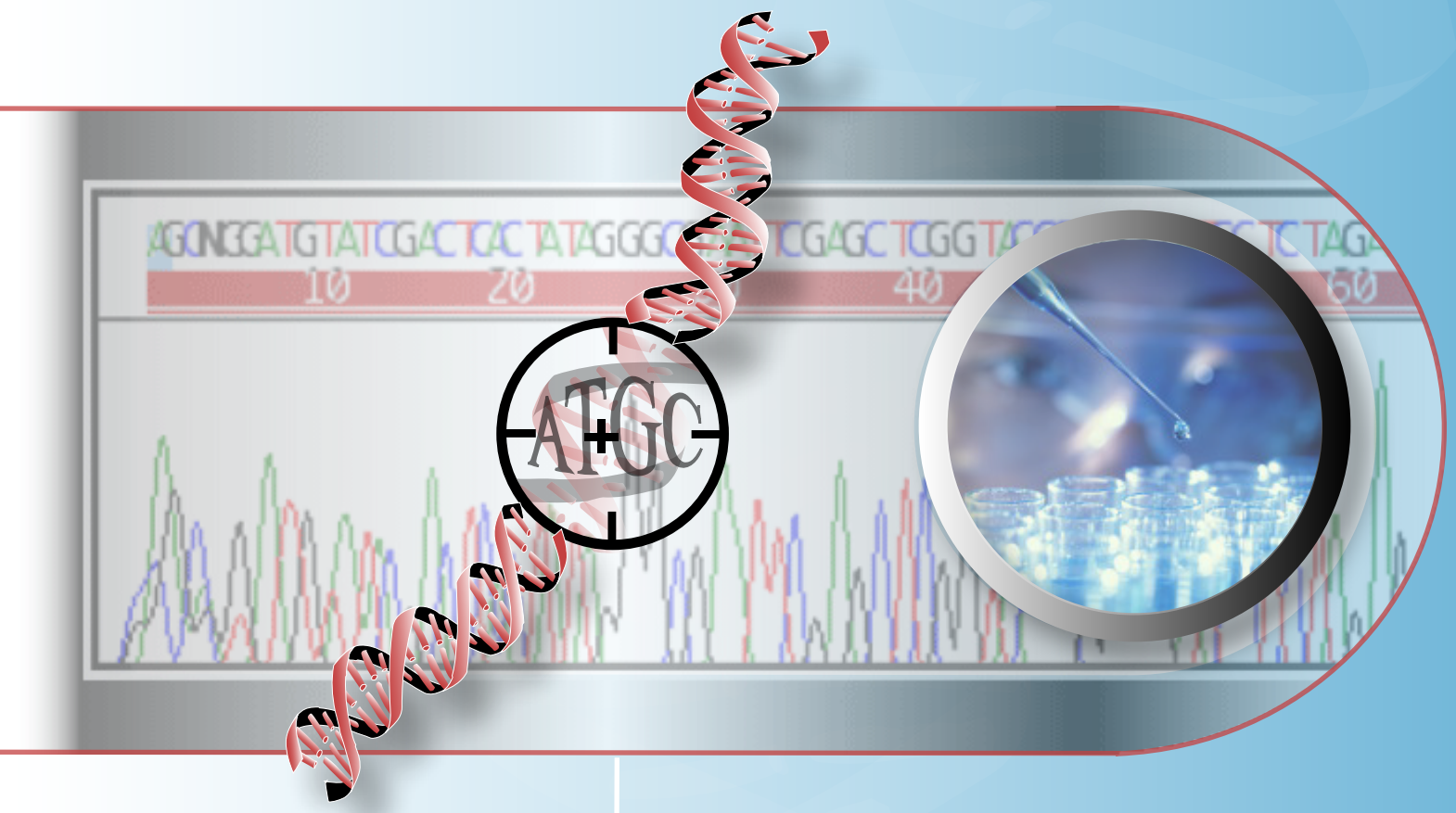
# Retrogen, Inc.

Solutions For Functional Genomics




www.retrogen.com

# Custom DNA Sequencing

Our sequencing facility is equipped with state-of-the-art technologies including next generation capillary ABI 3730 sequencers and advanced bioinformatics systems which enable us to provide you with consistent and accurate sequencing data at very competitive prices. You can also count on our dedicated technical staff, with years of experience in genomics and genetic analysis research, to assist you in accomplishing your projects faster. Without any additional expenses, you receive the following on every project:

- Highest quality sequencing data - up to 1,000 bases per reaction
- Longest reads allow you to finish your project faster with few custom primers
- Fully automated sample loading and data tracking
- Consistent data from every sequencing run
- Maximum sensitivity with a smaller amount of template
- Fast turnaround service with data delivered within 24 hours
- High throughput capacity with more than 20,000 reactions per week
- Dedicated & friendly customer service and FREE pickup for local customers
- Get professional consultation from our highly experienced genomics staff
- Please call for special discounts on large orders

## Submission Template Requirement

**Plasmid DNA Preparation:**

In order to obtain good sequence data, the quality of the template DNA is extremely important. The following methodologies are recommended for preparing plasmid DNA for automated sequencing: Cesium chloride (CsCl) banding, Retrogen plasmid kit, Qiagen plasmid kit, Wizard Plus plasmid kit, and other commercial kits available. Magnesium ions are essential for DNA polymerase activity. Template DNA and primer should be re-suspended in water or a buffer containing no more than 0.1 mM EDTA. The introduction of large amounts of EDTA in template DNA or primer will result in weak signals or short reads. The optimal concentration of the template should be at 0.2 µg/µl.

**PCR Product Preparation:**

The purity of the PCR product is very crucial to obtaining good sequence data. Any PCR primers and/or dNTPs remaining in the PCR product will adversely affect the quality of the sequence data. If the PCR product has a unique band, it can be purified by the size exclusion method, such as PCR purification kit from Retrogen or Qiagen and Pharmacia offers. If the PCR product has more than one band, the PCR product should be run on the agarose gel in order to isolate the desired band needed for sequencing. The band can be purified by the Gel Extraction kit from Retrogen or Qiagen. The purified PCR products can then be quantitated either on the gel or spectrophotometer.

**Recommended DNA Template and Primer Quantities for Each Reaction:**
*We recommend 2x the amount of DNA in case we need to repeat the reactions.*

DNA and Primer Separated

| DNA | Amount | 2X Amount | Concentration |
|---|---|---|---|
| PCR product | 50 ng | 100 ng | 5-20 ng/µl |
| Single Stranded Plasmid | 250 ng | 500 ng | 50-100 ng/µl |
| Double Stranded Plasmid | 300 ng | 600 ng | 50-200 ng/µl |
| BAC | 2000 ng | 4000 ng | 100-200 ng/µl |
| Custom Primer | 5 µl | 10 µl | 10 picomoles/µl |

DNA and Primer Combined

| DNA | Amount | Primer | Total Vol. | 2X DNA | 2X Primer | 2X Total Vol. |
|---|---|---|---|---|---|---|
| PCR product | 50 ng | 10 pmoles | 10 µl | 100 ng | 20 pmoles | 20 µl |
| Single stranded plasmid | 250 ng | 10 pmoles | 10 µl | 500 ng | 20 pmoles | 20 µl |
| Double stranded plasmid | 300 ng | 10 pmoles | 10 µl | 600 ng | 20 pmoles | 20 µl |
| BAC | 2000 ng | 10 pmoles | 10 µl | 4000 ng | 20 pmoles | 20 µl |



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# Quality Assurance

Since its inception, Retrogen has been dedicated to customer satisfaction. We handle both large and small projects while providing expert technical advice. Our highly experienced technical staff will help you determine the strategy that best suits your particular needs. If you are not completely satisfied with the results after consultation with the laboratory director, the process will be repeated at no additional charge.

**Repeat Policy:** If you request a failed reaction repeated with a different primer, new DNA, or special conditions, Retrogen will bill you for a new reaction. If samples consistently fail, Retrogen will repeat one failed reaction to confirm reactions were initially set up correctly. The following are examples of such cases:

- The same template fails with different primers or the same primers.
- Results are inconsistent with same template and different primers i.e., some succeed and others fail.
- A particular primer consistently fails with multiple templates.

For 96-well plates, Retrogen uses automated procedures to set up reactions. For optimal results, submit DNA concentrations that are consistent throughout the plate. For plasmid DNA, the concentration should be submitted at approximately 0.2 µg/µl. For PCR products, concentration should be submitted at approximately 0.02 µg/µl. Please refer to Submission Template Requirements for more information.

Since sequencing procedures for 96-well plates are automated, we cannot optimize conditions for each well. For 96-well plates that fail to meet an 85% success rate in accordance with Retrogen's QC/QA criteria, 1 µl of 8 randomly picked samples will be run out on an agarose gel. After inspection of DNA concentration and quality, Retrogen will determine whether to repeat the reactions. If the repeated reactions are successful, the remainder of the failed reactions will also be repeated.

**Customer satisfaction** is very important to us. Retrogen has a great deal of experience in sequencing difficult templates. If your DNA contains a GC-rich region, repetitive sequence, or secondary structure, contact Retrogen for service information and pricing. In addition, feel free to contact customer service with any questions concerning our repeat policy or troubleshooting.

**Confidentiality:** Retrogen guaranties the complete confidentiality of all client information, project data, and biological samples. We provide a confidentiality agreement for each customer stating that Retrogen will not disclose any data or other information concerning the DNA samples to be sequenced to third parties, without prior express written approval from the client.

**Ordering:**
DNA sequencing samples be can be shipped overnight at ambient temperature. In order to protect your samples from getting crushed during transportation, please put them in a secondary container.

For local customers, we can arrange for our courier to pick up your samples at no additional charge. You can call, email, or request a pickup through our LIMS System or our website at http://www.retrogen.com/index/pickup_request2.htm

North San Diego County: Please have your pick up scheduled electronically or by telephone by 1:00 pm and have your samples in the appropriate pick up location by 1:30 pm. We will pick up your samples between 1:30 and 2:30.

South San Diego County: Please have your pick up scheduled electronically or by telephone and have your samples in the appropriate pick up location by 11:00 am for the first pick up. We will pick up your samples between 11:00 am and 1:00 pm. For the second pick up have your samples ready by 3:00 pm and we will pick up your samples between 3:00 and 5:00.

**You will receive your results the following morning.**

# Types of Sequencing Services

## Primer Extension Sequencing

**Result:** 800-1000 bp sequence data per reaction
**Starting Material:** Templates provided by customer
Primers provided by customer
Custom primers can be synthesized by Retrogen for faster results.

The following universal primers are available to you at no cost: T3, T7, Sp6, M13R, M13F, pGEX3', pGEX5', and BGH reverse.

**Method:** Primer-extension by using big-dye chemistry or other related chemistry from Applied Biosystems.

If the sequence data is unsatisfactory, the sequencing reaction is repeated with different conditions. The better result will be sent to the customer.

