ab185907 – Bisulfite-Seq High Sensitivity Kit (For Illumina®)

Instructions for Use

For carrying out bisulfite conversion, followed by a "post-bisulfite" library preparation process for Illumina® platform-based bisulfite sequencing, all in one kit.

This product is for research use only and is not intended for diagnostic use.

Version 1 Last Updated 1 September 2014

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1. BACKGROUND

DNA methylation occurs by the covalent addition of a methyl group (CH_3) at the 5-carbon of the cytosine ring, resulting in 5-methylcytosine (5-mC). DNA methylation is essential in regulating gene expression in nearly all biological processes including development, growth, and differentiation. Alterations in DNA methylation have been demonstrated to cause a change in gene expression. For example, hypermethylation leads to gene silencing or decreased gene expression while hypomethylation activates genes or increases gene expression. Aberrant DNA methylation is also associated with pathogenesis of diseases such as cancer, autoimmune disorders, and schizophrenia. Thus genome-wide analysis of DNA methylation could provide valuable information for discovering epigenetic markers used for disease diagnosis and potential therapeutic targets.

Several methods such as whole genome bisulfite sequencing (WGBS) or reduced representation bisulfite sequencing (RRBS) are currently used for genome-wide DNA methylation analysis. These methods convert unmethylated cytosine to uracil while 5-mC remains unmodified by the bisulfite treatment. This allows epigenetic differences to be interpreted as genetic differences, which can then be detected by sequencing at single-base resolution and on a genome-wide scale. However, traditional methods to achieve this still do not have practical use because (1) such methods require large amounts of DNA (>1 µg) as input material, which is difficult to prepare from limited biological samples such as tumor biopsy samples, early embryos, embryonic tissues and circulating DNA; (2) such methods require that DNA is first sheared and then ligated to adaptors followed by bisulfite conversion (post-ligation bisulfite conversion). This procedure causes most of the DNA fragments contained in the adaptor-DNA fragment constructs to be broken, and thereby form mono-tagged templates that will be removed during library enrichment. Thus, incomplete coverage and bias occur when performing whole genome bisulfite sequencing; and (3) such methods are time-consuming (2 days). To overcome the weaknesses of these methods,

Abcam offers the Bisulfite-Seq High Sensitivity Kit (For Illumina®). The kit has the following features:

This kit has the following features:

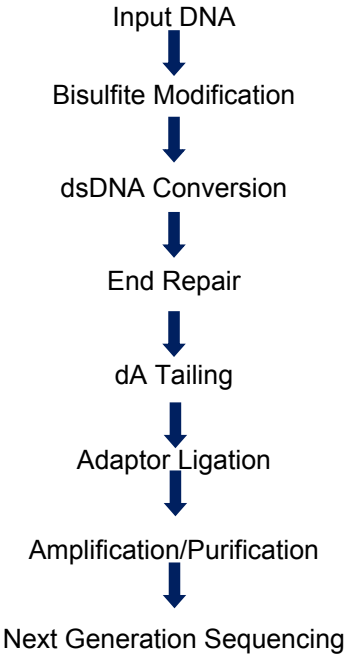
- Innovative method - Allows for simultaneous bisulfite conversion and size-appropriate DNA fragmentation. The bisulfite DNA can be directly ligated to adaptors thereby eliminating the possibility of breaking adaptor-ligated fragments, which often occurs with traditional WGBS and RRBS methods.
- Fast and streamlined procedure - the procedure from DNA bisulfite treatment to PCR amplification can be finished within the same day (<8 hours). Gel-free size selection/purification saves time and prevents handling errors, as well as loss of valuable samples.
- Complete conversion - The innovation reagent composition converts unmethylated cytosine into uracil at a level greater than 99.9%, with negligible inappropriate- or error-conversions of methylcytosine to thymine (<0.1%).
- High sensitivity, efficiency and flexibility- Adaptor ligation after bisulfite treatment eliminates loss of fragments and selection bias, which enables input DNA to be as low as 0.5 ng. The kit can be used for both non-barcoded (singleplexed) and barcoded (multiplexed) DNA library preparation.
- Extremely convenient - the kit contains all the required components for each step of the DNA library preparation process, which are sufficient for bisulfite conversion, ligation, clean-up, size selection, and library amplification, thereby allowing the bisulfite DNA library preparation to be streamlined for the most reliable and consistent results.
- Minimal bias - Ultra HiFi amplification enables achievement of reproducibly high yields of DNA libraries with minimal sequence bias and low error rates.
- Broad sample suitability - Starting materials can be genomic DNA isolated from various tissue/cell samples such as fresh and frozen tissues, cultured cells from a flask or microplate, microdissection samples, paraffin-embedded tissues, biopsy samples, embryonic cells,

plasma/serum samples, and body fluid samples, etc. DNA enriched from various enrichment reactions such as ChIP, MeDIP/hMeDIP, or exon capture may also be used as starting materials.

