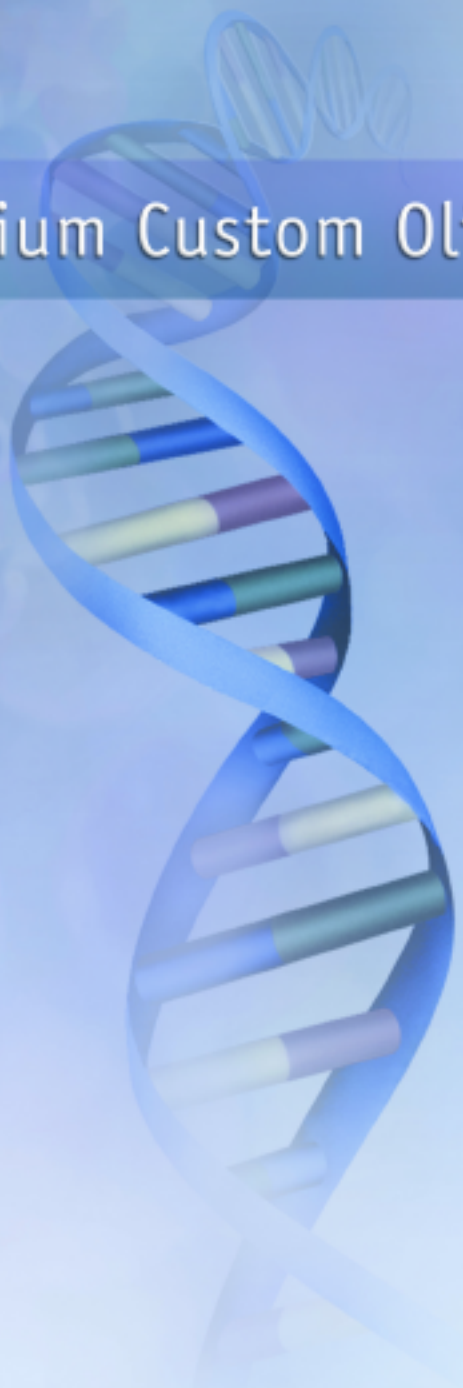
2011-2012 CATALOG

Premium Custom Oligonucleotides



Long Oligos Up to 250 mer

Modifications

Antisense Oligos

Fluorescent Molecular Probes

RNA Interference

Genetic Tools & Reagents

Gene Detection Systems

Gene Link™

Quality • Consistency • Confidence

For over a decade Gene Link has been providing researchers with the finest critical genetic research tools. Consistently maintaining our reputation and responsibility to supply quality products, we present our entire line with unrivaled confidence. Our products and services are supported and ensured by our commitment to premium quality and our constant efforts to introduce innovative products and cutting-edge technology to the research community worldwide.

Gene Link fosters customer satisfaction and loyalty by stressing personal relationships with our customers. Our dedicated and expertly trained customer service and technical support staff are motivated to serve our customers in any way possible. Routinely assisting our customers in the design of their experiments and other technical inquiries, we stay committed and connected to our customers who have entrusted us with supplying the tools they need in furthering their exciting and groundbreaking research. Gene Link has developed, and will continue to preserve, a reputation for "Quality, Consistency, and Confidence."

A leading supplier of premium custom oligonucleotides, researchers turn to Gene Link for demanding applications and consistent results. Gene Link services include genotyping, sequencing and gene construction; as well as a wide variety of other molecular biology products such as, siRNA, fluorescent probes, genetic tools and reagents, and non-radioactive gene detection systems for human genetic disorders.

Specializing in Long Oligos up to 250 mer

Contents

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RNA Interference

Genetic Tools & Reagents

Gene Detection Systems

General Information

Premium Oligonucleotide Synthesis

QUALITY • CONSISTENCY • CONFIDENCE

Gene Link oligos are for demanding applications and consistent results. We believe that investigators who value time and have no room for an experiment to fail due to oligo quality should consider Gene Link. Our numerous quality control steps for each oligo assure confidence.

GOLD STANDARD

Actual Gel Photo

An actual gel photo of each oligo is affixed on the oligo report. An absolute testimony of quality. Gene Link has raised the standard since inception over a decade ago. *We have the pictures to prove it!*

Superior to "Mass-Produced Factory Oligos"

Gene Link is not an oligo factory. Each oligo is synthesized, processed and quality assured to Gene Link's absolute standards. This includes coupling efficiency monitoring of each base during synthesis and electrophoretic analysis of each oligo on a polyacrylamide gel to visually assess quality.

Coupling Efficiency

We maintain a coupling efficiency threshold of greater than 99.5% for all oligos by using premium reagents of exacting specifications, membrane synthesis, state-of-the-art instruments and optimized software-driven protocols. This may not be evident when comparing short oligos, as 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.

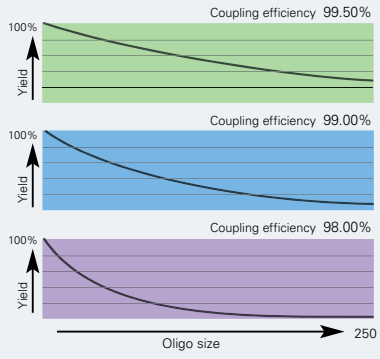
Trityl Monitoring

All Gene Link DNA synthesizers are equipped with trityl monitors for monitoring coupling efficiency of each added base. The instruments are programmed to halt when it falls below the threshold.

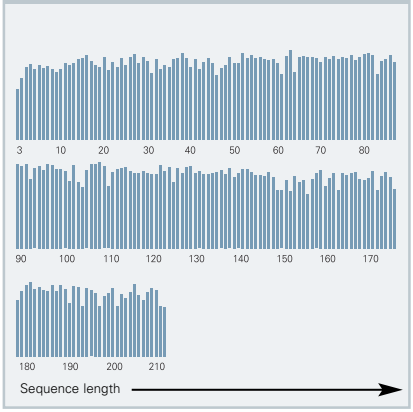
See example of routine trityl bars.

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



Routine Trityl Coupling Efficiency



Actual trityl coupling efficiency of a 210 mer.

The Gene Link Advantage

- Stringent Quality Control Measures
- Specializing in Long Oligos up to 250 mer
- Trityl Monitoring of All Oligos
- Polyacrylamide Gel Photograph of Each Oligo
- All Modifications Available
- All Oligo Types Available
- Easy Online Ordering System
- Online Design and Analysis Tools
- Knowledgeable Technical Support
- Personalized, Friendly Customer Service

GOLD STANDARD

Long Oligos

Ask our competitors how often they synthesize 200 to 250 mer oligonucleotides. Gene Link specializes in long oligos.

You are invited to compare.

Oligo Design and Analysis Tools

Gene Link has been leading the way by providing the most user friendly online experience in oligo ordering. From oligo design and analysis, to the convenient ordering system and the assurance of a secured transaction, Gene Link provides the most comprehensive web resource in the industry.

Features include convenient NCBI blasting and secondary structure analysis, simple import tools for large orders in spreadsheet or text file format, and three levels of review and editing.

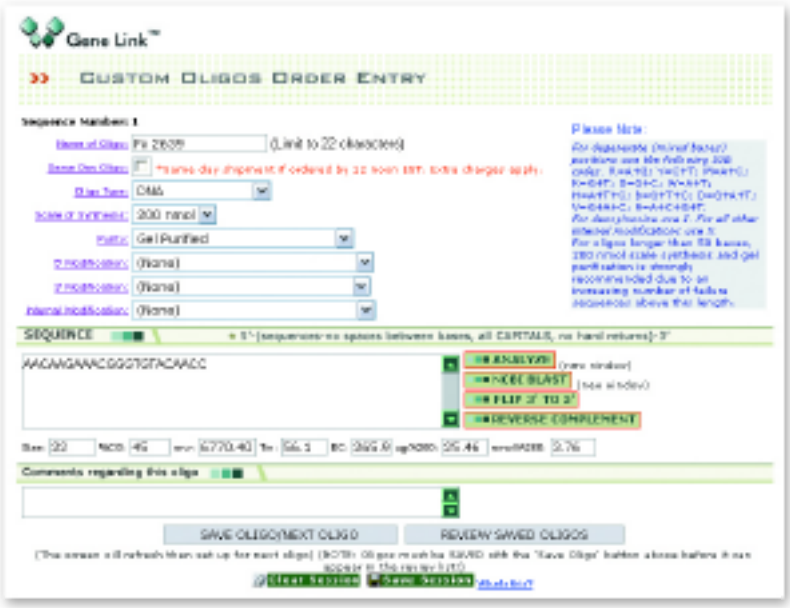
Applications include RNAi Explorer™, a robust siRNA search and design tool, and a standalone Oligo Explorer™ application for online acquisition of sequences and oligo design.

Custom Oligo Ordering System

- Classic ordering system with extensive analysis features
- Timesaver multi oligo import from spreadsheet and text files
- Ability to handle mixed oligo types, purity and modifications in a single order
- Selection of oligo type (DNA, RNA, Phosphorothioate, Chimeric etc.)
- Simple drop down menu selection for 5', internal or 3' modifications
- Analyze for oligo hairpin and loops
- Integrates with NCBI Blast for homology checks
- Flip 3' to 5' and reverse complement

Online Oligo Analysis

- Simulate annealing, loops and hairpin formations
- Calculate MW, EC, T_m, A_{260}, etc.



Save Session

Too busy to order all of your oligos in one session? Gene Link's answer to the multitasking researcher with endless interruptions is the "Save Session" feature. Enter as many oligos as you wish, click the "Save Session" button and resume at your will. Your oligos will be saved. What's more, you'll save money on shipping by consolidating your multiple orders into one.

Multiple Oligo NCBI Blast

Click NCBI BLAST to ascertain homologies to other sequences.

Perform NCBI Blast of multiple sequences at once by using Gene Link's online MultiBlast application. Import all the sequences using a spreadsheet or a text file. All of your sequences will be blasted and results retrieved. Gene Link offers a very convenient approach to perform multiple blast searches.

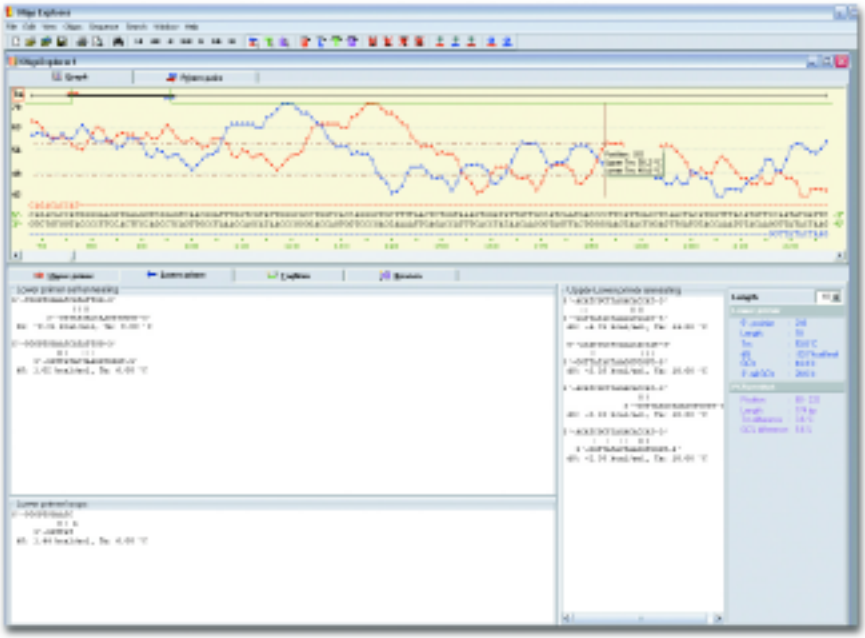
Oligo Explorer™

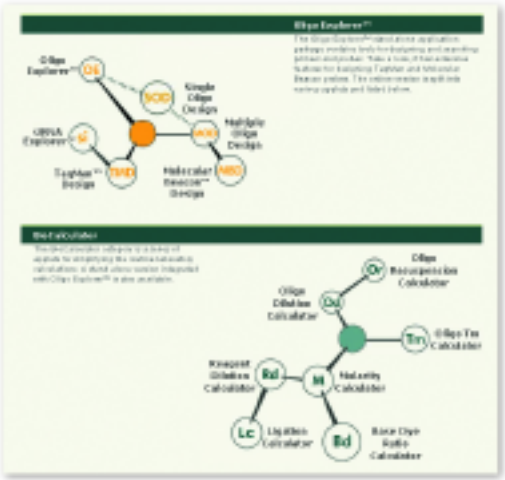
A PC-based application for standalone DNA sequence retrieval and oligo design.

Oligo Explorer™ was developed to design PCR and sequencing primers. Oligo Explorer™ is an efficient easy-to-use tool to determine primer properties like T_m, GC%, primer loops and primer dimers.

Oligo Explorer™ also includes a powerful "Primer Wizard" tool that helps you to find suitable primer pairs. You can set your own parameters for the primer pair search engine or use the default parameters. "Primer Wizard" suggests primer pairs that amplify PCR product of the given length. Individual primer pairs are suggested that theoretically will not form stable primer dimers or primer loops.

