Appendices

Appendix-I

Preparation of reagents and other chemicals

All the reagents were prepared in type-I water. Sterilization by autoclaving was done at 15 lbs pressure, 121°C, 15 min.

1 M Tris-HCl, pH 8.0	121.14 g of Tris powder (Sigma) was added in 600 mL of water and pH was adjusted to 8.0 with conc. HCl. The volume was made up to 1000 mL using water and sterilized by autoclaving.
0.5 M EDTA, pH 8.0	186.12 g of EDTA powder (Amresco) was added in 600 mL of water and pH was adjusted to 8.0 with NaOH. The volume was made up to 1000 mL using water and sterilized by autoclaving.
1X TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA)	10 mL of 1 M Tris-HCl, pH 8.0 and 2 mL of 0.5 M EDTA, pH 8.0 were added to 800 mL of water and the volume was finally made up to 1000 mL using water.
2 M $MgCl_2$	19.04 g of $MgCl_2$ (Sigma) was dissolved in 70 mL of water and the volume was made up to 100 mL with type-I water. The reagent was sterilized by autoclaving.
1 M $MgSO_4$	12.03 g of $MgSO_4$ (Sigma) was dissolved in 70 mL of water and the volume was made up to 100 mL with water. The reagent was sterilized by autoclaving.
5 M NaCl	29.22 g of NaCl (Amresco) was dissolved in 50 mL of water and the volume was made up to 100 mL with water. The reagent was sterilized by autoclaving.
10 N NaOH	40 g of NaOH (Amresco) was dissolved in 50 mL of water and the volume was made up to 100 mL with water. The reagent was sterilized by autoclaving.
10% SDS	10 g of SDS (Sigma) was dissolved in 50 mL of warm water and the volume was made up to 100 mL with water.
IPTG	23.8 mg of IPTG (Sigma) was dissolved in 1 mL water and filter sterilized using 0.2 micron syringe filter (Fisher

	Scientific).
X- Gal	80 mg of X-Gal (Sigma) was dissolved in 1 mL dimethylformamide (Qualigens) and filter sterilized using 0.2 micron syringe filter.
70% Ethanol	70 mL of ethanol (Amresco) was added to 30 mL of water and mixed well.
dNTPs (2.5 mM each)	10 µL of each dNTP (100 mM each of ATP, GTP, CTP, TTP) (Fermentas) were added to the volume of 40 µL and then diluted to 400 µL using sterile water.
1 M Glucose	18.01 g of glucose (Sigma) was dissolved in 70 mL of water and the volume was made up to 100 mL with water. The reagent was filter sterilized using 0.2 micron syringe filter.
50% Glycerol	50 mL of glycerol (Sigma) was added to 50 mL of water and mixed well. The reagent was sterilized by autoclaving.
Primer stock solution	The lyophilized oligos were dissolved in 1X TE to get a final concentration of 1 nmol/µL stock solution. The primer working solution was made by diluting (about 20X) the above stock using sterile water.
PNA working stock	The lyophilized PNA was dissolved in sterile water to get a final concentration of 100 µM stock solution and stored as aliquots in -20°C.
Washing buffer (for preparation Electro-competent cells)	50 mL of glycerol was dissolved in 450 mL of water and sterilized by autoclaving.
0.1M $CaCl_2$ solution	14.702 g of $CaCl_2$ dihydrate powder (Aldrich) was dissolved in a final volume of 1 L water and sterilized by autoclaving.
0.1M $CaCl_2$ +20% glycerol solution	14.702 g of $CaCl_2$ dihydrate powder and 200 mL glycerol were dissolved in water and the volume was made up to 1 L using water. The buffer was sterilized by autoclaving.

Appendix-II

Preparation of Media

All the media were prepared in type-I water. Sterilization by autoclaving was done at 15 lbs pressure, 121°C, 15 min.

