Reagents

Red Cell Lysis Buffer (RCLB):
Dissolved the following constituents in 800ml distilled water

- 0.32 M Sucrose  ≡  54.80g
- 1% Triton-X-100  ≡  5ml
- 1mM MgCl₂  ≡  0.51gms
- 12mM Tris  ≡  0.73 gms
Final volume adjusted to 500 ml.

Proteinase K buffer:

- EDTA (0.12mM)  ≡  22.4gm
- Sodium Chloride(0.375)  ≡  11gm
Constituted the volume to 500ml in the double distilled water.

10% SDS (Sodium dodecyl sulphate or Sodium Lauryl sulphate):
Dissolved 100gms of SDS in 900ml of H₂O, heated to 68°C to assist dissolution. Adjusted the pH to 7.2 by adding a few drops of concentrated HCl. Adjusted the volume to 1000 ml with double distilled H₂O.

5M NaCl:
Dissolved 292.2 gms of NaCl in 800ml of H₂O. Adjusted the volume to 1 liter with H₂O, sterilized by autoclaving.

Phenol-Chloroform:
Equal amounts of phenol and chloroform was mixed. The mixture was equilibrated by extracting several times with 0.1M Tris-Cl (pH = 7.6). This
equilibriated mixture was stored under an equal volume of 0.01M Tris-Cl (pH = 7.6) at 4°C in dark glass bottles.

**Phenol-Chloroform: Isoamyl Alcohol (25:24:1):**
A mixture consisting of equal parts of equilibrated phenol and chloroform:isoamyl-alcohol (24:1) was prepared and the mixture was stored under 100mM Tris.Cl (pH 8.0) in a dark brown tight bottle at 4 °C for periods upto one month.

**Bromophenol Blue (Loading Dye) 6x**
0.25% Bromophenol blue ≡ 25mg
40 % Sucrose ≡ 4gms
Above constituents dissolved in 10.0ml of double distilled water.

**Ethidium bromide (10mg/ml):**
Added 100mg of ethidium bromide to 10ml of DDW water, stirred on a magnetic stirrer for several hours to ensure that dye was fully dissolved. Wrapped the container in aluminum foil or transferred the solution to a dark bottle and stored at room temperature.

**0. 5 M EDTA**
186.1gms of EDTA.2H₂O was added to 800ml of double distilled water. It was mixed vigorously on a magnetic stirrer. pH was adjusted to 8.0 with NaOH (~20g of NaOH pellets) Final volume was adjusted to 1000ml by adding remaining DDW, aliquoted and sterilized by autoclaving.
Magnesium Chloride (1M) 500ml

(1M) MgCl\textsubscript{2} 101.6 gm

Dissolve MgCl\textsubscript{2} in double distilled water and autoclaved it.

10X PCR buffer 1.0mM

Supplied by manufacturers (Ms Bangalore Genei, India).

Tris Borate Buffer (5x)

Tris base 54gm
boric acid 27.5gm
5M EDTA (pH 8.0) 20ml

Dissolved into 1000 ml of double distilled water.

10X PCR buffer 1.0mM

Supplied by manufacturers (Ms Bangalore Genei, India).

1 M Tris:

Added 121.4 g Tris (hydroxymethyl Aminomethane) in 800ml DDW. Adjusted the pH to 7.4 or 8.0 with 1N HCl and made up the volume to 1000 ml.