OLIGO EXPLORER 1.2





Molecular Biology Convenience Applets

Gene Link has numerous online applets for quick calculations. The BioCalculator is a series of applets for simplifying the routine laboratory calculations. The following convenient calculators are available:

- Oligo Resuspension
- Oligo Dilution
- Oligo T_m
- Reagent Dilution
- Molarity Determination
- Ligation
- Base/Dye Ratio

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

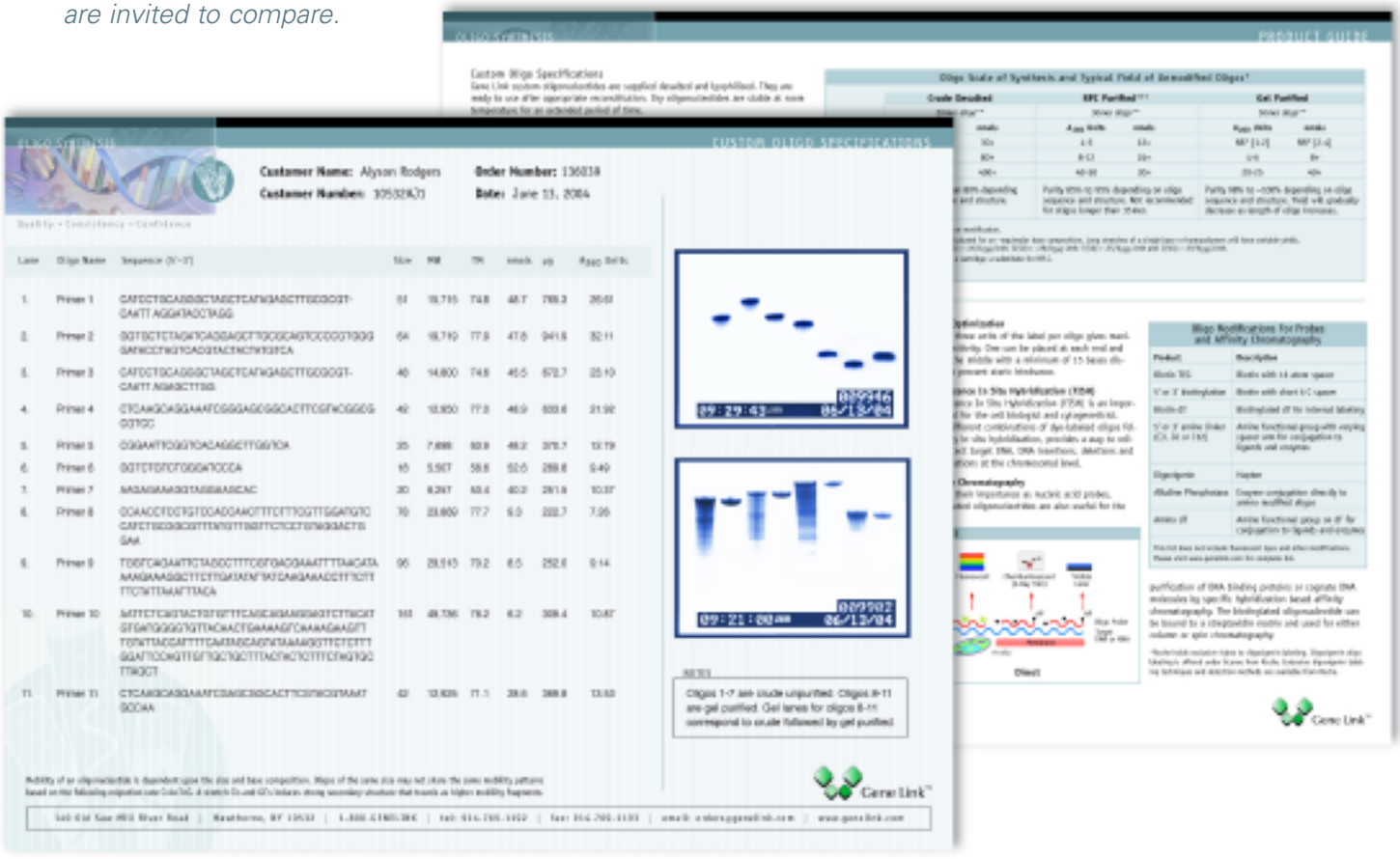
The crude, desalted oligonucleotide supplied is suitable for all amplification and sequencing protocols. Gel purification is advised for all oligos used for cloning applications and for oligos longer than 50 mer.

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.
50 nmol	26-6400-05
200 nmol	26-6400-02
1 µmol	26-6400-01
2 µmol	26-6400-03
10 µmol	26-6400-10
15 µmol	26-6400-15

*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 / purification					
Product	**Catalog No.**	**50 nmol**	**200 nmol**	**1 µmol**	**2 µmol**	**10 µmol**	**15 µmol**
Gel Purification	26-6400-XX	26-6400-77	26-6400-77	26-6400-55	26-6400-88	26-6400-44	26-6400-99
Reverse Phase Cartridge	26-6400-XX	26-6400-11	26-6400-11	26-6400-22	26-6400-23	26-6400-33	26-6400-34

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
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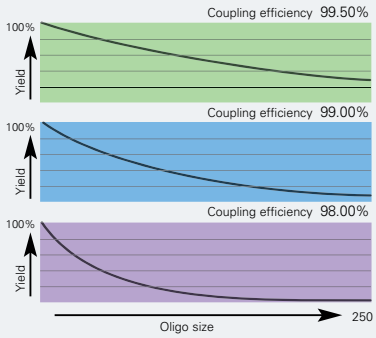


PCR and sequencing reactions are very robust and can tolerate up to 50% failure/truncated sequence oligos. However, you are clearly taking a chance by using long oligos synthesized at anything below 99.5% coupling efficiency.

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

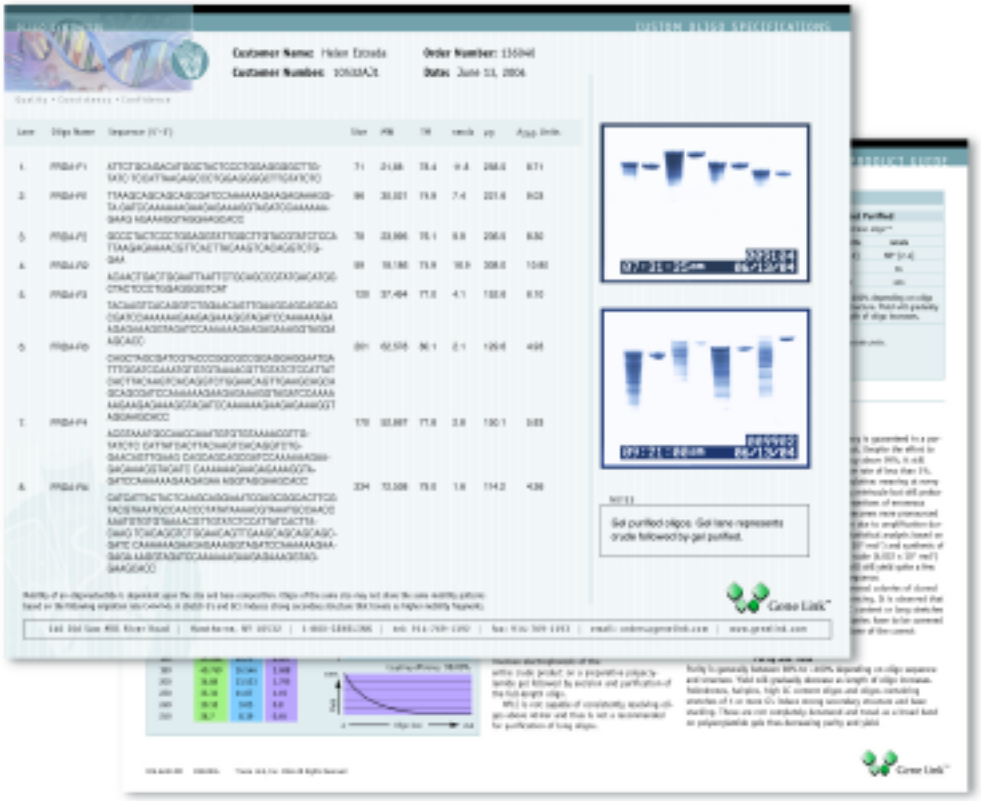
Scale	A_{260} Units	nmols	mg
1 µmol	4–6	4+	0.13–0.2
2 µmol	8–12	8+	0.26–0.8

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	Catelog No.
200 nmol scale	26-6400-02
1 µmol scale	26-6400-01
2 µmol scale	26-6400-03

*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 Catalog No/purification					
Product	**50 nmol**	**200 nmol**	**1 µmol**	**2 µmol**	**10 µmol**	**15 µmol**
Gel Purification	26-6400-77	26-6400-77	26-6400-55	26-6400-88	26-6400-44	26-6400-99

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)

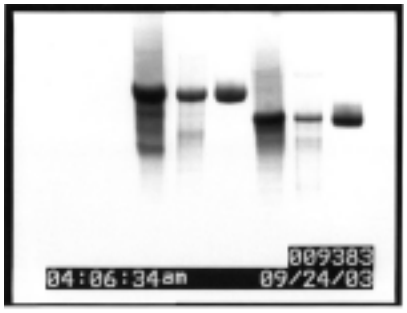
Purification by this method is considered the Gold Standard for oligonucleotide purification and yields 99%+ purity. Gel purification can be used for any length of oligonucleotide (as compared to HPLC and RPC cartridges which are limited to oligonucleotides below 40 mer). Gel purification is strongly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

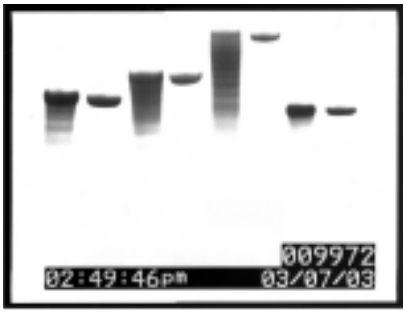
Ever wonder why we have not yet discovered a polymerase that can breeze through a stretch of G's? A stretch of three or more G's in an oligo sequence induces strong secondary structure. A string of G's and C's can exhibit internal Hoogsteen base pairing, non-Watson-Crick triple base pairing and should 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 Catalog No/purification					
Product	**50 nmol**	**200 nmol**	**1 µmol**	**2 µmol**	**10 µmol**	**15 µmol**
Gel Purification	26-6400-77	26-6400-77	26-6400-55	26-6400-88	26-6400-44	26-6400-99
Reverse Phase Cartridge	26-6400-11	26-6400-11	26-6400-22	26-6400-23	26-6400-33	26-6400-34

GOLD STANDARD

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.

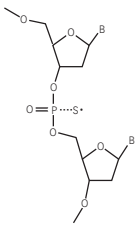
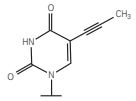
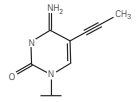
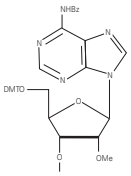
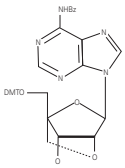
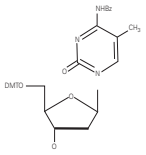
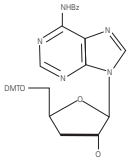
Antisense research requires short oligonucleotides that are complementary in sequence, and upon specific hybridization to its cognate gene product, induce inhibition of gene expression.

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'-0-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'-0-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'-0Me-nucleotides (2'-0Me-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

	Catalog No.	
Product	**200 nmol scale**	**1 µmol scale**
Phosphorothioates	26-64XX-XX	26-64XX-XX
5-Propyne pdC, pdU	26-650X-XX	26-650X-XX
5-Me-dC	26-6413-02	26-6413-01
2'-0-methyl bases	26-64XX-XX	26-64XX-XX
2'-5' linked bases	26-649X-02	26-649X-01
2-Amino-dA	26-6525-XX	26-6525-XX
Chimeric linkage	27-6420-02	27-6420-01

*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.

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.

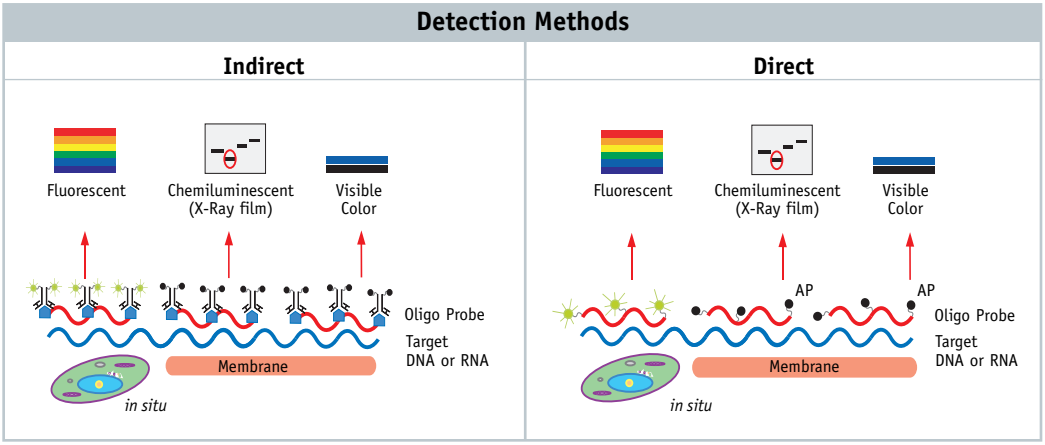
Biotin and Digoxigenin

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

	Catalog No.		
Product	**50 nmol scale**	**200 nmol scale**	**1 µmol scale**
Biotin dT, Biotin multi	27-642X-05	27-642X-02	27-642X-01
5' or 3' Biotinylation	27-642X-05	27-642X-02	27-642X-01
Biotin-TEG	26-6407-05	26-6407-02	26-6407-01
Digoxigenin*	26-6429-05	26-6429-02	26-6429-01
Alkaline Phosphatase	26-6511-05	26-6511-02	26-6511-01
2-Amino dA	26-6525-05	26-6525-02	26-6525-01
5' or 3' Phosphorylation	26-642X-05	26-642X-02	26-642X-01
3' and 5' Amino Linker C3, C6, C12	26-6418-05	26-6418-02	26-6418-01
Spacer C3, C9, C12, C18 & dSpacer	26-644X-05	26-644X-02	26-644X-01

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

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

	Catalog No		
Product	**50 nmol**	**200 nmol**	**1 µmol**
Gel Purification	26-6400-77	26-6400-77	26-6400-55

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.

A wide array of stock adaptors, linkers and primers for sequencing, cDNA synthesis and insert amplification are available including dye-labeled primers for fluorescent detection.

Gene Link stocks various random primers, including an array of fluorescent dye-labeled primers for genetic analysis using fluorescent detection instruments.

APPLICATION:
High yield cDNA synthesis, Microarray cDNA labeling, Genetic Analysis

Random Primers

Random primers are a mixture of oligonucleotides representing all possible sequences for that size. Random primers of other sizes can be also used similar to using hexamers in cDNA synthesis in combination with oligo d(T) to yield more 5' end cDNA sequences.

