# Premium Oligonucleotide Synthesis

**QUALITY • CONSISTENCY • CONFIDENCE**

**Gene Link oligos are for demanding applications and consistent results. We believe that investigators who value time and have no room for an experiment to fail due to oligo quality should consider Gene Link. Our numerous quality control steps for each oligo assure confidence.**

GOLD STANDARD

## Actual Gel Photo

An actual gel photo of each oligo is affixed on the oligo report. An absolute testimony of quality. Gene Link has raised the standard since inception over a decade ago. *We have the pictures to prove it!*

## Superior to "Mass-Produced Factory Oligos"

Gene Link is not an oligo factory. Each oligo is synthesized, processed and quality assured to Gene Link's absolute standards. This includes coupling efficiency monitoring of each base during synthesis and electrophoretic analysis of each oligo on a polyacrylamide gel to visually assess quality.

## Coupling Efficiency

We maintain a coupling efficiency threshold of greater than 99.5% for all oligos by using premium reagents of exacting specifications, membrane synthesis, state-of-the-art instruments and optimized software-driven protocols. This may not be evident when comparing short oligos, as PCR and sequencing reactions are very robust and can tolerate up to 50% failure/truncated sequence oligos. However, you are clearly taking a chance by using long oligos synthesized at anything below 99.5% coupling efficiency.

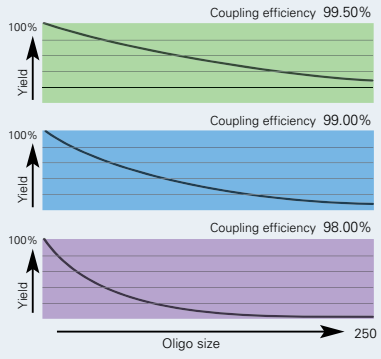
## Trityl Monitoring

All Gene Link DNA synthesizers are equipped with trityl monitors for monitoring coupling efficiency of each added base. The instruments are programmed to halt when it falls below the threshold.

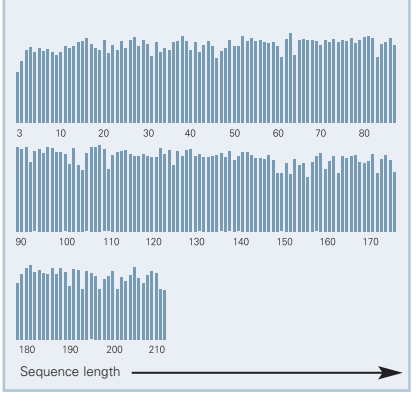
See example of routine trityl bars.

### Coupling Efficiency and Full Length Oligo Yield

| Oligo Size | 99.50% | 99.00% | 98.00% |
|---|---|---|---|
| 20 | 90.916 | 82.617 | 68.123 |
| 40 | 82.243 | 67.573 | 45.48 |
| 60 | 74.398 | 55.268 | 30.363 |
| 80 | 67.301 | 45.204 | 20.27 |
| 100 | 60.881 | 36.973 | 13.533 |
| 120 | 55.074 | 30.24 | 9.034 |
| 140 | 49.821 | 24.734 | 6.031 |
| 160 | 45.068 | 20.23 | 4.027 |
| 180 | 40.769 | 16.546 | 2.688 |
| 200 | 36.88 | 13.533 | 1.795 |
| 220 | 33.36 | 11.07 | 1.19 |
| 240 | 30.18 | 9.05 | 0.8 |
| 250 | 28.7 | 8.19 | 0.65 |




### Routine Trityl Coupling Efficiency




Actual trityl coupling efficiency of a 210 mer.

## The Gene Link Advantage

- Stringent Quality Control Measures
- Specializing in Long Oligos up to 250 mer
- Trityl Monitoring of All Oligos
- Polyacrylamide Gel Photograph of Each Oligo
- All Modifications Available
- All Oligo Types Available
- Easy Online Ordering System
- Online Design and Analysis Tools
- Knowledgeable Technical Support
- Personalized, Friendly Customer Service

GOLD STANDARD

### Long Oligos

Ask our competitors how often they synthesize 200 to 250 mer oligonucleotides. Gene Link specializes in long oligos.

