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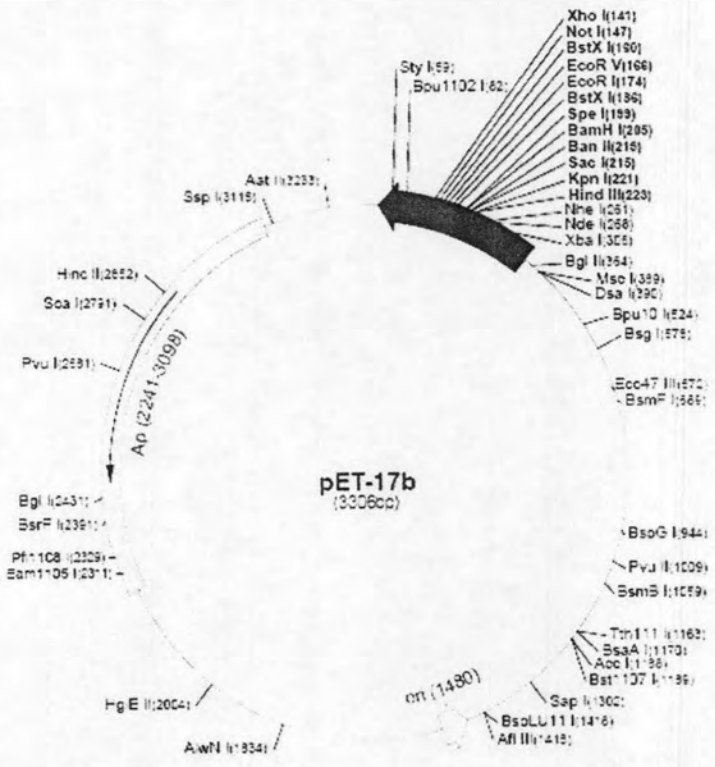
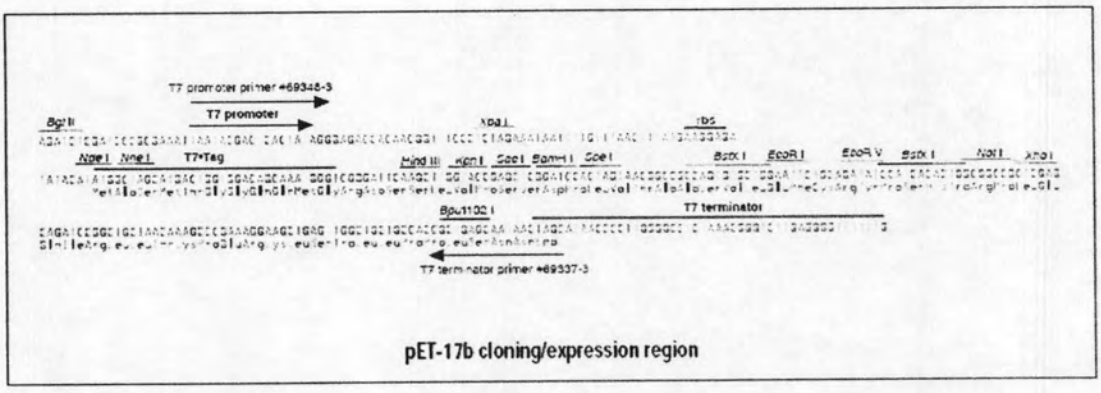
APPENDICES

APPENDIX A

Restriction map of pET-17b

pET-17b sequence landmarks	
T7 promoter	333-349
T7 transcription start	332
T7•Tag coding sequence	237-269
Multiple cloning sites (*Hind* III - *Xho* I)	141-228
T7 terminator	28-74
pBR322 origin	1480
bla coding sequence	2241-3098





pET-17b cloning/expression region

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 gel was incubated at 50°C for 10 min (or until the gel slice has completely dissolved) and mixed by vortexing the tube every 2-3 minutes during the incubation.
4. After the gel slice has 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 bind DNA, the sample was applied to the QIAquick column and centrifuged at 10,000xg 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 10,000xg for1 minute.
9. Buffer PE 0.75 ml was added to QIAquick column to wash and further centrifuged at 10,000xg for 1 minute.
10. The flow-through was discarded and QIAquick column was centrifuged at 12,000xg for an additional 1 minute.
11. To elute DNA, 50 µl of buffer EB (10 mM Tris-Cl, pH 8.5) or H_2O was added to the center of QIAquick membrane and centrifuged at 10,000xg for 1 minute.

APPENDIX C

Preparation for protein determination

Reagent for determination of protein concentration (modified from Lowry *et al.*, 1951)

Solution A (0.5% copper sulfate and 1% potassium tartate, pH 7.0)

Potassium tartate	1	g
Copper sulfate	0.5	g

Adjusted pH to 7.0 and adjust the solution volume to 100 ml.

Solution B (2% sodium carbonate and 1 N sodium hydroxide)

Sodium carbonate	20	g
Sodium hydroxide	4	g

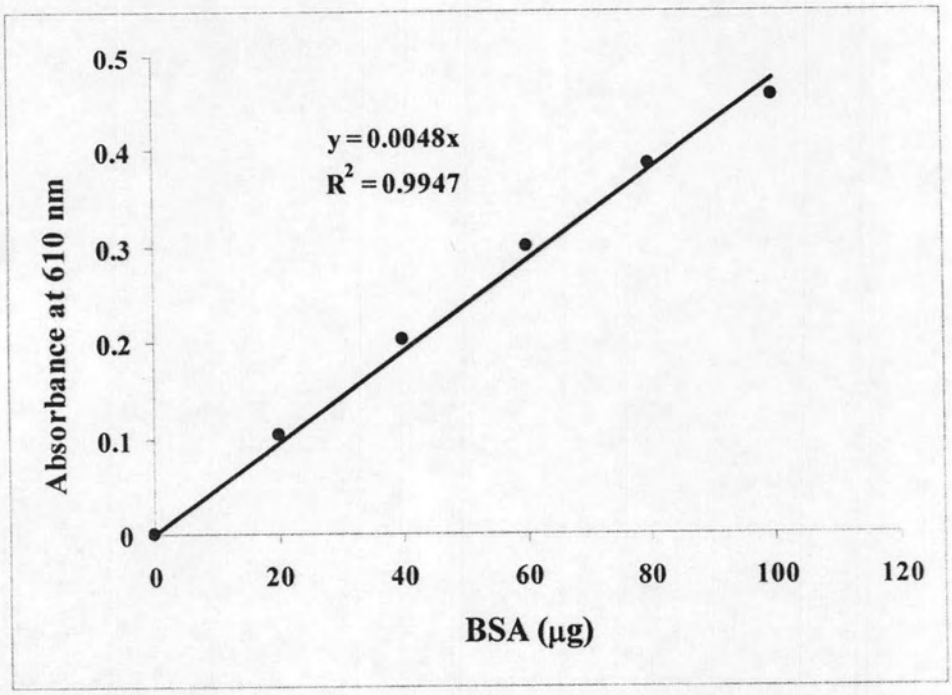
Dissolved in distilled water to 1 liter.

Solution C (phenol reagent)

Folin-Ciocalteu phenol reagent used in this work was reagent grade from Carlo Erba, Italy.

