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.

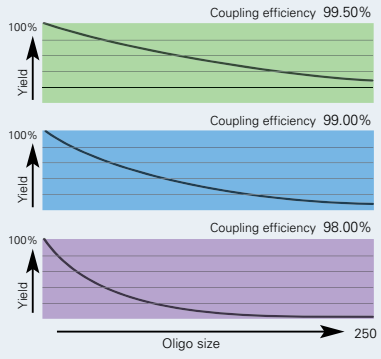
Trityl Monitoring

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.

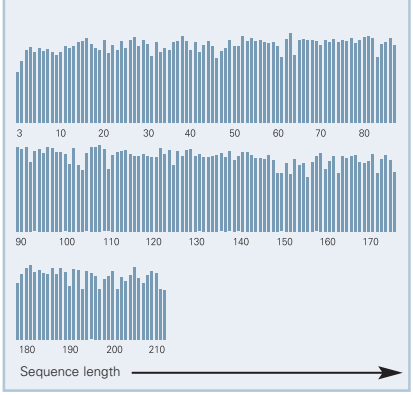
See example of routine trityl bars.

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 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

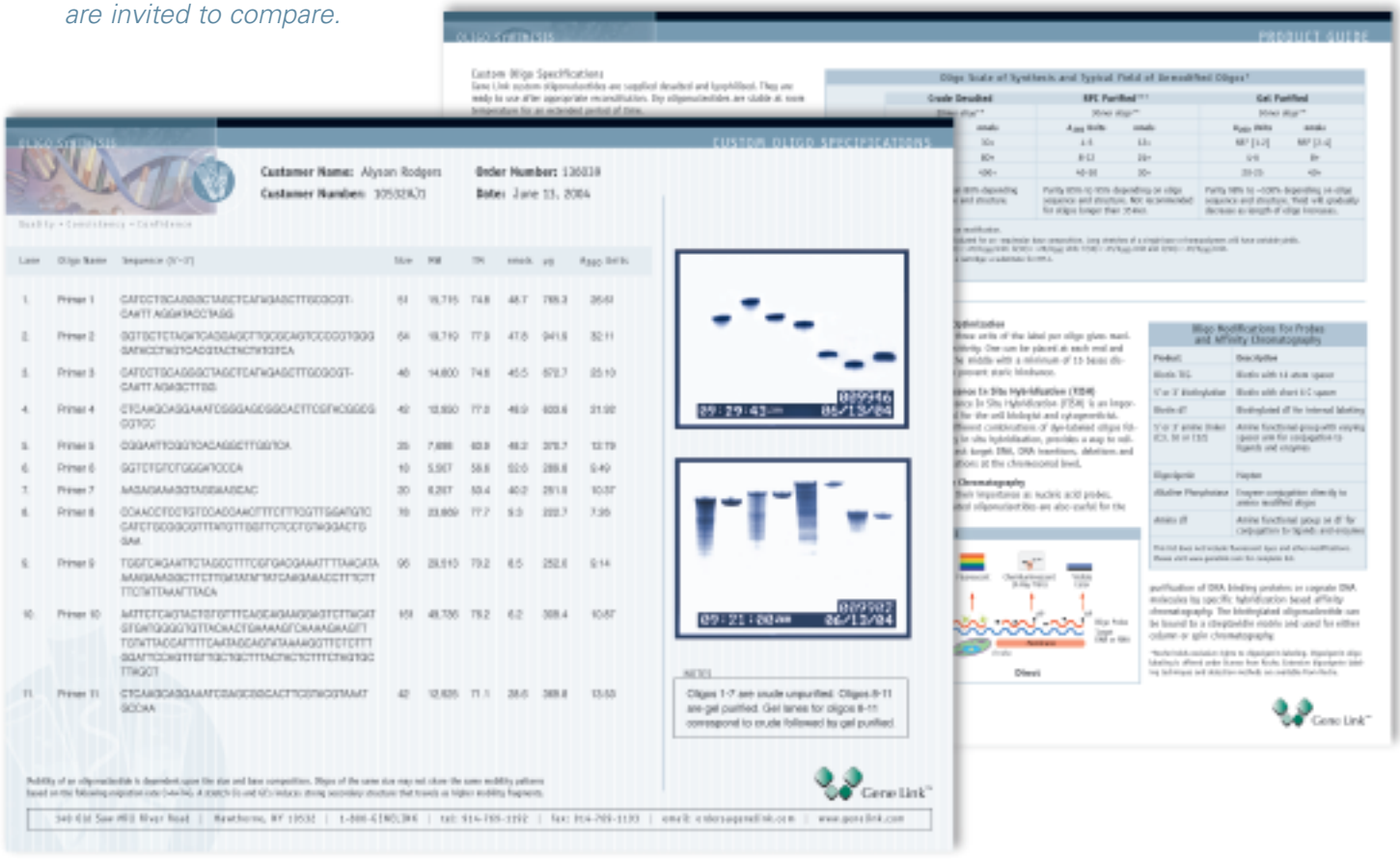
The crude, desalted oligonucleotide supplied is suitable for all amplification and sequencing protocols. Gel purification is advised for all oligos used for cloning applications and for oligos longer than 50 mer.