*You are invited to compare.*

## Oligo Design and Analysis Tools

Gene Link has been leading the way by providing the most user friendly online experience in oligo ordering. From oligo design and analysis, to the convenient ordering system and the assurance of a secured transaction, Gene Link provides the most comprehensive web resource in the industry.

Features include convenient NCBI blasting and secondary structure analysis, simple import tools for large orders in spreadsheet or text file format, and three levels of review and editing.

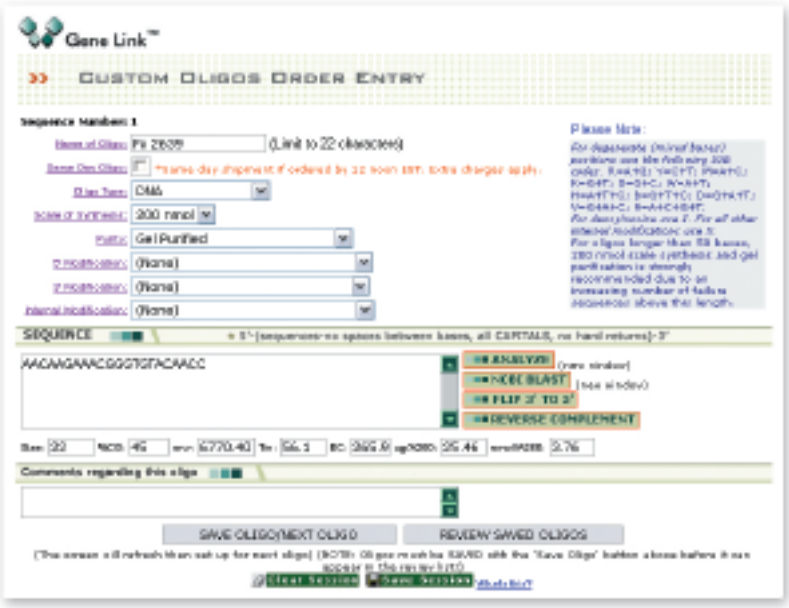
Applications include RNAi Explorer™, a robust siRNA search and design tool, and a standalone Oligo Explorer™ application for online acquisition of sequences and oligo design.

### Custom Oligo Ordering System

- Classic ordering system with extensive analysis features
- Timesaver multi oligo import from spreadsheet and text files
- Ability to handle mixed oligo types, purity and modifications in a single order
- Selection of oligo type (DNA, RNA, Phosphorothioate, Chimeric, etc.)
- Simple drop down menu selection for 5', internal or 3' modifications
- Analyze for oligo hairpin and loops
- Integrates with NCBI Blast for homology checks
- Flip 3' to 5' and reverse complement

### Online Oligo Analysis

- Simulate annealing, loops and hairpin formations
- Calculate MW, EC, $T_m$, $A_{260}$, etc.


### Save Session

**Too busy to order all of your oligos in one session?** Gene Link's answer to the multitasking researcher with endless interruptions is the "Save Session" feature. Enter as many oligos as you wish, click the "Save Session" button and resume at your will. Your oligos will be saved. What's more, you'll save money on shipping by consolidating your multiple orders into one.

## Multiple Oligo NCBI Blast

Click NCBI BLAST to ascertain homologies to other sequences.

Perform NCBI Blast of multiple sequences at once by using Gene Link's online MultiBlast application. Import all the sequences using a spreadsheet or a text file. All of your sequences will be blasted and results retrieved. Gene Link offers a very convenient approach to perform multiple blast searches.

