## 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.**

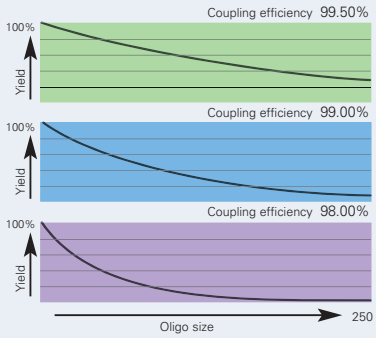
Gene Link specializes in long oligos. Our description of a long oligo is 180 mer to 250 mer. *You are invited to compare.*

### 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

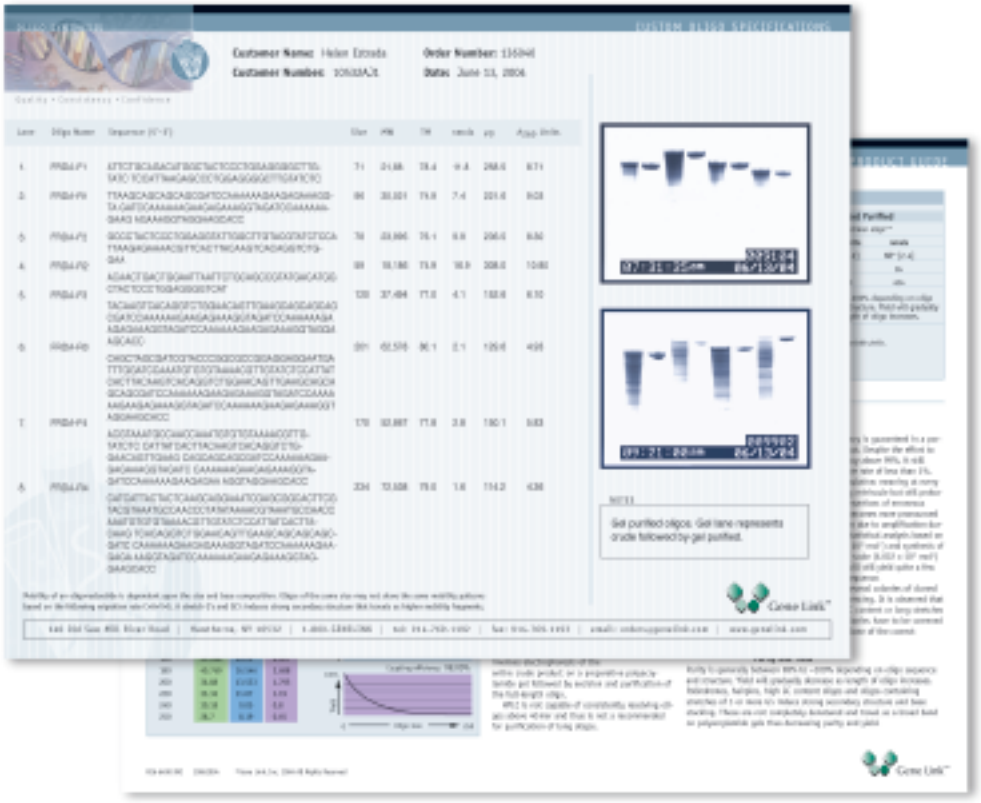
| Scale | $A_{260}$ Units | nmols | mg |
|---|---|---|---|
| 1 µmol | 4–6 | 4+ | 0.13–0.2 |
| 2 µmol | 8–12 | 8+ | 0.26–0.8 |

**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 µmol | Gel purification |
| 200-250 mer | 2 µmol | Gel purification |



## 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 for current list prices. Call for institutional discount pricing structure.

GOLD STANDARD

## 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.**

| | 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 |

**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)

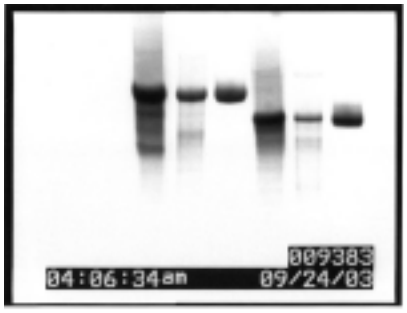
Purification by this method is considered the Gold Standard for oligonucleotide purification and yields 99%+ purity. Gel purification can be used for any length of oligonucleotide (as compared to HPLC and RPC cartridges which are limited to oligonucleotides below 40 mer). Gel purification is strongly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

