Ampicillin (100 mg/ml): weighed 100.0 mg ampicillin, dissolved in sterile water to a volume of 1.0 ml, sterilized by filtration through a 0.22 μm filter and stored in small aliquots at -20°C.

DEPC-treated water: added DEPC to a final concentration of 0.1% to distilled water, mixed well, incubated overnight at room temperature and autoclaved.

6X DNA loading dye: 30% Glycerol; 0.25% Bromophenol blue; 0.25% Xylene cyanol in autoclaved distilled water.

10X DNase I reaction buffer: 100 mM Tris-Cl (pH 8.4); 500 mM potassium chloride (KCl); 15 mM Magnesium chloride (MgCl₂); 0.01% Gelatin.

RNase A (10 mg/ml): Dissolved 10.0 mg RNase A (from 20 mg/ml stock) in 1 ml of sterile water containing 10 mM Tris-Cl (pH 7.5) and 15 mM NaCl. Heated for 15 min at 100 °C, cooled to room temperature and stored in aliquots at -20°C.

Ethidium bromide: stock 10 mg/ml in autoclaved distilled water.

EDTA (0.5 M): added 93.05 g EDTA to 400 ml of DEPC treated water and adjust pH to 8.0 with the help of 10 N NaOH. Made final volume to 500 ml with DEPC treated water. Sterilized the solution by autoclaving. For working with DNA, plain deionized water was used.

Formaldehyde gel loading dye: 1 M EDTA (pH 8.0); 0.25% Bromophenol 0.25% Xylene cyanol FF blue; 50% Glycerol.

Kanamycin (100 mg/ml): dissolved 100.0 mg kanamycin sulphate in 1.0 ml of sterile water, sterilized by filtration through a 0.22 μm filter and store in small aliquots.

Luria Broth (LB) medium (for 1.0 L): 10.0 g Bactotryptone; 5.0 g Bacto-yeast extract; 10.0 g NaCl.

LB agar plates (for 1.0 L): 10.0 g Bacto-tryptone; 5.0 g Bacto-yeast extract; 10.0 g NaCl; 15.0 g Bacto-agar. Sterilized by autoclaving and then cooled to 55 to 60°C in water bath. Dispensed into sterile disposable plastic petri plate on a laminar flow bench, covered the plates and allowed to solidify. The plates were dried overnight in an incubator at 37°C and stored inverted at 4°C after being covered with aluminium foil.

5X MOPS buffer: dissolved 20.9 g MOPS and 13.3 ml 3M sodium acetate solution in DEPC-treated autoclaved water, adjusted pH to 7.0, added 10.0 ml 0.5 M EDTA (pH, 8.0), made up the volume to 1.0 l and filter through 0.2 μm filter paper.

Murashige Skoog (MS) medium (Murashige and Skoog, 1962)
Preparation of stock solutions:

(a) Micronutrient stock: 70 mM boric acid (H₃BO₃), 14 mM Manganese chloride (MnCl₂). 0.5 M Copper sulphate (CuSO₄), 1 mM Zinc sulphate (ZnSO₄), 10 mM Sodium
Chloride (NaCl), 0.2 mM Sodium molybdate (NaMo₄) and 0.01 mM Cobaltous chloride (CoCl₂). Dissolved the chemicals in series in deionized water and adjusted the final volume to 100 ml.

(b) Macronutrient stocks: 1 M CaCl₂, 1 M Magnesium sulphate (MgSO₄), 1 M potassium nitrate (KNO₃), 1 M Ammonium nitrate (NH₄NO₃), 1 M, potassium di-phosphate (KH₂PO₄; pH 5.6), 1 M FeEDTA.

(c) Preparation of Fe-EDTA: added 2.5 g ferric sulphate (FeSO₄) and 3.36 g NaEDTA in 400 ml deionized water. Set pH to 5.5 to 6.0 with NaOH and adjusted final volume to 500 ml.

Preparation of 1X MS: for the preparation of 1.0 L solution, added 4.0 ml of CaCl₂, 1.5 ml of MgSO₄, 19.0 ml of KNO₃, 20.6 ml of NH₄NO₃, 1.25 ml KH₂PO₄, 2.5 ml FeEDTA, and 1.0 ml from micronutrient stock to 800 ml of deionized water. Set pH to 5.6 to 5.8 with KOH and adjusted the final volume to 1.0 L.

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

Phenol: melted solid phenol at 68°C. Added 0.1% of hydroxyquinoline and equal volume of 0.5 M Tris-Cl (pH 8.0). Stirred the mixture on a magnetic stirrer for 15 min. Allowed to stand and collect the lower phase. Equilibrated again with 0.1 M Tris-Cl (pH 8.0). Aspirate the lower phase. Add 0.1 volume of 0.1 M Tris-Cl (pH 8.0) containing 0.2% beta-mercaptoethanol and stored in dark colored bottle at 4°C.


Rifampicin (25 mg/ml): dissolved 25.0 mg rifampicin in 1.0 ml of ethanol and store in small aliquots.

RNase A (10 mg/ml): dissolved 10.0 mg RNase A in 1.0 ml of 10 mM Tris-Cl (pH 7.5) solution containing 15 mM NaCl. Heat for 15 min at 100°C cooled to room temperature and stored in aliquots at -20°C.

