ABI PRISM dRhodamine Terminator Cycle Sequencing Kit

- **Higher accuracy**
- **Longer reads**
- **More uniform peaks**

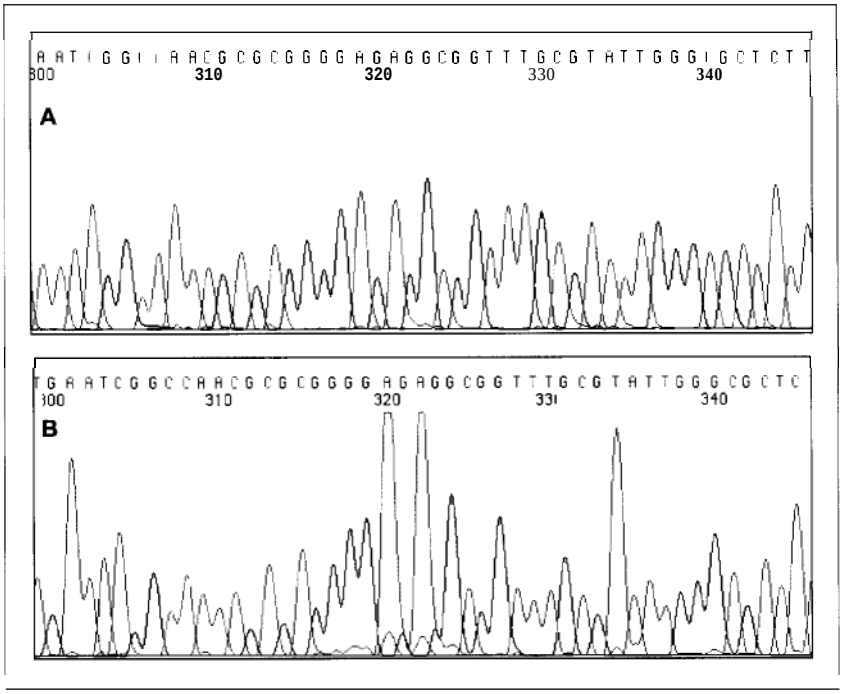


*Figure 1. DNA sequence of **pGEM®-3Zf(+)** from approximately bases 300-345 using the ABI **PRISM®** dRhodamine Terminator Cycle Sequencing Kit (Panel A) and the ABI **PRISM®** Dye Terminator Cycle Sequencing Kit (Panel B). The more uniform peak heights and reduced background noise generated with the dRhodamine terminators enable superior basecalling.*

Higher Accuracy, Longer Reads

Cycle sequencing using dye-labeled terminators is a rapid and convenient method for DNA sequence determination. Dye terminators allow single tube reaction set-up, reduced pipeting and liquid handling, and the ability to increase sample throughput.

The ABI PRISM® dRhodamine Terminator Cycle Sequencing Kit combines a newly developed set of dye terminators with AmpliTaq® DNA Polymerase, FS, the enzyme of choice for fluorescent sequencing. These new dichlororhodamine (dRhodamine) terminators provide more even peak patterns and less background noise than conventional rhodamine terminators. The result is higher accuracy, longer read lengths and greater productivity in automated sequencing.

