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APPENDICES

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_2HPO_4	0.5 g
$MgSO_4.7H_2O$	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 petridishes 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 $\mu g.ml^{-1}$. The medium was autoclaved at 121°C for 15 min.

Yeast Extract Glycerol Broth (YGB)

Glycerol	10.0 ml
K_2HPO_4	0.5 g
$MgSO_4.7H_2O$	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.

N-free Nutrient Solutions

Stock Solutions	Chemicals	g/liter
1	$CaCl_2.2H_2O$	294.1
2	KH_2PO_4	136.1
3	$FeC_6H_5O_7.3H_20$	6.7
	$MgSO_4.7H_2O$	123.3
	K_2SO_4	87.0
	$MnSO_4.H_2O$	0.338
4	H_3BO_3	0.247
	$ZnSO_4.7H_2O$	0.288
	$CuSO_4.5H_2O$	0.100
	$CoSO_4.7H_2O$	0.056
	$Na_2MoO_2.7H_2O$	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% KNO3 was added to give final N concentration of 70 ppm.

APPENDIX B

CHEMICALS AND SOLUTIONS

1. Solutions for DNA extraction

   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.

APPENDIX C

ANALYSIS OF SOIL SAMPLES

	Organic Matter (%)	Available P (ppm)	Available K (ppm)	Water Holding Capacity (%)	Moisture Content (%)
Sub Districts in Chat Trakarn District					
Chat Trakarn	2.15	23	46	36.95	1.94
Pa Daeng	1.31	15	61	27.89	1.21
Suan Meing	1.31	14	59	34.61	1.83
Sub Districts in Bang Rakam District					
Bang Rakam	1.24	60	139	34.98	1.42
Pluk Raed	1.54	48	136	32.66	1.63
Pan Sao	2.25	91	142	40.73	3.09
Bung Kok	0.84	52	172	35.09	1.21
Nong Ku-la	1.81	170	252	35.96	1.83
Chum Saeng					
Songkram	1.14	44	127	31.34	1.11
Bou Thong	1.31	77	143	33.57	1.42
Kui Muang	1.41	64	166	32.96	1.32
Sub Districts in Prom Piram District					
Wong Kong	1.34	59	54	33.36	1.52
Ta Look Taem	1.91	38	73	43.01	3.73
Wang Won	2.11	28	87	43.01	3.73
Dong Pa Kam	1.78	56	60	38.06	2.67

	Na (ppm)	Cl^- (ppm)	S (ppm)	Ca (ppm)	Mg (ppm)	Fe (ppm)	Mn (ppm)	Zu (ppm)	Cu (ppm)
Sub Districts in Chattrakarn District									
Chat Trakarn	116	53.25	3	680	681	93.3	15.5	1.1	0.4
Pa Daeng	79	26.62	19	848	884	6.5	3.7	0.2	0.2
Suan Meing	82	82	ND	521	1431	43.9	16.3	0.5	0.4
Sub Districts in Bang Rakam District									
Bang Rakam	85	ND	ND	1053	166	106.1	35	1.6	1.6
Pluk Raed	81	ND	17	1630	171	34.9	47	1.9	1.1
Pan Sao	141	31.95	4	1562	265	213.2	110.8	3.8	3
Bung Kok	93	ND	14	914	161	32.5	41.3	1.7	0.7
Nong Ku-la	117	53.25	38	1622	225	58.8	44	7.3	1.3
Chum Saeng									
Songkram	61	ND	ND	565	131	106.9	46.6	1.4	1.1
Bou Thong	62	ND	ND	1051	156	73.5	50.6	3.1	1
Kui Muang	79	ND	ND	571	122	131.9	79.6	1.9	1.3
Sub Districts in Prom Piram District									
Wong Kong	17	53.25	17	1035	230	64.5	17.5	0.9	1
Ta Look Taem	70	ไม่พบ	1	1579	333	58.4	32.2	1.3	1.6
Wang Won	73	ไม่พบ	48	2714	387	11.1	37.6	1.5	1.2
Dong Pa Kam	81	26.62	19	1621	303	82.8	26.6	1.6	1.5

ND = Not detected

Information on number soybean grower, soybean producitity, and soybean cultivation areas in 15 subdistricts of Phitsanulok province in 2005.

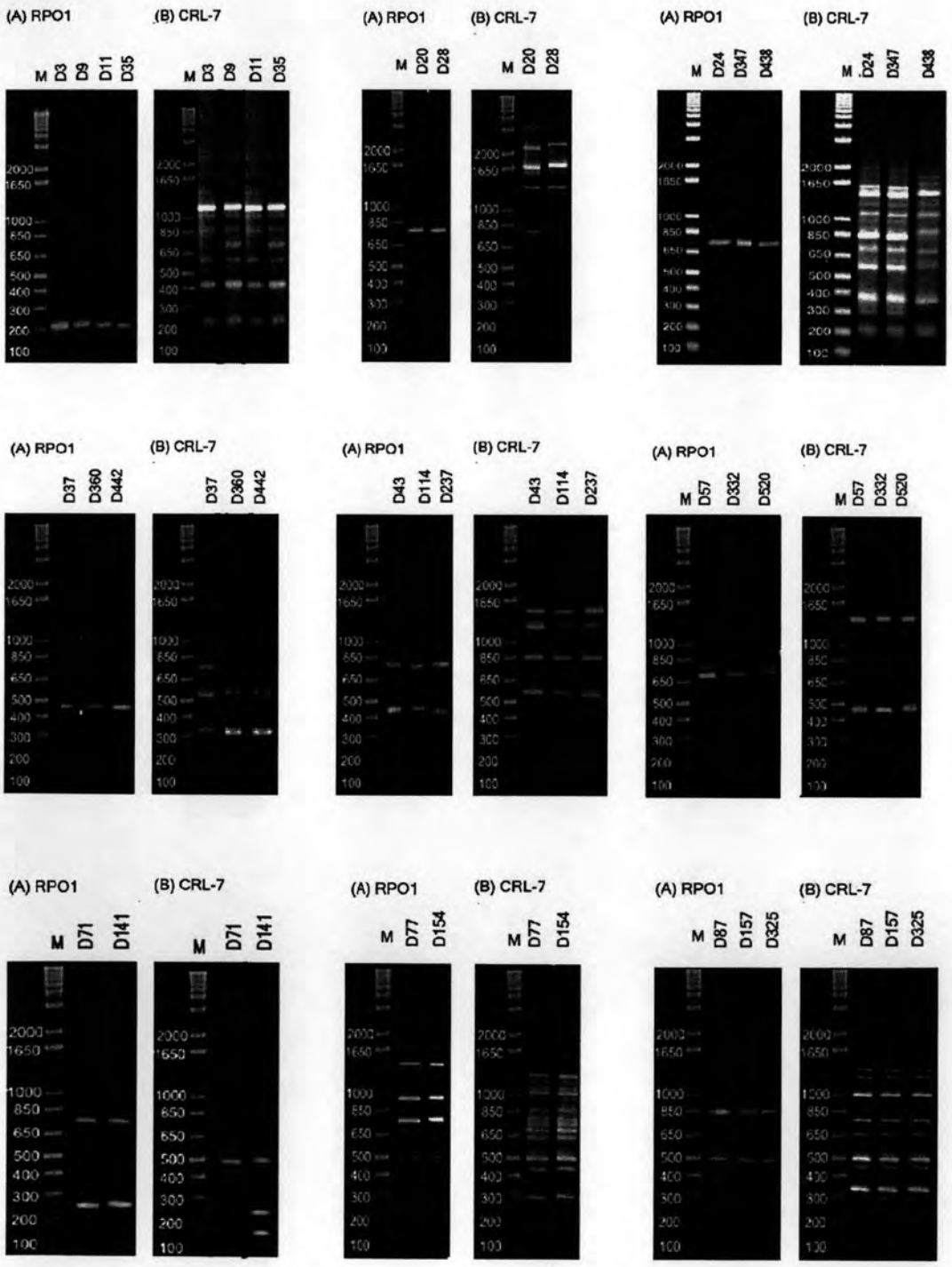
Soybean cultivars	Number of soybean grower	Number of soybean grower	Productivity (kg.rai^{-1})	Productivity (kg.rai^{-1})	Soybean area (*rai*)	Soybean area (*rai*)
Districts	summer crop	rainy season crop	summer crop	rainy season crop	summer crop	rainy season crop
Sub Districts in Chattrakarn District						
Chat Trakarn	No data					
Pa Daeng	127	1	203,563	16,743		
Suan Meing	150	1	415,301	3	2,112	10
Ban Dong	146	53	385,801	173,867	2,417	919
Sub Districts in Bang Rakam District						
Bang Rakam	70	49	105,827	6,018	868	50
Pluk Raed	77	177	146,020	315,783	859	1,882
Pan Sao	39	94	64,160	218,850	345	1,138
Bung Kok	109	228	267,060	546,724	1,228	2,554
Nong Ku-la	186	431	483,102	1,018,156	2,250	4,611
Chum Saeng	No					
Songkram	data					
Bou Thong	18	21	50,550	49,100	200	209
Kui Muang	52	117	97,673	13,414	513	244,717