The Bisulfite-Seq High Sensitivity Kit (For Illumina®) is designed to carry out bisulfite conversion, followed by a "post-bisulfite" library preparation process for Illumina® platform-based bisulfite sequencing, all in one kit. Intended applications include whole genome bisulfite sequencing, oxidative bisulfite sequencing, reduced representative bisulfite sequencing, and various other bisulfite-based next generation sequencing techniques. The optimized protocol and components of the kit allow the DNA to be bisulfite converted and fragmented simultaneously followed by quick non-barcoded (singleplexed) and barcoded (multiplexed) library construction using sub-nanogram quantities of bisulfite converted DNA.

This kit includes all reagents required for a successful preparation of a DNA library by directly using bisulfite-converted DNA generated from a small amount of input DNA (500 pg to 500 ng). In this preparation, DNA is bisulfite converted and fragmented to the appropriate length simultaneously during the bisulfite process. The bisulfite-treated DNA, which is in single stranded form, is then converted to dsDNA and directly used for ligation with BisDNA-specific adaptors that are necessary for amplification and sequencing. The fragments are size selected and purified using MQ binding beads, which allows for quick and precise size selection of DNA. Size-selected DNA fragments are amplified using a high- fidelity PCR Mix which ensures maximum yields from minimum amounts of starting material and provides highly accurate amplification of library DNA with low error rates and minimal bias.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit as given in the table upon receipt.

Observe the storage conditions for individual prepared components in sections 9 & 10.

For maximum recovery of the products, centrifuge the original vial prior to opening the cap.

5. MATERIALS SUPPLIED

Item	12 Tests	24 Tests	Storage Condition (Before Preparation)
Modification Buffer	3 mL	6 mL	RT
Modification Powder	2 vials	4 vials	RT
DNA Binding Solution	6 mL	12 mL	RT
Desulfonation Solution	70 µL	140 µL	RT
Elution Solution	0.5 mL	1 mL	RT
F-Spin Column	15	30	RT
F-Collection Tube	15	30	RT
5X Conversion Buffer	50 µL	100 µL	–20°C
Conversion Enzyme Mix	15 µL	30 µL	–20°C
Conversion Primer	26 µL	52 µL	–20°C
10X End Repair Buffer	40 µL	80 µL	–20°C
End Repair Enzyme Mix	25 µL	50 µL	–20°C
10X dA-Tailing Buffer	40 µL	80 µL	–20°C
Klenow Fragment (3'-5' exo-)	15 µL	30 µL	–20°C
2X Ligation Buffer	250 µL	500 µL	–20°C
T4 DNA Ligase	15 µL	30 µL	–20°C
Adaptors (50 µM)	15 µL	30 µL	–20°C
MQ Binding Beads	1.8 mL	3.6 mL	4°C
2X HiFi PCR Master Mix	160 µL	320 µL	–20°C
Primer U (10 µM)	15 µL	30 µL	–20°C
Primer I (10 µM)	15 µL	30 µL	–20°C
Elution Buffer	1 mL	2 mL	–20°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Vortex Mixer
- Method to assess the quality of DNA library
- Thermocycler
- Centrifuge including desktop centrifuge (up to 14,000 rpm)
- Magnetic stand (96-well format)
- Pipettes and pipette tips
- PCR tubes or plates
- 1.5 mL microcentrifuge tubes
- 100% Ethanol
- Distilled water
- Bisulfite-treated DNA sample

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not use kit or components if it has exceeded the expiration date on the kit labels
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding

8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

9.1 **Modification Solution**

Add 1 mL of Modification Buffer to 1 vial of Modification Powder to generate Modification Solution. Mix by inverting and shaking the vial repeatedly for 3-4 min (trace amount of undissolved Modification Powder may remain, which is normal as Modification Powder is saturated in solution).

Prepared Modification Solution can be stored at -20°C for up to 2 weeks without significant loss of efficiency. For the best results, the mixed solution should be used immediately.

