

Purification

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.

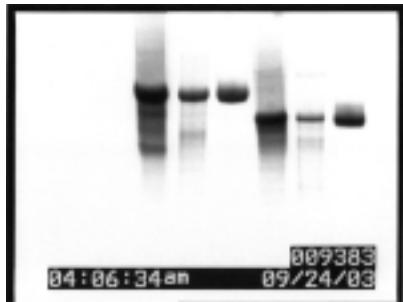
Polyacrylamide Gel Purification (PAGE)

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							
Scale	RPC Purified**				Gel Purified		
	A ₂₆₀ Units	nmols	mg	30mer oligo Typical yield	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 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		

G's: The Unresolved Dilemma

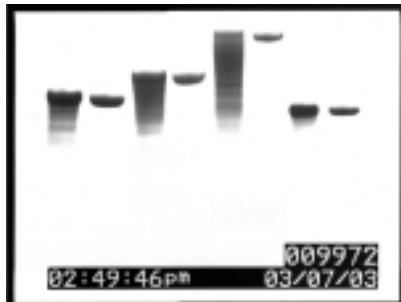
Ever wonder why we have not yet discovered a polymerase that can breeze through a stretch of G's? A stretch of three or more G's in an oligo sequence induces strong secondary structure. A string of G's and C's can exhibit internal Hoogsteen base pairing, non-Watson-Crick triple base pairing and should 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.



Comparison of 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 were loaded to show the truncated failure sequences. Approximately 8 µg of purified oligo were loaded. Lanes 1-3: 68 mer; lanes 4-6: 56 mer. Lanes 1 & 4: crude unpurified; lanes 2 & 5: RPC purified; lanes 3 & 6: gel purified.

Results: The above gel picture shows the lack of purification efficiency of RPC as compared to gel purification. Notice the remaining truncated oligo sequences that the RPC method failed to purify.



Comparison of Unpurified and Gel Purified Oligos

Polyacrylamide gel electrophoresis of crude and gel purified oligos in adjacent lanes. Lanes 1 & 2: 63 mer; lanes 3 & 4: 96 mer; lanes 5 & 6: 175 mer; lanes 7 & 8: 43 mer.

Results: At Gene Link we recommend gel purification of all long oligos and oligos used in cloning applications. Gel purification is the "gold standard" method of purification as the denaturing polyacrylamide gel resolution approaches single base and the major band is clearly visible to be excised and purified.

Oligo Size & Purification Recommendations		
Length	PAGE	HPLC/RPC
8-40 mer	Yes	Yes
41-250 mer	Yes	No
All Gene Link oligos shorter than 40 mer usually do not require any further purification if the application is for PCR or sequencing.		

Application Based Purification Recommendations	
Application	Purification
PCR & Sequencing	Not Required
Cloning & Gene Construction	Yes
Mutagenesis	Yes
Modified Oligos	Yes
Probes	Yes

Purification

All Gene Link oligos shorter than 40 mer usually do not require any further purification if the application is for PCR or sequencing. Gene Link recommends gel purification of oligos longer than 50 mer and all oligos destined to be cloned.

Product	Scale of Synthesis Price (\$)/purification					
	50 nmol	200 nmol	1 µmol	2 µmol	10 µmol	15 µmol
Gel Purification	75.00	75.00	150.00	280.00	1500.00	1800.00
Reverse Phase Cartridge	30.00	30.00	90.00	170.00	750.00	900.00