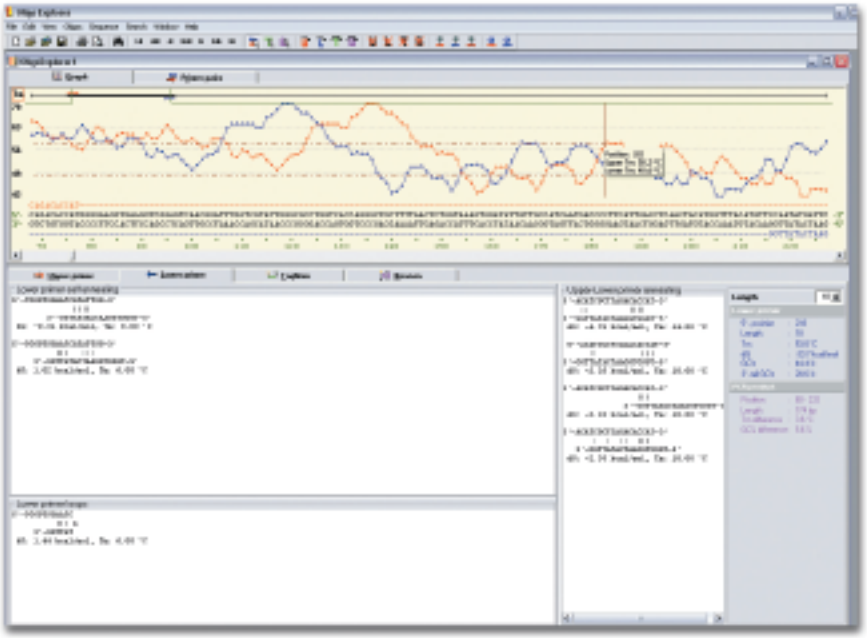

## Oligo Explorer™

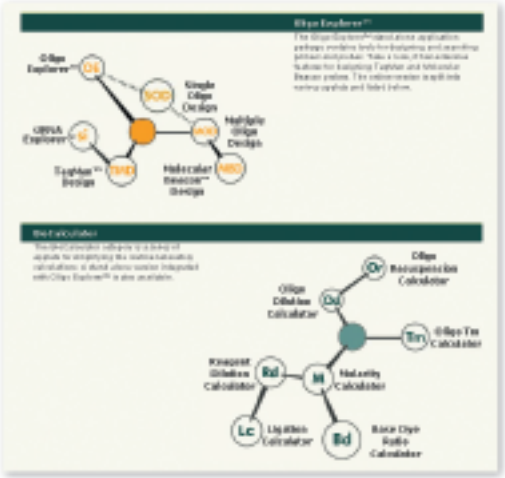
**A PC-based application for standalone DNA sequence retrieval and oligo design.**

Oligo Explorer™ was developed to design PCR and sequencing primers. Oligo Explorer™ is an efficient easy-to-use tool to determine primer properties like $T_m$, GC%, primer loops and primer dimers.

Oligo Explorer™ also includes a powerful "Primer Wizard" tool that helps you to find suitable primer pairs. You can set your own parameters for the primer pair search engine or use the default parameters. "Primer Wizard" suggests primer pairs that amplify PCR products of the given length. Individual primer pairs are suggested that theoretically will not form stable primer dimers or primer loops.

OLIGO EXPLORER 1.2





## Molecular Biology Convenience Applets

Gene Link has numerous online applets for quick calculations. The BioCalculator is a series of applets for simplifying the routine laboratory calculations. The following convenient calculators are available:

- Oligo Resuspension
- Oligo Dilution
- Oligo $T_m$
- Reagent Dilution
- Molarity Determination
- Ligation
- Base/Dye Ratio

## Oligo Specifications Report

Gene Link's Custom Oligonucleotide Synthesis Report specifies each oligo name and sequence along with its pertinent physical properties such as MW, %GC, $T_m$, $A_{260}$ units, etc. Our report is also unique in that we affix an actual polyacrylamide gel electrophoresis photograph onto each report, so that you also may visually attest to the quality of our product.

