APPENDIX

APPENDIX

Stock solutions :

Phosphate Buffered Saline (PBS) pH 7.2/pH 7.6

Sodium phosphate (anhydrous)	1.52 gm
Potassium dihydrogen phosphate	0.58 gm
NaCl	8.50 gm
Sterile ddH_2O	1000 ml

The pH was adjusted with 3M NaOH according to the experiment and the solution, filter-sterilized.

Proteinase K 20 mg/ml

Proteinase K	20 mg
Sterile ddH_2O	1 ml

Solution was filter-sterilized and stored at -20°C.

20X SSC pH 7.0

NaCl	3.0 M
Sodium citrate	0.3 M

The pH was adjusted with 10N NaOH.

Solutions for DNA extraction:

Paraffin-embedded tissue:

Proteinase K mix

Tris-HCl pH 7.5	10.0 mM
$MgCl_2$	1.5 mM
Tween 20	0.45 %
Proteinase K	60 µg/ml

Throat washes:

RPMI 1640

RPMI 1640	1 sachet
Sodium bicarbonate	2.0 gm
HEPES	1.0 gm
Sterile ddH_2O	1000 ml

The solution was filter-sterilized and stored at 4°C.

Digestion buffer

Tris-HCl pH 8.0	0.05 M
EDTA pH 8.0	0.005 M
SDS	1 %
Proteinase K (added fresh)	200 µg/ml

RNase 10 mg/ml

Ribonuclease	100.0 mg
Sterile ddH_2O	10.0 mg

Solutions for plasmid isolation:

Luria-Bertani broth (LB)

Trypton	1.0 gm
NaCl	1.0 gm
Yeast extract	0.5 gm
ddH_2O	100 ml

Broth was autoclaved and cooled before using. LB agar was prepared by adding 1.0 gm of Agar to the broth prior to autoclaving.

Ampicillin 20 mg/ml

Ampicillin	20 mg
Sterile ddH_2O	1 ml

Solution was filter-sterilized and stored at -20°C.

Solution I

Glucose	50 mM
Tris-HCl pH 8.0	25 mM
EDTA pH 8.0	10 mM
ddH_2O	100 ml

Solution was stored at 4°C.

Solution II

NaOH	0.2N
SDS	1 %

Solution was freshly prepared as necessary.

Solution III

5 M Potassium acetate	60.0 ml
Glacial acetic acid	11.5 ml
Sterile ddH_2O	28.5 ml

Solution was stored at 4°C.

Solutions for agarose gel electrophoresis:

5X TBE

Trizma base	54.0 gm
Boric acid	27.5 gm
EDTA 0.05 M	20.0 ml

Solution was made up to 1 litre with distilled water.

6X Gel loading dye

Bromophenol blue	0.25 %
Glycerol	40 % (w/v)
ddH_2O	100 ml

Solution was stored at 4°C.

Ethidium bromide 10 mg/ml

Ethidium bromide	100 mg
ddH_2O	10 ml

Solutions for nonradioactive Southern blot hybridization:

Denaturation buffer

NaCl	1.5 M
NaOH	0.5 M

Neutralization buffer

Tris-HCl	1.0 M
NaCl	1.5 M

Hybridization solution

5X SSC	22.5 ml
N-lauroylsarcosine, Na-salt	0.1 % (w/v)
SDS	0.02% (w/v)
Blocking reagent	1.0 % (w/v)

Wash Solution 1

2X SSC	100.0 ml
SDS	0.1 % (w/v)

Wash Solution 2

0.1X SSC	100.0 ml
SDS	0.1 % (w/v)

Solutions for detection of hybridized products

Buffer 1 pH 7.5

Tris-HCl	100 mM
NaCl	150 mM

Buffer 2

0.5 % (w/v) Blocking reagent in buffer 1.

Buffer 3

Tris-HCl	100 mM
NaCl	100 mM
$MgCl_2$	50 mM

Buffer 4

Tris-HCl	10 mM
EDTA	1 mM

Colour solution

4-Nitro blue tetrazolium chloride	45 µl
5-Bromo-4-chloro-3-indoyl phosphate	35 µl
Buffer 3	10 ml

Solutions for treatment of slides:

50X Denhardt's solution

Ficoll	5 gm
Polyvinylpyrrolidone	5 gm
Bovine serum albumin	5 gm
ddH_2O	500 ml

Solution was filter-sterilized and stored in aliquots at -20°C.

Organosilane solution pH 3.45

1 % (v/v) 3-amino-propyl-trithoxysilane in double distilled water. pH was adjusted with 1M HCl.

Glutardialdehyde

10 % (v/v) Glutardialdehyde in PBS.

Sodium-m-periodate

0.1 M Sodium-m-periodate in double distilled water.

Solutions for *in situ* hybridization:

0.2 % Glycine

Glycine	0.2 gm
PBS	100 ml

4 % Paraformaldehyde pH 7.2

Paraformaldehyde	4 gm
PBS	100 ml

Solution was freshly prepared as necessary by dissolving at 60-70°C and adding a few drops of 2N NaOH to give a clear liquid.

100 % deionized Formamide

Formamide	100 ml
Resin	10 gm

Solution was stirred vigorously for 30 minutes in a fume chamber and filtered twice through Whatman #1 paper. Aliquots were stored at -20°C.