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APPENDIX

APPENDIX I

I.I Equipment

Adjustable safelight lamp 1521541 Kodak 6 B, Eastman Kodak Company: U.S.A.

Autoclave HA-30, Hirayama Manufacturing Coporation: Japan.

Automicropipette Pipetman P20, P100 and P200, Gilson Medical Electronics S.A.: France.

Fluorescence lamp 2859 SHANDON, Shandon Scientific Co. Ltd.: London, England.

High speed microcentrifuge MC-15A, Tomy Seiko: Japan.

Horizontal electrophoresis unit and Power supply EPS 3200 or EPS 5100 for agarose gel electrophoresis.

Incubator BM 600, Memmert Gmb H, W: Germany.

Magnetic stirrer 0188 GMS, Scientific Instrument Development And Service Center: Faculty of Science, Chulalongkorn University.

pH meter PHM 83 Autocal, Radiometer Copenhagen: Denmark.

Refrigerated centrifuge J-21 C, Beckman Instrument Inc: U.S.A.

Shaking Waterbath 01PF623, New Brunswick Scientific Co., Inc.: U.S.A.

Standard cassette, Okamoto, Japan.

Ultracentrifuge L8-70, Beckman, California, U.S.A.

UV transilluminator 2011 MACROVUE, San Gabriel: California, U.S.A.

Vortex K 550-G, Scientific Industries Inc.: U.S.A.

Waterbath A466, Charlies Hearson & Co. Ltd.: England.

I.II Material/Supplies

Chromatography paper (Whatman 3 MM Chr.), Whatman International Ltd: England.

Film Kodak Tri-X pan400, Eastman Kodak Company: U.S.A.

Millipore filter HA 0.45 μm, Millipore Coporation: U.S.A.

Nylon membranes, positively charged, Boehringer Mannheim GmbH : Germany.

X-ray film RX-100, Fuji Photo Film Co. Ltd.: Japan.

X-ray film XK-5, Eastman Kodak Company: U.S.A.

