2005 Edition

Products, Protocols and Applications Guide

Custom Oligo Synthesis

Fluorescent Molecular Probes

siRNA

Genetic Tools & Reagents

Gene Link™

"Quality, Consistency, Confidence"

Gene Link has acquired a reputation for quality, consistency and confidence in the critical gene research tools and technology we supply to the research community worldwide. Our products and services are supported and ensured by our commitment to premium quality and our constant efforts to introduce new and improved products.

Gene Link fosters customer satisfaction and loyalty by maintaining personal relationships with our customers. We routinely assist our customers regarding the technical inquiries, the design of their experiments and the solving of their application problems. Our dedicated and highly trained staff of customer service and technical support employees is strongly motivated to serve our customers. Gene Link has endeavored to develop, and will continue to preserve, our reputation for "Quality, Consistency, and Confidence."

Gene Link ™
140 Old Saw Mill River Road Hawthorne, NY 10532
Tel: 914.769.1192 www.genelink.com Fax: 914.769.1193

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General Information

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About Us

Gene Link, Inc. is a dynamic biotechnology company and research organization. Our mission is to be one of the most reliable suppliers of reagents and reagent systems used in genetic research technology applications worldwide.

Gene Link, Inc. is privately held biotechnology company incorporated in the State of New York dedicated to fundamental and applied research on genetic/biomedical projects relating to gene mapping, localization, detection and marketing of our services and products in these areas. Since inception, Gene Link has acquired a reputation for quality, consistency and confidence in the services and products it provides to the research communities worldwide. Our services and products are supported and ensured by our commitment and constant efforts to excel. Gene Link is situated in Westchester County of New York, a location ideally suited for growth and strong interaction with prestigious nearby universities, biotechnology and pharmaceutical companies in New York, New Jersey and Connecticut.

Gene Link fosters customer satisfaction and loyalty by maintaining close personal relationships with our customers, and by offering technical support for the applications of our products. We routinely assist our customers regarding the design of their experiments and the solving of their application problems to help researchers reach their goals faster and with greater success. Gene Link customers are Universities, Research Institutes, Pharmaceutical companies and Hospitals involved in research. Understanding the needs of our customers and helping them achieve success is a priority at Gene Link. These customers are located worldwide.

Gene Link is a leading supplier of custom oligonucleotides for use in PCR, sequencing, cloning, and mutagenesis. Gene Link services include genotyping, sequencing and gene construction. Gene Link offers as well a wide variety of other molecular biology products such as, siRNA, fluorescent probes and non-radioactive gene detection systems for human genetic disorders.

Gene Link's research and development activities focus on developing novel gene detection systems. Gene Link is in the process of developing research products to aid scientists with detection of genetic disorders, specifically triple repeat disorders (Fragile X, Myotonic Dystrophy, Huntington etc.). Also available from Gene Link are kits for research purposes designed for genotyping other genetic disorders. To complement our genetic disorder detection systems we have recently introduced detection systems for infectious disorders.

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Confidentiality
Nondisclosure Agreement

At Gene Link, we respect your privacy and are committed to protecting it. We are committed to building customer trust by demonstrating this respect in every aspect of our marketing activities. If at any time you feel that Gene Link is not following its stated privacy policy, please feel free to contact us at 1-800-GENE-LINK with your concerns. The points below summarize our promise to you.

- The information you provide to Gene Link through our website or any other channel will be used only to provide you with communications that are relevant to Gene Link products and services that we feel may suit your needs and preferences.

- This information is held in strict confidence and will never be sold, traded or rented to other companies, individuals or entities for their marketing use.

- Every communication message that you receive from Gene Link will provide you with the opportunity to be removed from future information exchanges.

- You may also send an email to cust_service@genelink.com to request removal from our email or postal mail list. Please type the word "REMOVE" in the subject line and include your name, company name and the list(s) from which you would like to be removed.

- Upon request, we will provide you with a summary of the information you have provided to Gene Link. This information will be provided only to the email address on file for the customer making the request. You will be able to change, correct or remove any information at any time. The contact for such requests is cust_service@genelink.com

- Following website registration our site will use "session cookies" to help provide relevant information to you during your visit. The cookies are only accessible by Gene Link while you are visiting our site and are deleted upon your signoff.

- You may terminate your website registration at any time by sending an email to cust_service@genelink.com

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General Information

Products
Gene Link is committed to providing the highest quality products at competitive prices. Gene Link warrants that all products meet or exceed the performance standards described in the product specification sheets. If you are not completely satisfied with our product, our policy is to replace the product as soon as possible. Gene Link provides no other warranties of any kind, expressed or implied. Gene Link's liability shall not exceed the purchase price of the product. Gene Link shall have no liability for direct, indirect, consequential or incidental damages arising from the use, results of use, or inability to use its products.

Privacy
At Gene Link, we respect your privacy and are committed to protecting it. We are committed to building customer trust by demonstrating this respect in every aspect of our marketing activities. If at any time you feel that Gene Link is not following its stated privacy policy, please feel free to contact us at 1-800-GENE-LINK with your concerns. The points below summarize our promise to you.

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- This information is held in strict confidence and will never be sold, traded or rented to other companies, individuals or entities for their marketing use.
- Every communication message that you receive from Gene Link will provide you with the opportunity to be removed from future information exchanges.
- You may also send an email to cust_service@genelink.com to request removal from our email or postal mail list. Please type the word "REMOVE" in the subject line and include your name, company name and the list(s) from which you would like to be removed.
- Upon request, we will provide you with a summary of the information you have provided to Gene Link. This information will be provided only to the email address on file for the customer making the request. You will be able to change, correct or remove any information at any time. The contact for such requests is cust_service@genelink.com
- Following website registration our site will use "session cookies" to help provide relevant information to you during your visit. The cookies are only accessible by Gene Link while you are visiting our site and are deleted upon your signoff.
- You may terminate your website registration at any time by sending an email to cust_service@genelink.com

Customer Service
Customer Service representatives are available to Monday through Friday from 9:00 AM to 6:00 PM Eastern Time. E-mail: cust_service@genelink.com. Gene Link does not require written confirmation for telephone, e-mail or Internet orders. To avoid duplication, be certain that any written confirmation of an order is clearly marked CONFIRMING.

Payment
Payment terms are net 30 days.

Credit Cards
Gene Link accepts credit card payments (Visa, MasterCard and American Express only via phone, fax, and mail). Please provide card number, expiration date, and card billing name and address.

Shipping
All unmodified custom oligos are regularly shipped in 24 hrs. and guaranteed to be shipped within 48 hrs. of order receipt by Standard Overnight for guaranteed afternoon delivery. In stock catalog items regularly ship same day of order receipt. Upon request, Priority Overnight shipment will be made for guaranteed morning delivery. Exceptions may be necessary for US holidays. A $10.00 handling charge is added to each order in addition to shipping charges. Please consult Customer Service for additional shipping information.

Blanket Orders
As a service to our customers, Gene Link accepts blanket orders. For your convenience, we encourage you to use this type of purchase order. For more information please contact your Purchasing department or Customer Service department.

Returns
Products may not be returned without proper authorization by Gene Link's Customer Service Department. Due to the custom nature and temperature sensitivity of most of our products, we are unable to restock and resell returned goods.

Intended Use
All products sold by Gene Link are intended for research only. These products are not suitable for diagnostic or drug purposes, nor are they suitable for administration to humans or animals.

International Orders
Gene Link has Distributors worldwide. Please see inside back cover to find a distributor in your area.

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Ordering Information

All customers are encouraged to place orders through our easy to use on-line ordering system at www.genelink.com.

Web Ordering Instructions
The design of the online ordering system is based on the premise of establishing a customer information database for the Principal Investigator or Director or Supervisor of individual laboratories. This is termed as the 'Customer'. All other investigators (Users) from that laboratory will use the same 'Customer Number' to access our database. There is a field in the 'Custom Oligos Order Entry' form to enter the individual 'User' placing that particular order. At the first order screen, please select new user. For 'Customer Information', please enter the name, address, and e-mail for the Principal Investigator. 'Bill to information' will be filled in automatically from the information entered in the 'Customer Information' fields. If necessary, please correct these fields by changing the name and address to the accounts payable department for your institution. After submitting this information you will be ready to place an online order. Your Gene Link assigned customer number will be emailed to you directly. Please use the assigned customer number for placing future orders.

Custom Oligos Order Entry
User information is for the person who is ordering the oligos. This name will appear on the synthesis report and oligo tubes. An email confirmation will also be sent to the email address for this person. In addition, please provide the phone number in case of any questions.

Classic Oligos Order Form is the Gene Link standard one-by-one oligo entry system. It offers detailed physical parameter values e.g. Size, Molecular Weight, Tm, EC etc. and several convenient oligo analysis features, e.g. Structure Analysis, NCBI Blast, Reverse Complement etc. This order form page also lists various synthesis options listed below:
1. Oligo Type (DNA, RNA, Phosphorothioate, Chimeric, Phosphonate etc.)
2. Scale of synthesis (50 nmols, 200 nmols, 1 micromolar etc.)
3. Purification Type (desalted, Reverse Phase, Gel)
4. 3', 5' and Internal Modifications.

Enter the name of the oligo, select scale of synthesis, select purity desired, any modification and type of oligonucleotide.
Multi Oligo Import Order Form enables you to import multiple oligos at once. This is an ideal timesaver for those who order many short, unmodified oligos at one time. Following the guidelines given below will ensure error-free and rapid ordering.

1. Prior to ordering, save all your oligos in an Excel sheet in the following format; Example:

1	My oligo name	GATGACGATGACGTGCGTGACGTGACCCCAAA	my very own comments

Column A: **oligo number**
Column B: **oligo name**
Column C: **sequence**
Column D: **Any comments**

2. Directly import the excel file using the browse button. Please include the header row.
Our default scale of synthesis and purity method will be applied based on oligo length. These are given below:
Defaults for scale of synthesis and purity are as follows:
1-49mer: 50 nmol scale, desalted
50-99mer: 200 nmol scale with gel purification
100-199mer: 1 umol scale with gel purification
200-250mer: 2 umol scale with gel purification

You may change from the defaults by clicking on 'Classic View' and 'Click to Edit '. You may edit these upon review. After entering sequence information there are two options:
1) Submit Order
2) Review Saved Oligos

When you click 'Submit Order' at this point, Gene Link will receive the order, and the user will receive a confirmation at his/her email address. At Gene Link we value customer feedback and welcome comments and suggestions regarding our online ordering system. Please direct all inquiries by email to **techsupport@genelink.com**

Orders by Phone: **1-800-GENE-LINK**
Orders by Fax: **1-888-GENE-LINK**
(Please note that custom oligo orders cannot be placed by phone)
Orders by E-mail: orders@genelink.com

When placing an order, please provide the following information:
1. Purchase order number or credit card number
2. Customer number
3. Billing address
4. Shipping address
5. Name of person to whose attention order should be shipped
6. Name and telephone number of contact person
7. Product catalog number, description, size and quantity
8. For custom oligo orders, please include oligo name, sequence, scale of synthesis and any purification or modifications required.

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Terms and Conditions of Sale

1. Acceptance – GENE LINK, INC., HEREIN REFERRED TO AS SELLER, RESERVES THE RIGHT TO ACCEPT OR REJECT A SALES ORDER AT ITS SOLE DISCRETION WITHOUT ASSIGNING ANY REASON. ALL SALES ARE SUBJECT TO AND EXPRESSLY CONDITIONED UPON THE TERMS AND CONDITIONS CONTAINED HEREIN, AND UPON BUYER'S ASSENT THERETO. NO VARIATION OF THESE TERMS AND CONDITIONS WILL BE BINDING UPON SELLER UNLESS AGREED TO IN WRITING AND SIGNED BY AN OFFICER OR OTHER AUTHORIZED REPRESENTATIVE OF SELLER.

2. Changes -Orders arising hereunder may be changed or amended only by written agreement signed by both Buyer and Seller, setting forth the particular changes to be made and the effect, if any, of such changes on the price and time of delivery. Buyer may not cancel this order unless such cancellation is expressly agreed to in writing by Seller. In such event, Seller will advise Buyer of the total charge for such cancellation, and Buyer agrees to pay such charges, including, but not limited to, storage and shipment costs, costs of producing non-standard materials, costs of purchasing non-returnable materials, cancellation costs imposed on Seller by its suppliers, and any other cost resulting from cancellation of this order by Buyer which is permitted by Seller. Certification of such costs by Seller's independent public accountants shall be conclusive on the parties hereto.

3. Delivery, claims, delays - All sales are FOB Seller's shipping point unless otherwise noted. If Shipping and Handling Charges are quoted or invoiced, they will include handling charges in addition to actual freight costs. Delivery of the goods to the carrier at Seller's shipping point shall constitute delivery to Buyer but Seller shall bear all risk of loss or damage in transit. The general method of shipment for each item is DHL next afternoon service unless otherwise specified. However, Seller reserves the right, in its discretion, to determine the exact method of shipment. Seller reserves the right to make delivery in installments, all such installments to be separately invoiced and paid for when due per invoice, without regard to subsequent deliveries. Delay in delivery of any installment shall not relieve Buyer of Buyer's obligations to accept remaining deliveries.

Immediately upon Buyer's receipt of any goods shipped hereunder, Buyer shall inspect the same and shall notify Seller in writing of any claims for shortages, defects or damages and shall hold the goods for Seller's written instructions concerning disposition. If Buyer shall fail to so notify Seller within five days after the goods have been received by Buyer, such goods shall conclusively be deemed to conform to the terms and conditions hereof and to have been irrevocably accepted by the Buyer.

Seller shall not be liable for any loss, damage or penalty as a result of any delay in or failure to manufacture, deliver or otherwise perform hereunder due to any cause beyond Seller's reasonable control, including, without limitation, unsuccessful reactions, act of Buyer, embargo or other governmental act, regulation or request affecting the conduct of Seller's business, fire, explosion, accident, theft, vandalism, riot, acts of war, strikes or other labor difficulties, lightning, flood, windstorm or other acts of God, delay in transportation, or inability to obtain necessary labor, fuel, materials, supplies or power at current prices.

4. Allocation of goods - If Seller is unable for any reason to supply the total demands for goods specified in Buyer's order, Seller may allocate its available supply among any or all Buyers on such basis as Seller may deem fair and practical, without liability for any failure of performance which may result there from.

5. Payment - Terms of sale are net 30 days of date of invoice, unless otherwise stated. If the financial condition of Buyer results in the insecurity of Seller, in its sole and unfettered discretion, as to the ultimate collect ability of the purchase price, Seller may, without notice to Buyer, delay or postpone the delivery of the products; and Seller, at its option, is authorized to change the terms of payment to payment in full or in part in advance of shipment of the entire undelivered balance of said products. In the event of default by Buyer in the payment of the purchase price or otherwise, of this or any other order, Seller, at its option, without prejudice to any other of Seller's lawful remedies, may defer delivery, cancel this Contract, or sell any undelivered products on hand for the account of Buyer and apply such proceeds as a credit, without set-off or deduction of any kind, against the contract purchase price, and Buyer agrees to pay the balance then due to Seller on demand. Buyer agrees to pay all costs, including, but not limited to, reasonable attorney and accounting fees and other expenses of collection resulting from any default by Buyer in any of the terms hereof.

6. Taxes and other charges - Any use tax, sales tax, excise tax, duty, custom, inspection or testing fee, or any other tax, fee or charge of any nature whatsoever imposed by any governmental authority, on or measured by the transaction between Seller and Buyer shall be paid by Buyer in addition to the prices quoted or invoiced. In the event Seller is required to pay any such tax, fee or charge, Buyer shall reimburse Seller therefore; or, in lieu of such payment, Buyer shall provide Seller at the time the order is submitted an exemption certificate or other document acceptable to the authority imposing the tax, fee or charge.

7. Pricing - Prices are subject to change without notice. Please inquire about volume discounts. Please call us for current prices if you require this information prior to placing your order. We guarantee our written domestic quotations for one (1) year unless otherwise specified. For guarantee information regarding quotations outside the US, please contact our distributor in your local area. When placing your order, please reference our quoted prices or our pro forma number. If you place your order by phone, we will confirm our current price at that time.

8. Price Changes - Shipment will be made promptly even if prices have been nominally increased. Price reductions will be automatically applied to your invoice.

9. Warranties - Seller warrants that its products shall conform to the description of such products as provided to Buyer by Seller through Seller's catalog, analytical data or other literature. **THIS WARRANTY IS EXCLUSIVE, AND SELLER MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE.** Seller's warranties made in connection with this sale shall not be effective if Seller has determined, in its sole discretion, that Buyer has misused the products in any manner, has failed to use the products in accordance with industry standards and practices, or has failed to use the products in accordance with instructions, if any, furnished by Seller.

Seller's sole and exclusive liability and Buyer's exclusive remedy with respect to products proved to Seller's satisfaction to be defective or nonconforming shall be replacement of such products without charge or refund of the purchase price, in Seller's sole discretion, upon the return of such products in accordance with Seller's instructions. SELLER SHALL NOT IN ANY EVENT BE LIABLE FOR INCIDENTAL, CONSEQUENTIAL OR SPECIAL DAMAGES OF ANY KIND RESULTING FROM ANY USE OR FAILURE OF THE PRODUCTS, EVEN IF SELLER HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGE INCLUDING, WITHOUT LIMITATION, LIABILITY FOR LOSS OF USE, LOSS OF WORK IN PROGRESS, DOWN TIME, LOSS OF REVENUE OR PROFITS, FAILURE TO REALIZE SAVINGS, LOSS OF PRODUCTS OF BUYER OR OTHER USE OR ANY LIABILITY OF BUYER TO A THIRD PARTY ON ACCOUNT OF SUCH LOSS, OR FOR ANY LABOR OR ANY OTHER EXPENSE, DAMAGE OR LOSS OCCASIONED BY SUCH PRODUCT INCLUDING PERSONAL INJURY OR PROPERTY DAMAGE UNLESS SUCH PERSONAL INJURY OR PROPERTY DAMAGE IS CAUSED

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BY SELLER'S GROSS NEGLIGENCE. All claims must be brought within one (1) year of shipment, regardless of their nature.

10. Compliance with laws and regulations - Seller certifies that to the best of its knowledge its products are produced in compliance with applicable requirements of the Fair Labor Standards Act, as amended, and the Occupational Safety and Health Standards Act of 1970 and regulations, rules and orders issued pursuant thereto.

11. Buyer's use of products - Seller's products are intended primarily for laboratory research purposes and, unless otherwise stated on product labels, in Seller's catalog or in other literature furnished to Buyer, are not to be used for any other purposes, including but not limited to, in vitro diagnostic purposes, in foods, drugs, medical devices or cosmetics for humans or animals or for commercial purposes. Buyer acknowledges that the products have not been tested by Seller for safety and efficacy in food, drug, medical device, cosmetic, commercial or any other use, unless otherwise stated in Seller's literature furnished to Buyer. Buyer expressly represents and warrants to Seller that Buyer will properly test, use, manufacture and market any products purchased from Seller and/or materials produced with products purchased from Seller in accordance with the practices of a reasonable person who is an expert in the field and in strict compliance with all applicable laws and regulations, now and hereinafter enacted. Buyer further warrants to Seller that any material produced with products from Seller shall not be adulterated or misbranded within the meaning of the Federal Food, Drug and Cosmetic Act and shall not be materials which may not, under Sections 404, 505, or 512 of the Act, be introduced into interstate commerce.

Buyer realizes that, since Seller's products are, unless otherwise stated, intended primarily for research purposes, they may not be on the Toxic Substances Control Act (TSCA) inventory. Buyer assumes responsibility to assure that the products purchased from Seller are approved for use under TSCA, if applicable.

Buyer has the responsibility to verify the hazards and to conduct any further research necessary to learn the hazards involved in using products purchased from Seller. Buyer also has the duty to warn Buyer's customers and any auxiliary personnel (such as freight handlers, etc.) of any risks involved in using or handling the products. Buyer agrees to comply with instructions, if any, furnished by Seller relating to the use of the products and not misuse the products in any manner. If the products purchased from Seller are to be repackaged, relabeled or used as starting material or components of other products, Buyer will verify Seller's assay of the products. No products purchased from Seller shall, unless otherwise stated, be considered to be foods, drugs, medical devices or cosmetics.

12. Buyer's Representations and Indemnity - Buyer represents and warrants that it shall use all products ordered herein in accordance with Paragraph No. 9 "Buyer's Use of Products", and that any such use of products will not violate any law or regulation. Buyer agrees to indemnify and hold harmless Seller, its employees, agents, successors, officers, and assigns, from and against any suits, losses, claims, demands, liabilities, costs and expenses (including attorney and accounting fees) that Seller may sustain or incur as a result of any claim against Seller based upon negligence, breach of warranty, strict liability in tort, contract, or any other theory of law brought by Buyer, its officers, agents, employees, successors or assigns, by Buyer's customers, by end users, by auxiliary personnel (such as freight handlers, etc.) or by other third parties, arising out of, directly or indirectly, the use of Seller's products, or by reason of Buyer's failure to perform its obligations contained herein. Buyer shall notify Seller in writing within fifteen (15) days of Buyer's receipt of knowledge of any accident, or incident involving Seller's products which results in personal injury or damage to property, and Buyer shall fully cooperate with Seller in the investigation and determination of the cause of such accident and shall make available to Seller all statements, reports and tests made by Buyer or made available to Buyer by others. The furnishing of such information to Seller and any investigation by Seller of such information or incident report shall not in any way constitute any assumption of any liability for such accident or incident by Seller.

13. Patent disclaimer - Seller does not warrant that the use or sale of the products delivered under will not infringe the claims of any United States or other patents covering the product itself or the use thereof in combination with other products or in the operation of any process.

14. Returns - Goods may not be returned for credit except with Seller's permission, and then only in strict compliance with Seller's return shipment instructions. Any returned items may be subject to a 20% processing fee.

15. Technical Assistance - At Buyer's request, Seller may, at Seller's discretion, furnish technical assistance and information with respect to Seller's products. SELLER MAKES NO WARRANTIES OF ANY KIND OR NATURE, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE, WITH RESPECT TO TECHNICAL ASSISTANCE OR INFORMATION PROVIDED BY SELLER OR SELLER'S PERSONNEL. ANY SUGGESTIONS BY SELLER REGARDING USE, SELECTION, APPLICATION OR SUITABILITY OF THE PRODUCTS SHALL NOT BE CONSTRUED AS AN EXPRESS WARRANTY UNLESS SPECIFICALLY DESIGNATED AS SUCH IN A WRITING SIGNED BY AN OFFICER OR OTHER AUTHORIZED REPRESENTATIVE OF SELLER.

16. Miscellaneous - Seller's failure to strictly enforce any term or condition of this order or to exercise any right arising hereunder shall not constitute a waiver of Seller's right to strictly enforce such terms or conditions or exercise such right thereafter. All rights and remedies under this order are cumulative and are in addition to any other rights and remedies Seller may have at law or in equity. Any waiver of a default by Buyer hereunder shall be in writing and shall not operate as a waiver of any other default or of the same default thereafter.

If any provision of this Agreement shall be held to be invalid, illegal or unenforceable, the validity, legality and enforceability of the remaining provisions shall not be affected or impaired thereby. The paragraph headings herein are for convenience only; they form no part of the terms and conditions and shall not affect their interpretation.

This Agreement shall be binding upon, inure to the benefit of, and be enforceable by, the parties hereto, and their respective heirs, personal representatives, successors and assigns.

17. Governing Law - All disputes as to the legality, interpretation, application, or performance of this order or any of its terms and conditions shall be governed by the laws of the State of New York including its conflict of laws principles. Each party to this order agrees that any dispute arising between them, which results in either party instituting court proceedings shall be litigated in either the Federal District Court for the New York area or in the Circuit Court for the City of Hawthorne.

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DNA SYNTHESIS OVERVIEW

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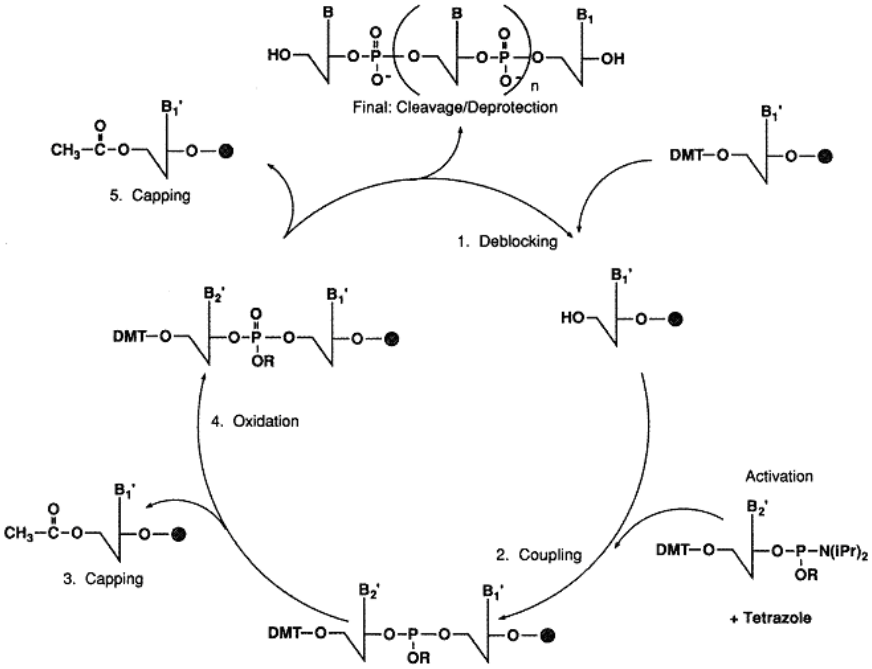
Beta-Cyanoethyl Phosphoramidite Chemistry

The synthesis of DNA from ß-cyanoethyl phosphoramidite monomers is currently the industry standard. With this method, high coupling efficiencies are easily attained. The absence of side reactions also confers high biological activity of the synthetic oligonucleotide. In the basic reaction cycle, a solid support, derivatized with the initial protected nucleoside, is contained in a reaction column. Reagents and solvents are pumped through the column to effect the addition of successive protected nucleotide monomers (phosphoramidites). Each addition cycle includes detritylation, activation, coupling, oxidation, and capping. Intervening wash steps remove excess reactants and by-products of reaction. After the chain elongation is complete, the oligomer must be removed from the support and fully deprotected. The crude product can be purified on an Oligo-Pak purification column, by gel electrophoresis, or by HPLC.



Solid Support

The synthesis of an oligonucleotide begins with the selection of the reaction column containing the initial support-bound protected nucleoside. The reactive 5'-hydroxyl group of the support-bound nucleoside is protected with a dimethoxytrityl (DMT) group. The 3'-hydroxyl group of the nucleoside is covalently attached, through an appropriate hydrocarbon spacer, to the Controlled Pore Glass (CPG) support. 500A CPG is recommended for oligonucleotides up to 50 bases in length; 1000A CPG is recommended for oligonucleotides more than 50 bases in length. Following this guideline provides better coupling efficiency by minimizing steric hindrance.

Monomers

The exocyclic amines of 2'-deoxyadenosine (dA), 2'-deoxycytidine (dC) and 2' deoxyguanosine (dG) have to be protected during synthesis to prevent side reactions from taking place at these sites. The dC and dA monomers are protected by benzoyl (bz) groups at the N4 and N6 positions, respectively, while the dG is protected at the N2 position by an isobutyryl (ibu) group. These groups are removed after synthesis of the oligonucleotide is complete, during deprotection. The phosphoramidite monomers are also protected at the 5'-hydroxyl position with a DMT group, while the 3'-phosphite is modified by ß-cyanoethyl and diisopropylamine groups.

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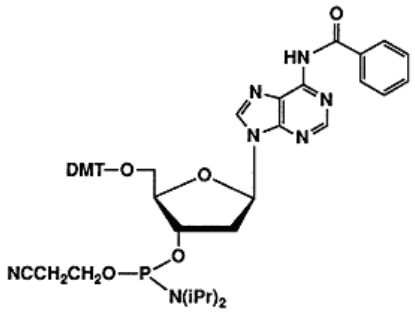
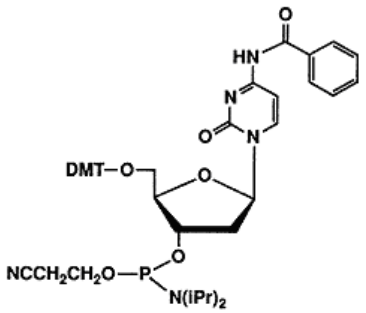
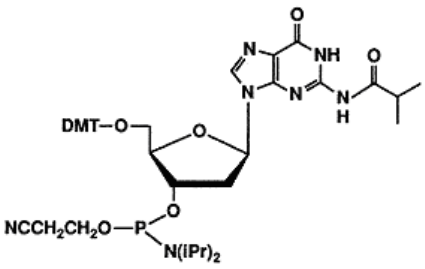


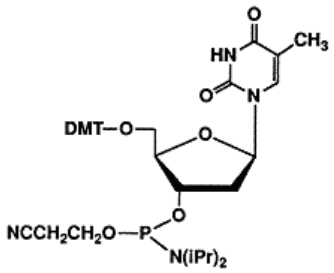
N-Benzoyl 5′-O-DMT-2′-deoxy-Adenosine-3′-(β-cyanoethyl)-Phosphoramidite



N⁴-Benzoyl-5′-O-DMT-2′-deoxy-Cytidine-3′-(β-cyanoethyl)-Phosphoramidite



N-Isobutyrl-5′-O-DMT-2′-deoxy-Guanosine-3′-(β-cyanoethyl)-Phosphoramidite



5′-O-DMT-Thymidine-3′-(β-cyanoethyl)-Phosphoramidite

Detritylation

In the first step of the synthesis cycle, the acid labile DMT group of the support-bound monomer is removed with a dichloroacetic acid solution (DCA). The resulting cation is orange. The yield of the DMT cation can be estimated spectrophotometrically and be used to determine step-wise coupling efficiency. As the DNA bases are acid-labile, the detritylation step must only be as long as is necessary to ensure complete detritylation.

Coupling

After detritylation the next protected phosphoramidite is delivered to the reaction column. Tetrazole is used to activate the phosphoramidite. The two reagents are mixed just prior to delivery to the reaction column. Tetrazole, a weak acid, protonates the tertiary nitrogen group of the phosphoramidite so that the diisopropylamine moiety becomes a good leaving group.

The Coupling Mechanism is a nucleophylic attack by the free 5′-hydroxyl group on the 3′-phosphorous of the incoming activated monomer. For this reason, it is important to have a totally hydroxyl-free environment in the column. To ensure this, dry acetonitrile is used as the general solvent, and all the reagents and solvents are maintained in the anhydrous state. Under these conditions the coupling efficiencies are very high, thereby permitting synthesis of long oligonucleotides.

Oxidation

The most recently added monomer is now linked to the chain by trivalent phosphite bond. However, phosphorus linkages are more stable when the oxidation state is pentavalent, as is the case in native DNA. Therefore, the bond is oxidized in an iodine solution.

Capping

Since 1-2% of the free 5′-hydroxyl groups do not undergo reaction, unreacted chains (failure sequences) must be capped to prevent further elongation in the next cycles. For this step, acidic anhydride and N-methyllimidazole are mixed to form an activated acetylating agent.

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Cycling
Following the capping step, the cycle of reactions is repeated, beginning with the detritylation step, until the chain elongation is complete.

Final Detritylation
If the oligonucleotide is to be purified by Oligo-Pak column methods or by Reverse Phase HPLC, the DMT group is left on the 5'-OH of the oligonucleotide and is removed only after purification. If the oligonucleotide is to be purified by gel electrophoresis or ion exchange HPLC, the oligonucleotide is detritylated at this stage.