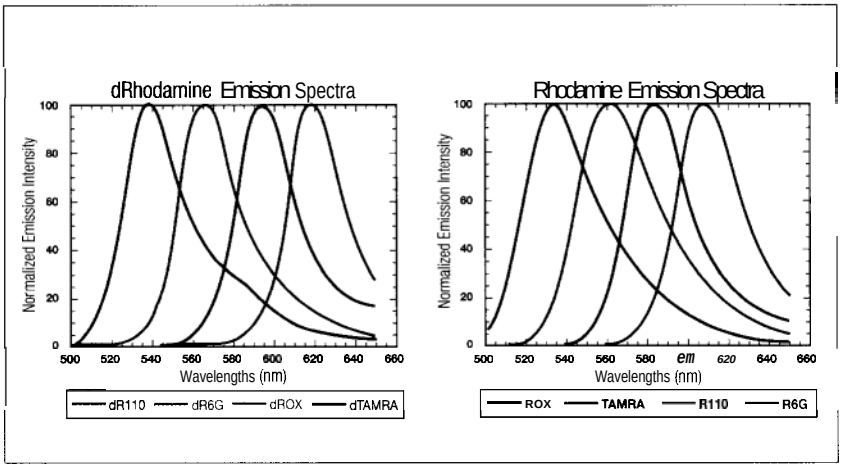


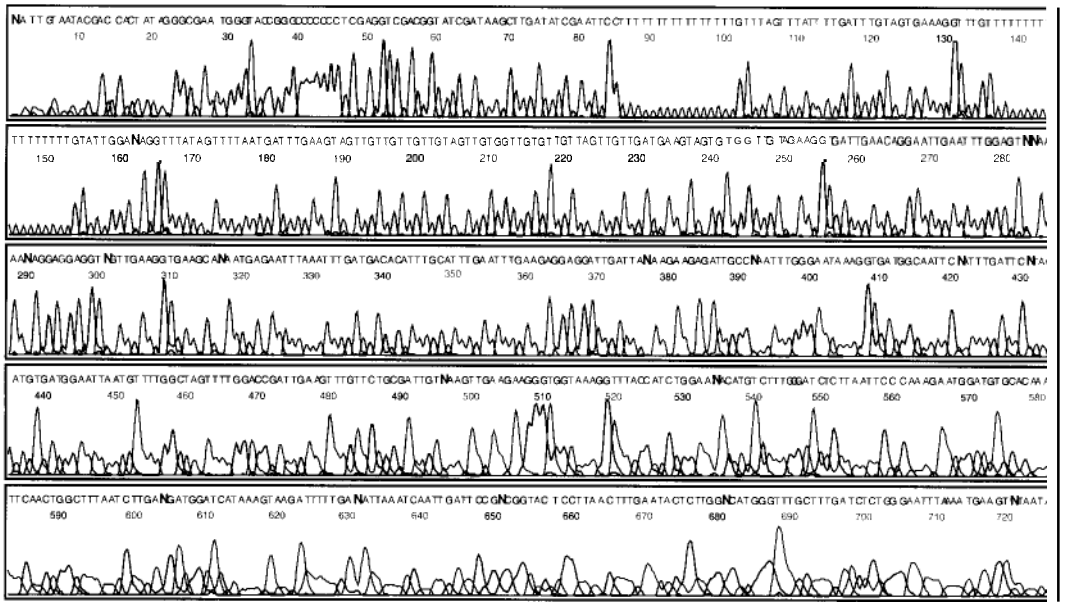
Figure 2. The dRhodamine dye set's emission spectra are more narrow than those of the Rhodamine dye set. This leads to less spectral overlap, which reduces background noise and generates cleaner signal.

dRhodamine Terminators

Dye terminator molecules used in fluorescent sequencing consist of a fluorescent dye attached to a dideoxynucleotide via a linker (Figure 6). The development of an improved set of dye terminators that provides the benefits of more even peak patterns, minimal mobility shifts and cleaner signal requires optimization of both the dyes themselves and the linker moieties.

Sequencing a *Dictyostelium* cDNA clone

Standard Rhodamine Terminators



dRhodamine Terminators

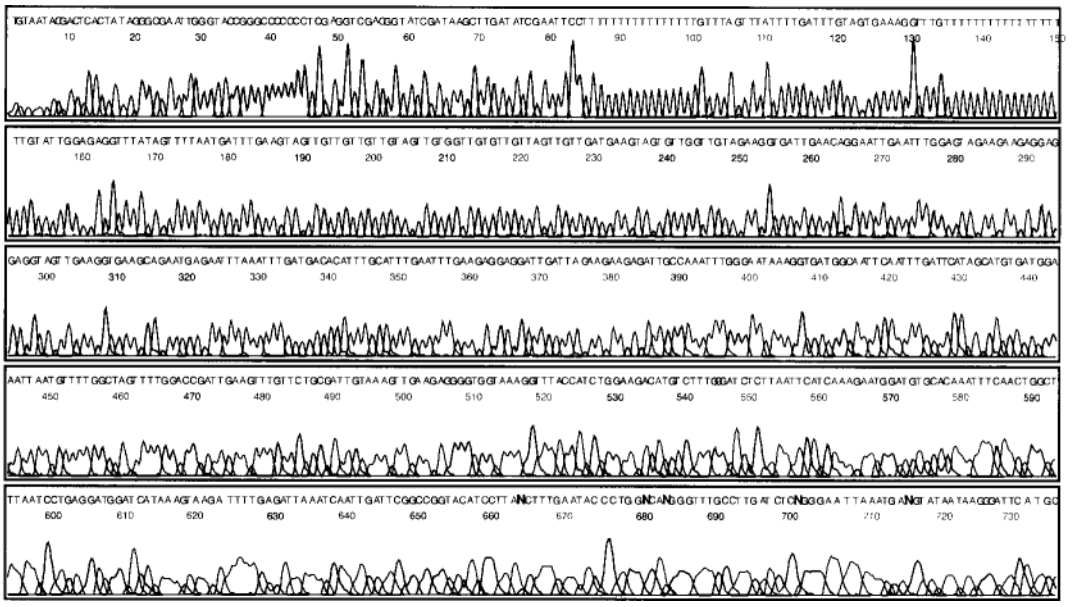
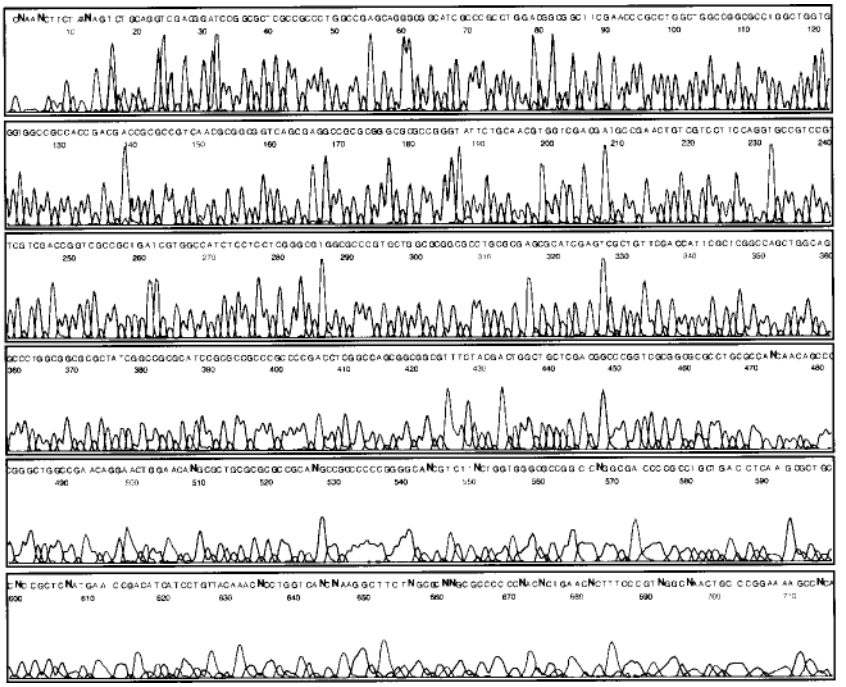


Figure 3. pDJ2 is a cDNA clone from Dictyostelium discoideum with unusual sequence motifs such as 300 contiguous bases with 1% "C," homopolymer "T" stretches, and "A" and "G" patterns that create the potential for base Calling errors. 250 ng of template pDJ2 was sequenced with both the ABI PRISM® Dye Terminator Cycle Sequencing Kit (Panel A) and the ABI PRISM® dRhodamine Terminator Cycle Sequencing Kit (Panel B). One-quarter volume of the ethanol-precipitated reaction mixtures was loaded onto a 5% Long Ranger™ gel and run using the 2X Run Module on an ABI PRISM® 377 DNA Sequencer. The dRhodamine terminator data shows more even peak heights and superior base calling.

Sequencing a "GC-rich" clone

Standard Rhodamine Terminators

A



dRhodamine Terminators

B

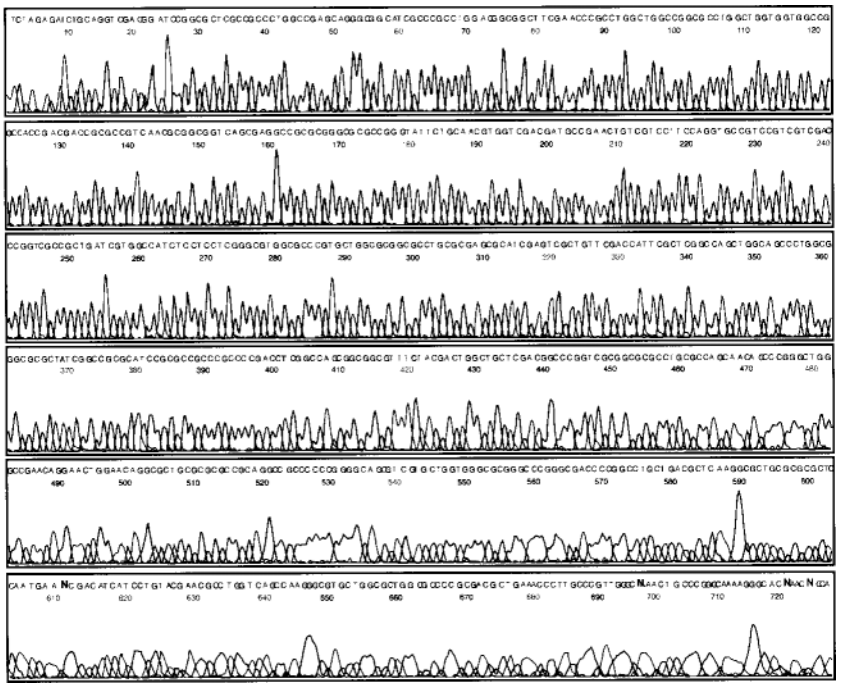


Figure 4. p4009-1 is greater than 65% GC-rich and notoriously difficult to sequence. 500 ng of template was sequenced with both the ABI PRISM® Dye Terminator Cycle Sequencing Kit (Panel A) and the ABI PRISM® dRhodamine Terminator Cycle Sequencing Kit (Panel B). One-quarter volume of the ethanol-precipitated reaction mixtures was loaded onto a 5% Long Ranger gel and run using the 2X Run Module on an ABI PRISM® 377 DNA Sequencer. The more even peak pattern characteristic of the dRhodamine terminators improves ambiguous basecalls caused by weak "G's" after "C's" and weak "G's" after "A's".

represents our commitment to providing automated, multicolor fluorescence-based genetic analysis systems, which include reagents, instruments and integrated software.

Specifications

ABI PRISM® sequencing kits provide all of the reagents needed for sequencing 100 or 1,000 single-stranded (ss) or double-stranded (ds) DNA templates. The minimum performance specification is 450 bases at 98% accuracy using the double-stranded control template provided in the kit. The reagents provided in each kit have been optimized for use with ABI PRISM' 377, 377XL, 310, and GeneAmp® PCR systems. The use of a GeneAmp PCR system is recommended to ensure optimal results.

References

1. Sandra L. Spurgeon, Shiaw-Min Chen and Sandy M. Koepf (1996). Improvements in Dye Primer and Dye Terminator Sequencing with AmpliTaq DNA Polymerase, FS. *Microbial & Comparative Genomics* Volume 1, Number 3, 254.

Ordering Information

Description	P/N
ABI PRISM® dRhodamine Terminator Cycle Sequencing Kit 100 ready reactions	403044
ABI PRISM® dRhodamine Terminator Cycle Sequencing Kit 1,000 ready reactions	403045
ABI PRISM® dRhodamine Terminator Cycle Sequencing Kit 5,000 ready reactions	4303143
dRhodamine Matrix Standards Kit	403047

Worldwide Sales Offices

Applied Biosystems vast distribution and service network, composed of highly trained support and applications personnel, reaches into 150 countries on six continents. For international office locations, please call the division headquarters or refer to our web site at **www.appliedbiosystems.com.**

Applera Corporation is committed to providing the world's leading technology and information for life scientists. Applera Corporation consists of the Applied Biosystems and Celera Genomics businesses.

Headquarters

850 Lincoln Centre Drive
Foster City, CA 94404 USA
Phone: 650.638.5800
Toll Free: 800.345.5224
Fax: 650.638.5884

For Research Use Only.
Not for use in diagnostic procedures.

Applied Biosystems

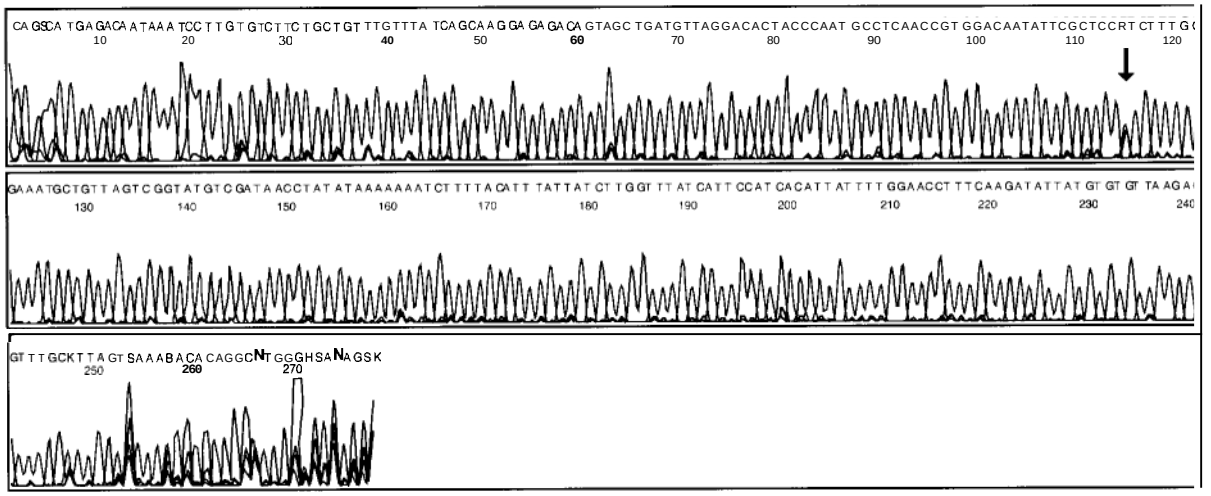
Publication 237629-002

Printed in U S A

Sequencing of a Heterozygous PCR Product

Dye-Labeled Primer

A



dRhodamine Terminators

B

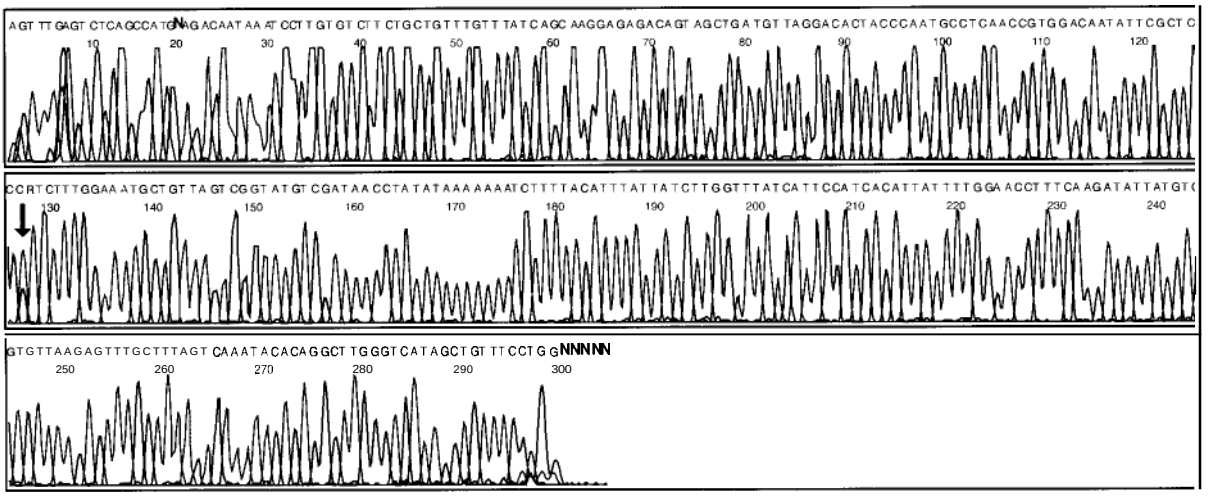


Figure 5. Exon 8 from MLH I was PCR amplified from genomic DNA, then was prepared and sequenced two different ways. In Panel A, the PCR product was diluted and sequenced using the ABI PRISM® Dye Primer Cycle Sequencing Kit. In Panel B, the PCR product was treated with Exonuclease I and Shrimp Alkaline Phosphatase and then sequenced using the ABI PRISM® dRhodamine Terminator Cycle Sequencing Kit. Following pooling and ethanol precipitation, both reactions were loaded onto a 36-cm well-to-read gel containing 5% Long Ranger and run on an ABI PRISM® 377 DNA Sequencer. Panel A shows a single heterozygote at base 115. Panel B shows a single heterzygote at base 128. Both panels show the same heterozygote, which is clearly called as an "R" by the base calling software.

dRhodamine terminators use four dichlororhodamine dyes (dR110, dR6G, dTAMRA and dROX) in place of our conventional rhodamine dyes (R110, R6G, TAMRA and ROX). The dRhodamine dyes are attached using an optimized propargyl/ethylene oxide/amino ("EO") linker in place of the conventional propargylamino linker (Figure 6).

