg 1303

Query: 1177 ctgatccgcattactagcgattccagcttcacgcactcgagttgcagagtgcgtccgg 1236  
 |||||  
 Sbjct: 1302 ctgatccgcattactagcgattccagcttcacgcactcgagttgcagagtgcgtccgg 1243

Query: 1237 actacgatcggttctggattggctccacctcgccgtggcgaccctctgtccgac 1296  
 |||||  
 Sbjct: 1242 actacgatcggttctggattggctccacctcgccgtggcgaccctctgtccgac 1183

Query: 1297 cattgtatgacgtgtgaagccctaccataaggccatgaggacttgcgtcatccccac 1356  
 |||||  
 Sbjct: 1182 cattgtatgacgtgtgaagccctaccataaggccatgaggacttgcgtcatccccac 1123

Query: 1357 cttctccgggttgcacccgcagtctccctggagtgcgtctgcgtcaactagggaca 1416  
 |||||  
 Sbjct: 1122 cttctccgggttgcacccgcagtctccctggagtgcgtctgcgtcaactagggaca 1063

**Query= gi|173| (1441 letters)**

Sequences producing significant alignments:	Score	E	
		(bits)	Value
gi 28894114 gb AY206687.1  Rhizobium rhizogenes strain 163C...	<u>2658</u>	0.0	
gi 464144 dbj D14504.1 ATU16SRD] Rhizobium sp. K-Ag-3 gene ...	<u>2654</u>	0.0	
gi 27261750 gb AY166841.1  Rhizobium tropici UPRM8033 16S r...	<u>2637</u>	0.0	
gi 1055273 gb U38469.1 RTU38469 Rhizobium tropici 16S ribos...	<u>2619</u>	0.0	
gi 28849772 gb AF514802.1  Rhizobium sp. ORS217 16S ribosom...	<u>2615</u>	0.0	
gi 464139 dbj D14505.1 ATU16SRDK Rhizobium sp. Ch-Ag-4 gene...	<u>2615</u>	0.0	
gi 464142 dbj D14501.1 ATU16SRDG Rhizobium rhizogenes gene ...	<u>2615</u>	0.0	
gi 464203 dbj D11344.1 RHM16SRDC Rhizobium tropici gene for...	<u>2609</u>	0.0	
gi 28849771 gb AF514801.1  Rhizobium sp. ORS214 16S ribosom...	<u>2605</u>	0.0	
gi 296410 emb X67224.1 AR16SRRNA A.rhizogenes (LMG 152) gen...	<u>2605</u>	0.0	

### Alignments

>gi|28894114|gb|AY206687.1| Rhizobium rhizogenes strain 163C 16S ribosomal RNA, partial sequence  
Length = 1475

Score = 2658 bits (1341), Expect = 0.0  
Identities = 1421/1444 (98%), Gaps = 4/1444 (0%)  
Strand = Plus / Minus

Query: 1 tacggctacccgttacgactttaccctagtcgctgaccctaccgtggtagctgcctcc 60  
Sbjct: 1446 tacggctacccgttacgacttaccccagtcgctgaccctaccgtggtagctgcctcc 1387

Query: 61 ttgcggtagcgcactaccctcggtaaaaccaactccatggtgtacggcggtgt 120  
Sbjct: 1386 ttgcggtagcgcactaccctcggtaaaaccaactccatggtgtacggcggtgt 1327

Query: 121 acaaggccccggaaacgtattcacccggcatgctgatccgcattactagcgattccaac 180  
Sbjct: 1326 acaaggccccggaaacgtattcacccggcatgctgatccgcattactagcgattccaac 1267

Query: 181 ttcatgcactcgagttgcagagtcaatccgaactgagatggctttggagattagctca 240  
Sbjct: 1266 ttcatgcactcgagttgcagagtcaatccgaactgagatggctttggagattagctca 1207

Query: 241 cactcgctgctcgccactgtcaccaccattgtacgtgttagccagccgt 300  
Sbjct: 1206 cactcgctgctcgccactgtcaccaccattgtacgtgttagccagccgt 1147

Query: 301 aagggccatgaggacttgacgtcatccccacccatccctctcggttatcaccggcagtccc 360  
Sbjct: 1146 aagggccatgaggacttgacgtcatccccacccatccctctcggttatcaccggcagtccc 1087

Query: 361 ctttagagtcccaacttaatgtggcaactaaggcgagggtgcgctcggtcgaaaaat 420  
 ||||| ||||| ||||| ||||| ||||| |||||  
 Sbjct: 1086 ctttagagtcccaactgaatgtggcaactaaggcgagggtgcgctcggtcgaaaaat 1027

Query: 421 taacccaaa--tct-acgacacgagctgacgacagccatgcgcacacgtcgtctgcgccac 477  
 ||||| ||| ||||| ||||| ||||| |||||  
 Sbjct: 1026 taacccaaacatctcacgacacgagctgacgacagccatgcgcacacgtcgtctgcgccac 967

Query: 478 cgaagtggaccatcatctacggtaacacaggatgtcaaggctggtaagggtctgc 537  
 ||||| ||||| ||||| ||||| |||||  
 Sbjct: 966 cgaagtggaccatcatctacggtaacacaggatgtcaaggctggtaagggtctgc 907

Query: 538 gcgttgcttcgaattaaaccacatgtccaccgcgtgtgcgggccccgtcaattcctt 597  
 ||||| ||||| ||||| ||||| |||||  
 Sbjct: 906 gcgttgcttcgaattaaaccacatgtccaccgcgtgtgcgggccccgtcaattcctt 847

