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APPENDICES

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APPENDIX A**Buffers for purification**

1) 10 mM Tris-HCl buffer with 10 mM CaCl₂ pH 8.5 (TB1)

Tris (hydroxymethyl)-aminomethane 1.21 g

CaCl₂ 1.11 g

Adjust pH to 8.5 with HCl and final volume to 1 litre

2) 15 mM Tris-HCl buffer pH 8.5 (TB2)

Tris (hydroxymethyl)-aminomethane 1.82 g

Adjust pH to 8.5 with HCl and final volume to 1 litre

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APPENDIX B

Preparation for polyacrylamide gel electrophoresis

1) Stock reagents30% Acrylamide 0.8% bis stock solution

Acrylamide	30.00	g
N, N'-methylene-bis-acrylamide	0.80	g

Adjust volume to 100 ml with distilled water

1.5 M Tris-HCl pH 8.8

Tris (hydroxymethyl)-aminomethane	18.17	g
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Adjust pH to 8.8 with 1 N HCl and adjust volume to 100 ml with distilled water

0.5 M Tris-HCl pH 6.8

Tris (hydroxymethyl)-aminomethane	6.06	g
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Adjust pH to 6.8 with 1 N HCl and final volume to 100 ml with distilled water

1.25 M Tris-HCl pH 6.8

Tris (hydroxymethyl)-aminomethane	15.14	g
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Adjust pH to 6.8 with 1 N HCl and final volume to 100 ml with distilled water

Tris-Glycine electrode buffer stock-solution x5 (25 mM Tris, 192 mM Glycine, pH 8.1)

Tris (hydroxymethyl)-aminomethane	3.03	g
Glycine	14.40	g

Dissolve in distilled water to 200 ml to give final pH of 8.1

2) Non-denaturing PAGE

7.5% Separating gel

30% acrylamide solution	5.0	ml
1.5 M Tris-HCl pH 8.8	5.0	ml
distilled water	10.0	ml
TEMED	7.5	μ l
10% $(\text{NH}_4)_2\text{S}_2\text{O}_8$	50.0	μ l
total volume	<u>20.0</u>	ml

3.0% Stacking gel

30% acrylamide solution	2.5	ml
0.5 M Tris-HCl pH 6.8	2.5	ml
distilled water	7.5	ml
TEMED	5.0	μ l
10% $(\text{NH}_4)_2\text{S}_2\text{O}_8$	50.0	μ l
total volume	<u>10.0</u>	ml

Sample buffer

1.25 M Tris-HCl pH 6.8 : glycerol: distilled water = 1: 2: 2 (v/v) were added with trace amount of bromophenol blue. For loading sample, three parts of sample was mixed with one part of sample buffer.

3) SDS-PAGE

7.5% Separating gel

30% acrylamide solution	5.0	ml
1.5 M Tris-HCl pH 8.8	7.5	ml
10% SDS	0.2	ml
distilled water	9.4	ml
TEMED	10.0	μ l
10% $(\text{NH}_4)_2\text{S}_2\text{O}_8$	50.0	μ l
total volume	<u>20.0</u>	ml

3.0% Stacking gel

30% acrylamide solution	1.0	ml
0.5 M Tris-HCl pH 6.8	2.5	ml
10% SDS	0.1	ml
0.2 M EDTA	0.1	ml
distilled water	6.3	ml
TEMED	5.0	μ l
10% $(\text{NH}_4)_2\text{S}_2\text{O}_8$	30.0	μ l
total volume	<u>10.0</u>	ml

Solubilizing medium

glycerol	2.0	ml
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20% SDS	1.0	ml
1.25 M Tris-HCl pH 6.8	1.0	ml
β -mercaptoethanol	1.0	ml
bromophenol blue		

One part of solubilizing medium was added to three parts of sample. The mixture was heated 1 minute in boiling water before loading to the gel.



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APPENDIX C

Stock solutions for colony hybridization

50x Denhardt's solution

Ficoll type 400	10.0	g/l
polyvinylpyrrolidone	10.0	g/l
BSA	10.0	g/l

Disolve Ficoll type 400, polyvinylpyrrolidone and BSA in double distilled water, filter and store at $-20\text{ }^{\circ}\text{C}$

20xSSC

3 M NaCl	175.3	g/l
0.3M $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$	88.2	g/l

Dissolve NaCl and $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ in double distilled water and adjust pH to 7.0 with a few drop of HCl. Sterilized by autoclaving and stored at room temperature.