From your custom oligo to the presentation of our oligo synthesis report, not a step of quality is overlooked. *You are invited to compare.*

### 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.

### Purity & Usage

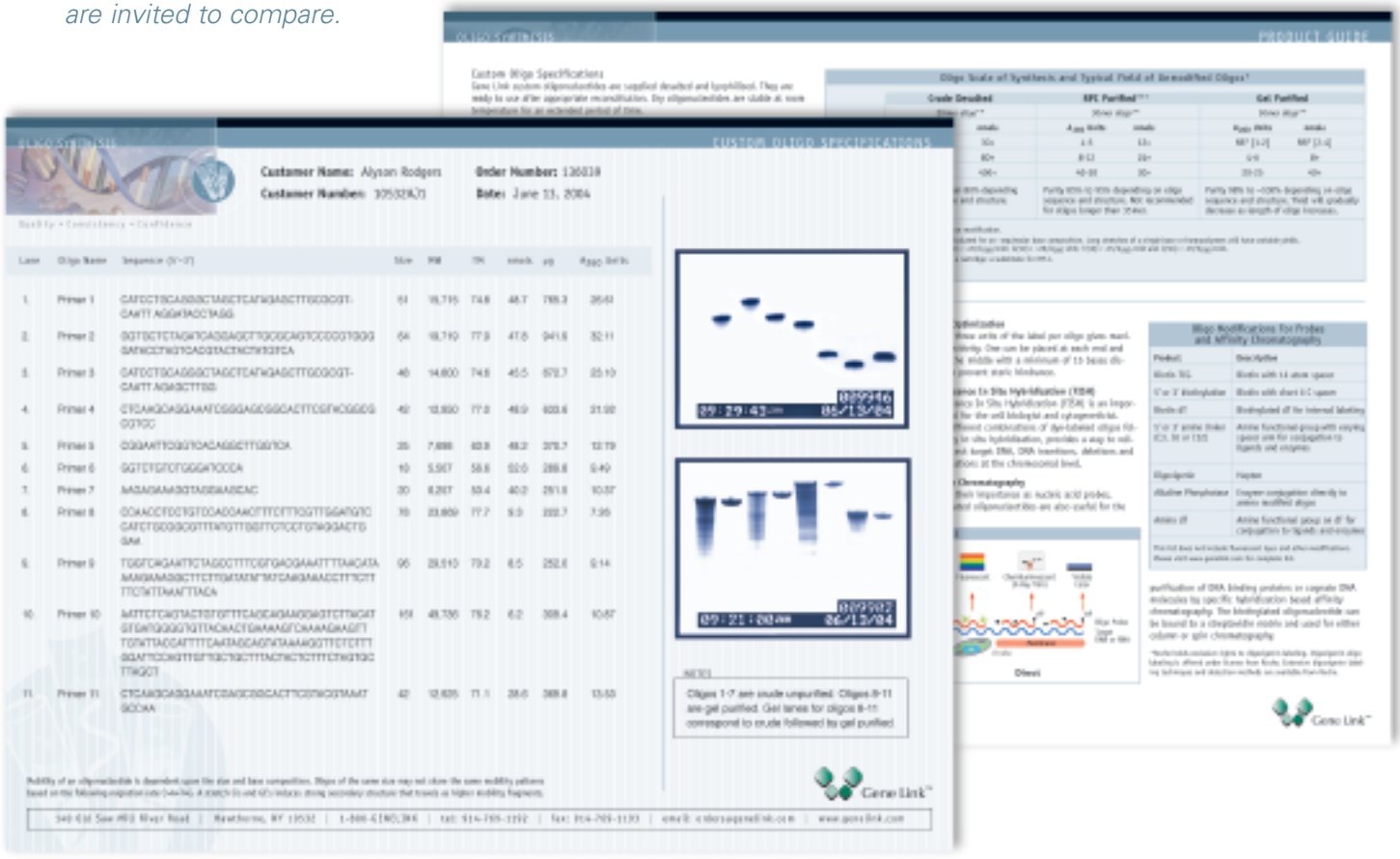
The crude, desalted oligonucleotide supplied is suitable for all amplification and sequencing protocols. Gel purification is advised for all oligos used for cloning applications and for oligos longer than 50 mer.

