• Brighter Signal
• Cleaner Data
• Longer Reads

Dye-labeled primer kits are ideal for sequencing inserts cloned into phage, plasmids, and bacterial artificial chromosomes (BACs), as well as PCR products that contain a universal primer binding site. Dye-labeled primers are also an excellent method for heterozygote or mutation detection in PCR-generated templates.

The ABI PRISM® BigDye™ Primer Cycle Sequencing Kit introduces a single-molecule energy transfer dye primer that provides improved performance over other dye-labeled primer chemistries, as well as greatly increased sensitivity.

**ABI PRISM® BigDye™ Primers**

ABI PRISM® BigDye™ primers are single energy-transfer molecules: an energy donor dye and an acceptor dye are connected by a highly efficient energy transfer linker.

In the case of the ABI PRISM® BigDye™ Primer Cycle Sequencing Kit, the acceptor is a dichlororhodamine dye. Dichlororhodamine dyes (ABI PRISM® dRhodamines) are an improvement over conventional rhodamine dyes. dRhodamines exhibit better spectral resolution—there is significantly less spectral overlap at their maximum excitation wavelength (Figure 1), and their sequencing products show much reduced background noise. This results in cleaner signal and greater base calling accuracy at longer read lengths.

An energy transfer linker couples the acceptor dRhodamine and donor fluorescein dyes for very efficient energy transfer in a single dye molecule. The entire dye complex is attached via an NHS ester to the 5' end of a sequencing primer (Figure 7). Thus, an optimally-designed sequencing primer can be used. Primer selection is not based upon any other sequence-dependent constraints.

**Advantages of ABI PRISM® BigDye™ Primers**

These improvements have been incorporated into the ABI PRISM® BigDye™ Primer Cycle Sequencing Kit with AmpliTaq® DNA Polymerase, FS. The results are very high sensitivity—reducing the need for large sample volumes and very even peak heights. Since the four BigDye primers have a three fold brighter signal and more equivalent emission intensities, reactions are done at a 1:1:1:1 ratio in terms of reaction volume and quantities of reagents. Reaction set-up is made simpler and the possibility of pipeting error is reduced.

The ABI PRISM® BigDye™ primers also show minimal mobility shifts. Low mobility shifts improve peak resolution and basecalling accuracy. There is less than one-third of a base constant shift for both BigDye primer sets. Thus, the same mobility file can be used for both the -21M13 and M13 reverse primers on both the ABI PRISM® 377 DNA Sequencer and 310 Genetic Analyzer.

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Figure 1. The BigDye™ primer dye set's emission spectra are brighter and narrower than those of the standard dye primer set. This leads to three-fold brighter signal and better spectral resolution.
Greater Flexibility in Experimental Design

The much higher signal-to-noise ratio generated by the ABI Prism® BigDye™ primers generates several options in experimental design. The amount of reagent used per reaction may be decreased, the amount of template can be decreased, the amount of product used in electrophoresis can be decreased (Figure 2) or, fewer cycles can be used. Due to the increase in signal generated with the ABI Prism® BigDye™ primers, it is possible to load reaction products directly onto an ABI Prism® 377 DNA Sequencer gel without ethanol precipitation (Figure 3). The above choices may also be balanced and combined into a single, more efficient strategy.

Improved Performance in Difficult Applications

Direct sequencing of the ends of a very large insert (greater than 100 kb) cloned into a BAC is very important to genome sequencing. “BAC-end sequencing” allows identification of an ordered contiguous set of BAC clones that overlap. Once the ordered set of BAC clones is identified, inserts can be further sub-cloned into plasmids for shotgun sequencing. Direct BAC sequencing has posed challenges in that there are very few copies of target molecules for priming within the limited volume of a sequencing reaction. With conventional labeled primers, the sequencing reaction can be signal-limited. ABI Prism® BigDye™ primers provide sufficient signal for direct BAC-end sequencing (Figure 4).
Templates generated by PCR are suitable for dye-labeled primer sequencing. The ABI Prism® BigDye™ primers have been used in sequencing of Extra Long PCR (XL PCR) products (Figure 5). PCR products can also be used for heterozygote determination in genes implicated in various cancers (Figure 6).

The ABI® Prism® BigDye™ Primer Cycle Sequencing Kit combines the benefits of AmpliTaq® DNA Polymerase, FS and BigDye primers in a ready-to-use format. BigDye primers, deoxynucleoside triphosphates, AmpliTaq® FS enzyme, magnesium chloride, and reaction buffer are premixed into ready reaction mixes. ABI Prism® BigDye primers are 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, Applied Biosystems expert field and telephone support teams are readily available, enabling you to confidently address a wide variety of sequencing applications.

ABI Prism® cycle sequencing kits are part of Applied Biosystems expanding line of fluorescent DNA analysis reagents. The ABI Prism® brand represents our commitment to providing automated, multicolor, fluorescence-based genetic analysis systems, which include reagents, instruments, and integrated software.

Figure 5. Instead of using a traditional template prep method, XL PCR was used to amplify a lambda clone. After PCR, the product was sequenced with the ABI Prism® BigDye™ Primer Cycle Sequencing Kit.

Figure 6. Exon 8 from MLH I was PCR amplified from genomic DNA with M13-tailed primers, then the PCR was diluted and sequenced using the ABI Prism® BigDye™ Primer Cycle Sequencing Kit. The data shows a single heterozygote at base 109.

Figure 7. BigDye™ Primer Structure.
Specifications

ABI Prism® sequencing kits provide all of the reagents needed for sequencing 100 or 5,000 single-stranded (ss) or double-stranded (ds) DNA templates. The minimum performance specification using the pGEM® control are as follows for the ABI Prism® 377 DNA Sequencer:

<table>
<thead>
<tr>
<th>Run Module</th>
<th>Specifications</th>
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<tbody>
<tr>
<td>Seq Run 36E-2400</td>
<td>450 bases at 98%</td>
</tr>
<tr>
<td>Seq Run 36E-1200</td>
<td>550 bases at 98%</td>
</tr>
<tr>
<td>Seq Run 48E-1200</td>
<td>650 bases at 98%</td>
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</table>

The reagents provided in each kit have been optimized for use with ABI Prism® 3700, 377, 3100, and 310 Genetic Analyzer and GeneAmp® PCR systems. When generating PCR products as templates for DNA sequencing, the use of a GeneAmp PCR System is recommended to ensure optimal results.

Ordering Information

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<thead>
<tr>
<th>Description</th>
<th>P/N</th>
</tr>
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<tbody>
<tr>
<td>The ABI Prism® BigDye™ Primer - 21M13 Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, FS 100 Ready Reactions</td>
<td>403051</td>
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<tr>
<td>The ABI Prism® BigDye™ Primer - 21M13 Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, FS 5000 Ready Reactions</td>
<td>403049</td>
</tr>
<tr>
<td>The ABI Prism® BigDye™ M13 Reverse Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, FS 100 Ready Reactions</td>
<td>403052</td>
</tr>
<tr>
<td>The ABI Prism® BigDye™ M13 Reverse Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, FS 5000 Ready Reactions</td>
<td>403050</td>
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<tr>
<td>dRhodamine Matrix Standards Kit for use with BigDye™ Primers, BigDye™ Terminators and dRhodamine Terminators</td>
<td>403047</td>
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References