Removal from Support and Deprotection
After the specified sequence has been assembled, the oligonucleotide must be removed (cleaved) from the support and fully deprotected prior to use. A 90-minute room temperature treatment with ammonium hydroxide is used to cleave the oligonucleotide from the support and to deprotect the phosphorus by ß-elimination of the cyanoethyl group. A 24-hour room temperature treatment or an 8 hour 55 ^{o}C treatment with ammonium hydroxide effectively removes the capping groups and the benzoyl and isobutyryl groups protecting the exocyclic amines. After cleavage/deprotection, the resulting crude mixture contains the tritylated product oligonucleotide, the truncated failure sequences with free 5'-hydroxyl ends, by-products of deprotection (benzamide, isobutyramide, acrylonitrile, and acetamide), and silicates from hydrolysis of the glass support.

Purification
The crude oligonucleotide product can be purified using reverse phase chromatography or by gel electrophoresis. Reverse phase purification can be either processed manually using cartridges or HPLC, both methods depend on the hydrophobic trityl group to separate the product from the failure sequences and the by-products of deprotection. Anion exchange HPLC and gel electrophoresis separate oligonucleotides by length. Normally the product is longer than the failure sequences.

References:
Agrawal, S. (1993). Protocols for Oligonucleotides and Analogs, 20. Agrawal S. (1994). Protocols for Oligonucleotide Conjugates, 26. Beaucage, S. L. and Caruthers, M. H. (1981). Tetrahedron Lett., 22, 1859. Eckstein, F. (1991). Oligonucleotides and Analogies. Letsinger, R. L. finnan, J. L., Heavner, G. A., and Lunsford, W. B. (1975). J. Am. Chem. Soc., 97, 3278. McBride, L. J. and Caruthers, M. H. (1983). Tetrahedron Lett., 24, 245. Matteucci, M. D. and Caruthers, M. H. (1980). Tetrahedron Lett. 21, 719.

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Oligo Reconstitution and Use

Gene Link oligos are supplied lyophilized. These are stable at room temperature for an extended period of time. We recommend the following reconstitution protocol. The solvent may be either sterile TE or sterile water depending on the established laboratory practice. At Gene Link we dissolve oligos in sterile water. After reconstitution store the stock solution at –80 °C or –20 °C.

The protocol given below is only to be used as a guideline and should not be substituted for any other specific protocol.

Reconstitution

Stock solution of 500 pmols/µl
[500 µM (micromolar)]

Gene Link provides the exact amount of nmols of each oligo supplied on the tube and on the Oligo Report. Multiply the 'nmol' amount by 2 to arrive at the volume of solvent to be added.

Example: 45.10 nmols x 2 = 90.2 µl
Dissolve the oligo in 90.2 µl to get 500 pmols/µl stock solution. Use as required.

Stock solution of 100 pmols/µl
[100 µM (micromolar)]

Gene Link provides the exact amount of nmols of each oligo supplied on the tube and on the Oligo Report. Multiply the 'nmol' amount by 10 to arrive at the volume of solvent to be added.

Example: 45.10 nmols x 10 = 451 µl
Dissolve the oligo in 451 µl to get 100 pmols/µl stock solution. Use as required.

Examples of Use

Polymerase Chain Reaction (PCR)

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 you would add 50 to 100 pmols. At Gene Link we use 0.5 pmol/µl; [0.5 µM (micromolar)].

Sequencing

The final concentration of the primer in automated sequencing is from 4 to 10 pmols (~0.05 - 0.1µg). Use the above dilution protocol to prepare a 100 pmols/µl [100 µM (micromolar)] solution and then dilute 10 fold to get 10 pmol/µl solution. Use 1µl. (10 pmols)

Quick Conversion Table

1 µM (µMolar) = 1 pmol/µl (pico moles/µl). Example: 20 µMolar primer solution is 20 pmol/µl

1 mM (mill Molar) = 1 nmols/µl (nano moles/µl)

The molecular weight of the oligo=mer x 330 dalton
For an 18 mer oligo, the MW=18x330=5940
i.e., 5940 g=1 mol, 5.940µg=1 nmol

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Oligonucleotide Analysis

The Custom Oligonucleotide Synthesis Report accompanying each custom oligonucleotide contains extensive thermodynamic and physical data for each oligo. This technical sheet explains each of the values and the method used for arriving at a particular physical data. The figures may differ for the same oligo sequence by using different software applications, the reason being the selection of particular details and constants for arriving at the calculated value. An example is the salt concentration for calculating Tm. Gene Link software uses 100 mM salt concentration, which closely approximates the total salt concentration in either PCR or sequencing reactions.

The size is the length of the oligo. This is exactly the number of bases in the oligonucleotide sequence. In case of modified bases including 3' and 5' modifications, the software assumes these as bases and is represented in the size of the oligonucleotide. This will be accurate if these were modified bases (e.g. inosine, deoxyuridine, etc.) and *inaccurate* if the modification is not a modified base (e.g. phosphate, amino linker etc.)

MW is the molecular weight of the oligonucleotide. The following weights are used for arriving at the calculation. To enter an oligonucleotide directly, type the oligo in the Oligo window. The maximum number of bases is 100. DNA or RNA may be entered. Inosine, I, is permitted as a base in the sequence. To select an oligonucleotide from a sequence, the sequence must be the currently active window. Highlight the desired oligo sequence then open this analysis. Any highlighted bases in the sequence are automatically entered 5' to 3' in the oligo box up to the limit of 100 bases. Alternatively, set the From: to the 5' base number of the oligo. Set the Length to the length of the oligo. Set the strand to Top or Cmp. The oligo will be displayed in the Oligo window. Note: The 3' bases of the oligo are displayed in the sequence window, use the Home key to display the 5' end on larger oligos. Mol Wt is the calculated molecular weight of the oligonucleotide. This is calculated from the table of molecular weights for each base found in the appendix A.

Tm is the melting temperature (temperature at which 50% of the oligo is a duplex) of the oligonucleotide calculated using the nearest-neighbor thermodynamic values methods of Breslauer et. al for DNA and Freier et. al for RNA. Note: this is not accurate for long sequences. The formula for the Tm is: Where H is the enthalpy, S is the entropy, R is 1.987 cal K-1 mol-1 , and CT is the total strand concentration. The formulas used for calculating H and S are described below. Filter Tm is a filter hybridization calculation. It is the thermodynamic Tm - 7.5, useful as an initial temperature when washing filters. %GC Tm is based on the %GC and is corrected for salt concentration and % formamide. The formula of Baldino et. al is 81.5 + 16.6 log[Na+] +0.41(%G+%C) - 0.65(% formamide) - 675/length. This formula is more accurate with longer oligos and is useful if hybridizing in formamide or high salt. The salt concentration is set with the Salt con (mMol) parameter. The default is 1 M. When calculating an oligo for PCR or sequencing, use 50 mMol. The % formamide concentration is adjusted with the % Formamide parameter. The default is 0.

GC + AT Tm is the traditional 2 for each A or T plus 4 for each G or C. This method is considered less accurate and is provided for reference. nmol/A260 is the concentration of the oligo in nanomoles per O.D. at 260 nm. This is calculated from the table of extinction coefficients and molecular weights for each base in the appendix A.

ug/A260 is the concentration of the oligo in micrograms per O.D. at 260 nm. This is calculated from the table of extinction coefficients in the appendix A. %GC is the percentage of G and C in the oligo. The calculation is the total number of G and C divided by the total number of bases in the oligo. It is used for the Tm calculations.

dG is the G (free energy) for the oligo as calculated by the nearest neighbor method of Breslauer et. al for DNA and Freier et. al for RNA. The G is calculated by the formula G = H-TS. Where H is the enthalpy, S is the entropy and T is the temperature set by the dG Temp. The values used to calculate the G for an oligo are listed in the appendix A. The G is a measure of stability, the greater the negative value, the more stable the duplex formed by the oligo. For example, G of the oligo ATGCTT is the G for AT + G for TG + G for GC + G for CT + G for TT. The value is corrected for temperature if the dG Temp is not 25. The initiation value of +5 for DNA and + 3.6 for RNA is added to the G. dH is the enthalpy of the oligo as calculated by the nearest neighbor method of Breslauer et. al for DNA and Freier et. al for RNA. The values to calculate the delta H (H) for an oligo are listed in the appendix A. For example, H of the oligo ATGCTT is the H for AT +H for TG + H for GC + H for CT + H for TT. dS is the entropy of the oligo as calculated by the nearest neighbor method of Breslauer et. al for DNA and Freier et. al for RNA. The values to calculate the delta S (S) for an oligo are listed in the appendix A. For example, delta S of the oligo ATGCTT is the S for AT + S for TG + S for GC + S for CT + S for TT. The initiation value of 10.8 is added to the calculation. 3'- End dG is the nearest-neighbor G for the last 7 (default value) bases of the 3' end. The number of bases may be changed with the 3'-end length parameter. The G is calculated by the formula G = H-TS. Where H is the enthalpy, S is the entropy and T is the temperature set by the dG Temp. The initiation value of +5 for DNA and + 3.6 for RNA is added to the G. Lower numbers are more stable.

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Quality Controlled Production Methods- Superior to "Mass-Produced Factory Oligos"

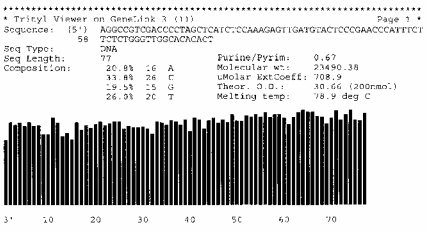
Gene Link is not an 'oligo factory'. Gene Link oligos are for demanding applications and consistent results. We believe that investigators who value time and have no room for an experiment to fail due to oligo quality should consider Gene Link. Each Gene Link oligo is synthesized, processed and quality assured to Gene Link's absolute standards. This includes coupling efficiency monitoring of each base during synthesis and electrophoretic analysis of each oligo side-by-side on a polyacrylamide gel to visually assess quality. A real gel picture is included as part of the oligo report.

Chemical DNA synthesis comprises of multiple reactions to complete a cycle of the appropriate base coupling. Maintaining the highest possible coupling efficiency necessitates the use of reagents of exacting specifications, state-of-the-art instruments and optimized software-driven protocols. This becomes a requirement for synthesizing a long oligo. Gene Link specializes in long oligos. *Our description of long oligos is 180mer to 250mer. You are invited to compare.*

PCR and sequencing reactions are very robust and can tolerate up to 50% failure/truncated sequence oligos. Coupling efficiency of 99.5% and 98% seems very good, but on closer examination the yield is almost half for a 40 mer!

You are clearly taking a chance by using oligos synthesized at anything below 99.5% coupling efficiency. Yes, the oligo will work most of the time. Would you not want the confidence of it working all the time?
Long oligos just cannot be synthesized at anything below 99.5% coupling efficiency. Please see the detailed coupling efficiency table and graph given below.

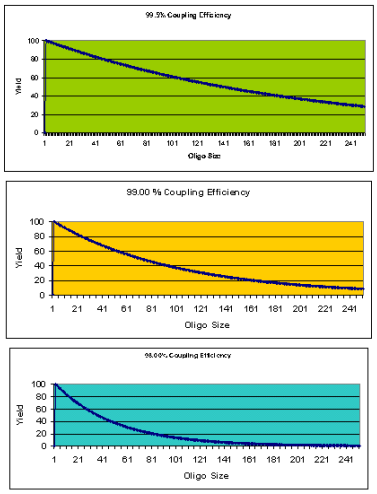
Routine Trityl Coupling Efficiency



Quality Control Real Gel Picture

Coupling Efficiency and Full Length Oligo Yield

Oligo Size	99.50%	99.00%	98.00%
20	90.916	82.617	68.123
25	88.665	78.588	61.578
30	86.471	74.717	55.862
35	84.331	71.055	50.314
40	82.243	67.573	45.48
45	80.208	64.261	41.11
50	78.222	61.112	37.16
55	76.286	58.117	33.59
60	74.398	55.268	30.363
65	72.557	52.56	27.445
70	70.761	49.984	24.808
75	69.009	47.534	22.425
80	67.301	45.204	20.27
85	65.635	42.989	18.323
90	64.011	40.882	16.562
95	62.427	38.878	14.971
100	60.881	36.973	13.533
110	57.905	33.438	11.057
115	56.472	31.799	9.995
120	55.074	30.24	9.034
130	52.381	27.349	7.382
140	49.821	24.734	6.031
150	47.385	22.369	4.928
160	45.068	20.23	4.027
170	42.865	18.296	3.29
180	40.769	16.546	2.688
190	38.776	14.964	2.196
200	36.88	13.533	1.795
210	35.08	12.24	1.47
220	33.36	11.07	1.19
230	31.73	10.01	0.98
240	30.18	9.05	0.8
250	28.7	8.19	0.65



Oligo Scale of Synthesis

Gene Link makes the following recommendations:

1-49mer:	50 nmol scale (purification dependent upon desired use)
50-99mer:	200 nmol scale with gel purification
100-199mer:	1 umol scale with gel purification
200-250mer:	2 umol scale with gel purification

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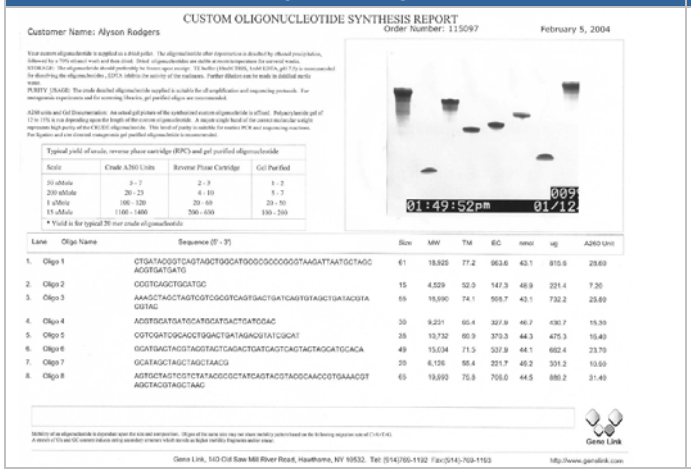
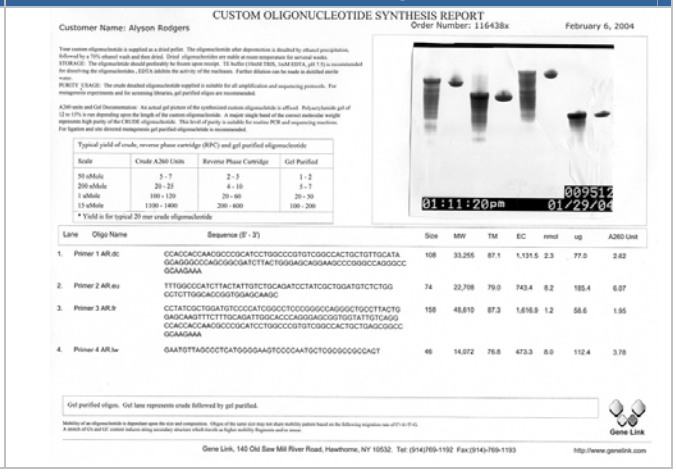
Oligo Specifications Report

All Gene Link oligos are accompanied by a detailed report. The specifications are also printed on the label of the tubes, which are cryo-resistant for years of freeze-thaw without the label disintegrating. The report includes:

- Complete Oligo Sequence
- Oligo physico-chemical parameters
- A real gel picture with oligos run side by side

Unpurified Oligos	Gel Purified Oligos
	

CUSTOM OLIGONUCLEOTIDE SYNTHESIS REPORT
Customer Name: Alyson Rodgers
Order Number: 115097
February 5, 2004

CUSTOM OLIGONUCLEOTIDE SYNTHESIS REPORT
Customer Name: Alyson Rodgers
Order Number: 116438x
February 6, 2004

Oligo Scale of Synthesis and Yield

Scale Of Synthesis	Crude desalted			RPC purified (HPLC substitute)			Gel Purified		
	Yield of a typical 20mer oligo*			Yield of a typical 30mer oligo*			Yield of a typical 50mer oligo*		
	A_{260} Units	nmols	mg	A_{260} Units	nmols	mg	A_{260} Units	nmols	mg
50 nmol	8-10	30+	0.2-0.3	4-5	12+	0.1-0.16	NR* [1-2]	NR* [2-4]	NR* [0.03-0.06]
200 nmol	20-25	80+	0.6-0.8	8-12	24+	0.26-0.40	4-6	8+	0.13-0.2
1 µmol	100-120	400+	3-4	40-50	30+	1.3-1.6	20-25	40+	0.6-0.8
10 µmol	1000-1200	4000+	33-40	400-500	1200+	13-16	200-250	400+	6.6-8
15 µmol	1100-1400	4400+	36-46	500-700	1500+	16-23	300-350	600+	9-11
Purity & Yield	Variable purity based on oligo length and sequence. Refer to coupling efficiency table. No further purification required for PCR and sequencing applications. Gel purification recommended for oligos above 50mer and all applications involving cloning and mutagenesis.			Purity 85% to 95% depending on oligo sequence and structure. Yield and purity will be lower for sequences with high GC content. Not recommended for oligos longer than 35mer.			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 30µg/A_{260} unit for oligos is calculated for an ~equimolar base composition. Long stretches of a single base or homopolymers will have variable yields. Example for homopolymeric 50mer: A (50)= ~20/A_{260} Unit; G (50)= ~28/A_{260} Unit; T (50)= ~35/A_{260} Unit and C (50)= ~39/A_{260} Unit

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Custom Oligonucleotide Synthesis

All Gene Link synthesized custom oligonucleotides are supplied desalted, lyophilized and ready to use after reconstitution. The "crude" desalted ethanol precipitated product is suitable for most PCR amplification, Short Tandem Repeat Polymorphism (STRP) repeat analysis for gene mapping and sequencing. An actual gel picture of quality assurance polyacrylamide gel electrophoresis is supplied in addition to technical information e.g. Tm, MW, base composition and the exact A_{260} units.

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. We maintain an absolute standard of coupling efficiency threshold of greater than 99.5% for all oligos by using the best reagents.

Trityl monitoring coupling efficiency of each base added during synthesis is accomplished with programmed 'halt' to seek user intervention if it falls below the threshold. This may not be evident when comparing short oligos, but is a requirement for long oligos. Ask our competitors how often they synthesize 200 to 250mer oligonucleotides. Please see the coupling efficiency table and graph on page 20. Gene Link specializes in long oligos. Our description of long oligos is 180mer to 250mer. You are invited to compare.

Unmodified Oligo Synthesis*			
Product	**Catalog No.**	**Size**	**Price $**
Custom Oligonucleotide Synthesis; 50 nmol scale	26-6400-05	50 nmol	0.90
Custom Oligonucleotide Synthesis; 200 nmol scale	26-6400-02	200 nmol	2.00
Custom Oligonucleotide Synthesis; 1 µmol scale	26-6400-01	1 µmol	3.75
Custom Oligonucleotide Synthesis; 2 µmol scale	26-6400-03	2 µmol	6.50
Custom Oligonucleotide Synthesis; 10 µmol scale	26-6400-10	10 µmol	32.00
Custom Oligonucleotide Synthesis; 15 µmol scale	26-6400-15	15 µmol	38.00

*minimum charge for 15mer applies. Please visit www.genelink.com for current list prices. Call for institutional discount pricing structure.

Same Day Oligo Service

Certain experiments cannot wait for an extra day. When such is the case, order our Same Day Oligo service. ***The custom oligo will be shipped the same day for next day am or pm delivery.*** Order should be received before 12 noon; this service is for oligos shorter than 30mer and will be synthesized only on a 200nmol scale. Gel picture and A_{260} will not be provided. Gel picture will be mailed if requested.

Same Day Oligo Service*		
Product	**Catalog No.**	**Price $**
Same Day Oligo Service (per oligo)	26-6400-00	20.00

*Oligos with modification and/or purification require additional time and may not ship same day. Call for additional information.

Same Day Oligos are on occasion shipped in water (see table below for amounts). This is primarily due to time constraints in meeting the shipping deadline. There will be no difference in stability if these are stored on receipt at –20 °C or dried in a speed vac. The quantity supplied is as written on the label.

Same Day Oligo*	
Scale of Synthesis	**Supplied In Sterile Water**
50 nmol scale	200 µL
200 nmol scale	400 µL
1 µmol scale	500 µL

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Purification

Automated chemical synthesis of DNA has improved rapidly, with substantial gains made in the chemistry enabling routine coupling yields in excess of 99% and very reliable automation with each synthesis cycle of less than 3 minutes. The final oligonucleotide product obtained in the 20-30mer range is substantially pure with very low truncated sequences thus requiring no further purification for most routine applications involving Polymerase Chain Reaction (PCR*) and DNA sequencing. Purification may be required for other applications and is recommended for cloning, site directed mutagenesis, ligation etc. Purification of oligonucleotides can be accomplished by various methods, the selection based on the particular requirement. The common techniques available and used frequently are polyacrylamide gel purification (PAGE), HPLC and Reverse Phase Cartridge (RPC). The table below summarizes the purification range of each of the above techniques.

Purification Recommendations			
Length	**PAGE**	**HPLC**	**RPC**
8-40mer	Yes	Yes	Yes
41-200mer	Yes	No	No

*All Gene Link oligos shorter than 40mer usually do not require any further purification if the application is for PCR or sequencing.

Polyacrylamide Gel Purification
Purification by this method is considered the Gold Standard for oligonucleotide purification. PAGE purification can be used for any length of oligonucleotide (as compared to HPLC and RPC cartridges which are limited to oligonucleotides preferably below 35mer). This technique is also the most labor-intensive method. Appropriate percentage of polyacrylamide gel (10-20%) is prepared and the oligonucleotide electrophoresed. The major product is the slowest migrating band, which is identified by UV shadowing and excised out. The gel slice is then processed for oligo elution commonly by crush and soak method. Gel purification is strongly advised for all applications involving cloning of the product, such as mutagenesis, cloning or gene construction applications.

HPLC
HPLC purification is usually based on reverse phase utilizing hydrophobic matrices. The oligonucleotide is synthesized with Trityl ON (the triphenylmethyl group at the 5' OH of the last base of the synthetic oligonucleotide) and the elution profile first elutes all non-tritylated truncated sequences followed by elution of the hydrophobically bound full-length oligonucleotide. This method yields greater than 95% purity depending upon the sequence and length of the oligonucleotide. Reverse phase based HPLC fails above 35-40mer oligonucleotide as longer oligos are inherently hydrophobic and bind non-specifically. **Gene Link does not offer HPLC purification at this time.**

Reverse Phase Cartridge Purification
This is an inexpensive alternate to HPLC reverse phase purification. The cartridge for reverse phase purification usually contains a hydrophobic matrix e.g. C18 silica, the principle of purification being the same as HPLC as well as the purification achieved of ~95% purity depending upon the sequence and length of the oligonucleotide. Reverse phase cartridge based purification also fails above 35-40mer oligonucleotides, as longer oligos are inherently hydrophobic and bind non-specifically.

Purification						
Product	**Catalog No.**	**50 nmol scale (XX=05)**	**200 nmol scale (XX=02)**	**1 µmol scale (XX=01)**	**10 µmol scale (XX=10)**	**15 µmol scale (XX=15)**
Gel Purification	26-6400-XX	75.00	75.00	150.00	1500.00	1500.00
Reverse Phase Cartridge	26-6400-XX	30.00	30.00	90.00	750.00	750.00

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ANTISENSE OLIGONUCLEOTIDES

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Antisense Oligonucleotides

Background

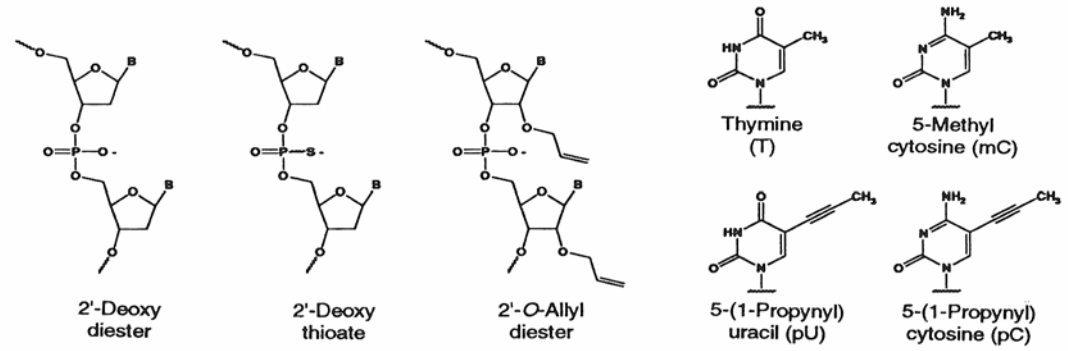
Antisense oligonucleotides refer to short, synthetic oligonucleotides, which are complementary in sequence, and upon specific hybridization to its cognate gene product, induce inhibition of gene expression. Oligonucleotides as short as a 15 mer have the required specificity to inhibit gene expression of a particular gene by annealing to the cellular mRNA (1,2). The mechanism of gene expression is based on two properties; the first is the physical blocking of the translation process by the presence of the short double stranded region, secondly the presence of the RNA-DNA duplex is susceptible to cellular RNase H activity. RNase H cleaves the RNA-DNA duplex region of the mRNA thus preventing the faithful translation of the mRNA (3).

Oligonucleotide Design

The driving force for the search for novel chemical modification groups compatible with Watson-Crick hybridization of oligonucleotides was based on the observation of the short stability of naturally occurring oligonucleotides with phosphodiester bonds. Oligonucleotides with natural phosphodiester bonds are highly susceptible to rapid degradation by cellular nucleases. Cellular nucleases have endonuclease activity as well such that 3' and 5' end caps are not sufficient to prevent from degradation.

Modification of the phosphodiester bond by replacing one of the non-bridging oxygens by sulfur imparts resistance to nuclease degradation, but in general the oligo hybridizes to the target sequence with lesser affinity than the phosphodiester counter part.

Internucleotide Linkages & C5 Propyne Analogs
Natural diester linkage, Thioate (S-Oligo) linkage & C5 propyne analogs of dC and dT



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The sulfur-substituted oligonucleotides have a phosphorothioate linkage and are termed as **phosphorothioates** or simply as **S-oligos.** Phosphorothioate oligos are synthesized by Gene Link using the Beaucage (4) sulfurizing reagent. The sulfurization reaction is rapid and is performed on automated DNA synthesizers yielding greater than 96% phosphorothioate linkages, the remainder are phosphodiester linkages. Custom phosphorothioate oligonucleotides synthesized by Gene Link can be specified to have all the diester bonds substituted or only some selected diester linkages depending upon the researchers experimental requirement. Substitution of all diester linkages is recommended to provide greater nuclease resistance.

Recently it has been shown that C-5 propyne analogs of dC and dT when substituted in a phosphorothioate oligonucleotide impart greater inhibition of gene expression due to increased binding affinity to the target mRNA and increased stability (5).

Based on the above information antisense oligonucleotides could either be phosphorothioated at all diester linkages or combined with substitutions of dC and dT by C-5 propyne analogs pdC and pdU.

References

1. Milligan, J.F., Matteucci, M.D. and Martin, J.C. (1993) Current concepts in antisense drug design. J. Medicinal Chem. 36:1923-1937.
2. Helene, C., Toulme, J. (1990) Specific regulation of gene expression by antisense, sense and antigene nucleic acids. Biochim. Biophys. Acta. 1049: 99-125.
3. Weintraub, H. M. (1990) Antisense RNA and DNA. Sci. Amer. 262:40-46.
4. Iyer, R.P., Egan. W., Regan, J.B and Beaucage, S.L. (1990) J. Am. Chem. Soc.112; 1253-1254.
5. Wagner, R.W., Matteucci, M.D., Lewis, J.G., Gutierrez, A.J., Moulds, C. and Froehler, B.C. (1993) Antisense gene inhibition by oligonucleotides containing C-5 propyne pyrimidines. Science 260:1510-1513.

Custom Antisense Oligo Synthesis (S-oligo)*						
Product	**Catalog No.**	**50 nmol scale (XX=05)**	**200 nmol scale (XX=02)**	**1 µmol scale (XX=01)**	**10 µmol scale (XX=10)**	**15 µmol scale (XX=15)**
Phosphorothioates	26-6401-XX	3.50	4.25	6.50	50.00	65.00
Chimeric linkage	27-6420-00	75.00	75.00	75.00	75.00	75.00

*minimum charge for 15mer applies.

Purification						
Product	**Catalog No.**	**50 nmol scale (XX=05)**	**200 nmol scale (XX=02)**	**1 µmol scale (XX=01)**	**10 µmol scale (XX=10)**	**15 µmol scale (XX=15)**
Gel Purification	26-6400-XX	75.00	75.00	150.00	1500.00	1500.00
Reverse Phase Cartridge	26-6400-XX	30.00	30.00	90.00	750.00	750.00

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RNA & 2’0 METHYL OLIGONUCLEOTIDES

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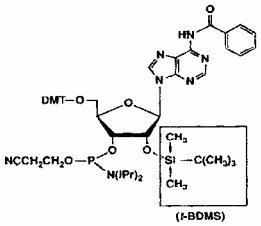
Custom RNA and 2' O Methyl Oligonucleotides

RNA and 2'-O-methyl RNA oligonucleotide synthesis is performed at Gene Link using the b–cyanoethyl chemistry and state-of-the-art synthesizers. These involve proprietary software protocols with long coupling times and specialized cycles to obtain ultra clean oligos.

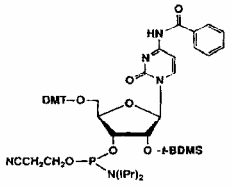
RNA oligos are susceptible to degradation to the same extent as native RNA extracted from various sources. An attractive alternate to prevent degradation from nucleases is the use of 2' O methyl RNA bases, when specific 2'OH is not required. The 2' O methyl oligonucleotides confer considerable nuclease resistance and are similar in hydrogen bonding properties to RNA/RNA than the lower RNA/DNA binding property. The coupling efficiency of 2' O methyl phosphoramidite is also higher than the RNA monomers resulting in higher yield of full-length oligos.

Gene Link also offers custom synthesis of RNA and DNA chimeric oligos with investigator specified ribo or deoxy bases or 2' O methyl bases. The chimeric oligos can also be synthesized with the regular phosphodiester bonds or substituted with phosphorothioate linkages. The combination of 2' O methyl RNA bases with phosphorothioate internucleotide linkages imparts these oligos greater nuclease resistance, which is particularly useful for antisense studies (please refer to our technical sheet on Antisense Oligonucleotides for other modifications). Custom phosphorothioate oligonucleotides synthesized by Gene Link can be specified to have all the diester bonds substituted or only some selected diester linkages depending upon the researchers experimental requirement. Substitution of all diester linkages is recommended to provide greater nuclease resistance.