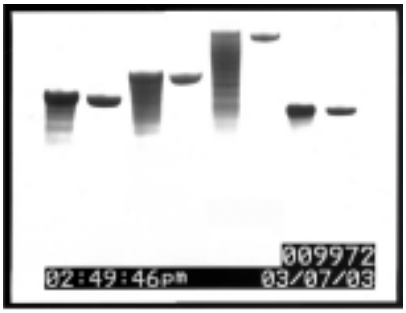
Ever wonder why we have not yet discovered a polymerase that can breeze through a stretch of G's? A stretch of three or more G's in an oligo sequence induces strong secondary structure. A string of G's and C's can exhibit internal Hoogsteen base pairing, non-Watson-Crick triple base pairing and should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

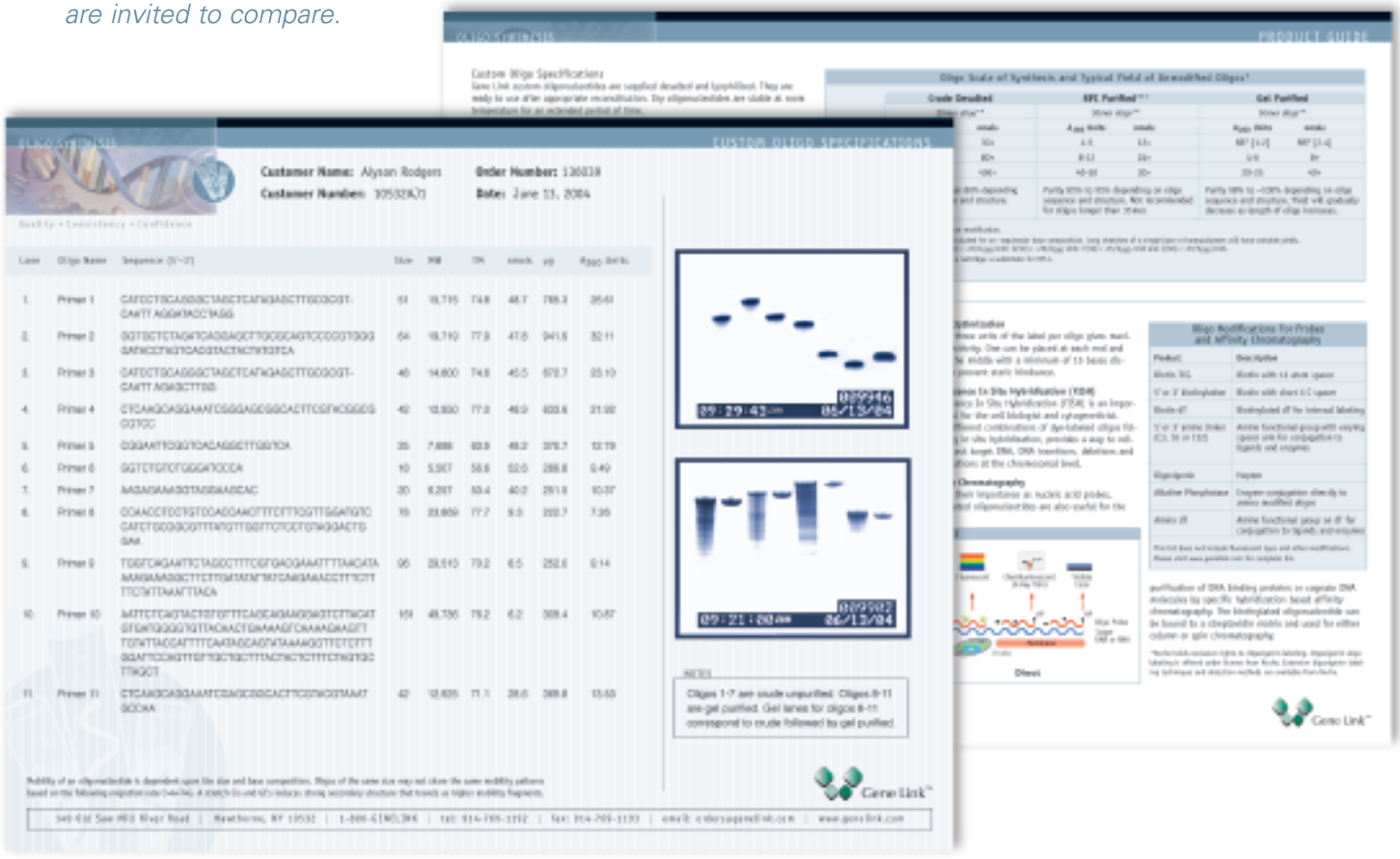
The crude, desalted oligonucleotide supplied is suitable for all amplification and sequencing protocols. Gel purification is advised for all oligos used for cloning applications and for oligos longer than 50 mer.

