APPENDIX II

Preparation of various solutions:

10% APS: Dissolved 100 mg of APS in 1 ml of ADW.

10X DNase reaction buffer: 100 mM Tris-Cl, pH 8.4, 500 mM KCl, 15 mM MgCl₂, 0.01% gelatin in ADW.

5X Formaldehyde gel running buffer for RNA: (1X-0.2 M MOPS; 20 mM sodium acetate; 10 mM EDTA) Dissolved 20.6 g of MOPS in 800 ml of DEPC treated 50 mM sodium acetate. Adjust pH 7.0 with 2 N NaOH and add 10 ml of DEPC treated EDTA (0.5M; pH 8.0). Make final volume to 1L with RNase free water.

5X MOPS buffer: Dissolved 20.9 g MOPS and 13.3 ml sodium acetate (3 M) solution in DEPC- treated ADW, adjusted pH to 7.0, added 10.0 ml 0.5M EDTA (pH 8.0). Made up the volume to 1 L and filter through 0.2 µm filter paper.

0.5M PIPES (piperazine-1, 2-bis[2-ethanesulfonic-acid]): Dissolve 15.1 g of PIPES in 80 ml Milli-Q water. Adjust pH of solution to 6.7 with KOH or HCl. Add Milli-Q water to final volume of 100 ml.

2X RNA loading mix: Mixed 95% formamide, 0.025% SDS, 0.025% bromophenol blue, 0.025% xylene cyanol FF, 0.025% ethidium bromide, 0.5 mM EDTA. Adjusted final volume with ADW and stored at -20°C.

20% SDS: Dissolved SDS (100.0g) in 300 ml of ADW and kept in water bath at 60-65°C to dissolve SDS. Final volume was made to 500 ml.

50X TAE buffer (1X 40 mM Tris-acetate, 1mM EDTA): Dissolved 242.1 g Tris-base, 57.1 ml of glacial acetic acid and 100 ml of EDTA (0.5 M; pH 8.0) in a total volume of 1 L ADW.

Acrylamide/Bis-acrylamide (40%): Add 80.0 g of acrylamide to 4.0 g of bis-acrylamide in ADW to a final volume of 200 ml. Filter sterilize and store at 4°C in dark.

Agarose Gel: Weigh appropriate amount of agarose and dissolve in appropriate volume of 1X TAE buffer (For example for 1% of agarose gel, 1 g of agarose dissolved in 100 ml of 1X TAE buffer). Heat in microwave oven to dissolve agarose and cool to 60°C. Add 0.5 µg of EtBr per ml of agarose solution. Pour into gel tray and allow it to solidify for 20-30 min.
**Ampicillin:** Dissolved 100.0 mg of ampicillin in 1 ml of ADW and sterilize by filtration through a 0.22-μm filter (MILLIPORE-Ireland). Aliquots were stored at -20°C.

**Colony lysis buffer:** Mixed 10 mM TE (pH 8.0) and 0.1% Tween 20.

**Coomasie Brilliant Blue:** 0.1% Coomasive (Brilliant blue R-250) + 40% methanol, 10% glacial acetic acid + 50% ADW.

**DEPC Water:** Added DEPC (0.1% final concentration) to ADW and mixed well. Kept undisturbed overnight.

**Destaining solution:** Dissolved 40% methanol and 10% glacial acetic acid in distilled water to a desired volume.

**DNA loading dye:** Dissolved in 10 mM Tris (pH 7.6-8.0), 0.03% bromophenol blue, 0.03% xylene cyanol FF, 60% glycerol (autoclaved), 60 mM EDTA (pH 8.0) in 10 ml water.

**dNTPs:** Prepared mixture of 10 mM of dATP, dCTP, dGTP and dTTP. Mixed well and stored at -20°C.

**EDTA (0.5 M):** Added 400 ml of DEPC water (unautoclaved) to 93.05 gm EDTA and adjusted pH to 8.0 with the help of 10 N NaOH. EDTA dissolves at a pH of around 8.0 only. Final volume was made to 500 ml with DEPC (unautoclaved) water and autoclaved. For working with DNA, non-DEPC water was used.