## Direct PCR Product Sequencing

**Result:** 700-1000 bp sequence data per reaction.
**Starting Material:** Purified PCR products from customer.
Primers provided by customer
**Method:** Primer-extension by using big-dye chemistry or other related chemistry from Applied Biosystems.

If the sequence data is unsatisfactory, the sequencing reaction is repeated with different conditions. The better result will be sent to the customer.

## Single Stranded DNA Sequencing

**Result:** Consensus sequence with > 99% accuracy.
A complete report of sequencing strategies and data.
**Starting Material:** Templates provided by customer
Initial primers provided by customer
Custom primers can be synthesized by Retrogen for faster results.

The following universal primers are available to you at no cost: T3, T7, Sp6, M13R, M13F, pGEX3', pGEX5', and BGH reverse.

**Method:** Design and synthesize internal primers at the optimal interval for better overlap.
Single stranded coverage of the template through multiple sequencing reactions.
Assemble and edit the contigs?
Resolve most problematic regions.
The consensus sequence is generated with accuracy greater than 99 %.



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# Types of Sequencing Services




- Double Stranded DNA Sequencing

**Result:** Finished consensus sequence with greater than 99.99% accuracy.
A complete report of sequencing strategies and data.

**Starting Material:** Templates provided by customer
Primers provided by customer
Custom primers can be synthesized by Retrogen for faster results.

The following universal primers are available to you at no cost: T3, T7, Sp6, M13R, M13F, pGEX3', pGEX5', and BGH reverse.

**Method:** Design and synthesize internal primers.
Double stranded coverage of the template through multiple sequencing reactions.
Assemble and edit the contigs?
The problematic regions are completely determined.
The consensus sequence is generated with accuracy greater than 99.99%.

- BAC, PAC and P1 Ends Sequencing

**Result:** 400-600 bp sequence data

**Starting Material:** Templates provided by customer
Primers provided by customer

**Method:** Proprietary protocols in conjunction with big-dye chemistry or other related chemistry from Applied Biosystems.

If the sequence data is unsatisfactory, the sequencing reaction is repeated with different conditions. The better result will be sent to the customer.

- cDNA EST Library Sequencing

**Result:** 700-1000 bp sequence data per reaction.

**Starting Material:** Bacterial cultures or templates provided by customer.
Primers provided by customer.
Custom primers can be synthesized by Retrogen for faster results.

The following universal primers are available to you at no cost: T3, T7, Sp6, M13R, M13F, pGEX3', pGEX5', and BGH reverse.

If the sequence data is unsatisfactory, the sequencing reaction is repeated with different condition. The better result will be sent to the customer.

**Method:** Primer-extension by using big-dye kit or other related chemistry from Applied Biosystems.
Use automated liquid sample handling instrument to prepare sequencing reactions.
The samples are cataloged and stored at -20°celcius.

# Types of Sequencing Services

## FDA Submission Sequencing

**Result:** Finished consensus sequence with 100 % accuracy.
A complete report of sequencing strategies and data.

**Starting Material:** Templates provided by customer
Primers provided by customer
Custom primers can be synthesized by Retrogen for faster results.

The following universal primers are available to you at no cost: T3, T7, Sp6, M13R, M13F, pGEX3', pGEX5', and BGH reverse.

**Method:** Use big-dye chemistry or other related chemistry from Applied Biosystems.
Complete double stranded coverage of the template through 4 fold-redundancy sequencing.
Resolve all problematic regions.
Assemble and edit the final sequence.

## Plasmid DNA Purification

**Result:** Purified plasmids suitable for automated sequencing, transfection, and other molecular methodologies.

**Starting Material:** Bacterial cultures or agar plates provided by customer.

**Method:** Protocols from RETROGEN Ultrapure MiniPrep Kit.

## PCR Product Purification

**Result:** Purified PCR fragments are suitable for automated sequencing.

**Starting Material:** Crude PCR products.
Expected size of the PCR product to be isolated and
Protocols from RETROGEN Ultrapure MiniPrep Kit.

## Shotgun Library Construction & Sequencing

**Result:** Original electropherograms
Consensus sequence on diskette

**Starting Material:** Shotgun sequencing strategies can be used to sequence a variety of samples including cosmids, BACs, PACs, and genomics.

**Method:** Randomly generated plasmid clones using nebulization techniques.

The random fragments (typically, 1-3 kb in length) are cloned into a standard vector with common restriction sites. Sequence data is obtained from each end of the random clones and assembled to provide up to 100% coverage of the entire clone. Contig alignment of each gel read.


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# Sequencing Prices and Turnaround

| Type of Service | Catalog No. | Price |
|---|---|---|
| Primer Extension Sequencing | 2000-01 | $3.40 - $15.00 per reaction |
| Direct PCR Product Sequencing | 2000-02 | $3.40 - $15.00 per reaction |
| Single Stranded DNA Sequencing | 2000-03 | $0.40 - $0.60 per base |
| Double Stranded DNA Sequencing | 2000-04 | $0.40 -$1.00 per bp |
| FDA Submission Sequencing | 2000-05 | $1.00 - $1.50 per bp |
| Sequencing Primer Synthesis | 2000-06 | $0.70 per base |
| Plasmid DNA Purification | 2000-08 | $2.00 - $5.00 per sample |
| Ready to Load Sequencing | 2000-10 | $5.00 per reaction |
| PCR Amplification and Purification | 2000-11 | $5.00 - $10.00 per sample |
| PCR Product Purification | 2000-12 | $5.00 - $10.00 per sample |
| Chromatogram Printout Sent by Mail | 2000-13 | $2.00 per chromatogram |
| BAC, PAC and P1 Ends Sequencing | 2000-15 | $10.00 - $20.00 per reaction |
| cDNA/Est Library Sequencing | 2000-16 | $3.40 - $15.00 per reaction |
| Oligo Design and Synthesis | 2000-21 | $25.00 - $40.00 per primer |
| Shotgun Library Construction | 2000-31 | Inquire |
| Subcloning | 2000-41 | $350.00 |
| Alignment and Editing | 2000-42 | $50.00 per kb |
| Transformations | 2000-43 | $5.00 per sample |
| Finishing and Assembly Services | 2000-44 | Inquire |
| Colony Picking 96 well | 2000-44 | Inquire |
| 384 Well Plate Sequencing | 2000-45 | Starts at $3.40 per reaction |
| 384 Well Plate PCR Purification | 2000-46 | $100 - $200 per plate |
| 96 Well Plate Sequencing | 2000-87 | $300 - $500 per plate |
| 96 Well Plate PCR Product Purification | 2000-88 | $100 - $200 per plate |

**Turnaround Times:**

| | |
|---|---|
| Ready to Load Reaction Service | 24 hours |
| Primer Extension Sequencing Service | 24 hours |
| Direct PCR Product Sequencing Service | 24 hours |
| Single Stranded Sequencing Service | 1 week per kb |
| Double Stranded Sequencing Service | 1-2 weeks per kb |
| Pac, Bac and P1 Ends Sequencing Service | 2-3 days |
| cDNA/EST Library Sequencing Service | Depends on library |
| FDA Submission Sequencing Service | 2 weeks per kb |
| Plasmid DNA Purification Service | 1 day |
| PCR Product Purification Service | 1 day |
| Shotgun Library Construction | Inquire |
| Subcloning | Inquire |





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# Custom Gene Synthesis

The availability of sequences of entire genomes has dramatically increased the number of protein targets, many of which will need to be overexpressed in cells other than the original source of DNA. Gene synthesis often provides a fast and economically efficient approach. The synthetic gene can be optimized for expression and constructed for easy mutational manipulation without regard to the parent genome. Yet design and construction of synthetic genes, especially those coding for large proteins, can be a slow, difficult and confusing process.

Through many years of experience in synthetic gene construction, Retrogen has developed a proprietary system by integrating software, instrumentation and protocols that allows us to complete any gene size at a very rapid turnaround time. With this proprietary system Retrogen can offer you complete gene synthesis service, from optimizing the codon to confirming the sequence of the synthetic gene at a very competitive price.

**Gene Synthesis Price:**
$1.25 - $1.50 per bp

**Gene Synthesis Turnaround:**

| | |
|---|---|
| Up to 1 kb | 2-3 Weeks |
| Larger than 1kb | Please call for turnaround |

Price depends on difficulty of gene and number of genes per order.
Discounts are available for mulitple-gene orders. Please call for custom pricing.

## Advantages to Retrogen's Custom Gene Synthesis:

- Optimized gene sequences
- All primers are designed and synthesized
- RNA secondary structures are minimized
- Restriction sites are added and/or removed
- We guarantee full sequence verification of both strands
- Fast Turnaround


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# Custom Gene Synthesis



## Specifications for Custom Gene Synthesis:

| | |
|---|---|
| Minimum Sequence Length:<br><br>Maximum Sequence Length: | 100 base pairs<br><br>15,000 base pairs |
| Gene Optimization Options: | Codon preference for host organism, secondary structure removal, GC content adjustment, addition or removal of restrictions sites. |
| Starting Material: | Email the sequence to gene@retrogen.com. |
| Gene Construction : | Proprietary gene synthesis software is used to design and construct the entire gene. The synthetic gene is cloned into a PCR blunt vector. Subcloning into a customer-provided vector is available at an additional charge. |
| DNA Sequencing: | Both strands are sequenced to verify the integrity of the synthetic gene. |
| Final Form of Construct: | 20 µg of lyophilized plasmid DNA containing the synthetic gene. |
| Results: | A complete report of gene construction. A plasmid map and printout of assembly report and chromatograms from automated sequencers. |

# Custom SNP Discovery & Resequencing Service

Single nucleotide polymorphisms (SNPs) are the most common variation responsible for genetic diversity between individuals, accounting for more than 85% of the variability. Recent advances in SNP identification and analysis have made SNP genotyping an invaluable tool to examine the variations responsible for disease susceptibility and drug responsiveness. SNP genotyping applications have recently extended into personalized health care, diagnostics, forensics, animal studies and agriculture. In order to address the needs of this rapidly growing SNP market, we at Retrogen employ a suite of integrated solutions to assist the customer accomplishing their SNP discovery projects from beginning to end.

Our SNP Discovery and Resequencing service starts out with the assay development by using commercial and proprietary informatics to align the cDNA sequence to the genomic sequence and choose a specific region for designing the primers. The primers are selected across the entire span of the targeted exon and are synthesized in our oligo synthesis department. All primer design and synthesis have to go through rigorous quality control to ensure that all the amplicons have sequencing data greater than Phred20 score. Exons are amplified from genomic DNA, and the resulting amplicons are purified for sequencing. The sequencing data is collected using fluorescent dye-terminator chemistry by fully automated ABI 3730xl sequencers. The electrophereograms are assembled with reference sequence and the potential SNPs are scored. Candidate SNPs with Phred20 score are validated by re-sequencing the opposite DNA strand to confirm the polymorphism.