Prehybridization solution

50x Denhardt's solution	20	ml
20xSSC	30	ml
Distilled water to 100 ml		

Hybridization solution

10 ml of prehybridization solution plus 4 ml of labelled-oligonucleotide probe.

APPENDIX D

Genotype markers of *E. Coli* strain DH5 α

DH5 α genotype: F ϕ 80d *lacZ* Δ M15 Δ (*lacZYA-argF*) U169 *deoR* *relA1*
endA1 *hsdR17*(*r_K⁻*, *m_K⁺*) *phoA* *supE44* λ :*thi*⁻¹ *gryA96* *recA1*

Symbol	Description	Effect
<i>lacZ</i> Δ M15	Partial deletion of β -D-galactosidase gene	Allow complementation of β -D-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⁻</i> , <i>m_K⁺</i>)	Methylate the DNA but do not restrict.
<i>supE</i>	Suppressor mutation	Suppress amber (UAG) mutation
<i>thi</i> ⁻¹	Mutation in thiamine metabolism	Thiamine required for growth in minimal media
<i>gryA96</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 in cloning vector.

APPENDIX E

Stock solutions for preparation of sequencing gel

20% Acrylamide solution

Acrylamide	193.0	g/l
N, N'-methylene-bis-acrylamide	6.7	g/l
Urea	46.7	g/l

Dissolve Acrylamide, N, N'-methylene-bis-acrylamide, and urea in double distilled water. Stir on magnetic stirrer for 30 minutes or until the mixture become homogenous. Filter and store at room temperature.

46.7% Urea solution

Urea	476.0	g/l
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Dissolve urea in double distilled water. Stir on manetic stirrer for 30 minutes or until the solution become homogenous. Filter and store at room temperature.

10xTBE

Tris base	121.0	g/l
EDTA	7.4	g/l
Boric acid	53.4	g/l

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

10% Ammonium persulfate

Dissolve 1g of ammonium persulfate in 10 ml of double distilled water. The solution may be stored for several weeks at 4 °C.

8% Acrylamide gel solution

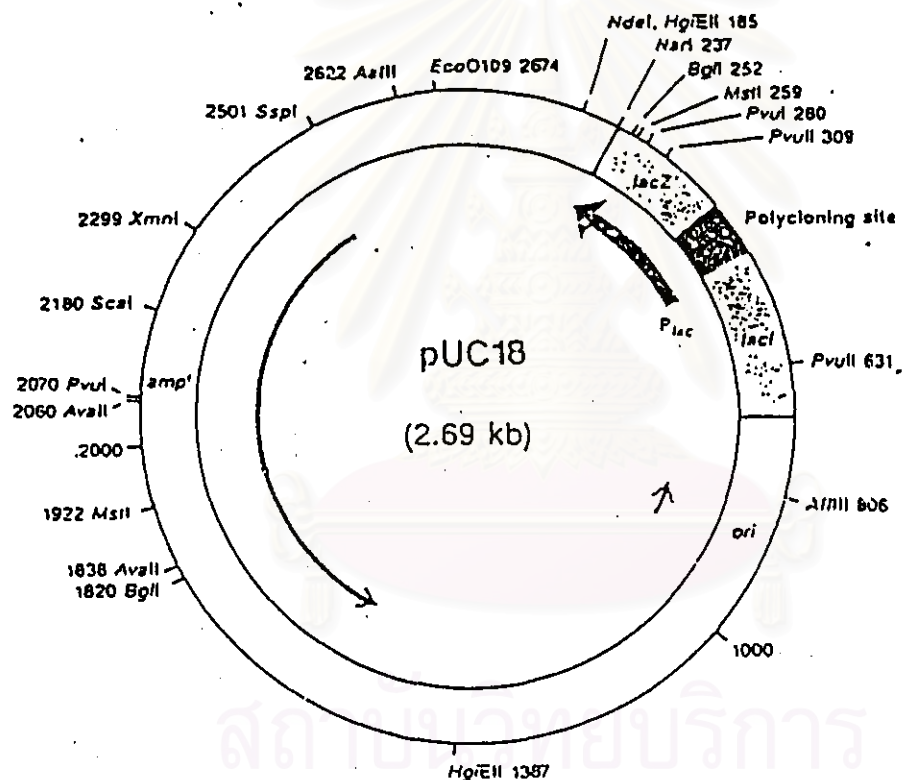
20% acrylamide solution	35	ml
46.7% urea solution	44	ml
10xTBE	9	ml
TEMED	85	μl
10% ammonium persulfate	425	μl

