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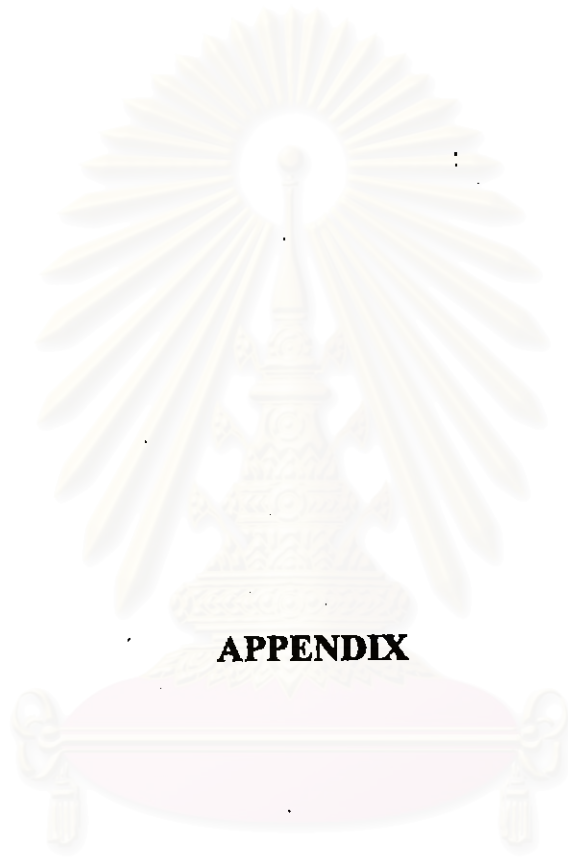
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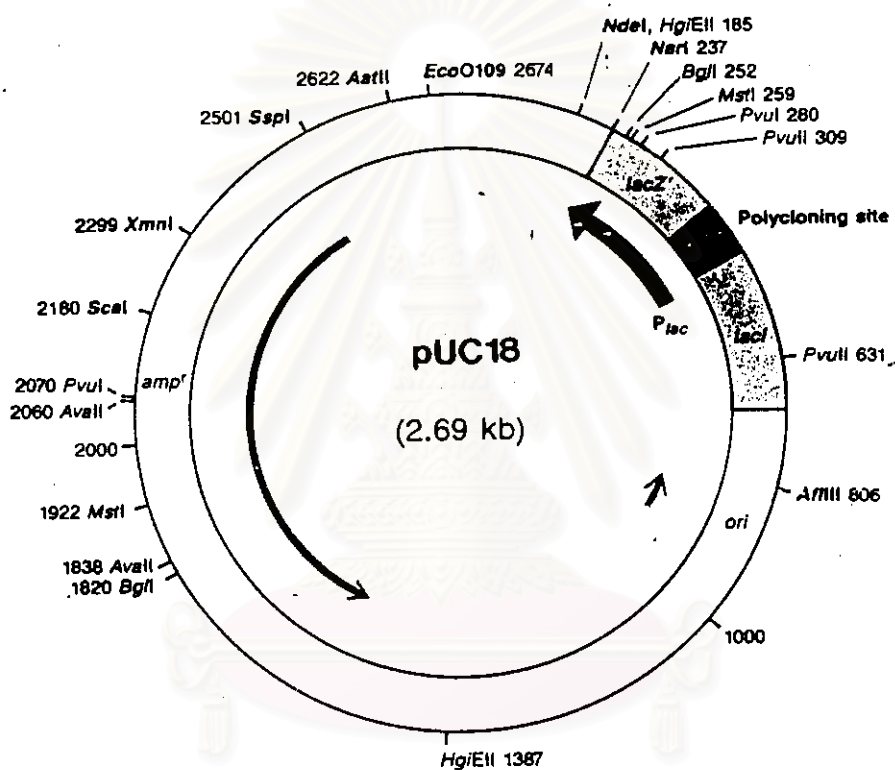
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APPENDIX A

Restriction mapping of plasmid pUC18



Polycloning Sites
pUC18

1	2	3	4	5	6	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	7	8	
Thr	Met	Ile	Thr	Asn	Ser	Ser	Ser	Val	Pro	Gly	Asp	Pro	Leu	Glu	Ser	Thr	Cys	Arg	His	Ala	Ser	Leu	Ala	Leu	Ala	
ATG	ACC	ATG	ATT	ACG	AAT	TCG	AGC	TCG	GTA	CCC	GGG	GAT	CCT	CTA	GAG	TCG	ACC	TGC	AGG	CAT	GCA	AGC	TTG	GCA	CTG	GCC
			EcoRI		SacI		KpnI		SmaI XmaI		BamHI		XbaI		SalI AccI HincII		PstI		SphI		HindIII					