APPLICATION:
Full length cDNA synthesis, Microarray cDNA labeling, Genetic Analysis

Oligo d(T) primers

Oligo $d(T)_{12\text{-}18}$ has conventionally been used to prime synthesis of first strand cDNA using the poly A tail to hybridize. The primer is a mixture of sizes from 12 to 18 mer. This is formulated by synthesis of individual oligos and then pooling them.

Gene Link provides various sizes of oligo d(T) and an array of fluorescent dye-labeled primers for genetic analysis using fluorescent detection instruments. The C-12 amino-labeled primers are ready to be conjugated to your choice of NHS-activated ligand.

APPLICATION:
Cloned Insert Sequencing, Insert Amplification, Genetic Analysis

Sequencing & Amplification Primers

Various primers (e.g. T7, M13, pUC) used for sequencing and amplifying inserts from popular and common cloning vectors are available. Please visit our web site for details and complete listing.

Sequencing & Amplification Primers*

Primer	Size	Catalog No
Sequence details on web site	25 µg	26-4000-XX

*Please visit www.genelink.com for complete and individual list of primers.

APPLICATION:
Fragment Restriction Site Addition and Conversion, Cloning

Adaptors

Gene Link supplies adaptors, short synthetic oligonucleotide preannealed duplexes with 5' blunt end phosphorylation. These have an internal restriction endonuclease site, which is created by ligation to fragments with complementary overhangs. The duplexes have an overhang and a blunt end.

Adaptors*

Adaptors	Size	Catalog No
Sequence details on web site	20 µg	26-3100-XX

*Please visit www.genelink.com for complete and individual list of adaptors.

APPLICATION:
Introduce Restriction Sites, Site Addition and Conversion, Cloning

Linkers

Linkers are used for various cloning strategies to introduce restriction

Random Primers Unlabeled & Dye Labeled

Primer	Size	Catalog No
Unlabeled random hexamer, heptamer, octamer, nonamer	100 µg	26-4000-XX
Unlabeled random 12, 24 & 36 mer	100 µg	26-4000-XX
5'-Phosphorylated random hexamer, heptamer, octamer, nonamer	50 µg	26-4000-XX
5'- dye labeled random hexamer, heptamer, octamer, nonamer	25 µg	26-4000-XX
5'-C-12 amino labeled random hexamer, heptamer, octamer, nonamer	25 µg	26-4000-XX
5'-Digoxigenin labeled random hexamer, heptamer, octamer, nonamer	25 µg	26-4000-XX

See complete list at www.genelink.com.

Oligo d(T) primers

Primer	Quantity	Catalog No
Oligo $d(T)_{12\text{-}18}$	100 µg	26-4000-XX
5'- dye labeled oligo $d(T)_{12\text{-}18}$ (Cy3, Cy5, Hex, Fam, Tet & Fluorescein labeled)	25 µg	26-4XXX-XX
5'- C-12 amino labeled oligo $d(T)_{12\text{-}18}$	25 µg	26-4XXX-XX

See complete list at www.genelink.com.

sites into the DNA after ligation. Linkers are short synthetic palindromic sequences that self anneal to form blunt ended double-stranded fragments. Linkers are supplied in phosphorylated and non-phosphorylated forms. All Gene Link supplied linkers pass stringent quality control protocols of ligation and cleavage.

Linkers

Linkers*	Size	Catalog No
Non-phosphorylated	20 µg	26-3200-XX
Phosphorylated	20 µg	26-3200-XX

*Please visit www.genelink.com for complete and individual list of linkers.

APPLICATION:

Probe for RNA Quality, Northern Blots, Gene Expression

OligoProber™

OligoProber™ are specific oligonucleotide probes for hybridization to their cognate species. These are specially suited for use in conjunction with RT-PCRmer™, as the complementary sequence lies in the amplified sequence. The OligoProber™ can also be used for all northern blots. OligoProber™ are available for use as hybridization probes with either 5' OH for ^{32}P labeling or with 3' biotin for non-radioactive detection.

OligoProber™

Product	Size	Catalog No
OligoProber™, Human, Rat & Mouse beta actin, GAPDH	2 nmols	40-1101-XX

*Please visit www.genelink.com for complete and individual list of OligoProber™.

APPLICATION:

Control for RNA Quality, Northern Blots, Gene Expression

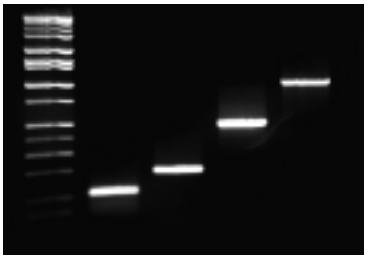
RT-PCRmer™

RT-PCRmer™ are primer pairs for specific amplification of cDNA. ß-actin is ubiquitously expressed and serves as a positive control for northern and other expression studies. ß-actin RT-PCRmer™ are generally used as controls for measuring cDNA synthesis efficiency by reverse transcription and as controls for mRNA (cDNA) quantitative expression studies. ß-actin RT-PCRmer™ are supplied in lyophilized powder form in aliquots of 10 nmols. The quantity supplied is sufficient for at least 400 regular 25 µl PCR reactions* for ethidium bromide stained visualization.

RT-PCRmer™

Product	Size	Catalog No
RT-PCRmer™, Human, Mouse & Rat beta actin, GAPDH, B2M	10 nmols	40-1022-10

*Please visit www.genelink.com for complete and individual list of RT-PCRmer™.



Amplification results of GAPDH RT-PCRmer™ using 50 ng of Omni-cDNA™ Human Pooled First Strand cDNA (Catalog No. 10-0100-05) as the template. The amplified fragment sizes are 109 bp, 208 bp, 505 bp and 1009 bp.

APPLICATION:

Polymorphism Studies, Gene Mapping, etc.

GL RAPD Decamer Sets

GL RAPD Decamer sets are arbitrary sequence decamer sets for rapid discerning of DNA polymorphism. The individual primers are constructed to have a GC content between 60-70% with no complementary ends to minimize primer dimers.

GL RAPD Decamer Sets

Product		Catalog No
GL RAPD Decamer	1 set	40-0001-05
30 Sets (01-AB to 30-BGBH)		

Details at www.genelink.com

The Gene Link Advantage

- A Wide Array of Stock Primers & Probes
- Adaptors & Linkers
- Optimized RT-PCRmer™ Primer Pairs
- RAPD Decamers for Polymorphic Genotyping
- Various Dye-labeled Stock Primers
- Custom Synthesis Available
- Easy Online Ordering System
- Knowledgeable Technical Support
- Personalized, Friendly Customer Service

GOLD STANDARD

Custom Design Service

Gene Link routinely designs and synthesizes custom adaptors, linkers and probes.

E-mail or call for details.

Fluorescent Molecular Primers & Probes

UNIQUE CAPABILITIES

Gene Link's proprietary synthesis and processing methods for fluorescent dyes yield primers and probes of superior quality. Gene Link offers synthesis of various forms of molecular primers and probes.

We provide technical service in the design of novel probes and synthesize numerous combinations of dyes, quenchers, RNA, phosphorothioate, 2'0 methyl and chimeric probes.

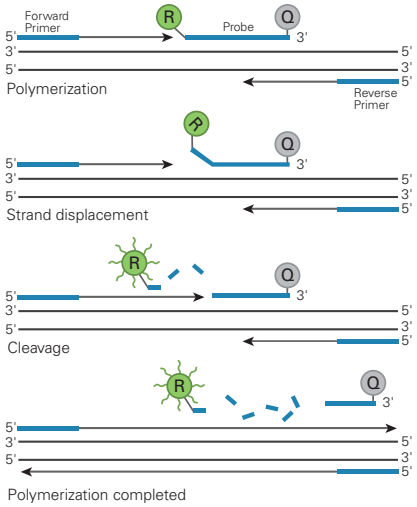
All fluorescent dye-labeled oligos are monitored for coupling efficiency. Many dye conjugations are available. Fluorescent primers are shipped in amber tubes to protect photosensitive primers.

Fluorescent Primers

Fluorescent primers are extensively used for genotyping, SNP genotyping, allelic discrimination and fragment analysis. Gene Link synthesizes all types of dye labeled primers and probes.

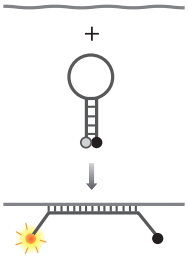
TaqMan Probes

TaqMan (also known as Fluorogenic 5' nuclease assay) probes contain two dyes, a reporter dye (e.g. 6-FAM) at the 5' end and a 3' acceptor dye, usually TAMRA or a Black Hole Quencher.



Molecular Beacons

Molecular Beacons synthesized by Gene Link have better than 50:1 signal to background ratio, usually in the range of 200. The purity is greater than 99% as judged by polyacrylamide gel electrophoresis. All dye conjugations are available. Please see our web site for complete details and specifications.



Purification

Gene Link recommends gel purification of all modified primers and probes.

Applications and Modifications

Application	Recommended Modifications
Real-Time PCR probes, QPCR	• C-5 methylated pyrimidine deoxynucleosides behave similar to LNA bases in imparting duplex stability. • The use of LNA bases renders 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 probes. • All types of fluorescent dyes and backbone modifications can be performed for *in situ* detection.
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. • C-5 methylated pyrimidine deoxynucleosides behave similar to LNA bases in imparting duplex stability.

Fluorophore Absorbance and Emission Data*

					Catalog Nos	
Dye	**Color**	**Absorbance Max (nm)**	**Emission Max (nm)**	**Extinction Coefficient**	**200 nmol scale**	**1 µmol scale**
6-FAM (Fluorescein)	Green	494	525	74850	26-6431-02	26-6431-01
TET	Orange	521	536	85553	26-6433-02	26-6433-01
HEX	Pink	535	556	95698	26-6432-02	26-6432-01
Cy 5	Violet	646	667	250000	26-6436-02	26-6436-01
Cy 5.5	Blue	683	707	190000	26-6460-02	26-6460-01
Cy 3	Red	552	570	150000	26-6437-02	26-6437-01
Cy 3.5	Purple	588	604	150000	26-6461-02	26-6461-01
Cy 7	Near IR	743	767	200000	26-6474-02	26-6474-01
Tamra	Rose	565	580	87000	26-6451-02	26-6451-01
ROX	Purple	587	607	105000	26-6430-02	26-6430-01
JOE	Mustard	528	554	105000	26-6467-02	26-6467-01
Alexa Dye Series	Varies	Varies	Varies	Varies	26-6XXX-02	26-6XXX-01
Dabcyl (Quencher)			453		26-6446-02	26-6446-01
BHQ-1,2,3** (Quencher)		534, 579, 672			26-6652-02	26-6652-02

*Color and fluorescence data vary with pH. Consult appropriate dye manufacturer for details.

Purification

	Catalog No		
Product	**50 nmol**	**200 nmol**	**1 µmol**
Gel Purification	26-6400-77	26-6400-77	26-6400-55
Reverse Phase Cartridge	26-6400-11	26-6400-11	26-6400-22

The Gene Link Advantage

- Stringent Quality Control Measures
- All combinations of Dyes, Modifications and Quenchers Available
- Chimeric Fluorescent Molecular Probes Synthesized
- Polyacrylamide Gel Picture of Each Primer and Probe
- All Oligo Types With Dyes and Quenchers Synthesized
- Easy Online Ordering System
- Shipped in Amber Tubes to Prevent Photo-bleaching
- Knowledgeable Technical Support
- Personalized, Friendly Customer Service

GOLD STANDARD

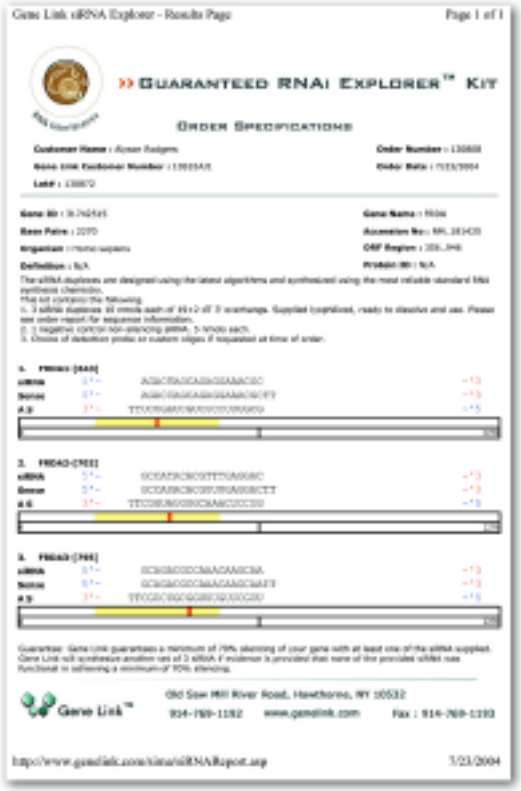
Expert Design Assistance

Gene Link routinely assists customers in designing novel probes with unique properties. Contact us if you require assistance.

RNA Interference

RNAi EXPLORER

RNAi Explorer™ from Gene Link is a series of products and services to aid researchers in exploring RNA interference. The online search and design tools for siRNA or shRNA are developed based on current known guidelines. You are encouraged to try the robust search and design algorithm.


Gene Link siRNA Explorer - Results Page

Page 1 of 1

» GUARANTEED RNAi EXPLORER™ KIT

ORDER SPECIFICATIONS

Gene Link™

1/23/2004

Guaranteed RNAi Explorer™ Kit

3+1 siRNAs and a detection probe. You simply supply the accession number or sequence for the gene to down regulate. Gene Link designs siRNAs using the latest published criteria and proprietary algorithms.

This kit contains three target siRNAs, a negative control and a detection probe of your choice OR 6 custom oligos.

