Annexure I

A. SOLUTIONS FOR DNA ISOLATION

1. Tris saturated phenol
   (a) Melt phenol at 68°C by keeping in water bath.
   (b) Measure the required volume of phenol, add 8-hydroxyquinoline to a final concentration of 0.1% (it is an anti-oxidant gives yellow colour to phenol).
   (c) Add equal volume of 1 M Tris (pH 8.0) and stir on magnetic stirrer for 15 min, allow the two phases to separate and remove the supernatant.
   (d) Repeat the extraction with 0.1 M Tris (pH 8.0) until pH of phenolic phase is greater than 7.6.
   (e) Add 0.2% β-mercaptoethanol.
   (f) Mix thoroughly and store in amber colored bottle at 4°C.

2. Chloroform: isoamyl alcohol preparation (24:1)
   Chloroform 24 mL
   Isoamyl alcohol 1 mL
   Mix thoroughly and store in amber coloured bottle at 4°C.

   Chloroform: Isoamyl alcohol (24:1) 25 mL
   Tris saturated phenol 25 mL
   Mix thoroughly and store in amber color bottle at 4°C.

4. 10 % Sodium Dodecyl sulphate (SDS)
   SDS 10 g
   Autoclaved double distilled water (up to 100 mL)
   Heat at 60°C to dissolve and then store at room temperature (adjust pH 7.2 using Conc. HCl).

5. Proteinase K (20 mg/mL)
   Proteinase K 20 mg
   Autoclaved distilled water 1 mL
   Store at -20°C.
   Proteinase K is highly active of the subtilisin type that is purified from the mold Trirachum album Limber, which has the function of digesting the proteins.
6. **0.5 M EDTA (pH 8.0)**

Disodium ethylene diamine tetra acetate  186.1 g  
Distilled water up to  1000 mL  
Dissolve EDTA in about 800 mL of autoclaved distilled water by keeping it on magnetic stirrer for 1 h, and then adjust the pH to 8.0 by NaOH pellets before making to final volume. Autoclave and store at room temperature.

7. **50X Tris acetate EDTA (TAE) buffer**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris base</td>
<td>242 g</td>
</tr>
<tr>
<td>Glacial acetic acid</td>
<td>57.1 mL</td>
</tr>
<tr>
<td>0.5 M EDTA (pH 8.0)</td>
<td>100 mL</td>
</tr>
</tbody>
</table>

8. **20,000 X Ethidium bromide (10 mg/mL)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethidium bromide</td>
<td>10 mg</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1 mL</td>
</tr>
<tr>
<td>Store at 4°C.</td>
<td></td>
</tr>
</tbody>
</table>

9. **6X Gel-loading dye**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
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<tbody>
<tr>
<td>Bromophenol blue</td>
<td>0.25 %</td>
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<tr>
<td>Sucrose in water</td>
<td>40 % (w/v)</td>
</tr>
<tr>
<td>Store at 4°C.</td>
<td></td>
</tr>
</tbody>
</table>

10. **70% ethanol**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
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</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>70 mL</td>
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<tr>
<td>Distilled water</td>
<td>30 mL</td>
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B. **SOLUTIONS FOR DECELLULARIZATION PROTOCOL**

1. **Phosphate Buffered Saline (PBS)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
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<tbody>
<tr>
<td>NaCl</td>
<td>8.00 g</td>
</tr>
<tr>
<td>KCl</td>
<td>0.20 g</td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>1.44 g</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>0.24 g</td>
</tr>
</tbody>
</table>

Add distilled water to one litre (1000 mL). Adjust pH to 7.4 with HCl. Autoclave it and store at room temperature.
2. **0.0205 % EDTA**
   
   EDTA Na. 2H₂O 0.0256 g  
   Distilled water 1000 mL  
   Store at 4 °C.

3. **0.1 % Sodium azide**
   
   Sodium azide 100 mg  
   Distilled water 100 mL

4. **10 % SDS**
   
   SDS 10 g  
   Autoclaved distilled water 1000 mL  
   Heat at 60°C to dissolve and then store at room temperature. To prepare 0.5 %, 1 %, 2 %, 3 % and 4 % SDS solution, mix prepared 10 % SDS stock solution to required distilled water calculating through, C₁V₁=C₂V₂ formula.

5. **0.25 % trypsin**
   
   Trypsin 250 mg  
   Autoclaved distilled water 100 mL  
   Store at room temperature.

C. SOLUTIONS FOR SEM

1. **2.5 % Gluteraldehyde**
   
   Gluteraldehyde 2.50 g  
   PBS (0.1 M) pH 7.2 100 mL  
   Store at 4 °C.

2. **0.1 M PBS (pH 7)**
   
   KH₂PO₄ 13.62 g  
   Na₂HPO₄.2H₂O 17.80 g  
   Add distilled water to one litre (1000 mL). Mix both solutions in the proportion 1: 1.5 (w/v) and adjust pH to 7 with HCl or NaOH. Store at room temperature.

D. SOLUTIONS FOR ANTI-OXIDATIVE TEST

1. **50 mM PBS (pH 7)**
   
   KH₂PO₄ 6.81 g
Na$_2$HPO$_4$.2H$_2$O 8.90 g
Add distilled water to one litre (1000 mL). Mix both solutions in the proportion 1: 1.5 (w/v) and adjust pH to 7.0 with HCl or NaOH. Store at room temperature.

2. **30 mM Hydrogen peroxide**

30 % H$_2$O$_2$ 0.34 mL
PBS 100 mL

E. SOLUTION FOR FIXATION OF TISSUE

1. **10 % Neutral buffer formalin**

Formalin 10 mL
Distilled water 90 mL
Store at room temperature.