VIII. APPENDIX

A. Solutions and buffers for DNA extraction

1. Red cells lysis buffer

1.0 M Tris (pH 7.65)	2.5	ml
1.0 M $MgCl_2$	1.25	ml
5.0 M NaCl	0.5	ml
distilled water to	250	ml

Sterilize by autoclaving and store at 4°C.

2. Nucleic lysis buffer

1.0 M Tris (pH 8.0)	12.5	ml
0.5 M EDTA (pH 8.0)	0.5	ml
5.0 M NaCl	5.0	ml
distilled water to	250	ml

Sterilize by autoclaving and store at 4°C.

3. Pronase K solution

0.5 M EDTA (pH 8.0)	40	µl
10 % SDS	1.0	ml
proteinase K	20	mg
distilled water to	10	ml

Dispense into aliquots and store at -20°C.

4. Acid citrate dextrose (ACD)

citric acid	0.48	g
sodium citrate	1.32	g
glucose	1.47	g
distilled water to	100	ml

Sterilize by autoclaving and store at 4°C.

B. <u>Solutions and buffers for plasmid preparation</u>

1. Sucrose buffer

sucrose	30	g
0.5 M EDTA (pH 8.0)	20	ml
1.0 M Tris (pH 8.0)	10	ml
distilled water to	200	ml

sterilize by autoclaving and store at 4°C.

2. Triton X-100 buffer

triton X-100	0.2	ml
1.0 M Tris (pH 8.0)	10	ml
0.5 M EDTA (pH 8.0)	20	ml
distilled water to	200	ml

sterilize by autoclaving and store at 4°C.

3. 30% PEG,1.5 M NaCl

polyethylene glycol	60	g
NaCl	17.54	g
distilled water to	200	ml

Sterilize by autoclaving and store at 4°C.

C. Reaction buffers for restriction endonuclease digestion.

1. Reaction buffer supplied for use with *Pst* I and *Hin* d III

final concentration:

50 mM Tris-HCl (pH 8.0)
10 mM $MgCl_2$
50 mM NaCl

2. Reaction buffer supplied for use with *Eco* R I and *Bgl* II

final concentration:

50 mM Tris-HCl (pH 8.0)
10 mM $MgCl_2$
100 mM NaCl

D. Solutions and buffers for agarose gel electrophoresis

1. 10x Tris-borate (TBE) buffer

Tris base	54	g
boric acid	27.5	g
0.5 M EDTA (pH 8.0)	20	ml

distilled water to 1000 ml

Mix throughly and store at room temperature.

2. Gel-loading buffer

bromophenol blue 0.25 %

sucrose in water 40 %(W/V)

Mix throughly and store at 4°C.

3. Stock ethidium bromide(10 mg/ml)

ethidium bromide 100 mg

distilled water 10 ml

Mix throughly until dissolve. Store at room temperature in a dark bottle.

E. <u>Solutions for Southern blotting</u>

1. 0.25 M HCl

Slowly add 21 ml concentrated HCl solution to 979 ml deionized water while stirring. Store at room temperature.

2. 1.5 M NaCl,0.5 M NaOH

NaCl 87.7 g

NaOH 20 g

deionized water to 1000 ml

Mix throughly and store at 4°C.

3. 1.5 M NaCl,1.0 M Tris (pH 7.5)

NaCl	87.7	g
Tris base	121.1	g
deionized water	800	ml

Adjust the pH to 7.5 with concentrated HCL. Adjust the volume to 1000 ml with deionized water. Mix throughly and store at 4 ^{o} C.

4. 20x SSC

NaCl	175.3	g
Sodium citrate,dihydrate	88.2	g
deionized water	800	ml

Adjust the pH to 7.0 with 4 N NaOH. ADjust the volume to 1000 ml with deionized water. Sterilize by autoclaving and store at 4^{o}C.