Guaranteed RNAi Explorer™ Kit*

Components	Unlabeled	Fluorescein Labeled
3 Target siRNAs (10 nmols each), 1 negative control siRNA (5 nmols unlabeled and 2 nmols fluorescein labeled) 1 QPCR probe (5 nmols) or 6 primers (50 nmol scale, up to 30 mer)	27-6402-06	27-6402-02

**Guarantee: Gene Link guarantees a minimum of 70% silencing of your gene with at least one of the siRNA supplied. Gene Link will synthesize another set of 3 siRNA if evidence is provided that none of the provided siRNAs were functional in achieving a minimum of 70% silencing.*

siRNA Explorer™

siRNA Explorer™ is an online tool for automated search and design of siRNAs. The search can be initiated by either entering the accession number, gene ID, uploading a sequence file or by pasting a sequence in the sequence window.

Several criteria options are provided for customer optimization. Custom siRNAs designed online can be conveniently ordered. These are supplied duplexed and lyophilized.

siRNA (duplexed)

Synthesis Scale	Catalog No.
20 nmol Synthesis Scale	27-6401-05
50 nmol Synthesis Scale	27-6401-05
200 nmol Synthesis Scale	27-6401-02
1 µmol Synthesis Scale	27-6401-10

shRNA

An alternate to individual chemical synthesis of siRNA is to construct a sequence for insertion in an expression vector. Several such transcription vectors are available. Typical shRNA design consists of two inverted repeats containing the sense and antisense target sequences separated by a loop sequence. Gene Link offers easy online design of shRNA. Visit www.genelink.com for details.

shRNA

Synthesis Scale	Desalted	Purification
200 nmol	27-6400-05	27-6400-77
1 µmol	27-6400-01	27-6400-55

Controls for RNAi experiments

A negative control should also be included when performing siRNA experiments to eliminate the possibility of nonspecific silencing effects. For this purpose, Gene Link provides a siRNA sequence that has been shown to have no effect on gene silencing. The negative control siRNA is also available labeled with fluorescein, which allows transfection efficiency to be tracked by fluorescent microscopy.

Control siRNA Duplexes

Product	Catalog No.	Size
Negative Control, non-silencing	27-6410-05	5 nmols
Human Vimentin; NM_003380	27-6412-05	5 nmols
Human Beta Actin; NM_001101	27-6413-05	5 nmols
Mouse Beta Tubulin; AF312873	27-6414-05	5 nmols
Rat Chromogranin-A; NM_021655	27-6415-05	5 nmols
Fluorescein Labeled Controls		
Negative Control, non-silencing	27-6410-02FL	2 nmols
Human Vimentin; NM_003380	27-6412-02FL	2 nmols
Human Beta Actin; NM_001101	27-6413-02FL	2 nmols
Mouse Beta Tubulin; AF312873	27-6414-02FL	2 nmols
Rat Chromogranin-A; NM_021655	27-6415-02FL	2 nmols

*RNAi and siRNA
RNA interference (RNAi) is a specific and sequence dependent targeted gene silencing activity. RNAi acts by post transcriptional degradation of mRNA by small interfering RNAs (siRNAs) of the same sequence. The silencing approaches 100% and has to be empirically determined and optimized. Not every siRNA can effectively down regulate a gene. The process of RNA interference varies by individual siRNA while some do not exhibit any interference at all.

Handling & Storage

Follow established stringent RNase free handling conditions. The lyophilized siRNA duplex should be stored immediately at -20°C. The lyophilized siRNA is stable for ~6 months at -20°C.

The Gene Link Advantage

- Guaranteed RNAi Explorer™ Kit Knocks Out Your Gene of interest!
- All Modifications Available
- Robust Online siRNA Design and Analysis Tool
- Easy Online Ordering System
- Negative and Positive Dye-labeled Silencing Controls
- Knowledgeable Technical Support
- Personalized, Friendly Customer Service

GOLD STANDARD

Gene Link siRNA Design Service

No charge siRNA design service. A wealth of professional expertise and robust design algorithms guarantee to silence your gene of interest.

FACILE AND RAPID PURIFICATION AND CONCENTRATION OF DNA

Gene Link offers a variety of DNA and RNA purification and concentration systems. Each system has been optimized for reproducible and consistent results, yielding ultra high quality DNA and RNA suitable for all molecular biology applications.

The Omni-Clean™ System

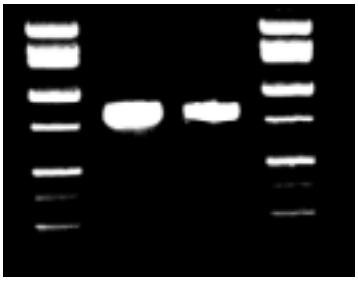
Purification of DNA from agarose gels and concentration of DNA by ethanol precipitation are routine protocols used in all molecular biology laboratories. The Omni-Clean™ system provides optimized reagents for rapid extraction of DNA from agarose gels, and for routine concentration of DNA. The DNA is concentrated, purified and completely salt-free.

APPLICATION

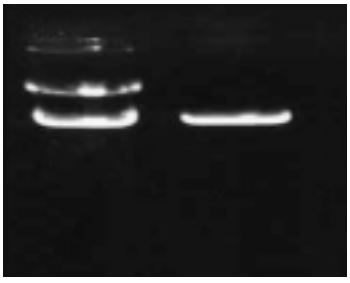
Purification of DNA fragments excised from agarose gels

Omni-Clean™ DNA Purification System

The Omni-Clean™ DNA Purification System takes advantage of the principle that DNA binds to powdered flint glass in the presence of chaotropic salts. This technique provides a rapid and efficient method for the purification of high quality DNA from solutions or agarose gel slices, and is suitable for cloning, sequencing, isotope labeling, and a host of other procedures.



Lanes 2 and 3 are fragments excised from agarose gel and purified using the Omni-Clean™ column based purification system.



Lane 1 is plasmid extracted using Omni-Pure™ plasmid purification system. Lane 2 is the lower fragment gel purified using the Omni-Clean™ gel glass bead based purification system.

Omni-Clean™ Gel DNA Purification Systems

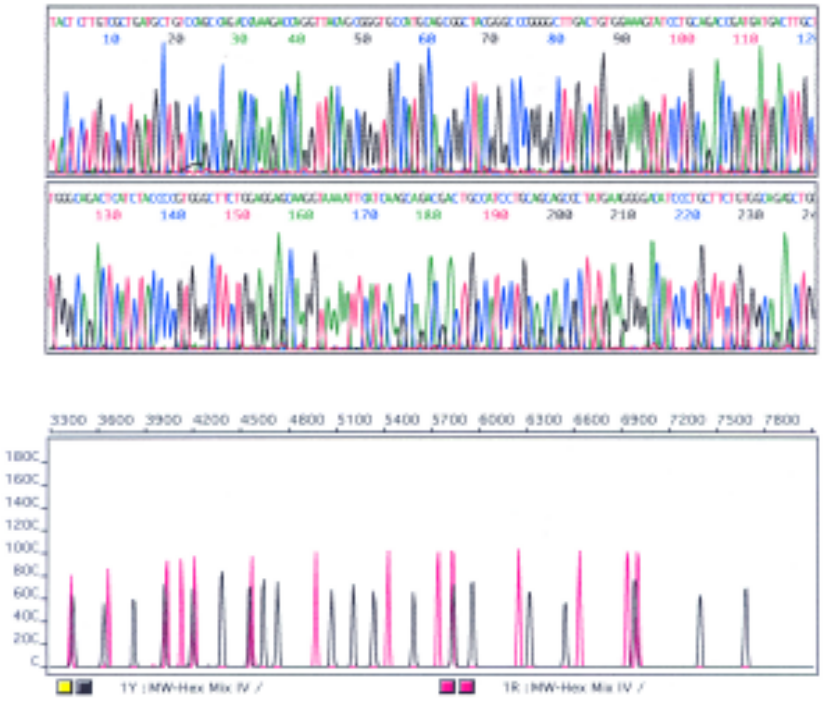
Product	Catalog No.	Size*
Omni-Clean™ Gel DNA Beads Purification System	40-4110-10	100
Omni-Clean™ Gel DNA Beads Purification System	40-4110-50	500
Omni-Clean™ Gel DNA Spin Column Purification System	40-4120-10	100
Omni-Clean™ Gel DNA Spin Column Purification System	40-4120-50	500

*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

APPLICATION:

Concentration and Cleanup of DNA in solution

The Omni-Clean™ DNA Concentration System yields higher quality DNA than with conventional ethanol precipitation. The samples are completely desalted, purified and concentrated. It is ideal for sequencing and genotyping samples with stringent purity requirements. The electropherogram shows samples purified using the Omni-Clean™ system.



Omni-Clean™ Gel DNA Concentration Systems

Product	Catalog No.	Size*
Omni-Clean™ DNA Beads Concentration System	40-4130-10	100
Omni-Clean™ DNA Beads Concentration System	40-4130-50	500
Omni-Clean™ DNA Spin Column Concentration System	40-4140-10	100
Omni-Clean™ DNA Spin Column Concentration System	40-4140-50	500

*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

Omni-Clean™ System

- Ultra Purified DNA in Less Than 20 minutes
- No Hazardous Reagents
- Suitable for All Molecular Biology Applications
- No More Ethanol Precipitations
- DNA Purification, Concentration and Desalting from Agarose Gel Slices Using Beads or Spin Columns

GOLD STANDARD

Ultra Pure Templates

Automated sequencing and genotyping requires high quality template DNA. The Omni-Clean™ system is the method of choice to reproducibly yield ultra high quality DNA.

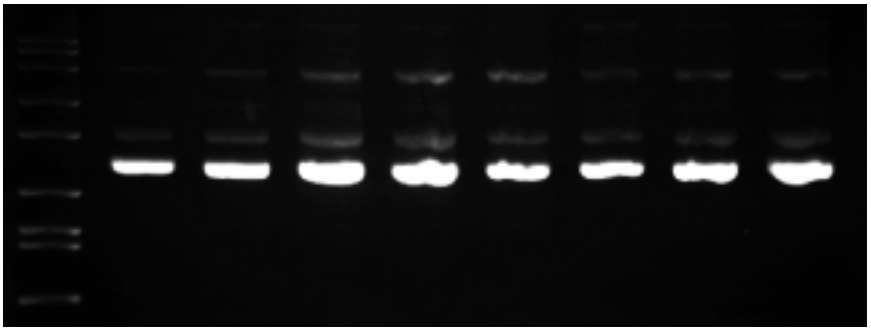
Facile and Rapid Extraction and Purification of DNA

Facile and rapid purification of DNA from varied sources can be performed using the Omni-Pure™ series of DNA, RNA and plasmid purification systems. Each purification system has been formulated, optimized and designed to yield the highest purity available with the starting sample volume specially geared towards the desired downstream application.

APPLICATION:

Mini-Prep Plasmid DNA Purification

Routine mini-preps of plasmid extraction are made even easier with consistent performance. Purification can be performed with a maximum of 3 ml of cells yielding up to 20 µg of purified DNA. The convenient spin column method can be scaled up by using multiple columns and processed in less than 30 minutes. The purified DNA is of high quality suitable for all molecular biology applications including direct use in fluorescent automated sequencing methods.

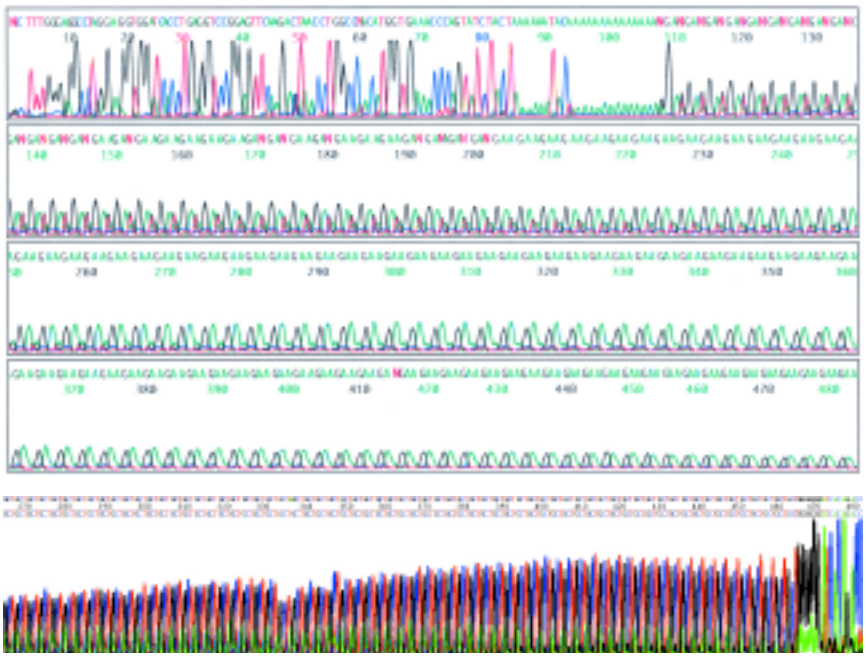


Replicates of plasmid purification using the Omni-Pure™ Plasmid DNA Purification System.

Omni-Pure™ Plasmid DNA Purification Systems

Product	Catalog No.	Size*
Omni-Pure™ Plasmid DNA Purification System	40-4020-01	100
Omni-Pure™ Plasmid DNA Purification System	40-4020-05	500

*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.



Friedreich's Ataxia Control DNA with 110 GAA repeats (top) and Myotonic Dystrophy Control DNA with 129 CTG repeats (bottom) sequencing electropherograms. These triple repeat sequencing requires ultra clean DNA

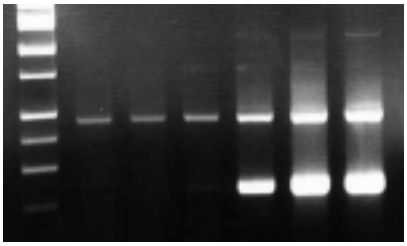
Plasmids were purified using the Omni-Pure™ system and processed for automated sequencing.

APPLICATION:

Viral DNA & RNA Purification

Gene Link provides a rapid purification system for extraction of viral DNA or RNA from human bodily fluids including blood. Viral DNA or RNA is captured on a special membrane and then eluted in a low volume for direct use in qualitative and quantitative amplification protocols for detection of a pathogen.

The Viral RNA purification system is ideal for small volumes of human bodily fluid samples, i.e., serum, plasma and CSF. Using the easy spin column format, purification of HIV, HCV and other RNA viruses is easily accomplished in less than 30 minutes and ready for RT-PCR amplification.