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

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APPENDIX F

Restriction map of pUC18

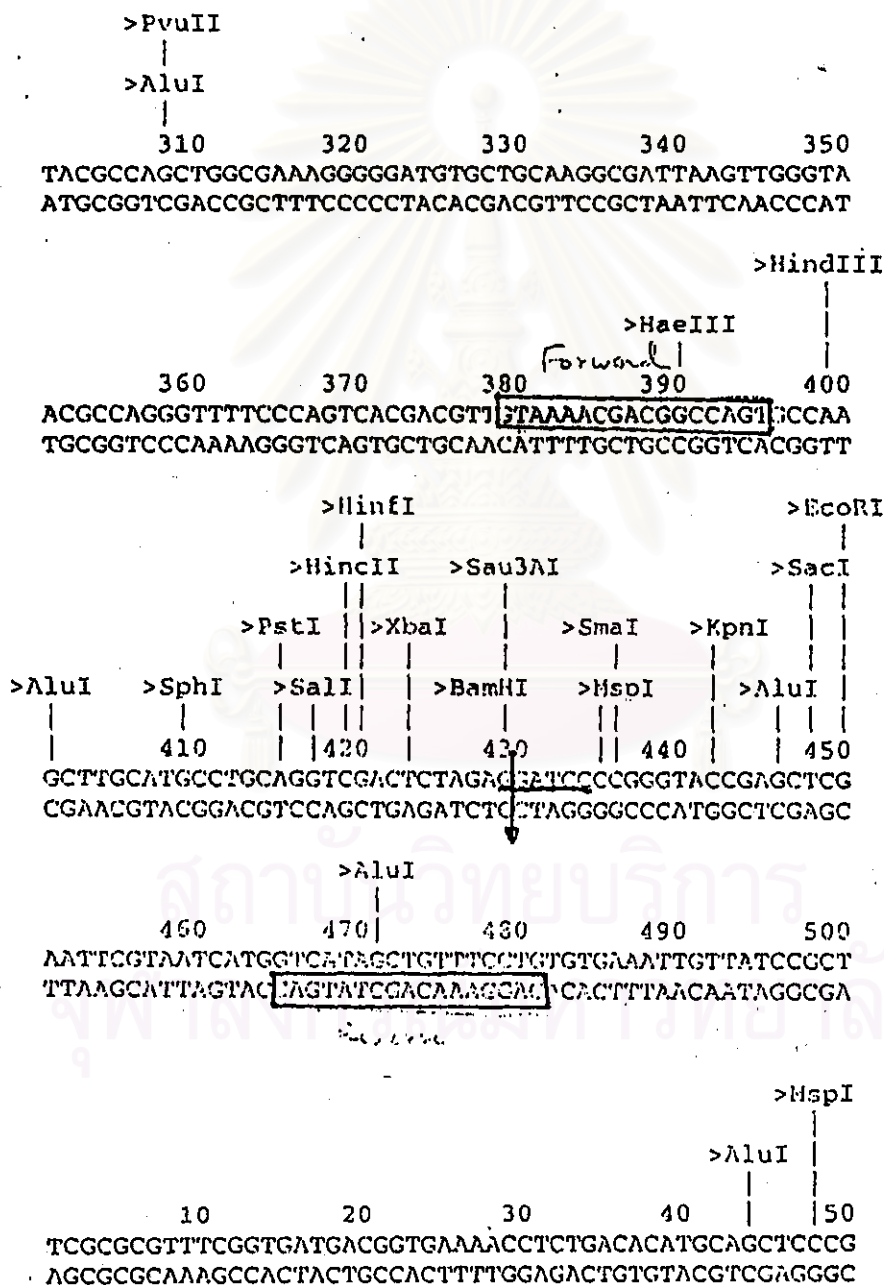


Cloning Sites

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	Phe	Thr	Asn	Ser	Ser	Ser	Val	Pro	Gly	Asp	Pro	Leu	Glu	Ser	Thr	Cys	Arg	His	Ala	Ser	Leu	Ala	Leu	Ala
ACC	ATG	ATT	ACG	AAT	TCG	AGC	TCG	GTA	ECC	GGG	GAT	CCT	CTA	GAG	TCG	ACC	TGC	AGG	CAT	GCA	AGC	TTG	GCA	CTG	GCC
EcoRI			SacI		KpnI		SmaI XbaI		BamHI		XbaI		SalI AccI HincII		PstI		SphI		HindIII						