APPENDIX B

Genotype markers of *E. coli* strain DH5 α

DH5 α genotype: F ϕ 80*lacZ* Δ M15 Δ (*lacZYA-argF*)U169 *deoR relA1 endA1*
hsdR17(*r_K⁻, m_K⁺*) *phoA supE44 λ ⁻ thi⁻¹ gyrA96 recA1*

Symbol	Description	Effect
<i>lacZ</i> Δ M15	Partial deletion of β -D-galactosidase gene	Allow complementation of β -galactosidase activity by α -complementation sequence in pUC vectors. Allow blue/white selection for recombinant colonies when plated on X-gal.
<i>deoR</i>		Involve the ability to grow on a minimal medium and enhance the uptake of larger plasmids.
<i>endA1</i>	Endonuclease mutation	Improve the yield and quality of plasmid DNA preparation.
<i>hsdR</i>	Restriction negative and Modification positive (<i>r_K⁻, m_K⁺</i>)	Methylate the DNA but do not restrict.
<i>supE</i>	Suppressor mutation	Suppress amber (UAG) mutation
<i>thi⁻¹</i>	Mutation in thiamine metabolism	Thiamine required for growth in minimal media
<i>gyrA96</i>	DNA gyrase mutation	This strain can be isolated by selecting for mutants resistant to nalidixic acid.
<i>recA1</i>	Recombination deficient	Product of <i>recA</i> gene serves as a master regulator of recombination. The <i>recA</i> mutation stabilizes the DNA insert carried in cloning vector.

APPENDIX C

Stock solutions for colony hybridization

50X Denhardt's solution

Ficoll type 400	10 g/l
polyvinylpyrrolidone	10 g/l
Bovine Serum Albumin (Fraction V)	10 g/l

: Dissolve Ficoll type 400, polyvinylpyrrolidone and Bovine Serum Albumin in dH₂O, filter and store at -20°C

20X SSC

3 M NaCl	175.3 g/l
0.3 M Na ₃ C ₆ H ₅ O ₇ ·2H ₂ O	88.2 g/l

: Dissolve NaCl and trisodium citrate·2H₂O in dH₂O and adjust pH to 7.0 with a few drop of HCl. Sterilize by autoclaving and store at room temperature.

20X SSPE, pH 7.4

3M NaCl	175.3 g/l
0.2 M NaH ₂ PO ₄ ·H ₂ O	27.6 g/l
0.02 M EDTA	7.4 g/l

: Dissolve NaCl, sodium phosphate and EDTA in dH₂O and adjust pH to 7.4 with NaOH (~ 6.5 ml of a 10 N NaOH). Dispense into aliquots, sterilize by autoclaving and stored at room temperature.



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APPENDIX E

Stock solutions for preparation of Sequencing gel

20% Acrylamide solution

Acrylamide	193 g/l
N,N' methylene bisacrylamide	6.7 g/l
urea	46.7 g/l

Dissolve acrylamide, N,N'methylene bisacrylamide and urea in dH₂O. Stir on magnetic stirrer for 30 min or until the mixture become homogenous. Filter and store at room temperature.

46.7% Urea solution

urea	467 g/l
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Dissolve urea in dH₂O. Stir on magnetic stirrer for 30 min or until the solution become homogenous. Filter and store at room temperature.

10X TBE

Tris base	121 g/l
EDTA	7.4 g/l
Boric acid	53.4 g/l

Dissolve Tris base, EDTA and Boric acid in dH₂O and adjust pH to 8.3. Store at room temperature.

10% Ammonium persulfate

Ammonium persulfate	1g/10 ml
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Dissolve ammonium persulfate in dH₂O. The solution may be stored for several weeks at 4°C.

8% Acrylamide gel solution

20% acrylamide solution	35 ml
46.7% urea solution	44 ml
10X TBE	9 ml
TEMED	85 μ l
10% ammonium persulfate	425 μ l

Mix all above except 10% ammonium persulfate until ready to pour gel.



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BIOGRAPHY

Miss Amonrat Tiptawonnukul was born on August 6, 1967 in Chantaburi, Thailand. She graduated with the Bachelor degree of Science (Maternity and Nursing), First degree of honor, from Mahidol University in 1989 and have further her study with the Master degree of Science in Biochemistry at Chulalongkorn University since 1993.



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