APPENDIX I

Chemicals

The following chemicals were from Sigma Chemicals, St. Louis, U.S.A.

- Agarose Type I : Low EEO, Mr = 0.13, No.A-6013
- Bovine serum albumin fraction V, No.A-4503
- β -mercaptoethanol, No. M-6250
- Deoxyribonucleic acid sodium salt Type III (from salmon testes), No. D-1626.
- Dextran sulfate sodium salt, M.W. 500,000, No. D-6001
- Dowex Mr-3, No. I-9005
- 8-Hydroxyquinoline, M.W. 145.2, No. # 6378
- Polyvinylpyrrolidone, M.W. 36,000, No. PVP 360
- Phenol, M.W. 94.11, No. P-3653
- RNase A Type I-AS (from Bovine Pancrease), No. R-5503
- Sodium Lauryl sulfate dodecyl sulfate, M.W. 288.4
- Spermidine free base, M.W. 145.3, No. S-2626

The following chemicals were from MERCK, May & Baker Ltd., ENGLAND

- D(+) Glucose, M.W. 198.17
- Ethidium bromide, M.W. 394.32, Art 11615
- Tri-sodium citrate dihydrate, M.W. 294.10, Art 6448
- Tris (hydroxymethyl) aminomethan, M.W. 121.14, Art 8382

The following chemicals were from SERVA

- Bromophenol blue sodium salt, M.W. 692.0
- Formamide, Mr - 45.1
- Lysozyme (chicken egg white), No. 28260

The following chemicals were from Pharmacia

- Ficoll type 400, Lot No. NE 04138
- Sephadex G-75
- Sephacryl S-1000

Agar (Bacteriological grade), casein hydrolysate (Peptone No.140 and yeast extract were from GIBCO, Madison, U.S.A. Multiprimer labelling kit code No. RPN1601Y and PB 10205 (α^{32}P dCTP) were from Amersham ENGLAND. Proteinase K, restriction endonuclease enzymes and low melting agarose were from Bethesda Research Laboratories. Nitrocellulose membrane (BAS5, 0.45 um) was from Schleicher and Schuell.

All chemicals were analytical grade.

APPENDIX II

Solutions and Buffers

Solutions and buffers for plasmid DNA isolation

LG broth : 10 g of casein hydrolysate, 10 g of yeast extract., 5g of NaCl and 1 g of glucose are dissolved in 1 litre of distilled water. The media solution is sterilized at 15 lb/in^2 for 15 min

Stock ampicillin : 25 mg/ml of ampicillin sodium salt is dissolved in distilled water. The solution is sterilized by filtration and stored in aliquots at -20°C.

Stock chloramphenicol : 200 mg/ml of chloramphenicol is dissolved in absolute ethanol. The solution is sterilized by filtration and stored in aliquots at -20°C.

Solution I : 25 nM Tris.Cl (pH 8.0), 50 mM glucose, and 20 nM EDTA (pH 8.0) are made up to 1 litre of distilled water and sterilized at 15 lb/in^2 for 15 min.
Powdered lyozyme in a concentration of 2 mg/ml should be dissolved in the solution just before use.

Solution II : 0.2 M NaOH, 1% SDS.

The solution should be made up from stock solutions of 10 M NaOH and 10% SDS just before use.

Solution III : 3.0 M NaOAc (pH 4.8)

TE buffer : 100 mM Tris.Cl and 10 mM EDTA in distilled water and the buffer is sterlized at 15 lb/in^2 for 15 min.

RNase A : 10 mg/ml of pancreatic RNase is dissolved in 10 mM Tris.Cl (pH 7.5) and 15 mM NaCl. The solution is boiled at 100°C for 15 min and allow to cool slowly to room temperature. Dispense into aliquots and stored at -20°C.

10% SDS : 100 g of SDS is dissolved in 900 ml of distilled water and heat to 68°C to assist dissolution. Adjust volume to 1 litre and dispense into aliquots.

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

Solutions and buffers for DNA preparation

Lysis buffer (pH 7.5) : 100 mM Tris.Cl, 0.32 M sucrose, and 1% of Triton X-100 are dissolved in 900 ml distilled water. The pH is adjusted with conc HCl.

Adjust volume to 1 litre and kept in the refrigerator.

STE buffer : 10 mM Tris.Cl (pH 7.5), 100 mM NaCl, and 1 mM EDTA are dissolved in distilled water. The buffer is sterilized at 15 lb/in^2 for 15 min.

Phenol : 250 g. of phenol crystals are is dissolved in 50 ml sterile distilled water. The dissolved phenol is extracted two times with 50 ml of 1 M Tris-Cl (pH 8.0). Then, 8-hydroxyquinoline is added to a final concentration of 0.1%. The phenol is extracted again with 0.1 M Tris. Cl (pH 8.0) and 0.2 M β-mercaptoethanol.

