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APPENDICES

APPENDIX I

Preparation of chemicals

A. Preparation of chemicals for enzyme assay

1.) Bradford solution

95 % Ethanol (5% v/v)	50.00	ml
85 % Phosphoric acid (8.5% v/v)	100.00	ml
Coomassie Brilliant blue G-250 (0.01% w/v)	0.10	g
Total volume	1,000.00	ml by ddH_2O

Filter before use

2.) Para- nitrophenol phosphate (PNPP) was concentrated to be 1 mg/ml. Fifteen mg of PNPP per tablet was diluted in 15 ml substrate buffer.

substrate buffer: 0.1 M Diethanolamine containing 5 mM $MgCl_2$ at pH 10.5

- 5 mM $MgCl_2$

$MgCl_2.6H_2O$	0.10	g
Total volume ddH_2O	100.00	ml by ddH_2O

- 0.1 M Diethanolamine containing 5 mM $MgCl_2$

Diethanolamine	10.20	ml
5 mM $MgCl_2$	89.80	ml

B. Preparation of chemicals for polyacrylamide gel electrophoresis

1.) Solution

1.1) 30 % Acrylamide/bis

Acrylamide	29.2	g
Bis	0.8	g
Total volume	100.00	ml by ddH_2O

Wrap a bottle with aluminum foil and filtrate the solution before use. Keep the solution at 4°C.

1.2) 10X running buffer for SDS-PAGE

Glycine (0.2 M)	75.07	g
Tris (Hydroxymetyl)-amminomethane (25 mM)	15.20	g
SDS (0.1% w/v)	5.00	mg
Total volume	500.00	ml by ddH_2O

1.3) 5X loading dye for SDS-PAGE

1 M Tris-HCl pH at 6.8 (0.312 M)	3.12	ml
glycerol (50% v/v)	5.00	ml
SDS (10% w/v)	1.00	mg
Bromophenol blue (0.25% w/v)	0.025	g
2-mercaptoethanol (0.0004% v/v)	0.0004	ml
Total volume	10.00	ml by ddH_2O

1.4) 10X running buffer for Native-PAGE

Glycine (0.2 M)	75.07	g
Tris (Hydroxymetyl)-amminomethane (25 mM)	15.20	g
Triton X-100 (0.1% v/v)	5.00	mg
Total volume	500.00	ml by ddH_2O

1.5) 5X loading dye for Native-PAGE

1 M Tris-HCl pH at 6.8 (0.312 M)	3.12	ml
glycerol (50% v/v)	5.00	ml
Triton X-100 (10% v/v)	1.00	mg
Bromophenol blue (0.25% w/v)	0.025	g
Total volume	10.00	ml by ddH_2O

2.) Polyacrylamide gel

2.1) 12 % separating polyacrylamide/bis gel

1 M Tris-HCl pH 8.8 (0.375 M)	5.60	ml
10% (w/v) SDS or 10% (v/v) Triton X-100 (0.1%)	150.00	μl
30% Acrylamide/bis (12%)	6.00	ml

10% Ammonium persulphate (0.05%)	75.00	µl
TEMED (0.05%)	7.50	µl
Total volume	15.00	ml by ddH_2O

2.2) 5 % Stacking polyacrylamide/bis gel

1 M Tris-HCl pH at 6.8 (0.125 M)	0.75	ml
10% (w/v) SDS or 10% (v/v) Triton X-100 (0.1%)	60.00	µl
30% Acrylamide/bis (4%)	0.80	ml
10% Ammonium persulphate (0.05%)	30.00	µl
TEMED (0.1%)	6.00	µl
Total volume	6.00	ml by ddH_2O

3.) Coomassie blue stain

3.1) Solution I

Methanol (50%)	50.00	ml
acetic acid (10%)	10.00	ml
Coomassie brilliant blue R-250 (1.25 mg/ml)	12.50	mg
Total volume	100.00	ml by ddH_2O

3.2) Solution II: 10 % methanol containing 10 % acetic acid

acetic acid (10%)	10.00	ml
methanol (10%)	10.00	ml
Total volume	100.00	ml by ddH_2O

C. Preparation of chemicals for histochemistry

1.) Alkaline phosphatase buffer (AP buffer) at pH 7.4

Hepes (0.1 M)	23.83	g
$MgCl_2.6H_2O$ (5 mM)	1.02	g
$CaCl_2.2H_2O$ (1 mM)	0.15	g
Total volume	1,000.00	ml by ddH_2O

