Labeling of microbial community DNA for microarray hybridization

1. **Prepare dNTP mix**: (using 100 mM dNTPs)
   - 5 μl each of dA/G/CTP
   - 2.5 μl of dTTP
   - 82.5 μl DEPC Water

2. **Prepare the DNA/Random primer mixture**:
   - Random primer (Life Technologies, random hexamers, 3 μg/μL) 5.5 μL
   - Target or control DNA 29.5 μL
   - Total volume 35 μL

   (1) Transfer 5.5 μL of Random primer to a PCR tube.

   (2) Transfer 29.5 μL DNA to the Random primer. Usually, 500 ng to 1000 ng of DNA should be used. Adjust volume to 29.5 μL with water; or if the original concentration of a sample is very low, the DNA can be concentrated.

   (3) Mix DNA and Random primer thoroughly and incubate at 99.9 °C for 5 min (using thermocycler)

   (4) Immediately chill tubes on ice.

3. **Prepare labeling premix**

   In a separate tube, for each reaction combine:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X buffer (included with the klenow)</td>
<td>5 μL</td>
</tr>
<tr>
<td>dNTP mix</td>
<td>2.5 μL</td>
</tr>
<tr>
<td>Klenow (imer)</td>
<td>1 μL</td>
</tr>
<tr>
<td>CyDye* (25 nM)</td>
<td>0.5 μL</td>
</tr>
<tr>
<td>H₂O</td>
<td>6 μL</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>15 μL</td>
</tr>
</tbody>
</table>

   * CyDyes are light sensitive – dispense in the dark room. This can be added last

   (1) Transfer 15 μL of the labeling premix to the DNA/Random primer mixture and mix well.

   (2) Incubate the reaction at 37 °C for 6 hrs.

   (3) Heat-inactivate the enzyme by incubating the reaction at 95 °C for 3 min and then cool at 4 °C

   * Labeled DNA can be kept at 4°C in the thermocycler if labeling is done overnight.
4. **Purification**

   (1) Purify labeled DNA with Qiagen QIAquick Kit as specified by the manufacturer;

   (2) Elute the DNA using 100 μL H₂O or EB buffer

   (3) Check CyDye incorporation using Nanodrop

   Minimum dye incorporation: pmol > 50 (pmol/μL * total μL)

   [for example: Labeled DNA eluted with 100 μL of EB buffer and the
   pmol/μL is 0.8; 100*0.8=80 – labeling is fine, proceed with hybridization]

5. **Dry using a Speed Vac**

   Program: Temp. 45 °C, Run 2 hours, Vacuum (Level) 5.1 (preset)

   Press “Auto Run”

   Pellet should be dry at the end of the run.