Oligo Modifications for Increased Duplex Stability & Nuclease Resistance

Increased duplex stability and nuclease resistance are underlying requirements for most oligonucleotide-based applications.

Gene Link offers several modifications that can render the oligo less susceptible to nuclease degradation as well as increase hybridization stability. In addition to the synthesis of these modified oligos, we routinely assist customers in the design of oligos that are particularly suited to their application.

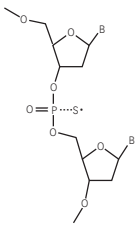
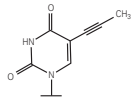
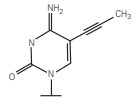
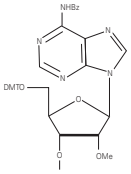
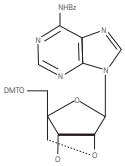
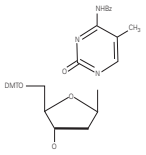
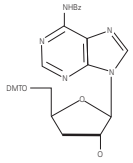
Antisense research requires short oligonucleotides that are complementary in sequence, and upon specific hybridization to its cognate gene product, induce inhibition of gene expression.

Increased stability of the RNA-DNA duplex in terms of hybridization and half-life is crucial to successful gene inhibition. These modifications can also be used for molecular probes and primers. Listed are some of the common modifications that impart these properties.

Antisense ODN Design Options

Traditionally phosphorothioate ODN's are used for antisense research. Consider inclusion of modified bases imparting increased duplex stability.

Oligo Modifications

Modification	Phosphorothioate	Propyne analogs	2'-O-methyl RNA	Locked Nucleic Acids	5-Me-dC	2'-5' Linked Oligonucleotides	Chimeric Linkages
Molecular Structure	 2'-Deoxy thioate	 5-(1-Propynyl) uracil (pU)  5-(1-Propynyl) cytosine (pC)	 2'-O-methyl A	 dA LNA	 5-Me-dC	 3' dA (2'-5' linked)	Structure varies
Chemical Characteristics	Modification of the phosphodiester bond by replacing one of the non-bridging oxygens by sulfur	C-5 propyne analogs of dC and dT	2'-O-methyl at the 2' hydroxyl position	Bicyclic nucleic acid where a ribonucleoside is linked between the 2'-oxygen and the 4'-carbon atoms with a methylene unit	C-5 methylated dC	2'-5' linked phosphodiester linkage, 3' deoxy bases	Mixed phosphorothioate and phosphodiester linkages and modifications
Duplex Stability	Hybridizes to the target sequences with lesser affinity than oligos with phosphodiester backbone	Increased binding affinity to the target mRNA and increased stability	Binding similar to DNA	Highest thermal stability of all available modifications	Increased	Increased binding efficiency to RNA	Increased
Nuclease Resistance	Imparts resistance to nuclease degradation	Increased nuclease resistance	Increased	Increased	Similar to DNA	Increased	Increased

APPLICATION	RECOMMENDED MODIFICATIONS
Antisense Gene Target	• 2'-O-Me-nucleotides (2'-O-Me-RNA) form more stable hybrids with complementary RNA strands than equivalent DNA and RNA sequences. • Phosphorothioate linkages confer resistance to nuclease degradation. • Locked Nucleic Acids (LNA) demonstrate unsur–passed duplex stability. Use phosphorothioate linkages to impart nuclease resistance and LNA bases to achieve the most stable hybridization. • Propyne modified with phosphorothioate linkages are 50x more effective than the corresponding phosphodiester oligo.
Real-Time PCR probes, QPCR	• 5-Me-dC enhances duplex stability, thus shorter probes can be synthesized. • LNA bases render the probe greater duplex stability than the use of single MGB (minor groove binders) at the 3' end. It is an excellent substitute for TaqMan MGB modifications. • All combinations of modifications, fluorescent dyes, and backbone modifications can be performed.
SNP Genotyping, Allelic Discrimination	• LNA substituted bases impart greater specificity with higher T_m. • All types of fluorescent dyes and backbone modifications can be performed. • 5-Me-dC behaves similar to LNA bases in imparting duplex stability.
Hybridization Probes and PCR Amplification Primers	• LNA substituted bases impart greater specificity with higher T_m. Substitute 4-6 DNA bases with LNA bases. • 5-Me-dC behaves similar to LNA bases in imparting duplex stability.

