**BIG-DYE SEQUENCING**

<table>
<thead>
<tr>
<th>Big Dye Version 1.1 Terminator RR mix</th>
<th>Aliquots:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applied Biosystems (cat# 4337450) 100rxns</td>
<td>concentrated to read up to 1100bp</td>
</tr>
<tr>
<td></td>
<td>dilute 1:1 to read ~500-600bp</td>
</tr>
<tr>
<td></td>
<td>dilute 1:4 to read ~250 bp</td>
</tr>
</tbody>
</table>

### 2.5X Big Dye Buffer (2.5X BD buffer)

Dye is diluted in buffer:

<table>
<thead>
<tr>
<th>Final concentration:</th>
<th>2.5X BD buffer</th>
<th>10ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>200mM Tris, pH 8.0</td>
<td>Tris, pH 8.0</td>
<td>2 ml</td>
</tr>
<tr>
<td>5mM MgCl₂</td>
<td>5mM MgCl₂</td>
<td>50 µl</td>
</tr>
<tr>
<td>in dH₂O</td>
<td>dH₂O</td>
<td>8 ml</td>
</tr>
<tr>
<td></td>
<td>filter sterilize</td>
<td></td>
</tr>
</tbody>
</table>

1. **Sequence Reaction**

   - Template (0.1ug -1ug / rxn) X µl
   - Primer (3.2 pmol / rxn) X µl
   - Big Dye 4 µl
   - 2.5X BD buffer 4 µl
   - q.s. to 20 µl with dH₂O

2. **Sequence Precipitation**

   1. Add the following to a 1.5ml microfuge tube:
      - 2 µl 3M NaOAC
      - 50 µl 95% EtOH, RT
   2. Add the sequencing reaction to the NaOAC/EtOH mixture and vortex.
   3. Place on ice for 10min (no longer or unincorporated dye will precipitate).
   4. Spin in a microcentrifuge at maximum speed for 15-30min.
   5. Wash with 70% EtOH (room temp).
   6. Vortex and spin for 5min.
   8. Resuspend in 10 µl formamide or store pellet at -20°C.

**Comments:**

1. Residual ethanol and salt will interfere with the sequencing reaction.
2. DNA should not be stored in formamide for long periods of time prior to sequencing (eg. the evening before a morning sequencing is ok but not a couple of days). If the pellets prepared are not to be sequenced immediately, it is best to store them dry at -20°C.