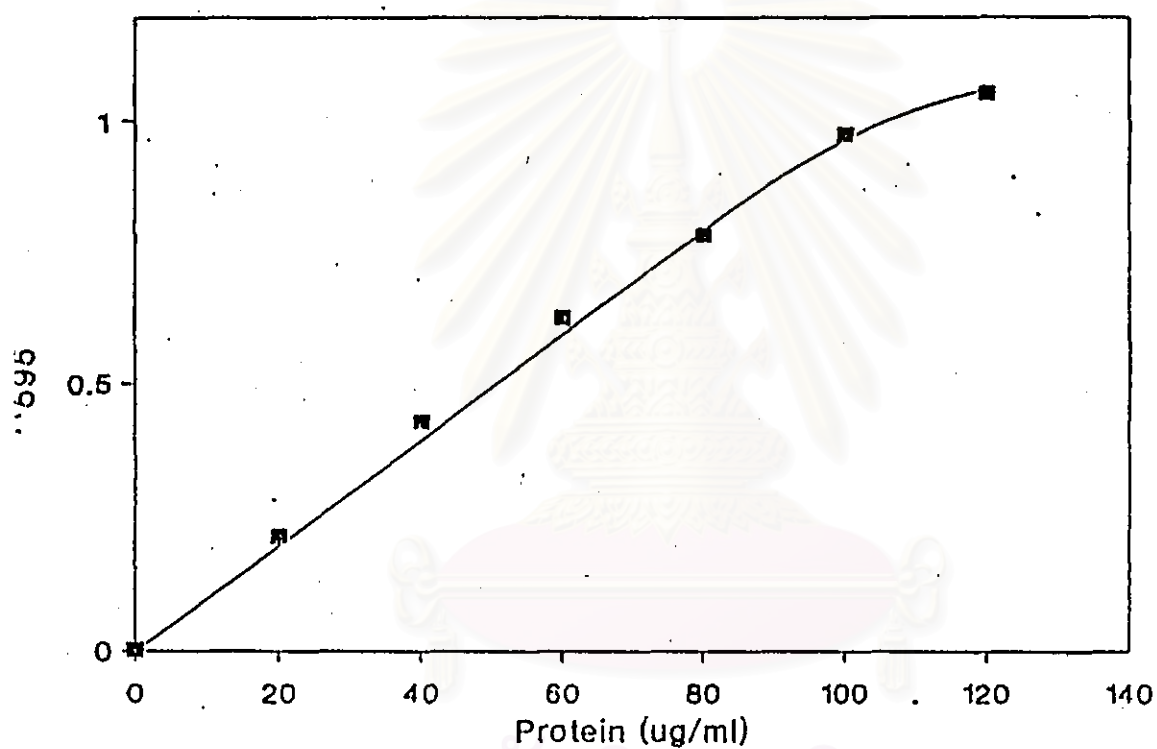
APPENDIX G

Annealing sites of forward and reverse universal primers in pUC18



APPENDIX H

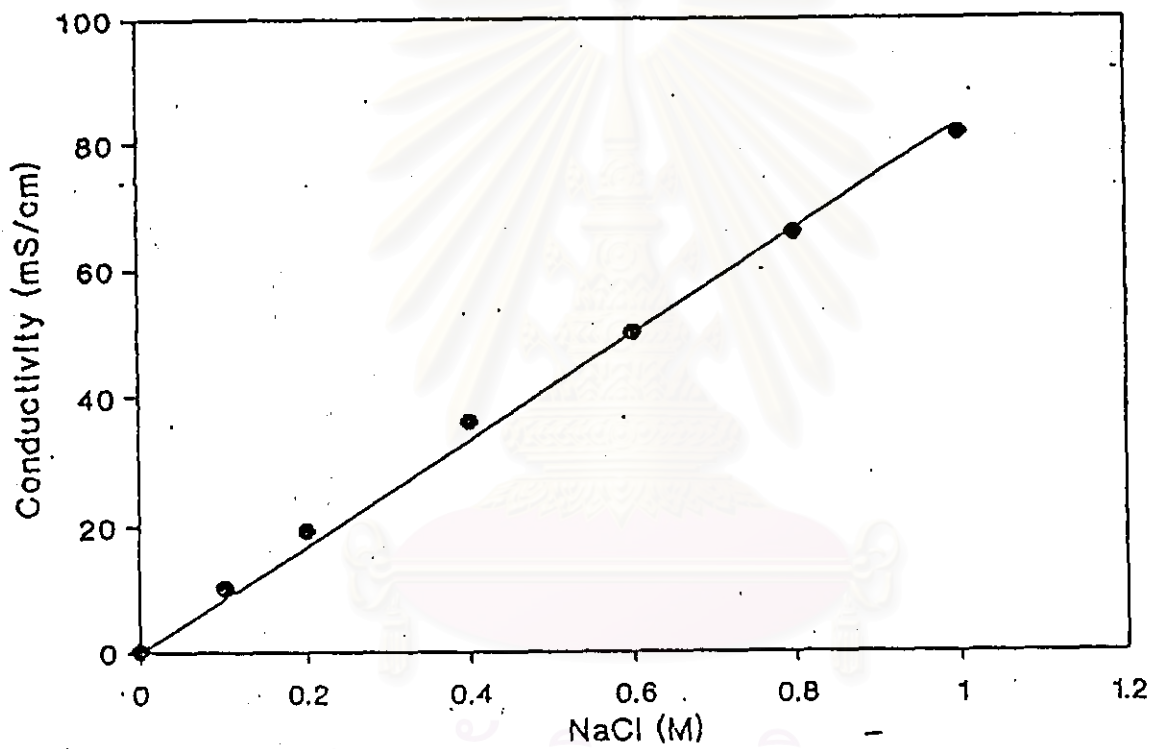
Standard curve for protein determination by Bradford's method.