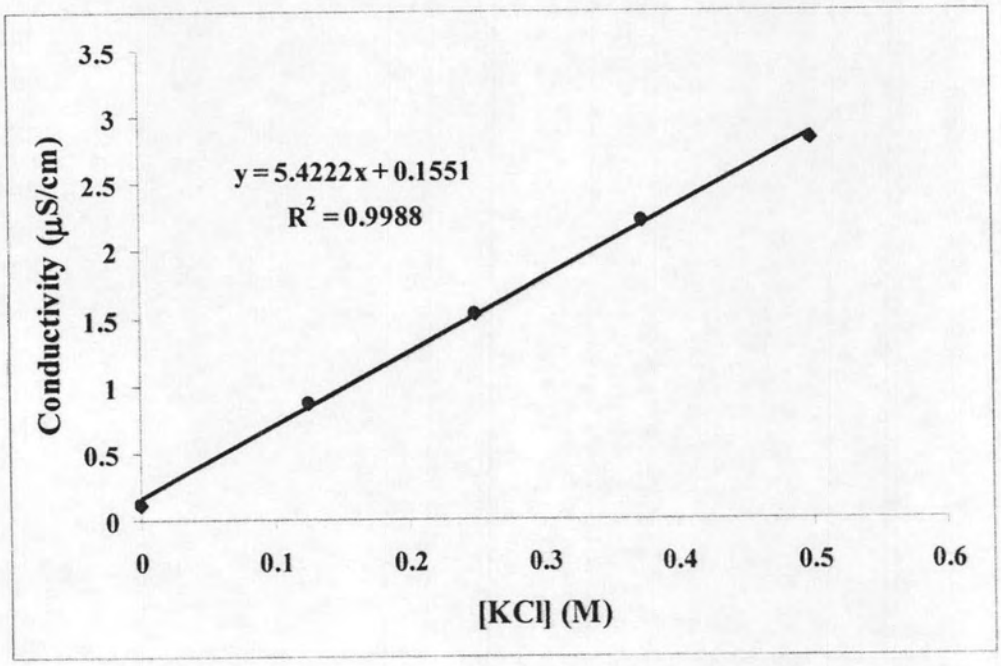
APPENDIX D

Standard curve for protein determination by Lowry' s method



APPENDIX E

Calibration curve for conductivity of potassium chloride



APPENDIX F

Preparation for non-denaturing polyacrylamide gel electrophoresis (Native-PAGE)

1. Stock solutions

2 M Tris-HCl (pH 8.8)

Tris (hydroxymethyl)-aminomethane	24.2	g

Adjusted pH to 8.8 with 1 N HCl and adjusted volume to 100 ml with distilled water.

1 M Tris-HCl (pH 6.8)

Tris (hydroxymethyl)-aminomethane	12.1	g

Adjusted pH to 6.8 with 1 N HCl and adjusted volume to 100 ml with distilled water.

1% (w/v) Bromophenol blue

Bromophenol blue	100	mg

Brought to 10 ml with distilled water and stirred until dissolved.
The aggregated dye was removed by filtration.

2. Working solutions

Solution A (30% (w/v) acrylamide, 0.8% (w/v) bis-acrylamide)

Acrylamide	29.2	g
N, N'-methylene-bis-acrylamide	0.8	g

Adjusted volume to 100 ml with distilled water.

Solution B (1.5 M Tris-HCl, pH 8.8)

2 M Tris-HCl (pH 8.8)	75	ml
Distilled water	25	ml

Solution C (0.5 M Tris-HCl, pH 6.8)

1 M Tris-HCl (pH 6.8)	50	ml
Distilled water	50	ml

APPENDIX F (continued)

10% (w/v) Ammonium persulfate

Ammonium persulfate	0.5	g
Distilled water	5.0	ml

Electrophoresis buffer (25 mM Tris, 192 mM glycine)

Tris (hydroxymethyl)-aminomethane	3.0	g
Glycine	14.4	ml

Dissolved and adjusted to total volunm 1 liter with distilled water

(final pH should be approximately 8.3)

5x Sample buffer (312.5 mM Tris-HCl pH 6.8, 50% (v/v) glycerol, 1% (v/v) bromophenol blue)

1 M Tris-HCl (pH 6.8)	0.6	ml
Glycerol	5.0	ml
1% Bromophenol blue	0.5	ml
Distilled water	1.4	ml

3. Native-PAGE

7.7% Separating gel

Solution A	2.6	ml
Solution B	2.5	ml
Distilled water	4.9	ml
10% (w/v) Ammonium persulfate	50	µl
TEMED	5.0	µl

5.0% Stacking gel

Solution A	0.67	ml
Solution C	1.0	ml
Distilled water	2.3	ml
10% (w/v) Ammonium persulfate	30	µl
TEMED	5.0	µl

APPENDIX F (continued)

4. Protein staining solution

Staining solution, 1 liter

Coomassie brilliant blue R-250	1.0	g
Glacial acetic acid	100	ml
Methanol	450	ml
Distilled water	450	ml

Destaining solution, 1 liter

Methanol	100	ml
Glacial acetic acid	100	ml
Distilled water	800	ml

5. Enzyme activity staining solution

1 M Tris-HCl, pH 8.5

Tris (hydroxymethyl)-aminomethane 6.06 g

Adjusted to pH 8.5 with 1 N HCl and made up volume to 100 ml with distilled water

40 mM L-phenylalanine

L-phenylalanine 0.066 g

Dissolved with 10 ml distilled water

50 mM NAD^+

NAD^+ 0.359 g

Dissolved with 10 ml distilled water

0.25 mg/ml phenazine methosulfate

Phenazine methosulfate 0.0025 g

Dissolved with 10 ml distilled water

2.5 mg/ml nitroblue tetrazolium

Nitroblue tetrazolium 0.025 g

Dissolved with 10 ml distilled water

Activity staining solution (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)

1 M Tris-HCl, pH 8.5	4.25	ml
40 mM L-phenylalanine	1.0	ml
50 mM NAD^+	1.0	ml
0.25 mg/ml phenazine methosulfate	1.0	ml
2.5 mg/ml nitroblue tetrazolium	1.0	ml
Distilled water	1.75	ml

APPENDIX G

Preparation for denaturing polyacrylamide gel electrophoresis

1. Stock solution

2 M Tris-HCl (pH 8.8)

Tris (hydroxymethyl)-aminomethane 24.2 g

Adjusted pH to 8.8 with 1 N HCl and adjusted volume to 100 ml with distilled water.

1 M Tris-HCl (pH 6.8)

Tris (hydroxymethyl)-aminomethane 12.1 g

Adjusted pH to 6.8 with 1 N HCl and adjusted volume to 100 ml with distilled water.

10% (w/v) SDS

Sodium dodecyl sulfate (SDS) 10 g

Added distilled water to a total volume of 100 ml.

50% (w/v) Glycerol

100% Glycerol 50 ml

Added distilled water to a total volume of 100 ml.

1% (w/v) Bromophenol blue

Bromophenol blue 100 mg

Brought to 10 ml with distilled water and stirred until dissolved.

The aggregated dye was removed by filtration.

2. Working solutions

Solution A (30% (w/v) acrylamide, 0.8% (w/v) bis-acrylamide)

Acrylamide 29.2 g

N, N'-methylene-bis-acrylamide 0.8 g

Adjusted volume to 100 ml with distilled water.