Viral DNA purification and amplification using zero, 1, 10 & 100 ng of template DNA. The top fragment is an internal control from human genomic DNA.

Omni-Pure™ Viral DNA & RNA Purification Systems

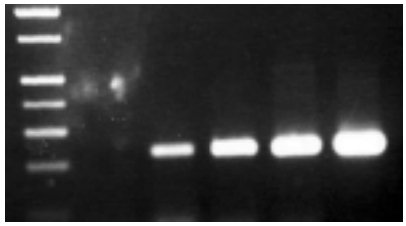
Product	Catalog No.	Size*
Omni-Pure™ Viral DNA Purification System	40-3720-01	100
Omni-Pure™ Viral DNA Purification System	40-3720-05	500
Omni-Pure™ Viral RNA Purification System	40-3650-01	100
Omni-Pure™ Viral RNA Purification System	40-3650-05	500

*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

APPLICATION:

Microbial DNA Purification

The microbial DNA purification system is ideal for DNA purification of pathogen DNA in the easy spin column format. Purification from sputum and other bodily fluids is rapidly performed using this system. The pathogen DNA can be directly used in qualitative and quantitative amplification protocols for detection of a pathogen.



Microbial DNA purification followed by amplification of a specific fragment using zero, 10, 20, 50 & 100 ng of template DNA.

Omni-Pure™ Microbial DNA Purification Systems

Product	Catalog No.	Size*
Omni-Pure™ Microbial DNA Purification System	40-3700-01	100
Omni-Pure™ Microbial DNA Purification System	40-3700-05	500

*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

Omni-Pure™ System

- Ultra Purified High Yield Plasmid DNA
- No Toxic Reagents
- Rapid Purification Protocols
- Suitable for All Molecular Biology Applications
- Convenient Optimized Reagents
- Easy Spin Column Format
- Genomic DNA Purification
- Viral DNA & RNA Purification
- Microbial DNA Purification

GOLD STANDARD

Pathogen Detection Made Easy

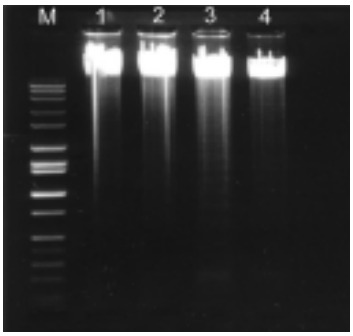
Purification of pathogen DNA or RNA is performed conveniently using the Omni-Pure™ system. The purified DNA or RNA can immediately be used for qualitative or quantitative detection.

See Gene Link's product line of Gene Detection Systems.

Facile and Rapid Extraction and Purification of Genomic DNA

The Omni-Pure™ Genomic DNA purification system is designed for a convenient volume of 300 µl whole blood (lower volumes can also be used) to yield an average of ~10 µg ultra pure DNA. This quantity is sufficient for restriction-based Southern blot analysis and hundreds of PCR-based analyses.

The Omni-Pure™ Genomic DNA purification system is designed for convenience and consistency. It is a universal genomic DNA purification system. Ultra pure genomic DNA can be purified from small amounts of almost all known sample types and sources. Samples from human blood, bodily fluids, animal and plant tissue and microbial and viral sources have been purified using the Omni-Pure™ Genomic DNA purification system. One purification is usually sufficient to yield enough DNA for all molecular biology applications.



Purified genomic DNA (~200 ng) was electrophoresed on a 0.8% agarose gel and stained with ethidium bromide. Observe high quality genomic DNA that ranges from ~30 to 50 kb in size. Lane M contains molecular size markers from 10 kb to 50 bp in length. Lanes 1-4 are genomic DNA samples obtained from blood samples of 4 different individuals using the Omni-Pure™ system.

APPLICATION:

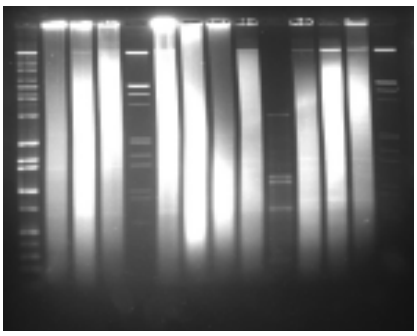
Tissue DNA Purification

An accompanying product manual contains a detailed protocol for the purification of genomic DNA from animal tissue. The protocol has been tested and yields high quality DNA. The gel picture shows genomic DNA extracted from mouse and rat skeletal muscle and liver. This system is geared towards minute tissue samples. From 2 mg of tissue an average yield of 2-10 µg is expected. The DNA is suitable for all molecular biology applications.

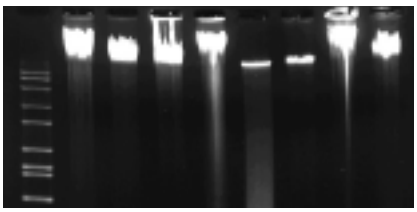
APPLICATION:

Blood DNA Purification

Each purification sample volume is specially geared towards the desired downstream application. A sample volume of 300 µl is recommended for human blood samples yielding on average from 5–15 µg of high molecular weight and high quality genomic DNA for two restriction digestions for Southern blot analysis. The yield is sufficient for hundreds of PCR amplification reactions. An accompanying product manual contains a detailed protocol for the extraction of genomic DNA from tissues and bodily fluids.



Human blood genomic DNA from different individuals purified using the Omni-Pure™ Genomic DNA Purification System. Approximately 5 µg were digested with different restriction enzymes and the samples were electrophoresed on a 0.7% agarose gel. Note the high molecular weight DNA and the consistency between different samples. The gel was processed for Southern blot analysis and chemiluminescent detection.

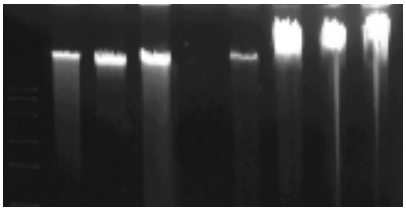


Samples from various animals were processed for DNA purification using the Omni-Pure™ Genomic DNA Purification System. Purified genomic DNA (~200 ng) was electrophoresed on a 0.7% agarose gel and stained with ethidium bromide. Observe high quality genomic DNA that ranges from ~30 to 50 kb in size. Lane 1 contains molecular weight markers followed by samples from human, rabbit, cat, mouse, guinea pig, sheep, pig and hamster.

APPLICATION:

Plant DNA Purification

An accompanying product manual contains a detailed protocol for the purification of genomic DNA from plant tissue. The protocol has been tested and yields high quality DNA. The gel picture shows genomic DNA extracted from plants such as ginger, green pepper, cilantro and carrot. This system is geared towards minute tissue samples. From 2 mg of tissue an average yield of 2-10 µg is expected. The DNA is suitable for all molecular biology applications.



Samples from various plant tissues were processed for DNA purification using the Omni-Pure™ Plant DNA Purification System. Purified genomic DNA (~200 ng) was electrophoresed on a 0.7% agarose gel and stained with ethidium bromide. Observe high quality genomic DNA that ranges from ~30 to 50 kb in size. Lane 1 contains molecular weight markers followed by plant samples from ginger, green pepper, cilantro, blank lane, carrot and animal genomic DNA comparison samples from human, mouse and pig.

Omni-Pure™ Genomic DNA Purification Systems

Product	Catalog No.	Size*
Omni-Pure™ Blood DNA Purification System	40-4010-01	100
Omni-Pure™ Blood DNA Purification System	40-4010-05	500
Omni-Pure™ Blood DNA Purification System	40-4010-10	1000
Omni-Pure™ Tissue DNA Purification System	40-4050-01	100
Omni-Pure™ Tissue DNA Purification System	40-4050-05	500
Omni-Pure™ Tissue DNA Purification System	40-4050-10	1000
Omni-Pure™ Plant DNA Purification System	40-4060-01	100
Omni-Pure™ Plant DNA Purification System	40-4060-05	500
Omni-Pure™ Plant DNA Purification System	40-4060-10	1000
Omni-Pure™ Universal DNA Purification System	40-4070-01	100
Omni-Pure™ Universal DNA Purification System	40-4070-05	500
Omni-Pure™ Universal DNA Purification System	40-4070-10	1000

*Sample volume for each purification system varies. Each purification yields sufficient quantity for desired applications.

Are You Genotyping?

Consider using Gene Link's comprehensive line of non-radioactive gene detection systems. We have genotyping solutions for triple repeat disorders, single base mutations and various pathogens.

The Omni-Pure™ Genomic DNA Purification System

- Ultra Purified Genomic DNA
- No Toxic Reagents
- ~30 Minute Protocols
- Blood & Bodily Fluid Genomic DNA
- Animal Tissue Genomic DNA
- Plant Tissue Purification
- Yeast DNA Purification
- Gram Positive & Negative Bacterial DNA
- Suitable for All Molecular Biology Applications
- Convenient Optimized Systems
- Detailed Manual Provided with Product

GOLD STANDARD

Convenience Perfected

All of the reagents, a simple 30 minutes protocol and ultra pure genomic DNA for all your gene detection applications. Get one today!

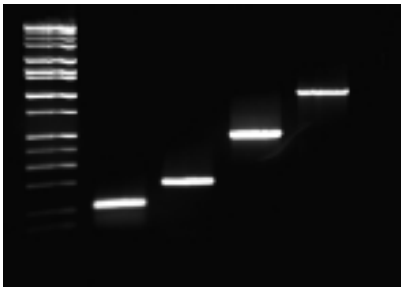
Omni-cDNA™ First Strand cDNA

First strand cDNA is useful for amplifying a particular cDNA using PCR. Gene Link provides a convenient source of high quality first strand cDNA prepared from freshly obtained tissue and appropriately frozen during transportation.

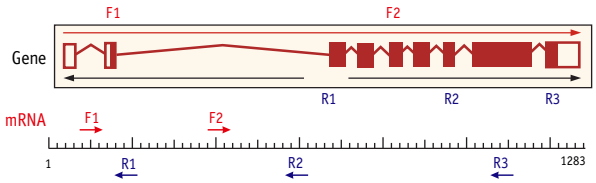
APPLICATION:

RT-PCR , cDNA amplification, Cloning, Tissue-specific gene expression, etc.

Tissue-specific individual first strand cDNA is available from Gene Link. These are of high quality and lot tested for amplification of ß -actin and/or GAPDH cDNA fragments up to 1.1 kb in length.



Amplification was performed using 50 ng of Omni-cDNA™ Human Pooled First Strand cDNA (Catalog No. 10-0100-05) as the template. A 10 µl aliquot of the amplified fragment was loaded on a 1% agarose gel. The amplified fragment sizes obtained with the RT-PCRmer™ primer pair used are as follows:
lane 2, F1/R1 = 109 bp;
lane 3, F2/R2 = 208 bp;
lane 4, F1/R2 = 505 bp and
lane 5, F1/R3 = 1009 bp.



GAPDH gene and mRNA sequences showing the position of different primers. The primers (RT-PCRmer™) were constructed to assess the quality of Gene Link Omni-cDNA™ first strand cDNA. Full length and near full length cDNA will amplify the 1.1 kb cDNA fragment signifying high quality. All Omni-cDNA™ lots undergo this qualification.

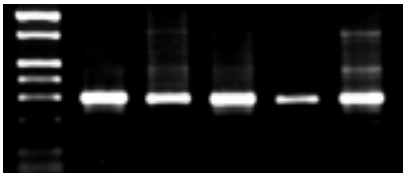
APPLICATION:

RT-PCR, cDNA cloning, etc.

The first strand cDNA has been prepared from pooled and or amplified mRNA obtained from different tissues. These are not from cultured cell lines. The assortment of tissues vary, including lung, heart, brain, spleen, skeletal muscle, smooth muscle, ovaries, pancreas, liver and kidney. The amount supplied is sufficient for at least 50 amplifications. Each lot is tested for amplification of ß -actin cDNA.

Omni-cDNA™ pooled first strand size distribution is from ~5 kb to 200 bp. These can also be used for cloning mRNA of interest by RT-PCR. A 1.3 kb and a ~500 bp amplified cDNA fragment of p53 is shown in the figure.

ß -actin amplified fragment of 289 bp. Lane 1 is molecular weight marker. Lanes 2-5 are ß -actin control PCR product from guinea pig, human, mouse and rat pooled first strand Omni-cDNA™.



An amplified fragment of 289 bp. Lane 1 is molecular weight markers. Lanes 2-6 are ß -actin control PCR products from brain, liver, intestine, skeletal muscle and spleen.

First Strand Pooled cDNA

Product	Catalog No.	Size
Omni-cDNA™ Human First Strand Pooled cDNA	10-0100-05	5 µg
Omni-cDNA™ Mouse First Strand Pooled cDNA	10-0200-05	5 µg
Omni-cDNA™ Rat First Strand Pooled cDNA	10-0300-05	5 µg
Omni-cDNA™ Guinea Pig First Strand Pooled cDNA	10-2100-05	5 µg

First Strand cDNA

Product	Catalog No.	Size
Guinea pig first strand cDNA; Various tissues; Brain, Heart,Liver, Kidney, etc.	10-21XX-05	5 µg

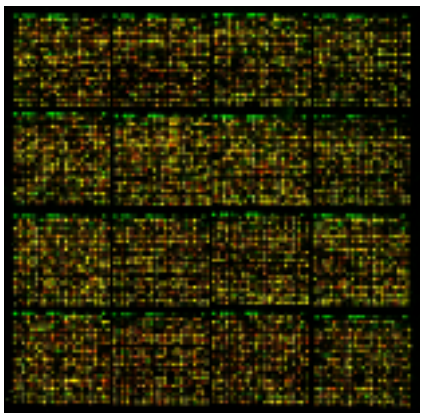
Various animal and plant tissue first strand cDNAs available.
Visit www.genelink.com for complete listing.

Omni-mRNA™ Pooled Reference mRNA

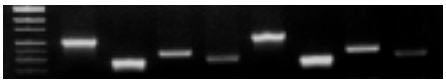
APPLICATION:

Microarray reference mRNA, RT-PCR, cloning, cDNA amplification

Omni-mRNA™ pooled reference mRNA is compatible with all commercially available labeling systems. Other applications of pooled reference mRNA include RNA ELISA, Quantigene, HPSA, and a number of other RNA amplification/detection systems.



