

## **CHAPTER VIII**

### **APPENDIXES**

#### **APPENDIX A**

#### **EQUIPMENTS**

- |  |                              |
|--|------------------------------|
| 1. Autoclave : Hirayama HEV-50                                 | Hiclave : Japan              |
| 2. Balance   | Mettler Toledo : Switzerland |
| 3. Centrifuge : Sorvall RT6000 D                               | Sorvall : Germany            |
| 4. DC Power Supply : EC 250-90                                 | E-C Minicell : USA           |
| 5. Deionization System : Nano Pure II                          | Barnstead : USA              |
| 6. DNA Thermal Cycler : Ampliton II                            | Thermolyne : USA             |
| 7. Dry Block Heater  | Thermoline : Australia       |
| 8. Freezer – 20° C   | Sanyo : Japan                |
| 9. Hemacytometer counting chambers                             | Improved neubauer : USA      |
| 10. High Speed Refrigerated Micro Centrifuge<br>: Tomy MRX-150 | Tomy Seiko Co.Ltd. : Japan   |
| 11. Horizontal Gel Electrophoresis System                      | E-C Minicell : USA           |
| 12. Hot Air Oven   | Memmert : Germany            |
| 13. Inverted phase contrast microscope<br>: Olympus CK 2       | Olympus : Japan              |
| 14. Magnetic Stirrer   | Barnstead/Thermolyne : USA   |
| 15. Micropipette : P10, P20, P200, P1000                       | Gilson : France              |
| 16. Microsyringe 250 µl  | Kloehm : USA                 |

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|--|-----------------------------------|
| 17. Microsyringe 50 $\mu$ l              | Hamilton : USA                    |
| 18. Microwave : Sharp                    | Sharp : Japan                     |
| 19. Mini centrifuge                      | National Labnet co. : USA         |
| 20. pH Meter : 500 pH                    | Cyberscan : Singapore             |
| 21. Photo documentation : Foto / Eclipse | Fotodyne : USA                    |
| 22. Refrigerator 4°C                     | Singer : Thailand                 |
| 23. Shaking Water Bath :                 | Memmert : Germany                 |
| 24. Spectrophotometer : Shimadzu UV      | Shimadzu Corporation : Japan      |
| 25. Transluminator : TVC-312A            | Spectroline : USA                 |
| 26. Vortex Mixer : VM-300                | Gemmy Industrial Corp :<br>Taiwan |

## APPENDIX B

### SUPPLIES

1. Conical tube 15 ml.	Nunc : Denmark
2. Disposable transfer pipettes	Elkay : USA
3. Disposable gloves	W.A.Rubbermate : Thailand
4. Filter paper No.1	Whatman : England
5. High Quality Printing Paper	Sony : Japan
6. Membrane filter 0.2 $\mu\text{m}$ .	Gelman : USA
7. Micro tube 1.5 ml	Costar : England
8. Microscope slide 1 $\times$ 3"	Sail brand : China
9. Pasteur pipette 5.75" long	Corning : USA
10. Pipette tip 100-1000 $\mu\text{l}$	Costar : USA
11. Pipette tip 1-200 $\mu\text{l}$	Costar : USA
12. Terasaki typing tray : 60 wells	Nunc : Denmark
13. Thin Wall tube 0.5 ml	Costar : England

## APPENDIX C

### CHEMICALS AND REAGENTS

1. Absolute ethanol	BDH : England
2. Agarose : ultra PURE	Gibco BRL : USA
3. Boric acid	Gibco BRL : USA
4. Bromphenol Blue : sodium salt	Sigma : USA
5. dATP, dGTP, dCTP, dTTP	AMRESCO : USA
6. Dextran	BDH : England
7. DNA Marker : Phi X 174 DNA RF Hae III Digest	Gibco BRL : USA
8. Eosin Y	Merck : Germany
9. Ethidium bromide	Sigma : USA
10. Ethylenediaminetetraacetic acid (EDTA) : Disodium Salt	BDH : England
11. Formaldehyde	Common group : Thailand
12. Glyceral	Sigma : USA
13. Guanidine HCl	Gibco BRL : USA
14. HCl	Merck : Germany
15. Isoprep	Robbin Scientific : Norway
16. KCl	Merck : Germany
17. $\text{KH}_2\text{PO}_4$	Merck : Germany
18. KOH	Merck : Germany
19. Mineral oil	Sigma : USA
20. $\text{Na}_2\text{HPO}_4$	Merck : Germany