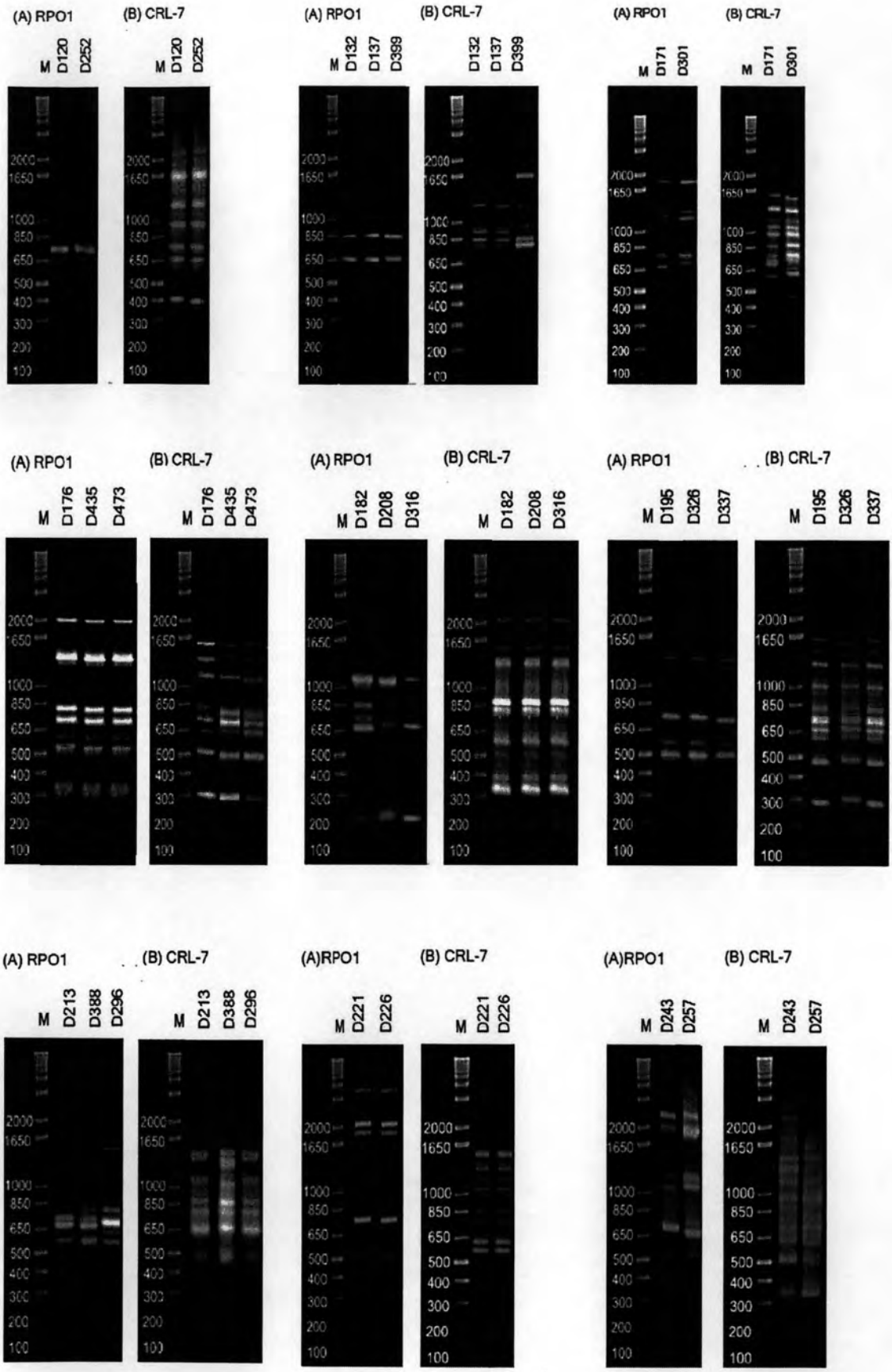
Soybean cultivars	Number of soybean grower	Number of soybean grower	Productivity ($kg.rai^{-1}$)	Productivity ($kg.rai^{-1}$)	Soybean area (*rai*)	Soybean area (*rai*)
Sub Districts in Prom Piram District						
Wong Kong	2	15	15,750	26,700	55	151
Ta Look						
Taem	33	18	5,080	3,900	343	203
Wang Won	1	1	3	1	30	10
Dong Pa Kam	3	26	2,700	42,817	24	254

APPENDIX D

RAPD-PCR FINGERPRINTS OF ISOLATES WITH IDENTICAL FINGERPRINTS

Identical sets of RAPD-PCR fingerprints were used to group soybean root nodule bacterial isolates from three sub-districts in Phitsanulok province into the same strains.

