BigDye® Terminator v3.1/ Sequencing Standard Kit 310/377/3130 and 3100 Series Systems

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Insert P/N 4363114 REV A
Printed in USA

For Research Use Only.
Not for use in diagnostic procedures.

This kit contains four vials, each of which contains enough DNA prepared with the BigDye® Terminator v3.1 Cycle Sequencing Kit to perform at least two control reactions on the ABI PRISM® 377 DNA Sequencer 36- or 48-cm well-to-read instruments or eight control reactions on the ABI PRISM® 310 Genetic Analyzer. This kit contains enough DNA prepared with the BigDye® Terminator v3.1 Cycle Sequencing Kit to perform at least four spectral calibrations or four control sequencing runs on the Applied Biosystems 3130 and 3100 Series Systems. The DNA contained in the preparation has been lyophilized to maximize stability.

Control Sequence

An electronic copy of the Control Sequence can be obtained by contacting your local Field Service Engineer.

AATTCCCTGC	AGGCGTGGCT	GCAGCCTGGT	TATGATTACT	GTTAATGTTG	CTACTACTGC	TGACAATGCT	**70**
GCTGCTGCTT	CTCCTCACTG	TCTCCACTTC	CTTGAACAAT	GCGCCGTCAT	GCTTCTTTTG	CCTCCCGCTG	**140**
CTCCAGAAAG	CTAGGCCGCA	GATCAGAACC	ACCACAGTCA	ATATCACCAC	CTTCCTCTTA	TAGATTCGGA	**210**
ATCTCATGAT	AGGGGCTCAG	CCTCTGTGCG	AGTGGAGAGA	AGTTTGCAGG	CGAGCTGAGG	AGCAATTGCA	**280**
GGTGATATGA	TGTGCTCGGC	TCAAGAAGCG	GGCCCGGAGA	GGAAGAAGTC	GTGCCGGGGC	TAATTATTGG	**350**
CAAAACGAGC	TCTTGTTGTA	AACATTGATC	CAACTGGAAT	GTCACTAATG	GCGAATCAAT	ATTCCATAAG	**420**
GCATGATGGT	TGCTCAGAGG	CAGGAGAAGA	GCAACGAATA	CGATCCTATA	AAAGATAAAA	CATAAATAAA	**490**
CAGTCTTGAT	TATATTCTGG	GTATTAAAGC	CACAATCAGA	ACAAATATAT	GCTTTGTATC	TTTTCTTGCC	**560**
TTCTTCATTA	CCAACTGCTT	CCGCGGCCAC	ATTAAGAGAA	CTTGTGGTAA	GATAAGAAGA	TATTTTATTC	**630**
GTTCTGCTGA	CTTGCTGGAT	GTCGGGAAAT	ATTCTGCATT	TGATAAGAGG	CGGTTAATTG	CAGATATAAT	**700**
TGGTAGTGAA	AAGGGTCGTT	GCTATGGTCA	CCGTGAAGCG	AGTACAGCAG	CACAAGAATG	TGTGCCGTTC	**770**
TCAGTTAATA	TTGTTTGAAT	ATGGTAACCT	GTTTTAGTCG	GTTTAAAGGT	AAGAAGATCT	AACCAAAAAC	**840**
AACACTGCAG	TGACTGATTG	TAGTATTTAT	TTTTTTACTT	AATCTTAATT	TTGGTGTAAA	CATCAACGGC	**910**
GCACTTCAAC	CAATACTCCA	ATGTTTTATC	CATCGACATG	ACGTTCGAGA	TAGGGTTGAG	TGTTGTTCCA	**980**
GTTTGGAACA	AGAGTCCACT	ATTAAAGAAC	GTGGACTCCA	ACGTCAAAGG	GCGAAAAACC	GTCTATCAGG	**1050**
GCGATGGCCC	ACTACGTGAA	CCATCACCCA	AATCAAGTTT	TTTGGGGTCG	AGGTGCCGTA	AAGCACTAAA	**1120**
TCGGAACCCT	AAAGGGAGCC	CCCGATTTAG	AGCTTGACGG	GGAAAGCCGG	CGAACGTGGC	GAGAAAGGAA	**1190**
GGGAAGAAAG							**1200**