GOLD **STANDARD**



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, A₂₆₀ 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 A₂₆₀. 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 SPECIFICATIONS										PURCHASE ORDER																														
<p>Customer Name: Alyson Rodgers Customer Number: 10532973 Order Number: 126018 Date: June 13, 2004</p>																																								
<p>CUSTOM OLIGO SPECIFICATIONS</p> <p>Customer 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.</p>										<p>Oligo Scale of Synthesis and Typical Yield of Synthesized Oligo*</p> <table border="1"> <thead> <tr> <th rowspan="2">Oligo Described</th> <th colspan="2">EFS Purified**</th> <th colspan="2">Gel Purified***</th> </tr> <tr> <th>Other oligo***</th> <th>Avg. Yield</th> <th>Other oligo</th> <th>Avg. Yield</th> <th>Other oligo</th> </tr> </thead> <tbody> <tr> <td>50mer</td> <td>~1.0</td> <td>1.0µg</td> <td>50mer</td> <td>~1.0</td> <td>1.0µg</td> </tr> <tr> <td>80mer</td> <td>~0.12</td> <td>20nmol</td> <td>80mer</td> <td>~0.12</td> <td>20nmol</td> </tr> <tr> <td>100mer</td> <td>~0.05</td> <td>10nmol</td> <td>100mer</td> <td>~0.05</td> <td>10nmol</td> </tr> </tbody> </table> <p>* Oligo scale depending on oligo sequence and structure. Not recommended for oligos longer than 100mers.</p> <p>** Purify 100 to 200% depending on oligo sequence and structure. Not recommended for oligos longer than 100mers.</p> <p>*** Purify 100 to 200% depending on oligo sequence and structure. May increase in length of oligo increases.</p> <p>** or more. Based on a minimum four nucleotide long section of a oligonucleotide that can be synthesized.</p> <p>*** Oligo scale depends on oligo sequence and structure. Not recommended for oligos longer than 100mers.</p> <p>**** Oligo scale depends on oligo sequence and structure.</p>			Oligo Described	EFS Purified**		Gel Purified***		Other oligo***	Avg. Yield	Other oligo	Avg. Yield	Other oligo	50mer	~1.0	1.0µg	50mer	~1.0	1.0µg	80mer	~0.12	20nmol	80mer	~0.12	20nmol	100mer	~0.05	10nmol	100mer	~0.05	10nmol
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										<p>Applications Use one of the listed per oligo scale, manufacturing. One can be placed at each end and be within with a minimum of 15 bases flanking each probe.</p> <p>Oligo to DNA Hybridization (ODH) Since the Oligo hybridization (ODH) is an important for cell biology and epigenetics. Oligo to DNA hybridization, of oligo-labeled oligo following in situ hybridization, provides a way to analyze longer RNA/DNA locations, definitions, and regions at the chromosomal level.</p> <p>Chromatography The separation of nucleic acid products, and oligonucleotides are also useful for the analysis of:</p> <ul style="list-style-type: none"> Protein: Protein Antibodies: Enzyme conjugation directly to antisera modified oligos Antisera: Affinity functional group or DTT for conjugation to specific antibodies <p>Method does not include fluorescent tags and other modifications. These will be provided upon request.</p> <p>Oligo Modifications For Protein and Antibody Chromatography</p> <table border="1"> <thead> <tr> <th>Product</th> <th>Description</th> </tr> </thead> <tbody> <tr> <td>Biotin-3'U</td> <td>Oligos with 3' biotin group</td> </tr> <tr> <td>5' or 3' biotinylation</td> <td>Oligos with short 5' C-terminus</td> </tr> <tr> <td>Biotin-ET</td> <td>Biotinylation of the internal labeling 5' or 3' amino linker (C3, M or T3).</td> </tr> <tr> <td>5' or 3' amino linker</td> <td>Amino functional group carrying spacer arm for conjugation to ligands and enzymes</td> </tr> </tbody> </table> <p>Hybridization Oligo hybridization is specific hybridization based on complementary DNA sequences. The hybridized oligonucleotides can be tested for a complementary probes and used for either column- or gel chromatography.</p> <p>Antisera: Oligo antisera is a specific antibody. Oligo antisera often used for Western blotting. Oligo antisera include two methods for synthesis:</p> <ul style="list-style-type: none"> Protein: Oligo protein DNA: Oligo DNA 			Product	Description	Biotin-3'U	Oligos with 3' biotin group	5' or 3' biotinylation	Oligos with short 5' C-terminus	Biotin-ET	Biotinylation of the internal labeling 5' or 3' amino linker (C3, M or T3).	5' or 3' amino linker	Amino functional group carrying spacer arm for conjugation to ligands and enzymes																		
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<p>NOTES: Oligos 1-7 are made unpurified. Oligos 8-11 are gel purified. Gel lanes for oligos 8-11 correspond to crude followed by gel purified.</p>										<p>Gene Link®</p>																														

Probability of an oligonucleotide is determined upon the size and base composition. Sizes of the same size may not share the same mobility patterns based on their following sequence code (A=red, G=green, C=blue, T=yellow). A (GAG) is a G-C rich double strand structure that travels at higher mobility frequency.

