## PCR Primer Design Guidelines

<table>
<thead>
<tr>
<th>Length</th>
<th>18 to 30 nucleotides</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC Content</td>
<td>40% to 60%</td>
</tr>
</tbody>
</table>
| **Annealing Temperature** | For annealing temperature, use 5°C to 10°C below melting temperature (Tm). Example Tm calculation:  
  \[ Tm \, (^\circ C) = 2( \#A + \#T ) + 4( \#G + \#C ) \]  
  Generally, a Tm between 55°C and 80°C will yield the best results. |
| **Sequence**    | Avoid polybase sequences (3 or more) Gs and Cs at the 3’ end.  
  Avoid mismatches at the 3’ end.  
  Avoid complementary sequences within the primer.  
  Avoid primer–dimer formation by complementarity at 3’ ends of primer pairs. |