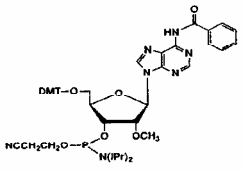
RNA and 2'-O-methyl phosphoramidite bases



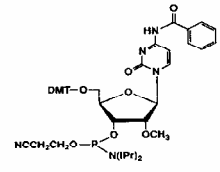
5'-DMT-Adenosine (Benzoyl) β-cyanoethyl Phosphoramidite



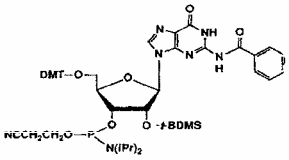
5'-DMT-Cytidine (Benzoyl) β-cyanoethyl Phosphoramidite



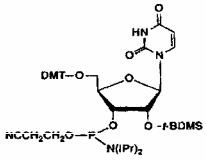
N⁶-Benzoyl-5'-O-DMT-2'-O-Methyl -Adenosine-3'-(β-cyanoethyl)-Phosphoramidite



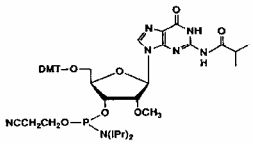
N⁴-Benzoyl-5'-O-DMT-2'-O-Methyl -Cytidine-3' -(β-cyanoethyl)-Phosphoramidite



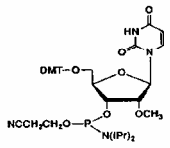
5'-DMT-Guanosine (Benzoyl) β-cyanoethyl Phosphoramidite



5'-DMT-Uridine β-cyanoethyl Phosphoramidite



N²-Isobutyryl-5'-O-DMT-2'-O-Methyl -Guanosine-3' -(β-cyanoethyl)-Phosphoramidite



5'-O-DMT 2'-O-Methyl-Uridine-3' -(β-cyanoethyl)-Phosphoramidite

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References

1. Goodwin,J.T., Stanick,W.A. and Glick,G.D. (1994) Improved solid phase synthesis of long oligoribonucleotides. Application to tRNA (phe) and tRNA(gly). J. Org. Chem. 59:7941-7943
2. Cotton,M., Oberhauser,B., Burnar, H. et al. (1991) 2'O methyl and 2'O ethyl oligoribonucleotides as inhibitors of the in vitro U7 snRNP-dependent messenger-RNA processing event. NAR 19:2629
3. Milligan, J.F., Matteucci, M.D. and Martin, J.C. (1993) Current concepts in antisense drug design. J. Medicinal Chem. 36:1923-1937.
4. Helene, C., Toulme, J. (1990) Specific regulation of gene expression by antisense, sense and antigene nucleic acids. Biochim. Biophys. Acta. 1049: 99-125.
5. Weintraub, H. M. (1990) Antisense RNA and DNA. Sci. Amer. 262:40-46.
6. Iyer, R.P., Egan. W., Regan, J.B and Beaucage, S.L. (1990) J. Am. Chem. Soc.112; 1253-1254.
7. Wagner, R.W., Matteucci, M.D., Lewis, J.G., Gutierrez, A.J., Moulds, C. and Froehler, B.C. (1993) Antisense gene inhibition by oligonucleotides containing C-5 propyne pyrimidines. Science 260:1510-1513.

RNA & 2'0 Methyl Oligonucleotides*			
Product	**Catalog No.**	**200 nmol scale (XX=02)**	**1 µmol scale (XX=01)**
RNA oligos	27-6400-XX	14.00	20.00
2'O methyl bases	27-6410-XX	14.00	20.00
DNA-RNA chimeric linkage	27-6420-00	75.00	75.00

*minimum charge for 20mer applies.

Purification						
Product	**Catalog No.**	**50 nmol scale (XX=05)**	**200 nmol scale (XX=02)**	**1 µmol scale (XX=01)**	**10 µmol scale (XX=10)**	**15 µmol scale (XX=15)**
Gel Purification	26-6400-XX	75.00	75.00	150.00	1500.00	1500.00
Reverse Phase Cartridge	26-6400-XX	30.00	30.00	90.00	750.00	750.00

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PHOSPHONATE OLIGONUCLEOTIDES

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Phosphonate Oligonucleotides

H-Phosphate Chemistry

H-phosphonate chemistry is of value when the internucleotide linkage required is other than the standard phosphodiester linkage. The H-phosphonate monomers shown below are used instead of the phosphoramidite bases. Using this method, the monomer that is able to be activated is a 5'-DMT-base-protected, nucleoside 3'-hydrogen phosphonate. The presence of the H-phosphonate moiety on these monomers renders phosphate protection unnecessary. The same base protecting groups are used in phosphite triester chemistry. The H-phosphate synthesis cycle is very similar to that of the phosphoramidite method. Slight differences result from the properties of the monomers utilized. For instance, a different activating agent is used. In addition, the H-phosphonate diesters generated by the coupling reactions are stable to the normal reaction conditions, so oxidation at every step is unnecessary. Instead, a single oxidation step can be performed at the end of the chain elongation. This single oxidation step makes it easy to produce modified DNA. For instance, if a sulfur-containing compound is used as the oxidizing agent, all of the internucleotide bonds will then contain sulfur instead if oxygen attached to the phosphorous atom. The most popular use of this chemistry is for the radioactive labeling of all phosphodiester linkages to phosphorothioate by using radioactive sulfur.

The Support

H-Phosphonate synthesis uses the same supports as does the ß-cyanoethyl phosphoramidite chemistry.

The Monomers

The H-Phosphonate monomers are protected at the 5'-hydroxyl position with dimethoxytrityl groups. The exocyclic amines on the bases are protected by the same groups as in the ß-cyanoethyl phosphoramidite analogues. The phosphonate group is attached to the 3' position.

Detritylation

This process is the same as in the ß-cyanoethyl phosphoramidite procedure.

Neutralization

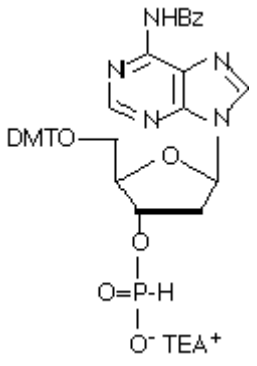
Residual DCA from the detritylation step is neutralized with a solution of pyridine in acetonitrile. This step also provides the proper solvent medium for the coupling reaction.

Coupling

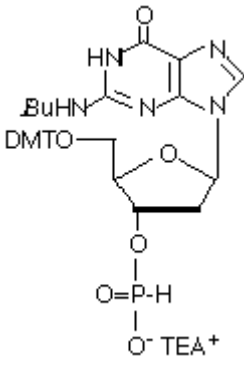
After detritylation and neutralization, the next protected H-phosphonate is delivered to the reaction column. Adamantoyl carboxyl acid chloride is used to activate the phosphonate by the addition of the adamantoyl moiety to the free oxygen of the H-phosphonate. The coupling mechanism is a nucleophilic attack by the free 5'-hydroxyl group on the phosphorous of the incoming activated monomer. For this reason, it is important that the environment be hydroxyl-free. The phosphonate diester linkages generated by this coupling are stable. Therefore, oxidation is performed at the end of the synthesis.

Cycling

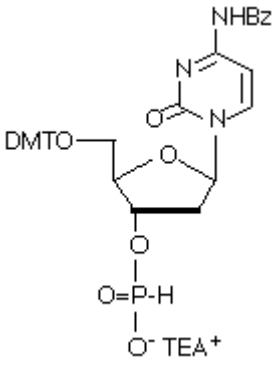
Following the coupling step, the cycle of reactions is repeated, beginning with the detritylation step, until chain elongation is complete.



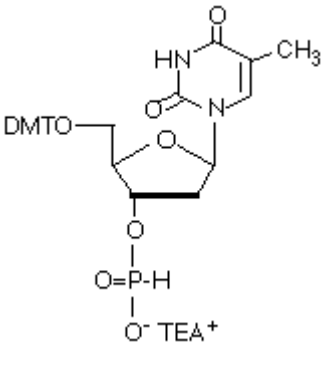
dA-H-Phosphonate



dG-H-Phosphonate



dC-H-Phosphonate



dT-H-Phosphonate

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Oxidation

The oxidation step is normally omitted during cyclic chain elongation and a single oxidation is performed later with the required oxidizer. In the oxidation step, the hydrogen atom that is bound to the phosphorous is replaced with an oxygen atom. Oxidation is accomplished with a mixture of two solutions. One solution contains iodine in tetrahydrofuran (THF), and the other N-methylmorpholine in water and THF. If required, the DNA can be modified at this step. For instance, phosphorothioates are generated by oxidizing with a sulfur-containing solution.

Final Detritylation

Regular detritylation cycle is used unless reverse phase purification is required. Final DMT should be kept 'ON' for purification by reverse phase cartridge or HPLC.

Purification

Purification by gel electrophoresis is recommended.

References:

1. Hall,R.H.,Todd,A.R., and Webb,R.F. (1957). Mixed anhydrides as intermediates in the synthesis of dinucleoside phosphates. J. Chem. Soc., 3291-3296.
2. Garegg,P.J., Regberg,T., Stawinski,J. and Stromberg,R. (1985). Formation of internucleotidic bonds via phosphonate intermediates. Chem. Scripta 25, 280-282.
3. Garegg,P.J., Regberg,T., Stawinski,J. and Stromberg,R. (1986). Nucleoside hydrogen phosphonates in oligonucleotide synthesis. Chem. Scripta 26, 59-62.
4. Garegg,P.J., Lidh,I.,Regberg,T., Stawinski,J. and Stromberg,R. (1986). Nucleoside H-phosphonates. Chemical synthesis of oligodeoxyribonucleotides by the hydrogen phosphonate approach. Tetrahedron Lett. 27, 4051-4054.
5. Froehler,B.C., Ng,P.G., and Matteucci,M.D. (1986). Synthesis of DNA via deoxynucleoside H-phosphonate intermediates. Nucleic Acids Res. 14, 5399-5407.
6. Froehler,B.C., and Matteucci,M.D. (1986). Nucleoside H-phosphonates : Valuable intermediates in the synthesis of oligonucleotides. Tetrahedron Lett. 27, 469-472.
7. Froehler,B.C. (1986). Nucleoside H-phosphonates : Deoxyoligonucleotide H-phosphonate diester intermediates in the synthesis of internucleotide phosphate analogues. Tetrahedron Lett. 27, 5575-5578.
8. Andrus,A.,Efcavitch,J.W.,McBride,L.T., and Giusti,B. (1988). Novel activating and capping reagents for improved hydrogen phosphonate DNA synthesis. Tetrahedron Lett. 29, 861-864.
9. Seliger,H. and Roesch,R. (1990). Simultaneous synthesis of multiple oligonucleotides using nucleoside H-phosphonate intermediates. DNA and Cell Biol., 9, 691-696.
10. Stawinski,J.,Stromberg,R.,Thelin,M., and Westman,E.. (1988). Studies on the t-butyldimethyl-silyl group as 2'-O-protection in oligoribonucleotide synthesis via the H-phosphonate approach. Nucl. Acids Res. 16, 9285-9288.
11. Stein,C.A.,Iversen,C.A.,Subashinge,C.,Cohen,J.S.,Stec,W.J. and Zon,G. (1990). Preparation of 35S-labeled polyphosphorothioate oligodeoxyribonucleotides by the use of H-phosphonate chemistry. Analytical Biochem. 188, 11-16.

Phosphonate Oligonucleotides*				
Product	**Catalog No**	**50 nmol scale (XX=05)**	**200 nmol scale (XX=02)**	**1 µmol scale (XX=01)**
Phosphonate Oligos	26-6600-01	na	na	40.00/base
Methyl Phosphonamidite	26-6510-XX	90.00	90.00	190.00

*minimum charge for 20mer applies.

Purification						
Product	**Catalog No.**	**50 nmol scale (XX=05)**	**200 nmol scale (XX=02)**	**1 µmol scale (XX=01)**	**10 µmol scale (XX=10)**	**15 µmol scale (XX=15)**
Gel Purification	26-6400-XX	75.00	75.00	150.00	1500.00	1500.00
Reverse Phase Cartridge	26-6400-XX	30.00	30.00	90.00	750.00	750.00

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MODIFICATIONS

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Custom Spiking & Custom Column (3' base spiking / mix)

Custom spiking is the addition of differing molar concentrations of bases at a single position. This is different from degeneracy at a position based on codons. Codon based degeneracy is usually equimolar concentration of each base at the same position (done at no extra charge for all internal and 5' position, see order form for single letter IUB codes). Custom spiking (example, 10% A, 75% G, 5% C & 10% T) has to be specified as required on the order form.

A custom column has to be prepared when the degeneracy and custom spiking is at the 3' position.

Custom Spiking & Custom Columns						
Product	**Catalog No.**	**50 nmol scale (XX=05)**	**200 nmol scale (XX=02)**	**1 µmol scale (XX=01)**	**10 µmol scale (XX=10)**	**15 µmol scale (XX=15)**
Custom spiking	26-6200-XX	35.00	35.00	50.00	60.00	60.00
Custom column	26-6201-XX	40.00	40.00	50.00	60.00	60.00

Minor Bases

Duplex stability and, therefore, melting temperature can be raised using the modified bases. For replacement of dC with 5-Me-dC, duplex melting temperature is increased by 1.3 º. Improved stacking in this case is believed to be brought about by elimination of water molecules from the duplex. The additional hydrogen bond formed by substituting dA with 2-amino-dA leads to a melting temperature increase of 3 º.

The design of primers is frequently complicated by the degeneracy of the genetic code. Three strategies are now available to confront this problem. In the first, a mixed base addition (N) is used to form the degenerate site. This approach is best if the number of degenerate sites is small. A second option is the use of 2'-deoxyInosine or 2'-deoxyNebularine, which exhibit low, but unequal, hydrogen bonding to the other four bases. The third option is the use of a universal nucleoside. In this strategy, the base analog does not hybridize significantly to the other four bases and makes up some of the duplex destabilization by acting as an intercalating agent. 3-Nitropyrrole 2'-deoxynucleoside (M) is the first example of a set of universal bases. Subsequently, 5-nitroindole was determined to be an effective universal base and to be superior to 3-nitropyrrole, based on duplex melting experiments.

The modified bases designated P and K show considerable promise as degenerate bases. The pyrimidine derivative P, when introduced into oligonucleotides, base pairs with either A or G, while the purine derivative K base pairs with either C or T. A dP+dK mix also can serve as a mixed base with much less degeneracy than dA+dC+dG+dT (N).

A standard Watson and Crick base pair is formed between iso-C and iso-G, but the hydrogen bonding pattern is quite different from the natural base pairs A-T and C-G (The 5-methyl analogue was chosen as the synthetic target due to the reported instability of 2'-deoxyisocytidine caused by deamination during oligonucleotide synthesis or deprotection).

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Minor Bases						
Product	Catalog No.	50 nmol scale (XX=05)	200 nmol scale (XX=02)	1 µmol scale (XX=01)	10 µmol scale (XX=10)	15 µmol scale (XX=15)
Deoxyinosine (dI),	26-6403-XX	25.00	25.00	55.00	440.00	550.00
Deoxyuridine (dU)	26-6408-XX	25.00	25.00	55.00	440.00	550.00
5-Br-dC	26-6411-XX	75.00	75.00	125.00	1000.00	1250.00
5 methyl dC	26-6413-XX	75.00	75.00	125.00	1000.00	1250.00
5 iodo dC	26-6414-XX	75.00	75.00	125.00	1000.00	1250.00
5 iodo dU	26-6415-XX	75.00	75.00	125.00	1000.00	1250.00
5 fluoro dU	26-6416-XX	75.00	75.00	125.00	1000.00	1250.00
O6 Me d-guanine	26-6409-XX	220.00	220.00	275.00	2200.00	2750.00
8-Oxoguanine	26-6434-XX	350.00	350.00	400.00	3200.00	4000.00
Amino dT-C6	26-6438-XX	320.00	320.00	390.00	3120.00	3900.00
2,6 diaminopurine	26-6504-XX	250.00	250.00	300.00	2400.00	3000.00
2-Aminopurine	26-6505-XX	200.00	200.00	250.00	2000.00	2500.00
Etheno dA	26-6506-XX	210.00	210.00	260.00	2080.00	2600.00
5-me iso dC	26-6513-XX	250.00	250.00	550.00	4400.00	5500.00
Iso dG	26-6514-XX	450.00	450.00	550.00	4400.00	5500.00
N6 Methyl dA	26-6601-XX	200.00	200.00	350.00	2800.00	3500.00
O4 Me d-thymine	26-6410-XX	250.00	250.00	300.00	2400.00	3000.00
2-F-U	26-6462-XX	250.00	250.00	310.00	2480.00	3100.00
2-F-C	26-6463-XX	250.00	250.00	310.00	2480.00	3100.00
8 BrdG	26-6464-XX	115.00	115.00	175.00	1400.00	1750.00
8 BrdA	26-6465-XX	115.00	115.00	175.00	1400.00	1750.00
Abasic modification	26-6471-XX	210.00	210.00	325.00	2600.00	3250.00
5-Br-dC	26-6411-XX	75.00	75.00	125.00	1000.00	1250.00
5-Br-dU	26-6412-XX	55.00	55.00	125.00	1000.00	1250.00
5-Me-dC	26-6413-XX	55.00	75.00	125.00	1000.00	1250.00
5-I-dC	26-6414-XX	75.00	75.00	125.00	1000.00	1250.00
5-I-dU	26-6415-XX	75.00	75.00	125.00	1000.00	1250.00
5-F-dU	26-6416-XX	75.00	75.00	125.00	1000.00	1250.00
Puromycin	26-6603-XX	75.00	75.00	125.00	1000.00	1250.00
Psoralen	26-6604-XX	250.00	250.00	325.00	2600.00	3250.00
Cholesterol	26-6602-XX	150.00	150.00	300.00	2400.00	3000.00
6 Thio-dG-CE Phosphoramidite	26-6417-XX	55.00	55.00	110.00	880.00	1100.00

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Spacers

Spacers are used to insert a spacer arm into an oligonucleotide. The compounds 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. PC Spacer is a photocleavable C3 spacer modifier.

Spacers						
Product	Catalog No.	50 nmol scale (XX=05)	200 nmol scale (XX=02)	1 µmol scale (XX=01)	10 µmol scale (XX=10)	15 µmol scale (XX=15)
5' amino linker C3	26-6466-XX	55.00	55.00	110.00	880.00	1100.00
Amino linker C6	26-6418-XX	55.00	55.00	110.00	880.00	1100.00
5' amino linker C12	26-6420-XX	55.00	55.00	110.00	880.00	1100.00
3' amino linker C3	26-6405-XX	55.00	55.00	110.00	880.00	1100.00
Amino linker C6	26-6418-XX	55.00	55.00	110.00	880.00	1100.00
3' amino linker C7	26-6406-XX	55.00	55.00	110.00	880.00	1100.00
Spacer C3	26-6439-XX	50.00	10.00	200.00	1600.00	2000.00
Spacer C12	26-6441-XX	150.00	150.00	200.00	1600.00	2000.00
Spacer C9	26-6440-XX	150.00	150.00	200.00	1600.00	2000.00
Spacer C18	26-6447-XX	150.00	150.00	200.00	1600.00	2000.00
Spacer dexoyribose	26-6442-XX	150.00	150.00	200.00	1600.00	2000.00
dSpacer	26-6435-XX	150.00	150.00	200.00	1600.00	2000.00

Linkers

Linkers are used to insert a spacer arm into an oligonucleotide. The compounds 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. PC Spacer is a photocleavable C3 spacer modifier.

Linkers						
Product	Catalog No.	50 nmol scale (XX=05)	200 nmol scale (XX=02)	1 µmol scale (XX=01)	10 µmol scale (XX=10)	15 µmol scale (XX=15)
5' amino linker C3	26-6466-XX	55.00	55.00	110.00	880.00	1100.00
5' amino linker C6	26-6418-XX	55.00	55.00	110.00	880.00	1100.00
5' amino linker C12	26-6420-XX	55.00	55.00	110.00	880.00	1100.00
3' amino linker C3	26-6405-XX	55.00	55.00	110.00	880.00	1100.00
3' amino linker C6	26-6418-XX	55.00	55.00	110.00	880.00	1100.00
3' amino linker C7	26-6406-XX	55.00	55.00	110.00	880.00	1100.00
Spacer C3	26-6439-XX	150.00	150.00	200.00	1600.00	2000.00
Spacer C12	26-6441-XX	150.00	150.00	200.00	1600.00	2000.00
Spacer C9	26-6440-XX	150.00	150.00	200.00	1600.00	2000.00
Spacer C18	26-6447-XX	150.00	150.00	200.00	1600.00	2000.00

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End Blockers

Gene Link end blockers are designed for use in DNA synthesizers to functionalize the 5′ or 3′ terminus of the target oligonucleotide. The 5′ Amino Linkers are available with a variety of chain lengths to exactly suit the desired application.

End Blockers						
Product	Catalog No.	50 nmol scale (XX=05)	200 nmol scale (XX=02)	1 µmol scale (XX=01)	10 µmol scale (XX=10)	15 µmol scale (XX=15)
5′ biotinylation	26-6423-XX	90.00	90.00	190.00	1520.00	1900.00
3′ biotinylation	26-6404-XX	90.00	90.00	190.00	1520.00	1900.00
Biotin-TEG	26-6407-XX	250.00	250.00	350.00	2800.00	3500.00
5′ amino linker C3	26-6466-XX	55.00	55.00	110.00	880.00	1100.00
5′ amino linker C6	26-6418-XX	55.00	55.00	110.00	880.00	1100.00
5′ amino linker C12	26-6420-XX	55.00	55.00	110.00	880.00	1100.00
3′ amino linker C3	26-6405-XX	55.00	55.00	110.00	880.00	1100.00
3′ amino linker C6	26-6418-XX	55.00	55.00	110.00	880.00	1100.00
3′ amino linker C7	26-6406-XX	55.00	55.00	110.00	880.00	1100.00
Spacer C3	26-6439-XX	150.00	150.00	200.00	1600.00	2000.00
Spacer C9	26-6440-XX	150.00	150.00	200.00	1600.00	2000.00
Spacer C12	26-6441-XX	150.00	150.00	200.00	1600.00	2000.00
Spacer C18	26-6447-XX	150.00	150.00	200.00	1600.00	2000.00
Dideoxy dC	26-6337-XX	150.00	150.00	200.00	1600.00	2000.00

End Modifiers

All modifications can be broadly termed as end modifiers as these modify the 5′ or 3′ end. This section contains a list of modifications that are used to modify the ends for conjugation to other modifications or to solid surfaces including magnetic beads. The end modifiers add a reactive functional group which can be used for conjugation. The common functional groups that can be added are amino, carboxyl and thiol. All of these can be combined with different length of spacers (C3, C12, Spacer 9 and 18) to add physical distance of the functional group from the end of the oligonucleotide sequence.

End Modifiers						
Product	Catalog No.	50 nmol scale (XX=05)	200 nmol scale (XX=02)	1 µmol scale (XX=01)	10 µmol scale (XX=10)	15 µmol scale (XX=15)
5′ biotinylation	26-6423-XX	90.00	90.00	190.00	1520.00	1900.00
3′ biotinylation	26-6404-XX	90.00	90.00	190.00	1520.00	1900.00
Biotin-TEG	26-6407-XX	250.00	250.00	350.00	2800.00	3500.00
5′ phosphorylation	26-6428-XX	55.00	55.00	110.00	880.00	1100.00
3′ phosphorylation	26-6427-XX	55.00	55.00	110.00	880.00	1100.00
5′ amino linker C3	26-6466-XX	55.00	55.00	110.00	880.00	1100.00
5′ amino linker C6	26-6418-XX	55.00	55.00	110.00	880.00	1100.00
5′ amino linker C12	26-6420-XX	55.00	55.00	110.00	880.00	1100.00
3′ amino linker C3	26-6405-XX	55.00	55.00	110.00	880.00	1100.00
3′ amino linker C6	26-6418-XX	55.00	55.00	110.00	880.00	1100.00
3′ amino linker C7	26-6406-XX	55.00	55.00	110.00	880.00	1100.00

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Spacer C3	26-6439-XX	150.00	150.00	200.00	1600.00	2000.00
Spacer C12	26-6441-XX	150.00	150.00	200.00	1600.00	2000.00
Spacer C9	26-6440-XX	150.00	150.00	200.00	1600.00	2000.00
Spacer C18	26-6447-XX	150.00	150.00	200.00	1600.00	2000.00
Spacer dexoyribose	26-6435-XX	150.00	150.00	200.00	1600.00	2000.00
Digoxigenin	26-6429-XX	275.00	275.00	350.00	2800.00	3500.00
Alkaline Phosphatase	26-6511-XX	550.00	550.00	1200.00	9600.00	12000.00
Dabcyl	26-6470-XX	290.00	290.00	350.00	2800.00	3500.00
Dabcyl dT	26-6446-XX	310.00	310.00	390.00	3120.00	3900.00
5' fluorescein	26-6426-XX	90.00	90.00	190.00	1520.00	1900.00
3' fluorescein	26-6425-XX	90.00	90.00	190.00	1520.00	1900.00
TET	26-6433-XX	150.00	150.00	200.00	1600.00	2000.00
HEX	26-6432-XX	150.00	150.00	200.00	1600.00	2000.00
6-FAM	26-6431-XX	150.00	150.00	200.00	1600.00	2000.00
ROX	26-6430-XX	250.00	250.00	325.00	2600.00	3250.00
TAMRA	26-6450-XX	250.00	250.00	325.00	2600.00	3250.00
Cy3	26-6437-XX	250.00	250.00	325.00	2600.00	3250.00
Cy3.5	26-6461-XX	250.00	250.00	325.00	2600.00	3250.00
Cy5	26-6436-XX	250.00	250.00	325.00	2600.00	3250.00
Cy5.5	26-6460-XX	250.00	250.00	325.00	2600.00	3250.00
Cy7	26-6474-XX	250.00	250.00	325.00	2600.00	3250.00

Affinity Ligands

Oligonucletides 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.

Besides 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. The biotinylated oligonucleotide can be bound to a streptavidin matrix and used for either column or spin chromatography.

** Roche holds exclusive rights to digoxigenin labeling. Digoxigenin oligo labeling is offered under license from Roche. Extensive digoxigenin labeling techniques and detection methods are available from Roche.*

Affinity Ligands						
Product	**Catalog No.**	**50 nmol scale (XX=05)**	**200 nmol scale (XX=02)**	**1 µmol scale (XX=01)**	**10 µmol scale (XX=10)**	**15 µmol scale (XX=15)**
Biotin dT	26-6424-XX	220.00	220.00	310.00	2480.00	3100.00
Biotin multi	26-6421-XX	220.00	220.00	310.00	2480.00	3100.00
5' biotinylation	26-6423-XX	90.00	90.00	190.00	1520.00	1900.00
3' biotinylation	26-6404-XX	90.00	90.00	190.00	1520.00	1900.00
Biotin-TEG	26-6407-XX	250.00	250.00	350.00	2800.00	3500.00
Digoxigenin	26-6429-XX	275.00	275.00	350.00	2800.00	3500.00
Alkaline Phosphatase	26-6511-XX	550.00	550.00	1200.00	9600.00	12000.00

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Photo Cleavable Monomers

PC Biotin Phosphoramidite exhibits similar properties to our popular 5'-biotin phosphoramidite.

1. It is fully compatible with all forms of DNA synthesis, cleavage and deprotection.
2. It contains a DMT group, which allows quantification of the coupling efficiency when removed on the synthesizer, or it can be used as a purification tag in the DMT-ON purification technique.
3. As an alternative to DMT-ON purification, simple capture of the PC Biotin oligo effects purification from failure sequences, which contain no biotin.
4. PC Biotin is rapidly and quantitatively cleaved from the 5'-terminus of the oligonucleotide using near-UV light at 300 þ 350nm.
5. After photocleavage, a 5'-phosphate is generated on the DNA, rendering it suitable for further biological transformations, like gene construction and cloning after ligation.

Amino- and thiol-modified oligonucleotides have proven to be very useful for the attachment of a variety of haptens and fluorophores, as well as for the tethering of the oligonucleotides to a diversity of beads and surfaces. Clearly, the addition of a photocleavable linkage to these products would be desirable. PC Amino-Modifier Phosphoramidite brings the same versatility as PC Biotin to its field of endeavor. For the final word in versatility, PC Spacer Phosphoramidite can be used as an intermediary to attach any modification reagent, available as a phosphoramidite, to the terminus of an oligonucleotide. And, as always in the PC family, subsequent photocleavage is fast and efficient, providing DNA appropriate for further biological determination or transformation.

Photo Cleavable Monomers						
Product	**Catalog No.**	**50 nmol scale (XX=05)**	**200 nmol scale (XX=02)**	**1 µmol scale (XX=01)**	**10 µmol scale (XX=10)**	**15 µmol scale (XX=15)**
PC Biotin phosphoramidite	26-64XX-XX	220.00	220.00	310.00	2480.00	3100.00
PC Amino phosphoramidite	26-6423-XX	220.00	220.00	310.00	2480.00	3100.00
PC Spacer phosphoramidite	26-6404-XX	220.00	220.00	310.00	2480.00	3100.00
PC Linker phosphoramidite	26-64XX-XX	270.00	270.00	380.00	3040.00	3800.00

Fluorescent Modifications

The use of fluorescent dyes in molecular biology has rapidly transformed from just single dye labeled primers for fragment analysis to the use of double labeled dyes and quenchers as probes for quantitative analysis. Fluorescence based detection offers a safe and sensitive method for quantitative detection. This also means that Molecular Biologists have to understand new terms like donors, acceptors, quenchers, FRET, Stokes shift etc. The molecular basis of some of the probe's designs is simply elegant and thus has led to an exponential use of molecular probes and consequently furthering new developments. It is essential to understand the basic concepts of fluorescence.

Gene Link offers synthesis of all different forms of molecular probes. We provide technical service in the design of novel probes and have synthesized numerous combinations of dyes, quenchers, RNA, phosphorothioate, 2'O methyl and chimeric probes.