340 E. Six Mile River Road | Hawthorne, NJ 07506 | 1-800-4-GENE LINK | fax: 973-765-1712 | fax: 973-765-1130 | email: customerservice@genelink.com | www.genelink.com



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.			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.			
				**RPC is reverse phase purification using a cartridge; a substitute for HPLC.			NR* Not Recommended			

*Yield of 30 μg/A₂₆₀ 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/A₂₆₀ Unit; G(50) = ~28/A₂₆₀ Unit; T(50) = ~35/A₂₆₀ Unit and C(50) = ~39/A₂₆₀ 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.

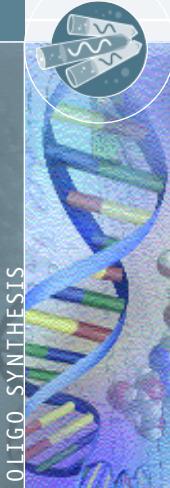
Same Day Oligo*

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

Product	Catalog No.	Scale of Synthesis Price (\$)/purification						
		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: Helen Estrada
Customer Number: 10532AJ1

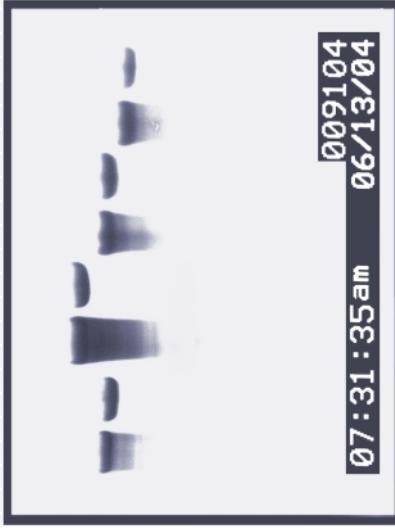
Order Number: 136040
Date: June 13, 2004

Lane Oligo Name Sequence (5'-3')

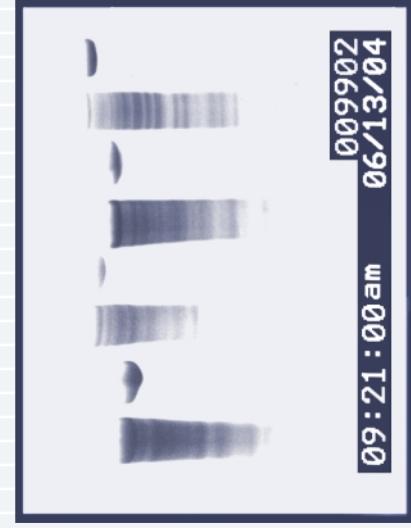
		Size	MW	T _M	nmoles	μg	A ₂₆₀ Units
1.	FRDA-F1	ATTCTGCAGACATGGCTACTCCCTGGAGGGGCTTGTATC TCCATTAAAGGCCCTGGAGGGCTTGTATCTC	71	21,88	78.4	11.8	258.5
2.	FRDA-R1	TTAAGCAGCAGCGATCaaaaaaaAGAGAGAAAGGTA GATCCAAAAAAAAGAAGAGAAAGGTAGATCCAAAAAGAAG AGAAAGGTAGGAAGCACC	96	30,021	74.9	7.4	221.6
3.	FRDA-F2	GCCCTACTCCCTGGAGGTATTGGCTTGTACGTATCTCCA TTAAGGAAAACGTTCACTACAAGTCACAGGCTTGAA CTACTCCCTGGAGGGTCA	78	23,995	76.1	9.9	236.5
4.	FRDA-R2	ACAACTGACTGGAATTAAITCTGCAGCGTATGACATGG CGATCCAAAAAGAAGAGAAAGGTAGATCCAAAAAAGA AGAGAAAGGTAGATCCAAAAAAGAAGAGAGAAAGTAGGA AGCACC	59	18,186	74.9	16.9	308.0
5.	FRDA-F3	TACAAGTCACAGGTCTGGAACAGTTGAAGCAGCAGCAG CGATCCAAAAAGAAGAGAAAGGTAGATCCAAAAAAGA AGAGAAAGGTAGATCCAAAAAAGAAGAGAGAAAGTAGGT AGCACC	120	37,494	77.0	4.1	152.6
6.	FRDA-R3	CAGCTAGCGATCGTACCCGGCGGGAGGAATGA TTTGGATCCAAATGTTGTAaaaACGTTGTATCTCATTAT CACTTACAAGTCACAGGTCTGGAACAGTTGAAGCAGCA GCAGCGATCCAAAAAAGAAGAGAAAGGTAGATCCAAA AAGAAGAGAAAGGTAGATCCAAAAAAGAAGAGAAAGGT AGGAAGCACC	201	62,578	80.1	2.1	129.6
7.	FRDA-F4	ACGTTAAATGCCAACCAAATGTTGTAaaaACGTTGTATCTC CATTATCACCTACAAGTCACAGGTCTGGAACAGTTGAAG CAGCGAGCGATCCAAAAAAGAAGAGAAAGGTAGATCCAAA AAGGTTAGGAAGCACC	170	52,867	77.8	2.8	150.1
8.	FRDA-R4	CATCAATTACTCAAGCAGAAAATCAGGGCACCTCG TACGTTAAATGCCAACCCATAAAACGTTAAATGCCAAC AAATGTGTGTAaaaACGTTGTATCTCATTATCACCTAACAG TCACAGGGTCTGAAACAGTTGAAGCAGCAGCGATC CAAAAGAAGAGAAAGGTAGATCCAAAAAAGAAGAGA AAGGTAGATCCAAAAAAGAAGAGAAAGTAGGAAGGCC	234	72,538	79.0	1.6	114.2