Omni-mRNA™ pooled reference mRNA size distribution is from ~5 kb to 200 bp. These can also be used for cloning mRNA of interest by RT-PCR. A 1.3 kb and a ~500 bp amplified cDNA fragment of p53 are shown in the figure.



Human and Mouse Omni-mRNA™ Amplified Pooled Reference mRNA (Catalog No. 08-0100-50 and 08-0200- 50) was used for synthesis of First Strand cDNA (Catalog No. 10-0100-05 and 10-0300-05) and used as a template for amplification of rare and abundant messages. PCR products generated from Human Omni-cDNA™ (lanes 2-5) and Mouse Omni-cDNA™ (lanes 6-9). Individual lanes correspond to the following products; lanes 2 & 6 = GAPDH (~500 bp); lanes 3 & 4 = granulocyte-macrophage colony stimulating factor (GMCSF) (~250 bp); lanes 4 & 7 = tumor necrosis factor alpha (TNF-α) (~325 bp); lanes 5 & 8 = Interleukin-1 receptor alpha (IL-1R α) (~300 bp). Lane 1 = molecular weight markers (200 bp-1500 bp).

Omni-mRNA™ Amplified Pooled Reference mRNA

Product	Catalog No.	Size*
Human Omni-mRNA™ Amplified Pooled Reference mRNA	08-0100-25	25 µg
Mouse Omni-mRNA™ Amplified Pooled Reference mRNA	08-0200-25	25 µg
Rat Omni-mRNA™ Amplified Pooled Reference mRNA	08-0300-25	25 µg
Guinea Pig Omni-mRNA™ Amplified Pooled Reference mRNA	08-2100-25	25 µg

*Quantity supplied is sufficient for direct hybridization of 20 microarrays

Omni-cDNA™ First Strand cDNA

- Near Full-length First Strand cDNA
- Average Size 500 bp to 5 kb
- Convenient Amplification Source of a cDNA Fragment
- Each Lot Tested for ß -actin cDNA and Primer Set Provided
- Tissue-specific Expression Studies

Omni-mRNA™ Pooled Reference mRNA

- Full-length and Near Full-length
- Reference Source of mRNA for Microarray
- Compatible with Other Labeling Systems
- Reference mRNA Among Different Research Groups

GOLD STANDARD

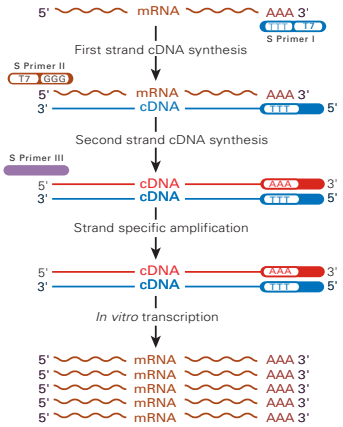
Get the Complete Message and More

We got it, so you can get it. Gene Link's Omni-cDNA™ and Omni-mRNA™ are your sources for high quality mRNA and cDNA for all your needs.

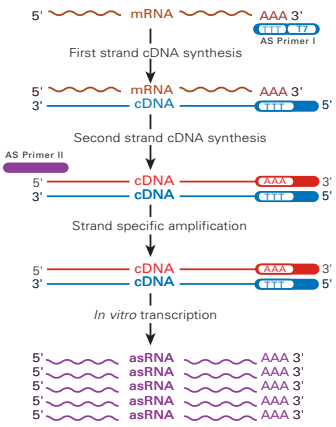
Omni-Array™ mRNA Amplification System

A universal method that performs global mRNA amplification, while maintaining the relative proportions comparable to the original sample is the Omni-Array™ mRNA amplification system. This system is compatible with all labeling and detection systems. Samples which were previously thought to be too small for microarray or other genome wide study can now be amplified using the Omni-Array™ mRNA amplification system.

Sense Strand Synthesis



Anti-Sense Strand Synthesis



APPLICATION:

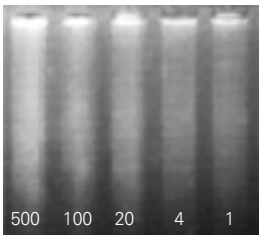
Microarray labeling, global mRNA amplification, cDNA synthesis, RT-PCR, cloning etc.

Omni-Array™ mRNA Amplification system

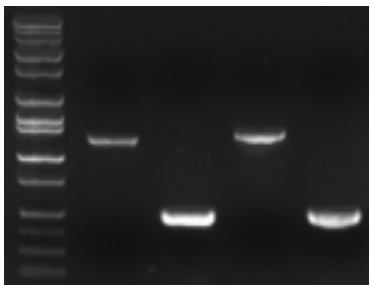
The Omni-Array™ mRNA amplification system provides a rapid and simple procedure for the generation of sufficient amounts of high quality sense or antisense strand RNA using nanogram quantities of starting RNA. The Omni-Array™ Amplification System is especially suited when the availability of total RNA becomes the limiting factor in performing certain experimental procedures.

System Format

The system contains enough reagents for 5 two round or 10 single round amplifications. Consistent results are obtained with 100 ng total RNA for single round amplification and with 2 ng total RNA for two round amplification. The final yield of mRNA is enough for labeling at least 2 microarrays. A single round of amplification results in 100-1000-fold amplification of the entire mRNA population from the sample.



One Round Amplification;
2 ng total RNA amplified



p53 cDNA amplification from human Omni-mRNA™ pooled reference mRNA. Lane 1, mw markers; lanes 2 and 4, ~1.3 kb 5' end fragment of p53; lanes 3 and 5, ~500 bp of middle portion of p53. Lanes 2-3 and 4-5 represent reproducible different preps.

Omni-Array™ mRNA Amplification Systems

Product	Catalog No.	Size
Omni-Array™ Sense strand mRNA Amplification System, 2 ng Version	08-0011-02	10 rxns
Omni-Array™ Antisense strand mRNA Amplification System, 2 ng Version	08-0021-02	10 rxns

Omni-Marker™ Unlabeled DNA Molecular Weight Markers

Omni-Marker™

Universal and Low unlabeled DNA markers contain a blend of fragments ranging from 50 base pairs to 10 kb. The universal contains fragments of the following sizes; 10 kb, 8 kb, 6 kb, 4 kb, 3 kb, 2 kb, 1.55 kb, 1.4 kb, 1 kb, 750 bp, 500 bp, and 400 bp. The "low" version contains fragments from 50 bp to 2 kb. The low Omni-Marker™ is ideal for routine PCR gels. A loading of 5 µl is sufficient per lane.

Molecular Weight Markers

Product	Catalog No.	Size
Omni-Marker™ Universal unlabeled	40-3005-01	100 µl
Omni-Marker™ Universal unlabeled	40-3005-05	500 µl
Omni-Marker™ Universal unlabeled	40-3005-10	1 ml
Omni-Marker™ Low unlabeled	40-3006-01	100 µl
Omni-Marker™ Low unlabeled	40-3006-05	500 µl
Omni-Marker™ Low unlabeled	40-3006-10	1 ml

*Shipped at room temperature. Store at –20°C. ** Normal recommended loading per lane is 5 µl.

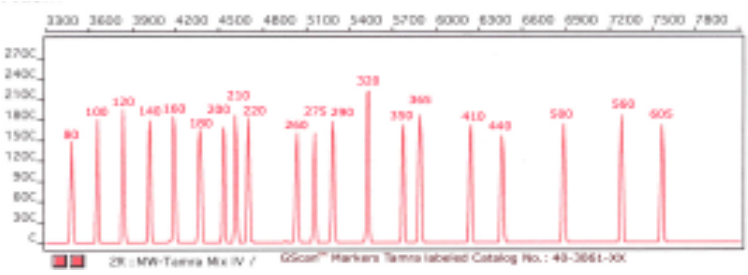
GScan™ Fluorescent Dye-labeled Molecular Weight Markers

APPLICATION:

Genetic Analyzer Fluorescent Polymorphism Studies

Genetic analysis of microsatellites, SNP, allelic discrimination, polymorphic fragment analysis and triple repeat amplification with fluorescent dyes require the parallel co-migration of appropriate molecular weight markers for accurate determination of fragment size.

GScan markers have been especially developed for the above applications. These are supplied ready to load and provide highly consistent fragment sizing.



GScan™ Dye-labeled Markers

Product	Catalog No.	Size
GScan™ Marker Tamra labeled 50 bp - 600 bp	40-3061-01	100 µl
GScan™ Marker Tamra labeled 50 bp - 600 bp	40-3061-05	500 µl
GScan™ Marker Hex labeled 50 bp - 600 bp	40-3081-01	100 µl
GScan™ Marker Hex labeled 50 bp - 600 bp	40-3081-05	500 µl

*A loading of 0.5 µl is suggested.

Omni-Array™ mRNA Amplification System

- As Low as 2 ng Amplification
- Amplified mRNA Suitable for Microarray, cDNA Synthesis, RT-PCR, Cloning, etc.
- Two Round Amplification From 2 ng Generates More Than 10 µg RNA
- Full-Length and Near Full-length Amplification

GScan™ Markers

- Precise & High Resolution Fragment Sizing
- Consistent and Reproducible
- Sharp Sensitive Peaks
- Available with Tamra and Hex Dyes
- Ready to Load

GOLD STANDARD

Not Enough mRNA?

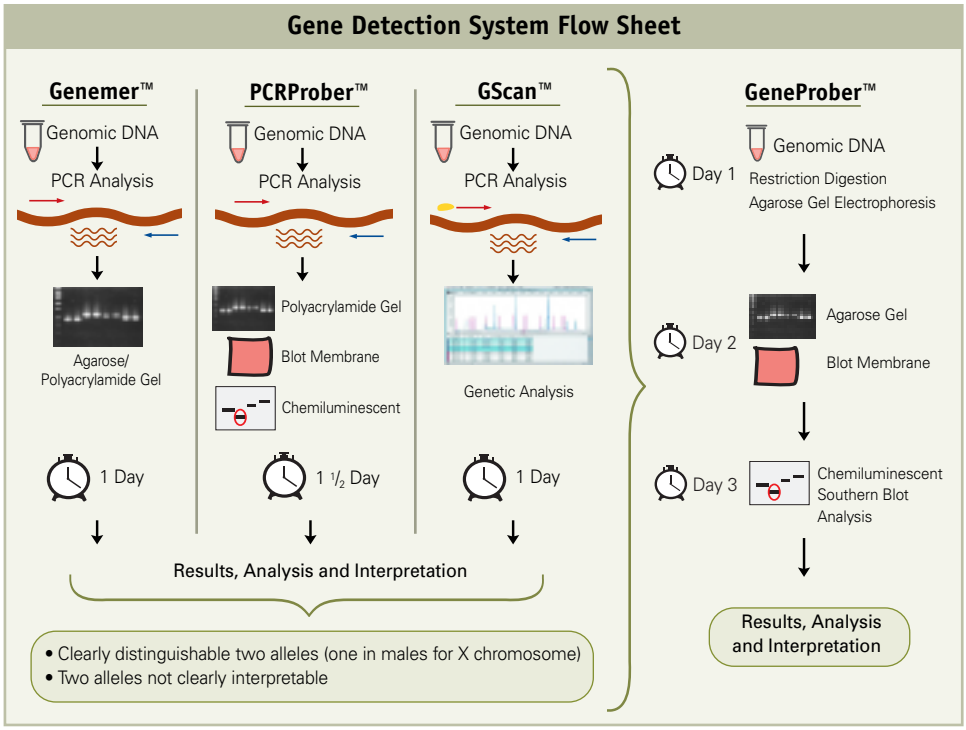
There is no barrier, as low as 2 ng, repeat, two nanograms; is sufficient to amplify and yield 10 micrograms of mRNA. Advance to the Omni-Array™ Amplification System.

Gene Detection Systems

Gene Link is the leader in triple repeat disorder genotyping using non-radioactive based methods. We have more than a decade of expertise and have developed facile non-radioactive detection methods for safe, sensitive and reliable genotyping of human genetic disorders. Take a look at the simple agarose and polyacrylamide gel based systems, the chemiluminescent Southern blot detection methods and the fluorescent systems for genotyping of triple repeat disorders.

The molecular basis of genetic disorders is as varied as clinical genetics itself. The molecular etiology of disorders may be fundamentally straightforward, such as in Sickle cell disease, as compared to a whole new class of diseases where anticipation is involved with apparently increasing disease severity in successive generations. Addressing the etiology and molecular diagnosis of the more complex disorders that involve anticipation (i.e., Fragile X syndrome, Myotonic dystrophy, and Huntington's disease) is often challenging.

This product profile of Gene Link's current gene detection product line spotlights non-radioactive detection products as well as conventional radioactive based methods for genotyping the challenging triple repeat disorders as well as single base mutations and various pathogens. Control DNA fragments with varying number of triple repeats, single base mutations and pathogen control DNA fragments are also available.

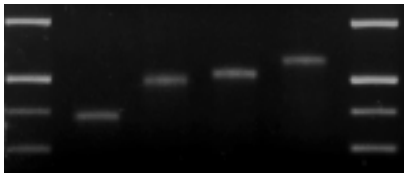


Genemer™

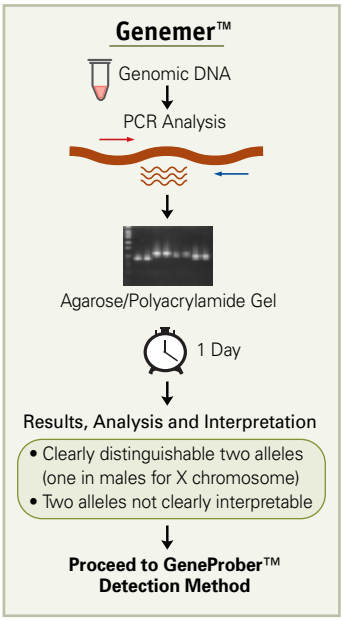
Genemer™ are optimized primer pairs for amplification of the gene fragment of interest, particularly those spanning a mutation. A wide range is available from single base pair mutation sites, the complex triple repeat disorders and various pathogens. Genemer™ kits are complete, easy-to-use systems for reliable genotyping of large repeats in certain triple repeat disorder.