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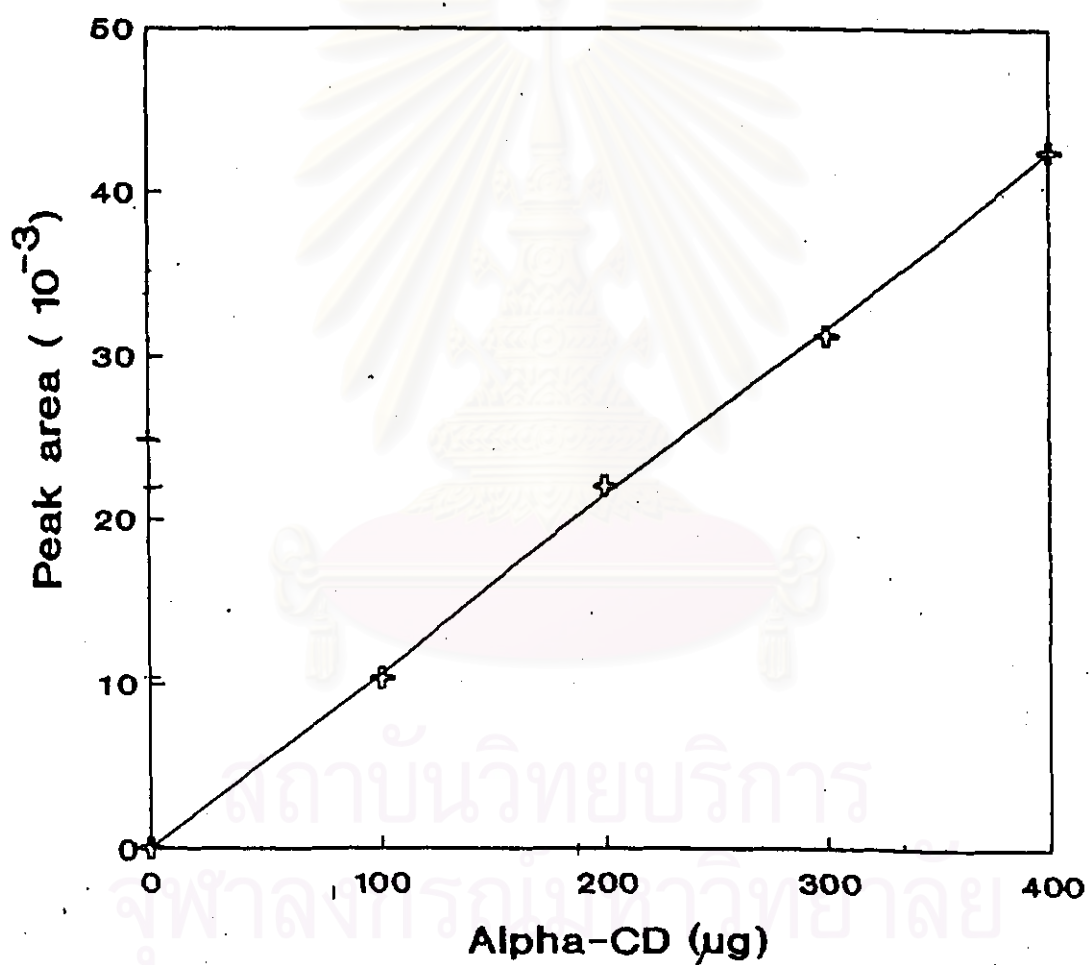
APPENDIX I