F. <u>Component of BioNick^{TM}Labelling System</u>

1. 10x dNTP Mix

0.2 mM each dCTP, dGTP,dTTP
0.1 mM dATP
0.1 mM biotin-14-dATP
500 mM Tris-HCl (pH 7.8)
50 mM $MgCl_2$
100 mM β–mercaptoethanol

100 µg/ml nuclease-free BSA

2. 10x Enzyme Mix

0.5 units/µl DNA Polymerase I

0.0075 units/µl DNase I

50 mM Tris-HCl (pH 7.5)

5 mM magnesium acetate

1 mM β-mercaptoethanol

0.1 mM phenylmethylsulfonyl fluoride

50% (v/v) glycerol

100 µg/ml nuclease-free BSA

3. Control DNA

5 µg pBR 322 in

10 mM Tris-HCl (pH 8.0)

1 mM EDTA

4. Stop buffer

300 mM EDTA

G. <u>Solutions and buffers for hybridization</u>

1. Prehybridization solution

Component	Final concentration	Amount
Formamide	50%(W/V)	50 ml
NaCl	0.9 M	5.26 g
$NaH_2PO_4.H_2O$	0.06 M	0.83 g
$Na_2EDTA.2H_2O$	0.006 M	0.22 g
Ficoll	0.1%(W/V)	0.1 g
Polyvinylpyrrolidone	0.1%(W/V)	0.1 g
Bovine serum albumin	0.1%(W/V)	0.1 g
Sodium dodecyl sulfate	1.0%(W/V)	1.0 g
Sheared,denatured salmon sperm DNA	200 µg/ml	20 mg

Dissolve solid components except DNA in 40 ml deionized water. Adjust pH to 7.4 with 4 M NaOH. Add 2.0 ml of 10 mg/ml sheard, denatured salmon sperm DNA. Adjust volume to 50 ml with deionized water. Add 50 ml formamide. Store at -20°C.

2. 20% dextran sulfate

Dissolve 10 g dextran sulfate in formamide to give a final volume of 50 ml. High molecular weight dextran sulfate dissolves very slowly. Stir or rock the solution slowly overnight. Store at 4°C.

3. 2x Hybridization solution

Component	Final concentration	Amount
NaCl	1.8 M	5.26 g
$NaH_2PO_4.H_2O$	0.12 M	0.83 g
$Na_2EDTA.2H_2O$	0.012 M	0.22 g
Ficoll	0.2%(W/V)	0.1 g
Polyvinylpyrrolidone	0.2%(W/V)	0.1 g
Bovine serum albumin	0.2%(W/V)	0.1 g
Sodium dodecyl sulfate	2.0%(W/V)	1.0 g
Sheared,denatured salmon sperm DNA	400 µg/ml	20 mg

Dissolve solid components except DNA in 40 ml deionized water. Adjust pH to 7.4 with 4 M NaOH. Add 2.0 ml of 10 mg/ml sheared,denatured salmon sperm DNA. Adjust volume to 50 ml with deionized water. Store at -20°C.

4. TBS-Tween 20

Component	Final concentration	Amount
Tris base	100 mM	12.1 g
NaCl	150 mM	8.77 g
Tween 20	0.05%(W/V)	0.5 ml

Adjust pH to 7.5 with 4 M HCl. Filter through a sterile 0.2-μ membrane. Store as a sterile solution at 4°C.

5. Blocking solution

Bovine serum albumin	3	g
TBS-Tween 20	100	ml

Adjust pH to 7.5 . Filter through a sterile 0.45-μ membrane. Store at 4°C.

6. Developer

Developer	1	part
distilled water	5	parts

Mix throughly and prepare before use.

7. Fixer

distilled water	3	parts
Fixative agent	1	part

Mix throughly.

distilled water to	5	parts

Again, mix throughly and prepare before use.

IX. BIOGRAPHY

NAME : **Miss ROONGSIRI MUANGMOONCHAI**

DATE OF BIRTH : **AUGUST 3 , 1968**

PLACE OF BIRTH : **RAYONG, THAILAND**

INSTITUTION ATTENED :

: PHADOONGPANYA SCHOOL, TAK

MARCH 1985, CERTIFICATE OF MATHAYOM VI

: FACULTY OF ASSOCIATED MEDICAL SCIENCES,

CHIANG MAI UNIVERSITY,CHIANG MAI

MARCH 1990, BECHELOR OF SCIENCE IN MEDICAL

TECHNOLOGY, B.Sc.(MED.TECH.)