BigDye® Direct Cycle Sequencing Kit

QUICK REFERENCE CARD

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 Sheet (SDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Perform PCR amplification

For optimal results, use high-quality gDNA with no degradation or contaminants. Use gDNA with an A_{260}/A_{280} ratio between 1.7 and 1.9 and appears as a single, high-molecular-weight band (>10 kb).

1. For each forward or reverse reaction, add the components to an appropriate reaction plate:

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

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

2. Pipet up and down to mix well, seal the plate with adhesive film or caps, then spin the plate briefly.

3. 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	∞

4. (Optional) For replicates or controls, assess the quality and quantity of PCR products by running them on an agarose gel.

STOPPING POINT (Optional) Store the amplified DNA at 4°C overnight or at –15°C or –25°C for long-term storage.

Perform cycle sequencing

The minimum quantity of PCR product to use for sequencing is 20 ng.

IMPORTANT! You need to use the BigDye® Direct M13 forward or reverse primers in your BigDye® Direct cycle sequencing reactions.

1. Prepare a forward or reverse sequencing reaction mix in a tube on ice:

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

2. For each sequencing reaction, add 3 µL of the sequencing reaction mix to the appropriate well in the respective forward or reverse reaction plate.
3. Seal the reaction plate with adhesive film or caps, then spin the plate briefly.
4. 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	∞

5. After the cycle sequencing reactions are complete, spin the plate briefly.

STOPPING POINT (Optional) Store the sequencing products at 4°C overnight or at –15°C or –25°C for long-term storage.

Purify the sequencing products

The purification methods described here are optimized for use with the BigDye® Direct Cycle Sequencing Kit at the specified sequencing volumes and are not recommended for other BigDye® products.

- BigDye XTerminator® Purification Kit (page 3)
- Spin columns (page 4)
- Spin plates (page 5)

Purify sequencing products using the BigDye XTerminator® Purification Kit

1. Spin the reaction plate at 100 *x g* for 1 minute, then remove the seal.
2. Prepare a premix with SAM™ Solution and XTerminator® Solution in an appropriately sized tube:

Component	Volume for 1 well	Volume for 96 wells
SAM™ Solution	45 µL	4752 µL
XTerminator® Solution	10 µL	1056 µL
Total volume	55 µL	5808 µL

   a. Add the SAM™ Solution to the tube using a conventional pipette tip.

   Note: If the SAM Solution contains particulates, heat the solution to 37°C and mix to resuspend. Cool to room temperature before using.

   b. Vortex the XTerminator® Solution bulk container at maximum speed for at least 10 seconds, until the solution is homogeneous.

   c. Using a wide-bore pipette tip, aspirate the XTerminator® Solution.

   IMPORTANT! Avoid pipetting from the top of the liquid.

   d. Mix the reagents until homogeneous.

3. Add 55 µL of SAM™ Solution/XTerminator® Solution premix to each well.
4. Seal the plate using MicroAmp® Clear Adhesive Films or a heat seal at 160°C for 1.5 seconds, then verify that each well is sealed:

   Note: For direct injections without a septa mat on the 3730/3730*xl* instrument, only the Heat Seal Film for Sequencing and Fragment Analysis Sample Plates are supported.

5. Vortex the reaction plate for 20 minutes, using the following conditions:

Vortexer	Speed
Digital Vortex-Genie® 2	1800 rpm
IKA MS3 Digital	2000 rpm†
IKA Vortex 3	Setting 5‡
Taitec MicroMixer E-36	Maximum
Union Scientific Vertical Shaker	Setting 100§

† Set the vortexer to Mode B.
‡ Use the maximum setting without allowing the vortexer to move across the bench.
§ Add more plates, if necessary, to meet mass requirements.

6. In a swinging-bucket centrifuge, spin the plate at 1000 × *g* for 2 minutes.

STOPPING POINT (Optional) Store the sample plates sealed with heat seal film or adhesive film for up to 48 hours at room temperature (20 to 25°C) or up to 10 days at 4°C or –20°C.

Purify sequencing products with Centri-SEP™ spin columns

1. Treat the samples with 2.2% sodium dodecyl sulfate (SDS):
   a. Prepare 2.2% SDS in deionized water. This SDS solution is stable at room temperature.
   b. Add 7 µL of water and 2 µL of 2.2% SDS to each 13-µL of completed cycle sequencing reaction to bring the final SDS concentration to 0.2%.
   c. Seal the plate, then mix thoroughly.
   d. Incubate the reaction plate in a thermal cycler:

Temp	Time
98°C	5 min
25°C	10 min

   e. Spin the plate briefly.
2. Hydrate the spin columns:
   a. Gently tap the column to cause the gel material to settle to the bottom of the column.
   b. Remove the upper end cap and add 0.8 mL of deionized water.
   c. Replace the upper end cap and vortex or invert the column a few times to mix the water and gel material.
   d. Allow the gel to hydrate at room temperature for at least 2 hours.

   Note: You can store hydrated columns for a few days at 2–6°C. Longer storage in water is not recommended. Allow columns stored at 2–6°C to warm to room temperature before use.
3. Assemble the spin columns:
   a. Invert or tap the column and allow the gel to settle to remove air bubbles.
   b. Remove the upper end cap first, then remove the bottom cap.
   c. Allow the column to drain completely by gravity.
      Note: If flow does not begin immediately, apply gentle pressure to the column with a pipette bulb.
   d. Insert the column into the wash tube provided.
   e. Spin the column in a microcentrifuge at 730 *x g* for 2 minutes to remove the interstitial fluid.
   f. Remove the column from the wash tube, then insert it into a sample collection tube.
4. Purify the sequencing products using the spin columns:
   a. Carefully transfer the cycle sequencing reaction treated with SDS from its tube to the center of the gel material.
   b. Spin the column in a microcentrifuge at 730 *x g* for 2 minutes to elute the sample into the sample collection tube.
      Note: If you are using a centrifuge with a fixed-angle rotor, place the column in the same orientation as it was in for the first spin. This is important because the surface of the gel will be at an angle in the column after the first spin.
   c. Discard the column.
5. Dry the sample in a vacuum centrifuge for 10–15 minutes without heat, or until dry.

