Appendices
APPENDIX

Ampicillin: Dissolve 100 mg of ampicillin in 1 ml of autoclaved distilled water and sterilize by filtration through a 0.22-μm filter and stored in small aliquots at -20°C.

Colony lysis buffer: Dissolve TE (10:0.1), 0.1% Tween-20 in 10 ml of ADW.

dNTPs: Prepare mixture of 10 mM of dATP, dCTP, dGTP and dTTP. Mix well and store at -20°C.

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

IPTG: Dissolve 1 g of IPTG in 4 ml of ADW. Adjust the volume of the solution to 5 ml with ADW. Sterilize by filtration through a 0.22-μm filter and stored in small 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 2.5 gm of LB and 1.5 gm of agar in 100 ml of ADW. Sterilized it by autoclaving and then cooled it to 50°C. Dispensed the solution into sterile disposable plastic petriplates. Covered plates and allowed them to solidify on the bench-top. Stored inverted at 4°C after covering with aluminium foil.

Ligation buffer: Mix 66 mM Tris-HCl (pH 7.6), 0.1 mM spermidine, 6.6 mM MgCl₂, 10 mM DTT and 150 mM NaCl in ADW.

NaOH (1 mol L⁻¹): Add 4 g of NaOH in 100 mL autoclaved water.

Phenol:Chloroform:isoamyl alcohol (PCI) (25: 24:1): Equilibrate phenol with 0.1 M Tris-HCL (pH 7.8). Take 25 ml of phenol from lower layer and add 24 ml chloroform and 1 ml isoamylalcohol, respectively. Store it at 4°C in dark.
Phosphate buffer (1M; pH 7.8): Dissolve 174.18 g of K$_2$HPO$_4$ and 136.09 g of KH$_2$PO$_4$ in 1 L of ADW, separately. Titrate K$_2$HPO$_4$ against KH$_2$PO$_4$ until the pH of the solution reaches to 7.8. Autoclave and keep for further use.

Picric acid (10mM) (MW= 229.11 g/mol): Dissolve 0.229 g of picric acid in 100 ml of autoclaved water and adjust pH to 7.0 with the help of 1 mol L$^{-1}$ of NaOH.

RNase A (10 mg/ml): Dissolve 10 mg RNase A in 1 ml of ddH$_2$O containing 10 mM Tris-Cl (pH 7.5) and 15 mM NaCl. Heat for 15 min at 100°C. Cool to RT and store in aliquots at −20°C.

SDS (20%): Dissolve SDS (100 g) in 300 ml ADW. Keep in water bath at 60-65°C to dissolve SDS. Final volume was made to 500 ml.

SOC Media: In 950 ml of dH$_2$O dissolved 20 gm bactotryptone, 5 gm bactoyeast, 0.5 gm NaCl. Added 10 ml of 250mM KCl and adjusted the pH to 7 with 5N NaOH. Adjusted the volume to 1 L and sterilized by autoclaving. Before use added 5 ml of sterile solution of 2 M MgCl$_2$ and 20 ml of sterile 1M solution of glucose (20mM).

Sodium acetate (3 M) (MW= 82.03): Dissolve 123.05 g of sodium acetate in 300 ml of autoclaved water and adjust pH to 5.2 or 5.5 with the help of glacial acetic acid. Make final volume to 500 ml with autoclaved water.

TAE buffer (50X) (1X- 40 mM Tris-acetate, 1 mM EDTA): Dissolve 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 of ADW.

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

Tris-HCl (1 M) (MW= 121.1): Add 60.55 g Tris base in 350 ml of DEPC treated autoclaved water. Adjust pH with 10 N NaOH (7.4, 7.6, or 8.0) depending upon the requirement. Make final volume to 500 ml with DEPC-treated ADW. For other purposes than RNA use ADW.
**X-gal**: Dissolve 10 mg of X-gal in 1 ml of dimethylformamide. Sterilize by filtration through a 0.22-µm filter. Store in small aliquots at -20°C.

**TB**: 10 mM Pipes, 55 mM MnCl₂, 250 mM KCl, 15 mM CaCl₂. Adjusted the pH to 6.7 with 5N KOH prior to adding the MnCl₂. Sterilized by filtration using 0.22 µm filter.