Modifications Increasing Nuclease Resistance and Duplex Stability

	Price ($)/site	
Product	**200 nmol scale**	**1 µmol scale**
Phosphorothioates	4.25	6.50
5-Propyne pdC, pdU	130.00	200.00
5-Me-dC	75.00	125.00
2'-O-methyl bases	14.00	20.00
2'-5' linked bases	275.00	300.00
LNA bases	190.00	250.00
Chimeric linkage	75.00	75.00

*minimum charge for 15-20 mer applies depending upon modification.
Visit www.genelink.com for complete conditions of use and licensing agreements.

The Gene Link Advantage

- Stringent Quality Control Measures
- Specializing in Challenging Combinations of Modifications
- Antisense ODN combinations
- SNP Genotyping & Allelic Discrimination
- Real Time PCR Probes
- All Oligo Types and Modifications Available
- Easy Online Ordering System
- Online Design and Analysis Tools
- Rapid Turn Around Time
- Knowledgeable Technical Support
- Personalized, Friendly Customer Service

GOLD STANDARD

Unique Modifications

Gene Link specializes in the design and synthesis of challenging combinations of modifications.

You are invited to compare.

Modifications for Cloning, Hybridization Probes and Affinity Chromatography

Gene Link encourages investigators to switch to non-radioactive detection methods. These provide a safe and sensitive alternative to radioactive methods.

Sensitive chemiluminescent, visible dye and fluorescence-based methods are available for qualitative and quantitative detection. The ligand modified oligos for non-radioactive detection can also be used for specific sequence-based affinity chromatography.

Phosphorylation for Ligation and Cloning

Oligonucleotides that are designed for eventual cloning are ligated to the appropriate vector. Efficient ligation requires that the 5' end of the fragment possess a phosphate group to form the phosphodiester linkage.

Custom oligos can be quantitatively modified to add a phosphate at the 5' end or the 3' end. This is a convenient way to kinase the oligos. 5' Phosphorylation should be requested as a modification at the time of order placement.

Non-Radioactive Hybridization Probes

Sensitivity

Chemiluminescent detection with alkaline phosphatase approaches 0.1 pg, equivalent to ~$1x10^6$ copies of the target; this is equivalent to less than 4 µg of human genomic DNA. Almost all Southern-based hybridizations can be switched to safe non-radioactive based methods.

Detection Strategy

The non-radioactive modification used must be complemented with a sensitive detection method. Please see technical details at www.genelink.com.

Modifications for Affinity and Hybridization Probes

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. Gene Link synthesizes all oligo types and modifications. Visit www.genelink.com for a complete listing and detailed information.

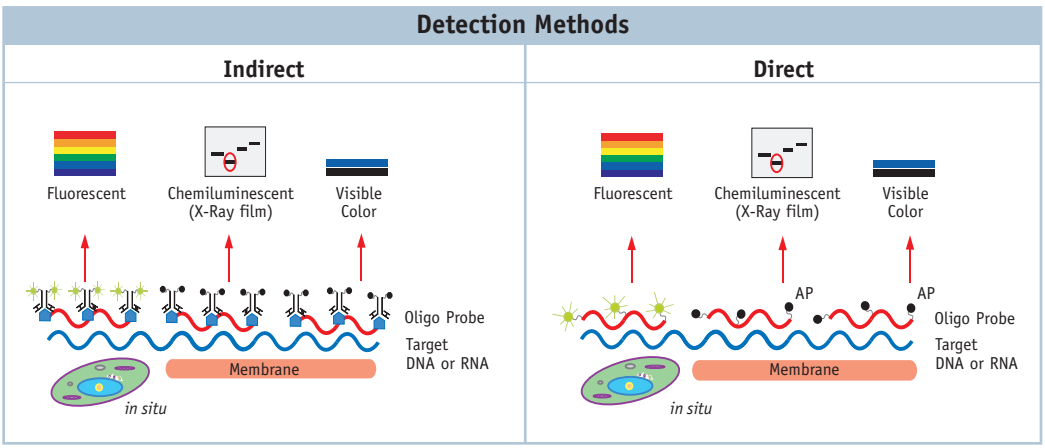
Biotin and Digoxigenin

Oligos can be labeled with biotin or conjugated with digoxigenin. These modifications can be placed at both the 5' and 3' ends and internally as a branch from a T residue.