21. Phenol Red solution	BDH : England
22. Polyxyethylene sorbitan monolaurate (Tween 20)	Sigma : USA
23. Sodium Chloride	BDH : England
24. Sodium Dodecyl Sulphate (SDS)	Sigma : USA
25. Sodium Hydroxide	Merck : Germany
26. Tris (hydroxymethyl) aminomethane	Merck : Germany
27. TRIZMA base	Sigma : USA
28. Trypan blue	Merck : Germany

## **APPENDIX D**

### **ENZYMES**

- |                       |                 |
|-----------------------|-----------------|
| 1. Proteinase K       | Gibco BRL : USA |
| 2. Taq DNA Polymerase | Gibco BRL : USA |

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## APPENDIX E

### REAGENTS FOR DNA EXTRACTION AND AMPLIFICATION

**1. 1 M Tris – HCl (pH7.6)**

Tris-HCl	121.1	gm
ddH <sub>2</sub> O	900	
adjust pH to 7.6 with conc. HCl		
add ddH <sub>2</sub> O up	1000	
sterilize by autoclaving		

**2. 0.5 M EDTA (pH 8.0)**

EDTA disodium salt	186.1	gm
ddH <sub>2</sub> O	900	ml
adjust pH to 8.0 with NaOH pellets		
add ddH <sub>2</sub> O up to	1000	ml
sterilize by autoclaving		

**3. 5 M NaCl**

NaCl	146.1	gm
ddH <sub>2</sub> O up to	500	ml
sterilize by autoclaving		

**4. RCLB (Red Cell Lysis Buffer)**

1 M Tris- HCL (pH7.6)	10	gm
0.5 M EDTA (pH 8.0)	2	ml
5M NaCl	2	ml
add ddH <sub>2</sub> O up to	1000	ml
sterilize by autoclaving		

## 5. 10 % SDS

SDS	100	gm
Add ddH <sub>2</sub> O up to	1000	ml
Dissolve and then filter through 0.2 µm filter		

## 6. 10 mg/ml. Proteinase K

Proteinase K	100	mg
add ddH <sub>2</sub> O up to	10	ml
aliquots and use immediately or store at -20 °C		

## 7. 5 % EDTA (pH 7.4)

EDTA disodium salt	50	gm
add ddH <sub>2</sub> O	900	ml
adjust pH to 7.4 with NaOH		
add ddH <sub>2</sub> O up to	1000	ml
sterilize by autoclaving		

## 8. 2mM dNTP Mix

100 mM dATP	20	µl
100 mM dCTP	20	µl
100 mM dGTP	20	µl
100 mM dTTP	20	µl
ddH <sub>2</sub> O up to	1000	µl

## 9. 80 % ETHANOL

Absolute Ethanol	80	ml
ddH <sub>2</sub> O up to	100	ml



**10. 5 mg/ml ETHIDIUM BROMIDE**

Ethidium Bromide	50	mg
ddH <sub>2</sub> O	10	ml

**11. 10X TBE BUFFER**

TRIZMA base	54	gm
Boric acid	27	gm
0.5 M EDTA pH 8.0	20	ml
add ddH <sub>2</sub> O up to	500	ml

**12. 1X TBE BUFFER**

10X TBE	100	ml
add ddH <sub>2</sub> O up to	1000	ml

**13. 2% AGAROSE GEL**

agarose	2	gm
1X TBE	100	ml
boil		
add 5 mg/ml Ethidium bromide	5	μl

**14. BUFFER FOR ELECTROPHORESIS (TBE)**

10X TBE	100	ml
add ddH <sub>2</sub> O up to	1000	ml

**15. GEL LOADING BUFFER**

Glycerol	30	ml
Bromphenol Blue	100	mg
1X TBE up to	100	ml

**16. 7.5 M Guanidine HCl**

Guanidine HCl	72	gm
1 M Tris-HCl (pH 7.6)	10	ml
add ddH <sub>2</sub> O up to	100	ml
dissolve and then filter through 0.2 $\mu$ m filter		

