

## Synthesis of Long Oligos

Synthesis of long oligos up to 250 mer requires greater than 99.5% coupling efficiency. This can only be attained by using reagents of exacting specifications, optimized protocols and state-of-the-art instruments. Gene Link has perfected and maintains these standards. *You are invited to compare.*

**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. See the coupling efficiency table and graph.**

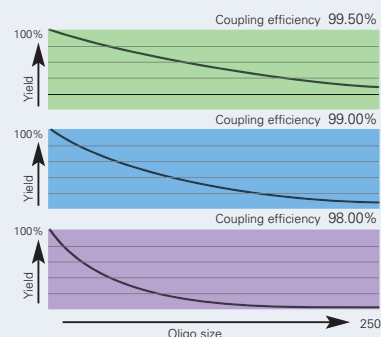
Gene Link specializes in long oligos. Our description of a long oligo is 180 mer to 250 mer. *You are invited to compare.*

### 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 70 mer is ~50% full-length and ~50% truncated sequences. Gel purification is strongly recommended for all oligos above 50 mer.

### 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



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.

### 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 becomes enormously important when synthesizing a long oligo. Coupling efficiency of 99% or 98% seems very good but on closer examination the yield is almost half for a 40 mer! See the coupling efficiency table.

### Long Oligo Scale of Synthesis and Typical Yield

Gel Purified 150 mer oligo typical yield			
Scale	A <sub>260</sub> Units	nmols	mg
1 $\mu$ mol	4-6	4+	0.13-0.2
2 $\mu$ mol	8-12	8+	0.26-0.8

**Purity & Yield 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.

### Oligo Size and Purification Recommendations

Scale	Synthesis Scale	Recommended Purification
1-49 mer	50 nmol	No purification required. Purification dependent upon desired application.
50-99 mer	200 nmol	Gel purification
100-199 mer	1 $\mu$ mol	Gel purification
200-250 mer	2 $\mu$ mol	Gel purification



**Customer Name:** Helen Estada  
**Customer Number:** 208342  
**Order Number:** 135041  
**Date:** June 11, 2006

Lane	Oligo Name	Sequence (5'-3')	Size	MW	%G	%C	%A	%T	App. Info.
1	PNBA-F1	ATCTCTGACAGTGGCTGCTGCTGGAAGGCTG... TAC TGGTTCAGAGCTGGAGAGGATGGATGTC	71	14,168	18.4	9.8	26.6	45.2	871
2	PNBA-F2	TAAAGCAGCAGCAGCAGCAGCAGCAGCAGCAG... TGGTTCAGAGCTGGAGAGGATGGATGTC	80	16,201	19.0	7.4	20.0	53.6	928
3	PNBA-F3	GCCCTGCTGCTGGAAGGCTGGCTTGGCTTGG... TAAAGCAGCAGCAGCAGCAGCAGCAGCAGCAG	78	15,896	19.1	9.8	29.6	41.5	836
4	PNBA-F4	AGAACTGACTGGATTTTGTGAGGCGGATGAG... TAAAGCAGCAGCAGCAGCAGCAGCAGCAGCAG	88	18,180	19.0	10.0	20.0	51.0	1080
5	PNBA-F5	TACAGTTCAGCAGTTCAGCAGCAGTTCAGCAG... TAAAGCAGCAGCAGCAGCAGCAGCAGCAGCAG	100	21,494	19.0	4.1	18.0	68.9	830
6	PNBA-F6	CAAGCAGCAGCAGCAGCAGCAGCAGCAGCAG... TAAAGCAGCAGCAGCAGCAGCAGCAGCAGCAG	101	21,510	19.1	2.1	12.0	76.8	491
7	PNBA-F7	AGGAAAGCAGCAGCAGCAGCAGCAGCAGCAG... TAAAGCAGCAGCAGCAGCAGCAGCAGCAGCAG	170	33,887	19.0	2.8	10.1	78.1	383
8	PNBA-F8	CATGCTGCTGCTGGAAGGCTGGCTTGGCTTGG... TAAAGCAGCAGCAGCAGCAGCAGCAGCAGCAG	204	41,028	19.0	1.8	11.0	77.2	438

