Custom Oligo Specifications
Gene Link custom oligonucleotides are supplied desalted and lyophilized. They are ready to use after appropriate reconstitution. Dry oligonucleotides are stable at room temperature for an extended period of time.

Storage & Reconstitution
The oligonucleotide should preferably be frozen upon receipt. TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) is recommended for dissolving the oligonucleotides. After reconstitution store the stock solution at -80°C or -20°C.

Gel Photo Documentation
An actual gel picture of the synthesized custom oligonucleotide is supplied. A major single band represents high purity of the crude oligonucleotide.

Purity & Usage
The crude, desalted oligonucleotide supplied is suitable for all amplification and sequencing protocols. Gel purification is advised for all oligos used for cloning applications and for oligos longer than 50 mer.

Biophysical Data
Each oligo after desalting is quantified by recording A260. Exact nmols and μg is determined by the extinction coefficient and molecular weight of the oligo.

OLIGO SYNTHESIS

Oligo Scale of Synthesis and Typical Yield of Unmodified Oligos*

<table>
<thead>
<tr>
<th></th>
<th>Crude Desalted</th>
<th>RPC Purified***</th>
<th>Gel Purified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scale</td>
<td>A260 Units</td>
<td>nmols A260 Units</td>
<td>A260 Units</td>
</tr>
<tr>
<td>50 nmol</td>
<td>8-10</td>
<td>30+</td>
<td>4-5</td>
</tr>
<tr>
<td>200 nmol</td>
<td>20-25</td>
<td>80+</td>
<td>8-12</td>
</tr>
<tr>
<td>1 μmol</td>
<td>100-120</td>
<td>400+</td>
<td>40-50</td>
</tr>
</tbody>
</table>

Purity & Yield
- Purity is more than 80% depending on oligo sequence and structure. Purity 85% to 95% depending on oligo sequence and structure. Not recommended for oligos longer than 35 mer.
- Purity 98% to ~100% depending on oligo sequence and structure. Yield will gradually decrease as length of oligo increases.

Hairpin Loop Formation and Primer Design*

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Hairpin Loops</th>
<th>Primer Design</th>
</tr>
</thead>
<tbody>
<tr>
<td>5'-CAGCGGACTACAGGCATGACGT-3’</td>
<td>2 22mer; dG = -47.5; Tm(NN)= 61.6°C</td>
<td>5'-GTCGCGCAGTACAGGCA-3’</td>
</tr>
<tr>
<td>3'- TAGCGATCGCATGACGTACAGGC-5’</td>
<td>stem=5 bp; loop=6</td>
<td>5'-GTCAGCCGACGTACAGGC-3’</td>
</tr>
<tr>
<td>5'-CAGCGGACTACAGGCATGACGT-3’</td>
<td>stem=5 bp; loop=6</td>
<td>5'-GTCAGCGGACTACAGGC-3’</td>
</tr>
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<td>3'- TAGCGATCGCATGACGTACAGGC-5’</td>
<td>stem=5 bp; loop=6</td>
<td>5'-GTCAGCCGACGTACAGGC-3’</td>
</tr>
</tbody>
</table>

Hairpin Structures
One essential element of efficient primer design is to minimize internal secondary structure, especially hairpin loops which tend to be deceptively stable at standard annealing temperatures. Hairpins are stable with as few as 4 bases stacked in the stem and a loop size of 4 to 6 bases. The stability declines as the loop size increases. The stem and loop size are related proportionally such that longer stem sizes can tolerate longer loop sizes (4).

As a general rule, avoid hairpins with more than 3 bases in the stem. Stable hairpin loop formation drastically reduces the primer concentration available for hybridization to the target sequence.

Base Composition
Higher GC content stabilizes hybridization, but a string of Gs and Cs can exhibit internal Hoogsteen base pairing, non-Watson-Crick base pairing and should be avoided (3,4). Although this anomalous behavior is difficult to predict, these structures can disrupt stable primer binding. In general, avoid runs of more than three consecutive Gs in primers. Also, examine potential primers for self-complementary and hairpin structures. Nuclear Magnetic Resonance (NMR) studies have shown that a stable hairpin can form with just four G-C basepairs in the stem and just three bases in the loop (5).

References
4. Gene Link, Inc. internal data.

Primer Design
Successful use of oligos as primers for amplification and sequencing starts with functional primer design followed by optimized PCR amplification conditions.

Fortunately, both PCR and sequencing reactions are inherently `robust’ and have been observed to tolerate wide variations in quality of primers when using unique templates. The same `tolerance’ can also lead to false priming, poor results and frustrating time loss with templates of higher complexity.

Primer specificity alone does not guarantee an optimum amplification yield. Numerous computer applications are available for primer search and design. Most of these applications do not consider the effect of hairpin structures which tend to be quite stable thermodynamically.

General guidelines for primer design are given below followed by a brief account of stable hairpin structure formation and non-Watson-Crick base pairing induced by a stretch of G’s and G’s interspersed with A’s or C’s (1-3).

General Guidelines
1. Specificity: Select an 18 to 24 mer stretch with perfect specificity.
2. Base Composition: Preferably maintain GC content below 60% with no stretches of more than 3G’s or 4 runs of the same base.
3. Tm: Select primer Tm within a few degrees of the pair.
5. Secondary Structure: Perform computer assisted analysis to view formation of stable dimers, loops and hairpins.