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(B) CRL-7
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(A) RPO1
(B) CRL-7
M D171 D301
M D171 D301
(A) RPO1
(B) CRL-7
M D176 D435 D473
M D176 D435 D473
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(B) CRL-7
M D182 D208 D316
M D182 D208 D316
(A) RPO1
(B) CRL-7
M D195 D326 D337
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(B) CRL-7
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(B) CRL-7
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M D221 D226
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M D243 D257
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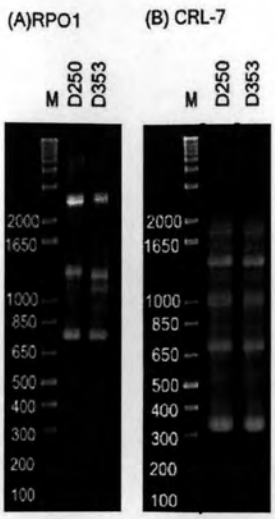
850
650
500
400
300
200
100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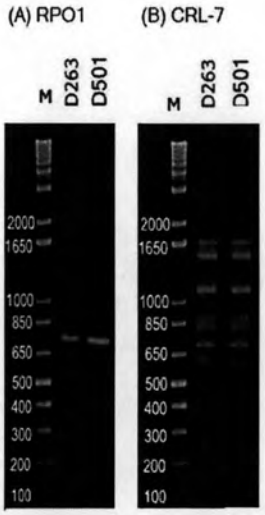
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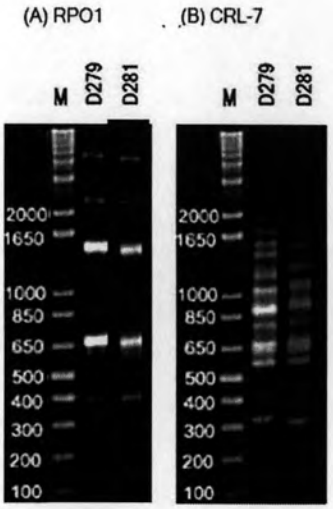
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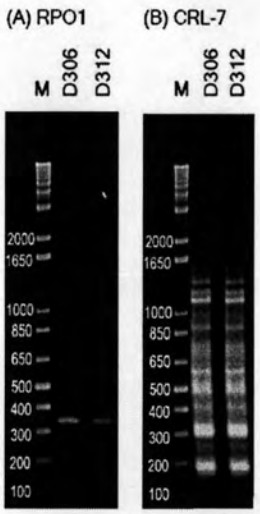
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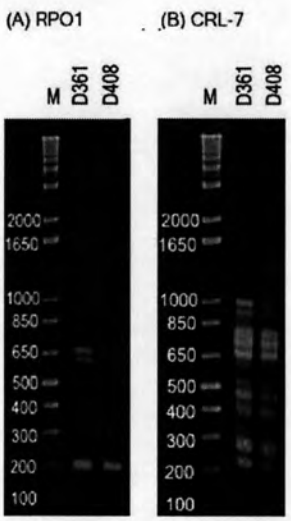
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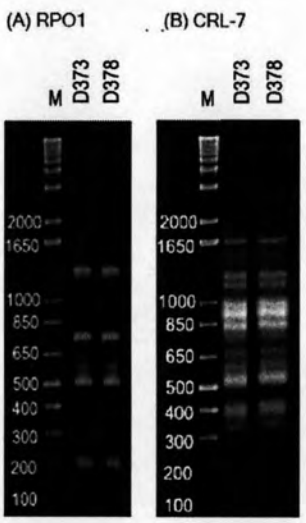
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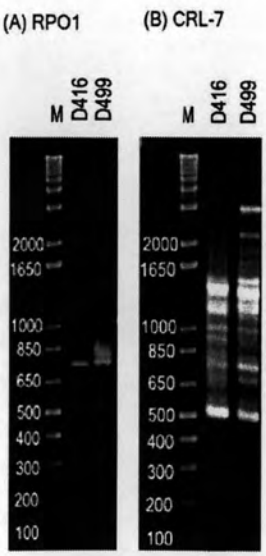
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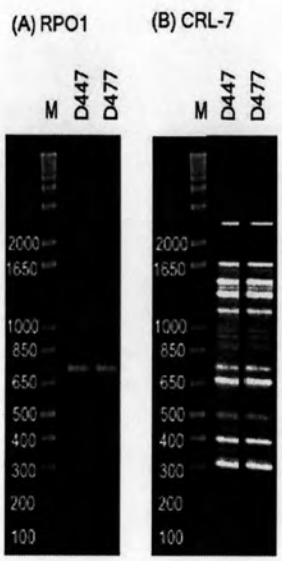
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300
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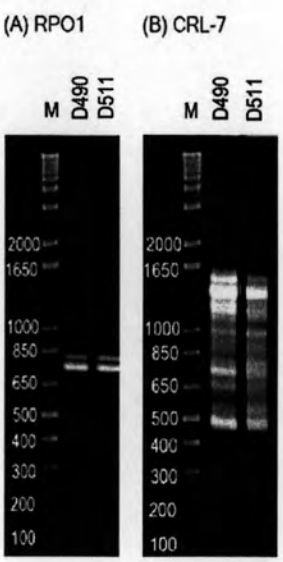
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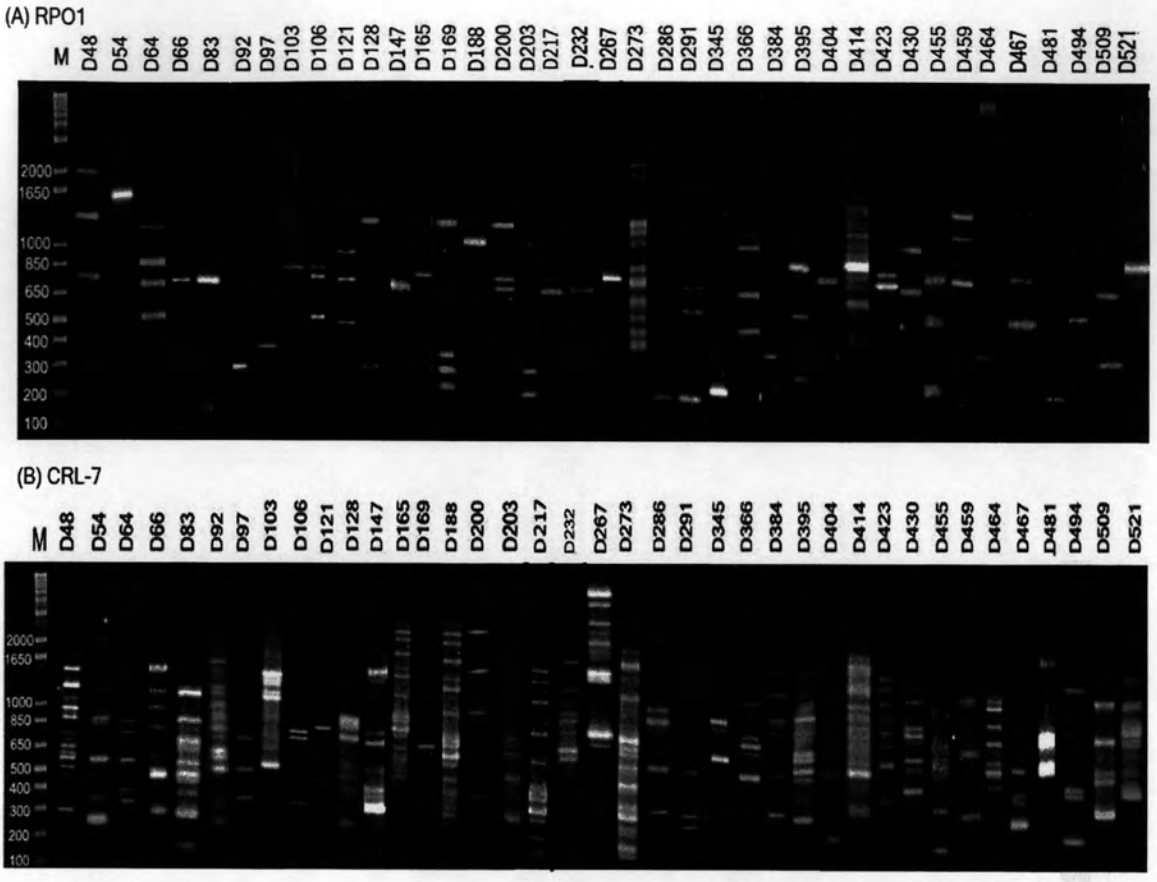
200
100

RAPD-PCR fingerprints of distinct root nodules bacteria isolates.



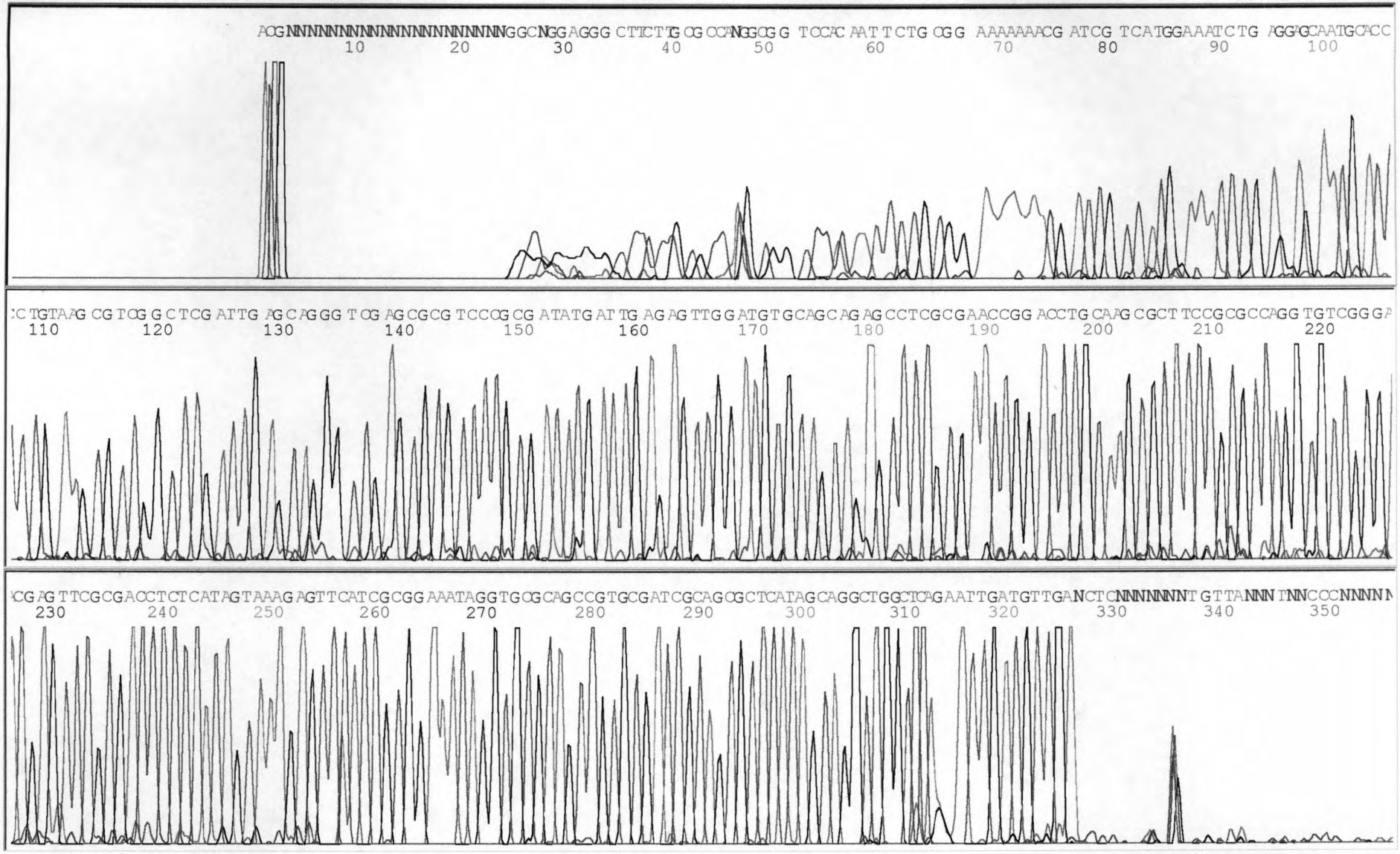
APPENDIX E

1. Sequencing data of 317 bp fragments obtained from multiplex PCR reactions when DNA of either strain D11 (fast- grower) or strain D345 (slow- grower) was used as the template.
2. Sequencing data of 657 bp fragment obtained from multiplex PCR reactions when DNA of strain D345 was used as the template.