Query: 598 gagtttaatcttcgaccgtactcccaggcgaaatgttaatgcgttagctgcgccac 657  
 ||||| ||||| ||||| ||||| |||||  
 Sbjct: 846 gagtttaatcttcgaccgtactcccaggcgaaatgttaatgcgttagctgcgccac 787

Query: 658 cgaacagtatactccgcacggtaacattcatgtttacggcgtggactaccaggat 717  
 ||||| ||||| ||||| |||||  
 Sbjct: 786 cgaacagtatactccgcacggtaacattcatgtttacggcgtggactaccaggat 727

Query: 718 ctaatcctgttgttccc-acgtttcgacccatctacgtttacggcgtggactaccaggat 776  
 ||||| ||| ||||| ||||| |||||  
 Sbjct: 726 ctaatcctgttgttcccacgtttcgacccatctacgtttacggcgtggactaccaggat 667

Query: 777 cttcgccactgggtttccgaatatctacgtttacggcgtggactaccaggat 836  
 ||||| ||||| ||||| |||||  
 Sbjct: 666 cttcgccactgggtttccgaatatctacgtttacggcgtggactaccaggat 607

Query: 837 ctcacccatccatactccagatcgacagtatcaaaggcgttccagggttggccctgg 896  
 ||||| ||||| ||||| |||||  
 Sbjct: 606 ctcacccatccatactccagatcgacagtatcaaaggcgttccagggttggccctgg 547

Query: 897 gatttcacccctgactgtcgatccgcctacgtgcgtttacggccgttaattccgaaca 956  
 ||||| ||||| ||||| |||||  
 Sbjct: 546 gatttcacccctgactgtcgatccgcctacgtgcgtttacggccgttaattccgaaca 487

Query: 957 acgctagcccccttagtattaccgcggctgtggaaaaagttggccgggatttttc 1016  
 ||||| ||||| ||||| ||||| |||||  
 Sbjct: 486 acgctagccccctcgattaccgcggctgtggcacgaagttggccgggatttttc 427

Query: 1017 cgataccgtattatcttccggtaaaagagttacaaccataggcctcatcact 1076  
 |||||  
 Sbjct: 426 cgataccgtattatcttccggtaaaagagttacaaccataggcctcatcact 367

Query: 1077 cacgcggcatggctggatcaggcttgcgcattgtccaatattccccactgctgcctcc 1136  
 |||||  
 Sbjct: 366 cacgcggcatggctggatcaggcttgcgcattgtccaatattccccactgctgcctcc 307

Query: 1137 ctaggagttggccgtgttcagtccaatgtggctgatcatccttcagaccagcta 1196  
 |||||  
 Sbjct: 306 ctaggagttggccgtgttcagtccaatgtggctgatcatccttcagaccagcta 247

Query: 1197 tggatcgccgttgcgttgcgtttacccaccaactagctaatccaacgcggcgtatc 1256  
 |||||  
 Sbjct: 246 tggatcgccgttgcgttgcgtttacccaccaactagctaatccaacgcggcgtatc 187

Query: 1257 tcttgcgataaaatcttctccgaaggacacatacgattagcacaagttccctgcg 1316  
 |||||  
 Sbjct: 186 ctcccgataaaatcttctccgaaggacacatacgattagcacaagttccctgcg 127

Query: 1317 ttattccgtagaaaaggtagattcccacgcgttactcacccgtctgccgtcccttc 1376  
 |||||  
 Sbjct: 126 ttattccgtagaaaaggtagattcccacgcgttactcacccgtctgccgtcccttc 67

Query: 1377 gggcgctcgacttgcattgttaagcctgcccgccagcggttgcgttgcggatcaa 1436  
 |||||  
 Sbjct: 66 gggcgctcgacttgcattgttaagcctgcccgccagcggttgcgttgcggatcaa 7

Query: 1437 actc 1440

||||

Sbjct: 6 actc 3

**Query= gi|174| (1277 letters)**

Sequences producing significant alignments:	Score	E	
		(bits)	Value
gi 464144 dbj D14504.1 ATU16SRD  Rhizobium sp. K-Ag-3 gene ...	<u>1752</u>	0.0	
gi 27261750 gb AY166841.1  Rhizobium tropici UPRM8033 16S r... 1735	0.0		
gi 21898743 gb AY117623.1  Rhizobium tropici strain PRF34 1... 1735	0.0		
gi 9837365 gb AF286362.1  Rhizobium sp. PRY71 16S ribosomal... 1735	0.0		
gi 14029734 gb AF364856.1 AF364856 Rhizobium sp. L28 115D 1... 1735	0.0		
gi 3724150 emb Z94806.1 RSPZ94806 Rhizobium genosp. Q 16S r... 1733	0.0		
gi 28894114 gb AY206687.1  Rhizobium rhizogenes strain 163C... 1725	0.0		
gi 1055273 gb U38469.1 RTU38469 Rhizobium tropici 16S ribos... 1725	0.0		
gi 286266 dbj D12798.1 RHM16SRRNG Rhizobium tropici 16S rRN... 1725	0.0		
gi 296479 emb X67233.1 RL16SRRN R.leguminosarum (LMG 9518) ... 1715	0.0		

### Alignments

>gi|464144|dbj|D14504.1|ATU16SRD| Rhizobium sp. K-Ag-3 gene for 16S rRNA, complete sequence

Length = 1468

Score = 1752 bits (884), Expect = 0.0

Identities = 971/1002 (96%)