Luria-Bertani (LB) Broth	25 g LB powder (Himedia) was dissolved in 1 L water and the dissolved medium was checked for its pH 7.5 ± 0.2. The medium was sterilized by autoclaving.
Luria-Bertani (LB) Agar	40 g LB agar powder (Himedia) was dissolved in 1 L water and the dissolved medium was checked for its pH 7.5 ± 0.2. The content was boiled to dissolve the medium completely. The medium was sterilized by autoclaving, cooled to 55°C and poured into sterile Petri plates.
Mueller-Hinton Broth (MHB)	22 g MHB powder (Himedia) was dissolved in 1 L water and the dissolved medium was checked for its pH 7.3 ± 0.2. The medium was sterilized by autoclaving.
Mueller-Hinton Agar (MHA)	38 g MHA powder (Himedia) was dissolved in 1 L water and the dissolved medium was checked for its pH 7.3 ± 0.2. The content was boiled to dissolve the medium completely. The medium was sterilized by autoclaving, cooled to 55°C and poured into sterile Petri plates.
Thiosulfate-Citrate-Bile salts-Sucrose (TCBS) Agar	89 g TCBS agar powder (Difco) was dissolved in 1 L water and the dissolved medium was checked for its pH 8.6 ± 0.2. The content was boiled to dissolve the medium completely. As there was no need to autoclave this medium, it was cooled to 55°C and directly poured into sterile Petri plates.
MacConkey Agar	50.03 g MacConkey agar powder (Himedia) was dissolved in 1 L water and the dissolved medium was checked for its pH 7.1 ± 0.2. The content was boiled to dissolve the medium completely. The medium was

	sterilized by autoclaving, cooled to 55°C and poured into sterile Petri plates.
Xylose-Lysine Deoxycholate (XLD) agar	56.68 g XLD agar powder (Himedia) was dissolved in 1 L water and the dissolved medium was checked for its pH 7.4 ± 0.2. The content was boiled to dissolve the medium completely. As there was no need to autoclave this medium, it was cooled to 55°C and directly poured into sterile Petri plates.
Hektoen Enteric Agar (HEA)	72.66 g HEA powder (Himedia) was dissolved in 1 L water and the dissolved medium was checked for its pH 7.5 ± 0.2. The content was boiled to dissolve the medium completely. As there was no need to autoclave this medium, it was cooled to 55°C and directly poured into sterile Petri plates.
Triple Sugar Iron (TSI) Agar	64.02 g TSI agar powder (Himedia) was dissolved in 1 L water and the dissolved medium was checked for its pH 7.4 ± 0.2. The content was boiled to dissolve the medium completely. The medium was sterilized by autoclaving, cooled to 55°C and poured into sterile test tubes to set the medium in sloped form with a butt about 1 inch long.
Super Optimal Broth (SOB) media	20 g of Tryptone (Difco), 5.0 g of yeast extract (Himedia), 0.584 g of NaCl (Amresco) and 0.186 g KCl (Fluka) were added to 700 mL water and the pH was adjusted to 7.0. The volume was made up to 1000 mL and sterilized by autoclaving.
Super Optimal Broth with Catabolite repression (SOC) media	2 mL of 1 M Glucose (sterile), 0.5 mL of 2 M $MgCl_2$ (sterile) and 1 mL of 1 M $MgSO_4$ (sterile) were added to 100 mL of SOB medium (sterile) at aseptic condition.

Appendix-III

Preparation of reagents for agarose gel electrophoresis

All the reagents were prepared in type-I water. Sterilization by autoclaving was done at 15 lbs pressure, 121°C, 15 min.

50 X TAE, pH 8.3	242 g of Tris and 18.6 g of EDTA were dissolved in 500 mL of water. 57.2 mL of glacial acetic acid (Merck) was added to the solution to mix well and the pH was adjusted to 8.3. The solution was finally made up to 1000 mL and sterilized by autoclaving.
1X TAE (40 mM Tris, 20 mM acetic acid and 1mM EDTA,	20 mL of 50X TAE was diluted using water to the final volume of 1000 mL.
EtBr (10 mg/mL)	1 g Ethidium Bromide (Sigma) was dissolved in 100 mL of type-I water in a dark bottle and stored at 4°C.
6X Dye (10mM Tris-HCl, 0.03% Bromophenol blue, 0.03% Xylene cyanol FF, 60% Glycerol, 60 mM EDTA)	500 µL of 1M Tris-HCl, pH 8.0, 15 mg of Bromophenol blue (Sigma), 15 mg of Xylene cyanol FF (Sigma), 30 mL of glycerol and 6.02 mL of 0.5 M EDTA, pH 8.0 were added and then volume was made up to 50 mL with water.