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## APPENDIX F

### REAGENTS FOR HLA SEROLOGICAL TYPING

#### 1. Phosphate buffered saline (PBS) pH 7.2

##### 1.1 Stock solution (10x PBS)

NaCl	80	gm
KCl	2	gm
Na <sub>2</sub> HPO <sub>4</sub>	11.5	gm
KH <sub>2</sub> PO <sub>4</sub>	2	gm
add ddH <sub>2</sub> O up to	1000	ml

##### 1.2 Working solution

PBS 10x	100	ml
add ddH <sub>2</sub> O	900	ml
adjust pH to 7.2, store at room temperature		

#### 2. 0.5% Phenol red solution

Phenol red	1	gm
2% KOH	2.8	ml
transfer mixture into ddH <sub>2</sub> O	100	ml
mix until Phenol red dissolved		
add ddH <sub>2</sub> O up to a final volume	200	ml
filter through 0.45 µm filter		
adjust pH to 7.2, store at 4°C		

**3. Formalin****3.1 Stock solution**

Formaldehyde	500	ml
0.5 % Phenol red	2	ml

**3.2 Working solution**

Stock solution	12.5	ml
add PBS to	100	ml
adjust pH to 7.2 and filter through # 1 filter paper		
store at room temperature		

**4. 0.5% Eosin Y**

Eosin Y	50	gm
add ddH <sub>2</sub> O to	1000	ml
filter through # 1 filter paper		
store at 4°C		

**5. 0.2% Trypan blue**

Trypan blue	0.2	gm
add PBS to	100	ml
dissolve trypan blue in PBS and store at room temperature		

## **CURRICULUM VITAE**

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**SCIENTIFIC PUBLICATIONS**

1. Sujirachato K, Leetrakool N, Rilermvanich L. Study of lymphocytotoxins in highly sensitized pregnant woman. In 13<sup>th</sup> Congress of Medical Technologist Association of Thailand, Abstract, 1989.
2. Sujirachato K, Chiewsilp P, Mongkolsuk T, Leetrakool N. The chance of end-stage kidney disease patients receiving HLA matched cadaveric donor kidneys. In Fourth National Congress of Pathological 1992. P.65.

3. Chiewsilp P, Sujirachato K, Jootar S, Leetrakool N, Mongkolsuk T. The value of HLA-ABC Common typing tray in relation to bone marrow transplantation. *Southeast Asian J Trop Med Public Health* 1993; 24 Suppl 1: 259-62.
4. Sujirachato K, Chiewsilp P, Tsuji K, Inoko H, Panyim S, Mongkolsuk T, Leetrakool N. HLA class II polymorphism in Thais detected by PCR-SSO and PCR-RFLP. *Tissue Antigens* 1994; 43: 224-8.
5. Chiewsilp P, Sujirachato K, Mongkolsuk T, Leetrakool N, Jootar S, Chuncharunee S, Hathirat P. DNA typing for related allogeneic bone marrow transplantation. *Transplant Proc* 1994; 26 1881-2.
6. Fongsatikul L, Nantachit N, Kamtorn N, Leetrakool N. HLA gene frequencies of northern Thais. *J Med Asso Thailand* 1997; 80 (suppl.1): 38-42.
7. Kunachiwa W, Leetrakool N, Stephens HAF. HLA-A11 subtyping by polymerase chain reaction-sequence specific primer (PCR-SSP): Phototyping. The 17<sup>th</sup> Annual Health Sciences Meeting. 20 July 1999. P.75.
8. Leetrakool N, Kunachiwa W, Sripueng S, Chomsook S. HLA-DR Polymorphism in Northern Thais. The Second Congress of the Federation of Immunological Societies of Asia-Oceania(FIMSA). 23-27 January 2000. P.175.
9. Leetrakool N, Kunachiwa W. The most common HLA-A locus in 3 ethnic groups of the northern Thai population. The 18<sup>th</sup> Annual Health Sciences Meeting. 8 June 2000. P.40.