Chloroform - Isoamyl alcohol : 24:1 (v/v)

3M sodium acetate (pH 6.0) : 408.1 g. of sodium acetate $3H_2O$ is dissolved in 800 ml distilled water. The pH is adjusted to 6.0 with glacial acetic acid. The volume is adjusted to 1 litre, dispensed into aliquots and sterilized by autochaving.

Solutions and buffers for Restriction endonuclease digestion

10x buffer for BglII and EcoRI : 50 mM Tris.Cl (pH 8.0), 10 mM $MgCl_2$

10x buffer for HindIII and PstI : 50 mM Tris-Cl (pH 8.0), 10 mM $MgCl_2$ and 50 mM NaCl.

5x loading dye : 15% Ficoll (Type 400), 1% SDS, 50 mM EDTA and 0.3% bromophenol blue are dissolved in 10 ml distilled water and stirred on a magnetic stirrer until dissolved.

Solutions and buffers for gel electrophoresis

TBE buffer (5x): 54 g of Tris. base, 27.5 g of boric acid, and 20 ml of 0.5 M EDTA (pH 8.0) are dissolved in 1 litre distilled water.

Stock Ethidium bromide : 10 mg of ethidium bromide is dissolved in 10 ml distilled water. Ethidium bromide solution is kept in a dark bottle and stored at 4°C.

Solutions and buffers for Southern transfer

20xSSC(pH 7.0) : 3.0 M NaCl/0.3 M Sodium citrate trihydrate. The pH is adjusted with conc HCl.

2 x SSC : dilute 100 ml of 20xSSC to 1 litre.

Solutions and buffers for radiolabelling

Multiprimer DNA labelling kit: the system is composed of

reaction buffer: dATP, dGTP, dTTP in Tris.Cl (pH 7.8), $MgCl_2$, and 2-mercaptoethanol.

hexaprimer solution	:	random hexanucleotide in an aqueous solution containing nuclease free BSA.
Klenow polymerase enzyme solution	:	1 unit/ul DNA polymerase I (Klenow fragment) in 50 mM $KHPO_4$ (pH 6.5), 10 mM 2-mercaptoethanol, and 50% glycerol.
Alpha ^{32}P-dCTP	:	3,000 Ci/mmol, PB 10205
Sephadex G-75	:	1.0 ml of Sephadex G-75 gel is packed in column in a Pasteur pipette and equilibrate with TE buffer.
Denatured	:	10 mg/ml of salmon sperm DNA in sterile salmon sperm DNA distilled water is sheared by passing it several times though an 18-gauge hypodermic needle. The DNA solution is boiled for 10 min and stored at -20°C in small aliquots.

Solutions and buffer for hybridization

Formamide	:	formamide is deionized with 5% (W/V) of Dowex mixed bed resin by inverting several times and stand on ice. The solution is prepared freshy just before use. Dowex is removed by filtration with filter paper.

Denhardt's solution (50x)	:	0.1 g Ficoll (Type 400), 0.1 g polyvinylpyrrolidone, and 0.1 g BSA are dissolved in 10 ml sterile distilled water by stirring on a magnetic stirrer.
Prehybridization: solution		3.0 ml 20xSSC, 0.65 ml stirile distilled water, 5.0 ml deionized formamide, 0.1 ml salmon sperm DNA solution (10 mg/ml), and 1.25 ml Denhardt's solution (50x) are mixed by stirring in 60°C water bath.
Hybridization solution	:	3.0 ml 20xSSC, 1 g dextran sulfate, 5.0 ml deionized formamide, 50 ul salmon sperm DNA solution (10mg/ml), and 200 ul Denhardt's solution (50x) are mixed by stirring in 60°C waterbath. The volume of solution is adjusted to 10 ml. The labelled probe is denatured by boiling for 5 min, then quickly chilled in ice-bath, and added into hybridization solution before hybridization.

APPENDIX III

Instruments

Autoclave, TYP 23, MELAG, W. Germany

Freezer -70°C, GFL, W. Germany

Hand-held, end-window geiger counter, series 900, mini-monitor, England

Horizontal gel electrophoresis chamber (home-made)

Incubator shaker, GFL, W. Germany

Micro-highspeed centrifugation, SIGMA-202 MC, USA.

Power supply, GELMAN instrument company, USA.

Refrigerated centrifugation, RC2-B, Ivan Sorvall Inc, USA.

Roller incubator, BACHOFER, LABORATORIUMSGERATE, W. Germany

Shaking waterbath, Dubnoff, Precision Scientific Co., USA.

Spectrophotometer, Spectronic 601, Milton Roy Company, USA.

Transilluminators (home-made)

VITA

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