Model 3100 24-8-49(B)_A05_D11(317bp)_nodD1F_01.ab1
Version 3.7
Basecaller-3100APOP4_D11(317bp)_nodD1F
BC 1.5.0.0 Cap 1

Signal G:309 A:204 T:171 C:168
DT3100POP4{BDv3}v1.mob
BSU_3100
Points 1200 to 15000 Pk 1 Loc: 1200

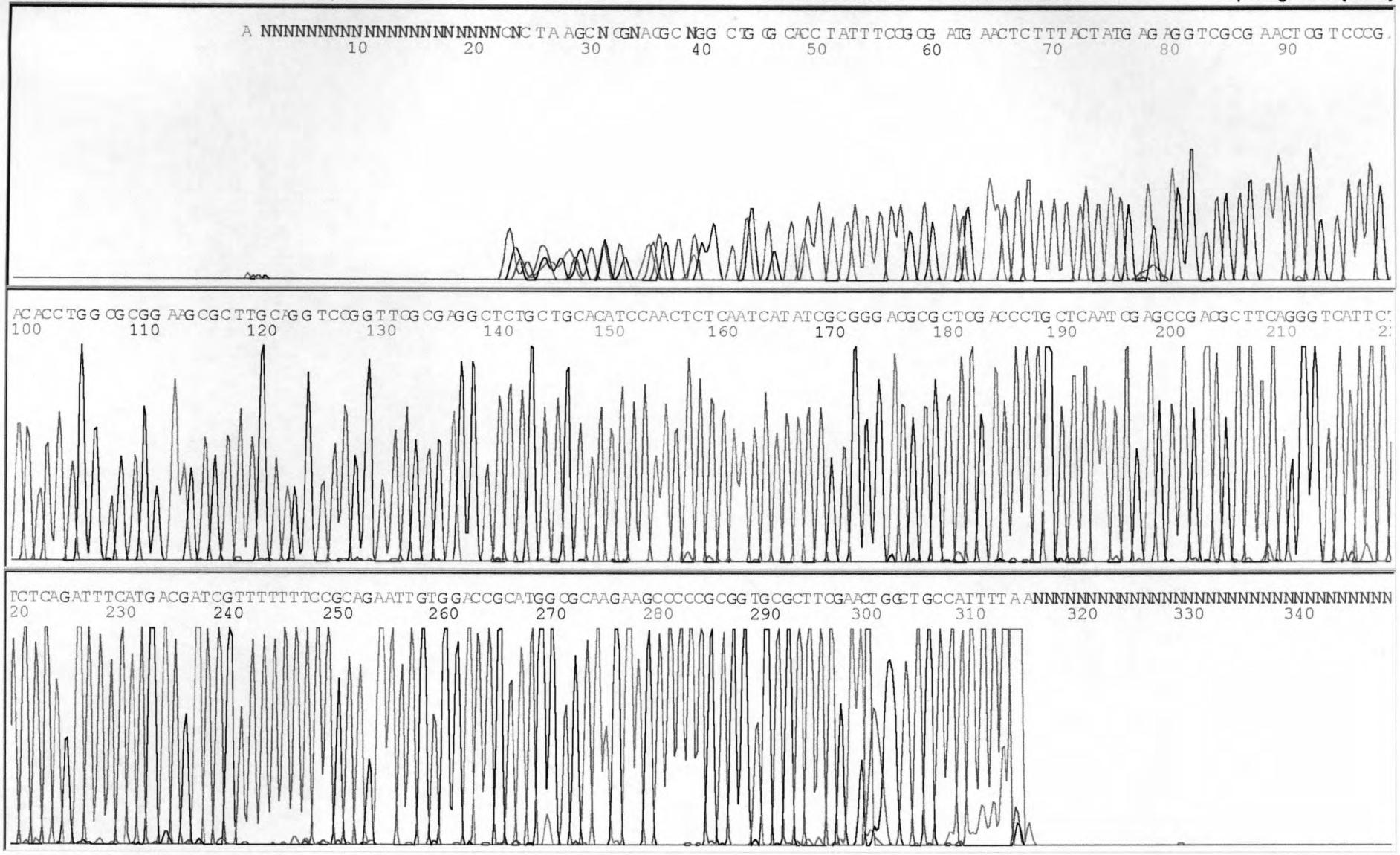
Fri, Aug 25, 2006 9:01 AM
Thu, Aug 24, 2006 10:31 PM
Spacing: 17.66{17.66}



Model 3100 24-8-49(B)_A06_D11(317bp)_nodD1R_02.ab1
Version 3.7
Basecaller-3100APOP4_D11(317bp)_nodD1R
BC 1.5.0.0 Cap 2

Signal G:163 A:115 T:137 C:126
DT3100POP4{BDv3}v1.mob
BSU_3100
Points 1200 to 15000 Pk 1 Loc: 1200

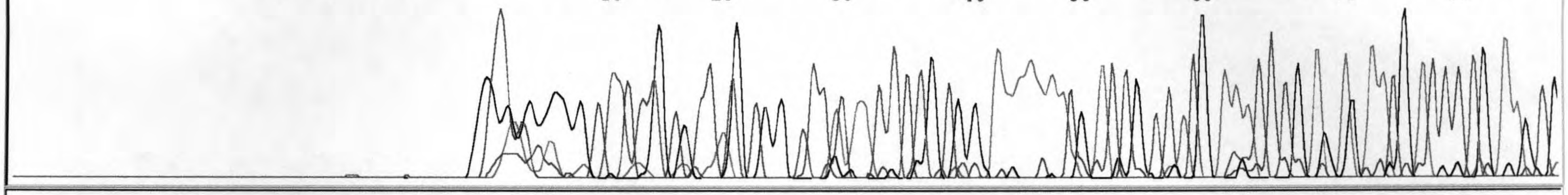
Fri, Aug 25, 2006 9:01 AM
Thu, Aug 24, 2006 10:31 PM
Spacing: 19.68{19.68}



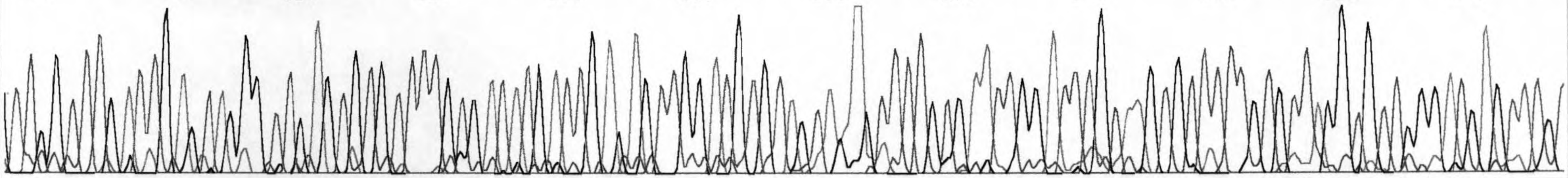
ABI PRISM

Model 3100 | 1-8-49(A)_H09_D345size317bp_nodD1F_15.ab1 | Signal G:255 A:175 T:136 C:140 |
Version 3.7 | DT3100POP4{BDv3}v1.mob | Wed, Aug 02, 2006 7:53 AM
Basecaller-3100APOP4_D345size317bp_nodD1F | BSU_3100 | Tue, Aug 01, 2006 4:54 PM
BC 1.5.0.0 | Cap 15 | Points 1200 to 15000 Pk 1 Loc: 1200 | Spacing: 16.78{16.78}

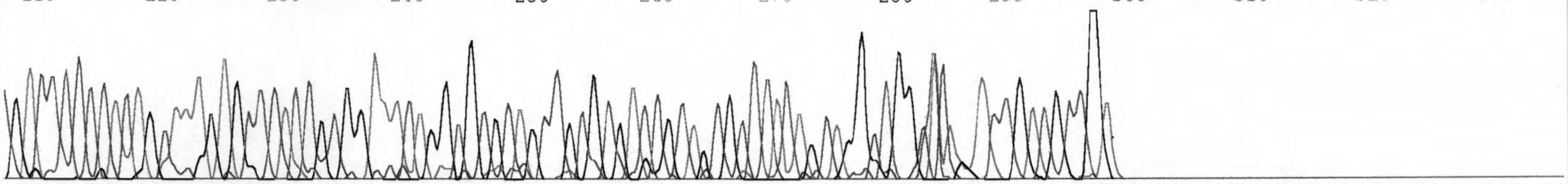
CAGNGGGG C TTCTTG CG CCATG CG G TCCAC AAT T CTG CG GGAAAAAAACG TAT CG TC ATG AAATC TG AG AGCAATG ACCC TG AAG CG
10 20 30 40 50 60 70 80