Strand = Plus / Minus

Query: 272 cgagctgacgacagccatgcagcacctgtctcgccaccgaagtggaccccctatctc 331

|||||||

Sbjct: 1002 cgagctgacgacagccatgcagcacctgtctcgccaccgaagtggaccccctatctc 943

Query: 332 tagaggttaacacaggatgtcaaggctgtaaggttctgcgcgttgcattaaacc 391

|||||||

Sbjct: 942 tagaggttaacacaggatgtcaaggctgtaaggttctgcgcgttgcattaaacc 883

Query: 392 acatgctccaccgcttgtcgggccccgtcaattccttgagtttaatcttcgaccg 451

|||||||

Sbjct: 882 acatgctccaccgcttgtcgggccccgtcaattccttgagtttaatcttcgaccg 823

Query: 452 tactccccaggcgaatgttaatgcgttagctgcgccaccgaacagtatactgcccac 511

|||||||

Sbjct: 822 tactccccaggcgaatgttaatgcgttagctgcgccaccgaacagtatactgcccac 763

Query: 512 ggctaacattcatcgttacggcgtggactaccaggatctaatcctgtttgtccca 571

|||||||

Sbjct: 762 ggctaacattcatcgttacggcgtggactaccaggatctaatcctgtttgtccca 703

Query: 572 cgcttcgcacccagcgtcagaatggaccagtggactaccaggatctaatcctgtttgtccca 631

|||||||

Sbjct: 702 cgcttcgcacccagcgtcagaatggaccagtggactaccaggatctaatcctgtttgtccca 643

Query: 632 ccgaatatctacgaattcacctcacactcggaaattccactcaccttccatactcca 691  
 |||||||  
 Sbjct: 642 ccgaatatctacgaattcacctcacactcggaaattccactcaccttccatactcca 583

Query: 692 gatgcacagtatcaaaggcagttccagggttagccctgggatttcaccctgactgatc 751  
 |||||||  
 Sbjct: 582 gatgcacagtatcaaaggcagttccagggttagccctgggatttcaccctgactgatc 523

Query: 752 gatccgcctacgtgcgttacgcccagtaattccgaacaacgctagccccctcgatt 811  
 |||||||  
 Sbjct: 522 gatccgcctacgtgcgttacgcccagtaattccgaacaacgctagccccctcgatt 463

Query: 812 accgcggctgtggcacnaanttagccgggcttctccggataccgtattatctc 871  
 |||||  
 Sbjct: 462 accgcggctgtggcacgaagttagccgggcttctccggataccgtattatctc 403

Query: 872 tccngtcaaagagcttacaaccctagggcctcatcactcacnccgatggctggatca 931  
 |||||  
 Sbjct: 402 tccggtaaaagagcttacaaccctagggcctcatcactcacgcggatggctggatca 343

Query: 932 ngcttcgcattgtccaatattccactgtgnctccgttagaaattggccgtgt 991  
 |||||  
 Sbjct: 342 ggcttcgcattgtccaatattccactgtgcctccgttagagttggccgtgt 283

Query: 992 ctcaatccaaatgtggctgatcatccttcagaacaactatggatcgtgccttggtagg 1051  
 |||||  
 Sbjct: 282 ctcagtccaaatgtggctgatcatccttcagaccagatggatcgtgccttggtagg 223

Query: 1052 gccttacccacccactagctaaccacgcggctcatlgtggatccgat 1111  
 |||||  
 Sbjct: 222 ccttacccaccaactagctaaccacgcggctcatlgtggatccgat 163

Query: 1112 cccgaaggacacatccgtttaatcacaagttccctgcgttattccgttagcaaaaggta 1171  
 |||||  
 Sbjct: 162 cccgaaggacacatccgttattccgttagcaaaaggta 103

Query: 1172 gattccacgcgttactcacccgtctgcgcctccctaaaggcgctgacttgcatgt 1231  
 |||||  
 Sbjct: 102 gattccacgcgttactcacccgtctgcgcctccctgcggcgctgacttgcatgt 43

Query: 1232 gttaagcctgccggccagcggttgcgttgcggatcaaac 1273  
 |||||  
 Sbjct: 42 gttaagcctgccggccagcggttgcgttgcggatcaaac 1

Score = 539 bits (272), Expect = e-150  
 Identities = 272/272 (100%)  
 Strand = Plus / Minus

Query: 1 tacggctacccgttacgacttcacccaggcgtgaccctaccgtggtagctgcctcc 60  
 |||||||

Sbjct: 1442 tacggctacccgttacgacttcacccaggcgtgaccctaccgtggtagctgcctcc 1383  
 |||||||

Query: 61 ttgcggtagcgcaactacccgtaaaaccaactccatggtgtgacggcggtgt 120  
 |||||||

Sbjct: 1382 ttgcggtagcgcaactacccgtaaaaccaactccatggtgtgacggcggtgt 1323  
 |||||||

Query: 121 acaaggcccggaaacgtattcacccggcatgctgatcccgattactagcattcaac 180  
 |||||||

Sbjct: 1322 acaaggcccggaaacgtattcacccggcatgctgatcccgattactagcattcaac 1263  
 |||||||

Query: 181 ttcatgcactcgagttcagagtcaatccgaactgagatggctttggagattagctca 240  
 |||||||