**Ethidium bromide:** Dissolved 10 mg of ethidium bromide in 1 ml of ADW by stirring overnight.

**Formaldehyde agarose gel:** For 1.2% agarose gel preparation (50 ml), dissolved 0.6 g agarose in 31.1 ml water by boiling. Readjusted volume to 31.1 ml and cooled down to 60°C. Added 5 ml 10X MOPS buffer and 8.9 ml formaldehyde 37% to get a final concentration of 2.2 M.

**Formaldehyde gel loading buffer:** 1 mM EDTA (pH 8.0); 0.25% (w/v) bromophenol blue; 0.25% xylene cyanol FF; 50% glycerol.

**IPTG:** Dissolved 238.3 mg of IPTG in 6 ml of ADW. Adjusted the volume of the solution to 10 ml with ADW. Mixed well and sterilized it by filtration through a 0.22 μm disposable filter. Dispensed the solution into 1 ml aliquots and stored them at -20°C.

**Kanamycin stock solution:** 25 mg/ml in H2O, sterile filter, store in aliquots at -20°C.

**LB broth (100 ml):** Dissolved 2.5 gm of LB in 100 ml of ADW. Sterilized it by autoclaving and stored at room temperature.
LB plates (100 ml): Dissolved 15 g of agar in 100 ml of ADW. Sterilized it by autoclaving and then cooled it to 50°C. Dispensed into sterile disposable plastic petriplates. Plates were covered and allowed to solidify and dry on the bench-top overnight. Stored inverted at 4°C after covering with aluminium foil.

Lysis buffer: Mix 10 mM Tris-Cl (pH 8.0) and 20 mM EDTA with 0.1% Tween 20.

SDS Gel Loading Buffer (5X): Mix 250 mM Tris-Cl, 500 mM β-Mercaptoethanol, 50% Glycerol, 0.5% Bromophenol blue in 10% SDS.

Sodium acetate (3M): Dissolved 123.05 g of sodium acetate in 300 ml of DEPC (unautoclaved) water and adjusted pH to 5.2 or 5.5 with the help of glacial acetic acid. Made the final volume to 500 ml with DEPC (unautoclaved) water. Sterilized by autoclaving.

Staining solution: Dissolved 0.25% (w/v) coomassie brilliant blue in 90 ml of 50% (v/v) methanol solution. After complete solubilization added 10 ml glacial acetic acid.

Transformation Buffer: Dissolve 10.88 g MnCl₂ 4H₂O, 18.65 g KCl and 2.20 g CaCl₂ 2H₂O in 800 ml of water. Then add 20 ml PIPES (0.5M, pH 6.7). Adjusted the pH to 6.7 with 5 N KOH prior to adding the MnCl₂. Sterilize Inoue transformation buffer by filtration through pre-rinsed 0.45 μm Nalgene filter.

Tris-EDTA (TE; pH 8.0): Mixed 10 mM Tris-Cl and 1 mM EDTA.

Tris-Glycine buffer (5X): Dissolved 15.1 g of Tris base and 94 g glycine in 900 ml of ADW. Added 50 ml of 10% w/v SDS. Final volume was adjusted to 1 L.

Tris-HCl (1M): Added 60.55 g Tris base in 350 ml of DEPC treated autoclaved water. Adjusted pH with 10 N NaOH (7.4, 7.6 or 8.0) depending upon the requirement. Final volume made to 500 ml with DEPC-treated ADW. For purposes other than RNA, ADW was used.

X-GAL: Dissolved 20 mg of X-GAL in 1 ml of dimethylformamide and mixed well. Sterilized it by filtration through a 0.22-μm disposable filter and was stored at –20°C.