Standard curve of conductivity against NaCl concentration

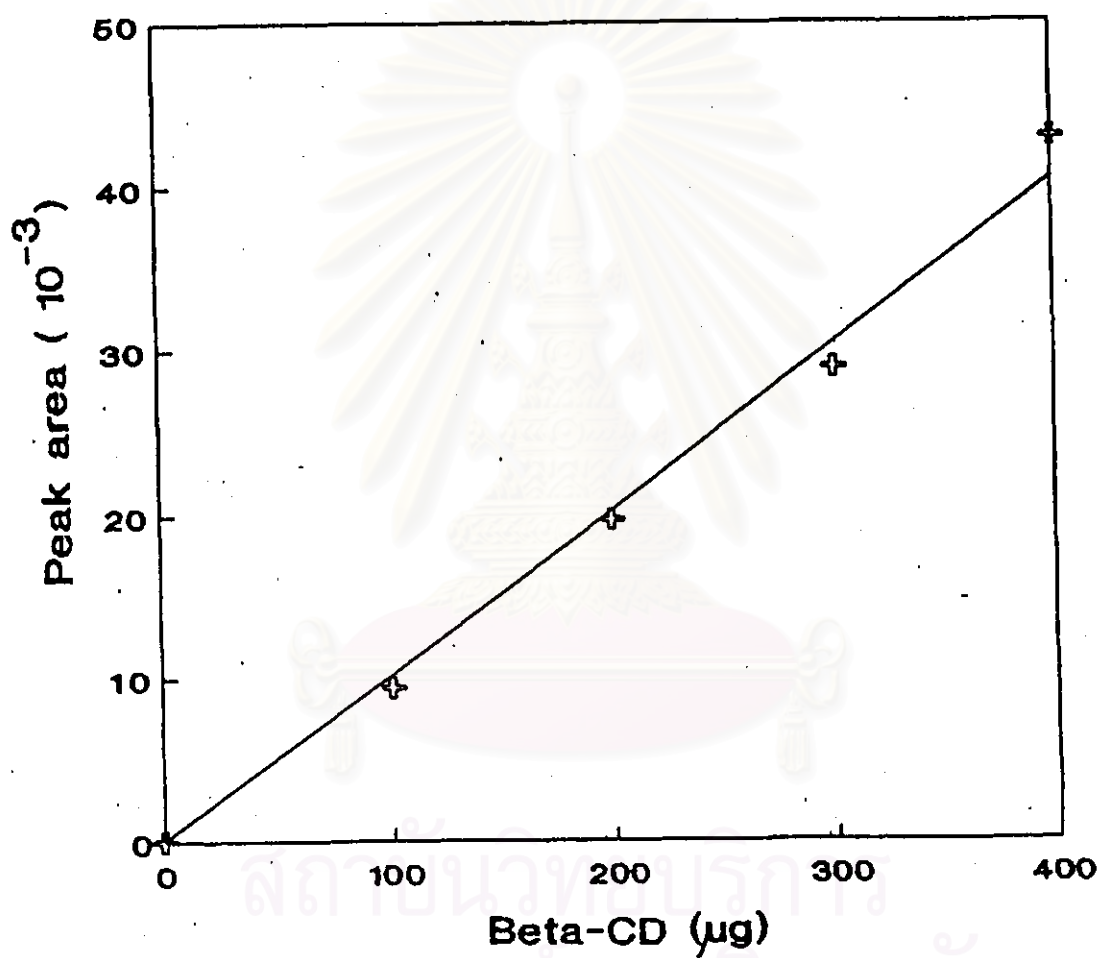


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APPENDIX