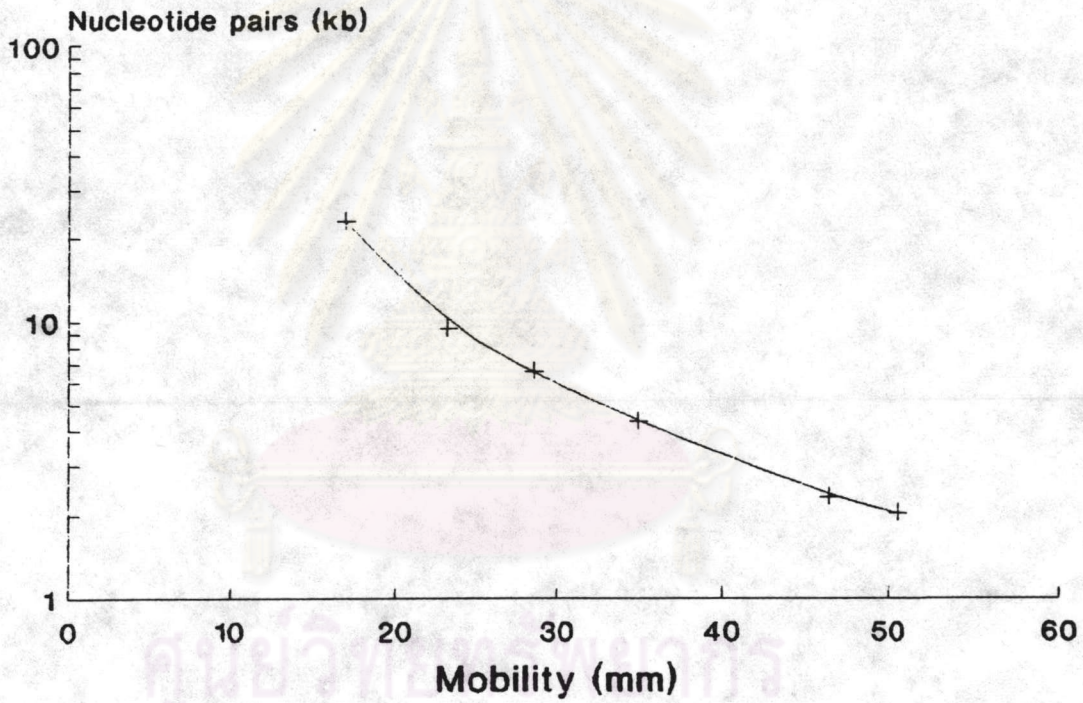
APPENDIX II

Chemicals	Product of company
Absolute ethanol	Merck
Acetic acid	Merck
Agarose(Type II)	Sigma
Agarose(Type VII)	Sigma
Bacto agar	Difco Laboratories, Ltd.
Bacto tryptone	Difco Laboratories, Ltd.
Boric acid	Merck
Bovine serum albumin(BSA)	Sigma
Bromphenol blue	Sigma
Butanol	BDH chemicals, Ltd.
Calcium chloride($CaCl_2.2H_2O$)	Merck
Cesium chloride	Sigma
Chloroform	BDH chemicals, Ltd.
Developer and fixer	Eastman Kodak Company
Diethyl ether	Merck
DIG-DNA labeling and detection kit nonradioactive	Boehringer Mannheim Coporation
Dipotassium hydrogen phosphate(K_2HPO_4)	Merck
Ethidium bromide	Sigma

Chemicals	Product of company
Ethylenediamine tetraacetic acid ,disodium salt dihydrate(Na_2EDTA)	Fluka
Ferric chloride($FeCl_3.6H_2O$)	BDH chemicals, Ltd.
Glucose	Fluka
Hydrochloric acid(HCl)	FARMITALIA CARLO ERBA
Isoamyl alcohol	Merck
N-lauroylsarcosine	Sigma
Lithium chloride(LiCl)	BDH chemicals, Ltd.
Lysozyme	Sigma
Malic acid	BDH chemicals, Ltd.
Magnesium chloride($MgCl_2$)	Merck
Magnesium sulphate($MgSO_4.7H_2O$)	BDH chemicals, Ltd.
D-mannitol	Merck
Phenol	FARMITALIA CARLO ERBA
Potassium dihydrogen phosphate(KH_2PO_4)	Merck
Potassium hydroxide(KOH)	EKA Nobel Ltd.
Pronase	Sigma
Ribonuclease A(RNase A)	Sigma
SDS	Sigma
Sodium acetate	Merck
Sodium chloride	Merck
Tri-sodium citrate	BDH chemicals, Ltd.
Sodium hydroxide(NaOH)	EKA Nobel Ltd.

Chemicals	Product of company
Sodium molybdate($NaMoO_4.2H_2O$)	BDH chemicals, Ltd.
Standard DNA marker	Sigma
Tris(hydroxymethyl)-aminomethane	Fluka
Trizma base	Sigma
Yeast extract	Oxoid

APPENDIX III



Standard mobility curve of linear DNA fragments.

APPENDIX IV

Effect of NaCl concentration on restriction endonuclease activity (Ausubel *et al.*, 1989).

Enzyme	0 mM NaCl	50 mM NaCl	100 mM NaCl	150 mM NaCl
*Bam*HI	+	++	+++	+++
*Bgl*II	++	+++	+++	+++
*Eco*RI	+	+++	+++	+++
*Hind*III	++	+++	+++	++
*Pst*I	+++	+++	+++	+++
*Sal*I	+	+	++	+++
*Sma*I	+	+	+	+
*Xho*I	++	+++	+++	+++

APPENDIX V

Calculation of the percent recovery of DNA fragment from low-melting temperature agarose gel electrophoresis.

-the percent recovery of *nod*D1 0.6 kb

The amount of *nod*D1 0.6 kb obtained after extraction of 20 μg pE39 3.3 kb cut with *Bam*HI from low melting temperature agarose gel was 1.80 μg.

Since 20 μg of DNA was the total DNA of pE39 before cutting, therefore the estimation amount of *nod*D1 in pE39 before extraction was $20\ \mu g \times \frac{0.6\ kb}{3.3\ kb} = 3.64\ \mu g$

So, the percent recovery of *nod*D1 0.6 kb was $\frac{1.80\ \mu g}{3.64\ \mu g} \times 100 = 49$

-the percent recovery of *nod*AB 2.2 kb

The amount of *nod*AB 2.2 kb obtained after extraction of 20 μg pRmSL42 8.5 kb cut with *Bam*HI, *Eco*RI and *Hind*III from low melting temperature agarose gel was 2.50 μg.

Since 20 μg of DNA was the total DNA of pRmSL42 before cutting, therefore the estimation amount of *nod*AB in pRmSL42 before extraction was $20\ \mu g \times \frac{2.2\ kb}{8.5\ kb} = 5.18\ \mu g$

So, the percent recovery of *nod*AB 2.2 kb was $\frac{2.50\ \mu g}{5.18\ \mu g} \times 100 = 48$

-the percent recovery of *nod*C 1.3 kb

The amount of *nod*C 1.3 kb obtained after extraction of 20 μg pRmSL42 8.5 kb cut with *Bam*HI, *Eco*RI and *Hind*III from low melting temperature agarose gel was 1.40 μg.