9.2 **Desulfonation Buffer**

Prepare final Desulfonation Buffer by adding 30 µL of Desulfonation Solution to every 1 mL of 90% Ethanol, and mix.

10. SAMPLE PREPARATION

10.1 Input DNA Amount: DNA amount can range from 500 pg to 1 µg per reaction. An optimal amount is 100 ng to 200 ng per reaction. Starting DNA may be in water or in a buffer such as TE. DNA should be of high quality and relatively free of RNA. RNAse I can be used to remove RNA

10.2 DNA Isolation: You can use your method of choice for DNA isolation. Abcam offers a Genomic DNA Isolation Kit (ab65358) for your convenience.

10.3 DNA Storage: Isolated genomic DNA can be stored at 4°C or -20°C until use.

11. ASSAY PROCEDURE

11.1 Bisulfite DNA Modification

11.1.1 For each 0.2 mL PCR tube, add 150 µL of the mixed Modification Solution followed by adding 1-5 µL of sample DNA.

Note: *Check if the sample DNA volume is large and the concentration is lower than 5 ng/µL. If so, it is recommended to concentrate DNA prior to bisulfite treatment.*

11.1.2 Tightly close the PCR tubes and place them in a thermal cycler with heated lid. Program and run the thermal cycler according to the following:

95°C 5 min
65°C 30 min
95°C 5 min
65°C 30 min
95°C 5 min
65°C 60 min
Hold 18-20°C up to 6 h

Meanwhile, insert the number of F-Spin Columns into F-Collection Tubes as needed by your experiment.

11.2 Modified DNA Clean up

11.2.1 Add 250 µL of DNA Binding Solution to each column. Then transfer the samples from each PCR tube (from Step 11.1) to each column containing the DNA Binding Solution. Centrifuge at 12,000 rpm for 45 sec. Remove columns from collection tubes and discard the flowthrough. Place columns back into collection tubes.

11.2.2 Add 250 µL of 90% Ethanol to each column. Centrifuge at 12,000 rpm for 45 sec.

11.2.3 Add 100 µL of the final Desulfonation Buffer (Desulfonation Solution and 90% Ethanol mixture) to each column. Allow columns to sit for 15 min at room temperature, then centrifuge at 12,000 rpm for 45 sec. Remove columns from collection tubes and discard the flowthrough. Place columns back into collection tubes.

11.2.4 Add 250 µL of 90% Ethanol to each column. Centrifuge at 12,000 rpm for 45 sec. Remove columns from collection tubes and discard the flowthrough. Place columns back into collection tubes. Add 250 µL of 90% ethanol to each column again and centrifuge at 12,000 rpm for 45 sec.

11.2.5 Insert each column into a new 1.5 mL tube. Add 10 µL of Elution Solution directly to each column's filter membrane. Centrifuge at 12,000 rpm for 60 sec to elute converted DNA.

11.2.6 The Modified DNA is now ready to use for post-bisulfite DNA library preparation, or storage at or below -20°C for up to 3 months. The peak size of converted DNA is 250-300 bp.

Note: *To ensure the DNA is properly modified, we recommend checking the bisulfite-treated DNA by real time methylation-specific PCR (MS-PCR).*

11.3 dsDNA Conversion Reaction

11.3.1 Prepare dsDNA Conversion reaction in a 0.2 mL PCR tube according to Table below:

Component	Sample (µL)
Bisulfite DNA	10(100-200ng)
5X Conversion Buffer	4
Conversion Primer	2
Distilled Water	3
Total Volume	19

11.3.2 Mix and incubate for 5 min at 95°C in a thermal cycler followed by 5 min at 4°C or on ice.

11.3.3 Add 1 µL of Conversion Enzyme Mix to the reaction tube. Mix and incubate for 60 min at 37°C in a thermocycler.

Note: *The optimal amount of input DNA should be 100 ng to 200 ng and eluted volume after bisulfite treatment should be < 20 µL.*

11.4 **Clean-Up of Converted dsDNA**

11.4.1 Resuspend MQ Binding Beads by vortex.

11.4.2 Add 36 µL of resuspended beads to the PCR tube of end repair reaction. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.

11.4.3 Incubate for 10 minutes at room temperature to allow DNA to bind to the beads.

11.4.4 Put the PCR tube on an appropriate magnetic stand until the solution is clear (about 2 minutes). Carefully remove and discard the supernatant. Be careful not to disturb or discard the beads that contain DNA.

