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APPENDICES

APPENDIX

1. Research Instruments

Automatic adjustable micropipette (Eppendorf, Germany)

Balance (Precisa, Switzerland)

Beaker (Pyrex)

DNA Thermal cycler 2400 (Perkin Elmer, Cetus USA)

Electrophoresis Chamber set (BIO-RAD, USA)

Flask (Pyrex)

Chemi Doc (BIO-RAD, USA)

Glass Pipette (Witeg, Germany)

Heat block (Bockel)

Parafilm (American National Can, USA)

Pipette boy (Tecnomara, Switzerland)

Pipette rack (Autopack, USA)

Pipette tip (Axygen, USA)

Plastic wrap

Polypropylene conical tube (Elkay, USA)

Power supply model

pH meter (Eutech Cybernataics)

Microcentrifuge (Eppendorf, USA)

Microcentrifuge tube (BIO-RAD, Elkay, USA)

Reagent bottle (Duran)

Spectrophotometer (BIO-RAD, USA)

Thermometer (Precision, Germany)

Vortex (scientific Industry, USA)

Water bath

2. General Reagents

Absolute ethanol (Merck)
Acetic acid (Merck)
Acrylamide:Bisacrylamide (Pharmacia Amersham)
Agar (Scharlau)
Agarose (USB)
Ampicillin (M&H manufacturing)
Calcium chloride (Merck)
Chloroform (Merck)
EDTA (Merck)
Ethidium bromide (Sigma)
Guanidine (USB)
Glucose (Merck)
Glycerol (Pharmacia Amersham)
Glycine (USB)
Hydrochloric acid (Merck)
Hydrogen peroxide (Sigma)
IPTG (USB)
Isoamyl alcohol (Merck)
Magnesium chloride (Fluka)
Magnesium sulphate (Sigma)
2-Mercaptoethanol (Pharmacia Amersham)
Methanol (Merck)
NZY (Gibco)
Phenol (Sigma)
Potassium acetate (BDH)
RNase A
Sodium acetate (Merck)

Sodium chloride (Scharlau)
SDS (Sigma)
Tris base (USB)
Tryptone (Scharlau)
Urea (USB)
Yeast extract (Scharlau)
100 bp DNA ladder (Biolabs)
1 kb DNA ladder (Gibco)

3. Bacterial Media

3.1 LB Medium (per liter)

10g	Bacto [®] -tryptone
5g	Bacto [®] -yeast extract
5g	NaCl

Adjust pH to 7.0 with NaOH.

3.2 LB Plates with Ampicillin

Add 15g agar to 1 liter of LB medium. Autoclave. Allow the medium to cool to 50 °C before adding ampicillin to a final concentration of 100 µg/ml. Pour 30-35 ml of medium into 85 mm petri dishes. Let the agar harden. Store at 4 °C for up to 1 month or at room temperature for up to 1 week.

3.3 LB Plates with Ampicillin/IPTG/X-Gal

Make the LB plates with ampicillin as above; then supplement with 0.5 mM IPTG and 80 µg/ml X-Gal and pour the plates. Alternatively, 100µl of 100 mM IPTG and 20 µl of 50 mg/ml X-Gal may be spreaded over the surface of an LB ampicillin plate and allowed to absorb for 30 minutes at 37 °C prior to use.

3.4 SOC Medium (100ml)

2.0 g	Bacto [®] -tryptone
0.5 g	Bacto [®] -yeast extract
1 ml	1M NaCl
0.25 ml	1M KCl
1 ml	2M Mg ²⁺ stock, filter sterilized
1 ml	2M glucose, filter sterilized

Add Bacto[®]-tryptone, Bacto[®]-yeast extract, NaCl and KCl to 97 ml distilled water. Stir to dissolve. Autoclave and cool to room temperature. Add 2 M Mg²⁺ stock and 2 M glucose, each to a final concentration of 20 mM. Bring to 100 ml with sterile, distilled water. The final pH should be 7.0.