## Features and Benefits:

- Comprehensive SNP Service
- Accurate Scoring SNPs
- Rapid Turnaround at a Competitive Price
- Assay Development and Validation
- Primer Design and Synthesis
- PCR Amplification and Purification
- Automated Fluorescent Dye-Terminator Sequencing
- Sequence Trace Alignment and Editing
- In Silico Mutation Identification
- Hard Copy Final Report


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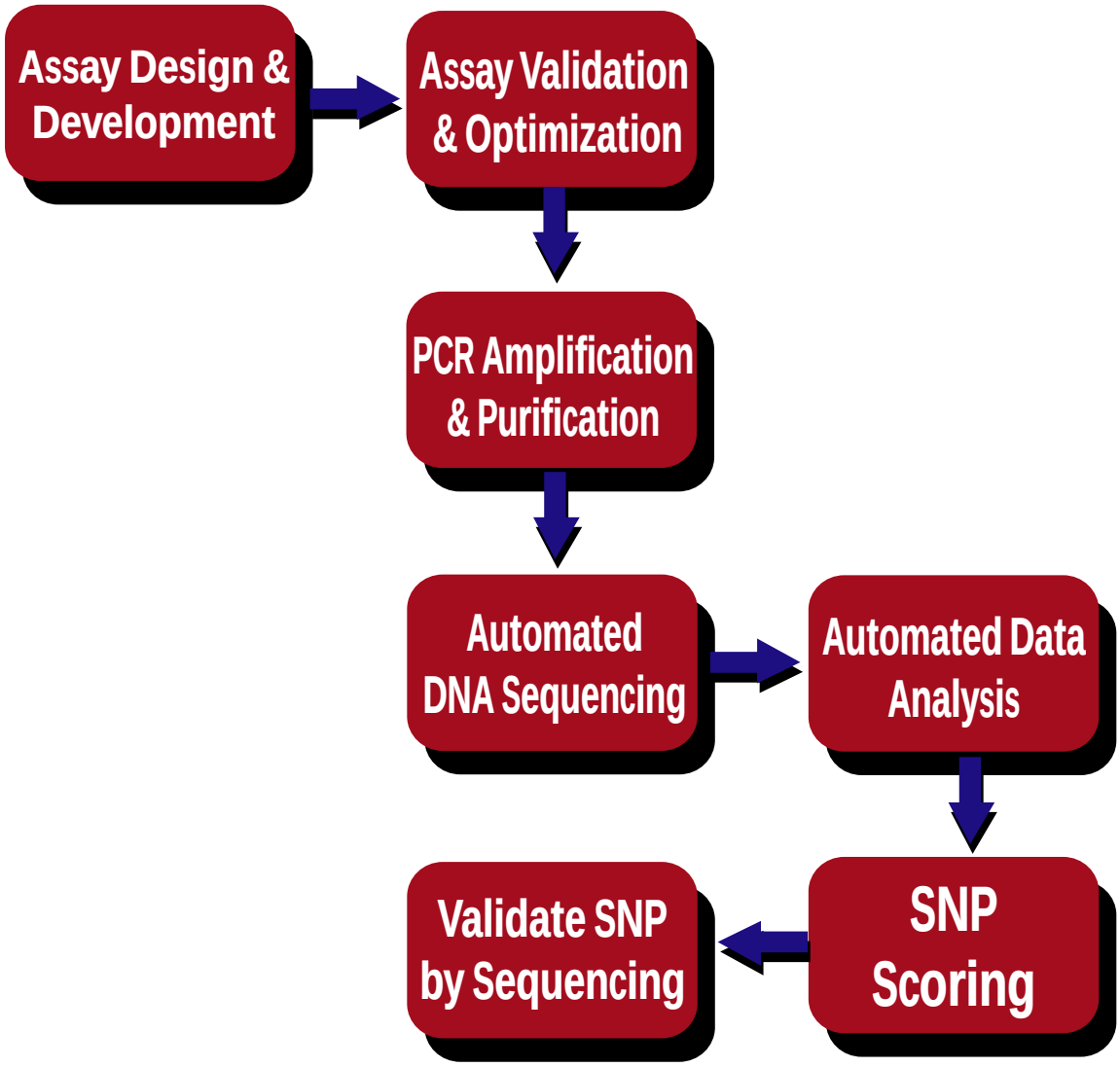
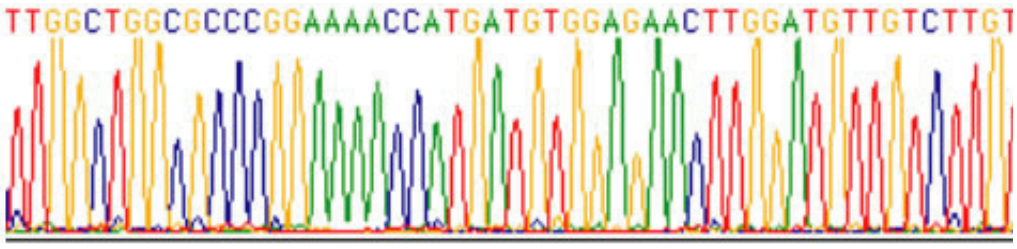
# Custom SNP Discovery & Resequencing Service



## SNP Discovery & Development

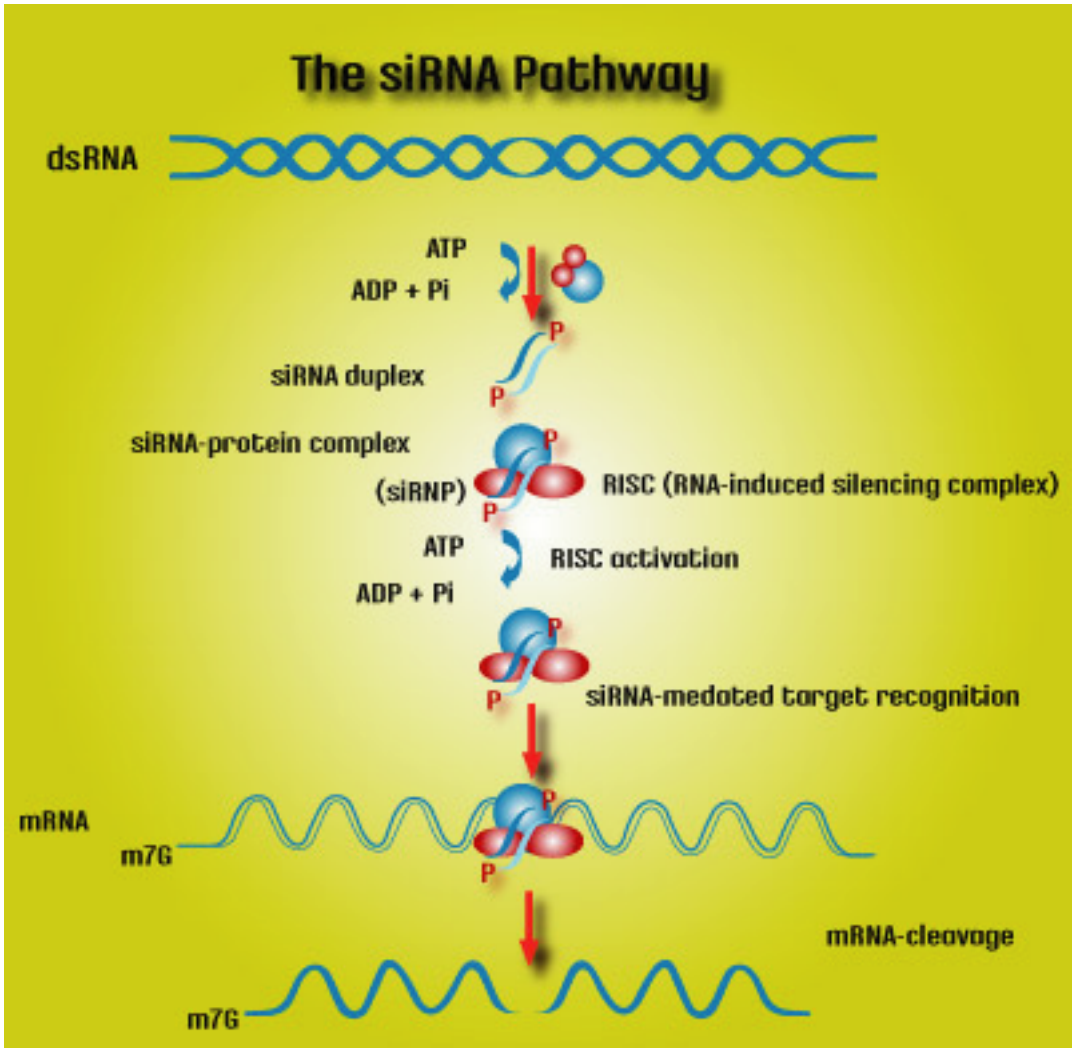



Example of Sequence Data:



# Custom siRNA Construction Services

Gene silencing of target mRNA by RNA interference (RNAi) has dramatically enhanced the arsenal of genetic tools that can be used to study gene function. RNAi is a process used by plants and invertebrates to specifically inactivate gene function. It has been shown that double-stranded RNA (dsRNA) complementary to particular messenger RNA (mRNA) can specifically inactivate gene function through the RNAi pathway in mammalian systems as well. Small interfering RNAs (siRNAs) are generated in vivo from long precursor molecules through the action of a specific RNAase III endonuclease called Dicer. Dicer initiates the cleavage of the precursor into ~22 nucleotide (nt) double-stranded duplexes with two nt 3'-overhangs and 5' phosphate termini termed short interfering RNAs (siRNAs). The siRNA is subsequently incorporated into the RNA induced silencing complex (RISC), a protein/RNA complex. The siRNA allows specific recognition of the mRNA target and the endonuclease function of the RISC results in cleavage of mRNA and thus loss of gene expression.