T CNG CTCG AT TG AG C AG GGCTCG AG CG CG TCCCG CG ATATG ATTG AG AGTTGG ATG TG CAG CANAG CCTCGCGAACCGG ACCTGCAAG CGCTTCCGCG CCAGGTG TCG GG ACGAG TTCGC
90 100 110 120 130 140 150 160 170 180 190 200



:GACCTCTCATAGTAA AG AGTTCATCGCGGAAATAGGTGCGCAGCCGTG CGATCGCAGCGCTCAT AGCAGGCTGGCTCAGAATTGATGTTG AANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
210 220 230 240 250 260 270 280 290 300 310 320 330

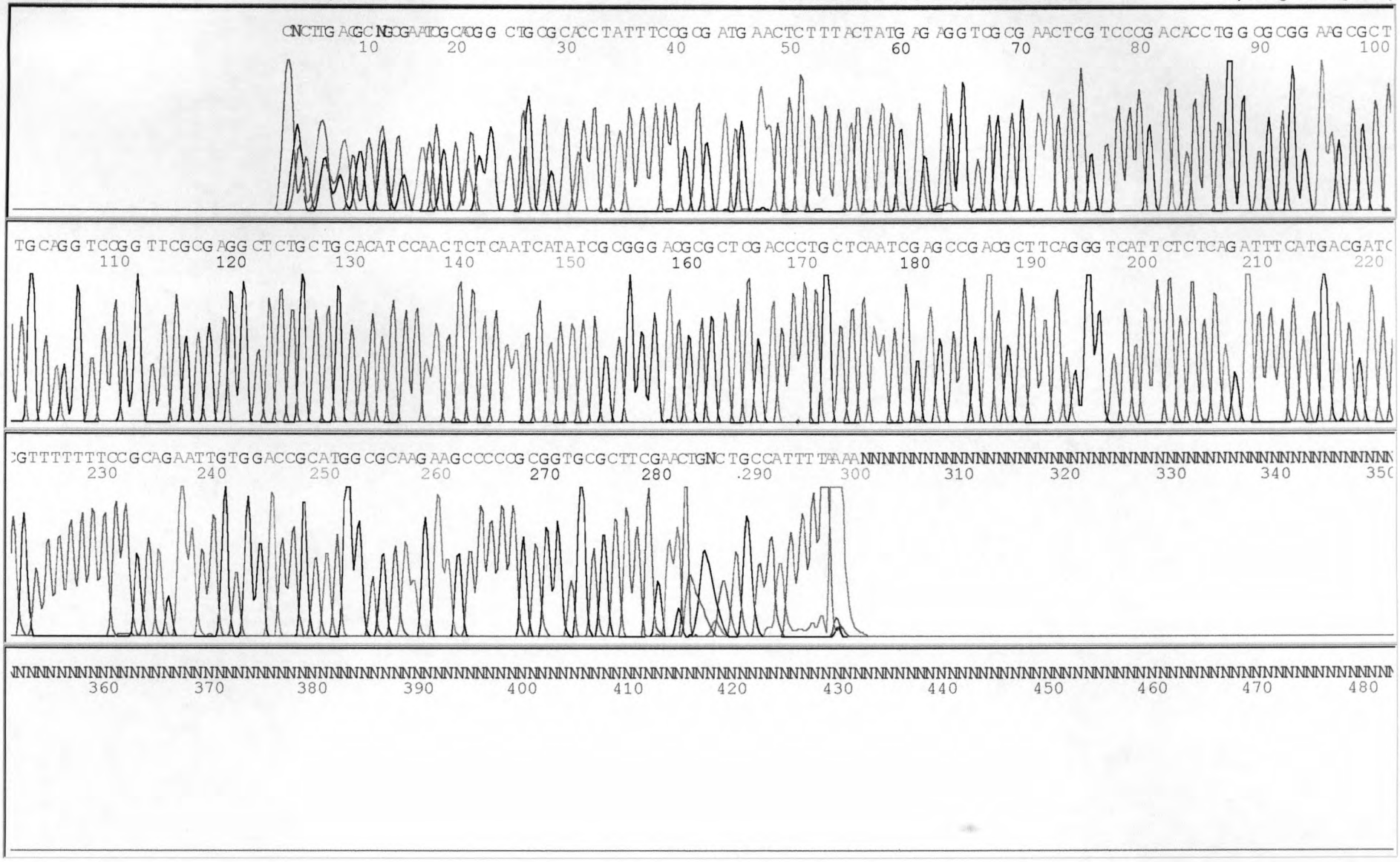


NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
340 350 360 370 380 390 400 410 420 430 440 450 460

Model 3100 1-8-49(A)_H10_D345size317bp_nodD1R_16.ab1
Version 3.7
Basecaller-3100APOP4_D345size317bp_nodD1R
BC 1.5.0.0 Cap 16

Signal G:274 A:169 T:199 C:207
DT3100POP4{BDv3}v1.mob
BSU_3100
Points 1200 to 15000 Pk 1 Loc: 1200

Wed, Aug 02, 2006 7:54 AM
Tue, Aug 01, 2006 4:54 PM
Spacing: 16.52{16.52}

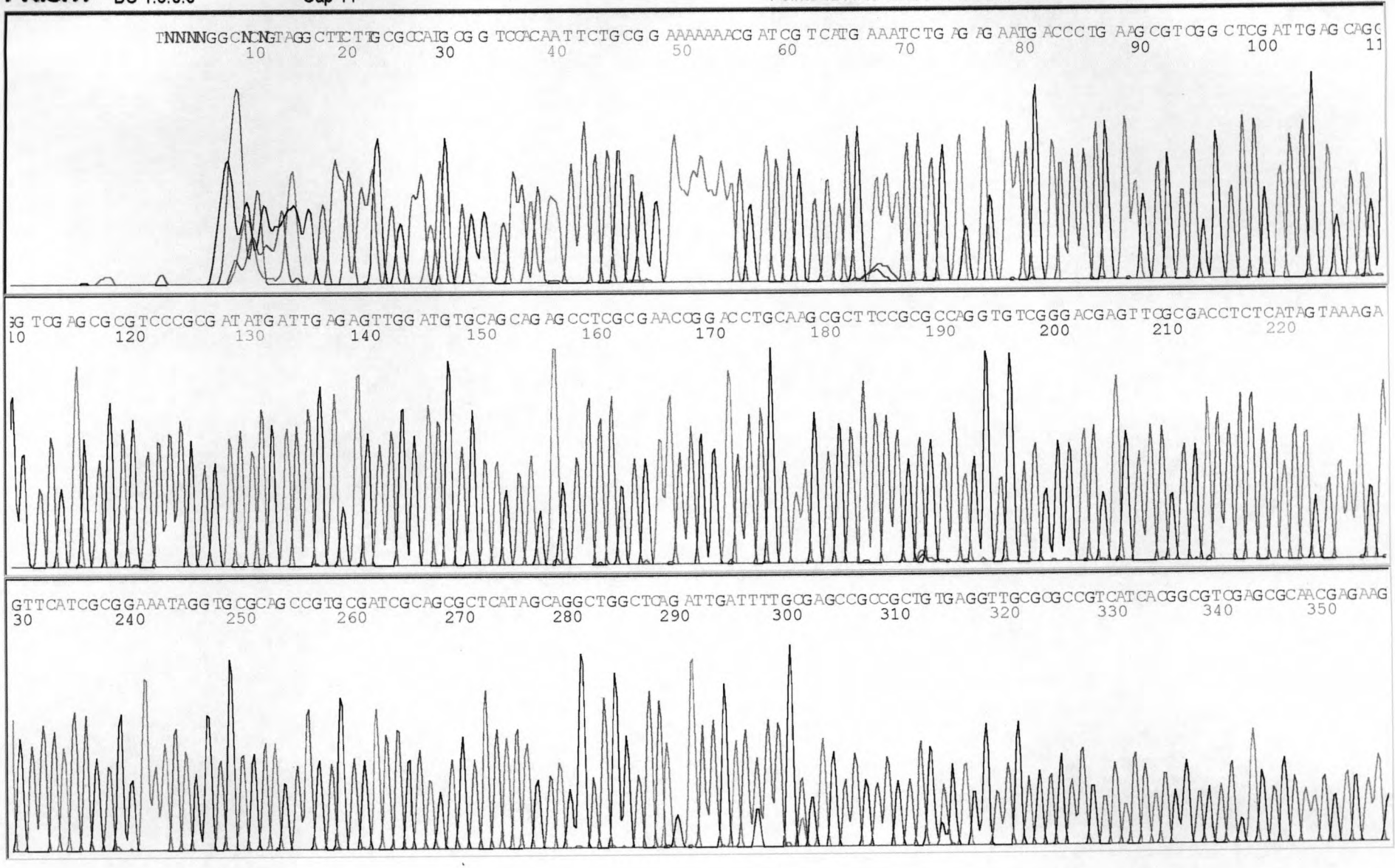


ABI PRISM

Model 3100 31-7-49(B)_F03_D345size657bp_nodD1F_11.ab1
Version 3.7
Basecaller-3100APOP4_D345size657bp_nodD1F
BC 1.5.0.0 Cap 11

