

BigDye® Direct Cycle Sequencing Kit Frequently Asked Questions (FAQs)

1. What components and quantities are included in the BigDye Direct Cycle Sequencing Kit?

P/N	Kit Component and quantity				
	BigDye® Direct PCR Master Mix	BigDye® Direct Sequencing Mix	BigDye® Direct M13 Forward Primer	BigDye® Direct M13 Reverse Primer	DNA Control
4458689	24 rxn	24 rxn	24 rxn	24 rxn	Sufficient for 200 rxn
4458687	100 rxn	100 rxn	100 rxn	100 rxn	Sufficient for 200 rxn
4458688	1000 rxn	1000 rxn	1000 rxn	1000 rxn	Sufficient for 200 rxn

2. On what platform is the BigDye® Direct Cycle Sequencing Kit supported?

Instrument	OS	Data Collection
3130/XL	XP SP2	3.0 and 3.1
3500/XL (DX as well)	Vista SP1	1.0
3730/XL	XP SP2	3.0 and 3.1

Secondary Analysis Software	Version
Seq A	5.4 and 5.3.1 and 5.3
SeqScape	2.7 and 2.6
Variant Reporter	1.1 and 1.0

3. With what plate format is the BigDye Direct Cycle Sequencing Kit compatible?

96-well and tubes (strip tubes or single tubes).

4. What are the BigDye Direct Cycle Sequencing Kit storage conditions?

Description	Storage Conditions	Stability	Freeze-Thaw -20°C ↔ Ambient
BigDye® Direct PCR Master Mix	-20°C	refer to expiration date	5 cycles
	4°C	refer to expiration date	
BigDye® Direct Sequencing Master Mix	-20°C	refer to expiration date	5 cycles
	4°C	4 weeks	
BigDye® Direct Sequencing Primers (frwd/Rev)	-20°C	refer to expiration date	5 cycles
	4°C	4 weeks	
Control CEPH DNA	-20°C	refer to expiration date	N/A

*if the BigDye® Direct Cycle Sequencing Kit has been left at room temp for >8hrs, it is recommended that the customer do not use the kit.

5. Under what conditions is the DNA stable in each of the BigDye Direct Cycle Sequencing steps?

Description	Storage Conditions	Stability
Formulated PCR reaction mix	Ambient	16 hrs
Amplified PCR	-20°C	4 weeks
	4°C	16 hrs
Formulated Sequencing reaction mix	Ambient	16 hrs
Sequencing product (no clean up)	-20°C	4 weeks
	4°C	16 hrs
Sequencing product (after BDx clean up)	-20°C	10 days
	4°C	10 days
	Ambient	48 hrs

6. Can customers combine other PCR mixes to use with the BigDye Direct Cycle Sequencing Kit?

No, all components of BigDye Direct Cycle Sequencing kit are specially formulated and optimized to work together, and cannot be combined with other PCR master mixes.

7. Can customers use standard gene specific PCR primers?

No, customers are not able to use standard gene specific PCR primers with the BigDye Direct Cycle Sequencing Kit. Customers are required to use universal primers, specifically M13 universal primers. Here is the sequence:

M13 universal sequences for primer design

- M13 forward primer sequence: 5' TGTAACGACGGCCAGT 3'
- M13 reverse primer sequence: 5' CAGGAAACAGCTATGACC 3'

8. Can customers use standard gene specific sequencing primers?

No, customers are not able to use standard gene specific sequencing primers with the BigDye Direct Cycle Sequencing Kit. Customers are required to use the M13 universal primers included in the kit.

9. What is the recommended template DNA concentration, pre-PCR?

The recommended DNA concentration is 4 ng

10. Under what temperature and time conditions is the DNA from the sample stable in the BigDye XTerminator® Purification Kit?

Samples can be stored in BigDye Xterminator Purification Kit, please refer to question #4 for storage conditions.

11. Is it preferable to use fresh PCR product or stored product for use with BigDye Direct Cycle Sequencing Kit? If stored, what temperature conditions should be used?

It is always recommended to use fresh PCR product, but if there is a need to store it, please refer to question #4 for storage conditions.

12. Is it better to store samples dry or in Hi-Di™ Formamide? What is the best temp?

It is recommended to store samples dry at -20°C.

13. Can customers dilute the BigDye Direct Cycle Sequencing Kit?

The BigDye Direct Cycle Sequencing Kit formulation has been optimized to work at full strength. Diluting the product will result in poor results.

14. Is an update to current Data Collection Software or Secondary Analysis Software needed?

Customers will need to download a BigDye Direct Cycle Sequencing Kit Installer to update the mobility files. This can be accessed either through the Applied Biosystems website with the following navigation path:

Applied Biosystems Home > Support > Software Downloads, Patches & Updates > DNA Sequencing > BigDye® Direct Cycle Sequencing Kit

Or, link directly to the following website:

<http://www.appliedbiosystems.com/bigdyedirectinstaller>

15. What Dye Set is used for the Spectral Calibration?

Please use the BigDye Terminator v3.1 Long Read Sequencing Standard to spectrally calibrate the instrument for dye set Z.

16. What can be done if pull-up peaks are experienced, caused by off-scale data?

It is recommended one of the following efforts be tried:

- Reduce the number of PCR cycles by 2-3 cycles
- Re-inject the affected samples
- Optimize injection parameters by reducing time or voltage

For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

© 2011 Life Technologies Corporation. All rights reserved.

The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners.