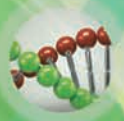


# A DNA/RNA Oligonucleotide Synthesis

Oligonucleotide - *AccuOligo®*
QC & Order system
Oligonucleotide FAQs
User Protocol

Life Science Product

01

# DNA/RNA Oligonucleotide Synthesis

## Overview

**High quality oligos and reasonable price.**
Founded in 1992, Bioneer is one of leading suppliers of synthetic oligonucleotides in the world (DNA and RNA). In-house production of raw materials (phosphoramidites, reagents & solvents) along with automated oligo synthesis and purification systems result in superior quality oligonucleotides at a reasonable price.
Since Bioneer manufactures all the components of our oligonucleotides, we manage the quality control every step of the way – ensuring that you receive only the highest quality product. Bioneer's oligonucleotides are purified through our unique Bio-RP cartridge purification technology, which offer 100% guarantee that they will work for your PCR or qPCR application! Most of all it is free of charge for Bio-RP purification!!
With high throughput oligo synthesis facilities around the world, Bioneer's capacity is unsurpassed and unrivalled with an ability to address the needs of customers requiring a few oligonucleotides on a regular basis. Of those customers that require very large numbers of oligonucleotides on a less frequent basis, we respond to your needs.

***AccuOligo*® technology**
*AccuOligo*® is free from oligo dislodging and potential loss during packing, shipping and opening, by the addition of an adhesive in the tube during the production process. Even the 96-deep well plate format orders have *AccuOligo*® technology option, preventing cross-contamination. (Dried oligonucleotide composition of the new patent application.)

- Prevention of dislodging and loss of oligos during production, packaging and shipping
- Prevents loss due to oligo "flaking" and tube cap attachment loss
- Prevents cross-contamination of multiple oligos in plate formats
- The adhesive does not affect PCR, Sequencing, restriction digests or other experimental methods
- Oligos could be seen by naked eye


**Contamination prevention through clean room production.**
All oligos manufactured at Bioneer are produced within a clean room facility, allowing us to provide nuclease/nucleic acid-free products to our customers.

**Molecular weight-level quality control with accurate MALDI- TOF QC.**
Every oligo produced goes through MALDI-TOF and 96-well CE QC systems, which means that the customer receives only the highest quality products.

**Bioneer's in-house developed Bio-RP purification system.**
Bioneer's R&D staffs have developed RP resin that tightly binds synthesized oligos and applied the resin to the Bio-RP purification system, allowing us to provide high-purity oligos to our customers.

**Why is Bio-RP purification better than desalting?**
Most oligos in the market are merely deprotected and desalted. The process of deprotection/desalting only partially purifies the oligo and leaves behind many impurities* and failure products such as truncated oligos and can even interfere with certain applications. This can lead to artificially inflated O.D. readings that increase with oligo length. Bio-RP purification removes all these contaminants and failure products, resulting in an oligo that is nearly at HPLC purity. The advantage of our product contains only quality oligos without impurities. To demonstrate this, plates of oligos of various sizes were tested for concentration in 2 steps: 1) After a deprotection / desalting step only, and 2) after Bio-RP purification (Figure 1).
The results of these tests show that failed sequences and exhibit presence of impurities* with increase in oligo length, which are not removed by deprotection/desalting process. This result shows inflated O.D. readings for oligos depend on oligo size (Table 1).
* Impurities in desalted oligos may include: Acetonitrile, Pyridine, Iodine, Ethylthiotetrazole, Dichloromethane, Acetic acid, Acrylonitrile, Benzamide and Isobutyramide.

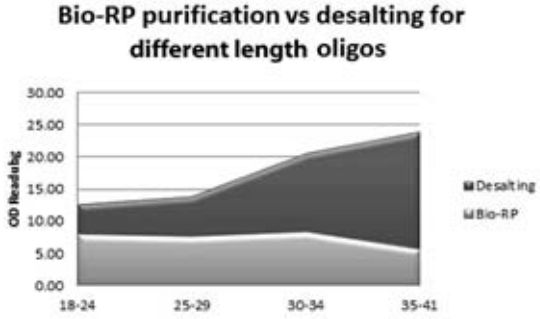



Figure 1. Plates of oligos of various lengths were tested for concentration after desalting and then by Bio-RP cartridge purification.

Note the inflated O.D. readings when deprotection / desalting alone is used

| Oligo Size | 18 - 24 | 24 - 29 | 30 - 34 | 35 - 41 |
|---|---|---|---|---|
| O.D. Inflation | 38 % | 45 % | 60 % | 77 % |

Table 1. Average O.D. increase is shown for oligos of various sizes when protection/desalting alone are used. Note for longer oligos, up to 77% of product does not consistently provide purified or functional oligo.

**Bio-RP purification:**
Phosphoramidite, which serves as the coupling monomer in oligonucleotide synthesis, has a DMT-protected 5'-OH group to prevent repetitive coupling reaction during the individual addition steps of synthesis. Only the n-mer oligos with the expected length, not n-1, n-2, n-3 … mer oligos, have the 5'-DMT group provided DMT was not removed after final round of synthesis (AKA "Trityl-On" mode). Because of this, Bioneer has developed unique Bio-RP resin, which attracts the hydrophobic DMT group, as well as an automated column purification process. In this process, we can purify n-mer oligo with high purity (>85%) using Bio-RP's efficient isolation of n-mer and removal of n-1, n-2, n-3…mer.

**HPLC purification:**
Highly purified oligonucleotide is essential for applications such as Site-directed mutagenesis or quantitative gene expression experiments. Thus, oligo purification only with desalting column or Bio-RP resin is not sufficient. HPLC purification provides oligo with significantly higher purity. Bioneer uses either anion-exchange resin or reverse-phase (RP) resin for the HPLC purification. Both Anion-exchange chromatography and RP-HPLC purification provide Oligos that are >90 % pure. It should be noted that oligos over 35 mer may have lower purity, because longer oligos reduce purification efficiency.

**PAGE purification:**
PAGE purification is needed for much longer oligos length. This process involves using electrophoresis on a polyacrylamide gel up to 130 mer, and purified > 95% purity. With that the level of separation is high but expects lower final yields due to losses in oligos that may occur during the gel based extraction process.

## Description

Standard oligonucleotide synthesis service includes synthesis of up to 130 mer oligonucleotides. A variety of synthesis scales, as well as a choice of purification methods, (Bio-RP, HPLC or PAGE), depending on your application, can be selected.

## Features and Benefits

- Free Bio-RP purification:
  Near HPLC purity at a standard oligo price
- Quick turnaround time:
  24 - 48 hr from order to shipping in most cases
- Broad range of modifications available:
  Nearly 300 modifications; If there is no modification listed that you want, you can still ask it to us.
- Competitive pricing:
  Great value for your research dollar

**Recommended Purification Methods by Application**

| Application | Purification | | |
|---|---|---|---|
| | Bio-RP | PAGE | HPLC |
| Standard PCR / RT -PCR | V | | |
| Multiplex PCR | V | V | |
| Real-Time PCR | | V | |
| Sequencing | V | | |
| Genotyping / SNP | | V | |
| Cloning (e.g. chemical linkers) | | V | V |
| Mutagenesis | | V | V |
| Gene Synthesis | | | V |
| DNA Microarray Probes | V | V | |
| Antisense | | V | |
| Primer Extension | V | V | |
| cDNA Library Generation | V | | |
| End Labelling & FISH | | V | |
| Northern & Southern Blotting | V | V | |
| Gel Shift Assay | | V | V |

## Specifications

| | |
|---|---|
| Synthesis Scale (Guaranteed quantity of starting materials) | 25 nmol, 50 nmol, 0.2 µmol, 1.0 µmol, 10 µmol, and 15 µmol |
| Length of Oligomer | 5 - 130 mer |
| Available Purification Methods | Bio-RP, HPLC and PAGE |
| QC & QA | MALDI-TOF Mass Spectrometer & CE (Capillary Electrophoresis) |

## Guaranteed Yield

- Yield of oligo synthesis is dependent upon composition of bases, purification method, oligo length, synthesis scale and the presence of modification(s).

## Guaranteed Quality

- Generally DMT-monitoring QC, PAGE QC, or HPLC QC is used for quality control of synthesized oligo, but its indirect nature does not provide the exact value of failed oligo amount or of successful modification.
- Unlike other methods, MALDI-TOF (Matrix-Assisted Laser Desorption / Ionization Time of Flight) mass spectrometer can get definite quality of synthesized oligo from the measurement of exact molecular weight of oligo.
- Bioneer provides high-quality oligos through MALDI-TOF QC of all products.
- QC Data sheet from MALDI-TOF is also provided for confirmation of oligo molecular weight.
- HPCE (High Performance Capillary Electrophoresis) 'Oligo-pro™ is used to analyze purity of oligos over 50 mer in length.

## Delivery

- All ordered oligos, except PAGE-purified or modified ones, will be shipped from Korea within 2 days of ordering. (Note that the Delivery of the final product is subject to delay, in the event of re-synthesis or shipping company's scheduling issues.)
- Delivery to customer's site will vary by country. Please contact your local distributor for a schedule.

## Guaranteed Yield and Delivery Time

| Synthesis Scale (μmole) | Base Limitation (mer) | Guaranteed Yield (O.D.) [ based on 20 bases ] | | | Working Day (day) | | |
|---|---|---|---|---|---|---|---|
| | | Bio-RP | PAGE | HPLC | Bio-RP | PAGE | HPLC |
| 0.025 | 15-60 | 2 | 1 | 1.5 | 2 | 3 | 3 |
| 0.05 | 10-75 | 4 | 2 | 2.5 | 2 | 3 | 3 |
| 0.2 | 5-110 | 8 | 6 | 7 | 2 | 3 | 3 |
| 1 | 5-130 | 30 | 18 | 25 | 2 | 3 | 3 |
| 10 | 5-50 | 300 | 150 | 200 | 2 | 3 | 3 |
| 15 | 5-50 | Inquire | Inquire | Inquire | 3 | 4 | 4 |

* Synthesis scales refer to the initial starting point for a synthesis. The starting synthesis scale and the length of the oligo will influence the final yield.

