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)

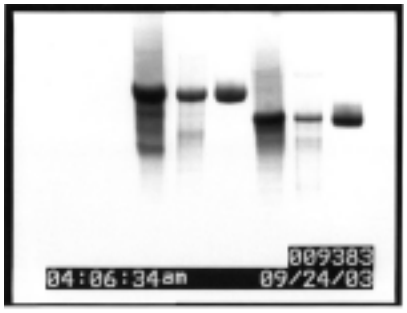
Purification by this method is considered the Gold Standard for oligonucleotide purification and yields 99%+ purity. Gel purification can be used for any length of oligonucleotide (as compared to HPLC and RPC cartridges which are limited to oligonucleotides below 40 mer). Gel purification is strongly 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

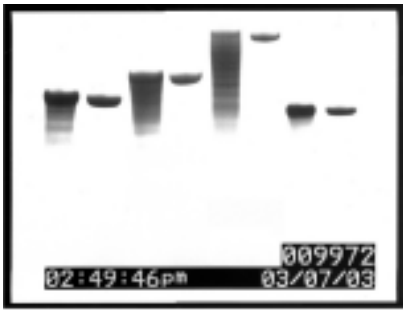
Ever wonder why we have not yet discovered a polymerase that can breeze through a stretch of G's? A stretch of three or more G's in an oligo sequence induces strong secondary structure. A string of G's and C's can exhibit internal Hoogsteen base pairing, non-Watson-Crick triple base pairing and should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



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.

	Scale of Synthesis Price ($)/purification					
Product	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, 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

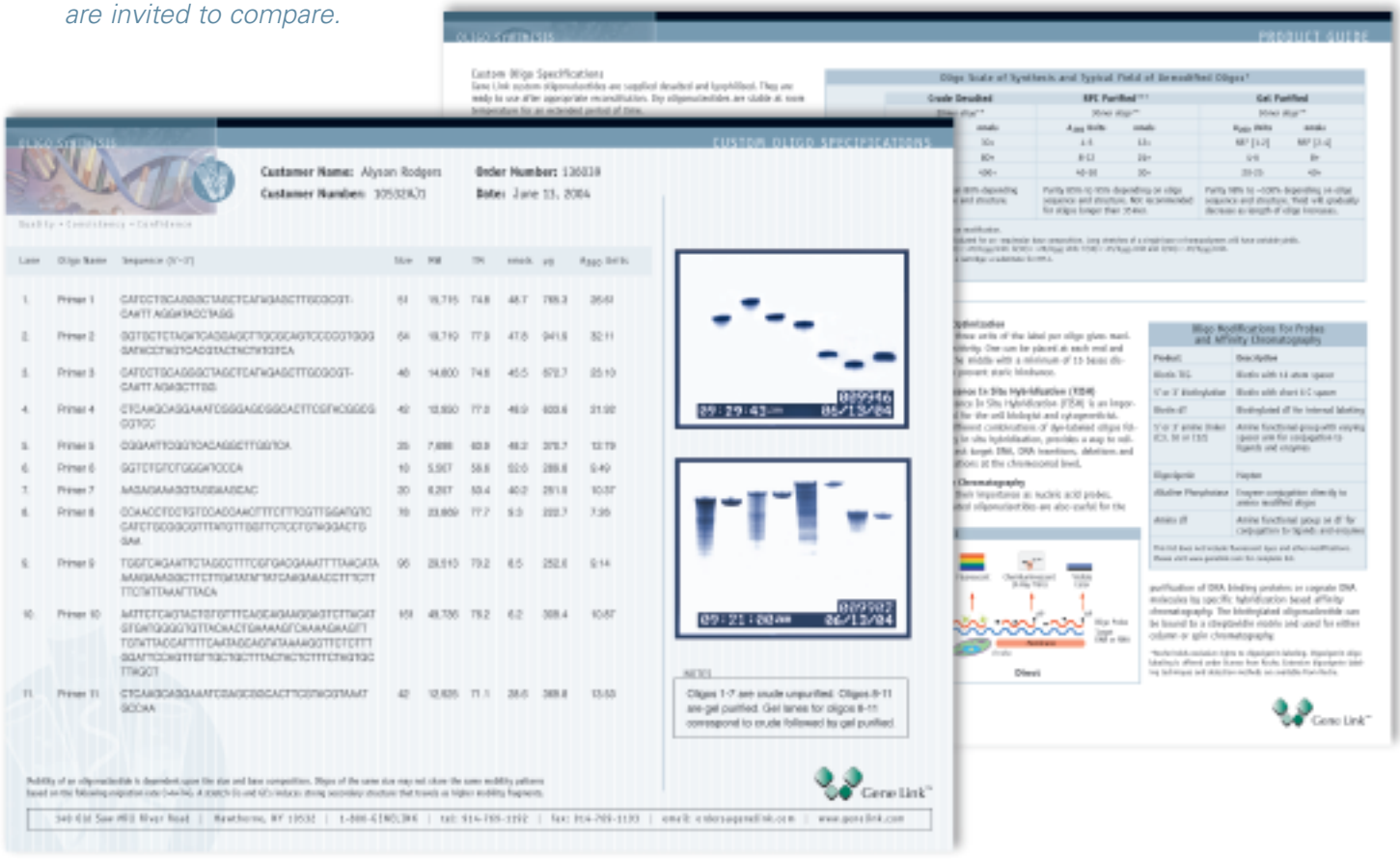
The crude, desalted oligonucleotide supplied is suitable for all amplification and sequencing protocols. Gel purification is advised for all oligos used for cloning applications and for oligos longer than 50 mer.

