VIII. APPENDIX

A. Solution for DNA preparation

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 MgCl2	10 ml
5 M NaCl	2 ml
Sterile distilled water to	1000 ml

Mix throughly and store at room temperature.

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.

3. Protinase K

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

4. Saturated sodium chloride

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

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.

6. Ethidium bromide (0.5 mg/ml)

Ethidium bromide 0.005 g

sterile distilled water to 10 ml

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

B. Solution for dot blot hybridization

1. 20 X 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.

2. 50 X Denhardt's solution

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

2.2. Cool down to room temperature.

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

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

3. Hybridization buffer

(6X SSPE, 5X Denhardt's solution, 0.1 % sarcosine sodium, 0.02 % SDS)

20X SSPE 300 ml

50X Denhardt's solution 100 ml

0.1 % sarcosine sodium 1 g

20 % SDS 1 ml

Mix throughly and store at 4°C.

4. Tetramethyammonium chloride (TMAC) solution

(50 mM Tris-HCl, pH 8.0, 3.0 TMAC (Sigma), 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.

5. 20X SSC

(3 M NaCl, 0.3M sodium citrate, pH 7.0)

NaCl	175.3 g
sodium citrate	88.2 g

Adjust pH to 7.0 with NaCl. Store at room temperature.

C. Solution for DIG-anti-DIG chemiluminescent detection

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.

2. Stock blocking solution (10 % W/V) (buffer 2)

blocking reagent	10 ml
buffer 1 to	100 ml

Stir on hot plate until dissolve. Sterile by autoclaving and store at 4°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 $MgCl_2$	100 ml

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

D. Instruments

1. DNA Thermal Cycler (Perkin Elmer Cetus)
2. Ultra Violet fluorescent tables (Vilber Lourmat, France)
3. Gel photography FCR-10 (Fotodyne Incorporated, U.S.A.)
4. Polaroid 667 (Polaroid Corporation, U.S.A.)
5. Dot-blotter (Bio-Rad)
6. Ultra Violet-Crosslinker (Fluo-link, BRL)
7. Flip-Flop shaker (Model FF 120 S, J.S.C. Instrument)
8. Microcentrifuge (Hermel)
9. pH/Millivolt meter model 661 (Orion Research Incorporated Laboratory Products Group, U.S.A.)
10. X-ray film cassette PUSH type L (Okamoto manufacturing Co., Ltd., Japan)
11. Microwave oven NN-6208 (Matsushita Electric Industrial CO., LTD., Japan)

IX. BIOGRAPHY

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