11.4.5 Keep the PCR tube in the magnetic stand and add 200 µL of freshly prepared 80% ethanol to the tube. Incubate at room temperature for 1 min, and then carefully remove and discard the ethanol.

11.4.6 Repeat Step 11.4.5 one time for a total of two washes.

11.4.7 Open the cap of the PCR tube and air dry beads for 10 minutes while the tube is on the magnetic stand.

11.4.8 Resuspend the beads in 12 µL Elution Buffer, and incubate at room temperature for 2 minutes to release the DNA from the beads.

11.4.9 Capture the beads by placing the tube in the magnetic stand for 2 minutes or until the solution is completely clear.

11.4.10 Transfer the clear solution to a new 0.2 mL PCR tube for the End Repair reaction.

11.5 DNA End Repairing

11.5.1 Prepare the End Repair reaction in a 0.2 mL PCR tube according to Table below:

Component	Sample (µL)
Converted dsDNA (from Step 11.4)	11-12
10X End Repair Buffer	2
End Repair Enzyme Mix	1
Distilled Water	5-6
Total Volume	20

11.5.2 Mix and incubate for 30 min at 20°C in a thermocycler.

11.6 Clean Up of End Repair DNA

11.6.1 Resuspend MQ Binding Beads by vortex.

11.6.2 Add 36 µL of resuspended beads to the PCR tube of end repair reaction. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.

11.6.3 Incubate for 10 minutes at room temperature to allow DNA to bind to beads.

11.6.4 Put the PCR tube on an appropriate magnetic stand until the solution is clear (about 2 minutes). Carefully remove and discard the supernatant. Be careful not to disturb or discard the beads that contain DNA.

11.6.5 Keep the PCR tube in the magnetic stand and add 200 µL of freshly prepared 90% Ethanol to the tube. Incubate at room temperature for 1 min, and then carefully remove and discard the ethanol.

11.6.6 Repeat Step 11.6.5 one time for a total of two washes.

11.6.7 Open the cap of the PCR tube and air dry beads for 10 minutes while the tube is on the magnetic stand.

11.6.8 Resuspend the beads in 12 µL Elution Buffer, and incubate at room temperature for 2 minutes to release the DNA from the beads.

11.6.9 Capture the beads by placing the tube in the magnetic stand for 2 minutes or until the solution is completely clear.

11.6.10 Transfer clear solution to a new 0.2 mL PCR tube for dA-tailing reaction.

11.7 DNA dA Tailing

11.7.1 Prepare the dA Tailing reaction in a 0.2 mL PCR tube according to Table below:

Component	Sample (µL)
End repaired DNA(Step 11.6)	12
10X dA-tailing Buffer	1.5
Klenow Fragment (3'-5' exo-)	1
Distilled Water	0.5
Total Volume	15

11.7.2 Mix and incubate for 30 min at 37°C followed by 10 min at 75°C in a thermocycler (without heated lid).

11.8 Adaptor Ligation

11.8.1 Prepare the Adaptor Ligation reaction in a 0.2 mL PCR tube according to Table below:

Component	Sample (µL)
End repaired/dA-tailing DNA(Step 11.7)	15
2X Ligation Buffer	17
T4 DNA Ligase	1
Adaptors	1
Total Volume	34

11.8.2 Mix and incubate for 10 min at 25°C in a thermocycler (without heated lid).

Note: *(1) The pre-annealed adapters included in the kit are suitable for both non-barcoded (singleplexed) and barcoded (multiplexed) DNA library preparation and are fully compatible with Illumina® platforms. (2) If using adaptors from other suppliers (both single-end and barcode adaptors), make sure they are compatible with Illumina® platforms and add the correct amount (final concentration 1.5-2 µM, or according to the supplier's instruction).*

11.9 **Size Selection of Ligated DNA (Optional)**

If the starting DNA amount is less than 200 ng, size selection is not recommended and alternatively, clean-up of ligated DNA can be performed prior to PCR amplification according to 11.10. protocol – “Clean-Up of Ligated DNA”

11.9.1 Resuspend MQ Binding Beads by vortex.

11.9.2 Add 14 µL of resuspended MQ Binding Beads to the tube of ligation reaction. Mix well by pipetting up and down at least 10 times.

11.9.3 Incubate for 5 minutes at room temperature.

11.9.4 Put the tube on an appropriate magnetic stand until the solution is clear (about 2 minutes). Carefully transfer the supernatant containing DNA to a new tube (Caution: Do not discard the supernatant). Discard the beads that contain the unwanted large fragments.