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

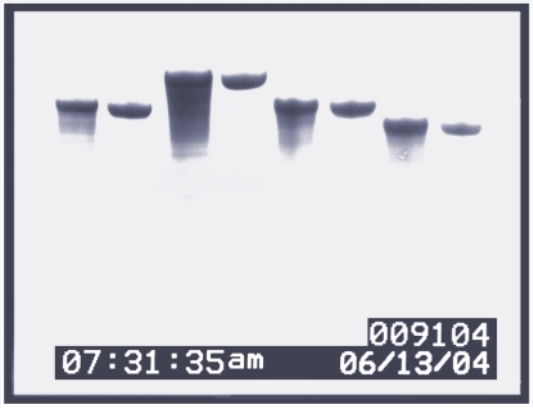
		Scale of Synthesis Price ($)/purification					
Product	**Catalog No.**	**50 nmol**	**200 nmol**	**1 µmol**	**2 µmol**	**10 µmol**	**15 µmol**
Gel Purification	26-6400-XX	75.00	75.00	150.00	280.00	1500.00	1800.00
Reverse Phase Cartridge	26-6400-XX	30.00	30.00	90.00	170.00	750.00	900.00

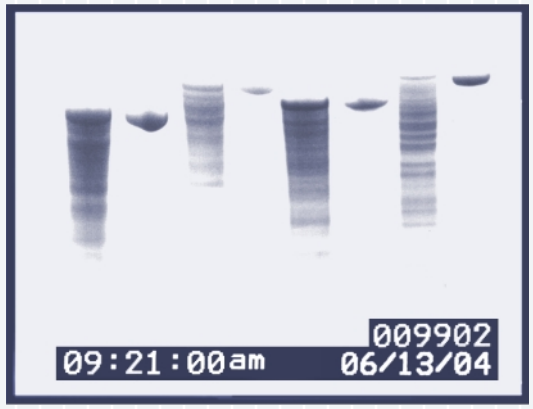
Quality • Consistency • Confidence

Customer Name: Helen Estrada **Order Number:** 136040

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

Lane	Oligo Name	Sequence (5'–3')	Size	MW	TM	nmols	µg	A_{260} Units
1.	FRDA-F1	ATTCTGCAGACATGGCTACTCCCTGGAGGGGCTTGTATC TCCATTAAGAGCCCTGGAGGGGCTTGTATCTC	71	21,88	78.4	11.8	258.5	8.71
2.	FRDA-R1	TTAAGCAGCAGCAGCGATCCAAAAAAGAAGAGAAAGGTA GATCCAAAAAAGAAGAGAAAGGTAGATCCAAAAAAGAAG AGAAAGGTAGGAAGCACC	96	30,021	74.9	7.4	221.6	9.03
3.	FRDA-F2	GCCCTACTCCCTGGAGGTATTGGCTTGTACGTATCTCCA TTAAGAGAAAACGTTCACTTACAAGTCACAGGTCTGGAA	78	23,995	76.1	9.9	236.5	8.30
4.	FRDA-R2	ACAACTGACTGGAATTAATTCTGCAGCCGTATGACATGG CTACTCCCTGGAGGGGTCAT	59	18,186	74.9	16.9	308.0	10.80
5.	FRDA-F3	TACAAGTCACAGGTCTGGAACAGTTGAAGCAGCAGCAG CGATCCAAAAAAGAAGAGAAAGGTAGATCCAAAAAAGA AGAGAAAGGTAGATCCAAAAAAGAAGAGAAAGGTAGGA AGCACC	120	37,494	77.0	4.1	152.6	6.10
6.	FRDA-R3	CAGCTAGCGATCGTACCCGGCGCCGGAGGAGGAATGA TTTGGATCCAAATGTGTGTAAAACGTTGTATCTCCATTAT CACTTACAAGTCACAGGTCTGGAACAGTTGAAGCAGCA GCAGCGATCCAAAAAAGAAGAGAAAGGTAGATCCAAAA AAGAAGAGAAAGGTAGATCCAAAAAAGAAGAGAAAGGT AGGAAGCACC	201	62,578	80.1	2.1	129.6	4.93
7.	FRDA-F4	ACGTAAATGCCAACCAAATGTGTGTAAAACGTTGTATCTC CATTATCACTTACAAGTCACAGGTCTGGAACAGTTGAAG CAGCAGCAGCGATCCAAAAAAGAAGAGAAAGGTAGATC CAAAAAAGAAGAGAAAGGTAGATCCAAAAAAGAAGAGAA AGGTAGGAAGCACC	170	52,867	77.8	2.8	150.1	5.83
8.	FRDA-R4	CATCATTACTACTCAAGCAGGAAATCGAGCGGCACTTCG TACGTAAATGCCAACCCTATATAAAACGTAAATGCCAACC AAATGTGTGTAAAACGTTGTATCTCCATTATCACTTACAAG TCACAGGTCTGGAACAGTTGAAGCAGCAGCAGCGATC CAAAAAAGAAGAGAAAGGTAGATCCAAAAAAGAAGAGA AAGGTAGATCCAAAAAAGAAGAGAAAGGTAGGAAGCACC	234	72,538	79.0	1.6	114.2	4.36





NOTES

Gel purified oligos. Gel lane represents 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		

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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™