

Take the direct route to DNA sequencing

BigDye® Direct Cycle Sequencing Kit

A fast PCR-sequencing workflow that delivers outstanding sequence quality

- Fewer steps—post-PCR purification and sequencing are combined in a single step
- Convenient sample handling—all steps from PCR to capillary electrophoresis can be performed in a single tube
- Simplified workflow—incorporates M13 universal sequencing primers
- Fast run times without compromise—POP-7™ polymer allows you to read as close as one base from the primer



DNA sequencing, like all other scientific endeavors, has undergone a dramatic transformation. From the days of the first Applied Biosystems® DNA sequencers more than twenty years ago, DNA sequencing using BigDye® chemistry is now routine in countless labs across the globe every day. And we're still innovating. The BigDye® Direct Cycle Sequencing Kit represents a significant improvement in the current cycle sequencing workflow (Figures 1 and 2)—fewer steps, less handling and hands-on time, developed using a set of reagents that are optimized to work together to give you accurate sequence data across the entire read—in less time.

Streamlined Workflow

The BigDye® Direct Cycle Sequencing Kit simplifies the existing Sanger sequencing workflow by providing post-PCR clean-up reagents and sequencing reagents in a single mix, which is added directly to the

PCR reaction (Figure 1). By combining PCR clean-up with cycle sequencing, a pipetting step is eliminated, which saves you time and reduces the possibility of errors during transfers. Also, performing all steps in a single tube allows easier traceability of your process.

Following the BigDye® Direct workflow, PCR primers* are designed with M13 tails and are used in conjunction with the BigDye® Direct PCR Master Mix, allowing the addition of the M13 primer binding sites to all PCR products. This means that all PCR products can be sequenced using the M13 forward and reverse universal sequencing primer pair provided in the kit. And because only one primer pair is required, sequencing set-up is less prone to error and is more amenable to higher-throughput strategies.

*PCR primer designs are specific to the user's DNA target regions of interest, and are not provided in the BigDye® Direct kit. Users are advised to design their PCR primers to include M13 tails.

