All Gene Link oligos shorter than 40 mer do not require any further purification if the application is for PCR or sequencing.

A 20 mer oligo synthesized at a coupling efficiency of 99.5% will contain ~90% full-length 20 mer and a mixture of truncated sequences comprising of ~10%.

As the length of the oligo increases, even at a coupling efficiency of 99.5%, the yield of the full-length oligo is reducing. See the table and graph on page 8. A 60 mer crude product will contain ~75% full-length oligo and similarly a 100 mer will contain ~60%.

Purification is strongly recommended for oligos longer than 50 mer.

The gold standard of long oligo purification is polyacrylamide gel electrophoresis.

HPLC/RPC

HPLC and RPC (Reverse Phase Cartridge) purification methods yield purity of 85% to 95% depending upon the sequence, GC content and length of the oligonucleotide. Reverse phase based HPLC fails above 40 mer as longer oligos are inherently hydrophobic and bind non-specifically.

Polyacrylamide Gel Purification (PAGE)

Purification by this method is considered the Gold Standard for oligonucleotide purification and yields 99%+ purity. Gel purification can be used for any length of oligonucleotide (as compared to HPLC and RPC cartridges which are limited to oligonucleotides below 40 mer). Gel purification is strongly advised for all applications involving cloning of the product, such as mutagenesis and gene construction applications.

Oligo Scale of Synthesis and Typical Yield

	RPC Purified** (30 mer oligo Typical yield)			Gel Purified (50 mer oligo Typical yield)		
Scale	**A_{260} Units**	**nmols**	**mg**	**A_{260} Units**	**nmols**	**mg**
50 nmol	4-5	12+	0.1-0.16	NR* [1-2]	NR* [2-4]	NR* [0.03-0.06]
200 nmol	8-12	24+	0.26-0.4	4-6	8+	0.13-0.2
1 µmol	40-50	30+	1.3-1.6	20-25	40+	0.6-0.8
Purity & Yield	**Purity 85% to 95%** depending on oligo sequence and structure. Yield and purity will be lower for sequences with high GC content. Not recommended for oligos longer than 35 mer. **RPC is reverse phase purification using a cartridge; a substitute for HPLC.			**Purity 98% to ~100%** depending on oligo sequence and structure. 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. NR* Not Recommended		

G's: The Unresolved Dilemma

Ever wonder why we have not yet discovered a polymerase that can breeze through a stretch of G's? A stretch of three or more G's in an oligo sequence induces strong secondary structure. A string of G's and C's can exhibit internal Hoogsteen base pairing, non-Watson-Crick triple base pairing and should be avoided. Although this anomalous behavior is difficult to predict, in general, avoid runs of more than three consecutive G's in primers. Also, examine potential primers for self-complementary and hairpin structures.

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

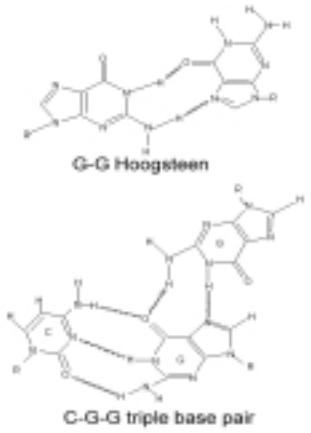
Hairpin Structures

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).



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