Filtered and stored in dark (brown bottle) at 4°C

APPENDIX G (continued)

Solution B (1.5 M Tris-HCl, pH 8.8 and 0.4% SDS)

2 M Tris-HCl (pH 8.8)	75	ml
10% (w/v) SDS	4	ml
Distilled water	21	ml

Solution C (0.5 M Tris-HCl, pH 6.8, 0.4% SDS)

1 M Tris-HCl (pH 6.8)	50	ml
10% (w/v) SDS	4	ml
Distilled water	46	ml

10% (w/v) Ammonium persulfate

Ammonium persulfate	0.5	g
Distilled water	5.0	ml

Electrophoresis buffer (25 mM Tris, 192 mM glycine and 0.1% (w/v) SDS)

Tris (hydroxymethyl)-aminomethane	3.0	g
Glycine	14.4	ml
SDS	1	g

Dissolved and adjusted to total volume to 1 liter with distilled water (final pH should be approximately 8.3)

5x Sample buffer (312.5 mM Tris-HCl pH 6.8, 50% (v/v) glycerol, 1% (w/v) bromophenol blue)

1 M Tris-HCl (pH 6.8)	0.6	ml
50% (v/v) Glycerol	5.0	ml
10% (w/v) SDS	2	ml
1% (w/v) Bromophenol blue	1	ml
β-Mercaptoethanol	0.5	ml
Distilled water	1.4	ml

APPENDIX G (continued)

3. SDS-PAGE

10% Separating gel

Solution A	3.3	ml
Solution B	2.5	ml
Distilled water	4.2	ml
10% (w/v) Ammonium persulfate	50	µl
TEMED	5	µl

5.0% Stacking gel

Solution A	0.67	ml
Solution C	1.0	ml
Distilled water	2.3	ml
10% (w/v) Ammonium persulfate	30	µl
TEMED	5	µl

4. Protein staining solution

Staining solution, 1 liter

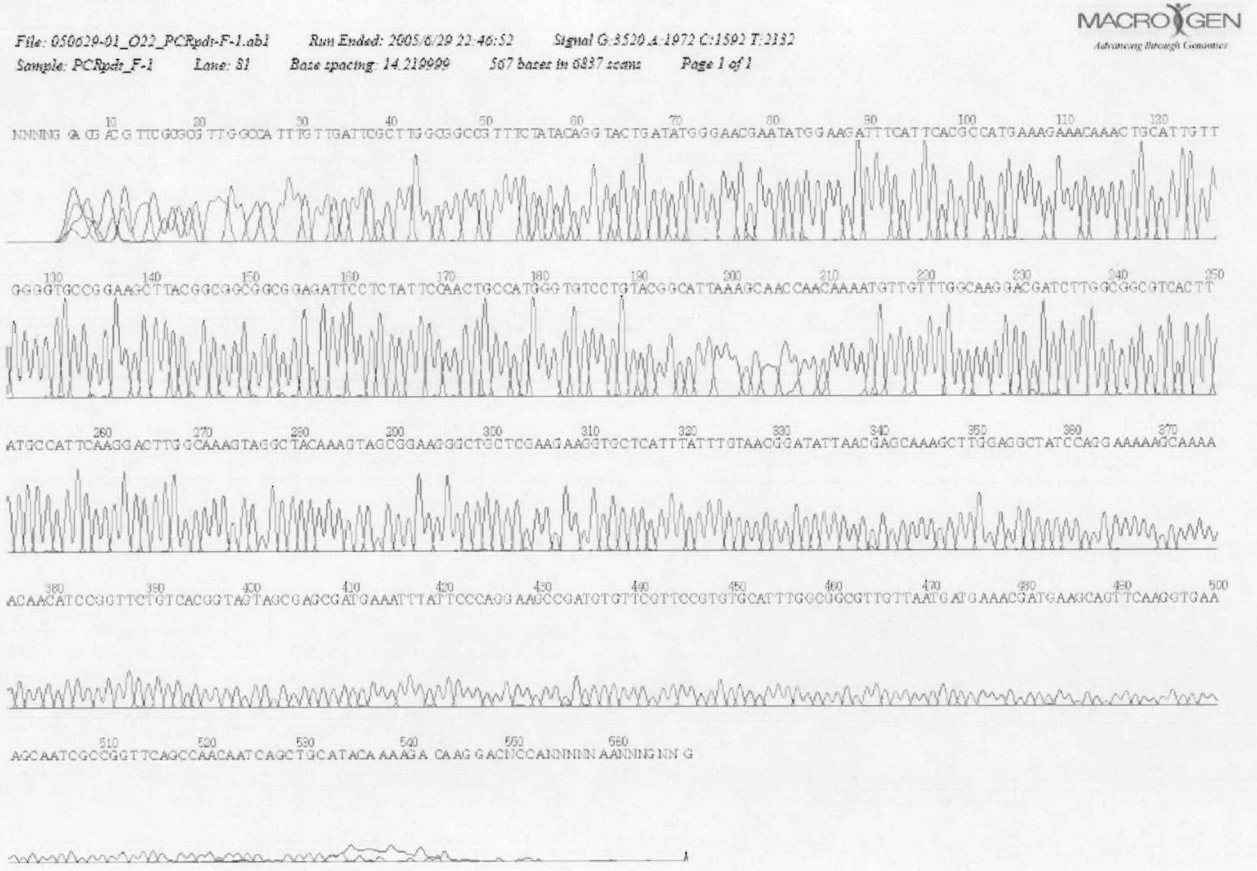
Coomassie brilliant blue R-250	1.0	ml
Methanol	450	ml
Distilled water	450	ml

Destaining solution, 1 liter

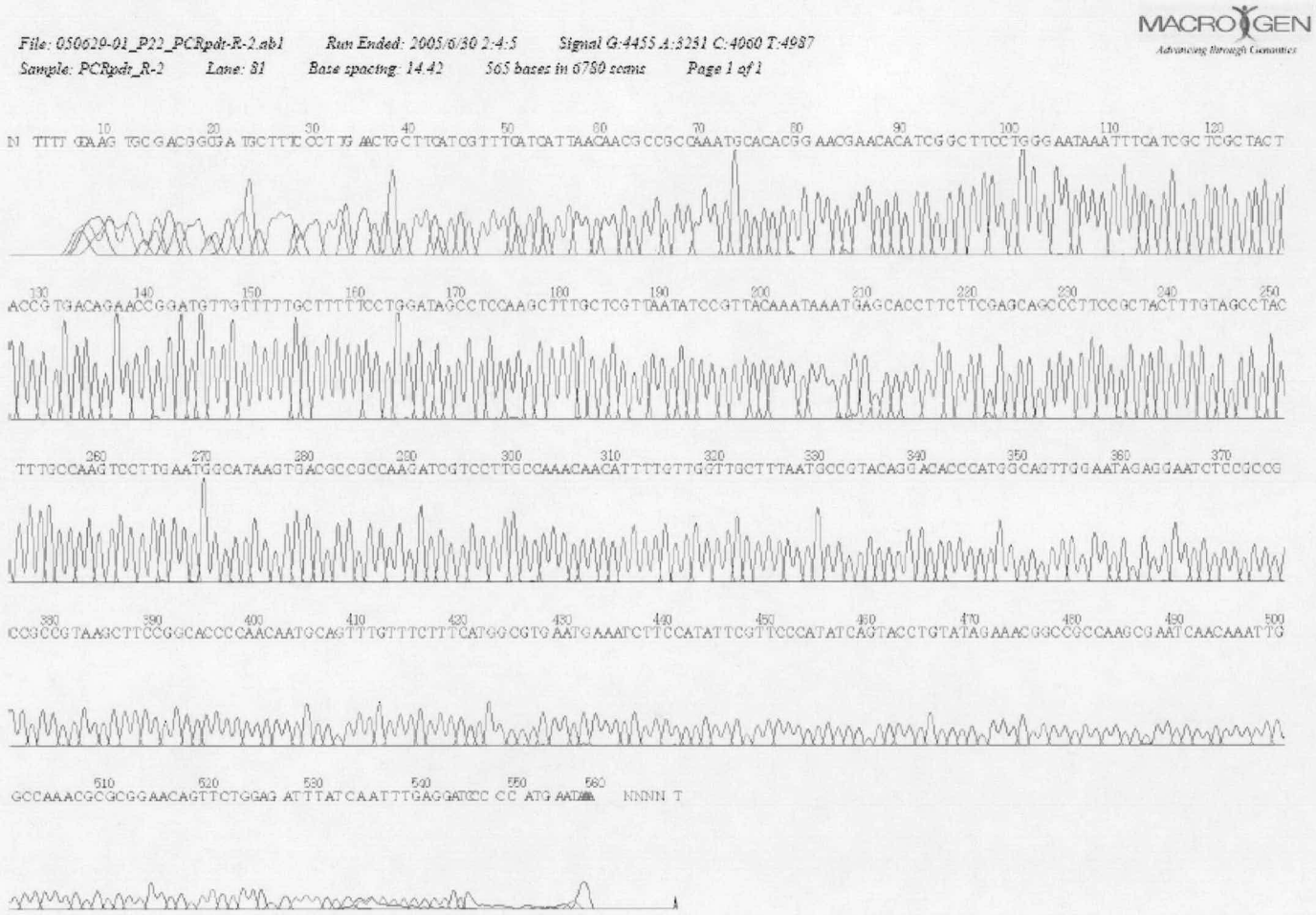
Methanol	100	ml
Glacial acetic acid	100	ml
Distilled water	800	ml