J

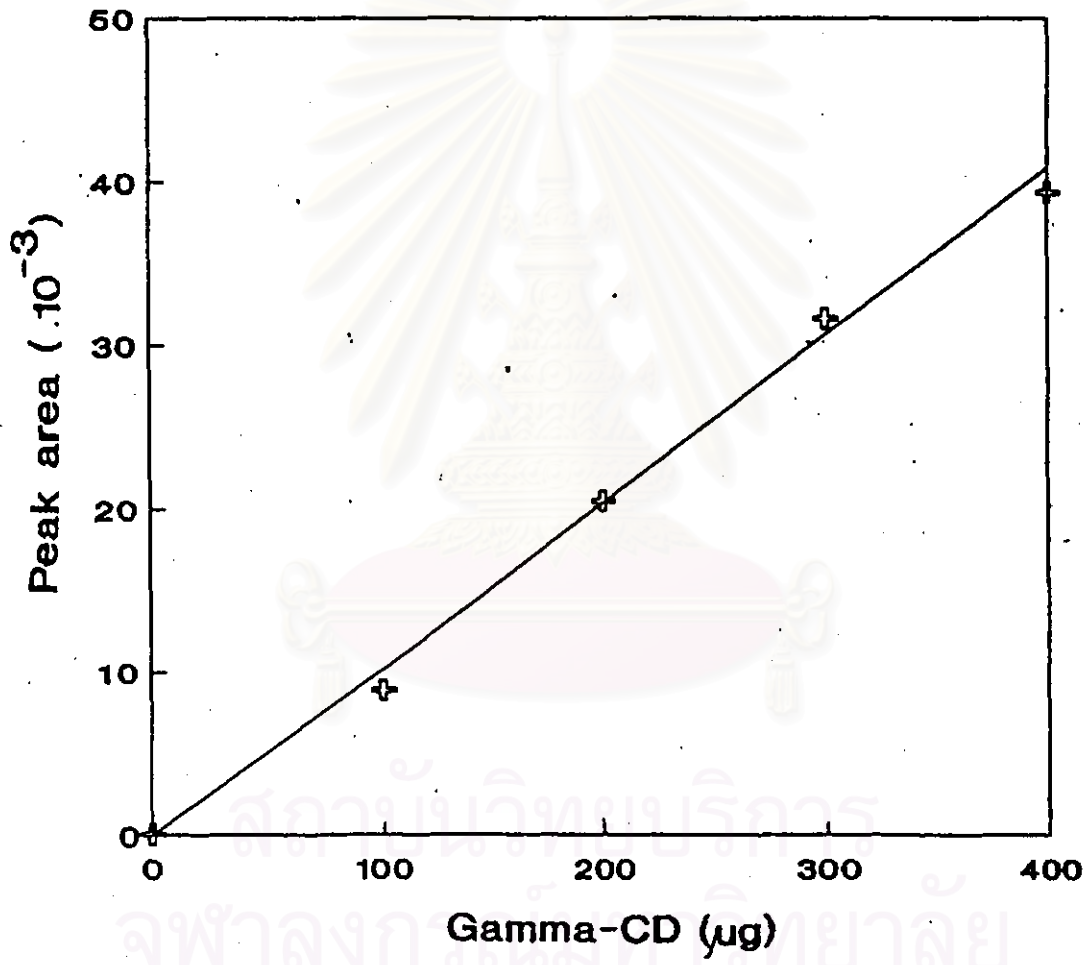
Standard curve of α -CD (by HPLC)

APPENDIX K

Standard curve of β -CD (by HPLC)