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. No further purification required for PCR and sequencing applications. 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. 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		

*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

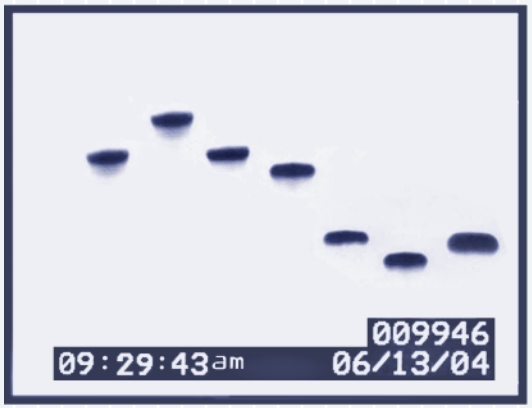
		Scale of Synthesis Price ($)/purification					
Product	**Catalog No.**	**50 nmol**	**200 nmol**	**1 µmol**	**2 µmol**	**10 µmol**	**15 µmol**
Gel Purification	26-6400-XX	75.00	75.00	150.00	280.00	1500.00	1800.00
Reverse Phase Cartridge	26-6400-XX	30.00	30.00	90.00	170.00	750.00	900.00

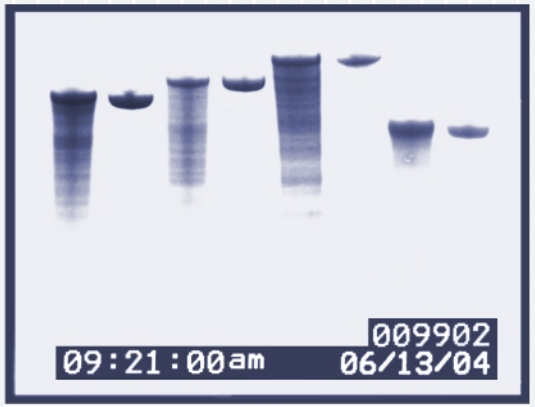
Quality • Consistency • Confidence

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; EDTA inhibits the activity of the nucleases. Further dilution can be made in distilled sterile water. 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. Polyacrylamide gels of 12 to 15% are run, depending upon the length of the custom oligonucleotide. 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 50mer.

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

	Crude Desalted 20mer oligo* Typical yield			RPC Purified** 30mer oligo* Typical yield			Gel Purified 50mer 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+	0.26-0.40	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 more than 80% depending on oligo sequence and structure. Refer to coupling efficiency table for oligo length dependent purity and yield. No further purification required for PCR and sequencing applications. Gel purification recommended for oligos above 50mer and all applications involving cloning and mutagenesis.			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 35mer. **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		

*Yield of 30µg/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 50mer: 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.

Oligo Reconstitution and Use

Gene Link oligos are supplied lyophilized. These are stable at room temperature for an extended period of time. TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) is recommended for dissolving the oligonucleotides; EDTA inhibits the activity of the nucleases. Further dilution can be made in distilled sterile water. After reconstitution store the stock solution at –80°C or –20°C.