More Even Peak Patterns

The EO linker designed for the dRhodamine terminators equalizes the incorporation rate of the four dye terminators, leading to a significantly more even peak pattern overall. dRhodamine terminators improve the characteristic weak G after A pattern observed with rhodamine terminators. With the new dRhodamine terminator kits, errors due to peak height variation are reduced and basecalling accuracy is improved (Table 1).

Longer Read Lengths

The new dRhodamine dyes are slightly red-shifted and have narrower emission spectra than conventional rhodamine dyes (Figure 2). This reduces the spectral overlap among the four dyes in the set and, therefore, reduces background noise, which can obscure signal at longer reads. The result is a much higher signal-to-noise ratio and improved basecalling accuracy (fewer miscalls and no calls), especially at longer read lengths. Compared with rhodamine terminators, read lengths increase as typically difficult-to-read-through regions yield cleaner signal. Increased accuracy of base calls, especially in difficult-to-sequence templates, such as homopolymer or repeat regions (Table 1 and Figure 1), is also a benefit of the dRhodamine terminators.

Improved Performance in All Applications

Improved peak patterns, higher signal-to-noise, and the ability to read through difficult regions make dRhodamine terminator chemistry the method of choice for most sequencing applications. The ability to use a one-tube chemistry can be translated into time and consumable cost savings.

Even peak heights without false terminations improve the outcome of many sequencing challenges, including heterozygote determination or mutation detection. Routine sequencing is also improved with the dRhodamine terminator system through more reliable terminator results and the ability to increase throughput and simplify sample handling.

Convenience of Ready Reaction Format

The ABI PRISM® dRhodamine Terminator Cycle Sequencing Kit combines the benefits of AmpliTaq® DNA Polymerase, FS and dRhodamine terminators in a ready-to-use format. dRhodamine dye terminators, deoxynucleoside triphosphates, AmpliTaq FS enzyme, magnesium chloride and reaction buffer are premixed into a single tube of ready reaction mix. This formulation is suitable for both single- and double-stranded DNA or PCR templates.

Guaranteed Performance

The reagents in our ABI PRISM® sequencing kits are tested twice to ensure quality—first for correct formulation and then for consistent, reliable performance on an ABI PRISM® DNA sequencer. In addition, PE Biosystems' expert field and telephone support teams are readily available, enabling you to confidently address a wide variety of DNA sequencing applications.

ABI PRISM® Cycle Sequencing Kits are part of PE Biosystems' expanding line of fluorescent DNA analysis reagents. The ABI PRISM™ brand

Errors + Ns

Template	21–220	221–420	421–620	Total	Accuracy (Y_0)
pGEM, R	0	0	3	3	99.5
pGEM, dR	0	0	1	1	99.8
pDJ2, R	2	7	9	18	97
pDJ2, dR	0	0	2	2	99.7
pCDNAII, R	6	8	11	24	96
pCDNAII, dR	1	3	1	5	99.2
p4009, R	0	0	16	16	97.3
p4009, dR	0	0	2	2	99.7

Table 1. Accuracy of dye terminator data obtained with rhodamine terminators (R) and ABI PRISM® dRhodamine Terminators (dR) on a variety of DNA template types (see Figures 3 through 5).

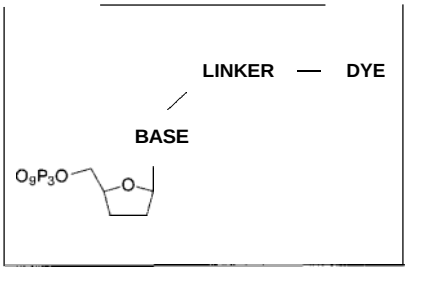


Figure 6. Basic structure of fluoresceni dye terminator molecule.