### Biophysical Data

Each oligo after desalting is quantified by recording $A_{260}$. Exact nmols and µg are determined by the extinction coefficient and molecular weight of the oligo.

### 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.



## Oligo Scale of Synthesis and Typical Yield

| | Crude Desalted | | | RPC Purified** | | | Gel Purified | | |
|---|---|---|---|---|---|---|---|---|---|
| | 20 mer oligo* Typical yield | | | 30 mer oligo* Typical yield | | | 50 mer oligo* Typical yield | | |
| **Scale** | **$A_{260}$ Units** | **nmols** | **mg** | **$A_{260}$ Units** | **nmols** | **mg** | **$A_{260}$ Units** | **nmols** | **mg** |
| **50 nmol** | 8-10 | 30+ | 0.2-0.3 | 4-5 | 12+ | 0.1-0.16 | NR* [1-2] | NR* [2-4] | NR* [0.03-0.06] |
| **200 nmol** | 20-25 | 80+ | 0.6-0.8 | 8-12 | 24+ | 0.26-0.4 | 4-6 | 8+ | 0.13-0.2 |
| **1 µmol** | 100-120 | 400+ | 3-4 | 40-50 | 30+ | 1.3-1.6 | 20-25 | 40+ | 0.6-0.8 |
| **Purity & Yield** | **Purity is greater than 80%** depending on oligo sequence and structure. Refer to coupling efficiency table for oligo length dependent purity and yield.<br><br>No further purification required for PCR and sequencing applications.<br><br>Gel purification recommended for oligos above 50 mer and all applications involving cloning and mutagenesis. | | | **Purity 85% to 95%** depending on oligo sequence and structure.<br><br>Yield and purity will be lower for sequences with high GC content.<br><br>Not recommended for oligos longer than 35 mer.<br><br>**RPC is reverse phase purification using a cartridge; a substitute for HPLC. | | | **Purity 98% to ~100%** depending on oligo sequence and structure.<br><br>Yield will gradually decrease as length of oligo increases. Palindromes, hairpins and high GC content oligos and oligos containing stretches of 3 or more G's induces strong secondary structure and base stacking thus decreasing purity and yield.<br><br>NR* Not Recommended | | |

*Yield of 30 µg/$A_{260}$ unit for oligos is calculated for an ~equimolar base composition. Long stretches of a single base or homopolymers will have variable yields. Example for homopolymeric 50 mer: A(50) = ~20/$A_{260}$ Unit; G(50) = ~28/$A_{260}$ Unit; T(50) = ~35/$A_{260}$ Unit and C(50) = ~39/$A_{260}$ Unit.

### Unmodified DNA Oligo Synthesis*

| Scale of Synthesis | Catalog No. | Price ($) |
|---|---|---|
| 50 nmol | 26-6400-05 | 0.90 |
| 200 nmol | 26-6400-02 | 2.00 |
| 1 µmol | 26-6400-01 | 3.75 |
| 2 µmol | 26-6400-03 | 6.50 |
| 10 µmol | 26-6400-10 | 32.00 |
| 15 µmol | 26-6400-15 | 38.00 |

*minimum charge for 15 mer applies. Please visit www.genelink.com for current list prices. Call for institutional discount pricing structure.

### Same Day Oligo*

**Design your oligos today and use them tomorrow morning!** Investigators who just can not wait order our rush service (order by 12 noon EST). We ship the same day for next early morning delivery in the US and 72 hours for most international destinations.