Standard PCR Set Up

Reagent	Final Concentration	Quantity/ 50µl Reaction
Sterile deionized water	–	variable
10X *PCR buffer	1X	5µl
2mM dNTP mix	0.2mM of each	5µl
Primer I, 10µM (10pmol/µl)	0.5µM	2.5µl
Primer II, 10µM (10pmol/µl)	0.5µM	2.5µl
Taq DNA Polymerase, 5U/µl	1.25u/50µl	0.25µl
Template DNA	10pg-1µg	variable

*Final $MgCl_2$ concentration is 1.5mM

Oligo Reconstitution

Stock solution of 500 pmols/µl [500 µM]

Gene Link provides the exact amount of nmols of each oligo supplied on the tube and on the Oligo Report. Multiply the 'nmol' amount by 2 to arrive at the volume of TE to be added.

Example: 45.10 nmols x 2 = 90.2 µl

Dissolve the oligo in 90.2 µl to get 500 pmols/µl stock solution. Use as required.

Dilute 10 fold to prepare a 50 pmols/µl [50µM]. Use as required.

Stock solution of 100 pmols/µl [100 µM]

Gene Link provides the exact amount of nmols of each oligo supplied on the tube and on the Oligo Report. Multiply the 'nmol' amount by 10 to arrive at the volume of TE to be added.

Example: 45.10 nmols x 10 = 451 µl

Dissolve the oligo in 451 µl to get 100 pmols/µl stock solution. Use as required.

Dilute 10 fold to prepare a 10 pmols/µl [10µM]. Use as required.

Examples of Use

Polymerase Chain Reaction (PCR)

The final concentration of primers in a PCR reaction is 0.2–1.0 µM. This is equivalent to 0.2–1 pmol/µl. At Gene Link, for a standard PCR we use 0.5 pmol/µl.

Sequencing

The final concentration of primer in automated sequencing is from 4 to 10 pmols (~0.05 - 0.1µg). Use the oligo reconstitution protocol to prepare a 100 pmols/µl [100µM] solution and then dilute 10 fold to get 10 pmol/µl solution. Use 1µl (10 pmols).

Quick Conversion Table

1 µM (µMolar) = 1 pmol/µl (picomoles/µl)
1 mM (milliMolar) = 1 nmols/µl (nanomoles/µl)

Example: 20 µMolar primer solution is 20 pmol/µl

Gene Link™

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Purity & Usage
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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' 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.

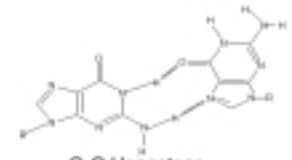
Hairpin Structures

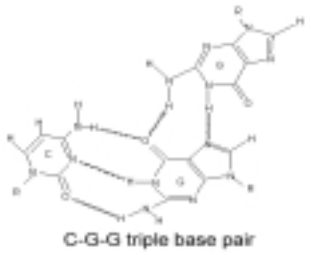
One essential element of efficient primer design is to minimize internal secondary structure, especially hairpin loops which tend to be deceptively stable at standard annealing temperatures. Hairpins are stable with as few as 4 bases stacked in the stem and a loop size of 4 to 6 bases. The stability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


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™

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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 35mer.		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 50mer: 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.

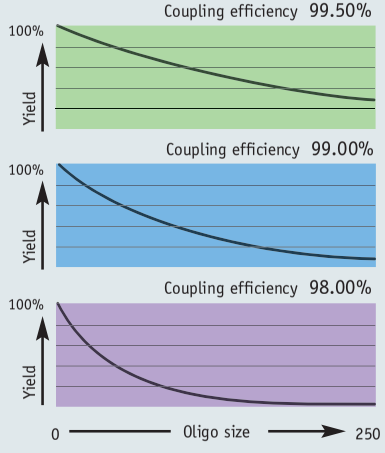
Synthesis of Long Oligos

Coupling Efficiency

Chemical DNA synthesis comprises of multiple reactions to complete a cycle of the appropriate base coupling. Thus the use of reagents of exacting specifications, state of the art instruments and optimized software driven protocols are necessary to maintain the highest possible coupling efficiency. This clearly becomes enormously important when synthesizing a long oligo. Gene Link specializes in long oligos. Our description of long oligos is 180mer to 250mer. PCR and sequencing reactions are very robust and can tolerate up to 50% failure/truncated sequence oligos. Coupling efficiency of 99.5% and 98% seems very good but on closer examination the yield is almost half for a 40mer! Please see the coupling efficiency table and graph.