4. Buffer**4.1 Alkaline Lysis Solution I**

50 mM	Glycine
25 mM	Tris-Chloride, pH 8.0
10 mM	EDTA, pH 8.0

4.2 Alkaline Lysis Solution II

0.2 N	NaOH
1 % (w/v)	SDS

4.3 Alkaline Lysis Solution III

60 ml	5 M Potassium Acetate
11.5 ml	Glacial Acetic Acid
28.5 ml	dH ₂ O

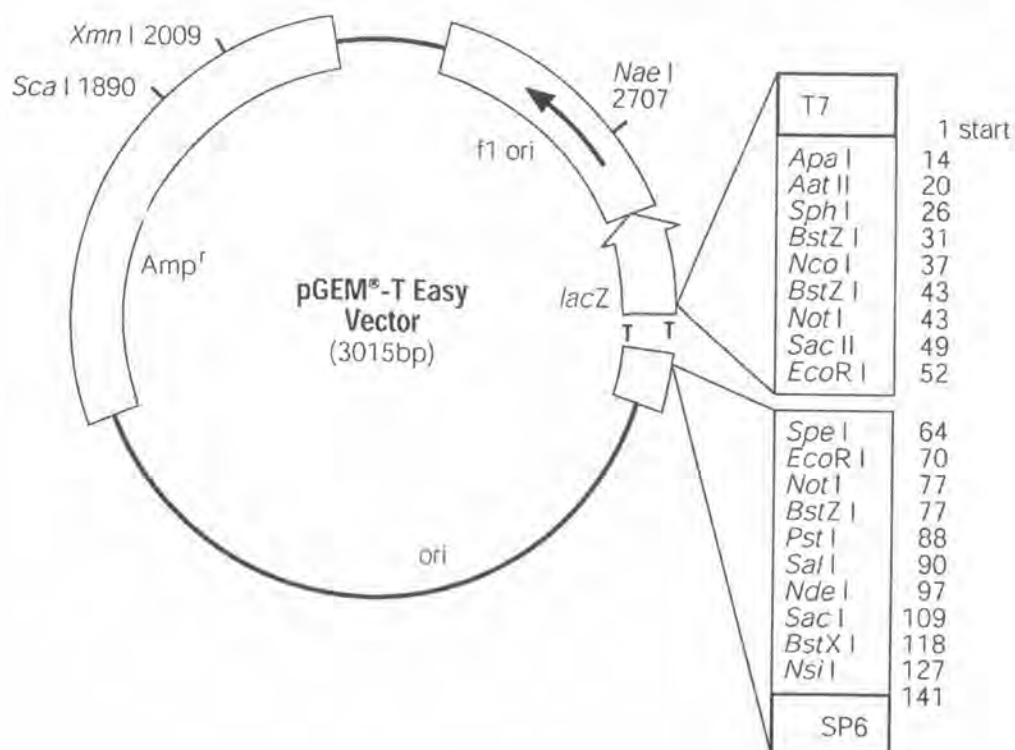
4.4 Buffer of enzymes

4.2.1 *Taq* DNA Polymerase 10X buffer (50 mM KCl, 10 mM Tris-HCl (pH9.0 at 25°C), 1.5 mM MgCl₂ and 0.1% TritonX-100 when diluted 1:10)

4.2.2 T4 DNA Ligase 10 X buffer (660 mM Tris-HCl (pH 7.6), 66 mM MgCl₂, 100 mM DTT, 660 μM ATP)

5. Vector

pGEM[®]-T Easy Vector Circle Map and Sequence Reference Points.



pGEM[®]-T Easy Vector sequence reference points:

T7 RNA polymerase transcription initiation site	1
multiple cloning region	10–128
SP6 RNA polymerase promoter (–17 to +3)	139–158
SP6 RNA polymerase transcription initiation site	141
pUC/M13 Reverse Sequencing Primer binding site	176–197

lacZ start codon	180
lacoperator	200–216
β -lactamase coding region	1337–2197
phage fl region	2380–2835
lacoperon sequences	2836–2996, 166–395
pUC/M13 Forward Sequencing Primer binding site	2949–2972
T7 RNA polymerase promoter (–17 to +3)	2999–3

BIOGRAPHY

Name	Miss Umaporn Methmaolee	Sex	Female
Birth date	Oct 22, 1981	Age	25
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Rachadapiseksompoch Research Fund, Chulalongkorn University	2005-2007
Snake bite and venom Research Unit supported by Rachadapiseksompoch Endowment Fund, Chulalongkorn University	2004-2007
The Affairs Thesis Grants for Graduate Students in public University, Graduate School, Chulalongkorn University	2004-2006