11.9.5 Add 10 µL of re-suspended beads to the supernatant, mix well and incubate for 5 minutes at room temperature.

11.9.6 Put the PCR tube on an appropriate magnetic stand until the solution is clear (about 2 minutes). Carefully remove and discard the supernatant. Be careful not to disturb or discard the beads that contain DNA.

11.9.7 Keep the PCR tube in the magnetic stand and add 200 µL of freshly prepared 80% Ethanol to the tube. Incubate at room

temperature for 1 min, and then carefully remove and discard the ethanol.

11.9.8 Repeat Step 11.9.7 one time, for a total of two washes.

11.9.9 Open the PCR tube cap and air dry beads for 10 minutes while the tube is on the magnetic stand.

11.9.10 Resuspend the beads in 12 µL Elution Buffer, and incubate at room temperature for 2 minutes to release the DNA from the beads.

11.9.11 Capture the beads by placing the tube in the magnetic stand for 2 minutes or until the solution is completely clear.

11.9.12 Transfer clear solution to a new 0.2 mL PCR tube for PCR amplification.

11.10 **Clean-Up of Ligated DNA**

11.10.1 Resuspend MQ Binding Beads by vortex.

11.10.2 Add 34 µL of resuspended beads to the PCR tube of ligation reaction. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.

11.10.3 Incubate for 5 minutes at room temperature to allow DNA to bind to beads.

11.10.4 Put the PCR tube on an appropriate magnetic stand until the solution is clear (about 2 minutes). Carefully remove and discard the supernatant. Be careful not to disturb or discard the beads that contain DNA.

11.10.5 Keep the PCR tube in the magnetic stand and add 200 µL of freshly prepared 80% ethanol to the tube. Incubate at room temperature for 1 min, and then carefully remove and discard the Ethanol.

11.10.6 Repeat Step 11.10.5 two times for a total of three washes.

11.10.7 Open the PCR tube cap and air dry beads for 10 minutes while the tube is on the magnetic stand.

11.10.8 Resuspend the beads in 12 µL Elution Buffer, and incubate at room temperature for 2 minutes to release the DNA from the beads.

11.10.9 Capture the beads by placing the tube in the magnetic stand for 2 minutes or until the solution is completely clear.

11.10.10 Transfer 11 µL of clear solution to a new 0.2 mL PCR tube for PCR amplification.

11.11 **Library Amplification**

11.11.1 Prepare the PCR Reactions:

Thaw all reaction components including master mix, DNA/RNA free water, primer solution and DNA template. Mix well by vortexing briefly. Keep components on ice while in use, and return to -20°C immediately following use. Add components into each PCR tube/well according to the following table.

Component	Sample (µL)
HiFi PCR Master Mix (2X)	12.5
Primer U	1
Primer I	1
Adaptor Ligated DNA	10.5
Total Volume	25

Important Note: *Use of Primer I included in the kit will generate a singleplexed library. For multiplexed library preparation, replace Primer I with user-defined barcodes (Illumina® compatible) instead of Primer I.*

11.11.2 Program the PCR Reactions:

Place the reaction plate in the PCR instrument and set the PCR conditions as follows:

Cycle Step	Temp (˚C)	Time (seconds)	Cycle #
Activation	98	30	1
Cycling	98	20	Variable*
	55	20	
	72	20	
Final Extension	72	120	1

Note: *PCR cycles may vary depending on the input DNA amount. In general, use 12 PCR cycles for 200 ng, 13 cycles for 100 ng, 15 cycles for 50 ng, 17 cycles for 10 ng, and 22 cycles for 1 ng DNA input. Further optimization of PCR cycle number may be required by the end user.*

11.12 **Clean-up Amplified Library DNA**

11.12.1 Resuspend MQ Binding Beads by vortex.

11.12.2 Add 25 µL of resuspended beads to the PCR tube of amplification reaction. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.

11.12.3 Incubate for 5 minutes at room temperature to allow DNA to bind to the beads.

11.12.4 Put the PCR tube on an appropriate magnetic stand until the solution is clear (about 2 minutes). Carefully remove and discard the supernatant. Be careful not to disturb or discard the beads that contain DNA.

11.12.5 Keep the PCR tube in the magnetic stand and add 200 µL of freshly prepared 80% Ethanol to the tube. Incubate at room temperature for 1 min, and then carefully remove and discard the ethanol.