2.) Phosphate buffer saline (PBS) at pH 7.4

Na_2HPO_4 (20 mM)	28.39	g

NaCl (150 mM)	4.38	g
Total volume	1,000.00	ml by ddH_2O

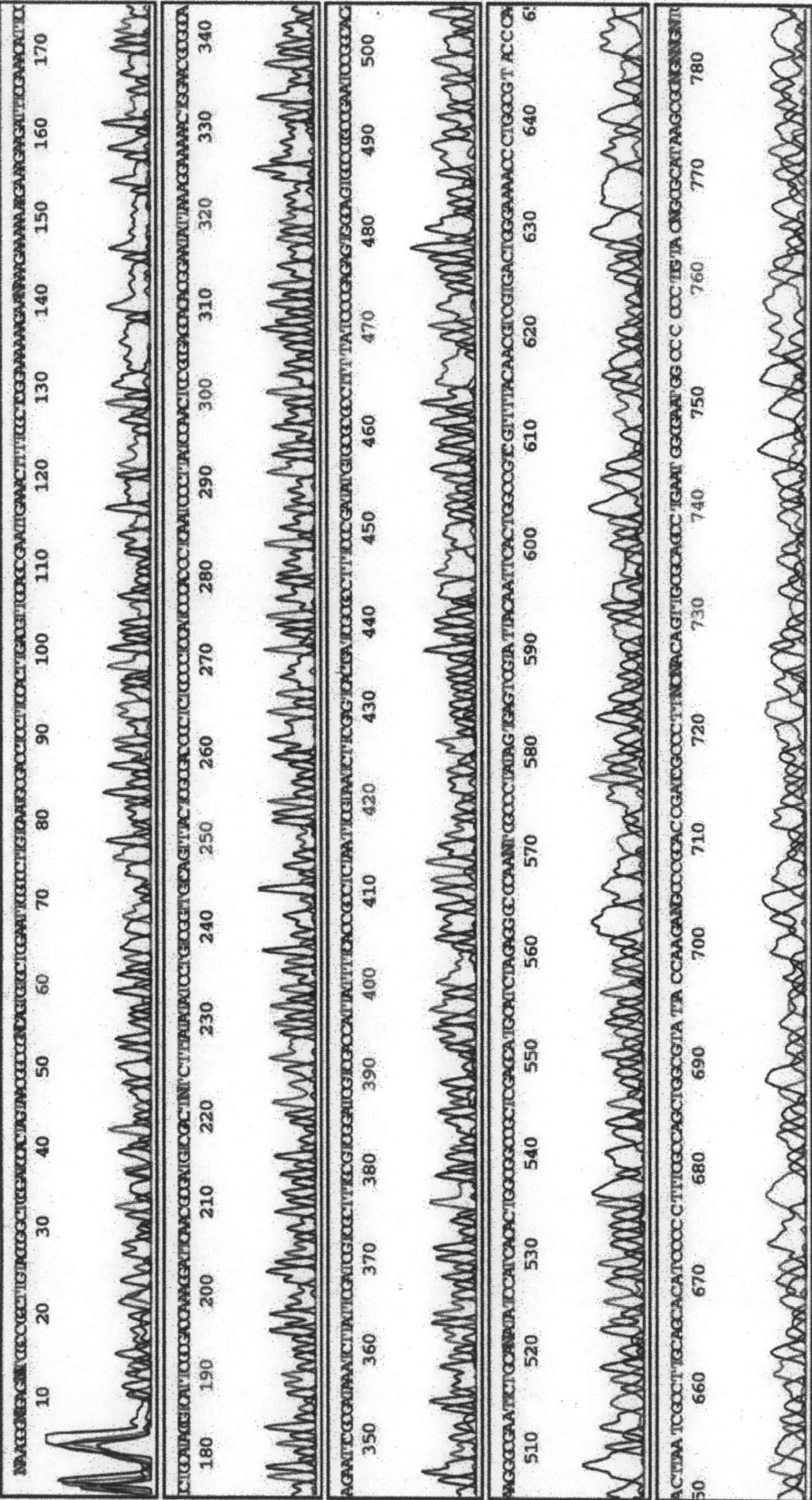
3.) 350 μg/ μl NBT and 175 μg/μl BCIP in Substrate buffer

[prepared as in (A), Appendix I]

NBT (350 μg)	0.0175	g
BCIP (175 μg)	0.0087	g
Total volume	50.00	ml by substrate buffer

APPENDIX II

Electropherogram from ABI Model 373 Genetic Analyzer


ABI PRISM
Model 373
Version 3.4
ABI50
Version 3.2
09-ap2.1
ap2.1
Lane 9
Signal G:53 A:48 T:28 C:35
373 BDT
BD20000906
Points 986 to 9560 Pk 1 Loc: 986
Page 1 of 2
Spacing: 8.77(8.77)