* Turn-around time stated is for unmodified oligos. Please inquire about purified and modified oligos

### Purification

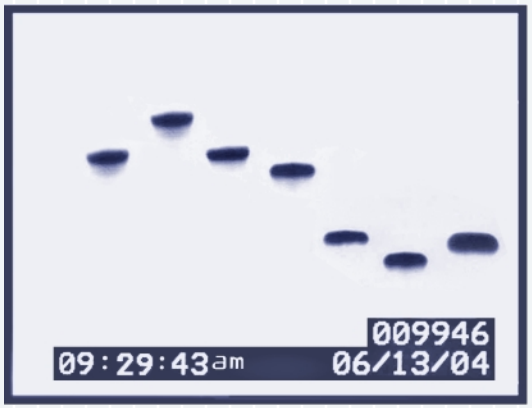
| | | Scale of Synthesis Price ($)/purification | | | | | |
|---|---|---|---|---|---|---|---|
| **Product** | **Catalog No.** | **50 nmol** | **200 nmol** | **1 µmol** | **2 µmol** | **10 µmol** | **15 µmol** |
| Gel Purification | 26-6400-XX | 75.00 | 75.00 | 150.00 | 280.00 | 1500.00 | 1800.00 |
| Reverse Phase Cartridge | 26-6400-XX | 30.00 | 30.00 | 90.00 | 170.00 | 750.00 | 900.00 |

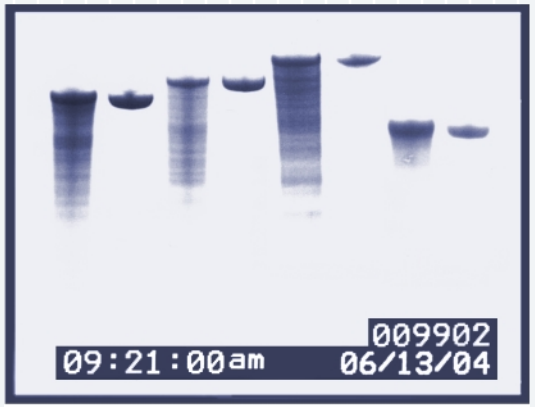
Quality • Consistency • Confidence

**Customer Name:** Alyson Rodgers **Order Number:** 136039

**Customer Number:** 10532AJ1 **Date:** June 13, 2004

| Lane | Oligo Name | Sequence (5'–3') | Size | MW | TM | nmols | µg | $A_{260}$ Units |
|---|---|---|---|---|---|---|---|---|
| 1. | Primer 1 | CATCCTGCAGGGCTAGCTCATAGAGCTTGCGCGTCAATTAGGATACCTAGG | 51 | 15,715 | 74.8 | 48.7 | 765.3 | 26.61 |
| 2. | Primer 2 | GGTGCTCTAGATCAGGAGCTTGCGCAGTCCCCGTGGGGATACCTAGTCACGTACTACTATGTCA | 64 | 19,719 | 77.9 | 47.8 | 941.9 | 32.11 |
| 3. | Primer 3 | CATCCTGCAGGGCTAGCTCATAGAGCTTGCGCGTCAATTAGAGCTTGG | 48 | 14,800 | 74.6 | 45.5 | 672.7 | 23.10 |
| 4. | Primer 4 | CTCAAGCAGGAAATCGGGAGCGGCACTTCGTACGGCGCGTCC | 42 | 12,950 | 77.0 | 48.9 | 633.6 | 21.92 |
| 5. | Primer 5 | CGGAATTCGGTCACAGGCTTGGTCA | 25 | 7,698 | 63.9 | 48.2 | 370.7 | 12.79 |
| 6. | Primer 6 | GGTCTGTCTGGGATCCCA | 18 | 5,507 | 56.6 | 52.6 | 289.8 | 9.49 |
| 7. | Primer 7 | AAGAGAAAGGTAGGAAGCAC | 20 | 6,257 | 53.4 | 40.2 | 251.5 | 10.37 |
| 8. | Primer 8 | CCAACCTCCTGTCCACCAACTTTCTTTCGTTGGATGTCCATCTGCGGCGTTTATGTTGGTTCTCCTGTAGGACTGGAA | 78 | 23,869 | 77.7 | 9.3 | 222.7 | 7.26 |
| 9. | Primer 9 | TGGTCAGAATTCTAGCCTTTCGTGACGAAATTTTAACATAAAAGAAAGGCTTCTTGATATATTATCAAGAAACCTTTCTTTTCTATTAAATTTACA | 96 | 29,513 | 70.2 | 8.5 | 252.0 | 9.14 |
| 10. | Primer 10 | AATTCTCAGTACTGTGTTTCAGCAGAAGGAGTCTTACATGTGATGGGGTGTTACAACTGAAAAGTCAAAAGAAGTTTGTATTACCATTTTCAATAGCAGTATAAAAGGTTCTCTTTGGATTCCAGTTGTTGCTGCTTTACTACTCTTTCTAGTGCTTAGCT | 161 | 49,736 | 76.2 | 6.2 | 309.4 | 10.87 |
| 11. | Primer 11 | CTCAAGCAGGAAATCGAGCGGCACTTCGTACGTAAATGCCAA | 42 | 12,925 | 71.1 | 28.6 | 369.8 | 13.53 |