11.12.6 Repeat Step 11.10.5 two times for total of three washes.

11.12.7 Open the PCR tube cap and air dry beads for 10 minutes while the tube is on the magnetic stand.

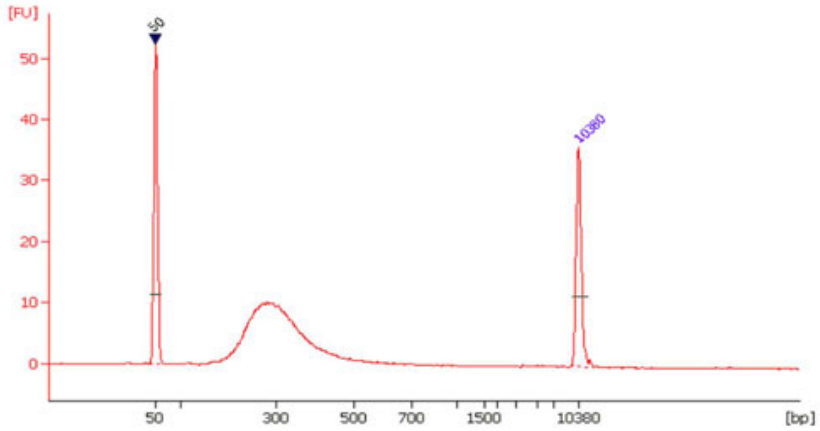
11.12.8 Resuspend the beads in 12 µL Elution Buffer, and incubate at room temperature for 2 minutes to release the DNA from the beads.

11.12.9 Capture the beads by placing the tube in the magnetic stand for 2 minutes or until the solution is completely clear.

11.12.10 Transfer 12 µL of clear solution to a new 0.2 mL PCR tube.

12. ANALYSIS

Typical Results



Size distribution of library fragments. Post-bisulfite DNA library was prepared from 10 ng of input DNA using ab185907.

13. TROUBLESHOOTING

Problem	Cause	Solution
DNA is Poorly Modified	Poor DNA quality (DNA is severely degraded)	Check if the sample DNA 260/280 ratio is between 1.8-1.9 and if DNA is degraded by running a gel. Ensure that RNA is removed by RNase treatment
	Too little DNA or too much DNA (i.e., < 500 pg or >1 µg)	Increase or decrease input DNA to within the correct range, or to the optimal amount of 100-200 ng
	Temperature or thermal cycling condition is incorrect	Check for appropriate temperature or thermal cycling conditions
	Insufficient DNA clean-up	Ensure that 30 µL of Desulfonation Solution is added into every 1 mL of 90% ethanol in Step 11.2.3

Elute Contains Little or No DNA	Poor input DNA quality (degraded)	Check if DNA is degraded by running a gel.
	DNA Binding Solution is not added into the sample	Ensure that DNA Binding Solution is added in Step 11.2.1
	Concentration of ethanol solution used for DNA clean-up is not correct	Use 90% ethanol for DNA clean-up
	Sample is not completely passed through the filter membrane of column	Centrifuge for 1 min at 12,000 rpm or until the entire sample has passed through the filter membrane
Low yield of library	Insufficient amount of bisulfite DNA	To obtain the best results, the optimized amount of input DNA for bisulfite treatment should be 100-200 ng
	Improper reaction conditions at each reaction step	Check if the reagents are properly added and incubation temperature and time are correct at each reaction step including Adaptor Ligation, Size Selection and Amplification
	Improper storage of the kit	Ensure that the kit has not exceeded the expiration. Standard shelf life, when stored properly, is 6 months from date of receipt

Unexpected peak size of trace: Presence of <150 bp adaptor dimers or presence of larger fragments than expected	Improper ratio MQ Binding Beads to DNA volume in size selection	Check if the correct volume of MQ Binding Beads is added to DNA solution accordingly. Proper ratios should remove the fragments with unexpected peak size. Ex: use 0.8X MQ Binding Beads to remove fragments below 150 bp or to remove fragments above 500 bp follow the protocol according to steps 1-12 of 11.9. Size Selection
	Insufficient ligation	Too much and too little input DNA may cause insufficient ligation, which can shift the peak size of the fragment population to be shorter or larger than expected. Make sure that the ligation reaction is properly processed using the proper amount of input DNA
	Over-amplification of library	PCR artifacts from over-amplification of the library may cause the fragment population to shift higher than expected. Make sure to use proper PCR cycles to avoid this problem

14. NOTES

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