Preparing and Loading the Sequencing Standard

WARNING! CHEMICAL HAZARD. Hi-Di™ Formamide. Exposure causes eye, skin, and respiratory tract irritation. It is a possible developmental and birth defect hazard. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

For the ABI PRISM® 377 DNA Sequencers

1. Prepare a loading buffer by combining deionized Hi-Di™ Formamide (P/N 4311320) and 25 mM EDTA (pH 8.0) containing 50 mg/mL blue dextran (P/N 402055) in a ratio of 5:1 (Hi-Di™ Formamide: EDTA/blue dextran).
2. Resuspend the Standard in the volume of loading buffer shown in the table below.
3. Vortex thoroughly, then spin briefly in a microcentrifuge.
4. Heat the Standard at 95°C for two minutes to denature. Place on ice until ready to load.
5. Load Standard as described in the table below.
6. Use the appropriate mobility file corresponding to the concentration and type of gel matrix used. Use run modules for Filter Set E when running the ABI PRISM® 377, 377XL, and 377-96 DNA Sequencers.

Instrument (well-to-read, cm)	Comb Size (wells)	Resuspension Volume (µL)	Loading Volume (µL)
36 and 48	36	5	2-3
36 and 48	48	5	1.5
36 and 48	64	6	1-1.5
36 and 48	96	6	1

Creating a matrix using Sequencing Standard on the 377 Sequencer (Macintosh Computer users only).

1. Refer to the ABI PRISM® 377 DNA Sequencer: User's Manual.

For 310 Instruments
The standard protocol requires the use of the Template Suppression Reagent (TSR: P/N 402844) or Hi-Di™ Formamide (P/N 4311320).

1. Add 100 µL of TSR or 100 µL Hi-Di™ Formamide to a tube containing the lyophilized Sequencing Standard.
2. Mix thoroughly on a vortex mixer.
3. Heat at 95°C for 2 minutes then chill on ice.
4. For Hi-Di™ Formamide samples add 10 µL of Standard to 90 µL of Hi-Di™ Formamide.
5. Hold on ice until ready to load on the instrument.
6. Use the appropriate mobility file and run module for Filter Set E corresponding to the type of polymer and capillary length used.

Creating a matrix with Sequencing Standard on the 310 Genetic Analyzer using POP-6™. To create a matrix with POP-4™ on the 310 Genetic Analyzer, it is recommended to use BigDye® Terminator v3.1 Matrix Standards (P/N 4336948).

1. For Windows NT® OS refer to the ABI PRISM® 310 Genetic Analyzer: User Guide (P/N 4317588).
2. For Macintosh® OS refer to the ABI PRISM® 310 Genetic Analyzer: User Guide (P/N 903565).

For 3130 and 3100 Series Systems:
Preparing the Sequencing Standard for a Spectral Calibration:

1. Resuspend one tube of the Sequencing Standard with 170 µL of Hi-Di™ Formamide.
2. Vortex thoroughly, then spin briefly in a microcentrifuge.
3. Heat the Standard at 95°C for 2 minutes to denature and immediately place on ice.
4. Dispense 10 µL of the denatured Standard into a 96-well microtiter plate, wells A1 through H2 for 16 capillaries or A1-D1 for 4 capillaries.
5. Centrifuge the plate to ensure the Standard is positioned at the bottom of the wells.
6. Assemble the plate and place the plate assembly on the autosampler.
7. For specifics on setting up a run, refer to your User's Guide or Getting Started Guide.

Preparing the Sequencing Standard for Sequencing:

1. Resuspend one tube of the Sequencing Standard with 170 µL of Hi-Di™ Formamide.
2. Vortex thoroughly, then spin briefly in a microcentrifuge.
3. Heat the Standard at 95°C for 2 minutes to denature and immediately place on ice.
4. Dispense 10 µL of the denatured Standard into a 96-well microtiter plate, wells A1 through H2 for 16 capillaries or A1-D1 for 4 capillaries.
5. Centrifuge the plate to ensure the Standard is positioned at the bottom of the wells.
6. Assemble the plate and place the plate assembly on the autosampler.
7. Run the samples using the appropriate default Sequencing Module and Dye Set Z.

NOTE: Discard any unused sample that has been resuspended in TSR or Hi-Di™ Formamide after 24 hours.

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