Signal G:244 A:151 T:118 C:135
DT3100POP4{BDv3}v1.mob
BSU_3100
Points 1200 to 15000 Pk 1 Loc: 1200

Tue, Aug 01, 2006 7:46 AM
Mon, Jul 31, 2006 9:16 PM
Spacing: 15.75{15.75}



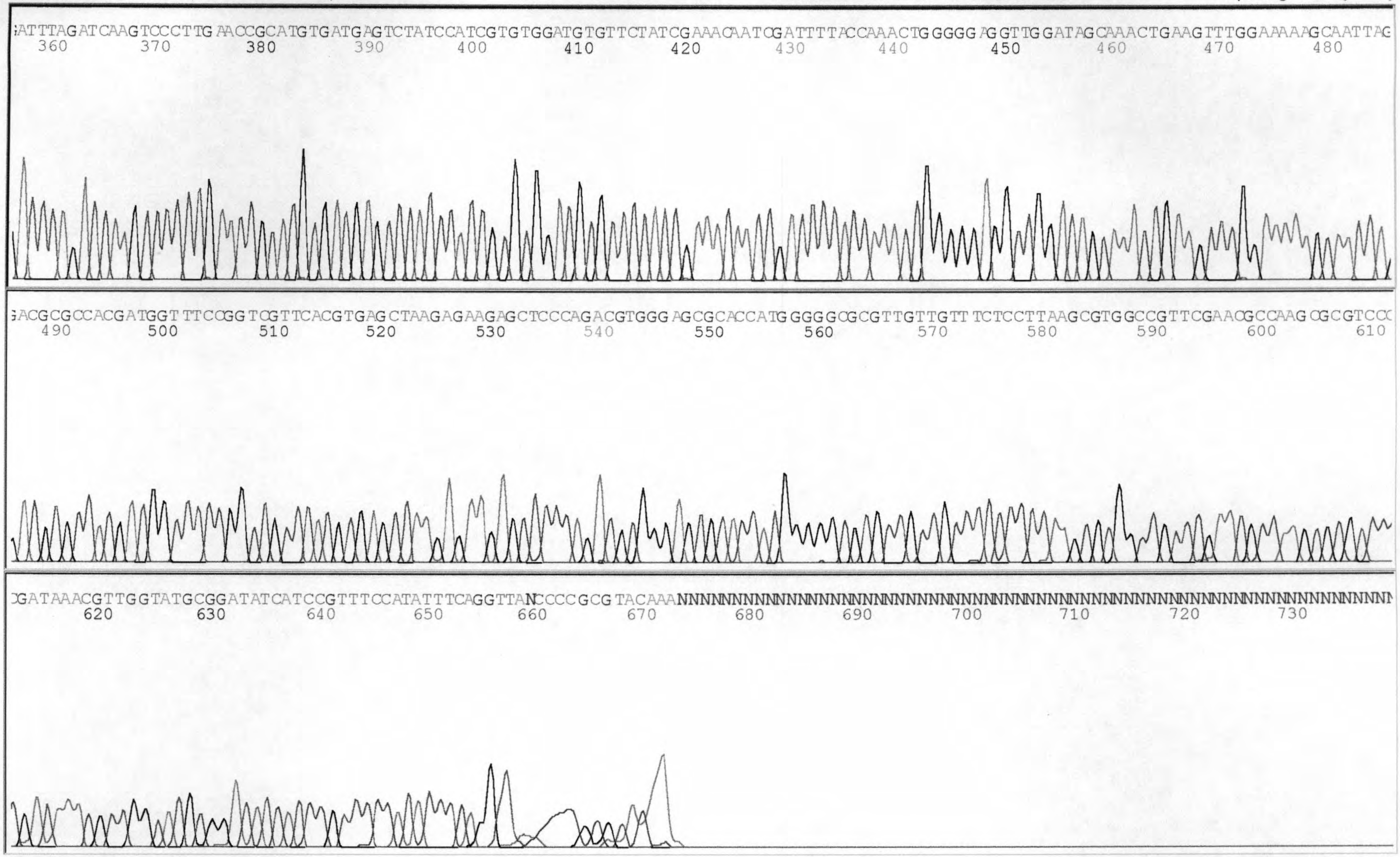
ABI PRISM

Model 3100
Version 3.7
Basecaller-3100APOP4_D345size657bp_nodD1F
BC 1.5.0.0

31-7-49(B)_F03_D345size657bp_nodD1F_11.ab1
Cap 11

Signal G:244 A:151 T:118 C:135
DT3100POP4{BDv3}v1.mob
BSU_3100
Points 1200 to 15000 Pk 1 Loc: 1200

Tue, Aug 01, 2006 7:46 AM
Mon, Jul 31, 2006 9:16 PM
Spacing: 15.75{15.75}

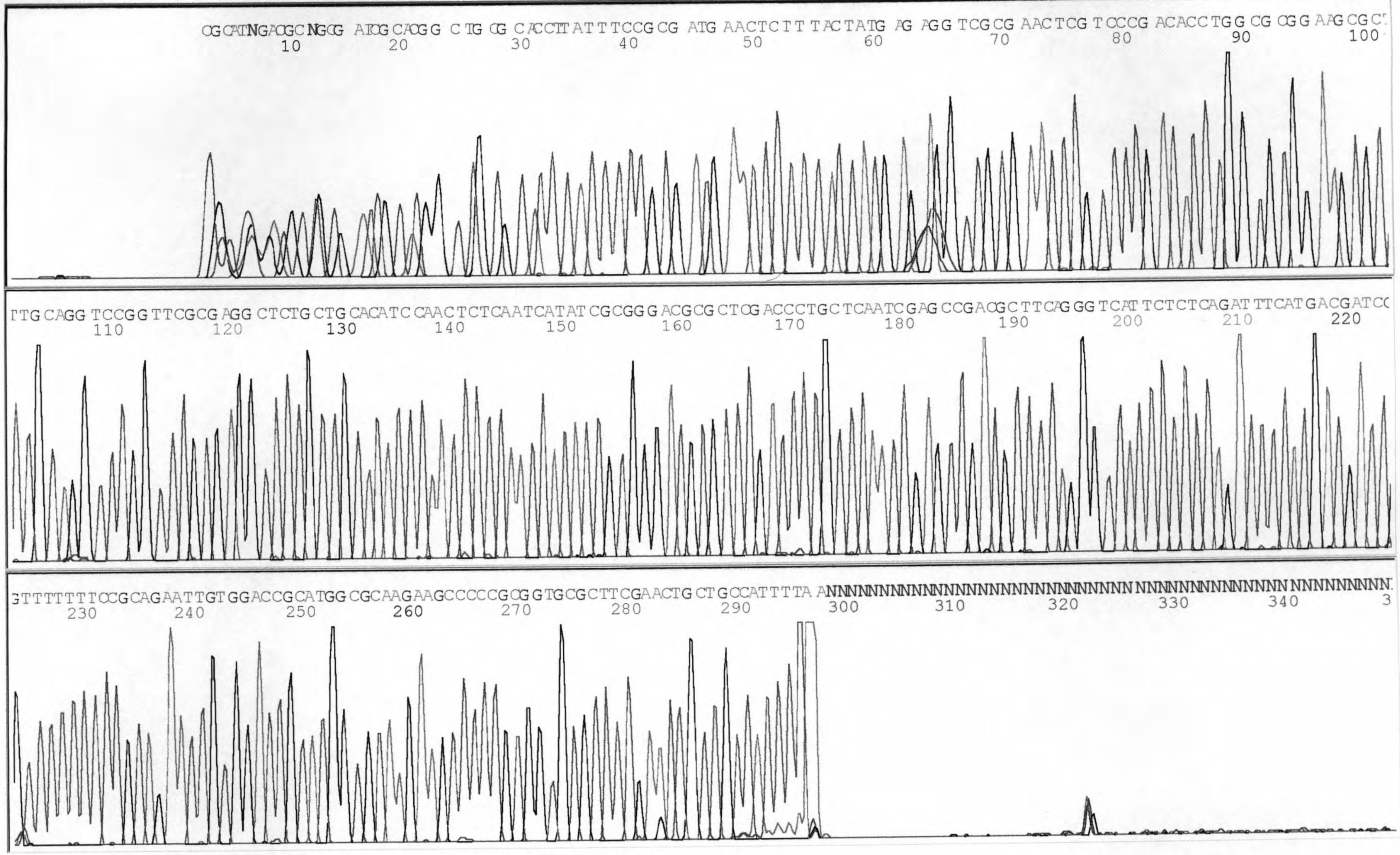


Model 3100
Version 3.7
Basecaller-3100APOP4_D345size657bp_nodD1R
BC 1.5.0.0

1-8-49(A)_A11_D345size657bp_nodD1R_01.ab1
Cap 1

Signal G:67 A:39 T:43 C:45
DT3100POP4{BDv3}v1.mob
BSU_3100
Points 1200 to 7500 Pk 1 Loc: 1200