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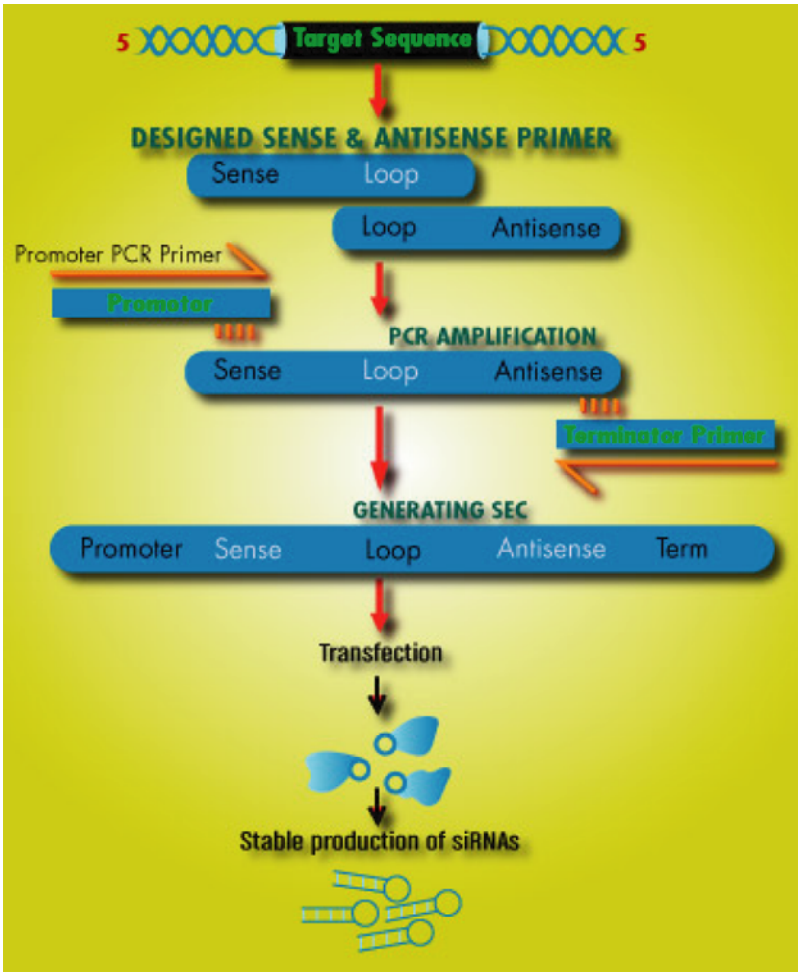
# siRNA Expression Cassette Construction

siRNA cassette is a PCR product which consists of a promoter and terminator sequence flanking a DNA insert encoding a hairpin siRNA. PCR can be used to rapidly produce different combinations of siRNA template and RNA polymerase promoter. The PCR products are directly transfected into mammalian cells resulting in functional expression of siRNA. This approach is the most cost-effective way for identifying the most potent and specific siRNA target sequences. This service includes constructing the siRNA expression cassette from your siRNA sequences and PCR amplification. At the completion of the project, you will receive 20 ug of siRNA. expression cassette and an experimental report.



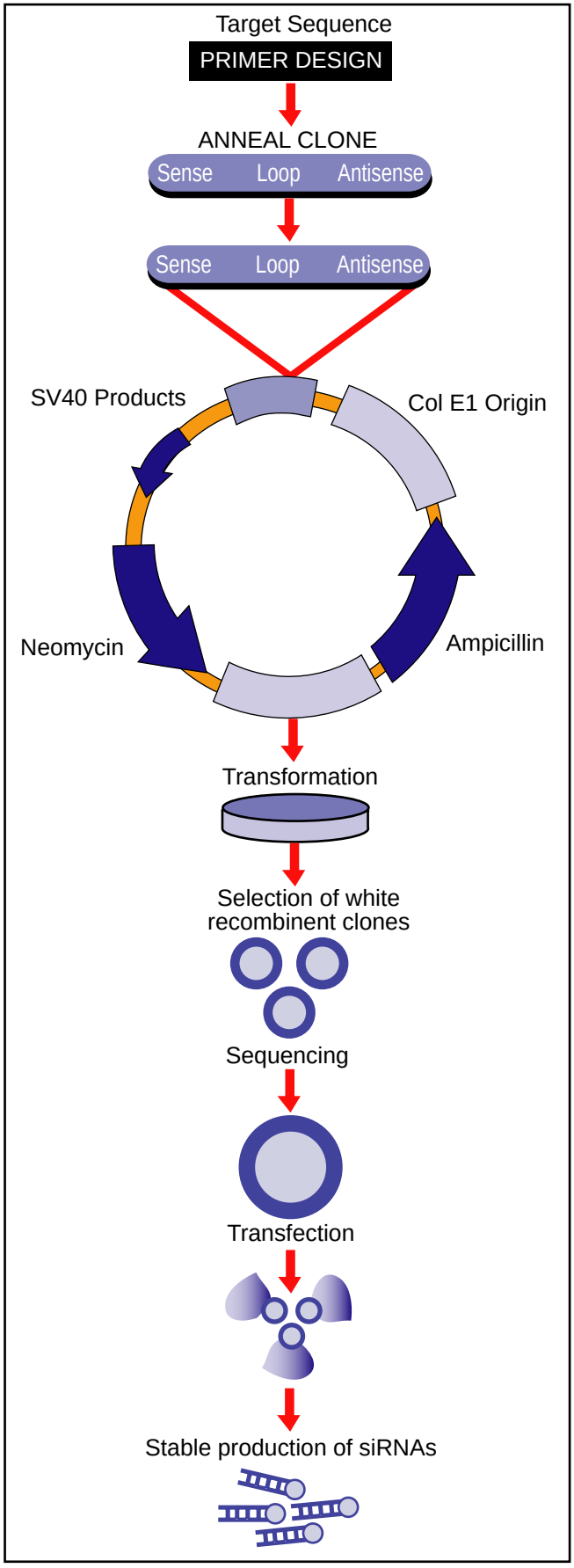

| Description | Catalog No. | Price |
|---|---|---|
| Custom siRNA ExpressionCassette | 300010 | $135.00 per SEC |

# siRNA Plasmid Construction

siRNA plasmid is another approach to express siRNA and inactivate gene function. Using DNA vector based siRNA approach, a small DNA sequence encoding a short hairpin RNA targeting the gene of interest is cloned into a commercially available vector.

The insert-containing vector can be transfected into the cells, and subsequently expresses the short hair-pin RNA. DICER processes the short hairpin into ~22 nt double-stranded duplexes and activates the RISC complex to cleave the mRNA target and inactivate the gene function. This service includes siRNA design, chemical synthesis, ligation, cloning, and double-stranded confirmation sequencing.

At the completion of the project, you will receive an experimental report and 20 ug of siRNA construct, ready for transfection.





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# Custom Mutagenesis

In vitro site-directed mutagenesis is an important methodology used to study protein structure-function relationships, identify intramolecular regions or amino acids that may mediate these functions, analyze gene expression and modify vectors. Many published mutagenesis methodologies labor intensive and technically difficult to perform. With many years of experience in mutational manipulation, Retrogen has developed its own methodology that allows us to finish any mutagenesis project at very fast turnaround time and at a very affordable price.

**The Benefits of Retrogen's Custom Mutagenesis:**

- Get Consultation from Our Experienced Scientists
- Get Your Mutagenesis Projects Done Fast at a Very Competitive Price
- We Sequence the Mutated Region to Ensure a 100% Accuracy
- 20 µg of Purified Plasmid Delivered is Ready to Use
- A Complete Report with a Chromatogram Printout is provided

**Starting Material:**
Please send 20 µg of plasmid DNA Email copies of vector map and sequence of the construct with indication of the bases that need to be changed to gene@retrogen.com

**Method:**
We use PCR site-directed method in conjunction with our proprietary protocols
We sequence the mutated region by automated fluorescent sequencing

**Final Form :**
20 µg of lyophilized plasmid DNA with the correct changes

**Documentation:**
A complete report of strategies and data
A printout of assembly report and chromatograms from automated sequencers

**Price:**
$200.00 - $500.00 per codon change
Includes both strands sequencing verification. Please call us for a discounted price if you have more than one mutant to be made

**Turnaround Time:**
Each mutant will take 2-3 weeks, including sequence verification

**Ordering:**
Make sure that the correct changes are indicated on the sequence printout of the construct
Email modified sequence and original sequence to gene@retrogen.com
Enclose 20 µg plasmid DNA and vector map
Send the package priority overnight to the below address:

# Custom Oligo Synthesis

Retrogen custom oligo synthesis is a premiere source for high quality oligos. Our proprietary high throughput platform allows us to produce thousands of quality oligos at a very competitive price. To ensure the quality, each oligo is checked by PAGE and delivered desalted and ready to use.

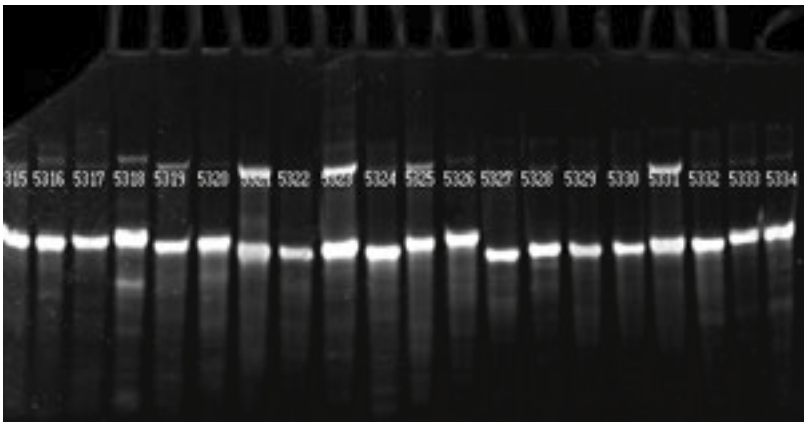
## Advantages of Ordering Oligos from Retrogen:

- Superior quality
- High capacity of producing 1000 oligos per day
- Cost effective solution for genomics research
- Fast turnaround time

**Quality Assurance**

Retrogen Custom oligo synthesis is checked by PAGE gel. The digital image of the acrylamide gel is stored in our database for future reference. Retrogen warrants that each product conforms with the specifications stated on the certificate of analysis. If the product does not meet these standards, this warranty assures re-synthesis, re-purification or credit issued. Notification of a potential problem must be made within 30 days of receipt of the oligo.

Example of digital image of the PAGE gel:





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# Oligo Modifications

**Introduction:**
The advantage of chemically synthesized oligonucleotides is the possibility of creating the artificial molecules that can be used in specific application. The increasing utilization of synthetic oligonucleotides in genetic analysis, diagnostic, and therapeutic applications in post genomics area has stimulated the development of a variety of reagents for the functionalization of oligonucleotides. Retrogen offers most of these modifications.