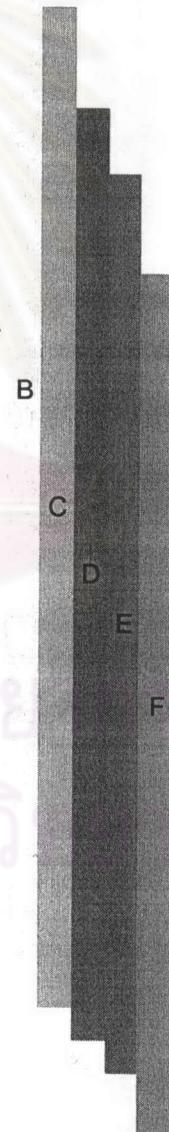
Sbjct: 1262 ttcatgcactcgagttcagagtcaatccgaactgagatggctttggagattagctca 1203  
 |||||||

Query: 241 cactcggtgtcgctgcccactgtcaccacc 272  
 |||||||

Sbjct: 1202 cactcggtgtcgctgcccactgtcaccacc 1171  
 |||||||

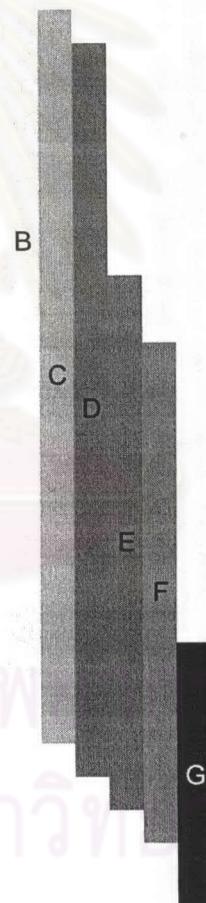
Data for table 4.1 Duncan's Multiple Range Test for plant dry weight when each of *Bradyrhizobium japonicum* strains S76, S78, S162, S178 was inoculated onto germinating seeds of soybean *Glycine max* cv. SJ 4, SJ 5, CM 2, CM 60, ST 1, ST 2, ST 3 in Leonard jars with nitrogen-free medium pH 5.0 for 28 days.

p	r	R mean
2	2.86	0.91
3	3.01	0.96
4	3.10	0.99
5	3.17	1.01
6	3.22	1.02
7	3.27	1.04
8	3.30	1.05
9	3.33	1.06
10	3.35	1.07
11	3.35	1.07
12	3.35	1.07
13	3.35	1.07
14	3.35	1.07
15	3.35	1.07
16	3.47	1.10
17	3.47	1.10
18	3.47	1.10
19	3.47	1.10
20	3.47	1.10
21	3.47	1.10
22	3.47	1.10
23	3.47	1.10
24	3.47	1.10
25	3.47	1.10
26	3.47	1.10
27	3.47	1.10
28	3.47	1.10
29	3.47	1.10
30	3.47	1.10
31	3.47	1.10
32	3.47	1.10
33	3.47	1.10
34	3.47	1.10
35	3.47	1.10
36	3.47	1.10
37	3.47	1.10
38	3.47	1.10
39	3.47	1.10
40	3.47	1.10
41	3.47	1.10
42	3.47	1.10
		0.61



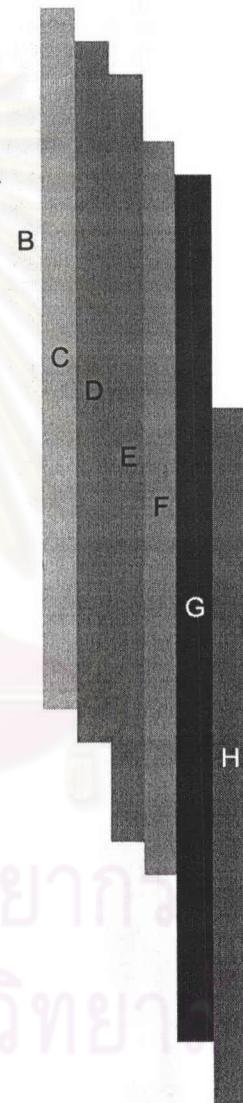
Data for table 4.3 Duncan's Multiple Range Test for plant dry weight when each of *Burkholderia* sp. S172, *Sinorhizobium fredii* S173, S174 was inoculated onto germinating seeds of soybean *Glycine max* cv. SJ 4, SJ 5, CM 2, CM 60, ST 1, ST 2, ST 3 in Leonard jars with nitrogen-free medium pH 5.0 for 28 days.

p	r	R	mean
2	2.89	0.74	2.38
3	3.04	0.78	2.00
4	3.13	0.80	1.95
5	3.20	0.82	1.92
6	3.25	0.84	1.90
7	3.29	0.85	1.83
8	3.32	0.85	1.79
9	3.35	0.86	1.75
10	3.37	0.87	1.68 A
11	3.39	0.87	1.67
12	3.41	0.88	1.64
13	3.42	0.88	1.61
14	3.43	0.88	1.60
15	3.44	0.88	1.59
16	3.45	0.89	1.59
17	3.45	0.89	1.57
18	3.46	0.89	1.56
19	3.47	0.89	1.45
20	3.47	0.89	1.42
21	3.47	0.89	1.41
22	3.47	0.89	1.40
23	3.47	0.89	1.40
24	3.47	0.89	1.39
25	3.47	0.89	1.37
26	3.47	0.89	1.37
27	3.47	0.89	1.34
28	3.47	0.89	1.34
29	3.47	0.89	1.16
30	3.47	0.89	0.89
31	3.47	0.89	0.86
32	3.47	0.89	0.83
33	3.47	0.89	0.69
34	3.47	0.89	0.57
35	3.47	0.89	0.47
			0.46



Data for table 4.4 Duncan's Multiple Range Test for plant dry weight when each of *Burkholderia* sp. S172, *Sinorhizobium fredii* S173, S174 was inoculated onto germinating seeds of soybean *Glycine max* cv. SJ 4, SJ 5, CM 2, CM 60, ST 1, ST 2, ST 3 in Leonard jars with nitrogen-free medium pH 6.8 for 28 days.