Coupling Efficiency and Full Length Oligo Yield

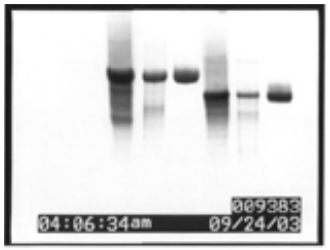
Oligo Size	99.50%	99.00%	98.00%
20	90.916	82.617	68.123
40	82.243	67.573	45.48
60	74.398	55.268	30.363
80	67.301	45.204	20.27
100	60.881	36.973	13.533
120	55.074	30.24	9.034
140	49.821	24.734	6.031
160	45.068	20.23	4.027
180	40.769	16.546	2.688
200	36.88	13.533	1.795
220	33.36	11.07	1.19
240	30.18	9.05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



Purification

Crude oligo is the total yield after chemical synthesis; this contains the full-length product as well as all truncated n-1 sequences. For example, at 99% coupling efficiency the crude yield of a 70mer is ~50% full length and ~50% truncated shorter sequences. Gel purification is strongly recommended for all oligos above 50mer. All Gene Link oligos shorter than 40mer usually do not require any further purification if the application is for PCR or sequencing without downstream cloning of the product. Gel purification involves electrophoresis of the entire crude product on a preparative polyacrylamide gel followed by excision and purification of the full-length oligo.

HPLC is not capable of consistently resolving oligos above 40mer and thus is not a recommended for purification of long oligos.



Unpurified, RPC and Gel Purified Oligos

Polyacrylamide gel electrophoresis of crude, reverse phase cartridge (RPC) and gel purified oligos.

Approximately 15 µg of crude unpurified oligo was loaded to show the truncated failure sequences.

Approximately 8 µg of purified oligos were loaded.

Lanes 1–3 is a 68mer oligo.
Lanes 4–6 is a 56mer oligo.

Lanes 1&4: crude unpurified.
Lanes 2&5: RPC purified.
Lanes 3&6: gel purified.

Sequence Accuracy

Statistically sequence accuracy is guaranteed in a portion of the full length product. Despite the effort to maintain a coupling efficiency above 99%, it still leaves the unavoidable failure rate of less than 1%. This ~1% failure rate is cumulative; meaning at every step of each cycle; including miniscule but still probable, error of deletions and insertions of erroneous bases. For long oligos this becomes more pronounced and exaggerated on occasion due to amplification during PCR. Mathematical and statistical analysis based on Avogadro's number (6.022×10^{23} mol^{-1}) and synthesis of a long oligo at 1 micromolar scale (6.022×10^{17} mol^{-1}) at 99% coupling efficiency will still yield quite a few million copies of the exact sequence.

It is imperative to pick several colonies of cloned inserts and confirm by sequencing. It is observed that on occasions due to high GC content or long stretches of bases, relatively more colonies have to be screened by sequencing to find the clone of the correct sequence.

Purity and Yield

Purity is generally between 98% to ~100% depending on oligo sequence and structure. Yield will gradually decrease as length of oligo increases. Palindromes, hairpins, high GC content oligos and oligos containing stretches of 3 or more G's induce strong secondary structure and base stacking. These are not completely denatured and travel as a broad band on polyacrylamide gels thus decreasing purity and yield.

Gene Link™

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 50mer.

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	
	20mer oligo**		30mer oligo**		50mer 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 35mer.		Purity 98% to ~100% depending on oligo sequence and structure. Yield will gradually decrease as length of oligo increases.	

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Oligo Labeling

Oligo labeling for Non Radioactive Hybridization Probes & Affinity Chromatography
Radioactive labeling is still used extensively despite the development of sensitive non-radioactive detection methods. Chemiluminescent, visible dye and fluorescence-based detection offer a safe and sensitive method for qualitative, as well as quantitative detection.

Gene Link offers synthesis of non-radioactive modified oligos for use as hybridization probes and affinity chromatography. We provide technical service in the design of novel probes and synthesize numerous combinations of fluorescent dyes and quenchers, ligands, modifications and direct conjugation to alkaline phosphatase.

Sensitivity
Chemiluminescent detection with alkaline phosphatase approaches 0.1pg, equivalent to ~1X10^6 copies of the target; this is equivalent to less than 4µg of human genomic DNA. Almost all Southern based hybridizations can be switched to safe non-radioactive based methods with no associated hazard or need to frequently label probes.

Detection Strategy
The non-radioactive modification used must be complemented with a sensitive detection method. There are two approaches. In the direct method, the oligo probe is directly labeled with fluorescent dye(s) for fluorescent in situ hybridization (FISH); or with alkaline phosphatase (AP). The blot is incubated with an AP substrate that either gives visible color or chemiluminescence detected by exposure to X-ray film or imaging systems. In the indirect method, the probe is labeled with a ligand such as biotin or digoxigenin*. The blot is then incubated with an AP or horseradish peroxidase (HRP) conjugated antibody specific for the ligand followed by color or chemiluminescent detection.

Modifications
Oligos can be labeled with biotin or digoxigenin* or directly labeled with alkaline phosphatase. A variety of linker arms are available as spacers to minimize steric hindrance. See table for a list of popular ligand modifications and spacer molecules.

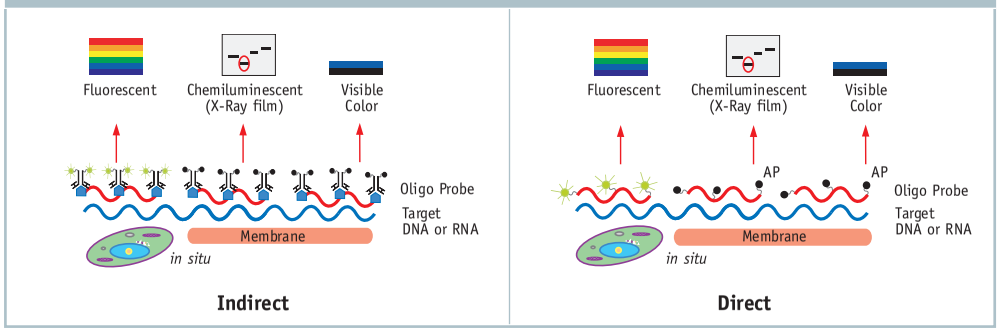
Signal Optimization
Placing three units of the label per oligo gives maximal sensitivity. One can be placed at each end and one in the middle with a minimum of 15 bases distance to prevent steric hindrance.

Fluorescence In Situ Hybridization (FISH)
Fluorescence In Situ Hybridization (FISH) is an important tool for the cell biologist and cytogeneticist. Using different combinations of dye-labeled oligos followed by in situ hybridization, provides a way to reliably detect target DNA, DNA insertions, deletions and translocations at the chromosomal level.

Affinity Chromatography
Besides their importance as nucleic acid probes, biotinylated oligonucleotides are also useful for the purification of DNA binding proteins or cognate DNA molecules by specific hybridization based affinity chromatography. The biotinylated oligonucleotide can be bound to a streptavidin matrix and used for either column or spin chromatography.

*Roche holds exclusive rights to digoxigenin labeling. Digoxigenin oligo labeling is offered under license from Roche. Extensive digoxigenin labeling techniques and detection methods are available from Roche.

Detection Methods



Oligo Modifications For Probes and Affinity Chromatography

Product	Description
Biotin TEG	Biotin with 16 atom spacer
5' or 3' biotinylation	Biotin with short 6 C spacer
Biotin dT	Biotinylated dT for internal labeling
5' or 3' amino linker (C3, C6 or C12)	Amino functional group with varying spacer arm for conjugation to ligands and enzymes
Digoxigenin	Hapten
Alkaline Phosphatase	Enzyme conjugation directly to amino modified oligos
Amino dT	Amino functional group on dT for conjugation to ligands and enzymes

This list does not include fluorescent dyes and other modifications. Please visit www.genelink.com for complete list.

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Fluorescent Molecular Primers & Probes

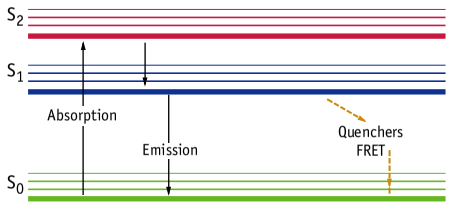
The use of fluorescent dyes in molecular biology has rapidly transformed from just single dye labeled primers for fragment analysis to the use of multiple labeled dyes and quenchers as probes for real time quantitative PCR analysis. Fluorescence based detection offers a safe and sensitive method for quantitative detection. Gene Link offers synthesis of all different forms of molecular primers and probes. We provide technical service in the design of novel probes and synthesize numerous combinations of dyes, quenchers, RNA, phosphorothioate, 2'O methyl and chimeric probes.