**Phosphorylation:**
Oligonucleotides can be phosphorylated enzymatically at the 5'-terminus by polynucleotide kinase. This reaction is reversible, and is difficult to control. For applications, like in gene assemblage, where complete phosphorylation is important, it is advisable to introduce the phosphate chemically. This can be achieved easily by using a chemical phosphorylating amidite at the last condensation cycle. The following phosphorylation is available at Retrogen:

| Modifications | Available Positions |
|---|---|
| 5'-Phosphorylation | 5' |
| 3'-Phosphorylation | 3' |

**Amination:**
The purpose of terminal amination is to introduce a non-hindered nucleophilic centre for the specific introduction of further functionalization with any desired group (e.g. biotin, fluorescein, rhodamine, Texas Red, digoxigenin, alkaline phosphatase, horseradish peroxidase). This primary amine reacts with N-hydroxy-succinimide esters (NHS esters) to form amide bonds. The terminal amino groups will react with mild nucleophilic reagents, approx. 100 times faster than the heterocyclic aminogroups at the bases. The reaction is favored at alkaline pH , because the primary amine is kept in an unprotonated state. The following are 5'-Amino-Modifers available at Retrogen:

| Modifications | Available Positions |
|---|---|
| 5'-Amino-Modifier C6 | 5' |
| 5'-Amino-Modifier C12 | 5' |
| 5'-Amino-Modifier 5 | 5' |
| 5'-Amino-Modifier C3-TFA | 5' |
| 5'-Amino-Modifier C6-TFA | 5' |
| Amino Modifier C2 dT | 5', Internal |
| Amino Modifier C6 dT | 5', Internal |
| Amino Modifier C3 | 3' |
| Amino Modifier C7 | 3' |

**Spacer Arm:**
The spacer phosphoramidite C16 attaches a lipophilic group to the 5'-terminus improving cellular uptake of oligonucleotides and therefore biological activity of antisense oligonucleotides. The following are spacers available at Retrogen:

| Modification | Available Positions |
|---|---|
| dSpacer | 5', Internal |
| Spacer 18 | 5', Internal |
| Spacer 9 | 5', Internal |
| Spacer C12 | 5', Internal |
| Spacer C3 | 5', Internal, 3' |


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# Oligo Modifications

**Thiolation:**
Thiolation can be used as an alternative to amination. Furthermore, it opens the possibility of specific coupling to a sulphydryl containing ligand through disulphide bridge. The thiol group is protected by a trityl group and separated by a six-carbon linker from the amidite group. The protecting trityl can be removed by treatment with silver nitrate and dithiothreitol. The following are 5'-Thiol-Modifiers available at Retrogen:

| Modifications | Available Positions |
|---|---|
| Thiol Mod C3 S-S | 3' |
| Thiol Modifier C6 S-S | 5' |

**Biotin:**
Biotinylated oligonucleotides have been used in a large number of molecular biology applications including quantification of PCR-amplified sequences, chemiluminescent sequencing, in situ hybridization, solid phase restriction site mapping, single base mutational analysis, genomic walking, and cloning of unknown DNA sequences. Once incorporated, the biotin label can be detected by standard streptavidin-based detection methods.

| Modifications | Available Positions |
|---|---|
| Biotin-TEG | 3', 5', internal |
| Biotin-dT | Internal |

**Fluorescein:**
Fluorescein is introduced for direct fluorescence detection. Fluorescein-labelled oligonucleotides have been used for non-isotopic fluorescent sequencing, PCR quantitation, colour PCR, and studies of cellular uptake and distribution of antisense olgonucleotides. The fluorescein label can be used either as a hapten for antibody binding or as a fluorophore for fluorescent signal generation.

| Modification | Available Positions |
|---|---|
| TAMRA | 3', 5' |
| 6-FAM | 3', 5' |
| Cy3 | 5' |
| Cy5 | 5' |
| Fluorescein | 3', 5' |
| Fluorescein-dT | 3', 5', internal |
| HEX | 5' |
| TAMRA-dT | 5', internal |
| TET | 5' |

**Phosphorothioate (S-oligo):**
Phosphorothioate analogues of DNA and RNA have sulphur in place of oxygen as one of the non-bridging ligands bound to the phosphorus. Phosphorothioates have been shown to be more resistant to nuclease degradation than the natural DNA and RNA and still to bind to complementary nucleic acid sequences. Phosphorothioate oligodeoxy-nucleotides have demonstrated their usefulness as antisense molecules inhibiting gene expression and as potential chemotherapeutic agents. Phosphorothioate is available at any position in an oligonucleotide, and can be used multiple times within a sequence.



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# Oligo Modifications

**DeoxyInosine:**
This is a deoxynucleoside with the base hypoxanthine (6-hydroxypurine). When used within a hybridization probe, deoxyinosine residues can form base-pairs with dA, dC, dG, or T residues on the target strand. DeoxyInosine can be used instead of wobbles and has the advantage that the hybridization probe is not "diluted" by the non-pairing components of the wobbles. DeoxyInosine is available at any position in an oligonucleotide, and can be used multiple times within a sequence.

**Degenerate Bases: (Wobbles)**
For some experiments the synthesis of sequences which have one or more degenerate positions may be required. This can be achieved using a mixture of the required phosphoramidites (2, 3 or 4 mixed together) in the addition step at the appropriate position. The resulting oligonucloetide will have a mixture of bases at the specified position in a ratio similar (but not equal) to the proportions in which the bases were mixed. When the desired redundancy is located at the 3'-end, a mixture of supports is used.

| Modifications | Available Positions |
|---|---|
| A = Adenosine | 3', 5', internal |
| C = Cytidine | 3', 5', internal |
| G = Guanosine | 3', 5', internal |
| T = Thymidine | 3', 5', internal |
| U = Uracil | 3', 5', internal |
| K = G or T | 3', 5', internal |
| M = A or C | 3', 5', internal |
| R = A or G | 3', 5', internal |
| S = G or C | 3', 5', internal |
| W = A or T | 3', 5', internal |
| Y = C or T | 3', 5', internal |
| B = C, G or T | 3', 5', internal |
| D = A, G or T | 3', 5', internal |
| H = A, C or T | 3', 5', internal |
| V = A, C or G | 3', 5', internal |
| N = any base | 3', 5', internal |

# Oligo Purifications

When ordering their oligo from Retrogen, many people wonder how pure is pure enough. Our high coupling efficiency proprietary synthesis platforms together with our rigorous quality control procedure result in high quality oligo that can be use in most basic molecular applications without additional purification. This technical information will help you select the best purification option for your oligo and application.

In the DNA synthesis, each nucleotide is coupled sequentially to the growing chain. In each couple cycle, a small percentage of the oligo chains will not be extended, resulting the mixture of full length and truncated oligo. After the oligo is cleaved from the support and the protecting groups are removed, there are different types of purification can be used to separate the truncated oligo from full-length oligo. For some applications, it is crucial that only full-length oligo is present in the final yield. For others, the presence of truncated oligo will not affect the experimental results.

Desalting Purification At Retrogen, every oligo is desalted by Sephadex column. Desalting procedure removes residual by-products from the synthesis, cleavage, and deprotecting procedures. This level of purify is adequate for most common applications, such as standard PCR, sequencing, and hybridization.

Reverse-phase cartridge purification (RP1) Separation on a reverse-phase cartridge purification offers the next level of typically 90-95 % purity. The level of purity for RP1 purification is almost equivalent to that provided by HPLC, but the recovery is much higher. The principle of this purification is based on the selection of full length product with the DMT group. The n-1 oligo without the DMT group will not be selected when the oligo is passed through the column. This purification is recommended for purifying the oligo less than 50 bases.

HPLC Purification is recommended for the oligo from 6-50 bases in length that requires additional purification. HPLC purification removes most truncated oligo sequences, resulting mostly full-length product in the final yield. The ion exchange HPLC is used to separate the oligo sequences on the basis of charge and it is performed on the Oigo3 column from Perseptive Biosystems company. From the chromatogram, the fractions with the highest purity are selects. The HPLC purified oligo is then desalted by Sephadex column.

PAGE Purification is recommended for the oligo longer than 50 bases that required additional purification. PAGE purification removes most the truncated oligo sequences resulting the highest level of 95-99% purity, but gives lower yield. PAGE separates the oligos on the basis of charge and molecular weight. PAGE purification is performed on a denaturing polyacrylamide gel prepared from stock solution of acrylamide, urea, and TBE buffer. The oligo is detected through UV shadowing onto the sensitive digital image system. The full-length product is excised and eluted from the gel. The PAGE purified oligo is then desalted by Sephadex column.


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# Custom Oligo Synthesis

## Estimated Yields & Turnaround:

| Scale of Synthesis | Purification | Yield | Turnaround |
|---|---|---|---|
| 0.02 µM 24 hour service | Desalt | 2-3 OD | 24 hours |
| 0.02 µM 48 hour service | Desalt | 2-3 OD | 48 hours |
| | | | |
| 0.05 µM 24 hour service | Desalt | 3-10 OD | 24 hours |
| 0.05 µM 48 hour service | Desalt | 3-10 OD | 48 hours |
| 0.05 µM 2-3 day service | Desalt | 3-10 OD | 2-3 days |
| | RP1 | 2-5 OD | 2 additional days |
| | PAGE | 0.5-1 OD | 3 additional days |
| | HPLC | 1-2 OD | 3 additional days |
| | | | |
| 0.2 µM 24 hour service | Desalt | 10-20 OD | 24 hours |
| 0.2 µM 48 hour service | Desalt | 10-20 OD | 48 hours |
| 0.2 µM 2-3 day service | Desalt | 10-20 OD | 2-3 days |
| | RP1 | 3-7 OD | 2 additional days |
| | PAGE | 1-2 OD | 3 additional days |
| | HPLC | 2-5 OD | 3 additional days |
| | | | |
| 1.0 µM 24 hour service | Desalt | 20-50 OD | 24 hours |
| 1.0 µM 48 hour service | Desalt | 20-50 OD | 48 hours |
| 1.0 µM 2-3 day service | Desalt | 20-50 OD | 2-3 days |
| | RP1 | 5-10 OD | 2 additional days |
| | PAGE | 3-5 OD | 3 additional days |
| | HPLC | 5-10 OD | 3 additional days |

## Recommended Scale of Synthesis and Purification Levels:

| Application | Scale of Synthesis | Purification |
|---|---|---|
| Antisense studies | 1 µM | RP1 |
| End labelling | .2 µM | HPLC, PAGE |
| Gel shift assay | .2 µM | HPLC, PAGE |
| Gene synthesis | .2 µM | PAGE |
| Hybridization | .2 µM | Desalt, RP1 |
| Kinasing | .2 µM | HPLC, PAGE |
| Mutagenesis | .5 µM, .2 µM | HPLC, PAGE |
| PCR .5 nM, | .2 µM | Desalted |
| Qualitative PCR | .5 µM, .2 µM | HPLC, PAGE |
| RT-PCR | .5 µM, .2 µM | Desalt |
| Sequencing | .5 µM, .2 µM | Desalt |
| Modified bases and chemical linkers | .5 µM, .2 µM | RP1 |
| Reporter groups (biotin, DIG or fluorescent dyes) | .5 µM, .2µM | RP1, HPLC |

# Custom Oligo Synthesis

## Melting Temperature Calculation

The Tm, or melting temperature, characterizes the stability of the DNA hybrid formed between an oligonucleotide and its complementary strand. The Tm is critical for determining the optimal temperature at which to use an oligonucleotide as a primer in PCR applications (annealing temperature), as a probe for in situ hybridization, and in Southern, Northern or Dot blot analyses.finition

Tm = Temperature at which 50% of a given oligonucleotide is hybridized to its complementary strand.
In the absence of destabilizing agents, like formamide or urea, Tm will depend on 3 major parameters:

1. The sequence: a GC-rich sequence has a higher melting temperature.
2. The strand concentration: high oligonucleotide concentrations favor hybrid formation, which results in a higher melting temperature.
3. The salt concentration: high ionic strength results in a higher Tm as cations stabilizes the DNA duplexes.

