BUFFERS AND SOLUTION FOR DNA EXTRACTION FROM FROZEN CERVICAL TISSUE, PAP SMEARS AND CELL CULTURE

Phosphate buffered saline (PBS), pH 7.2

Sodium phosphate (anhydrous)	1.52 g
Potassium dihydrogen phosphate	0.58 g
NaCl	8.50 g
dH_2O	made up to 1 liter

20X SSC, pH 7.0

NaCl	175.3 g
Sodium Citrate trihydrate	88.2 g
dH_2O	made up to 1 liter

1M Tris - HCL, pH 8.0

Trizma base	121.1g
dH_2O	made up to 1 liter

The pH was adjusted with concentrated HCL

SSC-buffered phenol

Phenol was melted at 68°C. An antioxidant, 8-hydroxyquinoline was added to a final concentration of 0.1%. The melted phenol was then extracted with with equal volume of 1 X SSC, pH 8 by stirring at 4°C, overnight, until the pH of the aqueous phase is more than 7.6. The SSC buffered phenol was stored in a dark bottle at 4°C for up to 1 month.

CELL CULTURE

All media were filter sterilized and stored at 4°C.

RPMI 1640 base medium

RPMI 1640 base media	1 sachet
Sodium bicarbonate	2.0 g
Hydroxyethylpiperazine ethanesulfonic Acid (HEPES)	1.0 g
dH_2O	made up to 1 liter

Reviving media

RPMI base media	77 ml
Fetal calf serum (FCS)	20 ml
Penicillin	2 ml
Kanamycin	1 ml
L - glutamine	0.012 g

Media was filtered sterilized with 0.22 μm milipore filter

Culture Media

Preparation of culture media was the same as reviving media with the addition of 10% FCS.

Freezing solution

FCS	10 ml
Dimethyl sulfoxide (DMSD)	4 ml
RPMI 1640 base medium	6 ml

The solution was prepared fresh and sterilized with 0.22μm millipore filter.

GEL ELECTROPHORESIS

Tris-borate EDTA (TBE) 5X

Trizma base	54g
Boric acid	27.5 g
EDTA 0.05M pH 8.0	20 g
dH_2O	made up to 1 liter

Loading Buffer 6 X

Bromophenol blue	0.25%
Sucrose	40% (w/r)
dH_2O	made up to 100 ml

20x SSC pH 7.0

SOUTHERN BLOT HYBRIDIZATION

Solutions are prepared as described by Boehringer Mannheim (1989). Reagents used were from the Non-radioactive DNA labelling and Detection kit.

Hybridization solution

5X SSC	22.5 ml
N-lauroylsarcosine, Na-salt (Sigma)	0.1%(w/v)
SDS	0.02% (w/v)
Blocking reagent (vial 11)	1% (w/v)

ISOLATION OF PLASMID

LB broth

Bacto-Tryptone	10 g
Bacto-yeast extract	5 g
NaCl	10 g
ddH_20	up to 1 litre

IN SITU HYBRIDIZATION

Denhardt's solution (50x)

Ficoll 400	5 g
Polyvinylpyrrolidone-360	5 g
BSA	5 g
dH_2O	added to 500 ml

The solution was sterilized using the disposable 0.22 µm Nalgene filter, dispensed into 25 ml aliquots and stored at -20°C.

4% paraformaldehyde

Paraformaldehyde	4 g
PBS	100 g

Paraformaldehyde was dissolved by heating the solution 60°C-70°C and by adding drops of 2 N NaOH while adjusting the pH to 7.2.

Phosphate Buffered Saline (PBS) pH 7.6

Sodium chloride	7.75 g
Potassium phosphate dibasic	1.5 g
Potassium phosphate, monobasic	0.2 g
dH_2O	to 1 litre of the solution

The pH of the solution was adjusted to 7.6 with 3 M NaOH or 8.5% phosphoric acid.

Prehybridization mixture

50x Denhardt's solution	0.2 ml
50% dextran sulphate	1.0 ml
Sonicated salmon sperm DNA (10 mg/ml)	0.2 ml
20X SSC	2.0 ml
Formamide	5.0 ml
Sterile dH_2O	added to 10 ml

Hybridization solution (probe-cocktail)

50x Denhardt's solution	0.2 ml
50% dextran sulphate	1.0 ml
Sonicated salmon sperm DNA (10mg/ml)	0.2 ml
20X SSC	2.0 ml
100% deionized formamide	5.0 ml
Digoxigenin labelled probe	500 ng/slide
Sterile dH_2O	to 10 ml

100% deionized formamide

Formamide	100 ml
Resin	10 g

The mixture was stirred vigorously for 30 minutes, filtered twice through Whatman filter paper and stored at -20°C.

IMMUNOSTAINING

The LSAB kit (Dako) contained the following reagents :-

1. Biotinylated anti-mouse and anti-rabbit immunoglobulin in 0.05 M Tris-HCL buffer, pH 7.6.
2. Streptavidin-HRP conjugate in 0.05 M Tris-HCL buffer pH 7.6.

AEC substrate system (K697,Dako) contains the following reagents:-

1. 0.1 M Acetate buffer, pH 5.2
2. 3% Hydrogen peroxide in water
3. 3% 3-amino-9-ethylcarbazote (AEC) in N,N dimenthylformamide.

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

0.1 M Carbonate buffer, pH 9.6

Na_2CO_3	1.65 g
$NaHCO_3$	3.14 g
dH_20	up to 500 ml

Phosphate buffered Saline (PBS), pH 7.3

Sodium chloride	8.0
Potassium phosphate monobasic	0.2
Sodium phosphate dibasic	1.15
Potassium chloride	0.2
dH_20	up to 1 litre

PBS-0.05% Tween 20 (PBS-T) was prepared by adding 0.05% of Tween 20 to the above solution.

Lamb serum

Lamp serum (Gibco) was removed from the freezer and thawed at 37°C afterwhich it was heat inactivated at 56°C for 30 minutes. The serum was then filtered through Whatman filter paper and 45 µm membrane filter. It was dispensed into aliquots and stored at -20°C.

ABTS (50x) (20mg/ml)

(2,2-azinodi-(3-ethylbenz thiazolin sulfate(Boeringer)	0.2 g
dH_20	10 ml

0.1 M Ciltrate Buffer, pH 4.0

Citric acid monohydrate	6.62 g
Sodium citrate dihydrate	5.44 g
dH_20	to 1 litre

Peroxidase substrate

50X ABTS	150 µl
30% Hydrogen peroxidase	1.8 µl
0.1M Citrate buffer	up to 6 ml

The solution was prepared fresh.

Diethanolamine buffer - pH 9.8

Diethanolamine	97 ml
Magnesium chloride	0.1 g
dH_20	up to 1 litre

The solution was filtered through Whatman paper and stored in a dark bottle at room temperature.

Phosphatase substrate

Phosphatase substrate 104 (Sigma)	0.1 g
Diethanolamine buffer	100 ml

20% glucose

Glucose	10 g
Sterile dH_2O	to 50 ml

The solution was transferred to a 50 ml tube and rotated end-over-end overnight at room temperature and stored at 4°C.

0.2 M NaH_2PO_4

NaH_2PO_4	27.8 g
dH_2O	to 1 litre

0.2 M Na_2HPO_4

Na_2HPO_4	53.65 g
dH_2O	to 1 litre

0.1 M Phosphate buffer, pH 6

0.2 M NaH_2PO_4	87.7
0.2 M Na_2HPO_4	12.3
dH_2O	to 100 ml