APPENDIX H

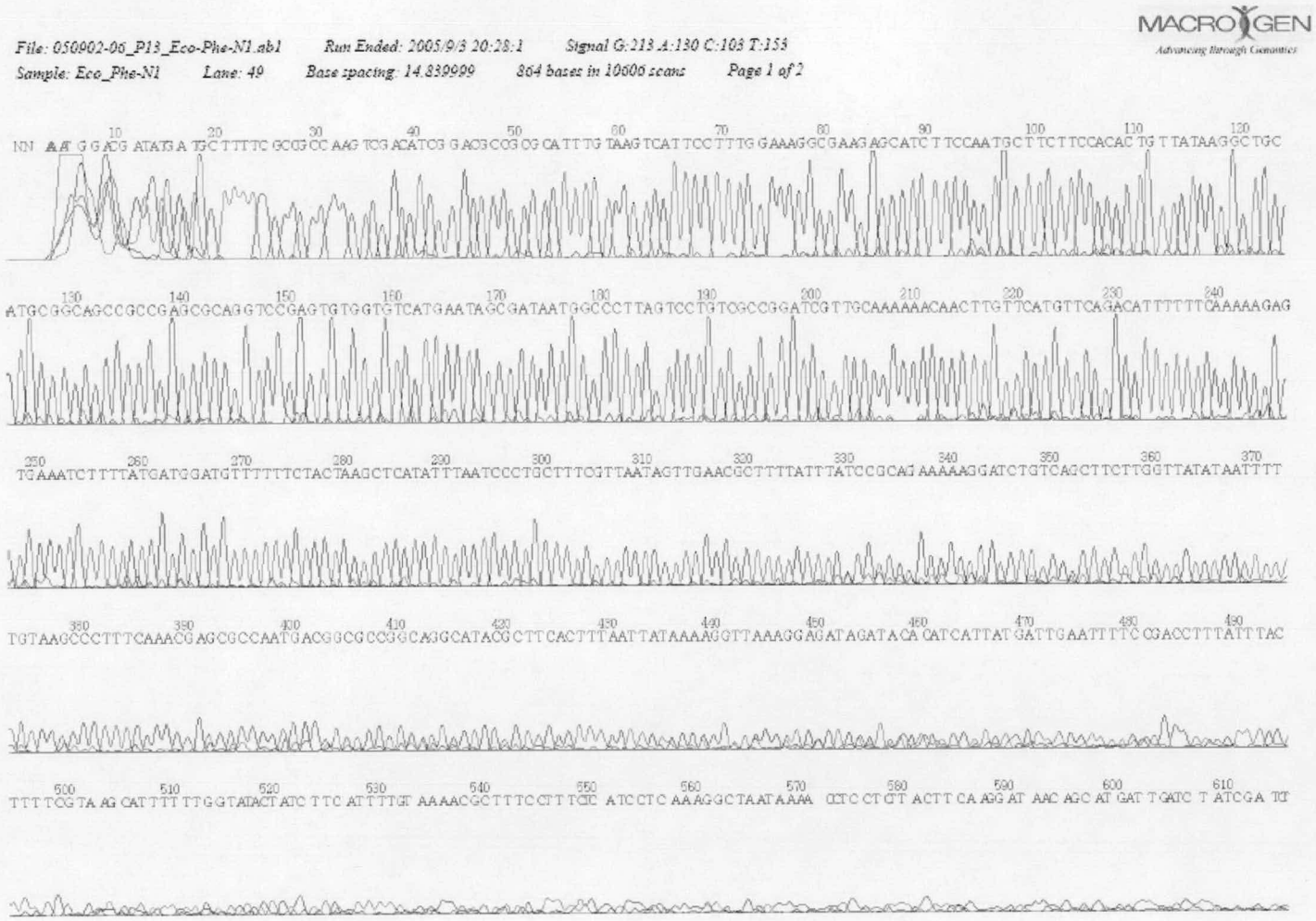
The chromatograms of the phenylalanine dehydrogenase gene from *Bacillus lentus*



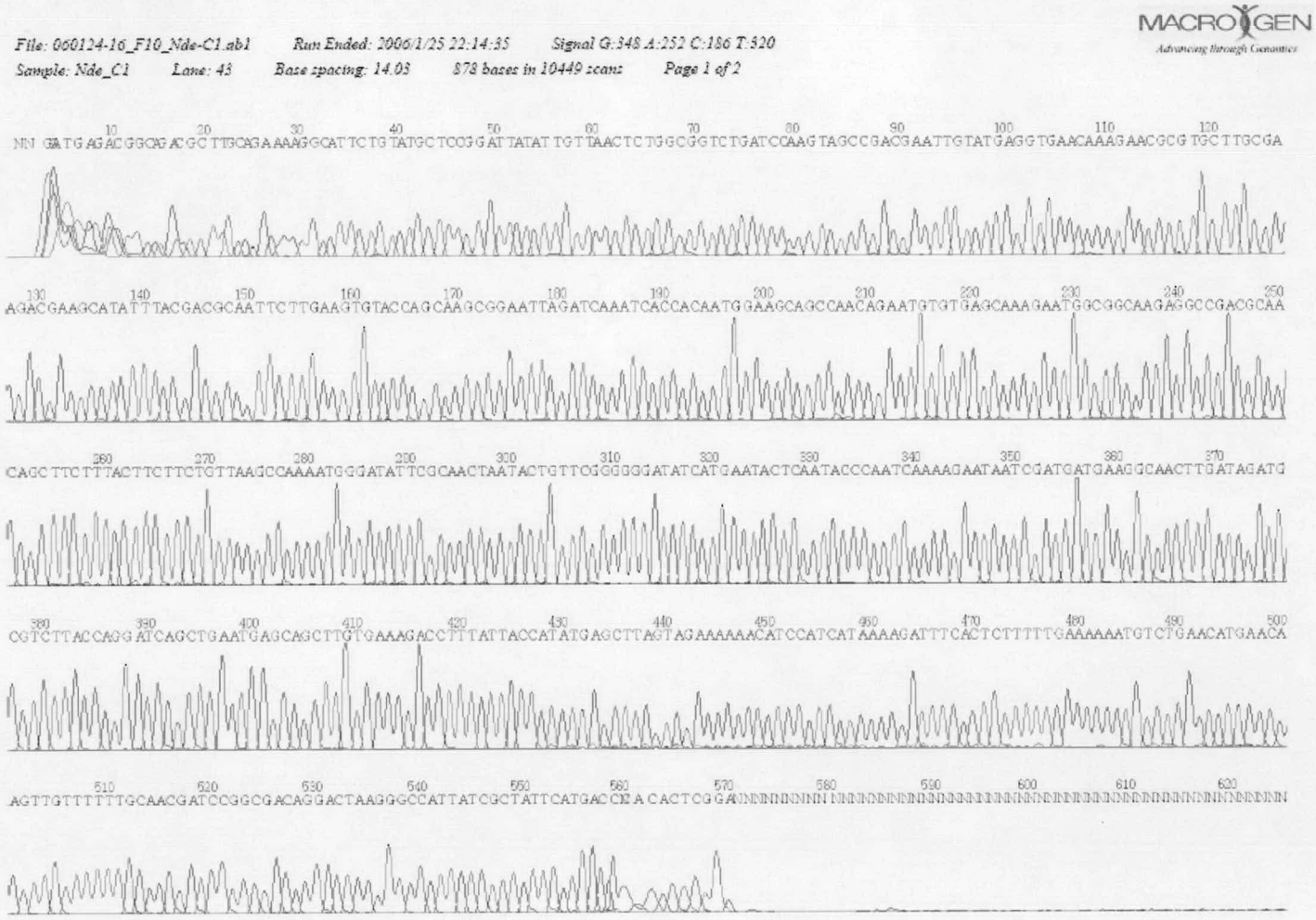
a) The chromatogram of the internal fragment of phenylalanine dehydrogenase gene using sense primer F1



