

## BigDye<sup>®</sup> Terminator v3.1 and v1.1 Cycle Sequencing Kits Frequently Asked Questions

**Q: How do these two new chemistries compare to BigDye<sup>®</sup> v1.0 and v3.0 kits? Which one is right for me?**

**A:**

- The v3.1 kit is based on the v3.0 kit, and is recommended for the majority of sequencing applications
- If you are currently using the v3.0 kit, you will most likely be interested in switching to the v3.1 kit
- The v1.1 kit is based on the original BigDye Terminator “v1” chemistry, and is designed for specialty applications such as sequencing short PCR products using rapid electrophoresis run modules
- If you are currently using the “v1” kit, you will most likely be interested in switching to the v1.1 kit
- See Table Below:

Application	BigDye <sup>®</sup> Terminator v3.1	BigDye <sup>®</sup> Terminator v1.1
de novo Sequencing	+	✓
Resequencing	+	✓
Sequencing difficult templates	+	+
Long-read sequencing	+	✓
Sequencing across all template types (plasmids, BACs, and fosmids)	+	✓
Mixed base detection	+	✓
Sequencing short PCR products using rapid electrophoresis run modules	✓	+

+ Recommended    ✓ Satisfactory

**Q: What if I am using the BigDye<sup>®</sup> v2.0 kit? Which one of these new chemistries is right for me?**

**A:**

- If you are interested in the most robust, flexible chemistry that will generate the longest reads, then you will prefer the v3.1 kit. If you are primarily sequencing

short PCR fragments with rapid electrophoresis modules, or if you would like to be confident that the new kit will plug directly into your v2.0 kit workflow, then you may prefer v1.1.

**Q: How does the v3.1 kit compare in performance to that of v3.0 kit?**

**A:**

- The v3.1 kit is more robust, and will sequence through templates that have previously been difficult to sequence
- Because of its optimized signal balance, and improved peak height uniformity through the entirety of the sequence, the v3.1 kit will generate longer, higher quality reads

**Q: What kind of DNA templates and sequence motifs will show improved success with the v3.1 kit?**

**A:**

- The v3.1 kit's enhanced robustness enables success with a wide variety of templates, including PCR products, plasmids, and large insert clones such as fosmids and bacterial artificial chromosomes (BACs). The v3.1 kit has shown improvements in sequencing difficult dinucleotide repeats (CT repeats in particular) and homopolymer regions.

**Q: How does the v1.1 kit compare in performance to that of the “v1” kit?**

**A:**

- The v1.1 kit is more robust than the “v1” kit, and will sequence through templates that have previously been difficult to sequence
- The v1.1 kit is more uniform than the “v1” kit, and therefore improves base calling accuracy
- Like the “v1” kit, the v1.1 kit demonstrates optimal basecalling just adjacent to the primer

**Q: What kind of DNA templates and sequence motifs will show improved success with the v1.1 kit?**

**A:**

- Like the v3.1 kit, the v1.1 kit has shown improvements in sequencing difficult dinucleotide repeats (CT repeats in particular) and homopolymer regions.

**Q: How do the new chemistry formulations compare to their predecessors? What is different, and what has remained the same?**

**A:**

- The v3.1 kit uses the same dyes as the v3.0 kit while the v1.1 kit uses the same dyes as the “v1” and v2 kits
- Other components of the formulation have been optimized
- The v3.1 and v1.1 kits feature a new enzyme

**Q: What is BigDye Terminator v1.1/v3.1 Sequencing Buffer (5X)? Why is it being bundled with the kits?****A:**

- Applied Biosystems recommends performing reactions consisting of 8 uL of ready reaction mix in a final volume of 20 uL. However, we are aware that some customers choose to use less than the recommended volume of ready reaction mix. When customers use less ready reaction mix, sequencing buffer is required to ensure proper reaction conditions are maintained.
- The sequencing buffer is being bundled with the kits because if customers decide to dilute their reactions with either a “home brew” buffer or one from another vendor, they may observe a deterioration in data quality.
- The decision to prepare your reactions using less than the recommended 8 ul of ready reaction mix can carry with it significant tradeoffs, including increased sample failure rate, shorter read lengths and decreased signal strength. Applied Biosystems does not support diluted reactions, and we do not guarantee the performance of BigDye chemistry when it is diluted.

**Q: How do I obtain a protocol manual?**

**A:** Protocol manuals are not included with the kits, but are available free of charge. You must either request the protocol or download it from the Applied Biosystems Web site. Some changes have been made to protocols that will enhance the performance of the new chemistries. Customers are encouraged to review and follow the new recommended protocols.

**Q: Are the new chemistries compatible with Trace Tuner™ Software and PHRED analysis software?**

**A:** New versions of Trace Tuner™ are calibrated to provide accurate quality values for data generated with v3.1 and v1.1 chemistries. Although PHRED has not been recalibrated specifically for these chemistries, we have found that it will also score the data accurately.

**Q: Which instruments support this chemistry?**

**A:** All Applied Biosystems DNA Analyzers, Genetic Analyzers, as well as the 373 DNA Sequencer are compatible with BigDye v3.1 and v1.1 chemistry.

**Q: What do I need to do to get started?**

**A:** Just the cycle sequencing kits themselves. No instrument calibration or new software is required to use v3.1 or v1.1 chemistry. We recommend that you consult and follow the new protocol manuals.

**Q: What are the plans for the availability of BigDye Terminator v1.0, v2.0, and v3.0 kits?**

**A:** Customers worldwide have adopted our flagship BigDye Terminator v3.1 and v1.1 kits enthusiastically, recognizing the range and magnitude of the benefits they provide. Because of the technological superiority of the BigDye Terminator v3.1 and v1.1 kits, in combination with their overwhelming acceptance by the customer base, we have chosen to discontinue the now redundant BigDye Terminator “v1.0”, v2.0, and v3.0 kits effective June 30, 2003. This streamlining of our chemistry portfolio will better enable us to continue to provide customers with the highest level of product innovation and support. The discontinuation will not affect BigDye<sup>®</sup> Primer, dRhodamine, or BigDye<sup>®</sup> Terminator dGTP kits

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