Appendix C:

Chemicals & Reagents

All the chemicals, media and reagents used were of Molecular Biology grade.

❖ 1M Tris: 12.11 g of Tris-base was dissolved in 80 ml water. Desired pH was adjusted with the help of concentrated HCL. The final volume was adjusted to 100 ml with the help of MQ water; pH was adjusted as per requirement and was sterilized by autoclaving.

❖ 0.5 M EDTA (pH 8.0): 18.61 g of Disodium salt of Ethylene diamine tetraacetic acid 2 H2O was added to 80 ml water. Stirred vigorously on a magnetic stirrer. NaOH was used to adjust the pH to 8.0. It was sterilized by autoclaving after making the volume to 100 ml.

❖ 5M NaCl: 29.22 g of sodium chloride was dissolved in 80 ml of water, volume raised to 100 ml with water and sterilized by autoclaving.

❖ Proteinase K: 0.15 g of powdered proteinase K was dissolved in 10 ml of autoclaved MQ water. It was filter sterilized.

❖ 10X Sera Lysis Buffer (50 ml):

\[
\begin{align*}
1M\ Tris\ pH\ 8.0 &= 0.5\ ml \\
0.5\ M\ EDTA &= 1.0\ ml \\
5.0\ NaCl &= 1.5\ ml \\
MQ\ H_2O &= 47.0\ ml
\end{align*}
\]
❖ TE buffer:

10mM Tris (pH 8.0)
1mM EDTA (pH 8.0)

❖ 10X TBE (per litre):

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris</td>
<td>121 g</td>
</tr>
<tr>
<td>Boric acid</td>
<td>61.8 g</td>
</tr>
<tr>
<td>EDTA</td>
<td>9.3 g</td>
</tr>
</tbody>
</table>

❖ Gel Loading buffer:

0.25% bromophenol blue

40% (w/v) Sucrose in water

❖ Proteinase K buffer

1M Tris·HCl (pH 7.4) 10 uL
1M CaCl2 2 uL
MQ H2O 988 uL

❖ Equilibrated Phenol: Phenol was melted at 68°C. 8-Hydroxyquinoline was added to make the final concentration at 0.1%. Then several times extraction was carried out with equal volumes of 0.1 M Tris (pH 8.0) till pH 7.5-7.8 was
reached. Finally two changes in 10mM Tris and 1mM EDTA (pH 8.0) was
given and stored at 4°C after the last change.

❖ Ethidium Bromide: 5 mg/ml stock solution was made in water.

❖ 20% Sodium Dodecyl sulphate (20% SDS): 20 gm of SDS was dissolved in
90 ml of water. Heated at 68°C to assist dissolution. pH was adjusted to 7.2
by adding 1-2 drops of concentrated HCl. Final volume was adjusted to 100
ml.

Molecular biology reagents:
❖ Taq DNA polymerase-PCR amplification consumables were used from
  Perkin Elmer, Promega and MBI Fermentas.
❖ PCR purification and Gel extraction kits were obtained from Qiagen.
❖ DNA ladders and PCR markers were bought from Promega and MBI
  Fermentas.