NOTES

Oligos 1-7 are crude unpurified. Oligos 8-11 are gel purified. Gel lanes for oligos 8-11 correspond to crude followed by gel purified.

Mobility of an oligonucleotide is dependent upon the size and base composition. Oligos of the same size may not share the same mobility patterns based on the following migration rate C>A>T>G. A stretch G's and GC's induces strong secondary structure that travels as higher mobility fragments.

Gene Link™

140 Old Saw Mill River Road | Hawthorne, NY 10532 | 1-800-GENELINK | tel: 914-769-1192 | fax: 914-769-1193 | email: orders@genelink.com | www.genelink.com

## 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 $A_{260}$. Exact nmols and µg is determined by the extinction coefficient and molecular weight of the oligo.

### Oligo Scale of Synthesis and Typical Yield of Unmodified Oligos*

| | Crude Desalted | | RPC Purified*** | | Gel Purified | |
|---|---|---|---|---|---|---|
| | 20 mer oligo** | | 30 mer oligo** | | 50 mer oligo** | |
| **Scale** | **$A_{260}$ Units** | **nmols** | **$A_{260}$ Units** | **nmols** | **$A_{260}$ Units** | **nmols** |
| **50 nmol** | 8-10 | 30+ | 4-5 | 12+ | NR* [1-2] | NR* [2-4] |
| **200 nmol** | 20-25 | 80+ | 8-12 | 24+ | 4-6 | 8+ |
| **1 µmol** | 100-120 | 400+ | 40-50 | 30+ | 20-25 | 40+ |
| **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. | |

*The yield of modified oligos varies based on modification.
**Yield of 30 µg/$A_{260}$ unit for oligos is calculated for an ~equimolar base composition. Long stretches of a single base or homopolymers will have variable yields. Example for homopolymeric 50 mer: A(50) = ~20/$A_{260}$ Unit; G(50) = ~28/$A_{260}$ Unit; T(50) = ~35/$A_{260}$ Unit and C(50) = ~39/$A_{260}$ Unit.
***RPC is reverse phase purification using a cartridge; a substitute for HPLC.
NR* Not Recommended.

## 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 3 G's or 4 runs of the same base.
3. **Tm:** Select primer Tm within a few degrees of the pair.
4. **Cross Homologies:** Perform NCBI blast to determine extent of cross homologies.
5. **Secondary Structure:** Perform computer assisted analysis to view formation of stable dimers, loops and hairpins.

