APPENDIX-I

1) **Sucrose (1M):** Dissolved 3.41gms of sucrose in 10ml of deionised water and sterilized by autoclaved.

2) **Magnesium chloride (MgCl₂)(100mM):** Dissolved 0.41gms of MgCl₂ in 20ml of deionised water and sterilized by autoclaved.

3) **TritonX-100 (10%):** Taken 100µl of TritonX-100 and mixed with 900µl of deionised water and mixed properly and then filtered sterilized by passing through a 0.22µm filter.

4) **100mM Tris Cl (pH 8.0):** Dissolved 0.32gms of Tris Cl in 10ml of deionised water, then adjusted the pH to 8.0 by 1M Sodium hydroxide. Sterilize the solution by autoclaving.

5) **100mM Tris Cl (pH 7.5):** Dissolved 0.32gms of Tris Cl in 10ml of deionised water, then adjusted the pH to 7.5 by 1M Sodium hydroxide. Sterilize the solution by autoclaving.

6) **10% SDS:** Dissolved 1gm of SDS in 10ml of deionised water, and then filtered sterilized by passing through a 0.22µm filter.

7) **0.5M EDTA:** Dissolved 9.306gm of disodium salt of EDTA in 20ml of deionised water, and then adjusted the pH to 8.0 by 1M Sodium hydroxide. Sterilize the solution by autoclaving.

8) **5M Sodium chloride (NaCl):** Dissolved 5.85gm of sodium chloride in 20ml of deionised water. Sterilize the solution by autoclaving.
9) **20mg/ml Pronase:** Dissolved 20mg of Pronase in 898μl of deionsed sterile water, 100μl of Tris Cl (pH 8.0) and 2μl of 5M sodium chloride. The solution was kept at 37°C for 1hour then stored in −20°C.

10) **TE buffer (pH 8.0):** Added 1ml of 100mM TrisCl (pH 8.0) and 200μl of 0.5M EDTA solution to 8.8ml of deionsed water. Sterilize the solution by autoclaving.

11) **50X TAE Buffer:** Dissolved 24.2gms of Tris base in 84.29ml of deionsed water, properly mixed the solution and then added 10ml of 0.5M EDTA and 5.71ml of glacial acetic acid. Sterilize the solution by autoclaving.

12) **1mg/ml BSA:** Dissolved 100mg of BSA in 100ml of deionsed sterile water and kept at 4°C overnight. Filtered sterilized the solution by passing through a 0.22μm filter and stored in −20°C.

13) **Ethidium Bromide (10mg/ml):** Dissolved 1gm of ethidium bromide in 100ml of water. Stir the solution in a magnetic stirrer. Wrapped the bottle with aluminum foil and stored at 4°C.

14) **50%DMSO:** Mixed 50ml of 100%DMSO in 50ml of deionsed sterile water, and filtered sterilized the solution by passing through a 0.22μm filter and stored in −20°C.

15) **6X Loading buffer solution:** Dissolved 0.25% of bromophenol blue, 0.25% xylene cyanolFF in 40% sucrose and stored the solution at 4°C.

16) **5X TBE buffer:** Dissolved 54gm of Tris base and 27.5gm of boric acid in 980ml of triple distilled water and then added 20ml of 0.5M EDTA. Sterilize the solution by autoclaving.
17) **β-Mercaptoethanol (ME):** Obtained as a 14.4M solution and stored at 4°C.

18) **100mM PMSF:** Dissolved 17.4mg of PMSF in 1ml of Isopropanol and filtered sterilized the solution by passing through a 0.22μm filter and stored in −20°C.

19) **1%CHAPS:** Dissolved 0.1gm of CHAPS in 1ml of deionized sterile DEPC water. Filtered sterilized the solution by passing through a 0.22μm filter and stored in −20°C.

20) **10mM EGTA:** Dissolved 0.38gm of EGTA in 100ml of deionized sterile DEPC water. Filtered sterilized the solution by passing through a 0.22μm filter and stored in −20°C.