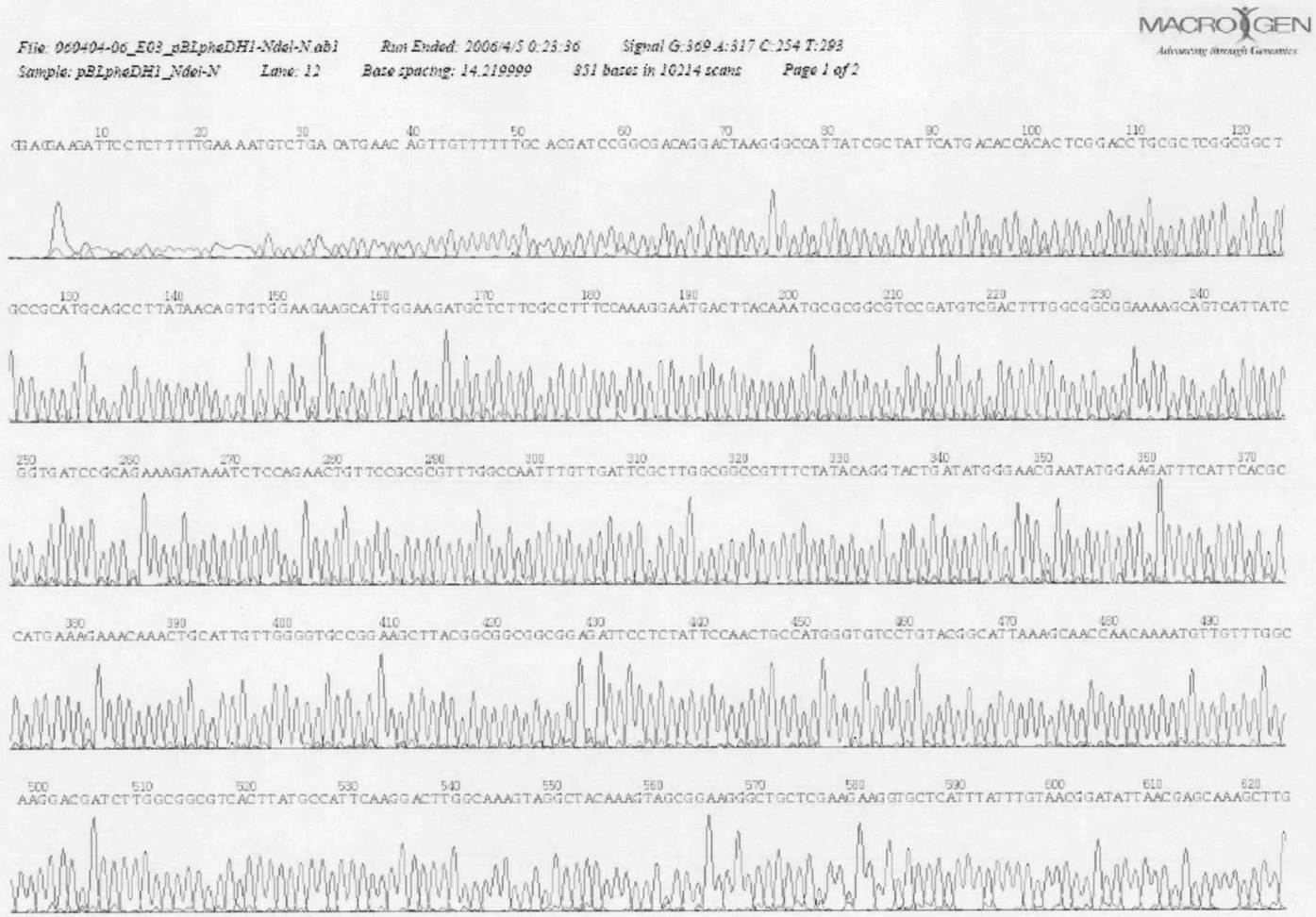
a) The chromatogram of the internal fragment of phenylalanine dehydrogenase gene using antisense primer R2



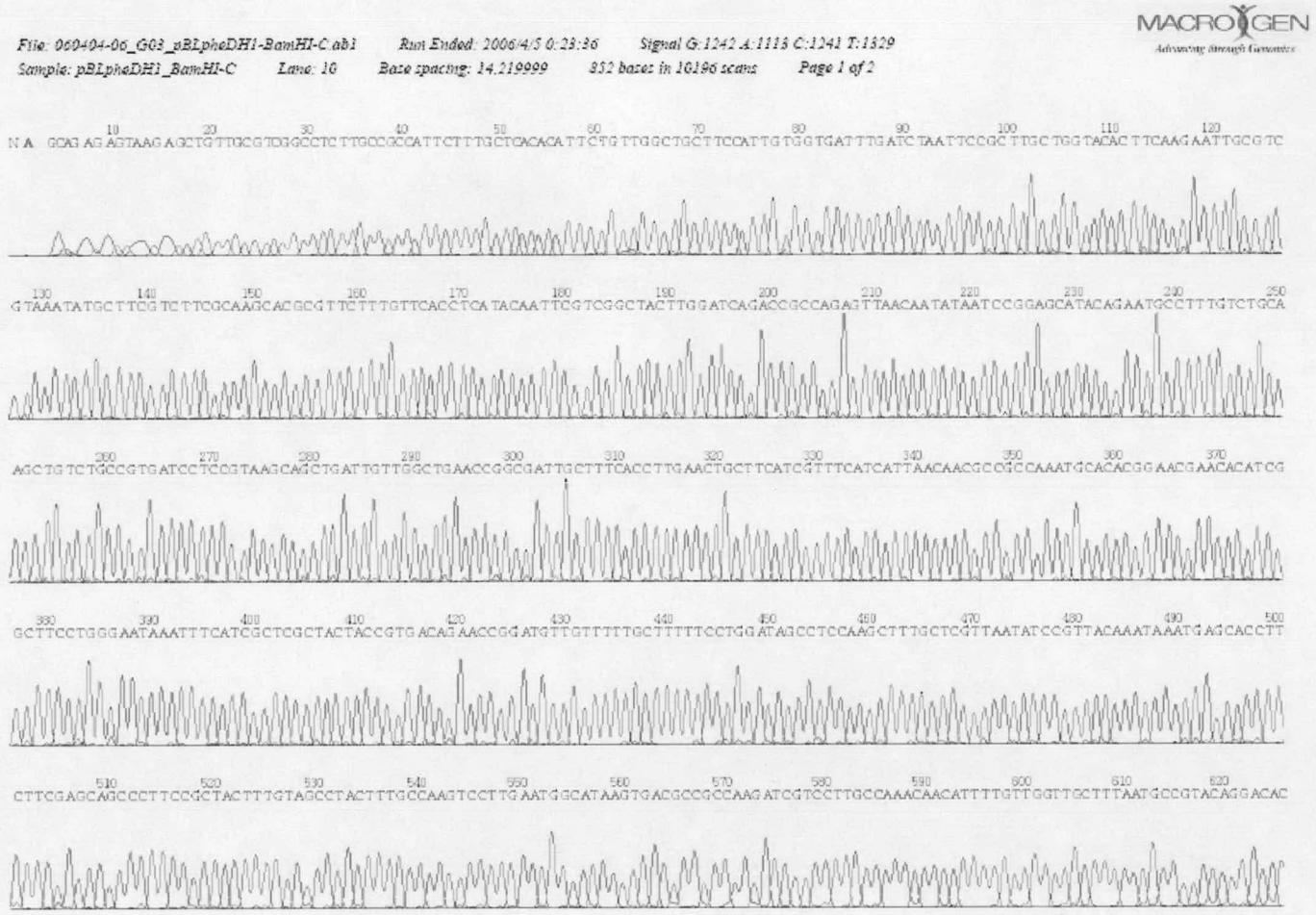
b) The chromatogram of the 5′-terminal fragment of phenylalanine dehydrogenase gene using antisense primer N2