Appendix-IV

Genomic DNA isolation

All the reagents were prepared in type-I water. Sterilization by autoclaving was done at 15 lbs pressure, 121°C, 15 min.

10% CTAB	10 g of hexadecyl trimethyl ammonium bromide (CTAB) (Sigma) was dissolved in 100 mL of 0.7 M NaCl.
0.7M NaCl	14 mL of 5 M NaCl was dissolved in 86 mL of pure water and sterilized by autoclaving.
RNase A	10 mg of Ribonuclease A (from bovine pancreas) powder (Sigma) was dissolved in 1 mL of sterile 0.01 M Tris-HCl, pH 8.0.
Proteinase K	20 mg proteinase K enzyme powder (Amresco) was dissolved in 1 mL of sterile water.
Chloroform: Isoamyl alcohol (24:1)	1 mL of isoamyl alcohol (Sigma) was added to 24 mL of chloroform (Amresco) and mixed well. Reagent was stored in a dark bottle.
Phenol:Chloroform: Isoamyl alcohol (25:24:1)	25 mL of phenol (saturated with 10 mM Tris-HCl, pH 8.0 and 1 mM EDTA) (Sigma) was added to 25 mL of chloroform:isoamyl alcohol mix (24:1) and the contents were mixed well by shaking. Reagent was stored in a dark bottle.

Appendix-V

Preparation of reagents for plasmid DNA isolation

All the reagents were prepared in type-I water. Sterilization by autoclaving was done at 15 lbs pressure, 121°C, 15 min.

Solution P 1 (50 mM Tris-HCl, 10 mM EDTA, 100 µg/mL RNase A)	5 mL of 1 M Tris-HCl (pH 8.0), 2 mL of 0.5 M EDTA (pH 8.0) and 1.0 mL of 10 mg/mL RNase A stock were mixed and the volume was made up to 100 mL using water.
Solution P 2 (0.2 N NaOH, 1% SDS)	2 mL of 10 N NaOH and 10 mL of 10% SDS were mixed and the volume was made up to 100 mL using water.
Solution P 3 (2.5 M potassium acetate, pH 4.8)	60 mL of 5 M potassium acetate and 11.5 mL of glacial acetic acid was mixed and the final volume was made up to 100 mL using water.
5 M Potassium acetate	49.07 g of Potassium acetate (Amresco) was dissolved in 70 mL of water and the volume was finally made up to 100 mL. The solution was sterilized by autoclaving.

Appendix-VI

Preparation of antibiotic stock solution

Ampicillin	100 mg of ampicillin sodium salt (Himedia) was dissolved in 1 mL of type-I water. The solution was filter sterilized using 0.2 micron syringe filter and stored as aliquots at -20^{o}C.
Kanamycin	100 mg of kanamycin acid sulphate powder (Himedia) was dissolved in 1 mL of type-I water. The solution was filter sterilized using 0.2 micron syringe filter and stored as aliquots at -20^{o}C.
Nalidixic Acid	10 mg of nalidixic acid (Sigma) was dissolved in 1 mL of 0.1 N NaOH. The solution was filter sterilized using 0.2 micron syringe filter and stored as aliquots at -20^{o}C.
Norfloxacin	1 mg/mL of norfloxacin powder (Fluka) was dissolved in 1 mL of glacial acetic acid. The solution was filter sterilized using 0.2 micron syringe filter and stored as aliquots at -20^{o}C.
Ciprofloxacin	1 mg of ciprofloxacin hydrochloride powder (Himedia) was dissolved in 1 mL of type-I water. The solution was filter sterilized using 0.2 micron syringe filter and stored as aliquots at -20^{o}C.
Ofloxacin	1 mg of ofloxacin powder (Sigma) was dissolved in 1 mL of type-I water having pH 4 (adjusted using glacial acetic acid). The solution was filter sterilized using 0.2 micron syringe filter and stored as aliquots at -20^{o}C.
Levofloxacin	1 mg of levofloxacin powder (Fluka) was dissolved in 1 mL of type-I water. The solution was filter sterilized using 0.2 micron syringe filter and stored as aliquots at -20^{o}C.
Sparfloxacin	1 mg of sparfloxacin (Fluka) was dissolved in 1 mL of 0.1 N NaOH. The solution was filter sterilized using 0.2 micron syringe filter and stored as aliquots at -20^{o}C
Moxifloxacin	10 mg of moxifloxacin hydrochloride (Sigma) was dissolved in 1 mL of 0.1 N NaOH. The solution was filter sterilized using 0.2 micron syringe filter and stored as aliquots at -20^{o}C

