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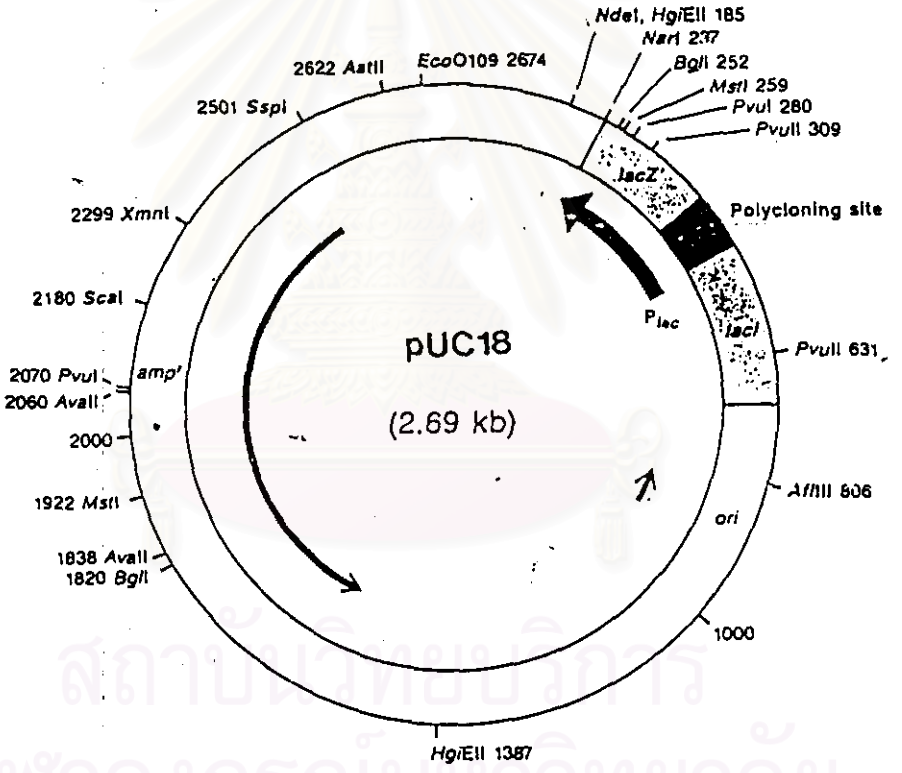
APPENDIX A

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	Se	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		BamHI		XbaI		SalI		PstI			SphI			HindIII		
										XmaI						AccI										
																HincII										

APPENDIX B

Genotype markers of E. coli strain DH5a and XL-1 Blue

DH5-α genotype: F$^-$ φ80d*lac*ZΔM15 Δ(*lac*ZYA-*arg*F)U169 *deo*R *rel*A1 *end*A1 hsdR17(r_K^-, m_K^+) *pho*A *sup*E44 λ$^-$ thi-^{1} *gyr*A96 *rec*A1

XL-1 Blue genotype: *recA1 end A1 gyrA 96 thi-1 hsd R17 supE-44 relA1 lac* [F'-*proAB* lac^q ZΔ *M15* Tn*10*(Tet^r)]

Symbol	Description	Effect
lacZ M15	Partial deletion of -D-galactosidase gene	Allow complementation of -galactosidase activity by -complementation sequence in pUC vectors. Allow blue/white selection for recombinant colonies when plated on X-gal.
DeoR		Involve the ability to grow a minimal medium and enhance the uptake of larger plasmids.
EndA1	Endonuclease mutation	Improve the yield and quality of plasmid DNA preparation.
HsdR	Restriction negative and Modification positive (rK-, mK+)	Methylate the DNA but do not restrict.
SupE	Suppressor mutation	Suppress amber (UAG) mutation

thi-1	Mutation in thiamine metabolism	Thiamine required for growth in minimal media
gyrA96	DNA gyrase mutation	This strain can be isolated by selecting for mutants resistant to nalidixic acid.
RecA1	Recombination deficient	Product of recA gene serves as a master regulator of recombination. The recA mutation stabilizes the DNA insert carried in cloning vector.
lac^q	over-expression of LacI repressor protein	
F'	M13 infection	Plaques appear small and
	Single-stranded DNA rescue	turbid
	Blue/While color selection	Yields will be reduced due to
	RC; regulation of RNA	slow growth
lac ZΔ*M15*	synthesis; stringent factor;	
rel A	ATP; GTP 3'-	
	pyrophosphotransferase	
	r-glutamylphosphate	
pro A	reductace	
pro B	r-glutamate kinase	
Tn 10	Transposition	

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, polyvinylpyrrolidon and Bovine Serum Albumin in dH_2O, filter sterilization and store at -20°C

20X SSC

3 M NaCl	175.3 g/l
0.3 M $Na_3C_6H_5O_7.2H_2O$:	88.2 g/l

: Dissolve NaCl and trisodium citrate.$2H_2O$ in dH_2O 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_2PO_4. H_2O	27.6 g/l
0.3 M EDTA	7.4 g/l

: Dissolve NaCl, sodium phosphate and EDTA in dH_2O 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.

APPENDIX D

Plasmid preparation medium

Terrific broth

Compound	Amount/liter	Final concentration
Solution a		
Bacto-tryptone	12 g	1.2 %
Bacto yeast extract	24 g	2.4 %
Redistilled glycerol	4 g	0.4 %
Dissolve in 900 ml water and autoclave		
Solution b		
KH_2PO_4	2.3 g	17 mM
K_2HPO_4	12.5 g	72 mM

Dissolve the two potassium phosphates in 100 ml water and autoclave. When solution (a) and (b) are cool, combine them (1:1) to give ferrific broth.

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 dH_2O. Stir on magnetic stirrer for 30 min or until the mixture become homogenous. Filter and store at room remperature.

46.7% Urea solution

urea	467 g/l

Dissolve urea in dH_2O. 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 and in dH_2O and adjust pH to 8.3. Store at room temperature.

10% Ammonium persulfate

Ammonium persulfate	1 g/10 ml

Dissolve ammonium persulfate in dH_2O. 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 µl
10% ammonium persulfate	425 µl

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

Biography

Miss Chaunchom Maunpasitporn was born on May 18, 1967 in Chainat. She graduated with the degree of Bachelor of Science from the Department of Medical Technology at Cheingmai University in 1989. In 1995, she has studied in Master degree of Science at the Department of Biotechnology, Chulalongkorn University.