### Hairpin Loop Formation and Primer Design*

| | | | | |
|---|---|---|---|---|
| Sequence | 5'-CAGCGCACTACAGGCATGACGT-3'<br>22 mer; dG = -47.5; Tm(NN) = 61.6°C | 5'-GTCCGCACGTACGGACAT-3'<br>18 mer; dG = -38.4; Tm(NN): 57.0°C | 5'-GTCAGCCGCACGTACGGACAT-3'<br>21 mer; dG: -46.3; Tm(NN): 61.70°C | 5'-AGTAACGCACTACGGACTTACGAC-3'<br>24 mer; dG = -47.1; Tm(NN) = 58.8°C |
| ***Dimers** | 5' CAGCGCACTACAGGCATGACGT 3'<br>3' TGCAGTACGGACATCACGCGAC 5'<br>STACK AT 3 IS 4 BP LONG.<br>dG = -4.8; Tm = -58.4°C | 5' GTCCGCACGTACGGACAT 3'<br>3' TACAGGCATGCACGCCTG 5'<br>STACK AT 8 IS 6 BP LONG.<br>dG = -5.7; Tm = -42.4°C | 5' GTCAGCCGCACGTACGGACAT 3'<br>3' TACAGGCATGCACGCCGACTG 5'<br>STACK AT 11 IS 6 BP LONG.<br>dG = -4.65; Tm = -28.2°C | 5' AGTAACGCACTACGGACTTACGAC 3'<br>3' CAGCATTCAGGCATCACGCAATGA 5'<br>STACK AT 2 IS 4 BP LONG.<br>dG = -2.05; Tm = -47.3°C |
| **Hairpin Loops** | None | 5' GTCCGCAC<br>3' TACAGGCATG<br>STEM AT 1 IS 5 BP LONG. LOOP = 6.<br>dG = -5.3; Tm = 87.3°C | 5' GTCAGCCGCAC<br>3' TACAGGCATG<br>STEM AT 6 IS 3 BP LONG. LOOP = 6<br>dG = -2.4; Tm = 68.9°C | 5' AGTAACGCACT<br>3' CAGCATTCAGGCA<br>STEM AT 2 IS 4 BP LONG. LOOP = 12.<br>dG = 0.8; Tm = 13.8°C |

*Secondary structure results are truncated to show the most stable structures. All thermodynamic values including Tm and secondary structures calculated and displayed solely indicate the relative stability of the secondary structures. They should only be used to compare the relative stability of the structures. dG value unit is kcal/mol.
Visit www.genelink.com/tools/gl-SOD.asp to design oligos or click on the 'Analyze' button while on the online oligo ordering page.

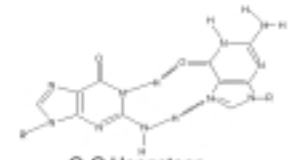
### Hairpin Structures

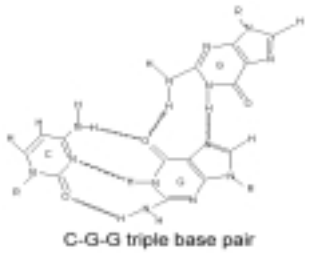
One essential element of efficient primer design is to minimize internal secondary structure, especially hairpin loops which tend to be deceptively stable at standard annealing temperatures. Hairpins are stable with as few as 4 bases stacked in the stem and a loop size of 4 to 6 bases. The stability declines as the loop size increases. The stem and loop size are related proportionately 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 G's and C's 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 G's 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).


G-G Hoogsteen


C-G-G triple base pair

### References


1. Michael Zuker (2003) *Nucleic Acids Res.*, 31, 3406–3415.
2. SantaLucia, J. (1998) *Proc. Nat. Acad. Sci. USA* 95, 1460.
3. Sarochi, M-T., Courtois, Y., Guschlbauer, W. 1970. *Eur. J. Biochem.* 14: 411.
4. Gene Link, Inc. internal data.
5. Summer, M.F., Byrd R.A., Gallo, K.A., Samson, C.J., Zon, G., Egan, W. 1985. *Nucleic Acids Res.*13: 6375.

Gene Link™