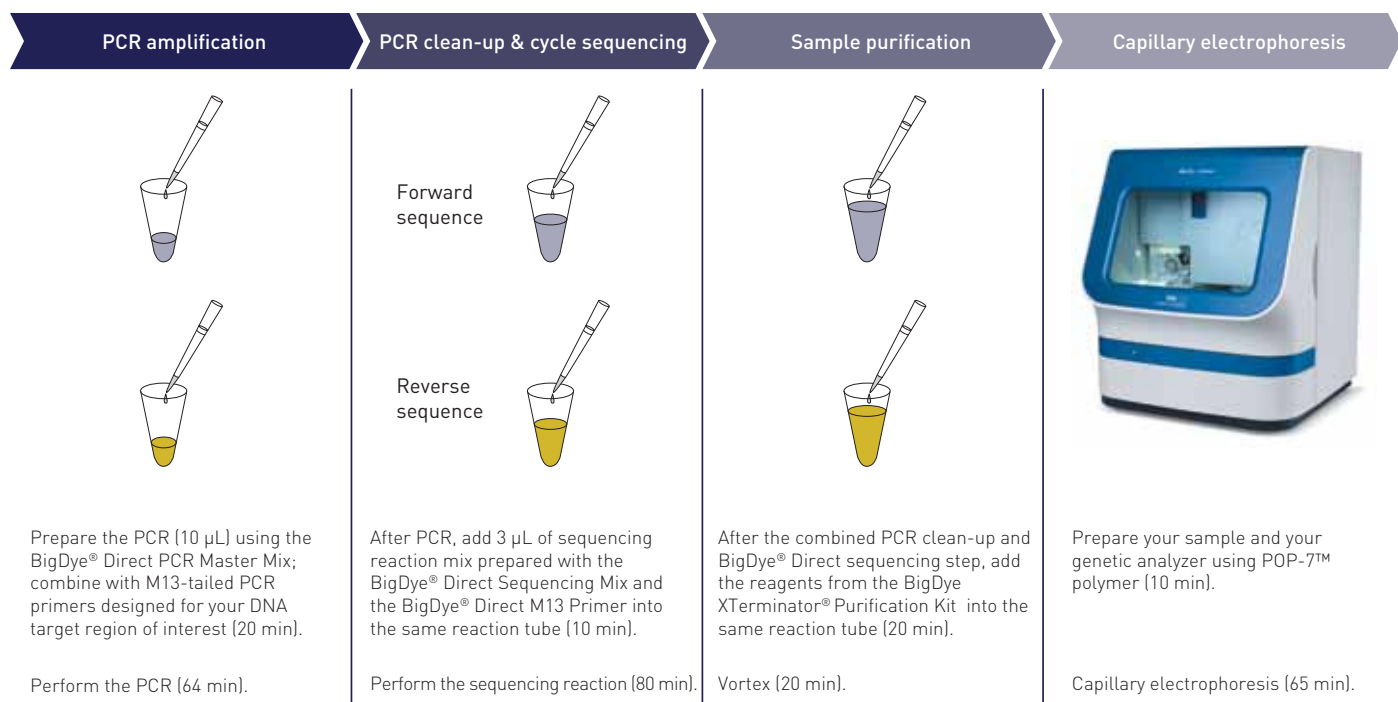


Figure 1. Simple Workflow in a Single-Tube Reaction. The BigDye® Direct sequencing workflow starts with a PCR reaction performed with the BigDye® Direct PCR Master Mix in a 10 μ L volume. Following PCR, 3 μ L of a sequencing mix (containing the BigDye® Direct sequencing mix and clean-up reagents) and M13 sequencing primer is added directly to the PCR tube—so that PCR clean-up and sequencing are achieved in a single step. Finally, the sequencing purification step can be performed in the same tube using the BigDye XTerminator® Purification Kit (sold separately), and the tube can then be placed directly in the sequence analyzer for analysis.

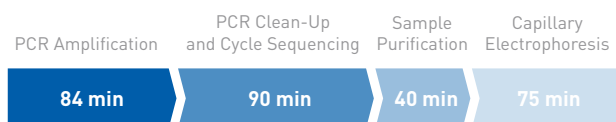
Uncompromised Performance

BigDye® Terminator cycle sequencing chemistries have set the standard for nearly two decades, and the BigDye® Direct Cycle Sequencing Kit is no exception. Employing the gold-standard AmpliTaq Gold® UP enzyme and hot-start capabilities to minimize nonspecific amplification, the BigDye® Direct Kit is optimized to complete the PCR step in about an hour.

Using POP-7™ polymer, the BigDye® Direct Cycle Sequencing Kit provides high-resolution sequence data—as close as 1 base after the primer—and rapid capillary electrophoresis times (Figure 3). Better 5' mobility improves basecalling accuracy, reducing the need for manual sequence editing (Figure 4). Combine this with the process efficiencies achievable by using a single set of M13 universal sequencing primers, and you can potentially achieve a doubling of your genetic analyzer's throughput.

BigDye® Direct Cycle Sequencing Kit workflow, run with POP-7™ Polymer

Four steps in approximately 5 process hours



A traditional cycle sequencing workflow, run with POP-6™ Polymer

Five steps in approximately 8 process hours

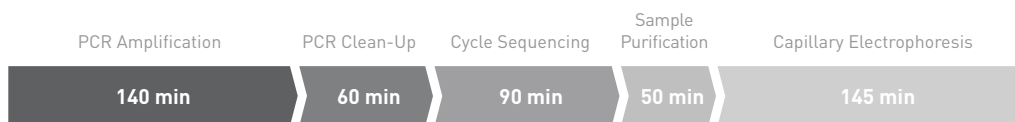


Figure 2. The BigDye® Direct Kit Delivers Significant Efficiencies Compared to Standard Sequencing. Traditional sequencing workflows can require more than 8 hours of process time and 5 steps to complete. In contrast, the BigDye® Direct workflow typically requires only 5 hours and 4 steps, producing sequence reads up to 40% faster and with less hands-on time. [Note: the times reported for the PCR and sequencing steps include hands-on time and thermal cycling time using the Veriti® Thermal Cycler.]

Designed for Convenience

Our entire BigDye® cycle sequencing product line is recognized as the gold standard among sequencing chemistries, consistently delivering quality data for a wide range of sequencing applications. The BigDye® Direct Cycle Sequencing Kit builds on this excellence in sequencing by delivering a workflow that includes a PCR master mix, a sequencing mix (enabling post PCR clean-up and cycle sequencing in one step), a DNA control, and M13-tailed universal sequencing primers (forward and reverse)—all designed to work optimally together. This frees you from the task of procuring multiple reagents and can potentially reduce the number of optimization experiments you may be required to perform in your laboratory. Also, using the quality-controlled BigDye® Direct PCR and Sequencing Master Mixes can help ensure reproducibility and minimize variability from experiment to experiment.