**Quantity of synthesis:** 1000 µmol (1000 µmol per oligo)  
**Scale of synthesis:** 200 nmol scale  
**Price (\$)/base:** 2.00

## Purity and Yield

Gel purified oligo purity is generally between 98% to ~99.9% depending on oligo sequence and structure. Yield will gradually decrease as oligo length 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 broad bands on a polyacrylamide gel thus decreasing purity and yield.

## Unmodified DNA Oligo Synthesis\*

Scale of Synthesis	Price (\$)/base
200 nmol scale	2.00
1 µmol scale	3.75
2 µmol scale	6.50

\*minimum charge for 15 mer applies. Please visit [www.genelink.com](http://www.genelink.com) for current list prices. Call for institutional discount pricing structure.

## 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 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



### 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 (10mM Tris, 1mM 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 50mer.

### 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.

### Biophysical Data

Each oligo after desalting is quantified by recording A<sub>260</sub>. Exact nmols and µg is determined by the extinction coefficient and molecular weight of the oligo.

## 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

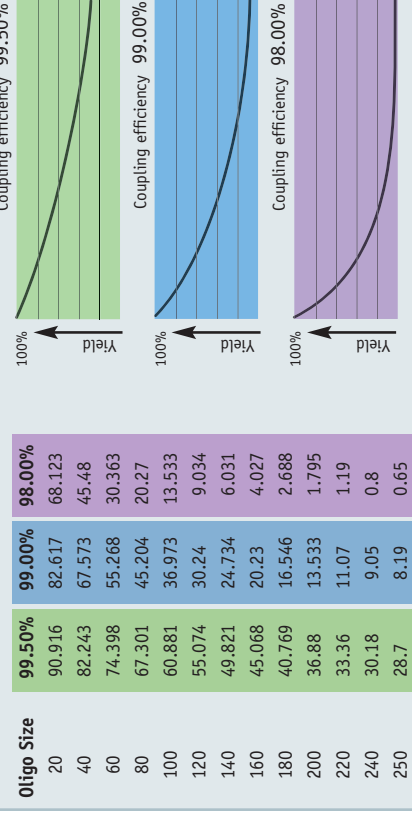
and can tolerate up to 50% failure/truncated

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.

### 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.

### Coupling Efficiency and Full Length Oligo Yield



## Oligo Scale of Synthesis and Typical Yield of Unmodified Oligos\*

Scale	Crude Desalted		RPC Purified***		Gel Purified	
	A <sub>260</sub> Units	nmols	A <sub>260</sub> Units	nmols	A <sub>260</sub> 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+
<b>Purity &amp; Yield</b>	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.	

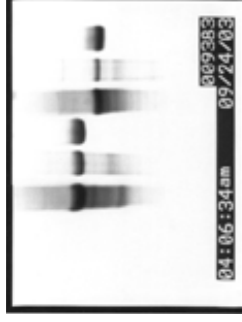
\*The yield of modified oligos varies based on modification.

\*\*Yield of 30µg/A<sub>260</sub> 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<sub>260</sub> Unit; G(50) = ~28/A<sub>260</sub> Unit; T(50) = ~35/A<sub>260</sub> Unit and C(50) = ~39/A<sub>260</sub> Unit.

\*\*\*RPC is reverse phase purification using a cartridge; a substitute for HPLC.

NR\* Not Recommended.



### Unpurified, RPC and Gel Purified Oligos

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

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

Approximately 8µg 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 x 10<sup>23</sup> mol<sup>-1</sup>) and synthesis of a long oligo at 1 micromolar scale (6.022 x 10<sup>17</sup> mol<sup>-1</sup>) 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.