## Additional Service

| Post-handling |
|---|
| Concentration normalized-standard (100 pmol/μl) |
| Concentration normalized-custom |
| Barcode labeling (Free of charge) |
| Lyophilization (Free of charge) |
| Aliquoting to daughter plates |
| Mixing primers |
| Individual tube plate-labeled |
| Custom plate |
| Double-strand annealing |
| MALDI-TOF QC (Free of charge) |
| MALDI-TOF Analysis (SNP or Genotyping) |
| Shipping |
| Hand mix service (MIX base code) |
| Remainder in plate service |
| Circular ssDNA service |

## Custom Analytical Service

| Items |
|---|
| I-E HPLC |
| RP HPLC |
| Capillary electrophoresis |
| PAGE analysis |
| MALDI-TOF analysis, spectrocheck |
| MALDI-TOF analysis, other mass system |

# 01 HT- Oligo™ (High Throughput Oligonucleotide)

## Description

If researchers require obtaining large number of oligonucleotide for high throughput use, then Bioneer has your answer. So far, Bioneer has maintained reputation to maintain a total capacity of producing 30,000 oligonucleotides per day. In-house production of raw materials along with proprietary high throughput oligo synthesis and purification systems result in much higher quality oligonucleotides at significantly lower cost. Bioneer understands that high quality and low cost is especially important to our high throughput screening oligo users.
Pricing can be flexible with order volume, so please contact us with any questions on discounts available.
Also, if you need any synthesis, purification, or post-handling services not mentioned on this page, please contacts oligo-support@bioneer.com and we will do our best to help you!

## Features and Benefits

- Free Bio-RP purification:
  Near HPLC purity at a standard Oligo price
- Quick turnaround time:
  48 hr from order to shipping in most cases
- Broad range of modifications available:
  If you don't see it, just ask, we can probably do it.
- Competitive pricing:
  Great value for your research dollar

## Guaranteed Yield and Delivery Time

| Synthesis Scale (μmole) | Base Limitation (mer) | Guaranteed Yield (O.D.) [ based on 20 bases ] | | | Working Day (day) | | |
|---|---|---|---|---|---|---|---|
| | | Bio-RP | PAGE | HPLC | Bio-RP | PAGE | HPLC |
| 0.025 | 15-60 | 2 | 1 | 1.5 | 2 | 3 | 3 |
| 0.05 | 10-75 | 4 | 2 | 2.5 | 2 | 3 | 3 |
| 0.2 | 5-110 | 8 | 6 | 7 | 2 | 3 | 3 |
| 1 | 5-130 | 30 | 18 | 25 | 2 | 3 | 3 |
| 10 | 5-50 | 300 | 150 | 200 | Inquire | Inquire | Inquire |
| 15 | 5-50 | Inquire | Inquire | Inquire | Inquire | Inquire | Inquire |

## Description

Bioneer provides the ideal tools for your specialized applications. With the advent of technologies such as Real-Time detection and quantification, as well as mass-scale genotyping projects, more and more scientists require the use of modified oligos. All common oligo modifications are available in Bioneer. Our fluorogenic oligos for qPCR are unsurpassed in quality, and priced for value. Please contact oligo-support@bioneer.com for more information on our modified oligos.

## Features and Benefits

- Free Bio-RP purification:
  Near HPLC purity at a standard oligo price
- Quick turnaround time:
  24 - 48 hr from order to shipping in most cases
- Broad range of modifications available:
  Nearly 300 modifications; If you don't see it, just ask, we can probably do it
- Competitive pricing:
  Great value for your research dollar

## Modifications

| 3' Modification | 5' Modification | Internal Modification |
|---|---|---|
| DIG | DIG | Internal Amino Modifier C6 dT |
| C6 Amine | C6 Amine | Internal Biotin-dT |
| Phosphate | Phosphate | Fluorescein dT |
| Biotin | Biotin | C3 spacer |
| C3 spacer | Thiol | C6 spacer |
| C6 spacer | C3 spacer | C12 spacer |
| C12 spacer | C6 spacer | 18 atom spacer |
| C18 atom spacer | C12 spacer | dS spacer |
| dS spacer | 18 atom spacer | Phosphorothioate (per insertion) |
| FAM | dS spacer | 5-Methyl dC |
| TAMRA | FAM | Inosine |
| Thiol | TAMRA | Deoxy Uridine |
| Texas Red | HEX | 2'-O-Methyl |
| JOE | TET | 5-Bromo dU |
| ROX | Texas Red | 8-Oxo-dA |
| Cy5 | JOE | 8-Oxo-dG |
| Cy3 | ROX | Ferrocene-dT |
| Dabcyl | Cy5 | 5-hydroxymethyl-dC |
| BHQ1 | Cy3 | 5-Hydroxy-dU |
| BHQ2 | IRD700 | 5-hydroxymethyl-dU |
| Cholesteryl | IRD800 | Ara-dC |
| Inverted dT | C2 Aldehyde | 5-F-dU |
| 3'-Inverted dA | Cy5.5 | N6-methyl-2'-deoxyadenosine |
| 3'-Inverted dC | Cholesteryl | PC(photo-cleavable) Linker |
| 3'-Inverted dG | Dabcyl | DNP(2,4-dinitrophenyl)-TEG |
| Ara-dC | Ara-dC | 3'-dA |
| 5-F-dU | 5-F-dU | 3'-dC |
| 2',3'-ddC | 2',3'-ddA | 3'-dG |
| AlexaFluor 488 | 2',3'-ddC | 3'-dT |
| AlexaFluor 532 | 2',3'-ddG | 5'-Nitroindole |
| AlexaFluor 546 | 2',3'-ddT | 2'-F-rA |
| AlexaFluor 594 | AlexaFluor 488 | 2'-F-rC |
| AlexaFluor 647 | AlexaFluor 532 | 2'-F-rG |
| AlexaFluor 660 | AlexaFluor 546 | 2'-F-rU |
| AlexaFluor 750 | AlexaFluor 594 | 2-Aminopurine |
| N6-methyl- 2'-deoxyadenosine | AlexaFluor 647 | 2,6-Diaminopurine |
| DNP (2,4-dinitrophenyl)-TEG | AlexaFluor 660 | Dithiol |
| Cy3.5 | AlexaFluor 750 | EDTA-C2-dT |
| Cy5.5 | N6-methyl- 2'-deoxyadenosine | Thymidine Glycol |
| Puromycine | PC(photo-cleavable) Amine Linker | Zebularine |

| | | |
|---|---|---|
| Yakima Yellow | PC(photo-cleavable) Biotin Linker | Biotin-TEG |
| 3′-dA | DNP (2,4-dinitrophenyl)-TEG | HEX-dT |
| 3′-dC | Cy3.5 | Methylene Blue |
| 3′-dG | 3′-dA | Tamra-dT |
| 3′-dT | 3′-dC | BHQ1-dT |
| 5-Nitroindole | 3′-dG | BHQ2-dT |
| 2′-F-rA | 3′-dT | Dabcyl-dT |
| 2′-F-rC | 5-Nitroindole | Deoxypurine (2′-DeoxyNebularine) |
| 2′-F-rG | 2′-F-rA | Azide |
| 2′-F-rU | 2′-F-rC | Trebler Branching |
| Maleimide | 2′-F-rG | O6-Methyl 2′-dG |
| 2-Aminopurine | 2′-F-rU | O4-Methyl-dT |
| 2,6-Diaminopurine | Maleimide | dT-Alkyne |
| Dithiol | 2-Aminopurine | Epoch Eclips Quencher |
| EDTA-C2-dT | 2,6-Diaminopurine | Thiol-dT |
| Thymidine Glycol | Dithiol | Cy3 dA |
| Zebularine | BHQ2 | Cy3 dC |
| 3′ Methylene Blue | EDTA-C2-dT | Cy3 dG |
| 3′ AMCA (amino-methyl-coumarin-acetate) | Thymidine Glycol | Cy3 dT |
| 3′ Deoxypurine (2′-DeoxyNebularine) | Zebularine | Cy5 dA |
| 3′ PEG-2000 | 5′ Methylene Blue | Cy5 dC |
| 3′ Azide | 5′ AMCA (amino-methyl-coumarin-acetate) | Cy5 dG |
| 3′ Acrylamide (acrydite) | 5′ Deoxypurine (2′-DeoxyNebularine) | Cy5 dT |
| O6-Methyl 2′-dG | 5′ PEG-2000 | Pyrene-dU |
| O4-Methyl-dT | 5′-BromoHexyl (Br) | Perylene-dU |
| dT-Alkyne | 5′ Acridine | C8-Alkyne-dT |
| Rhodamine 6G | 5′ Acrylamide (acrydite) | C8-Alkyne-dC |
| Epoch Eclips Quencher | 5′-Yakima Yellow | Desthiobiotin TEG |
| EBQ | O6-Methyl 2′-dG | N3-Methyl dC |
| Pyrene-dU | O4-Methyl-dT | N4-Ethyl dC |
| Perylene-dU | 5′ C3-Amine | Azobenzene |
| C8-Alkyne-dT | 5′ C12-Amine | 8-Br-dA |
| C8-Alkyne-dC | dT-Alkyne | 8-Br-dG |
| Desthiobiotin TEG | Rhodamine 6G | 8-Br-dC |
| N3-Methyl dC | Pyrene-Cap | 6-thio-dG |
| N4-Ethyl dC | Epoch Eclips Quencher | 4-thio-dT |
| Marina blue | 5′-Hexynyl | EBQ-dT New |
| Azobenzene | Pyrene-dU | |
| | Perylene-dU | |
| | C8-Alkyne-dT | |
| | C8-Alkyne-dC | |
| | AminoOxy | |
| | Desthiobiotin TEG | |
| | N3-Methyl dC | |
| | N4-Ethyl dC | |
| | 5′-OMe-dT | |
| | C6-Psoralen | |
| | Marina blue | |
| | DBCO TEG | |
| | Azobenzene | |

## Description

Widely-used for real-time qPCR, a dual-labeled probe has a reporter dye at the 5′ end and a quencher dye at the 3′end. Probes can be used for the sensitive quantitative or qualitative detection of genes. With this known feature, attaching different types of fluorophores can be used for multiplex qPCR reactions. Bioneer's offering on the dual-labeled probes are synthesized are at high quality controlled environment through MALDI-TOF analysis and provided at a lower cost and faster in delivery in time vs. other competitors.

## Features and Benefits

- Free Bio-RP purification:
  Near HPLC purity at a standard oligo price
- Quick turnaround time:
  4 - 5 days from order to shipping in most cases
- Broad range of modifications available:
  If you don't see it, just ask, we can probably do it
- Competitive pricing:
  Great value for your research dollar

## Characteristics of Fluorescent Dyes

| Dye | Excitation Max (nm) | Extinction Coefficient (L/mole·Cm) | Emission Max (nm) |
|---|---|---|---|
| 6-FAM | 494 | 83,000 | 520 |
| Fluorescein-dT | 494 | 83,000 | 522 |
| TET | 521 | 73,000 | 541 |
| HEX | 535 | 73,000 | 553 |
| TAMRA | 556 | 91,000 | 580 |
| Cy3 | 546 | 150,000 | 563 |
| Cy3.5 | 581 | 150,000 | 596 |
| Cy5 | 646 | 250,000 | 662 |
| Cy5.5 | 675 | 250,000 | 694 |
| Cy7 | 743 | 250,000 | 767 |
| JOE | 529 | 71,000 | 555 |
| ROX | 588 | 82,000 | 608 |
| Texas Red | 598 | 116,000 | 617 |
| NED | 546 | - | 575 |
| VIC | 538 | - | 554 |
| IR700 | 685 | 170,000 | 705 |
| IR800 | 787 | 200,000 | 807 |
| Rhodamine 6G | 524 | 116,000 | 550 |
| DABCYL | 478 | 32,000 | - |
| BHQ-1 | 534 | 34,000 | - |
| EBQ | 570 | 41,360 | - |
| BHQ-2 | 579 | 38,000 | - |

Table 2. Characteristics of fluorescent dyes available in Bioneer.

## Combination of Dual-Probe

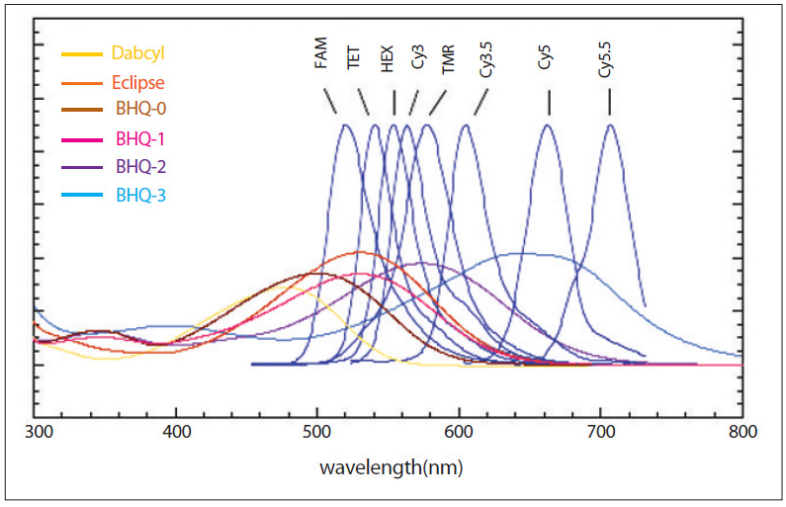



Figure 2. UV/Vis absorption curves from fluorescent dyes and quenchers.

| Dye | Excitation Max (nm) | Emission Max (nm) | Compatible Quencher | | | | |
|---|---|---|---|---|---|---|---|
| | | | DABCYl | TAMRA | BHQ1 | BHQ2 | EBQ |
| 6-FAM | 494 | 520 | | | | | |
| JOE | 529 | 555 | | | | | |
| TET | 521 | 541 | | | | | |
| HEX | 535 | 553 | | | | | |
| VIC | 538 | 554 | | | | | |
| Cy3 | 546 | 563 | | | | | |
| NED | 546 | 575 | | | | | |
| TAMRA | 556 | 580 | | | | | |
| Cy3.5 | 581 | 596 | | | | | |
| ROX | 588 | 608 | | | | | |
| Texas Red | 598 | 617 | | | | | |
| Cy5 | 646 | 662 | | | | | |
| Cy5.5 | 675 | 694 | | | | | |

Table 3. List of fluorescent dyes and their comparable quenchers.

| Modification | | |
|---|---|---|
| 5'-FAM-3'-TAMRA | 5'-FAM-3'- EBQ | 5'-FAM-3'-BHQ1 |
| 5'-HEX-3'-TAMRA | 5'-HEX-3'- EBQ | 5'-HEX-3'-BHQ1 |
| 5'-TET-3'-TAMRA | 5'-TET-3'- EBQ | 5'-TET-3'-BHQ1 |
| 5'-JOE-3'-TAMRA | 5'-JOE-3'- EBQ | 5'-JOE-3'-BHQ1 |
| 5'-FAM-3'-DABCYL | 5'-TAMRA-3'- EBQ | 5'-TAMRA-3'-BHQ1 |
| 5'-HEX-3'-DABCYL | 5'-ROX-3'- EBQ | 5'-ROX-3'-BHQ1 |
| 5'-TET-3'-DABCYL | 5'-Texas Red-3'- EBQ | 5'-Texas Red-3'-BHQ1 |
| 5'-TAMRA-3'-DABCYL | 5'-Cy5-3'-EBQ | 5'-Cy3-3'-BHQ1 |
| 5'-JOE-3'-DABCYL | 5'-Cy3-3'- EBQ | 5'-FAM-3'-BHQ2 |
| | | 5'-HEX-3'-BHQ2 |
| | | 5'-TET-3'-BHQ2 |
| | | 5'-JOE-3'-BHQ2 |
| | | 5'-TAMRA-3'-BHQ2 |
| | | 5'-ROX-3'-BHQ2 |
| | | 5'-Texas Red-3'-BHQ2 |
| | | 5'-Cy5-3'-BHQ2 |
| | | 5'-Cy3-3'-BHQ2 |
| | | 5'-FAM-BHQ1-dT-Amine-3' |
| | | 5'-FAM-Tamra-dT-PO4-3' |

Table 4. List of Dual-labeled probes Bioneer provides

# EBQ – Next Generation Dark Quencher Modifications

## Description

Fluorescence spectroscopy is a valuable tool for detecting fluorescence levels of biological molecules with fluorophores (dyes) such as Marina Blue, Pacific Blue, Oregon Greens, Cy dyes, AMCA, Bodipy derivatives, Fluorescein derivatives, FAM, JOE, TET, HEX, VIC, Cy3, NED, TAMRA, Cy3.5, ROX, Texas Red, Cy5, and Cy5.5 Quenchers are generally used for quenching fluorescent signals from the fluorescent dyes. These quenching effects have been widely using for detecting the level of the molecule which is of interest in cells or in samples.

Previously, BHQ quenchers (BHQ1 & BHQ2) are commonly used because they have a high potency of quenching activity. However, BHQ1 and BHQ2 are only active in a narrow range of wavelength (BHQ1; ca 400-570 nm, BHQ2; ca 560-700 nm). In order to overcome this, Bioneer has developed EBQ (Excellent Bioneer Quencher) that provides better quenching effects in wider range of wavelength (400-700 nm). Now experience the benefits of EBQ; wide wavelength coverage, superior quality, and less cost for your fluorescence spectroscopy experiment!

## Features and Benefits

- Broad range wavelength coverage:

  A wide absorbance range of 400 - 700 nm for effective quenching of emission signal from dyes. You can unify quenchers into EBQ (Maximum absorption occurred at 570 nm).

- High Stability:

  A structurally stable as a quencher against changes in temperature or pH

- Variety option of selectable dyes:

  EBQ effectively quenches most commonly used reporter-dyes with emission of 400-700 nm wavelength range such as Marina Blue, Pacific Blue, Oregon Greens, Cy dyes, AMCA, Bodipy derivatives, Fluorescein derivatives, FAM, JOE, TET, HEX, VIC, Cy3, NED, TAMRA, Cy3.5, ROX, Texas Red, Cy5, Cy5.5, etc.

- Fast and reliable Customer Service:

  Quick turnaround time: 4-5 days from order to bench in most cases

- Competitive pricing:

  Great value for your research dollar.

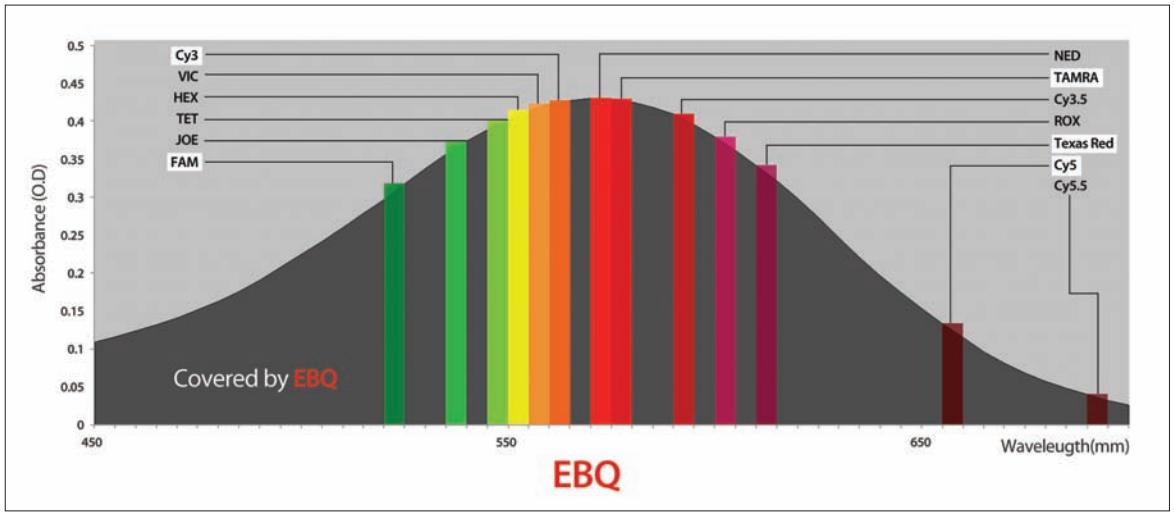



Figure 1. EBQ effectively quenched most commonly used reporter-dyes (Absorption spectrum of EBQ with the emission spectra of reporter-dyes).

# EBQ – Next Generation Dark Quencher Modifications

## Description

- Comparison of quenching effect of FAM-EBQ and FAM-BHQ1 probes
  - EBQ quenches FAM dye more efficiently than BHQ1 does.

| Dual-Labeled Probe | Fluorescence Signal Intensity | | | Efficiency (%) |
|---|---|---|---|---|
| | Before | After | Difference | |
| FAM-EBQ | 4.41 | 93.17 | 88.76 | 113.0 |
| FAM-BHQ1 | 4.20 | 82.74 | 78.54 | 100.0 |

Table 1. Measurement of quenching efficiency between EBQ quencher and BHQ1 through S1 nuclease treatment.

EBQ absorbance range for effective quenching: 400-700 nm

BHQ1 absorbance range for effective quenching: 450-570 nm

FAM Excitation: 494 nm, Emission: 520 nm

- Comparison of quenching effect of ROX-EBQ and ROX-BHQ2 probes
  - EBQ quenches ROX dye more efficiently than BHQ2 does.

| Dual-Labeled Probe | Fluorescence Signal Intensity | | | Efficiency (%) |
|---|---|---|---|---|
| | Before | After | Difference | |
| ROX-EBQ | 1.89 | 65.25 | 63.37 | 124.7 |
| ROX-BHQ2 | 1.14 | 51.94 | 50.80 | 100.0 |

Table 2. Measurement of quenching efficiency efficiency between EBQ quencher and BHQ1 through S1 nuclease treatment.

EBQ absorbance range for effective quenching: 400-700 nm

BHQ2 absorbance range for effective quenching: 540-670 nm

ROX Excitation: 588 nm, Emission: 608 nm

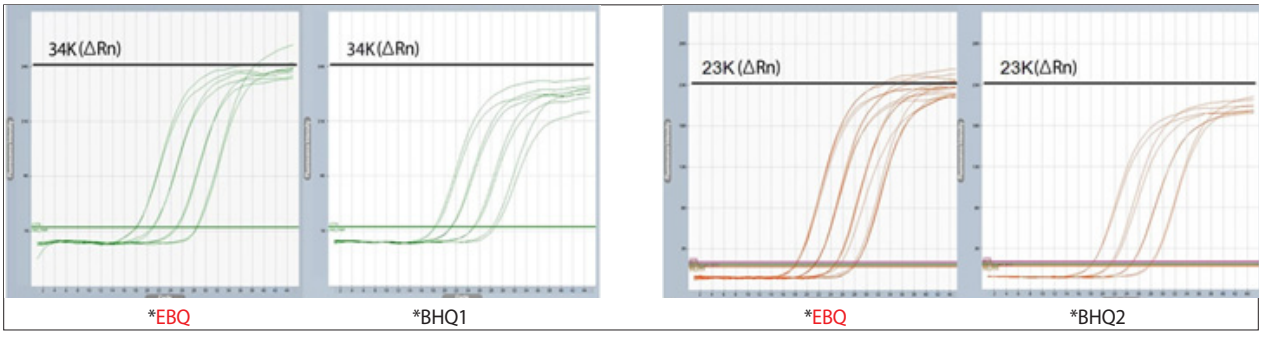



Figure 2. Measurement of quenching efficiency through ΔRn measurement.

ΔRn value= a fluorescence quenching capacity of Quencher at Real-Time PCR

## Description

Extendamers are oligonucleotides from 130 - 200 bases. Bioneer specializes in long oligo synthesis and provides Bio-RP or PAGE purification. Extendamers are QC tested by PAGE after oligo synthesis, ensuring the highest quality products for your research needs. Bio-RP (or PAGE) purification also ensures you have only full length oligonucleotides for your experiments. Use Extendamers for cloning, mutagenesis and other demanding applications.

## Features and Benefits

- Bio-RP purification or PAGE purification:
  Only full length oligo is in final product
- Use for a variety of applications:
  Ideal for cloning, gene construction and ddRNAi
- Competitive pricing:
  Great value for your research dollar

## Modifications available for Extendamers™

| Modification |
|---|
| 5′-Phosphate |
| 5′-Biotin |
| 5′-Amine |
| 5′-Fluorescein |
| 5′-Tamra |
| Inosine |

## Guaranteed Yield and Delivery Time

| Base Limitation (mer) | Coupling Efficiency | QC | Guaranteed Yield (nmole) | | Working Day (day) | |
|---|---|---|---|---|---|---|
| | | | Bio-RP | PAGE | Bio-RP | PAGE |
| 130 - 200 | >99.0% | PAGE Gel or MALDI-TOF MS | 3 - 4 | 0.25 - 0.3 | 5 - 6 | 7 - 8 |

# Large Scale Oligonucleotide

## Description

Bioneer's production facilities can accommodate large scale oligonucleotide synthesis orders ranging from milligrams to tens of grams of the purest DNA and RNA oligos, including both standard and modified. A large portion of our bulk oligo customers are involved in research requiring antisense oligos, providing new approaches in the development of pharmaceuticals and target validation. Some of the frequently used modifications are:

**Phosphorothioates and Chimeric Oligos:**
General oligonucleotides are subject to rapid degradation by nucleases. Therefore, oligos for antisense application are usually synthesized with a phosphorothioate bond modification to make them resistant to nuclease activity. In phosphorothioates, a sulfur atom replaces a binding oxygen in the oligo phosphate backbone.

**2'O-Methyl RNA oligos:**
2'O-Methyl RNA increases nuclease stability and affinity of the antisense oligo to the target RNA.

## Features and Benefits

- Free Bio-RP purification:
  Near HPLC purity at a standard oligo price
- Broad range of modifications available:
  If you don't see it, just ask, we can probably do it
- Competitive pricing:
  Great value for your research dollar

## Specification

| | |
|---|---|
| Synthesis Scale (Final yield) | 50 mg - 1 g |
| Base Limitation | 5 - 35 mer |
| Available Purification Methods | Bio-RP, HPLC |
| QC | MALDI-TOF Mass Spectrometer |

## Description

Bioneer's core competency also consists of providing industry's highest quality synthesis of Custom RNA oligos. Like our standard oligonucleotides, our siRNAs or RNA oligos synthesis also includes our patented Bio-RP purification, which is provides at close to HPLC quality. For your most stringent applications, we offer a wide array of modification options and services including bar-code labeling and annealing chimeric RNA/DNA oligos. Please find the chart in the ordering information tab detailing our guaranteed yield, available modifications, purification, and service options.

## Features and Benefits

- Free Bio-RP purification:
  Near HPLC purity at a standard oligo price
- Broad range of modifications available:
  If you don't see it, just ask, we can probably do it
- Competitive pricing:
  Great value for your research dollar

## Guaranteed Yield and Modifications

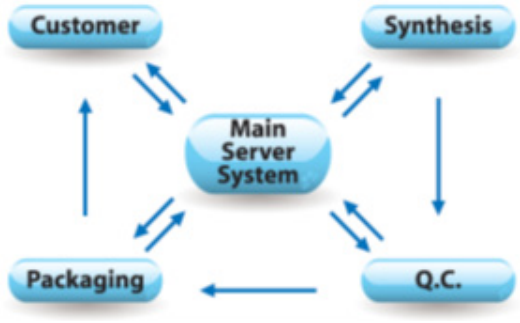
Custom RNA Synthesis

| Cat. no. | Guaranteed Yield | Base Limitation |
|---|---|---|
| SR-1001 | 10 nmole | 30 mer |
| SR-1002 | 20 nmole | |
| SR-1003 | 50 nmole | |
| SR-1004 | 100 nmole | |
| SR-1005 | 10 nmole | 31 - 35 mer |
| SR-1006 | 20 nmole | |
| SR-1007 | 50 nmole | |
| SR-1008 | 100 nmole | |

Modification available for Custom RNA

| 3' Modification | 5' Modification | Internal Modification |
|---|---|---|
| 3'-Amine | 5'-Amine | Internal 2'-F(A) |
| 3'-Biotin | 5'-Biotin | Internal 2'-F(C) |
| 3'-Cholesterol | 5'-Cy3 | Internal 2'-F(G) |
| 3'-DABCYL | 5'-Cy5 | Internal 2'-F(U) |
| 3'-Fluorescein | 5'-Fluorescein | Internal 2'-OMe |
| 3'-PEG 2000 | 5'-PEG 2000 | Chimeric DNA Bases |
| 3'-Phosphorylation | 5'-Phosphorylation | Internal Deoxy-abase |
| 3'-TAMRA | 5'-TAMRA | Internal Inosine |
| 3'-Thiol | 5'-Thiol | Internal Phosphorothioate |

## MALDI-TOF QC System




The customer order data is initially saved on the main server system and then transferred to synthesis. Following synthesis, oligonucleotides are spotted on MALDI-TOF mass plates using a proprietary, fully automated, 384-well sample O.D. quantification/ dispensing robot developed by Bioneer. The MALDI plates are then transferred to QC Division. Any transfer of oligo samples and plates between different divisions and/or equipment requires the bar-code on each sample racks to be checked by a production specialist to confirm the oligo data. Then, bar coding ensures compliance and allows related divisions to easily retrieve important oligo data from the main server system.

After receiving the oligonucleotides and all the related information, the QC department checks the quality of the oligonucleotides. The mass spectrum of each oligo is saved and the QC program checks whether the oligonucleotides have been synthesized appropriately. Upon completion, the final QC data is transferred to the main server. The Bioneer QC Program is also used to confirm oligo contaminants (including truncated oligonucleotides) present in the MALDI spectra. Another key advantage of Bioneer's QC system is its ability to automatically insert mass spectra (0.6X mass - 1.3X mass) of each oligo into the oligo data sheet. Mass spectrum for all ordered oligonucleotides will be provided to customers at no extra charge.

After this, MALDI data is delivered from QC to the main server, and subsequently all the related information is delivered to Packaging so that the correctly synthesized samples will be delivered in the appropriate format as requested by the customer. Finally, Bioneer delivers oligonucleotides in a selection of different tubes, 96-well plates, or 384-well plates as per the customers' preferences. After packaged completely, all the oligonucleotides will be shipped by FedEx or UPS, and via their tracking systems, customers may monitor the exact place where the ordered oligonucleotides are in transportation.

In QC, data on all failed samples is automatically returned to synthesis and the re-synthesis of the failed oligonucleotides proceeds whilst QC examines the failed oligo further. This rapid exchange of related information is a key to Bioneer's rapid turnaround time.

### Automatic MALDI-TOF QC:

Bioneer employs multiple MALDI-TOF mass spectrometers that are fully automated from loading to mass determination. The mass spectrometry data for each sample is automatically inserted into the oligo information sheet. Bioneer is one of the few oligo producers that checks all oligonucleotides (single and high throughput orders) by MALDI-TOF and provides mass data with each oligo, at free of charge.

Typical MALDI-TOF QC Data

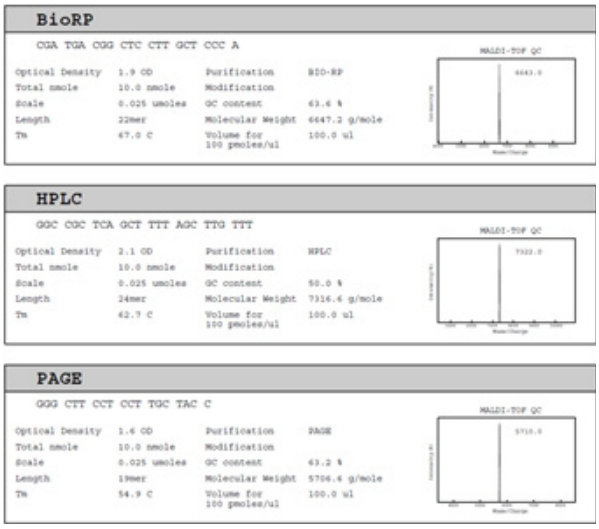



Figure 1. Typical Oligo Datasheet with MALDI-TOF Information.

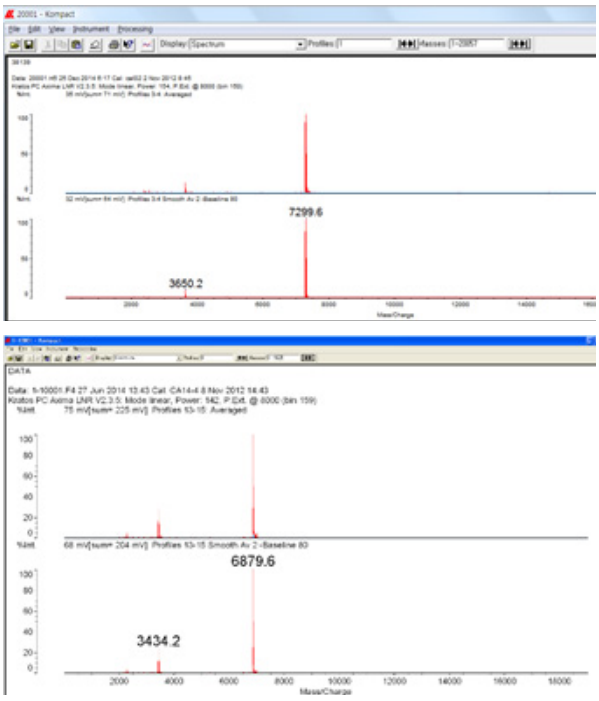



Figure 2. Examples of a typical 30 mer and 23 mer oligo spectrum, in this case employing a Kratos MALDI-TOF system.

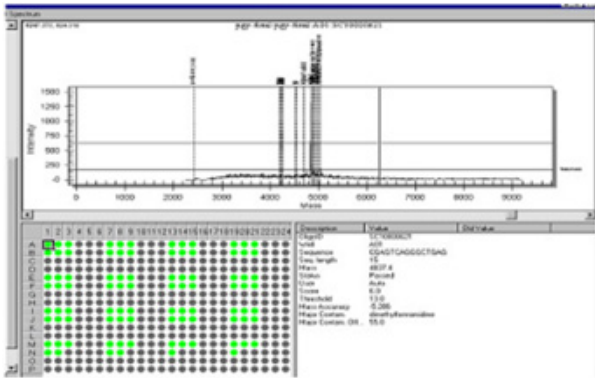


Figure 3. Example of High Throughput Plate QC Analysis.

The mass spectrum and result of 92 (plate) oligonucleotides - Bioneer can also provide "spectrochecked" oligo QC data for users of the Sequenom SNP Analysis System.

**The Use of Matrix-Assisted Laser Desorption / Ionization Time of Flight Mass Spectrometry of Synthetic Oligonucleotide QC**

At Bioneer MALDI-TOF (Matrix Assisted Laser Desorption-Ionization Time of Flight) is the technology used extensively for failure detection and other problems that cannot be resolved by other methods. Bioneer's fully automated, high throughput QC systems allow the company to provide superior, high quality product superior to that of our competitors. The QC systems installed at Bioneer currently can check the quality of 35,000 synthetic oligonucleotides per day. Each and every oligo is supplied with an oligo data sheet that includes MALDI-TOF mass spectrum.

**Interpreting MALDI-TOF Mass QC for Oligonucleotide:**

A MALDI-TOF mass spectrometer accurately measures molecular weight of a sample. The technique is most useful because it compares the theoretical mass calculated on the basis of oligonucleotide sequence to actual measured data. MALDI-TOF can also be used to check for sequence errors that may occur while inputting sequences. Such QC method is an absolute requirement for sequence dependent experiments, such as PCR, cloning and sequencing, which can also be used to check whether an oligo has been modified correctly. MALDI-TOF is also used to check for the presence of truncated oligonucleotides and salt contamination.

In addition, A MALDI-TOF mass system is most suitable for the QC of oligonucleotides less than 50 bases long. Longer oligonucleotides (>50 mer) cannot be ionized effectively (100%) by the laser, therefore they cannot be easily detected and therefore will show a poor detection signal that may fail QC. At Bioneer, any oligo greater than 50 bases in length are checked for quality by PAGE. PAGE QC data sheets are provided with each oligo >50 bases

**HPLC Analysis of Oligo Purity:**

- Reverse Phase HPLC

At Bioneer, a Reverse Phase HPLC is mostly used to QC of intermediates or single stranded DNA produced in the oligo synthesis process. It is a simple QC technique for modified oligo with hydrophobic groups. Reverse Phase is faster and cheaper than Ion Exchange methods and requires less sample.

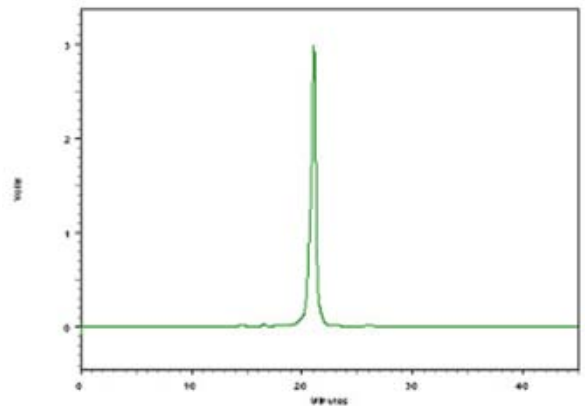


Figure 4. Example of oligo (26 mer) purity analysis using a Reverse Phase (C-18) Column.

- Purity Analysis using Ion-exchange Chromatography Method (using Anion-exchange column)

HPLC, equipped with a DIONEX's DNAPac column, is used to QC of oligonucleotides, in particular - Decoy oligonucleotides. The high resolution capability of Ion-exchange can easily separate single stranded DNA and double stranded DNA. At Bioneer Ion-exchange chromatography is commonly used to QC decoy oligonucleotides, and plays a key role in QC confirmation with strict QC standards required for gene therapy.

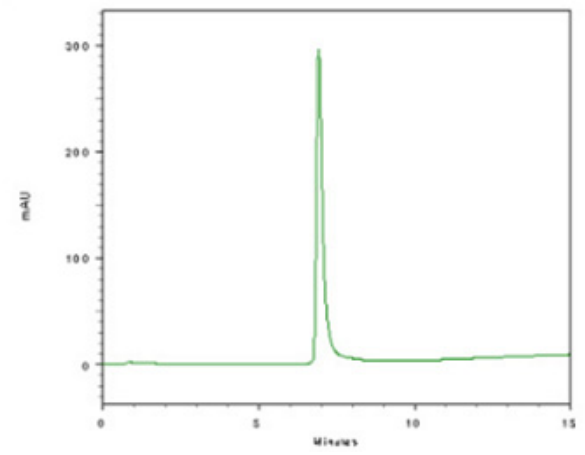



Figure 5. Double stranded DNA (DS DNA) test. Double stranded oligonucleotides are used for decoy or EMSA experiments.

Prior to any experiments, the formation of DS DNA must be checked. For decoy experiments used in the development of new drugs, it is necessary to check the ratio of DS DNA in the decoy. In order to guarantee the medical efficacy, the medicine should be formed in decoy like API from the start of drug development. dsDNA confirmation is an FDA requirement.

### GC Analysis of Oligo Purity

Gas Chromatograph (GC) is used to QC for solvent content in Decoy oligonucleotides and S-oligos used in gene therapy. Prior to administering any oligo based drug to humans, one vital importance is to check for the presence of residual organic solvents that may remain after synthesis and purification. Solvent content may compromise efficacy and cause unwanted side effects. The types of residual organic solvents that may be present include acetonitrile, pyridine and toluene etc. Concentrations should be minimally less 0.1%.

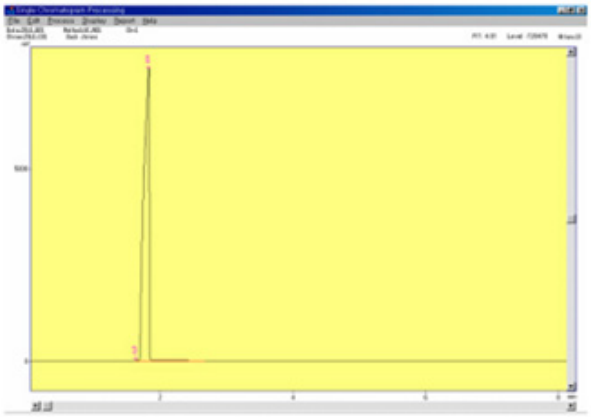


Figure 6. Standard solvent data.

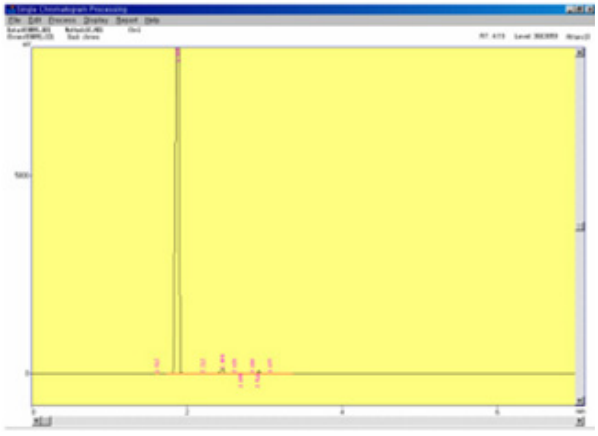


Figure 7. Solvents confirmation data in antisense oligonucleotide.

## QC of Large Scale, Antisense and Decoy Oligonucleotides

The high-quality oligonucleotides used in gene therapy and other therapeutic-type applications obviously require more stringent QC. Bioneer has developed specialized QC methods for large scale, antisense and decoy oligonucleotides. These specialized QC procedures comprise HPLC, CE, gel electrophoresis, NMR and additional specific tests such as endotoxin, bioburden, moisture content and etc. Bioneer's wide array of QC procedures satisfies all of our users' specific QC requirements. The array of QC steps available from Bioneer is as follows:

1. Ion-Exchange HPLC analysis
2. Reverse Phase HPLC analysis
3. Capillary Electrophoresis
4. NMR analysis
5. Moisture content analysis
6. Sodium content analysis
7. Heavy metal content analysis
8. Solvent content analysis
9. Endotoxin test
10. Bioburden test

### NMR – Spectroscopy

Nuclear Magnetic Resonance (NMR) spectrometer plays a very important role in understanding 3-dimensional structures of molecules. With increasing interests in the structure of biological materials, the use of NMR spectrometer is expanding into new areas, such as drug development, DNA analysis, human genomic and proteomic research and so on. NMR is commonly used to determine physical structure at the molecular level.

At Bioneer NMR is used for 31P-NMR measurement to compare typical frequency values for phosphates present in DNA backbones. By comparing actual frequencies with theoretical it is possible to check the state and purity of phosphates in synthetic oligonucleotides.

### Heavy Metal Testing

For antisense and decoy oligonucleotides that are directly injected into animals or humans as medicines in the pre-clinical or clinical phase, it is necessary for check for heavy metal groups that may influence the efficacy or may cause side effects. The types of heavy metals that require QC may differ in each oligo. Inductively Coupled Plasma-Optical Emission Spectrometers (I.C.P), Atomic Absorbance Spectrophotometers (AAS) and I.C.P Mass Spectrometers are routinely employed to QC oligonucleotides for heavy metal groups. Upon request, Bioneer's oligonucleotides can be checked quantitatively/qualitatively for metals such as Lead, Nickel and Fe etc.

### Water Content Analysis

Bioneer can also QC oligonucleotides for water content. A Sartorius' Water Content Measurement instrument (MA-30) is employed to measure water contents that may remain in synthesized antisense oligo following the final drying step of the oligo purification process.

### Bioburden Testing

Bioneer confirms the sterility of an aseptic oligo production environment by routinely conducting microbial testing of the water used in the synthesis process and final aliquoting steps. Susceptible areas of potential microbial contamination in the synthesis process and the environment, including operators are also checked periodically. Prevention ensures that the final oligonucleotides will be proven to be safe and free from microbial contaminations.

### Endotoxin Test

Bioneer also utilizes a Kinetic Chromogenic Analysis (KCA) method to confirm that the oligonucleotides are free of any exothermic materials. Generally exothermic materials present in injectable therapeutics are endotoxins from microbial contamination, especially from Gram negative bacterial contamination and must be avoided.

Kinetic Chromogenic Analysis (KCA) is based on an enzyme linked color reaction (limulus Amoebocyte Lysate reaction). The presence of endotoxins is quantified by measuring color of the reaction against known standards. Many samples can be quantified simultaneously using a standard micro-plate reader. KCA is a fast, cost-effective and short measurement. With such a method, Bioneer only provides oligonucleotides with less than 0.25 EU/ml for therapeutic applications.

### Oligonucleotide Ordering Systems

Orders from customers are gathered on a main production server system prior to synthesis. To eliminate re-entry errors, on-line and e-mail orders are recommended. Orders are automatically distributed (batched) to an appropriate synthesizer according to the length of oligo, the type of modification, and users' plate choice. Every lot to be synthesized is labeled with its own Barcode ID, which is used for identifying the oligonucleotide plate through the synthesis process. Bioneer's Quality Assurance Staff can monitor all procedures from synthesis to liquating using our proprietary Automatic Oligonucleotide Production System (AOP System).

# 01 Oligonucleotide FAQs

Contents

## Handling and Storage

**1. How should I store my oligo?**

Normally, oligos should be stable at -20˚C and can be stored at that temperature for more than a year. Although stable in solution, oligos will be degrade if the storage solution is contaminated with nucleases. Therefore, we recommend that oligos be stored in the dried form. If you want to store oligos in solution, it is best to aliquot the oligo into several tubes and store them separately. Oligos can also be subject to degradation due to the 'Freezing and Thawing Effect' when the oligo solutions are frozen and thawed repeatedly. For storage of oligodeoxyribonucleotide (DNA), pH value should be maintained at neutrality. Under acidic conditions, DNA can become depurinated. On the other hand, the phosphodiester bond of oligoribonucleotides (RNA) can be hydrolyzed under basic conditions.

Oligo Shelf life

| Storage Condition | Shelf Life (Month) |
|---|---|
| at RT in water | 2 |
| at 4˚C in water | 9 |
| at -20˚C in water | 18 |
| at -20˚C (Dry) | 24 |

**2. How should I resuspend my oligo?**

For long-term storage, we recommend that the oligos be dissolved in a buffer, such as TE (10 mM Tri-HCl, 0.1 mM EDTA, pH 8.0), instead of just sterilized water. Once resuspended, oligos should be kept frozen at -20˚C. Since some oligos may not be easily dissolve in sterilized water the addition of NaOH does help dissolve oligos in water.

**3. Do I have to treat fluorescent dye modified oligos differently in storage and handling?**

If exposed to light, fluorescent dye modified oligos are more fragile than unmodified oligos and their fluorescence intensity will decrease over time. To maintain their fluorescence efficiency, fluorescent dye modified oligos should be stored in the dark at -20°C.

| Preferred TE Buffer for Reconstitution & Storage pH for Fluorescent Probes | |
|---|---|
| 6-FAM, HEX, TET, ROX, and TAMRA | TE Buffer pH 7.5 or 8.0 |
| Cy3, Cy3.5, Cy5, and Cy5.5 | TE Buffer pH 7.0 or 7.5 |
| Cy dyes rapidly degrade in acidic pH | |

**4. If oligos were left at room temperature for more than a week, would they still work?**

Once dried, oligos are supposed to have tremendous stability. Even in solution, they are reasonably stable. Therefore, in most cases, without contamination by materials which can cause decomposition of oligos, they should still work well, even if they were left at room temperature for more than a week.

## Quantity, Concentration, and Tm

**1. How does Bioneer quantify my oligo?**

The quantity of oligo we provide is based on its UV optical density (O.D.) measured at a wavelength of 260 nm.

**2. How do I calculate the oligo quantity from the measured O.D. value?**

After measuring the amount of 260 nm UV light absorbed by the synthesized oligonucleotide, the following formula is used to calculate the actual amount.

$O.D. = \varepsilon C$

ε is the extinction coefficient which is a material-unique constant signifying the amount of light that can be absorbed by a specific material, and C is the concentration of the oligonucleotides. If one knows the extinction coefficient and the O.D. value of the oligonucleotide, the concentration can be calculated by substituting those values in the formula above. The ε value of an oligonucleotide can be calculated in two ways: 1) the sum of extinction coefficients of each base (Table. 1, and 2) an extinction coefficient value calculated by considering sequence interference. The extinction coefficients for each method at 260 nm UV are as below:

Table 1. (Unit: L/mole.cm)

| | |
|---|---|
| dA | 15,400 |
| dC | 7,400 |
| dG | 11,500 |
| dT | 8,700 |

Table 2. (Unit: L/mole.cm)

| 5'-> 3' | dA | dC | dG | dT |
|---|---|---|---|---|
| dA | 27,400 | 21,200 | 25,000 | 22,800 |
| dC | 21,200 | 14,600 | 18,000 | 15,200 |
| dG | 25,200 | 17,600 | 21,600 | 20,000 |
| dT | 23,400 | 16,200 | 19,000 | 16,800 |

Therefore, the extinction coefficient may be different, depending on the calculation method.

**3. If the O.D. value of an 18 mer oligo containing 3dG, 4dC, 5dA and 6T (GGGCCCCAAAAATTTTTT) is 0.7, how much oligo is there?**
The calculated amount of oligonucleotide depends on the method of extinction coefficient calculation.

Extinction coefficient based on Table 1
ε = Number of G bases *11500 + Number of C bases *7400 + Number of A bases *15400+ Number of T bases *8700
= 11500 x 3 + 7400 x 4 + 15400 x 5 + 8700 x 6 = 193.3 (L/mmole)

Therefore, substituting the values in the formula O.D. = εC leads to
C = 0.7 /193.3 = 0.003621 (mmole/L) = 3.6 (pmole/μl)

Extinction coefficient based on Table 2

$$\varepsilon = \sum_{1}^{n-1} \times \text{nearestneighbor} - \sum_{2}^{n-1} \times \text{individual}$$

ε = (GG + GG +GC +CC +CC +CC +CA +AA +AA +AA +AA +AT +TT +TT +TT +TT +TT)-(G + G + G + C + C + C + C + A + A + A + A + A + T + T + T + T + T)
= (21,600 + 21,600 + 17,600 + 14,600 + 14,600 + 14,600 + 21,200 + 27,400 + 27,400 + 27,400 + 27,400 + 22,800 + 16,800 + 16,800 + 16,800 + 16,800 + 16,800) - (11,500 + 11,500 + 11,500 + 7,400 + 7,400 + 7,400 + 7,400 + 15,400 + 15,400 + 15,400 + 15,400 + 15,400 + 8,700 + 8,700 + 8,700 + 8,700 + 8,700)
= (342,200) - (173,100)
= 169,100 (L/mmole)

Therefore, substituting the values in the formula O.D. = εC leads to
C = 0.7 /169.1 = 0.004140(mmole/L) = 4.14 (pmole/μl)
Our company implements the method of Table 2 for concentration calculation.

**4. What is the method for adjusting the oligonucleotide concentration?**
On the data sheet that Bioneer provides for each oligo, the volume of TE buffer or distilled water necessary to make a 100 pmole/μl oligo solution appears by the "volume for 100 pmole/μl" heading.

For example, if 189.0 is indicated on the sheet, add 189 μL of TE buffer to the tube.
Such a solution would be 100 μM in concentration:
100 pmole/μl = 10 x $10^6$ pmole/$10^6$ μl
= 100 x $10^6$x $10^{-12}$ mole/L = $10^{-4}$ mole/L
= $10^{-4}$ x $10^6$ μmole/L
= $10^2$ μmole/L
= 100 μM

**5. When I ordered the 50 nmole scale, I got less than 50nmoles. What happened?**
50 nmole scale synthesis of oligos doesn't mean we can guarantee 50 nmole of final oligos. Instead, 50 nmole scale refers to the loading amount of solid support used at the beginning of oligonucleotide synthesis. Since oligos are usually ordered by the reaction scale not the final yield, the amounts of oligos which customers could get is naturally less than ordered. The final yields can vary with oligo length, base composition and coupling efficiency.

**6. When I do not know the exact base composition, is there any method to quantify the synthesized oligo?**
Nor Approximately - a single stranded oligo with 1 O.D. value contains 33 μg while double stranded oligo contains 50 μg. For short oligos, however, there would be big deviations from the above values.

**7. Unit conversions**
System of scientific units:
$10^{-1}$ = deci[d], $10^1$ = deca[da]
$10^{-2}$ = centi[c], $10^2$ = hecto[h]
$10^{-3}$ = milli[m], $10^3$ = kilo[k]
$10^{-6}$ = micro[μ], $10^6$ = mega[M]
$10^{-9}$ = nano[n], $10^9$ = giga[G]
$10^{-12}$ = pico[p], $10^{12}$ = tera[T]
$10^{-15}$ = femto[f], $10^{15}$ = peta[P]

Example for unit exchange of oligonucleotides
1 pmole/μl
= 1x$10^{-12}$ mole / 1x$10^{-6}$ L
= 1x$10^{-6}$ mole / L
= 1 μmole/ L
= 1 μM

**8. How do we calculate the molecular weight of an oligonucleotide?**
The molecular weight of an oligo can be calculated with the following equation:
M.W. =(NA x 249.2) + (NC x 225.2) + (NG x 265.2) + (NT x 240.2) + (oligolength-1) x 63.98) + 2.02
NA = Total number of A
NC = Total number of C
NG = Total number of G
NT = Total number of T

**9. How do I convert oligo quantity expressed in nmole into weight?**
Normally, the amount of synthetic oligonucleotide is described in number of moles, usually nmole. The amount of oligo can easily be calculated from the following equation:
Amount of oligo (ng) = Molecular Weight (M.W. in g) X Number of moles (nmole)

**10. How do I measure Tm of the synthesized oligo?**
Tm (melting temperature) refers to the temperature where 50% of oligonucleotides exist in duplex form and the rest in single-strand form. There are several ways to calculate Tm. At Bioneer, we use the nearest-neighbor method (PNAS 83, 3746-50). It is believed that the

effect of hybridization is different for every sequence and that through thermodynamic measurements; you can estimate Tm values more accurately. For example, the sequences of 5′-GC-3′ and 5′-CG-3′ are different in thermodynamic measurements. The method for nearest-neighbor calculation is as follows:

$$Tm = \frac{Enthalpy \times 1000}{Entropy(dS) + (R \times \ln[Oligo])} - 273.15 + 12.0 \times \log([salt])$$

Through thermodynamic measurements, enthalpy and entropy values are determined between 2 bases. [Salt] is the concentration of monovalent cations and [Oligo] is the oligo concentration. R is the gas constant (1.987 cal•$K^{-1}$$mole^{-1}$). Bioneer's Tm value is calculated by the nearest-neighbor method with 50 mM for salt concentration and 1 nM for oligo concentration.

Please note that there are other ways of estimating the Tm. For oligos shorter than 15 mer, the Wallace rule can be used:
Tm = 2°C (A + T) + 4°C (G + C)

Another estimation method based on the GC content for long sequences is:
Tm = 81.5 + 0.41 (%GC) – 500 /[L] + 16.6 log [M]
([L]; oligonucleotide length, [M]; monovalent cation concentration)

However, these methods do not consider the base stacking effect and usually the estimation is not as accurate as the nearest neighbor method. Nonetheless, there are still some disadvantages in the nearest neighbor method for 60-70 or under 15 bp estimation.
Bioneer provides the Tm values of every oligo, but the values are estimations and we cannot guarantee the exact values. Therefore, the Tm value should be used only as a reference. If the experiment does not yield anticipated results, it is recommended to lower the annealing temperature by 4-5 degrees from the Tm value. If there are many non-specific products, trial-and-error approach should be taken to obtain the optimum annealing temperature.

**11. Why are there differences in Tm value that Bioneer provided and mine?**
The TM Calculator that BIONEER uses is different from, and more accurate than, the more commonly used calculators based on the Wallace rule.

**12. What are the symbols denoting degenerate bases.**

| | |
|---|---|
| R: | A or G |
| Y: | C or T |
| M: | A or C |
| K: | G or T |
| S: | G or C |
| W: | A or T |
| V: | A, C, or G |
| H: | A, T, or C |
| B: | G, T, or C |
| D: | G, A, or T |
| N: | A, C, G, or T |

## Modified Oligo

**1. Are the list of modified oligos and their structure available?**
Please refer the following Bioneer website;

http://eng.bioneer.com/products/Oligo/Modified-ordering.aspx (International except North America)
http://us.bioneer.com/products/Oligonucleotides/ModifiedOligosordering.aspx (US & Canada)

## Synthesis and Order

**1. Does the oligo synthesized have phosphate group at 5′ or 3′ position?**
If not ordered separately, the oligos synthesized do not contain phosphate group at 5′ or 3′ position. If you want to have oligo phosphorylated at 5′or 3′, you should specify 5′or 3′phosphorylation modification when ordering.

**2. Can you make the oligos having a high percentage of G residues?**
It is known that oligos having a high percentage of "G" residues are difficult to synthesize, especially if sequence contains several "G" in a row. It is also reported if there are "G"s existed four or more in a row, oligos tend to aggregate and form "guanine tetraplex". (Poon and MacGregor, Biopolymers, 1998, 45, 427-434) By substitution of inosine for some of "G", the formation of "guanine tetraplex" can be disrupted.

**3. Do you provide oligoribonucleotide (RNA) synthesis?**
Yes, we do. We can offer oligoribonucleotide with 2′ -OH and/or 2′ -O-methyl structure at the desired site. We can also synthesize the chimeric oligos which have DNA and RNA structures mixed.

**4. What are base limitations on each synthesis scale?**
0.025 µmole synthesis scale: 15 – 60 mer
0.05 µmole synthesis scale: 10 – 75 mer
0.2 µmole synthesis scale: 5 – 110 mer
1.0 µmole synthesis scale: 5 – 130 mer
10 µmole synthesis scale: 5 – 50 mer
15 µmole synthesis scale: 5 – 50 mer

**5. How are oligonucleotides synthesized?**

The most popular method for synthesizing oligonucleotides is to form natural 3'-5' phosphodiester bonds between monomers by using 'phosphite triester' protocols. ß-cyanoethyl phosphoramidites, the building monomers, were developed by Koster and used most often to synthesize oligonucleotides (Nucl. Acids Res. 1984, 12, 4539; Tetrahedron Lett. 1983, 24,5843). Through the 'phosphite triester' method using ß-cyanoethyl phosphoramidite, high coupling efficiency is achieved ( > 98%) and the time consumed for coupling is much shorter than that of other methods of oligo synthesis. Moreover, since the monomers, ß-cyanoethyl phosphoramidites, are quite stable prior to the activation, which is necessary for oligo synthesis, and means they can be stored for a long period of time.

The oligonucleotide is synthesized while attached covalently to a solid support. Excess soluble protected nucleoside β-cyanoethyl phosphoramidites and coupling reagent can drive the reaction near to completion. Among the solid supports, controlled pore glass (CPG), which consists of a glass matrix prepared uniformly with pores of defined size, has been used predominantly over the last few years.

The whole synthesis of oligonucleotides can be accomplished by the chain reactions where four different reaction cycles - deblocking, coupling, oxidation and capping are performed repeatedly (Figure 1)

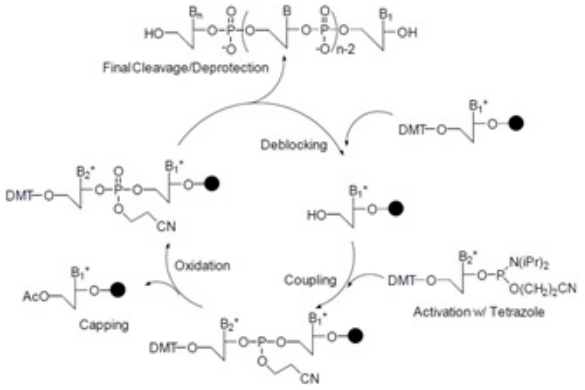



Figure 1 . Oligo Synthesis Cycle.

**A. Deblocking**

In the first step in of synthesis - deblocking - the 5' protecting group, DMT, is cleaved from the CPG. For deblocking, acidic condition is necessary, and trichloroacetic acid (3% in dichloromethane) is used in most of cases. It is reported that oligos can be depurinated in acidic conditions, especially more severe for adenosine. Since trichloroacetic acid is very acidic (pKa : ~1.5), deblocking solution with trichloroacetic acid should not be left too long in the reaction. Instead of trichloroacetic acid, dichloroacetic acid, which is less acidic than trichloroacetic acid, can be used for deblocking to avoid the depurination problem in certain cases. Since the DMT cation, which is produced after deblocking cycle, shows a very strong orange color, it can be used to monitor the coupling efficiency by measuring its light absorbance.

