**Appendix B**

REAGENTS

All solutions are made up using reagent grade water obtained from Milli-Q water system (Millipore) with a resistance greater than 18 MΩ per $cm^2$.

When handling DNA or tissue sections great care is taken not to contaminate any of the surfaces or equipment. This is because of the presence of DNases (deoxyribonucleases) and RNases (ribonucleases) which can destroy the experimental materials (Leibowitz & Young 1989).

**B1: Phosphate buffered saline (PBS)-calcium and magnesium free (10X concentrate)**

To 800 ml Milli-Q water, add

2.0 g KCL

80 g NaCl

11.5 g $Na_2HPO_4$

2.0 g $KH_2PO_4$

Make up the volume to 1 litre

Dilute stock 1/10 with Milli-Q water.

**B2: Fixative**

*10% buffered formalin*

10 ml formaldehyde solution (36-42%) (AnalaR BDH from Merck, Australia)

10 ml of 10 X PBS

Make up the volume to 100 ml with Milli-Q water

Store in a capped bottle.

## B3: Reagents used in plasmid purification

*Luria Broth (LB) Agar plates*

To 800 ml Milli-Q water add

10 g Tryptone

5 g Yeast extract

10 NaCl

15 g Agar (Bacto)

Make up the volume to 1 litre and autoclave, and when the solution has cooled, add antibiotic and pour into plates.

Wrap in parafilm and store at 4°C.

*Luria Broth (LB)*

To 800 ml Milli-Q water add

10 g Tryptone

5 g Yeast extract

10 g NaCl

Make up the volume to 1 litre and autoclave, and when the solution has cooled, add antibiotic.

*Cell resuspension solution*

| | |
|---|---|
| 50 mM | Tris-HCL, pH 7.5 |
| 10 mM | EDTA (Boehringer-Mannheim, Germany, # 200-0081) |
| 100 µg/ml | RNase |

*Cell lysis solution*

| | |
|---|---|
| 0.2 M | NaOH |
| 1 % | sodium dedocyl sulphate (SDS) |

*Tris-EDTA (TE) Buffer*

| | |
|---|---|
| 10 mM | Tris-HCL, pH 7.5 |
| 1 mM | EDTA (Boehringer-Mannheim, # 200-0081, Germany) |

Column wash solution (concentrate prior to ethanol addition)

| | |
|---|---|
| 200 mM | NaCl |
| 20 mM | Tris-HCL, pH 7.5 |
| 5 mM | EDTA (Boehringer-Mannheim, # 200-0081, Germany) |

Neutralizing solution

| | |
|---|---|
| 1.32 M | Potassium acetate, pH 4.8 |

**B4: Hybridization buffer**

The hybridization buffer is prepared as follows:

Dissolve 1.0 g dextran sulphate (Sigma, USA, # D-8906), in 2.5 ml 20X SSC, 1 ml herring sperm DNA (Sigma, USA # D 7290), 2.5 mg/ml in 10 mM EDTA (pH 7) and 5 ml distilled water; then add 5.0 ml formamide (Promega, USA, # 200-0100) and mix well: store at -20°C.

**B5: Reagents used in *in situ* hybridization and in immunocytochemistry assays**

*Sodium citrate solution (SSC) (10X concentrate)*

To 800 ml Milli-Q water, add

87.7 g NaCl

44.1 g Na citrate

Make up the volume to 1 litre.

*Tris Buffer 1*

0.1 M Tris, 0.5 M NaCl, 5 mM $MgCl_2$, 0.05% (v/v) Triton X-100, 0.05% (w/v) BSA, pH 7.5.

(for 1L: 12.1 g Tris, 58.4 g NaCl, 0.4 g $MgCl_2$, 0.5 ml Triton X-100. 0.05% (w/v) BSA.)

*Tris Buffer 2*

20.1 M Tris, 0.1 M NaCl, 10 mM $MgCl_2$, ph 9.5.

(for 1L: 12.1 g Tris, 5.8 g NaCl, 2.0 g $MgCl_2$, 0.5 ml Triton X-100.)

*Dithanolamine buffer*

Dissolve 48.5 g diethanolamine in 400 ml water, add 1.0 g $NaN_3$ and 0.05 g $MgCl_2 \cdot 6H_2O$. Adjust pH to 9.8 and make up volume to 500 ml in Milli-Q water. Store refridgerated and wrapped in foil.

*p-nitrophenyl phosphate (p-NPP) substrate*

5 mg tablets of p-nitrophenyl phosphate (Sigma # 104) in 5 ml pre-heated to 37°C diethanolamine buffer (prepared as follows: dissolve 48.4 mg diethanolamine in 400 ml water, add 0.1 g $NaN_3$ and 0.05 mg $MgCl_2 \cdot 6H_2O$. Adjust pH to 9.8 and make up volume to 500 ml. Store refridgerated wrapped in foil).

## B6: Stain used in routine histology

*Mayer's Haematoxylin*

Reference: Lilli and Fullmer 1976.

| | 1000 ml | 200 ml |
|---|---|---|
| Hematoxylin crystals C.I. 75290 | 5.0 g | 1 g |
| Distilled water | 800.0 ml | 160 ml |
| Aluminium ammonium sulphate | 50.0 g | 10.0 g |

## B7: Reagents used in cell culture

*Preparation of culture medium*

Quantity sufficient to make up 1 litre:

α-Minimum Essential Medium (α-MEM), 1 packet of powdered medium (Gibco BRL, # 12000-022)

30 µg/ml penicillin, and 100 µg/ml streptomycin (Gibco Laboratories, USA, # 15070-063)

Sodium bicarbonate $NaHCO_3$ (0.85 g/litre) (Sigma, USA # S 5761)

Hepes Buffer (25 mM) (Gibco Laboratories, USA, # 15630-080)

Add approximately 600 ml distilled water

Stir with spin bar for 10-15 mintues

pH to 7.3

Make up volume to 1 litre

Sterilise through 0.2 µm pore size bell filter

Store in tightly capped glass bottles at 4° C

Add L-glutamine (2 mM) (Gibco Laboratories, # 25030-081), L-ascorbic acid phosphate (0.1 M) (Wako Pure Chemicals, Osaka, Japan, # 013-12061), and 10 % foetal bovine serum (FCS) (Gibco Laboratories, # 10440-022), freshly before use.

*Trypsin-EDTA*

To make 100 ml of cell harvesting solution

0.1 g powdered trypsin (Sigma, USA, # T 4665)

0.02 g EDTA (Boehringer-Mannheim, # 200-0081, Germany)

Make up to 100 ml with 1X PBS

Stir with spin bar for 20-30 minutes

Sterilise through 0.2 µm pore size bell filter.

**B8: Reagents used in scanning electron microscopy**

*Sodium cacodylate buffer 0.2 M, pH 7.2*

To 300 ml destilled water, add

21.4 g sodium cacodylate salt x $3H_2O$ (Merck # 820670)

pH to 7.2

Make up the volume to 500 ml.

*fixative for SEM:*

*Glutaraldehyde in sodium cacodylate buffer 0.1 M, pH 7.2*

To 10 ml 0.2 M sodium cacodylate buffer (pH 7.2) add

6.8 ml destilled water and

3.2 ml glutaraldehyde solution (grade I, 25% aqueous solution, Sigma # G 5882),

store at 4°C.