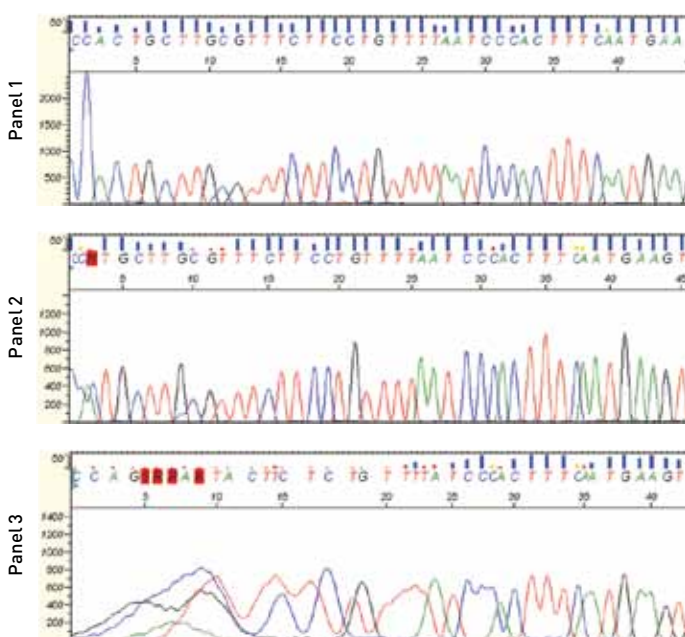


Figure 3. The BigDye® Direct Cycle Sequencing Kit Delivers Longer Reads and Excellent 5' Resolution. Though POP-7™ polymer is developed to yield faster sequencing runs than those performed with POP-6™ polymer (75 min vs. 145 min), the resolution of the first 20 to 30 bases after the sequencing primer can be affected (**Panel 3**). The BigDye® Direct kit's M13-tailed universal sequencing primer pair and optimized chemistry deliver high-quality base calls close to the sequencing primer when capillary electrophoresis is performed using POP-7™ polymer (**Panel 1**), with resolution typically equivalent or superior in quality to sequencing data generated using the BigDye® Terminator Cycle Sequencing Kit v1.1 and POP-6™ polymer (**Panel 2**).

The BigDye® Direct Cycle Sequencing Kits are supported on Applied Biosystems® 3130, 3130xl, 3500, 3500xl, 3730, and 3730xl sequencing platforms and are compatible with our BigDye XTerminator® Purification Kits for post-sequencing purification. The BigDye® Direct Cycle Sequencing Kit is compatible with your existing laboratory equipment, so there is no additional capital investment needed. Only a simple software update is needed for your genetic analyzer. This makes it easy for you to integrate the BigDye® Direct Cycle Sequencing Kit into your existing work routines and to take advantage of all the benefits this new formulation provides.

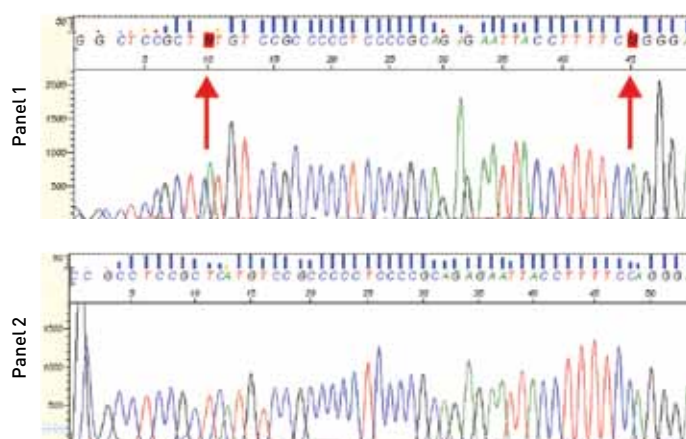


Figure 4. More Accurate Basecalling With POP-7™ Polymer. Samples analyzed with the BigDye® Terminator Cycle Sequencing Kit v1.1 and analyzed using POP-6™ polymer sometimes exhibit 5' C/A and A/G mobility shifts that can lead to an incorrect base call (**Panel 1**). Samples analyzed with the BigDye® Direct kit can improve 5' mobility and, consequently, basecalling accuracy (**Panel 2**).

Reactions per Kit

Reaction number	P/N
24 rxn	4458689
100 rxn	4458687
1000 rxn	4458688

Note: Each kit contains BigDye® Direct PCR Master Mix, BigDye® Direct Sequencing Master Mix, BigDye® Direct M13 Forward Sequencing Primer, BigDye® Direct M13 Reverse Sequencing Primer, and Control DNA.

Documentation

Description	P/N
BigDye® Direct Sequencing Kit Protocol*	4458040
BigDye® Direct Sequencing Kit Quick Reference Card*	4458017

*Available online

Software Update (free of charge)

Description	
BigDye® Direct Installer	www.appliedbiosystems.com/bigdyedirectinstaller

Go to www.appliedbiosystems.com/bigdyedirect to learn more, or contact your local Sales Representative for more information.

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Printed in the USA. C013911 0211

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