**B. Coupling**

The 5'-hydroxyl group on the CpG, which is exposed after the deblocking step, is coupled to the nucleoside β-cyanoethyl phosphoramidites to form triphosphite ester which is subsequently oxidized to a phosphotriester bond. For nucleoside β-cyanoethyl phosphoramidites, to avoid the unwanted side reaction during the whole oligo synthesis, exocyclic amino groups in base moiety are protected to result amide structure. Benzoyl groups are used for both adenosine and cytidine. On the other hand, isobutyryl group is used for guanosine base protection. Since thymidine doesn't have exocyclic amine group in base there is no need for extra protection. 5'-Hydroxyl groups are protected with DMT for all nucleoside βcyanoethyl phosphoramidites (Figure 2).

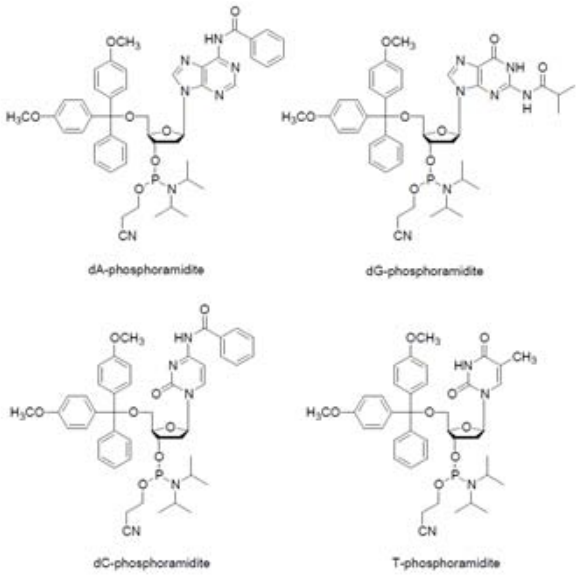



Figure 2. Nucleoside β-cyanoethyl phosphoramidites.

Since nucleoside β-cyanoethyl phosphoramidites are quite stable under normal conditions, they cannot react directly with a free 5' hydroxyl function on a growing chain. They must first be activated by treatment with an activator usually a type of weak acid.

Among a variety of candidates, tetrazole has shown a great efficiency and has been used as a standard activator. Tetrazole has been thought to play a dual role: it protonates the diisopropylamino group of the phosphoramidite function; and then comes in as a nucleophile, generating a very reactive tetrazolophosphane intermediate. Coupling reactions with these activated nucleoside phosphoramidite reagents are very fast (less than 2 min) and are almost quantitative

**C. Oxidation**

The newly formed phosphite internucleotide linkage is unstable and susceptible to both acidic and basic cleavage. Therefore, the trivalent phosphite triester is oxidized to a stable pentavalent phosphate triester.

Iodine is used as a mild oxidant in basic tetrahydrofuran solution with water as the oxygen donor. The reaction is extremely fast, being quantitative in 30 sec.

**D. Capping**

Since the coupling reaction cannot be quantitative in a finite time period, a small percentage of truncated sequences are produced at every coupling step. If these failure sequences were allowed to react further, it would be difficult to isolate the product from the sequence mixture. This problem is overcome largely by capping the remaining free hydroxyls through acetylation.

Acetylation is achieved with the strong acetylation reagent which forms on reaction of equimolar amounts of acetic anhydride and N-methylimidazole. The reaction is almost quantitative in 30 sec. After oxidation, the nucleotide addition cycle is complete. Oligonucleotide synthesis can continue removing the DMT group at the 5′-end of the growing chain and repeating another cycle of nucleotide addition. At the end of whole synthesis of oligonucleotides, cleavage from support and simultaneous base and phosphate deprotection are achieved by treatment with concentrated ammonium hydroxide.

**6. Standard Oligo structure**

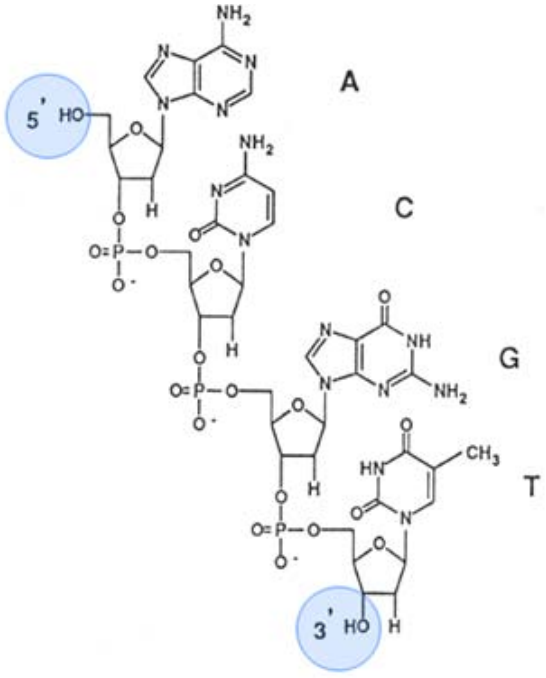


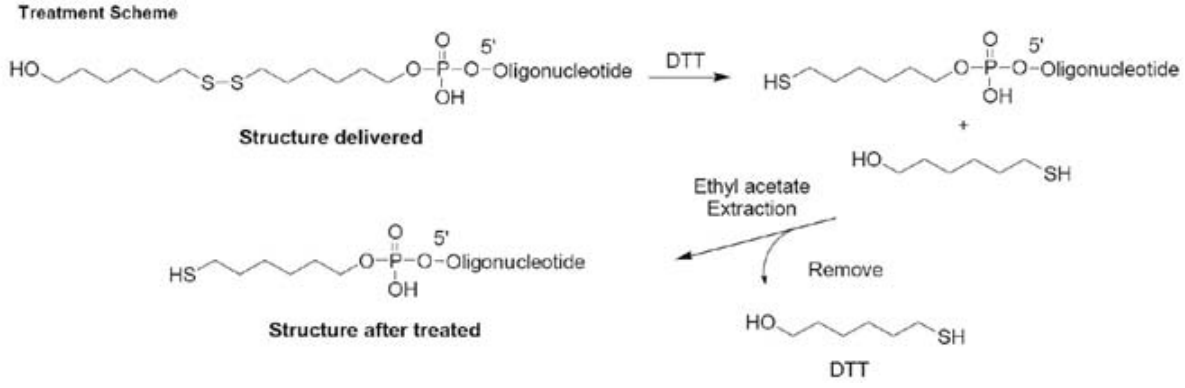

Figure 3. Standard oligo structure.

## Treatment for thiol modified oligonucleotides

1. Dissolve the dried thiol-modified oligonucleotide (5 O.D. based) in distilled water or an appropriate buffer, e.g. 0.1M TEAA pH 7.5 (50 µl)

2. Add 10 µl of 1.0 N dithiothreitol (DTT), vortex, then incubate at room temperature for 15 min.
(1.0 N DTT: 0.01 M Sodium acetate (pH 5.2) 20 ml + 1.545 g DTT dissolve (filtration))

3. Remove excess DTT and unwanted thiol fragments from the thiol-modified oligonucleotide mixture by extracting with ethyl acetate 3 times, using 50 µl per extraction. Discard the upper layer after vortexing the mixture. You must immediately proceed to the next step since the free sulfhydryl group becomes unstable after the removal of DTT.



## Anneal complementary pairs of oligonucleotides

General Procedure

1. Mix the concentrated complementary oligonucleotides together at 1:1 molar ratio in a micro centrifuge tube.

2. Dilute the oligonucleotide mixture to a final concentration of 1 pmol/μl with Tris or phosphate buffer containing salts, e.g.10 mM Tris, 0.1 mM EDTA, 50 mM NaCl (pH 8.0) or 100 mM sodium phosphate, and 150 mM NaCl, 0.1 mM EDTA (pH 7.5 or 8.0).

3. Anneal the oligonucleotides using one of the annealing methods described below.

4. Aliquot and store at -20˚C. The double-stranded DNA probes may be stored at 4˚C for several weeks, given that care is taken to protect the probes from nuclease degradation.

**Annealing Methods**

- Option 1: Anneal with a heating block
  1. Incubate the oligonucleotides at 95˚C for 5 min.
  2. Gradually reduce the heat until the oligonucleotides have reached room temperature.

- Option 2: Anneal with a water bath
  1. Boil 400 ml of water in a large glass beaker on a hotplate.
  2. Incubate the tube of oligonucleotides in the boiling water for 5 min.
  3. Turn off the hotplate, leaving the oligonucleotides in the beaker on the hotplate to slowly cool to room temperature.

- Option 3: Anneal with a thermal cycler
  1. A thermal cycler allows for convenient and reproducible annealing of oligonucleotides.
  2. Use Table 1 as a guide to program your thermal cycler for either a simple or advanced protocol.
  3. The notation "-1˚C/cycle" indicates a 1˚C decrease in temperature per cycle.
  4. Refer to your thermal cycler's Operation Manual or consult the manufacturer for information about programming your particular instrument.

Table 1. Thermo cycler programs for annealing complementary oligonucleotides.

| | | | | |
|---|---|---|---|---|
| Simple Protocol | | Cycles | Temperature | Time |
| | Step 1 | 1 | 95˚C | 5 min |
| | Step 2 | 70 | 95˚C (-1˚C/cycle) | 1 min |
| | Step 3 | | 4˚C | HOLD |
| Advanced Protocol (example in which the oligonucleotide pair has a Tm of 55˚C) | | Cycles | Temperature | Time |
| | Step 1 | 1 | 95˚C | 5 min |
| | Step 2 | 40* | 95˚C (-1˚C/cycle) | 1 min |
| | Step 3 | 1 | 55˚C | 30 min |
| | Step 4 | 20* | 55˚C (-1˚C/cycle) | 1 min |
| | Step 5 | | 4˚C | HOLD |

* The number of cycles in step and 4 depends on the Tm of the oligonucleotides to be annealed.