p	r	R	mean
2	2.89	0.7196	0.53
3	3.04	0.757	0.59
4	3.13	0.7794	0.69
5	3.20	0.7968	0.79
6	3.25	0.81	0.87
7	3.29	0.8192	0.88
8	3.32	0.8267	0.91
9	3.35	0.8342	1.25
10	3.37	0.8391	1.29
11	3.39	0.8441	1.3
12	3.41	0.8491	1.34
13	3.42	0.8516	1.36
14	3.43	0.8541	1.37
15	3.44	0.8566	1.37
16	3.45	0.8591	1.4
17	3.45	0.8591	1.42
18	3.46	0.8615	1.42
19	3.47	0.864	1.57
20	3.47	0.864	1.57
21	3.47	0.864	1.57
22	3.47	0.864	1.6
23	3.47	0.864	1.62
24	3.47	0.864	1.62
25	3.47	0.864	1.65
26	3.47	0.864	1.67
27	3.47	0.864	1.69
28	3.47	0.864	1.72
29	3.47	0.864	1.74
30	3.47	0.864	1.79
31	3.47	0.864	1.83
32	3.47	0.864	1.89
33	3.47	0.864	1.95
34	3.47	0.864	2.06
35	3.47	0.864	2.25
			2.17



**Table A9. Critical values for Duncan's multiple range test**

The body of the table represents  $D_{\square, (r, v)}$  the critical value for Duncan's multiple range test for significance level  $\alpha$ ,  $v$  degrees of freedom and range  $r$ .

Example: The  $D$  value to compare two means on the 4-range at  $\alpha = 0.05$  with 10 degrees of freedom is 3.37

$v$	$\alpha$	Range of sample means																		$\alpha$	$v$
		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
1	.100	8.929	8.929	8.929	8.929	8.929	8.929	8.929	8.929	8.929	8.929	8.929	8.929	8.929	8.929	8.929	8.929	8.929	8.929	.100	1
1	.050	17.97	17.97	17.97	17.97	17.97	17.97	17.97	17.97	17.97	17.97	17.97	17.97	17.97	17.97	17.97	17.97	17.97	17.97	.050	1
1	.010	89.98	89.98	89.98	89.98	89.98	89.98	89.98	89.98	89.98	89.98	89.98	89.98	89.98	89.98	89.98	89.98	89.98	89.98	.010	1
2	.100	4.129	4.129	4.129	4.129	4.129	4.129	4.129	4.129	4.129	4.129	4.129	4.129	4.129	4.129	4.129	4.129	4.129	4.129	.100	2
2	.050	6.085	6.085	6.085	6.085	6.085	6.085	6.085	6.085	6.085	6.085	6.085	6.085	6.085	6.085	6.085	6.085	6.085	6.085	.050	2
2	.010	14.03	14.03	14.03	14.03	14.03	14.03	14.03	14.03	14.03	14.03	14.03	14.03	14.03	14.03	14.03	14.03	14.03	14.03	.010	2
3	.100	3.328	3.330	3.330	3.330	3.330	3.330	3.330	3.330	3.330	3.330	3.330	3.330	3.330	3.330	3.330	3.330	3.330	3.330	.100	3
3	.050	4.501	4.516	4.516	4.516	4.516	4.516	4.516	4.516	4.516	4.516	4.516	4.516	4.516	4.516	4.516	4.516	4.516	4.516	.050	3
3	.010	8.260	8.321	8.321	8.321	8.321	8.321	8.321	8.321	8.321	8.321	8.321	8.321	8.321	8.321	8.321	8.321	8.321	8.321	.010	3
4	.100	3.015	3.074	3.081	3.081	3.081	3.081	3.081	3.081	3.081	3.081	3.081	3.081	3.081	3.081	3.081	3.081	3.081	3.081	.100	4
4	.050	3.927	4.012	4.033	4.033	4.033	4.033	4.033	4.033	4.033	4.033	4.033	4.033	4.033	4.033	4.033	4.033	4.033	4.033	.050	4
4	.010	6.511	6.677	6.740	6.756	6.756	6.756	6.756	6.756	6.756	6.756	6.756	6.756	6.756	6.756	6.756	6.756	6.756	6.756	.010	4
5	.100	2.894	2.934	2.964	2.969	2.969	2.969	2.969	2.969	2.969	2.969	2.969	2.969	2.969	2.969	2.969	2.969	2.969	2.969	.100	5
5	.050	3.635	3.749	3.796	3.814	3.814	3.814	3.814	3.814	3.814	3.814	3.814	3.814	3.814	3.814	3.814	3.814	3.814	3.814	.050	5
5	.010	5.702	5.893	6.040	6.065	6.074	6.074	6.074	6.074	6.074	6.074	6.074	6.074	6.074	6.074	6.074	6.074	6.074	6.074	.010	5
6	.100	2.748	2.846	2.890	2.907	2.911	2.911	2.911	2.911	2.911	2.911	2.911	2.911	2.911	2.911	2.911	2.911	2.911	2.911	.100	6
6	.050	3.460	3.586	3.649	3.680	3.694	3.697	3.697	3.697	3.697	3.697	3.697	3.697	3.697	3.697	3.697	3.697	3.697	3.697	.050	6
6	.010	5.243	5.439	5.549	5.615	5.655	5.680	5.694	5.701	5.703	5.703	5.703	5.703	5.703	5.703	5.703	5.703	5.703	5.703	.010	6
7	.100	2.679	2.785	2.838	2.864	2.876	2.878	2.878	2.878	2.878	2.878	2.878	2.878	2.878	2.878	2.878	2.878	2.878	2.878	.100	7
7	.050	3.344	3.477	3.548	3.588	3.611	3.622	3.625	3.625	3.625	3.625	3.625	3.625	3.625	3.625	3.625	3.625	3.625	3.625	.050	7
7	.010	4.948	5.145	5.260	5.333	5.383	5.416	5.439	5.454	5.464	5.470	5.472	5.472	5.472	5.472	5.472	5.472	5.472	5.472	.010	7
8	.100	2.630	2.741	2.800	2.832	2.849	2.857	2.858	2.858	2.858	2.858	2.858	2.858	2.858	2.858	2.858	2.858	2.858	2.858	.100	8
8	.050	3.261	3.398	3.475	3.521	3.549	3.566	3.575	3.579	3.579	3.579	3.579	3.579	3.579	3.579	3.579	3.579	3.579	3.579	.050	8
8	.010	4.745	4.939	5.056	5.134	5.189	5.227	5.256	5.276	5.291	5.302	5.309	5.313	5.316	5.317	5.317	5.317	5.317	5.317	.010	8
9	.100	2.592	2.708	2.771	2.808	2.829	2.840	2.845	2.846	2.846	2.846	2.846	2.846	2.846	2.846	2.846	2.846	2.846	2.846	.100	9
9	.050	3.199	3.339	3.420	3.470	3.502	3.523	3.556	3.544	3.547	3.547	3.547	3.547	3.547	3.547	3.547	3.547	3.547	3.547	.050	9
10	.100	2.563	2.682	2.748	2.788	2.813	2.827	2.835	2.839	2.839	2.839	2.839	2.839	2.839	2.839	2.839	2.839	2.839	2.839	.100	10
10	.050	3.151	3.293	3.376	3.430	3.465	3.489	3.505	3.516	3.522	3.525	3.525	3.525	3.525	3.525	3.525	3.525	3.525	3.525	.050	10
10	.010	4.482	4.671	4.789	4.871	4.931	4.975	5.010	5.036	5.058	5.074	5.087	5.098	5.106	5.117	5.120	5.122	5.123	5.124	.010	10
11	.100	2.540	2.660	2.729	2.772	2.799	2.817	2.827	2.833	2.835	2.835	2.835	2.835	2.835	2.835	2.835	2.835	2.835	2.835	.100	11
11	.050	3.113	3.256	3.341	3.397	3.435	3.462	3.480	3.493	3.501	3.506	3.509	3.510	3.510	3.510	3.510	3.510	3.510	3.510	.050	11
11	.010	4.392	4.579	4.697	4.780	4.841	4.887	4.923	4.952	4.975	4.994	5.009	5.021	5.031	5.039	5.045	5.054	5.057	5.059	.010	11