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Fluorescent Modifications						
Product	Catalog No.	50 nmol scale (XX=05)	200 nmol scale (XX=02)	1 µmol scale (XX=01)	10 µmol scale (XX=10)	15 µmol scale (XX=15)
5′ fluorescein	26-6426-XX	90.00	90.00	190.00	1520.00	1900.00
3′ fluorescein	26-6425-XX	90.00	90.00	190.00	1520.00	1900.00
Fluorescein dT	26-6422-XX	250.00	250.00	390.00	3120.00	3900.00
TET	26-6433-XX	150.00	150.00	200.00	1600.00	2000.00
HEX	26-6432-XX	150.00	$150.00	200.00	1600.00	2000.00
6-FAM	26-6431-XX	150.00	$150.00	200.00	1600.00	2000.00
ROX	26-6430-XX	250.00	250.00	325.00	2600.00	3250.00
5′ TAMRA	26-6450-XX	250.00	250.00	325.00	2600.00	3250.00
3′ TAMRA	26-6451-XX	250.00	250.00	325.00	2600.00	3250.00
Tamra dT	26-6449-XX	350.00	350.00	450.00	3600.00	4500.00
JOE	26-6467-XX	350.00	350.00	450.00	3600.00	4500.00
Coumarin NHS	26-6483-XX	190.00	190.00	250.00	2000.00	2500.00
Cy3	26-6437-XX	250.00	250.00	325.00	2600.00	3250.00
Cy3.5	26-6461-XX	250.00	250.00	325.00	2600.00	3250.00
Cy5	26-6436-XX	250.00	250.00	325.00	2600.00	3250.00
Cy5.5	26-6460-XX	250.00	250.00	325.00	2600.00	3250.00
Cy7	26-6474-XX	250.00	250.00	325.00	2600.00	3250.00
Alexa Fluor 350	26-6477-XX	410.00	410.00	460.00	3680.00	4600.00
Alexa Fluor 405	26-6485-XX	410.00	410.00	460.00	3680.00	4600.00
Alexa Fluor 430	26-6486-XX	410.00	410.00	460.00	3680.00	4600.00
Alexa Fluor 488	26-6448-XX	410.00	410.00	460.00	3680.00	4600.00
Alexa Fluor 532	26-6487-XX	410.00	410.00	460.00	3680.00	4600.00
Alexa Fluor 546	26-6488-XX	410.00	410.00	460.00	3680.00	4600.00
Alexa Fluor 555	26-6489-XX	410.00	410.00	460.00	3680.00	4600.00
Alexa Fluor 568	26-6490-XX	410.00	410.00	460.00	3680.00	4600.00
Alexa Fluor 594	26-6491-XX	410.00	410.00	460.00	3680.00	4600.00
Alexa Fluor 633	26-6478-XX	410.00	410.00	460.00	3680.00	4600.00
Alexa Fluor 647	26-6479-XX	410.00	410.00	460.00	3680.00	4600.00
Alexa Fluor 660	26-6492-XX	410.00	410.00	460.00	3680.00	4600.00
Alexa Fluor 680	26-6480-XX	410.00	410.00	460.00	3680.00	4600.00
Alexa Fluor 700	26-6481-XX	410.00	410.00	460.00	3680.00	4600.00
Alexa Fluor 750	26-6484-XX	410.00	410.00	460.00	3680.00	4600.00
Cascade Blue	26-6493-XX	350.00	350.00	450.00	3600.00	4500.00
Marina Blue	26-6494-XX	350.00	350.00	450.00	3600.00	4500.00
Oregon Green 500	26-6495-XX	350.00	350.00	450.00	3600.00	4500.00
Oregon Green 514	26-6496-XX	350.00	350.00	450.00	3600.00	4500.00
Oregon Green 488	26-6507-XX	350.00	350.00	450.00	3600.00	4500.00
Oregon Green 488X	26-6508-XX	350.00	350.00	450.00	3600.00	4500.00
Pacific Blue	26-6509-XX	350.00	350.00	450.00	3600.00	4500.00
Rhodamine Green	26-6515-XX	350.00	350.00	450.00	3600.00	4500.00
Rhodol Green	26-6516-XX	350.00	350.00	450.00	3600.00	4500.00

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Rhodamine Green X	26-6517-XX	350.00	350.00	450.00	3600.00	4500.00
Rhodamine Red X	26-6518-XX	350.00	350.00	450.00	3600.00	4500.00
Texas Red X	26-6469-XX	350.00	350.00	450.00	3600.00	4500.00
DNP (2,4-dinitrophenyl)	26-6512-XX	350.00	350.00	450.00	3600.00	4500.00
Dabcyl (Quencher)	26-6470-XX	350.00	350.00	450.00	3600.00	4500.00
Dabcyl dT	26-6446-XX	350.00	350.00	400.00	3200.00	4000.00
BHQ-1* (Quencher)	26-6472-XX	390.00	390.00	500.00	4000.00	5000.00
BHQ- 2* (Quencher)	26-6468-XX	390.00	390.00	500.00	4000.00	5000.00
BHQ-3* (Quencher)	26-6473-XX	390.00	390.00	500.00	4000.00	5000.00

Dye-Labeled Oligonucleotide Excitation and Emission Spectra

Dye	Color	Absorbance max (nm)	Emission max (nm)	Extinction Coefficient
6-FAM (Fluorescein)	Green	494	525	74850
TET	Orange	521	536	85553
HEX	Pink	535	556	95698
Cy 5	Violet	646	667	250000
Cy 5.5	Blue	683	707	190000
Cy 3	Red	552	570	150000
Cy 3.5	Purple	588	604	150000
Cy7	Near IR	743	767	200000
Tamra	Rose	565	580	87000
ROX	Purple	587	607	105000
JOE	Mustard	528	554	105000
Alexa Dye Series	Varies	Varies	Varies	Varies

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FLUORESCENT MOLECULAR PROBES

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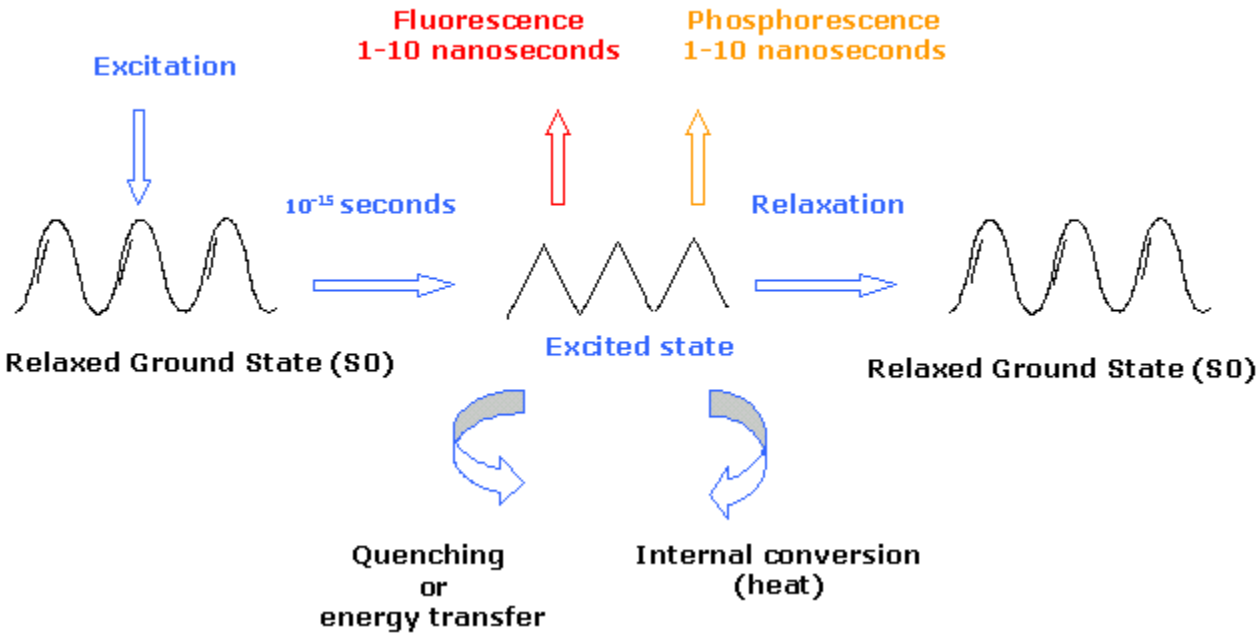
The use of fluorescent dyes in molecular biology has rapidly transformed from just single dye labeled primers for fragment analysis to the use of double labeled dyes and quenchers as probes for quantitative analysis. Fluorescence based detection offers a safe and sensitive method for quantitative detection. This also means that Molecular Biologists have to understand new terms like donors, acceptors, quenchers, FRET, Stokes shift etc. The molecular basis of some of the probe's designs is simply elegant and thus has led to an exponential use of molecular probes and consequently furthering new developments. It is essential to understand the basic concepts of fluorescence.

Gene Link offers synthesis of all different forms of molecular probes. We provide technical service in the design of novel probes and have synthesized numerous combinations of dyes, quenchers, RNA, phosphorothioate, 2'O methyl and chimeric probes.

Luminescence, Phosphorescence & Fluorescence Defined

Molecules absorb energy as photons of light are shined on them. The property to absorb photons of light depends on the atomic configuration of the molecules, leading to an excited state of energy level. The energy that is absorbed can be translated into rotational, vibrational or electronic modes. The exact fate of the energy depends on the wavelength of the incoming light. The longer the wavelength, the lower the energy. The vibrational and rotational energy levels are closer together than the electronic levels. Thus changes in these levels are often associated with the absorption of infrared radiation and release of energy as heat. However, light in the visible and UV regions of the spectrum has enough energy to cause changes in the electronic states of a molecule without a relative elevation of temperature.

- Luminescence — The release of energy as light at low temperature is termed as luminescence. The molecules emitting light are relatively cool. It is in contrast to light bulbs, fire etc. Luminescence is a general term not limited to the duration of the emitted light.
- Phosphorescence — Delayed luminescence after absorption of energy. The wavelength of the released light energy is at a different wavelength and it continues for an extended period of time, even after excitation has ceased.
- Fluorescence — Instantaneous luminescence after absorption of energy. The fluorescence is usually at a different wavelength and it ceases almost at once when excitation has ceased.



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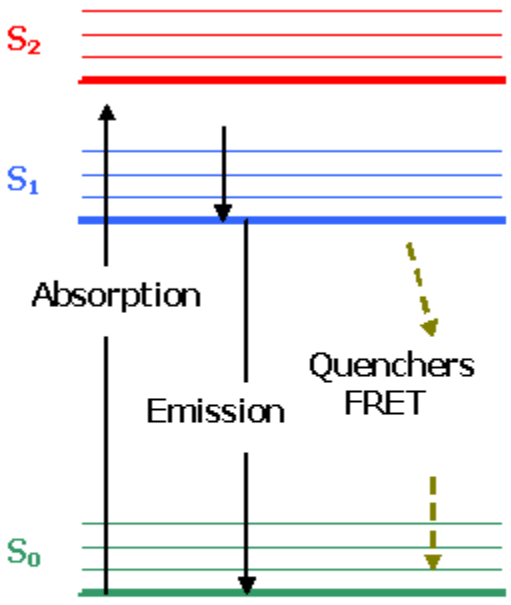
Excitation and Emission

The excitation level of molecules varies at different wavelengths. Molecules exposed to a beam of light absorb more at a particular wavelength. This specific wavelength is termed as the Excitation Maxima. The emission maxima is the wavelength at which the maximum amount of light is released. The molecule stays in the excited state for a finite time, usually <1-10 nanoseconds and returns to the relaxed state upon emission of energy. The lifetime is measured in nanoseconds, e.g. the lifetime of Cy3 is <0.3ns and Cy5 is 1.0ns. Excitation and Emission is a cyclic process and consequently can be repeated to an extent before it starts to fade, termed as photobleaching.

We will now focus our discussion referring to fluorescent dyes used for molecular probes and primer design. The popular ones in use are Fluorescein, 6-FAM (6-fluorescein amidite), HEX (hexachloro fluorescein), TET (tetrachloro fluorescein), all fluorescein derivatives, Cy series dyes, the Alexa series dyes and rhodamine derivatives. The dyes are selected based on the excitation and emission wavelengths, bleaching, quenching and various other biophysical factors. All dyes when excited undergo a conformational change based on the interaction with other molecules, bases and dyes in close proximity. The emission is thus bound by these variables of the microenvironment interaction. The amount of emission will vary and not all the energy consumed and absorbed during excitation is released as fluorescence.

The excitation time is usually in picoseconds to nanoseconds; Similarly, the emission time is measured in nanoseconds. The energy used for excitation elevates the dye from a stable and relaxed state, termed as S_0 to S_1-S_2. The transition from S_2 to S_1 is without any release of light energy. The photons emitted from the excited S_1 state on its return to the relaxed S_0 state are not equal to the difference between the excitation and emission maxima. This is termed as the Stokes shift. The Stokes shift represents the energy lost while the molecule was in the excited state.



Absorption of a photon and excitation to S_1 or S_2. Radiationless energy loss and return to S_1. Return to S_0 from S_1 with emission of fluorescence or by energy transfer to quenchers or other acceptor dye (FRET).

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Quenching

Reduction in the expected fluorescence emission is termed as quenching. Generally, it would be an impediment if the emission was reduced. The phenomenon of quenching forms the basis of the mode of action of molecular probes; the designed and controlled fluorescence based on hybridization to the target sequence.

Natural quenching occurs due to 'fading' after repeated cycles of excitation and relaxation. The decrease in the ability of further excitation of a proportion of molecules is termed as photobleaching. Some dyes are much more sensitive than others to photobleaching, for example fluorescein photobleaches very easily. Often the rate of decomposition is proportional to the intensity of illumination. So a simple practical way to overcome this is to reduce the incident radiation. It is sometimes possible to introduce antioxidants such as phenylalanine or azide to reduce bleaching. Quenching is also observed when the concentration of the dye is too high and the overall brightness decreases. This is 'self quenching'. It is observed that multiple labeling of an oligo with the same dye does not always lead to an increase in fluorescence.

Placing a molecule that absorbs light in close proximity to the fluorophore can induce quenching. The quenching effect is exhibited by fluorescent as well non-fluorescent molecules. A non-fluorescent quencher is the basis of the design of Molecular Beacons. This molecule could be non-fluorescent and acts as energy sink, and termed as a quencher. In other instances this molecule could itself be a dye with overlapping spectral absorption and emission spectra, in such cases energy is transferred from one to another without any emission of light energy. This is termed as resonance energy transfer. Generally, the term 'quencher' is used for non-fluorescent molecules in probe design and 'double dye' or 'dual dye' used for probes with two dyes with spectral overlap.

Fluorescence Resonance Energy Transfer (FRET)

Resonance energy transfer, often known as fluorescence resonance energy transfer (FRET) or Förster energy transfer. It is the radiationless transfer of excitation energy from a donor to an acceptor. An important consequence of this transfer is that there is no emission of light by the donor. The acceptor may or may not be fluorescent. FRET is a distance-dependent interaction where the energy transfer occurs typically over a distance of 1-10 nm. The distance-dependent nature of FRET is highlighted by the fact that it is proportional to the inverse sixth power of the intermolecular separation. The fact that FRET typically occurs in the 1-10 nm region means that these separation distances are comparable with the dimensions of biological macromolecules. This means that FRET can be a valuable tool in studying proximity events in biological systems.

FRET varies based on the degree of spectral overlap of the donor and acceptor. That is the degree to which the emission band of the donor and the absorption band of the acceptor overlap. This is called the "spectral overlap" or sometimes the "Förster overlap integral". This describes the amount of overlap where resonance can occur, i.e. where the donor and acceptor have the same frequencies.

There are many other applications and consequences arising from these equations. For example if the donor and acceptor are the same molecular species it is still possible to observe FRET. This is called homotransfer and could be thought of as energy migration. The obvious conclusion from this is that the observed fluorescence would not be changed. However, at high concentration of dye it is possible to observe concentration quenching. In this case the transfer of the energy does not result in emission, the explanation for this is that the transfer is occurring at a less than critical distance and some of the dyes are acting as energy sinks (Source: Amersham Biosciences Website).

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. Recent designs substitute the 3' TAMRA fluorescent acceptor quencher dye with non-fluorescent quencher, e.g. Black Hole Quencher. The proximity of the quencher to the reporter in an intact probe allows the quencher to suppress, or "quench" the fluorescence signal of the reporter dye through FRET. If the target of interest is present, these probes specifically anneal between the forward and reverse primer sites. During the reaction, the 5' to 3' nucleolytic activity of Taq polymerase cleaves the probe between the reporter and the quencher only if the probe hybridizes to the target. The probe fragments are displaced from the target, separating the reporter dye from the quencher dye and thus resulting in increased fluorescence of the reporter. Accumulation of PCR products is detected directly by monitoring the increase in fluorescence of the reporter dye. Because increase in fluorescence signal is detected only if the target sequence is complementary to the probe, nonspecific amplification is not detected.

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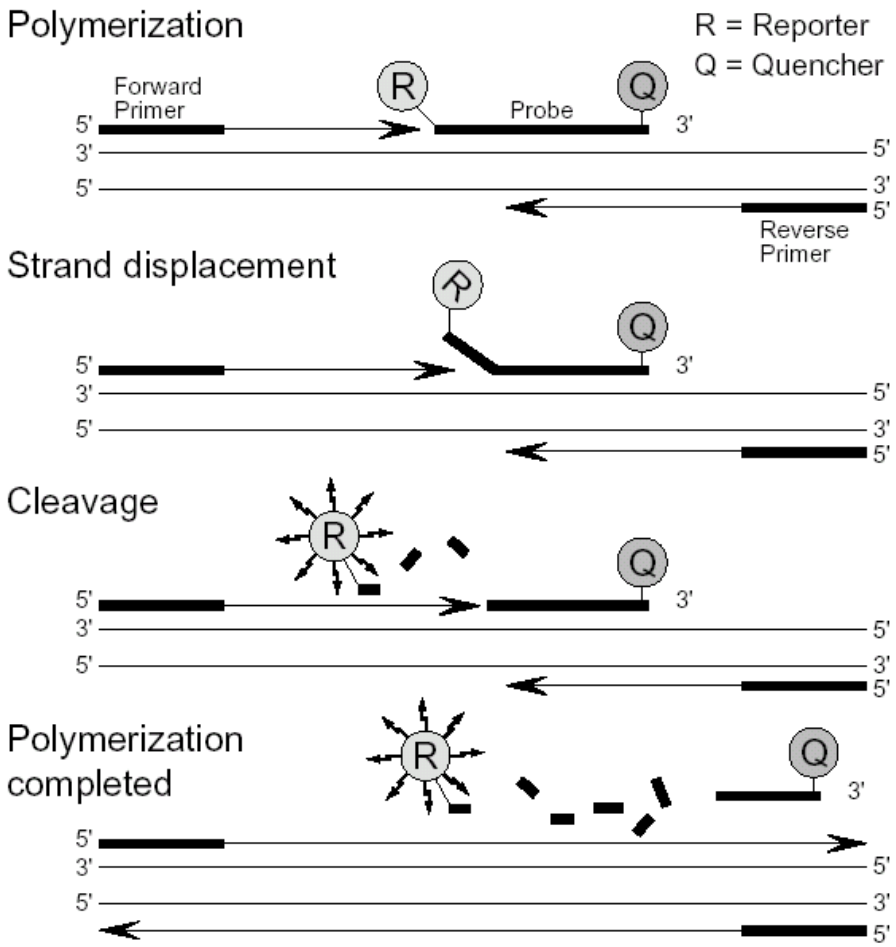

Design of TaqMan Primers and Probes

Some guidelines for TaqMan probes and primers selection are as follows:

- G-C content between 20% and 80%
- Avoid runs of an identical nucleotide, especially guanine
- Avoid G to be on the 5' end
- Probes and primers should contain more C than G
- Melting temperature (Tm) should be 68-70 °C for probes and 58-60 °C for primers
- The five nucleotides at the 3' end of each primer should have no more than two Gs and/or Cs
- Give precedence to better probes over primers
- Probe should be as close to 5' primer as possible without overlapping

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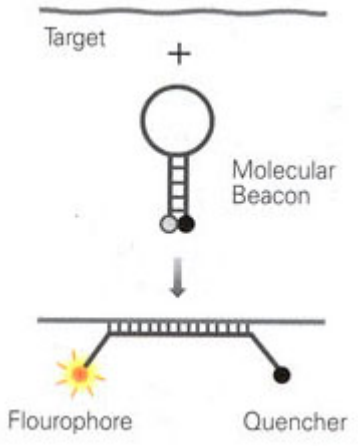
Molecular Beacons

Molecular beacons are oligonucleotide probes that can report the presence of specific nucleic acids in homogenous solutions (Tyagi S, Kramer FR. Molecular beacons: probes that fluoresce upon hybridization, Nature Biotechnology 1996; 14: 303-308.) They are useful in situations where it is either not possible or desirable to isolate the probe-target hybrids from an excess of the hybridization probes, such as in real-time monitoring of polymerase chain reactions in sealed tubes or in detection of RNAs within living cells. Molecular beacons are hairpin-shaped molecules with an internally quenched fluorophore whose fluorescence is restored when they bind to a target nucleic acid (Figure 1). They are designed in such a way that the loop portion of the molecule is a probe sequence complementary to a target nucleic acid molecule. The stem is formed by the annealing of complementary arm sequences on the ends of the probe sequence. A fluorescent moiety is attached to the end of one arm and a quenching moiety is attached to the end of the other arm. The stem keeps these two moieties in close proximity to each other, causing the fluorescence of the fluorophore to be quenched by energy transfer. Since the quencher moiety is a non-fluorescent chromophore and emits the energy that it receives from the fluorophore as heat, the probe is unable to fluoresce. When the probe encounters a target molecule, it forms a hybrid that is longer and more stable than the stem and its rigidity and length preclude the simultaneous existence of the stem hybrid. Thus, the molecular beacon undergoes a spontaneous conformational reorganization that forces the stem apart, and causes the fluorophore and the quencher to move away from each other, leading to the restoration of fluorescence.



Operation of molecular beacons: On their own, these molecules are non-fluorescent, because the stem hybrid keeps the fluorophore close to the quencher. When the probe sequence in the loop hybridizes to its target, forming a rigid double helix, a conformational reorganization occurs that separates the quencher from the fluorophore, restoring fluorescence.

In order to detect multiple targets in the same solution, molecular beacons can be made in many different colors utilizing a broad range of fluorophores (Tyagi S, Bratu DP, Kramer FR. Multicolor molecular beacons for allele discrimination, Nature Biotechnology 1998; 16: 49-53.) DABCYL, a non-fluorescent chromophore, serves as the universal quencher for any fluorophore in molecular beacons. Owing to their stem, the recognition of targets by molecular beacons is so specific that single-nucleotide differences can be readily detected.

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Molecular Beacon Example Sequence

Fluorophore at 5' end; 5'-GCGAGCTAGGAAACACCAAAGATGATATTTGCTCGC -3'-DABCYL,

```
5' GCGAGCTAGGAAACACCA
   ||||||             ]
3' CGCTCGTTTATAGTAGAA
```
STEM AT 1 IS 6 BP LONG. LOOP = 24.

The vertical lines at the 5' and 3' ends identify the arm sequences that are complementary.

The length of the probe sequence (10-40 nt) is chosen in such a way that the probe target hybrid is stable in the conditions of the assay. The stem sequence (5-7 nt) is chosen to ensure that the two arms hybridize to each other but not to the probe sequence. Folding of the designed sequence with the help of a computer program can indicate whether the intended stem-and-loop conformation will occur. The computer program can also predict the melting temperature of the stem.

Signal to background ratio

1. Determine the fluorescence (F_{buffer}) of 200 µl of molecular beacon buffer solution using 491 nm as the excitation wavelength and 515 as the emission wavelength. If the fluorophore is not fluorescein, choose wavelengths that are optimal for the fluorophore in the molecular beacon.
2. Add 10 µl of 1 µM molecular beacon to this solution. Record the new level of fluorescence (F_{close})
3. Add a two-fold molar excess of the oligonucleotide target and monitor the rise in fluorescence until it reaches a stable level (F_{open}).
4. Calculate the signal to background ratio as $(F_{open}-F_{buffer})/(F_{close}-F_{buffer})$.

Thermal denaturation profiles

1. Prepare two tubes containing 50 µl of 200 nM molecular beacon dissolved in 3.5 mM $MgCl_2$ and 10 mM Tris-HCl, pH 8.0 and add the oligo target to one of the tubes at a final concentration of 400 nM.
2. Determine the fluorescence of each solution as a function of temperature using a thermal cycler with the capacity to monitor fluorescence. Decrease the temperature of these tubes from 80 °C to 10 °C in 1 °C steps, with each hold lasting one minute, while monitoring the fluorescence during each hold.

Real time monitoring of polymerase chain reactions

Utilize molecular beacons that are complementary to a sequence in the middle of the expected amplified target fragment. The length of their arm sequences should be chosen so that a stem is formed at the annealing temperature of the polymerase chain reaction. The length of the loop sequence should be chosen so that the probe-target hybrid is stable at the annealing temperature. Whether a molecular beacon actually exhibits these designed features is determined by obtaining thermal denaturation profiles. The molecular beacons with appropriate thermal denaturation characteristics are included in each reaction at a concentration similar to the concentration of the primers. During the denaturation step, the molecular beacons assume a random coil configuration and fluoresce. As the temperature is lowered to allow annealing of the primers, stem hybrids form rapidly, preventing fluorescence. However, at the annealing temperature, molecular beacons also bind to the amplified target fragment and generate fluorescence. When the temperature is raised to allow primer extension, the molecular beacons dissociate from their targets and do not interfere with polymerization. A new hybridization takes place in the annealing step of every cycle, and the intensity of the resulting fluorescence indicates the amount of accumulated amplified target fragment.

Procedure
1. Set up six 50 µl reactions so that each contains a different number of targets, 0.34 µM molecular beacon, 1 µM of each primer, 2.5 units of Taq polymerase, 0.25 mM of each deoxyribonucleotide, 3.5 mM $MgCl_2$, 50 mM KCl, and 10 mM Tris-HCl, pH 8.0.
2. Program the thermal cycler to incubate the tubes at 95 °C for 10 minutes to activate Amplitaq Gold DNA polymerase, followed by 40 cycles of 30 seconds at 95 °C, 60 seconds at 50 °C and 30 seconds at 72 °C. Monitor fluorescence during the 50 °C annealing steps.

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Troubleshooting

- The assay medium may contain insufficient salt. There should be at least 1 mM $MgCl_2$ in the solution, in order to ensure that the stem hybrid forms.

- The molecular beacon may fold into an alternate conformation that results in a sub-population that is not quenched well. Change the stem sequence (and probe sequence, if necessary) to eliminate the possibility of incomplete restoration of fluorescence at low temperatures.

- If the stem of a molecular beacon is too strong, at low temperatures it may remain closed while the probe is bound to the target. This may happen inadvertently if the probe sequence can participate in the formation of a hairpin that results in a stem longer and stronger than originally designed. Change the sequence at the edges of the probe and the stem sequence to avoid this problem.

Molecular Beacon Design

The PCR primers themselves should have been optimized in a regular PCR to see that it performs well. Assuming the melting temperature of the primers are ~55 °C.

There are two independent features to control in the design of the MB probe, the stem and the target loop sequence. Design the probe sequence and see that there is minimal secondary structures, loop formation and dimers and the Tm is ~5 ° higher than the PCR primer annealing temperature. For a good guideline keep it at ~60 °C. Add the stem sequence of 5-7 bp. The Tm of the stem itself will be ~ 60-70 °C. You are done!

General guidelines for MB design at Gene Link are as follows:

1. Design regular 18-24mer PCR primers for amplification with a Tm around 55 ^{0}C. The optimal amplified fragment should be between 100-300 bp. Perform PCR, optimize conditions and you should obtain clean amplification product visible on ethidium bromide stained gels.

2. Design target probe sequence with a Tm ~8-10 ^{0} higher than the PCR primers annealing temperature, example 60-65 ^{0}C. The probe should be designed near the center of the amplified fragment avoiding stretches of strong secondary structure. [Taqman probes are designed ~ 5-10 bases near the primer of the same strand]

3. Add the stem 5- 7 bp stem sequence with a GC content of 70-80%. Avoid a G at the 5' end next to the fluorophore. G's seem to quench. Hairpin Stem Tm should be 7-10 ^{0} higher than the PCR annealing temperature, example 65-70 ^{0}C.

Caution: See that by adding the stem you have not created secondary structures with the loop sequence. Try variation of the stem sequence to avoid secondary structure with the loop sequence.

The Hairpin stem Tm is based on free energy stabilization and folding. The following is a good guideline:

GC rich stem
5 bp = 55 ^{0}C- 60 ^{0}C
6 bp = 60 ^{0}C-65 ^{0}C
7 bp = 65 ^{0}C-70 ^{0}C

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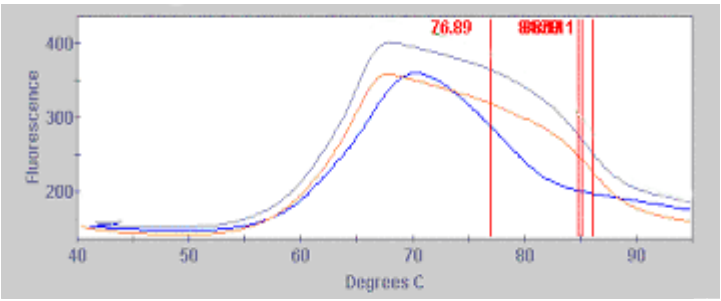
Gene Link Molecular Beacon Melt Curve Protocol

1. Prepare Molecular Beacon stock solution at 100 pmols/µl [100 µM (micromolar)] in 1 X PCR Buffer. Gene Link provides the exact amount of nmols of each oligo supplied on the tube and on the Oligo Report. Multiply the 'nmol' amount by 10 to arrive at the volume of solvent to be added.
2. Prepare Molecular Beacon working solution at 5 pmols/µl [5 µM (micromolar)] in 1 X PCR Buffer.
3. Set up two 25 µl reactions, one with probe alone, one with target + probe as follows:

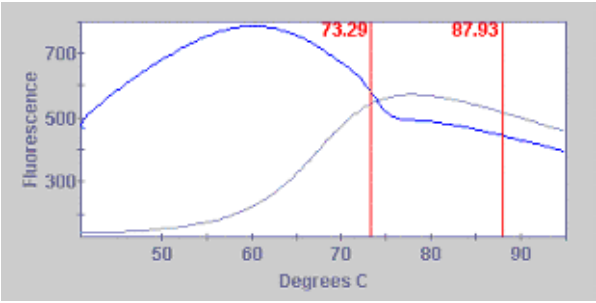
Molecular Beacon Probe Alone	*Molecular Beacon Probe plus Target*
2.5 µl 10X PCR buffer	2.5 µl 10x PCR buffer
3 µl 25 mM $MgCl_2$	3 µl 25 mM $MgCl_2$
1 µl 5 pmol/ul probe [0.2 pmol/µl final or 200 nM]	1 µl 5 pmol/ul probe [0.2 pmol/µl final or 200 nM]
	3 µl 5 pmol/ul target [0.6 pmol/µl final or 600 nM]
18.5 µl H_20	15.5 µl H_2O

The protocol for Molecular Beacon Melt Curve ramps from 40 to 95 °C at 0.2 °/second

Screen capture of graphs from a Cepheid SmartCycler



Multiple Molecular Beacon Probe Alone Melt Curve



Molecular Beacon Probe plus Target and Probe Alone Melt Curve

Notes:
-$MgCl_2$ needs to be higher for MB reactions than for regular PCR as it helps to stabilize the stem structure of the probe during the high ramp rate. Final concentration of $MgCl_2$ should be between 2.5 and 4 mM. Here we use 3 mM final. This is the same range of concentration used in an actual amplification reaction.
-Final concentration of probe should be 200-600 nM. Here we use 200 nM. This is also the same concentration range used for the real time reaction.
-For a melt curve it is important to saturate the probe with target. Use 2-3 X the *Molar* amount of target. Here we use 3 X target for a final concentration of 600 nM. For real time monitoring, 500 ng genomic DNA, diluted 10X to various concentrations can be used as a starting point.

QPCR
Once you have your melt curve you want to select an annealing temperature for your real time PCR where the probe alone is completely closed (shows no fluorescence), and the probe+target is completely open (shows maximal fluorescence). This temperature should be about 5-8 ° below the Tm of the probe/target hybrid (red vertical line on melt curve of probe+target). It is important to test your primers at the annealing temperature to ensure that you will have strong, clean amplification at this temperature.

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In a small-scale synthesis (eg. ~5-10 nmol; 2000-5000 assays), what is the amount of fluorophore-conjugated oligonucleotide typically obtained after purification?
The smallest scale of synthesis offered by Gene Link for Molecular Beacons is at the 200 nmol scale. Synthesis at the lower scale does NOT appreciably reduce cost, as cost of synthesis is NOT directly proportional to the scale of synthesis. Whereas, the higher scale affords Gene Link the ability to comfortably purify the product by polyacrylamide gel, and thus provide our customers with a product of the highest quality. **Gene Link provides between 7-15 nmols of purified product at the 200 nmols scale synthesis, which is sufficient for 2000-5000 assays.**

How are these purified (eg. PAGE or HPLC)?
Answered partly above. Gene Link performs and strongly recommends gel purification and believes it to be the "gold standard" of purification. All other forms of purification depend on either hydrophobic (Reverse Phase HPLC) or ionic affinity (Ion-exchange HPLC); both of these techniques are sequence-dependent and do not yield the purity achieved by gel purification. Gene Link provides a before-and-after gel picture, of the crude and the gel purified oligo.

