

PART NO. 4458016 REV. C | PRODUCT INSERT

Part number	Quantity	Component	Storage Conditions
• 4458689	• 24 reactions	BigDye® Direct PCR Master Mix	-20°C until first use, then 4°C
• 4458687	• 100 reactions	BigDye® Direct Sequencing Master Mix	-20°C
• 4458688	• 1000 reactions	BigDye® Direct M13 Fwd Primer	-20°C
		BigDye® Direct M13 Rev Primer	-20°C
		Control DNA CEPH 1347-02, 20 µL, 50 ng/µL	-20°C

Note: For safety and biohazard guidelines, refer to the “Safety” section in the *BigDye® Direct Cycle Sequencing Kit Protocol* (PN 4458040). For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Refer to the *BigDye® Direct Cycle Sequencing Kit Protocol* (PN 4458040) for detailed instructions and troubleshooting. Go to the BigDye® Direct product page at www.appliedbiosystems.com/bigdyedirect to download PDF versions of the protocol and other product documentation.

Prepare and run the PCR reactions

- For each forward or reverse reaction, add to an appropriate reaction plate:

Component	Volume
Genomic DNA (4 ng/µL)	1.0 µL
M13-tailed PCR primers (0.8 µM each) [†]	1.5 µL
BigDye® Direct PCR Master Mix	5.0 µL
Deionized water	2.5 µL
Total volume for each PCR reaction	10.0 µL

[†] The PCR primers must include the M13 forward and reverse sequences.

- Pipet up and down to mix well, seal the plate with adhesive film, then spin the plate briefly.
- Run the reactions in a thermal cycler:

Stage	Veriti® thermal cyclers		9700 thermal cycler	
	Temp	Time	Temp	Time
Hold	95°C	10 min	96°C	5 min
Cycle (35 cycles)	96°C	3 sec	94°C	30 sec
	62°C	15 sec	62°C	45 sec
	68°C	30 sec	68°C	45 sec
Hold	72°C	2 min	72°C	2 min
Hold	4°C	∞	4°C	∞

Prepare and run the cycle sequencing reactions

- Prepare a forward or reverse sequencing reaction mix in a tube once:

Component	Volume
BigDye® Direct Sequencing Master Mix	2.0 µL
One sequencing primer:	1.0 µL
• BigDye® Direct M13 Fwd Primer or	
• BigDye® Direct M13 Rev Primer	
Total volume to add to each sequencing reaction	3.0 µL

- For each sequencing reaction, add 3 µL of the sequencing reaction mix to the appropriate well of amplified DNA.
- Seal the plate with adhesive film, then spin the plate briefly.
- Run the reactions in a thermal cycler:

Stage	Veriti® thermal cyclers		9700 thermal cycler	
	Temp	Time	Temp	Time
Hold	37°C	15 min	37°C	15 min
Hold	80°C	2 min	80°C	2 min
Hold	96°C	1 min	96°C	1 min
Cycle (25 cycles)	96°C	10 sec	96°C	10 sec
	50°C	5 sec	50°C	5 sec
	60°C	75 sec	60°C	4 min
Hold	4°C	∞	4°C	∞

- After the reactions are complete, spin the plate briefly.

Purification of sequencing products

Refer to the *BigDye® Direct Cycle Sequencing Kit Protocol* (PN 4458040) for instructions on purification methods developed specifically for the BigDye® Direct Cycle Sequencing Kit:

- BigDye XTerminator® Purification Kit
- Spin columns or spin plates

Capillary electrophoresis conditions

For spectral calibration, use Dye Set Z and the Sequencing Install Standard, BigDye® Terminator v3.1 Kit.

Refer to your instrument user guide for instructions on setting up and performing the capillary electrophoresis run and refer to the *BigDye® Direct Cycle Sequencing Kit Protocol* (PN 4458040) for instructions on installing the basecaller files and mobility files.

Instrument	Polymer	Array	Basecaller file	Mobility file
3500/3500xl DNA Analyzer	POP-7™ polymer	50 cm	KB_3500_POP7_BDTv3direct.bcc	KB_3500_POP7_BDTv3direct.mob
3730/3730xl DNA Analyzer	POP-7™ polymer	36 cm	KB_3730_POP7_BDTv3direct.bcc	KB_3730_POP7_BDTv3direct.mob
	POP-7™ polymer	50 cm	KB_3730_POP7_BDTv3direct.bcc	KB_3730_POP7_BDTv3direct.mob
3130/3130xl Genetic Analyzer	POP-7™ polymer	36 cm	KB_3130_POP7_BDTv3direct.bcc	KB_3130_POP7_BDTv3direct.mob
	POP-7™ polymer	50 cm	KB_3130_POP7_BDTv3direct.bcc	KB_3130_POP7_BDTv3direct.mob

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