APPENDIX-I

Preparation of solutions

The various solutions and reagents used in the experiments were prepared as described below:

REAGENTS AND SOLUTIONS:

Ethidium Bromide:
10 mg was dissolved in 1ml of distilled water. It is carcinogenic and hence gloves and mask were worn during preparation.

Loading dye:
Bromophenol Blue- 0.25per cent (w/v)
Glycerol-30per cent (v/v)

RNase A (10mg/ml):
10 mg was dissolved in 1 ml sterile distilled water.

50 X TAE:
Trizma base 242g
Glacial acetic acid 57.1ml
0.5 M EDTA (pH-8.0) 100ml

Tris, acetic acid and EDTA were dissolved in 600ml and the volume was made upto1000 ml.

0.5 M EDTA (pH 8.0):
186.12 g of EDTA was added to 800 ml water and few pellets of NaOH were also added. 10 N NaOH was used. Adjust the pH to 8.0 and the volume was made up to1000 ml.
5 M NaCl:

292.2 g of NaCl was dissolved in 600 ml and the volume was made upto 1000 ml. The solution was sterilized by autoclaving.

10 per cent CTAB:

10 g CTAB was added to 80 ml sterile distil water and left overnight. Next day, volume was made up to 100 ml.

3 M KCl:

223.65 g KCl was dissolved in 600 ml and the volume was made up to 1000 ml. The solution was sterilized by autoclaving.

70 per cent Ethanol:

70 ml absolute alcohol was added to 30 ml of water.

3 M Sodium acetate (pH 7.0)

102 g sodium acetate was added to 150 ml and pH was adjusted to 7.0 with glacial acetic acid and volume was made up to 250 ml.

3 M NaOH:

120 g Sodium hydroxide was dissolved in 700 ml and volume was made up to 1000 ml.

dNTP (2mM):

The working stock was 2mM and it was prepared from 10mM stock solution of dNTP by five fold dilution.

**Extraction Buffer used in the protocol by CTAB:**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris (1M)</td>
<td>20</td>
</tr>
<tr>
<td>EDTA (0.5M)</td>
<td>4</td>
</tr>
<tr>
<td>NaCl(5M)</td>
<td>28</td>
</tr>
<tr>
<td>β-mercaptoethanol</td>
<td>0.2</td>
</tr>
<tr>
<td>CTAB (10 per cent)</td>
<td>20</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>27.8</td>
</tr>
</tbody>
</table>

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100 ml