Is there mass spectroscopic confirmation of the purified product?
Not applicable. Gene Link performs gel purification and supplies a real gel picture. Molecular Beacons provided by Gene Link should have better than 50:1 signal to background ratio, usually in the range of 200. The purity is greater than 98%.

Do beacons undergo reverse complement (i.e. target) verification and is a close to open ratio analysis performed?
Gene Link currently does not perform these analyses as they are considered as "after the fact". These analyses do NOT constitute quality of oligo synthesis but rather of design of oligonucleotide. Customers designing their probes should keep the parameters in mind. Gene Link can guide customers in the design of the stem, the loop and the Tm. A good initial reading of Fred Kramer's articles suffices the need, just as designing oligos for PCR, sequencing etc.

What fluorophores are available for conjugation?
The most popular ones are FAM, Fluorescein, HEX and TET, Cy5 and Cy3 in that order; others available as post synthesis conjugation are Alexa Series Dyes, Tamra, Texas Red, Coumarin, etc.

What quenchers are available for conjugation?
In additional to Dabcyl, BHQ-1, BHQ-2 and BHQ-3 are also available.

How quickly and in what form are beacons shipped?
Gel purified Beacons are routinely shipped in 4-5 business days. These are shipped lyophilized ready to use.

What type of guarantees could be provided in the event of an inferior or incorrect beacon synthesis (eg. free replacement)?
All Gene Link products are replaced at no charge for not meeting specifications. This rarely happens. This means free replacement for the same sequence. We usually suggest designing a new probe/sequence or to avoid stretches of G's and C's etc.

Would a free beacon sample be available for testing by our laboratory?
Yes. New customers are offered "Buy One, Get One Free" of the same fluorophore.

Would there be on-going technical support in the form of updating us on new developments in beacon synthesis?
Yes. We get to know our customers and talk frequently. The latest development is the wavelength shift, incorporating a different fluorophore in the stem in addition to the 5' end. This is similar to FRET.

Gene Link is not an "oligo factory." We believe as scientists and researchers that each oligo/probe/gene construction/genotyping etc. is an experiment to be well performed. We believe in quality, consistency and gaining confidence ourselves in our products, and ultimately gaining our customer's total confidence in Gene Link products. We hope we have answered most of the frequently asked questions. Please feel free to call (1-800-436-3546) or email (techsupport@genelink.com) if you require more information.

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Common Fluorophore Absorbance and Emission Data

Dye	Color	Absorbance max (nm)	Emission max (nm)	Extinction Coefficient
6-FAM (Fluorescein)	Green	494	525	74850
TET	Orange	521	536	85553
HEX	Pink	535	556	95698
Cy 5	Violet	646	667	250000
Cy 5.5	Blue	683	707	190000
Cy 3	Red	552	570	150000
Cy 3.5	Purple	588	604	150000
Cy 7	Near IR	743	767	200000
Tamra	Rose	565	580	87000
ROX	Purple	587	607	105000
JOE	Mustard	528	554	105000
Alexa Dye Series	Varies	Varies	Varies	Varies

Other Molecular Probes Fluorescent Dyes

Dye	Color	Absorbance max (nm)	Emission max (nm)	Extinction Coefficient
Cascade Blue	Blue	396	410	29000
Marina Blue	Blue	362	459	19000
Oregon Green 500	Green	499	519	78000
Oregon Green 514	Green	506	526	85000
Oregon Green 488	Green	495	521	76000
Oregon Green 488-X	Green	494	517	84000
Pacific Blue	Blue	416	451	36000
Rhodamine Green	Green	504	532	78000
Rhodol Green	Green	496	523	63000
Rhodamine Green-X	Green	503	528	74000
Rhodamine Red-X	Red	560	580	129000
Texas Red-X	Red	583	603	136000

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Alexa Dye Series

Dye	Color	Absorbance max (nm)	Emission max (nm)	Extinction Coefficient
Alexa Fluor 350	Blue	346	442	19000
Alexa Fluor 405	Green	401	421	34000
Alexa Fluor 430	Green	433	541	16000
Alexa Fluor 488	Green	495	519	71000
Alexa Fluor 532	Yellow	532	553	81000
Alexa Fluor 546	Red	556	573	104,00
Alexa Fluor 555	Red	555	565	150000
Alexa Fluor 568	Red	578	603	91000
Alexa Fluor 594	Red	590	617	73000
Alexa Fluor 633	Violet	632	647*	100000
Alexa Fluor 647	Violet	650	665*	239000
Alexa Fluor 660	Purple	663	690*	123000
Alexa Fluor 680	Blue	679	702*	184000
Alexa Fluor 700	Near IR	702	723*	192000
Alexa Fluor 750	Near IR	749	775*	240000

Spectral characteristics of the Alexa Fluor dyes. Extinction coefficient at l max in cm -1m-1.
*Human vision is insensitive to light beyond~650nm, and therefore it is not possible to view the far-red fluorescent dyes by looking through the eyepiece of a conventional fluorescent microscope. Source: Molecular Probes www.probes.com

Email support@genelink.com or call 1-800-GENE LINK for more technical information.

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*PHRI Molecular Beacon License Agreement
"This product is sold under license from the Public Health Research Institute. It may be used under PHRI Patent Rights only for the purchaser's research and development activities".

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Fluorescent Molecular Probes*					
Fluorophore*	Color	Absorbance max (nm)	Emission max (nm)	200 nmol scale	1 µmol scale
Dabcyl (Quencher)		453		290.00	350.00
BHQ-1** (Quencher)		534		290.00	350.00
BHQ-2** (Quencher)		579		290.00	350.00
BHQ-3** (Quencher)		672		290.00	350.00
6-FAM (Fluorescein)	Green	494	525	390.00	450.00
TET	Orange	521	536	390.00	450.00
HEX	Pink	535	556	390.00	450.00
Cy 7	Near IR	743	767	490.00	550.00
Cy 5	Violet	643	667	490.00	550.00
Cy 5.5	Blue	683	707	490.00	550.00
Cy 3	Red	552	570	490.00	550.00
Cy 3.5	Purple	588	604	490.00	550.00
Tetramethylrhodamine (TAMRA)	Rose	565	580	540.00	600.00
Alexa Fluors	Varies	Varies	Varies	540.00	600.00

Fluorescent Modifications

The use of fluorescent dyes in molecular biology has rapidly transformed from just single dye labeled primers for fragment analysis to the use of double labeled dyes and quenchers as probes for quantitative analysis. Fluorescence based detection offers a safe and sensitive method for quantitative detection. This also means that Molecular Biologists have to understand new terms like donors, acceptors, quenchers, FRET, Stokes shift etc. The molecular basis of some of the probe's designs are simply elegant and thus has led to an exponential use of molecular probes and consequently furthering new developments. It is essential to understand the basic concepts of fluorescence.

Gene Link offers synthesis of all different forms of molecular probes. We provide technical service in the design of novel probes and have synthesized numerous combinations of dyes, quenchers, RNA, phosphorothioate, 2'O methyl and chimeric probes.

Fluorescent Modifications						
Product	Catalog No.	50 nmol scale (XX=05)	200 nmol scale (XX=02)	1 µmol scale (XX=01)	10 µmol scale (XX=10)	15 µmol scale (XX=15)
5' fluorescein	26-6426-XX	90.00	90.00	190.00	1520.00	1900.00
3' fluorescein	26-6425-XX	90.00	90.00	190.00	1520.00	1900.00
Fluorescein dT	26-6422-XX	250.00	250.00	390.00	3120.00	3900.00
TET	26-6433-XX	150.00	150.00	200.00	1600.00	2000.00
HEX	26-6432-XX	150.00	$150.00	200.00	1600.00	2000.00
6-FAM	26-6431-XX	150.00	$150.00	200.00	1600.00	2000.00
ROX	26-6430-XX	250.00	250.00	325.00	2600.00	3250.00
5' TAMRA	26-6450-XX	250.00	250.00	325.00	2600.00	3250.00
3' TAMRA	26-6451-XX	250.00	250.00	325.00	2600.00	3250.00
Tamra dT	26-6449-XX	350.00	350.00	450.00	3600.00	4500.00
JOE	26-6467-XX	350.00	350.00	450.00	3600.00	4500.00
Coumarin NHS	26-6483-XX	190.00	190.00	250.00	2000.00	2500.00
Cy3	26-6437-XX	250.00	250.00	325.00	2600.00	3250.00

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Cy3.5	26-6461-XX	250.00	250.00	325.00	2600.00	3250.00
Cy5	26-6436-XX	250.00	250.00	325.00	2600.00	3250.00
Cy5.5	26-6460-XX	250.00	250.00	325.00	2600.00	3250.00
Cy7	26-6474-XX	250.00	250.00	325.00	2600.00	3250.00
Alexa Fluor 350	26-6477-XX	410.00	410.00	460.00	3680.00	4600.00
Alexa Fluor 405	26-6485-XX	410.00	410.00	460.00	3680.00	4600.00
Alexa Fluor 430	26-6486-XX	410.00	410.00	460.00	3680.00	4600.00
Alexa Fluor 488	26-6448-XX	410.00	410.00	460.00	3680.00	4600.00
Alexa Fluor 532	26-6487-XX	410.00	410.00	460.00	3680.00	4600.00
Alexa Fluor 546	26-6488-XX	410.00	410.00	460.00	3680.00	4600.00
Alexa Fluor 555	26-6489-XX	410.00	410.00	460.00	3680.00	4600.00
Alexa Fluor 568	26-6490-XX	410.00	410.00	460.00	3680.00	4600.00
Alexa Fluor 594	26-6491-XX	410.00	410.00	460.00	3680.00	4600.00
Alexa Fluor 633	26-6478-XX	410.00	410.00	460.00	3680.00	4600.00
Alexa Fluor 647	26-6479-XX	410.00	410.00	460.00	3680.00	4600.00
Alexa Fluor 660	26-6492-XX	410.00	410.00	460.00	3680.00	4600.00
Alexa Fluor 680	26-6480-XX	410.00	410.00	460.00	3680.00	4600.00
Alexa Fluor 700	26-6481-XX	410.00	410.00	460.00	3680.00	4600.00
Alexa Fluor 750	26-6484-XX	410.00	410.00	460.00	3680.00	4600.00
Cascade Blue	26-6493-XX	350.00	350.00	450.00	3600.00	4500.00
Marina Blue	26-6494-XX	350.00	350.00	450.00	3600.00	4500.00
Oregon Green 500	26-6495-XX	350.00	350.00	450.00	3600.00	4500.00
Oregon Green 514	26-6496-XX	350.00	350.00	450.00	3600.00	4500.00
Oregon Green 488	26-6507-XX	350.00	350.00	450.00	3600.00	4500.00
Oregon Green 488X	26-6508-XX	350.00	350.00	450.00	3600.00	4500.00
Pacific Blue	26-6509-XX	350.00	350.00	450.00	3600.00	4500.00
Rhodamine Green	26-6515-XX	350.00	350.00	450.00	3600.00	4500.00
Rhodol Green	26-6516-XX	350.00	350.00	450.00	3600.00	4500.00
Rhodamine Green X	26-6517-XX	350.00	350.00	450.00	3600.00	4500.00
Rhodamine Red X	26-6518-XX	350.00	350.00	450.00	3600.00	4500.00
Texas Red X	26-6469-XX	350.00	350.00	450.00	3600.00	4500.00
DNP (2,4-dinitrophenyl)	26-6512-XX	350.00	350.00	450.00	3600.00	4500.00
Dabcyl (Quencher)	26-6470-XX	350.00	350.00	450.00	3600.00	4500.00
Dabcyl dT	26-6446-XX	350.00	350.00	400.00	3200.00	4000.00
BHQ-1* (Quencher)	26-6472-XX	390.00	390.00	500.00	4000.00	5000.00
BHQ- 2* (Quencher)	26-6468-XX	390.00	390.00	500.00	4000.00	5000.00
BHQ-3* (Quencher)	26-6473-XX	390.00	390.00	500.00	4000.00	5000.00

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RNA INTERFERENCE / siRNA

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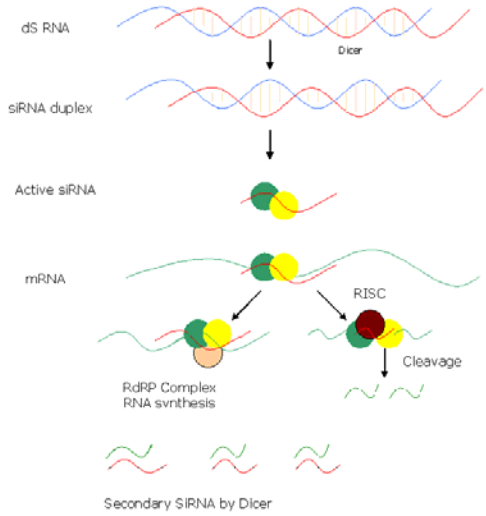
RNAi Explorer™

RNAi Explorer™ from Gene Link is a series of product 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. After selection of appropriate siRNA or shRNA, you can place an order. You are encouraged to try the robust search and design algorithm.

How does RNAi work?

Recent advances in molecular biology have shown that gene expression can be effectively silenced in a highly specific manner through the addition of double stranded RNA (dsRNA) (1-3). The term RNA interference (RNAi) was coined to describe this phenomenon and, while the mechanism was originally observed in plants and later in the worm *Caenorhabditis elegans*, subsequent studies have shown that RNAi is present in a wide variety of eukaryotic organisms including mammals (4-6). For the most part, it is believed that RNAi serves as an antiviral defense mechanism although there is preliminary evidence that it also plays a role in the formation and maintenance of heterochromatin during mitosis and meiosis (7,8).



Once dsRNA enters the cell, it is cleaved by an RNase III–like enzyme, Dicer, into double stranded small interfering RNAs (siRNA) 21-23 nucleotides in length that contain 2 nucleotide overhangs on the 3' ends (9-11). In an ATP dependent step, the siRNAs become integrated into a multi-subunit protein complex, commonly known as the RNAi induced silencing complex (RISC), which guides the siRNAs to the target RNA sequence (12). At some point the siRNA duplex unwinds, and it appears that the antisense strand remains bound to RISC and directs degradation of the complementary mRNA sequence by a combination of endo- and exonucleases (13).

Preliminary studies in mammalian systems using long dsRNAs to initiate the RNAi response failed because they led to the induction of a non-specific Type I interferon response that produced extensive changes in protein expression and eventually resulted in cell death (14,15). Subsequent studies, however, using synthetic, short double-stranded RNAs that mimic the siRNAs produced by the enzyme dicer, sequence specific gene silencing could be achieved in mammalian cells without inducing the interferon response (6,16). siRNA technology is now extensively recognized as a powerful tool for the specific suppression of gene expression and is presently being used by researchers in a wide range of disciplines for the assessment of gene function.

Guaranteed RNAi Explorer™ Kit

3+1 siRNAs and a detection probe. You simply give us the accession number or sequence for the gene to down regulate. Gene Link designs siRNAs using latest published criteria's and proprietary algorithms. Three target and a negative control siRNA will be supplied and a detection probe of your choice. This kit contains the following.

1. 3 siRNA duplexes 10 nmols each of 19+2 dT 3' overhangs. They are supplied lyophilized, ready to dissolve and use. Detailed position and sequence information is provided in the order report.

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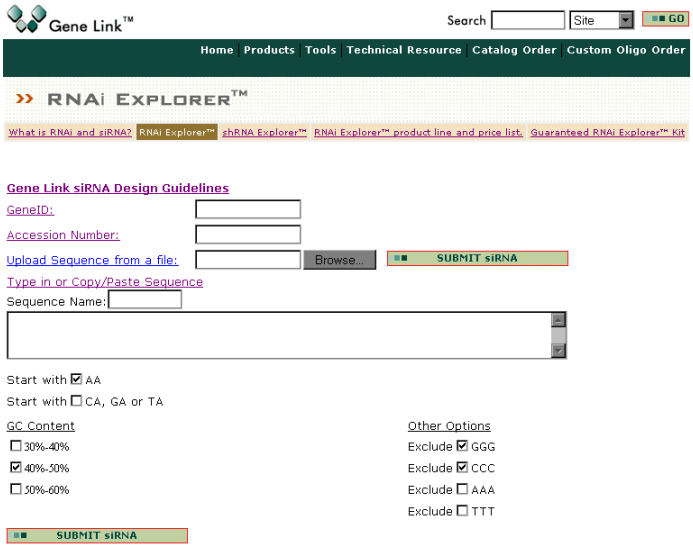
2. 1 negative control non-silencing siRNA duplex; 5 nmols supplied lyophilized, ready to dissolve and use.
3. Choice of detection probe or 6 custom oligos up to 30 mer if requested at time of order.

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.



How are effective siRNA designed?

siRNA Design Strategies:

Default parameters: AA(19mer)

The default criteria selects target sequences of 21 nucleotides that begin with AA and are located within a region of the coding sequence that is within 50-100 nucleotides of the AUG start codon and within 50-100 nucleotides from the termination codon. The presence of AA at the start of the sequence allows for the use of dTdT at the 3'-end of the antisense sequence. The sense strand can also be synthesized with dTdT at the 3' end, because only the antisense strand is involved in target recognition. The use of dTdT reduces the cost of synthesis and also makes the siRNA duplex more resistant to exonuclease activity. Because a number of reports have demonstrated that the presence of AA at the beginning of the target sequence is not an absolute requirement, the selection program includes the option to search for sequences that begin with other nucleotide pairs.

GC Content

The G-C content of the sequence is also used as a condition for selecting target sequences. Ideally the GC content will be less than 50%, although successful gene silencing has been reported with siRNAs that have G-C contents between 50 and 60%. The default parameter selects for a G-C content in the 40-50% range, however, options are available that allow for selection over wider ranges.

Stretches of Nucleotide Repeats

The default mode avoids sequences with repeats of three or more G's or C's, as their presence initiates intra-molecular secondary structures preventing effective siRNA silencing hybridization. As an option, repeat stretches of A's and T's can also be eliminated, as they tend to reduce the specificity of the target sequence. If possible, this option is highly recommended.

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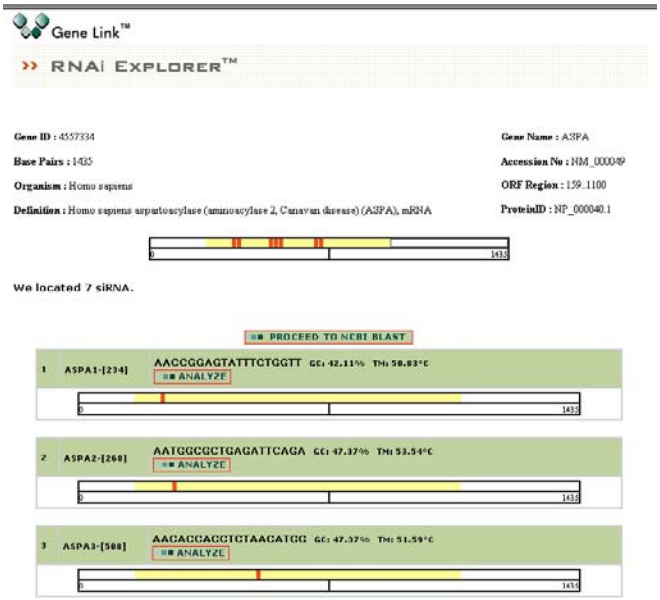
Blast Search
Once a target sequence has been chosen, a BLAST search is initiated to ensure that your target sequence is not homologous to other gene sequences. Target sequences that have more than 15 contiguous bases pairs of homology to other genes in the NCBI database are eliminated.



Why use fluorescent and modified siRNA's?

It is not essential to monitor the subcellular localization of siRNA after transfection. When desired, the siRNA can be labeled with fluorescent dyes to track the delivery and uptake of siRNA. Usually after uptake the siRNA are present free in the cytoplasm and in complex formation with proteins in the endosomes.

The siRNA can be modified with various other modifications like 2'O methyl RNA, biotin or digoxigenin based on the researcher's need. All of these modifications are available from Gene Link.

What are my siRNA delivery options?

Delivery of siRNA directly in cells can be achieved by using microinjection or electroporation. Another popular option is the use of transfection reagents. Several companies offer specialized siRNA-delivery reagents. Please consult the transfection reagent vendor's protocol for detailed information for the exact requirements and procedure. Careful optimization of variable factors should be ensured for all initial transfection experiments. It is based on this and further optimization that reproducible gene knock out results will be obtained. Usually the RNAi effect is seen within 4 hours and the maximum down regulation observed in 24-48 hrs. The effect lasts several cell generations and from 4-10 days depending on cell culture type.

How does purity of synthetic siRNAs affect RNAi?

RNAi is a sequence-specific chain of events. Chemical synthesis of siRNAs is based on coupling of bases to yield a particular sequence. The yield and purity depends on the coupling efficiency. Gene Link siRNAs can be used without further purification, but Gene Link recommends purified siRNAs for use in transfection.

What concentration of siRNA is most effective?
As low as 1 nM concentration of siRNA has been shown to be effective in exhibiting RNAi. Initial experiments should be done at varying concentrations from 1-10 nM. Some reports have used as high as 25nM concentrations. High quality siRNAs should be used.

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What is the optimal cell density for transfection?

This is another variable that has to be optimized and then maintained. A good starting point is 60-70% confluent cells. Time points should be taken after transfection to determine the maximum inhibition. Start at 4 hours and end at 72 hours initially.

How do I quantify down regulation?

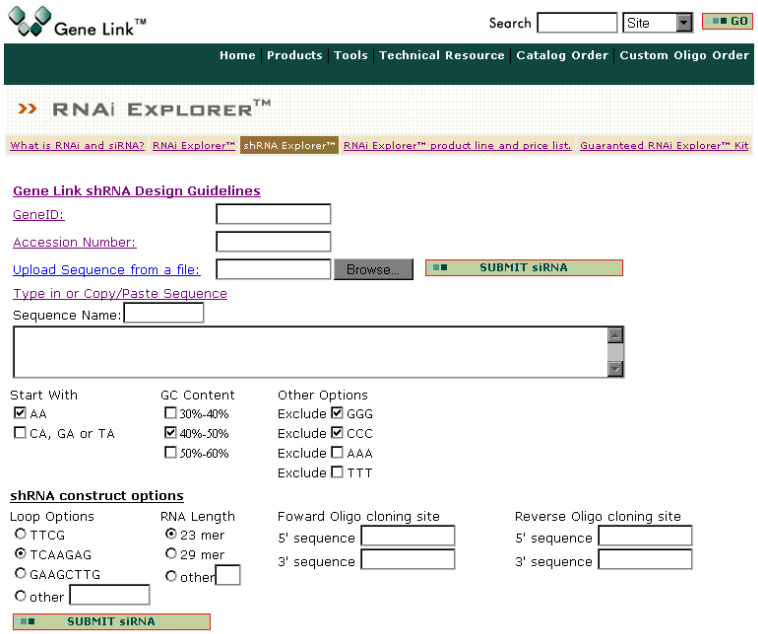
RNAi down regulates a gene function without actually interacting with the gene. The subtle action is by mRNA degradation. Thus the degree of RNA interference achieved is directly proportional to the level of mature mRNA and the translated proteins. The options are:

1. Measurements of target protein (enzyme) activity. This option is suitable if a robust assay is available or has been in prior use. The assay would vary by the nature of the protein product.
2. Measurement of target mRNA level. This is the preferred method as it directly quantifies the level of mRNA. Quantitative PCR is very effective in measuring relative amount of target sequence. This can be achieved simply by SYBR green or by the use of TaqMan Probes or Molecular Beacons.

What are shRNA?

An alternate to individual chemical synthesis of siRNA is to construct a sequence for insertion in an expression vector. Several companies offer RNAi vectors for the transcription of inserts. Some use an RNA polymerase III (Pol III) promoter to drive expression of both the sense and antisense strands separately, which then hybridize in vivo to make the siRNA. Other vectors are based on the use of Pol III to drive expression of short "hairpin" RNAs (shRNA), individual transcripts that adopt stem-loop structures, which are processed into siRNAs by the RNAi machinery.

shRNA Explorer™
Designing Oligonucleotides for RNAi Expression Vectors
shRNA Explorer™ Design Strategy



1. **Search for candidate siRNA sequences based on the following default parameters*.**
   shRNA size parameters: AA>23-29. Default is 23 nt.
   The default criteria selects target sequences of 23 nucleotides that begins **after** the AA and are located within a region of the coding sequence that is within 50-100 nucleotides of the AUG start codon and within 50-100 nucleotides from the termination codon. The AA sequence is NOT included in the shRNA sequence. The selection program includes the option to search for sequences that begin with other nucleotide pairs.

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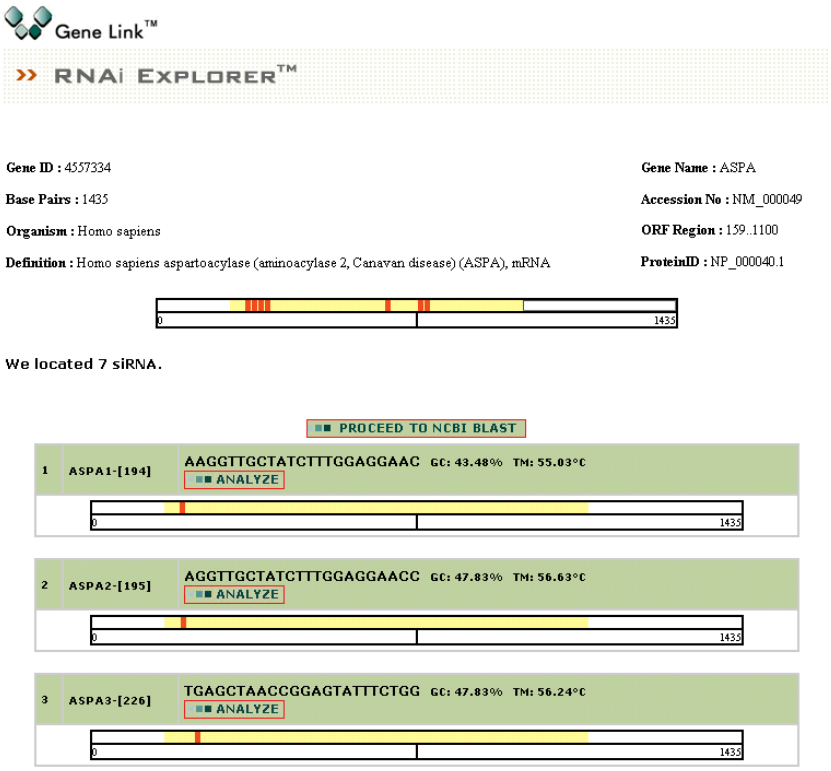
The upper strand of the target sequence should start with a G or an A, as RNA polymerase III prefers to initiate transcription with a purine. If a G or A is not present, then it must be inserted immediately upstream of the target sequence. A 'G" is added to the sequence at the 5' end if it is not present. A terminator sequence consisting of 6 dTs is added immediately downstream of the target sequence.

GC Content

The G-C content of the sequence is also used as a condition for selecting target sequences. Ideally the GC content will be less than 50%, although successful gene silencing has been reported with siRNAs that have G-C contents between 50 and 60%. The default parameter selects for a G-C content in the 40-50% range, however, options are available that allow for selection over wider ranges.

Stretches of Nucleotide Repeats

The default mode avoids sequences with repeats of three or more G's or C's, as their presence initiates intra-molecular secondary structures preventing effective siRNA silencing hybridization. As an option, repeat stretches of A's and T's can also be eliminated, as they tend to reduce the specificity of the target sequence. If possible, this option is highly recommended.



2. **Select Loop Sequence**

A loop sequence is necessary for construction of shRNA. Based on reported findings the default sequence for the loop is TCAAGAG. Other options are TTCG and GAAGCTTG (HindIII site) and there is also an option to enter your own sequence. **Enter Cloning Site Sequence.** Option is provided to enter cloning site sequence for 'forward' and 'reverse' oligo.

3. **Submit for siRNA search**

After selecting all the options, select 'Submit siRNA Search". This will initiate the program to download the sequence based on the accession number, Gene ID or to upload sequence you provided. Initially the ORF is determined and on the basis of the criteria suitable sequence is selected and a NCBI BLAST search is initiated. A list of siRNA sequences are presented for selection. The selected siRNA sequences are then converted for shRNA constructs.

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4. **View shRNA Construct**
   Annealed View: All selected features are incorporated in the construct and are presented for viewing and editing if required. This is presented in 'annealed form' for visual inspection of the sequence.
   shRNA view: Use this feature to view the forward oligo construct in a short hairpin view.

5. **Final Reviewing and or Editing before placing order**
   Final reviewing of all oligo sequences is presented before actual submission of the order. Two 'view' formats are available; 'Simple View' and 'Classic View'.

6. **Submit Order**
   Pressing 'Submit Order' places an order. An immediate email confirmation with an order number is sent. Please call or email if you do not receive an email confirmation.

*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.

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References
1. Fire,A., Xu,S., Montgomery, M.K., Kostas, S.A., Driver, S.E. and Mello, C.C. (1998) Nature, 391, 806 811.
2. Napoli, C., Lemieux, C. & Jorgensen, R. (1990) Plant Cell 2, 279 289.
3. Hannon, G.J. (2002) RNA interference. Nature, 418, 244 251
4. Billy,E., Brondani,V., Zhang,H., Muller,U. and Filipowicz,W. (2001) Proc. Natl. Acad. Sci. USA, 98, 14428 14433
5. Paddison, P.J., Caudy, A.A. and Hannon, G.J. (2002) Proc. Natl Acad. Sci. USA, 99, 1443 1448
6. Elbashir, S.M., Harborth, J., Lendeckel, W., Yalcin, A., Weber, K. and Tuschl,T. (2001) Nature, 411, 494 498
7. Volpe TA, Kidner C, Hall IM, Teng G, Grewal SI, Martienssen RA. (2002) Science.,297, 1833-1837.
8. Allshire R. (2002) Science, 297, 1818-1819.
9. Elbashir, S.M., Lendeckel, W. and Tuschl, T. (2001) Genes Dev., 15, 188 200.
10. Bernstein, E., Caudy, A.A., Hammond, S.M. and Hannon, G.J. (2001) Nature, 409, 363 366.
11. Hammond, S.M., Bernstein, E., Beach, L. and Hannon, G.J. (2000) Nature, 404, 293 296.
12. Nykanen, A., Haley, B. and Zamore, P.D. (2001) Cell, 107, 309 321.
13. Martinez, J., Patkaniowska, A., Urlaub, H., Luhrmann, R. and Tuschl, T. (2002) Cell, 110, 563 574.
14. Caplen, N.J., S. Parrish, F. Imani, A. Fire and R.A. Morgan. 2001. Proc Natl Acad Sci U S A 98:9742-9747.
15. Ullu E, Djikeng A, Shi H, Tschudi C., 2002, Philos Trans R Soc Lond B Biol Sci. 357, 65-70.
16. Ui-Tei, K., S. Zenno, Y. Miyata and K. Saigo, 2000, FEBS Lett 479:79-82.

RNAi Explorer™ Control siRNA Duplexes

The negative and positive control siRNA duplexes serve as validation for your experiments including monitoring transfection and silencing efficiencies. These are supplied lyophilized.