Gene Link has developed a wide array of specific amplification primers and optimized conditions for gene fragments and specifically for the triple repeat disorders. For example, the Huntington Genemer™ kit is capable of routinely amplifying greater than 150 CAG repeats. Other detection systems available are for single base mutations and various pathogens.

The Genemer™ product contains 10 nmols of the primer pair that is sufficient for 400 regular 50 µl amplifications. The Genemer™ Kit contains reagents as well as control DNA sufficient for 100 amplifications.



Friedreich's ataxia GAA repeat genotyping was performed using FRDA Genemer™ (Catalog No. 40- 2027-10) and various FRDA Genemer™ Control DNAs (Catalog No. 40-2027-XX). Optimized reagents from the FRDA Genemer™ Kit were used to amplify long repeats. Lanes 1 & 6 are molecular weight markers; lanes 2–5 are 64, 102, 110 and 125 GAA repeat amplification products.



Genemer™

Product	Size	Catalog No.
Fragile X, Huntington Disease, Myotonic Dystrophy, Friedreich's Ataxia, Kennedy Disease, Sickle Cell, Rh, Sry, and other Genemer™ Kits available	1 Kit	40-20XX-11
HIV, HCV, HBV, MTB and MTB Genemer™ Kit	1 Kit	60-20XX-11
Fragile X, Huntington Disease, Myotonic Dystrophy, Friedreich's Ataxia, Kennedy Disease, Sickle Cell, Rh, Sry, and other Genemer™ primer pairs available	10 nmols	40-20XX-10

Visit www.genelink.com for complete listing of Gene Detection Systems product line.

The Gene Link Advantage

- No More Hazardous Radioactive Exposure
- No More Decayed Probes
- Safe, Sensitive and Reliable Genotyping
- Optimized Kits for Reproducible Results
- Triple Repeat Disorder Genotyping
- Wide Array of Detection Methods
- Chemiluminescent Detection
- Fluorescent Based Genetic Analysis
- Control DNA Standards for Varying Triple Repeat Lengths
- Wide Selection of Disease Genotyping
- Knowledgeable Technical Support

GOLD STANDARD

First Round Genotyping

The Genemer™ and GScan™ products are for initial screening. All samples yielding disease causing triple repeats should be confirmed by Southern blot analysis using the GeneProber™ product line to confirm genotype.

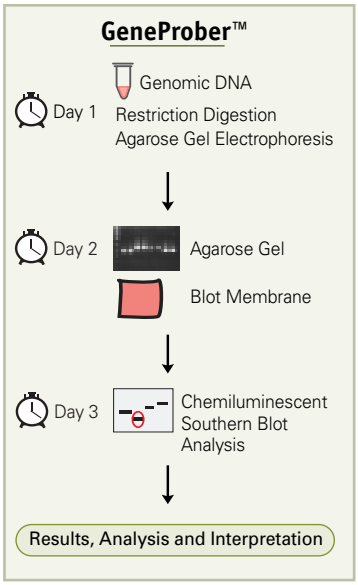
A specific gene fragment probe for Southern blot based hybridization of genomic DNA. The GeneProber™ is available unlabeled for radioactive based methods and labeled with digoxigenin for chemiluminescent detection. One tube is supplied containing 500 ng of the lyophilized unlabeled GeneProber™ probe.

The quantity supplied is sufficient for at least 5 random prime labeling reactions using 100 ng for each reaction. Gene Link recommends using 25 ng of probe for each labeling reaction. The digoxigenin labeled probe supplied is sufficient for five 20 x 20 blots.

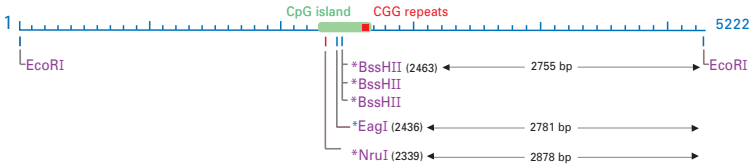
The GeneProber™ product line is based on the chemiluminescent Southern blot detection method. These probes are specially developed to detect the triple repeat amplifications. Fragile X, Huntington disease, Myotonic dystrophy, Friedreich's ataxia and Kennedy disease GeneProber™ unlabeled and digoxigenin labeled are available.

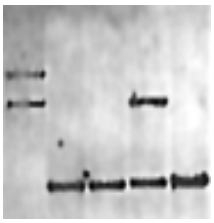
Gene Link's non-radioactive detection systems for genotyping of triple repeat disorders are rapid, reliable and as sensitive as the ^{32}P labeled southern blots. No more decayed probes and radioactive exposure.

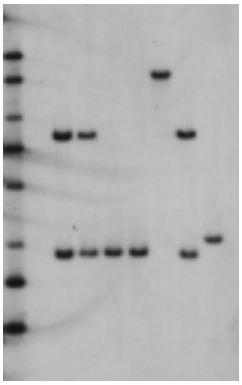
A detailed manual accompanies the product giving a step by step protocol, results and interpretation guidelines.



Fragile X Southern Blot Analysis







Fragile X CGG repeats genotyping results using Gene Link's GeneProber™ products. Fragile X GeneProber™ GLFXDig1 Digoxigenin-labeled Probe (Catalog No. 40-2004-41) and GeneProber™ GLFX1 Unlabeled Probe (Catalog No. 40-2004-40) were used to probe human blood genomic DNA digested with Eco RI and Eag I. Left blot was hybridized with digoxigenin labeled probe and processed for chemiluminescent detection. The blot on the right was hybridized with ^{32}P labeled GLFX1 and processed for radioactive Southern blot detection. Both blots were exposed overnight to X-ray films. Normal fragment sizes for females are between 2.8 kb and 5.2 kb, and a single fragment of 2.8 kb in males. The blot shows samples with CGG repeats in the affected range.

GeneProber™

Product	Size	Catalog No.
Fragile X, Huntington Disease, Myotonic Dystrophy, Friedreich's Ataxia and Kennedy Disease GeneProber™ unlabeled probe	500 ng	40-20XX-40
Fragile X, Huntington Disease, Myotonic Dystrophy, Friedreich's Ataxia and Kennedy Disease GeneProber™ digoxigenin labeled probe	110 µl	40-20XX-41

Visit www.genelink.com for complete listing of Gene Detection Systems product line.

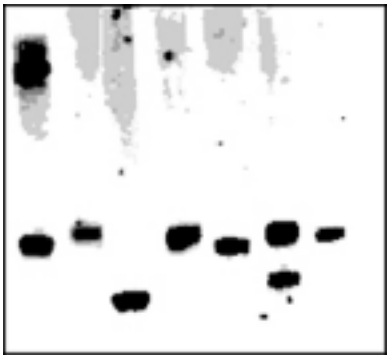
PCRProber™ Gene Detection Kits

PCRProber™ alkaline phosphatase labeled probe is for amplification and non-radioactive detection of a trinucleotide repeat region amplified PCR product. The PCRProber™ Kit comprises of a primer pair for PCR amplification followed by gel blot and chemiluminescent detection using the alkaline phosphatase oligonucleotide probe. Quantity supplied is sufficient for 100 amplifications.

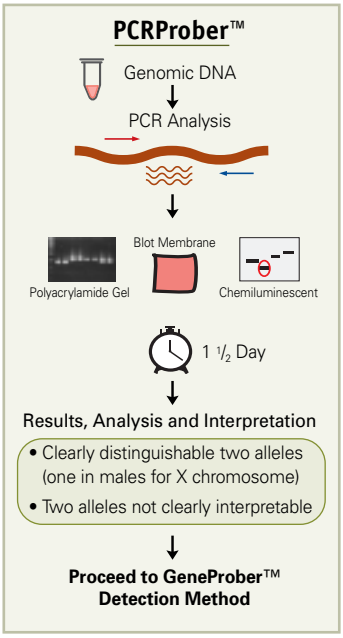
Gene Link's PCRProber™ Kit is based on PCR amplification followed by Southern blot chemiluminescent detection using an alkaline phosphatase labeled oligonucleotide probe. This kit is a safe and sensitive alternate to radioactive-based detection methods. The amplified products are resolved on a sequencing polyacrylamide gel, and then blotted and processed for chemiluminescent detection.

The PCRProber™ Kit is simple and robust for routine triple repeat detection of greater than 100 repeats of all triple repeat disorders listed, except Fragile X. The CGG repeat in Fragile X can be detected up to ~50 repeats.

It is strongly recommended that the genotyping be followed up by using GeneProber™ Southern blot detection methods when two alleles are not clearly discernable.



Fragile X CGG repeats genotyping results using Gene Link's PCRProber™. Various human genomic DNA samples were amplified using the reagents supplied in the PCRProber™ Kit (Catalog No. 40-2004-32) and processed for chemiluminescent detection following the protocol in the manual provided. Fragile X CGG repeat genotyping as an initial screening using the PCRProber™ is rapid as it amplifies CGG repeat sizes up to ~50 repeats. Lane 1 in the blot shows a female sample containing 29 and ~60 CGG repeats respectively.



PCRProber™ Kits

Product	Size	Catalog No.
Fragile X, Huntington Disease, Myotonic Dystrophy, Friedreich's Ataxia and Kennedy Disease PCRProber™ Kits available.	1 kit	40-20XX-32
Fragile X, Huntington Disease, Myotonic Dystrophy, Friedreich's Ataxia and Kennedy Disease PCRProber™ available.	12 µl	40-20XX-31

Visit www.genelink.com for complete listing of Gene Detection Systems product line.

GScan™ Gene Detection Kits

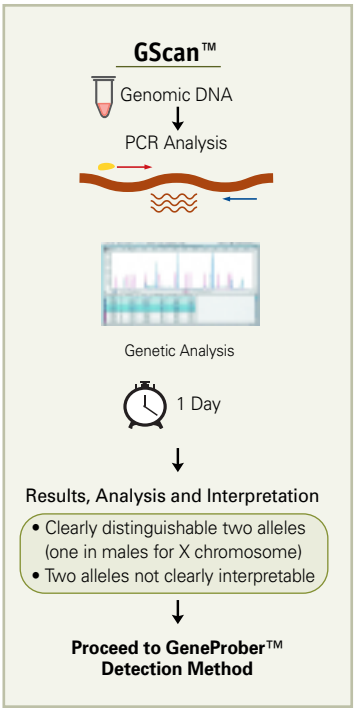
GScan™ Kits contain optimized PCR amplification reagents and a wide selection of fluorescent-labeled primers for genotyping after PCR using fluorescent genetic analyzer instrument(s). Kit includes sufficient reagents for 100 detections.

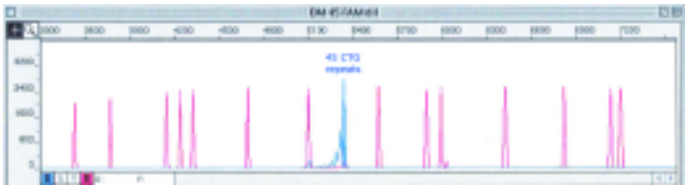
Genotyping using this kit requires use of the appropriate fluorescent genetic analyzer instrument(s) and software capable of detection of fluorescently labeled fragments of varying lengths. These kits have been optimized for an ABI310 genetic analyzer.

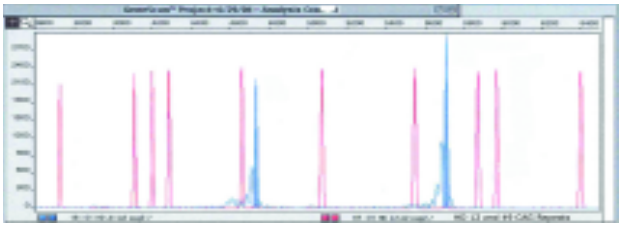
Gene Link's GScan™ gene detection kits are safe, convenient and sensitive, and afford automated compilation of data. The kits contain optimized PCR amplification reagents and a wide array of fluorescent-labeled primers for genotyping after PCR using fluorescent genetic analyzer instrument(s). Included in these kits are ready-to-run control samples of various repeats of the triple repeat disorder. These control samples are for calibration with the molecular weight markers for accurate size determination of the amplified fragments.

The GScan™ Kits are simple and robust for routine triple-repeat detection of greater than 100 repeats of all triple repeat disorders listed, except Fragile X. The CGG repeat in Fragile X can be detected up to ~50 repeats.

It is strongly recommended that the genotyping be followed up by using Southern blot detection methods when two alleles are not clearly discernable.







Above:Myotonic Dystophy CTG genotyping
Right: Huntington Disease CAG genotyping

GScan™ Gene Detection Kits

Product	Size	Catalog No.
Fragile X, Huntington Disease, Myotonic Dystrophy, Friedreich's Ataxia and Kennedy Disease GScan™ Kits available.	1 Kit	40-20XX-15

Visit www.genelink.com for complete listing of Gene Detection Systems product line.

GScan™ Dye Labeled Markers

Product	Catalog No.	Size
GScan™ Marker Tamra labeled 50 bp - 600 bp	40-3061-01	100 µl
GScan™ Marker Tamra labeled 50 bp - 600 bp	40-3061-05	500 µl
GScan™ Marker Hex labeled 50 bp - 600 bp	40-3081-01	100 µl
GScan™ Marker Hex labeled 50 bp - 600 bp	40-3081-05	500 µl

*A loading of 0.5 µl is suggested.

Genemer™ Control DNA

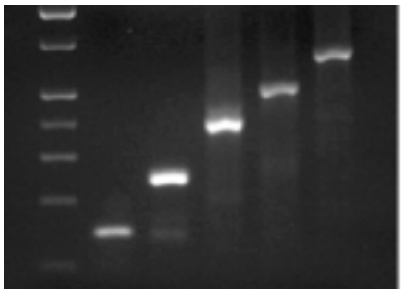
Genemer™ Control DNA are cloned fragments of a particular gene for use with gene or mutation specific Genemer™ and GScan™ products. These control DNAs are ideal genotyping templates for optimizing and performing control amplification with unknown DNA. One tube is supplied containing 500 ng of lyophilized DNA segment of the specified fragment spanning the mutation region. The quantity supplied is sufficient for 1000 regular 50 µl PCR reactions.