ABI PRISM

Model 373
Version 3.4
ABI50
Version 3.2

06-ap1.6

ap1.6
Lane 6

Signal G:39 A:36 T:25 C:27
373 BDT
BD20000906
Points 988 to 9560 Pk 1 Loc: 988

Page 1 of 2
2001IN 12âé 30ï (ï) 1:44 PM
2001IN 12âé 29ï (ïy) 3:56 PM
Spacing: 9.00(-9.00)

Model 373
Version 3.4
ABI50
Version 3.2

10-ap2.2

ap2.2
Lane 10

Signal G:104 A:98 T:53 C:67
373 BDT
BD20000906
Points 985 to 9560 Pk 1 Loc: 985

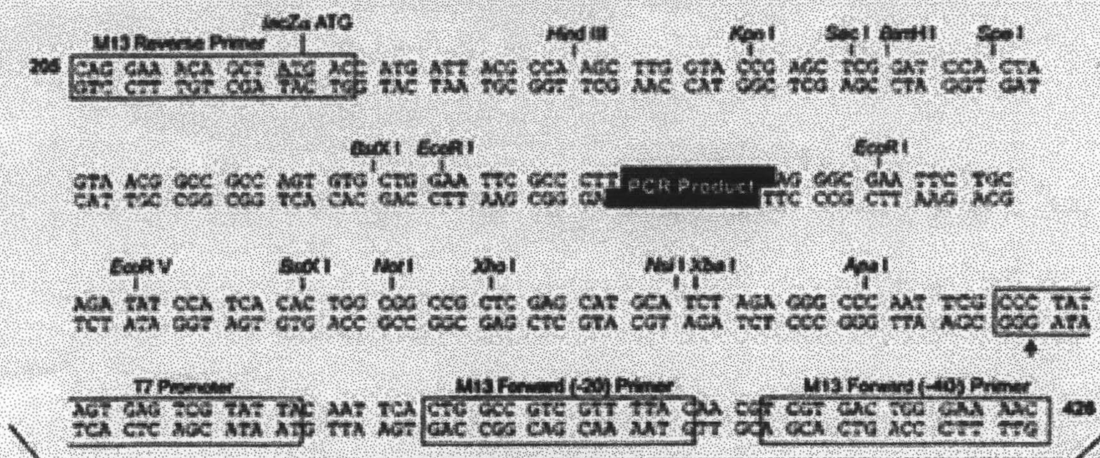
2001ÎN 12âé 30ì (ì) 1:44 PM
2001ÎN 12âé 29ì (ìy) 3:56 PM
Spacing: 12.67{12.67}

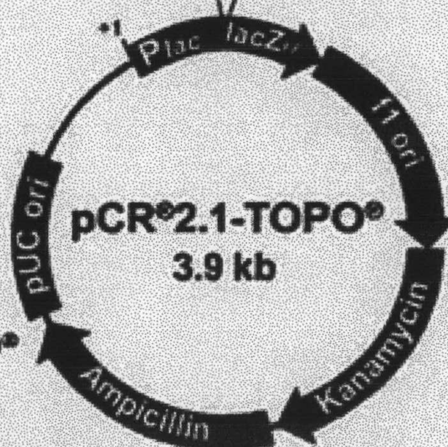
APPENDIX III

Map of pCR®2.1-TOPO®

pCR®2.1-TOPO® Map

The map below shows the features of pCR®2.1-TOPO® and the sequence surrounding the TOPO® Cloning site. Restriction sites are labeled to indicate the actual cleavage site. The arrow indicates the start of transcription for T7 polymerase. For the full sequence of the vector, you may download it from our web site or call Technical Service (page 19).





Comments for pCR®2.1-TOPO®
3908 nucleotides

LacZα fragment: bases 1-571
M13 reverse priming site: bases 205-221
Multiple cloning site: bases 234-357
T7 promoter/priming site: bases 364-383
M13 Forward (-20) priming site: bases 391-406
M13 Forward (-40) priming site: bases 411-426
f1 origin: bases 548-962
Kanamycin resistance ORF: bases 1296-2090
Ampicillin resistance ORF: bases 2108-2968
pUC origin: bases 3113-3786

Biography

Miss Tipwan Suppasat was born on the 12th of October (1977) in Chantaburi Province, Thailand. She finished her secondary school level at Sriyanusorn in 1995 in Chantaburi Province. She got a Bachelor's Degree in Biology from Faculty of Science, Chulalongkorn University in 1999. Then, she has been a graduate student in the Master's Degree in Zoology, Department of Biology, Faculty of Science, Chulalongkorn University since 2000. During her graduate study, she has been a scholar of University Affairs. After graduation, she will be a lecturer at Naraesuan University in Pitsanulok.