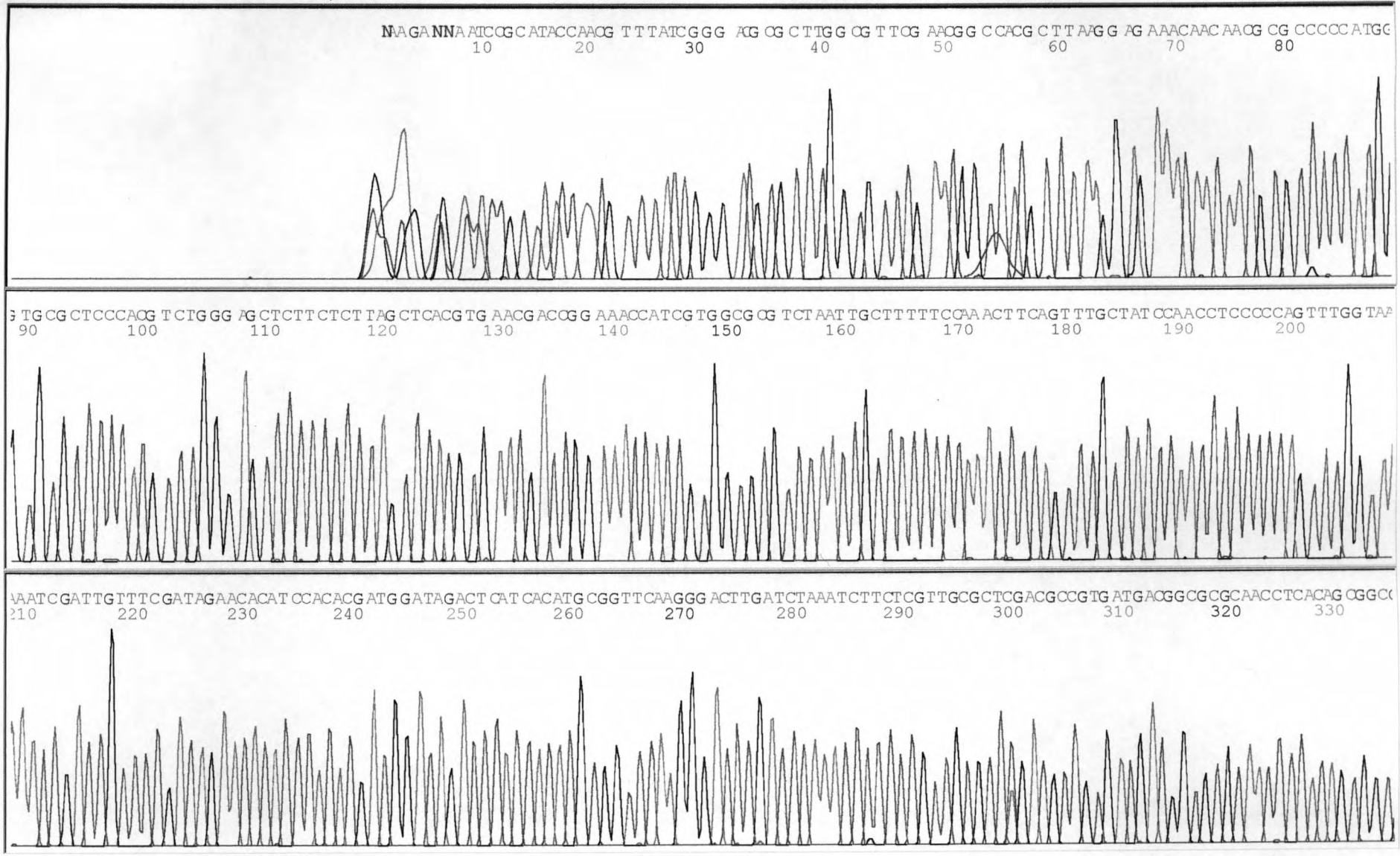
Wed, Aug 02, 2006 8:34 AM
Tue, Aug 01, 2006 8:38 PM
Spacing: 15.06{15.06}



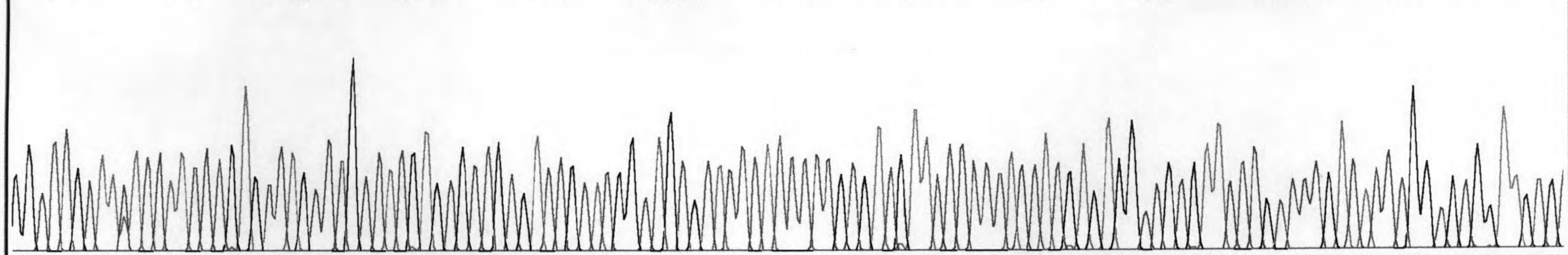
Model 3100 22-8-49_D03_D345(657bp)_nodYF_07.ab1
Version 3.7
Basecaller-3100APOP4_D345(657bp)_nodYF
BC 1.5.0.0 Cap 7

Signal G:82 A:49 T:47 C:49
DT3100POP4{BDv3}v1.mob
BSU_3100
Points 1200 to 15000 Pk 1 Loc: 1200

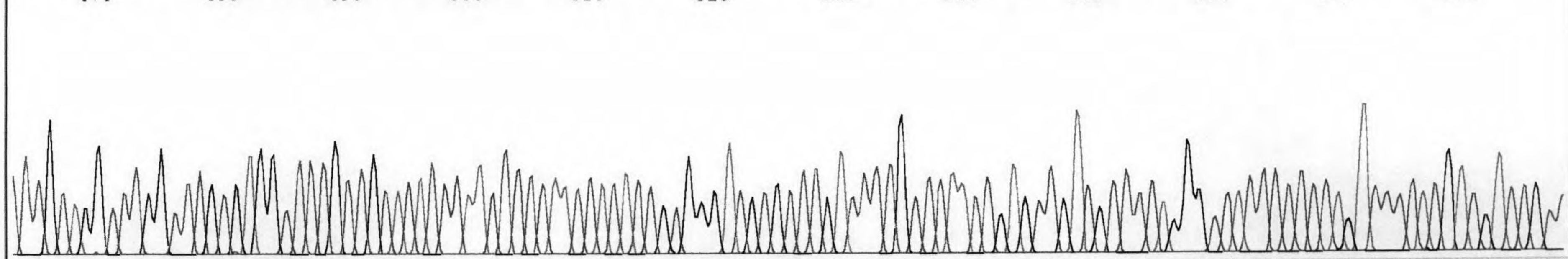
Wed, Aug 23, 2006 7:47 AM
Tue, Aug 22, 2006 5:41 PM
Spacing: 16.52{16.52}