NOTES

Gel purified oligos. Gel lane represents
crude followed by gel purified.



07:31:35 am **06/13/04**



09:21:00 am **06/13/04**

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 Gs and GCs induces strong secondary structure that travels as higher mobility fragments.



Custom Oligo Specifications
Gene Link custom oligonucleotides are supplied desaltsed 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 A₂₆₀. Exact nmols and µg is determined by the extinction coefficient and molecular weight of the oligo.

Oligo Scale of Synthesis and Typical Yield

		Crude Desaltsed				RPC Purified**				Gel Purified			
		20mer oligo* Typical yield				30mer oligo* Typical yield				50mer oligo* Typical yield			
Scale	A ₂₆₀ Units	nmols	mg	A ₂₆₀ Units	nmols	mg	A ₂₆₀ Units	nmols	mg	NR* [1:2]	NR* [2:4]	NR* [0.03-0.06]	
50 nmol	8-10	30+	0.2-0.3	4-5	12+	0.1-0.16	NR*	NR*	NR*	4-6	8+	0.13-0.2	
200 nmol	20-25	80+	0.6-0.8	8-12	24+	0.26-0.40	20-25	40+	40+	6-8	10+	0.6-0.8	
1 µmol	100-120	400+	3-4	40-50	30+	1.3-1.6							
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.				Purity 85% to 95% depending on oligo sequence and structure.				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.				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.				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				Not recommended for oligos longer than 35mer.				NR* Not Recommended			
		No further purification required for PCR and sequencing applications.				**RPC is reverse phase purification using a cartridge; a substitute for HPLC.				No further purification required for PCR and sequencing applications.			
		Get purification recommended for oligos above 50mer and all applications involving cloning and mutagenesis.				Get purification recommended for oligos above 50mer and all applications involving cloning and mutagenesis.				Get purification recommended for oligos above 50mer and all applications involving cloning and mutagenesis.			

*Yield of 30µg/A₂₆₀ 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/A₂₆₀ Unit; G(50) = ~28/A₂₆₀ Unit; T(50) = ~35/A₂₆₀ Unit and C(50) = ~39/A₂₆₀ Unit.

Oligo Reconstitution and Use**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 500pmols/µl stock solution. Use as required.

Stock solution of 100 pmols/µl [100 µM]

Dilute 10 fold to prepare a 50 pmols/µl [50 µM]. 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 [10 µM]. 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 – 0.1 µg). Use the oligo reconstitution protocol to prepare a 100 pmols/µl [100 µM] solution and then dilute 10 fold to get 10 pmol/µl solution. Use 1 µl (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