Control siRNA Duplexes		
Product	**Size**	**Catalog Number**
Non-Silencing Controls		
Negative Control unlabeled, non-silencing	5 nmols	27-6410-05
Negative Control FL labeled, non-silencing	2 nmols	27-6410-05FL
Unlabeled Positive Controls		
Human Vimentin; NM_003380	5 nmols	27-6412-05
Human Beta Actin; NM_001101	5 nmols	27-6413-05
Mouse Beta Tubulin; AF312873	5 nmols	27-6414-05
Rat Chromogranin-A; NM_021655	5 nmols	27-6415-05
Fluorescein Labeled Positive Controls		
Human Vimentin; NM_003380; FL labeled	2 nmols	27-6412-05FL
Human Beta Actin; NM_001101; FL labeled	2 nmols	27-6413-05FL
Mouse Beta Tubulin; AF312873; FL labeled	2 nmols	27-6414-05FL
Rat Chromogranin-A; NM_021655;FL labeled	2 nmols	27-6415-05FL

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Guaranteed RNAi Explorer™ Kit*		
Components	**Qty**	**Price**
3 Target siRNAs	10 nmols each	$995.00
1 negative control siRNA	5 nmols	
1 QPCR probe or 6 free oligos*	5 nmols	

*Select choice when placing order

27-6402-01	Guaranteed RNAi Explorer kit with Fluorescein/Dabcyl Molecular Beacon
27-6402-02	Guaranteed RNAi Explorer kit with Fluorescein/Tamra TaqMan
27-6402-06	Guaranteed RNAi Explorer kit with 6 oligos up to 30mer at 50 nmol scale

siRNA (duplexed)				
Catalog Number	**27-6401-06**	**27-6401-05**	**27-6401-02**	**27-6401-10**
Purification	**20 nmol scale**	**50 nmol scale**	**200 nmol scale**	**1 µmol scale**
Crude	$230.00	$320.00	$380.00	$800.00
RPC	$325.00	$380.00	$470.00	$900.00
Gel	$410.00	$480.00	$570.00	$955.00

Control siRNA Duplexes		
Product	**Size**	**Catalog Number**
Non-Silencing Controls		
Negative Control unlabeled, non-silencing	5 nmols	27-6410-05
Negative Control FL labeled, non-silencing	2 nmols	27-6410-05FL
Unlabeled Positive Controls		
Human Vimentin; NM_003380	5 nmols	27-6412-05
Human Beta Actin; NM_001101	5 nmols	27-6413-05
Mouse Beta Tubulin; AF312873	5 nmols	27-6414-05
Rat Chromogranin-A; NM_021655	5 nmols	27-6415-05
Fluorescein Labeled Positive Controls		
Human Vimentin; NM_003380; FL labeled	2 nmols	27-6412-05FL
Human Beta Actin; NM_001101; FL labeled	2 nmols	27-6413-05FL
Mouse Beta Tubulin; AF312873; FL labeled	2 nmols	27-6414-05FL
Rat Chromogranin-A; NM_021655;FL labeled	2 nmols	27-6415-05FL

shRNA (DNA oligonucleotides)		
Product	**200 nmol scale**	**1 µmol scale**
DNA Oligo Synthesis	2.00	3.75
Gel Purification	75.00	150.00

*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 (siRNA's) 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.

Prices subject to change without notice All Gene Link products are for research use only

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DNA SEQUENCING

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DNA Sequencing

Gene Link offers custom DNA sequencing as a service. Gene Link is committed to provide superior quality products and custom services. The Custom Sequencing Service will soon establish itself as an exceptional service in Quality, Consistency and Confidence. Gene Link uses ABI sequencers.

Catalog No.	Description	Price $
15-1001-01	CloneIDSeq	34.00/run
15-1001-02	DSSeq	1.15/base pair
15-1001-03	Bac/Pac/P1Seq	100/run
15-1000-00	Sequencing Hard Copies	12.50
15-1001-10	Failed Runs	25.00
15-1010-10	Rush Sequencing Service	20.00

CloneIDSeq Catalog No. 15-1001-01 $34.00/run

This service is for plasmid clone and PCR product identification. Unidirectional sequencing is performed using the standard primers or a primer sequence specified by the investigator. There is no additional charge for standard primers (T7, T3, M13, SP6, SK, KS). For custom primers there will be a charge for oligo synthesis. Accuracy of 97% is typically achieved for this service with a read length of 300-500 bases. Sequence data files will be emailed. Hard copies of electropherograms and sequence files and electronic files on disk compatible with ABI sequence analysis software will be mailed upon request at an additional cost of $12.50.

Turn around time for this service is 48-72 hours. A 24-hour rush service is available for an additional charge of $20.00. Failed runs or runs with less than 97% accuracy are repeated at no extra charge. Duplicate failed runs are attributed to DNA and a minimum charge of $25.00 is applied.

DSSeq Catalog No. 15-1001-02 $1.15/base pair

This service provides the complete sequence of the plasmid clone or PCR product. Double stranded sequencing is performed using primer walk. The service costs include primer design, construction of contig maps and the final aligned sequence. The sequence accuracy is greater than 99%. There is an additional charge for all primers synthesized for sequencing. Typical turnaround time is 1kb/week. Sequence data files will be emailed. Hard copies of electropherograms and sequence files, electronic files on disk compatible with ABI sequence analysis software, and all primers custom synthesized for the project will be mailed upon completion of the project.

Bac/Pac/P1Seq Catalog No. 15-1001-03 $100/run

This service provides terminal end sequencing of the Bac/Pac/P1 clones. A read length of 300 bases is guaranteed. The sequence accuracy is greater than 95%. There is an additional charge for all primers synthesized for sequencing. Typical turnaround time is one week. Sequence data files will be emailed. Hard copies of electropherograms and sequence files, electronic files on disk compatible with ABI sequence analysis software will be mailed upon completion of the project.

**Institutional discounts available.

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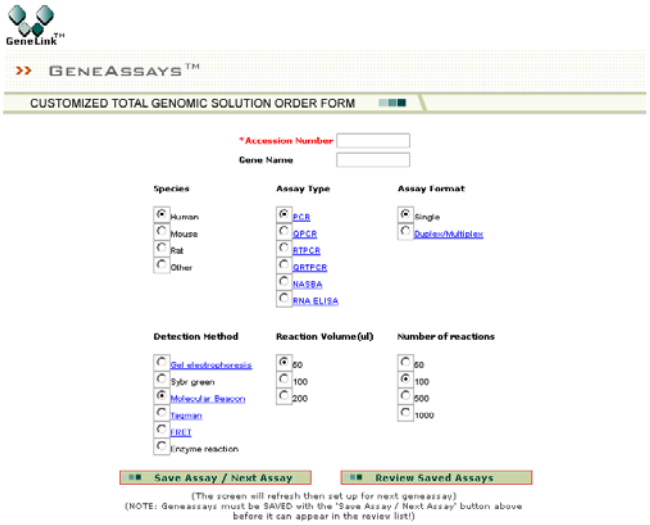

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Introducing GeneAssays™, a comprehensive and customized gene expression analysis service. In this era of high throughput analysis Gene Link stands next to you as your most trusted partner in gene expression analysis. Our experienced staff will help you design the most cost-effective solution to your gene expression analysis needs. The philosophy of GeneAssays™ is unlike any other service in the market. Our scientists work closely with you in trying to offer, "what you need" and not just "what is available". Our range includes any known gene in the entire human, mouse and rat genome and most other genomes. Depending on your research application, equipment, reagents and training available in your laboratory, our scientists will customize a suitable assay that will offer you the most economic solution to reaching your research goal. The types of assays involved in this system are PCR, RT-PCR, Single tube RT-PCR, NASBA, Single tube NASBA, RNA ELISA, etc.. Detection and quantitation technologies include gel electrophoresis, Sybr green, Taqman, Molecular Beacon and FRET probes. Our personalized GeneAssays™are designed totally with your application in mind.

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- We customize a number of solutions for the group and offer a quotation
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- We design all the assays and execute the order
- The scientist performs the tests and with close co-operation with our scientists and achieves his/her scientific goals

What is so unique about this system?

- This is the first time any company has offered all of these solutions under one roof
- The flexibility of the system allows the customer to totally customize an assay for himself/herself. For most companies, the relationship with a customer finishes with the completion of the sale, for Gene Link Geneassays™, it is only the beginning of a long and fruitful relationship.

**The polymerase chain reaction (PCR) process is covered by patents owned by Hoffmann-La Roche. A license to perform is automatically granted by the use of authorized reagents.

Prices subject to change without notice

All Gene Link products are for research use only.

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STOCK PRIMERS AND PROBES

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Random Primers

Random Primers are a mixture of oligonucleotides representing all possible sequences for that size. Random Primers can be used to prime synthesis in oligo-labeling similar to using hexamers (1,2) and cDNA synthesis. Random prime labeling yields high specific activity labeled DNA probes, which can be used for all southern, northern and in situ hybridization studies. Random Primers can be also used similar to using hexamers in cDNA synthesis in combination with oligo d(T) to yield more 5' end cDNA sequences.

Recently random primers have been used to detect DNA polymorphism. These polymorphisms, simply detected as DNA segments which amplify from one parent but not the other, are inherited in a Mendelian fashion and can be used to construct genetic maps in a variety of species. The authors suggested that these polymorphisms be called RAPD (pronounced RAPID) markers, after Random Amplified Polymorphic DNA (3).

Gene Link stocks various random primers, including an array of fluorescent dye-labeled primers for genetic analysis using fluorescent detecting instruments. The C-12 amino-labeled primers are ready to be conjugated to the investigator's choice of NHS-activated ligand.

The product is supplied as a lyophilized powder. After reconstitution store at –20 °C. Oligo purity is greater than 98% as determined by denaturing polyacrylamide gel electrophoresis.

References:
1. Feinberg, A.P. & Vogelstein, B. (1983) Anal. Biochem. 132:6-13.
2. Feinberg, A.P. & Vogelstein, B. (1984) Anal. Biochem. 137:266-267.
3. Williams J.G, Kubelik A.R, Livak K.J, Rafalski J.A, & Tingey S.V. (1990) Nucleic Acids Res. 18(22):6531-5

Random Unlabeled

Catalog #	Primer	Sequence	Quantity	Price
26-4000-03	Random Hexamer	5'-(NNNNNN)-3'	100 µg	45.00
26-4000-11	Random Heptamer	5'-(NNNNNNN)-3'	100 µg	45.00
26-4000-12	Random Octamer	5'-(NNNNNNNN)-3'	100 µg	45.00
26-4000-06	Random Nonamer	5'-(NNNNNNNNN)-3'	100 µg	45.00
26-4000-13	Random 12mer	5'-(NNNNNNNNNNNN)-3'	100 ug	45.00
26-4000-14	Random 24mer	5'-(NNNNNNNNNNNNNNNNNNNNNN)-3'	100 ug	45.00
26-4000-16	Random 36mer	5'-(NNNNNNNNNNNNNNNNNNNNNNNNNNNNNN)-3'	100 ug	45.00

Random Cy3 Labeled

Catalog #	Primer	Sequence	Quantity	Price
26-4000-21	5'-Cy3-Hexamer	5'-Cy3-(NNNNNN)-3'	25 µg	150.00
26-4000-22	5'-Cy3-Heptamer	5'-Cy3-(NNNNNNN)-3'	25 µg	150.00
26-4000-23	5'-Cy3-Octamer	5'-Cy3-(NNNNNNNN)-3'	25 µg	150.00
26-4000-24	5'-Cy3-Nonamer	5'-Cy3-(NNNNNNNNN)-3'	25 µg	150.00

Random Cy5 Labeled

Catalog #	Primer	Sequence	Quantity	Price
26-4000-31	5'-Cy5-Hexamer	5'-Cy5-(NNNNNN)-3'	25 µg	150.00
26-4000-32	5'-Cy5-Heptamer	5'-Cy5-(NNNNNNN)-3'	25 µg	150.00
26-4000-33	5'-Cy5-Octamer	5'-Cy5-(NNNNNNNN)-3'	25 µg	150.00
26-4000-34	5'-Cy5-Nonamer	5'-Cy5-(NNNNNNNNN)-3'	25 µg	150.00

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Random HEX Labeled				
Catalog #	Primer	Sequence	Quantity	Price
26-4000-41	5'-HEX-Hexamer	5′-Hex-(NNNNNN)-3′	25 µg	150.00
26-4000-42	5'-HEX-Heptamer	5′-Hex-(NNNNNNN)-3′	25 µg	150.00
26-4000-43	5'-HEX-Octamer	5′-Hex-(NNNNNNNN)-3′	25 µg	150.00
26-4000-44	5'-HEX-Nonamer	5′-Hex-(NNNNNNNNN)-3′	25 µg	150.00

Random TET Labeled				
Catalog #	Primer	Sequence	Quantity	Price
26-4000-61	5'-TET-Hexamer	5′-TET-(NNNNNN)-3′	25 µg	150.00
26-4000-62	5'-TET-Heptamer	5′-TET-(NNNNNNN)-3′	25 µg	150.00
26-4000-63	5'-TET-Octamer	5′-TET-(NNNNNNNN)-3′	25 µg	150.00
26-4000-64	5'-TET-Nonamer	5′-TET-(NNNNNNNNN)-3′	25 µg	150.00

Random 6-FAM Labeled				
Catalog #	Primer	Sequence	Quantity	Price
26-4000-51	5'-FAM-Hexamer	5′-Fam-(NNNNNN)-3′	25 µg	150.00
26-4000-52	5'-FAM-Heptamer	5′-Fam-(NNNNNNN)-3′	25 µg	150.00
26-4000-53	5'-FAM-Octamer	5′-Fam-(NNNNNNNN)-3′	25 µg	150.00
26-4000-54	5'-FAM-Nonamer	5′-Fam-(NNNNNNNNN)-3′	25 µg	150.00

Random Fluorescein Labeled				
Catalog #	Primer	Sequence	Quantity	Price
26-4000-71	5'-FI-Hexamer	5′-Fl-(NNNNNN)-3′	25 µg	150.00
26-4000-72	5'-FI-Heptamer	5′-Fl-(NNNNNNN)-3′	25 µg	150.00
26-4000-73	5'-FI-Octamer	5′-Fl-(NNNNNNNN)-3′	25 µg	150.00
26-4000-74	5'-FI-Nonamer	5′-Fl-(NNNNNNNNN)-3′	25 µg	150.00

Random C-12 Amino Labeled				
Catalog #	Primer	Sequence	Quantity	Price
26-4000-91	5'-Amino C-12-Hexamer	5′-Amino C-12 (NNNNNN)-3′	25 µg	150.00
26-4000-92	5'-Amino C-12-Heptamer	5′-Amino C-12 (NNNNNNN)-3′	25 µg	150.00
26-4000-93	5'-Amino C-12-Octamer	5′-Amino C-12 (NNNNNNNN)-3′	25 µg	150.00
26-4000-94	5'-Amino C-12-Nonamer	5′-Amino C-12 (NNNNNNNNN)-3′	25 µg	150.00

Random Digoxigenin Labeled				
Catalog #	Primer	Sequence	Quantity	Price
26-4000-81	5'-Dig-Hexamer	5′-Dig-(NNNNNN)-3′	25 µg	200.00
26-4000-82	5'-Dig-Heptamer	5′-Dig-(NNNNNNN)-3′	25 µg	200.00
26-4000-83	5'-Dig-Octamer	5′-Dig-(NNNNNNNN)-3′	25 µg	200.00
26-4000-84	5'-Dig-Nonamer	5′-Dig-(NNNNNNNNN)-3′	25 µg	200.00

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Random Phosphorylated				
Catalog #	Primer	Sequence	Quantity	Price
26-4000-10	Random Hexamer Phosphorylated pd(N)6	5′-p(NNNNNN)-3′	50 µg	100.00
26-4000-07	Random Heptamer Phosphorylated pd(N)7	5′-p(NNNNNNN)-3′	50 µg	100.00
26-4000-08	Random Octamer Phosphorylated pd(N)8	5′-p(NNNNNNNN)-3′	50 µg	100.00
26-4000-09	Random Nonamer Phosphorylated pd(N)9	5′-p(NNNNNNNNN)-3′	50 µg	100.00

Oligo dT Primers

Oligo dT Primers				
Catalog #	Primer	Sequence	Quantity	Price
26-4000-01	Oligo d(T)16	5′ TTTTTTTTTTTTTTTT	100 ug	45.00
26-4000-02	Oligo d(T)18	5′-TTTTTTTTTTTTTTTTTT-3'	100 ug	45.00
26-4000-04	Oligo d(T)12	5′-TTTTTTTTTTTT-3'	100 ug	45.00
26-4000-05	Oligo d(T)12-18		100 ug	45.00

Oligo dT C-12 Amino				
Catalog #	Primer	Sequence	Quantity	Price
26-4100-02	5′-C-12 amino-Oligo d(T)12-18	5'-C-12- d(T)12-18-3′ (varies)	25 µg	150.00
26-4112-02	5'-C-12 amino-Oligo d(T)12	5′-C-12-TTTTTTTTTTTT-3'	25 µg	150.00
26-4113-02	5′-C-12 amino-Oligo d(T)13	5′-C-12-TTTTTTTTTTTTT-3'	25 µg	150.00
26-4114-02	5′-C-12 amino-Oligo d(T)14	5′-C-12-TTTTTTTTTTTTTT-3'	25 µg	150.00
26-4115-02	5′-C-12 amino-Oligo d(T)15	5′-C-12-TTTTTTTTTTTTTTT-3'	25 µg	150.00
26-4116-02	5′-C-12 amino-Oligo d(T)16	5′-C-12-TTTTTTTTTTTTTTTT-3'	25 µg	150.00
26-4117-02	5′-C-12 amino-Oligo d(T)17	5′-C-12-TTTTTTTTTTTTTTTTT-3'	25 µg	150.00
26-4118-02	5′-C-12 amino-Oligo d(T)18	5′-C-12-TTTTTTTTTTTTTTTTTT-3'	25 µg	150.00
26-4119-02	5′-C-12 amino-Oligo d(T)19	5′-C-12-TTTTTTTTTTTTTTTTTTT-3'	25 µg	150.00
26-4120-02	5′-C-12 amino-Oligo d(T)20	5′-C-12-TTTTTTTTTTTTTTTTTTTT-3'	25 µg	150.00
26-4121-02	5′-C-12 amino-Oligo d(T)21	5′-C-12-TTTTTTTTTTTTTTTTTTTTT-3'	25 µg	150.00

Oligo dT Alexa Labeled				
Catalog #	Primer	Sequence	Quantity	Price
26-4200-02	5'-Alexa-Oligo d(T)12-18	5'-Alexa- d(T)12-18-3′ (varies)	25 µg	150.00
26-4212-02	5'-Alexa-Oligo d(T)12	5′-Alexa-TTTTTTTTTTTT-3'	25 µg	150.00
26-4213-02	5'-Alexa-Oligo d(T)13	5′-Alexa-TTTTTTTTTTTTT-3'	25 µg	150.00
26-4214-02	5'-Alexa-Oligo d(T)14	5′-Alexa-TTTTTTTTTTTTTT-3'	25 µg	150.00
26-4215-02	5'-Alexa-Oligo d(T)15	5′-Alexa-TTTTTTTTTTTTTTT-3'	25 µg	150.00
26-4216-02	5'-Alexa-Oligo d(T)16	5′-Alexa-TTTTTTTTTTTTTTTT-3'	25 µg	150.00
26-4217-02	5'-Alexa-Oligo d(T)17	5′-Alexa-TTTTTTTTTTTTTTTTT-3'	25 µg	150.00
26-4218-02	5'-Alexa-Oligo d(T)18	5′-Alexa-TTTTTTTTTTTTTTTTTT-3'	25 µg	150.00
26-4219-02	5'-Alexa-Oligo d(T)19	5′-Alexa-TTTTTTTTTTTTTTTTTTT-3'	25 µg	150.00
26-4220-02	5'-Alexa-Oligo d(T)20	5′-Alexa-TTTTTTTTTTTTTTTTTTTT-3'	25 µg	150.00
26-4221-02	5'-Alexa-Oligo d(T)21	5′-Alexa-TTTTTTTTTTTTTTTTTTTTT-3'	25 µg	150.00

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Oligo dT Cy5 Labeled				
Catalog #	Primer	Sequence	Quantity	Price
26-4400-02	5'-Cy5-Oligo d(T)12-18	5'-Cy5- d(T)12-18-3' (varies)	25 µg	$150.00
26-4412-02	5'-Cy5-Oligo d(T)12	5'-Cy5- TTTTTTTTTTTT-3'	25 µg	$150.00
26-4413-02	5'-Cy5-Oligo d(T)13	5'-Cy5-TTTTTTTTTTTTT-3'	25 µg	$150.00
26-4414-02	5'-Cy5-Oligo d(T)14	5'-Cy5-TTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4415-02	5'-Cy5-Oligo d(T)15	5'-Cy5-TTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4416-02	5'-Cy5-Oligo d(T)16	5'-Cy5-TTTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4417-02	5'-Cy5-Oligo d(T)17	5'-Cy5-TTTTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4418-02	5'-Cy5-Oligo d(T)18	5'-Cy5-TTTTTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4419-02	5'-Cy5-Oligo d(T)19	5'-Cy5-TTTTTTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4420-02	5'-Cy5-Oligo d(T)20	5'-Cy5-TTTTTTTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4421-02	5'-Cy5-Oligo d(T)21	5'-Cy5-TTTTTTTTTTTTTTTTTTTTT-3'	25 µg	$150.00

Oligo dT Cy3 Labeled				
Catalog #	Primer	Sequence	Quantity	Price
26-4300-02	5'-Cy3-Oligo d(T)12-18	5'-Cy3- d(T)12-18-3' (varies)	25 µg	$150.00
26-4312-02	5'-Cy3-Oligo d(T)12	5'-Cy3- TTTTTTTTTTTT-3'	25 µg	$150.00
26-4313-02	5'-Cy3-Oligo d(T)13	5'-Cy3-TTTTTTTTTTTTT-3'	25 µg	$150.00
26-4314-02	5'-Cy3-Oligo d(T)14	5'-Cy3-TTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4315-02	5'-Cy3-Oligo d(T)15	5'-Cy3-TTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4316-02	5'-Cy3-Oligo d(T)16	5'-Cy3-TTTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4317-02	5'-Cy3-Oligo d(T)17	5'-Cy3-TTTTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4318-02	5'-Cy3-Oligo d(T)18	5'-Cy3-TTTTTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4319-02	5'-Cy3-Oligo d(T)19	5'-Cy3-TTTTTTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4320-02	5'-Cy3-Oligo d(T)20	5'-Cy3-TTTTTTTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4321-02	5'-Cy3-Oligo d(T)21	5'-Cy3-TTTTTTTTTTTTTTTTTTTTT-3'	25 µg	$150.00

Oligo dT Dig Labeled				
Catalog #	Primer	Sequence	Quantity	Price
26-4500-02	5'-Dig-Oligo d(T)12-18	5'-Dig- d(T)12-18-3' (varies)	25 µg	$200.00
26-4512-02	5'-Dig-Oligo d(T)12	5'-Dig- TTTTTTTTTTTT-3'	25 µg	$200.00
26-4513-02	5'-Dig-Oligo d(T)13	5'-Dig-TTTTTTTTTTTTT-3'	25 µg	$200.00
26-4514-02	5'-Dig-Oligo d(T)14	5'-Dig-TTTTTTTTTTTTTT-3'	25 µg	$200.00
26-4515-02	5'-Dig-Oligo d(T)15	5'-Dig-TTTTTTTTTTTTTTT-3'	25 µg	$200.00
26-4516-02	5'-Dig-Oligo d(T)16	5'-Dig-TTTTTTTTTTTTTTTT-3'	25 µg	$200.00
26-4517-02	5'-Dig-Oligo d(T)17	5'-Dig-TTTTTTTTTTTTTTTTT-3'	25 µg	$200.00
26-4518-02	5'-Dig-Oligo d(T)18	5'-Dig-TTTTTTTTTTTTTTTTTT-3'	25 µg	$200.00
26-4519-02	5'-Dig-Oligo d(T)19	5'-Dig-TTTTTTTTTTTTTTTTTTT-3'	25 µg	$200.00
26-4520-02	5'-Dig-Oligo d(T)20	5'-Dig-TTTTTTTTTTTTTTTTTTTT-3'	25 µg	$200.00
26-4521-02	5'-Dig-Oligo d(T)21	5'-Dig-TTTTTTTTTTTTTTTTTTTTT-3'	25 µg	$200.00

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Oligo dT FAM Labeled				
Catalog #	Primer	Sequence	Quantity	Price
26-4600-02	5'-FAM-Oligo d(T)12-18	5'-FAM- d(T)12-18-3' (varies)	25 µg	$150.00
26-4612-02	5'-FAM-Oligo d(T)12	5'-FAM- TTTTTTTTTTT-3'	25 µg	$150.00
26-4613-02	5'-FAM-Oligo d(T)13	5'-FAM-TTTTTTTTTTTT-3'	25 µg	$150.00
26-4614-02	5'-FAM-Oligo d(T)14	5'-FAM-TTTTTTTTTTTTT-3'	25 µg	$150.00
26-4615-02	5'-FAM-Oligo d(T)15	5'-FAM-TTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4616-02	5'-FAM-Oligo d(T)16	5'-FAM-TTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4617-02	5'-FAM-Oligo d(T)17	5'-FAM-TTTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4618-02	5'-FAM-Oligo d(T)18	5'-FAM-TTTTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4619-02	5'-FAM-Oligo d(T)19	5'-FAM-TTTTTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4620-02	5'-FAM-Oligo d(T)20	5'-FAM-TTTTTTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4621-02	5'-FAM-Oligo d(T)21	5'-FAM-TTTTTTTTTTTTTTTTTTTT-3'	25 µg	$150.00

Oligo dT Fluorescein Labeled				
Catalog #	Primer	Sequence	Quantity	Price
26-4700-02	5'-Fl-Oligo d(T)12-18	5'-Fl- d(T)12-18-3' (varies)	25 µg	$150.00
26-4712-02	5'-Fl-Oligo d(T)12	5'-Fl- TTTTTTTTTTT-3'	25 µg	$150.00
26-4713-02	5'-Fl-Oligo d(T)13	5'-Fl-TTTTTTTTTTTT-3'	25 µg	$150.00
26-4714-02	5'-Fl-Oligo d(T)14	5'-Fl-TTTTTTTTTTTTT-3'	25 µg	$150.00
26-4715-02	5'-Fl-Oligo d(T)15	5'-Fl-TTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4716-02	5'-Fl-Oligo d(T)16	5'-Fl-TTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4717-02	5'-Fl-Oligo d(T)17	5'-Fl-TTTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4718-02	5'-Fl-Oligo d(T)18	5'-Fl-TTTTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4719-02	5'-Fl-Oligo d(T)19	5'-Fl-TTTTTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4720-02	5'-Fl-Oligo d(T)20	5'-Fl-TTTTTTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4721-02	5'-Fl-Oligo d(T)21	5'-Fl-TTTTTTTTTTTTTTTTTTTT-3'	25 µg	$150.00

Oligo dT HEX Labeled				
Catalog #	Primer	Sequence	Quantity	Price
26-4800-02	5'-HEX-Oligo d(T)12-18	5'-HEX- d(T)12-18-3' (varies)	25 µg	$150.00
26-4812-02	5'-HEX-Oligo d(T)12	5'-HEX- TTTTTTTTTTT-3'	25 µg	$150.00
26-4813-02	5'-HEX-Oligo d(T)13	5'-HEX-TTTTTTTTTTTT-3'	25 µg	$150.00
26-4814-02	5'-HEX-Oligo d(T)14	5'-HEX-TTTTTTTTTTTTT-3'	25 µg	$150.00
26-4815-02	5'-HEX-Oligo d(T)15	5'-HEX-TTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4816-02	5'-HEX-Oligo d(T)16	5'-HEX-TTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4817-02	5'-HEX-Oligo d(T)17	5'-HEX-TTTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4818-02	5'-HEX-Oligo d(T)18	5'-HEX-TTTTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4819-02	5'-HEX-Oligo d(T)19	5'-HEX-TTTTTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4820-02	5'-HEX-Oligo d(T)20	5'-HEX-TTTTTTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4821-02	5'-HEX-Oligo d(T)21	5'-HEX-TTTTTTTTTTTTTTTTTTTT-3'	25 µg	$150.00

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Oligo dT TET Labeled				
Catalog #	Primer	Sequence	Quantity	Price
26-4900-02	5'-TET-Oligo d(T)12-18	5'-TET- d(T)12-18-3' (varies)	25 µg	$150.00
26-4912-02	5'-TET-Oligo d(T)12	5'-TET- TTTTTTTTTTT-3'	25 µg	$150.00
26-4913-02	5'-TET-Oligo d(T)13	5'-TET-TTTTTTTTTTTT-3'	25 µg	$150.00
26-4914-02	5'-TET-Oligo d(T)14	5'-TET-TTTTTTTTTTTTT-3'	25 µg	$150.00
26-4915-02	5'-TET-Oligo d(T)15	5'-TET-TTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4916-02	5'-TET-Oligo d(T)16	5'-TET-TTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4917-02	5'-TET-Oligo d(T)17	5'-TET-TTTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4918-02	5'-TET-Oligo d(T)18	5'-TET-TTTTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4919-02	5'-TET-Oligo d(T)19	5'-TET-TTTTTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4920-02	5'-TET-Oligo d(T)20	5'-TET-TTTTTTTTTTTTTTTTTTT-3'	25 µg	$150.00
26-4921-02	5'-TET-Oligo d(T)21	5'-TET-TTTTTTTTTTTTTTTTTTTT-3'	25 µg	$150.00

Sequencing Primers

Sequencing Primers				
Catalog #	Primer	Sequence	Quantity	Price
26-3000-01	M13/pUC (-20) 17 mer	5' GTAAAACGACGGCCAGT	25 µg	35.00
26-3000-02	M13/pUC Reverse(-24) 16 mer	5' AACAGCTATGACCATG	25 µg	35.00
26-3000-03	M13/pUC (-40) 17 mer	5' GTTTTCCCAGTCACGAC	25µg	35.00
26-3000-04	M13/pUC Reverse(-48) 24 mer	5' AGCGGATAACAATTTCACACAGGA	25 µg	35.00
26-3000-05	T7 Promotor primer 23 mer	5' TAATACGACTCACTATAGGGAGA	25 µg	35.00
26-3000-06	T3 Promotor primer 20 mer	5' ATTAACCCTCACTAAAGGGA	25 µg	35.00
26-3000-07	SP6 Promotor primer 24 mer	5' CATACGATTTAGGTGACACTATAG	25 µg	35.00
26-3000-08	Lambda gt11 forward primer 24 mer	5' GGTGGCGACGACTCCTGGAGCCCG	25 µg	35.00
26-3000-09	Lambda gt11 reverse primer 24 mer	5' TTGACACCAGACCAACTGGTAATG	25 µg	35.00
26-3000-10	Lambda gt10 forward primer 24 mer	5' AGCAAGTTCAGCCTGGTTAAG	25 µg	35.00
26-3000-11	Lambda gt10 reverse primer 24 mer	5' CTTATGAGTATTTCTTCCAGGGTA	25 µg	35.00
26-3000-12	SP6 Promotor primer; 18 mer	5'-ATTTAGGTGACACTATAG-3'	25 µg	35.00
26-3000-21	T7 Promotor/Bluescript primer; 22 mer	5'-GTAATACGACTCACTATAGGGC-3'	25 µg	35.00
26-3000-22	T3 Promotor/Bluescript primer 20mer	5'-AATTAACCCTCACTAAAGGG-3'	25 µg	35.00

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Adaptors

Gene Link supplies adaptors, which are 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 are supplied as lyophilized powder. After reconstitution, store at -20 °C. Oligo purity is greater than 98% as determined by denaturing polyacrylamide gel electrophoresis.