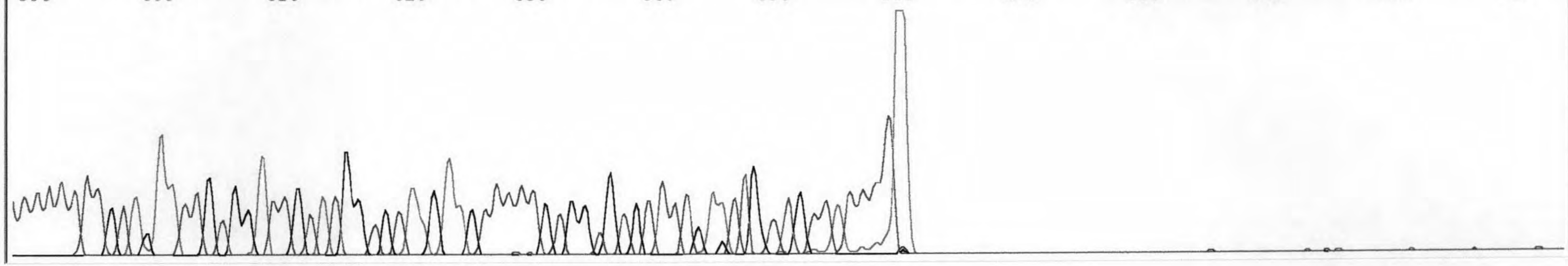
GGCTCGCAACATCAATCTGAGCCAGCCTGCTATGAGCGCTGCGATCGCACGGCTGCGCACCTATTTCCGCGATGAACTCTTTACTATGAGAGGTCGCGAACTCGTCCCGACACCTGGCGCGGAAGCGC
340 350 360 370 380 390 400 410 420 430 440 450 460

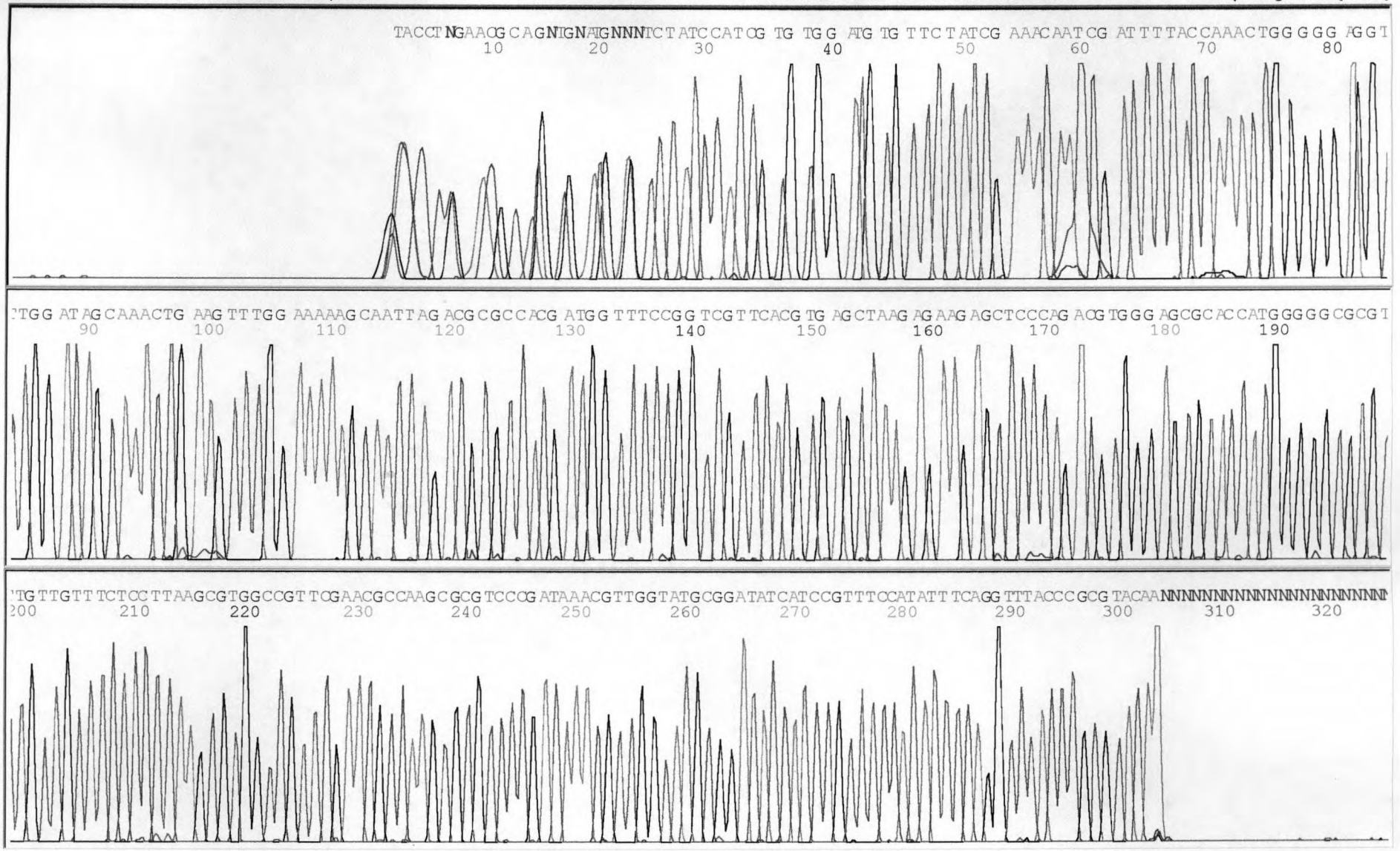


TTGCAGGTCCGGTTCGCGAGGCTCTGCTGCACATCCAACTCTCAATCATATCGCGGGACGCGCTCGACCCTGCTCAATCGAGCCGACGCTTCAGGGTCATTCTCTCAGATTTCATGACGATCGTT
470 480 490 500 510 520 530 540 550 560 570 580



TTTTTCCGCAGAATTGTGGACCGCATGGCGCAAGAAGCCCCCGCGGTGCGCTTCGAACTGGCTGCCATTTTAANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
590 600 610 620 630 640 650 660 670 680 690 700 710




TACCTNGAACGCAGNTGNATGNNNTCTATCCATCG TG TGG ATG TG TTCTATCG AAACAATCG ATTTTACCAAACTGGGGG AGGT
10 20 30 40 50 60 70 80
TGG ATAGCAAACTG AAG TTTGG AAAAAGCAATTAG ACG CGCCACG ATGG TTTCCGG TCGTTCACG TG AGCTAAG AG AAG AGCTCCCAG ACG TGGG AGCGCACCATGGGGG CGCGT
90 100 110 120 130 140 150 160 170 180 190
TG TTGTTTCTCCTTAAGCGTGGCCGTTCGAACGCCAAGCGCGTCCCGATAAACGTTGGTATGCGGATATCATCCGTTTCCATATTTCAGG TTTACCCGCG TACAANNNNNNNNNNNNNNNNNNNNNNNN
200 210 220 230 240 250 260 270 280 290 300 310 320

BIOGRAPHY

Miss Duangporn Emampaiwong was born on August 21, 1982. She obtained a Bachelor of Science Degree with Second Class Honours in Applied Biology from Rajabhat Suandusit Institute, Thailand, in 2004.

Publication

1. Chansa-ngavej, K., Chongfuengprinya, W., Ly Kim Pheng, Emampaiwong, D., Sulanchupakorn, S., and Sawangdee, S. 2006. Use of RAPD-PCR fingerprints to monitor changes in DNA and field distribution of soybean rhizobia biofertilizers. Proceedings of The 14th World Fertilizer Congress. Held at Lotus Kad Suan Kaew Hotel, January 23-27, 2006. Chiangmai, Thailand. 6 Pages (in press).

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1) ดวงพร เอมอำไพวงศ์ และ กาญจนา ชาญสง่าเวช. 2549. การพัฒนาไพรเมอร์ที่มีความจำเพาะต่อการจำแนกแบคทีเรียตรึงไนโตรเจนประเภทเพิ่มจำนวนเร็วและประเภทเพิ่มจำนวนช้าที่ปมรากถั่วเหลืองโดยวิธีมัลติเพล็กซ์พีซีอาร์. หนังสือรวมบทคัดย่อการประชุมวิชาการ คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย. ครั้งที่ 14 : หน้า 11.
2) Emampaiwong, D. and Chansa-ngavej, K. 2005. Development of primers specific for the detection of fast-growing *Sinorhizobium fredii* and slow-growing *Bradyrhizobium japonicum*. Abstract Book, The 2nd AgBiotech Graduate Conference II. May 16-17, 2005. Bangkok, Thailand. p. 116
3) Chansa-ngavej, K., Chongfuengprinya, W., Ly Kim Pheng, Emampaiwong, D., Sawangdee, S., Sulanchupakorn, S., and Yamada, M. 2006. RAPD-PCR fingerprinting , strain selection and greenhouse-scale testing of thermotolerant soybean rhizobial biofertilizers. Abstract Book. The 5th JSPS-NRCT Joint seminar on Development of Themotolerant Microbial Resources and Their Applications. November 7-10, 2006. Pattaya. Thailand (in press).