### 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.<br><br>No further purification required for PCR and sequencing applications.<br><br>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.<br><br>Yield and purity will be lower for sequences with high GC content.<br><br>Not recommended for oligos longer than 35 mer.<br><br>**RPC is reverse phase purification using a cartridge; a substitute for HPLC. | | | **Purity 98% to ~100%** depending on oligo sequence and structure.<br><br>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.<br><br>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

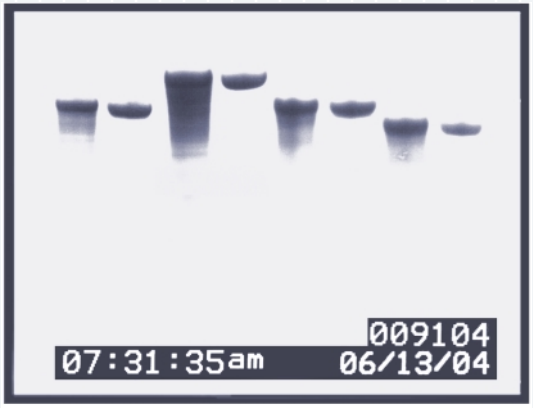
| | | Scale of Synthesis Price ($)/purification | | | | | |
|---|---|---|---|---|---|---|---|
| **Product** | **Catalog No.** | **50 nmol** | **200 nmol** | **1 µmol** | **2 µmol** | **10 µmol** | **15 µmol** |
| Gel Purification | 26-6400-XX | 75.00 | 75.00 | 150.00 | 280.00 | 1500.00 | 1800.00 |
|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

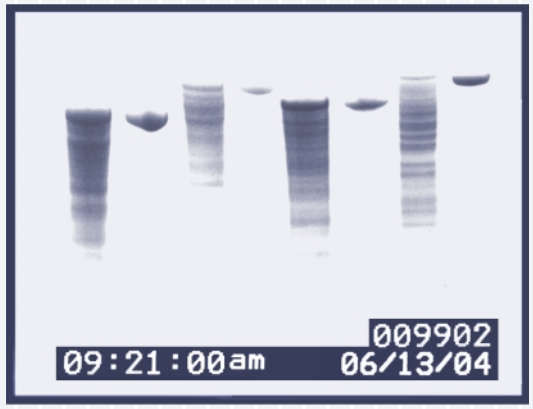
Quality • Consistency • Confidence

**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. 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. | |

*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.

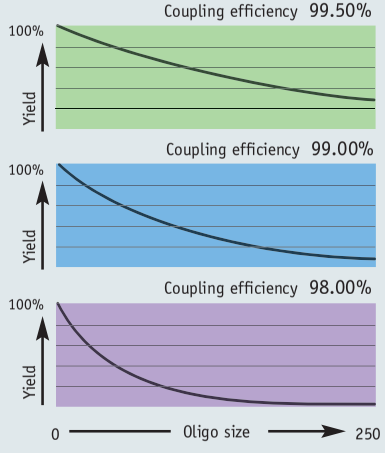
## Synthesis of Long Oligos

### 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

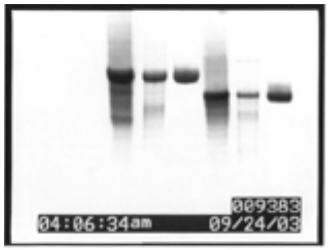
| Oligo Size | 99.50% | 99.00% | 98.00% |
|---|---|---|---|
| 20 | 90.916 | 82.617 | 68.123 |
| 40 | 82.243 | 67.573 | 45.48 |
| 60 | 74.398 | 55.268 | 30.363 |
| 80 | 67.301 | 45.204 | 20.27 |
| 100 | 60.881 | 36.973 | 13.533 |
| 120 | 55.074 | 30.24 | 9.034 |
| 140 | 49.821 | 24.734 | 6.031 |
| 160 | 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 ($6.022 \times 10^{23}$ $mol^{-1}$) and synthesis of a long oligo at 1 micromolar scale ($6.022 \times 10^{17}$ $mol^{-1}$) 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™