Quality • Consistency • Confidence

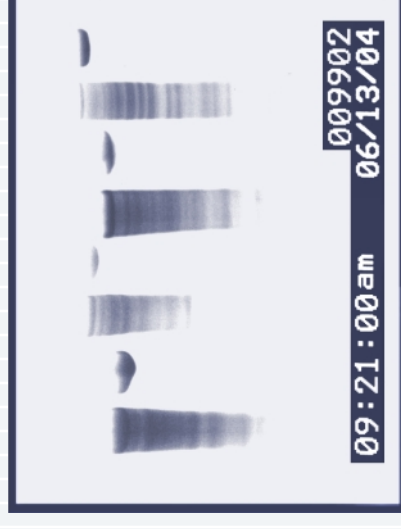
**Customer Name:** Helen Estrada  
**Customer Number:** 10532AJ1

**Order Number:** 136040  
**Date:** June 13, 2004

Lane	Oligo Name	Sequence (5'-3')	Size	MW	TM	nmols	µg	A <sub>260</sub> Units
1.	FRDA-F1	ATTTCGACAGCATGGTACTCCCTGGAGGGCTTGATCTCCATTAAAGAGCCCTGGAGGGCTTGATCTC	71	21,88	78.4	11.8	258.5	8.71
2.	FRDA-R1	TTAAGCAGCAGCAGCGATCCAAAAAAGAAAGAGAAAGGTA GATCCAAAAAAGAAAGAGAAAGGTAGATCCAAAAAAGAAAG AGAAAGGTAGGAAGCACC	96	30,021	74.9	7.4	221.6	9.03
3.	FRDA-F2	GCCTACTCCCTGGAGGTATTGGCTTGACGTATCTCCA TTAAGAGAAAACGTTCACTTACAAGTCACAGGCTGGAA	78	23,995	76.1	9.9	236.5	8.30
4.	FRDA-R2	ACAACGTACTGGAATTAATCTGCAGCCGTATGACATGG CTACTCCCTGGAGGGTTCAT	59	18,186	74.9	16.9	308.0	10.80
5.	FRDA-F3	TACAAATCACAGGCTCGAAACAGTTGAAGCAGCAGCAG CGATCCAAAAAAGAGAGAAAGGTAGATCCAAAAAAGA AGAGAAAGGTAGATCCAAAAAAGAAAGAGAAAGGTAGGA AGCACC	120	37,494	77.0	4.1	152.6	6.10
6.	FRDA-R3	CAGTAGCGATCGTACC GGCCGGGAGGGAATGA TTTGGATCCAAATGTGTAAACGTTGTATCTCCATTAT CACTTACAAGTCACAGGCTGGAACAGTTGAAGCAGCA GCAGCGATCCAAAAAAGAAAGAGAAAGGTAGATCCAAAA AAGAAAGAAAGGTAGATCCAAAAAAGAAAGAGAAAGGT AGGAAGCACC	201	62,578	80.1	2.1	129.6	4.93
7.	FRDA-F4	ACGTAATGCCAACCAATGTGTAAACGTTGTATCTC CATTATCACTTACAAGTCACAGGCTGGAACAGTTGAAG CAGCAGCAGGATCCAAAAAAGAAAGAGAAAGGTAGATC CAAAAAAGAAAGAGAAAGGTAGATCCAAAAAAGAAAGAGA AGGTAGGAAGCACC	170	52,867	77.8	2.8	150.1	5.83
8.	FRDA-R4	CATCATTACTACTCAAGCAGGAAATCGAGGGCCTTCG TACGTAATGCCAACCCATATAAACGTAATGCCAACCC AAATGTGTAAACGTTGTATCTCCATTATCACTTACAAG TCACAGGCTGGAACAGTTGAAGCAGCAGCAGCGATC CAAAAAAGAAAGAGAAAGGTAGATCCAAAAAAGAAAGAGA AAGGTAGATCCAAAAAAGAAAGAGAAAGGTAGGAAGCACC	234	72,538	79.0	1.6	114.2	4.36



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## 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™