Adaptors*				
Catalog #	**Adaptors**	**Sequence**	**Quantity**	**Price**
26-3100-01	EcoR I /EcoR I	5′ AATTCGAATTC GCTTAAG 5′	20 μg	50.00
26-3100-02	EcoR I /Not I	5′ AATTCGCGGCCGC GCGCCGGCG 5′	20 μg	50.00
26-3100-03	EcoR I /Sma I	5′ AATTCCCCGGG GGGGCCC 5′	20 μg	50.00
26-3100-04	EcoR I /Xho I	5′ AATTCGGCTCGAG GCCGAGCTC 5′	20 μg	50.00
26-3100-05	Xho I /EcoR I	5′ TCGAGGAATTC CCTTAAG 5′	20 μg	50.00
26-3100-06	Xho I /Not I	5′ TCGAGGCGGCCGC CCGCCGGCG 5′	20 μg	50.00
26-3100-07	BamH I/Sma I	5′ GATCCCCCGGG GGGGCCC 5′	20 μg	50.00
26-3100-08	Hind III/Not I adaptor	5'-AGGTTGCGGCCGC-3' 3'-ACGCCGGCGp-5'	20 μg	50.00
26-3100-09	Sph I/Spe I adaptor	5'-CACTAGT-3' 3'-GTACGTGATCAp-5'	20 μg	50.00
26-3100-10	Sac I/ Not I adaptor	5'-CGCGGCCGC-3' 3'-TCGAGCGCCGGCGp-5'	20 μg	50.00
26-3100-11	Xho I/Pst I adaptor	5'-TCGAGCTGCAGG-3' 3'-CGACGTCCp-5'	20 μg	50.00
26-3100-12	Xho I/Sma I adaptor	5'-TCGAGCCCGGG-3' 3'-CGGGCCCp-5'	20 μg	50.00
26-3100-13	Sph I/Not I adaptor	5'-CGCGGCCGC-3' 3'-GTACGCGCCGGCGp-5'	20 μg	50.00
26-3100-14	Not I/Kpn I adaptor	5'-GGCCGCGGTACC-3' 3'-CGCCATGGp-5'	20 μg	50.00
26-3100-15	Xba I/EcoRI adaptor	5'-CTAGAGAATTC-3' 3'-TCTTAAGp-5'	20 μg	50.00
26-3100-16	Eag l/Eco Rl adaptor	5'-GGCCGGAATTC-3' 3'-CCTTAAGp-5'	20 μg	50.00
26-3100-17	Apa L l/Sac II adaptor	5'-pTGCACCCGCGGACGT-3' 3'-GGGCGCCTGCAp-5'	20 μg	50.00

*Please inquire about other adaptors

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Linkers

Linkers are used for various cloning strategies to introduce restriction 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. The product is supplied as a lyophilized powder. After reconstitution, store at -20 °C. Oligo purity is greater than 98% as determined by denaturing polyacrylamide gel electrophoresis.

Linkers*				
Catalog #	Linkers	Sequence	Quantity	Price
26-3200-02	Bam HI linker; non-phosphorylated	5'-CGGGATCCCG-3'	20 µg	40.00
26-3200-03	BamH I phosphorylated linker	5'-pCGGGATCCCG-3'	20 µg	50.00
26-3200-04	Bgl II non-phosphorylated linker	5'-GAAGATCTTC-3'	20 µg	40.00
26-3200-05	Bgl II phosphorylated linker	5'-pGAAGATCTTC-3'	20 µg	50.00
26-3200-06	Cla I non-phosphorylated linker	5'-CCATCGATGG-3'	20 µg	40.00
26-3200-07	Cla I phosphorylated linker	5'-pCCATCGATGG-3'	20 µg	50.00
26-3200-08	EcoR I non-phosphorylated linker	5'-CCGGAATTCCGG-3'	20 µg	40.00
26-3200-09	EcoR I phosphorylated linker	5'-pCCGGAATTCCGG-3'	20 µg	50.00
26-3200-10	Hind III non-phosphorylated linker	5'-CCAAGCTTGG-3'	20 µg	40.00
26-3200-11	Hind III phosphorylated linker	5'-pCCAAGCTTGG-3'	20 µg	50.00
26-3200-12	Nco I non-phosphorylated linker	5'-CATGCCATGGCATG-3'	20 µg	40.00
26-3200-13	Nco I phosphorylated linker	5'-pCATGCCATGGCATG-3'	20 µg	50.00
26-3200-14	Not I non-phosphorylated linker	5'-AGCGGCCGCT-3'	20 µg	40.00
26-3200-15	Not I phosphorylated linker	5'-pAGCGGCCGCT-3'	20 µg	50.00
26-3200-16	Pst I non-phosphorylated linker	5'-GCTGCAGC-3'	20 µg	40.00
26-3200-17	Pst I phosphorylated linker	5'-pGCTGCAGC-3'	20 µg	50.00
26-3200-18	Pvu I non-phosphorylated linker	5'-ATCGATCGAT-3'	20 µg	40.00
26-3200-19	Pvu I phosphorylated linker	5'-pATCGATCGAT-3'	20 µg	50.00
26-3200-20	Sma I non-phosphorylated	5'-TCCCCCGGGGGA-3'	20 µg	40.00
26-3200-21	Sma I phosphorylated linker	5'-pTCCCCCGGGGGA-3'	20 µg	50.00
26-3200-22	Xba I non-phosphorylated linker	5’-CTAGTCTAGACTAG-3’	20 µg	40.00
26-3200-23	Xba I phosphorylated linker	5'-pCTAGTCTAGACTAG-3'	20 µg	50.00
26-3200-24	Xho I non-phosphorylated linker	5'-CCGCTCGAGCGG-3'	20 µg	40.00
26-3200-25	Xho I phosphorylated linker	5'-pCCGCTCGAGCGG-3'	20 µg	50.00
26-3200-26	Nhe I non-phosphorylated linker	5'-GGCTAGCC-3'	20 µg	40.00
26-3200-27	Nhe I phosphorylated linker	5'-pGGCTAGCC-3'	20 µg	50.00
26-3200-28	Apa I non-phosphorylated linker	5’-GGCTAGCC-3’	20 µg	40.00
26-3200-29	Apa I phosphorylated linker	5’-pGGGCCCC-3’	20 µg	50.00
26-3200-30	Eag I non-phosphorylated linker	5’-CCGGCCGG-3’	20 µg	40.00
26-3200-31	Eag I phosphorylated linker	5’-pCGGCCGG-3’	20 µg	50.00
26-3200-32	Pac I non-phosphorylated linker	5’-GTTAATTAAC-3’	20 µg	40.00
26-3200-33	Pac I phosphorylated linker	5’-pTTAATTAAC-3’	20 µg	50.00
26-3200-34	Kpn I non-phosphorylated linker	5’-GGGTACCC-3’	20 µg	40.00
26-3200-35	Kpn I phosphorylated linker	5’-pGGTACCC-3’	20 µg	50.00
26-3200-36	Mlu I non-phosphorylated linker	5’-CGACGCGTCG-3’	20 µg	40.00

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26-3200-37	Mlu I phosphorylated linker	5'-pGACGCGTCG-3'	20 µg	50.00
26-3200-38	Spe I non-phosphorylated linker	5'-GGACTAGTCC-3'	20 µg	40.00
26-3200-39	Spe I phosphorylated linker	5'-pGACTAGTCC-3'	20 µg	50.00

*Please inquire about other linkers

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™ is 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 10n mols. The 10 nmols of primer when dissolved in 500 µl sterile water or TE will give a solution of 20 µMolar, i.e. 20 pmols/µl. The quantity supplied is sufficient for at least 400 regular 25 µl PCR reactions* for ethidium bromide stained visualization. This primer set will amplify a fragment of 289 bp from human and rat cDNA. The fragments can be distinguished from rat or human source by digestion with Pvu II; the rat amplified 289 bp fragment is digested to give a 132 and 157 fragments whereas the human amplified fragment is not digested due to the absence of the Pvu II (1).

The product is supplied as a lyophilized powder, after reconstitution store at –20 °C. Oligo purity is greater than 98% as determined by denaturing polyacrylamide gel electrophoresis.

Reference

1. du Breuil, R. M., Patel, J.M. and Mendelow, B.V. (1993) PCR methods and applications. 3:57-59.

**The polymerase chain reaction (PCR) process is covered by patents owned by Hoffmann-La Roche. A license to perform is automatically granted by the use of authorized reagents.*

RT-PCRmer			
Product	**Catalog No.**	**Quantity**	**Price $**
RT-PCRmer; Human beta actin	40-1001-10	10 nmols	100.00
RT-PCRmer; Rat beta actin	40-1002-10	10 nmols	100.00
RT-PCRmer; Mouse beta actin	40-1003-10	10 nmols	100.00
RT-PCRmer; beta 2 microglobulin	40-1004-10	10 nmols	100.00
RT-PCRmer; GAPDH H/M/R	40-1005-10	10 nmols	100.00
RT-PCRmer; Beta actin control PCR mix (human &	40-1002-00	10 nmols	100.00

*Please see our First Strand cDNA section for related products.

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OligoProber™

OligoProber™ are specific oligonucleotide probes for hybridization to their cognate species. These are specially suited for use in conjunction with RT-PCRmers™, 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. The OligoProber™ is supplied as a lyophilized powder in aliquots of 2 nmols. The 2 nmols of primer when dissolved in 100μl sterile water or TE will give a solution of 20 μMolar i.e. 20 pmols/μl. Oligo purity is greater than 98% as determined by denaturing polyacrylamide gel electrophoresis.

Reference
1. du Breuil, R. M., Patel, J.M. and Mendelow, B.V. (1993) PCR methods and applications. 3:57-59.

**The polymerase chain reaction (PCR) process is covered by patents owned by Hoffmann-La Roche. A license to perform is automatically granted by the use of authorized reagents.*

OligoProber			
Product	**Catalog No.**	**Quantity**	**Price $**
OligoProber™; Human beta actin	40-1101-02	2 nmols	55.00
OligoProber™; Rat beta actin	40-1102-02	2 nmols	55.00
OligoProber™; Mouse beta actin	40-1103-02	2 nmols	55.00
OligoProber™; GAPDH H/M/R	40-1105-02	2 nmols	55.00
OligoProber™; Biotinylated Human beta actin	40-1111-02	2 nmols	150.00
OligoProber™; Biotinylated Rat beta actin	40-1112-02	2 nmols	150.00
OligoProber™; Biotinylated Mouse beta actin	40-1113-02	2 nmols	150.00
OligoProber™; Biotinylated GAPDH H/M/R	40-1115-02	2 nmols	150.00

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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. Selected sequences representing some of the combinations of unique sequences without runs are spread over 30 sets, each containing 40 primers. The total number of primers spread over 30 sets is 1200. These primers are used in amplification reactions for determining Random Amplified Polymorphic DNA (RAPD) fragments.

Polymorphism
The vast amount of phenotypic differences between individuals may be either due to genetic differences, meaning differences encoded at the DNA level, or due to non-genetic differences particularly dependent on environmental interaction. The condition of the existence of two or more forms of DNA sequence for a particular stretch or forms of proteins that are genetically distinct and structurally different is known as polymorphism.

Genetic Polymorphism is defined as the occurrence of multiple alleles at a locus, where at least two alleles exist with frequencies greater than one percent. Examples of polymorphism at the protein level include: (a) Blood group systems ABO, MNSs, Rh, (b) Galactosemia, galactose-1-phosphate uridyl transferase locus, and (c) alpha-1-antitrypsin (1).

Polymorphisms are principally of value for their use as genetic "markers" to distinguish different inherited forms of a gene in family studies. The human haploid genome consists of $\sim 3x10^9$ bases. Not all individuals have exactly the same DNA sequences. Differences in DNA sequence termed as polymorphism exist in the human population. These differences in DNA sequence may either create or abolish a site recognized by a restriction endonuclease thereby upon digestion, electrophoretic separation and southern analysis may give rise to differing hybridizing fragment lengths. DNA based variations at restriction sites detected in this manner are called restriction fragment length polymorphism (RFLP). The differing fragment lengths constitute co-dominant alleles at a DNA locus. RFLPs may also arise from deletion or insertion of DNA, rather than from single nucleotide changes (1).

Polymerase Chain Reaction has afforded rapid progress in genetic research, Various techniques have essentially replaced the use of RFLP based analysis of polymorphism. The use of Short Tandem Repeat (STR), VNTR and microsatellite markers has generated enormous amount of polymorphic data by incorporating multi loci high throughput screening (2). The limiting factor in microsatellites is the initial generation and establishing of these markers.

A PCR based RFLP analysis is termed. Other modified approaches include AFLP (Amplification fragment length polymorphism) for DNA fingerprinting (3) and genetic characterization in plants (4). Other techniques have utilized the fact that a large number of primer combination based amplification has to yield polymorphic amplified fragments. A robust random approach of using random sequence decamer primers to amplify DNA is named RAPD (Random Amplified Polymorphic DNA, (pronounced RAPID)) (5-7).

Polymorphisms are principally of value for their use as genetic "markers" to distinguish different inherited forms of a gene in family studies. Genetic markers are of enormous practical use in medical genetics for:

- Mapping genes to individual chromosomes by linkage analysis
- Pre-symptomatic and prenatal diagnosis of genetic disease
- Detection of heterozygous carriers of genetic disease
- Evaluation of high- and low-risk persons with a predisposition to common adult disorders, such as coronary heart disease, cancer, and diabetes
- Paternity testing and forensic application
- Matching of donor-recipient pairs for tissue and organ transplantation

The main context of this manual is focused on human and medical genetics. The same protocols have been used for all DNA polymorphism studies and the same implications can be extended to bacterial, plant and other organisms.

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RAPD Decamer Primers

Williams et al first reported the use of arbitrary sequence short primers (5). They described a new assay for DNA polymorphism based on the amplification of random DNA segments with *single* primers of arbitrary sequence. These fragments amplify from one parent DNA and not from the other, signifying Mendelian inheritance. Such polymorphism can be valuable for construction of polymorphic genetic maps (5-7).

Probability of Polymorphic Fragments

The probability of finding these short decamer primer cognate sequences in the target DNA varies and may be numerous, such that a single primer generates multiple amplification fragments. Discerning a polymorphic site is simply based on the presence in one and absence in another of the same size fragment. Repeating the amplification *should* give the same result if it is truly polymorphic. The fragment pattern generated for a particular species' DNA should be constant in reproducible experimental conditions. A primer's ability to generate amplification products is dependent on its sequence and the genome size of the template. The technique is actually based on the premise of 'randomness,' the primers finding its complementary sequence randomly and possibly fortuitously polymorphic and not present in the 'other' template.

Probability calculation of the frequency of a decamer predicts between 5-10 in a genome size of 2 X 10^9 base pairs. Smaller genome size still generates amplification products, possibly due to mismatch miss-priming.

Nomenclature of Amplified Fragment

Gene Link RAPD decamer sets follow the same numbering pattern adopted by Operon. The similar numbering will assist customers with a universal numbering system. The primer sets are named using letters and the individual primers are named using numerals. Gene Link recommends using the convention of Paran et al. (8). Use the primer name followed by polymorphic fragment size as the suffix.

Results and Interpretation

Obtaining favorable and reliable results requires meticulous and careful experimental conditions. The template quality is of paramount importance. The DNA should be predominantly of high molecular weight with very few sheared DNA. Highly sheared DNA may lose the polymorphic site by breakage and thus not yield informative results. Thorough consistency is required to enable reproducing useful results obtained. Due to the arbitrary nature of this technique, polymorphic fragments may not be obtained even by using all the primers under stringent conditions. Under such eventuality, it is recommended that the annealing temperature be decreased gradually.

The amplified fragment gel profile may seem daunting with numerous bands. Closer visualization should clearly reveal a pattern of presence and absence of a particular band. This should be noted as possibly polymorphic and should be reproducible. Several published reports indicate the use of RAPD markers yielding polymorphic markers from template DNA from plants, bacteria and mice (10-12).

Smeared Fragment Pattern is generally a sign of mismatched priming and excess non-stringent amplification. A smear pattern is almost always converted to distinct fragments by optimizing the concentration of the primers, annealing temperature, polymerase and the template.

Use of Multiple RAPD Primers has been shown to improve discrimination over one primer used individually. This increased discrimination is limited to profiles that already show a difference. Two virtually identical patterns obtained by using one primer indicates highly related strains and cannot be more informative by using two primers (13).

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References

1. Javed, A. A., Huang. Y. and Bombard, A. T. (1995) Molecular analysis of genetic diseases. An overview for clinicians. J. Assoc. Acad. Min. Phys. 6:15-27.
2. Javed, A. A., Stambolian, D., and Huang, Y. (1995) The gene of Nance-Horan syndrome maps to Xp22.12. Am. J. Hum. Genet. 57:A194.
3. Vos, P., Hogers, R., Bleeker, M. et al. (1995) AFLP: A new technique for DNA fingerprinting. Nucl. Acids Res. 23: 4407-4414.
4. Ranamukhaarachchi, D. G., Kane, M.E., Guy, C.L. and Li, Q.B. (2000) Modified AFLP technique for rapid genetic characterization in plants. Biotechniques 29: 858-866.
5. Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., and Tingey, S.V. (1990) *DNA polymorphisms amplified by arbitrary primers are useful as genetic markers*, Nucl. Acids Res. 18, 6531-6535.
6. Williams, J. G., Reiter, R.S., Young, R.M. and Scolnik, P.A. (1993) Genetic mapping of mutations using phenotypic pools and mapped RAPD markers. Nucl. Acids Res. 21:2697-2702.
7. Welsh, J. and McClelland, M. (1990) *Fingerprinting genomes using PCR with arbitrary primers*, Nucl. Acids Res. 19, 303-306.
8. Paran, I., Kesseli, R., and Michelmore, R. (1991) *Identification of RFLP and RAPD markers linked to downy mildew resistance genes in lettuce using near-isogenic lines*, Genome 34, 1021-1027.
9. Atienzer, F., Evenden, A., Jha, A., Savva, D. and Depledge, M. (2000) Optimized RAPD analysis generates high quality geneomic DNA profiles at high annealing temperature. Biotechniques 28:52-54.
10. Martin, G.B., Williams, J.G.K., and Tanksley, S.D. (1991) *Rapid identification of markers linked to a Pseudomonas resistance gene in tomato by using random primers and near-isogenic lines*, Proc. Natl. Acad. Sci. USA 88, 2336-2340.
11. Reiter, R.S., Williams, J.G.K., Feldman, K.A., Rafalski, J.A., Tingey, S.V., and Scolnik, P.A. (1992) *Global and local genome mapping in Arabidopsis thaliana by using recombinant inbred lines and random amplified polymorphic DNAs*, Proc. Natl. Acad. Sci. USA 89, 1477-1481.
12. Woodward, S.R., Sudweeks, J., and Teuscher, C. (1992) *Random sequence oligonucleotide primers detect polymorphic DNA products which segregate in inbred strains of mice*, Mammalian Genome 3, 73-78.
13. Hopkins, K.L and Hilton, A.C.(2001) Use of multiple primers in RAPD analysis of clonal organisms provides limited improvement in discrimination. Biotechniques 30: 1262-1267.

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Each tube contains 10 nmols of lyophilized primer. This is sufficient for thousands of amplification reactions.

GL RAPD Decamer Sets			
Product	**Size**	**Catalog No.**	**Price, $**
GL RAPD Decamer 01–AB Set	10 nmols x 40 tubes	40-0001-01	350.00
GL RAPD Decamer 02–CD Set	10 nmols x 40 tubes	40-0001-02	350.00
GL RAPD Decamer 03–EF Set	10 nmols x 40 tubes	40-0001-03	350.00
GL RAPD Decamer 04–GH Set	10 nmols x 40 tubes	40-0001-04	350.00
GL RAPD Decamer 05–IJ Set	10 nmols x 40 tubes	40-0001-05	350.00
GL RAPD Decamer 06–KL Set	10 nmols x 40 tubes	40-0001-06	350.00
GL RAPD Decamer 07–MN Set	10 nmols x 40 tubes	40-0001-07	350.00
GL RAPD Decamer 08–OP Set	10 nmols x 40 tubes	40-0001-08	350.00
GL RAPD Decamer 09–QR Set	10 nmols x 40 tubes	40-0001-09	350.00
GL RAPD Decamer 10–ST Set	10 nmols x 40 tubes	40-0001-10	350.00
GL RAPD Decamer 11–UV Set	10 nmols x 40 tubes	40-0001-11	350.00
GL RAPD Decamer 12–WX Set	10 nmols x 40 tubes	40-0001-12	350.00
GL RAPD Decamer 13–YZ Set	10 nmols x 40 tubes	40-0001-13	350.00
GL RAPD Decamer 14–AAAB Set	10 nmols x 40 tubes	40-0001-14	350.00
GL RAPD Decamer 15–ACAD Set	10 nmols x 40 tubes	40-0001-15	350.00
GL RAPD Decamer 16–AEAF Set	10 nmols x 40 tubes	40-0001-16	350.00
GL RAPD Decamer 17–AGAH Set	10 nmols x 40 tubes	40-0001-17	350.00
GL RAPD Decamer 18–AIAJ Set	10 nmols x 40 tubes	40-0001-18	350.00
GL RAPD Decamer 19–AKAL Set	10 nmols x 40 tubes	40-0001-19	350.00
GL RAPD Decamer 20–AMAN Set	10 nmols x 40 tubes	40-0001-20	350.00
GL RAPD Decamer 21–AOAP Set	10 nmols x 40 tubes	40-0001-21	350.00
GL RAPD Decamer 22–AQAR Set	10 nmols x 40 tubes	40-0001-22	350.00
GL RAPD Decamer 23–ASAT Set	10 nmols x 40 tubes	40-0001-23	350.00
GL RAPD Decamer 24–AUAV Set	10 nmols x 40 tubes	40-0001-24	350.00
GL RAPD Decamer 25–AWAX Set	10 nmols x 40 tubes	40-0001-25	350.00
GL RAPD Decamer 26–AYAZ Set	10 nmols x 40 tubes	40-0001-26	350.00
GL RAPD Decamer 27–BABB Set	10 nmols x 40 tubes	40-0001-27	350.00
GL RAPD Decamer 28–BCBD Set	10 nmols x 40 tubes	40-0001-28	350.00
GL RAPD Decamer 29–BEBF Set	10 nmols x 40 tubes	40-0001-29	350.00
GL RAPD Decamer 30–BGBH Set	10 nmols x 40 tubes	40-0001-30	350.00

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GENETIC TOOLS AND REAGENTS

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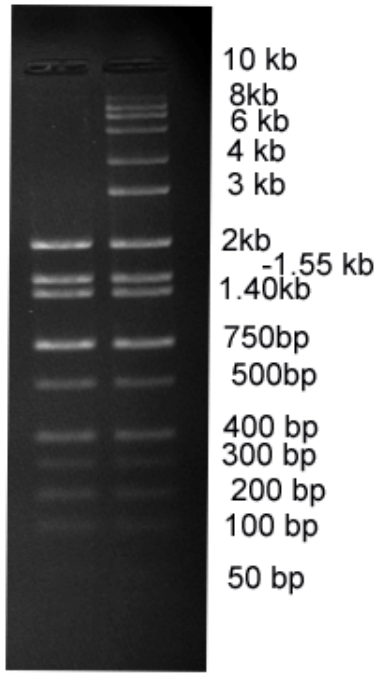
Omni-Ladder™ Unlabeled DNA Molecular Weight Markers

Omni-Marker™ Universal and Low are unlabeled DNA markers containing 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 2kb. The low Omni-Marker™ is ideal for routine PCR gels. A loading of 5 µl is sufficient per lane.

Omni-Marker™ Universal unlabeled		Omni-Marker™ Low unlabeled	
Fragment Size	Approx. conc.	Fragment Size	Approx. conc.
10 kb	30 ng	2 kb	150 ng
8 kb	30 ng	1.55 kb	100 ng
6 kb	45 ng	1.40 kb	100 ng
4 kb	60 ng	1.00 kb	120 ng
3 kb	85 ng	750 bp	30 ng
2 kb	150 ng	500 bp	60 ng
1.55 kb	100 ng	400 bp	20 ng
1.40 kb	100 ng	300 bp	40 ng
1.00 kb	120 ng	200 bp	30 ng
750 bp	30 ng	100 bp	20 ng
500 bp	60 ng	50 bp	15 ng
400 bp	20 ng		
300 bp	40 ng		
200 bp	30 ng		
100 bp	20 ng		
50 bp	15 ng		



The gel picture shows the fragments obtained by electrophoresing in 1.5% agarose gel. The low and universal Omni-Markers are provided pre-mixed with or without dye. The marker and dye both contain 0.02% sodium azide.

Molecular Weight Markers			
Product	**Catalog No.**	**Size**	**Price $**
Omni-Marker™ Universal unlabeled	40-3005-01	100 µl	15.00
Omni-Marker™ Universal unlabeled	40-3005-05	500 µl	50.00
Omni-Marker™ Universal unlabeled	40-3005-10	1 ml	90.00
Omni-Marker™ Low unlabeled	40-3006-01	100 µl	15.00
Omni-Marker™ Low unlabeled	40-3006-05	500 µl	50.00
Omni-Marker™ Low unlabeled	40-3006-10	1 ml	90.00

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

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Omni-Ladder™ Labeled DNA Molecular Weight Markers

Omni-Marker™ dye labeled, Alkaline Phosphatase, biotin and digoxigenin labeled MW Markers are also available.

Omni-Marker™ dye labeled, Alkaline Phosphatase, biotin and digoxigenin labeled MW Markers			
Product	**Catalog No.**	**Size***	**Price $**
Omni-Marker™ GScan-1 Tamra labeled 50 bp-1kb	40-3061-01	100 µl	95.00
Omni-Marker™ GScan-1 Tamra labeled 50 bp-1kb	40-3061-05	500 µl	395.00
Omni-Marker™ GScan-2 Tamra labeled 50 bp- 600 bp	40-3062-01	100 µl	95.00
Omni-Marker™ GScan-2 Tamra labeled 50 bp- 600 bp	40-3062-05	500 µl	395.00
Omni-Marker™ GScan-1 Tet labeled 50 bp-1kb	40-3071-01	100 µl	95.00
Omni-Marker™ GScan-1 Tet labeled 50 bp-1kb	40-3071-05	500 µl	395.00
Omni-Marker™ GScan-2 Tet labeled 50 bp- 600 bp	40-3072-01	100 µl	95.00
Omni-Marker™ GScan-2 Tet labeled 50 bp- 600 bp	40-3072-05	500 µl	395.00
Omni-Marker™ Biotin labeled 50 bp-1kb	40-3081-01	100 µl	95.00
Omni-Marker™ Biotin labeled 50 bp-1kb	40-3081-05	500 µl	395.00
Omni-Marker™ Digoxigenin labeled 50 bp-1kb	40-3091-01	100 µl	95.00
Omni-Marker™ Digoxigenin labeled 50 bp-1kb	40-3091-05	500 µl	395.00
*A loading of 0.5µl is suggested.			

Loading Buffers

Gene Link also supplies loading buffers. We recommend Orange G for very low molecular weight DNA, as it usually runs around the 20-30bp range on 1% agarose. Bromophenol Blue /Xilene Cyanol DNA loading buffer is better for DNA of larger molecular weight.

Loading Buffers			
Product	**Catalog No.**	**Size**	**Price $**
5X BPB/XC non-denaturing loading buffer	40-3002-01	100 µl	5.00
5X BPB/XC non-denaturing loading buffer	40-3002-10	1ml	10.00
5X Orange G/XC non-denaturing loading buffer	40-3004-01	100 µl	5.00
5X Orange G/XC non-denaturing loading buffer	40-3004-10	1ml	10.00
2X BPB/XC Sequencing loading buffer	40-5027-01	100 µl	5.00
2X BPB/XC Sequencing loading buffer	40-5027-10	1ml	10.00

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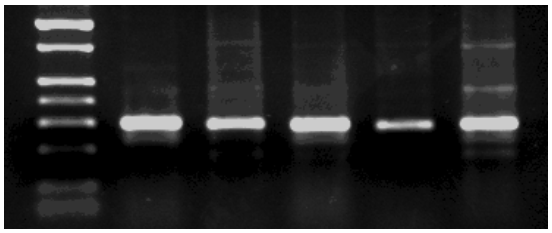
Guinea Pig First Strand cDNA

Background

First strand cDNA is useful for amplifying a particular cDNA using PCR. The PCR reaction must be optimized using varying amounts of the cDNA. This optimization is particularly important when the target mRNA species is of low abundance. The protocol given is for amplifying β-actin as a control to validate the quality of the 'first strand cDNA' supplied. The PCR conditions to amplify the target cDNA will be based on the primers selected. It should be noted that specific sequence primers as well as degenerate sequence primers can be used successfully to amplify the target sequence.

The first strand cDNA has been prepared from freshly obtained tissue and appropriately frozen during transportation. RNA was extracted using the widely used and published method (1). Oligo dT has been used to prime the synthesis of the first strand using Moloney Murine leukemia Virus (MMLV) Reverse Transcriptase. The amount supplied, 5 μg (lyophilized) first strand cDNA and 200 μl of β-actin control PCR mix, is sufficient for at least 50 amplifications. Each lot is tested for amplification of β-actin cDNA.

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



References

1. Chomczynski,P. and Sacchi, N. (1987) Anal. Biochem. 162:156-159.

First Strand cDNA			
Product	**Catalog No.**	**Size**	**Price $**
Guinea pig first strand pooled cDNA	10-2100-05	5 μg	425.00
Guinea pig first strand cDNA, Brain	10-2101-05	5 μg	425.00
Guinea pig first strand cDNA, Heart	10-2102-05	5 μg	425.00
Guinea pig first strand cDNA, Liver	10-2103-05	5 μg	425.00
Guinea pig first strand cDNA, Kidney	10-2104-05	5 μg	425.00
Guinea pig first strand cDNA, Intestine	10-2105-05	5 μg	425.00
Guinea pig first strand cDNA, Skeletal muscle	10-2106-05	5 μg	425.00
Guinea pig first strand cDNA, Lungs	10-2107-05	5 μg	425.00
Guinea pig first strand cDNA, Spleen	10-2108-05	5 μg	425.00
Guinea pig first strand cDNA, Ovaries	10-2109-05	5 μg	425.00
Guinea pig first strand cDNA, Pancreas	10-2110-05	5 μg	425.00
Guinea pig first strand cDNA, Eye	10-2111-05	5 μg	425.00

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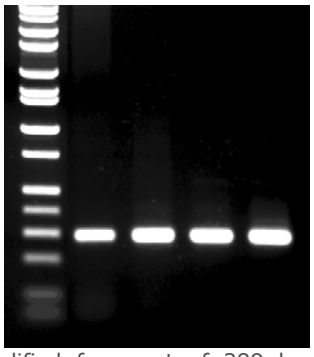
Omni-cDNA™ Pooled First Strand cDNA

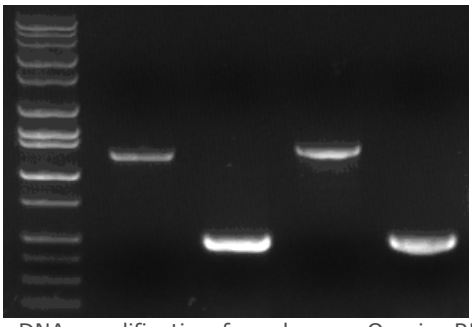
First strand cDNA is useful for amplifying a particular cDNA using PCR. The PCR reaction must be optimized using varying amounts of the cDNA. This optimization is particularly important when the target mRNA species is of low abundance. The protocol given is for amplifying β-actin as a control to validate the quality of the 'first strand cDNA' supplied. The PCR conditions to amplify the target cDNA will be based on the primers selected. It should be noted that specific sequence primers as well as degenerate sequence primers can be used successfully to amplify the target sequence.