The most accurate method of estimating the Tm of oligonucleotides, and that used at Retrogen, is based upon a thermodynamic analysis of the melting process from which it can be shown that

$$Tm = \frac{\Delta H}{(\Delta S + R\ln(C))} - 273.15 - 12.0\log[Na+]$$

The changes in enthalpy (H) and entropy (S) of duplex formation are calculated from nearest-neighbor thermodynamic parameters. R is the molar gas constant (1.987 cal.K-1mole-1), and C is the molar concentration of oligonucleotide.

*Reference*

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# Oligo Synthesis Price List

## Column purified oligo-24 hour turnaround service

| Synthesis Scale | Price per Base | Maximum Length |
|---|---|---|
| 0.02 µmole | $0.60 / base | 35mer |
| 0.05 µmole | $0.70 / base | 60mer |
| 0.2 µmole | $1.40 / base | 80mer |
| 1.0 µmole | $2.20 / base | 100mer |
| 10.0 µmole | $17.00 / base | 100mer |

## Column purified oligo - 48 hour turnaround

| Synthesis Scale | Price per Base | Maximum Length |
|---|---|---|
| 0.02 µmole | $0.40 / base | 35mer |
| 0.05 µmole | $0.60 / base | 60mer |
| 0.2 µmole | $1.30 / base | 80mer |
| 1.0 µmole | $2.00 / base | 100mer |
| 10.0 µmole | $16.00 / base | 100mer |

## Column purified oligo - 2-3 day turnaround

| Synthesis Scale | Price per Base | Maximum Length |
|---|---|---|
| 0.05 µmole | $0.50 / base | 60mer |
| 0.2 µmole | $1.20 / base | 80mer |
| 1.0 µmole | $1.90 / base | 100mer |

## Purifications

### RP1 Purification

| Synthesis Scale | Estimate Yield | Cost per oligo | Turnaround |
|---|---|---|---|
| 0.05 µmole | RP1 2-5 OD | add $20.00 | 2 additional days |
| 0.2 µmole | RP1 3-7 OD | add $20.00 | 2 additional days |
| 1.0 µmole | RP1 5-10 OD | add $40.00 | 2 additional days |

### PAGE Purification

| Synthesis Scale | Estimate Yield | Cost per oligo | Turnaround |
|---|---|---|---|
| 0.05 µmole | PAGE 0.5-1OD | add $40.00 | 3 additional days |
| 0.2 µmole | PAGE 1-2 OD | add $60.00 | 3 additional days |
| 1.0 µmole | PAGE 3-5 OD | add $100.00 | 3 additional days |

### HPLC Purification

| Synthesis Scale | Estimate Yield | Cost per oligo | Turnaround |
|---|---|---|---|
| 0.05 µmole | HPLC 1-2 OD | add $40.00 | 3 additional days |
| 0.2 µmole | HPLC 2-5 OD | add $60.00 | 3 additional days |
| 1.0 µmole | HPLC 5-10 OD | add $100.00 | 3 additional days |



# Oligo Synthesis Price List

## Modifications:

| Modifications | 0.05 & 0.2 µmole | 1.0 µmol |
|---|---|---|
| 5' Phosphorylation | add $25.00 | add $50.00 |
| 3' Phosphorylation | add $25.00 | add $50.00 |
| 5' Biotin | add $75.00 | add $125.00 |
| 3' Biotin | add $75.00 | add $125.00 |
| 5' Flourescein | add $75.00 | add $125.00 |
| 3' Flourescein | add $75.00 | add $125.00 |
| 6-FAM | add $90.00 | add $150.00 |
| HEX | add $90.00 | add $150.00 |
| TET | add $90.00 | add $150.00 |
| 5' Amine | add $50.00 | add $75.00 |
| 3' Amine | add $50.00 | add $75.00 |
| 5' Thiol | add $50.00 | add $75.00 |
| 3' Thiol | add $50.00 | add $75.00 |
| Inosine | add $10.00 per site | add $15.00 |
| 2'-deoxyUridine | add $10.00 per site | add $15.00 |

## Phosphorothioated Oligo (S-oligo)

| Synthesis Scale | Price per Base | Set-up | Maximum Length |
|---|---|---|---|
| 0.05 µmole | $1.70 / base | $20.00 | 60mer |
| 0.2 µmole | $2.40 / base | $25.00 | 80mer |
| 1.0 µmole | $3.20 / base | $30.00 | 100mer |
| 10.0 µmole | $18.00 / base | $35.00 | Inquire |

## RNA Synthesis - Column purified synthetic products

| Synthesis Scale | Price per Base | Set-up | Maximum Length |
|---|---|---|---|
| 0.2 µmole | $20.00 / base | $25.00 | 80mer |
| 1.0 µmole | $25.00 / base | $30.00 | 100mer |
| 10.0 µmole | $30.00 / base | $35.00 | Inquire |

Shipping:
Column and RP1 purified oligos are guaranteed to ship within the times indicated above. PAGE and HPLC oligos are shipped within 5 working days with Overnight Express. All orders must be placed by 1:00 PM Pacific time.

| | |
|---|---|
| Local Shipping | Add $8.00 |
| Overnight Express | Approximately $25.00 (check DHL website for shipping costs) |


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# Oligo Synthesis Frequently Asked Questions (FAQ)

***1. What is scale of synthesis?***

Scale of synthesis refers to the amount of starting CPG (controlled-pore glass) support-bound monomer used to initiate the DNA synthesis, not the amount of final material synthesized. As an example, a 20 mer synthesized at a 200 nanomole scale of synthesis will produce approximately 80 nanomoles. The losses occur during synthesis, post-synthetic processing, transfer of material, and quality control.

***2. Do I need to have my oligo purified?***

It depends on whether or not modifications are requested and what the application will be. Failure sequences may be generated both during the synthesis and post-synthesis processing. We recommend that all modifications be purified either by cartridge or HPLC. For recommended purity and scale (based upon application), please see below.

Recommended Scale of Synthesis and Purification Levels

| **Application** | **Scale of Synthesis** | **Purification** |
|---|---|---|
| Antisense studies | 1 µM | RP1 |
| End labelling | .2 µM | HPLC, PAGE |
| Gel shift assay | .2 µM | HPLC, PAGE |
| Gene synthesis | .2 µM | PAGE |
| Hybridization | .2 µM | Desalt, RP1 |
| Kinasing | .2 µM | HPLC, PAGE |
| Mutagenesis | .5 µM, .2 µM | HPLC, PAGE |
| PCR | .5 µM, .2 µM | Desalted |
| Qualitative PCR | .5 µM, .2 µM | HPLC, PAGE |
| RT-PCR | .5 µM, .2 µM | Desalt |
| Sequencing | .5 µM, .2 µM | Desalt |
| Modified bases and chemical linkers | .5 µM, .2 µM | RP1 |
| Reporter groups (biotin, DIG or fluorescent dyes) | .5 µM, .2µM | RP1, HPLC |

***3. How much do I get or what scale of synthesis should I order?***

Estimated Yields for Different Purifications and Turnaround

| **Scale of Synthesis** | **Purifications** | **Yield** | **Turnaround** |
|---|---|---|---|
| 0.02 µM 24 hour service | Desalt | 2-3 OD | 24 hours |
| 0.02 µM 48 hour service | Desalt | 2-3 OD | 48 hours |
| 0.05 µM 24 hour service | Desalt | 3-10 OD | 24 hours |
| 0.05 µM 48 hour service | Desalt | 3-10 OD | 48 hours |
| 0.05 µM 2-3 day service | Desalt | 3-10 OD | 2-3 days |
| | RP1 | 2-5 OD | 2 additional days |
| | PAGE | 0.5-1 OD | 3 additional days |
| | HPLC | 1-2 OD | 3 additional days |
| 0.2 µM 24 hour service | Desalt | 10-20 OD | 24 hours |
| 0.2 µM 48 hour service | Desalt | 10-20 OD | 48 hours |
| 0.2 µM 2-3 day service | Desalt | 10-20 OD | 2-3 days |
| | RP1 | 3-7 OD | 2 additional days |
| | PAGE | 1-2 OD | 3 additional days |
| | HPLC | 2-5 OD | 3 additional days |
| 1.0 µM 24 hour service | Desalt | 20-50 OD | 24 hours |
| 1.0 µM 48 hour service | Desalt | 20-50 OD | 48 hours |
| 1.0 µM 2-3 day service | Desalt | 20-50 OD | 2-3 days |
| | RP1 | 5-10 OD | 2 additional days |
| | PAGE | 3-5 OD | 3 additional days |
| | HPLC | 5-10 OD | 3 additional days |

# Oligo Synthesis Frequently Asked Questions (FAQ)

***4. What do I re-suspend my oligo in and what concentration should I make it?***

Purified water, PBS or any biological buffers are acceptable as diluents. The recommended diluent volume is 100 µl - 1 ml, the concentration depending on the application to be used and the yield of the resulting product. Standard concentration for PCR primers is 0.1 mM.

***5. How do I determine my concentration?***

Concentration is determined by measuring the OD260 of the diluted oligo. Prepare a dilution of the resuspended oligo and measure the OD260 . Determine the concentration as follows:

(µg or pmoles/ OD260 ) X dilution factor = final concentration / mL.

***6. How stable is my oligo once I have resuspended it?***

If sterile diluent is used to resuspend the oligo, it will be stable at 4°C for about a month. If stored frozen at -20°C or -70°C, it will remain stable for 2-3 months. Repeated freeze-thaw should be avoided, as it will denature the oligo. Avoid the use of distilled water, since solution pH may be as low as 4-5.

***7. Does my oligo have a phosphate on the 5' end?***

Unless requested, oligos are synthesized without either 3' or 5'phosphate. The 5' phosphate modification is available, normally as an additional charge.

***8. My annealed oligos will not ligate. What is the problem?***

Ligation reactions require a 5' phosphate. If your oligos do not contain a 5'phosphate, ligation will not occur. The problem can be addressed without ordering an additional oligo pair: phosphorylate your oligos enzymatically with kinase before use in ligation reactions.

***9. How do you calculate the molecular weight of my oligo?***

The molecular weights for oligos is the sum of the component molecular weights of all bases, with mixed bases contributing proportionately. The component molecular weights of the bases vary as to their salt form. Many times desalted oligos are ammonium salts, while cartridge, HPLC and PAGE purified oligos are sodium salts. The molecular weights used in the calculations are listed in the following Table III.