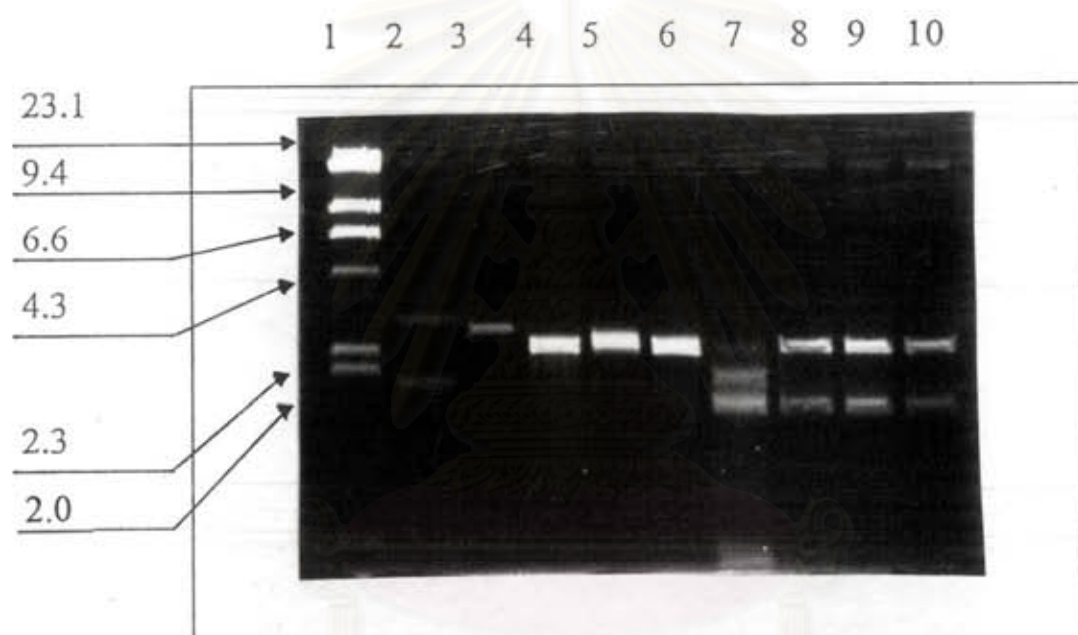
APPENDIX L

Standard curve of γ -CD (by HPLC)



APPENDIX M

Restriction analysis of recombinant plasmid 909 by various restriction endonuclease



Lane 1. size standard marker λ /HindIII

2. pUC18 uncut

3. pUC18/BamHI

4. 909/ NdeI

5. 909/ScaI

6. 909/PvuII

7. 909/PvuII+ScaI

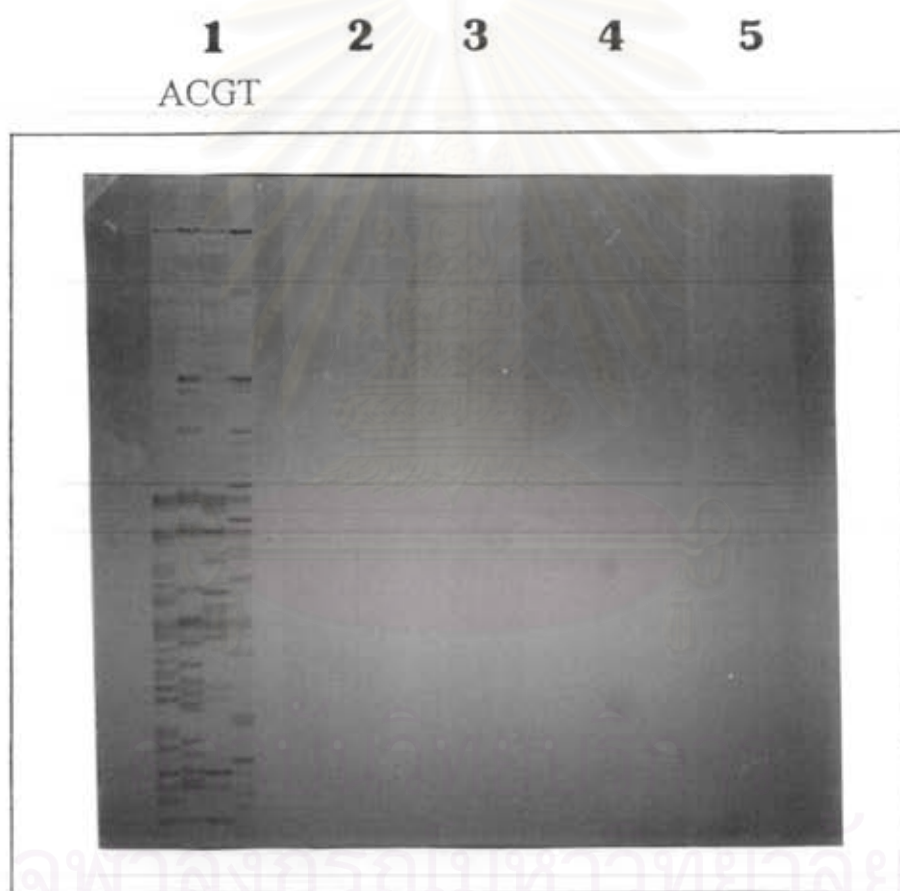
8. 909 uncut

9. 909/EcoRI

10. 909/HindIII

APPENDIX N

DNA sequencing of pUC18 and plasmid 909, using synthetic oligonucleotide probes as primers as well as universal forward and reverse primer



Lane 1. pUC18 with universal forward primer

2-5. 909 with universal forward primer , reverse primer, PNB, and PCC, respectively.

BIOGRAPHY

Mr. Surasak Laloknam was born on May 23 (1972) in Bangkok, Thailand. He graduated with a Bachelor of Science Degree in Biology Faculty of Science, Srinakarinwirot at Bangkok in 1995. He was enrolled in the M.Sc.Biochemistry Program since 1995.



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