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 various tissues vary, but are representative of different organs and tissue types. These include lung, heart, brain, spleen, skeletal muscle, smooth muscle, ovaries, pancreas, liver and kidney. There is lot-to-lot variation but an overall representation of tissue type is maintained. Oligo dT has been used to prime the synthesis of the first strand using Moloney Murine leukemia Virus (MMLV) Reverse Transcriptase or AMV reverse transcriptase. The amount supplied is sufficient for at least 50 amplifications. Each lot is tested for amplification of β-actin cDNA.

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



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



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

First Strand pooled cDNA

Product		Catalog No.	Size	Price $
Omni-cDNA™	Human first strand pooled cDNA	10-0100-05	5 μg	425.00
Omni-cDNA™	Mouse first strand pooled cDNA	10-0200-05	5 μg	425.00
Omni-cDNA™	Rat first strand pooled cDNA	10-0300-05	5 μg	425.00
Omni-cDNA™	Guinea Pig first strand pooled cDNA	10-2100-05	5 μg	425.00

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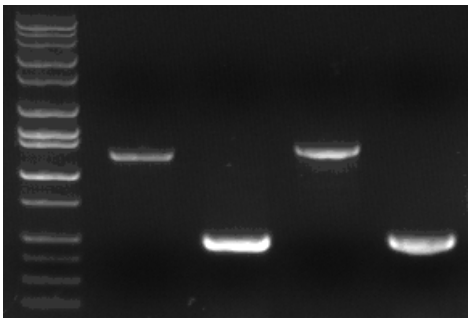
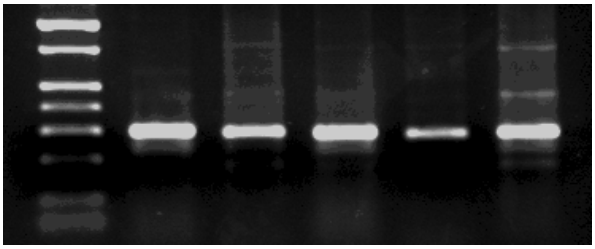
Gene Link introduces *Omni-mRNA*™ pooled reference mRNA. Commercially available for the first time, *Omni-mRNA*™ is a unique blend of amplified high-quality mRNA purified from various tissues. Using the same reference mRNA in different microarray experiments provides a common denominator for accurate and reproducible comparison of gene expression data. In addition, use of the same reference mRNA among different research groups allows inter-laboratory comparisons as well. Gene Link recommends using pooled reference mRNA as a reference sample in any multicolor hybridization experiment using cDNA or oligonucleotide microarrays.



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

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



Guinea Pig β-actin amplification. An amplified fragment of 289 bp. Lane 1 is molecular weight markers. Lanes 2-6 are β-actin control PCR product from brain, liver, intestine, skeletal muscle and spleen first strand cDNA.

Omni-mRNA™ amplified pooled reference mRNA

Quantity supplied 50 μg in 25 μg x 2 tubes is sufficient for direct hybridization of 20 microarrays

Product	Catalog No.	Size	Price $
Human Omni-mRNA™ amplified pooled reference mRNA	08-0100-50	50μg (25μg x 2 tubes)	395.00
Mouse Omni-mRNA™ amplified pooled reference mRNA	08-0200-50	50μg (25μg x 2 tubes)	395.00
Rat Omni-mRNA™ amplified pooled reference mRNA	08-0300-50	50μg (25μg x 2 tubes)	395.00
Guinea Pig Omni-mRNA™ amplified pooled reference mRNA	08-2100-50	50μg (25μg x 2 tubes)	395.00

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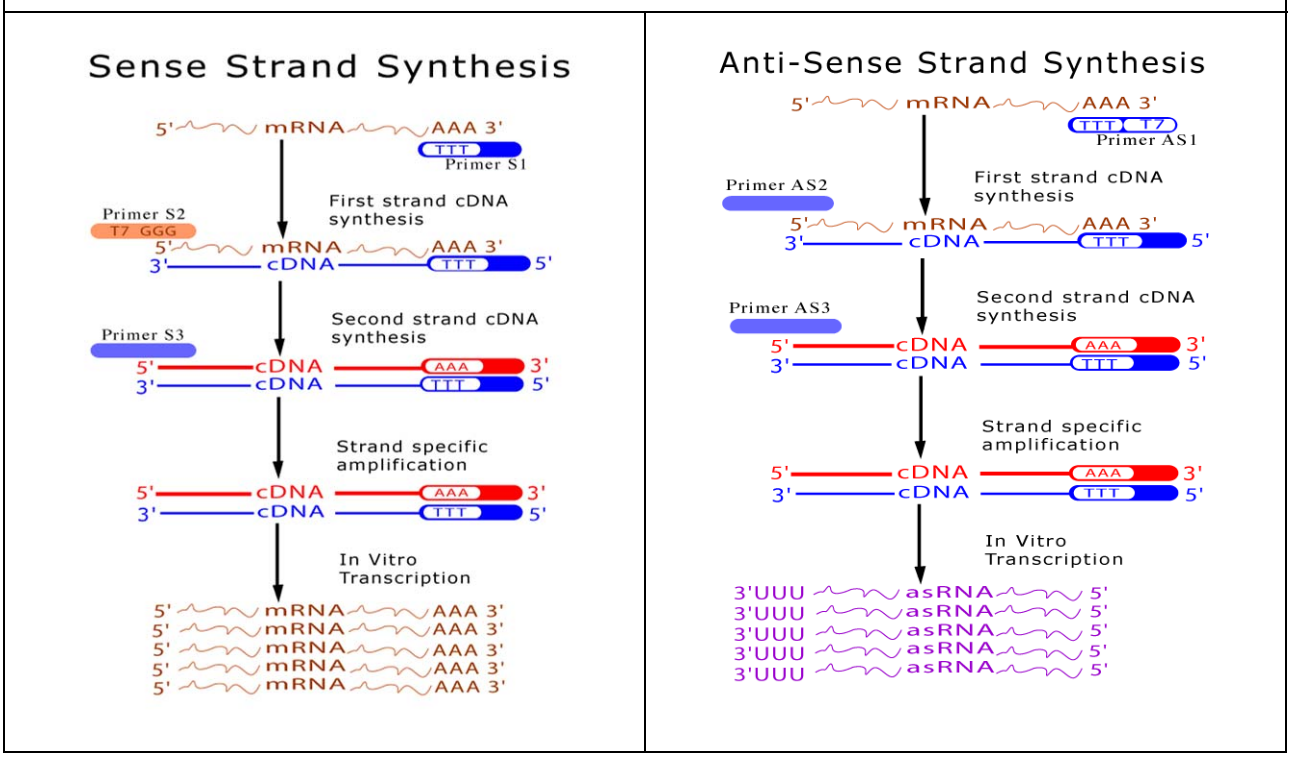
Omni-Array™ Amplification Kits

When the availability of total RNA becomes the limiting factor in performing certain experimental procedures, the Omni-RNA Amplification Kit provides a rapid and simple procedure for the generation of usable amounts of high quality sense or antisense strand RNA. The amplified RNA is suitable for microarrays, RT-PCR, cloning, *in vitro* transcription, and a multitude of other applications. Using this amplification protocol, microgram quantities of sense or antisense RNA can be produced from as little as 2 ng of total RNA in a single round of amplification.

The Omni-Array system offers the user two protocols for amplification of sense or antisense strand RNA depending on the initial amount of total RNA present. A single round protocol is sufficient to generate >10 μg of sense or antisense strand RNA from 100 ng of total RNA. When the initial amount of total RNA is less than 100 ng, a two round amplification protocol is recommended. Using two rounds of amplification >10 μg of sense or Antisense strand RNA can be generated from as little as 2 ng of total RNA. The single round protocol can easily be performed in less than 1 day while the 2 round protocol requires approximately 1 ½ days.

Omni-Array™ Amplification Strategy



Omni-Array™ mRNA Amplification Kits			
Product	**Catalog No.**	**Size**	**Price $**
Omni-Array ™ Sense strand mRNA amplification kit, 2 ng Version	08-0011-02	10 rxns	495.00
Omni-Array ™ Antisense strand mRNA amplification kit, 2ng Version	08-0021-02	10 rxns	495.00

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Appendix

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Amino Acids Abbreviations

Amino acid	3letter abrv.	1letter abrv.	MW
Alanine	Ala	A	89
Arginine	Arg	R	174
Asparagine	Asn	N	132
Aspartic Acid	Asp	D	133
Cysteine	Cys	C	121
Glutamic Acid	Glu	E	147
Glutamine	GlN	Q	146
Glycine	Gly	G	75
Histidine	His	H	155
Isoleucine	Ile	I	131
Leucine	Leu	L	131
Lysine	Lys	K	146
Methionine	Met	M	149
Phenylalanine	Phe	F	165
Proline	Pro	P	115
Serine	Ser	S	105
Threonine	Thr	T	119
Tryptophan	Trp	W	204
Tyrosine	Tyr	Y	181
Valine	Val	V	117

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The Standard DNA Genetic Code

First Position (5' end)	Second Position				Third Position (3' end)
	T	C	A	G	
T	TTT Phe [F]	TCT Ser [S]	TAT Tyr [Y]	TGT Cys [C]	T
	TTC Phe [F]	TCC Ser [S]	TAC Tyr [Y]	TGC Cys [C]	C
	TTA Leu [L]	TCA Ser [S]	TAA Stop [end]	TGA Stop [end]	A
	TTG Leu [L]	TCG Ser [S]	TAG Stop [end]	TGG Trp [W]	G
C	CTT Leu [L]	CCT Pro [P]	CAT His [H]	CGT Arg [R]	T
	CTC Leu [L]	CCC Pro [P]	CAC His [H]	CGC Arg [R]	C
	CTA Leu [L]	CCA Pro [P]	CAA Gln [Q]	CGA Arg [R]	A
	CTG Leu [L]	CCG Pro[P]	CAG Gln [Q]	CGG Arg [R]	G
A	ATT Ile [I]	ACT Thr [T]	AAT Asn [N]	AGT Ser [S]	T
	ATC Ile [I]	ACC Thr [T]	AAC Asn [N]	AGC Ser [S]	C
	ATA Ile [I]	ACA Thr [T]	AAA Lys [K]	AGA Arg [R]	A
	ATG Met [M] Start	ACG Thr [T]	AAG Lys [K]	AGG Arg [R]	G
G	GTT Val [V]	GCT Ala [A]	GAT Asp [D]	GGT Gly [G]	T
	GTC Val [V]	GCC Ala [A]	GAC Asp [D]	GGC Gly [G]	C
	GTA Val [V]	GCA Ala [A]	GAA Glu [E]	GGA Gly [G]	A
	GTG Val [V]	GCG Ala [A]	GAG Glu [E]	GGG Gly [G]	G

Start Codon
Stop Codon
Nonpolar Side Chain
Uncharged Polar Side Chain
Charged Polar Side Chain

IUB Standard Amino Acid Codes			
[A] Ala: Alanine	[C] Cys: Cysteine	[D] Asp: Aspartic acid	[E] Glu: Glutamic acid
[F] Phe: Phenylalanine	[G] Gly: Glycine	[H] His: Histidine	[I] Ile: Isoleucine
[K] Lys: Lysine	[L] Leu: Leucine	[M] Met: Methionine	[N] Asn: Asparagine
[P] Pro: Proline	[Q] Gln: Glutamine	[R] Arg: Arginine	[S] Ser: Serine
[T] Thr: Threonine	[V] Val: Valine	[W] Trp: Tryptophan	[Y] Tyr: Tyrosine

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Common Conversions of Nucleic Acids

Molar Conversions
1µg of 1000 bp DNA = 1.52pmol
1µg of pUC18/19 DNA (2686 bp) = 0.57pmol
1µg of pBR322 DNA (4361 bp) = 0.35pmol
1µg of SV40 DNA (5243 bp) = 0.29pmol
1µg of PhiX174 DNA (5386 bp) = 0.28pmol
1µg of M13mp18/19 DNA (7250 bp) = 0.21pmol
1µg of lambda phage DNA (48502 bp) = 0.03pmol
1pmol of 1000 bp DNA = 0.66µg
1pmol of pUC18/19 DNA (2686 bp) = 1.77µg
1pmol of pBR322 DNA (4361 bp) = 2.88µg
1pmol of SV40 DNA (5243 bp) = 3.46µg
1pmol of PhiX174 DNA (5386 bp) = 3.54µg
1pmol of M13mp18/19 DNA (7250 bp) = 4.78µg
1pmol of lambda phage DNA (48502 bp) = 32.01µg

Spectrophotometric Conversions
1 A_{260} of dsDNA = 50µg/ml = 0.15mM (in nucleotides)
1 A_{260} of ssDNA = 33µg/ml = 0.1mM (in nucleotides)
1 A_{260} of ssRNA = 40µg/ml = 0.12mM (in nucleotides)
1mM (in nucleotides) of dsDNA = 6.7 A_{260} units
1mM (in nucleotides) of ssDNA = 10.0 A_{260} units
1mM (in nucleotides) of ssRNA = 8.3 A_{260} units
The average MW of a deoxyribonucleotide base = 333 Daltons
The average MW of a ribonucleotide base = 340 Daltons

Reference

1. *Sambrook, J. et al,. (1989) Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Laboratory. Cold Spring Harbor, N.Y.*

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Estimation of Ends (3' or 5') Concentration

Circular DNA
pmol ends = pmol DNA x number of cuts x 2
Linear DNA
pmol ends = pmol DNA x (number of cuts x 2 + 2)
1µg of 1000 bp DNA = 3.04pmol ends
1µg of linear pUC18/19 DNA = 1.14pmol ends
1µg of linear pBR322 DNA = 0.7pmol ends
1µg of linear SV40 DNA = 0.58pmol ends
1µg of linear PhiX174 DNA = 0.56pmol ends
1µg of linear M13mp18/19 DNA = 0.42pmol ends
1µg of lambda phage DNA = 0.06pmol end

Common Conversions of Oligonucleotides

Molecular Weight
MW = 333 x N
Concentration of Oligonucleotides
C (µM or pmol/µl) = A_{260} / (0.01 x N) C (ng/ml) = (A_{260} x MW) / (0.01 x N)

MW - molecular weight, Da
A_{260} - absorbance at 260nm
N - number of bases

Melting Temperature of Duplex DNA and Oligonucleotides

For Duplex Oligonucleotide shorter than 25 bp (1)
T_m = 2(A+T) + 4(C+G)
A, T, C, G - number of respective bases.

Presence of m^5C in oligonucleotide increases the melting temperature of duplex. m^4C and m^6A have an opposite effect (2, 3).
For Duplex DNA longer than 25 bp (4)
T_m=81.5°C+16.6log(M_{NaCl})+0.41(%GC)-(500/N)-0.65(%formamide)
N - number of bp
M_{NaCl} - molar concentration of NaCl

References

1. *Thein, S.L., Wallance, R.B., Human Genetic Diseases: a practical approach, IRL Press, Herndon, Virginia, 33-50, 1986.*
2. *Butkus, V., Klimasauskas, S., Petrauskiene, L., Maneliene, Z., Janulaitis, A., Minchenkova, L.E. and Schyolkina, A.K., Nucleic Acids Res., 20, 8467-8478, 1987.*
3. *Jurgaitis, A., Butkus, V., Klimasauskas, S., Janulaitis, A., Bioorganicheskaya Khimiya, 14, 158-165, 1988.*
4. *Bolton, E.T., McCarthy, B.J., Proc. Natl. Acad. Sci. USA 48, 1390-1397, 1962.*

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Commonly Used Media, Stock Solutions and Buffers

Growth Media

LB Medium, per liter:

Tryptone	10g
Yeast extract	5g
NaCl	10g
H_2O	to 1 liter
Adjust pH to 7.0	

Low Salt LB Medium, per liter:

Tryptone	10g
Yeast extract	5g
NaCl	5g
H_2O	to 1 liter
Adjust pH to 7.0	

Terrific Broth Medium, per liter:

Tryptone	12g
Yeast extract	24g
Glycerol	4ml
Add H_2O	to 900ml
Autoclave, cool to 60°C or less before adding 100ml of filter sterilized 10X TB phosphate (0.17M KH_2PO_4, 0.72M K_2HPO_4).	

SOB Medium, per liter:

Tryptone	20g
Yeast extract	5g
NaCl	0.5g
250mM KCl	10ml
H_2O	to 900ml
Adjust pH to 7.0 and add H_2O to 990ml.	
Autoclave, cool to room temperature and add 10ml of sterile solution of 1M $MgCl_2$ before use.	

SOC Medium, per liter:

SOB Medium (1 liter) with the addition of 20ml filter sterilized 1M glucose.

M9 Minimal Medium, per liter:

5X M9 salts	200ml
Sterile H_2O	to 1 liter
1M $MgSO_4$	2ml
20% glucose	20ml
1M $CaCl_2$	0.1ml

5X M9 Salts, per liter:

$Na_2HPO_4x7H_2O$	64g
KH_2PO_4	15g
NaCl	2.5g
NH_4Cl	5g

Stock Solutions

10M Ammonium Acetate:

Ammonium acetate	385.4g
H_2O	to 500ml

1M $CaCl_2$:

$CaCl_2x2H_2O$	147g
H_2O	to 1 liter

100X Denhardt Solution:

Ficoll 400	10g
Polyvinylpyrrolidone	10g
Bovine serum albumin	10g
H_2O	to 500ml
Filter sterilize and store at -20°C in 25ml aliquots.	

1M Dithiothreitol (DTT):

DTT	15.45g
H_2O	to 100ml
Store at -20°C	

0.5M EDTA (ethylenediamine tetraacetic acid) (pH 8.0):

$Na_2EDTAx2H_2O$	186.1g
H_2O	to 700ml
Adjust pH to 8.0 with 10M NaOH	(~50ml)
H_2O	to 1 liter

10mg/ml Ethidium Bromide:

Ethidium bromide	0.2g
H_2O	to 20ml
Mix well and store at 4°C in dark.	
CAUTION: Ethidium bromide is a mutagen and must be handled carefully.	

1M KCl:

KCl	74.6g
H_2O	to 1 liter

Buffers

10X Stock Phosphate-buffered Saline (PBS), per liter:

NaCl	80g
KCl	2g
Na_2HPO_4	14.4g
KH_2PO_4	2.4g
H_2O	to 800ml
HCl	to pH 7.4
H_2O	to 1 liter

20X SSC, per liter:

NaCl	175.3g (3M)
$Na_3citratexH_2O$	88.2g (0.3M)
H_2O	to 800ml
Adjust pH to 7.0 with 1M HCl	
H_2O to 1 liter	

20X SSPE, per liter:

NaCl	175.3g (3M)
$NaH_2PO_4xH_2O$	27.6g (0.2M)
Na_2EDTA	7.4g (0.02M)
H_2O	to 800ml
Adjust pH to 7.4 with 10M NaOH	
H_2O to 1 liter	

5X SDS Electrophoresis Buffer, per liter:

Tris base	15.1g
Glycine	72.0g
SDS	5.0g
H_2O	to 1 liter
Dilute to 1X or 2X for working solution, as appropriate.	
Store up to 1 month at 0°C to 4°C.	
Do not adjust the pH of the solution, as the solution is pH 8.3 when diluted.	

50X TAE (Tris/acetate/EDTA) Electrophoresis Buffer, per liter:

Tris base	242g
Glacial acetic acid	57.1ml
0.5M EDTA (pH 8.0)	100ml
H_2O	to 1 liter
Adjust pH to ~8.5	

10X TBE (Tris/borate/EDTA) Electrophoresis Buffer, per liter:

Tris base	108g
Boric acid	55g
0.5M EDTA (pH 8.0)	40ml
H_2O	to 1 liter

10X TPE (Tris/phosphate/EDTA) Electrophoresis Buffer, per liter:

Tris base	108g
Phosphoric acid (85%)	15.5ml
0.5M EDTA (pH 8.0)	40ml
40ml	to 1 liter

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Additives		1M $MgCl_2$:		TE (Tris/EDTA) Buffer, pH 7.4, 7.6 or 8.0, per liter:	
Antibiotics: Ampicillin Chloramphenicol Kanamycin Tetracycline	 to 50µg/ml to 20µg/ml to 30µg/ml to 12µg/ml	$MgCl_2x6H_2O$ H_2O	20.3g to 100ml	1M Tris, pH 7.4, 7.6 or 8.0 0.5M EDTA (pH 8.0) H_2O	10ml (10mM) 2ml (1mM) to 1 liter
Galactosides: X-Gal IPTG	 to 20µg/ml to 0.1mM				
Media containing agar or agarose:		1M $MgSO_4$:			
Agar (for plates) Agar (for top agar) Agarose (for plates) Agarose (for top agarose)	15g per liter 7g per liter 15g per liter 7g per liter	$MgSO_4x7H_2O$ H_2O	24.6g to 100ml		
		5M NaCl:			
		NaCl H_2O	292g to 1 liter		
		10M NaOH: NaOH H_2O	 400g to 1 liter		
		1M Tris-HCl [tris(hydroxymethyl)aminomethane]:			
		Tris base H_2O Adjust to desired pH with concentrated HCl. Mix and add H_2O to 1 liter	121g to 800ml		
		3M Sodium Acetate (pH 5.2 and 7.0) (1):			
		Sodium acetate. $3H_2O$ H_2O. Adjust the pH to 5.2 with glacial acetic acid or adjust the pH to 7.0 with dilute acetic acid. H_2O to 1 liter	408.1g to 800ml		

References

1. *Sambrook, J., Fritch, E.F., Maniatis, T.,Molecular Cloning: A Laboratory Manual, second edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, A.1-B.25, 1989.*
2. *Current Protocols in Molecular Biology, vol. 1 (Ausubel, F.M., et al., ed.), John Wiley & Sons, Inc., Brooklyn, New York, 1.1.1-1.1.4, 1999.*
3. *Current Protocols in Molecular Biology, vol. 4 (Ausubel, F.M., et al., ed.), John Wiley & Sons, Inc., Brooklyn, New York, A.2.1-A.2.6, 1999.*

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DNA Migration in Agarose and Polyacrylamide Gels

Recommended Gel Percentages for Separation of Linear DNA			
Agarose gel, %	Range of separation,bp	Polyacryl-amide gel, %	Range of separation,bp
0.5	1,000-30,000	3.5	100-1,000
0.7	800-12,000	5.0	80-500
1.0	500-10,000	8.0	60-400
1.2	400-7,000	12.0	40-200
1.4	200-4,000	20.0	5-100
2.0	50-2,000		

Dye Migration in Polyacrylamide Non-denaturing Gels		
Gel, %	Bromophenol blue (Size of the fragments in nucleotides)	Xylene cyanol
3.5	100	460
5.0	65	260
8.0	45	160
12.0	20	70
15.0	15	60
20.0	12	45

Dye Migration in Polyacrylamide Denaturing Gels		
Gel, %	Bromophenol blue (Size of the fragments in nucleotides)	Xylene cyanol
5.0	35	140
6.0	26	106
8.0	19	75
10.0	12	55
20.0	8	28

DNA Size Migration with Sample Loading Dyes			
Agarose concentration, %	Xylene cyanol	Bromophenol blue	Orange G
0.7-1.7	~4000bp	~300bp	~50bp
2.5-3.0	~800bp	~100bp	~30bp

Reference

1. *Sambrook, J., et al., Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Laboratory. Cold Spring Harbor, N.Y., 1989.*

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International System of Unit Prefixes

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

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Physical Constants of the Nucleoside Triphosphates and Related Compounds

Compound	MW	lambda$_{max}$*,nm	e**
ATP	507	259	15400
CTP	483	271	9000
GTP	523	253	13700
UTP	484	262	10000
dATP	491	259	15200
dCTP	467	271	9300
dGTP	507	253	13700
dTTP	482	267	9600
ddATP	475	261	15200
ddCTP	451	281***	13100
ddGTP	491	253	13600
ddTTP	466	267	9600
NAD	664	260	18000
NADH	665	338****	6200
NADP	743	260	18000
NADPH	745	260	18000

* determined at pH 7.0
** extinction coefficient (absorbance at lambda$_{max}$ for 1M solution at pH 7.0)
*** determined at pH 2.0
**** determined at pH 10.0

Conversion Formula
C = A / e x 10E3
C - mM concentration of compounds
A - observed absorbance at lambda$_{max}$
e - extinction coefficient

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Physical Properties of Some Common Radioisotopes

Radioisotope	Half-life	Specific Activity (MBq/mmol)
^{32}P	14.3 days	10E2-10E7
^{33}P	25.4 days	10E2-10E7
^{35}S	87.4 days	10-10E7
^{131}I	8.06 days	10E3-10E5
^{125}I	60 days	10E3-10E7
^{14}C	5730 years	10-10E3
^{3}H	12.43 years	10E3-10E6

Summary of Useful Conversion
1Becquerel (Bq) = 1 disintegration per second = 2.7x10E-11Curies (Ci)
1Ci = 3.7x10E10Bq = 37GBq = 2.22x10E12 disintegrations per minute (dpm)
1mCi = 37MBq = 2.22x10E9dpm
1μCi = 37kBq = 2.22x10E6dpm
1GBq = 27mCi
1MBq = 27μCi
1kBq = 27nCi

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Size and MW of Various Nucleic Acids

Nucleic acid	Length in bases or base pairs	MW, Daltons
RNA		
tRNA (E.coli)	75	2.5 x 10E4
5S rRNA	120	3.6 x 10E4
16S rRNA	1700	5.5 x 10E5
18S rRNA	1900	6.1 x 10E5
23S rRNA	3700	1.2 x 10E6
28S rRNA	4800	1.6 x 10E6
DNA		
pBR322 DNA	4361	2.8 x 10E6
SV40	5243	3.5 x 10E6
PhiX174	5386	3.6 x 10E6
Adenovirus 2 (Ad2)	35937	2.8 x 10E7
Lambda phage	48502	3.1 x 10E7
Escherichia coli	4.7 x 10E6	3.1 x 10E9
Saccharomyces cerevisiae	1.5 x 10E7	9.9 x 10E9
Dictyostelium discoideum	5.4 x 10E7	3.6 x 10E10
Arabidopsis thaliana	7.0 x 10E7	4.6 x 10E10
Caenorhabditis elegans	8.0 x 10E7	5.3 x 10E10
Drosophila melanogaster	1.4 x 10E8	9.2 x 10E10
Gallus domesticus (chicken)	1.2 x 10E9	7.9 x 10E11
Mus musculus (mouse)	2.7 x 10E9	1.8 x 10E12
Rattus norvegicus (rat)	3.0 x 10E9	2.0 x 10E12
Xenopus laevis	3.1 x 10E9	2.0 x 10E12
Homo sapiens	3.3 x 10E9	2.2 x 10E12
Zea mays	3.9 x 10E9	2.6 x 10E12
Nicotiana tabacum	4.8 x 10E9	3.2 x 10E12

Reference

1. *Ausubel, F.M., et al., Current Protocols in Molecular Biology, John Wiley and Sons, New York, 1988.*

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Temperature Dependence of the pH for Commonly Used Buffers

Buffer System	pK_a / 20°C	[Delta]pK_a / 10°C
MES	6.15	-0.110
ADA	6.60	-0.110
PIPES	6.80	-0.085
ACES	6.90	-0.200
BES	7.15	-0.160
MOPS	7.20	-0.013
TES	7.50	-0.200
HEPES	7.55	-0.140
TRICINE	8.15	-0.210
TRIS	8.30	-0.310
BICINE	8.35	-0.180
GLYCYLGLYCINE	8.40	-0.280

Reference

1. Good, N.E., Biochemistry 5, 467-476, 1986.

Temperature Dependence of the pH of 50mM Tris-HCl Solutions

4°C	25°C	37°C
8.1	7.5	7.2
8.2	7.6	7.3
8.3	7.7	7.4
8.4	7.8	7.5
8.5	7.9	7.6
8.6	8.0	7.7
8.7	8.1	7.8
8.8	8.2	7.9
8.9	8.3	8.0
9.0	8.4	8.1
9.1	8.5	8.2
9.2	8.6	8.3
9.3	8.7	8.4
9.4	8.8	8.5

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DNA Extinction Coefficients

Nucleotide	(A260 / mol)
A	15.4
T	8.7
G	11.5
C	7.4
I	7.2**
I	10.7*
Neighbors	**(A260 / mol)**
AA	13.7
AT	11.4
AG	12.5
AC	10.6
AI	9.3**
AN	12.2*
TA	11.7
TT	8.4
TG	9.5
TC	8.1
TI	8.1**
TN	9.4*
GA	12.60
GT	10.0
GG	10.8
GC	8.8
GI	8.8**
GN	10.5*
CA	10.6
CT	7.6
CG	9.0
CC	7.3
CI	7.2**
CN	8.6*
IA	9.3**
IT	8.4**
IG	8.8**
IC	7.1**
II	6.8**
IN	8.4*
NA	12.1*
NT	9.4*
NG	9.4*
NC	8.7*
NI	8.7*
NN	9.9*

Handbook of Biochemistry and Molecular Biology (1975) Fasman G.D., ed., 3rd edition, Nucleic Acids - Vol. 1, pp 589, CRC Press, Cleveland, OH.

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MW and Tm Calculation

Base	Base Abbreviation	MW	EC	Notes
DeoxyAdenosine	A	313.21	15.4	
DeoxyCytosine	C	289.19	7.4	
DeoxyGuanosine	G	329.21	11.5	
Thymidine	T	304.2	8.7	
Inosine	I	314.2	7.2	
A+G+T+C	N	308.95	10.70	
A+G	R	321.21	13.45	
C+T	Y	296.69	8.05	
A+C	M	301.2	11.40	
G+T	K	316.7	10.10	
G+C	S	309.2	9.45	
A+T	W	308.71	12.05	
A+T+C	H	302.2	10.5	
G+T+C	B	307.53	9.20	
G+A+T	D	315.54	11.86	
G+A+C	V	310.53	11.43	
phosphate	P	79.98	0	
Other	X	0	0	add mw of the Modification
deoxy uridine	U	290.17	9.9	

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Compound	MW	$lambda_{max}$*,nm	e**
Size= Total number of bases.			
%GC= (G+C)/Size			
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) + (Yx 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 x315.54) + (V x 310.53) + (P x 79.98) + (U x 290.17) –62			
Tm For Oligos shorter than 25 bp = 2(A+T) + 4 (C+G)			
For longer oligos: Reference Bolton, Et and McCarthy, B.J. (1962) PNAS 48: 139-1397			
Tm=81.5 – 16.6 + (0.41 x %GC)) – 600 / size			
EC =			
Formula for Tm Calculation			
Tm = 81.5 + 16.6 x Log10[Na+] + 0.41 (%GC) – 600/size			
[Na+] is set to 100 mM			
Example: 5'-ATGCATGCATGCATGCATG3' 20mer; GC=50%; AT= 50%			
Tm = 81.5 + 16.6 x Log10[0.100] + 0.41 x 50 – 600/20			
Tm = 81.5 - 16.6 + 0.41 x 50 – 600/20			
Tm = 81.5 - 16.6 + 20.5 – 30			
Tm = 64.9 + 20.5 – 30			
Tm = 85.40 – 30			
Tm = 55.4°C			
Tm 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
Follow IUB 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 Y=C+T M=A+C K=G+T
S=G+C W=A+T H=A+T+C B=G+T+C
D=G+A+T V=G+A+C N=A+C+G+T.
Inosine=I

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