VIII. APPENDIX

A. Solution for DNA preparation

A. 1 Red cell lysis solution (10 mM Tris-HCl, pH 7.6; 5 mM MgCl2; 10 mM NaCl)

1 M	Tris-HCl, pH 7.6	10	ml
0.5 M	$MgCl_2$	10	ml
5 M	NaCl	2	ml

Sterile distilled water to 1000 ml

Mix throughly and store at room temperature

A. 2 Nucleic lysis buffer (10 mM Tris-HCl, pH 8.0; 400 mM NaCl; 2 mM EDTA)

1 M	Tris-HCl, pH 8.0	10	ml
5 M	NaCl	80	ml
0.5 M	EDTA	4	ml

Sterile distilled water to 1000 ml

Mix throughly and store at room temperature

A. 3 Proteinase K

10 mg/ml of proteinase K is dissolved in sterile distilled water, dispended into aliquots and stored at -20 ° C.

A. 4 Saturated sodium chloride

6 M NaCl is dissolved in in sterile distilled water and stirred on hot plate. Sterile by autoclaving and store at room temperature.

A. 5 Tris-HCl / EDTA (TE) pH 8.0 (10 mM Tris-HCl, pH 8.0; 0.1 mM EDTA)

1 M	Tris-HCl pH 8.0	10	ml
0.5 M	EDTA	0.2	ml

Sterile distilled water to 1000 ml

Mix throughly and store at room temperature.

A. 6 Ethidium bromide (0.5 mg/ml)

Ethidium bromide	0.005	g
Sterile distilled water to	10	ml

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

B. <u>Solution for dot blot hybridization</u>

B. 1 20X SSPE (3 M NaCl, 0.2 M NaH_2PO_4; 20 mM EDTA)

NaCl	175.5	g
NaH_2PO_4	24	g
0.5 M EDTA	30	ml
Sterile distilled water to	1000	ml

Adjust pH to 7.4 with NaOH. Store at room temperature.

B. 2 50 X Denhardt's solution

B. 2.1 Prepare 100 ml of 2 % polyvinylprolidone (PVP) (Sigma) and 2 % Ficoll 400 and autoclave it for 10 min at 120 ° C.

B. 2.2 Cool down to room temperature.

B. 2.3 Add 2 g of BSA (fraction V) and sterile distilled water to make up 200 ml of solution.

B. 2.4 Filter through a 0.45 μm filter and store in aliquot at -20 ° C until use.

B. 3 Hybridization buffer (6 X SSPE; 5X denhardt's solution, 0.1 % sarcosine sodium, 0.02 % SDS)

20 X SSPE	300	ml
50 X denhardt's solution	100	ml
0.1 % sarcosine sodium	1	g
20 % SDS	1	ml

Mix throughly and store at 4 ° C.

B. 4 Tetramethylammonium chloride (TMAC) solution (50 mM Tris-HCl, pH 8.0; 3 M TMAC, 2 mM EDTA, 0.1 % SDS)

1 M	Tris-HCl, pH 8.0	50	ml
5 M	TMAC	600	ml
0.5 M	EDTA	4	ml
20 %	SDS	5	ml
	Sterile distilled water to	1000	ml

Mix throughly and store at 4 ° C in a dark bottle.

B. 5 20 X SSC (3 M NaCl; 0.3 M tri-Sodium citrate, pH 7.0)

NaCl	175.5	g
tri-Sodium citrate	88.2	g

Adjust pH to 7.5 with NaOH, sterile by autoclaving and store at room temperature.

C. Solution for DIG-anti-DIG chemiluminescent detection

C. 1 Buffer 1 (100 mM maleic acid; 150 mM NaCl, pH 7.5)

Maleic acid	11.61	g
NaCl	8.78	g
Sterile distilled water to	1000	ml

Adjust pH to 7.5 with NaOH, sterile by autoclaving and store at room temperature.

C. 2 Buffer 2 (Stock blocking solution, 10% W/V)

Blocking reagent	10	ml
buffer 1	100	ml

Stir on hot plate until dissolve. Sterile by autoclaving and store at 4 ° C.

C. 3 Buffer 3 (100 mM Tris-HCl, pH 9.5; 100 mM NaCl, 50 mM $MgCl_2$)

1 M	Tris-HCl, pH 9.5	100	ml
5 M	NaCl	20	ml
0.5 M	$MgCl_2$	50	ml

Mix throughly, A new solution should be prepared fresh daily unless the solution will precipitate.

D. Instrument

D. 1 DNA Thermal Cycle (Perkin Elmer Cetus, USA)
D. 2 Ultra Violet fluorescent table (Viber Lourmat, France)
D. 3 Gel Photography FCR-10 (Fotodyne Incorperated, USA)
D. 4 Polaroid 667 (Polaroid Corperation, USA)
D. 5 Dot-blotter (Schleicher & Schuell, USA)
D. 6 Ultra Violet-crosslinker (Fluo-link, BRL)
D. 7 Flip-flop shaker (Model FF 120 S, J.S.C. Instrument)
D. 8 Hybridization incubator (Robbin Scienctific Corperation, USA)
D. 9 pH/Millivolt meter model 661 (Orion Research Incorporated Laboratory product Group, USA)
D. 10 Microcentrifuge (Hermele)
D. 11 X-ray film cassette PUSH type L (Okamoto manufacturing Co., Ltd., Japan)
D. 12 Microwave oven NN-6208 (Matsushita Electric Industrial Co., Ltd., Japan)

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Publications

1. Viputtigul, K., Kanjanahaluethai, A., Maneekarn, N. and Sittisombut, N. (1994). Improvements and comparison of peroxidase anti-peroxidase staining method with plaque assay for the titration of dengue type 2 virus. Chiang Mai Medical Bulletin 32: 123-128.
2. Sittisombut, N., Maneekarn, N., Kanjanahaluethai, A., Kasinrerk, V., Viputtigul, K. and Supawadee, J. (1995). Lack of augmenting effect of interferon-γ on dengue virus replication in human peripheral blood monocytes. J. Med. Virol. 45(1): 43-49.

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