   Note: Do not overdry.

Purify sequencing products with Centri-SEP™ spin plates

1. Treat the samples with 2.2% sodium dodecyl sulfate (SDS):
   a. Prepare 2.2% SDS in deionized water. This SDS solution is stable at room temperature.
   b. Add 7 µL of water and 2 µL of 2.2% SDS to each 13 µL of completed cycle sequencing reaction (0.2% final SDS concentration).
   c. Seal the plate, then mix thoroughly.
   d. Heat the reaction plate then allow the plate to cool to ambient temperature in the thermal cycler:

Temp	Time
98°C	5 min
25°C	10 min

   e. Spin the plate briefly.
2. Prepare the spin plate and perform the purification according to the manufacturer's instructions.

Perform capillary electrophoresis

Refer to your instrument user guide for instructions on setting up and performing the capillary electrophoresis run.

- Use Dye Set Z and the Sequencing Install Standard, BigDye® Terminator v3.1 Kit, to create the BigDye® Direct spectral calibration information.
- Use the BigDye® Direct mobility and calibration files for optimal basecalling with the BigDye® Direct Cycle Sequencing Kit.

  Note: For instructions on installing the mobility and calibration files, see the *BigDye® Direct Cycle Sequencing Kit Protocol* (PN 4458040).

Table 1 Run conditions for 3500/3500*xl* DNA Analyzers

Polymer	Array	Run module	Mobility file
POP-7™ polymer	50 cm	StdSeq50_POP7	KB_3500_POP7_BDTv3direct.mob
POP-7™ polymer	50 cm	FastSeq50_POP7	KB_3500_POP7_BDTv3direct.mob
POP-7™ polymer	50 cm	RapidSeq50_POP7	KB_3500_POP7_BDTv3direct.mob
POP-7™ polymer	50 cm	ShortReadSeq50_POP7	KB_3500_POP7_BDTv3direct.mob
POP-7™ polymer	50 cm	BDX_StdSeq50_POP7	KB_3500_POP7_BDTv3direct.mob
POP-7™ polymer	50 cm	BDX_FastSeq50_POP7	KB_3500_POP7_BDTv3direct.mob
POP-7™ polymer	50 cm	BDX_RapidSeq50_POP7	KB_3500_POP7_BDTv3direct.mob
POP-7™ polymer	50 cm	BDX_ShortReadSeq50_POP7	KB_3500_POP7_BDTv3direct.mob

Table 2 Run conditions for 3130/3130*xl* Genetic Analyzers

Polymer	Array	Run module	Mobility file
POP-7™ polymer	36 cm	RapidSeq36_POP7	KB_3130_POP7_BDTv3direct.mob
POP-7™ polymer	36 cm	UltraSeq36_POP7	KB_3130_POP7_BDTv3direct.mob
POP-7™ polymer	50 cm	StdSeq50_POP7	KB_3130_POP7_BDTv3direct.mob
POP-7™ polymer	50 cm	FastSeq50_POP7	KB_3130_POP7_BDTv3direct.mob
POP-7™ polymer	36 cm	BDX_RapidSeq36_POP7	KB_3130_POP7_BDTv3direct.mob
POP-7™ polymer	36 cm	BDX_UltraSeq36_POP7	KB_3130_POP7_BDTv3direct.mob
POP-7™ polymer	50 cm	BDX_StdSeq50_POP7	KB_3130_POP7_BDTv3direct.mob
POP-7™ polymer	50 cm	BDX_FastSeq50_POP7	KB_3130_POP7_BDTv3direct.mob

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Table 3 Run conditions for 3730/3730*xl* DNA Analyzers

Polymer	Array	Run module	Mobility file
POP-7™ polymer	36 cm	StdSeq50_POP7	KB_3730_POP7_BDTv3direct.mob
POP-7™ polymer	36 cm	RapidSeq36_POP7	KB_3730_POP7_BDTv3direct.mob
POP-7™ polymer	36 cm	TargetSeq36_POP7	KB_3730_POP7_BDTv3direct.mob
POP-7™ polymer	50 cm	LongSeq50_POP7	KB_3730_POP7_BDTv3direct.mob
POP-7™ polymer	50 cm	FastSeq50_POP7	KB_3730_POP7_BDTv3direct.mob
POP-7™ polymer	36 cm	BDX_StdSeq50_POP7	KB_3730_POP7_BDTv3direct.mob
POP-7™ polymer	36 cm	BDX_RapidSeq36_POP7	KB_3730_POP7_BDTv3direct.mob
POP-7™ polymer	50 cm	BDX_LongSeq50_POP7	KB_3730_POP7_BDTv3direct.mob
POP-7™ polymer	50 cm	BDX_FastSeq50_POP7	KB_3730_POP7_BDTv3direct.mob

Available BigDye® Direct Cycle Sequencing Kits

Kit	Part no.
BigDye® Direct Cycle Sequencing Kit, 24 reactions	4458689
BigDye® Direct Cycle Sequencing Kit, 100 reactions	4458687
BigDye® Direct Cycle Sequencing Kit, 1000 reactions	4458688

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