RT-PCR – Primer Resuspension and Dilution:

Resuspension and O.D. Measurement:

- Resuspend into 100 µl TE
- 2 µl of primer + 78 µl TE
- Measure 80 µl TE as blank and measure primer O.D. at 260
  Calculation as following:
  O. D. Concentration (µg/ml) = O.D.\textsubscript{260} x 37 x Dilution Factor (40)

Final Dilution of Working Concentration for Primer:

Normally, final working concentration for primer is 10 pmol/µl (µM)

Calculate primer concentration as follow

Method 1:

\[
\text{O.D. Concentration (µg/ml)} = x \times 10^{-3} \text{ µg/µl}
\]

Primer Concentration = O.D. Concentration (µg/µl)/ MW
\[
= \frac{\mu\text{mol/µl}}{x} \times 10^6 \text{ pmol/µl (µM)}
\]

Example:

O.D.\textsubscript{260} = 0.325
Molecular Weight = 6214.15

O.D. Concentration = 0.325 x 37 x 40 = 481 µg/ml
Primer Concentration = 481 x 10^{-3}/6214.15 = 77.404 x 10^{-6} µmol/µl
\[
= 77.404 \text{ pmol/µl (µM)}
\]

Method 2:

O.D.\textsubscript{260} = Sum of Extinction Coefficient Contributions x Cuvette Pathlength (1cm) x Primer Concentration / Dilution Factor (40)

Extinction Coefficient Calculation:

<table>
<thead>
<tr>
<th>Chromophore</th>
<th>Extinction Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>15,200</td>
</tr>
<tr>
<td>C</td>
<td>7,050</td>
</tr>
<tr>
<td>G</td>
<td>12,010</td>
</tr>
<tr>
<td>T</td>
<td>8,400</td>
</tr>
</tbody>
</table>

Extinction Coefficient Contributions
\[
= \text{Extinction Coefficient x number of Chromophore}
\]
Example:
A 18 nucleotide primer has 1 of A, 6 of C, 5 of G and 6 of T
O.D.\textsubscript{260} = 0.325

Sum of Extinction Coefficient Calculation as below

<table>
<thead>
<tr>
<th>Chromophore</th>
<th>Extinction Coefficient</th>
<th>Number</th>
<th>Extinction Coefficient Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>15,200</td>
<td>1</td>
<td>15,200</td>
</tr>
<tr>
<td>C</td>
<td>7,050</td>
<td>6</td>
<td>42,300</td>
</tr>
<tr>
<td>G</td>
<td>12,010</td>
<td>5</td>
<td>60,050</td>
</tr>
<tr>
<td>T</td>
<td>8,400</td>
<td>6</td>
<td>50,400</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td><strong>167,950</strong></td>
</tr>
</tbody>
</table>

O.D.\textsubscript{260} = Sum of Extinction Coefficient Contributions (M\textsuperscript{-1}cm\textsuperscript{-1}) x Cuvette Pathlength (1cm) x Primer Concentration (M)/Dilution Factor (40)

\[
0.325 = 167,950 \text{ (M}^{-1}\text{cm}^{-1}) \times 1 \text{ (cm)} \times \text{Primer Concentration (M) / 40}
\]

\[
\text{Primer Concentration} = \frac{0.325 \times 40}{167,950} = 77.404 \times 10^{-6} \text{ M}
\]

= 77.404 \text{ µM}

Working concentration primer:
Primer concentration (µM) x taking volume from original primer (µl)
= Final working concentration (µM) x (H\textsubscript{2}O volume + taking volume from original primer)(µl)

Example:
Take 10 µl from original prime tube
Primer Concentration is 77.404 µM
Final working concentration wants to be 10 µM
Calculate how much autoclaved water has to be added as follow

\[
77.404 \text{ µM} \times 10 \text{ µl} = 10\text{µM} \times (H_2O \text{ Volume (µl)} + 10\text{µl})
\]

\[
H_2O \text{ Volume} = \frac{77.404 \times 10}{10} - 10 = 67.404 \text{ µl}
\]