Spacers and Linkers

Spacers and linkers are used to insert a spacer arm into an oligonucleotide. These may be added in multiple additions when a longer spacer is required. 3' Spacer C3 CPG may also act as a blocker of exonuclease and polymerase activity at the 3'-terminus. dSpacer is used to introduce a stable abasic site within an oligonucleotide.

Detection Methods



Alkaline Phosphatase Conjugation

Alkaline phosphatase conjugation is similar in chemistry to digoxigenin labeling. The oligo is synthesized with an amino modification and then conjugated with activated alkaline phosphatase. Amino C-12 is recommended as the modification.

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

In addition to 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.

Locked Nucleic Acids (LNA)

LNA bases can be incorporated into oligos to increase probe specificity and thermal stability. LNA exhibit unprecedented thermal stabilities towards complementary DNA and RNA. The higher binding affinity of oligos containing LNA bases allows for the design and use of shorter probes in hybridization assays.

Oligo Modifications For Cloning, Hybridization Probes and Affinity Chromatography

Product	Price ($)/site		
	50 nmol scale	200 nmol scale	1 µmol scale
Biotin dT, Biotin multi	220.00	220.00	310.00
5' or 3' Biotinylation	90.00	90.00	190.00
Biotin-TEG	250.00	250.00	350.00
Digoxigenin*	275.00	275.00	350.00
Alkaline Phosphatase	550.00	550.00	1200.00
Locked Nucleic Acid (LNA)	190.00	190.00	250.00
5' or 3' Phosphorylation	55.00	55.00	110.00
3' and 5' Amino Linker C3, C6, C12	55.00	55.00	110.00
Spacer C3, 9, C12, 18 & dSpacer	150.00	150.00	200.00

See website for complete listing. Please inquire about volume discounts.

*Boehringer Mannheim/Roche holds exclusive rights to digoxigenin labeling. Digoxigenin oligo labeling is offered under license from Roche.

Purification

The coupling efficiency of most modifications range between 80%-95% and certain modifications require specialized synthesis and processing protocols. All modified oligos should preferably be gel purified to obtain greater than 98% purity.

Purification

Product	Price ($)/purification		
	50 nmol	200 nmol	1 µmol
Gel Purification	75.00	75.00	150.00

The Gene Link Advantage

- Stringent Quality Control Measures
- Specializing in Challenging Combinations of Modifications
- Non-Radioactive Probe Alternatives
- Affinity Ligands
- All Oligo Types and Modifications Available
- Easy Online Ordering System
- Rapid Turn Around Time
- Knowledgeable Technical Support
- Personalized, Friendly Customer Service

GOLD STANDARD

Take a Fresh Look

Switch to non-radioactive. Gene Link specializes in the design and synthesis of non-radioactive modifications for use as probes.