c) The chromatogram of the 3′-terminal fragment of phenylalanine dehydrogenase gene using sense primer C2



d) The chromatogram of the 5′-terminal fragment of pBLPheDH using sense primer Nde-N



d) The chromatogram of the 3′-terminal fragment of pBLPheDH using antisense primer Bam-C

APPENDIX I

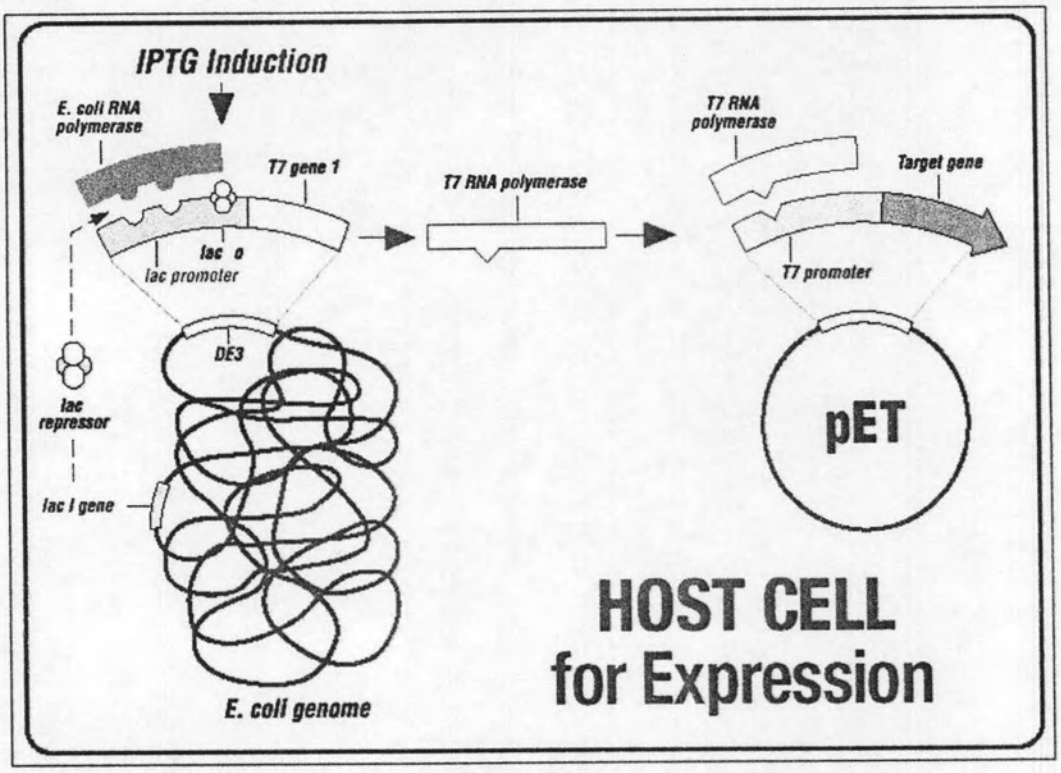
Abbreviation for amino acid residues

Amino acid	3 Letters-Abbreviation	1-Letter-Abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Source: Voet, 2004

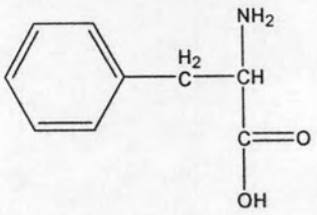
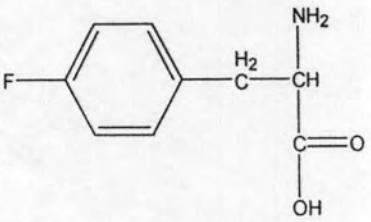
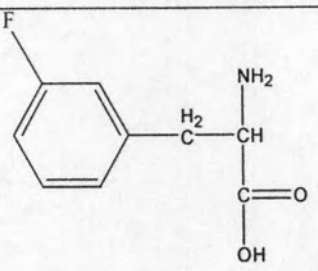
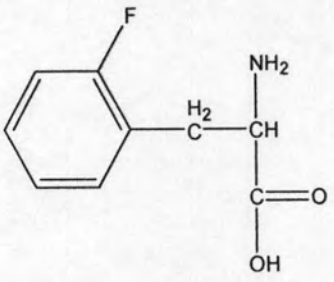
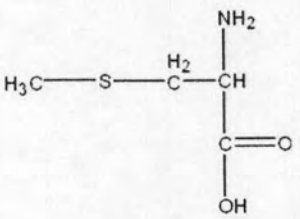
APPENDIX J

Control element of the pET system

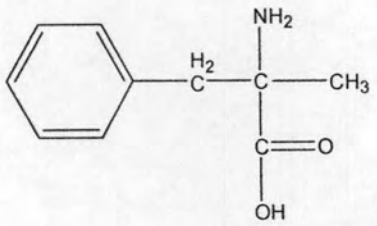
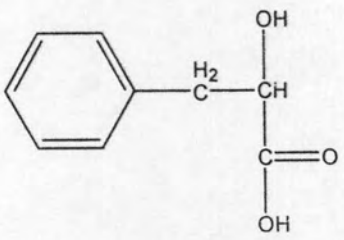
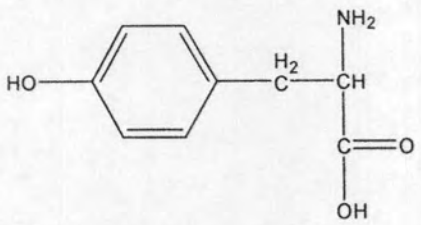
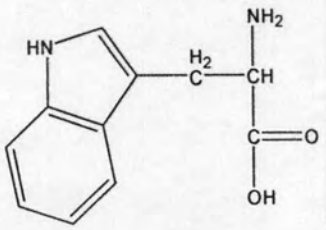
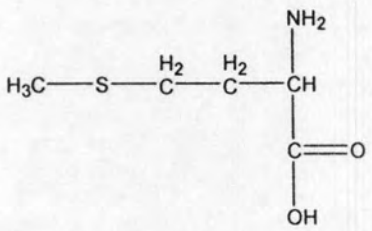