Since 20 μg of DNA was the total DNA of pRmSL42 before cutting, therefore the estimation amount of *nod*C in pRmSL42 before extraction was 20 μg x $\frac{1.3 \text{ kb}}{8.5 \text{ kb}}$ = 3.06 μg

So, the percent recovery of *nod*C 1.3 kb was $\frac{1.40\ \mu g}{3.06\ \mu g}$ x 100 = 46

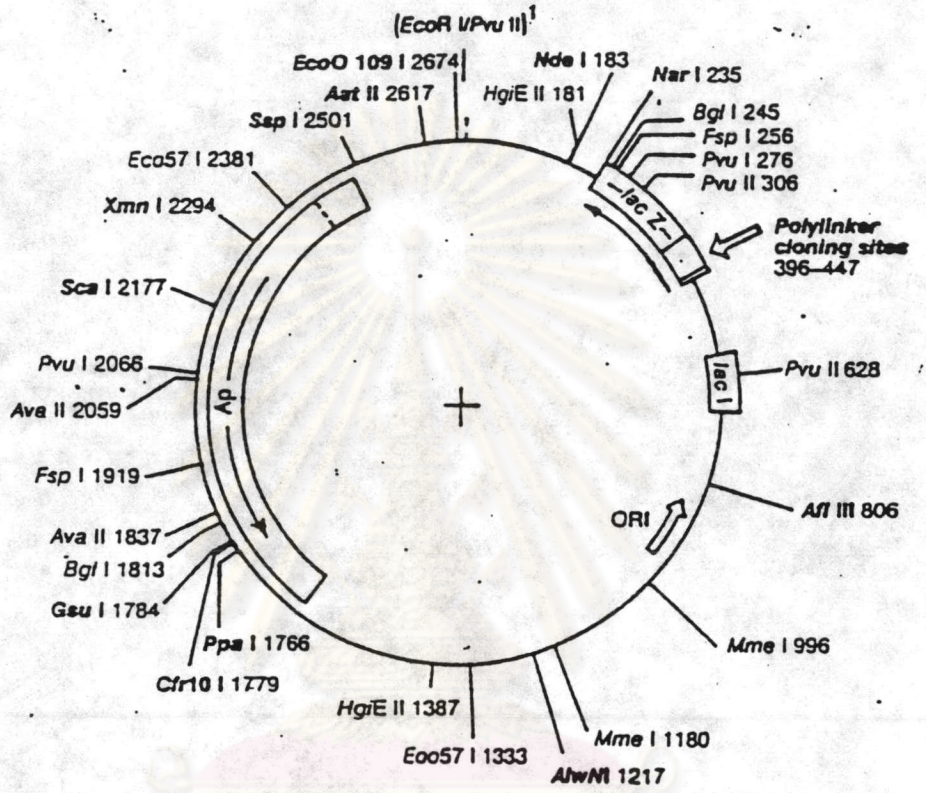
-the percent recovery of *nif*HDK 6.1 kb

The amount of *nif*HDK 6.1 kb obtained after extraction of 20 μg pSA30 10.3 kb cut with *Eco*RI from low melting temperature agarose gel was 5.46 μg.

Since 20 μg of DNA was the total DNA of pSA30 before cutting, therefore the estimation amount of *nif*HDK in pSA30 before extraction was 20 μg x $\frac{6.1 \text{ kb}}{10.3 \text{ kb}}$ = 11.84 μg

So, the percent recovery of *nif*HDK 6.1 kb was $\frac{5.46\ \mu g}{11.84\ \mu g}$ x 100 = 46

APPENDIX VI



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*

pUC19

	1	2	3	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	5	6	7	8
	Thr	Met	Ile	Thr	Pro	Ser	Leu	His	Ala	Cys	Arg	Ser	Thr	Leu	Glu	Asp	Pro	Arg	Val	Pro	Ser	Ser	Asn	Ser	Leu	Ala
ATG	ACC	ATG	ATT	ACG	CCA	AGC	TTG	CAT	GCC	TGC	AGG	TCG	ACT	CTA	GAG	GAT	CCC	CGG	GTA	CCG	AGC	TCG	AAT	TCA	CTG	GCC

HindIII *SphI* *PstI* *SalI* *AccI* *HincII* *XbaI* *BamHI* *SmaI* *XmaI* *KpnI* *SacI* *EcoRI*

In pUC18, the *EcoRI* site lies immediately downstream from P_{lac}.
In pUC19, the *HindIII* site lies immediately downstream from P_{lac}.

The physical map of pUC18 (Messing, 1983; Yanisch-Perron *et al.*,1985)

BIOGRAPHY

Miss Netnaphis Chinanonwait was born on March 20, 1969 in Uthaithani, Thailand. She graduated with the Bachelor degree of Science in Medical Technology (2^{nd} class honours) from Chiang Mai University in 1991.