The control DNAs were developed to complement Gene Link's gene detection system product line. The control DNAs for a single base mutation are helpful to optimize the protocol and in setting up positive control standards.

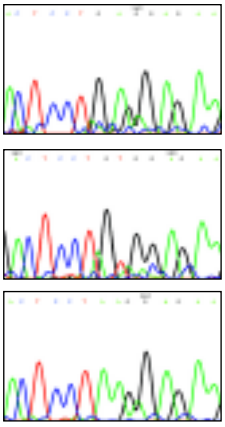
The control DNA series for various triple repeat disorders were generated by amplification. These serve as controls for the amplification of various triple repeats and to run as positive controls. The size of the triple repeats has been determined by sequencing and gel electrophoresis.

The stability of size repeats upon cloning and amplification has not been determined. Thus, the size should be considered approximate and there is no claim for each fragment to contain the exact number of triple repeats.

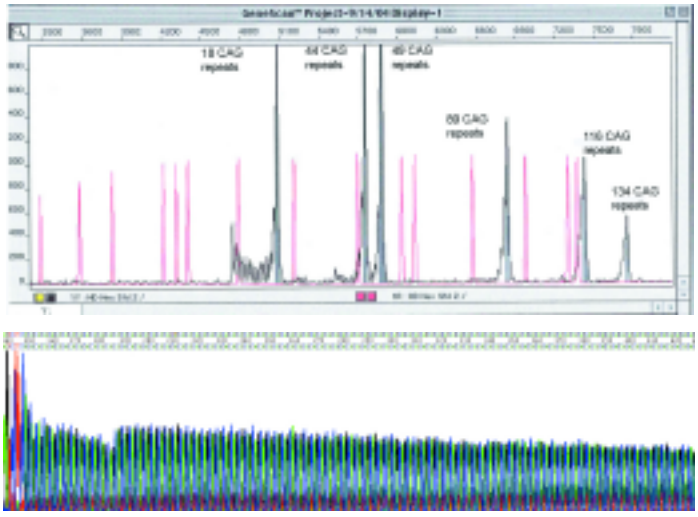
These control DNAs are sold with the expressed condition that these not be used for exact triple repeat size determination of DNA of unknown genotype. The control DNA should be used for determining the performance of specific Genemer™, GScan™ and PCRProber™ Gene Link products.



Lane M is molecular weight markers. Lanes 1-5 represent PCR products from DM genomic clones that contain 12, 45, 93, 129, and 182 CTG repeats respectively.



Sickle Cell Genemer™ control DNA for Hb-A, Hb-S and Hb-C.



Huntington's Disease Genemer™ control DNA of various CAG repeats shown here used for GScan™ analysis.

Genemer™ Control DNA Kits

Product	Size	Catalog No.
Fragile X, Huntington Disease, Myotonic Dystrophy, Friedreich's Ataxia and Kennedy Disease control DNA available with varying number of triple repeats. Control DNA also available for single base mutation disorders.	500 ng	40-20XX-XX
HIV, HBV, HCV, MTB, and WNV pathogen control Genemer™ DNA.	500 ng	60-20XX-06

Please visit www.genelink.com for other Genemer™ Control DNA not listed here.

Appendix

PCR Components and Analysis

PCR buffer conditions vary and it is imperative to optimize buffer conditions for each amplification reaction. At Gene Link most amplification reactions have been optimized to work with the following standard buffer condition, unless otherwise indicated.

Recipe

Standard Gene Link PCR Buffer Composition

10 X PCR buffer	1 X PCR buffer
100 mM Tris-HCl pH 8.3	10 mM
500 mM KCl	50 mM
15 mM $MgCl_2$	1.5 mM
0.01% Gelatin	0.001%

dNTP Concentration

Standard dNTP concentration of 0.2 mM of each base is used. See section on PCR additives when dNTP concentration is changed.

Recipe

2.0 mM dNTP Stock Solution Preparation*

Component	Volume
100 mM dGTP	100 µl
100 mM dATP	100 µl
100 mM dTTP	100 µl
100 mM dCTP	100 µl
Water	4.6 ml
Total Volume	5 ml

*Aliquot and freeze.

$MgCl_2$ Concentration

The concentration of Mg^{2+} will vary from 1-5 mM, depending upon primers and substrate. Since Mg^{2+} ions form complexes with dNTPs, primers and DNA templates, the optimal concentration of $MgCl_2$ has to be selected for each experiment. Low Mg^{2+} ion concentration results in a low yield of PCR product, and high concentrations increase the yield of non-specific products and promote mis-incorporation. Lower Mg^{2+} concentrations are desirable when fidelity of DNA synthesis is critical. The recommended range of $MgCl_2$ concentration is 1-4 mM, under the standard reaction conditions specified. At Gene Link, using the standard PCR buffer with KCl, and a final dNTP concentration of 0.2 mM, a $MgCl_2$ concentration of 1.5 mM is used in most cases. If the DNA samples contain EDTA or other chelators, the $MgCl_2$ concentration in the reaction mixture should be raised proportionally. Given below is a $MgCl_2$ concentration calculation and addition table using a stock solution of 25 mM $MgCl_2$.

Primer Concentration

The final concentration of primers in a PCR reaction is usually 0.5 to 1 µM (micromolar). This is equivalent to 0.5 to 1 pmol/µl. For a 100 µl reaction, add 50 to 100 pmols. At Gene Link we use 0.5 pmol/µl in the final reaction.

⚠ Always use filter barrier pipette tips to prevent cross contamination.

Primer Reconstitution

Stock Primer Mix: Prepare a primer stock solution of 100 µM, i.e., 100 pmols/µl in sterile TE.

Primer Mix: Prepare a 10 pmols/µl Primer Mix solution by a ten fold dilution of the stock primer mix.

Example: Add 160 µl sterile TE to a new tube, and to this tube add 20 µl of each primer stock solution. Label this tube as Primer Mix 10 pmols/µl.

Recipe

TE Buffer pH 7.5 Composition

1 X TE Buffer pH 7.5
10 mM Tris-HCl pH 7.5
1 mM EDTA

PCR Additives

DNA polymerases need to elongate rapidly and accurately to function effectively *in vivo* and *in vitro*, yet certain DNA regions appear to interfere with their progress. One common problem are pause sites, at which DNA polymerase molecules cease elongation for varying lengths of time. Many strong DNA polymerase pauses are at the beginnings of regions of strong secondary structure such as template hairpins (1). Taq polymerase used in PCR suffers the same fate and GC-rich DNA sequences often require laborious work to optimize the amplification assay. The GC-rich sequences possess high thermal and structural stability, presumably because the high duplex melting temperature permits stable secondary structures to form, thus preventing completion of a faithful replication (2).

Nucleotide analog 7-deaza dGTP is effective in reducing the secondary structure associated with the GC rich region by reducing the duplex stability (4). Betaine, DMSO and formamide reduce the T_m and the complex secondary structure, thus the duplex stability (1-5). Tetramethyl ammonium chloride (TMAC) actually increases the speci-

$MgCl_2$ Concentration & Addition Table

Final concentration of $MgCl_2$ in 50 µl reaction mix, (mM)	1.0	1.25	1.5	1.75	2.0	2.5	3.0	4.0
Volume of 25 mM $MgCl_2$, (µl)	2	2.5	3	3.5	4	5	6	8

ficity of hybridization and increases the T_m. The use of TMAC is recommended in PCR conditions using degenerate primers.

These PCR additives and enhancing agents have been used to increase the yield, specificity and consistency of PCR reactions. These additives may have beneficial effects on some amplification and it is impossible to predict which agents will be useful in a particular context. Therefore, they must be empirically tested for each combination of template and primers.

PCR Additives

Additive	Purpose & Function	Concentration
7-deaza-2'-deoxyguanosine; 7-deaza dGTP	GC rich region amplification. Reduces the stability of duplex DNA.	Totally replace dGTP with 7-deaza dGTP; or use 7-deaza dGTP: dGTP at 3:1.
Betaine (N,N,N-trimethylglycine = [carboxymethyl]trimethylammonium)	Reduces T_m facilitating GC rich region amplification. Reduces duplex stability.	Use 3.5 M to 0.1 M Betaine. Be sure to use Betaine or Betaine (mono)hydrate and not Betaine HCl.
BSA (bovine serum albumin)	Proven particularly useful when attempting to amplify ancient DNA or templates, which contain PCR inhibitors such as melanin.	BSA concentration of 0.01 µg/µl to 0.1 µg/ µl can be used.
DMSO (dimethyl sulfoxide)	Reduces secondary structure and is particularly useful for GC rich templates.	DMSO at 2-10% may be necessary for amplification of some templates, however 10% DMSO can reduce Taq polymerase activity by up to 50% so it should not be used routinely.
Formamide	Reduces secondary structure and is particularly useful for GC rich templates.	Formamide is generally used at 1-5%. Do not exceed 10%.
Non-ionic detergents e.g. Triton X-100, Tween 20 or Nonidet P-40 (NP-40)	Stabilizes Taq polymerase and may also suppress the formation of secondary structure.	0.1-1% Triton X-100, Tween 20 or NP-40 may increase yield but may also increase non-specific amplification. As little as 0.01% SDS contamination of the template DNA (left- over from the extraction procedure) can inhibit PCR by reducing Taq polymerase activity to as low as 10%, however, inclusion of 0.5% Tween-20 or -40 will effectively neutralize this effect.
TMAC (tetramethyl ammonium chloride)	Reduces potential DNA-RNA mismatch and improves the stringency of hybridization reactions. It increases T_m and minimizes mis-pairing.	TMAC is generally used at a final concentration of 15-100 mM to eliminate non-specific priming.

References

1. Kovarova, M. and Draber, P. (2000) New Specificity and yield enhancer for polymerase chain reactions (2000) Nucl. Acids. Res. 28: e70.
2. Henke, W., Herdel, K., Jung, K., Schnorr, D. and Loening, S. (1997) Betaine improves the PCR amplification of GC-rich DNA sequences. Nucl. Acids Res. 25: 3957-3958.
3. Daniel S. Mytelka, D.S., and Chamberlin, M.J.,(1996) Analysis and suppression of DNA polymerase pauses associated with a trinucleotide consensus. Nuc. Acids Res., 24:2774–278.
4. Keith, J. M., Cochran, D.A.E., Lala, G.H., Adams, P., Bryant, D.and Mitchelson, K.R. (2004) Unlocking hidden genomic sequence. Nucl. Acids Res. 32: e35.
5. Owczarzy, R., Dunietz, I., Behlke, M.A., Klotz, I.M. and Walde, J.A.. (2003) Thermodynamic treatment of oligonucleotide duplex–simplex equilibria. PNAS, 100:14840-14845.

Formulas

%GC= (G+C)/length

MW = (A x 313.2) + (C x 289.19) + (G x 329.21) + (T x 304.2) + (I x 314.2) + (N x 308.95) + (R x 321.21) + (Y x 296.69) + (M x 301.2) + (K x 316.7) + (S x 309.2) + (W x 308.71) + (H x 302.2) + (B x 307.53) + (D x 315.54) + (V x 310.53) + (P x 79.98) + (U x 290.17) – 62

T_m for oligos shorter than 25 bp = 2(A+T) + 4(C+G)

For longer oligos:
T_m = 81.5 – 16.6 + (0.41 x %GC)) – 600/size
(Reference Bolton, E.T and McCarthy, B.J. (1962) PNAS 48: 139-1397)

Formula for T_m Calculation

T_m = 81.5 + 16.6 x Log10[Na^+] + 0.41 (%GC) – 600/size
[Na^+] is set to 100 mM

Example: 5'-ATGCATGCATGCATGCATGC-3' 20 mer; GC=50%; AT= 50%

T_m = 81.5 + 16.6 x Log10[0.100] + 0.41 x 50 – 600/20

T_m = 81.5 - 16.6 + 0.41 x 50 – 600/20

T_m = 81.5 - 16.6 + 20.5 – 30

T_m = 64.9 + 20.5 – 30

T_m = 85.40 – 30

T_m = 55.4°C

T_m for same oligo using 2(A+T) + 4(C+G)

= 2(5+5) + 4(5+5)

= 2(10) + 4(10)

= 20 + 40

= 60°C

Degenerate Bases in Sequence

International union of biochemistry (IUB) recommends the use of single letter nomenclature for degenerate/mixed bases. The use of inosine is recommended to reduce the number of degeneracies. For degenerate (mixed bases) positions use the following IUB codes:

R = A+G	H = A+T+C
Y = C+T	B = G+T+C
M = A+C	D = G+A+T
K = G+T	V = G+A+C
S = G+C	N = A+C+G+T
W = A+T	I = Inosine

MW and Extinction Coefficient

Base	MW	EC
deoxy adenosine	313.21	15.4
deoxy cytosine	289.19	7.4
deoxy guanosine	329.21	11.5
thymidine	304.2	8.7
deoxy inosine	314.2	7.2
A+G+T+C	308.95	10.70
A+G	321.21	13.45
C+T	296.69	8.05
A+C	301.2	11.40
G+T	316.7	10.10
G+C	309.2	9.45
A+T	308.71	12.05
A+T+C	302.2	10.5
G+T+C	307.53	9.20
G+A+T	315.54	11.86
G+A+C	310.53	11.43
phosphate	79.98	0.00
deoxy uridine	290.17	9.9

International System of Unit Prefixes

Prefix	Symbol	Multiple
exa	(E)	10^{18}
peta	(P)	10^{15}
tera	(T)	10^{12}
giga	(G)	10^{9}
mega	(M)	10^{6}
kilo	(k)	10^{3}
hecto	(h)	10^{2}
deka	(da)	10^{1}
deci	(d)	10^{-1}
centi	(c)	10^{-2}
milli	(m)	10^{-3}
micro	(μ)	10^{-6}
nano	(n)	10^{-9}
pico	(p)	10^{-12}
femto	(f)	10^{-15}
atto	(a)	10^{-18}

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