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

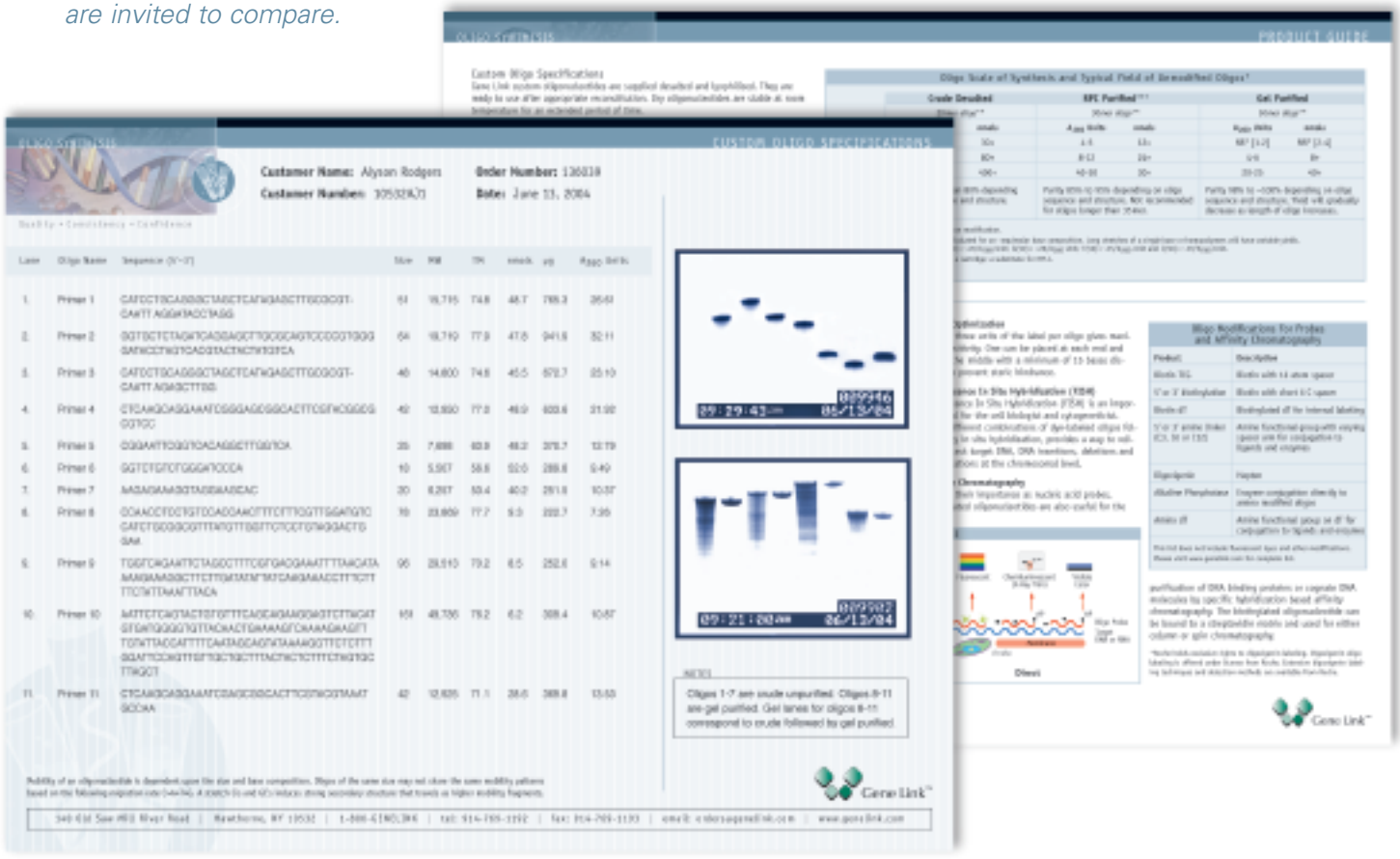
The crude, desalted oligonucleotide supplied is suitable for all amplification and sequencing protocols. Gel purification is advised for all oligos used for cloning applications and for oligos longer than 50 mer.

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

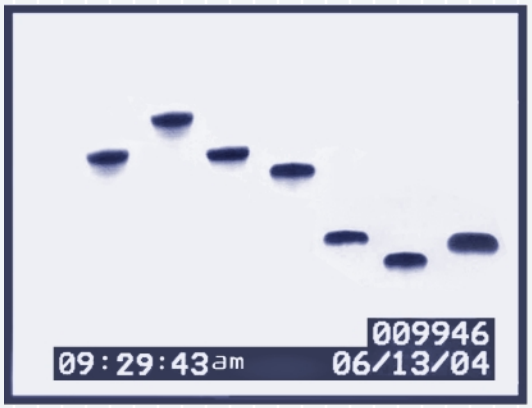
		Scale of Synthesis Price ($)/purification					
Product	**Catalog No.**	**50 nmol**	**200 nmol**	**1 µmol**	**2 µmol**	**10 µmol**	**15 µmol**
Gel Purification	26-6400-XX	75.00	75.00	150.00	280.00	1500.00	1800.00
Reverse Phase Cartridge	26-6400-XX	30.00	30.00	90.00	170.00	750.00	900.00

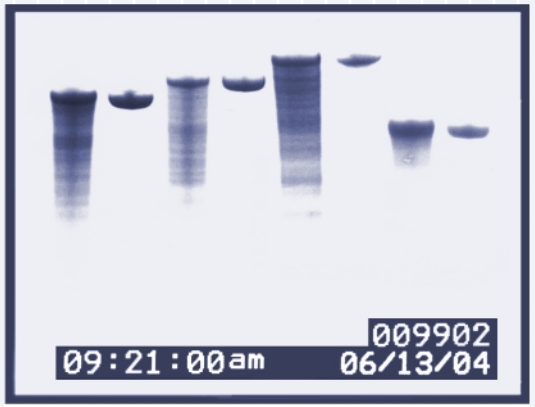
Quality • Consistency • Confidence

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™