Calculated with the FprobMC function of The SAS System, Vs. 6.12.

Table A9. Critical values for Duncan's multiple range test (Continued ...)

$v$	$\alpha$	Range of sample means																			$\alpha$	$v$
		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20		
12	.100	2.521	2.643	2.714	2.759	2.788	2.808	2.820	2.832	2.833	2.833	2.833	2.833	2.833	2.833	2.833	2.833	2.833	2.833	.100	12	
12	.050	3.081	3.225	3.312	3.370	3.410	3.439	3.459	3.474	3.484	3.491	3.495	3.498	3.498	3.498	3.498	3.498	3.498	3.498	.050	12	
12	.010	4.320	4.504	4.622	4.705	4.765	4.812	4.850	4.882	4.907	4.927	4.944	4.957	4.969	4.978	4.986	4.993	4.998	5.002	5.005	.010	12
13	.100	2.504	2.628	2.701	2.748	2.779	2.800	2.814	2.824	2.829	2.832	2.832	2.832	2.832	2.832	2.832	2.832	2.832	2.832	.100	13	
13	.050	3.055	3.200	3.288	3.348	3.389	3.419	3.441	3.458	3.470	3.478	3.484	3.488	3.490	3.490	3.490	3.490	3.490	3.490	.050	13	
13	.010	4.261	4.442	4.560	4.643	4.706	4.754	4.793	4.824	4.850	4.871	4.889	4.904	4.917	4.927	4.936	4.944	4.950	4.955	4.960	.010	13
14	.100	2.491	2.616	2.689	2.739	2.771	2.794	2.810	2.820	2.827	2.831	2.833	2.833	2.833	2.833	2.833	2.833	2.833	2.833	.100	14	
14	.050	3.033	3.178	3.268	3.328	3.371	3.402	3.426	3.444	3.457	3.467	3.474	3.479	3.482	3.484	3.484	3.484	3.484	3.484	.050	14	
14	.010	4.210	4.390	4.508	4.591	4.654	4.703	4.743	4.775	4.802	4.824	4.843	4.859	4.872	4.884	4.894	4.902	4.909	4.916	4.921	.010	14
15	.100	2.479	2.605	2.681	2.730	2.764	2.788	2.817	2.825	2.830	2.833	2.834	2.834	2.834	2.834	2.834	2.834	2.834	2.834	.100	15	
15	.050	3.014	3.160	3.250	3.312	3.356	3.389	3.413	3.432	3.446	3.457	3.465	3.471	3.476	3.478	3.480	3.480	3.480	3.480	.050	15	
15	.010	4.167	4.346	4.463	4.547	4.610	4.660	4.700	4.733	4.760	4.783	4.803	4.820	4.834	4.846	4.856	4.866	4.874	4.887	.010	15	
16	.100	2.469	2.596	2.672	2.723	2.759	2.784	2.802	2.814	2.824	2.830	2.833	2.835	2.836	2.836	2.836	2.836	2.836	2.836	.100	16	
16	.050	2.998	3.144	3.235	3.297	3.343	3.376	3.402	3.422	3.447	3.464	3.484	3.499	3.517	3.537	3.545	3.545	3.545	3.545	.050	16	
16	.010	4.131	4.308	4.425	4.508	4.572	4.622	4.662	4.696	4.724	4.748	4.768	4.785	4.800	4.813	4.825	4.835	4.843	4.858	.010	16	
17	.100	2.460	2.587	2.665	2.717	2.753	2.779	2.798	2.812	2.822	2.829	2.834	2.837	2.838	2.838	2.838	2.838	2.838	2.838	.100	17	
17	.050	2.984	3.130	3.222	3.285	3.331	3.365	3.392	3.412	3.441	3.451	3.459	3.465	3.469	3.472	3.474	3.475	3.475	3.475	.050	17	
17	.010	4.099	4.275	4.391	4.474	4.538	4.589	4.630	4.664	4.692	4.717	4.737	4.755	4.771	4.785	4.797	4.807	4.816	4.824	4.832	.010	17
18	.100	2.452	2.580	2.659	2.711	2.749	2.776	2.795	2.810	2.821	2.829	2.834	2.838	2.840	2.840	2.840	2.840	2.840	2.840	.100	18	