21) **1M DTT:** Dissolved 3.09gm of DTT in 20ml of 0.01M Sodium acetate (pH 5.2). Filtered sterilized the solution by passing through a 0.22μm filter and stored in 4°C.

22) **1M Sodium hydroxide (NaOH):** Dissolved 2gm of sodium hydroxide pellet in 50ml of deionized sterile water.

23) **10% Tween-20:** Taken 100μl of Tween-20 and mixed with 900μl of deionized DEPC water and mixed properly and then filtered sterilized by passing through a 0.22μm filter.

24) **10% Glycerol:** Mixed 10ml of 100% glycerol in 90ml of deionized sterile water, and filtered sterilized the solution by passing through a 0.22μm filter and stored in −20°C.
25) **Hypertonic solution**: Dissolved 0.4g KCl, 5.795g Na$_2$HPO$_4$·12H$_2$O and 0.4g KH$_2$PO$_4$ to 1L DEPC water. Sterilized the solution by autoclaving and then filtered the solution by passing through a 0.22μm filter and stored in 4°C.

26) **Hypotonic solution**: Dissolved 1.2114g Tris base, 0.7456g KCL and 0.2033g MgCl$_2$ to 1L DEPC water. Sterilized the solution by autoclaving and then filtered the solution by passing through a 0.22μm filter and stored in 4°C.

27) **SSCP solution**: Mixed 95% Formamide, 10mM EDTA, 5mM NaOH 0.01% xylene cyanolFF and 0.01% bromophenol blue.

28) **10mg/ml Sodium Thiosulphate solution**: Dissolved 100mg of sodium Thiosulphate in 10ml of triple distilled water and stored at 4°C.

29) **200mg Silver nitrate**: Dissolved 200mg silver nitrate in 200ml of deionized water and stored the solution in a dark brown bottle.

30) **37% Formaldehyde solution**: Mixed 3.7ml of 100% formaldehyde in 6.3ml of deionized water and stored the solution in dark brown bottle.

31) **3% Sodium carbonate**: Dissolved 6gm of sodium carbonate in 200ml of deionized water.

32) **100mM HEPES solution**: Dissolved 2.38gms of HEPES in 100ml of in 100ml of DEPC deionized water. Filtered sterilized the solution by passing through a 0.22μm filter and stored in 4°C.
33) **200mM Potassium chloride (KCL):** Dissolved 0.7456gm of potassium chloride in 100ml of DEPC deionized water. Sterilized the solution by autoclaving and then filtered the solution by passing through a 0.22μm filter and stored in 4°C.

34) **100mM Potassium chloride (KCL):** Dissolved 1.4912gm of potassium chloride in 100ml of deionized water. Sterilized the solution by autoclaving and then filtered the solution by passing through a 0.22μm filter and stored in 4°C.

35) **Polyacrylamide gel (PAGE):** Dissolved 29.29gms of acrylamide and 0.5gms of bis-acrylamide in 100ml of deionized water and then kept on magnetic stirrer until it was dissolved. Filtered sterilized the solution by passing through a 0.22μm filter and stored in dark bottle at 4°C.

36) **1.5M Tris (pH 8.8):** Dissolved 18.15gms of Tris base in 80ml of deionized water, and then adjusted the pH to 8.8 with 6N HCL. Filtered sterilized the solution by passing through a 0.22μm filter and stored at 4°C.

37) **10% Ammonium persulphate (APS):** Dissolved 0.1gm of ammonium persulphate in 900μl of sterile water and stored at 4°C.

38) **Phenol for nucleic acid extraction:** Phenol of molecular biology grade was triple distilled and the pH of the phenol was calibrated with 1M Tris buffer, with the buffer repeatedly changed every few hours till pH is 8.0. Antioxidant like 8-hydroxyquinoline (0.1%) was added to increase the stability of the solution. The phenol was stored in dark brown bottles and kept at 4°C.