Solution for RNA isolation:

Extraction buffer I (Ghawana et. al., 2011): phenol saturated with Tris buffer to a pH of 6.7±0.2 (Sigma-Aldrich Inc., USA). To phenol, SDS; 0.1% (w/v), sodium acetate [NaOAc; 0.32 M (w/v)] and EDTA (0.01 M final concentration from a stock solution of 0.5 M, pH 8.0) was added.
3 M sodium acetate: dissolved 123.0 g of sodium acetate in 300 ml of DEPC-treated water (unautoclaved) and adjusted pH to 4.8, 5.2 or 5.5 with glacial acetic acid. Made final volume to 500 ml with DEPC-treated water (un-autoclaved) following by autoclaving the solution. For working with DNA, plain deionized water was used.

Extraction buffer II (Chang et al. 1993) consisted of 2% (w/v) cetyltrimethylammonium bromide, 2% (w/v) polyvinylpyrrolidone (PVP), 100 mM (hydroxymethyl)aminomethane [Tris-HCl (pH 8.0) (used from a stock of 1 M Tris-HCl; pH, 8.0)], 25 mM ethylenediaminetetra acetic acid [EDTA (used from a stock of 0.5 M; pH, 8.0)], 2 M sodium chloride [NaCl (used from a stock of 5 M)]. All these five components were mixed and autoclaved. Beta-mercaptoethanol (2% final concentration) and filter sterilized spermidine (0.5 g/l) were added just before use. Other reagents and solutions included: suspension sodium Tris buffer (SSTE buffer consisting of 1 M NaCl, 10 mM Tris-HCl and 1 mM EDTA).

CIA [24:1 (v:v)]: add 1 ml isoamyl alcohol into 24 ml chloroform.

10% LiCl: dissolve 10 g LiCl in 100 ml DEPC treated autoclaved water.

CTAB-based DNA isolation protocol

Extraction buffer (Chang et al. 1993): consisted of 2% (w/v) cetyltrimethylammonium bromide, 2% (w/v) polyvinylpolypyrrolidone (PVPP), 100 mM (hydroxymethyl)aminomethane [Tris-HCl (pH 8.0) (used from a stock of 1 M Tris-HCl; pH, 8.0)], 25 mM ethylenediaminetetra acetic acid [EDTA (used from a stock of 0.5 M; pH, 8.0)], 2 M sodium chloride [NaCl (used from a stock of 5 M)]. All these five components were mixed and autoclaved. Beta-mercaptoethanol (2% final concentration) was added just before use.

50X TAE buffer: added 242.1 g Tris-base; 57.1 ml glacial acetic acid; 100 ml of EDTA (0.5 M; pH 8.0) in a total volume of 1 L sterile water.

1 M Tris-HCl: added 60.55g Tris base in 350 ml of DEPC treated autoclaved water. Adjust the pH to 7.4, 7.6 and 8.0 with 1 N HCl or 10 N NaOH depending upon the requirement and make up the volume to 500 ml with DEPC treated autoclaved water. For working with DNA use sterile water.

Tris-EDTA buffer (TE; pH 8.0): Tris-EDTA contains 10 mM Tris-HCl and 1 mM EDTA (pH 8.0) in sterile water.

X-Gal: dissolved 10.0 mg of X-Gal in 1.0 ml of dimethyl formamide and store in small aliquot at -20°C.
Appendix II

**YEM broth (1 L):** Dissolved 0.50 g of K2HPO4, 0.40 g of yeast extract, 10.0 g of mannitol, 0.20 g of MgCl2 and 0.10 g of NaCl in 1 L of ADW. Adjusted pH to 7.0. Sterilized by autoclaving and stored at RT.

**YEM-agar plates (1 L):** Dissolved 0.50 g of K2HPO4, 0.40 g of yeast extract, 10.0 g of mannitol, 0.20 g of MgCl2 and 0.10 g of NaCl in 1 L of ADW. Adjusted pH to 7.0 and 1.8% agar was added. Sterilized by autoclaving and then cooled to 50 °C in water bath. Appropriate filter sterilized antibiotics were added. Dispensed into sterile disposable plastic petri plates inside laminar flow cabinet. Media in the plates was allowed to solidify, covered and allowed to dry on the benchtop overnight. Stored inverted at 4 °C after covering with aluminium foil.

**YE-agar plate (1.0 L):** dissolved 0.50 g of K2HPO4, 0.40 g of yeast extract, 0.20 g of MgCl2 and 0.10 g of NaCl in 1 L of water. Adjusted pH to 7.0 and 1.8 % agar was added, sterilized by autoclaving, and then cooled to 50°C in water bath. Dispensed into sterile disposable plastic petri plates inside laminar flow cabinet. Media in the plates was allowed to solidify, covered and allowed to dry on the bench top overnight. Stored inverted at 4°C after covering with aluminium foil.

**Betaine (5M):** The addition of betaine is reported to reduce the formation of secondary structure in GC-rich regions by eliminating the base pair composition dependence of DNA melting. Prepare the betaine as follows:

1. Placed 400 ml water into a large beaker, added 585.75 g betaine in ~50 g batches while mixing with a magnetic stirrer.
2. Stirred until the betaine was completely dissolved and incubated at 37°C for 1 h.
3. Adjusted the volume to 1 l with water, filtered the solution with a 0.2 μm cellulose acetate filter and stored at -20°C.

**Elution buffer:** 10 mM Tris-Cl (pH 8.5).

**Resuspension buffer:** 100 ml 0.5 X MS, 5 μl BAP (10 mM stock), MES (0.5 g/l), 2 % sucrose and Silwet-77 (200 μl/l).