APPENDIX K

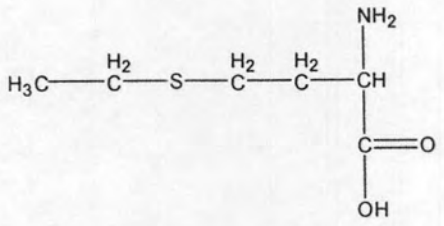
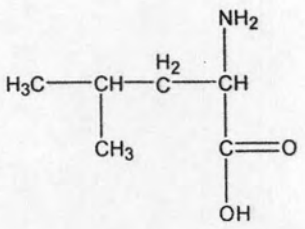
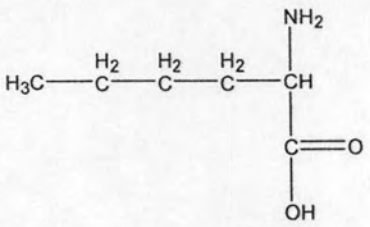
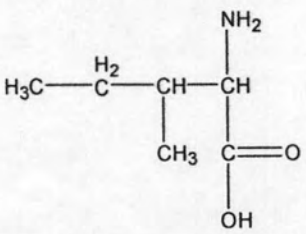
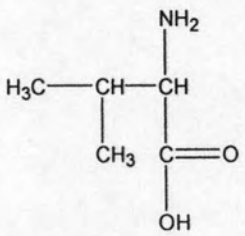
Structure of amino acids and their analogs

Amino acids and analogs	Structure
L-phenylalanine	
p-fluoro-DL-phenylalanine	
m-fluoro-DL-phenylalanine	
o-fluoro-DL-phenylalanine	
S-methyl-L-cysteine	

APPENDIX K (continued)

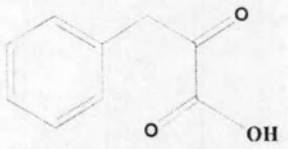
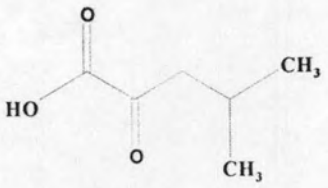
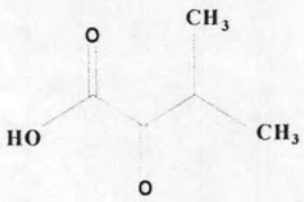
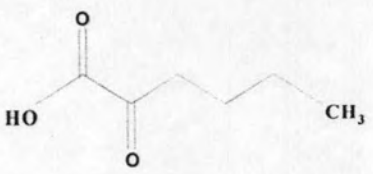
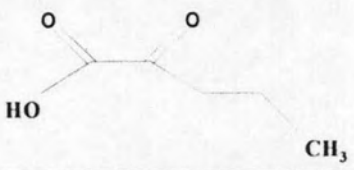
Amino acids and analogs	Structure
α-methyl-DL-phenylalanine	
L-α-phenyllactate	
L-tyrosine	
L-tryptophan	
L-methionine	

APPENDIX K (continued)

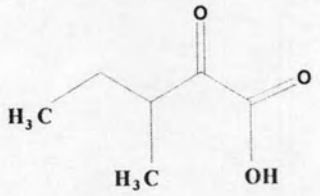
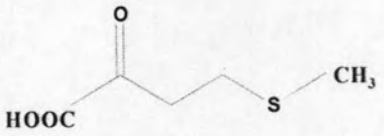
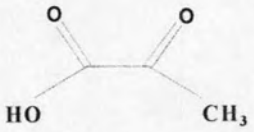
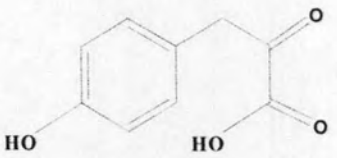
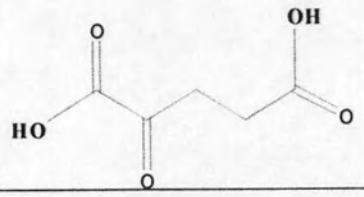
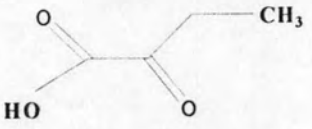
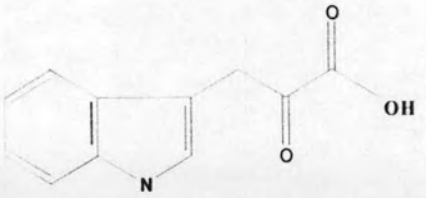
Amino acids and analogs	Structure
L-ethionine	
L-leucine	
L-norleucine	
L-isoleucine	
L-valine	

APPENDIX L

Structure of keto acids and their analogs

Keto acid	Structure
phenylpyruvate	
α-ketoisocaproate	
α-ketoisovalerate	
α-ketocaproate	
α-ketovalerate	

APPENDIX L (continued)

Keto acid	Structure
α-keto-β-methyl-n-valerate	
α-keto-γ-methiol-butyrate	
pyruvate	
p-hydroxyphenylpyruvate	
α-ketoglutarate	
α-keto-n-butyrate	
indole-β-pyruvic acid	

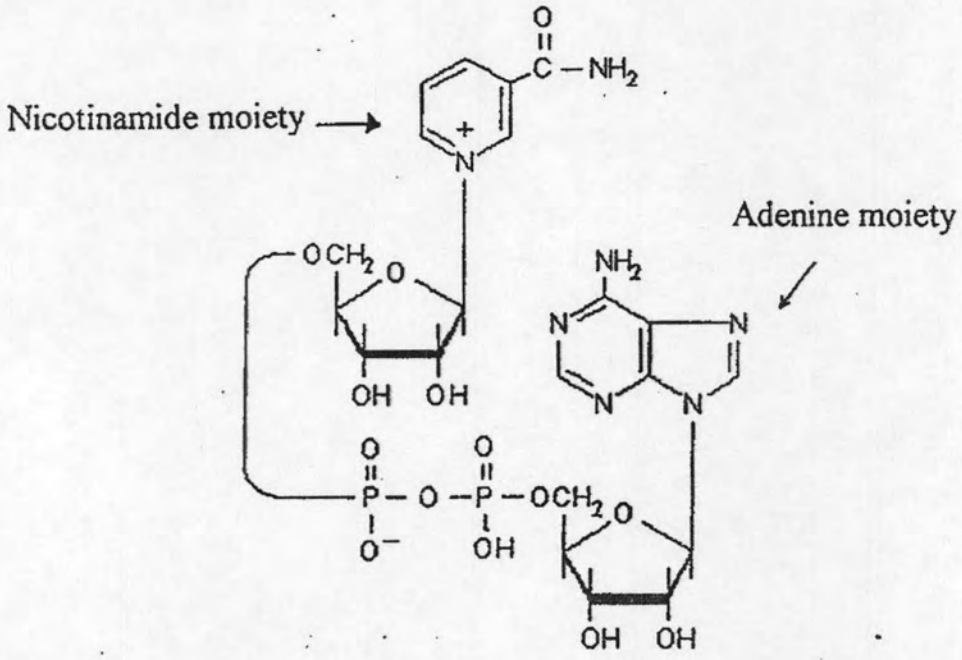
APPENDIX M

Amino acids and their corresponding keto acids

Amino acid	Keto acid
L-phenylalanine	β-phenylpyruvate
L-norleucine	α-ketocaproate
L-leucine	α-ketoisocaproate
L-norvaline	α-ketovalerate
L-valine	α-ketoisovalerate
L-methionine	α-keto-γ-methiol-n-butyrate
α-aminobutyrate	α-keto-n-butyrate
L-isoleucine	α-keto-β-methylvalerate
L-glutamic acid	α-ketoglutarate