Appendix-VII

Preparation of SDS-PAGE reagents

All the reagents were prepared in type-I water. Sterilization by autoclaving was done at 15 lbs pressure, 121°C, 15 min.

30% Acrylamide	290 g of acrylamide (MP biomedicals) and 10 g of N, N'-Methylene-bis-acrylamide (MP biomedicals) were added in 600 mL of water. The content was dissolved by mildly heating the solution. The volume was finally made up to 1 L. The solution was filter sterilized using 0.2 micron syringe filter and stored in a dark bottle at the refrigerator.
1.5 M Tris-HCl, pH 8.9	18.17 g of Tris powder (Sigma) was added in 60 mL of water and pH was adjusted to 8.9 with conc. HCl. The volume was made up to 100 mL using water and sterilized by autoclaving.
1 M Tris-HCl, pH 6.8	12.114 g of Tris powder was added in 60 mL of water and pH was adjusted to 6.8 with conc. HCl. The volume was made up to 100 mL using water and sterilized by autoclaving.
0.25 M EDTA	50 mL of 0.5 M EDTA, pH 8.0 was diluted in 50 mL of water and sterilized by autoclaving.
10% APS	1 g of Ammonium persulfate (MP biomedicals) was dissolved in 10 mL of water and saved as aliquots in 4°C.
4X sample buffer	10 mL of β- mercaptoethanol (Sigma), 22.4 mL of glycerol, 1.4 mL of 1M Tris-HCl, pH 6.8, 6 g of SDS and 0.02 g bromophenol blue were dissolved in 25 mL of water and the final volume was made up to 50 mL.
1X SDS running buffer	3 g of Tris powder, 14.4 g of glycine (Merck) and 1 g of SDS were dissolved in 750 mL of water and the final volume was made up to 1L.
Coomassie blue stain	2.5 g of Coomassie blue R-250 (Propure), 92 mL of acetic acid and 450 mL of methanol (Nice) were added to water and the final volume was made up to 1 L.
Destaining solution	50 mL of acetic acid and 100 mL of methanol were added to 350 mL of water and mixed well.

Appendix-VIII

Preparation of reagents for RNA isolation

All the reagents were prepared in type-I water. Sterilization by autoclaving was done at 15 lbs pressure, 121°C, 15 min.

DEPC treated water	1 mL of Diethylpyrocarbonate (Amresco) was added to 1 L of water and mixed vigorously. The solution was incubated at 37°C for 12 h and autoclaved for 15 min to remove any trace of DEPC.
10X FA gel buffer (200 mM MOPS, 50 mM Sodium acetate and 10 mM EDTA)	10.463 g of 3-[N-morpholino] propane sulfonic acid (MOPS) (Sigma), 1.02 g of sodium acetate (Sigma) and 0.93 g of EDTA were dissolved in 200 mL of DEPC treated water and the pH was adjusted to 7.0 using NaOH. The final volume was made up to 250 mL using DEPC treated water.
1X FA gel buffer	100 mL of 10X FA gel buffer and 20 mL of 37% formaldehyde (Sigma) were added to 880 mL of DEPC treated water and mixed well.
5X RNA loading dye	16 µL of saturated aqueous bromophenol blue solution, 80 µL of 0.5 M EDTA (pH 8.0), 720 µL of 37% (12.3 M) formaldehyde, 2 mL of 100% glycerol, 3.084 mL of formamide and 4 mL of 10X FA gel buffer were added and the final volume was made up to 10 mL using DEPC treated water.