18	.050	2.971	3.117	3.210	3.274	3.320	3.356	3.383	3.404	3.421	3.435	3.445	3.454	3.460	3.465	3.469	3.472	3.473	3.474	.050	18	
18	.010	4.071	4.246	4.361	4.445	4.509	4.559	4.601	4.635	4.664	4.689	4.710	4.729	4.745	4.759	4.771	4.782	4.792	4.801	4.808	.010	18
19	.100	2.445	2.574	2.653	2.706	2.744	2.772	2.793	2.808	2.820	2.828	2.834	2.839	2.841	2.843	2.843	2.843	2.843	2.843	.100	19	
19	.050	2.960	3.106	3.199	3.264	3.311	3.347	3.375	3.397	3.415	3.429	3.440	3.449	3.456	3.462	3.466	3.469	3.472	3.474	.050	19	
19	.010	4.046	4.220	4.335	4.418	4.483	4.533	4.575	4.610	4.639	4.664	4.686	4.705	4.722	4.736	4.749	4.760	4.771	4.780	.010	19	
20	.100	2.439	2.568	2.648	2.702	2.741	2.769	2.791	2.807	2.819	2.828	2.835	2.839	2.843	2.845	2.845	2.845	2.845	2.845	.100	20	
20	.050	2.950	3.097	3.190	3.255	3.303	3.339	3.368	3.390	3.409	3.423	3.435	3.445	3.452	3.459	3.463	3.467	3.470	3.473	.050	20	
20	.010	4.024	4.197	4.312	4.395	4.459	4.510	4.552	4.587	4.617	4.642	4.664	4.684	4.701	4.716	4.729	4.741	4.751	4.761	.010	20	
25	.100	2.416	2.546	2.628	2.685	2.726	2.758	2.782	2.800	2.815	2.827	2.836	2.843	2.849	2.853	2.856	2.858	2.860	2.860	.100	25	
25	.050	2.913	3.059	3.154	3.221	3.271	3.310	3.341	3.366	3.386	3.403	3.417	3.429	3.439	3.447	3.454	3.459	3.464	3.471	.050	25	
25	.010	3.942	4.112	4.224	4.307	4.371	4.423	4.466	4.502	4.532	4.559	4.582	4.603	4.621	4.638	4.652	4.665	4.677	4.688	.010	25	
30	.100	2.400	2.532	2.615	2.674	2.717	2.750	2.776	2.796	2.813	2.826	2.837	2.846	2.853	2.859	2.863	2.867	2.869	2.873	.100	30	
30	.050	2.888	3.035	3.131	3.199	3.250	3.290	3.321	3.349	3.371	3.389	3.405	3.418	3.429	3.439	3.447	3.454	3.466	3.470	.050	30	
30	.010	3.889	4.056	4.168	4.250	4.314	4.366	4.409	4.445	4.477	4.504	4.528	4.550	4.569	4.586	4.601	4.615	4.628	4.640	4.650	.010	30
50	.100	2.370	2.504	2.590	2.652	2.698	2.735	2.764	2.788	2.808	2.828	2.851	2.862	2.871	2.879	2.885	2.891	2.896	2.901	.100	50	
50	.050	2.841	2.988	3.084	3.154	3.208	3.251	3.286	3.315	3.340	3.362	3.380	3.396	3.410	3.423	3.434	3.444	3.453	3.461	.050	50	
60	.100	2.363	2.497	2.584	2.646	2.693	2.731	2.761	2.786	2.807	2.825	2.840	2.853	2.864	2.874	2.883	2.890	2.897	2.903	.100	60	
60	.050	2.829	2.976	3.073	3.143	3.198	3.241	3.277	3.307	3.333	3.355	3.374	3.391	3.406	3.419	3.431	3.441	3.451	3.460	.050	60	
60	.010	3.762	3.922	4.031	4.111	4.174	4.226	4.270	4.307	4.340	4.368	4.394	4.417	4.437	4.456	4.474	4.489	4.504	4.518	4.530	.010	60
100	.100	2.348	2.483	2.571	2.635	2.684	2.723	2.755	2.782	2.805	2.824	2.856	2.869	2.881	2.891	2.901	2.909	2.917	2.924	.100	100	
100	.050	2.806	2.953	3.050	3.122	3.177	3.222	3.259	3.290	3.317	3.341	3.361	3.380	3.396	3.411	3.424	3.436	3.447	3.457	.050	100	
100	.010	3.714	3.871	3.978	4.057	4.120	4.172	4.215	4.253	4.286	4.315	4.341	4.364	4.385	4.405	4.422	4.439	4.454	4.468	.010	100	