Excitation and Emission
The excitation level of molecules varies at different wavelengths. Molecules exposed to a beam of light absorb more at a particular wavelength. This specific wavelength is termed as the Excitation Maxima. The emission maximum is the wavelength at which the maximum amount of light is released. The molecule stays in the excited state for a finite time, usually <1-10 nanoseconds and returns to the relaxed state upon emission of energy. Excitation and Emission is a cyclic process and consequently can be repeated to an extent before it starts to fade, termed as photo-bleaching.

Different fluorescent dyes are used for molecular probes and primer design. The dyes are selected based on the excitation and emission wavelengths, bleaching, quenching and various other biophysical factors.

Quenching
Reduction in the expected fluorescence emission is termed as quenching. The phenomenon of quenching forms the basis of the mode of action of molecular probes; the designed and controlled fluorescence based on hybridization to the target sequence.

Detection Methods



Absorption of a photon and excitation to S_1 or S_2. Radiationless energy loss and return to S_1. Return to S_0 from S_1 with emission of fluorescence or by energy transfer to quenchers or other acceptor dye (FRET).

Quenchers

Dye	Absorbance max (nm)
Dabcyl	453
*BHQ-1	534
*BHQ-2	579
*BHQ-3	672

Placing a molecule that absorbs light in close proximity to the fluorophore can induce quenching. The quenching effect is exhibited by fluorescent as well as non-fluorescent molecules. A non-fluorescent quencher is the basis of the design of Molecular Beacons.

Fluorescence Resonance Energy Transfer (FRET)
Resonance energy transfer, often known as fluorescence resonance energy transfer (FRET) or Förster energy transfer. It is the radiation-less transfer of excitation energy from a donor to an acceptor. An important consequence of this transfer is that there is no emission of light by the donor. The acceptor may or may not be fluorescent.

FRET varies based on the degree of spectral overlap of the donor and acceptor. This is called the "spectral overlap" or sometimes the "Förster overlap integral". This describes the amount of overlap where resonance can occur, i.e. where the donor and acceptor have the same frequencies.

TaqMan Probes
TaqMan (also known as Fluorogenic 5' nuclease assay) probes contain two dyes, a reporter dye (e.g. 6-FAM) at the 5' end and a 3' acceptor dye, usually TAMRA. Recent designs substitute the 3' TAMRA fluorescent acceptor quencher dye with non-fluorescent quencher, e.g. BHQ-1. The proximity of the quencher to the reporter in an intact probe allows the quencher to suppress, or "quench" the fluorescence signal of the reporter dye through FRET.

Molecular Beacons
Molecular beacons are hairpin shaped oligos with a fluorophore and a quencher at either ends. The loop serves as the specific target sequence. The stem is formed by the annealing of complementary arm sequences on the ends of the probe sequence. The stem keeps the fluorophore and the quencher in close proximity to each other, causing the fluorescence of the fluorophore to be quenched by energy transfer. When the probe encounters a target molecule, it forms a hybrid that is longer and more stable than the stem leading to the restoration of fluorescence.

Fluorophore Absorbance and Emission Data*

Dye	Color	Absorbance Max (nm)	Emission Max (nm)	Extinction Coefficient
6-FAM (Fluorescein)	Green	494	525	74850
TET	Orange	521	536	85553
HEX	Pink	535	556	95698
Cy 5	Violet	646	667	250000
Cy 5.5	Blue	683	707	190000
Cy 3	Red	552	570	150000
Cy 3.5	Purple	588	604	150000
Cy 7	Near IR	743	767	200000
Tamra	Rose	565	580	87000
ROX	Purple	587	607	105000
JOE	Mustard	528	554	105000
Alexa Dye Series	Varies	Varies	Varies	Varies

*Color and fluorescence data vary with pH. Consult appropriate dye manufacturer for details.

*BHQ is a registered trademark of Biosearch Technologies. Complete disclaimer of license statement for Molecular Beacons products, PHRI Molecular Beacon and BHQ license agreement can be viewed at the following link: www.genelink.com/newsite/products/MBPriceList.asp

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