APPENDIX N

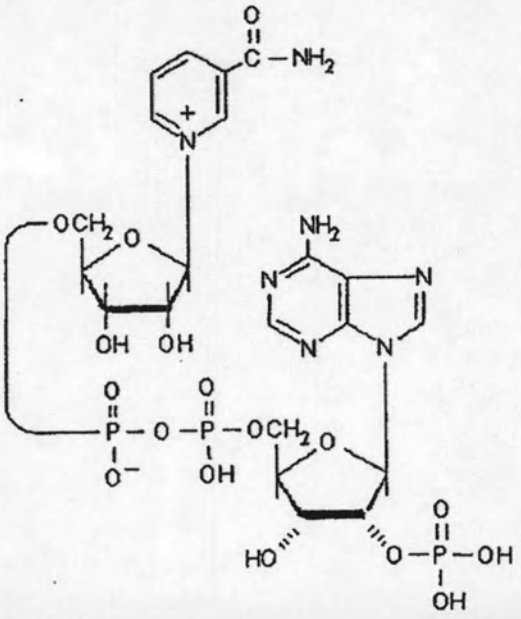
NAD^+ analogs



Nicotinamide adenine dinucleotide (NAD^+)

The NAD^+ analogs used in this work can be divided into 3 groups based on their modified structure.

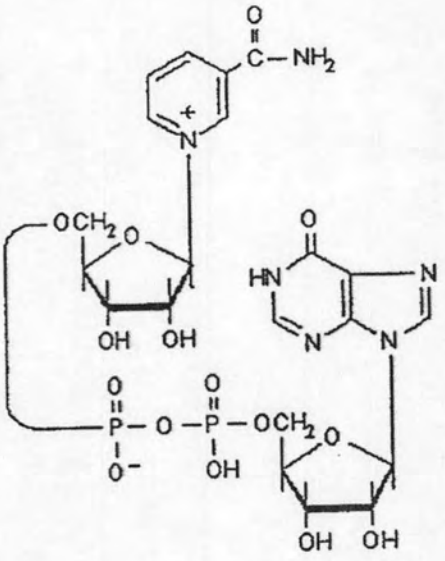
1. Coenzyme analog modified at C-2 position of the adenosylribose



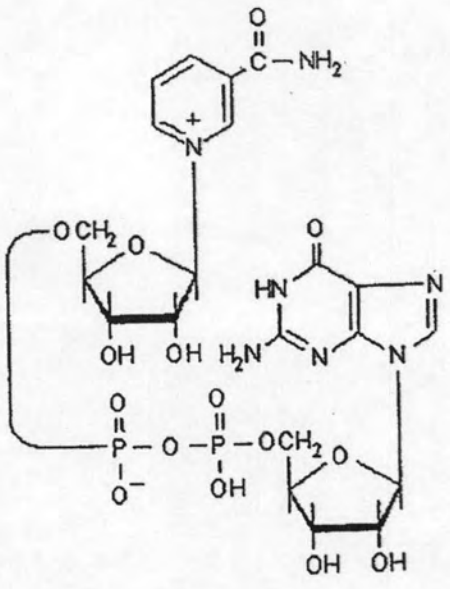
Nicotinamide adenine dinucleotide phosphate ($NADP^+$)

APPENDIX N (continued)

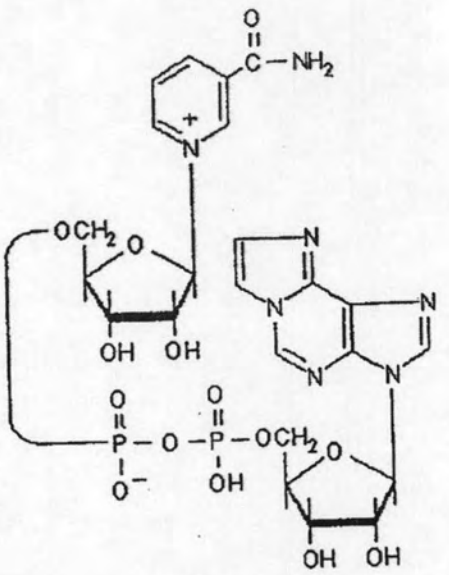
2. Coenzyme analog modified at the amino group in the adenine moiety



Nicotinamide hypoxanthine dinucleotide (Deamino-NAD$^+$)



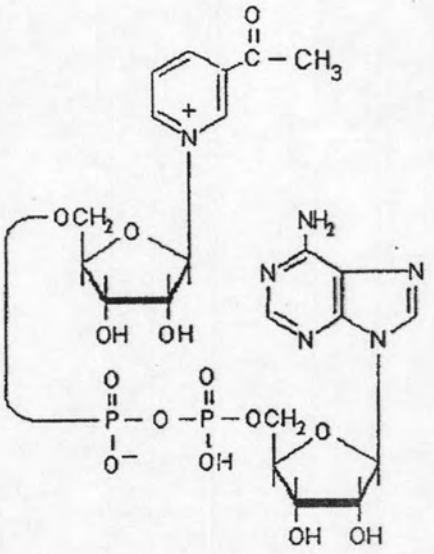
Nicotinamide guanine dinucleotide



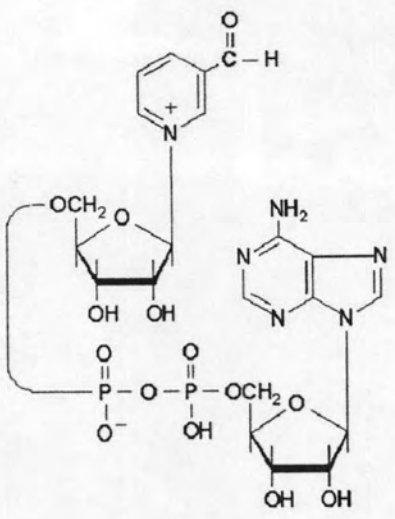
Nicotinamide 1, N^6-ethenoadenine dinucleotide

APPENDIX N (continued)

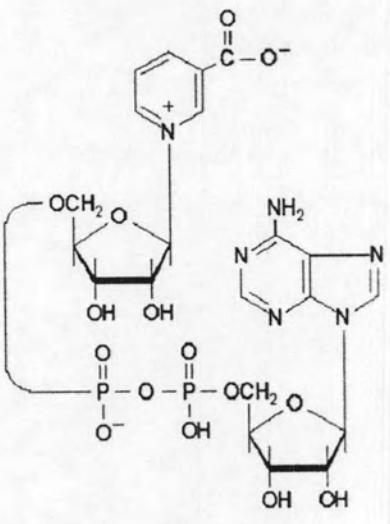
3. Coenzyme analog modified at the nicotinamide moiety



3-Acetylpyridine adenine dinucleotide



3-Pyridinealdehyde adenine dinucleotide



Nicotinic acid adenine dinucleotide (deamido-NAD$^+$)

BIOGRAPHY

Miss Mayura Thongchuang was born on June 19th, 1982 in Suphanburi. After graduating with degree of Bachelor of Science from the Department of Biochemistry at Chulalongkorn University in 2003, she keeps on studying for Master of Science at the Biotechnology Program, Faculty of Science at Chulalongkorn University in that year.