## BIOGRAPHY

Miss Patima Permpoonpattana was born on April 25, 1978. She obtained a Bachelor of Science Degree in Biotechnology from King Mongkut's Institute of Technology Lardkrabang, Bangkok, Thailand, in 2000.

### Publications

- 1) สรณญา พันธุ์พุกษ์, บางช. ยะกีบ, ปฏิมา เพิ่มพูนพัฒนา, ปรางปะໄພ รอดบำรุง, และ อรัญ อินเจริญศักดิ์. 2545. การค้นหาเชิงไอล์ดอจีนในไซาโนแบคทีเรียโดยวิธีปฏิกริยาลูกฟูก. วารสารวิทยาศาสตร์ลาดกระบัง. 11(1) : 22-34.
- 2) Nonthicha Jamkangwan, Wanvisa Boonsri, Charasporn Suralai, Sirikarn Charoenbhakdi, Patima Permpoonpattana and Kanjana Chansa-ngavej. 2002. RAPD-PCR Fingerprints of Cyanobacteria and Microalgae. Proceedings of the 14<sup>th</sup> Annual Meeting of the Thai Society for Biotechnology. 3 pages. CD-ROM format.
- 3) Patima Permpoonpattana, Siroj Srisarakorn, Salisa Jumpa and Kanjana Chansa- ngavej. 2002. Heat, pH and Nodulation Responses of Fast-growing *Sinorhizobium fredii* S174 and Slow-growing *Bradyrhizobium japonicum* S178. Proceedings of the 14<sup>th</sup> Annual Meeting of the Thai Society for Biotechnology. 4 pages. CD-ROM format.

### Presentation at Scientific Conferences

- 1) ปฏิมา เพิ่มพูนพัฒนา และ กานุจนา ชาญส่งเวช. 2545. *Burkholderia* sp.S172 เป็นแบคทีเรียที่ต้องในโครงเจนแบบพิงพาอาศัยในปมหากถัวเหลืองหรือเป็นแบคทีเรียที่ต้องในโครงเจนแบบอิสระ. หนังสือรวมบทคัดย่อการประชุมทางวิชาการ คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย. ครั้งที่ 10 : หน้า 7.
- 2) ศิริจัน ศรีสวัสดิ์, ปฏิมา เพิ่มพูนพัฒนา, สุวรรณ์ แสงเกิดทรัพย์ และ กานุจนา ชาญส่งเวช. 2545. การสร้างพลาสมิดที่มี *nodbox::lacZ* ยืนพิวัชเพื่อใช้เป็นตัวรายงานภัยในเซลล์เกี่ยวกับการเนี่ยวนำโดยสารสกัดจากเมล็ดถัวเหลืองในการแสดงออกของยีน *nodAB* ใน *Sinorhizobium fredii* S174. หนังสือรวมบทคัดย่อการประชุมทางวิชาการ คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย. ครั้งที่ 10 : หน้า 161.
- 3) วันวิสาข บุญศรี, จรัสพร สรາລัย, สิริกานต์ เจริญภักดิ์, ปฏิมา เพิ่มพูนพัฒนา และ กานุจนา ชาญส่งเวช. 2545. ลายพิมพ์ดีเอ็นเอในอนุกรมวิธานของไซาโนแบคทีเรียและสาหร่ายขนาดเล็ก. หนังสือรวมบทคัดย่อการประชุมทางวิชาการ คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย. ครั้งที่ 10 : หน้า 164.
- 4) ปฏิมา เพิ่มพูนพัฒนา และ กานุจนา ชาญส่งเวช. 2546. โพลีเปปไทด์ 38 และ 25 กิโลคาลตันเพิ่มขึ้นใน *Sinorhizobium fredii* S173 และ S174 ซึ่งเลี้ยงที่อุณหภูมิสูง. หนังสือรวมบทคัดย่อการประชุมวิชาการ คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย. ครั้งที่ 11 : หน้า 5.
- 5) Patima Permpoonpattana., Suwat Saengkerdsub and Kanjana Chansa-ngavej. 2001. Preliminary identification of fast-growing *Bradyrhizobium japonicum* isolated from soybean root nodules in acid soil. Abstract Book, BioThailand 2001 : From Research to Market. November 7-10, 2001. Bangkok, Thailand. p. 234.
- 6) Kanjana Chansa-ngavej, Patima Permpoonpattana, Weerasak Chongfungprinya, Suwat Sangkerdsub, Pattareeya Theerarajanapong, Siripen Vetchgarun, Hirohida Toyama and Mamoru Yamada. 2002. Development of thermotolerant *Bradyrhizobium japonicum* for biofertilizers and halotolerant *Bacillus subtilis* K1 for production of protease for use in soy sauce production. Abstract Book, The 3<sup>rd</sup> JSPS-NRCT Joint Seminar on Development of Thermotolerant Microbial Resources and Their Applications. November 17-21, 2002. Chiang Mai, Thailand. p.75.