Molecular Weight Calculations:

*Molecular Weights of the Bases*

| | **Sodium Salt** | | **Ammonium Salt** |
|---|---|---|---|
| | DNA | Thioate | DNA |
| WA | 313.21 | 329.27 | 330.24 |
| WC | 289.18 | 305.25 | 306.24 |
| WG | 329.21 | 345.27 | 346.24 |
| WT | 304.19 | 320.26 | 321.23 |
| WCORR | 61.96 | 61.96 | 96 |

Molecular Weight Calculation: (PA * WA) + (PC * WC) + (PG * WG) + (PT * WT) + (Pmod * Wmod) - WCORR

Where PA is the number of As and WA is the component weight of A and Pmod is the number of Modifications, and Wmod is the component weight of the added modification.



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# Oligo Synthesis Frequently Asked Questions (FAQ)



Molecular Weights of Common Modifications

| Modification | Molecular Weight | Modification | Molecular Weight |
|---|---|---|---|
| 5'-Biotin | 405.45 | 3'-TAMARA | 623.60 |
| 5'-(6 FAM) | 537.46 | 3'-Dabsyl | 498.49 |
| 5'-HEX | 744.13 | 3'-Fluorescein-dT | 815.71 |
| 5'-TET | 675.24 | 3'-(6 FAM) | 569.46 |
| 5'-Cy5 | 533.63 | 3'-Amino Modifier C3 | 153.07 |
| 5'-Cy3 | 507.59 | 3'-Amino Modifier C7 | 209.18 |
| 5'-Dabcyl | 430.18 | 3'-Thiol Modifier C3 | 154.12 |

***11. What is coupling efficiency?***

Coupling efficiency is a measure of the DNA synthesizer's ability to couple each new monomer to the growing chain. If all the monomers coupled completely to the growing chain, the coupling efficiency would be 100%. If 1% of the growing monomer chain fails to react, then the coupling efficiency of that step is only 99%. The coupling efficiency for the complete synthesis of the oligo is usually determined from the yields of full-length sequence after the first and last cycle. Coupling efficiencies greater than 99.0% are essential for good oligo product with minimum purification.

***12. How is the coupling efficiency determined?***

Following the first coupling step, the amount of Trityl released during deblocking is directly proportional to the amount of full-length oligo made in the previous cycle. When the Trityl is cleaved during the deblocking step, the resulting Trityl cation is orange in color. The intensity of this color can be measured by UV spectrophometry. By comparing the intensities of the Trityl produced after the first and last coupling, one can calculate the average successful base coupling per cycle and hence the coupling efficiencies.

***13. I sequenced a clone I prepared with your primer and the sequence for the primer region was different from the one I ordered. Why?***

Base insertions are attributed to a small amount of detritylated amidite present during coupling, while deletions are probably due to failure sequences that don't get capped and are subsequently extended.

However, a better explanation for the observation of altered sequences is the incomplete deprotection of the oligo. With a deprotecting group still on a few positions when the annealed and ligated oligos were transformed into E. coli, the host mismatch repair system would try to resolve these bumps with the results sometimes being the wrong base. The most likely culprit for incomplete deprotection is the isobutyryl protected dG's. These are the hardest deprotection groups to remove. If the oligos were vigorously deprotected a second time, mostly likely the new clones would have sequenced correctly. Also, in general, the longer the oligo, the greater the probability of side reactions accumulating along with increased chances of incomplete deprotection.

# Oligo Synthesis Frequently Asked Questions (FAQ)

***14. Why are some modified oligos so expensive in relation to the cost of the modifying reagent?***

The limited reagent stability (most <48 hours) and lower coupling efficiencies of the reagent requires that excess modifying reagent be used to insure adequate quantities of full length product is made. As a result, higher cost incurred in synthesis.

***15. Why are the yields lower for modified bases?***

Many of the modified amidites are unstable and do not couple as efficiently as the unmodified bases (even though longer coupling procedures may be used), thus failure sequences are more abundant than in normal synthesis. Consequently, all modified oligos should be purified either by cartridge or HPLC to remove the more abundant failure sequences. Yields are reduced as a result of purification.. The end product, although with a lower yield, is much more pure.

***16. Why isn't the yield for 1 µm scale syntheses five times greater than 0.2 µm scale syntheses?***

For 0.2 µm scale, the monomer coupling is done at a 40-50-fold excess. To do so for larger scale syntheses (such as 1.0 µm scale) would be cost-prohibitive. Large-scale syntheses are done at 10-fold mole excess of amidites. However, to increase the yields for these larger scale syntheses, the coupling times are extended to increase coupling efficiencies.

***17. What is the longest length an oligo can be synthesized?***

The real answers lies in the limit of resolution of the purification method and the coupling efficiency of the DNA synthesizer. It is not unusal to synthesize oligo in excess of 150 bases and to obtain sufficient quantities by PAGE purification to do successful gene construction. It should be remembered that the longer the oligo, the greater the chance of accumulated sequence errors.


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# RetroPrep PCR Purification Kit

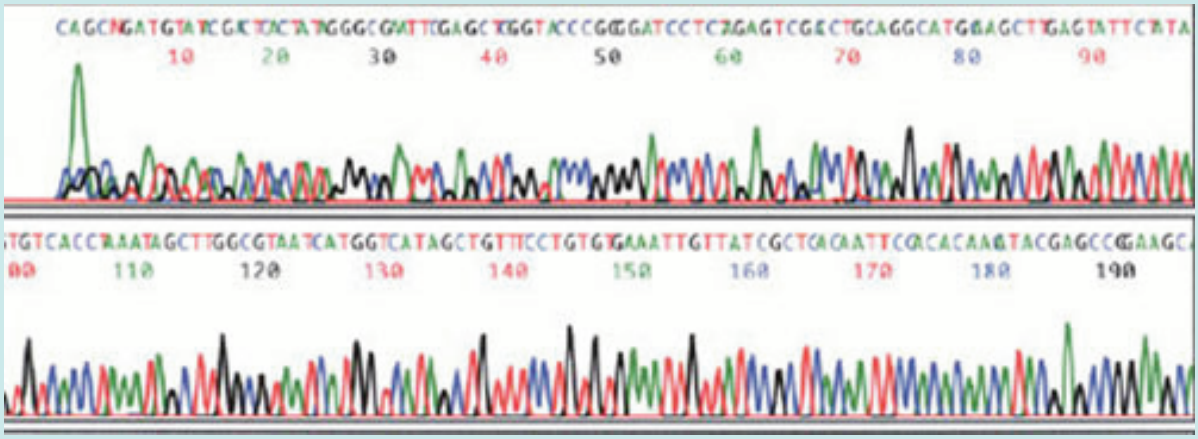
**Description:**

The PCR Purification system is designed for fast cleanup of DNA fragments from enzymatic PCR reactions. The Retrogen PCR Purification columns contain a silica-gel membrane for binding of up to 10 µg DNA in high-salt buffer and elution in low-salt buffer. Impurities are washed away, and pure DNA is eluted in a small volume of water, ready for use in any subsequent application. The purification procedure removes nucleotides, primers, enzymes, mineral oil, salts, detergents and other impurities from DNA samples. Retrogen silica-membrane technology eliminates the problems and inconvenience associated with loose resins and slurries. Binding buffers promote selective adsorption of DNA molecules within the size range of 70 bp to 10 kb.

**Key Benefits:**

- ► Efficient removal of contaminants
- ► Up to 95% recovery of ready-to-use DNA
- ► Generates high quality of automated sequencing data
- ► DNA cleanup in 3 simple steps: just bind, wash, and elute
- ► No resins, no phenol, no alcohol precipitation

***Get quality automated squencing data from RetroPrep PCR purification kit***



QC analysis of RetroPrep PCR Purification kit. The sequence data was generated from ABI 3730 DNA sequencer, using Bigdye terminator kit from Applied Biosystems.

Ordering Information:

| Product | Contents | Cat.No. | Price |
|---|---|---|---|
| RetroPrep PCR Purification (100) | 100 RetroPrep spin columns | 14001 | $100.00 |
| RetroPrep PCR Purification (250) | 250 RetroPrep spin columns | 14002 | $225.00 |

# RetroPrep Ultrapure Miniprep Kit

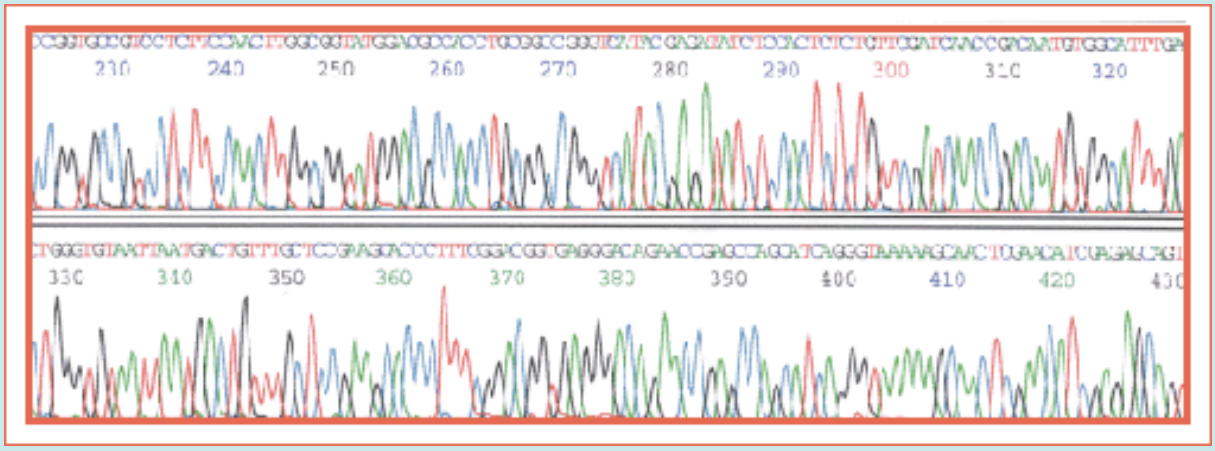
Description:

The Retrogen Miniprep DNA Purification System eliminates many of the problems associated with standard miniprep procedures, by providing a simple and reliable method for rapid isolation of plasmid DNA. This system can be used to isolate any plasmid, but works most efficiently when the plasmid is <20,000bp. The entire miniprep procedure can be completed in 30 minutes or less, depending on the number of samples processed. The purified plasmid can be used directly for automated fluorescent DNA sequencing or restriction enzyme digestion without further manipulation (when high copy number plasmids are used).

