Method: DIG 3'-end labeling

Protocol: Oligonucleotide 3'-end labeling:

1. Add 100pmol of oligonucleotide to a reaction vial.
2. Bring the volume to 10uL using sterile double distilled water.
3. For the control reaction: add 5uL of control oligonucleotide (vial 5) and 5uL of sterile double distilled water to a reaction vial.
4. On ice, add the following:
   a. 4uL of 5X Reaction Buffer (vial 1)
   b. 4uL of CoCl₂ solution (vial 2)
   c. 1uL of DIG-ddUTP solution (vial 3)
   d. 1uL of Terminal Transferase (400units, vial 4).
5. Mix and centrifuge briefly.
6. Incubate at 37°C for 15mins then place on ice.
7. Stop the reaction by adding 2uL of 0.2M EDTA (pH 8).
8. If you are using the labeled oligonucleotide as a probe in a northern blot, it is not necessary to clean up the reaction before diluting the probe in hybridization buffer.

Protocol: Determining labeling efficiency:

1. Dilute a portion of the labeled oligonucleotide (both the control and your probe) to a starting concentration of 2.5 pmol/uL.
   a. The initial reaction had a theoretical yield of 100 pmol labeled oligo/22uL. Add 2.75uL labeled oligo to 2.25uL Dilution Buffer (vial 9) to produce 5uL of stock at a starting concentrations of 2.5 pmol/uL.
2. Using a 96 well plate, prepare a dilution series for both the control and your labeled oligo according to the following table. All dilutions should be prepared using Dilution Buffer (vial 4) or with TE buffer.

<table>
<thead>
<tr>
<th>Well</th>
<th>Vol of Oligo (uL)</th>
<th>From well #</th>
<th>Vol of Dilution Buffer (uL)</th>
<th>Dilution</th>
<th>Final Conc</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>Original stock (2.5 pmol/uL)</td>
<td>48</td>
<td>1:25</td>
<td>100 fmol/uL</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>1</td>
<td>7</td>
<td>1:3.3</td>
<td>30 fmol/uL</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>1</td>
<td>18</td>
<td>1:10</td>
<td>10 fmol/uL</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>2</td>
<td>18</td>
<td>1:10</td>
<td>3 fmol/uL</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>3</td>
<td>18</td>
<td>1:10</td>
<td>1 fmol/uL</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

3. To a strip of positively charged nylon membrane, apply 1uL spots of each dilution.
4. Fix the labeled oligonucleotide to the membrane by baking for 30 mins at 120°C. (This step can be omitted but detection is significantly less robust without fixation).
5. Follow the protocol for detection of DIG labeled oligo.
6. Compare the intensity of the spots from the labeling reacting to the intensity of the control spots. You can comparatively determine the initial concentration of DIG labeled probe in your original sample.