**Key Benefits:**

- Finish a batch of 24 in 30 minutes
- High quality plasmid for automated sequencing
- No resins or phenol extractions
- Economical cost for each prep

***High Quality Data from RetroPrep Ultrapure Miniprep Kit***




Ordering Information:

| Product | Contents | Cat.No. | Price |
|---|---|---|---|
| RetroPrep Ultrapure Miniprep Kit (100) | 100 RetroPrep spin columns | 12001 | $80.00 |
| RetroPrep Ultrapure Miniprep Kit (250) | 250 RetroPrep spin columns | 12002 | $180.00 |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# RetroPrep Gel Extraction Kit

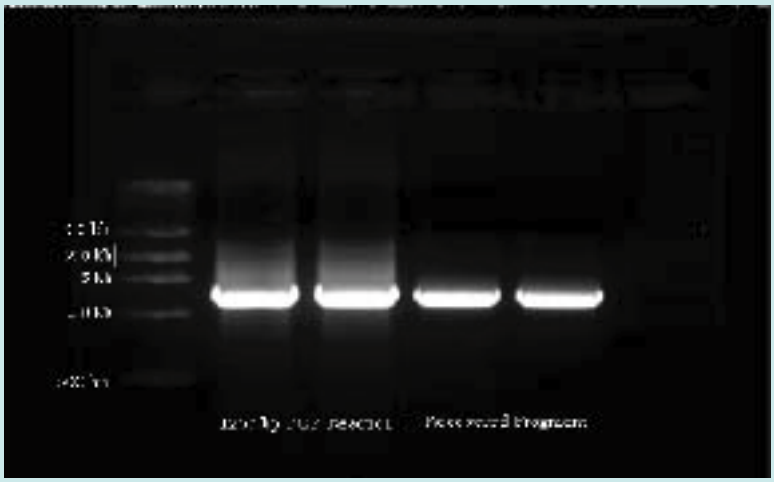
**Description:**

The Gel extraction system is designed for fast cleanup of DNA fragments from enzymatic reactions and agarose gels. The Retrogen extraction columns contain a silica-gel membrane for binding of up to 10 µg DNA in high-salt buffer and elution in low-salt buffer. Impurities are washed away, and pure DNA is eluted in a small volume of water, ready for use in any subsequent application. The purification procedure removes nucleotides, enzymes, mineral oil, salts, agarose, polyacrylamide, ethidium bromide, dyes, detergents and other impurities from DNA samples. Retrogen silica-membrane technology eliminates the problems and inconvenience associated with loose resins and slurries. Binding buffers promote selective adsorption of DNA molecules within the size range of 70 bp to 10 kb.

**Key Benefits:**

- ► Efficient removal of contaminants
- ► Up to 95% recovery of ready-to-use DNA
- ► DNA cleanup in 3 simple steps: just bind, wash, and elute
- ► No resins, no phenol, no alcohol precipitation

**DNA Recovery:** The purity and yield of DNA following cleanup is illustrated below. DNA recovery from agarose gel is typically 60-90%. Up to 10µg DNA in a maximum of 400mg agarose can be processed per gel extraction column.



Ordering Information:

| Product | Contents | Cat.No. | Price |
|---|---|---|---|
| RetroPrep Gel Extraction Kit (100) | 100 RetroPrep spin column | 12003 | $80.00 |
| RetroPrep Gel Extraction Kit (250) | 250 RetroPrep spin columns | 12004 | $200.00 |

# RetroClean Dye Removal Kit

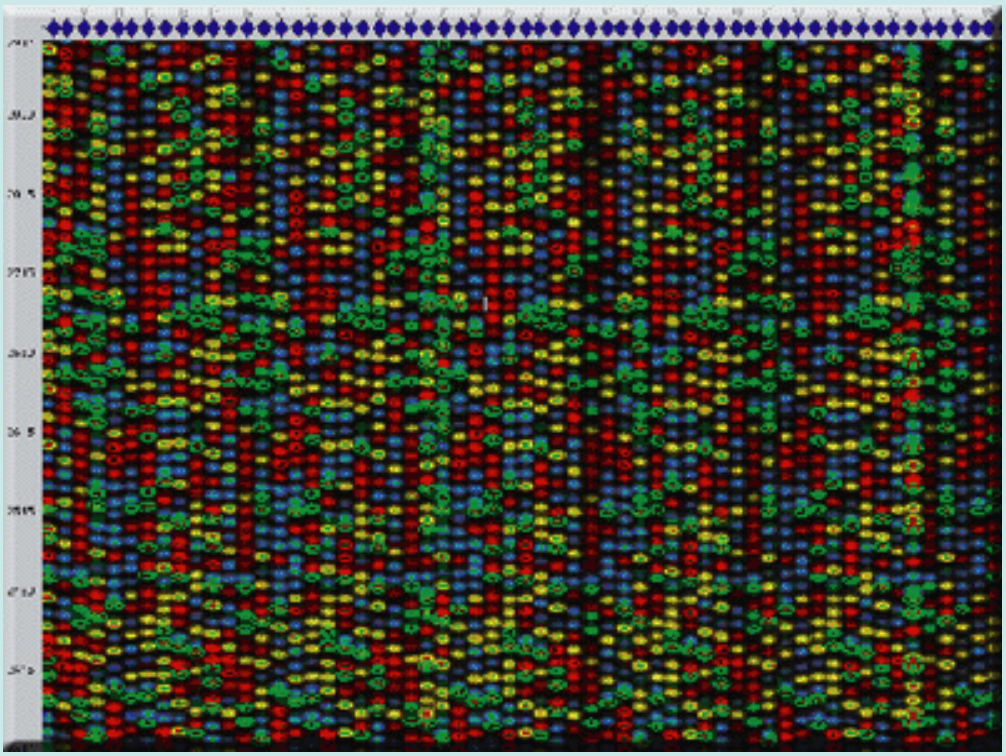
Description:

RetroClean Dye Removal kit is designed to efficiently remove unincorporated dye terminators from sequencing reactions. The removal of dye terminators is important to prevent the unincorporated dye from interfering with the sequencing results. RetroClean uses gel filtration technology that is generally acknowledged as the best method of removing salts, dye terminators, and any other small molecule contaminants. When the sequencing reaction mixtures are applied to the RetroClean columns, the dye terminator diffuses into the pores and is retained in the gel-filtration material, while the sequencing DNA fragments are excluded and collected in the flow-through. This method is faster, more reproducible, and generates better quality sequence data than most ethanol precipitation methods.

Key Benefits:

- Fast procedure with few centrifugation steps
- Efficient removal of all unincorporated dye from sequencing reactions
- High quality automated sequencing data

***High Quality Data from RetroClean Dye Removal Kit***



Ordering Information:

| Product | Contents | Cat.No. | Price |
|---|---|---|---|
| RetroClean Dye Removal Kit (100) | 100 RetroClean spin columns | 13001 | $100.00 |
| RetroClean Dye Removal Kit (250) | 250 RetroClean spin columns | 13002 | $225.00 |



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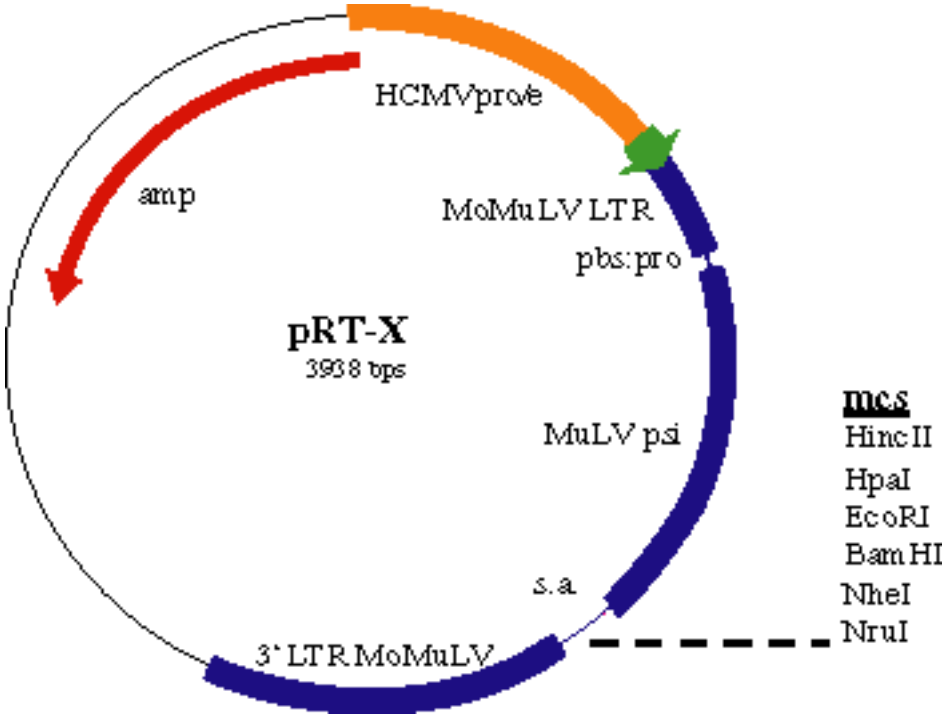
# Retrovirus Kit

Retrogen retroviral vectors are Moloney Murine Leukemia Virus (MoMuLV)-based vectors. We have constructed a foundation vector, pRT-X, which has been trimmed to contain the minimal amount of retroviral sequence required to produce an infectious, replication deficient, retrovirus. A minimized retroviral vector allows the insertion of larger cDNAs of interest. All vectors contain the CMV promoter/enhancer fused to a minimal fragment of the 5'LTR for high level transcription initiation and retroviral genome synthesis. A modified packaging region was constructed to add the wild-type MoMuLV splice acceptor sequence. The addition of the splice acceptor increases mRNA stability and the expression of the inserted gene. Using the 293 gag-pol cell line retroviral packaging system, high titers of viral particles can be routinely obtained with all pRT-X-based vectors. We provide the MoMuLV envelope gene expressed from the CMV promoter/enhancer for high level envelope expression and use in ecotropic infections. Retroviral particles containing an ecotropic envelope are able to infect mouse and rat, but not human cells. For amphotropic envelope expression, we provide the 4070A MuLV envelope gene expressed from the CMV promoter/enhancer vector. Amphotropic envelope containing particles can infect cells of most animal species except hamsters.




pRT-X is the minimal retroviral vector devoid of any internal gene sequences. This vector may be used as a retroviral expression vehicle where drug selection to produce stable cell lines is not required. For the pRT-X vector, we have inserted the b-galactosidase or luciferase genes for use as positive controls.

Ordering Information:

| Product | Contents | Cat.No. | Price |
|---|---|---|---|
| pRT-X Retroviral Kit | pRT-X, pRT-Z, pRT-L | 12007 | $350.00 |
| pRT-X Neo Retroviral Kit | pRT-X Neo, pRT-Z Neo, pRT-L Neo & G418 | 12008 | $375.00 |

# Retrogen, Inc.

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